Fusarium stem rot of vanilla in north Sulawesi

by Arthur Pinaria 25

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Abstracts of Presentations

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Integrating morphological and molecular characterization for the identification of *Pythium* species: The case of *Pythium christmatum* from fraser fir and *Pythium pseudointermedium* from corn. Z. G. ABAD (1,2), J. Phillips (1,2), and J. A. Abad (2). (1) Plant Pathogen Identification Laboratory; (2) Dept. Plant Pathology, North Carolina State University, Raleigh, NC. Phytopathology 94:S1. Publication no. P-2004-0001-AMA.

The Genus *Pythium* with over 200 reported species is an exceptional group of straminipiles in terms of ecology and plant pathology. Many *Pythium* spp. occupy a high level of niche diversity that cannot be surpassed by any other straminipile or fungi. From our collection of isolates, two heterothallic putative new species have been identified. *Pythium christmatum*, isolated from fraser fir, produces sporangia spherical, pyriform, ellipsoid, terminal and intercalary. *Pythium pseudointermedium*, isolated from corn, produces spherical, terminal and intercalary sporangia as well as cylindrical intercalary sporangia. Most morphological features of both species do not fit with descriptions of any described taxa. The phylogenetic analyses of the ITS rDNA region from our two isolates along with 120 other *Pythium* spp. (PPIL data base) support that both organisms are in fact different species. *Pythium christmatum* is in the same cluster with *P. macrosporum* and *P. suppressorium*. *Pythium pseudointermedium* groups with *P. intermedium*. Sequences of the ITS rDNA region of both species are different and have been deposited at the GenBank. Based on the integration of morphological and ITS rDNA sequence analyses, we conclude that these are putative new species.

Comparison of sequences amplified by N gene and universal *Tospovirus* primers. J. A. ABAD (1), J. Speck (1), A. M. Harness (2), M. D. Bandla (2), and J. W. Moyer (1). (1) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC; (2) Agdia Inc., Elkhart, IN. Phytopathology 94:S1. Publication no. P-2004-0002-AMA.

Tospoviruses (Genus *Tospovirus*, Family *Bunyaviridae*) have emerged as one of the most important causes of viral diseases in cultivated plants worldwide. Precise identification of viruses is vital for disease diagnosis to impede dissemination and to prevent epidemics. Most assays utilize RT-PCR targeting the N gene for detection and genotyping of tospoviruses. We investigated the use of universal Tospoprimers (AGDIA, Elkhart, IN), which amplify a 415 nt fragment of the viral RNA-dependent RNA polymerase gene (L RNA), and determined that they equally differentiate *Tospovirus* species when compared with the use of N gene (S RNA) specific primers. Phylogenetic analyses of the L RNA segment (130 deduced amino acids from 35 sequences generated by Tospoprimers in this work and 5 from databanks) and the N gene segment (255 deduced amino acids from 35 sequences from this work and 20 from databanks) revealed seven clusters corresponding to seven different *Tospovirus* species including *Tomato spotted wilt virus*, *Impatiens necrotic spot virus*, *Iris yellow spot virus*. The amino acid sequence identities ranged from 59% to 84% and 30% to 85% for the L RNA and N gene segments, respectively. The variability within species is being analyzed. In conclusion, identical grouping of species by using both primer sets shows the suitability of Tospoprimers to identify tospoviruses to the species level.

The abstracts are published as submitted. They were formatted but not edited at the APS headquarters office.

Assessing root health by a soil bioassay with beans as an indicator of soil health. G. S. ABAWI (1), J. W. Ludwig (1), and C. H. Petzoldt (2). (1) Plant Path. Dept.; (2) IPM Program, NYSAES, Cornell Univ., Geneva, NY 14456. Phytopathology 94:S1. Publication no. P-2004-0003-AMA.

The emerging concept of soil health deals with integrating the soil physical, chemical and biological properties for improving soil quality and crop productivity in a sustainable and environmentally friendly manner. Practical and informative assessments of soil biological properties remain a challenge. Healthy and productive soils are expected to contain high populations of diverse beneficial microbial communities and low populations and diversity of parasitic soil organisms. We have successfully used a soil bioassay with snap beans as a measure of a general soil suppressive capacity of soils to root pathogens. The latter was conducted in collaboration with the Soil Health Program Work Team for vegetable production systems at Cornell. The protocol involves thoroughly mixing each composite soil sample, planting the soil in 2 or more 10-cm clay pots, planting 7 seeds/pot and growing for 4-6 weeks in a greenhouse. Washed roots were then rated for root rot severity (RRS, root health) on a scale of 1 (no visible disease symptoms) to 9 (>75% of roots are affected, reduced in size and at different stages of decay). For example, the RRS ratings of bean roots grown in soils from 9-year-old plots maintained under conventional, organic, present-IPM, and future-IPM vegetable production systems (IPM Program, Cornell) averaged 7.3, 4.1, 3.3, and 2.7, respectively in 2002. Similar results were obtained in 2003. Observed differences in root health status in the large number of soils tested will be related to other soil properties as well as crop yield and quality.

Effect of heat stress on aflatoxin and fumonisin production in corn (maize, *Zea mays*) in Arkansas. H. K. ABBAS (1), W. T. Shier (2), and R. D. Cartwright (3). (1) USDA-ARS, CG&PRU, Stoneville, MS; (2) College of Pharmacy, University of Minnesota, Minneapolis, MN; (3) Cooperative Extension Service, University of Arkansas, Little Rock, AR. Phytopathology 94:S1. Publication no. P-2004-0004-AMA.

A severe infestation by aflatoxin-producing fungi diminished the food quality of the southern US corn (maize) crop in 1998. Commercial corn hybrids planted at the same and other locations in Arkansas (21 in 1998; 29 in 1999; 15 in 2001; some planted multiple years) were evaluated for resistance to mycotoxin contamination from natural infection by *Fusarium* spp. and *Aspergillus* spp. At harvest, kernel corn samples were evaluated for the presence of aflatoxins and fumonisins. In 1998, samples from all hybrids exceeded 20 ppb aflatoxin (range: 21-699 ppb) and 2 ppm fumonisins (23-79 ppm), the maximum levels permitted for some uses by United States Food and Drug Administration guidelines. In 1999 aflatoxin levels ranged from none detected in most hybrids to 255.3 ppb, and fumonisin levels from 0.3-8.1 ppm. In 2001 the fumonisin levels were very high in all hybrids (range: 8-83.6 ppm), whereas aflatoxin levels were low (<5 in most hybrids, ranging to 131 ppb). The presence of aflatoxin (AFB1 and AFB2) in samples was confirmed by TLC and LC/APCI/MS and fumonisins (FB1, FB2, FB3, FB4 and FC4) by LC/ESI/MS. Arkansas experienced unusually high day and nighttime temperatures in 1998, but nearly normal temperatures in 1999 and 2001. The results are consistent with heat stress having an important affect on mycotoxin production in corn by *A. flavus* and *Fusarium* spp.

Novel use of fish emulsion as a disease control product: Management of soilborne and foliar plant pathogens. P. A. ABBASI, K. L. Conn, and G. Lazarovits. SCPFRC, Agriculture and Agri-Food Canada, 1391 Sandford St. London, ON N5V 4T3. Phytopathology 94:S2. Publication no. P-2004-0005-AMA.

Soil amendments provide a simple, reduced risk, and cost competitive management of soilborne plant pathogens. They can provide effective disease control while increasing, but not eliminating, populations of beneficial microbes. Fish emulsion is a liquid by-product of the processing of menhaden fish into fish meal. We have demonstrated that fish emulsion added to peat-based substrate or soil acts as a disease control product. Effective reductions in incidence and severity of seedling damping-off diseases caused by *Pythium* and *Rhizoctonia* were obtained in both peat-based mix and soil. The disease protection increased with the length of incubation of the organic material in the substrate suggesting that disease control was biologically-based. In micro-plot experiments, fish emulsion amendment to various soils from commercial potato fields with low to high scab levels also reduced potato scab severity in some soils and increased yield in all soils. We also found that foliar sprays of diluted solutions of fish emulsion can reduce bacterial spot severity on tomatoes and peppers while increasing the fruit yield. Fish emulsion not only acted as a disease management product but was also found to be an excellent source of fertilizer.

Variability of Kentucky bluegrass cultivars' field reactions to necrotic ring spot in WI. S. Abler, J. Gregos, and G. JUNG. University of Wisconsin, Department of Plant Pathology, Madison, WI. Phytopathology 94:S2. Publication no. P-2004-0006-AMA.

Kentucky bluegrass (*Poa pratensis* L.) is an important cool season turfgrass species that is used extensively in the Midwest for home lawns, golf courses, and athletic fields. There are hundreds of Kentucky bluegrass cultivars available to consumers, however, there are no reports of their field reaction to necrotic ring spot (NRS) incited by the root and crown-infecting fungus *Ophiosphaerella korrae*. Therefore, our study was initiated to evaluate 100 commercial cultivars and experimental lines of Kentucky bluegrass for their susceptibility to a WI isolate of *O. korrae* obtained from Dr. Ned Tisserat of Kansas State University. The cultivars were established in 5 × 5 ft plots in a randomized complete block design with three replications. The center of each plot was artificially inoculated in November, 2001 by removing three soil plugs, inserting inoculum, and replacing the plugs. Initial symptoms of NRS were noticed in August of 2002, while distinct "frog-eye" symptoms were first noticed in July of 2003. Visual ratings (0-5) of disease severity were recorded in August, 2002 and July, 2003. The number of individual "frog-eyes" per plot was also recorded in July, 2003. There were significant differences in disease expression among the cultivars of Kentucky bluegrass. In most plots, the disease symptoms were limited to the center, inoculated area. However, some plots had diseased patches throughout the plot. Furthermore, the susceptibility of the cultivars used in this study showed a strong correlation with the NRS rating for the 2000 NTEP Kentucky bluegrass trial in WI.

Molecular characterization of two viruses involved in the grapevine leafroll complex. N. Abou Ghanem-Sabanadzovic, S. Sabanadzovic, A. ROWHANI, and D. Golino. Department of Plant Pathology, University of California, Davis, CA 95616. Phytopathology 94:S2. Publication no. P-2004-0007-AMA.

A consistent genomic portion comprising the ORFs encoding for partial viral replicase and complete HSP70 homologue, p55, coat protein and p23 was obtained for Grapevine leafroll-associated virus 4 (GLRaV-4) from the original accession LR106. Similar part of genome was also sequenced for GLRaV-6 isolated from an ELISA-positive source. Sequence analysis has shown that the 2 viruses are similar and more closely related to GLRaVs-5 and -9. The level of amino acid identity was almost similar independent of putative products used for comparison and varied from 80 to 85%. Similar degree of identity was also observed when comparing the corresponding regions of GLRaVs-5 and -9. All four viruses shared highly conserved 3′ termini of viral coat protein gene. In vitro expression of viral coat proteins is under way in order to investigate the serological correlation among these viruses.

Preliminary molecular data on a putative new grapevine leafroll associated virus. N. ABOU GHANEM-SABANADZOVIC, S. Sabanadzovic, and A. Rowhani. Department of Plant Pathology, University of California, Davis, CA 95616. Phytopathology 94:S2. Publication no. P-2004-0008-AMA.

Symptoms resembling leafroll disease were observed on a grapevine cv. Carnelian. The disease was confirmed on the leafroll-specific indicator host, *Vitis vinifera* cv. Cabernet Franc. The diseased grapevine repeatedly tested negative by RT-PCR and/or ELISA for any of the known Grapevine leafroll associated viruses (GLRaVs). Analysis of infected tissue has shown the presence of multiple dsRNA bands, similar to those of GLRaVs. Purified dsRNAs were used as template for cDNA cloning and sequencing. The partial sequence data obtained indicated that the virus present in diseased Carnelian has genome organization similar to GLRaVs-4, -5, -6 and -9 and seemed to be a new member of the family *Closteroviridae*. Further study is under way in order to clarify its taxonomic identity.

Detection of the *Crinivirus*, *Cucurbit yellow stunting disorder virus* Using antibodies produced against its recombinant coat protein. Y. ABOU-JAWDAH and H. Hourani. Department of Plant Sciences, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Beirut, Lebanon. Phytopathology 94:S2. Publication no. P-2004-0009-AMA.

Cucurbit yellow stunting disorder virus (CYSDV), family *Closteroviridae* and genus *Crinivirus*) is transmitted by the whitefly *Bemisia tabaci* and causes a major disease of cucurbits in the Middle East and Mediterranean Europe. More recently CYSDV was reported in Portugal, Morocco and North America. At present no serological kit is available for its detection. CYSDV coat protein (CP) gene was cloned into a bacterial expression vector, expressed as a fusion protein with an N-terminal hexa-histidine tag, purified by affinity chromatography and used as an antigen to produce CYSDV CP polyclonal antiserum. The resulting antiserum was successfully assayed in serological tests. Tissue blot immunoassay (TBIA) provided a fast, sensitive and reliable test, showed the virus localization in the phloem tissue and is recommended for large-scale surveys, while indirect ELISA is recommended for determining virus concentration.

Susceptibility of tubers from potato varieties and advanced breeding lines to different genotypes of *P. infestans* at three storage temperatures. F. M. ABU-EL SAMEN (1), D. S. Douches (2), R. Hammerschmidt (1), and W. W. Kirk (1). (1) Plant Pathology; (2) CSS, Michigan State University, E. Lansing, MI 48824. Phytopathology 94:S2. Publication no. P-2004-0010-AMA.

This study was carried out to evaluate tuber susceptibility of potato varieties and advanced breeding lines (ABL) which demonstrated acceptable levels of resistance to foliar late blight infection. Potato tubers from two varieties and eight ABL developed in four states (MI, MN, ND, and WI) were inoculated with five genotypes of *Phytophthora infestans*. Tubers were incubated at 3, 7 and 10°C for 30 days post-inoculation. Tuber rot was evaluated by measuring the average reflective intensity (ARI) of diseased tissue from three cut surfaces of each tuber. Tuber rot severity was expressed relative to the ARI of the non-inoculated treatments for each variety/ABL. Average tuber rot severity was lowest at 3°C in all varieties/ABL and *P. infestans* genotypes combinations. Tuber rot severity was greatest at 10°C in most variety/ABL and *P. infestans* genotype combinations. However, tuber rot severity was greater at 7°C compared to 10°C in some variety/ABL and *P. infestans* genotype combinations. These results suggest that different *P. infestans* genotypes and varieties/ABL have different temperature optima for tuber rot. The US-8 genotype caused greatest tuber rot followed by US-1, US-14, US-6 and US-11, respectively. Some potato varieties/ABL with foliar resistance exhibited susceptibility in their tubers to *P. infestans*. These results support the hypothesis that foliar and tuber resistance is not strongly correlated.

Quantification of *Mycosphaerella graminicola* in wheat by real-time PCR. T. B. ADHIKARI (1), B. Balaji (2), J. Breeden (1), J. M. Anderson (1), and S. B. Goodwin (1). (1) USDA-ARS, Purdue University, West Lafayette, IN 47907; (2) Department of Agronomy, Purdue University, West Lafayette, IN 47907. Phytopathology 94:S2. Publication no. P-2004-0011-AMA.

Septoria tritici blotch, caused by *Mycosphaerella graminicola*, is a serious fungal disease of wheat worldwide. Developing wheat cultivars resistant to *M. graminicola* is complicated by the long latent period of the disease, environmental effects on symptom expression, and variation among evaluators in the use of disease-scoring systems. To circumvent these problems, we used quantitative real-time PCR to calculate the biomass of *M. graminicola* in inoculated resistant and susceptible wheat cultivars. A set of specific primers was designed from beta-tubulin gene sequences, which amplified a PCR product of 100 bp from pure cultures and infected wheat leaves but not the uninoculated controls. The real-time PCR analysis showed that, by 12 d after inoculation, biomass of *M. graminicola* and disease severity were highly correlated and significantly less in two resistant cultivars compared to the susceptible controls. Real-time PCR appears to be a valuable tool for discriminating between septoria-resistant and susceptible lines of wheat.

Real-time PCR analysis of genes expressed during wheat-*Mycosphaerella graminicola* interactions. T. B. ADHIKARI (1), B. Balaji (2), J. Breeden (1), L. Crane (1), J. M. Anderson (1), and S. B. Goodwin (1). (1) USDA-ARS, Purdue University, West Lafayette, IN 47907; (2) Department of Agronomy,

Purdue University, West Lafayette, IN 47907. Phytopathology 94:S3. Publication no. P-2004-0012-AMA.

A previous large-scale cDNA-AFLP profiling experiment to determine global gene-expression responses of wheat against two fungi, two viruses and Hessian fly identified many genes with differential expression specific to each interaction. We used the interaction between wheat and the fungus *Mycosphaerella graminicola* at several time points after inoculation to further test whether expression of selected host genes was altered in response to the pathogen. Thirty genes that were up regulated in the profiling experiment, including phenylalanine ammonia lyase, heat-stress genes, transcription factors, and peroxidases plus some with no useful annotation, were chosen for the study. Analysis of these genes by quantitative real-time PCR confirmed increased expression in the resistant cultivars compared to the susceptible controls. However, expression levels of each gene varied by cultivar. The findings will be discussed in relation to gene expression incited by other pests and pathogens.

Characterization of a new tobamovirus species from hibiscus. S. ADKINS (1), I. Kamenova (1), and D. J. Lewandowski (2). (1) USDA-ARS-USHRL, Fort Pierce, FL 34945; (2) Univ. of Florida, CREC, Lake Alfred, FL 33850. Phytopathology 94:S3. Publication no. P-2004-0013-AMA.

A new tobamovirus species, *Hibiscus latent Fort Pierce virus* (HLFPV) was recently isolated from the malvaceous plant hibiscus (*Hibiscus rosa-sinensis*) in the Florida landscape. Virion morphology and genome organization are typical of tobamoviruses. The experimental host range of HLFPV is mostly limited to the *Malvaceae*, which includes economically important ornamental crops like hibiscus, in addition to fiber and food crops such as cotton, kenaf and okra. Another new tobamovirus species, *Hibiscus latent Singapore virus* (HLSV), was recently reported infecting hibiscus in Singapore. Sequence comparisons indicate that HLFPV is distinct from but related to HLSV. The coat protein (CP) genes and deduced CPs are 68% and 73% identical, respectively, whereas the movement protein (MP) genes and deduced MPs are 70% and 73% identical, respectively. However, the HLFPV and HLSV CP genes are only 45-53% and 37-47% identical at the nucleotide and amino acid levels, respectively, to all other tobamoviruses and the MP genes are even more dissimilar, suggesting the existence of a malvaceous-infecting subgroup of tobamoviruses.

Biolistic inoculation of gladiolus with *Cucumber mosaic cucumovirus*. J. A. Aebig, K. Kamo, and H. T. HSU. Floral and Nursery Plants Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD. Phytopathology 94:S3. Publication no. P-2004-0014-AMA.

A new method of inoculating gladiolus with *Cucumber mosaic virus* (CMV) was developed using the Bio-Rad Helios Gene Gun System. This method circumvents the traditional use of aphids to transmit CMV, a virus that is mechanically transmissible to many plant species but generally not to gladiolus. Cartridges containing virus-coated gold microcarriers were prepared and the virus shot into *Nicotiana benthamiana* leaves and gladiolus corms and cormels. The biolistic procedure successfully transmitted three CMV isolates, two from serogroup I and one from serogroup II. Survival rates of two cultivars of gladiolus cormels and corms in sterile and non-sterile environments were compared. Infection rates of 100% were obtained when as little as 2 micrograms of virions were used in cartridge preparation. CMV remained viable after the cartridges were stored for many months.

Resistance to pitch canker in Monterey pine: Will it save the forest? B. J. AEGERTER and T. R. Gordon. University of California, Davis, CA. Phytopathology 94:S3. Publication no. P-2004-0015-AMA.

Pitch canker, caused by *Fusarium circinatum*, causes canopy dieback and mortality in native and planted stands of Monterey pine (*Pinus radiata*). The pitch canker pathogen can also kill seedlings. When clean seeds were planted under naturally-infested leaf litter (1500 CFU/g needles), only 18% of germinating seedlings survived to 6 weeks. In artificially-infested soil (100 CFU/g soil), 30% of seedlings survived to 10 weeks. Mortality increased significantly at higher inoculum levels. Additional experiments were conducted to determine the susceptibility of seedlings from families with differing frequencies of resistant progeny as determined by stem inoculation of 2 year old trees. Seedlings were challenged by either seed inoculation or by sowing seed in infested leaf litter. The results indicate no correlation between a family's mortality rate at seedling emergence and its resistance at 2 years old. Thus, it cannot be assumed that seedlings capable of developing into pitch canker-resistant trees will reach this stage of development if challenged by the pathogen at or soon after emergence. This has implications for nursery production, reforestation efforts, and natural regeneration.

Effect of fungicide treatments and storage temperature on postharvest symptom development of citrus black spot caused by *Guignardia citricarpa*. J. P. AGOSTINI (1), N. A. R. Peres (2), J. E. Adaskaveg (3), and L. W. Timmer (4). (1) INTA, Montecarlo, Misiones, Argentina; (2) University of Florida, GCREC, Dover, FL; (3) Dept. of Plant Pathology, University of California, Riverside, CA; (4) University of Florida, CREC Lake Alfred, FL. Phytopathology 94:S3. Publication no. P-2004-0016-AMA.

Symptoms of black spot occur on fruit in the field and may continue to develop in storage, transport, or at the destination. The effects of preharvest fungicide programs, drench treatments at harvest, packingline treatments, and storage temperature on postharvest symptom development was evaluated on Murcotts, Valencia oranges, and lemons stored for 50 days after harvest in Misiones, Argentina. An effective preharvest fungicide program reduced disease incidence at harvest by 80-90% and also greatly reduced symptom development on stored asymptomatic fruit. Drench treatments at harvest with a benzimidazole fungicide had little or no effect on postharvest symptom development. Storage at 8°C greatly slowed symptom development compared to storage at ambient temperature. Packingline treatments with imazalil, thiabendazole, fludioxonil, or pyrimethanil produced variable results but never greatly reduced symptom development. A rigorous field fungicide program and cold storage are the most effective means to prevent postharvest development of black spot and reduce the risk of introduction of this disease to new areas.

Defense mechanism of rice against *Cochliobolus miyabeanus* is distinct from that against *Magnaporthe grisea*. I.-P. AHN (1), S. Kim (1), S. Kang (2), and Y.-H. Lee (1). (1) School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; (2) Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA. Phytopathology 94:S3. Publication no. P-2004-0017-AMA.

Magnaporthe grisea and *Cochliobolus miyabeanus* infect rice (*Oryza sativa* L.), and they utilize two distinct mechanisms. In Tetep, a rice cultivar resistant to both fungi, pathogen infection triggered hypersensitive reaction (HR). In a rice cultivar susceptible to both pathogens, Nakdong, while *M. grisea* did not cause HR, *C. miyabeanus* caused rapid cell death. Conidial germination fluid (CGF) from *C. miyabeanus* induced cell death in both cultivars, and induced resistance against *M. grisea*. Benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) also increased resistance to *M. grisea*. Methyl jasmonate (MeJA) was ineffective against either fungus. Resistance of Nakdong to *M. grisea* by BTH or CCF correlated with *PR* gene expression. In contrast, the expression pattern of the *PR* and *JAmyb* genes in response to *C. miyabeanus* was nearly identical between Nakdong and Tetep. Together, rice employs distinct defense mechanisms against *M. grisea* and *C. miyabeanus*.

Pyrenophora tritici-repentis races in the Czech Republic and Poland. S. ALI (1), J. Sarova (2), W. Wakulinsky (3). (1) North Dakota State University, Fargo, ND; (2) Research Institute of Crop Production, Prague, Czech Republic; (3) Agriculture University of Warsaw, Warsaw, Poland. Phytopathology 94:S3. Publication no. P-2004-0018-AMA.

P. tritici-repentis, the causal organism of tan spot, is an important foliar pathogen of wheat in Czech Republic and Poland. The fungal population occurs as multiple races based on their ability to induce chlorotic (chl+) and necrotic (nec+) lesions on wheat differentials. The *P. tritici-repentis* population race structure from Czech Republic and Poland has not yet been published. Fifty-one *P. tritici-repentis* single-spore isolates, recovered from diseased leaves of wheat, triticale, and non-cereal grasses were race typed. Of 51 isolates tested, 33 were race 1; three were race 2; nine were race 3; and six were race 4. The results indicate that the fungal population prevalent in the two countries is comprised of multiple races and is similar to the races that occur in other regions of the world. Wheat germplasm should be screened with races 1, 2, and 3 for breeding for tan spot resistance in the region.

Effect of foliar disease on epiphytic yeast populations of creeping bentgrass and tall fescue. T. W. ALLEN, L. L. Burpee, and J. W. Buck. University of Georgia, Griffin, GA. Phytopathology 94:S3. Publication no. P-2004-0019-AMA.

The effect of foliar diseases caused by *Sclerotinia homoeocarpa* or *Rhizoctonia solani* on the yeast populations on creeping bentgrass and tall fescue were determined by leaf washing and dilution plating. Leaves with lesions caused by either pathogen supported significantly larger yeast populations than symptomless leaves. Total yeast populations on symptomless leaves of bentgrass and tall fescue ranged from 7.9×10^3 to 1.4×10^5 colony forming units per centimeter^2 (cfu/cm^2) and 2.4×10^3 to 1.6×10^4 cfu/cm^2, respectively. Total yeast populations on *S. homoeocarpa*-infected or *R. solani*-infected bentgrass leaves were 3.6 to 10.2 times and 6.2 to 6.4 times larger, respectively, than on symptomless leaves. In general, symptomless and diseased bentgrass leaves supported larger yeast populations

than symptomless or diseased tall fescue leaves. Yeasts categorized as white pigmented species were identified as *Cryptococcus laurentii*, *C. flavus*, *Pseudozyma antarctica*, *P. aphidis*, and *P. parantartica*. The percentage of pink yeasts in the total yeast population ranged from 2.6 to 9.9% on symptomless leaves and increased to 32.0 to 44.7% on *S. homoeocarpa*-infected leaves. Pink yeasts included *Rhodotorula glutinis*, *R. mucilaginosa*, *Rhodosporidium dacryoideum*, and *Sporidiobolus pararoseus*. Foliar disease significantly affected population size and composition of epiphytic yeasts on bentgrass and tall fescue.

Effect of overhead irrigation on phylloplane yeast populations on bentgrass and tall fescue. T. W. ALLEN, L. L. Burpee, and J. W. Buck. University of Georgia, Griffin, GA. Phytopathology 94:S4. Publication no. P-2004-0020-AMA.

Maintaining high populations of epiphytic yeasts on plant surfaces is one prerequisite to using yeasts as biocontrol agents in the field; however, the effects of host maintenance practices like irrigation on phylloplane yeasts is unknown. The effect of overhead irrigation (0, 5, and 15 minutes) on the yeast populations on creeping bentgrass and tall fescue were determined by leaf washing and dilution plating. Total yeast populations on bentgrass and tall fescue ranged from 19,232 to 50,733 colony forming units per centimeter squared (cfu/cm^2) and 1,023 to 7,467 cfu/cm^2, respectively. Five minutes of irrigation on field plots significantly reduced the yeast populations on bentgrass by 31% and on tall fescue by 26%. Total yeast populations on irrigated bentgrass (15 minutes) were reduced by 45% compared to non-irrigated bentgrass, however, no differences in yeast populations sizes were observed between the treatments two hours after the irrigation event. Yeast populations on turfgrass were observed to follow a cyclic pattern; on successive days the yeast populations on bentgrass were higher in the mornings compared to eight hours later in the afternoons. Population decreases during the day were observed to be similar to the reductions observed with irrigation.

Detection of hibiscus latent Fort Pierce virus in New Mexico. J. A[illegible]n (1), I. Kamenova (2), S. Adkins (2), and S. F. HANSON (1). (1) New Mexico State University, Departme[illegible] Entomology Plant Pathology and Weed Science, Las Cruces, NM; (2) USDA-ARS Horticultural Research Laboratory, Ft. Pierce, FL. Phytopathology 94:S4. Publication no. P-2004-0021-AMA.

Hibiscus Latent Fort Pierce virus (HLFPV) is a newly described tobamovirus recently isolated from landscape plantings of *Hibiscus rosasinensis* in Florida. Consistent with members of the tobamovirus group, HLFPV is mechanically transmitted and causes economic losses by damaging the leaves and flowers of the host. Because Hibiscus is a popular ornamental planted nationwide, we sought to determine if HLFPV was present in areas outside of FL. Dot blot immunoassay (DBIA) and tissue blot immunoassay (TBIA) were used to identify potentially infected plants in New Mexico. Electron microscopy showed the presence of tobamovirus like rods in immuno-positive samples. [illegible]acts from infected and uninfected tissue were used in immunocapture-reverse-transcription-polymerase chain reaction (IC-RT-PCR) using primers specific for the HLFPV coat protein ORF. PCR products were detected only in immuno-positive samples. Sequence analysis showed that the PCR products were 100% identical to HLFPV indicating that this virus is present in New Mexico.

Long-term survival of different genotypes of 2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas fluorescens* i[illegible]oil. R. ALLENDE-MOLAR (1), B. B. Landa (2), and D. M. Weller (1,3). (1) Washington State University, Pullman, WA; (2) Universidad de Cordoba, Cordoba, Spain; (3) USDA-ARS, Pullman, WA. Phytopathology 94:S4. Publication no. P-2004-0022-AMA.

DAPG-producing *P. fluorescens* are often enriched in soils that have undergone pea or wheat monoculture. Seventeen genotypes (A-Q) of such strains have been distinguished by repetitive sequence-based-polymerase chain reaction (rep-PCR); some of which are exceptionally aggressive root colonizers. In a greenhouse experiment, the long-term survival in soil of strains representative of genotypes A, B, D, L and K was tested. Soils inoculated with bacteria at 10^4 CFU/g of soil were sown to wheat for 10 successive cycles of 3 weeks each and then were stored dry at room temperature. After one year, the soils were again planted with wheat and root colonization by the bacteria evaluated during five three-week growth cycles. Strains F1[illegible] and L5.1-96 (D) were detect[illegible] the roots in all five cycles indicating their ability to survive in soil over a long period of time and then rebound in population size when wheat is again grown. L[illegible]6 (D) was the most persistent strain and reached population densities of up to 10^7 CFU/g of root. Strains of genotypes A, B and L were not detected when wheat was sown in the stored soils.

The effect of *Ustilago hordei* mating inhibitors on expression of farnesyltransferase genes in *U. hordei*, *Tilletia tritici*, and *T[illegible]ica*. T. S. AL-NIEMI, P. J. Kosted, S. A. Gerhardt, and J. E. Sherwood. Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717. Phytopathology 94:S4. Publication no. P-2004-0023-AMA.

Mating is required for the smut and bunt fungi to become pathogenic. Mating must occur via the interaction of sexually compatible sporidia to form dikaryotic mycelia that in turn infects the host plant. Central to fungal mating systems is the production of sexual pheromones, members of the farnesylated family of proteins, which drive the regulation of genes involved in the various steps of the mating process. In previous work on *Ustilago hordei*, the cause of barley covered smut, we established that analogs of the *U. hordei* pheromones, ranging from simple amino acids to farnesylated peptides, inhibit mating in this fungus and teliospore germination of *Tilletia*. We have cloned the beta-subunit of farnesyltransferase from *U. hordei*, *T. indica* (Karnal bunt) and *T. tritici* (wheat common bunt) using polymerase chain reaction. The genes have been sequenced and the relatedness of these genes examined. Expression of the farnesyltransferase genes has been examined in *U. hordei* and *T. indica* grown alone, in mixtures of compatible mating types, or in the presence of mating inhibitors, and germinating teliospores of *T. tritici* with and without mating inhibitors.

Immunofluorescence localization by confocal microscopy of *Maize mosaic virus* (*Rhabdoviridae*) in organs and tissues of [illegible]planthopper vector *Peregrinus maidis*. E.-D. AM[illegible]R (1,2) and S. A. Hogenhout (1). (1) Department of Entomology; (2) Molecular and Cellular Imaging Center, Ohio State University, Wooster, OH. Phytopathology 94:S4. Publication no. P-2004-0024-AMA.

Previous ultrastructural studies indicated that virions of *Maize mosaic virus* (MMV, *Rhabdoviridae*) bud mainly through the nuclear membrane in maize cells and in most tissues of its planthopper vector *Peregrinus maidis*. However, in secretory cells of the principle salivary glands, MMV virions bud predominantly through cytoplasmic membranes facilitating the release of virions with salivary secretions. Recent confocal microscopy-immuno-fluorescence localization of MMV in organs and tissue sections confirmed the perinuclear accumulation of MMV in maize leaf cells and in most tissues of the vector *P. maidis*, except in the principle salivary glands where MMV was found also near the periphery of secretory cells. Furthermore, although most of the vector organs and tissues appeared infected with MMV, infection was most extensive in cells of the nervous and tracheal systems. Implications of these findings in suggesting the route of MMV infection in the vector/host insect will be discussed.

Population diversity of the *Fusarium oxysporum* f. sp. *koae*, the pathogen of Koa dieba[illegible] Hawaii. R. C. Anderson (1), T. C. Bentley (2), and J. C. CORRELL (2). ([illegible] USGS-Pacific Island Ecosystems Research Center, Honolulu, HI; (2) Department of Plant Pathology, University of Arkansas, Fayetteville, AR. Phytopathology 94:S4. Publication no. P-2004-0025-AMA.

Koa (*Acacia koa*) is an important endemic Hawaiian tree species. *Fusarium oxysporum* f. sp. *koae* has been implicated in several recent and widespread dieback maladies of Koa in the Hawaiian islands. Although originally identified as a pathogen of Koa some 25 years ago in Hawaii, the recent outbreaks have caused considerable alarm. Samples of symptomatic Koa trees were collected from several locations on Hawaii. [illegible]ates of *Fusarium oxysporum* were recovered from symptomatic tissue. Isolates were examined for pathogenicity on Koa seedlings in standardized inoculation tests. Isolates were also characterized based on colony morphology, vegetati[illegible]mpati-bility, and mtDNA RFLPs. The majority of the isolates examined belonged to a single mtDNA RFLP haplotype and a single vegetative compatibility group (VCG). Also, isolates within the predominant VCG had a similar colony morphology. Isolates of *F. o. koae* are being examined further and also being compared to isolates from other leguminous hosts. These data may reveal if *F. o. koae* is an introduced or endemic pathogen to the Hawaiian islands.

Chemical control strategies for Late blight management in Argentine. A. ANDREU (1) and D. Caldiz (2). (1) Mar del Plata National University, CC1245, Argentina; (2) McCain Argentina SA. Phytopathology 94:S4. Publication no. P-2004-0026-AMA.

The Late blight caused by the oomycete *Phytophthora infestans* is considered the most important disease of potato crops worldwide. Yield reductions are caused by premature foliage infections and tuber infections. Update information on Late blight susceptibility of commercial cultivars is needed by crop managers and growers in order to optimize a successful use of integrated crop management strategies. Laboratory, greenhouse, and field test showed that cv. Shepody is highly susceptible to the most agressive Argentinian isolate, while cvs. Kennebec and Russet Burbank are medium susceptible,

Ranger Russet and Santana (foliage) low susceptible and Innovator is resistant at foliage and tuber level. Tubers of Kennebec and Santana resulted more susceptible than their foliage. Seed treatments with contact fungicides (propineb, mancozeb) protect the healthy tubers of susceptible cultivars from infections during the handling process but do not protect the plant after crop emergence. However, seed treatments with systemic fungicides (propineb + iprovilocarb, dimetomorf + mancozeb) protect the tuber during handling and the plant up to 40 days after crop emergence. Early foliar applications of compounds with basipetal translocation, fosfites (Alliete) or aminobutiric acid (BABA), enhance plant resistance (SAR) at tuber level during harvest and storage. A high level of phytoalexin and phenol compounds were found in these tubers, hence, these compounds can be considered molecular markers of the defense response. All these results are part of an integrated Late blight management approach that is being carried out in Argentina.

Integrated management strategies for Rhizoctonia crown and root rot. J. C. ANSLEY, B. J. Jacobsen, N. K. Zidack, and M. R. Johnston. Department of Plant Science and Plant Pathology, Montana State University, Bozeman, MT 59717. Phytopathology 94:S5. Publication no. P-2004-0027-AMA.

Rhizoctonia crown and root rot caused by the fungus, *Rhizoctonia solani* AG 2-2 is one of the most damaging sugarbeet diseases worldwide. Losses are highest in warm, irrigated production areas where sugarbeets are planted intensively. A range of fungicides were tested for Rhizoctonia crown and root rot control efficacy. Research was conducted with a randomized complete block design with six replications at Huntley, MT from 1998-2003. Plots were single rows 9.14m long and all plots except the uninoculated controls were inoculated with 14g per plot of ground barley infected with *Rhizoctonia solani* AG 2-2 at the 4 leaf stage. Fungicide applications were made just prior to application of inoculum at the 4 leaf stage or at the 8, 10, 12-14 leaf stage using a 15.24cm band applied over the row. Overall, azoxystrobin at 2.13g–4.25g ai./304.8 row meter treatment applied at the 4 and 4 plus 8 leaf stage provided the lowest disease index and greatest extractable sucrose/ha. Treatments after the 8 leaf stage were not effective. The effect of temperature on infection and disease development was tested by growing plants in controlled environment growth chambers at 10, 16, and 24°C. Plants were inoculated at the 4 leaf stage with ground barley inoculum followed by application of soil over the crown of the plant. At 10°C, very little disease developed while at 16°C only 8% of roots had greater than 50% decay compared to 68% for plants grown at 24°C.

Nursery survey for sudden oak death in Texas. D. N. APPEL, S. Service, and T. Kurdyla. Texas A&M University, College Station, TX. Phytopathology 94:S5. Publication no. P-2004-0028-AMA.

A pilot nursery survey for the sudden oak death (SOD) pathogen, *Phytophthora ramorum*, was conducted in Texas. Plants were sampled from 36 nurseries in 27 counties distributed throughout the state, with visits to additional nurseries made for educational purposes. A systematic sample of plants with suspect symptoms was collected, returned to the laboratory, and processed for isolation of *Phytophthora* spp. on a selective medium (PARP). The sample included 519 plants from 58 species consisting mostly of known hosts of the pathogen. Thirty–two suspected *Phytophthora* isolates were obtained. Twenty-one proved to be *Phytophthora* spp. Five known species were identified, including *P. parasitica*, *P. palmivora*, *P. drechsleri*, *P. cinnamomi*, and *P. megasperma*. A few isolates have yet to be identified, but none are considered to be likely *P. ramorum* candidates. SOD was therefore not found in Texas. Through the creation of a website, the development of fact sheets, and presentations at workshops, the survey has significantly raised the awareness of nursery operators, landscape maintenance personnel, and natural resource managers throughout the state of the threat posed by SOD.

Relationship of disease resistance and stand persistence in alfalfa cultivars from the 1940's to the 1990's. J. J. ARISS (1), L. H. Rhodes (1), R. M. Sulc (1), and J. F. S. Lamb (2). (1) The Ohio State University, Columbus, OH; (2) USDA-ARS, St. Paul, MN. Phytopathology 94:S5. Publication no. P-2004-0029-AMA.

Over the past six decades cultivated alfalfa has been bred to incorporate desirable agronomic traits, including improved yield, forage quality and resistance to disease and insect pests. However, some studies have suggested that limited progress has been made in alfalfa yield and persistence, regardless of improvements in disease resistance. A four-year field trial, seeded in 1999, compared yield, percent ground cover, and disease incidence and severity of several diseases among sixteen alfalfa cultivars. Entries were selected to include the top yielding varieties by decade from the 1940's to the present. Additional modern cultivars were included as checks. In September 2003, percent ground cover was less in stands of cultivars with older release dates than stands of modern varieties. As expected, more plants affected by Fusarium wilt (*Fusarium oxysporum* f. sp. *medicaginis*) and anthracnose (*Colletotrichum trifolii*) were present in varieties with lower reported resistance. There were highly significant negative correlations between Fusarium wilt incidence and percent ground cover. Anthracnose incidence and percent ground cover also showed a significant negative correlation. There were no significant correlations between percent ground cover and spring black stem, summer black stem, Rhizoctonia stem blight or Sclerotinia crown and stem rot. While increased resistance to the major alfalfa diseases may not account wholly for increased yield and persistence, disease incidence data from Ohio suggests that resistance to specific diseases enhances stand persistence.

Vector incompetence of *Frankliniella tritici* is not associated with a barrier to midgut infection by *Tomato spotted wilt virus*. F. M. ASSIS FILHO (1), J. Stavisky (2), S. R. Reitz (3), C. M. Deom (4), and J. Sherwood (4). (1) AGDIA, Inc., County Road 6, Elkhart, IN 46514; (2) NYS Integrated Pest Management Program, NYSAES, Geneva, NY; (3) USDA-ARS Center for Biological Control, 6383 Mahan Dr., Tallahassee, FL 32308; (4) Department of Plant Pathology, University of Georgia, Athens, GA 30602. Phytopathology 94:S5. Publication no. P-2004-0030-AMA.

Tomato spotted wilt virus (TSWV) is the type species of the genus *Tospovirus*. TSWV is transmitted by thrips in a propagative manner. The mechanism leading to vector competence of several thrips species to transmit TSWV has been investigated, but it is not yet well characterized. In this study the interaction of TSWV and the non-vector species *Franklinella tritici* was studied to determine if insight on the dynamics of vector competence could be ascertained. First instar larva, up to 24-h old, were given a 24h acquisition access period. Immunofluorescence microscopy was used to evaluate TSWV distribution within the alimentary canal of larvae and adults. It was found that TSWV replicated in the thrips midgut but did not move to the salivary glands, suggesting that vector incompetence of *F. tritici* may be associated with the absence of virus movement to the salivary glands. Although *F. tritici* is not reported to transmit TSWV, its ability to transmit other tospoviruses has not been examined. Hence, classification of thrips species as "vectors" and "non-vectors" of specific tospoviruses is dependent upon comprehensive transmission assays and not virus detection assays.

Differential responses of self-fertile *Phytophthora infestans* isolates to metalaxyl. C. AVILA-ADAME and J. B. Ristaino. North Carolina State University, Raleigh, NC. Phytopathology 94:S5. Publication no. P-2004-0031-AMA.

Sexual reproduction of *P. infestans* occurs primarily through a heterothallic process that involves the two compatibility types A1 and A2. However, under certain conditions isolates of *P. infestans* produce sexual structures by selfing. Eight isolates originating from single leaf lesions produced oospores in the absence of the opposite mating type when grown on metalaxyl amended media. Single zoosporic cultures prepared from these isolates were tested for sensitivity to metalaxyl and the ability to produce oospores by selfing. The ED_{50} values for mycelial growth inhibition ranged from less than 0.01 microgram to more than 100 micrograms per milliliter. Four isolates maintained selfing potential. One isolate of A1 mating type and another of A2, which were resistant to metalaxyl, produced more oospores in the presence of 1 microgram and 10 micrograms per milliliter of fungicide. However, the number of oospores formed by sensitive isolates (an A1 and an A2) in the presence of metalaxyl increased in one test but not in the other. Oospores have not been detected in potato leaf discs pretreated with metalaxyl and infected by either sensitive or resistant isolates.

Fungicides for control of Phytophthora blight (*Phytophthora capsici*) of vegetables. M. BABADOOST and S. Z. Islam. Department of Crop Sciences, University of Illinois, Urbana, IL. Phytopathology 94:S5. Publication no. P-2004-0032-AMA.

Laboratory and field trials were conducted to evaluate fungicides for control of *Phytophthora capsici*, causal agent of Phytophthora blight in vegetable crops. A1662 (480SC), cyazofamid (Ranman 400SC), dimethophorph (Acrobat 50WP), phosphorous acid (Agrifos 400LS), metalaxyl + copper hydroxide (Ridomil Gold Copper 65WP), and famoxadone + cymoxanil (Tanos 50WDG), incorporated into culture medium of *P. capsici* (20 µg a.i./ml of lima bean agar), reduced colony growth by 48–100% and sporangium germination by 2–99%. In a processing pumpkin field trial at Pekin, IL with natural infestation with *P. capsici*, weekly sprays of 1) A1662, 2) Agrifos, 3) Acrobat + copper sulfate (Cuprofix 36.9DF) alternated with Cuprofix alone, 4) Ranman alternated with Acrobat + Cuprofix, or 5) Tanos + Cuprofix alternated with Acrobat + Cuprofix reduced vine infection by 52-81% and fruit infection by 30-57% compared with disease incidence in untreated plots. In a similar field trial at Shawneetown, IL with bell pepper, six weekly applications of Ranman alone, Ranman alternated with Acrobat, or Ranman alternated with Ridomil Gold Copper significantly reduced incidence of Phytophthora blight in treated plots when compared to untreated controls.

Specific stylet activities by sharpshooters are involved in inoculation of *Xylella fastidiosa*. E. A. BACKUS (1), [illegible]. Joost (2), F. Yan (3), and H. Shugart (1). (1) USDA-ARS, Parlier, CA; (2) University of California, Riverside, CA; (3) Peking University, Beijing, China. Phytopathology 94:S6. Publication no. P-2004-0033-AMA.

Glassy-winged sharpshooter (GWSS) feeding behavior is being studied to determine the exact behaviors associated with inoculation of the xylem-limited bacterium, *Xylella fastidiosa* (Xf) in plants. To do this, we are using electropenetration graph (EPG) monitoring of feeding, videomicrography of stylet movements in transparent diet and histology of Xf aggregations in vector precibaria. Studies on grape described last year showed preliminary correlations of GWSS waveforms A1 and A2 with salivary sheath formation, B1 and B2 with other pathway activities, and C with ingestion. They also implicated the B1 waveform in inoculation of Xf near EPG-identified probes. Work this year has emphasized understanding the biological meanings of sub-patterns in the B1 waveform and correlation of waveforms with bacterial populations in the vector precibarium. Results presented describe stylet activities during the 'spikelet burst' sub-pattern of B1, which may represent expulsion of bacteria-laden fluid from the precibarium. The ultimate goal of our research is to develop a Stylet Penetration Index using EPG monitoring of feeding, which could provide a rapid means of screening grape varieties for host plant resistance to the vector.

Gene expression of *Theobroma cacao* leaves responding to wounding, ethylene, or methyl jasmonate. B. BAILEY (1), M. Strem (1), H. Bae (1), and M. Guiltinan (2). (1) BARC-W, Beltsville, MD; (2) Penn State Univ., University Park, PA. Phytopathology 94:S6. Publication no. P-2004-0034-AMA.

Yield of *Theobroma cacao* (cacao), is limited by disease and insect pests. Wounding, ethylene, and methyl jasmonate induce resistance to pests in many plant species. The effects of wounding, ethylene, and methyl jasmonate on gene expression in cacao leaves were studied. Differential expression was observed for genes putatively encoding a DNA binding protein (*TcWRKY-1*), a protein regulating cell division (*TcORFX-1*), a Type III peroxidase (*TcPer-1*), an endo-1,4-beta-glucanase (*TcGlu-1*), a class VII chitinase (*TcChiB*), a caffeine synthase (*TcCaf-1*), and a light harvesting complex protein (*TcLhca-1*). *TcWRKY-1* and *TcORFX-1* were induced by wounding in young red (YRL) and mature green (MGL) leaves. Elevated *TcPer-1* mRNA levels were detected after wounding in YRL and MGL. Wounding induced *TcChiB* in YRL and repressed *TcLhca-1* in MGL. Ethylene (12 mL/L) induced *TcPer-1* in YRL and MGL but induced *TcGlu-1* in MGL only. Ethylene repressed *TcLhca-1* and *TcCaf-1* in YRL. Ethylene repressed *TcLhca-1* and *TcChiB* in MGL. Methyl jasmonate (0.2 mM) induced *TcCaf-1* and *TcChiB* in YRL and *TcPer-1* and *TcChiB* in MGL. Ethylene/methyl jasmonate combined induced *TcChiB* in YRL and *TcGlu-1* in MGL. The response of cacao leaves to wounding, ethylene, or methyl jasmonate was influenced by the stage of leaf development. There was evidence of genetic crosstalk between the actions of ethylene and methyl jasmonate in cacao leaves.

Real-time quantitative PCR of wheatgrass-specific markers to confirm alien translocations and BYDV/CYDV resistance in wheat. B. Balaji (1), [illegible] THOMPSON (2), and J. M. Anderson (2). (1) Department of Agronomy, Purdue University, West Lafayette, IN; (2) Crop Production and Pest Control Research, USDA-ARS, West Lafayette, IN. Phytopathology 94:S6. Publication no. P-2004-0035-AMA.

Barley yellow dwarf virus (BYDV) and Cereal yellow dwarf virus (CYDV) are the most severe viral diseases in cereal crops, and at this time there is little or no effective control. However, introgression of alien chromatin from wheatgrass into wheat has increased the BYDV/CYDV resistance in some wheat lines. To identify lines with YDV resistance in a large population, use of wheatgrass-specific molecular markers are indispensable. Various techniques such as C-banding, FISH and RFLP were used to determine the relative location of translocation breakpoints and size of the transferred alien chromatin segments in a number of wheatgrass-wheat translocation lines. In our laboratory, FISH was used to determine the number of sub-telomeric (pAW161) signals in each of the translocation lines, relative to their susceptibility or resistance to BYDV/CYDV. Real-time PCR using SYBR Green I was also used to detect and quantify pAW161 signals in these translocation lines. Our results show that the amplification cycles (Ct) obtained were inversely correlated to the number of pAW161 signals identified by FISH. Real-time PCR was also used to analyze other wheatgrass-specific markers that are uniquely present in BYDV/CYDV-resistant plants. By using these markers in real-time PCR, we now have a high-throughput system that can efficiently screen a large number of translocation and BYDV/CYDV resistant lines from among the populations.

Spinach curly top virus: A new *Curtovirus* species revealing a history of recombination among curtoviruses. S. Baliji (1), M. C. Black (2), R. French (3), D. C. STENGER (3), and G. S[illegible] (1). (1) University of Texas at San Antonio, San Antonio, TX 78249; (2) Texas A&M University, Uvalde, TX 78802; (3) USDA-ARS, University of Nebraska, Lincoln, NE 68583. Phytopathology 94:S6. Publication no. P-2004-0036-AMA.

A curtovirus of spinach was isolated in southwest Texas during 1996. Disease symptoms included severe stunting and chlorosis, with younger leaves curled, distorted, and dwarfed. Symptoms produced in spinach agroinoculated with cloned viral DNA were similar to those observed in the field. Viral single-stranded and double-stranded DNA forms typical of curtovirus infection were detected in both field-collected and experimental host plants by Southern blot hybridization. The complete nucleotide sequence of the infectious clone comprised 2925 nucleotides, with seven open reading frames encoding proteins homologous to those of other curtoviruses. Complete genome comparisons revealed that the spinach curtovirus shared 64.2-83.9% nucleotide sequence identity relative to four previously characterized curtovirus species: *Beet curly top virus*, *Beet severe curly top virus*, *Beet mild curly top virus*, and *Horseradish curly top virus*. Phylogenetic analysis of individual open reading frames indicated that the evolutionary history of the three virion sense genes was different from that of the four complementary sense genes, suggesting that recombination among curtoviruses may have occurred. Collectively, these results indicate that the spinach curtovirus characterized here represents a new species of the genus *Curtovirus*, for which we propose the name Spinach curly top virus.

Association of bacteriophages with citrus canker and their use for control. B. BALOGH (1), J. B. Jones (1), R. E. Stall (1), B. I. Canteros (2), and A. M. Gochez (2). (1) University of Florida, Gainesville, FL; (2) INTA EEA Bella Vista, Corrientes, Argentina. Phytopathology 94:S6. Publication no. P-2004-0037-AMA.

Asiatic citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*), is a worldwide menace to the citrus industry. Bacteriophages may be considered as biological control agents of the disease. A phage collection was established and included (i) 13 *Xac* phages from other collections, (ii) 4 phages from other *Xanthomonas* sp. that are pathogenic on *Xac*, and (iii) 64 from isolations from diseased plant tissue. Phages were recovered from 27% and 48% of diseased plant samples collected in Florida and Argentina, respectively. These phages varied in host specificity, chloroform sensitivity and plaque morphology. In greenhouse experiments foliar application of a mixture of four phages significantly reduced disease severity caused by a "Miami" strain of *Xac*. The area of diseased plant tissue was reduced from 55% to 16% on plants where the phage mixture was applied when the wild type bacterium was used for inoculation, whereas no reduction in disease severity was observed when a phage-resistant mutant of the pathogen was treated with the phage mixture.

Reduction in Phytophthora disease with increased inoculum application pressure. T. J. Banko, C. X. Hong, and P. A. RICHARDSON. Virginia Tech, Virginia Beach. Phytopathology 94:S6. Publication no. P-2004-0038-AMA.

Water retention ponds have been shown to be a source of inoculum for Phytophthora diseases in ornamental nurseries that recycle irrigation water. However, water discharged from irrigation risers has been found to contain less viable inoculum than water collected directly from the retention pond source. In this study, we investigated one possible explanation for this discrepancy, that is, irrigation water pressure. *Catharanthus roseus* plants were grown in a greenhouse and then inoculated with zoospores of *Phytophthora nicotianae* by spraying the inoculum over the plants at application pressures of 0, 0.21, 0.42, or 0.63 MPa (0, 30, 60 or 90 psi). The 0 MPa treatment applied with an aspirator-type hand mister. The pressurized inoculum treatments were applied with a CO_2-pressurized sprayer. After inoculation, the plants were placed in a humidity tent overnight, then returned to greenhouse benches. Three days later the plants were evaluated for disease by counting the numbers of diseased leaves, shoot tips, and stems. In three separate experiments, there was a significant linear reduction in disease with increased application pressure. This suggests that pressure may be a factor in the reduction of viable inoculum in irrigation systems. Other possible factors are currently under investigation.

Detection of four rust pathogens of cereals and grasses using real-time PCR. C. W. BARNES, L. J. Szabo, and K.-P. Nguyen. USDA-ARS, Cereal Disease Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, MN. Phytopathology 94:S6. Publication no. P-2004-0039-AMA.

Puccinia rust species are widespread pathogens of cereals and grasses that annually cause significant crop losses worldwide. Duplex real-time PCR assays were developed for rapid detection of four rust pathogens, *P. graminis*, *P. recondita*, *P. striiformis*, and *P. triticina*, using TaqMan chemistry. Nuclear rDNA provided a variable region (ITS1) to distinguish between

species, and a conserved region (28S) as an internal control. Specific ITS1 primer/probe sets exhibited high specificity and sensitivity with detection limits below 1 pg of rust DNA. Specificity of the assays was tested across multiple isolates representing a range of races and *forma specialis* within a species. Additionally, assay specificity was evaluated by testing a range of other grass rusts, as well as other fungi. The 28S primer/probe combination was successful in detecting all rust species tested within the duplex assays, validating the integrity of each assay. Rust species were identified within 30 min from the beginning of the assay, demonstrating the advantages of real-time PCR for detection of cereal rust pathogens.

Resistance in soybean cultivars to *Pythium* damping-off and root rot. G. D. BATES (1), C. S. Rothrock (1), J. C. Rupe (1), and P. Chen (2). (1) Dept. of Plant Pathology; (2) Dept. of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701. Phytopathology 94:S7. Publication no. P-2004-0040-AMA.

Pythium damping-off and root rot can be a serious problem in alluvial soils with poor internal drainage used for rice and soybean production. The flood tolerant soybean cultivar Archer has shown resistance to several *Pythium* spp. isolated from soybean in Arkansas when compared to Hutcheson, a popular cultivar. Archer contains two resistance genes for *Phytophthora sojae* (*Rps* 1k and 6), which were speculated to play a role in resistance to *Pythium* spp. A set of differential cultivars containing specific resistance genes for *Phytophthora sojae* were planted in vermiculite infested with *Pythium aphanidermatum* and assessed for disease. The cultivar Williams 82 (*Rps* 1k) demonstrated resistance to *P. aphanidermatum* similar to Archer. PRX 145-48 (*Rps* 3c) also demonstrated some resistance, but significantly less than *Rps* 1k. Further assays with isolines containing *Rps* 1k and *Rps* 3c reiterated the resistance for both of these genes compared to other isolines. Hypocotyl injections of cultivars containing these genes also demonstrated the association between *Rps* 1k and *Rps* 3c and resistance to *Pythium* spp. Breeding lines from Archer × Pioneer 9641 and Archer × Asgrow 5403 were evaluated for *Pythium* resistance. Results suggest the *Phytophthora* resistance gene *Rps* 1k is important for resistance to *Pythium* damping-off and root rot.

Cultural control of Armillaria root disease of grape. K. BAUMGARTNER. USDA, ARS, Davis, CA. Phytopathology 94:S7. Publication no. P-2004-0041-AMA.

Root collar excavation for control of Armillaria root disease of grape was investigated in two California vineyards (vineyard K1, vineyard N1) from 2002 to 2004. When timed in early stages of root collar infection, root collar excavation may cause mycelial fans of *Armillaria mellea* to recede from the root collar before xylem is decayed. In 2002, soil was removed to expose root collars of 50 healthy and 50 symptomatic vines/vineyard. Nonexcavated vines (50 healthy, 50 symptomatic) were randomly chosen, for a total of four treatments. In 2002 and 2003, yields of 30 vines/treatment/vineyard were measured. In 2004, pruning weights were measured from the same vines. Symptomatic vines had significantly fewer clusters ($P < 0.0001$), lower yields ($P < 0.0001$), lower cluster weights ($P < 0.0001$), fewer shoots ($P < 0.0001$), lower pruning weights ($P < 0.0001$), and smaller shoots ($P < 0.0001$) than healthy vines in both vineyards. In vineyard N1, excavation had a significant positive effect on yield ($P = 0.0231$) and cluster weight ($P = 0.0042$) of symptomatic vines, compared to symptomatic, nonexcavated vines. Symptomatic, excavated vines in vineyard N1 had the same high cluster weights as healthy vines and there were no significant effects of excavation on yields or pruning weights of healthy vines. In vineyard K1, excavation had no effect on yields or pruning weights of symptomatic vines and had significant negative effects on pruning weight ($P = 0.0008$) and shoot weight ($P = 0.0373$) of healthy vines, compared to healthy, nonexcavated vines. Differences between the vineyards are likely due to frequent re-filling of excavated root collars in vineyard K1, where root collar excavation was ineffective.

Efficacy comparison between seed-coated and soil-applied nematicides in root-knot nematode-infested cucumber fields. J. O. BECKER (1) and D. Hofer (2). (1) Department of Nematology, University of California, Riverside, CA; (2) Syngenta Crop Protection, Basel, CH. Phytopathology 94:S7. Publication no. P-2004-0042-AMA.

Greenhouse trials demonstrated that abamectin-seed coating protected young cucumber seedlings against root-knot nematodes. In fall of 2003, field performance of abamectin-coated cucumber seeds (0.3 mg a.i./seed) was compared to pre-season soil application of oxamyl or fosthiazate (2.03 kg a.i./ha) as well as to a non-treated control. Two trials were conducted near Irvine, CA, one with cucumber cv. Imanol and one with cv. Kahina. The sandy loam was infested with 16 and 24 J2 of *Meloidogyne incognita* per 100cc soil, respectively. Three weeks after seeding cv. Kahina, all nematicide treatments resulted in a more vigorous growth compared to the non-treated control. With cv. Imanol, similar differences were visible only between the abamectin seed coating and the non-treated control. Both trials were harvested twice at the end of the 6-week growing season. For the first harvest of cv. Kahina, all nematicide treatments resulted in a 23-34% increase in fruit numbers and total fruit weight compared to the non-treated control. None of the treatments differed in yield, root galling or final J2 population at the second harvest. For the first harvest of cv. Imanol, only the abamectin seed-coating resulted in significantly higher total fruit weight than the non-treated control. At the second harvest, none of the treatments differed in yield, root galling or final J2 population. Seed coating with abamectin was at least as effective as soil-applied non-fumigant nematicides at a 100 times lower a.i. application rate per ha.

Population structure of seedborne *Stagonospora nodorum* on New York wheat. R. S. BENNETT, M. G. Milgroom, and G. C. Bergstrom. Department of Plant Pathology, Cornell University, Ithaca, NY. Phytopathology 94:S7. Publication no. P-2004-0043-AMA.

High incidences of seed infected by *Stagonospora nodorum* are common in commercial seedlots of wheat, and infected seed has been shown to be a potentially important source of inoculum for foliar epidemics of Stagonospora nodorum blotch. The seedborne aspect of *S. nodorum's* biology was examined within a population genetic framework. We examined 330 seedborne isolates from seven field populations at 153 AFLP loci and compared them to a foliar population. Virtually every seedborne isolate (326/330) had a unique multilocus haplotype. Seedborne populations were similar to the foliar population in having high levels of genotypic diversity, low levels of gene diversity, and many loci with rare alleles. We found little evidence of genetic differentiation among the three locations, and no cultivar specialization was detected. Estimates of multilocus linkage disequilibrium were low, but rejection of the null hypothesis of random mating was possible in several populations. Because seedborne populations are genetically similar to the foliar population, infected seed may be a source of genetically diverse isolates of *S. nodorum* for foliar epidemics.

***Phytophthora ramorum* not detected in a survey of North Carolina nurseries.** D. M. BENSON and C. Y. Warfield. N.C. State University, Raleigh, NC. Phytopathology 94:S7. Publication no. P-2004-0044-AMA.

A statewide survey of 14 nurseries in NC that grow *Rhododendron*, *Pieris*, and *Viburnum* was conducted May–early June 2003 to detect the presence of the cool-season pathogen *P. ramorum*, cause of Sudden Oak Death in California. The entire crop of a given host species at a nursery was surveyed with foliage samples collected from plants that had dieback and blight symptoms. The two detection methods used included culture on PARP medium at 20°C and nested PCR. Across all nurseries, over 230,000 plants were surveyed and 339 samples collected. There was no evidence of *P. ramorum* in any PCR reactions, nor was the fungus isolated in culture. At seven nurseries, 43 isolates of *P. citricola* were recovered from hybrid rhododendron and 18 isolates from *Pieris*. *P. cactorum* was recovered at two nurseries from either rhododendron or *Pieris*. At four nurseries, 36 isolates collected from rhododendron and two from *Pieris* were identified as *Phytophthora* sp, but could not be further identified. The frequency and diversity of other *Phytophthora* spp. recovered may have been higher had the survey been conducted later in the season when conditions are more favorable for warm-season *Phytophthora* spp.

Genetic variation in field populations of the crown rot fungus *Fusarium pseudograminearum*. A. R. BENTLEY (1), L. W. Burgess (1), B. A. Summerell (2), and J. L. Smith-White (1). (1) Faculty of Agriculture, Food and Natural Resources, The University of Sydney, 2006, NSW, Australia; (2) Royal Botanic Gardens and Domain Trust, Mrs Macquaries Road, Sydney, 2000, NSW, Australia. Phytopathology 94:S7. Publication no. P-2004-0045-AMA.

Crown rot of wheat and barley, caused primarily by the fungus *Fusarium pseudograminearum* is a limiting disease of winter cereal production in many parts of the world. Changes in farming practices over the past two decades, including the widespread adoption of no-tillage farming have led to a significant increase in the incidence of this disease in Australia. Significant efforts are currently underway to integrate possible sources of genetic resistance into commercial cereal cultivars. Assessment of the potential effectiveness of such sources requires an understanding of the existence and nature of genetic variability within the natural pathogen population. The current study was designed to assess the extent of genetic variation within *F. pseudograminearum*. Field populations of *F. pseudograminearum* were isolated and morphologically identified from a number of sites in eastern, northern and western Australia. The level of genetic variation was assessed using Amplified Fragment Length Polymorphism analysis, and sequencing of the Elongation Factor gene. The results of these analyses suggest that genetic variation exists within and between field populations of the morphological

species *F. pseudograminearum* in Australia. Further work is required to assess the genetic and pathogenic variation in these, and additional geographic populations of *F. pseudograminearum*.

Evaluation of cowpea germplasm for seedling resistance to *Rhizoctonia solani*. P. A. BERLAND, J. A. Thies, and R. L. Fery. USDA, ARS, Charleston, SC. Phytopathology 94:S8. Publication no. P-2004-0046-AMA.

Rhizoctonia solani is a major pathogen of cowpea (*Vigna unguiculata*) and causes substantial yield losses worldwide. Resistant cultivars would be the preferred control measure. A greenhouse method to evaluate cowpea germplasm for seedling resistance was developed using inocu cultured on dent corn kernels. A total of 360 U.S. Plant Introductions (PI) from the core subset of the USDA Cowpea Germplasm Collection and a moderately resistant check, Kentucky Wonder-191 pole bean (*Phaseolus vulgaris*), were evaluated. Five seeds per PI were planted individually in 0.2-L cells containing pasteurized soil (2 sand:1 soil) mixed with 0.5g of infested kernels ped to a particle size of 0.6 × 0.6 × 0.2 cm^3. Two weeks later, seedlings were rated for disease on a 1 to 5 scale with 1 = 0 to 3, 2 = 4 to 25, 3 = 26 to 50, 4 = 51 to 80, and 5 = 81 to 100% of hypocotyl circumference covered with lesions. Disease indices ranged from 2.4 to 5.0 with nine PI exhibiting moderate resistance 3.0). Moderately resistant PI were re-evaluated in a replicated test using a randomized complete block design. Disease indices were lower in the moderately resistant PI (mean = 2.4) than in the susceptible PI (mean = 4.2) ($P < 0.0001$).

Functional analysis of the nuclear genome of Ophiostomatoid fungi. L. Bernier (1), C. Breuil (2), W. E. Hintz (3), P. A. Horgen (4), V. JACOBI (1), J. Dufour (1), G. Bouvet (1), M. Aoun (1), S. Diguistini (2), P. de astide (3), and M. Pinchback (3). (1) Laval University, Ste-Foy, QC; (2) University of British Columbia, Vancouver, BC; (3) University of Victoria, Victoria, BC; (4) University of Toronto at Mississauga, Mississauga, ON. Phytopathology 94:S8. Publication no. P-2004-0047-AMA.

Despite decades of intensive research on the Dutch Elm Disease pathogen *Ophiostoma novo-ulmi*, little is known about its genome and the functions of specific genes. The Canadian Ophiostoma Genome Project is a collaborative effort of four research teams to conduct a large-scale identification and analysis of genes controlling important aspects of the life cycle of two Ophiostomatoid fungi: *O. novo-ulmi* and the phylogenetically related sapstain fungus *O. piceae*. An expressed sequence tag (EST) library from *O. novo-ulmi* yeast cells was established. It contains 4386 readable sequences and serves as a reference library for Ophiostomatoid fungi. More specific EST libraries were prepared from *O. novo-ulmi* mycelium (grown at suboptimal temperatures), fruiting bodies, and from *O. piceae* mycelium grown on different carbon sources using the suppression subtractive hybridization (SSH) technique. Each SSH library contains distinct populations of fungal genes that are important for substrate colonization and propagation. Preparation of a SSH library enriched for *O. novo-ulmi* genes expressed in American elm callus tissue is in progress. The results of this project will allow the construction of EST microarrays for Ophiostomatoid fungi and, thus, monitoring of expression in thousands of genes.

Environmental fate of a genetically marked endophyte in grapevines. B. BEXTINE (1), C. Lauzon (2), D. Lampe (3), and T. Miller (1). (1) University of California, Riverside; (2) California State University, Hayward; (3) Duquesne University, Pittsburgh, PA. Phytopathology 94:S8. Publication no. P-2004-0048-AMA.

Xylella fastidiosa, which es Pierce's disease in grapevine, is the target of symbiont therapy which employs symbiotic bacteria to deliver anti-pathogen compounds to disrupt disease progression. A permit was obtained from the EPA in late spring 2003 to conduct field trials in covered grapevines at three commercial vineyards and one experiment station in Californ The putative delivery agent, *Alcaligenes xylosoxidans denitrificans* (*Axd*), which expresses a fluorescent protein (DsRed), was applied to grapevines by needle inoculation, foliar spray application, and soil drench. The plants were covered with insect-free screening, to exclude arthropods from test plants. Samples were taken throughout the growing season and marked Axd was detected in several grapevines 2 wks post-inoculation, fewer grapevines 4 wks post-inoculation, and no grapevines 6 wks post-inoculation, ind g that transgenic bacterial populations declined over time. Marked *Axd* was not detected in any grape berries or soil samples from *Axd* positive grapevines.

Single aphids transmit multiple genotypes of *citrus tristeza virus*, but often with changed population dynamics. K. K. Biswas (1), K. L. MANJUNATH (1), L. J. Marais (1), and R. F. Lee (2). (1) Univ. of Florida, Plant Path. Dept., Gainesville, FL 32611; (2) USDA-ARS, NCGRCD, Riverside, CA 92507. Phytopathology 94:S8. Publication no. P-2004-0049-AMA.

The Nartia strain of *citrus tristeza virus* (CTV) has been widely used during last fifty years for cross protection against stem pitting CTV in South African grapefruit. We have previously shown the presence of several genotypes of CTV in this isolate by use of a heteroduplex mobility assay of a 400 bp region (nt 1074-1478) using primers which amplify a product in all CTV isolates tested. Single aphid transmitted (SAT) isolates of Narita show a wide array of symptoms as well as serological diversity. CTV populations of the source and the SAT plants were analyzed by RT-PCR to amplify the 400 bp region, then cloned and the clones screened by PCR using CTV genotype-specific primers. Multiple populations of CTV were present in most of the SAT isolates, but in different ratios which may be responsible for the phenotypes differences and serological diversity.

Development of wild *Helianthus annuus* populations with multiple disease resistance. C. C. BLOCK. USDA-ARS, Regional Plant Introduction Station, Ames, IA. Phytopathology 94:S8. Publication no. P-2004-0050-AMA.

Two populations of wild annual sunflower (*Helianthus annuus*) with multiple disease resistance were developed by recurrent phenotypic selection. Individual plants were selected for resistance to Alternaria leaf blight, caused by *Alternaria helianthi* and to Septoria leaf blight, caused by *Septoria helianthi*. Multiple sunflower accessions were pooled to form the parental sets for each population because superior resistance was infrequently found within any individual accession. Resistance was characterized by pinpoint flecks on leaves, or by small leaf spots showing little of the typical chlorosis and necrosis observed with a susceptible reaction. Plants were concurrently selected for resistance to natural infection by powdery mildew, caused by *Erysiphe cichoracearum*. The *A. helianthi* resistance found in these populations is similar to that previously reported only from perennial species such as *H. tuberosus* (Jerusalem artichoke). These populations provide the opportunity to transfer resistance to cultivated *H. annuus* without the need for difficult interspecific hybridization.

rest fires influence the abundance of Armillaria root disease in ponderosa pine. J. T. Blodgett (1) and J. E. LUNDQUIST (2). (1) USDA-Forest Service, 1730 Samco Rd., Rapid City, SD; (2) USDA - Forest Service, 240 West Prospect, Fort Collins, CO. Phytopathology 94:S8. Publication no. P-2004-0051-AMA.

Forest tree diseases influence forest fires by changing stand structure and generating fuels, bu tle is known about how fires influence fuel-generating diseases. This field study was designed to examine the effects of fire on the abundance and potential spread of *Armillaria* root disease in ponderosa pine (*Pinus ponderosa*) forests. Five plots were established in an area of the Black Hills National Forest, SD that burned 3 years previous to this study. Each plot consisted of four subplots that varied in fire intensity (*i.e.*, low, medium, and high fire intensity, and unburned). The abundance of *Armillaria* rhizomorphs on root collars increased ($P < 0.001$) as fire intensity increased. Pair-wise comparisons were significant among fire intensities except between unburned and low intensity subplots. Our results show that *Armillaria* can survive intense fires and can readily colonize roots of trees killed by fire.

Population dynamics and non-target effects of transgenic *Pseudomonas fluorescens* in the rhizosphere of wheat and pea. S. BLOUIN BANKHE (1), A. Brown (1), M. Y. Son (2), L. S. Thomashow (2), and D. M. Weller (2). (1) Washington State University, Pullman, WA; (2) USDA-ARS, Pullman, WA. Phytopathology 94:S8. Publication no. P-2004-0052-AMA.

Pseudomonas fluorescens strain Q8r1-96 produces 2,4-diacetylphloroglucinol (DAPG). Transgenic strains Z30-97, Z32-97, Z33-97, and Z34-97, constructed by inserting the phenazine-1-carboxylic acid (PCA) biosynthetic locus into Q8r1-96, produce both antibiotics. Both PCA and DAPG have been shown to suppress root diseases such as take-all of wheat The population dynamics of these strains were monitored in the rhizosphere of field grown wheat during two seasons after application as a seed treatment and in the rhizosphere of greenhouse-grown pea during multiple growth cycles after introduction into soil. The non-target effects of the introd strains on the indigenous rhizosphere bacteria were determined by using terminal restriction fragment length polymorphism (T-RFLP) analysis. In general, the transgenic strains did not differ from Q8r1-96 in their ability to colonize the rhizosphere of wheat and pea. However, when Q8r1-96 and a transgenic strain were present on the same root, the wild type often out-competed the transgenic strain.

Increased incidence of seed transmission of High Plains virus disease in sweet corn. T. D. BLUNT and J. P. Hill. Colorado State University, Fort Collins, CO. Phytopathology 94:S8. Publication no. P-2004-0053-AMA.

High Plains virus ease (HPD) was first discovered in sweet corn (*Zea mays* L.) plants in the Central and Western United States in 1993. Symptoms in

susceptible sweet corn plants include severe stunting, chlorotic flecking and/or streaking, yellowing and little or no ear set. The pathogen is now considered to be a new virus in the genus Tenuivirus. HPD is often found in conjunction with *Wheat streak mosaic virus* (WSMV) and vectored by the wheat curl mite, *Aceria tosichella* Keifer (Acari: Eriophyidae). HPD has been reported to be seed-transmitted, but at very low incidences. However, observations of a field in Colorado suggested that the virus was seed-transmitted at a very high incidence (approximately 20%) in 2001. ELISA tests (indirect method) with sweet corn seed recovered from the same seed lot as that used to plant the infected field showed positive results. A much higher seed incidence (4%) was obtained for that seed source, and contrasts sharply with previous reports of less than 0.0001%.

The effect of wind speed on dispersal of bacteria from canker-infected citrus trees. C. H. BOCK (1), P. E. Parker (2), and T. R. Gottwald (1). (1) USDA-ARS-USHRL, 2001 S. Rock Rd., Ft. Pierce, FL 34945; (2) USDA-APHIS, Moore Air Base, Edinburg, TX 78539. Phytopathology 94:S9. Publication no. P-2004-0054-AMA.

The effect of wind speed on dispersal of citrus canker (*Xanthomonas axonopodis* pv. *citri*) was investigated. Wind was simulated with an electric fan to produce winds of 0 [control], 4, 6 and 15 ms^{-1} at the canopy face. Spray generated by nozzles was used to simulate rain, and was fed into the wind 1 m upwind of (and directed at) the inoculum source. Samples were taken using panel (at 1, 2, 3 and 5 m) and funnel samplers (at 0, 1 and 2 m) downwind from the inoculum source. Bacteria were deposited at all distances, but most were deposited 1 m downwind (86% of the sample at 1 m vs <1% at 5 m). Greater wind speed increased the quantity of bacteria deposited at all distances. At 1 m downwind and no wind, 6,168 bacteria were deposited on the panels, but at 15 ms^{-1} over 320,700 were deposited. The concentration of bacteria rose from 9 to 138 ml^{-1}, respectively. The results show that greater wind speed increases the quantity of bacteria dispersed. The effect of wind speed on dispersal of *X. axonopodis* pv. *citri*) is relevant where storms with strong winds can increase wind blown inoculum (as in Florida).

Effects of *Alternaria brassiciola* infection on the reproductive fitness of the littoral ruderals *Cakile maritima* and *C. edentula* in SE Australia. C. H. BOCK (1), P. H. Thrall (2), and J. J. Burdon (2). (1) USDA-ARS-USHRL, 2001 S. Rock Road, Ft. Pierce, FL 34945; (2) CSIRO, GPO 1600, Canberra, ACT 2601. Phytopathology 94:S9. Publication no. P-2004-0055-AMA.

Cakile maritima (CM) and *C. edentula* (CE) are hosts of *Alternaria brasicicola* along the New South Wales coast. The effect of disease severity and duration on growth and reproduction of these plants was investigated under greenhouse conditions. Differences between the two species were evident in mean plant height, leaf number, mean plant weight, pod weight, seed weight and other agronomic measures of yield. Species differed in their reaction to infection. CM developed more severe disease than CE (Area Under the Disease Progress Curve = 639 vs 446, respectively). Growth and reproductive potential of both species was significantly reduced by the infection. For example, CM plant height was reduced by up to 30%, and leaf number by 50%. The number of pods was reduced by 70%, and up to 100% of seed was infected with *Alternaria* (CE responded similarly). Infection with *A. brassicicola* reduces growth and reproductive potential of CM and CE, and disease can result in seed infection that reduces fitness of the subsequent generation.

In vitro biofilm formation by *Erwinia carotovora* subs. *atroseptica* can increased when grown on a cellulose-coated surface. T. Bolduc (1), L. L. R. MARQUES (1,2), M. E. Olson (1,2), and H. Ceri (1,2). (1) University of Calgary; (2) MBEC Bioproducts Inc., AB, Canada. Phytopathology 94:S9. Publication no. P-2004-0056-AMA.

Erwinia carotovora subsp. *Atroseptica* (Eca) causes a vascular disease in potato known as blackleg. Vascular plant pathogens often form biofilms during the colonization and/or pathogenicity to the host. Characterization of biofilm formation by Eca in a high-throughput assay, the MBEC™ device, indicated that biofilm growth on plastic pegs was not efficient, even after 48 hours. Biofilm production was better when the bacteria were grown on balsa wood chips, which, however, are not ideal for high throughput testing. To enhance Eca biofilm formation on the MBEC™ device, we coated the pegs with various forms of cellulose. Pegs coated with carboxymethyl (CM) cellulose allowed for enhanced biofilm formation by *Eca* in vitro. Our findings indicate that the coating of inert surfaces with natural materials may be a good approach for enhancing biofilm formation by plant pathogens in vitro, by mimicking surface characteristics of the actual hosts.

Phytoplasma disease of watercress in Hawaii. W. B. BORTH (1), R. P. P. Almeida (1), S. K. Fukuda (2), R. T. Hamasaki (1), and J. S. Hu (1). (1) Department of Plant and Environmental Protection Sciences; (2) Department of Tropical Plant and Soil Sciences, University of Hawaii. Phytopathology 94:S9. Publication no. P-2004-0057-AMA.

A yellows disease affecting watercress (*Nasturtium officinale*) in Hawaii has symptoms of reduced leaf size, leaf yellowing, and occasionally witches' brooms. This disease is found on all the watercress farms on Oahu, but has not yet been found on other Hawaiian Islands. Watercress plants were tested for phytoplasma infection by polymerase chain reaction (PCR) assays using phytoplasma-specific primers. Amplicons of the expected sizes were produced from all symptomatic plants, but not from healthy plants raised from seed. Sequence analysis of the cloned PCR products indicate that the watercress is infected with a phytoplasma nearly identical to the severe strain of North American aster yellows (SAY). Six weed species, including *Amaranth* sp., *Eclipta prostrata*, *Emilia sonchifolia*, *Plantago major*, *Myriophyllum aquaticum* and *Sonchus oleraceus* are also hosts of this phytoplasma in Hawaii. Leafhoppers collected from affected watercress farms have been tentatively identified as *Macrosteles* sp. nr. *severini*, which had not been previously recorded in Hawaii. Leafhoppers collected from symptomatic watercress transmitted SAY to watercress, plantain (*Plantago major*), and lettuce (*Lactuca sativa*) in greenhouse experiments. Leafhoppers can also breed on watercress, as demonstrated by large numbers of nymphs and adults collected from watercress farms during one year of surveys and oviposition experiments in the laboratory.

Effect of seed and foliar insecticide treatments on barley yellow dwarf disease of wheat in Alabama. K. L. BOWEN, K. L. Flanders, and K. B. Burch. Dept. Entomology and Plant Pathology, Auburn University, AL. Phytopathology 94:S9. Publication no. P-2004-0058-AMA.

Barley yellow dwarf of wheat is caused by *Barley yellow dwarf virus* and *Cereal yellow dwarf virus*, both of which are vectored by several aphid species. This disease is important in winter wheat production throughout the state of Alabama. A study was conducted in north Alabama to determine the effects of seed and foliar insecticide treatments for managing barley yellow dwarf in wheat. Seed were treated with thiamethoxam (Cruiser 5FS) at 39 g a.i./100 kg or left untreated. Foliar treatments included a control and lambda-cyhalothrin (Warrior) at 0.028 g a.i./ha in 2002, as well as zetacypermethrin (MustangMax) at 0.020 g a.i./ha in 2003. Seed treatment reduced incidence of barley yellow dwarf and tended to reduce stand counts. No yield gain was obtained with the seed treatment. Foliar insecticides reduced incidence of barley yellow dwarf in one year, and improved yields in both years.

Forest structure changes in the spruce-fir ecosystem of the Black Mountains NC. T. A. BOWERS (1), R. I. Bruck (1), and W. P. Robarge (2). (1) North Carolina State University, Dept. of Plant Pathology, Raleigh, NC 27695; (2) North Carolina State University, Dept. of Soil Science, Raleigh, NC 27695. Phytopathology 94:S9. Publication no. P-2004-0059-AMA.

Montane spruce-fir forests of the Southern Appalachians are remnants of the last ice age existing now only as isolated islands at elevations above 1,400 meters. The spruce-fir ecosystem throughout the region was shown to be in decline from the early 1960s into the late 1980s. This trend was determined following extensive surveys in high elevation red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* (Pursh) Poir.) forests conducted during the 1980's as part of the Spruce-fir Research Cooperative and National Acid Precipitation Assessment Program. In 2002-3, we resurveyed 28 permanent spruce-fir forest plots in the Black Mountains of North Carolina installed in 1985. Following previous protocols, we measured forest structure parameters such as basal area, stem density, and tree crown damage. Insect and disease anomalies were also recorded when found. Results show an overall increase in live basal area and live stem density at all elevations for spruce and fir populations, with a continued downward trend in overall crown condition for both species. Data suggests vigorous regeneration is occurring at many sites, especially in areas of former severe mortality. However, significant decline in crown condition continues to manifest especially in older trees of previous surveys. Causal factors behind the current trend in crown decline remain unknown; no correlation to balsam woolly adelgid (*Adelges piceae* Ratzburg) or other biotic diseases was found.

Molecular and virulence characterization of *Pyricularia grisea* in Arkansas in 2000-2003. E. J. BOZA, J. C. Correll, R. D. Cartwright, and F. N. Lee. Department of Plant Pathology, University of Arkansas, Fayetteville, AR. Phytopathology 94:S9. Publication no. P-2004-0060-AMA.

Rice blast, caused by *Pyricularia grisea* is an economically important fungal disease of rice worldwide. Rice blast is a particularly important constraint in the southern rice-producing states of the United States. Previous analysis of the blast pathogen population in the U.S. indicated that there were eight genetic families (A through H) based on the commonly used MGR586 fingerprinting probe, but only four groups occur in the contemporary

population (1990–present) in Arkansas (A, B, C, and D). Populations of the rice blast pathogen are routinely recovered from rice each growing season and characterized for their MGR586 DNA fingerprint, vegetative compatibility group, and pathogenicity. Ongoing genetic examination and pathogenicity tests have indicated that the blast pathogen population structure in 2000-2003 is predominated by isolates which belong to a single genetic group (Group A, >95% of the isolates), and these isolates consistently correspond to race IB-49. Isolates in groups B and D were also recovered in 2000-2003 (<3% for each group). No group C isolates were recovered in 2000-2003. The cultivars sampled, or possibly differential fitness in MGR586 fingerprint groups, may partially explain the bias for certain fingerprint groups.

Foliar fungicides for control of Sclerotinia stem rot of canola. C. A. BRADLEY (1), G. Endres (1), M. Halvorson (1), B. Johnson (1), B. Henson (1), K. McKay (1), P. Porter (2), and D. LeGare (2). (1) North Dakota State University; (2) University of Minnesota. Phytopathology 94:S10. Publication no. P-2004-0061-AMA.

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, can be a devastating disease to canola grown in North Dakota (ND) and Minnesota (MN). Field trials located in ND and MN were established in 2003 to evaluate foliar fungicides for control of SSR. The trials were artificially inoculated with *S. sclerotiorum* ascospores and grown under mist-irrigation systems to promote infection and disease progression. Twenty-one fungicide treatments and an untreated control were evaluated. Fungicides were applied when canola was at 30 or 50% bloom. The fungicides boscalid, iprodione, PCNB, prothioconazole, thiophanate-methyl, vinclozolin, V-10114, and V-10116 were shown to decrease SSR compared to the untreated control depending on location. In general, where disease pressure was high, yield was increased with efficacious fungicides.

Teaching pest risk assessment: A case study in Iowa. J. Bradshaw (1,2), P. ESKER (1,3), S. Fernandez (1), A. J. Moreira (1), A. Murillo-Williams (1,4), F. W. Nutter, Jr. (1), and J. Tollefson (2). (1) Dept. Plant Pathology; (2) Dept. Entomology; (3) Dept. Statistics; (4) Dept. Agronomy, Iowa State University, Ames, IA 50011. Phytopathology 94:S10. Publication no. P-2004-0062-AMA.

During the fall semester 2003, a colloquium was taught in Plant Pathology and Entomology at Iowa State entitled, "Issues in Crop Bioterroism and Food Security." As part of this class, graduate students were asked to attend seminars from leading experts in crop biosecurity, develop pest risk criteria of importance to Iowa agriculture, and apply these criteria to assess exotic pests that could negatively impact Iowa agriculture. We created a flowchart with six nodes to pre-screen corn and soybean pests to determine potential risk: Pr[introduction], economic importance, Pr[detection], Pr[eradication], Pr[establishment], and ability to control the pest. If at each criterion the result was "yes", we quantified the risk at all nodes except Pr[detection] using a 1-5 scale (1 = low, 5 = high). Node values were summed and averaged (n = 8) to rank pests from highest to lowest. Our class tested this system against 35 exotic pests (preliminary rankings will be presented). This exercise has tremendous potential to be incorporated into agricultural courses as a teaching tool on pest risk assessment.

Methyl bromide alternatives for control of black root rot of strawberry in Georgia. P. M. BRANNEN (1), L. M. Ferguson, and F. J. Louws (2). (1) Univ. of Georgia, Athens, GA; (2) North Carolina State Univ., Raleigh, NC. Phytopathology 94:S10. Publication no. P-2004-0063-AMA.

Key fungi associated with the black root rot complex of strawberry in GA were documented as *Rhizoctonia fragariae*, *Pythium* spp. and *Fusarium* spp. Fumigants, including 1,3-dichloropropene, chloropicrin, and metam-sodium, were tested over three years as potential alternatives to methyl bromide (MeBr), an ozone-depleting compound traditionally used to manage this complex. Non-fumigated plots reduced yields to 62%, 57%, and 63% compared to MeBr in yrs 1-3, respectively. Two application methods were also compared: conventional shank injection versus drip application. When using shank injection, three-way combinations of alternative fumigants resulted in comparable disease control and yields to those provided by MeBr. Shank-applied Telone-C35 (T-C35; 35 gal/A) + Vapam HL (MS; 37.5 gal/A) generated yields with values of 99%, 95%, and 93%. T-C35 (35 gal/A) + MS (75 gal/A), tested in yrs 2 and 3, generated yields of 113% and 107%, respectively. Drip-applied Inline (32 gal/A) + MS (75 gal/A), generated yields of 86% and 83% in yrs 1 and 2, respectively. Drip applications generally provided lower yields than those of shank-injected applications, possibly indicating poor distribution of the fumigant in the bed when using drip application methods. Drip application technologies require additional research to ensure enhanced and consistent efficacy. Shank-applied three-way combinations generated consistent yields and disease control, and these need to be broadly evaluated to address concerns associated with weed management and plant-back issues.

Sugar beet seedling age and susceptibility to *Aphanomyces cochlioides*. J. R. BRANTNER and C. E. Windels. University of Minnesota, Northwest Research and Outreach Center, Crookston, MN. Phytopathology 94:S10. Publication no. P-2004-0064-AMA.

Sugar beet (*Beta vulgaris*) is highly susceptible to *Aphanomyces cochlioides*, but cultivars with partial resistance are available. Our objective was to determine the effect of seedling age on susceptibility to *A. cochlioides*. Seedlings of a susceptible and a partially resistant cultivar were inoculated with zoospores (50,000 or 100,000) per pot (10 × 10 × 9 cm, 10 plants) at 1, 2, 3, or 4 weeks after planting (10 replicates/treatment). Seedlings began dying within 4 days and root rot indices (0 = 0–100 scale, 0 = healthy and 100 = severe disease) were determined 2 weeks after inoculation. There were no significant differences in RRI for the two inoculum concentrations, so data were combined. Disease severity varied significantly by plant age at inoculation; RRI averaged 91, 96, 81, and 32 for seedlings inoculated 1, 2, 3 or 4 weeks after planting, respectively. Both cultivars responded similarly following inoculation and RRI were high, but lower (P = 0.05) for the partially resistant (73) than the susceptible (77) cultivar. Results indicate sowing a partially resistant cultivar may be inadequate in controlling *A. cochlioides* if infection occurs during the seedling stage.

Control of postharvest Alternaria fruit rot with fungicides applied to blueberry during the growing season. P. R. BRISTOW and G. E. Windom. Washington State University, Puyallup 98371-4998. Phytopathology 94:S10. Publication no. P-2004-0065-AMA.

Fruit rot caused by *Alternaria* spp. has become an increasingly serious problem in fresh blueberries grown in the Pacific Northwest. Fresh berries now represent 65% of the annual WA harvest compared with 45% a few years ago. Some berries picked during the peak of the harvest season are held in refrigerated storage for a several weeks to command a higher price. Alternaria fruit rot reduces fruit quality in storage. Several fungicides were compared in a commercial field (cv. 'Reka'). In the same field, plants were entered into or removed from a fungicide spray program (tank mixture of Rovral + Dithane DF) on selected dates to protect flowers/berries for different periods during the growing season. When sound ripe berries were incubated individually in a moist chamber at 16-18°C, the most effective fungicides were BAS 516 and Dithane DF followed by Omega, Rovral and Reason. Serenade was not effective. The mixture was most effective when applied about the time berries began to turn blue. Applications made during bloom or immediately before harvest did not lower disease incidence. The results of these field trials indicate that several new fungicides, including some that are already labeled for use on blueberry, will control the disease when applied at the proper time.

Aphid transmission of stem pitting *Citrus tristeza virus* from mixed virus infections. R. H. BRLANSKY (1), A. Roy (1), and V. D. Damsteegt (2). (1) University of Florida, CREC, Lake Alfred, FL 33850; (2) FDWSRU, ARS, USDA, Ft. Detrick, MD 21702. Phytopathology 94:S10. Publication no. P-2004-0066-AMA.

Citrus tristeza virus (CTV), a phloem-limited closterovirus, contains a single-stranded, 20 kb RNA. Numerous isolates of the virus exist which produce a variety of symptoms in various citrus hosts. One of these symptoms is stem pitting (SP). SP does not occur in all citrus hosts but may cause low tree vigor, decline and an economic reduction in fruit size and yield. Historically, the first appearance of CTV-SP follows the introduction of the efficient CTV vector, *Toxoptera citricida*. Hypotheses for this association range from the introduction of these strains in new planting materials to the increased ability of *T. citricida* to selectively vector SP strains from existing CTV mixtures. CTV exists as a complex of isolates or subisolates and *T. citricida* has been shown to separate the complex. A study was initiated to determine what *T. citricida* transmits when a severe SP CTV isolate is mixed with a non-SP isolate from Florida. Biological and molecular data was generated from grafted mixtures and from aphid transmissions from these mixtures showed that the stem pitting CTV was predominant.

A new corn and sorghum infecting *Cercospora* sp. isolated from smooth brome grass. K. BRODERS and J. E. Partridge. Department of Plant Pathology, University of Nebraska-Lincoln. Phytopathology 94:S10. Publication no. P-2004-0067-AMA.

An undergraduate research project was initiated during the 2003 crop season to isolate fungi from lesions on weed species. In Southeast and Northeast Nebraska a fungus was isolated from lesions on all smooth brome grass (*Bromus inermis*) adjacent to corn and soybean fields. A single spore colony isolate was selected for Koch's postulates analysis. The pathogen had the characteristics of being a member of the genus *Cercospora*. There are no reports of *B. inermis* as a host for any species of *Cercospora*. The morphology and size of the conidia of this isolate are within the size param-

eters of *Cercospora zeae-maydis* (CZ-M) (60-100 m × 5-9 m versus 30-95 m × 5-9 m in the literature for CZ-M). However, this isolate grows much faster in culture (V-8) than CZ-M. Unlike the typical CZ-M, a 7 day culture grew to 87 mm diameter versus 13.5 mm for our culture of CZ-M. A zone of inhibition is produced when paired in culture with CZ-M. Inoculations on corn (B73 and Golden Cross Bantam), sorghum (TX623) and smooth brome grass produced characteristic symptoms. Koch's postulates were performed on corn and smooth brome grass. However, though small disease lesions would be produced consistently on sorghum, we have been unable to reisolate it from sorghum lesions. Current research is seeking to accurately determine the species of this isolate.

Risk fro *irococcus conigenus* to understory red pine seedlings. J. J. Bronson and G. R. STANOSZ. Dept. Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 94:S11. Publication no. P-2004-0068-AMA.

Changes (for aesthetic reasons) in red pine (*Pinus resinosa*) management practices include creation of irregular, longer edges of harvest sites and leaving residual overstory trees, instead of complete clearcut harvesting before replanting. Also, in a departure from traditional even-aged plantations in which trees of one age are grown, there is increasing interest in development of uneven-aged or multicohort red pine stands, in which trees of several ages would grow together. To confirm the risk of damage from the shoot blight pathogen *Sirococcus conigenus* (which inhabits crowns of overstory trees) to regeneration in these situations, red pine seedlings were planted in ten 50-seedling plots in the understory of a maturing red pine stand in northcentral Wisconsin in spring 2002 and 2003. Initial seedling establishment and symptom development was recorded during each growing season and in fall of each year seedlings were harvested to determine the presence of the pathogen. By fall 2002 and 2003, respectively, mean shoot blight incidence on established seedlings was 89% and 98%. Most of these seedlings were dying or dead and pynidia with conidia of *S. conigenus* were present on almost all of the seedlings. The shoot blight pathogen *Sphaeropsis sapinea* also was detected, though much less frequently. These results support previous research and concern about the risk that shoot blight pathogens pose to understory red pine seedlings.

Validation of a grape *Botrytis cinerea* infection risk index for California strawberries. J. C. BROOME (1), J. F. Strand (2), J r (3), S. L. Swezey (3), E. J. Morgan (2), and F. O. Martino (2). (1) University of California Sustainable Agriculture Research and Education Program; (2) University of California Integrated Pest Management Program; (3) University of California Center for Agroecology and Sustainable Food Systems. Phytopathology 94:S11. Publication no. P-2004-0069-AMA.

A grape *Botrytis cinerea* infection model is being validated for strawberries. Campbell Scientific dataloggers were placed in commercial strawberry fields in Prunedale and Watsonville. Fifteen-minute averages of temperature, relative humidity and leaf wetness were downloaded twice daily, checked and posted on the Internet. The model was validated at 2 locations over 2 years with 3 treatments (untreated control, calendar, and model b fungicide applications) and 3 replications (3 beds of 50 plants) in a randomized complete block design. Disease incidence (DI) was analyzed using the SAS GLM procedure for repeated measures. In 2002 and 2003, Prunedale model treatment received 6 and 7 applications, calendar 9 and10 applications, respectively. In 2002, DI for the control was 4.22%, higher than 2.4% for the model or 2.73% for the calendar treatments ($P > 0.01$). In 2003, DI was not significantly different. For both years, similar disease control resulted from 3 fewer model-guided sprays. At Watsonville, in 2002 and 2003, the model treatment received 8 and 7 applications and the calendar 5 and 6 applications, respectively. In 2002, DI was not significantly different. In 2003, DI for the control was 10%, 9% in calendar plots and 6.5% in model plots ($P > 0.004$). At Watsonville, with a more tolerant variety to *B. cinerea*, further study is needed.

Experimental host range and evidence for molecular recombination f infectious clones of *Squash leaf curl* and *Squash mild leaf curl* viruses. J. K. BROWN, K. Baumann, and A. M. Idris. Department of Plant Sciences, University of Arizona, Tucson, AZ 85721. Phytopathology 94:S11. Publication no. P-2004-0070-AMA.

Squash leaf curl (SLC) disease was first reported in the southwestern USA in squash in 1978 and in watermelon during 1981, and the associated begomoviruses were provisionally named SLCV and Watermelon curly mottle virus (WCMoV), respectively. SLCV was shown to have a narrow host range (*Cucurbita* s while WCMoV infected all cucurbit species examined and bean. Two DNA-A and DNA-B components each, were cloned independently from SLC-affected plants, and two closely related but distinct strains, SLCV-E (extended) and SLCV-R (restricted), were identified. However, only a partial experimental host range was determined for each, and the complete sequence for SLCV-R was not available. An extensive host range study for WCMoV suggested that it might be synonymous with SLCV-E but a complete WCMoV sequence was not available. In this study, infectious clones were obtained for the three isolates and their sequences were determined. Nucleotide sequence analysis indicated that SLCV-E and WCMoV are isolates of the same virus (at >98%) (now SLCV), and SLCV-R was a distinct species (now *Squash mild leaf curl virus* (SMLCV)), at 84%. The experimental host range for SLCV/WCMoV was determined to include common bean, cantaloupe, cucumber, *Nicotiana benthamiana*, pumpkin, watermelon, and zucchini squash, compared to SMLCV, which infects only bean, pumpkin, and zucchini squash. Analysis of the DNA-A sequence for SLCV and SMLCV revealed that multiple recombination events have occurred in the diversification of the two species.

The USDA Regulated Plant Pest List: A transparent window to pests of regulatory significance. L. G. BROWN. USDA\APHIS\PPQ. Phytopathology 94:S11. Publication no. P-2004-0071-AMA.

USDA\Animal Plant Health and Inspection Service\Plant Protection and Quarantine (USDA\APHIS\PPQ) use the Regulated Plant Pest List to notify U.S. trading partners of specific exotic pests that we wish to exclude. Pests are considered any species, strain or biotype of plant or pathogenic agent injurious to plants or plant products. Countries that export goods to the U.S. use the list to certify that shipments are free of listed pests. Currently, plant pests listed are derived primarily from Title 7, Code of Federal Regulations, and Parts 300–399. Most additions to the list result from new interceptions at ports and ever-widening research on PRAs, foreign pest outbreaks, scientific society activities, and through federal and state survey activities. Pests currently regulated by must meet the definition of regulated quarantine pests stipulated by the International Standards for Phytosanitary Measures of the Food and Agricultural Organization, International Plant Protection Convention (FAO\IPPC). To meet these requirement pests are categorized by a decision process. The sources for candidate pests and the risk assessment method are explained to give transparency to the decision process described in the poster.

The role of kernel water relations in resistance t atoxin production in corn. R. L. BROWN (1), Z.-Y. Chen , and T. E. Cleveland (1). (1) USDA-ARS-SRRC, New Orleans, LA; (2) Louisiana State University Ag. Center, Baton Rouge, LA. Phytopathology 94:S11. Publication no. P-2004-0072-AMA.

Corn genotypes resistant to aflatoxin production have been identified, and several investigations are now focused on the identification of resistance traits in these genotypes. Enhanced knowledge pertaining to the mode of operation of resistance mechanisms would lay a solid foundation for successful deployment of resistance genes into desired germplasm. Preliminary studies have indicated a potential link between resistance and kernel ability to both imbibe water and germinate in a relatively shorter time. In previous studies, aflatoxin-susceptible genotypes accumulated significantly and drastically less aflatoxin when allowed to imbibe water prior to inoculation with *Aspergillus flavus*. This was demonstrated even when imbibition was facilitated at 0°C. To determine an association between kernel water relations and resistance, further investigations of kernel imbibing and germinating ability were conducted. Kernel hormone levels under constitutive, imbibed and infected conditions were also determined. Previous studies have demonstrated the induction of specific antifungal proteins during imbibition. A recent comparative proteome analysis of kernels revealed 5-fold higher constitutive levels of stress-related proteins in resistant over susceptible genotypes. Therefore, proteomics was also performed to assess the effect of imbibition/infection on constitutive levels of kernel antifungal and stress-related proteins. Results of these investigations will be discussed.

Effects of pre- nt fallow and crop rotations on severity of Prunus replant disease. G. T. BROWNE (1), S. M. Schneider (2), and T. J. Trout (2). (1) USDA-ARS, University of California, Davis; (2) USDA-ARS WMRL, Parlier, CA. Phytopathology 94:S11. Publication no. P-2004-0073-AMA.

Prunus replant disease (PRD) caus unting (mild cases) and tree death (severe cases) in young almond trees planted on sites with a recent history of *Prunus* cultivation. Pre-plant fumigation can prevent PRD, but is known about controlling it with cultural or biological approaches. We evaluated short-term fallow and cover crop rotations for PRD control near Parlier, CA. Microplots (60 cm dia., 120 cm deep) were filled with soil from a PRD-affected orchard in Apr 2002. Treatments (2002) included: 1) almond on Nemaguard peach rootstock (G) Jun-Nov; 2) A/NG Jun-Nov + methyl bromide:chloropicrin (MBP) (50:50, 448 kg/ha, Nov); 3) bare fallow Apr-Nov; 4) fallow Apr-Nov + MBP Nov; 5) field corn Jun-Nov; 6) Piper sudan grass Jun-Nov; 7) Penewawa wheat Nov-Mar; and 8) Piper an Jun-Nov + Penewawa wheat Nov-Mar; each on five replicate plots. After each crop's

growth period, the roots (sudan, A/NG) or roots and shoots (corn, wheat) were chopped and incorporated into the soil. Treatment efficacy was assessed according to growth of Nemaguard peach seedlings, planted four per plot Apr 2003, weighed Nov 2003. Treatment effects were significant ($P < 0.0001$); the means were separated with 95% CIs. Compared to the non-fumigated, non-fallowed A/NG control (Trt. 1), which had stunted plants, fallow+MBP increased peach shoot mass by 286%. Also, MBP alone, or sudan or wheat increased shoot mass (157, 132, and 135%, respectively), but fallow alone and the other rotations did not. Sudan or wheat rotation may help manage PRD, and confirmation is underway.

Status of *Puccinia jaceae* in 2004 for biological control of yellow starthistle in California. W. L. BRUCKART (1), D. G. Luster (1), and D. Woods (2). (1) USDA-ARS-FDWSRU, Ft. Detrick, MD; (2) California Dept. Food & Agriculture, Sacramento, CA. Phytopathology 94:S12. Publication no. P-2004-0074-AMA.

Yellow starthistle (YST, *Centaurea solstitialis*), an invasive weed infesting 5.8 million hectares in California, is a target of classical biological control. Development of *Puccinia jaceae*, an host specific rust fungus, has been pursued to complement insect agents already in place. Following an extensive risk analysis (65 species of plants, 10 families), release of a Turkish isolate of *P. jaceae* into California was approved on April 4, 2003 (PPQ 526 Permit No. 47497), and the first field inoculation of YST was made in North America outside of containment on July 9, 2003 in Napa County, California. Infection in the field was confirmed on July 30, 2003, and included both uredinia and telia. After the release in 2003, environmental conditions for spread in the field were unfavorable, but inoculum was increased in a CDFA greenhouse in Sacramento, CA. Plans are in place to monitor infection and spread of the fungus as well as to make additional releases in CA, pending extension and expansion of the permit. Events pertinent to biological control of YST in 2004 will be presented.

Evaluation of fludioxonil and azoxystrobin for control of Rhizoctonia root rot of soybean. E. S. BUCHER and W. L. Pedersen. University of Illinois, Urbana-Champaign, IL. Phytopathology 94:S12. Publication no. P-2004-0075-AMA.

Rhizoctonia root rot is a common, widespread disease of soybean. This study was designed to evaluate three seed treatments (mefanoxam with fludioxonil, azoxystrobin, and a combination of mefanoxam, fludioxonil and azoxytrobin) for control of this disease. Plots were inoculated with three levels of *Rhizoctonia solani* AG 2-2, including a non-infested ground sorghum treatment and control. Inoculum consisted of ground sorghum infested with *Rhizoctonia solani* AG 2-2 then mixed with dry sand at three levels (3:1, 1:1, 1:3 volume/volume) and applied with a 1.2 m Gandy applicator at 33.6 kg/ha and lightly incorporated. Soybean cultivar S-35A6 was planted with a 4-row planter with 38 cm rows. Plant stand, vigor, yield, and root disease incidence and severity were determined at V3 and R3. The combination of mefanoxam, fludioxonil, and azoxystrobin significantly lowered incidence of seedling disease at the 1:3 and 1:1 inoculation levels. Mefanoxam with fludioxonil was effective in reducing disease under normal conditions, while azoxystrobin alone was not. The combination of mefanoxam, fludioxonil, and azoxystrobin was the most effective in reducing disease.

Characterization of Sclerotinia stem rot resistance in double haploid *Brassica napus* for mapping purposes. BUCHWALDT, F. Q. Yu, R. Li, D. H. Hegedus, and S. R. Rimmer. Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatchewan, Canada. Phytopathology 94:S12. Publication no. P-2004-0076-AMA.

Sclerotinia sclerotiorum causes yield losses in diverse crops and resistance is rare. A Chinese *Brassica napus* cultivar, Zhongyou 821, known for its stem rot resistance under field condition was crossed with a susceptible *B. napus* and 225 double haploid lines were developed. These were characterized for stem rot resistance in a greenhouse as follows. A virulent clone of *S. sclerotiorum*, common in western Canada, was grown on agar containing glucose and sources of P, K plus Mg. Stems of flowering plants were inoculated with two (4 mm) plugs taken from the edge of a growing colony and attached with parafilm to the mid-stem section 10-15 cm apart. Lesion length was measured 7, 14 and 21 days after inoculation, and stem appearance was noted as either firm or collapsed. The average length of 10-20 lesions per line was continuous over plant genotype. The most resistant lines, with lesions less than 50 mm and firm stems, were significantly different from susceptible lines with lesions longer than 100 mm and collapsed stems. Mid-stem inoculation gave good differentiation and will be used for QTL mapping. Inoculation at the stem base or above the first side branch resulted in shorter and longer lesions, respectively. When mycelium was produced on V8 agar, cell wall degrading polygalacturonases were induced, which resulted in less differentiation.

Expanded host and geographic range of *Pseudomonas syringae* pv. *alisalensis*. C. T. BULL (1), P. Goldman (1), N. C. Morris (1), S. T. Koike (2), and D. Y. Kobayashi (3). (1) USDA/ARS, Salinas, CA; (2) Univ. Calif. Coop. Extension, Salinas, CA; (3) Dept. Plant Pathology, Rutgers Univ., New Brunswick, NJ. Phytopathology 94:S12. Publication no. P-2004-0077-AMA.

Pseudomonas syringae pv. *alisalensis* is an emerging pathogen that causes bacterial blight on crucifers in California. In 1995 a bacterial disease of arugula (*Eruca sativa*) in California was reported to be caused by *P. syringae* pv. *maculicola* and a similar disease was observed in New Jersey. Further characterization of pathogens from both locations indicated they were *P. syringae* pv. *alisalensis*. REP-PCR banding patterns of the arugula isolates were identical to those of *P. syringae* pv. *alisalensis* but distinct from *P. syringae* pv. *maculicola* patterns. Additionally, a bacteriophage isolated using *P. syringae* pv. *alisalensis* as a host lysed the arugula strains but not *P. syringae* pv. *maculicola* strains. Although these data indicate that the pathogens from arugula were *P. syringae* pv. *alisalensis*, additional host range studies are in progress to confirm this conclusion. While all the *P. syringae* pv. *alisalensis* strains from rappini and broccoli tested to date were positive for ice nucleation, the strains from arugula were negative. These data expand the geographic and host range of *P. syringae* pv. *alisalensis* which currently is known to be pathogenic on other crucifers and monocots and indicate that this pathogen is variable for ice nucleation. These data expand the geographic and host range of *P. syringae* pv. *alisalensis* which currently is known to be pathogenic on other crucifers and monocots and indicate that this pathogen is variable for ice nucleation.

Characterization of *Acidovorax avenae* subsp. *citrulli* strains isolated from watermelon and melon fruits in Israel. S. BURDMAN (1), N. Kots (1), and G. Kritzman (2). (1) Hebrew University of Jerusalem, Rehovot, Israel; (2) The Volcani Center, Bet Dagan, Israel. Phytopathology 94:S12. Publication no. P-2004-0078-AMA.

Acidovorax avenae subsp. *citrulli* (Aac) is the causal agent of bacterial fruit blotch (BFB) of cucurbits. BFB gained attention with outbreaks in watermelon fields in the Mariana Islands and Florida in the late 80's. Aac was also found to cause disease on other cucurbits such as cantaloupe, honeydew and pumpkin. In Israel, the bacterium was detected in imported seeds and in diseased seedlings grown from imported seeds during the 90's. Although the occurrence of BFB in Israel has been sporadic, in 2003, the disease caused yield losses in a few fields, where plants were produced from imported seeds. Here we report the isolation of several strains from diseased watermelon and melon plants. The strains were confirmed as Aac using Koch postulates, followed by GC-FAME analysis and sole carbon substrate utilization profiles. Differences were found among strains in their pathogenicity on watermelon, melon and cucumber varieties. Also, several differences were observed in carbon substrate utilization profiles. Aac strains isolated from similar hosts shared the most similarities in pathogenicity and carbon substrate utilization profiles. Walcott et al previously suggested the existence of at least two Aac groups, one including strains from cantaloupe and pumpkin, the other representing the typical BFB-causing strains. Results from this study indicate that both groups may have been imported to Israel.

The genetics of Luteovirus transmission in the aphid vector, *Schizaphis graminum*. M. BURROWS, D. Smith, E. Benson, and S. Gray. USDA-ARS, Cornell University, Ithaca, NY. Phytopathology 94:S12. Publication no. P-2004-0079-AMA.

The circulative, persistent transmission of viruses that cause barley yellow dwarf is well described, but the genetics of transmission in aphids is not well understood. Clonal variants of *Schizaphis graminum* were identified that differ in transmission phenotype. Sexual forms of clone Sg-F, which transmits BYDV-SGV and CYDV-RPV, and clone Sg-SC, which does not transmit either virus, were used to generate 11 F1 hybrids. F1 hybrids segregated independently for their ability to transmit SGV (0% to 83%) and RPV (0% to 90%). Sexual forms of the F1 hybrids were randomly mated and 46 F2 hybrids obtained. The F2 hybrids also segregate independently for their ability to transmit SGV (0% to 100%) and RPV (0% to 94%). These data suggest transmission of RPV and SGV is not controlled by the same loci. Purified RPV was injected directly into the hemocoel of three F1 hybrids that do not transmit RPV. Transmission efficiency was not significantly improved, suggesting the gut is not a major barrier to transmission, although it may reduce efficiency. A real time PCR method was developed to quantify RPV in whole aphids and aphid tissues during the transmission process. Sg-F individuals ingested an average of 1.9×10^8 copies of RNA while Sg-SC contained an average of 8.9×10^8 copies of RNA during a 48 hour acquisition access period. Sg-SC aphids ingested and retained more virus than Sg-F aphids, but they were unable to transmit RPV to a susceptible host. Current efforts are focused on identifying and quantifying virus movement across the different barriers within the vector.

Influence of environment on atmospheric concentrations of *Peronospora antirrhini* conidia in field-grown snapdragons. J. M. Byme, M. K. Hausbeck, and L. E. SCONYERS. Michigan State University, East Lansing, MI. Phytopathology 94:S13. Publication no. P-2004-0080-AMA.

Atmospheric concentrations of *Peronospora antirrhini* conidia in a commercial snapdragon field were monitored using a Burkard spore sampler over three growing seasons to investigate the influences of environment on the concentration of airborne conidia. Atmospheric conidial concentrations followed a diurnal pattern, and peak conidial concentrations occurred between 0700 and 0900 hours. Minimum daily temperatures below 10.0°C appeared to have a moderate limiting effect on atmospheric conidia concentrations, while temperatures below 6.0°C had more severe limiting effects. Maximum daily temperatures higher than 30.0°C limited concentrations of atmospheric conidia. Long dew periods (>6 hours) were associated with relatively large conidia releases. Consecutive days with short leaf wetness periods suppressed atmospheric conidial concentrations. Significant positive correlations were found between total rainfall and total spore count in 2000, which may explain large spore concentrations for that year. These findings, combined with current management practices, can aid in reducing downy mildew.

Population structure of *Cercospora kikuchii* from soybean. G. Cai and R. W. SCHNEIDER. Dept. Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803. Phytopathology 94:S13. Publication no. P-2004-0081-AMA.

Random amplified polymorphic DNA and microsatellite-primed PCR were used to characterize 161 isolates of *Cercospora kikuchii* collected from three soybean fields in Louisiana and three isolates from outside sources. Analysis of molecular variance showed that the Louisiana population was significantly different from outside isolates ($P = 0.01$), but isolates from different locations within the state were not significantly different. This analysis also provided evidence of tissue specificity (leaf vs. seed). Clustering analysis grouped most Louisiana isolates into clade (ABC) that was well separated from clade D. The latter clade included seven Louisiana isolates and the three outside isolates. Clade (ABC) was further divided into three clades, A, B and C, with clades A and B containing most isolates. Multilocus gametic disequilibrium tests failed to reject the null hypothesis of random mating in clade B, but it was rejected in other clades and the total collection. Pathogenicity tests were conducted on six soybean cultivars with representative isolates in clades A, B, and D. Clade D was significantly more virulent than the other two clades.

Biology of powdery mildew (*Podosphaera clandestina*) on sweet cherry (*Prunus avium*). J. M. CALABRO and R. A. Spotts. Oregon State University, Hood River, OR. Phytopathology 94:S13. Publication no. P-2004-0082-AMA.

Powdery mildew (PM) on sweet cherry, caused by *Podosphaera clandestina*, is a serious fungal pest in regions of Oregon and Washington. Studies were conducted to examine the relationship between PM infection and fruit pitting. After harvest, mildewed fruit were brought to 1°C, bruised with a standard instrument, and then held at 3-4°C. After two weeks all fruit were rated for pit development. Mildew infected fruit were no more sensitive to pitting following a bruise incident than fruit not infected with PM. Studies were also conducted to evaluate late season foliar PM levels under various management practices. Ten shoots per tree were collected; the outermost, fully expanded ten leaves of each shoot were rated for PM. Of the five cultivars compared, Sweetheart had the greatest PM incidence followed by Staccato, Lapins, Bing, and Regina with the least PM ($P = 0.05$). Of the twelve training system/rootstock combinations all on cultivar Bing, PM was greatest on Spanish bush training system/Mazzard rootstock and was the least on trees with steep leader training system/Edabriz rootstock ($P = 0.05$).

Pathogenicity of selected fungi on *Spartina alterniflora* and their possible role in Louisiana's marsh dieback. S. D. CALLAHAN and R. W. Schneider. Dept. Plant Pathol. & Crop Physiol., Louis. State Univ. Agric. Center, Baton Rouge, LA 70803. Phytopathology 94:S13. Publication no. P-2004-0083-AMA.

Approximately 158,000 hectares of *Spartina alterniflora* (smooth cordgrass), the predominant plant species in Louisiana's salt and brackish marsh, died in 2000-2001 from unknown causes. A severe drought and unusually high summer temperatures were recorded during this time. The term "brown marsh syndrome" (BMS) was coined to describe these large expanses of dead plants and bare mud flats. Several studies demonstrated that certain abiotic causes may be involved with BMS, but the entire syndrome has not been reproduced, and the role plant pathogens had not been explored. Fungi were isolated from roots and shoots of *S. alterniflora* that had been collected at BMS and nonaffected sites. Pathogenicity tests were conducted at two salinity levels by inoculating with colonized toothpicks and by placing colonized agar blocks at nodes beneath leaf sheaths. A species of *Fusarium* caused stalk rot-like symptoms in inoculated plants. These isolates also will be assessed in water stressed plants at different salinity levels.

The Texas cooperative oak wilt suppression project completes 16th year. K. S. CAMILLI (1) and R. F. Billings (2). (1) Texas Forest Service, Austin, TX; (2) Texas Forest Service, College Station, TX. Phytopathology 94:S13. Publication no. P-2004-0084-AMA.

Oak wilt, *Ceratocystis fagacearum*, infects and kills both live oaks (*Quercus virginiana*, *Q. fusiformis*) and red oaks (*Quercus shumardii*, *Q. marlandica*, *Q. velutina*). In Texas, due to extensive root grafting in stands of live oak this fungus can spread up to 100 feet per year. Therefore, uncontrolled disease centers may cover hundreds of acres and affect thousands of trees. Oak wilt has been confirmed on a diversity of sites in 59 counties in central Texas and 6 counties in west Texas. To address this devastating problem, the Texas Forest Service, in cooperation with the USDA Forest Service (Forest Health Protection) and the City of Austin, initiated the Texas Cooperative Oak Wilt Suppression Project in 1988. The primary objectives are to increase public awareness, provide technical assistance (including financial aid), detect, evaluate, and suppress oak wilt infection centers. Oak wilt centers are controlled by; disrupting the root systems through trenching, using fungicide injections, and removing diseased red oaks. Federal cost shares have been used to install 2.96 million of feet of trench (560 miles) as the primary means to halt disease spread in 2,126 expanding oak wilt centers within 34 counties. Trenches installed have a 65% success rate following a single treatment. Over 7,400 live oaks have been treated with fungicide and 3,650 infected red oaks have been identified and eliminated with project assistance. As the project enters its 17th year detection, prevention, suppression, and public education will continue.

Evaluation of Abound 2.08SC in calendar and AU-Pnut leaf spot advisory programs for disease control on peanut. H. L. CAMPBELL, A. K. Hagan, and K. L. Bowen. Dept. of Entomology and Plant Pathology, Auburn University 36849. Phytopathology 94:S13. Publication no. P-2004-0085-AMA.

In 2002 and 2003, Abound 2.08SC was evaluated on 'Georgia Green' peanut for control of early and late leaf spot caused by *Cercospora arachidicola* and *Cercosporidium personatum*, respectively and southern stem rot (SSR) caused by *Sclerotium rolfsii* using standard 14, 21, and 28-d calendar programs and standard (6/3) and modified (8/4, and 10/5) AU-Pnut leaf spot advisory rules. Chlorothalonil was applied at a rate of 1.26 kg ai/ha followed by Abound 2.08SC at 0.34 kg ai/ha. In 2002, peanuts treated under the standard 14-d calendar schedule gave significantly better leaf spot control than the other programs according to AUDPC. SSR control and pod yield was similar across all programs. In 2003, AUDPC for the 10/5 AU-Pnut schedule were significantly higher than those in the other programs. SSR incidence was highest in plots treated according to the 10/5 AU-Pnut schedule. Despite significant differences in leaf spot and SSR control, yield response with the modified 6/3 and 10/5 AU-Pnut advisories was similar.

Impact of strip-till into various cover crops on disease development and yield in peanut. H. L. CAMPBELL, J. R. Weeks, and A. K. Hagan. Dept. of Entomology and Plant Pathology, Auburn University, AL 36849. Phytopathology 94:S13. Publication no. P-2004-0086-AMA.

In 2001, 2002, and 2003, impact of various cover crops was evaluated in a strip-till production system for its effect on diseases of peanut. Selected winter forage crops were planted followed in spring by strip-till planting of peanut into forage residue. *Tomato spotted wilt virus* (TSWV) and southern stem rot (SSR) were evaluated in all plots and soil samples for nematode assay were taken. In 2001, there was no significant difference in the incidence of TSWV or SSR between cover crops. Ryegrass/wheat plots yielded significantly higher than the other forage crops except for ryegrass/oats. In 2002, there were no significant differences in SSR incidence but peanut in the wheat and rye/ryegrass plots had significantly less TSWV compared with those planted behind the oats. Root knot nematode counts were highest in the rye plots and lowest in the fallow plots. In 2003, TSWV incidence was lower in peanut planted behind rye and wheat than behind oats and fallow. Again, SSR counts were similar across all forage crops. Nematode population counts were highest in the rye plots and lowest in the fallow plots.

Epidemic analysis of early leaf spot suppression by strip-tillage. E. G. CANTONWINE and A. K. Culbreath. University of Georgia, Tifton, GA. Phytopathology 94:S13. Publication no. P-2004-0087-AMA.

Early leaf spot (ELS), caused by *Cercospora arachidicola*, is an important disease of peanut (*Arachis hypogaea*). Epidemics are suppressed by strip-tillage in fields under crop rotation. Experiments were carried out to identify

the effect of strip-tillage on epidemics and mechanisms responsible for suppression. Peanut was planted to conventional and strip-tilled fields. ELS intensity was assessed weekly as % incidence or with the Florida 1-10 scale to compare epidemic onsets and rates. Host resistance was assessed using a detached leaf inoculation procedure. Non-inoculated detached leaves and Rotorod samplers were used to monitor natural inoculum. Infection periods were determined using HOBO data loggers. Epidemic onset was delayed in strip-tillage by 6 days in 2002, and 11 days in 2003; rates were not affected. Tillage did not affect resistance. Inoculum was detected earlier with detached leaves from conventional tillage. Spore concentrations in the air were correlated with disease. Fewer infection periods occurred in strip-tillage in 2002, but not in 2003. These data suggest that strip-tillage delays the onset of ELS epidemics due to a reduction in the amount of initial inoculum.

Characterization of fungicide resistance and pathogenic fitness in a population of *Pythium dissotocum* isolated from nurs[10]rrigation water. K. M. CARLSON, C. X. Hong, and P. A. Richardson. Virginia Polytechnic Institute and State University, Virginia Beach, VA. Phytopathology 94:S14. Publication no. P-2004-0088-AMA.

Plant disease caused by *Pythium* spp. is a serious concern in nurseries that utilize recycled irrigation water. Fungicide use is the most commonly employed control method, and the available chemistries must be used prudently to reduce the risk of fungicide-resistance. The most frequently used chemicals in[10]e the acyclalanine, mefenoxam, and the carbamate, propamocarb. The objective of this study was to characterize the *in-vivo* and *in-vitro* resistance to mefenoxam and propamocarb in a population of *Pythium dissotocum* isolated from nursery irrigation water, and to compare the pathogenic fitness of the sensitive and resistant isolates. *In-vitro* resistance to both fungicides was characterized in over 200 isolates by measuring isolate growth on fungicide amended media. Over 35% of these isolates were resistant to mefenoxam, 5% were resistant to propamocarb, and 7% were resistant to both fungicides. *In-vivo* resistance was not highly correlated with *in-vitro* resistance in isolates with resistance to propamocarb or low resistance to mefenoxam, but typically was correlated with isolates having high resistance to mefenoxam. Realized pathogenic fitness, based upon a replacement series assay, is currently being compared in isolates designated as sensitive or resistant to either or both fungicides.

Deletion mutagenesis of eight *avr* gene homologues in *X. campestris* pv. *campestris*. A. CA[58]AÑEDA, Y. P. Duan, B. El Yacoubi, J. D. Reddy, and D. W. Gabriel. Plant Molecular and Cellular Biology Program, Plant Pathology Dept., University of Florida, Gainesville, FL. Phytopathology 94:S14. Publication no. P-2004-0089-AMA.

Xanthomonas campestris pv. *campestris* (Xcc) causes black rot of a very wide range of cruciferous hosts. Its genomic DNA sequence was recently published. Despite the fact that there limited race specificity of Xcc, eight genes with significant similarity to avirulence (*avr*) genes were found in the genome. *avr* genes encode effectors that determine pathogen race specificity and sometimes host-specific pathogenicity. Marker-interruption was used to individually knock out all eight of the potential *avr* genes, with no evident effect on pathogenicity on cabbage. Splice overlap PCR was then used to delete several combinations of two, three, four and five of the *avr* genes, again with no evident effect on pathogenicity on cabbage. Three of the *avr* genes were cloned and expressed in both *X. citri* and in *Pseudomonas fluorescens* carrying pHIR11. One of the *avr* genes suppressed both host and non-host hypersensitive responses (HRs), indicating a role in plant defense suppression.

Selective genetic suppression of hypersensitive-response-related cell death in resistant *Nicotiana* spp. during infection with CaMV. J. [24]CAWLY (1), A. B. Cole (1), W. Qiu (2), L. Kiraly (1), and J. Schoelz (1). (1) University of Missouri, Columbia, MO; (2) Southwest Missouri State University, Mountain Grove, MO. Phytopathology 94:S14. Publication no. P-2004-0090-AMA.

[25]
The P6 protein of *Cauliflower mosaic virus* (CaMV) strain W260 elicits a hypersensitive response (HR) on inoculated leaves of *Nicotiana edwardsonii*. This HR, common to many plant pathogens, has two key characteristics: cell death within the initially infected tissues and local restriction of the pathogen. Here we show that a cell death suppressor gene designated *CCD1*, originally identified in *N. bigelovii*, selectively blocks the cell death pathway during HR, whereas the resistance pathway against W260 remains intact. Cell death suppression was evident both macroscopically and microscopically. The suppression of HR-m[25]ed cell death was specific to CaMV, as *Tobacco mosaic virus* elicited HR in the plants that contained the suppressor gene. *CCD1* also blocks the development of a systemic cell death symptom induced specifically by the P6 protein of W260 in *N. clevelandii*. Introgression of *CCD1* from *N. bigelovii* into *N. clevelandii* blocked the development of systemic cell death in response to W260 infection, but did not prevent syst[25] cell death induced by *Tomato bushy stunt virus* or *Tomato spotted wilt virus*. Thus, *CCD1* blocks both local and systemic cell death induced by P6 of W260, but does not act as a general suppressor of cell death induced by other plant viruses.

[158]
Altered structure, composition, and biological activity of root exudates in transgenic roots with [157]ed root cap gene expression. R. CELOY, F. Wen, S. Gardais, and M. C. Hawes. University of Arizona, Tucson, AZ. Phytopathology 94:S14. Publication no. P-2004-0091-AMA.

[239]
Microbiology and ecology of the rhizosphere play a key role in plant health and are strongly influenced by plant genotype. Although mechanisms for genotype specificity in community population dynamics remain unclear, exudates released from roots into the external environment underlie the plant's capacity to stimulate microbial growth in the rhizosphere. In healthy, unwounded roots of most crop species, the majority of root exudates (up to 90%) are released from the root tip region by active secretion from the root cap. Such exudates include a high molecular weight polysaccharide mucilage, and thousands of detached living cells (root 'border' cells). A combination of approaches was used to characterize root cap gene expression patterns and to isolate specific genes required for production of border cells. Gene specific silencing was used to inhibit expression of genes controlling the release of border cells and associated exudates produced by roots, and to show that such plants exhibit marked changes in their ability to stimulate growth, gene expression, and behavioral responses in soilborne bacteria and fungi.

[6]
Construction of linkage map in creeping bentgrass and QTL mapping for dollar spot resistance in greenhouse and field inoculations. [3]CHAKRABORTY (1), J. J. Bae (1), J. Curley (1), S. Warnke (2), and G. Jung (1). (1) Dept. of Plant Pathology, Univ. of Wisconsin, 1630 Linden Dr., Madison, WI 53706, USA; (2) US Nat'l Arboretum, Washington D.C., 20002, USA. Phytopathology 94:S14. Publication no. P-2004-0092-AMA.

Dollar spot caused by *Sclerotinia homoeocarpa* F. T. Bennett is the most economically important turf disease in North America. Dollar spot resistance in a creeping bentgrass cultivar would greatly reduce the c[10] and environmental impacts of fungicide application. Not much work has been done to understand the genetic mechanism of resistance to dollar spot in creeping bentgrass. A genetic mapping population having the F_1 full-sib structure was developed from the cross between the outcrossing clones 372 and 549 showing difference in disease response. A linkage map with fourteen linkage groups has been constructed so far, with one hundred and twenty-two RAPD and fifty-three RFLP markers. Twenty-nine of the RFLP markers are from sequenced probes from cDNA libraries constructed from leaf tissue of the two mapping parents. These ESTs have the potential for correlating genes to traits like disease resistance. Some of the cDNA clones with putative functions like protein kinase and *N. tabacum* wound inducive protein have been mapped. For mapping QTL for dollar spot resistance, two greenhouse experiments using one isolate have been done on three hundred progenies. Transgressive segregation was seen in both, with the parents being intermediate. Both single factor ANOVA and interval mapping have been used to detect multiple QTLs over the experiments. One QTL in linkage group 4 is consistent in both the experiments with high LOD, whereas two additional QTLs are specific to certain experiments. QTL analysis based on field inoculations in summer 2004 at two locations will be presented.

[52]
Status of the citrus canker eradication program in Florida and development of a citrus canker extention education program of the University of [8]rida. H. L. CHAMBERLAIN (1), L. W. Timmer (2), and P. D. Roberts (1). (1) University of Florida, SWFREC, Immokalee, FL 34142; (2) University of Florida, CREC, Lake Alfred, FL 33850. Phytopathology 94:S14. Publication no. P-2004-0093-AMA.

[52]
Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri*, was detected in Florida for the third time in 1995 near the Miami International Airport on a residential citrus tree. Since detection, citrus canker has spread to sixteen different counties in central and south Florida. Various legal battles in the residential sector have halted eradication efforts in some areas of Florida. However, recent decisions from the Florida State Supreme court have upheld the eradication process and procedures. Over two million commercial citrus trees and nearly 800,000 residential trees have been removed. Eradication continues in residential areas and there has been one recent large infestat[52]n a commercial grove, but quarantines have been lifted from some areas following successful eradication. A citrus canker extension program was developed to lead and coordinate education for the commercial citrus industry, homeowners, and non-citrus commercial businesses. The mission of the program is to reduce spread by eliminating transport of infected citrus plant material and encouraging decontamination of vehicles and personnel. The

Division of Plant Industry continues to address legal issues where necessary and conduct extensive survey and control efforts. Public and private agencies have partnered to continue statewide education activities meeting the needs of various audiences.

Plasmid-borne *nor-nir* genes in the microsymbiont *Rhizobium meliloti* JJ1c10. Y.-K. CHAN and W. A. McCormick. Agriculture and Agri-Food Canada, Ottawa, ON, Canada. Phytopathology 94:S15. Publication no. P-2004-0094-AMA.

The sequential dissimilatory reduction of nitrite and nitric oxide to nitrous oxide is the key reaction in denitrification. It requires the expression of the *nir* and *nor* genes. Southern hybridization using specific structural gene probes for *nirK* and *norCB* confirmed the location of *nir* and *nor* genes on the pSymA megaplasmid of *R. meliloti*, the N_2-fixing endosymbiont of alfalfa. Nucleotide sequence analysis revealed a 20-kb *nor-nir* region, which was linked to the *nap* genes encoding periplasmic nitrate reduction. The *nos* region encoding nitrous oxide reduction is situated within 30 kb downstream from *nir*, with a *fix* cluster intervening between them. Most of the *nor-nir* genes appeared to be well conserved among related proteobacterial denitrifiers. Tn*5* mutants of *R. meliloti* were generated for functional tests, which confirmed the requirement of NirV and one unidentified protein for nitrite reduction; NorB, NorD and another unidentified protein for nitric oxide reduction. The high amino acid sequence identity of the predicted *R. meliloti* gene products with those of *Pseudomonas* G-179 suggests that these two soil bacteria share a common source for their denitrification genes. However, their location and arrangement in *R. meliloti* are different from those of G-179. This is likely a result of lateral gene transfer, rearrangement, and recombination.

Pierce's disease severity in relation to various rootstocks. C. J. CHANG (1) and R. E. Scott, Jr. (2). (1) University of Georgia, Griffin, GA; (2) Montmorenci Vineyard, Aiken, SC. Phytopathology 94:S15. Publication no. P-2004-0095-AMA.

One hundred Carignane grapevines with St. George as rootstocks were planted at Montmorenci Vineyard, Aiken, SC in 1995. In 1998, 1147 Cabernet Sauvignon grapevines with three various rootstocks were planted; 179 vines with rootstock 101-14, 463 with 420A, and 505 with 3309. As of today, there were 59 (59%) Carignane alive, and 84 (47%), 128 (28%), and 65 (13%) Cabernet Sauvignon alive with its respective rootstocks of 101-14, 420A, and 3309. The rootstocks seemed to play a role in the survival of Carignane and Cabernet Sauvignon to Pierce's disease (PD). None of the leaf petioles from 21 St. George rootstocks was tested positive for the presence of PD bacterium either in ELISA testing or in bacterial isolation on PW medium. Of the 58 Carignane leaf petioles tested, 53 (91%) were positive in ELISA testing, while only 15 (26%) gave positive isolation on PW. Of the surviving Cabernet Sauvignon, leaf petioles from 21, 19, and 20 vines with rootstocks 101-14, 420A, and 3309 respectively were tested. The vines gave positive results in ELISA were 17 (81%), 14 (74%), and 15 (75%) and in isolation 6 (29%), 3 (16%), and 6 (30%) in respect to rootstocks 101-14, 420A, and 3309.

Distribution of *Typhula* species in Wisconsin, Michigan, Minnesota, and Utah using species-specific PCR primers. S. V. CHANG (1), E. Scheef (1), S. Thomson (2), P. G. Johnson (2), and G. Jung (1). (1) University of Wisconsin, Madison, WI; (2) Utah State University, Logan, UT. Phytopathology 94:S15. Publication no. P-2004-0096-AMA.

Snow mold, caused by *Typhula* spp. is one of the most important winter diseases of perennial grasses and winter cereals in the northern regions of the United States. *Typhula* species that cause snow mold include *Typhula incarnata* (TIN), *T. phacorrhiza* (TPH), and *T. ishikariensis* (TISH) *ishikariensis* (TISI), var. *canadensis* (TISC) and var. *idahoensis* (TISD). The objective of this study was to investigate the geographical distribution of *Typhula* species in Wisconsin, Michigan, Minnesota, and Utah so that the effects of environmental factors on their adaptation can be understood. The diseased samples were collected from golf courses in WI (2001 and 2002), UT (2001), MI (2002), and MN (2002). *Typhula* species and varieties were identified through the use of species-specific PCR primers. The TIN was distributed throughout WI, whereas fungi of the TISHs were detected in a higher frequency where there was longer snow cover in all states. Within the TISHs, TISI was most frequently identified throughout the sampling areas in WI (2001), UT, and MI. However, in 2002, a winter with shorter snow cover duration, the frequency of TISC in MN and WI was higher than the other varieties. This result suggests that TISC is a good opportunist and better competitor, growing more actively in conditions that the other varieties could not tolerate. In contrast, TISD was much less frequently found throughout the collection areas except UT (none). In addition, TPH was detected in the lowest frequency ranging from 0 to 10% in each of the four states.

Pathogenicity of *Microdochium nivale* isolates collected in Wisconsin and evaluation of virulence to bentgrass cultivars. T. H. CHANG, S. W. Chang, and G. Jung. University of Wisconsin, Madison, WI. Phytopathology 94:S15. Publication no. P-2004-0097-AMA.

Pink snow mold caused by *Microdochium nivale* is a major disease of turfgrasses during the winter season in Wisconsin. One hundred sixty isolates of *M. nivale*, collected from golf courses throughout Wisconsin, were analyzed for their genetic diversity, pathogenicity, and virulence to bentgrasses. In growth chamber experiments, 86% of the isolates were pathogenic to both a creeping bentgrass 'Penncross' and a colonial bentgrass 'Tiger'. Virulence of eight different isolates of *M. nivale* from four genetically diverse groups estimated from RAPD marker-derived genetic distance, were evaluated on 80 day-old cultivars of bentgrasses (nine creeping, five colonial and three velvet) under controlled environmental conditions. The virulence and host resistance of *M. nivale* isolates among cultivars and species of bentgrass were statistically significant ($P < 0.0001$), however, no significant interaction between cultivars and isolates was detected. Of the three bentgrass species, creeping bentgrass was the most resistant to *M. nivale* isolates. Of the 17 cultivars, 'Pennlinks' creeping bentgrass was the most resistant, while 'Bardot' colonial bentgrass was the most susceptible to the pathogen. Increasing the inoculum concentrations (fresh mycelium weight/volume) of *M. nivale* significantly increase the amount of disease development in each bentgrass species. However, a slope of the dose response disease curve was steeper in virulent isolates than less virulent isolates. Comparison of field and in vitro evaluations of virulence and pathogenicity of *M. nivale* on bentgrasses will be discussed.

Role of *Pseudomonas putida* alginate production in biofilm development and stress tolerance in low-water-content habitats. W.-S. Chang, M. van de Mortel, and L. J. HALVERSON. Iowa State University, Ames, IA. Phytopathology 94:S15. Publication no. P-2004-0098-AMA.

Bacteria colonize soil and plant surfaces as biofilms, which are aggregates of cells encapsulated in an exopolymeric substance (EPS) of their own making. The hygroscopic nature of EPS may improve bacterial growth and survival in low-water-content habitats. We assessed whether alginate is a component of the *P. putida* EPS under matric stress (low water content) conditions, and whether it protects the cell from the damaging effects of cellular dehydration. Carbohydrate composition analysis showed that alginate was a significant component of EPS under matric stress, but not solute stress or unstressed, conditions. With increasing matric stress severity, there was a corresponding increase in alginate production by the wild type, but not by an alginate-deficient (*algD*) mutant. The architecture of biofilms formed by the *algD* mutant under matric stress conditions was distinct from that of the wild type. Alginate production also enhanced cell survival under severe desiccation stress and reduced the rate of biofilm drying. These results suggest that alginate production increases the hydration of the immediate microenvironment of the cell and protects biofilm cells from the damaging effects of desiccation stress.

Programmed cell death and structural defense mechanisms in pepper fruit against *Xanthomonas axonopodis* pv. *glycines* infection. S. P. CHANG, Y. H. Jeon, S. G. Kim, and Y. H. Kim. School of Agricultural Biotechnology and Center for Plant Molecular Genetics and Breeding Research, Seoul National University, Seoul 151-742, Korea. Phytopathology 94:S15. Publication no. P-2004-0099-AMA.

Plants defend themselves against pathogens by structural and biochemical reactions. Defense structures act as physical barriers and inhibit pathogen from gaining entrance and spreading through the plant. *Xanthomonas axonopodis* pv. *glycines*, the causal pathogen of bacterial pustule of soybean, causes hypersensitive response on pepper plant. When pepper fruits were inoculated with *X. axonopodis* pv. *glycines*, time-series defense-related structural changes occurred in the inoculated sites. Early programmed cell death (PCD) responses characterized by condensation and vacuolization of the cytoplasm, membrane separation from the cell wall, condensation and compartmentalization of nuclear materials, and membrane blebbing were observed by transmission electron microscopy. Nuclear fragmentation proved by TUNEL (TdT-mediated dUTP nick end labeling) method under confocal laser scanning microscopy and DNA laddering futher supported PCD responses in inoculated sites. Cell elongation and cell division forming a periderm-like boundary layer that demarcated healthy tissues from the inoculation sites were observed at later stages. Using several stains such as toluidine blue, sudan, annexin V and phloroglucinol-HCl, defense-related materials and structural changes were examined. Accumulation of defense-related materials such as lignin, cutin, suberin and phenolic compounds around the inoculation sites were observed. Altogether, these results suggest that non-host pepper plant against *X. axonopodis* pv. *glycines* infection defend itself by PCD responses and structural and biochemical reactions.

Effect of Annosus root rot on the keepability of noble fir Christmas trees. G. A. CHASTAGNER and P. Kaufmann. Washington State University, Puyallup, WA. Phytopathology 94:S16. Publication no. P-2004-0100-AMA.

Annosus root rot, caused by *Heterobasidion annosum*, has become a significant problem in second and third rotation Pacific Northwest noble and Fraser fir Christmas tree plantations. In some fields, over 30% of the trees are killed prior to harvest. In fields with high levels of disease, growers will begin harvesting trees once the trees reach a minimum marketable size in an effort to minimize disease losses. Various levels of staining are often evident on the cut trunks of these trees. A study was done to determine if there was a relationship between the percentage of stem surface area stained at harvest and the postharvest keepability of noble fir trees. Freshly cut healthy and healthy-appearing trees with various levels of staining were displayed in water. Within 4 to 7 days, trees with the highest degree of staining had noticeably lower moisture levels than the other trees. After 21 days, the percent moisture contents (MC) of the trees without any staining ranged from 127.4 to 157.2%. Trees with the lowest level of staining (1.9 to 19.8% of the trunk surface stained) had MC's similar to the healthy trees. However, the MC's of the trees with the highest degree of staining (36.2 to 56.7% of the trunk stained) ranged from 19.7 to 96.8%. These results indicate that a high level of staining on healthy-appearing trees has a significant, adverse affect on the postharvest keepability of Christmas trees.

Susceptibility of conifer shoots to infection by *Phytophthora ramorum*. G. A. CHASTAGNER (1), E. M. Hansen (2), K. L. Riley (1), and W. Sutton (2). (1) Washington State University, Puyallup, WA; (2) Oregon State University, Corvallis, OR. Phytopathology 94:S16. Publication no. P-2004-0101-AMA.

Large numbers of different types of conifers are grown as Christmas trees and in conifer nurseries in the Pacific Northwest. Two conifers, Douglas-fir and coast redwood, have been reported among the naturally-infected hosts of *Phytophthora ramorum* in California. This pathogen also recently has been isolated from grand fir Christmas trees in California. In an effort to better understand the potential impact this pathogen might have on the Christmas tree and conifer nursery industries, a series of inoculation studies were conducted to determine the potential susceptibility of foliage and shoots from 25 conifers to *P. ramorum*. Twenty of the conifers tested, including many of the important species that are used as Christmas trees, were susceptible to *P. ramorum*. Some true firs were highly susceptible. Symptoms included needle blight, a shoot blight resulting from needle infections, and stem lesions resulting from the growth of the pathogen from infected needles into the stem. Growth stage had a significant effect on susceptibility. Needles on Douglas-fir shoots were only susceptible to infection when inoculated just after bud break. The results indicate that many different types of conifers are potentially susceptible to *P. ramorum*.

Effectiveness of fungicides in protecting Douglas-fir shoots from infection by *Phytophthora ramorum*. G. A. CHASTAGNER (1), E. M. Hansen (2), K. L. Riley (1), and W. Sutton (2). (1) Washington State University, Puyallup, WA; (2) Oregon State University, Corvallis, OR. Phytopathology 94:S16. Publication no. P-2004-0102-AMA.

The effectiveness of 20 fungicides in protecting Douglas-fir seedlings from infection by *Phytophthora ramorum* was determined. Some systemic products were applied about a week prior to bud break, while most treatments were applied just after bud break. One day after the pre-bud break treatment applications, all the seedlings were inoculated and then incubated under optimal conditions for disease development. The only pre-bud break treatment that completely prevented infection was the drench application of Subdue MAXX. Pre-bud break drench applications of Stature, Insignia, and Terrazole had no affect on the number of infected seedlings. The reduction in infection by the pre-bud break applications of Heritage and Chipco Signature was variable and applications of Phostrol reduced infections by about 71 to 75%. Post-bud break applications of Dithane, Gavel, Maneb, and Polyram provided 100% control. Although not as consistently effective, applications of Champ Formula 2F, Reason, Daconil Ultrex, Stature, and IKF – 916, reduced the number of infected seedlings by 70 to 100%. Most of the other fungicides included in these tests provided more limited or variable reductions in the number of infected seedlings. These results indicate that several fungicides have the potential to provide excellent control of *P. ramorum* on Douglas-fir, including several common "protectant" types of fungicides.

Quorum sensing in *Erwinia carotovora* subspecies: *rsmA* is the primary target of ExpR, a LuxR homolog. A. CHATTERJEE, Y. Cui, H. Hasegawa, and A. K. Chatterjee. University of Missouri, Columbia, MO. Phytopathology 94:S16. Publication no. P-2004-0103-AMA.

The quorum sensing signal, *N*-[3-oxohexanoyl]-L-homoserine lactone (AHL), is required by *Erwinia carotovora* subspecies for the production of extracellular proteins (exoproteins) and secondary metabolites, the expression of pathogenicity genes and the Hrp (hypersensitive response and pathogenicity) regulon. Although ExpR, a LuxR homolog, has been detected in Ecc strains, its role in regulation of AHL or exoproteins is not understood. Studies with *expR* from Ecc strain SCC3193 revealed that it prevented exoprotein production in strains Ecc71 and SCRI193. These negative effects resulted from activation of *rsmA* expression by ExpR. RsmA, a RNA binding protein, is a translational repressor and also promotes message decay [Rsm = regulator of secondary metabolism]. In *E. coli* MC4100 carrying *expR*, the expression of an *rsmA-lacZ* transcriptional fusion, as in an Ecc71 derivative, was strongly activated in the absence of AHL. In its presence, the ExpR effect was repressed. Studies in *E. coli* also demonstrated ExpR-mediated activation of expression of *rsmA* from *E. carotovora* ssp. *atroseptica* strain Eca12 and Ecc strains EC153 and SCC3193. We conclude that ExpR activates the *rsmA* promoter and AHL prevents this activation. Our findings for the first time document regulation of a gene for an RNA-binding protein by a LuxR-like protein and AHL, link quorum sensing signaling via post-transcriptional regulation and explain the basis for the pleiotropic effect of AHL in Ecc.

Functional genomics of *Pseudomonas syringae* pv. tomato: A protocol for rapid isolation of gene-specific Tn5-insertion mutants. A. CHATTERJEE (1), D. Tian (1), H. Hasegawa (1), Y. Cui (1), A. Collmer (2), and A. K. Chatterjee (1). (1) University of Missouri, Columbia, MO; (2) Cornell University, Ithaca, NY. Phytopathology 94:S16. Publication no. P-2004-0104-AMA.

The availability of the *P. syringae* pv. tomato strain DC3000 genome sequence has paved the way to systematic analysis of gene function. However, realization of this potential requires a high throughput method for gene inactivation and a method to identify mutations in specific genes. We describe a protocol that allows rapid screening for desired bacterial mutants by PCR. By mating, we recovered ca. 7000 potential mini-Tn5 (Km^r) insertion mutants and screened them for random insertions. Two types of PCR primers were used: one set of primers consisted of Tn5 sequences and the other set represented gene specific sequences. For amplification, gene-specific primers were used in conjunction with Tn5 primers as pairs such that amplicons would be produced only if the Tn5 insertion occurred in the target gene. Using this protocol, we obtained several regulatory mutants including $AhlR^-$, Crp^-, $GacS^-$, $PhoP^-$ and gene for tail tape measure protein. Inactivation of the cognate genes was confirmed by RNA analysis and phenotypic analysis. These studies are being extended to generate a collection of well-characterized mutants for community use.

Transition from methyl bromide use on commercial vegetable farms. D. O. CHELLEMI (1), J. Mirusso (2), and J. Nance (3). (1) USDA, ARS, Ft. Pierce, FL 34945; (2) Mirusso Enterprises, Delray Beach, FL 33446; (3) Dow AgroSciences, Winter Haven, FL 33884. Phytopathology 94:S16. Publication no. P-2004-0105-AMA.

Alternatives to methyl bromide were monitored on six commercial farms in southeastern Florida. Broadcast application of a mixture of 1,3-dichloropropene and chloropicrin using a deep placement coulter system with a subsequent application of the herbicides napropamide and trifluralin and a final application of chloropicrin in the planting bed resulted in levels of soilborne diseases similar to adjacent areas fumigated with a mixture of methyl bromide and chloropicrin. Yield of fresh market pepper in areas treated with the alternative program ranged from 12% below to 18% above yields in adjacent methyl bromide treated areas. Yield of fresh market tomato ranged from 9% below to 10% above yields with methyl bromide. After three consecutive years of use on a pepper farm, disease incidence remained similar to levels in adjacent methyl bromide treated areas and a 15% increase in yield was documented. After four consecutive years of use on a tomato farm, disease incidence also remained at a level similar to adjacent methyl bromide treated areas and a 10% increase in yield was observed. The results demonstrate that a technically feasible alternative to methyl bromide is available for fresh market tomato and pepper production in Florida.

L-proline, a potent antioxidant, is able to suppress ROS-induced apoptosis-like programmed cell death in *Colletotrichum trifolii*. C. CHEN and M. B. Dickman. University of Nebraska-Lincoln, Lincoln, NE. Phytopathology 94:S16. Publication no. P-2004-0106-AMA.

The small GTP-binding protein Ras is known to play a crucial role in regulating cellular signal transduction processes leading to cell growth, differentiation and apoptosis. Previous studies in *Colletotrichum trifolii*, the causal agent of alfalfa anthracnose, have shown mutants expressing a dominant active form of Ras (DARas) exhibit severe defects in polarized growth, aberrant hyphal morphology and significantly reduced conidiation when grown on minimal media. Interestingly, this aberrant hyphal phenotype can be completely restored by L-proline. Here, we extended our analyses on

the mechanism(s) by which L-proline restores the wild type hyphal phenotype in the DARas mutant. We found that aberrant hyphal growth in DARas mutants was accompanied by highly increased ROS levels and this ROS production was significantly inhibited by L-proline with a result of phenotypic reversion. Moreover, our results indicated that the high levels of ROS in DARas mutant triggered an apoptosis-like programmed cell death, as assessed by TUNEL labeling and Annexin V staining, and this apoptotic response was also inhibited by L-proline. Thus, our data reveal a previously unrecognized function of L-proline: its ability to suppress ROS-induced apoptosis-like programmed cell death, as a potent antioxidant. The ability of L-proline to scavenge intracellular ROS and thereby inhibit ROS-mediated apoptosis may be an important and broad based function of this amino acid in responding to cellular stress, in addition to its well established as an osmolyte.

Osmosensitivity and osmoprotection of selected *Pseudomonas syringae* strains. C. CHEN and G. A. Beattie. Iowa State University, Ames, IA. Phytopathology 94:S17. Publication no. P-2004-0107-AMA.

The osmotolerance of a *P. syringae* strain may influence its growth in and on plants. This tolerance is a function of its osmosensitivity and its response to osmoprotectant compounds, which may be produced by the host or other microorganisms. We evaluated the tolerance of three *P. syringae* strains for which the genome sequence is, or soon will be, available: *P. s.* pv. *tomato* DC3000, *P. s.* pv. *syringae* B728a, and *P. s.* pv. *phaseolicola* 1448A. We included *P. aeruginosa* PAO1 for comparison. In a low osmolarity medium amended with NaCl, the strains exhibited significant differences in their osmosensitivity, with DC3000 being the most sensitive (DC3000 > 1448A > B728a > PAO1). Glycine betaine, choline, and to a lesser extent acetylcholine, were effective osmoprotectants for all 4 strains when provided exogenously at 1 mM. Proline, carnitine, DL-pipecolate, and glutamate conferred protection in a strain-dependent manner, whereas trehalose, sucrose, mannitol, maltose and taurine conferred no protection. These differences in osmotolerance among the strains may impact their phyllosphere fitness and interactions with the host plant.

Critical evaluation of green fluorescent protein-based bioreporters deployed in stressful environments. C. CHEN and G. A. Beattie. Iowa State University, Ames, IA. Phytopathology 94:S17. Publication no. P-2004-0108-AMA.

We evaluated the performance of whole-cell biosensors under stressful conditions encountered by epiphytic bacteria in the phyllosphere. These biosensors contained a fusion between an osmotic stress-responsive promoter and the *gfp* gene. For time-course studies, we used chloramphenicol to inhibit protein synthesis during the incubation period required for GFP folding. We found that solar radiation decreased the fluorescence of GFP-expressing cells, probably by inactivating GFP, and thus was a barrier to the use of GFP-based biosensors as quantitative bioreporters on leaves in the field. For the biosensor and a control that constitutively expressed *gfp*, conditions that were stressful enough to significantly reduce the bacterial populations (e.g., exposure to rapid desiccation) also reduced the mean fluorescence of living cells. This result indicates that highly stressful conditions may interfere with the quantitative response of GFP-based biosensors to the target signal.

US crop grouping and international harmonization. H. CHEN (1), B. Schneider (2), D. C. Thompson (1), D. L. Kunkel (1), J. J. Baron (1), and R. E. Holm (1). (1) IR-4 Project, Rutgers University, North Brunswick, NJ; (2) US EPA, Washington, DC. Phytopathology 94:S17. Publication no. P-2004-0109-AMA.

Crop grouping was first defined in 1971 in "Food and Feed Crops of the United States" (the "Green Book"). The current US crop grouping scheme was published in 40CFR, 180.41 in 1995. The second edition of the "Green Book" published in 1998 discussed the use of crop groups for conducting residue studies in the US. The "Green Book" has become a great tool to facilitate pesticide registrations in many crops with fewer residue studies and lower cost. Even though the current crop grouping scheme has enabled a 5-fold increase in US minor use registration, there are still many orphan crops that are not included in any crop group, and there has been continued importation and introduction of ethnic crops into the US since 1995. An expansion of the US crop grouping scheme has become a great challenge for improving the efficiency of the registration process, and for facilitating establishment of import tolerances and international harmonization. The USDA/IR-4 International Crop Grouping Symposium held in October of 2002 produced a significant number of new crop group proposals. To bring these proposals to federal approval as new crop group regulations, a long-term joint effort between IR-4 and the EPA with input from national and international researchers and crop experts has been initiated. This effort will not only potentially double the number of food use clearances per tolerance petition, but also facilitate the coordination between US and Codex classifications and international harmonization.

Simultaneous association of two *Xylella fastidiosa* genotypes with almond leaf scorch disease in California. J. CHEN (1), R. Groves (1), L. L. Civerolo (1), M. Viveros (2), M. Freeman (3), and Y. Zheng (4). (1) USDA-ARS, Parlier, CA, University of California Cooperative Extension; (2) Bakersfield; (3) Fresno, CA; (4) P.O. Box 27853, Fresno, CA. Phytopathology 94:S17. Publication no. P-2004-0110-AMA.

Almond leaf scorch disease (ALSD) has recently reemerged in San Joaquin Valley of California threatening almond production. ALSD is caused by *Xylella fastidiosa*, a nutritionally fastidious bacterium. To analyze the bacterial population, we identified single nucleotide polymorphisms in the 16S rRNA gene from *X. fastidiosa* genome sequence database and used the information to design primers for multiplex PCR assays. These muliplex PCR assays simultaneously detected two genotypes, one represented by strain Temecula (causing grapevine Pierce's disease) and the other by strain Dixon (causing ALSD), from early passage of *X. fastidiosa* cultures from infected almond. The two genotypes were correlated with colony morphology variations. This is the first report of mixed strain infection of almond by different *X. fastidiosa* genotypes under natural environmental conditions.

Application of PCR-based detection methods for monitoring curly top viruses in beet leafhoppers and tomato plants in California. L. CHEN (1), M. J. Soto-Aguilar (2), and R. L. Gilbertson (1). (1) Dept. Plant Pathology, UC Davis, CA; (2) Donald Danforth Plant Science Center, St. Louis, MO. Phytopathology 94:S17. Publication no. P-2004-0111-AMA.

Epidemics of curly top disease (CTD) continue to plague tomato production in California (e.g., hundreds of acres affected in a Kern County epidemic in 2003). A rapid PCR-based method for detection of the curly top viruses (CTVs) *Beet mild curly top virus* (BMCTV), *Beet severe curly top virus* (BSCTV), and *Beet curly top virus* (BCTV) was developed and used to monitor these viruses in beet leafhoppers and tomato crops. Monthly samples of leafhoppers were collected by California Department of Food and Agriculture personnel for PCR testing. A high incidence of virus-carrying leafhoppers was detected early in the season (March-May), whereas the incidence decreased markedly during the growing season (June-November). This indicates that leafhoppers are acquiring significant amounts of virus from reservoir hosts in the foothills. BMCTV was predominant CTV associated with CTD in tomatoes in 2001-2003 in California, although BSCTV was also involved around Fresno, CA. These molecular tools are providing further insight into CTD and its management in California.

Characterization of *Botrytis*-resistance of *Lilium speciosum* Thunb. var. *gloriosoides* Baker. R. S. CHEN and J. L. Jong. Graduate Institute of Biotechnology, National Chiayi University, Chiayi 600, Taiwan. Phytopathology 94:S17. Publication no. P-2004-0112-AMA.

Gray mold of ornamental lilies, caused by *Botrytis elliptica* (Berk.) Cooke., was resulted in considerable economic losses worldwide. In this study, the screening for resistance to *B. elliptica* was made by the leaf-disk method. The results showed that *Lilium speciosum* var. *gloriosoides*, a native variety in Taiwan, was highly resistant to *B. elliptica*. While *L. speciosum* var. *gloriosoides* was inoculated with *B. elliptica*, the formation of phenolic compounds was observed, and mycelial growth of *B. elliptica* was found to be significantly inhibited. Several resistance-related proteins, including chitinase, beta (b)-1,3-glucanase, peroxidase and superoxide dismutase, were detectable after inoculation. The significantly high level of activities of chitinase, peroxidase and superoxide dismutase activities from *L. speciosum* var. *gloriosoides* inoculated with *B. elliptica* were observed. Only one isoform of chitinase was found by sodium dodecylsulfate-polyacrylamide gel with 0.01% of glycol chitin, and the molecule weight was about 25 kilodalton. The results obtained in this study could be useful to understand the resistance mechanisms of *L. speciosum* var. *gloriosoides*, and to develop a better strategy for the control of gray mold disease.

Chickpea rootstocks have no effect on chickpea scions in reaction to *Ascochyta rabiei*. W. CHEN, K. E. McPhee, and F. J. Muehlbauer. USDA-ARS, Washington State University, Pullman, WA 99164. Phytopathology 94:S17. Publication no. P-2004-0113-AMA.

Ascochyta blight caused by *Ascochyta rabiei* is an important disease of chickpea. Genotype-dependent interactions between chickpea and *A. rabiei* separate isolates of the pathogen into two pathotypes, and the pathogen is known to produce several phytotoxins. Reciprocal grafting between resistant and susceptible chickpea genotypes was employed to elucidate possible mechanisms of resistance by detecting translocations of disease-mediating agents such as toxin receptors or detoxifying enzymes across the graft junction. Six chickpea genotypes Burpee, Dwelley, Myles, Spanish White, UC-27, and Yuma were grafted in all possible combinations including self-grafting, and inoculated with isolates of either pathotype I or pathotype II of *A. rabiei*

using a mini-dome technique. Results showed that the scions of a given genotype grafted onto either resistant or susceptible genotype rootstocks reacted similarly to infection by *A. rabiei*. The genotype of the rootstock showed no detectable effect on the disease phenotype of the scion. The disease phenotype is conditioned locally by the scion genotype.

Melanin production in *Ascochyta rabiei* is involved in pathogenicity on chickpea. W. CHEN (1), K. D. Sharma (1), M. H. Wheeler (2), and F. J. Muehlbauer (1). (1) USDA-ARS, Washington State University, Pullman, WA 99164; (2) USDA-ARS, College Station, TX 77840. Phytopathology 94:S18. Publication no. P-2004-0114-AMA.

Ascochyta rabiei, the cause of chickpea blight, produces melanin in culture and in infected plants. The role of melanin in pathogenicity of *A. rabiei* on chickpea was investigated using spontaneous albino mutants of the pathogen. Unlike wild-type strains, the albino mutants were not pathogenic on chickpea. Pyroquilon and tricyclazole, specific inhibitors of 1,8-Dihydroxynaphthalene (DHN) biosynthesis, blocked melanin synthesis by wild types in culture, and reduced disease severity by the wild type when applied onto chickpea plants. Transcripts of scytalone dehydratase, an enzyme in the DHN-melanin pathway, were detected in germinating spores using RT-PCR; also the albino mutants converted the DHN-melanin precursor scytalone to a dark pigment. This study demonstrated: 1) that *A. rabiei* uses the DHN pathway for melanin biosynthesis; 2) that the melanin biosynthesis pathway is operative during spore germination and the infection process; and 3) that melanin production appears to be required for pathogenicity of *A. rabiei* on chickpea.

Stripe rust epidemics and races of *Puccinia striiformis* in the United States in 2003. X. M. CHEN (1,2), M. K. Moore (2), and D. A. Wood (1). (1) USDA-ARS and (2) Dept. of Plant Pathology, Washington State Univ., Pullman, WA. Phytopathology 94:S18. Publication no. P-2004-0115-AMA.

In the US in 2003, stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (PST), occurred in 25 states, reduced yield by 88.5 million bushels, and cost multimillion dollars for application of fungicides. Stripe rust of barley, caused by *P. striiformis* f. sp. *hordei* (PSH), occurred in California and the Pacific Northwest and reduced yield by 150,000 bushels. Without resistant cultivars, losses of wheat and barley would have been much greater. More than 400 wheat stripe rust samples from the 25 states were tested on 20 wheat genotypes used to differentiate PST races, and 30 barley stripe rust samples from California, Idaho, Oregon, and Washington were tested on 12 barley genotypes used to differentiate PSH races. Of 35 PST races detected, 10 have been identified as new races. Races PST-98 (virulent on Lemhi, Heines VII, Produra, Stephens, Lee, Fielder, Express, Yr8, Yr9, Clement, and Compair) and PST-100 with the same virulences as PST-98 plus virulence on Yamhill were the most common each with a 30% frequency. Races with common virulences on Lemhi, Lee, Fielder, Express, Yr8, Yr9, Clement, and Compair accounted for more than 95% of the PST samples. Of nine PSH races detected, two were new. PSH-54 (virulent on Topper, Abed Binder 12, Trumpf, and Bancroft), PSH-48 (only virulent on Topper), and PSH-56 (virulent on Topper, Hiproly, Abed Binder 12, Trumpf, and Bancroft) accounted for 26, 22, and 22%, respectively, of the PSH samples. PST races with more virulences were more common, while PSH races with fewer virulences were more common.

Genetic diversity of blackleg strains of canola in western Canada identified through phenotype and genotype characterization. Y. CHEN, P. S. Parks, and W. G. D. Fernando. Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2. Phytopathology 94:S18. Publication no. P-2004-0116-AMA.

Leptosphaeria maculans causes blackleg disease of canola in Canada and many other parts of the world. In 2002, a new pathogenicity group (PG), PG3, was isolated, identified and characterized based on the interaction phenotype on differential cultivars Glacier and Quinta. In Manitoba, *L. maculans* populations consist mainly of PG2 and PG1 isolates. The discovery of PG3 may have serious implications on the canola industry, as most, if not all, cultivars developed in western Canada carry resistance to the PG2 group only. The PG3 strain has been identified in eighteen percent of field locations. New strains that do not conform to any of the known PG groups have been found, indicating that high sexual recombination and/or mutation may exist. Preliminary results from sequence-related amplified polymorphism (SRAP), showed genetic diversity of strains within a field; however, the isolates causing infection at different growth stages on the same plant were not genetically different. The evolution, population dynamics and genetic relatedness of these strains are under investigation in an effort to understand the pathogen's ability to overcome resistance.

Investigating the role of an aflatoxin resistance-associated protein in maize using RNAi. Z.-Y. CHEN (1), R. L. Brown (2), T. E. Cleveland (2), and K. E. Damann (1). (1) Louisiana State Univ. Ag. Center, Baton Rouge, LA; (2) SRRC, USDA-ARS, New Orleans, LA. Phytopathology 94:S18. Publication no. P-2004-0117-AMA.

Aflatoxins are carcinogens produced by *Aspergillus flavus* and *A. parasiticus* during infection of susceptible crops such as maize. Although resistant maize genotypes and resistance-associated proteins (RAPs) have been identified, the direct involvement of these proteins in resistance has been difficult to demonstrate until the recent discovery of a sequence-specific gene silencing phenomena called RNA interference (RNAi). In this study, RNAi technology is used to specifically silence a RAP gene and the resulting changes of kernel resistance in the silenced transformants will be evaluated. As a first step, a binary RNAi vector is being constructed with Gateway technology to eliminate several cloning steps. Afterwards, immature maize embryos will be transformed with the newly constructed RNAi vector through collaboration with Iowa State University. These RNAi studies should uncover the roles of RAP gene(s) in kernel resistance. Genes confirmed as important to resistance can then serve as markers for marker-assisted breeding.

Post-harvest aflatoxin accumulation in transgenic peanut lines containing anti-fungal genes. K. D. CHENAULT (1), H. A. Melouk (1), and C. C. Holbrook (2). (1) USDA-ARS Plant Science Laboratory, Stillwater, OK; (2) USDA-ARS, Tifton, GA. Phytopathology 94:S18. Publication no. P-2004-0118-AMA.

The fungus *Aspergillus flavus* is one of several Aspergillus species which produces aflatoxins upon colonization of cultivated peanut (*Arachis hypogaea* L.). Aflatoxins are carcinogenic to humans and other animals and the accumulation of these toxins on peanut kernels can influence product marketing. Pre and post-harvest aflatoxin accumulation of peanut are influenced by many factors making the management of aflatoxin contamination in peanut very complex. The problem of aflatoxin management could be solved if peanut cultivars were resistant to *A. flavus* colonization. In this study, seven peanut (*Arachis hypogaea* L.) lines containing anti-fungal transgenes and three runner cultivars (Okrun, Georgia Green, and Tifton 8) were evaluated in field plots in Tifton, Georgia for their levels of post-harvest aflatoxin contamination (PAC) upon colonization by the fungus *Aspergillus flavus*. Georgia Green demonstrated the highest level of resistance to PAC followed by genotypes Tifton 8, K24 and K34. Transgenic genotypes K24 and K34 also accumulated significantly less toxin than their parent genotype Okrun. These data confirm that at least two of the transgenic genotypes tested may have useful resistance to *A. flavus*.

Enzyme profiles associated with a cold tolerant *Trichoderma atroviride* during low temperature biocontrol of pathogenic fungi. M. Cheng, P. A. Gay, and J. H. MCBEATH. Plant Pathology & Biotechnology Laboratory and Agriculture, Forestry Experiment Station, University of Alaska Fairbanks, AK 99775. Phytopathology 94:S18. Publication no. P-2004-0119-AMA.

T. atroviride, a cold tolerant biocontrol agent that parasites a wide range of plant pathogenic fungi, produces cell-wall degrading enzymes at low temperature in response to plant pathogenic fungi. *T. atroviride* 901 was grown at 7°C in minimal medium broth amended with or without autoclaved mycelia of *Botrytis cinerea*, *Phytophthora capsici*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Characteristic substrates and enzyme standards were used to detect the presence of cell wall degrading enzymes. The results showed enhanced beta-1,4-N-acetylglucosaminidase (NAGase), exochitinase, endochitinase, beta-1,3-endoglucanase and proteinase activity when compared to *T. atroviride* grown in the absence of pathogens. Total activity of all enzymes produced increased over a 30-day period. NAGase, exochitinase and endochitinase were detected 5 days after induction and specific activities were pathogen dependent. The timing of beta-1,3-endoglucanase and proteinase production was also pathogen dependent. Beta-1,3-glucanase activity for *R. solani*, *B. cinerea* and *P. capsici* was detected at 15, 20, and 30 days, respectively. Specific proteinase activity peaked at 10 and 20 days for *S. sclerotiorum* and *P. capsici*, respectively. No detectable proteinase activity was observed when the autoclaved mycelia of *R. solani* and *B. cinerea* were used as inducer. Beta-1,3-endoglucanse activity was also not detected when *S. sclerotiorum* was used as an inducer.

Survival of sclerotia of *Botrytis* sp., cause of onion neck rot, in red-ferrosol soil in northern Tasmania, Australia. M. I. CHILVERS (1), F. S. Hay (2), and C. R. Wilson (2). (1) Washington State University, Pullman, WA; (2) University of Tasmania, Australia. Phytopathology 94:S18. Publication no. P-2004-0120-AMA.

Botrytis aclada and *B. allii* are the primary causal agents of onion neck rot. To determine the duration of sclerotium survival of these fungi, sclerotia were collected from infected onion bulbs, placed into nylon mesh bags (20 sclerotia/10 cm^2 bag) and buried 6 cm deep in soil in 20 cm-diameter pots (1

bag/pot). The pots were buried so that the soil inside and out of the pot was level, in a pasture in January 1999. Three replicate bags were retrieved at each of 0, 1, 3, 5, and 29 months after burial. To assess viability at each sampling period, sclerotia were washed in distilled water, incubated on water agar at 20°C for 5 days, and assessed for germination. All 20 sclerotia/bag were recovered and 72, 73 and 75% of the sclerotia were viable at the 0-, 1-, and 3-month sampling periods, respectively. Five months after burial, 88% of the sclerotia buried were recovered and 63% were viable. Twenty-nine months after burial, 73% of the sclerotia were recovered, and 13% were viable. The results demonstrate the importance of a minimum 3- to 4-year rotation between onion crops to reduce the likelihood of soilborne sclerotia serving as a source of inoculum.

Genetic and physiological basis of partial resistance to *Sclerotinia sclerotiorum* in *Phaseolus coccineus*. T. J. Chipps, B. Gilmore, J. R. Myers, and H. U. STOTZ. Department of Horticulture, Oregon State University, Corvallis, OR. Phytopathology 94:S19. Publication no. P-2004-0121-AMA.

Scarlet runner beans (*Phaseolus coccineus*) are a potential genetic resource to transfer partial white mold (*Sclerotinia sclerotiorum*) resistance to common bean (*P. vulgaris*). We have identified the *P. coccineus* accessions PI 255956 and PI 535278 as the most promising resource for mapping and introgressing resistance traits. The F2 generation of a cross between susceptible 'Wolven Pole' and PI 255956 segregated into 50 resistant and 138 susceptible plants. Partial white mold resistance is quantitatively inherited and mapping suggests QTL to be located on linkage groups 3, 7, 8, 11, 12, and 14. Physiological analysis of partial resistance in PI 255956 and PI 535278 suggests a role of oxalate tolerance in resistance to *S. sclerotiorum*. Specifically, PI 255956 is significantly less sensitive to exogenous oxalate than 'Wolven Pole'. Oxalate concentrations were similar in infected stem tissues of the partially resistant lines and lower than in 'Wolven Pole'. PI 255956 and PI 535278 express more oxalate oxidase than 'Wolven Pole'. Thus, oxalate oxidase expression can only partially explain the elevated oxalate tolerance of PI 255956.

Identification of differentially expressed genes from a resistant soybean-rust interaction using suppressive subtractive hybridization. J. J. CHOI and R. D. Frederick. USDA-ARS-FDWSRU, Ft. Detrick, MD. Phytopathology 94:S19. Publication no. P-2004-0122-AMA.

Soybean rust caused by *Phakopsora pachyrhizi* has spread to many parts of the world, most recently to Africa and South America. Although the disease is not in the continental United States, there are concerns that significant yield losses would occur if the pathogen were introduced and became established. Four single genes have been described for rust resistance, *Rpp1-4*, but no commercially available U.S. soybean cultivars are resistant to all isolates of rust. To understand the molecular mechanisms of rust resistance, a suppressive subtractive hybridization (SSH) was employed. A SSH cDNA library was constructed using mRNA from soybean line PI200492 (*Rpp1*) inoculated with *P. pachyrhizi* isolate HW94-1 (resistant interaction). Sequence was evaluated from 1011 cDNA clones. Using the BLAST sequence comparison algorithms, cDNA clones were classified into functional categories based on the Munich Information Center for Protein Sequences (MIPS), with unclassified/unknown function as the largest, followed by: metabolism; cell rescue, defense, cell death and ageing; energy; and communication and signaling.

Screening of resistant tomato germplasm to *Tomato yellow leaf curl virus*, Thailand isolate (TYLCTHV-[2]). O. CHOMDEJ (1), O. Chachawankanpanich (2), W. Kositratana (3), and J. Chunwongse (4). (1) Center for Agricultural Biotechnology, Kasetsart University, Thailand; (2) National Center for Genetic Engineering and Biotechnology, Kasetsart University, Thailand; (3) Dept. of Plant Pathology, Kasetsart University, Thailand; (4) Dept. of Horticulture, Kasetsart University, Thailand. Phytopathology 94:S19. Publication no. P-2004-0123-AMA.

Tomato yellow leaf curl virus (TYLCV) is the most damaging disease of tomato throughout Thailand. The objective of this study was to screen and breed a new resistant cultivar. Sixteen-tomato accessions from the Asian Vegetable Research Development Center (AVRDC), Taiwan were screened for resistance to Thailand isolate (TYLCTHV-[2]). The accessions expressing the resistant genotyp were then crossed to the TYLCV-susceptible female parent, Seeda3 (SD3), to generate F1 progenies. Tomato parents and their F1 progenies were inoculated with TYLCTHV-[2] at 3 weeks old using whitefly (Bemisia tabaci) as the inoculation vector. Disease response of the plants was rated according to the incidence and severity of the development of viral yellowing and curling symptoms. The presence of protein in the inoculated plants was confirmed by Enzyme-Linked Immunosorbent Assay (ELISA). AVRDC tomato parental lines; FLA591-15, H24, CLN2443C, TLB111, TLB111-F6-4-1, TLB130-F6-3-1 and F1 progenies of SD3 X TLB130-F6-3-1 expressed little or no symptom at all at one month after inoculation. Serological detection by ELISA readings correlated perfectly with physical observation of the genotype. Test cross will be done to determine the mode of action of the gene(s).

Biological control of bacterial soft rot of Chinese cabbage by the lactonase-producing *Bacillus* sp. 5-3. J.-E. Chung, S.-E. Lee, S.-Y. Park, B.-S. Kim (1), and J.-S. CHA. Department of Plant Medicine, Chungbuk National University, Cheongju, Chungbuk; (1) Department of Applied Plant Science, Kangnung National University, Kangnung, Kangwon, Republic of Korea. Phytopathology 94:S19. Publication no. P-2004-0124-AMA.

Pathogenicity of *Erwinia carotovora* subsp. *carotovora* (*Ecc*), a pathogen of bacterial soft rot disease, is controlled by quorum sensing. Breakdown of quorum-sensing signal compounds, *N*-acyl homoserine lactones (AHLs), reduce pathogenicity of the pathogen. *Bacillus* sp. 5-3, isolated from a Chinese cabbage field, was found to produce a lactonase that breaks down *N*-Octanoyl-DL-homoserine lactone (OHL). Molecular charactracterizations indicated the strain contained a lactonase biosynthesis gene homologous to *aiiA* of *Bacillus thuringiensis* serovar *thompsoni*. Production of the pathogenicity-related exo-enzymes, pectate lyase, polygalacturonase and cellulase of *Ecc* was suppressed by *Bacillus* sp. 5-3 and lesion length on a detached Chinese cabbage leaf was reduced when *Ecc* was inoculated with *Bacillus* sp. 5-3. In field experiments, *Bacillus* sp. 5-3 controlled bacterial soft rot of Chinese cabbage at levels equivalent to or better than Biokeeper®, streptomycin, and oxolinic acid, all of which are registered as control methods for bacterial soft rot of Chinese cabbage.

Phytoparasitic nematodes associated with field-grown floricultural crops in southern Florida. G. T. CHURCH. USDA, ARS, US Horticultural Research Laboratory, Fort Pierce, FL 34945. Phytopathology 94:S19. Publication no. P-2004-0125-AMA.

The U.S. floricultural industry was valued at $4.62 billion in 2002 with the value of cut flowers and caladiums grown in Florida valued at $41 million. The production of cut flowers and *Caladium* tubers takes place in field soil and requires intensive pest management strategies not required in ornamental nursery plant production. Soil borne pests are currently controlled through pre-plant soil fumigation with methyl bromide and other alternative fumigants. A survey of commercial farms in southern Florida was conducted in 2002 and 2003 to determine the phytoparasitic nematodes associated with cut flowers and *Caladium*. The genera *Paratrichodorus*, *Hopoliamus*, and *Scutellanema* were identified at relatively low numbers. Multiple species of *Meloidogyne* were identified at high levels. *Meloidogyne arenaria* was identified from both cut flower and *Caladium* farms, while *Meloidogyne incognita* was identified from cut flower farms. Morphology and enzyme phenotypes were used in the identification of phytoparasitic nematodes. Increasing the information available on phytoparasitic nematodes associated with cut flowers and *Caladium* will assist in the development of more effective pest management strategies.

Bean pod mottle virus effects on yield of ten soybean lines. C. L. CIHLAR-STRUNK and M. A. C. Langham. Plant Science Department, South Dakota State University, Brookings, SD. Phytopathology 94:S19. Publication no. P-2004-0126-AMA.

Bean pod mottle virus (BPMV) (genus: *Comovirus*, family: *Comoviridae*), a widespread pathogen of legumes, was evaluated on ten soybean lines during 2002 and 2003 at two locations in South Dakota. The lines included in this study were A2302 (Asgrow), DK B28-51 (DeKalb), SD00-1312R, SD93-828E, SD99-026R, SD99-048R, SD99-085R, SD99-096R, SD99-99R, and T3205RR (Thompson). Plants were spray-inoculated at the V3-V5 stage with sap extract (1:10 dilution of BPMV-infected *Phaseolus vulgaris* cv. Provider macerated in 0.02 M Na and K PO_4 buffer, pH 7.2) with 1% silica carbide (w/v) added at 552 kPa. Yield, test weight, node, pod, and seed characteristics were recorded. Analysis of yield data indicated significant differences among cultivars, as well as, between locations and years. Yield effects were significant for the main effects cultivar ($P < 0.0001$), site ($P < 0.0419$), and year ($P < 0.0002$). Comparisons of inoculated versus non-inoculated treatments ($P < 0.0001$) indicated that all soybean lines were significantly affected by BPMV infection. Yield losses ranged from 1% to 19% in 2002 and 16% to 56% in 2003.

Yield decline of sweetpotato cultivars and virus infection. C. A. CLARK, M. W. Hoy, and C. D. Kokkinos. Dept. Plant Pathology & Crop Physiology, Louisiana State Univ. AgCenter, Baton Rouge, LA. Phytopathology 94:S19. Publication no. P-2004-0127-AMA.

Sweetpotato (*Ipomoea batatas*) cultivars decline in yield and quality after years of commercial production. Accumulation of mutations and pathogens, especially viruses, are thought to be the primary causes. A group of sweet-

potato cultivars from different countries was propagated from virus-tested (VT) plants and grown in a field for 2 years, surrounded by the farmer's crop. Infection by the potyviruses known to occur in commercial stock (SPFMV, SPVG, and IVMV) was determined at the end of each season by graft indexing storage roots to *Ipomoea setosa* followed by ELISA tests of indicator plants that developed symptoms. In 2000, all cultivars had similarly high rates of infection, while in 2001, NC-262, Picadito, and Tanzania had lower rates of infection than Beauregard, Bienville, Jonathan, or Xushu-18. Yield of G0, G1, and G2 plants was compared in 2002 and decline was precipitous for Jonathan, negligible for Xushu-18 and intermediate for Beauregard and Bienville. In separate tests, yields of VT plants of Picadito and Xushu-18 were not affected by SPLCV inoculation but yields of inoculated plants of Beauregard, Bienville, Jonathan and NC-262 were reduced by 38-75%. Sweetpotato cultivars vary significantly in rate of yield decline, infection by potyviruses and susceptibility to SPLCV.

Suppression of Rhizoctonia root rot and increased recovery of NOS+ *Streptomyces* spp. in rapeseed meal amended soils. M. F. Cohen (1), H. Yamasaki (2), and M. MAZZOLA (1). (1) USDA-ARS, Wenatchee, WA; (2) Univ. of the Ryukyus, Japan. Phytopathology 94:S20. Publication no. P-2004-0128-AMA.

Rapeseed seed meal (RSM) amendment to orchard soils suppressed apple root infection by *R. solani* AG-5, and resulted in significantly higher populations of *Streptomyces* spp. In soil and recovered from apple roots. Although a preponderance of *Streptomyces* spp. Resident to treated soils inhibited in vitro growth of *R. solani*, the vast majority resident to the rhizosphere were not antagonistic toward the pathogen and RSM amendment did not deter saprophytic growth of *R. solani*. Preliminary results from split-root studies indicate that seedlings planted in RSM-amended soil display heightened systemic resistance against *R. solani*. A large proportion of *Streptomyces* spp. Recovered from the apple rhizosphere produced nitric oxide (NO) via NO synthase and production of nitrogen oxides by bacterial nitrification was greater than 100-fold higher in RSM-amended soils than control or glucose-amended soils. Based on known roles of NO in plant defense mechanisms and the long-term nature of *Streptomyces* root colonization we have observed in the field, we hypothesize functions for NO in RSM-mediated disease suppression.

Silencing of rubisco activase, an up-regulated protein during *Tobacco mosaic virus* infection, does not attenuate TMV infection in *Nicotiana benthamiana*. A. B. COLE, S. Y. Folimonova, B. S. Watson, L. W. Sumner, and R. S. Nelson. The Samuel Roberts Noble Foundation. Phytopathology 94:S20. Publication no. P-2004-0129-AMA.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) activase, a member of the AAA+ superfamily of ATPases and potential chaperone, was associated with purified *Tobacco mosaic virus* (TMV) inclusion body complexes in TMV-infected tobacco plants. In addition, we determined that rubisco activase transcript levels were up-regulated during TMV infection. To investigate the possible role of rubisco activase in TMV accumulation and plant defense responses, we used a *Tobacco rattle virus* (TRV) vector to silence rubisco activase in *Nicotiana benthamiana* plants prior to challenge with TMV. We report here that silencing of rubisco activase enhances TMV accumulation and pathogenicity. Several possible explanations for the involvement of rubisco activase with the TMV replicase complex are presented.

Plant pathology education, extension and outreach at Southern University and A&M College. D. J. COLLINS (1), D. J. Morgan-Collins (2), Y. Qi (1), and O. Bandele (3). (1) Urban Forestry Program, College of Agricultural Family and Consumer Sciences, and Southern University Agricultural Research and Extension Center, Baton Rouge, LA 70813; (2) Berean SDA Church School, Baton Rouge, LA 70805; (3) Southern Univ. Agri. Research and Ext. Ctr., Baton Rouge, LA. Phytopathology 94:S20. Publication no. P-2004-0130-AMA.

Southern University is a Historically Black 1890 Land Grant Institution in Baton Rouge, Louisiana. As such, our three fold mission is to educate, conduct research, and assist clientele through outreach, extension programs. Recently, plant pathology has been introduced into each of these three facets of the Urban Forestry program. Some highlights and accomplishments in plant pathology education have included starting an introductory plant pathology class, graduate plant pathology class, mentoring and supervising research projects for both undergraduate students and students in the M.S. thesis program. Our program conducts outreach in community K–12 schools by assisting with school gardens, mentoring science fair participants, career day, providing hands-on experiential learning exercises, giving tours of the horticultural research farm, tree farm, and laboratories in the Agcenter of Southern University. Extension plant pathology activities include educational programs in urban plant pathology, assistance to limited resources farmers, writing print and web based extension publications, and educational videos.

The development of new Gene Ontology (GO) terms for annotation of genes of bacterial pathogens implicated in plant pathogenesis. C. W. COLLMER (1,4), N. T. Perna (2), M. D. D'Ascenzo (3), and A. Collmer (4). (1) Wells College, Aurora, NY; (2) University of Wisconsin, Madison, WI; (3) Boyce Thompson Institute, Ithaca, NY; (4) Cornell University, Ithaca, NY. Phytopathology 94:S20. Publication no. P-2004-0131-AMA.

The increasing availability of complete bacterial genomes offers new opportunities for asking broad questions about plant pathogenesis. While examining sequence similarities across genomes can address some questions, searching for gene products with similar functions (regardless of structure) in different organisms also could be enlightening. This is difficult without carefully defining terms, and the relationships among terms, to ensure interoperability across genomes. The GO Consortium has been developing three ontologies that describe gene products in terms of molecular function, biological process, and cellular component; GO annotation of both eukaryotic and prokaryotic genomes is increasing. We are developing terms to add to the GO ontologies that can annotate genes in *Pseudomonas syringae* and *Erwinia chrysanthemi* that are implicated in plant pathogenesis; community input is essential. Once accepted by the broader community and the GO Consortium, the new GO terms can be used to annotate genes of these two species through their respective community annotation websites.

Effect of scion bud source and irrigation on incidence of almond union mild etch. J. H. CONNELL (1), J. K. Uyemoto (2), and R. Rosecrance (3). (1) University of California Cooperative Extension, Oroville; (2) USDA ARS, University of California Dept. of Plant Pathology, Davis; (3) California State University Chico, Chico. Phytopathology 94:S20. Publication no. P-2004-0132-AMA.

Union mild etch (UME) is a disorder of young almond trees propagated on Marianna 2624 plum rootstock. Mildly affected trees exhibit pale foliage. Shoot growth ceases, leaves yellow and droop, leaf margins roll up lengthwise and develop necrotic patches, and leaves abscise prematurely on severely affected trees. Some trees die. Etching of the stem at the scion-rootstock junction is apparent when bark is removed. Symptoms show in late spring and become more pronounced as the season progresses. In 2000, 20 different bud-line sources of 'Butte' almond were planted in an attempt to identify bud sources with low potential for UME. Healthy and UME bud sources of 'Mission' almond were also included. Randomized complete blocks replicated six times with 8-tree plots per bud source were split into two irrigation regimes; adequate water applied at the ETc level; and, excess water application. Stem water potential was measured with a pressure bomb and soil moisture was monitored with moisture sensors. The 20 'Butte' bud sources were not significantly different from one another in incidence of UME. Excess irrigation significantly increased UME incidence in the 'Butte' cultivar compared to the adequate irrigation regime. There were no significant differences in UME incidence between the two 'Mission' bud sources regardless of irrigation. Over-irrigation appears to be a factor that increases UME incidence in cultivars that are more susceptible to the disorder.

Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit. W. S. CONWAY (1), B. Leverentz (1), W. J. Janisiewicz (3), R. A. Saftner (1), and M. J. Camp (2). (1) USDA-ARS, PQSL, Beltsville, MD 20705; (2) USDA-ARS, BCS, Beltsville, MD 20705; (3) USDA-ARS, AFRS, Kearneysville, WV 25430. Phytopathology 94:S20. Publication no. P-2004-0133-AMA.

'Golden Delicious' apples were wound inoculated with either *Colletotrichum acutatum* or *Penicillium expansum* and then treated in various combinations with heat (38°C) for four days, 2% sodium bicarbonate, and two biocontrol agents alone or combined. The fruit were stored for four months at 0°C and then at 20°C for two weeks. Antagonists alone reduced decay caused by *P. expansum* but were more effective when combined. Sodium bicarbonate increased the effectiveness of each antagonist alone or in combination. Heat alone, heat in combination with the antagonists, or a combination of the two antagonists plus sodium bicarbonate eliminated decay. Either heat or the antagonists reduced decay caused by *C. acutatum*, but a combination of the two was required to eliminate decay caused by this pathogen. Sodium bicarbonate alone had no effect on either pathogen. The research goal is to develop a strategy for control of apple decays that combines several alternate methods of control to replace the use of synthetic fungicides.

Molecular mechanisms conferring reduced sensitivities to triazoles in UK isolates of *Septoria tritici*. H. J. COOLS, B. A. Fraaije, and J. A. Lucas. Rothamsted Research, Harpenden, UK. Phytopathology 94:S20. Publication no. P-2004-0134-AMA.

Reliable control of Septoria leaf blotch, the most important foliar disease of wheat in the UK, caused by *Septoria tritici*, is now, after the emergence of

widespread resistance to strobilurin (QoI) fungicides, heavily dependent on the application of triazole fungicides. Increased dependence on triazoles has prompted concerns over the possible development of resistance. In 2003, triazole sensitivity bioassays, performed at Rothamsted Research, demonstrated that *S. tritici* isolates obtained from a field in Kent, UK, are up to 20-fold less sensitive to triazoles. Sequencing of the target-encoding *CYP51* gene has identified mutations in isolates from Kent (2003) and Rothamsted Research (2001-2003) which may contribute to differences in triazole sensitivity. Mutations at equivalent codons are responsible for triazole resistance in clinical isolates of *Candida albicans*, and field isolates of *Uncinula necator*, and *Blumeria graminis* f. sp. *hordei*. Furthermore, we have shown both constitutive and induced over-expression of known and novel genes encoding ATP-binding cassette (ABC) transporters in some, but not all, *S. tritici* isolates tested. None of the isolates tested over-expressed the *CYP51* gene. We suggest reduced triazole sensitivity in *S. tritici* can be conferred by a combination of mechanisms, including mutations in the *CYP51* gene and up-regulation of ABC transporters. Further studies are in progress to determine the precise contribution of individual mechanisms to the less sensitive phenotype.

Plant spacing effect on microclimate and Rhizoctonia web blight development in container-grown azalea. W. E. COPES (1) and H. Scherm (2). (1) USDA-ARS, Poplarville, MS; (2) University of Georgia, Athens, GA. Phytopathology 94:S21. Publication no. P-2004-0135-AMA.

Rhizoctonia web blight is a reoccurring problem in compact varieties of container-grown azalea (*Rhododendron* sp.) in the Gulf Coast States. During the summers of 2002 and 2003, disease severity was measured weekly in the inoculated center plant of plots consisting of 49 'Gumpo' azalea plants. Plant spacing within plots was set at 0, 6, 12, 18, or 24 cm, and plots were arranged in three randomized complete blocks. Evaporative potential (EP), leaf wetness (LW), relative humidity (RH), and temperature were monitored in each plot. EP increased significantly with plant spacing, but LW, RH, and temperature, summarized to reflect environmental requirements of *R. solani*, were not significantly different among treatments. Plant spacing also had no significant effect on disease severity in the 2 years. Disease increased steadily from mid-July to late August or early September, then decreased. In southern Mississippi, periods conducive for web blight appear to exist weekly during most of the summer. Daily irrigation and compact plant form likely contributed to the lack of effect of spacing on disease development.

DNA sequence analyses reveals the phylogenetic relationship of the *Eucalyptus* stem pathogen *Coniothyrium zuluense* within the genus *Mycosphaerella* and the polyphyletic nature of this group. M. N. CORTINAS (1), P. W. Crous (2), B. D. Wingfield (1), and M. J. Wingfield (1). (1) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; (2) Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. Phytopathology 94:S21. Publication no. P-2004-0136-AMA.

Coniothyrium zuluense causes a serious stem canker disease of *Eucalyptus*. Very little is known regarding the taxonomy of this asexual fungus, which was provided with a name based on morphological characteristics. *Coniothyrium zuluense* lacks definitive morphological characters and cultures are pleomorphic. Therefore, DNA sequence comparisons are essential for identification. It has recently also been found in other countries including those in Africa, Asia and South America. In this study we considered the phylogenetic position of this important pathogen including DNA sequences of the internal trascribed spacers, the ribosomal small subunit and intron sequences of elongation factor1 alpha and beta tubulin genes. Distance studies revealed that *C. zuluense* is clearly separated from the type species of *Coniothyrium ie*, *Coniothyrium palmarum* and other *Coniothyrium* species by 37 to 39% nucleotide sequence divergence. *C. zuluense* was also shown to be closely related to *Mycosphaerella* spp. In phylogenetic trees including *C. zuluense* and a wide range of species of *Mycosphaerella*, two distinct clades were observed. Bootstrap values were high for this division in *Mycosphaerella* and challenge the monophyletic status of the genus.

Expression of defense genes in root tissues of two soybean cultivars with different levels of partial resistance to *Phytophthora sojae*. S. COSTANZO (1), M. G. Redinbaugh (2), and A. E. Dorrance (1). (1) The Ohio State University/OARDC, Wooster, OH; (2) USDA Agricultural Research Service, Wooster, OH. Phytopathology 94:S21. Publication no. P-2004-0137-AMA.

Phytophthora sojae is the causal agent of root and stem rot of soybean, and is considered a major constraint to the production of this crop worldwide. In partially resistant soybeans, the damage caused by the pathogen is restricted to the tap root and lower stem. An earlier study suggested that the interface between the expanding lesion and healthy tissue is the site where active lesion-limiting mechanisms and defense responses are important. To confirm this initial finding, we used Northern blot analysis to determine the expression level of nine defense-related genes including pathogenesis-related (PR) proteins and enzymes of the phenylpropanoid pathway during the course of infection. After artificial inoculation of soybean cultivars Conrad (partially resistant) and OX2 (susceptible) with *P. sojae* isolate 34.S.5.1, 1.5 cm long root sections were collected at 0, 6, 12, 24, 48, 72 and 120 h after inoculation (h.a.i.). Analogous root tissue samples were collected from non-inoculated and mock inoculated control plants. No difference in the expression of basic peroxidase and 4-coumarate Co-A ligases 1 and 2 was noted between the two cultivars, but levels of beta-1,3-endoglucanase (EGL) and matrix metalloproteinase (MMP) were significantly greater in Conrad at 72 h.a.i. These results suggest a possible involvement of EGL and MMP as factors involved in the expression of partial resistance to *P. sojae* in soybean.

Temporal dynamics of *Clonostachys rosea* isolates and suppression of *Botrytis cinerea* sporulation in strawberry leaves. L. V. Cota, L. A. MAFFIA, E. S. G. Mizubuti, and C. C. Alfenas. Universidade Federal de Viçosa, 36570-000 Viçosa, MG, Brazil. Phytopathology 94:S21. Publication no. P-2004-0138-AMA.

In a research program aiming to manage Botrytis blight, four *C. rosea* (Cr) isolates, capable of establishing in rose, strawberry, eucalyptus, and tomato leaves as well as suppressing *B. cinerea* (Bc) sporulation on these organs were selected. The capacity of these isolates in colonizing strawberry leaf tissues and inhibiting Bc was further investigated. All four isolates survived in active leaves but leaf colonization decreased from 16.3-18.3% to 3.9-5.5% from 1 to 49 days after application. Each isolate was applied on strawberry leaves either before or after Bc inoculation, at 24h intervals, ranging from 0 to 288h. For all application times before Bc inoculation, reduction of pathogen sporulation varied from 47.3 to 97.0%. For most application times after inoculation, reduction of pathogen sporulation was larger than 95.0%. In another experiment (conducted twice), each isolate was applied on leaves, Bc was inoculated after 1 to 49 days (treatments weekly spaced), and pathogen sporulation was evaluated. In the 1st run, reduction of pathogen sporulation was 52.3 and 9.7%, 1 and 7 days after Cr application, respectively; Bc sporulation was not reduced at the other treatments. In the 2nd run, reduction of pathogen sporulation was 62.6 and 19.4%, 1 and 49 days after antagonist application, respectively. Regarding leaf colonization and Bc antagonism, the four isolates were as effective as a Cr isolate provided by J.C. Sutton and will be further studied. Research funded by CNPq and FAPEMIG.

Variability among vegetative compatibility groups of *Aspergillus flavus* in crop colonization and overwintering. P. J. COTTY. USDA-ARS, University of Arizona, Tucson, AZ. Phytopathology 94:S21. Publication no. P-2004-0139-AMA.

Aflatoxin contamination costs U.S. cotton industries millions of dollars annually by limiting use of cottonseed in the lucrative dairy market. Certain native atoxigenic vegetative compatibility groups (VCGs) of *Aspergillus flavus* competitively exclude aflatoxin producers during crop development and thus reduce crop aflatoxin content. In order to assess variability among VCGs in crop colonization and ability to over-winter, small-scale field tests were performed on cotton at 8 locations in South Texas from the Upper Coast to the Rio Grande Valley. Eight isolates belonging to distinct VCGs were obtained from soils and cottonseed in South Texas. Sterile wheat seed colonized with each VCG was applied to soil beneath the cotton canopy at 10 lbs per acre in mid-May. All VCGs in a test were applied to the same area. Compositions of *A. flavus* communities in soils prior to treatment, on crops at harvest, and in soils one year after treatments were assessed by vegetative compatibility analyses. In five tests (3 in 2000 and 2 in 2001), three VCGs were evaluated. In three tests in 2001, eight VCGs were compared. Variability among *A. flavus* strains was observed in crop colonization, dispersal from treatment areas, and in overwintering ability. Ability to colonize and disperse was not necessarily related to over-wintering ability. Applied VCGs moved from soils onto crops in all test areas suggesting that atoxigenic strain technology might be valuable in aflatoxin management across both irrigated and non-irrigated areas of South Texas.

Frequency of the teleomorph of *Phaeosphaeria nodorum* on winter wheat in North Carolina, USA. C. COWGER (1) and H. V. Silva-Rojas (2). (1) USDA-ARS, Raleigh, NC; (2) Colegio de Postgraduados, Montecillo, Mexico. Phytopathology 94:S21. Publication no. P-2004-0140-AMA.

Ascocarps of *Phaeosphaeria nodorum*, which causes Stagonospora nodorum blotch of winter wheat, have not been previously reported in the northeastern or southeastern U.S. despite prolonged searching. We sampled tissues from living wheat plants or wheat debris in Kinston, North Carolina, each month except June from May to October 2003. Altogether, over 1,000 fruiting bodies were examined microscopically and tallied as *P. nodorum* pycnidia or ascocarps, "empty," or "other fungi." *P. nodorum* ascocarps were present each month after May at a frequency of 0.8%-5.4%, and comprised a sig-

nificantly higher percentage of fr g bodies from wheat heads than of those from lower stems and leaves. Because the reproductive structures of *P. nodorum* are easily confused with those of the morphologically similar *P. arenaria*, the internally transcribed spacer (ITS) regions of *Phaeosphaeria* isolates from Kinston and Plymouth, NC, were sequenced and compared to known sequences of both species. The results w e presented. The mating type of each isolate in the sequencing sample was also determined, and an approximate balance was found between mating type 1 and mating type 2. We conclude that in the North Carolina *P. nodorum* population, sexual reproduction plays a role in initiation of new epidemics and the creation of adaptively useful genetic variability, although its relative importance in structuring this population is still unknown.

Identification and mapping of host resistance genes to Septoria tritici blotch of wheat. S. G. COWLING (1), A. L. Brule-Babel (1), D. J. Somers (2), and L. Lamari (1). (1) University of Manitoba, Winnipeg, MB; (2) Agriculture and Agri-Food Canada, Winnipeg, MB. Phytopathology 94:S22. Publication no. P-2004-0141-AMA.

Septoria tritici blotch, caused by *Mycosphaerella graminicola*, is a foliar disease that affects wheat crops worldwide. Salamouni, a hexaploid wheat line, was previously identified to have three incompletely dominant resistance genes to isolate MG2. of these resistance genes also control resistance to isolate MG96-36. The objective of this study is to isolate the three resistance genes in Salamouni and map their chromosome location using microsatellite markers. Double haploid lines from the Salamouni/Katepwa (resistant/susceptible) cross were evaluated for resistance to both isolates. Resistant double haploid lines were crossed back to Katepwa to generate F1 and F2 generations, and F2:3 families. F2 screening identified which crosses contained a single gene segregating for reaction to isolate MG96-36 or MG2. F2:3 families will be screened to identify homozygous resistant, segregating, and homozygous susceptible families. Bulk segregant analysis will be used to identify potentially linked microsatellite markers. Once potential markers have been identified, fine mapping of the resistance genes will be conducted. This inform will allow plant breeders to efficiently identify resistance genes using marker-assisted selection, which will facilitate the incorporation of multiple resistance genes into new and existing cultivars.

Interaction dynamics between saprophytic wood-inhabiting fungi and *Armillaria* spp. K. D. COX and H. Scherm. University of Georgia, Athens, GA. Phytopathology 94:S22. Publication no. P-2004-0142-AMA.

Armillaria root rot, caused by *Armillaria tabescens* and *A. mellea*, limits peach tree longevity in the southeastern U.S. Colonized roots of dead trees serve as inoculum sources for adjacent living trees and provide considerable inoculum levels in subsequent plantings. To investigate potential for minimizing or preventing root colonization by the pathogen, both *Armillaria* species were challenged individually with the wood-inhabiting saprophytes *Phanerochaete velutina*, *Hypholoma fasciculare*, *Schizophyllum commune*, *Ganoderma lucidum*, and *Xylaria hypoxylon* on glass slides, wood blocks, and peach root segments in laboratory microcosms. In the latter system, the saprophytic fungi reduced internal and external *Armillaria* colonization by 30 to 100% with *S. commun G. lucidum*, and *X. hypoxylon* having the most pronounced reductions. There were no significant differences between *Armillaria* species in their response to the presence of the saprophytes. In experiments where root segments were pre-colonized by the saprophytes, *Armillaria* was unable to colonize the roots.

A fu nal genomics approach to investigate the Alternaria-Brassica- pathosystem as a model for necrotrophic fungal–plan nteractions. K. D. CRAVEN (1), R. Cramer (2), C. B. Lawrence (3), T. K. Mitchell (1). (1) North Carolina State University, Raleigh, NC; (2) Colorado State University, Fort Collins, CO; (3) Virginia Bioinformatics Institute, Blacksburg, VA. Phytopathology 94:S22. Publication no. P-2004-0143-AMA.

Black spot disease, caused by the necrotrophic toxin producing fungus *Alternaria brassicicola* is a major pre and post harvest problem on cultivated *Brassica* species. No satisfactory level of resistance has been identified among cruciferous crop plants, although resistant and susceptible ecotypes of *A. thaliana* have been reported. We initiated a functional genomics based approach for elucidating the mechanisms of pathogenicity in the fungus and defense responses in resistant and susceptible Brassicaceae hosts. To identify candidate fungal genes for targeted knockout, three separate cDNA (EST) libraries were generated and sequenced from: 1) infected cabbage tissue; 2) infected rape-seed tissue; and 3) *A. brassicicola* spores germinated under conditions of nitrogen starvation. From the EST sequences, targets with inferred homology to toxin biosynthesis genes, secreted pathogenicity factors, and putative signaling components of the ceramide biosynthesis pathway were selected for functional analysis. We will present results of targeted gene disruptions and effects on pathogenicity.

Phylogenetic relationships between the rapid ght pathogen of turf and aquatic protists in the genus *Labyrinthula*. K. D. CRAVEN (1), P. D. PETERSON (2), T. K. Mitchell (1), and S. B. Martin (2). (1) North arolina State University, Fungal Genomics Laboratory, Raleigh, NC; (2) Clemson University, Pee Dee Research and Education Center, Florence, SC. Phytopathology 94:S22. Publication no. P-2004-0144-AMA.

The labyrinthulids are common marine protists, some of which cause devastating diseases on seagrasses and other marine organisms. On the basis of morphological characteristics that typify the genus, a *Labyrinthula* sp. has recently been reported as the causal agent of an emerging disease of land plants, namely "rapid blight" in cool-season turfgrasses. Here, we utilized ssRNA gene sequences to determine the phylogenetic relationships of 11 "rapid blight" pathogens isolated from four species of diseased turfgrass collected across the U.S. All of the isolates group very closely to other *Labyrinthula* spp., confirming their genetic identity and supporting previous morphological characterization. Further, our analysis ows that all of the turf pathogens examined form a monophyletic clade, suggesting they share a recent common ancestor and that colonization of land plants may have occurred once in the evolution of these unique organisms.

Analysis of beet curly top virus (BCTV) in weeds in New Mexico. R. CREAMER, A. Lewis nd J. Rascon. New Mexico State University. Phytopathology 94:S22. Publication no. P-2004-0145-AMA.

Beet curly top virus (BCTV) infects many dicotyledonous weeds. These infected weeds serve as inoculum sources for transmission by the beet leafhopper to crop plants, including chile. To understand the role of weeds in BCTV epidemiology in New Mexico, weeds were collected from the margins of 10 chile fields bimonthly during 2003. Leaf samples were tested for BCTV by PCR and sequencing of portions of the coat protein and rep protein genes. The weeds did not show obvious symptoms of disease. Of the 1,881 weeds tested, 68 (3.6%) were infected with BCTV. Weeds with the highest levels of infection (8-10%) included London rocket, *Kochia*, *Datura*, and spurred anoda. Infected plants of these species were found in four to six months of the year. Sequences of the BCTV isolates showed that similar biotypes were more likely to be found in plants collected on the same date from the same field, regardless of the type of plant collected. Similar biotypes were also found in different plant species collected from different fields on different dates. These results suggest that there was no viral strain specificity in the coat protein and rep protein genes due to host plant species.

Effect of wound position, auxin concentration and *Agrobacterium vitis* strain F2/5 on wound healing in woody grapevine tissue. J. E. CREASAP, C. L. Reid, M. C. Goffinet, and T. J. Burr. Cornell University, Geneva, NY. Phytopathology 94:S22. Publication no. P-2004-0146-AMA.

A. vitis is the causal agent of crown gall disease in grape, which can be severe in many regions worldwide. *Vitis vinifera* L. cultivars are highly susceptible to freeze injury, providing the necessary wounds for infection. Wound position in relation to the uppermost bud of cuttings was determined to be important in tumor development. Inoculated wounds below buds developed tumors, while wounds opposite the bud did not, implying that auxin flow contributes to tumor formation. If auxin was applied to wounds prior to inoculation with a tumorigenic *A. vitis* strain, they all developed tumors and increased amounts of callus in the cambium. Wounds inoculated with an *A. vitis* biological control strain, F2/5, prior to application of the pathogen did not develop galls. A closer examination of these wounds determined that callus cells formed in the cambium during wound healing are susceptible to transformation by the pathogen. Although the mechanism by which F2/5 prevents transformation is unknown, our observations suggest F2/5 inhibits normal wound healing by inducing necrosis in the cambium.

Epiphytic survival of *Erwinia tracheiphila* on *Cucumis melo*. E. E. CROMER, S. H. Helland, D. S. Mueller, P. D. Dixon, and M. L. Gleason. Iowa State University, Ames, IA. Phytopathology 94:S22. Publication no. P-2004-0147-AMA.

Bacterial wilt of muskmelon, caused by *Erwinia tracheiphila*, is a vascular disease that is vectored by striped and spotted cucum beetles. Epiphytic survival of the pathogen has received little study. The objective of this study was to contrast epiphytic survival of *E. tracheiphila* on muskmelon leaves under wet and dry conditions. All studies used a rifampicin-resistant *E. tracheiphila* strain that was spray-inoculated on leaves of 6-wk-old 'Athena' muskm After sonication of leaves for 30 seconds in pH 7 phosphate buffer, a 0.1 ml aliquot of the resulting suspension was placed on nutrient agar amended with peptone and rifampicin to culture the pathogen. In a preliminary study on wet leaves, *E. tracheiphila* was detected on 30% of leaves sampled after 24 h. To compare survival in wet and dry environments, seedlings were spray-inoculated and placed either in a dew or growth chamber at

28°C. Leaves were sampled periodically and epiphytic populations were determined 0 to 48 h after inoculation. In an initial trial, *E. tracheiphila* survived for up to 48 h under both dry and wet conditions. In a second trial, however, *E. tracheiphila* was detected on both dry and wet leaves 6 h after inoculation, but not at 12, 24, or 48 h. These data suggest that epiphytic survival of *E. tracheiphila* on muskmelon leaves is highly variable, but that it can continue for 48 h, far longer than the 6 h reported in previously published studies.

Application of molecular techniques to characterise *Cyclaneusma minus*. T. M. CROWLEY (1), M. S. Muralitharan (1), and T. W. Stevenson (2). (1) Deakin University, Geelong VIC, Australia; (2) RMIT University, Bundoora VIC, Australia. Phytopathology 94:S23. Publication no. P-2004-0148-AMA.

Pinus radiata is the most significant plantation softwood used for structural timber and long fiber manufacture in Australia and other parts of the Southern Hemisphere. However, profit and productivity of *P. radiata* plantations can be reduced due to the needle cast pathogen *Cyclaneusma minus*. *Cyclaneusma minus* is a relatively difficult trait to assess, and to date, minimal molecular research has been undertaken, highlighting the need to study and understand this pathogen. Our laboratory has dedicated the past three years to gaining a better understanding of both the biology and the genetics of *C. minus*. We have found there to be considerable cultural and genetic variation between isolates within Australia and New Zealand. Cultural variation has been documented and investigated using techniques such as incompatibility testing. Several molecular techniques have been explored including RAPDs, microsattelites, ITS and IGS regions. So far it appears that two separate clades may exist within *C. minus*. In addition, we have utilized PCR techniques to allow rapid detection of *C. minus* on *P. radiata*.

Real-time PCR for detection and quantification of *Xanthomonas campestris* pv. *vesicatoria* in samples from transplant seedling greenhouses. D. A. CUPPELS and T. Ainsworth. Agriculture and Agri-Food Canada, London, Ontario. Phytopathology 94:S23. Publication no. P-2004-0149-AMA.

Bacterial spot, which affects all above-ground parts of the host plant, is a devastating disease of tomatoes that has caused significant decreases in marketable yield for Ontario growers. Infested seed is thought to be the primary source of infection for greenhouse-grown transplant seedlings; these plants may harbor large pathogen populations (100,000 CFU/g) yet remain symptomless until they have been transported to grower fields. Our objective was to develop a rapid and reliable means of detecting and quantifying *Xanthomonas campestris* pv. *vesicatoria* on these seedlings so that the severity of the disease threat presented by this inoculum source can be more adequately assessed. Employing the Roche LightCycler system and SYBR Green I, we have tested a number of protocols and primers derived from the bacteria spot diagnostic DNA probe KK1750 (Kuflu and Cuppels, 1997). Specificity was confirmed using DNA from the four bacterial spot groups (A, B, C, and D) and from other plant-associated bacteria as template. Although crude DNA lysates showed a linear relationship between log input CFU and threshold cycle number, the amplification efficiency and square regression coefficients improved substantially with purified DNA. However, a significant amount of DNA was lost during purification raising the detection limit of the assay from 100 to 500 CFU/assay. Thus we were able to determine the pathogen population in 10-g samples of seedling leaves (approximately 1,000) if it exceeded 25,000 CFU.

QTL mapping of resistance to a ryegrass isolate and a rice-infecting lab strain in ryegrass, and comparison with blast resistance genes in rice. J. URLEY (1), S. C. Sim (1), S. Warnke (2), R. Barker (3), S. Leong (1), and G. Jung (1). (1) Dept. of Plant Pathology, University of Wisconsin, Madison, WI; (2) US National Arboretum, Washington, D.C.; (3) USDA-ARS Nat'l Forage Seed Production Research Ctr., Oregon State University, Corvallis, OR. Phytopathology 94:S23. Publication no. P-2004-0150-AMA.

Gray leaf spot (GLS) is a serious fungal disease on the important turf and forage species, perennial ryegrass (*Lolium perenne*) caused by the rice blast fungus *Magnaporthe grisea*. Early reports suggest little resistance is present in perennial ryegrass cultivars. However, greenhouse inoculations in our using one ryegrass isolate suggests some resistance is segregating in an annual × perennial ryegrass (MFA × MFB) pseudo-F_2 population. A well-saturated genetic linkage map has been constructed for this population using RFLP, RAPD, AFLP, and SSR markers, and the segregation seen allows quantitative trait locus (QTL) analysis in this population. Potential QTL for resistance to one ryegrass isolate, detected on at least three linkage groups, will be discussed. This population has also been inoculated with a rice-infecting lab strain of *M. grisea* that has previously shown a different segregation pattern on selected progeny. The phenotypic and QTL results from this strain will also be discussed. To confirm that QTL detected in the current population are still detected in the next generation, a resistant segregant, MF8, has been crossed with a susceptible perennial clone, L4B-5, from a different genetic background, to form a new pseudo-F_2 mapping population segregating for GLS resistance. Locations and effects of QTL for GLS resistance can be compared between rice and ryegrass via comparative QTL mapping, which can then lead to map-based cloning of novel resistance genes as well as marker-assisted breeding for gray leaf spot resistance in perennial ryegrass.

Etiology of Phaeosphaeria leaf spot of maize in Brazil. F. K. DALLIO, A. L. do Amaral, M. L. De Carli, and J. F. Barbosa Neto. Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Phytopathology 94:S23. Publication no. P-2004-0151-AMA.

Phaeosphaeria leaf spot (PLS), which has been attributed to *Phaeosphaeria maydis*, is a major maize disease in Brazil. However, there are doubts about its causal agent. Aiming to identify and to characterize fungi related to PLS in Brazil, maize leaves with typical lesions were collected in Goias (GO) and Rio Grande do Sul (RS) States, in two growing seasons, early and late season, in 2002 and 2003. Segments of leaves with lesions were disinfested and placed in moist chambers. Fungi were isolated in PDA and sent to CABI – Bioscience, UK, for identification. Pathogenicity tests were carried out in different environments (acclimatized room, plastic greenhouse and in field conditions). *Phyllosticta* sp. (anamorph of *P. maydis*), *Phoma sorghina* and *Sporormiella* sp. were isolated and proved pathogenic to maize. Whether *Phoma sorhina* occurred in all environments, *Sporormiella* sp. And *Phyllosticta* sp. Were restricted, respectively, to GO and to RS. According to these results, different fungi species, especially *P. sorghi* are associated to PLS symptoms in Brazil, and environmental conditions play an important role in the predominance of a specific agent at different locations.

Spread of Sharka disease (PPV-M strain) in peach orchards submitted to roguing in southern France. S. DALLOT (1,2), T. Gottwald (1), G. Labonne (2), and J. B. Quiot (2). (1) USDA-ARS-USHRL, Fort Pierce, FL 34945; (2) UMR BGPI-INRA, Montpellier, 34398 France. Phytopathology 94:S23. Publication no. P-2004-0152-AMA.

Nineteen peach blocks infected by the aggressive *Plum pox virus* strain M were monitored visually during 7 to 10 years and symptomatic trees were removed every year. Annual disease incidence was low (2 to 6%) in all orchard blocks but new symptomatic trees were continuously detected, even after 7 to 10 years of uninterrupted roguing. An exploratory approach using survival modeling was developed to evaluate to what extent, tree location within orchards, orchard characteristics and disease status within the vicinity of the orchards influenced the risk for a tree to become infected through time. Twelve variables were selected from survey data and from databases created using a geographic information system. The extended Cox model fitted to our data showed a significant effect of four of the variables tested on the risk for a tree to become infected through time: The area of the orchard block, the density of planting, the distance of a tree from the edge of the orchard block sharing a boundary with another infected orchard as well as the distance to the nearest previously detected symptomatic tree within the block. These results suggest that new PPV-M infections within orchards submitted to roguing resulted from exogenous sources of inoculum, disease development of latent infected trees as well as infected trees overlooked within the orchards during visual surveys. A revision of the control measures to more effectively remove potential sources of inoculum will be discussed in the context of the French agro-ecosystem.

Frequency of colonization of corn kernels by atoxigenic *Aspergillus flavus* applied as a potential biocontrol agent. K. DAMANN, R. Sweany, and C. DeRobertis. Department of Plant Pathology & Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA. Phytopathology 94:S23. Publication no. P-2004-0153-AMA.

Aflatoxin contamination of corn is a chronic problem among Southern states. Others have demonstrated the efficacy of using atoxigenic isolates of this fungus to lessen aflatoxin contamination of cotton by "competitive exclusion" of toxigenic strains from cotton soils. This decreases the inoculum potential of toxigenic strains, presumably allowing the atoxigenic strains to out compete the toxigenic strains at the infection court. To successfully use this approach with corn, it is necessary to demonstrate that the biocontrol isolate can colonize corn kernels. A proprietary *A. flavus* isolate (Circle One Global, Inc., Shellman, GA), fixed to barley seed, was distributed atop soil between 16 corn rows at the rate of 20lbs/A on June 4, 2003. The plot was combine-harvested on September 4, and kernels from each row were bagged separately. Randomly collected subsamples (200 kernels/row) were plated on AFPA selective medium. *A. flavus* was recovered from approximately 50% of the sampled kernels. Single NIT mutants of the isolates were selected and paired with CNX and Nir A mutants of the biocontrol fungus which is VCG 24, one not previously recovered in Louisiana. Approximately 60% of the kernels colonized by *A. flavus*, or 30% of the kernels sampled, were identified as VCG 24 and presumed to be the biocontrol isolate originally applied between the rows.

Molecular cloning and characterization of the *GIP* gene family from *Phytophthora infestans*. C. M. B. DAMASCEN[24]), D. R. Ripoll (1), S. Kamoun (2), J. Bishop (3), and J. K. C. Rose (1). (1) Cornell University, Ithaca, NY; (2) Ohio State University, [1]oster, OH; (3) Washington State University Vancouver, Vancouver, WA. Phytopathology 94:S24. Publication no. P-2004-0154-AMA.

[99]

Phytophthora pathogens secrete glucan[99] inhibitor proteins (GIPs) into the plant cell wall during infection. GIPs bind and inhibit the activity of plant extracellular endo-beta-1,3-glucanases (Egases), blocking the release of glucan elicitors. GIPs were first identified in *P. sojae* and appear to show a high specificity for particular Egase isoforms. GIP1 from *P. sojae* was shown [99]ind specifically to EgaseA from soybean but not to isoform EgaseB. Despite their potential importance as suppressors of Egase-mediated defense responses, th[99]olecular basis of GIP action and specificity are not well understood. We have recently identified a 4-member *GIP* gene family [11]*GIP1-4*) from *P. infestans*, and detected GIP isozymes that are secreted by *P. infestans* in culture and *in vivo* into the apoplast of infected tomato leaves using Western analysis. Molecular modeling has been used to predict putative docking sites on the surfaces of Egases and GIPs that may be involved in the high affinity binding between these proteins and some of these amino acid residues showed evidence of positive evolutionary selection, suggesting the existence of a 'molecular arms race' between GIPs and Egases. We are in the process of identifying the GIP-Egase complexes that occur *in vivo* during pathogenesis and progress will be presented.

[1]

Comparative host range of U.S. isolates of Plum pox virus among Prunus and other woody plant species following graft inoculation or aphid transmission. V. D. DAMSTEE[2]T (1), R. Scorza (2), F. E. Gildow (3), W. L. Schneider (1), A. L. Stone (1), and D. G. Luster (1). (1) USDA, ARS, FDWSR, Ft. Detrick, MD 21702; (2) USDA, ARS, AFRS, Kearneysville, WV 25430; (3) Dept. Plant Pathology, PSU, State College, PA 16802. Phytopathology 94:S24. Publication no. P-2004-0155-AMA.

[1]

Plum pox virus (PPV) was identified in the U.S. in 1999. Despite extensive national surveys PPV has been found only in a four county area of PA. Many wild Prunus species have the potential to serve as reservoirs for PPV, if found to be susceptible to the virus. Identifying susceptible wild and ornamental host species is essential for success of the eradication effort. Susceptibility to PPV infection was evaluated by graft-inoculation and aphid transmission of PPV-PENN isolates to rooted cuttings or seedlings of more than 30 Prunus species. Plants were observed for symptoms for 30-90 days, assayed by ELISA and PCR, back-inoculated to peach seedlings, vernalized, and re-assayed following regrowth. Several important commercial, wild, and ornamental species including black cherry, chokecherry, dwarf-flowering almond, flowering almond, and ornamental cherry were identified as potential reservoir species of PPV-PENN isolates.

[2]

Population biology of *Heterobasidion annosum* infecting Christmas trees in the Pacific Northwest. N. L. DART, G. A. Chastagner, and T. L. Peever. Washington State University, Pullman, WA. Phytopathology 94:S24. Publication no. P-2004-0156-AMA.

[22]

Historically, the forest pathogen *Heterobasidion annosum* has seldom been found in PNW Christmas tree plantations. However, with the increased production of *Abies procera*, Annosus root rot disease incidence has increased from <4% in 1980 to 24% in 2000. To determine the population structure of *H. annosum* in PNW Christmas tree plantings and infer sources of inoculum, mode of spread and host specificity, approximately 1150 isolates have been obtained from 5 different hosts in 21 plantations. Hosts included *A. fraseri*, *A. grandis*, *A. procera*, *A. nordmanniana*, and *Pseudotsuga menziesi* trees ranging from 2 to 5 years in age. Taxon-specific competitive priming PCR and somatic compatibility tests were used to classify a subset of the isolates as S or P strain. Both methods indicated that all of the classified isolates, which represented all 5 hosts and a broad geographic range, were S-type. Future research will focus on estimation of population structure of the fungus using microsatellite markers including mapping of multilocus genotypes sampled from disease foci to determine the relative importance of basidiospore and root-to-root spread of the pathogen.

Fungicide efficacy and timing trials for the control of downy mildew of baby lima bean. J. F. DAVEY, R. P. Mulrooney, T. A. Evans, and R. B. Carroll. University of Delaware, Newark, DE. Phytopathology 94:S24. Publication no. P-2004-0157-AMA.

[3]

Downy mildew of baby lima bean caused by *Phytophthora phaseoli* is the number one disease on lima beans in Delaware. This pathogen has recently evolved into new races that have overcome resistance in lima bean cultivars. In 2003, field experiments tested the efficacy and timing of applications of fungicides for downy mildew control. Baby lima beans were grown and artificially inoculated with a sporangial suspension of *Phytophthora phaseoli* race E at the I of Delaware's Experiment Station Farm in Newark, DE. Fungicides were applied throughout the season at different rates and timing schedules. Ridomil Gold/Copper WP 2.0 lb and Phostrol 4.0 pt applied four times every seven days were the best treatments in the efficacy trial. Both treatments significantly increased yield and decreased disease severity. Ridomil Gold/Copper WP 2.0 lb applied three times every seven days and Ridomil Gold/Copper WP 2.0 lb followed by two applications of Champ DP 2.0 lb every seven days performed the best in the preventative fungicide application trial. These treatments significantly increased yield and decreased disease severity. In the post-infection fungicide trial Ridomil Gold/Copper WP 2.0 lb applied twice every seven days and Ridomil Gold/Copper WP 2.0 lb followed by two applications of Champ DP 2.0 lb every seven days performed significantly better than the untreated control for yield and disease severity. These experiments have identified both effective and affordable control options for downy mildew of lima bean.

Analysis of beta-tubulin in *Cercospora beticola* with differing benzimidazole-sensitivity characteristic[24] M. Davidson (1), L. E. HANSON (1), G. D. Franc (2), R. M. Spence (2), and L. Panella (1). (1) USDA-ARS, Fort Collins, CO; (2) University of Wyoming, Laramie, WY. Phytopathology 94:S24. Publication no. P-2004-0158-AMA.

[17]

Cercospora beticola causes Cercospora leaf spot of sugar beet and crops in the genus *Beta*. On sugar beet, Cercospora leaf spot is the most important foliar disease worldwide. The primary controls are host resistance and foliar fungicide treatments. Benzimidazole fungicides are used for disease control, even though benzimidazole-resistance [17]creasingly widespread in the pathogen population in the United States. We examined isolates of *C. beticola* from different years and geographic regions for sensitivity to benzimidazole fungicides and for their beta-tubulin gene sequence. Two homologous PCR primer pairs for the beta-tubulin gene developed for ascomycetes gave inconsistent results in *C. beticola*, but a third set amplified well. This indicates some [17]ability in areas of the gene proposed to be highly conserved. However, isolates with benzimidazole resistance had a mutation in the beta-tubulin gene that corresponded to mutations prev[17]ly determined to confer benzimidazole resistance in other ascomycetes. A PCR-based method for detection of benzimidazole resistance is being developed.

[6]

The occurrence of a mixed infection of a virus related to clover [180] mosaic potexvirus and unknown potyvirus in *Verbena canadensis*. D. A. DAVISON, M. Annamalai, and C. A. Baker. FDACS / DPI – Bureau of Entomology, Nematology, and Plant Pathology. Phytopathology 94:S24. Publication no. P-2004-0159-AMA.

In 2004, mildly stunted plants of *Verbena canadensis* ('Homestead purple') in Florida were reported to harbor a po[18]irus related to clover yellow mosaic (ClYMV) (*Plant Disease* 88:223). Reverse-transcription polymerase chain reaction (RT-PCR) using degenerate primers to the Nib gene of the family *Potyvir*[132]evealed that these plants were also infected with a potyvirus. The ~1.7 kb PCR product was cloned into pCR 2.1-TOPO plasmid and sequenced. A Blast search in the GenBank database with this sequence indicates that the verbena potyvirus may be a new species. Further sequencing of this virus is continuing. A limited survey of *V. canadensis* from US propagation sources was conducted. The results so far show that these viruses are not yet widespread in this host in the ornamental industry. Other *Verbena* spp. Are being surveyed as potential hosts of these viruses. Potyvirus antiserum (Agdia, Elkart, IN) can detect the potyvirus therefore adding ClYMV to the virus screen list for verbena indexing could eliminate both viruses from the industry. Antiserum for ClYMV is available from DSMZ Plant Virus Collection, Braunschweig, Germany.

[7]

Dynamics of rhizosphere competition among genotypes of 2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas fluorescens* depends on the host crop. L. DE LA FUE[35] (1), B. B. Landa (2), L. S. Thomashow (3), and D. M. Weller (3). (1) Washington State University, Pullman, WA 99164; (2) Dept. de Agronomia, Universidad de Cordoba, 14080 Cordoba, Spain; (3) USDA-ARS, Pullman, WA. Phytopathology 94:S24. Publication no. P-2004-0160-AMA.

DAPG-producing *P. fluorescens* strains are responsible for the natural suppressiveness of certain soils to root pathogens. Seventeen distinct rep-PCR genotypes (A-Q) of DAPG producers have been identified, some of which differ in ability to colonize the rhizosphere. Alfalfa, bean, barley, flax, lentil, lupine, oat, pea and wheat were grown in a pea wilt suppressive soil containing indigenous A-, D-, L-, O-, P- and Q-genotype isolates. After six consecutive 3-week cycles, population densities of DAPG producers differed quantitatively in the rhizospheres of the nine crops. When a nonsuppressive soil was inoculated with strains from three genotypes that aggressively

colonize wheat or pea, the P-genotype displaced D- and K-genotype strains in the pea rhizosphere. K- and D-genotype strains were equally competitive in the pea rhizosphere when co-inoculated, but the K genotype displaced genotypes D and P on wheat roots.

Phylogenetic analysis of *Trichoderma stromaticum*, a mycoparasite of *Crinipellis perniciosa*, the cause of witches' broom disease of cacao. J. T. DE SOUZA (1,2), S. A. Rehner (2), G. J. Samuels (2), A. W. Pomella (3), and P. K. Hebbar (4). (1) University of Maryland, Wye Research and Education Center; Queenstown, MD; (2) USDA, ARS, Beltsville, MD; (3) Almirante Cacau, Itajuípe, BA, Brazil; (4) Mars Inc., Hackettstown, NJ. Phytopathology 94:S25. Publication no. P-2004-0161-AMA.

Cocoa production in Central and South America is severely limited by the basidiomycete *Crinipellis perniciosa*, which causes the disease known as witches' broom. In Brazil, a commercial formulation of *T. stromaticum*, a co-evolved mycoparasite of *C. perniciosa*, is being used in the field to manage this disease. DNA fingerprinting and physiological studies have demonstrated that there are two genetically distinct groups in *T. stromaticum*, one of which is more effective for biological control of *C. perniciosa*. We present further evidence, based on multilocus phylogenetic analyses of seven nuclear loci, that *T. stromaticum* in Bahia State, Brazil is partitioned into two discrete phylogenetic groups.

Evaluation of chemical inducers and PR protein reporters for induced systemic resistance to citrus bacterial diseases. M. H. Dekkers, J. H. GRAHAM, J. K. Burns, J. Cubero, and G. C. Colburn. University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850. Phytopathology 94:S25. Publication no. P-2004-0162-AMA.

Foliar sprays of several compounds were evaluated on Swingle citrumelo for their ability to induce systemic resistance (ISR) for control of *Xanthomonas axonopodis* pv. *citrumelo* (citrus bacterial spot) and *X. axonopodis* pv. *citri* (citrus bacterial canker). Three pathogenesis-related (PR) proteins from citrus were evaluated as reporters of ISR: chitinase PR-4, chitinase-1 (possible PR-3) and beta-glucanase (PR-2). Up-regulation of gene expression for these PR proteins was monitored by real-time PCR in plants treated with acibenzolar-s-methyl and/or inoculated with bacteria. PR-2 expression was most up-regulated in response to acibenzolar-s-methyl and bacterial infection, PR-4 and chitinase-1 induced less consistent responses. A strong positive relationship between up-regulation and the suppression of bacterial lesions indicated the potential of PR-2 as a reporter for ISR. However, some PR-2 response was found for all compounds, suggesting PR-2 is only indirectly related to ISR-mediated reduction of bacterial disease.

Identification of a possible new anthracnose race on dry beans. L. E. del Río and R. S. LAMPPA. Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105. Phytopathology 94:S25. Publication no. P-2004-0163-AMA.

A *Colletotrichum lindemuthianum* isolate was retrieved from seeds of a pink bean cultivar grown in Manitoba, Canada in 2002. This "pink isolate" produced a virulence pattern, on a set of 12 standard differentials and 11 commercial cultivars, that differed from race 73, which was previously know from Canada. Spores of each isolate, grown on Mathur's medium, were suspended in water plus Tween 80 (0.01% v/v), adjusted to 10^6 spores/mL, and sprayed until runoff on the primary leaves of two-week old seedlings using a Paasche airbrush. Inoculations were replicated three times and the experiments repeated twice for the differentials and once for the cultivars. Inoculated plants were incubated in moist chambers for five days at 22°C under 14-hr fluorescent light and then moved back to the greenhouse. Disease reaction was assessed 5 days later. The pink isolate produced sporulating lesions on differentials Michelite, Cornell 49242, PI 207262, and AB 136, a reaction profile unknown in the US. 'Vista' a navy bean susceptible to race 73 was resistant to the pink isolate while 'Topaz' a pinto bean resistant to race 73 was susceptible. Molecular work is under way to verify the distinctive nature of the presumed new *C. lindemuthianum* race.

Blueberry anthracnose: From bud infection to fruit rot. A. DeMARSAY and J. Oudemans. Dept. of Plant Biology and Pathology, Rutgers University Blueberry and Cranberry Research and Extension Center, Chatsworth, NJ 08019. Phytopathology 94:S25. Publication no. P-2004-0164-AMA.

In 2001, we began a study of the overwintering *Colletotrichum acutatum* infections on highbush blueberry that give rise to primary inoculum for anthracnose fruit rot. We found that inflorescence buds become infected as they form in the summer and serve as the pathogen's main reservoir during dormancy. Infections overwinter in the outer bud scales covering inflorescence buds and may migrate after budbreak to the inner scales enclosing individual flower bud primordia. We observed *C. acutatum* infections on inner bud scales attached to fruit clusters during bloom and fruit development. Moreover, we noted varietal differences in the persistence of inner bud scales that seem to correlate with relative resistance to anthracnose fruit rot and fruit phenology. An easily scouted feature such as dormant infection levels or bud scale drop could be incorporated into an IPM strategy for timing fungicide applications to control fruit rot with fewer, more effective sprays. Postharvest fungicide applications may prevent or eradicate overwintering infections in buds, reducing disease pressure in later years.

***Agrobacterium*-mediated transformation of *Phoma medicaginis*.** M. DHULIPALA, J. N. Enis, and S. M. Marek. Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078. Phytopathology 94:S25. Publication no. P-2004-0165-AMA.

Phoma medicaginis causes spring black stem and leaf spot of alfalfa and can be an important foliar pathogen of many other legumes. The objective of this study was to assess the genomic tractability of *P. medicaginis* as a fungal pathosystem for the model legume, *Medicago truncatula*. Conidia from 3 isolates of *P. medicaginis* from alfalfa were successfully transformed to co-express green fluorescent protein (GFP) and resistance to hygromycin B (*hph*) using *Agrobacterium tumefaciens* LBA 4404 carrying the binary vector, pPTGFPH. The conidia and hyphae of hygromycin-resistant transformants fluoresced brightly and facilitated live imaging of the infection of *M. truncatula* leaf cells. T-DNA integration into genome of *P. medicaginis* was confirmed by PCR. Analysis of the transgene insertion frequency and the genomic integration sites of transformants will be presented.

Functional analysis of a novel esophageal parasitism gene from *Heterodera glycines*. H. G. DIAB (1), B. Gao (1), T. J. Baum (3), R. S. Hussey (2), X. Wang (1), and E. L. Davis (1). (1) North Carolina State University, Raleigh, NC; (2) University of Georgia, Athens, GA; (3) Iowa State University, Ames, IA. Phytopathology 94:S25. Publication no. P-2004-0166-AMA.

Secretions from esophageal gland cells of soybean cyst nematode (SCN) *H. glycines* drastically manipulate the plant genome during parasitism. Greater than 60 unique parasitism genes encoding different esophageal gland secretions have been identified through expressed sequence tag analyses of SCN gland-cell cDNA libraries. Clone 4E02 is one of these SCN parasitism gene candidates with no database homologue (a pioneer) that is expressed exclusively within the subventral gland cells of SCN in preparasitic second-stage juveniles and all developmental stages of SCN that parasitize soybean roots. Since parasitism gene expression in the subventral gland cells is relatively unique in later parasitic stages of SCN, 4E02 has been chosen for further characterization. Development of antibodies specific to the 4E02 product is in process to assay for the 4E02 protein within SCN specimens and secreted into plant tissues. Functional analysis of pioneer parasitism genes is inherently difficult, but includes expression of the SCN 4E02 gene in transgenic plant tissues to assess effects on plant cell ontogeny and morphology. A second functional approach includes potential gene knockout of 4E02 in SCN using RNA interference and analysis of its effects on parasitism.

Temperature-wetness relationships for *Colletotrichum acutatum* infections on almond blossoms and leaves. J. DIEGUEZ-URIBEONDO, H. Förster, and J. E. Adaskaveg. Dept. Plant Pathology, University of California, Riverside, CA 92521. Phytopathology 94:S25. Publication no. P-2004-0167-AMA.

The effect of temperature and wetness duration (WD) on early development of the almond anthracnose fungus, *Colletotrichum acutatum*, was studied on almond blossom petals and leaves in laboratory and growth chamber experiments. The temperature range for conidial germination was 10 to 35°C. Germination occurred faster on petals than on leaves. After 12 h at 20°C, germination was 70% on leaves and 90% on petals, and 90% of the germ tubes differentiated into appressoria on leaves, whereas on petals it was 30%. Below 15°C the development was considerably delayed and appressoria required 24 h to develop. At 10°C, there was no germination after 24 h, and after 96 h, germination was 40% on leaves and 60% on petals, and only 5% of the germ tubes on leaves and 1% on petals formed appressoria. The relationships between WD and appressoria formation was studied on leaves. The WD required for symptom development depended on temperature and correlated with the period required for appressoria formation. Thus, at 20°C, the minimum WD for symptom development was 12 h, while at 15 and 10°C, it was 24 and over 96 h, respectively. Disease severity increased linearly with the number of appressoria formed (R^2 = 0.93). The results are in agreement with our previous studies where we established that the lower threshold for disease manifestation is 10°C and the WD-threshold is temperature-dependent. Thus, the results presented here represent the biological explanation for the effect of temperature and WD on anthracnose development on almond.

Russet on snap beans, a new disease caused by *Plectosporium tabacinum* in New York and Maryland. H. R. DILLARD and A. C. Cobb. Cornell University, NYSAES, Dept. Plant Pathology, Geneva, NY 14456. Phytopathology 94:S26. Publication no. P-2004-0168-AMA.

An unofficially described russet of snap beans has been observed in commercial fields sporadically in NY and MD. In 2000, an unusual significant rain event in MD interrupted the harvest of snap beans in some areas. When harvesting resumed, mature pods in some fields showed severe symptoms of russet and the remainder of the crop was lost. Initial symptoms of russet appear on pods as diffuse superficial light brown necrotic flecks with undefined borders. Lesions are microscopically associated with root hairs. Isolates of an unknown fungus were obtained from very thin symptomatic epidermal tissue sections. Tissue sections were triturated in a drop of sterile distilled water, and streaked on potato dextrose agar amended with 50 mg/l each of streptomycin sulfate and chloramphenicol, and incubated at 23°C. Koch's postulates were satisfied in mist chamber experiments. Disease severity was greater on pods that were mature at the time of inoculation than on younger pods. The pathogen was identified by W. Gams as *Plectosporium tabacinum*. This is the first time a pathogen has been identified as the cause of russet in snap beans.

Characterization of a new esterase in *Streptomyces scabiei*, the causal agent of common scab. I. DIONNE and C. Beaulieu. Biology Department, Sherbrooke University, Quebec, Canada. Phytopathology 94:S26. Publication no. P-2004-0169-AMA.

Streptomyces scabiei is the main causal agent of common scab of potato. Thaxtomins are phytotoxins that have been shown to be essential to the pathogenicity of *S. scabiei*. Thaxtomins are produced in culture media containing plant extracts or suberin. Suberin does not only induce the production of thaxtomins in *S. scabiei* strain EF-35, it also induces the biosynthesis of an extracellular esterase having suberin as a substrate. To recover this esterase, *S. scabiei* strain EF-35 was grown in a minimal media supplemented with 0.2% suberin. Proteins from the culture supernatants were precipitated successively with ammonium sulphate and with ethanol and then, were concentrated using an Amicon centrifugal filter unit. An activity gel allowed the identification of a new esterase in *S. scabiei* strain EF-35. Indeed, this esterase exhibited a higher molecular weight than another esterase that has been previously characterized by another research group. Our esterase, however, shared similar characteristics with a *S. coelicolor* esterase. The *S. coelicolor* esterase gene was cloned and sequenced. This gene will be used as probe to screen a partial genomic library of *S. scabiei* EF-35.

Functional characterization of SM1, a 16-kDa protein from *Trichoderma virens* involved in plant-associated interactions. S. D. [illegible]OVIC (1), L. J. Dangott (1), C. R. Howell (2), and C. M. Kenerley (1). (1) Texas A&M University, College Station, TX; (2) USDA-ARS, College Station, TX. Phytopathology 94:S26. Publication no. P-2004-0170-AMA.

Several *Trichoderma* species are known to induce plant defense responses in different hosts. We have identified a small protein (SM1) of 16-kDa from culture filtrates of *Trichoderma virens*. SM1 was purified in a native form to homogeneity by anion-exchange and gel filtration chromatography. Purified protein induced HR-like lesions and autofluorescence response on *Oryza sativa* and *Nicotiana tabacum* leaves. SM1 was bioassayed for toxicity against several plant pathogenic fungi and bacteria, however no inhibition of growth was observed. Antibodies for SM1 have been raised and strains disrupted in the *sm1* gene were obtained. The targeted gene disruption was conducted via fungal transformation of an arginine auxotroph, and confirmed by Southern, Northern and SDS-PAGE. By conducting various plant bioassays, we are currently testing the ability of SM1 to induce plant defense responses and to protect plants challenged with soilborne fungi *Pythium ultimum* and *Rhizoctonia solani*.

Global gene expression changes in *Magnaporthe grisea* under nitrogen-starved and appressoria-inductive conditions. N. M. DONOFRIO, H. Pan, J. S. Jeung, Y. Oh, T. K. Mitchell, and R. A. Dean. North Carolina State University. Phytopathology 94:S26. Publication no. P-2004-0171-AMA.

Rice blast, caused by the fungus *Magnaporthe grisea*, is the most devastating disease of rice. We are interesting in identifying molecular determinants controlling pathogenicity in the rice blast patho-system, and a major objective of this goal is to better understand the biology of the pathogen during the infection process. We have been employing several different functional genomic approaches to identify molecular components of both host and fungus. One experimental approach required designing a novel oligonucleotide microarray chip in collaboration with Agilent Technologies, which includes sequences from both rice and *M. grisea*. Thus far, we have utilized our chip to study global transcriptional changes in *M. grisea* in two different experiments. The first experiment analyzes transcriptional changes in the fungus during nitrogen starvation, which is thought to mimic early and late conditions during pathogenesis of the rice plant. The second experiment examines global gene expression during appressorial formation. We have analyzed the data from these experiments using the commercial software package Genespring, and identified major genes and pathways associated with primary metabolism, and early infection. Future experiments will include real-time PCR to confirm the expression of genes of interest from the microarray, as well as mutant knockout experiments.

Incidence of the atoxigenic *Aspergillus flavus* strain AF36 in pistachio and fig orchards in California. M. A. DOSTER (1), T. J. Michailides (1), and P. J. Cotty (2). (1) Dept. Plant Pathology, University of California, Davis/Kearney Agricultural Center, Parlier, CA 93648; (2) USDA-ARS/University of Arizona, Tucson, AZ 85721. Phytopathology 94:S26. Publication no. P-2004-0172-AMA.

Aflatoxin, produced by the fungi *Aspergillus flavus* and *A. parasiticus*, can contaminate many crops including almonds, figs, pistachios, and walnuts in California. Certain strains of *A. flavus* are unable to produce aflatoxins. One such atoxigenic strain, AF36, has been used successfully as a biocontrol agent to substantially reduce aflatoxin contamination in commercial cotton fields in Arizona and Texas. The objective of the current study was to determine the natural occurrence of AF36 and other atoxigenic strains in commercial orchards in California using vegetative compatibility analyses. Out of 431 *A. flavus* isolates from commercial pistachio orchards, 6.3%, 2.1%, and 1.6% belonged to atoxigenic strains AF36, A564, and A815, respectively. In addition, out of 97 isolates from fig orchards, 6.2%, 1.0%, and 1.0% belonged to strains AF36, A564, and A815, respectively. So far, AF36 has been detected in commercial pistachio orchards in five counties, which contain approximately 89% of the bearing acreage in California. In conclusion, the atoxigenic strain AF36 occurs naturally in pistachio and fig commercial orchards in California and is widespread throughout the major pistachio growing areas.

Bacterial blight of carrot seed crops in the Pacific Northwest. L. J. DU TOIT, F. J. Crowe (2), M. L. Derie (1), R. B. Simmons (2), and G. Q. Pelter (3). (1) Washington State University, Mount Vernon, WA; (2) Oregon State University, Madras, OR; (3) Washington State University, Ephrata, WA. Phytopathology 94:S26. Publication no. P-2004-0173-AMA.

Carrot seed crops in [illegible]d WA were surveyed in 2001/02 and 2002/03 to monitor development of *Xanthomonas campestris* pv. *carotae* (Xcc), causal agent of bacterial blight. In addition to assays of seed and stecklings, 20 plants were sampled twice in the fall from each direct-seeded crop (16 to 24 fields) and three times from April to August from each direct-seeded and steckling crop (4 to 8 fields). The pathogen was detected in 6 of 23 and 0 of 7 stock seed lots, and 2 of 6 and 5 of 6 steckling shipments in WA and OR, respectively. In early fall 2001 and 2002, Xcc was detected in 1 of 22 and 10 of 14 crops, respectively. The prevalence of Xcc increased with time, but symptoms were only observed during summer. By August 2002 and 2003, Xcc was detected in 11 of 12 and 7 of 10 crops in WA and 10 of 10 and 13 of 13 crops in OR, respectively. In general, Xcc was less prevalent in steckling vs. direct-seeded crops, and in furrow- and drip-irrigated vs. overhead irrigated crops. The pathogen was detected in 34 of 42 harvested seed lots, at populations ranging from 1.3×10^1 to 1.4×10^8 CFU/g seed. Xcc was also detected in airborne dust/debris generated during threshing, but was not recovered from honey bee pollinators sampled from an infected crop.

Role of *pthA* homologues in *Xanthomonas citri* pv. citri A^W in hyperplasia and necrosis of Key lime and grapefruit. Y. P. DUAN, A. Al-Saadi, and D. W. Gabriel. Plant Molecular and Cellular Biology Program, Plant Pathology Dept., University of Florida, Gainesville, FL. Phytopathology 94:S26. Publication no. P-2004-0174-AMA.

Xanthomonas citri pv. citri A^W (A^W) caused typical canker symptoms on Key lime, but on grapefruit, initial canker symptoms progress rapidly to a strong necrosis, a phenotype unique to A^W strains. The necrosis did not appear to reflect a defense or hypersensitive response (HR). In the A^W strains tested, two homologues of *pthA* were identified, both of which were found on one of the two plasmids present in these strains. Marker-interruption of one of the *pthA* homologues resulted in greatly reduced pathogenicity on both Key lime and grapefruit. This *pthA* homologue, named *pthAW*, was cloned and sequenced. *pthAW* is 99% identical to *pthA* at the DNA level. Heat treatment of the A^W strains by progressively increasing temperatures 1° per day from 32°C to 36°C resulted in plasmid rearrangements and deletions. Twenty heat-treated isolates were inoculated onto Key lime and grapefruit. None of the isolates were evidently affected in pathogenicity on Key lime, but all of them lost varying degrees of pathogenicity on grapefruit.

Cytochrome *b*561-like genes are useful in identification of *Xanthomonas* strains. Y. P. [illegible]AN and D. W. Gabriel. Plant Molecular and Cellular Biology Program, Plant Pathology Dept., University of Florida, Gainesville, FL. Phytopathology 94:S27. Publication no. P-2004-0175-AMA.

The cytochrome *b*561 (*b*561) genes in eukaryotes are known to be involved in detoxification of reactive oxygen species. Such genes are reportedly not present in bacteria, but the recent availability of many bacterial genomic sequences has resulted in the identification of genes with some similarity to *b*561. The bacterial genes have strikingly similar structural features to the eukaryotic *b*561 genes, including highly conserved transmembrane helices and histidine residues. Based on published genomic DNA sequences, most bacteria contain two or three paralogous *b*561-like genes. We cloned and sequenced additional *b*561 genes from more than 30 strains of *Xanthomonas* and found that the *b*561 gene sequences were surprisingly reliable for use in strain identification and in typing strains at the pathovar and species level. The bacterial *b*561 genes may also be useful for taxonomic purposes.

Fungi resident in chickpea de[illegible] and competitive interactions with *Ascochyta rab*[illegible]. F. M. DUGAN (1), T. L. Peever (2), and W. Chen (1). (1) USDA-ARS; (2) Department of Plant Pathology; Washington State University, Pullman, WA. Phytopathology 94:S27. Publication no. P-2004-0176-AMA.

Fungi resident in chickpea (*Cicer arietinum*) debris from Pullman, WA, were identified and enumerated with the objective of finding species potentially useful for biological control of *Ascochyta rabiei*, causal agent of Ascochyta blight. In addition to *A. rabiei*, primary colonizers were, in order of highest abundance, *Alternaria tenuissima*, *Al. infectoria*, *Ulocladium consortiale*, *Epicoccum purpurascens*, *Fusarium graminearum*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *F. solani*, *Clonostachys* cf. *levigata* and two Phoma-like coelomycetes. Present in lower numbers were *Al. malorum*, *Alternaria* sp., *Botryotrichum keratinophilum*, *Curvularia inaequalis*, *Fusarium* spp., *Pithomyces chartarum* and *U. atrum*. *Stemphylium/Pleospora* and *Au. Pullulans* were commonly isolated from debris of previous harvests. Compared to *A. rabiei*, *Au. Pullulans* grew 19-52% more rapidly on autoclaved chickpea stems at 20°C and relative humidities >80% or >90%. *Au. Pullulans* excluded growth of *A. rabiei* from stem segments receiving prior inoculation with *Au. Pullulans*. Results suggest that *Au. Pullulans* can be used to inhibit *A. rabiei* and its sexual stage, *Didymella rabiei*, on chickpea debris.

Quorum sensing contributes to virulence and epiphytic fitness of *Pseudomonas syringae*. G. DULLA, B. Quiñones, and S. Lindow. University of California, Berkeley, CA. Phytopathology 94:S27. Publication no. P-2004-0177-AMA.

Pseudomonas syringae pv. *syringae* (*Pss*) exhibits quorum sensing (QS), in which it expresses genes conferring extracellular polysaccharide production, motility, and factors contributing to virulence to bean only when cells reach a relatively high local concentration. QS in *Pss* involves the production of the diffusible signal molecule, 3-oxohexanoyl homoserine lactone (AHL). Mutants deficient in production of AHL are hypermotile, enter moist bean leaves more readily and incite formation of more lesions but are less tolerant of dessication stress after topical application to bean leaves than the parental strain. About 18% of culturable epiphytic bacteria can produce small diffusible molecules that interfere with QS in *Pss*. About 7% of bacterial epiphytes produce the same AHL, often in amounts more than 20-fold higher, as *Pss*. While coinoculation of AHL-producing strains with *Pss* reduce lesion size in inoculated bean pods and reduce the number of lesions when sprayed together on leaves compared with that of plants inoculated with *Pss* alone, increased lesion size and number occur on bean coinoculated with *Pss* and QS-inhibiting strains. Mutants of QS-interfering strains deficient in this phenotype do not increase disease when coinoculated with *Pss*. Premature induction of QS in *Pss* thus inhibits disease initiation and can be exploited for disease control.

***Fusarium oxysporum* f. sp. *niveum* race 2 of wat[illegible]melon in Indiana.** D. S. EGEL (1), R. Harikrishnan (2), and R. Martyn (1). (1) Purdue University, West Lafayette, IN.; (2) North Dakota State University, Fargo, ND. Phytopathology 94:S27. Publication no. P-2004-0178-AMA.

Fusarium wilt of watermelon, *Citrullus lanatus*, caused by *Fusarium oxysporum* f. sp. *niveum* (FON), is a major disease management problem in the U.S. Almost all commercial cultivars have at least partial resistance to two of the known races (FON-0 and FON-1), but there are no cultivars or hybrids available with resistance to race 2 (FON-2). FON-1 is uniformly distributed throughout the watermelon growing regions but FON-2 has only a limited known distribution (Texas, Oklahoma, Maryland, Delaware). In recent years, significant wilt has occurred in southern Indiana in cultivars previously considered resistant; thus the presence of race 2 was suspected. Two isolates of FON obtained from wilted watermelon plants in two different commercial fields in southern Indiana and one isolate obtained from a wilted seedling in a transplant house, along with known isolates of races 0, 1, and 2, were used to root-dip inoculate (10^5 microconidia/ml) 2-wk-old seedlings of the differential cultivars, Black Diamond, Charleston Gray, and Calhoun Gray. Both isolates from commercial field plants caused greater than 60% wilt in all three differentials suggesting they were race 2. The isolate obtained from the seedling grown in the transplant house wilted Black Diamond and Charleston Gray but not Calhoun Gray and, thus, was considered to be race 1. This is the first report of FON race 2 in Indiana and the first report from the Midwest region of the United States.

Disease development on lisianthus (*Eustoma grandiflorum*) following aerial transmission of *F[illegible]rium avenaceum* by adult shore flies, fungus gnats, and moth flies. Z. A. EL-HAMALAWI and M. E. Stanghellini. Department of Plant Pathology, University of California, Riverside 92521. Phytopathology 94:S27. Publication no. P-2004-0179-AMA.

Fusarium stem rot of lisianthus (*Eustoma grandiflorum*), caused by *Fusarium avenaceum*, is a destructive disease in California. The pathogen produces large masses of orange-colored macroconidia on stem lesions that extend up to 35 cm in length from the soil surface. Populations of macroconidia (97% viability) range from 1.1×10^8 to 1.9×10^8 per cm of infected stem. An above-ground life stage for a soilborne pathogen could serve as the source for airspora and/o[illegible]quisition and subsequent aerial dissemination by insect vectors. Adult shore flies, fungus gnats and moth flies have been implicated as aerial vectors of soilborne fungal pathogens of several vegetable crops in which the pathogen reproduces above-ground. The role of these insects in the epidemiology of *Fusarium* on lisianthus was studied. Twenty adults of each insect were placed in separate screen cages containing naturally-infected stems of lisianthus bearing macroconidia of *F. avenaceum* and 20 healthy lisianthus seedlings (1 mo.) in 6-cm-diam. Pots. Controls consisted of healthy plants caged with insects without infected stems and healthy plants caged with infected stems without insects. After 4 days exposure to insects and/or infected stems, plants were removed from the cages and incubated in a greenhouse. Disease symptoms developed on 45, 55.5, and 75% of the plants caged with moth flies, fungus gnats, and shore flies, respectively, over the next 66 days. None of the plants in the various control treatments developed disease symptoms.

Role of the phytotoxin coronatine in pathogenesis of *Pseudomonas syringae* pv. *tomato* DC3000 in edibl[illegible]*ssica* spp. S. V. ELIZABETH and C. L. Bender. Dept. Entomology & Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078. Phytopathology 94:S27. Publication no. P-2004-0180-AMA.

Coronatine (COR) is a non host-specific, chlorosis-inducing toxin produced by several pathovars of *P. syringae* and is c[illegible]sidered a virulence factor on certain hosts. COR ha[illegible]o components: coronafacic acid (CFA) and coronamic acid (CMA). *P. syringae* pv. *tomato* strain DC3000 has been recently sequenced and hence is a model organism for investigating plant-microbe interactions. The pathogen is genetically t[illegible]able, is pathogenic on Arabidopsis, tomato and crucifers and can cause significant loss on edible *Brassica* spp. Hence, it is important to investigate the role of coronatine in the pathogenesis of the bacterium on edible *Br[illegible]a* spp. Including collard and turnip, which are major crops in Oklahoma. These hosts were inoculated with defined mutants of DC3000 that were defective in CFA, CMA and/or COR production and were monitored for symptoms, colonization, chlorophyll and anthocyanin content. Our results suggest that COR is required for symptom development in both hosts and for multiplication of DC3000 in turnip. This indicates that there are differences in the COR-host interaction. It is hypothesized that COR could suppress plant defense pathways and hence promote bacterial colonization of host tissue. Therefore, gene expression analysis will be conducted to monitor defense signal transduction pathways in collard and turnip.

A new species of *Fusicoccum* causing a canker disease of Pacific ma[illegible]e. M. ELLIOTT (1), A. Rossman (2), D. Farr (2), and R. L. Edmonds. (1) College of Forest Resources, University of Washington, Seattle, WA; (2) Systematic Botany & Mycology Laboratory, USDA-ARS, Beltsville, MD. Phytopathology 94:S27. Publication no. P-2004-0181-AMA.

Pacific madrone (*Arbutus menziesii*) is a broadleaf evergreen tree native to Western North America and has been suffering a decline during the past thirty years. A fungus, previously identified as *Nattrassia mangiferae*, causes a canker disease that is primarily responsible for the decline. Isolates of the pathogen were studied using morph[illegible]cal and molecular methods. Only asexual spores were observed, but sequencing of the ITS region of the ribosomal rDNA places the fungus in the genus *Botryosphaeria*. The madrone canker fungus resembles *Fusicoccum* anamorphs of closely related *Botryo-*

sphaeria species *B. ribis* and *B. parva*, and has a similar pathology. Based on rDNA sequences, isolates of *Nattrassia mangiferae* from a culture collection were shown to have teleomorphs in *Botryosphaeria* in a clade with *B. mamane* and *B. corticis*. However, the madrone fungus is not identical or even closely related to *Nattrassia* and should be considered a new species of *Fusicoccum*.

Distribution of tan spot and race structure of *Pyrenophora tritici-repentis* in Ohio. J. S. ENGLE (1), P. E. Lipps (1), and T. L. Friesen (2). (1) The Ohio State University, Dept. Plant Pathology, Wooster, OH 44691; (2) USDA-ARS, Red River Valley Ag. Research Center, Fargo, ND 58105-5677. Phytopathology 94:S28. Publication no. P-2004-0182-AMA.

Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, has been a major economic problem in the Northern Great Plains, but is considered less of a problem in the eastern soft wheat growing region of the Midwest. *P. tritici-repentis* was reported in Ohio in the 1980's, the race structure and the distribution of the pathogen has not been previously investigated. During 2002 flag leaf samples were collected from 361 wheat fields in 37 counties to determine the leaf blotching pathogens present in the state. Within each county, 15 fields were randomly chosen and from each field, isolations were obtained from 2 flag leaves. A total of 218 *P. tritici-repentis* isolates were obtained from 30 counties. A sub-sample of 37 isolates representing all counties was inoculated onto a set of differential wheat lines. Results indicated that races 1, 2 and 3 were present, with race 1 being predominant. This study was repeated in 2003 with a total of 374 fields sampled in 35 counties. Preliminary results indicate flag leaves from most counties had tan spot. Weather conditions in 2002 and 2003 were very different, indicating that conditions for tan spot development may occur frequently in Ohio and that tan spot may be more important than previously thought.

Greenhouse and field reaction of soft red winter wheat cultivars to Stagonospora leaf and glume blotch, *Stagonospora nodorum*. J. S. ENGLE and P. E. Lipps. The Ohio State University, Dept. of Plant Pathology, Wooster, OH 44691. Phytopathology 94:S28. Publication no. P-2004-0183-AMA.

Resistance to *Stagonospora nodorum*, Stagonospora leaf and glume blotch, has been shown to be independent in different plant organs and the level of resistance response varies with plant maturity. The reaction of 13 wheat cultivars was evaluated in the greenhouse and field in 2002. In the greenhouse, fully expanded flag leaves and emerged heads were inoculated and rated for disease severity. The same cultivars were planted in one inoculated and two uninoculated field plots. These were rated weekly for disease severity from flag leaf emergence to hard dough stage and glumes were assessed at hard dough stage. Coker 9663 had the least amount of leaf blotch on the flag leaf disease in the greenhouse (mean (x) = 7.4%) and field (x = 14.9%), while Patterson had a susceptible reaction in the greenhouse (x = 43.2%) and field (x = 66.7%). Coker 9025 had the least amount of glume blotch in the field (x = 2.1%), while Coker 9663 had lowest glume blotch rating in the greenhouse (x = 25.7%). AGI 535 had the most glume blotch in the field (x = 7.3%), while Honey had the highest rating in the greenhouse (x = 89.7%). Cultivars with intermediate reactions for leaf and glume blotch had rank differences indicating that greenhouse inoculations may only differentiate the most resistant from the most susceptible.

Spore release of *Phaeomoniella chlamydospora* associated with grapevine cordons in California. A. ESKALEN, S. R. Latham, and W. D. Gubler. University of California, Davis, CA. Phytopathology 94:S28. Publication no. P-2004-0184-AMA.

The fungus *Phaeomoniella chlamydospora* causes Petri disease (syn. Young vine decline) and is also associated with esca (Black measles) of grapevines in California and many other grape production countries. Spore traps were placed in selected vineyards where Petri disease was known to occur. Spore trapping data were collected weekly throughout 2002 and 2003. Results showed that spores of *Pa. Chlamydospora* were trapped in Napa, Sonoma, Mendocino, Tulare, San Joaquin, San Luis Obispo, Madera and Solano Counties. Successful trapping of *Pa. Chlamydospora* in each case was correlated with rainfall events in each location. Pycnidia of *Pa. Chlamydospora* were found under exfoliating bark of grapevine cordons and on 2-3 year-old pruning wounds on cordon spurs. Spores from pycnidia were shown to be viable and pathogenic. These results showed conclusively that *Pa. Chlamydospora* has the ability to act as airborne inoculum in California vineyards during winter and spring rainfall. Pruning wounds were shown to be susceptible for up to 4 months.

Survival analysis of phytoplasma-infected papaya causing yellow crinkle disease in Australia. P. D. ESKER (1,2), P. M. Dixon (2), and F. W. Nutter, Jr. (1). (1) Department of Plant Pathology; (2) Department of Statistics, Iowa State University, Ames, IA. Phytopathology 94:S28. Publication no. P-2004-0185-AMA.

To investigate the effects of phytoplasmas on survival of papaya plants, we examined different hypotheses regarding the influence of the tomato big bud (TBB) and sweet potato little leaf V4 (SPLL-V4) strains, papaya plant age, and seasonal (wet/dry) effects of time of infection and their influence on the time-to-death (TTD) in papaya. Survival analysis methods were applied to monthly incidence data collected over a 36-month period in a papaya plantation in the Northern Territories, Australia. These methods included the use of Kaplan-Meier estimates of the survival function, accelerated failure time analysis, and Cox proportional hazards. Based on these analyses, no differences in TTD were detected between TBB and SPLL-V4 infected papaya. Also, neither plant age nor seasonal effects influenced TTD, which ranged from ~ 4 to 5.5 months. This information shows that pathogenesis of both phytoplasma strains causing yellow crinkle is similar.

Suppression of root knot and lesion nematodes by cover crops, poultry litter and compost. K. L. EVERTS, S. Sardanelli, R. J. Kratochvil, and D. K. Armentrout. University of Maryland, College Park, MD. Phytopathology 94:S28. Publication no. P-2004-0186-AMA.

Increased potato production in Maryland has led to resurgent parasitic nematode populations. Experiments were established in microplots infested with *Meloidogyne incognita* or *Pratylenchus* spp. To evaluate twelve 3-year rotational sequences. For the first two years, potato (2000) and cucumber (2001) each were followed by cyst nematode resistant 'Manokin', or susceptible 'Pioneer 93B01' soybean cultivars; castor bean 'Mall', grain sorghum 'NKKS585', sorghum sudangrass 'Green grazer' that was followed by rapeseed 'Dwarf Essex'; or left fallow and amended with poultry litter (PL). Additional 'Green grazer' and 'Pioneer 93B01' plots also were amended with PL or PL compost. Plant biomass was soil-incorporated each fall. In 2002, all plots were planted with potatoes followed by soybeans. 'Manokin', 'Mall', 'Green grazer', and fallow plots amended with PL decreased populations of *M. incognita* in the fall of 2000 and 2001, but not the following summers. 'NKKS585', fallow plots amended with PL, and 'Green grazer' amended or not with PL or compost, reduced *Pratylenchus* spp. In 2001. Carry-over suppression was observed the following summer, where 'Green grazer' plots had been amended with 8.2, 2.8 Mg/ha PL or 11.7 Mg/ha compost. Cover crops reduced nematode populations, however the effect was transient.

The 14-3-3 homolog MAFA interacts with VERB synthase in *Aspergillus flavus*. A. M. FAKHOURY (1) and G. A. Payne (2). (1) Southern Illinois University, Carbondale, IL; (2) North Carolina State University, Raleigh, NC. Phytopathology 94:S28. Publication no. P-2004-0187-AMA.

The filamentous fungus *Aspergillus flavus* is a ubiquitous pathogen of several plant species such as corn, cotton and peanut. Fungal infections result in the production of aflatoxins, a series of structurally related compounds known to be carcinogenic. A thorough understanding of the regulation of aflatoxin biosynthesis has proven difficult given the diversity of factors involved. These include nutritional, physiological and developmental factors. We have previously reported that a 14-3-3 homolog (*mafA*) is involved in aflatoxin biosynthesis in *A. flavus*. The disruption of *mafI* in a mycotoxigenic strain of *A. flavus* abolishes aflatoxin production. 14-3-3 proteins are ubiquitous in eukaryotes with reported functions ranging from regulating primary metabolism in plants, to controlling trafficking in cells. We used a yeast two hybrid assay to identify proteins that interact with MAFA under conditions conducive to aflatoxin biosynthesis. We are reporting here that MAFA interacts with versicolorin B synthase (VBS), the enzyme that governs the conversion of versiconal (VHOH) to versicolorin B (VERB) in the aflatoxin biosynthetic pathway.

Biological control of monilia pod rot (*Moniliophthora roreri*) on "high flavor" cocoa's field using biopesticidas based on *Bacillus subtillis* and *Pseudomonas cepacea*. C. E. FALCONI, A. R. Oleas, and V. R. Yánez. Center of Biological Control, Faculty of Agropecuarian Science, The Army Polythechnic School, Telefax: (593) 02 2870187, E-mail: cfalconi@espe.edu.ec, vivi983@hotmail.com, P.O. Box 171-5-231B, Sangolquí, Ecuador. Phytopathology 94:S28. Publication no. P-2004-0188-AMA.

Monilia pod rot is the most devastating cocoa disease, which is causing 40–80% of production losses. Multiple aplication increse steadely the cost of production, chemicals are mainly washed during raining season. This research aims to determine the effect of biopesticides peet formulated on the disesase reduction. Two biopesticides based on the epiphytic bacteria *P. cepacia* and *B. subtilis* were evaluated to control *M. roreri* on "high flavor" cocoa's, variety EET – 103. The study was carried on the Limón farm, located in the Cotopaxi Province, Ecuador. The experiment was established in a Randomized Block Design with four replications, six trees were considered as

an experimental unit. 50 g of peet fermeted bioproducts were washed in 20 liters of water. Four treatments were included, two bioproducts (bacteria suspension, 0.05% sweetened water, 0.05% Carrier), a positive (sweeetened water and carrier), a negative (absolute control). The treatments were applied every 15 days, from September 2002 to February 2003. The evaluated variable was the external severity on the developing pod. With the data obtained, the Area Under Disease Progress Curve was calculated (AUDPC). The variance analysis demonstrated significative differences among treatmets. Tukey test 0.5% determined differences among biopesticides and controls. From the data obtained, the treatments based on *P. cepacia* y *B. subtilis* reduced the incidence of *M. roreri* in 76 and 86%, respectively, in comparison with the negative control. Data collected suggest that the application of these biopesticides is an efficient strategy to control monilia pod rot in an integrated pest management of cocoa. Project sponsored by Programa de Modernización de los Servicios Agropecuarios-PROMSA.

Molecular variation in *Pennisetum mosaic virus*. Z. F. FAN, C. L. Deng, H. Chen, S. S. Cai, W. J. Wang, X. M. Liang, X. Jiang, and H. F. Li. Department of Plant Pathology and State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100094, P.R. China. Phytopathology 94:S29. Publication no. P-2004-0189-AMA.

Pennisetum mosaic virus (PenMV) is a newly identified potyvirus (accession number AY172336), which is closely related to other members of *Sugarcane mosaic virus* subgroup. PenMV was originally isolated from a perennial grass (*Pennisetum centrasiaticum*) in Shanxi Province of northern China. PenMV could infect maize (*Zea mays*) naturally and caused mosaic in all the cultivars of maize and sorghum tested. The nucleotide sequences of the coat protein (CP) gene for 8 PenMV isolates (5 from the perennial grass and 3 from maize) have been determined. The CP genes of all these isolates comprised 909 nucleotides and encoded 302 amino acid residues. The similarity among the deduced CP amino acid sequences of the 3 maize isolates was 98.5% to 100%, and that among the CPs of the 5 grass isolates was 98.3% (between AY083684 and AY172336) to 100%. The CP amino acid sequence similarity between the grass isolates and maize isolates ranged from 98.7% to 99.0%. Therefore, the extent of variation among the PenMV isolates from maize was slightly less than that among the PenMV grass isolates. Since many plants of this perennial grass were infected by PenMV in some areas of Shanxi Province, and the virus was isolated from maize in a field with many infected grass plants, one can speculate that the virus in maize likely came from the infected grass through transmission by vector aphids.

Efficacy of fungicides for control of downy mildew of lettuce. J. J. FARRAR. California State University, Plant Science Dept., Fresno, CA 93740. Phytopathology 94:S29. Publication no. P-2004-0190-AMA.

An evaluation of fungicides for lettuce downy mildew control was conducted at the University of California Westside Research Station. Plots consisted of one 40-inch bed 30-feet long with two seed lines. Experimental design was a randomized complete block with four replications. Plots were treated every 7 days starting on 21 Jan. Treatments included an untreated control, two rates of Reason, Reason plus Previcur, Quadris, Acrobat plus Maneb, Acrobat plus Aliette alternated with Acrobat plus Maneb, Cabrio, and Cabrio alternated with Acrobat plus Maneb. Disease severity was rated as percentage of leaf area diseased on 10 whole plants per plot. Evaluations in Feb and early Mar indicated that all fungicide treatments resulted in equivalent disease control and significantly reduced disease compared to the untreated check. Therefore, the last fungicide applications were on 6 Mar and disease evaluations were continued weekly in order to examine the longevity of control. Plots were harvested on 10 Apr. The lowest disease rating and highest yield was obtained in the Acrobat plus Aliette alternated with Acrobat plus Maneb and the Cabrio alternated with Acrobat plus Maneb treatments.

Molecular phylogenetic relationships among the anamorphic *Septoria* species from woody perennials. N. FEAU (1), R. C. Hamelin (2), and L. Bernier (1). (1) Laval University, Sainte-Foy, QC; (2) Service Canadien des Forêts, Centre de Foresterie des Laurentides, Sainte-Foy, QC. Phytopathology 94:S29. Publication no. P-2004-0191-AMA.

We recovered 25 species of *Septoria* anamorphs and 21 species with a *Mycosphaerella* teleomorphic state pathogenic on various woody perennials. We carried out phylogenetic analyses using nuclear rDNA, the mitochondrial rDNA small subunit (SSU), the beta-tubulin gene and two group-I introns located in the SSU rDNA gene. Phylogenetic trees were generated using maximum parsimony and neighbor-joining analysis. Separate analyses of the ITS, beta-tubulin and the mitochondrial rDNA sequences resulted in low resolution among the isolates tested. Analysis of the consensus alignment obtained with these three sequences resulted in higher phylogenetic resolution and revealed that the *Septoria* species constitute polyphyletic groups within the *Mycosphaerella* genus. Concordance among gene genealogies is currently being analysed and indicates incongruence between nuclear and mitochondrial genes and the group-I introns.

Isolation of disease resistance genes associated with the salicylic acid pathway in *Citrus*. V. J. FEBRES and G. A. Moore. University of Florida, PO Box 110690, Gainesville, FL. Phytopathology 94:S29. Publication no. P-2004-0192-AMA.

Activation of inducible defenses in plants is contingent upon recognition of an invasion. An important component of this defense system is systemic acquired resistance (SAR). After a hypersensitive response to invading pathogens, plants show elevated accumulation of salicylic acid (SA), induced expression of pathogenesis-related proteins, and SAR to subsequent infection by a broad range of pathogens. Through genomics and other more traditional techniques, several of the genetic components of the plant-pathogen signal transduction pathways have been elucidated for herbaceous annual plants such as *Arabidopsis*, tomato and tobacco, among a few other species. However, little is known at the genetic level about the interactions between woody perennial plants and microorganisms. Through BLAST analysis and sequence comparisons, we have identified some of the potential genes in the SA pathway of citrus types. Using the GenBank EST database, we have identified homologous sequences in different citrus species for the following genes: *EDR1*, *EDS1*, *NDR1*, *NPR1*, *PBS1*, *PR1*, *RAR1* and *SGT1*. So far, we have cloned complete or partial sequences for these genes indicating that orthologs of at least some of the genes found in annual species are present in *Citrus*. We also evaluated their induction by SA.

The effect of two melanin biosynthesis inhibitors on growth and reproduction of *Monosporascus cannonballus*. D. M. FERRIN and M. E. Stanghellini. Dept. of Plant Pathology, University of California, Riverside, CA 92521. Phytopathology 94:S29. Publication no. P-2004-0193-AMA.

Melanins play a variety of roles in fungal survival and pathogenesis. Perithecial and outer ascospore walls of *Monosporascus cannonballus* are both highly melanized. The effects of two melanin biosynthesis inhibitors (tricyclazole and carpropamid) on growth and reproduction of *M. cannonballus* were examined in vitro. At a concentration of 100 mg/L, tricyclazole reduced mycelial growth by 60%, but carpropamid had no effect. Tricyclazole reduced perithecium production by 41% at a concentration of 10 mg/L, and no perithecia were formed at concentrations of 50 or 100 mg/L. In contrast, carpropamid reduced perithecium production by 52% at a concentration of 5 mg/L. However, it did not completely prevent perithecium production even at 100 mg/L. Both compounds visibly reduced perithecium and ascospore maturation at 5 mg/L, the lowest concentration tested. These fungicides may have potential for use in the management of root rot and vine decline of melons through inhibition of reproduction of *M. cannonballus* in roots, thereby reducing the amount of inoculum returned to soil.

The effect of osmotic water potential on growth and reproduction of *Monosporascus cannonballus*. D. M. FERRIN and M. E. Stanghellini. Department of Plant Pathology, University of California, Riverside, CA 92521. Phytopathology 94:S29. Publication no. P-2004-0194-AMA.

Monosporascus cannonballus causes root rot and vine decline of melons in the desert production regions of southern California. The effect of osmotic water potential on growth and reproduction of *M. cannonballus* was examined on media amended with KCl, NaCl or sucrose in order to establish a range of osmotic potentials. Patterns of growth responses of four isolates to decreasing water potential were similar for each of the three osmoticum. Compared to growth on nonamended PDA (–0.3 Mpa), growth of all four isolates increased as water potential was reduced to –0.8 Mpa. Maximum growth occurred from –0.6 to –0.8 Mpa. Growth was not reduced below that on nonamended PDA until the water potential had been reduced to at least –1.8 Mpa, and a 50% reduction in growth did not occur until the water potential had been reduced to less than –2.5 Mpa. Reproduction was more sensitive to reduced water potential than mycelial growth. Mature perithecia were produced only at water potentials greater than –1.0 Mpa. Irrigation water quality and salt accumulation in soil may enhance growth of *M. cannonballus* and subsequent disease development.

Soybean partial resistance and tolerance to *Phytophthora sojae*. C. R. FERRO (1), C. B. Hill (1), M. R. Mill[illegible] (2), and G. L. Hartman (1,2). (1) University of Illinois; (2) USDA-ARS. Phytopathology 94:S29. Publication no. P-2004-0195-AMA.

Phytophthora root rot, caused by *Phytophthora sojae*, is a major soybean disease in the USA. Sixteen maturity group II and III soybean cultivars were used to evaluate differences in tolerance or partial resistance to two isolates of *P. sojae* that defeat the *Rps 1k* resistance gene. An inoculum layer test was used to test partial resistance to *P. sojae* isolates pathotype 28 and pathotype

30, with a mock-inoculated control. Out of the sixteen cultivars tested, ten cultivars showed partial resistance to pathotype 28 and three of these had partial resistance to pathotype 30. In the field, tolerance was evaluated by comparing yields and heights of cultivars with seed treated or not treated with the fungicide mefenoxam in experimental plots infested or not infested with *P. sojae* inoculum. Tolerance to *P. sojae* pathotypes 28 and 30 was found in 5 of 14 commercial cultivars with the *Rps 1k* gene. One cultivar had partial resistance and tolerance to both *P. sojae* pathotypes. With appropriate selection of pathotypes, the greenhouse and field evaluations successfully identified cultivars with partial resistance or tolerance to *P. sojae*.

Biological control of watermelon seed infection by *Acidovorax avenae* subsp. *citrulli*. A. FESSEHAIE and R. R. Walcott. The University of Georgia, Department of Plant Pathology, Athens, GA 30602. Phytopathology 94:S30. Publication no. P-2004-0196-AMA.

A. avenae subsp. *citrulli* (Aac), causes bacterial fruit blotch (BFB), a devastating disease of cucurbits. Cucurbit seed represent the most important source of inoculum for BFB; however, effective seed treatment options are limited. Recently, it was shown that watermelon blossom inoculation with Aac could lead to seed infection and it was hypothesized that protecting blossoms could prevent seed infection. To test this hypothesis, three bacteria with *in vitro* antagonistic activity against Aac (*A. avenae* subsp. *avenae* 99-2, *P. fluorescens* A502 and an unidentified Gram-positive bacterium recovered from watermelon seed, (WS1)) were evaluated for their ability to prevent seed infection via blossoms. Under greenhouse conditions, female watermelon blossoms were pollinated and inoculated with each biological control agent. Four hours later, the blossoms were challenged with Aac at 10^9 CFU/ml. Blossoms were also protected with Kocide 101 and 0.1M PBS as negative and positive controls, respectively. At maturity, seeds were harvested from each fruit and tested for Aac by the seedling grow-out assay. None of the seedlots produced from blossoms protected with AAA99-2 and A506 were positive for Aac. In contrast, 55 and 33% of the seedlots from blossoms protected with WS1 and Kocide, respectively, were infested. Additionally, 87.5% of the seedlots from blossoms protected with PBS were infested with Aac. These results indicate the potential for biological control to prevent seed infestation by Aac.

Influence of environment on wheat infection efficiency by *Tilletia indica* Mitra artificially inoculated in Northwest Mexico. P. FIGUEROA-LOPEZ, G. Fuentes-Davila, and M. A. Camacho-Casas. INIFAP, CEVY, Cd. Obregon, Mexico. Phytopathology 94:S30. Publication no. P-2004-0197-AMA.

Variability of disease severity within single wheat genotypes artificially inoculated and sown on a single planting date might result in unreliable scores when screening for Karnal bunt (Kb) resistance. It has been attributed, *inter alia*, to pathogen genetic variability, segregation of genotypes evaluated, and to environment. Although several predictive models for Kb ocurrence are available, they have not been used to explain variability shown by genotypes during screening. Our objective was to explain the variability of disease severity observed on twenty and twelve genotypes of bread and durum wheat, respectively, established on four planting dates. Thirty spikes per genotype per planting date were inoculated by injection at Zadoks stage 49, with 10,000 allantoid sporidia. After harvesting, disease severity was evaluated on every line as percentage of infected grain. Data for every planting date was averaged I lines and correlated with the number of days with conducive environmental conditions for infection, according to the Jhorar *et al.* model, considering the period from the first inoculation up to five days after the last inoculation for every planting date. The number of days with conducive infection conditions explained the observed variation in disease severity among planting dates on both bread ($R^2 = 0.99$) and durum wheat nurseries ($R^2 = 0.94$). Considering the effect of environment on expression of resistance will allow a more accurate evaluation of resistance and its stability.

Blue light represses conidiation in *Exserohilum turcicum*, the causal agent of northern leaf blight of maize. J. E. FLAHERTY and L. D. Dunkle. USDA-ARS, Crop Production and Pest Control Research Unit, Purdue University, West Lafayette, IN. Phytopathology 94:S30. Publication no. P-2004-0198-AMA.

We examined the effects of light on growth and development of *Exserohilum turcicum*, the fungal pathogen causing northern leaf blight of maize. Cultures grown under continuous light were developmentally arrested after the formation of conidiophores, whereas cultures maintained in continuous darkness or a light/dark cycle produced mature conidia. Vegetative growth was more extensive under light/dark cycles than under constant light or darkness, both visually and based on ergosterol content. In cultures exposed to various periods of dark before a shift back to continuous light, a 2-hour dark period was sufficient to initiate the conidiation program. Only wavelengths in the blue light range (425-485 nm) repressed conidiation. This light-repressive phenomenon contrasts with other fungal species in which blue light induces or enhances conidiation. Because previous studies have shown that long photoperiods reduce colonization of maize by *E. turcicum*, we propose that the repressive effect of light on conidiation, growth, and pathogenicity involves signal transduction pathways mediated by a blue-light receptor. The goal of our study is to identify genes involved in light-regulated conidiation and to characterize their involvement in growth, development, and pathogenicity. To this end, we are constructing subtractive cDNA libraries from mRNA isolated from cultures grown under conidiation-permissive and repressive conditions.

Consistent inoculation method for corn (*Zea mays* L.) hybrids to *Cercospora zeae-maydis* in greenhouse environments. J. M. FLEER and J. E. Partridge. Department of Plant Pathology, University of Nebraska-Lincoln. Phytopathology 94:S30. Publication no. P-2004-0199-AMA.

Cercospora zeae-maydis (CZ-M) is the causal fungus of gray leaf spot of corn. A consistent method for inoculating CZ-M in greenhouse systems is essential for studying this disease. Field inoculations show varying degrees of success with dependency upon temperature, relative humidity, dew periods, location, and year. Resistant (company defined) (NC+5172B, PIO33V15, DK687, RX718YG) and susceptible (company defined) (NC+4990B, PIO3394, DKC57-84, RX752YG) hybrids were provided by four seed companies to develop a consistent inoculation procedure for greenhouse environments. A 10^3 conidia/mL suspension was sprayed onto V3 stage corn plants. Polyethylene bags were placed over the plants and incubated for four days. During this time relative humidity was 100% but decreased to 23-75% upon bag removal. Average temperature and dew point were 23.6°C and 10.4°C respectively for the entire experiment. Lesion progression was observed from cotyledon to V5 from 7 to 21 days after inoculation (DAI). Stromata, conidiophore, and conidia development varied but were observed from 6-21 DAI. The third true leaves (V4 stage) were removed from each of 16 replicates. Lesions were counted, lesion area measured, and comparison pictures were taken for each hybrid. Resistance and susceptibility can be determined by observing lesion type (A-D in literature), lesion length vs. width, and disease progression up the plant. This is a consistent year-round method for inoculation of CZ-M on corn in greenhouse environments.

GFP-tagging of *Citrus tristeza virus* in citrus trees. A. S. FOLIMONOV, S. Y. Folimonova, and W. O. Dawson. Department of Plant Pathology, CREC, University of Florida, Lake Alfred, FL 33850. Phytopathology 94:S30. Publication no. P-2004-0200-AMA.

Citrus tristeza virus (CTV), a member of the *Closteroviridae*, is an economically important pathogen of citrus. Bipolar particles of CTV contain a 19.5 kb single stranded RNA genome, which encodes at least 12 open reading frames (ORF). Ten ORFs are expressed from 3′ co-terminal subgenomic RNAs. CTV is transmitted by aphids and is limited to phloem-associated cells in infected trees. Several approaches were examined to label the CTV infection in citrus trees, using the green fluorescent protein (GFP) as a reporter, including the construction of self-processing fusions in-frame with the coat protein (CP), the substitution of an existing ORF, and the insertion of an additional gene into the infectious cDNA plasmid. Systemic distribution of CTV in infected seedlings was readily detectable by specific green fluorescence in phloem-associated cells of leaves, stems, roots, and fruit. Like its parental strain, the GFP-expressing CTV produced stem pitting. Application of the GFP-expressing virus for studying the interactions of CTV with citrus trees will be discussed.

Mapping determinants of stem pitting in *Citrus tristeza virus*. S. Y. FOLIMONOVA (1), A. S. Folimonov (1), C. J. Robertson (1), S. M. Garnsey (1), M. E. Hilf (2), and W. O. Dawson (1). (1) University of Florida, Citrus Research and Education Center, Lake Alfred, FL; (2) USDA-ARS-USHRL, Fort Pierce, FL. Phytopathology 94:S30. Publication no. P-2004-0201-AMA.

Citrus tristeza virus (CTV), a type member of the *Closteroviridae*, possesses a single-stranded positive sense RNA genome of 19.5 kb, which encodes at least 12 open reading frames. CTV is limited to phloem-associated cells in citrus trees; in nature CTV is transmitted by aphids in a semi-persistent manner. Severe strains of CTV cause disorders in tree trunk formation that result in stem-pitting (SP) symptoms: decreased fruit yield, size, and quality. In attempt to map SP determinants of CTV, we created hybrid viruses of a SP strain of CTV (T68) and a non-SP strain (T36) by substituting 3′ fragments of T68 into a T36 infectious cDNA clone. The resulting recombinant constructs were passaged in *N. benthamiana* protoplasts to achieve sufficient virus inoculum for infection of citrus seedlings. Subsequent inoculations were made from initial infections by grafting to sweet orange and grapefruit seedlings to assess SP expression.

Biocontrol of silver leaf disease of almond in California. H. FÖRSTER (1), R. A. Duncan (2), D. F. Thompson (1), and J. E. Adaskaveg (1). (1) Dept. Plant Pathology, Univ. of California, Riverside, CA 92521; (2) UCCE, Modesto, CA 95358. Phytopathology 94:S31. Publication no. P-2004-0202-AMA.

Silver leaf disease of *Prunus* species is caused by *Chondrostereum purpureum*. The basidiomycete infects woody tissues through fresh wounds and causes wood decay. Leaves of diseased trees become silvery in appearance due to a toxin produced by the pathogen. Although the fungus occurs on many non-cultivated tree species, and in the past has occasionally been found on temperate tree fruit crops in California, recent outbreaks of the disease caused serious damage in almond orchards in Madera, San Joaquin, and Stanislaus Counties. In management studies, freshly cut branches were inoculated at selected times after spray-treatment with fungicides (myclobutanil, tetraconazole) that were highly effective *in vitro* or with biocontrol agents (*Trichoderma harzianum*, *T. viride*). Treatment efficacy was evaluated after 2 months. Internal wood discoloration was measured and the presence of the pathogen was verified by isolation. Growth rates of the fungus in control branches ranged from 5 to 18 cm/month for summer/fall and spring inoculations, respectively. Tetraconazole had little effect on the disease and the efficacy of myclobutanil was variable, not exceeding 50% control. Treatments with both biocontrol agents were highly effective. When branches were inoculated 14 days after treatment, disease incidence, severity, and recovery of the pathogen were reduced to very low or zero levels. Our studies demonstrate that a highly effective protective treatment is commercially available for the management of silver leaf disease of almond.

Bayer CropScience fungicides – We've got you covered. L. FOUGHT, G. Musson, J. Bloomberg, M. R. Schwartz, and R. Kaiser. Bayer CropScience, Research Triangle Park, NC. Phytopathology 94:S31. Publication no. P-2004-0203-AMA.

The agriculture industry faces a multitude of challenges and opportunities. Today's growers are not just farmers. Each grower practices a variety of professions including small business owner, economist, environmental steward, mechanic, plant physiologist, weed scientist, entomologist, and plant pathologist. Bayer CropScience provides solutions for weed, insect, and fungal pest problems through a diverse portfolio of products for food and fiber crops. Bayer's fungicides protect crops from economic damage caused by fungi from all fungal classes. Resistance in pathogen populations is managed through fungicide programs that include products with single-site and multi-site modes of action, systemic and non-systemic translocation, and other physiochemical properties that ultimately deposit the fungicide at the site of the infection. Bayer CropScience remains committed to the development of tools for growers and the scientific community that provides improvements in agriculture and food quality.

Electron microscopy studies of velvetleaf and corn root colonization by a weed deleterious rhizobacterium. C. L. Foune and R. E. ZDOR. Andrews University, Berrien Springs, MI. Phytopathology 94:S31. Publication no. P-2004-0204-AMA.

The ability of *Pseudomonas putida* ATH2-1RI/9 to colonize velvetleaf roots and reduce plant growth was examined in sterilized soil. Inoculation of velvetleaf resulted in reducing root and shoot weights to 50% of uninoculated plants. Inoculation of corn plants did not cause a reduction in plant growth. As measured by viable cell counts, rhizosphere levels of inoculum were approximately 10^9-10^{10} CFU/g dry root for both plant species. Both scanning and transmission electron microscopy were used to visualize bacteria on the root surface of inoculated plants. Bacteria were distributed unevenly over the upper and lower root surfaces with no apparent tissue damage. Thin sections of velvetleaf root tissue were treated with antibodies generated against *P. putida* ATH2-1RI/9 cells. Labelled cells were only detected within the cortical region of upper root tissue. These microscopy studies confirm the root colonization ability of *P. putida* ATH2-1RI/9 but due to the low frequency of labelled cells found within root tissue suggest that this bacterium is not an endophyte of velvetleaf.

Evolution of QoI resistance in populations of *Mycosphaerella graminicola*. B. A. FRAAIJE and J. A. Lucas. Rothamsted Research, Harpenden, UK. Phytopathology 94:S31. Publication no. P-2004-0205-AMA.

Septoria leaf blotch, caused by *Mycosphaerella graminicola*, is the most important foliar disease of wheat in NW-Europe. Good farming practices, including the use of resistant cultivars, can reduce infection, but fungicides are the main measure for disease control. Due to their persistent broad-spectrum disease control and potential extra yield benefits through increased green canopy duration, Qo inhibitors have become a key component of disease management strategies on cereals. However, in 2002, five years after introduction, control failures were reported for Ireland, and a mutation in cytochrome *b* (G143A) was identified in resistant strains isolated from a fungicide-treated field at Rothamsted. The same mutation was previously detected in resistant wheat powdery mildew populations in N-Germany. Advances in DNA diagnostics now allow routine detection of specific alleles using real-time PCR. Where a clear phenotype-to-genotype relationship exists, the evolution of fungicide resistance at the population level can be measured directly in samples without the need for laborious fungicide sensitivity tests on large numbers of isolates. Retrospective DNA testing of populations present in archived leaf samples revealed the G143A mutation was already present in 2001, albeit at a low frequency of <5%. Using real-time PCR, we are now testing different anti-resistance strategies based on alternation/mixing of fungicides with different modes of action. Samples of spore-traps are also being analysed to establish the role of ascospores in the spread of resistant alleles in populations of *Mycosphaerella graminicola*.

PCR-based systems for the detection, differentiation and quantification of *Xylella fastidiosa* strains. M. FRANCIS (1), E. Civerolo (2), and H. Lin (2). (1) University of California, Davis, CA; (2) USDA-Agricultural Research Service, Parlier, CA. Phytopathology 94:S31. Publication no. P-2004-0206-AMA.

The overall goal of this work is to develop reliable clinical detection systems protocols for identification and quantification of *Xylella fastidiosa* (Xf) strains in naturally-occurring single or mixed infections in different hosts, as well as in insect vectors. Five PCR systems were developed based on the currently available genomic sequences of four Xf strains: Pierce's disease of grapevine (PD), almond leaf scorch (ALS), oleander leaf scorch (OLS) and citrus variegated chlorosis (CVC). One system, based on primer pair HL5/HL6 and a probe labeled with FAM as a fluorescent dye, detected and differentiated three Xf strains (PD, ALS, OLS), and Xf-CVC DNA. Four additional primer systems were developed for standard PCR and Real Time PCR using Taq-Man probes labeled with fluorochromes with different wavelength emissions. Each pair of primers can be used for standard PCR detection or combined with fluorochrome-labeled probes for quantitative assay. These systems were able to specifically differentiate each strain in suspensions containing a mixture of Xf strains (whole bacteria or DNA) and also in DNA extractions from field collected samples.

Association of a disease in *Fraxinus* in Colombia with the presence of phytoplasmas. L. FRANCO-LARA, S. L. Gaitán, J. J. Filgueira, and E. Salcedo. Universidad Militar Nueva Granada, Carrera 11 No. 101-80, Bogota, Colombia. Phytopathology 94:S31. Publication no. P-2004-0207-AMA.

Fraxinus sp (Urapan), an introduced species is common the Colombian Andes between 1800 and 2700 meters of altitude. In Bogota, *Fraxinus* represent about 75% of the tree coverage of the city. In the last decade, symptoms such as yellowing, defoliation, dieback, epicormic growth, tufted foliage, small leaves and deliquescent branching appeared on trees. In Bogota, a recent survey showed that 99% of the trees had symptoms. Colombian *Fraxinus* also sustain heavy populations of *Tropidosteptes chapingoensis* that contribute to their unhealthy condition. In the present work a sample of 100 trees from Bogota was studied: symptoms were recorded and plants tested by DAPI, PCR and dot blot analyses. All of the sampled trees showed at least two of the symptoms mentioned above. Nested PCR tests on leaf extracts with primers P1/Tint followed by R16F2/R2 were positive for 22% of the samples. Of these, 10 were also positive by DAPI and dot blotting using a probe against the 16SrRNA gene of the phytoplasma Ash yellows. The amplification products of two samples of the above mentioned nested PCR were sequenced. A fragment of 520 base pairs was BLASTed to GenBank and 94% of homology was found with six accessions corresponding to the 16SrRNA region of Ash yellows phytoplasma. Considering that the observed symptoms resemble those of the phytoplasmal disease Ash yellows in North American *Fraxinus* and the fact that phytoplasmas are detected in Colombian *Fraxinus*, an association has been established between the presence of phytoplasmas and the disease of *Fraxinus* in Colombia.

Evaluation of insecticide applications to reduce corn leafhopper populations and corn stunt disease. C. A. FRAZIER (1), C. Summers (2), S. M. Mueller (1), C. Collar (1), and A. Newton (2). (1) University of California Cooperative Extension, Division of Agriculture and Natural Resources, Oakland, CA; (2) University of California, Davis, CA. Phytopathology 94:S31. Publication no. P-2004-0208-AMA.

Corn stunt disease, caused by *Spiroplasma kunkelii* and vectored by the corn leafhopper (*Dalbulus maidis*), has become a significant problem in silage corn production in the southern portion of the San Joaquin Valley, California, since 1996. In large, replicated field trials, systemic seed treatments (imidacloprid in 2002 and clothianidin in 2003) reduced leafhopper numbers by about 50% compared to control plots. Disease incidence, based on symptom expression,

was significantly reduced in each trial but not as much as leafhopper populations. Yields in the seed treatments were not significantly higher than the control treatment. In three large-scale replicated field trials in 2003, foliar pyrethroid insecticides applied after leafhopper populations were established were more effective than organophosphates in reducing leafhopper counts. Disease incidence was not significantly reduced in any of these foliar trials. However in two of the trials, insecticide treatments as a group had higher yields ($P = 0.02$ and $P = 0.06$) than the control with increases of 1.0 and 0.6 tons per acre (30% dry matter basis) respectively.

Reciprocal monophyly of Potyvirus and Rymovirus genera, family Potyviridae, is supported by genome sequences of *Agropyron mosaic virus* and *Hordeum mosaic virus*. R. FRENCH and D. C. Stenger. USDA-ARS, University of Nebraska, Lincoln, NE. Phytopathology 94:S32. Publication no. P-2004-0209-AMA.

Assignment of mite-transmitted virus species to the genus *Rymovirus*, family *Potyviridae* has changed several times, and the status of the genus has been questioned. To address this issue, complete nucleotide sequences of *Agropyron mosaic virus* (AgMV) and *Hordeum mosaic virus* (HoMV) were determined. AgMV (9540 nt) and HoMV (9463 nt) each encode a single polyprotein with predicted cleavage products typical of potyviruses, and share 68.5% (nt) and 71.6% (aa) sequence identity. Both are equidistant (53% nt, 49% aa sequence identity) from a third rymovirus, *Ryegrass mosaic virus* (RGMV). Phylogenetic analyses by neighbor joining, maximum parsimony, and Bayesian inference each group the three *Rymovirus* species in an exclusive clade with a most recent common ancestor basal to a clade containing 34 species of the genus *Potyvirus*. Because AgMV, HoMV, and RGMV share a reciprocal monophyletic relationship with species of the genus *Potyvirus*, and are divergent in sequence and type of vector, the genus *Rymovirus* remains to be a useful taxonomic unit within the family *Potyviridae*.

Mapping of a major net blotch resistance gene in the Q21861 X SM89010 doubled haploid population. T. L. FRIESEN (1), J. D. Faris (1), and B. J. Steffenson (2). (1) USDA-ARS Northern Crop Science Laboratory, Fargo, ND; (2) University of Minnesota, St. Paul, MN. Phytopathology 94:S32. Publication no. P-2004-0210-AMA.

Pyrenophora teres Drechs. *F. teres* Smedeg causal agent of net blotch on barley is an economically important pathogen worldwide. The objective of this study was to evaluate resistance to net blotch in segregating barley populations and identify molecular markers linked to major resistance genes. The Q21861 X SM89010 doubled haploid population consisting of 125 barley lines was used to evaluate the genetics of resistance. The barley population was inoculated with two *P. teres* isolates (15A and 0-1) which differ in virulence based on a barley differential set. Q21861 was highly susceptible and SM89010 was highly resistant to both pathotypes. Phenotypic analysis showed that the same resistance locus was effective against both pathotypes. The population segregated in a 79:46 resistant: susceptible ratio which is significantly different than the expected 1:1 segregation. AFLPs and SSRs along with bulked segregant analysis were used to identify markers associated with resistance. Markers flanking a single major resistance locus were identified, and these markers also showed skewed segregation indicating that the single major resistance gene lies within a genomic region of skewed segregation.

Distributional dynamics of *Verticillium dahliae* in potato plant tissue through the growing season. K. E. FRENT (1), D. I. Rouse (1), and S. H. Jansky (2). (1) University of Wisconsin, Dept. of Plant Pathology; (2) Dept. of Horticulture, Madison, WI 53706. Phytopathology 94:S32. Publication no. P-2004-0211-AMA.

To better understand the relationships that occur between stem colonization of *Verticillium dahliae* (Vd) and disease in potato, there is a need to quantify the colonization of the pathogen within host tissue. Methods commonly used to quantify Vd populations in potato rely on plating onto Vd selective medium either (1) sap squeezed from the base of a fresh stem sample or (2) a stem sample that has been dried and milled. In both methods, fungal populations are then counted and expressed in terms of colony forming units (cfu) per unit volume of sap or per unit weight of dried stem. The above methods were used to quantitatively examine the variability and distribution of Vd populations in individual potato stems within a plant of a given genotype through time. Our host population consisted of clones from the Wisconsin potato breeding program and two clones highly resistant to Vd. Overall, the methods correlated (0.255, $P < 0.001$, n = 892), but there was high within-plant stem-to-stem variation at any given time point. We found that Vd populations are log-normally distributed within a clone, and their mean and variance change through time. As a result, small sample size and sample bulking can bias the estimation of means and variances, which is misleading for the breeder. Eight to 25 individual stems are needed to estimate the variance with 90% confidence. In addition, important information useful for describing disease progression in a clone can be lost due to unsatisfactory sampling.

The role of symbiotic genes in *Rhizobium* biofilm formation. N. A. FUJISHIGE, N. N. Kapadia, and A. M. Hirsch. University of California, Los Angeles, CA. Phytopathology 94:S32. Publication no. P-2004-0212-AMA.

Rhizobia are nitrogen-fixing soil bacteria that establish symbiotic relationships with legume plants; rhizobia provide ammonia to the plant in exchange for sugar and housing within specialized organs, called root nodules. Although the symbiosis is well known, relatively little has been done to understand how rhizobia exist under non-symbiotic conditions. In their natural habitats, microbes attach to surfaces and assemble into structured communities, called biofilms. Our goal is to characterize factors involved in rhizobial attachment and aggregation during biofilm formation and the infection phase of symbiosis. These two different lifestyles share common features, including requirements for attachment and aggregation, leading us to hypothesize that many genes involved in establishing biofilms are also involved in establishing infections on legume roots. Biofilms, which are much simpler to analyze than root infections, will serve as a model to study legume root colonization. We show that many symbiotic genes, especially certain nodulation (*nod*) genes, are critical for biofilm formation. We will discuss how *nod* genes might be involved in biofilm formation.

Association of pericarp color, plant color and grain hardness with fungal colonization and susceptibility to grain mold. D. L. FUNNELL and J. F. Pedersen. USDA-ARS, Wheat, Sorghum and Forage Research, University of Nebraska, Lincoln, NE. Phytopathology 94:S32. Publication no. P-2004-0213-AMA.

Sorghum lines with a tan necrotic wound response that produce white grain yield desirable consumer products, but plants with these traits can be more susceptible to pathogens. We previously have reported the favorable vigor and yields of lines near isogenic for plant (purple or tan) and pericarp (red or white) color. All lines had seed of medium hardness, and variations within this range were not associated with plant or pericarp color or with pathogen susceptibility. Enumeration of fungi from seed collected from field grown plants revealed that colonization by *Alternaria* was approximately 20% higher in red seed. When enriching for *Fusarium* spp., it appeared that field-grown white seed may be colonized by *Fusarium verticillioides* to a greater extent than red seed. Controlled inoculation assays with a pathogenic *F. verticillioides* isolate indicated that lesion length was highly significantly greater in purple lines when compared with all tan lines, and purple lines with red seed had significantly greater lesions when compared with the other 3 color combinations. Among tan lines, there were no significant differences in lesion length between red and white pericarp lines.

Effects of sorghum cultivars with different levels of sorgoleone production, and wheat rotation, on soil microorganism communities. D. L. FUNNELL and J. F. Pedersen. USDA-ARS, Wheat, Sorghum and Forage Research, University of Nebraska, Lincoln, NE. Phytopathology 94:S32. Publication no. P-2004-0214-AMA.

Sorghum produces sorgoleone, a toxic compound exuded from the roots with allelopathic properties. Cultivar 'Redlan' (a high sorgoleone producer) and cultivar 'RTx433' (a low sorgoleone producer) are being used in *in vitro* rotations with the wheat cultivar 'Lewjain' to assess possible affects on soil microorganism populations. Using soil collected from a nearby fallow sorghum field, the wheat and 2 sorghum cultivars were planted in the greenhouse for 4 cycles of 4 weeks each then rotated. During the first cycle, fungal and bacterial populations increased, with the greatest increases occurring in soils planted with wheat or with sorghum cultivar RTx433. Wet weight of aerial plant parts declined through the 4 cycles but those of sorghum cultivar RTx433 increased following rotation from wheat. Numbers of fluorescent pseudomonads increased following planting with wheat and sorghum over those found in fallow soil but were variable in following cycles. However, numbers of fluorescent pseudomonads were consistently highest in soil planted with sorghum cultivar RTx433. Characteristics of fluorescent *Pseudomonas* species will be assessed for potential antimicrobial activity.

Culturable biofilms isolated from 'Jewel' sweetpotato with antagonism to *Rhizopus stolonifer* in vitro. A. E. Fyffe (1), C. STEVENS (1), V. A. Khan (1), C. L. Wilson (2), and J. W. Williams (3). (1) Dept. Agr. Sci. Tuskegee Univ., AL 36088; (2) USDA-ARS Appalachian Fruit Research Station Kearneysville, WV 25430; (3) Dept. of Biology Tuskegee Univ., AL 36088. Phytopathology 94:S32. Publication no. P-2004-0215-AMA.

Culturable biofilms were isolated on nutrient yeast dextrose agar (NYDA) and potato dextrose agar (PDA) by direct impression on 'Jewel' sweetpotato storage roots, this revealed the presence of amorphous biofilms-like particles,

and culturable bacteria-like biofilms within 24 hours on PDA and NYDA, with different colony morphological types identified within 12 days, were observed using stereo microscope image digitized by a Kodak Microscopy Documentation System. Each of the colony morphotypes contained a mixture of solitary and biofilm bacteria, were streaked out on both media, while yeast (*Rhodotorula* spp.) and Trichoderma isolates only grew on PDA. Culturable biofilm bacteria isolated from these morphotypes was identified mainly as *Bacillus cereus* from storage roots disk washate method. Antagonists of the microflora present on the root surfaces, played an important role in suppression of *R. stolonifer*. Simultaneous growth of *R. stolonifer* and bacterial biofilms, suppressed and deteriorated aerial hyphae growth of *R. stolonifer* on NYDA. The suppression of aerial hyphae growth and disintegration of *R. stolonifer* by Trichoderma isolates were also apparent on PDA. Results revealed that suppression of aerial hyphae growth of *R. stolonifer* occurred as a result of mycoparasitism of Trichoderma isolates.

Preliminary studies of signaling and sporulation in *Uncinula necator*. D. M. Gadoury (1), L. M. WAKEFIELD (1), R. C. Seem (1), L. Cadle-Davidson, and I. B. Dry (2). (1) Cornell University, NYSAES, Geneva, NY 14456; (2) CSIRO, Glen Osmond, SA 5064 Australia. Phytopathology 94:S33. Publication no. P-2004-0216-AMA.

Initiation of cleistothecia by *U. necator* results in near-total cessation of asexual sporulation atypical of source-sink relationships. Conidiophore production ceased 48 hr after hyphal contact between compatible mating types, and conidiation ceased on extant conidiophores at places remote from the site of ascocarp initiation. In nascent mildew colonies, the sequence and nature of morphological changes strongly implies involvement of a biochemical signal, promulgated within the mycelium, that operates to generate conidiophores synchronously. Normal condiation occurred after micro-surgical excision of the colony center on day 5 after inoculation, but not day 4, which essentially seemed to reset a developmental clock. Timing of signal promulgation was directly proportional to density of inoculation. Preliminary biochemical and genetic studies of signaling in this system will be described.

A novel secretory ubiquitin extension protein from the soybean cyst nematode. B. [illegible]O (1), R. Allen (1), E. Davis (2), T. Baum (3), and R. Hussey (1). (1) Dept. of Plant Pathology, (1) University of Georgia, Athens, GA 30602; (2) North Carolina State University, Raleigh, NC 27695; (3) Iowa State University, Ames, IA 50011. Phytopathology 94:S33. Publication no. P-2004-0217-AMA.

Parasitism genes expressed in the esophageal gland cells have a central role in nematode parasitism of plant. A gene encoding a novel secretory ubiquitin (UBQ) extension protein was isolated from an esophageal gland-specific library from the soybean cyst nematode (SCN) and was strongly expressed in the dorsal gland. The deduced UBQ protein sequence has several novel features including a signal peptide, an UBQ domain identical to soybean UBQ, and a novel 19 amino acid extension peptide. Antibodies specific for the extension peptide immunolocalized the extension protein in the dorsal gland cell and detected the peptide in stylet secretions on a Western blot. We hypothesis that the novel UBQ might display a chaperone-like function to delivery a SCN signal molecule to parasitzed soybean root cells for a critical role in disease development.

Differential gene expression in *Ustilago maydis*. M. D. GARCIA-PEDRAJAS, D. L. Andrews, and S. E. Gold. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274. Phytopathology 94:S33. Publication no. P-2004-0218-AMA.

Ustilago maydis displays dimorphic growth alternating between a budding haploid and a filamentous dikaryon formed by the fusion of two haploid cells. This transition in morphology coincides with a change from saprophytic to pathogenic development; only the filamentous dikaryon is pathogenic on maize. Previously we identified a role for the cAMP signal transduction pathway in dimorphism and pathogenicity. In the present work we used suppression subtractive hybridization PCR (SSHP) to produce cDNA libraries enriched for genes upregulated in either budding or filamentous cells to facilitate identification of novel genes involved in dimorphism. Screening of these libraries led to the identification of a total of 63 genes, 26 upregulated during filamentous growth and 37 upregulated in budding cells, the majority of which had not previously been reported in *U. maydis*. Differential expression in budding versus filamentous cells was confirmed by northern blot analysis. We are now involved in the optimization and production of deletion mutants of some of the highly differential genes. The effect of these mutations on morphology and/or pathogenicity will be discussed.

Molecular genetics of Fusarium head blight susceptibility associated with chromosome 2A of wild emmer. D. F. Garvin (1) and R. W. STACK (2). (1) USDA-ARS, Univ. of Minnesota, St. Paul, MN; (2) North Dakota State Univ., Fargo, ND. Phytopathology 94:S33. Publication no. P-2004-0219-AMA.

The LDN(DIC) set of disomic substitution lines, in which chomosomes of durum wheat cv. Langdon (LDN) are replaced by corresponding ones from the wild emmer "Israel A", have been useful in locating genes for resistance to Fusarium head blight (FHB). The line LDN(DIC-2A) shows increased FHB, suggesting the presence of a susceptibility factor. A population of 95 recombinant inbred lines (RICL) from the cross LDN(DIC-2A) × LDN was screened for FHB resistance. DNA from the parents was isolated and screened with microsatellite markers to identify polymorphic loci. The FHB disease index of the RICL ranged from 18 to 95, with a mean of 61. The disease index means for LDN and LDN(DIC-2A) were approximately 45 and 80, respectively. Molecular markers spanning over 100 cM of chromosome 2A were scored in the RICLs. QTL analysis revealed at least one region of chromosome 2A that was associated with increased susceptibility to FHB. Disease index means for individuals with alternate alleles at the microsatellite marker most strongly associated with FHB susceptibility differed significantly ($P = 0.01$) (71 vs 54).

Effects of sub-lethal fungicide doses on the incidence of post-emergence damping-off of seedlings caused by *Pythium*. C. D. GARZON, J. E. Molineros, and G. W. Moorman. The Pennsylvania State University, University Park, PA. Phytopathology 94:S33. Publication no. P-2004-0220-AMA.

Fungicide resistance in plant pathogenic *Pythium* species is a serious threat to greenhouse crops irrigated by re-circulating systems. Dilution of fungicides in irrigation solutions exposes pathogens to sublethal doses with unknown effects. A greenhouse grower reported that Pythium root rot incidence increased after application of fungicide. In addition, isolates with resistance to mefenoxam and/or propamocarb have been observed to grow faster in media amended with fungicide than on fungicide-free media. These observations raised the question as to whether exposure of isolates with resistance to fungicides at sub-lethal doses of such chemicals may have a stimulating effect on the pathogen, inducing an increase in disease severity or rate of disease development. To test this hypothesis, geranium seedlings were treated with fungicide solutions at four concentrations and inoculated with two *Pythium* spp. Isolates, one resistant and one sensitive to mefenoxam and/or propamocarb, or not inoculated. Area under the disease progress curve was calculated and compared among treatments. Preliminary experiments suggested that sub-lethal doses of fungicides may induce increased disease severity caused by resistant and intermediate resistant isolates under conducive conditions. Our results using a statistically representative population and the importance of maintaining recommended fungicide concentrations in the management of Pythium diseases in greenhouse crops will be discussed.

Managing resistance risks with more complementary chemistry for plant disease control. R. GEDDENS, C. Shepherd, and S. Soehner. DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE. Phytopathology 94:S33. Publication no. P-2004-0221-AMA.

Tanos™ is a new fungicide from DuPont introduced in the U.S. in 2004 for use in potatoes, tomatoes and vegetables. Tanos™ contains the active ingredients Famoxate™ (famoxadone) and Curzate™ (cymoxanil). Famoxate™ is a novel oxazolidinedione fungicide that inhibits energy production in plant pathogenic fungi and is highly active against spore germination and mycelial growth. Famoxate™ is classified as a Qo complex III respiration inhibitor considered at risk for resistance development. For this reason, DuPont requires Tanos™ use both in combination and alternation with companion fungicides with a different target site. DuPont also limits the number of Tanos™ applications as recommended by the global Fungicide Resistance Action Committee (FRAC). Tanos™ programs are reliable and effective because they combine complementary fungicides to optimize disease control as well as reduce the risk of resistance development.

Inoculum sources of *Xanthomonas axonopodis* pv. *allii* in Colorado. D. H. GENT, H. F. Schwartz, and C. A. Ishimaru. Dept. of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO. Phytopathology 94:S33. Publication no. P-2004-0222-AMA.

Xanthomonas leaf blight, caused by *Xanthomonas axonopodis* pv. *allii* (Xaa), is an emerging disease of onion worldwide, but inoculum sources of Xaa are largely unknown. Weeds, alternate hosts, volunteer onion, irrigation water, and crop debris were sampled for Xaa in Colorado in 2003 and 2004. Epiphytic populations of Xaa on weeds and leguminous crops were quantified by leaf rinsing and subsequent plating onto modified MXP medium. Populations of the spontaneous rifampcin mutant Xaa strain RO177 were monitored in irrigation water and crop debris. Pathogenic Xaa, either naturally occurring or strain RO177, were recovered from all sources evaluated, but were not found in onion growing regions of Colorado free from Xanthomonas leaf blight the previous year. Xaa populations were maintained throughout the winter in infected leaves on the soil surface, but decreased over 100,000 fold in buried

leaves. Field studies are ongoing to further quantify Xaa survival and dissemination, and their implications for Xanthomonas leaf blight management.

Managing *Iris yellow spot virus* of onion with cultural practices, host genotype, and novel chemical treatments. D. H. GENT, H. F. Schwartz, and R. Khosla. Colorado State University. Phytopathology 94:S34. Publication no. P-2004-0223-AMA.

Iris yellow spot tospovirus (IYSV) is an emerging threat to onion worldwide and an immediate threat to sustainable and profitable production in the western U.S. No management strategies have been developed for IYSV. Field studies were conducted to determine the individual and combined effects of cultural practices, host genotype, and novel chemical treatments on disease incidence, severity, and onion yield and grade. Plant density was strongly associated with disease incidence with a moderately resistant cultivar, but not with two susceptible cultivars. Disease incidence among 43 cultivars varied from 16 to 100 percent; red market class cultivars were uniformly susceptible to IYSV. Acibenzolar-S-methyl and imidacloprid reduced disease incidence 34 and 38 percent, respectively, with a corresponding 34 and 38 percent increase in jumbo grade bulbs. A multi-faceted management approach appears essential for effective IYSV management.

Managing Xanthomonas leaf blight of onion with copper bactericides and acibenzolar. D. H. GENT and H. F. Schwartz. Dept. of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO. Phytopathology 94:S34. Publication no. P-2004-0224-AMA.

Xanthomonas leaf blight, caused by *Xanthomonas axonopodis* pv. *allii* (Xaa), is a potentially devastating disease in several onion production regions of the world, but few management strategies have been developed. Laboratory and field studies evaluated the ability of copper bactericides and acibenzolar (Actigard) to reduce *in planta* bacterial multiplication and Xanthomonas leaf blight severity. Xaa multiplication *in planta* was not significantly different between copper bactericides and acibenzolar treatments. In field studies, two or four applications of acibenzolar were as or more effective than 10 weekly sprays of copper hydroxide alone or in combination with mancozeb, reducing disease severity as much as 46% compared to copper hydroxide/mancozeb tank-mixes. In the absence of disease, however, acibenzolar reduced total yield as much as 17% as compared to copper hydroxide. Careful use of acibenzolar should reduce traditional copper bactericide reliance and improve suppression of Xanthomonas leaf blight.

Drip applied soil fumigants for calla lily production. J. SERIK (1) and I. D. Greene (2). (1) USDA-ARS, Parlier, CA 93648; (2) Golden State Bulb Growers, Moss Landing, CA 95039. Phytopathology 94:S34. Publication no. P-2004-0225-AMA.

A trial was established in Marina, CA during the spring of 2002 to test alternatives to methyl bromide for calla lily production. The pest targets were weeds and soilborne pathogens. Chemicals were applied through the drip irrigation tape in either 33mm or 67mm of water. Chemical treatments included chloropicrin, furfural + metham sodium, sodium azide, iodomethane + chloropicrin, 1, 3-dichloropropene and 1, 3-dichloropropene + chloropicrin and a water control. Data pertaining to weed control and pathogen populations were previously reported. During the summer of 2003, flower counts indicated that treatments containing chloropicrin produced the healthiest plants. Disease ratings and plant height measurements during this same period reaffirmed the benefits of chloropicrin. Calla rhizomes were harvested during the fall of 2003 and the bulbs were graded according to size and salability. Again, treatments containing chloropicrin as well as metham sodium produced the greatest yield and dollar value. Results indicate that a successful calla lily crop may be produced with chloropicrin fumigation in combination with one or more additional fumigants.

Pathogenicity, mitochondrial DNA RFLPs, and colony morphology comparisons of isolates of *Fusarium virguliforme* and *F. tucumaniae* capable of causing SDS of soybean. S. L. GIAMMARIA, M. L. Rosso, J. C. Correll, and J. C. Rupe. Department of Plant Pathology, University of Arkansas, Fayetteville, AR. Phytopathology 94:S34. Publication no. P-2004-0226-AMA.

Sudden death syndrome (SDS) is a recurrent problem in soybean growing areas of South America and the US. Recent reports have concluded that SDS is caused by two different species within the *"Fusarium solani"* complex, *Fusarium virguliforme* and *F. tucumaniae* (syn. *F. solani* f. sp. *glycines*) (Aoki et al. 2003). The objective of this study was to compare ten representative isolates of each species for pathogenicity on soybean and greenbean, mtDNA RFLPs, colony morphology, and growth rate. The isolates examined included 10 of *F. tucumaniae* from South America and 10 of *F. virguliforme* from the US. Pathogenicity tests were performed on the green bean cv. Top Crop and the SDS-susceptible soybean cv. Essex by scoring for root-rot and SDS-foliar symptoms. Significant differences in aggressiveness were measured for foliar SDS symptoms within each species. Conversely, no significant differences in aggressiveness were measured for root-rot. All SDS isolates failed to cause disease on the green bean cultivar. Minor differences in pigmentation and growth rate were observed among US isolates of *F. virguliforme*, while a considerable variation in pigmentation and growth rates were observed among the isolates of *F. tucumaniae*. MtDNA RFLP profiles were different between species while similar within each species. The results provide further support of the genetic differences between the two taxa capable of causing SDS symptoms.

Distribution of *Xanthomonas campestris* pv. *pelargonii* after leaf surface inoculation of geranium. C. Giannetti and M. A. SULZINSKI. University of Scranton, Scranton, PA. Phytopathology 94:S34. Publication no. P-2004-0227-AMA.

Known quantities of a rifampicin resistant strain of *Xanthomonas campestris* pv. *pelargonii* (*Xcp*) were inoculated to either the leaf surface or cut petiole of geraniums (*Pelargonium* spp.). Plants were incubated for 8 weeks in a growth chamber under conditions conducive for bacterial replication and symptom development for bacterial wilt disease. Inoculated and non-inoculated leaves were then collected, and extracts were plated for viability testing on YDC agar containing rifampicin. Likewise, plant tissues were extracted and underwent PCR amplification using previously described primer pairs specific for *Xcp* DNA and for the geranium 18s rRNA gene. Plants on which *Xcp* was inoculated to the leaf surface appeared healthy throughout the 8 week incubation. Viability testing showed no significant spread of *Xcp* from initially inoculated leaves, and PCR analysis showed no detectable movement of *Xcp* from inoculated leaves. Mock inoculated plants continued to appear healthy and yielded no detectable *Xcp* by either viability testing or PCR. Control plants that were petiole inoculated with *Xcp* showed systemic symptoms of bacterial wilt and *Xcp* was shown to be widely distributed, both by viability testing and by PCR. These results suggest that even under optimum conditions for bacterial replication and movement, the mere presence of *Xcp* on the phylloplane does not necessarily portend that the bacteria will gain entry to reproduce or to cause disease in the plant.

Development of a grid RT-PCR method for detection of *Peanut mottle* (PeMV) and *Peanut stripe viruses* (PStV) in peanut germ plasm. A. G. GILLASPIE, M. L. Wang, D. L. Pinnow, and R. N. Pittman. USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, GA 30223. Phytopathology 94:S34. Publication no. P-2004-0228-AMA.

A high-throughput method, using three dimensional pooling in a 96-well format, has been developed for detection of PeMV and PStV from peanut seed lots. Samples from seeds and leaves were pooled by rows and columns and extracted by Qiagen miniprep method for total RNA. The plate-pooled sample was a proportional sample from all rows and columns. Plate-pooled samples testing negative by RT-PCR were assumed free of virus requiring no further testing. The pooled-row and –column samples were tested when the plate-pooled sample was positive so that virus-infected samples could be located in the grid at the intersect of the row and the column. The results from RT-PCR are consistent with the results from individual seed samples tested by DAS-ELISA. Similar results were obtained with leaf tissue. The results obtained by RT-PCR and DAS-ELISA reflect the symptoms observed in the seedlings arising from the tested seeds. The method is sensitive enough to detect and select one infected seed among the 96 plate-pooled samples. The method will be useful for testing newly introduced seed lots.

A lognormal distribution of phytopathogenic bacteria in corn, cowpea, tomato and watermelon seeds. R. D. GAITIS (1), R. R. Walcott (2), F. H. Sanders (1), and C. C. Block (3). (1) University of Georgia, Tifton, GA; (2) University of Georgia, Athens, GA; (3) USDA-ARS, Iowa State University-Plant Introduction Station, Ames, IA. Phytopathology 94:S34. Publication no. P-2004-0229-AMA.

Numbers of colony-forming units (CFU) of *Xanthomonas axonopodis* pv. *vignicola*, *Clavibacter michiganensis* subsp. *michiganensis* and *Acidovorax avenae* subsp. *citrulli* were counted on MXP, CNS, and WFB 68 semi-selective media, respectively, from single seeds of cowpea (*Vigna unguiculata*), tomato (*Lycopersicon esculentum*) and watermelon (*Citrullus lanatus*) washed in phosphate-buffered saline (1 seed ml^{-1}). Bacterial populations ranged from 10 CFU $seed^{-1}$ to 1.3×10^6 CFU $seed^{-1}$. Cumulative probability plots of CFU $seed^{-1}$ produced substantial curves with raw data, but a log-transformation demonstrated a linear relationship. Evidence of bacteria distributed lognormally also was obtained from ELISA assays for *Pantoea stewartii* subsp. *stewartii* by comparing absorbance values obtained from individual corn (*Zea mays*) seed to standard curves created from a *P.s. stewartii* dilution series. A lognormal distribution of bacteria in infected seeds has implications for determining sample sizes for seed health testing.

Taxonomic diversity of Erysiphales (powdery mildew fungi) in the Pacific Northwest. D. A. GLAWE. Plant Pathologist, Puyallup Research and Extension Center, Washington State University, 7612 Pioneer Way East, Puyallup, WA 98371-4998. Phytopathology 94:S35. Publication no. P-2004-0230-AMA.

Powdery mildews caused by species of Erysiphales (Fungi, Ascomycetes) rank among the world's most damaging plant diseases. As part of a taxonomic study of Erysiphales in the Pacific Northwest (AK, BC, ID, MT, OR, and WA), in 2002-2003 1200 specimens of powdery mildew fungi were collected from 64 host families. Specimens included 141 new host species records for the region, distributed among 53 host families. Families with 4 or more new host species records included (followed by number of new host records): Asteraceae (25), Lamiaceae (12), Rosaceae (9), Berberidaceae (5), Cucurbitaceae (5), Ericaceae (5), Fabaceae (5), Boraginaceae (4), Brassicaceae (4), and Papaveraceae (4). Preliminary taxonomic assessment of the specimens and comparison with host-fungus indices suggests that approximately 150 species of Erysiphales may occur in the region, about 7 times as many as reported previously. These findings, and comparison of taxonomic literature for North American species with recent taxonomic work in Asia and Europe, suggest that nearly all of the names traditionally used for powdery mildew fungi in the region need revision.

The biotechnological method against weeds and illegal sowing of *Papaver somniferum* L. L. A. GLUKHOVA, A. A. Abdukarimov, and I. A. Orlova. Institute of Genetics & Plant Experimental Biology, Uzbek Academy of Sciences of Uzbekistan Republic. Phytopathology 94:S35. Publication no. P-2004-0231-AMA.

Drug addiction for a long time became the world disaster. The method of the biocontrol of opium poppy, *Papaver somniferum* L. by using microorganisms with raised virulence has been developed. The biotechnological semi-industrial method of preparation *Pleospora papaveracea* (*De Not.*) *Sacc.* includes: reisolation of pathogen from infected plants of poppy; purification of monoclones; preparation of mycelium in liquid media, collecting and drying of pathogen spores. On the basis of this technology together with the UN expert Dr. M. Greaves, composition of mycoherbicides, which is highly effective for the biocontrol of illegal crops of opium poppy in natural conditions has been created. Address: Yukori – Yuz, Kibray district, Tashkent, 702151, Uzbekistan, E – mail: ludag@tkt.uz, Phone: (011998712)646426 (h), (011998712)647646 (w); fax: (011998712)642230.

An eradication strategy for *Phytophthora ramorum* in Oregon coastal forests. E. Goheen (1), E. Hansen (2), A. Kanaskie (3), M. McWilliams (3), N. OSTERBAUER (4), W. Sutton (2), and L. Rehms (4). (1) USDA Forest Service, Central Point, OR; (2) Oregon State University, Corvallis; (3) Oregon Dept. of Forestry, Salem; (4) Oregon Dept. of Agriculture, Salem. Phytopathology 94:S35. Publication no. P-2004-0232-AMA.

A multi-agency task force has been trying to eradicate *Phytophthora ramorum* in Oregon's coastal forests near Brookings (Curry Co.) since finding the pathogen there in 2001. In 2001 and 2002, a total of 21 sites (19.4 ha) containing infected tan oaks, rhododendrons and huckleberries were treated in an attempt to eradicate *P. ramorum*. Treatment consisted of cutting and burning all host plants on each site and within a 15-m buffer of non-symptomatic hosts. The sites were monitored for *P. ramorum* periodically post-treatment. Surveys within the sites showed that *P. ramorum* was surviving at a low level (1.1% of all samples) within 12 of the sites, mainly on sprouts from infected tan oak stumps. Surveys around these sites suggest *P. ramorum* may have moved from these sites into neighboring susceptible hosts. On other sites, the treatment appeared to eliminate *P. ramorum*. We will modify our treatment strategy to include the prevention of sprouting from all host stumps within the treated sites.

Population history of *Phytophthora infestans* inferred from nuclear and mitochondrial DNA sequences. L. GOMEZ, J. Thorne, I. Carbone, and J. B. Ristaino. North Carolina State University, Raleigh, NC. Phytopathology 94:S35. Publication no. P-2004-0233-AMA.

Nuclear and mitochondrial DNA variability was used to examine the population history of *P. infestans*. DNA sequence data from three nuclear regions and two mitochondrial regions were obtained from seventy-seven isolates collected from various locations including Brazil, Bolivia, Ecuador, Peru, Costa Rica, Mexico, USA, and Ireland. Gene genealogies were constructed for the Ras, Intron Ras, and Beta-tubulin nuclear regions as well as for the mitochondrial P4 (*cox 1*) and P3 (*rp114*, *rp15*, and *tRNAs*) regions. Ras, Intron Ras and Beta-tubulin genealogies consisted of six, three and two haplotypes, respectively. For P4 and P3 regions two main haplotypes were resolved corresponding to the I and II mtDNA haplotypes described elsewhere. Population subdivision tests showed significant genetic differentiation between the Bolivia-Brazil subpopulation and the Mexico-Costa Rica subpopulation (P = 0.0000), the Peru-Ecuador subpopulation (P = 0.0000), and the USA-Ireland subpopulation (P = 0.0080). Neutrality tests indicated that the Ras locus could be under selection or that population growth or genetic hitchhiking occurs. Private haplotypes were found in Mexican and Ecuadorian populations. Five Ecuadorian isolates from *Solanum tetrapetalum* were highly polymorphic within the regions analyzed and may be a new species. Peruvian, USA and Irish populations shared the same haplotypes across the nuclear and mitochondrial regions suggesting a common ancestry among these populations.

Spatial pattern of citrus tristeza in Tamaulipas, Mexico. C. Góngora-Canul (1), P. RIVAS-VALENCIA (1), N. Ruiz-García (1), E. Loeza-Kuk (1), G. Mora-Aguilera (1), D. Ochoa-Martínez (1), M. A. Gutiérrez-Espinosa (2), and R. Álvarez-Ramos (3). (1) Instituto de Fitosanidad, Colegio de Postgraduados; (2) Instituto de Recursos Genéticos y Productividad, Colegio de Postgraduados; (3) Comité para el Fomento e Investigación Citrícola del Estado de Tamaulipas, Campo Exp. "Gral. Fco. Villa". Phytopathology 94:S35. Publication no. P-2004-0234-AMA.

The spatial pattern of CTV dispersion was studied in two orchards of Valencia orange in Mante and Llera counties of Tamaulipas, Mexico in January, 2004 with the purpose of assisting in the sampling design for CTV detection in eradication programs. The restrictive sampling scheme was a data set of 15 rows per 100 trees and 9 rows per 49 trees, respectively. The CTV detection was made by direct ELISA and inmunoprinting. A 102/1500 and 10/441 of incidence were detected in Mante and Llera, respectively. The data matrix was analyzed with the spatial autocorrelation technique using LCOR ver. 1.3C. The spatial pattern in Mante Orchard was aggregated, the direction of the spread was North East–South West, continuous, and positively correlated (P = 0.01) three trees away across rows (24 m) and also discontinuous, 8, 9 and 10 trees away across rows or 80 m (P = 0.01). The spread direction was also South East – North West, continuous, one tree within row or 6 m (P = 0.01) and discontinuous 4 and 5 trees away within row (30 m) (P = 0.01). The maximum spread in this direction was 94 trees away within row (564 m) (P = 0.01). In Llera Orchard CTV spread was in the North East-South West direction, discontinuous, five trees away across rows (40 m) (P = 0.05), and South East - North West, discontinuous, nine trees away within row or 72 m (P = 0.05), with maximum of 32 trees away within row (256 m) (P = 0.01). The longer distance of spreads both in trees across rows and within row was for the orchard in Mante as well as the higher strength of spatial dependence. The aggregated pattern is attributed to an aphid(s) species other than T. citricida, which has not been detected in the region. The apparent wind effect on aphids dispersal was the same in both orchards.

PCR detection of citrus greening bacterium (CGB) disease in commercial citrus species by addition of sodium sulphite in DNA extraction. K. GOPAL (1), M. K. Reddy (2), G. Prasadbabu (1), V. K. Baranwal (3), S. Khayum (1), C. Phaneendra (1), V. Gopi (4), and C. Palanivel (4). (1) ICRP on Tropical Fruits, ANGR Agricultural University, Tirupati-517 502, A.P., INDIA; (2) IIHR, Bangalore-89, INDIA; (3) Plant Virology Unit, IARI, New Delhi-12; (4) J.J. College of Arts & Science, Pudukkotai-4, T.N. INDIA. Phytopathology 94:S35. Publication no. P-2004-0235-AMA.

Citrus greening bacterium (CGB) disease caused by fastidious prokaryotic bacterium. The diseased trees shows yellowing of veins, leaf mottling, small misshapen fruits with pale green peel, dieback of twigs, decay of roots. The disease is diagnosed by biological indexing on indicator hosts, which is time taking and symptoms depend on temperature. Therefore, a rapid and reliable detection method by PCR was developed using CTAB method for DNA isolation. Phenolics and tannins in citrus leaves generally interfered with obtaining good quality of DNA, which affects the reliable detection of CGB by PCR. To reduce the interference of the inhibitors, addition of sodium sulphite (SS) in DNA extraction was evaluated in comparison with the CTAB method. Addition of SS @ 6 µl/ml solution yielded good quality of DNA and good amplification in PCR compared to CTAB method. Numbers of CGB infected trees were detected by adopting the method in Sathgudi sweet orange, acid lime and Rangpur lime.

Genomic analysis of host–pathogen interactions between *Fusarium graminearum* and its gramineous hosts. R. S. GOSWAMI (1), J. R. [illegible] (2), and H. C. Kistler (1,3). (1) University of Minnesota, St. Paul, MN; (2) Purdue University, West Lafayette, IN; (3) USDA-ARS, CDL, St. Paul, MN. Phytopathology 94:S35. Publication no. P-2004-0236-AMA.

The *Fusarium graminearum* species complex is comprised of strains belonging to at least nine biogeographically structured cryptic species differing significantly in their aggressiveness and mycotoxin production as shown by our initial studies. With an aim to elucidate gene function related to patho-

genicity, three cDNA libraries were created by suppression subtractive hybridization using wheat heads inoculated with a highly aggressive strain (tester) and either a low aggressive strain or water (driver) 48 hours after inoculation. Fungal ESTs were identified by matches to the *F. graminearum* whole genome sequence. Based on comparisons with sequences in other fungal genomic databases and predicted function of genes corresponding to ESTs, candidate sequences potentially involved in pathogenicity have been identified eg. Genes coding for an ABC transporter, amino acid permease and polyketide synthase. Some of these are being targeted for site directed mutagenesis using gene replacement.

Bacterial leaf scorch of in New Jersey: Incidence and economic impact. A. B. GOULD, G. Hamilton, M. Vodak, J. Grabosky, and J. Lashomb. Rutgers University, New Brunswick, NJ. Phytopathology 94:S36. Publication no. P-2004-0237-AMA.

Bacterial leaf scorch (BLS) of amenity trees, caused by the bacterium *Xylella fastidiosa*, is associated with water stress that results in symptoms of leaf scorch and decline of affected trees. BLS is widespread on trees in the red oak group in residential neighborhoods, municipal parks, golf courses, and woodlots throughout the eastern United States. In a 2002-2003 baseline census of oak trees in several New Jersey communities, oak comprised 25-35% of the total street tree resource, and 88-95% of these oaks were highly susceptible red (*Quercus rubra*) and pin (*Q. palustris*) species. Of these susceptible trees, 30-35% were symptomatic for BLS. Analysis of the economic impact of this disease indicates that the affected communities will sustain, and must plan for, losses from $0.7-1.6 million during the next 10-year period.

Effect of mycofumigation on soilborne and ed pathogens. E. GRIMME, D. Ezra, N. K. Zidack, G. A. Strobel, and B. J. Jacobsen. Montana State University, Bozeman, MT. Phytopathology 94:S36. Publication no. P-2004-0238-AMA.

Muscodor albus, an endophytic fungus obtained from *Cinnamomum zeylanicum*, produces volatile compounds which effectively inhibit and kill certain bacteria and plant pathogenic fungi. The antimicrobial use of *M. albus* and its volatile compounds is coined mycofumigation. Control of soilborne fungi by mycofumigation was examined in field trials by placing nylon bags with *Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani* infested soil at different soil depths (0, 10, 20 & 30cm). *M. albus* grown on barley was spread over the top and covered with a 5cm soil layer and black plastic mulch. The nylon bags were removed after 7 days of mycofumigation and sugarbeet seeds were planted in the mycofumigated soil. Based on seedling establishment, mycofumigation with *M. albus* appears to be most effective at depths of 20 to 30cm. In other experiments, a chemical cocktail of the volatile compounds emitted by *M. albus* and a stabileze formulation of the fungus were tested in greenhouse experiments by treating infested soil with the cocktail or stabileze formulation and monitoring the establishment of sugarbeet seedlings. Results showed significantly better emergence of seedlings in the cocktail and stabileze treatments compared to the pathogen control. The influence of *M. albus* and artificial mixtures derived from the compounds emitted by *M. albus* were tested on several seed pathogens in storage conditions (dry and moist). Results indicated reduction in seed infection while preserving germination ability in comparison to non-treated controls.

A genetic shift in the predominant virus strain sing mosaic in Louisiana sugarcane. M. P. GRISHAM and Y.-B. Pan. USDA, ARS, SRRC, Sugarcane Research Unit, Houma, LA. Phytopathology 94:S36. Publication no. P-2004-0239-AMA.

Leaf samples were collected from 658 sugarcane plants showing mosaic symptoms in 2002 and 2003 at locations within the Louisiana sugarcane industry. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine if the diseased plants were infected with sugarcane mosaic virus (SCMV) or sorghum mosaic virus (SrMV), the two viruses reported to cause mosaic in Louisiana. Virus strains were identified by restriction fragment length polymorphism (RFLP) analysis of the RT-PCR product. In most leaf samples, only one virus strain was identified; however, in approximately 5% of the samples, two strains were present and in one sample three strains. Among the strains identified, 67%, 10% and 3% were SrMV strains I, H and M, respectively. In surveys conducted between 1978 and 1995, over 95% of the virus isolates associated with sugarcane mosaic were SrMV strain H. Most of the remaining isolates were SrMV strain I, except for an occasional isolate of SrMV strain M. SCMV was not found in either survey. Approximately 13% of the SrMV isolates identified in 2002-2003 produced an RFLP banding pattern that did not match that of any described strain. Neither SCMV- nor SrMV-specific RT-PCR products were produced by 7% of the plants showing mosaic symptoms, suggesting that another virus may cause mosaic in sugarcane in Louisiana.

Almond leaf scorch disease in the San Joaquin Valley of California: Factors affecting pathoge distribution. R. GROVES (1), J. Chen (1), E. Civerolo (1), M. Viveros (2), and M. Freeman (3). (1) USDA-ARS, Parlier, CA; (2) University of California Cooperative Extension (UCCE), Bakersfield, CA; (3) UCCE, Fresno, CA. Phytopathology 94:S36. Publication no. P-2004-0240-AMA.

Almond leaf scorch disease (ALSD), caused by the xylem-limited bacterium *Xylella fastidiosa* (*Xf*), has long been present in the San Joaquin Valley (SJV). Recently, the incidence of ALSD has reemerged as a significant disease threat in many almond producing regions of the SJV. ALSD incidence was monitored within affected orchards of Fresno and Kern Counties, in California to determine patterns of disease distribution, symptom expression among affected varieties, and to determine the genotypes of *Xf* strains associated with ALSD. Disease incidence ranged from <1.0% to 10.2% in four surveyed orchards. With increasing disease incidence, significant aggregations of diseased trees occurred within rows in three of four orchards surveyed using ordinary runs analysis. Almond varieties including Sonora, Price, and Carmel displayed more severe leaf scorching symptoms than Nonpareil, Peerless, Norman, and Fritz. Two genomic populations of *Xf* were simultaneously detected in affected trees within orchards of Kern County representing both grape and almond strains. Taken together, results from these studies improve our understanding of the factors responsible for movement and distribution of the pathogen and will provide a more complete characterization of ALSD epidemiology.

Evaluating the potential utility of partial resistance to *Bremia lactucae* m the lettuce cultivars 'Grand Rapids' and 'Iceberg'. R. C. GRUBE. USDA-ARS, Salinas, CA 93905. Phytopathology 94:S36. Publication no. P-2004-0241-AMA.

Single dominant *Dm* genes that confer race-specific resistance to downy mildew (*Bremia lactucae*) have historically had short effective life spans once deployed in lettuce cultivars. Partial or 'field resistance' (FR) has been investigated as an alternative and possibly more durable host resistance strategy. Most studies of FR, including ongoing mapping efforts, have focused on the cultivar 'Iceberg'. We sought to evaluate the utility of the cultivar 'Grand Rapids' as a source of FR, particularly in comparison to 'Iceberg'. Field trials were conducted in the Salinas Valley of California over a three-year period to examine responses of both cultivars to endemic populations of *B. lactucae*. Both cultivars remained nearly free of symptoms, with a level of resistance comparable to cultivars with effective *Dm* genes. Both FR cultivars were crossed with susceptible cultivars and evaluated for response to *B. lactucae*. Susceptibility of F1 progeny established that FR was recessive, and similar segregation of FR in F2 and other populations suggested similar inheritance for both cultivars. A large proportion of 'Grand Rapids' × 'Iceberg' F2:4 families were susceptible, implying that FR genes in the two cultivars were not allelic. Based on the susceptibility of cultivars with known genes in field experiments as well as on seedling screens using characterized isol FR from Grand Rapids could not be attributed to any known *Dm* genes. Our results suggest that 'Grand Rapids' may be useful as a source of one or more novel *B. lactucae* resistance genes.

Detection and characterization of Euphorbia ringspot potyvirus. M. A. Guaragna, J. Ambrose, and R. L. JORDAN. US National Arboretum, USDA-ARS, Beltsville, MD. Phytopathology 94:S36. Publication no. P-2004-0242-AMA.

Euphorbia milii var. *splendens* ('Crown of Thorns') is a low-growing succulent evergreen shrub with very thorny grooved stems and branches and has thick fleshy leaves. The recent development of more compact varieties in a range of colors has provided the floral indus th another popular potted plant. A commercially-grown plant exhibiting ring-shaped chlorotic spots and leaf and flower deformations was shown to contain 650-850 nm flexuous particles and tested positive for potyvirus in ELISA using our genus *Potyvirus* broad spectrum reacting PTY-1 monoclonal antibody. The only reported virus known to infect 'Crown of Thorns' is Euphorbia ringspot potyvirus (EuRSV). Total RNA extracts from infected leaves were used in RT-PCR assays with generic potyvirus (and, later, EuRSV-specific) primers which amplify various highly conserved 335, c.700, or c.1600bp fragments from the 3′ terminus of most potyviruses which includes the potyviral coat protein (CP) and the 3′ non-coding region (3′NCR). Sequence analysis of cloned amplicons revealed potyvial similarities, but less than 64% CP amino acid identity and less than 55% 3′NCR identity with any potyvirus currently in the GenBank database, including other ornamental-infecting potyviruses. Pairwise alignments and Clustal W analysis confirm the classification of this poorly described virus as a potyvirus member. Since no antisera against this virus is available, synthetic peptides and bacterially-expressed His-tagged cloned CP from this virus are under evaluation as sources of antigen for EuRSV-specific antisera production for future use in diagnosis.

Ultrastructural and molecular characterization of *Citrus leprosis virus* indicates possible association of two distinct viruses. A. S. GUERRA-MORENO, L. Manjunath (1), R. H. Brlansky (2), D. Howd (2), and R. F. Lee (3). (1) University of Florida, Dept. Plant Path., Gainesville, FL 32611; (2) Univ. of Florida, CREC, Lake Alfred, FL 33850; (3) USDA-ARS, NCGRCD, Riverside, CA 92507. Phytopathology 94:S37. Publication no. P-2004-0243-AMA.

Citrus leprosis virus (CiLV) has been present in South American countries for several decades. The recent occurrence of this virus in Central American countries poses a serious threat to the U.S. citrus industry where it does not occur. Rapid diagnostic methods are essential for both prevention and management. Two virus types were identified in CiLV-infected tissues collected at different locations: one with virus particles occurring in the cytoplasm and the second with virus occurring predominantly in the nucleus. Putative viral sequences have been determined by screening and sequencing of a large number of cDNA clones from tissue infected with cytoplasmic-type virus particles. RT-PCR and hybridizations have shown consistent association of this sequence with cytoplasmic-leprosis from several countries, but not with the nuclear type. These data suggest that symptoms commonly associated with leprosis may be caused by two distinct viruses.

***Sclerotinia sclerotiorum* produces oxalic acid to deregulate guard cells during infection.** R. L. GUIMARAES and H. U. Stotz. Department of Horticulture, Oregon State University, Corvallis, OR. Phytopathology 94:S37. Publication no. P-2004-0244-AMA.

Sclerotinia sclerotiorum, which infects a broad range of plant species, uses oxalate to damage plant tissues and to cause wilting and necrosis. In this work we show that *S. sclerotiorum* alters stomatal movement by means of oxalate. Using a GFP tagged strain we show that infected *Vicia* faba leaves are wide open during darkness in the vicinity of the fungus, and that growing hyphae penetrate open stomata. This suggests that the fungus alters stomatal behavior to facilitate infection. Stomata of *V. faba* infected with oxalate-deficient *S. sclerotiorum* are significantly less open in darkness in comparison with a wild type isolate. We also demonstrate that oxalate induces stomatal opening in detached epidermal peels from *V. faba* leaves, and that at physiological concentrations of 10 mM, oxalate treatment 1) triggers guard cell potassium ion accumulation and starch breakdown; and 2) interferes with ABA-induced stomatal closure. In whole leaves, a wild type *Sclerotinia* strain caused significantly higher transpiration rates and reduced plant biomass when compared with an oxalate-deficient mutant. The above results suggest that oxalate may act by two different mechanisms, one that triggers accumulation of osmotica in guard cells and another that inhibits ABA-induced stomatal closure. Simultaneous regulation of these two mechanisms by oxalate may explain the dramatic degree of stomatal opening in leaf lesions infected by wild type strains of *S. sclerotiorum*.

Identification and fungicide sensitivity of fungal pathogens causing tomato fruit rot in Ohio. L. J. GUTIERREZ and B. B. McSpadden Gardener. The Ohio State University, Dept. of Plant Pathology, Wooster, OH 44691. Phytopathology 94:S37. Publication no. P-2004-0245-AMA.

Tomato fruit rot limits marketable yield in fresh market and processing tomato fields every year. Nineteen fields throughout Ohio were sampled for fruit with sunken lesions. Fungi were isolated from these samples, classified using RFLP analysis, and identified by ITS sequence analysis. *Colletotrichum* were the most abundant, representing 89 of the 134 isolates. In addition, there were 16 *Alternaria*, 11 *Fusarium*, 6 *Phomopsis*, and 12 unidentified isolates. A representative sample was tested for pathogenicity and fungicide sensitivity. These isolates were capable of causing dark, sunken, circular lesions on wounded tomato fruit. Azoxystrobin, chlorothalonil, fixed copper, and mancozeb were tested for the ability to inhibit pathogen growth *in vitro* and decrease lesion development *in situ*. Three times the labeled rate of fixed copper was ineffective at inhibiting growth of any isolate. *Colletotrichum* isolates were sensitive to the labeled rate of azoxystrobin, chlorothalonil, and mancozeb. However, greater than or equal to 50% of the *Fusarium* isolates were not inhibited by mancozeb nor azoxystrobin, respectively. Similarly, greater than 50% of the *Alternaria* isolates were not inhibited by chlorothalonil nor mancozeb. These non-*Colletotrichum* isolates were found to be widely distributed and capable of causing fruit rot in Ohio. The chemical fungicides used in tomato production are not effective at inhibiting growth of these isolates.

Isolating and screening antagonists to *Hemileia vastatrix*. F. Haddad, L. A. MAFFIA, E. S. G. Mizubuti, and R. S. Romeiro. Universidade Federal de Viçosa, 36570-000 Viçosa, MG, Brazil. Phytopathology 94:S37. Publication no. P-2004-0246-AMA.

Brazil is the largest coffee producing country. Organically-grown coffee (OGC) plantings are increasing fast, notably in Minas Gerais State. Leaf rust (*Hemileia vastatrix*), is a serious disease in either conventionally grown or OGC. Leaf rust control is largely based on fungicide applications, however for OGC, alternatives to disease management are needed. We hypothesized that there are naturally occurring antagonists to *H. vastatrix* in OGC. From samples collected in OGC fields, we isolated 154 bacteria and 239 fungi (respectively, 28 and 36 from leaf washate, 18 and 69 from debris washate, 2 and 37 from leaf discs on paraquat medium, 36 and 97 from soil washate). We got 70 bacteria from all washates treated at 80°C/20 min. To screen for potential antagonists, spore or cell suspension of each fungal or bacterial isolate was sprayed on leaves of 'Catuai' seedlings and after 48h inoculated with *H. vastatrix*. Efficient antagonists to rust were isolated from all sampled places and materials. For one bacterial isolate, rust latent period (LP) was not completed. All other isolates were not efficient in reducing LP or urediniospore germination. Seventeen isolates reduced infection frequency and number of urediniospores produced per leaf by more than 70%. They were species of *Bacillus* (I10, I25, I157, I175, I205, I281, I400), *Pseudomonas* (I286), *Fusarium* (I109, I143, I171, I173), *Penicillium* (I334, I432), *Aspergillus* (I300), *Acremonium* (I31), and *Cladosporium* (I411), and will be further evaluated. Research funded by FAPEMIG.

Biocontrol of coffee leaf rust with antagonists isolated from organic crops. F. Haddad, L. A. Maffia, E. S. G. MIZUBUTI, and R. S. Romeiro. Universidade Federal de Viçosa, Viçosa, MG, Brazil. Phytopathology 94:S37. Publication no. P-2004-0247-AMA.

Leaf rust (*Hemileia vastatrix*) (Hv) is a major constraint for coffee production in Brazil and alternatives for disease management are needed, mainly for organic farmers. Seventeen biocontrol agents, 7 *Bacillus* sp. (I10, I25, I157, I175, I205, I281, and I400), 4 *Fusarium* sp. (I109, I171, I143, and I173), 2 *Penicillium* sp. (I334 and I432), and one of each *Pseudomonas* sp. (I286), *Aspergillus* sp. (I300), *Acremonium* sp. (I31), and *Cladosporium* sp. (I411), were applied on coffee plants either at 0, 4, 8, 12, or 16 days before or after Hv inoculation. Reduction of infection frequency (RIF) and of the number of urediniospores/leaf (RUPL) were quantified. When applied before inoculation, isolates I10, I25, I157, I175, I281, and I286 were the most effective, and did not differ from copper sulfate for both RIF and RUPL at all application times. In post inoculation, for RIF 16 and 12 isolates were as effective as epoxiconazol at times 0 and 4 days, respectively. At 8 days, isolates I25 and I286 did not differ from the fungicide. At 12 and 16 days all isolates were less effective than the fungicide. For RUPL, 15, 11, and 11 isolates were as effective as epoxiconazol at 0, 4, and 8 days, respectively. At 12 days, only I143, I157, and I300 did not differ from the fungicide, and at 16 days all isolates were less effective than the fungicide. Isolates I10, I25, I157, and I286 were the most effective in reducing rust intensity, particularly when applied before pathogen inoculation. Research funded by FAPEMIG.

Comparison of fungicides for control of powdery mildew on dogwood. A. HAGAN (1), J. W. Olive (2), J. Stephenson (2), and M. Rivas-Davila (1). (1) Dept. of Entomology and Plant Pathology, Auburn University, AL 36849; (2) Ornamental Horticulture Research Center, Mobile, AL 36689. Phytopathology 94:S37. Publication no. P-2004-0248-AMA.

Efficacy of fungicides for control of powdery mildew (*Microsphaera pulchra*) was assessed on flowering dogwood (*Cornus florida*). Also, impact of Compass application rate and treatment interval on powdery mildew control and growth of flowering dogwood was evaluated. Container-grown trees were maintained in shade and were irrigated as needed with overhead sprinklers. While reductions in disease were obtained with all fungicides, Banner Maxx, Eagle, Heritage, and to a lesser extent SunSpray Oil and Phyton 27 gave the best control of powdery mildew over a minimum of 3 yr. Generally, Bayleton T/O, 3336, and Domain were less effective. In 2002, tree caliper and height were similar across all fungicide-treated and unsprayed trees. At the 1.0 and 2.0 oz rates, Compass consistently gave better control when applied at 1 than at 4 wk intervals but was equally effective at the 4.0-oz rate across all treatment intervals. In one of four trials, tree caliper of the Compass-treated dogwood was larger than that for the unsprayed controls. No phytotoxicity was observed.

***Cucurbit leaf crumple virus* in Imperial Valley of California: A newly emergent cucurbit-infecting geminivirus.** C. HAGEN (1), E. Natwick (2), T. Turini (2), and R. L. Gilbertson (1). (1) Department of Plant Pathology, 1 Shields Ave., University of California-Davis 95616; (2) University of California Cooperative Extension, 1050 East Holton Rd., Holtville, CA 92250-9615. Phytopathology 94:S37. Publication no. P-2004-0249-AMA.

Cucurbit leaf crumple virus (CuLCrV) is an emergent and potentially economically important bipartite begomovirus first identified in cucurbits in southern California in 1998. To determine the threat CuLCrV poses to California melon production, a specific PCR test was developed, field plots established with various crop plants and surveys of melon production was

conducted in southern California during the 2000-2003 growing seasons. Surveys revealed that CuLCrV was established in Imperial County, but only reached high levels in fall melons. A CuLCrV agrobacterium-mediated inoculation system was developed and used to screen squash, watermelon, cantaloupe and honeydew melon varieties for susceptibility. Both in the field and in the laboratory, cantaloupe and watermelon plants underwent a recovery phenotype. Recovering tissues had lower viral titers than symptomatic tissues and we are investigating the role of RNA silencing in this phenotype.

Elicitor function of Soybean mosaic virus-G7 provoking Rsv1-mediated lethal systemic hypersensitive response maps to P3. M. R. HAJIMORAD, A. L. Eggenberger, and J. H. Hill. Plant Path. Dept., Iowa State University, Ames, IA 50011. Phytopathology 94:S38. Publication no. P-2004-0250-AMA.

Rsv1, a single dominant resistance gene in soybean PI 96983, confers extreme resistance against all known American strains of Soybean mosaic virus (SMV), except G7 and G7d. SMV-G7 provokes a lethal systemic hypersensitive response (LSHR); whereas SMV-G7d, an experimentally evolved variant of SMV-G7, induces systemic mosaic. To identify the elicitor of Rsv-mediated LSHR, chimeras were constructed by exchanging fragments and their elicitor functions were assessed on PI 96983. SMV-G7 derived chimera containing only P3 of SMV-G7d lost the elicitor function, while the reciprocal chimera of SMV-G7d gained the function. The P3 of the two viruses differ by six nucleotides, of which two are translationally silent. The four amino acid (aa) differences are located at positions 823, 915, and 1112 of the precursor polypeptide. Analyses of site-directed mutants revealed that nucleotide substitutions leading to translationally silent mutations as well as reciprocal aa exchanges at position 915 did not influence the loss or gain of the elicitor function. SMV-G7 mutants with aa substitutions at any of the other three positions lost the ability to provoke LSHR, but induced SHR instead. Two concomitant aa substitutions at positions 823 and 953 abolished SMV-G7 elicitor function. Conversely, SMV-G7d gained the elicitor function by a single exchange at position 823, and mutants with aa exchanges at positions 953 or 1112 induced SHR instead of mosaic. Taken together, the data suggest that strain-specific P3 of SMV is the elicitor of Rsv1-mediated LSHR.

Identification of potexvirus isolates from phlox and portulaca as strains of *Alternanthera mosaic virus*, and comparison of the genomic 3′ region. J. HAMMOND, M. D. Reinsel, and C. J. Maroon-Lango. USDA-ARS, USNA, FNPRU, Beltsville, MD. Phytopathology 94:S38. Publication no. P-2004-0251-AMA.

Potexvirus isolates from creeping phlox (*Phlox stolonifera*) and trailing portulaca (*Portulaca grandiflora*) from commercial nurseries reacted strongly to polyclonal antiserum against *papaya mosaic virus* (PapMV). Cloning and sequencing showed that the coat protein (CP) gene of a phlox isolate from Pennsylvania was c.97% identical at the amino acid level to that of *Alternanthera mosaic virus* (Geering and Thomas, 1999; Arch. Virol. 144:577-592), and only c.75% to PapMV. The full genomic sequence of the phlox isolate was determined. The regions 5′ of the CP gene were found to be more closely related to (but distinct from) PapMV than to any other characterized potexvirus. This phlox isolate was therefore named AltMV-PA. The host range of AltMV-PA was found to be similar to that of the Australian isolate, AltMV-Au. The 3′-terminal portion of the genome of the portulaca potexvirus isolate, and of additional potexvirus isolates from two phlox cultivars from commercial nurseries in Maryland, were also cloned and sequenced. The sequence of these isolates were similar to those of AltMV-Au and AltMV-PA, and the isolates were designated AltMV-Po (portulaca), AltMV-SF (phlox cv. Sherwood Forest), and AltMV-BR (phlox cv. Blue Ridge). Each of the phlox and portulaca isolates reacted strongly with antiserum to AltMV-Au, and also with anti-PapMV antiserum. The host range of AltMV includes several ornamentals; these findings suggest that AltMV may be quite widespread, and may be misdiagnosed as PapMV.

Genetic and molecular analysis of the RNA interference pathway in *Aspergillus*. T. M. HAMMOND, T. R. McDonald, J. W. Bok, and N. P. Keller. Department of Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 94:S38. Publication no. P-2004-0252-AMA.

Our lab is investigating the post-transcriptional gene silencing mechanism of RNA interference (RNAi) in *Aspergillus* species with a goal of using RNAi to control mycotoxin production by filamentous fungi. Our studies show that RNAi can be directed to block the production of two highly toxic and carcinogenic compounds by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nidulans*. Creation of an RNAi-based mycotoxin control strategy will require a thorough understanding of this mechanism in these organisms. Therefore, a genetic and molecular analysis of RNAi in *Aspergillus* species is underway. Our work with *A. nidulans* suggests that the Aspergilli require a PAZ and PIWI domain protein, characteristic of RNAi in other eukaryotes, but do not require an RNA dependent RNA polymerase (RDRP), an enzyme often found to play an important role in RNAi. We are currently in the process of determining if RDRPs have a supplementary role in *Aspergillus* RNAi, such as in 'spreading' or 'amplification' of the silencing effect. Additionally, we are testing two putative RNAse III enzymes in *A. nidulans* for RNAi functionality.

Dollar spot control on ultradwarf bermudagrass putting greens with curative and preventive fungicide applications. D. Y. HAN (1), J. B. Unruh (2), and S. B. Davis (3). (1) Auburn University, Auburn, AL; (2) University of Florida, Jay; (3) Bayer Environmental Science, Gulf Breeze, FL. Phytopathology 94:S38. Publication no. P-2004-0253-AMA.

Dollar spot is an increasing problem on ultradwarf bermudagrass putting greens in the spring and fall, especially greens mowed at very low heights or fertilized very sparingly. Dollar spot infects bermudagrass in the fall, as the grass slows growth before winter dormancy, and is most severe the following spring. The disease is not a problem during the summer, while bermudagrass is growing at its maximum rate and the weather is to hot to be conducive for the growth of *Sclerotinia homoeocarpa*. In tests during 2002 and 2003, curative treatments with a variety of fungicides during the spring while a TifEagle bermudagrass putting green was emerging from dormancy resulted in healing of bermudagrass greens from dollar spot three to five weeks faster than untreated controls. Addition of slow-release nitrogen fertilizer enhanced the activity of fungicides and healing of the turfgrass. Iprodione, chlorothalonil and several DMI fungicides also provided some degree of control of dollar spot in the spring when applied as preventive treatments before the onset of symptoms the pervious fall, delaying the need for a curative treatment in the spring.

Some tropical foliicolous fungal pathogens. R. T. HANLIN (1) and M. C. Gonzalez (2). (1) Dept. of Plant Pathology, University of Georgia, Athens, GA; (2) Depto. de Botanica, Instituto de Biologia, UNAM, Mexico City, Mexico. Phytopathology 94:S38. Publication no. P-2004-0254-AMA.

Collecting trips in the American tropics have yielded a number of foliicolous fungi representing several families and orders of Ascomycota. The purpose of this report is to present a summary of some members of this diverse group of fungi, which occur on a wide variety of hosts. Genera considered include *Apiosphaeria*, *Coccodiella*, *Diatractium*, *Lembosia*, *Myriogenospora*, *Phyllachora* and *Ophiodothella*. These genera are separated on the characteristics of the ascoma (superficial or immersed), the asci (unitunicate or bitunicate), and the ascospores (shape, cell number and color). All except *Lembosia* have unitunicate asci. In *Coccodiella*, *Diatractium*, *Myriogenospora*, *Phyllachora* and *Ophiodothella* the ascospores are hyaline, but in *Apiosphaeria* and *Lembosia* they are brown. *Myriogenospora* has filiform ascospores that separate into part-spores, *Diatractium* has isthmoid ascospores, and in the other genera they are ellipsoid to subglobose.

Phylogeography of *Armillaria ostoyae* in the western United States. J. W. HANNA (1,2), N. B. Klopfenstein (1), M.-S. Kim (1), G. I. McDonald (1), and J. A. Moore (2). (1) USDA Forest Service-RMRS, Moscow, ID; (2) University of Idaho, Moscow, ID. Phytopathology 94:S38. Publication no. P-2004-0255-AMA.

Using a direct-PCR method, sequences of the nuclear ribosomal DNA (i.e., large subunit, internal transcribed spacer, 5.8S, and intergenic spacer) were obtained from *Armillaria ostoyae* genets collected from the western United States. Phylogenetic analyses using Bayesian methods defined several groups of *A. ostoyae*. Analysis of *A. ostoyae* from outside the western United States indicates the presence of a circumboreal group of *A. ostoyae* that also occurs in Utah. Phylogeographically unique groups were also present in the Rocky Mountain and Pacific Northwest regions. Other *Armillaria* species were used as outgroups to examine evolutionary relationships among the groups of *A. ostoyae*. The occurrence of these groups allows inferences about paleogeographic and paleoclimatic influences on phylogeography of *A. ostoyae*. Additionally, hybridization has occurred among groups that may have been previously isolated for millions of years. Hybridization has potential implications on species evolution and could contribute to variation in pathogenicity and virulence.

Barriers to implementing Integrated Pest Management (IPM) and safe pesticide use in developing countries. M. A. HANSEN. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Phytopathology 94:S38. Publication no. P-2004-0256-AMA.

The increasing occurrence of epidemics related to pesticide resistance in agricultural crops in developing countries in recent years has stimulated

outreach projects focused on educating farmers of these nations about both IPM and pesticide safety. One such project was initiated by the non-governmental organization, Opportunities Industrialization Centers International, with a tomato-grower cooperative in a village in southern Ghana in 2003. The immediate goals of the project were to diagnose diseases present in the farmers' tomato fields, provide control recommendations, and teach farmers how to calibrate their backpack sprayers. Long-term goals included decreasing pesticide use while increasing tomato yield and farmer income. In the fall of 2003 I diagnosed 7 diseases in the farmers' tomato crops and provided cultural and chemical control recommendations for these diseases. In the course of this work, many barriers to successful implementation of IPM and pesticide safety became apparent. These included lack of the following: education about pests and pesticides, basic math skills that would allow farmers to calibrate sprayers, and proper calibration and safety equipment. Limited availability of low toxicity pesticides and incomplete pesticide labels were additional barriers. Lack of interest in implementing IPM practices was not a barrier. Increased availability of educational programs in rural areas has the potential to increase the implementation of IPM and pesticide safety, as well as environmentally friendly disease control practices, in developing countries.

***AvhR* in *Agrobactrium vitis* is associated with of induction of a tobacco hypersensitive response and grape necrosis.** G. HAO, H. Zhang, and T. J. Burr. Cornell University, Dept. of Plant Pathology, Geneva, NY 14456. Phytopathology 94:S39. Publication no. P-2004-0257-AMA.

A quorum-sensing system in *A. vitis* was recently reported to be associated with induction of a tobacco hypersensitive response and grape necrosis. A second *luxR* homolog *avhR* has now been identified in Tn5 mutant M1320 which has lost its abilities to induce HR. The deduced AvhR protein has characteristic autoinducer binding and helix-turn-helix DNA binding domains and shares greatest identity to AGR-c-1279 (50.6%) from *A. tumefaciens*. Thin layer chromatograms overlaid with an autoinducer biosensor revealed no differences between profiles of N-acyl-homosering lactone autoinducers from M1320 and F2/5. Site-directed mutagenesis of *avhR* and a downstream open reading frame, ORF8 (ATP-binding Cassette (ABC) transporter homolog) confirmed that functional *avhR* is essential for HR and full necrosis. M1320 was fully complemented with a cosmid clone carrying *avhR* and with the gene cloned separately into an expression vector. A 14kb region of the cosmid flanking *avhR* was sequenced and ten open reading frames (ORFs) were identified and compared with homologous regions from genomes of *A. tumefaciens* C58 and *Sinorhizobium meliloti* Rm1021. It was also determined that the expression of *avhR* is dependent on a functional *aviR* indicating that a hierarchical quorum-sensing system that regulates induction of HR and necrosis in *A. vitis*.

Alternatives to methyl bromide for management of Verticillium wilt in strawberry. J. J. HAO, J. M. Duniway, and D. M. Dopkins. Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616. Phytopathology 94:S39. Publication no. P-2004-0258-AMA.

Verticillium wilt of strawberry in California has been controlled largely by soil fumigation with mixtures of methyl bromide and chloropicrin. Because of the pending phase-out of methyl bromide, alternatives to methyl bromide for management of Verticillium wilt in strawberry were researched in field experiments during 1993-2004 near Watsonville, CA. Among the chemical alternatives used, 1,3-dichloropropene mixed with chloropicrin was effective for controlling *Verticillium dahliae* in soil, as was chloropicrin alone at higher rates; metam sodium was only partially effective. The efficacy of chemical fumigants was generally improved by drip application and impermeable plastic mulch. While all the main varieties of strawberry currently used in California are susceptible to Verticillium wilt, the extent to which they were damaged varied significantly. One-year rotation with broccoli or rye reduced subsequent wilt incidence in strawberry, but not by as much as soil fumigation. Incorporation of high-nitrogen organic amendments (e.g. blood, feather, meat and bone meals) into soil at 0.45-1.34 kg/m^2 reduced *V. dahliae* in soil, but did not reduce disease incidence for more than one crop cycle. Isolates of *Pseudomonas* spp. from strawberry reduced the incidence of Verticillium wilt when inoculated onto strawberry at transplanting. An effective integration of methods for management of Verticillium wilt is likely to be needed following further restrictions on methyl bromide.

Prevalence of dry bean diseases in North Dakota-2003. R. HARIKRISHNAN, L. E. del Río, and R. S. Lamppa. North Dakota State University, Fargo, ND 58105. Phytopathology 94:S39. Publication no. P-2004-0259-AMA.

Diseases are an important limiting factor affecting dry bean (*Phaseolus vulgaris* L.) production in North Dakota, the largest bean producer in the US. However, data from systematic field surveys that characterize the prevalence of diseases affecting this crop are not available. A survey was conducted in 2003 with this goal. Fungal and bacterial disease prevalence (+ or –) and incidence (# of infected plants /field) were estimated in 90 fields spread through Grand Forks, Pembina, Steele, Traill, and Walsh counties. These counties account for approximately 60% of the total acreage planted in North Dakota. Common bacterial blight was prevalent in 98% of the fields inspected with a mean incidence of 73%. The second most prevalent disease was white mold affecting 72% of the fields scouted with an average incidence of 15%. Rust and bacterial brown spot were prevalent in 26 and 28% of the fields surveyed with mean incidence of 12 and 11%, respectively. Root rots, caused predominantly by *Fusarium solani*, affected 21% of the fields scouted. Other diseases noticed during the survey with minor prevalence and incidence were anthracnose and halo blight.

Effects of temperature (TEMP) and relative humidity (RH) on ascospore infection, and viability of pre-colonized flowers by *Sclerotinia sclerotiorum* on white mold development (WM) in dry beans. R. HARIKRISHNAN and L. del Río. Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105. Phytopathology 94:S39. Publication no. P-2004-0260-AMA.

Studies were conducted to test the influence of two TEMP and RH levels on infection of senescent dry bean flowers (SDBF) by *S. sclerotiorum* (*Ss*) ascospores, and on the viability of pre-colonized senescent flowers (PC) by *Ss* and on WM. In the first study, SDBF were dip inoculated for 30-40 seconds in a 10^4 ascospores/ml suspension and placed individually on the axis of the first inter-node of two-week old plants. Inoculated plants were incubated at all combinations of TEMP (22 and 18 ± 1°C) and RH (90 and 25 ± 5%). For the second study, SDBF were colonized by placing them on a 2-day old *Ss* culture at 20 ± 1°C overnight. The PC were incubated at 18 ± 1°C and 25 ± 5% (TEMP/RH) for drying. At 24 h intervals, seedlings were inoculated with individual PC and incubated as described above. Results of these studies indicate that plants incubated at 18°C and 90% RH reached 100% WM incidence earlier than other TEMP/RH combinations, and that PC can withstand 96 hours of drying and still be viable. Implications of these results will be discussed.

Isolation and PCR amplification of plant viral nucleic acid from immunochromatographic devices. A. M. HARNESS (1), B. P. Kulemeka (1), J. A. Abad (2), and M. D. Bandla (1). (1) Agdia, Inc., Elkhart, IN; (2) North Carolina State University, Raleigh, NC. Phytopathology 94:S39. Publication no. P-2004-0261-AMA.

Immunochromatographic devices (ICDs) provide a rapid, easy to use diagnostic method for the identification of suspected pathogens in the field. Confirmation of positive results by other tests, such as PCR, is often necessary. The diagnostic labs that use these portable devices sometimes do not have the equipment or the staff to perform PCR in their facility, necessitating the shipment of the material to another lab for confirmation. We have developed a simple extraction method to recover the nucleic acids from the test line or sample pad from ICDs, which can then be used as a template for RT-PCR. This method is useful when no material is available for further testing and eliminates the need to ship potentially infected plant material. Several extraction methods were evaluated for consistency and reliability using CMV, TMV, and TSWV ImmunoStrips™. The influence of storage conditions over time on the stability of the nucleic acids on these ICDs is examined. This methodology can also be used to confirm the positive signal from immunoprints or dot blots.

Fatty acid methyl ester (FAME) profile analysis of *Phytophthora cactorum*. L. A. HARRISON, M. B. Riley, and S. N. Jeffers. Clemson University, Clemson, SC. Phytopathology 94:S39. Publication no. P-2004-0262-AMA.

In addition to morphological traits, species of *Phytophthora* can be characterized by FAME profile analysis. Sixty-eight isolates of *P. cactorum* were examined. Isolates were collected over 30 years from several hosts (primarily strawberry but also apple and woody ornamental plants) that came from various geographical locations in North America and Europe. Isolates were grown under standard incubation conditions before fatty acids were extracted and then identified by gas chromatography. Sixteen fatty acids were common to all isolates, and these were used to generate FAME profiles, which were analyzed using MIDI and SAS software. In a dendrogram produced by cluster analysis, isolates formed numerous terminal groups, but, overall, these groups did not appear to be associated with any known character—e.g., host, geographical origin, or mefenoxam sensitivity. A Euclidean Distance of approximately 1.2 delimited FAME profile diversity for *P. cactorum* based on these 68 isolates. Additional isolates will be used to further define the diversity among FAME profiles for this species and to examine the utility of this approach for species identification.

Global regulators control antibacterial metabolite production in *Pseudomonas syringae* pv. tomato DC3000. H. HASEGAWA (1), A. Chatterjee (1), D. Tian (1), Y. Cui (1), A. Collmer (2), and A. K. Chatterjee (1). (1) University of Missouri, Columbia, MO; (2) Cornell University, Ithaca, NY. Phytopathology 94:S40. Publication no. P-2004-0263-AMA.

We have shown that *P. syringae* pv. tomato strain DC3000 produces an antibiotic that is effective against *E. coli* and other bacteria. We have also determined that environmental conditions regulate antibiotic production. To determine genetic regulation of antibiotic production we examined antibiotic production by an assortment of well-characterized DC3000 mutants. In these assays, *E. coli* was used as the test strain. Antibiotic production was not affected in AhlR$^-$, Crp$^-$ and PhoP$^-$ mutants. By contrast, antibiotic production was severely inhibited in both GacA$^-$ and GacS$^-$ mutants. This inhibition in the GacA$^-$ strain was reversed by the DC3000 *gacA* gene. Our previous work has shown that GacA regulation occurs at least in part via *rsmB*, a non-translatable RNA that functions as global positive regulator. The findings reported here show that (a) multiple copies of the *rsmB* gene markedly stimulate antibiotic production in DC3000 and (b) the *rsmB* gene reverses the effects of GacA deficiency on antibiotic production.

Small metabolites control antibiotic production in *Pseudomonas syringae* pv. tomato DC3000. H. HASEGAWA (1), A. Chatterjee (1), D. Tian (1), A. Collmer (2), and A. K. Chatterjee (1). (1) University of Missouri, Columbia, MO; (2) Cornell University, Ithaca, NY. Phytopathology 94:S40. Publication no. P-2004-0264-AMA.

Pseudomonas syringae pv. tomato DC3000 produces antibacterial antibiotic(s) effective against many Gram negative bacteria including *E. coli*. To understand the biosynthesis and regulation of antibiotic production in DC3000, we isolated mini-Tn5 insertion mutants altered in antibiotic production. Sequence analysis of DNA flanking mini-Tn5 insertions revealed that (a) inactivation of *pqqA*, *pqqF* and *gntV* resulted in loss of antibiotic production (Ant$^-$ phenotype); and (b) insertions in *nadC* and a locus for IF2 (initiation factor 2 subunit) resulted in antibiotic over-production (Ant^{hyper} phenotype). Based upon studies in other systems, *pqqA* and *pqqF* are presumed to specify products required for pyrroloquinoline quinone (PQQ) biosynthesis, *gntV* to encode a gluconokinase required for gluconate catabolism, and *nadC* to code for quinolinic acid phosphoribosyltransferase, an enzyme responsible for the biosynthesis of nicotinamide adenine dinucleotide (NAD) and its derivatives. Further evidence for the roles of *pqq* and *nad* genes comes from feeding experiments with PQQ and nicotinic acid. Antibiotic production was restored in PqqA$^-$ and PqqF$^-$ mutants in medium supplemented with PQQ (500 nM). Likewise, addition of nicotinic acid to growth medium reversed the Ant^{hyper} phenotype of the NadC$^-$ mutant. Our findings demonstrate a tight metabolic regulation of antibiotic production in DC3000.

Cellular distribution of RNA of seed-transmitted viruses in soybean embryos. J. S. HAUDENSHIELD and L. L. Domier. USDA-ARS, Urbana, IL. Phytopathology 94:S40. Publication no. P-2004-0265-AMA.

Soybean mosaic virus (SMV) and *Tobacco ringspot virus* (TRSV) cause serious virus diseases of soybean, and both viruses are transmitted vertically through infected seed. TRSV seed transmission approaches 90% in seed from infected plants. SMV is transmitted at a lower frequency (1% or less), although it has been reported to be as high as 30%, depending upon the virus isolate and soybean cultivar. We examined the cellular distribution of viral RNA in paraffin-embedded, thin sections of embryos from sytemically infected soybean plants by *in situ* RNA hybridization, using RNA probes derived from cloned cDNA of the 5′ terminus of SMV and TRSV (RNA-2). Both TRSV and SMV invaded many cell types in stage 2, 3 and 4 soybean embryos, including cells of cotyledons, embryonic leaves, procambial tissue, and shoot apexes. Viral infection was observed in over 80% of the embryos examined for both viruses, suggesting that the lower frequencies of seed transmission reported for SMV must be the result of events occurring after developmental stage 4, possibly a reduced viability of the seed and/or SMV at maturity.

Quantification of *Phytophthora ramorum in planta* using real-time PCR. J. HAYDEN and M. Garbelotto. University of California, Berkeley, CA. Phytopathology 94:S40. Publication no. P-2004-0266-AMA.

Phytophthora ramorum, a recently-described pathogen that infects a wide variety of plant hosts causes the disease complex called sudden oak death. We used real-time PCR and previously developed primers specific to the rDNA internal transcribed spacer region to quantify pathogen colonization and identify presence of spores of *P. ramorum* in infected host tissue. We inoculated leaves of *Umbellularia californica*, and documented the change over time in detectable pathogen DNA. We measured the difference in amount of DNA in leaves with living *P. ramorum* from those in which the pathogen has been heat-killed, and we quantified the pathogen DNA present in sporulating, thus potentially infectious, samples versus those without spores. We also compared quantification using TaqMan chemistry with an alternate method using SYBR green, the detection technique currently in use by USDA-APHIS. This work is expected to assist regulatory agencies and researchers for whom the difference between infectious and non-infectious samples is crucially important, and to further the ongoing study of the epidemiology of this emerging forest pathogen.

Forecasting late blight in potato crops of southern Idaho using logistic regression analysis. D. HENDERSON (1), C. Williams (1), and J. S. Miller (2). (1) Univ. of Idaho, Moscow, ID; (2) Univ. of Idaho, Aberdeen, ID. Phytopathology 94:S40. Publication no. P-2004-0267-AMA.

Late blight forecasts, such as BLITECAST, predict the threat of disease based on the presence or absence of weather favorable for disease. However, previous forecasting models have not been effective in the semi-arid potato producing areas of the Pacific Northwest. Research was conducted to identify weather variables to forecast late blight in Idaho. Weather data were collected from Agrimet weather stations at five locations over a nine year period. Weather variables were divided into four categories: factors affecting the formation of over-wintering inoculum; factors affecting the over-wintering of inoculum and development of disease on refuse piles; factors enhancing disease development during the growing season; and factors limiting disease development during the growing season (Zwankhuizen and Zadoks, 2002, Plant Pathology 51:413-423). Late blight incidence and severity were categorized for each year/location combination using a 0-4 disease index scale. A preliminary binary logistic regression model indicated that three variables were significant disease predictors: number of days with precipitation from April-September, number of hours with unfavorable conditions (T greater than or equal to 27°C) from April-September; and total global radiation from April-September. Logistic regression analysis using the disease index as an ordinal scale is being investigated.

Characteristic analysis of genetic variation in *Plasmodiophora brassicae* from Korea. S. H. Heo, H. K. Lee, W. C. Lee, and H. G. KIM. Department of Agricultural Biology, Chungnam National University, Daejeon, 305-764, Korea. Phytopathology 94:S40. Publication no. P-2004-0268-AMA.

Fundamental informations were derived to apply for the development of resistant cultivars of Chinese cabbage against clubroot by clarifying the mycological and genetic characteristics associated with this fungus. In order to distinguish representative race and their pathogenicity, single spore isolation of *Plasmodiophora brassicae* from diseased plants was carried out to collect galls from severely infested areas in Korea. Pathogenicity of race 4 showed highly strong and widespread than other races in virulence analysis. Also RAPD and RFLP analysis were conducted to assay their genetic variation and diversity. RAPD analysis with single spore isolates clearly showed genetic differences between different races but not distinct variation within the same race. More than 2 races, however, were confirmed in a single gall when DNA of spores from naturally developed galls was assayed. The result of DNA analysis from single gall induced by artificial inoculation with same races revealed little genetic variations. Analysis of progeny identified the stable transfer of genetic characteristics to progenies. And there was shown relatively high (at least 70%) genetic similarity between isolates collected from different locations within an identical field assayed when we considered the long cultivation period of Chinese cabbage in Korea. This result will be useful for breeding program of resistant cultivars on Chinese cabbage against clubroot caused by *Plasmodiophora brassicae* should be primarily focused on the race 4 to get more benefit.

Post-harvest removal of sooty blotch and flyspeck on apples by combining dipping and brushing treatments. S. M. HERNANDEZ (1), J. Batzer (1), M. L. Gleason (1), D. S. Mueller (1), and P. M. Dixon (2). (1) Dept. of Plant Pathology; (2) Dept. of Statistics, Iowa State University, Ames, IA. Phytopathology 94:S40. Publication no. P-2004-0269-AMA.

Sooty blotch and flyspeck (SBFS) fungi colonize the cuticle and reduce the quality and value of apples. One potential option to reduce SBFS to mark acceptable levels is post-harvest washing. The objective of this study was to compare post-harvest dip treatments (NaOCl at 200 and 500 µg/ml, ClO(2) at 1 and 5 µg/ml, or fruit soap) followed by brushing on a grading line for removal of SBFS. After 7-min dip treatments, apples were brushed for 15, 30, 60, or 90 s on a grading line. Percent disease was determined before and after treatment of 'Honey Gold' apples from Iowa and Wisconsin in 2002 and 'Golden Delicious' apples from Kentucky and North Carolina in 2003. Removal of SBFS in all treatments was variable, but generally exceeded the no-dip control. Increasing the brushing time significantly increased removal of SBFS signs. The only situation where brushing apples probably increased the fresh-market value of apples was on 'Honey Gold', which had averaged

6% coverage with SBFS prior to dip treatments and about 1% after dip treatments and brushing for 90 s.

Temperature optima for mycelial growth of newly discovered fungi in the sooty blotch and flyspeck complex on apples. S. M. HERNANDEZ (1), J. C. Batzer (1), M. L. Gleason (1), S. Mueller (1), P. M. Dixon (2), V. Best (3), and P. S. McManus (3). (1) Dept. of Plant Pathology; (2) Dept of Statistics, Iowa State University, Ames, IA; (3) Dept. of Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 94:S41. Publication no. P-2004-0270-AMA.

Several fungal species cause sooty blotch and flyspeck (SBFS), a disease complex resulting in cosmetic damage that reduces the value of apples. The optimal temperature for mycelial growth of two isolates of each of five putative SBFS species (*Colletogleum* sp. FG2.2, *Dissoconium* sp. DS1, *Peltaster* sp. P2, *Peltaster* sp. P6, and *Pseudocercosporella* sp. RH1) and one previously described species (*Peltaster fructicola* P1) was determined in growth chambers in IA and WI. Plugs from 3-wk-old cultures of all 12 isolates were transferred to water agar in 22-mm-diameter wells and incubated at 10, 15, 20, 25, 30 and 35°C for 7 wk in darkness. The experimental design was a randomized complete block with six replications. Colony diameters were measured every 7 to 11 days. Optimum growth for each isolate occurred at either 20 or 25°C, with slower growth at 10 and 15°C and no growth at 30 or 35°C. At 10 and 15°C, *Dissoconium* sp. DS1 isolates and one *Peltaster fructicola* P1 isolate at 15°C grew more rapidly than the other isolates. At 20 and 25°C, *Dissoconium* sp. DS1 and *Peltaster fructicola* P1 isolates grew most rapidly. Determining the temperature requirements for SBFS fungi will increase ecological understanding of these pathogens and ultimately contribute to development of more effective management strategies.

Genetics of host specificity in *Ascochyta* species infecting legumes. M. A. HERNANDEZ-BELLO and T. L. Peever. Department of Plant Pathology, Washington State University. Phytopathology 94:S41. Publication no. P-2004-0271-AMA.

Ascochyta spp. (teleomorphs: *Didym* spp.) infect a number of economically important legumes including chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), pea (*Pisum sativum*), and hairy vetch (*Vicia villosa*). Greenhouse inoculations have demonstrated that *A. fabae*, *A. pisi*, *A. viciae-villosae*, and *A. lentis* are host-specific on faba bean, pea, hairy vetch, and lentil, respectively. These pathosy represent an excellent opportunity to determine the molecular basis of host specificity at the species level and the role of host specificity in fungal speciation. Phylogenetic analysis of glyceraldehyde-3-phosphate-dehydrogenase, translation elongation factor alpha, and chitin sythase revealed that isolates from each host were monophyletic and identified *A. fabae* and *A. pisi* as closely related taxa. Forty ascospore progeny and the parents of a cross between *A. fabae* and *A. pisi* were inoculated on both pea and faba bean in growth chambers. Typical disease symptoms developed when each parental isolate was inoculated on its respective host but most progeny isolates were non-pathogenic on both hosts. Nine progeny isolates infected pea and two infected faba bean, whereas all others did not infect either host. We are developing a high-resolution genetic map for this cross using AFLP markers. Mapping and possible alternative approaches to elucidate the genetic control of specificity on these hosts will be discussed.

Development of methods to distinguish between oleander leaf scorch and Pierce's disease strains of *Xylella fastidiosa*. R. HERNANDEZ-MARTINEZ, C. K. Dumenyo, and D. A. Cooksey. University of California, Riverside, Riverside, CA 92521. Phytopathology 94:S41. Publication no. P-2004-0272-AMA.

Pierce's disease (PD) of grapes and oleander leaf scorch (OLS) are two diseases in southern California caused by *Xylella fastidiosa*, a bacterium vectored by xylem feeding insects. PD and OLS strains are genetically distinct; while OLS strains have been associated only with oleander, PD strains appear to have a broader host range. We report the development of methods to distinguish between the two strains. A 750 bp product was PCR-amplified using RST31 and RST33 primers followed by *Eco*47III digestion. PCR products from OLS strains produced two fragments while those from PD strains remain uncut. A second method used primers gumB-P1 and gumB-P2 to amplify a 1.2 Kb PCR product that was subsequently digested with *Hae*III or *Ban*II. *Hae*III digestion of the amplified product generated two fragments only from PD strains while *Ban*II digestion resulted in the production of two fragments only from OLS strains. These methods can be used among others; to detect cross infection in hosts and to identify strain-specific vectors and new inoculum sources.

Isolation and characterization of genes involved in the initial stages of biofilm development in *Pantoea stewartii* subsp. *stewartii*. C. M. HERRERA, M. Koutsoudis, and S. B. von Bodman. University of Connecticut, Storrs, CT. Phytopathology 94:S41. Publication no. P-2004-0273-AMA.

Pantoea stewartii subsp. *stewartii* causes Stewart's bacterial wilt and leaf blight disease in sweet corn and maize. The wilting condition results from the production of an extracellular polysaccharide (EPS) virulence factor that blocks the xylem of infected plants. EPS synthesis in *P. stewartii* is governed by quorum sensing, and loss of regulation leads to abnormal biofilm development and attenuated infectivity. Based on these observations, we predict that the fundamental steps of biofilm development are a driving force for bacterial host invasion. We will present data from mutant studies to show that QS regulation is essential for the bacterium to attach to surfaces under non-inducing conditions, while inducing conditions at higher cell density promote EPS synthesis, swarming motility, microcolony and biofilm formation, and parallel loss of attachment. Microscopic imaging will be used to establish a functional relationship between steps of biofilm formation and plant host invasion.

Identification of genes involved in resistance to cercospo, a toxin produced by *Cercospora* spp. S. HERRERO (1), K. R. Chung (2), and M. E. Daub (1). (1) North Carolina State University, Raleigh, NC; (2) University of Florida, Lake Alfred, FL. Phytopathology 94:S41. Publication no. P-2004-0274-AMA.

Some members of the genus *Cercospora* produce and are resistant to cercosporin, a non-host specific toxin and photosensitizer that reacts with molecular oxygen to produce singlet oxygen and superoxide. The damaging effects caused by these reactive oxygen species ultimately lead to cell death. In *Cercospora* spp., cercosporin levels can accumulate up to 1mM. Since growth is never compromised at such a high toxin concentration, these fungi must possess the genetic and biochemical machinery that render them resistant to this potent photosensitizer. To better understand the mechanism of cercosporin resistance in *C. nicotianae* and ultimately resistance to singlet oxygen, we constructed both forward (F) and reverse (R) cDNA subtraction libraries between the cercosporin-resistant wild type and a mutant lacking the transcription factor *CRG1*. The *crg1*-disrupted mutants are sensitive to cercosporin toxicity and have reduced cercosporin production. Poly(A)+ RNA isolated from 3,4 and 5 day-old cultures of *C. nicotianae* was subtracted from Poly(A)+ RNA from a *crg1*-disrupted strain. To date, a total of 341 genes have been sequenced: 82 unique EST's are expressed only in wild type (F) while 46 are expressed only in the *crg1* mutant (R). Blast analysis of these sequences revealed that 51% (F) and 63% (R) of the clones show high similarity ($P < 10^{-5}$) to genes in the GenBank database. Further characterization of these genes and additional sequencing is in progress.

Oxygen competition as a biological control mechanism. L. G. HJELJORD and A. Tronsmo. Agricultural University of Norway, N-1432 Ås, Norway. Phytopathology 94:S41. Publication no. P-2004-0275-AMA.

Efforts to develop biological control of gray mold in strawberries have demonstrated the necessity of a better understanding of interactions among pathogenic and antagonistic fungi. Since *Botrytis cinerea* conidia germinate in <6 h at 20°C, antagonism must occur rapidly. *B. cinerea* germination and infection is induced by nectar at the base of the receptacle in strawberry flowers. Concentrated suspensions of *Trichoderma* spp., *Ulocladium* spp. or *Epicoccum nigrum* prevented *B. cinerea* germination in liquid media with glucose and fructose concentrations equivalent to those in nectar. Dissolved oxygen measurements showed that when *B. cinerea* was coinoculated with 1×10^7 *T. atroviride* conidia ml^{-1}, the medium became depleted of oxygen within 2 h, and the pathogen failed to germinate. Introduction of oxygen reversed the inhibition. This antagonist concentration also prevented infection of detached flowers by coinoculated *B. cinerea*. The larger, fast-germinating conidia of *E. nigrum* and *Ulocladium* spp. were effective at lower concentrations. Effectiveness of all increased with temperature. When *T. atroviride* conidia were treated to increase respiration several hours before contact with *B. cinerea*, their inhibitory activity increased 10-fold. Oxygen competition in flower microsites may therefore be an important unrecognized biocontrol mechanism.

A survey of *Pythium* species in Taiwan. H. H. HO. State University of New York, New Paltz, NY. Phytopathology 94:S41. Publication no. P-2004-0276-AMA.

A comprehensive study was initiated to study the genus *Pythium* in Taiwan to assess the potential threat of *Pythium* spp. to local agriculture and forestry. To date, 34 species of *Pythium* have been isolated from primarily soil samples: *Py. acanthicum*, *Py. aphanidermatum*, *Py. arrhenomanes*, *Py. carolinianum*, *Py. catenulatum*, *Py. coloratum*, *Py. debaryanum*, *Py. deliense*, *Py. Dissotocum*, *Py. graminicola*, *Py. helicoides*, *Py. inflatum*, *Py. intermedium*, *Py. Irregulare*, *Py. marsipium*, *Py. middletonii*, *Py. monospermum*, *Py. Myriotylum*, *Py.*

oedochilum, *Py. oligandrum*, *Py. paroecandrum*, *Py. periplocum*, *Py. Perplexum*, *Py. pulchrum*, *Py. rostratum*, *Py. salpingophorum*, *Py. spinosum*, *Py. splendens*, *Py. sylvaticum*, *Py. tardicrescens*, *Py. torulosum*, *Py. ultimum*, *Py. vexans* and *Py. volutum*. They are mostly potentially plant pathogens and widely distributed throughout Taiwan with *Py. vexans*, *Py. splendens*, *Py. aphanidermatum*, *Py. spinosum* and *Py. acanthicum* as the dominant species.

Evolution of fungal pathogenicity on plant: Model studies with *Alternaria* species and *Arabidopsis thaliana*. S. G. HONG and B. M. Pryor. Dept. of Plant Sciences, Univ. of Arizona, Tucson, AZ. Phytopathology 94:S42. Publication no. P-2004-0277-AMA.

Alternaria brassicicola and *Arabidopsis thaliana* have been used as a model system to study fungal-plant interactions. To facilitate comparative studies of fungal pathogenicity and plant defense mechanisms, additional *Alternaria* species were evaluated for pathogenicity on *Arabidopsis*. From pathogenicity screening of 23 *Alternaria* species on three ecotypes of *A. thaliana*, *A. arborescens*, *A. longipes*, *A. brassicicola*, *A. japonica*, *A. brassicae*, and *A. solani* displayed high level of pathogenicity on *Arabidopsis*. Phylogenetic analyses using sequence and AFLP fingerprint data revealed that these species represent diverse lineages of *Alternaria*. Other closely related species were greatly reduced in their ability to cause disease. These results, together with different responses of *Arabidopsis* for different pathogenic species, suggest that diverse pathogenic mechanisms arose several times independently. In addition, the level of sensitivity and defense response were different among the three ecotypes of *Arabidopsis*.

Use of alt a 1 gene sequences in phylogenetic study of *Alternaria* and related genera. S. G. HONG (1), R. A. Cramer (2), C. B. Lawrence (2), and B. M. Pryor (1). (1) Dept. of Plant Sciences, Univ. of Arizona, Tucson, AZ; (2) Dept. of Bioagricultural Sciences and Pest Management, Colorado State Univ., Fort Collins, CO. Phytopathology 94:S42. Publication no. P-2004-0278-AMA.

A gene for the human major allergen, alt a 1, was amplified from 52 species of *Alternaria* and related genera and sequence information was used for phylogenetic study. Compared to glyceraldehyde-3-phosphate dehydrogenase (gpd) gene sequences, alt a 1 sequences are 3.8 times more variable and contains 3.5 times more parsimony informative sites than gpd sequences. Analyses of alt a 1 and gpd sequences strongly supported grouping of *Alternaria* spp. and related taxa into several species-groups suggested in previous studies, especially the alternata, infectoria, porri, and radicina sp.-groups. The sonchi sp.-group was newly suggested in this study. Although support for the brassicicola sp.-group and the *Ulocladium* group were not strong, they were maintained using different analysis methods. Relationships among species-groups and closely related species of the same species-group were not fully resolved. However, higher resolution could be obtained using alt a 1 sequences or a combined dataset than using gpd sequences.

Characterisation of damage and organisms associated with the decline of Eucalyptus wandoo B[illegible]ly in Western Australia. R. J. HOOPER and K. Sivasithamparam. The University of Western Australia, Crawley WA 6009, Australia. Phytopathology 94:S42. Publication no. P-2004-0279-AMA.

Crown decline of Eucalyptus wandoo has escalated over the last ten years so very few unaffected stands remain in the York area. Canopy damage characteristics were assessed and severity of aerial cankers and borer damage were compared for healthy and declining stands. Neither fusiform or cracking cankers or Type-1 borer damage were found on healthy branches. Relationships between foliage density and canker index were significant for intermediate and unhealthy classes but negligible for the healthy class indicating that branch cankers are causing foliage loss. Considering; several Type-1 borer infestations of varying age were consistently found per sampled branch; a constant association was found between Type-1 borer damage and cankers of greater severity in intermediate and unhealthy classes; and the high likelihood of branch cankers causing foliage loss in declining stands; there is strong evidence to support an interaction between Type-1 borer infestations and invasion by opportunistic fungal pathogen (s) is responsible for declining canopies of E. wandoo.

Development of a method using allometric ratios for d[illegible]osing tree decline syndromes. R. J. HOOPER and K. Sivasithamparam. The University of Western Australia, Crawley WA 6009, Australia. Phytopathology 94:S42. Publication no. P-2004-0280-AMA.

The use of allometric ratios was assessed to be the most objective methodology available for a canopy decline where there has been conjecture over key factors including: tree recovery and the relationship between foliage loss and decline symptoms. Quantitative methodology was employed to investigate differences between canopy density of healthy and declining stands of Eucalyptus wandoo. A technique using a destructive, partial harvest of trees, sampling branches from a variety of tree age, canopy position and stand density was used to compare relationships for healthy and actively declining branches ie. with branches showing foliage chlorosis or wilting. Branch sapwood cross-sectional area was a more effective predictor than total cross-sectional area. Leaf fresh-weight gave more reliable results than leaf dry-weight and leaf area so ratios using sapwood cross-sectional area and leaf fresh-weight were used as a measure of foliage density for each branch order when relating to the disease index in future work.

Systemic acquired resistance (SAR) in cucumber against infection by *Colletotrichum orbiculare* is enhanced by systemic fungicide and SAR activator applications. B. J. HORVATH and R. Hammerschmidt. Michigan State University, East Lansing, MI. Phytopathology 94:S42. Publication no. P-2004-0281-AMA.

Plant defense mechanisms such as systemic acquired resistance (SAR) are involved in the protection of plants against pathogens. Recent research has shown that fungicide and SAR activator compounds may enhance the SAR response by the plant and, therefore, enhance the protection against the pathogen. In this study, our objective was to evaluate the response of a locally systemic fungicide, trifloxystrobin, and a SAR activator, acibenzolar-S-methyl (ASM) to determine if these compounds affect the SAR response in the presence or absence of a *C. orbiculare* inducing treatment. Application of these compounds results in a decrease in the severity of disease, and the combination of these two compounds further decreases disease severity. These results suggest application of these compounds may enhance the plant's own defense mechanisms and increase plant protection in the field during large disease outbreaks.

Comparative genomics and core fungal genes. T. HSIANG. University of Guelph, Guelph, ON, Canada. Phytopathology 94:S42. Publication no. P-2004-0282-AMA.

Biologists face an overwhelming richness of nucleotide and protein sequence data. By the end of 2003, there were over 100 complete or almost complete non-viral genomes in publicly available databases. Most of these were bacterial, since prokaryotic genomes are generally much smaller than eukaryotic genomes. Among eukaryotes, fungi have some of the smallest genome sizes and hence represent the highest number among eukaryotic genomes sequenced, with most of these released within the last three years. With the advent of high throughput genomic sequencing and the availability of several complete genomes, we can begin to address questions concerning the number of genes that fungi share in common; the number of fungal genes that are also found in plants, animals or bacteria; and which genes are exclusively fungal. This talk will discuss these issues as well as present the methodology by which this "cottage industry" comparative genomics can be done on a desktop computer.

Analysis of molecular responses in feeding cells of so[illegible]n cyst nematode. X. HU, T. Bourett, R. Howard, R. [illegible]f, and G. Lu. Pioneer Hi-Bred International, Inc., A DuPont Company, P. O. Box 1004, Johnston, IA 50131, USA. Phytopathology 94:S42. Publication no. P-2004-0283-AMA.

Soybean cyst nematode (SCN) is an obligate, sedentary endoparasite of soybean that is responsible for approximately $1 billion in annual crop losses in North America. SCN second-stage juveniles (J2) penetrate into soybean roots and inject secretions that modify root cells and transform them into a specialized feeding structure called a syncytium. The syncytium provides nourishment essential for nematode growth and reproduction. We are trying to develop an understanding of the regulation of the syncytium formation. One approach in our research is focusing on the molecular responses to the formation of syncytial cells. RNA profiling and Laser Capture Microdissection (LCM) techniques were used to study molecular responses in SCN syncytia. Promoters driving the *GUS* gene in transgenic soybean hairy roots were used to study expression levels in SCN syncytia. Results showed that SCP1 promoter activity was repressed, whereas UCP3 promoter activity was significantly induced in syncytia. These results indicate that changes of molecular signals are associated with syncytium formation during the nematode/root interaction in plants.

Root cell proliferation induced by a secretory root-knot nematode signaling peptide. G. HUANG (1), R. Dong (1), R. Allen (1), P. Davis (2), T. Baum (3), and R. Hussey (1). Depts. of Plant Pathology, (1) Univ. of Georgia, Athens, GA 30602; (2) N.C. State Univ., Raleigh, NC 27695; (3) Iowa State Univ., Ames, IA 50011. Phytopathology 94:S42. Publication no. P-2004-0284-AMA.

Secretory proteins encoded by parasitism genes mediate nematode parasitism of plants. A parasitism gene that encodes a novel secretory peptide (13 amino acids) has been cloned from root-knot nematode species. This gene was

strongly expressed in the subventral glands of parasitic nematode stages and its product appears to be secreted into host roots during parasitism. The peptide contained a functional domain similar to that of *Arabidopsis* CLAVATA3-like proteins. *In planta* expression analyses revealed that the peptide enhanced root growth and induced root-cell proliferation. A putative target of this bioactive peptide was identified by yeast two-hybrid analysis to be a plant SCARECROW-like transcription factor, which controls root growth and development. This nematode peptide may function as a signal molecule to reprogram the fate of host root cells, thereby contributing to the formation of giant-cells and/or galls.

Natural occurrence of *Xylella fastidiosa* in a commercial nursery in Maryland. Q. HUANG. Floral and Nursery Plants Research Unit, US National Arboretum, USDA-ARS, Beltsville, MD 20705. Phytopathology 94:S43. Publication no. P-2004-0285-AMA.

Xylella fastidiosa causes many economically important plant diseases and occurs in many important landscape trees and shrubs including oak, maple, elm, sycamore and oleander. The natural occurrence of the bacterium in commercial nurseries is currently unknown. Knowledge of the presence of the disease in native vegetation and nursery stock plants is needed to facilitate control measures to ensure the production of disease-free plant materials. A survey was conducted in a wholesale and production nursery in Maryland for the occurrence of *X. fastidiosa* on nursery plants and surrounding vegetation using enzyme-liked immunosorbent and polymerase chain reaction assays. Our results indicate possible association of *X. fastidiosa* with the nursery plants Japanese stewartia and crape myrtle, as well as the surrounding plants mimosa and sassafras, previously unknown hosts of the bacterium. The significance and implication of our findings will be discussed.

Pomegranate wilt and its potential biocontrol. Q. HUANG, H. R. Chen, Y. Y. Zhu, Y. Y. Wang, J. H. Fan, and Z. S. Mao. College of Plant Protection, Yunnan Agricultural University, Kunming 650201, China. Phytopathology 94:S43. Publication no. P-2004-0286-AMA.

Pomegranate wilt caused by *Ceratocystis fimbriata* has been a persistent disease in *Punica granatum*. It contributed 11.2% incidence in 2003, which was much higher compared to 4% in 2002 and resulted economic loss about 1.5 million USD. The older pomegranate bushes were particularly susceptible to infection, and replanting young seedling died soon after pulled out the wilted one. All local cultivars can be infected, no cultivar was resistant to infection and required wound sites for infection. it has remained a major problem in Yunnan pomegranate production. There are no chemical options available for *C. fambriata* controlling, so effective control measures against *C. fambriata* on pomegranate need to be developed and implemented. The *Bacillus subtilis* has been reported to be associated with and antagonistic to this fungi. So alternatively biological control seems to be very promising. Strains of bacteria were isolated from the soil and the natural infected pomegranate and screened for their inhibitory capabilities. The most inhibitory strains belong to *Bacillus subtilis* and fluorescent Pseudomonas. In assays involving each and combinations of them are undergoing at greenhouse. This information will be useful to design strategies to enhance crop protection against Ceratocystis diseases.

Development of a PCR-based detection assay for foliar nematodes (*Aphelenchoides* spp.). L. C. HUDSON, X. Wang, E. L. Davis, and C. Y. Warfield. N.C. State University, Raleigh, NC. Phytopathology 94:S43. Publication no. P-2004-0287-AMA.

Foliar nematodes (*Aphelenchoides* spp.) are becoming increasingly common and injurious on a wide range of ornamental plants grown in nurseries. These organisms can cause plants to become unthrifty or defoliate. Foliar nematodes generally go undetected until physical damage is observed. Visual detection is labor intensive, and nematodes are easily missed when populations are low. There is no cure for f[illegible]nematodes, and exclusion is currently the best option for control. The goal of this project was to develop a practical, high-throughput diagnostic assay for use in a greenhouse or nursery production system. The assay uses *Aphelenchoides* spp.-specific primers designed to amplify unique regions of foliar nematode DNA using PCR. We have confirmed specificity of the primers to genus level and the assay is being optimized for sensitivity, reproducibility, and capacity to detect foliar nematodes within plant tissues. The detection assay could be used in a production setting to minimize the risk of foliar nematode spread through propagation or movement of infested, asymptomatic nursery stock from nursery-to-nursery, or for final use in the landscape.

Interaction between *Aphanomyces euteiches* and *Phytophthora medicaginis* in resistant and susceptible alfalfa cultivars evaluated using real-time PCR. T. J. HUGHES and G. J. Vandemark. USDA-ARS, Prosser, WA 99350. Phytopathology 94:S43. Publication no. P-2004-0288-AMA.

Root rot diseases of alfalfa caused by *Aphanomyces euteiches* (Ae) and *Phytophthora medicaginis* (Pm) are controlled through a combination of cultural practices. Because Ae and Pm have been implicated in a damping-off disease com[illegible]x, breeders often screen concurrently for resistance to both pathogens. Real-time PCR assays have been developed to quantify the amount of pathogen DNA present in roots of inoculated alfalfa. Results indicate that for both Ae and Pm, a negative and significant correlation exists between resistance and the amount of pathogen DNA present. Cultivars Saranac and WAPH-1 (susceptible and resistant, respectively, to Ae and Pm) were co-inoculated with spore suspensions of Ae, Pm, or both. The quantity of pathogen DNA detected was significantly lower for WAPH-1 than for Saranac when inoculated with either Ae or Pm. When inoculated with both Ae and Pm, the quantity of Pm DNA detected was significantly less than the amount of Pm DNA detected in plants inoculated with Pm alone. Results of these experiments suggest that co-inoculation with Ae and Pm reduces the colonization efficacy of Pm.

Effect of coir extracts on growth of *Phytophthora capsici* and *Botrytis cinerea in vitro*. N. HYDER, S. N. Wegulo, M. Vilchez, and J. J. Sims. University of California, Riverside, CA 92521. Phytopathology 94:S43. Publication no. P-2004-0289-AMA.

Phytophthora spp. are major plant pathogens in recycled irrigation water in nurseries and *Botrytis cinerea* is one of the most damaging stem and foliar pathogens in greenhouses. Coir is becoming increasingly popular as a potting medium in containerized nursery production. The effect of coir extracts on growth of *Phytophthora capsici* and *Botrytis cinerea* were investigated *in vitro*. On half-strength PDA, *p*-coumaric acid, known to be present in coir, reduced mycelial area of *P. capsici* by 25% at 0.04 mg/ml and 81% at 0.24 mg/ml after 8 days. The acetone-soluble portion of a methanol coir extract at 2.4 mg/ml reduced mycelial area of *P. capsici* and *B. cinerea* by 56% after 8 days and 19% after 5 days, respectively. Results from these studies suggest that coir extracts have potential for use in controlling both below- and above-ground plant pathogens.

Characterization of infectious clones for *Melon chlorotic leaf curl virus*, and evidence of [illegible]ecular recombination with other New World begomoviral species. A. M. IDRIS, K. Mills, and J. K. Brown. Department of Plant Sciences, University of Arizona, Tucson, AZ 85721. Phytopathology 94:S43. Publication no. P-2004-0290-AMA.

The whitefly-transmitted *Melon chlorotic leaf curl virus* (MCLCV) is a begomovirus (Geminiviridae) that causes patchy foliar chlorosis, leaf curling, and fruit lesions in *Cucumis melo* L. cultivars, and was first reported in Guatemala in 2001. Total nucleic acids were extracted from symptomatic leaves and aliquots were incubated with select restriction enzymes. Analysis of digestion products by Southern hybridization using PCR-labeled, component specific probes for MCLCV revealed that Cla I linearized viral dsDNA. Hence, Cla I was used to clone both viral components. The DNA sequence for MCLCV DNA-A (pMCLCV-C160) and DNA-B (pMCLCV-C151) was determined. Phylogenetic analysis with well-studied begomo[illegible]uses indicated MCLCV is distinct species, with its closest relative being Squash yellow mild mottle virus (AY064391) from Costa Rica, at 90%. Recombination analysis of the DNA-A sequence indicated that MCLCV shared numerous genome segments with other begomoviruses from the Western Hemisphere. The MCLCV DNA-A and DNA-B clones were shown to be infectious when biolistically inoculated to muskmelon plants. The experimental host range for MCLCV includes bean, cantaloupe, cucumber, *Datura stramonium*, *Nicotiana benthamiana*, pumpkin, and watermelon.

Genetic diversity among *Clavibacter michiganensis* subsp. *michiganensis* and subsp. *sepedonicus* in Russian Federation. A. N. IGNATOV (1,2), E. V. Matveeva (1), E. Sh. Pekhtereva (1), and N. W. Schaad (3). (1) Russian Res. Inst. Phytopath., Moscow, Russia; (2) Centre Bioeng., Russian Acad. Sci., Moscow, Russia; (3) USDA-ARS, FD-WSRU, Ft. Detrick, MD, USA. Phytopathology 94:S43. Publication no. P-2004-0291-AMA.

Clavibacter michiganensis subsp. *michiganensis* (Cmm) and subsp. *Sepedonicus* (Cms), the causal agents of bacterial canker of tomato and ring rot of potato, respectively, are present in the Russian Federation (RF) and Northern and Eastern Europe. The highly regulated Cms has been found in Denmark, Finland, France, Germany, Greece, Spain, and The Netherlands, countries with extensive trade with RF. Ring rot is favored by cool climates common to potato-growing regions of the RF. To determine the genetic diversity within the Russian population of these two pathogens, over 100 suspect strains of Cms and Cmm were collected from diseased potatoes and tomatoes from different regions of RF in 2002/2003. After cloning and confirming the identification by pathogenicity tests, 32 strains of Cms and 39 strains of Cmm were analyzed by AP- and rep-PCR. The Russian strains of Cmm and Cms were easily distinguished from each other and from their respective type strains.

Efficacy of phosphorous acid against pink rot and Pythium leak on potato. D. A. INGLIS (1), J. S. Miller (2), and D. A. Johnson (3). (1) Washington State University-Mount Vernon REU, Mount Vernon, WA 98273; (2) University of Idaho REC, Aberdeen, ID 83210; (3) Department of Plant Pathology, Washington State University, Pullman, WA 99164. Phytopathology 94:S44. Publication no. P-2004-0292-AMA.

Phosphorous acid at 9.37 kilograms active ingredient per hectare was applied to the foliage of potato cultivars three times at 2 week-intervals beginning at tuber bulking in field trials in western and eastern WA, and ID. Control of *Phytophthora erythroseptica* (*Pe*) was evaluated by inoculating harvested tubers by: i) wounding and inserting a 0.5 centimeter^2 colonized agar plug, ii) dipping the bud end in a zoospore suspension (zsp) at 10^4/ml, iii) pipetting a zsp onto the stem end and placing a zsp-saturated 1-centimeter-diameter filter paper disc onto the bud end, or iv) pipetting a zsp onto the bud end only. Inoculation with *Pythium ultimum* (*Pu*) was by wounding since *Pu* is not capable of penetrating non-damaged tuber periderm tissue. In 2002 phosphorous acid changed incidence and severity of pink rot from 100 to 88% ($P = 0.05$) and 41 to 46% for western WA tubers that were wounded, from 99 to 92% and 85 to 57% ($P = 0.05$) for eastern WA tubers that were wounded, and from 94 to 80% ($P = 0.05$) and 45 to 27% for ID tubers that were dip-inoculated, respectively. In 2003 pink rot was reduced from 60 to 0% incidence and 56 to 0% severity for western WA tubers ($P = 0.05$) that were inoculated with zsp on both the stem and bud end, but only from 84 to 66% ($P = 0.05$) incidence and 20 to 16% severity for ID tubers that were inoculated on the bud end. Tuber infections by *Pe* generally occur via stolons. Phosphorous acid may provide more effective pink rot control when infections occur naturally via stolons, eyes or lenticels rather than by wounds. Control of Pythium leak was not evident in this study.

Genetic structure of benzimidazol-resistant population of the soybean purple stain fungus *Cercospora kikuchii* in Japan. I. INOUE (1), K. Ishikawa (2), N. Yasuda (1), A. Miyasaka (1), and S. Koizumi (1). (1) National Agricultural Research Center, Tsukuba, Japan; (2) Niigata Crop Research Center, Nagaoka, Japan. Phytopathology 94:S44. Publication no. P-2004-0293-AMA.

Soybean disease management largely depends on applications of benzimidazol fungicides in Japan. Recently, benzimidazol-resistant *C. kikuchii* has appeared in several places, and the disease management has become increasingly difficult. Here, we examined genetic structure of benzimidazol-resistant population of the fungus using amplified fragment length polymorphism (AFLP) markers. Among 232 fungal isolates collected from 14 prefectures in Japan, 150 isolates from 13 prefectures were identified as resistant to the fungicides. DNA fingerprinting based on AFLP patterns divided the 232 isolates into 103 genotypes. A phenogram of the 103 genotypes constructed by the unweighted pair group method using arithmetic average revealed four phylogenic groups (I-IV). All of the benzimidazol-resistant isolates were involved in the group I. These results indicate that genetically similar clones resistant to benzimidazol are widely distributed in Japan.

Molecular characterization of spinach germplasm and a marker linked to downy mildew resistance using AFLPs. M. IRISH (1), J. C. Correll (1), B. de los Reyes (2), and C. Feng (2). (1) Department of Plant Pathology; (2) Department of Crop Soils and Environmental Science, University of Arkansas, Fayetteville, AR. Phytopathology 94:S44. Publication no. P-2004-0294-AMA.

AFLPs have been used to characterize genetic diversity in 332 spinach genotypes in the USDA spinach germplasm collection. A total of 64 AFLP primer combinations were screened for their ability to identify polymorphisms among six spinach genotypes initially selected from diverse genetic pedigrees. The AFLP procedure yielded 16-124 reliably scorable fragments per primer combination. Several primer combinations, which yielded a high number of polymorphic fragments, were selected to evaluate the accessions in the germplams collection. The AFLP technique was a robust method for characterizing genetic diversity of spinach. The AFLP procedure was also used to identify polymorphisms among progeny of a cross between a downy mildew (*Peronospora farinosa* f. sp. *spinaciae*) susceptible and resistant parent (BC_3). A single AFLP marker was found to be linked to the gene(s) controlling resistance to race 5 and 6 of the pathogen.

An aerobiological and epidemiological risk assessment for the aerial incursion of soybean rust into the United States. S. A. Isard (1), R. D. MAGAREY (2), and J. M. Russo (3). (1) University of Illinois, Urbana-Champaign; (2) USDA-APHIS, Raleigh, NC; (3) ZedX Inc., Bellefonte, PA. Phytopathology 94:S44. Publication no. P-2004-0295-AMA.

Soybean rust (*Phakopsora pachyrhizi*) was first detected in Brazil in 2001 and is likely to be aerially transported into the Unites States. A risk assessment of the potential for aerial incursion was prepared for the USDA Economic Research Service (ERS). There are four components to this composite spatial risk assessment: I) source area strength in South America, ii) aerial transport potential using a complex 3-D atmospheric model, iii) host area strength, and iv) epidemic potential using the NAPPFAST predictive modeling tool. An index was constructed for each component ranging in value from 0.0 to 1.0. The composite risk index was the product of the four component indexes. Risk was determined for each of the ten USDA agricultural regions during each of six bi-monthly periods. The results showed that the risk of aerial incursion was greatest in the Southeastern and Appalachian regions during April to July.

Glutamine is an essential component of the defined medium 3G10-R for *Xylella fastidiosa*. M. L. ISHIDA, P. C. Andersen, and B. Leite. University of Florida, Quincy, FL. Phytopathology 94:S44. Publication no. P-2004-0296-AMA.

Simple chemically-defined media are required for studies addressing the nutritional basis for growth of *X. fastidiosa*. Nutritional studies can be used to reveal interactions between bacterial cells, xylem fluid and xylem cell walls. The formulation of a xylem fluid-based medium, with (3G10-R) increased the capacity of *X. fastidiosa* to form biofilm and decreased the number of cells in the planktonic state (Leite et al, 2004 - FEMS 230:283-290). Deletion studies using 3G10-R medium selectively removed the following compounds: biotin, glutamine, glutathione, glucose, phenol red, ferric pyrophosphate and magnesium chloride. The buffer system was maintained in all formulations. The only essential component for growth was found to be L-glutamine. In the absence of reduced glutathione, less biofilm formation was observed compared to results obtained with the original 3G10-R formulation. These results may have relevance on how we currently view the metabolism of *X. fastidiosa* in culture.

Optimization of red-light irradiation in inducing resistance in vegetable seedlings against *Phytophthora capsici*. S. Z. ISLAM (1), M. Babadoost (1), and Y. Honda (2). (1) Dept. Crop Sci., Univ. of Illinois, Urbana, IL 61801; (2) Fac. Life & Env. Sci., Shimane Univ., Matsue, Japan. Phytopathology 94:S44. Publication no. P-2004-0297-AMA.

Induction of resistance against *Phytophthora capsici* in vegetable seedlings by exposure to red-light (600-700 nm, 287 µW/cm^2) for four weeks has been previously reported. This study was conducted in the greenhouse to optimize red-light treatment of vegetable seedlings for inducing resistance against *P. capsici*. Red-light irradiation of plants during daytime did not induce significant resistance against *P. capsici*. Red-light irradiation at night induced an equal or higher degree of resistance against *P. capsici* than continuous red-light irradiation (day and night). Thirty days post-inoculation, 80-85% of pepper, tomato, and pumpkin seedlings that received red-light at night remained uninfected. The rates of seedling survival for those receiving continuous red-light irradiation (day and night) or daytime-only irradiation, were 70-75% and 35-40%, respectively. In controls (grown under natural day light), 75% of seedlings became infected and died within 15 days of inoculation. Doubling the red-light intensity induced greater resistance in tomato seedlings but had the opposite effect in pepper.

Soybean rust in Argentina. A. IVANCOVICH (1), G. Botta (1), S. Vallone (2), E. Guillin (2), N. Formento (3), G. Guerra (4), and I. Bonacic (5). (1) INTA Pergamino, Pcia. de Buenos Aires, Argentina; (2) INTA Marcos Juárez, Pcia. de Córdoba, Argentina; (3) INTA Paraná, Pcia. de Entre Ríos, Argentina; (4) INTA Manfredi, Pcia. de Córdoba, Argentina; (5) INTA Saenz Peña, Pcia. de Chaco, Argentina. Phytopathology 94:S44. Publication no. P-2004-0298-AMA.

Soybean rust is a destructive fungal disease causing severe losses to soybean on several continents. Soybean rust is caused by two fungal species, *Phakopsora pachyrhizi*, (Asian form), and *P. meibomiae*, (American form). The Asian form is more aggressive and causes more damage than the American form. In Brazil and Paraguay, *P. pachyrhizi* was detected in 2001 and 2002. In Argentina, soybean rust was reported in 2002, in the northern provinces of Chaco and Misiones. During 2003 soybean rust was detected in field trials at INTA Cerro Azul Experimental Station (province of Misiones), and in two fields plots at Gobernador Virasoro (province of Corrientes). Molecular diagnose (PCR) confirmed the presence of *P. pachyrhizi*, not only on the soybean samples but also on several alternative hosts found in those areas, specifically Cajan (*Cajanus cajan*), Kudzu (*Pueraria lobata*) and Mucuna (*Styzolobium niveun*). The disease is restricted to the northern region of Argentina, more than 1000 km north of the main soybean area of Argentina.

Physiological effects of the strobilurin fungicide pyraclostrobin on plants. T. JABS (1), H. L. Ypema (2), and H. Köhle (1). (1) BASF AG, Agricultural Center, Global Research Agricultural Products, Limburgerhof, Germany; (2) BASF Corp., Research Triangle Park, NC 27709. Phytopathology 94:S44. Publication no. P-2004-0299-AMA.

In addition to its broad-spectrum fungicidal activity, the BASF strobilurin fungicide pyraclostrobin had beneficial effects on crop growth and yield in the absence of pathogen challenge. These effects were partly related to increased carbohydrate content in the plant and increased activity of the enzyme nitrate reductase, allowing for a higher uptake and metabolism of nitrogen. Other physiological effects on the plants were especially apparent under conditions of environmental stress. Foliar application of pyraclostrobin reduced the stress-induced production of reactive oxygen intermediates in plant leaf tissues, activated the plant antioxidative system and prevented the release of ethylene and premature senescence. Since environmental stresses are caused by changes in the genetic and metabolic regulation of reactive oxygen intermediates, we postulate that both the antioxidative and the anti-senescence effects of pyraclostrobin contribute to its ability to improve stress tolerance. The described processes help the plants to store assimilates for a longer time and ensure optimal maturation and yield.

Physiological effects of the new fungicide boscalid on crop quality. T. JABS (1), C. Gunkel (1), J. Pfirrmann (1), M. Scherer (1), and H. L. Ypema (2). (1) BASF AG, Agricultural Center, Global Research Agricultural Products, Limburgerhof, Germany; (2) BASF Corp., Research Triangle Park, NC 27709. Phytopathology 94:S45. Publication no. P-2004-0300-AMA.

Boscalid is a new broad-spectrum fungicide of the anilide class developed by BASF. Boscalid interferes with mitochondrial respiration and due to its mode of action and efficacy spectrum it has a very good fit for use in fruits, vines, vegetables and turfgrass. In addition to its fungicidal effects, boscalid was shown to influence physiological processes of plants not infected or challenged by pathogens. Following use of boscalid, yield and quality parameters of crops such as vegetables and fruits were found to be positively affected. Application of boscalid also was associated with a reduction of stress-induced release of ethylene and premature senescence. These observations are discussed in relation to the mode of action of boscalid.

Inheritance of resistance to phomopsis seed decay in PI 80837 and MO/PSD-0259 soybean. E. W. Jackson, P. Fenn, S. E. SMITH, P. Chen, C. Feng, and P. K. Miller. University of Arkansas, Fayetteville, AR. Phytopathology 94:S45. Publication no. P-2004-0301-AMA.

Phomopsis seed decay (PSD) can limit production and affect the quality of soybean seed and grain in the South. Previous studies indicated that resistance in PI 417479 and in PI 80837 was simply inherited. The PSD-resistant PI 417479 was used to develop the resistant line MO/PSD-0259. The objectives of this research were to; a) study the inheritance of PSD-resistance in MO/PSD-0259, b) determine if genes for PSD resistance in PI 80837 and MO/PSD-0259 are different, and c) identify SSR markers linked to PSD resistance in both lines. PSD-susceptible 'Agripro 350' and PI 80837 were crossed with MO/PSD-0259. Populations (F_2,$F_{2:3}$) were developed, grown and inoculated in field plots, and seed assayed for infection. DNA from all plants in F_2 populations was used to screen SSR markers. Segregation for seed infection in the 'Agripro 350' × MO/PSD-0259 F_2 population fit a 3:1(R:S) ratio, indicating that resistance in MO/PSD-0259 is conferred by a single dominant gene. F_2 segregation in PI 80837 × MO/PSD-0259 fit a 15:1 (R:S) ratio in two populations, and the $F_{2:3}$ lines showed a 7:8:1 (R:H:S) segregation indicating that resistance in the two lines is conferred by two different dominant genes. SSR primers producing polymorphisms were used to screen F_2 populations, and SSR markers linked to PSD resistance in PI 80837 and MO/PSD-0259 were identified.

Annual burning increases tree mortality, *Armillaria* and canker incidence. K. A. JACOBS, M. L. Bowles, and K. A. Bolger. The Morton Arboretum, Lisle, IL. Phytopathology 94:S45. Publication no. P-2004-0302-AMA.

Prescribed burning is used in oak woodlands to selectively thin invasive plants and restore native plant biodiversity. However, frequent fires may injure plants and compromise tree health. Forty, 0.125 hectare circular plots, twenty in stands that have been annually burned for 17 years, were inventoried in an oak woodland at The Morton Arboretum. Stem density, species richness and cover, canker and Armillaria root disease incidence were determined and each tree (n = 1247) was assigned a disease severity rating (DSR). The annual burn treatment reduced by ten-fold the density of small diameter trees compared with prebum conditions. Average native species coverage per plot was an order of magnitude higher for summer flowering herbs in burned (26.3) versus unburned (3.8) plots, but the reverse was true for woody plants (1.8 versus 21.6). Average DSR in which 1 = healthy and 4 = dead, was significantly (P = 0.01) greater in burned (2.4) versus unburned (1.6) plots as was the annual mortality rate between 1998 and 2002 (6% and 1.6%, respectively). The causes of mortality include *Armillaria mellea* and *A. gallica* in several species and canker disease of basswood (*Tilia americana*). Rhizomorphs were found on 60% of excavated trees in burned plots compared to 27% in unburned plots. Overall canker incidence (including fire-scar associated cankers) was almost double in the burned (38%) versus unburned plots (21%), due in part to Botryosphaeria canker of basswood. The potential for annual burning to increase tree disease ought to be weighed with long-term woodland management goals.

Evolution of fungicide resistance in experimental populations of fungi. M. D. L. A. JAIME, J. B. Anderson, and L. M. Kohn. Dept of Botany, University of Toronto, Mississauga, ON, Canada L5L 1C6. Phytopathology 94:S45. Publication no. P-2004-0303-AMA.

Fungicide resistance impedes control of plant disease. To identify the conditions that favor the emergence of fungicide resistance in fungal plant pathogens, we are studying the evolution of benomyl resistance in *Saccharomyces cerevisiae*. We are measuring the rates of mutation for resistance, the genetic mechanisms of resistance, and the fitness costs associated with resistance. *S. cerevisiae* populations were evolved under two different selection regimens: i) a stepwise increase in benomyl concentration and ii) a single, supra-lethal concentration. Under the two regimens, the trajectories of adaptation and the fitness costs of resistance differed. We are now in the process of identifying the underlying mutations responsible benomyl resistance in the artificial populations of *S. cerevisiae*. We also plan to examine the evolution of fungicide resistance in the filamentous plant pathogenic fungi *Sclerotinia sclerotiorum* and *Botrytis cinerea* and have developed an *in-planta* system for experimental evolution. Our ultimate goal is to compare the evolution of resistance in the yeast model system and in the two plant pathogens.

Spatial patterns of *Aspergillus flavus* strain S in soils of south Texas. R. JAIME-GARCIA and P. J. Cotty. USDA-Agricultural Research Service, University of Arizona, Tucson, AZ. Phytopathology 94:S45. Publication no. P-2004-0304-AMA.

Aspergillus flavus, the causal agent of aflatoxin contamination of cottonseed is a natural inhabitant of soils. *A. flavus* can be divided into two strains, S and L, with S strain isolates producing greater quantities of aflatoxins than L strain isolates. Aflatoxin contamination can be severe in several crops in South Texas. The structure of *A. flavus* communities residing in soils of South Texas was determined by analyzing 326 soil samples from 152 fields located from the Rio Grande Valley in the south to Fort Bend county in the north from 2001 through 2003. Analysis of variance indicated significant differences among regions and among fields within regions. The Coastal Bend (30.7%) and Upper Coast (25.5%) regions had significantly higher S strain incidences than the Rio Grande Valley (4.8%). Maps of S strain patterns show that the S strain constitutes over 30% of the overall *A. flavus* community in the area extending from the central Coastal Bend to the central Upper Coast regions. The western Rio Grande Valley had the lowest S strain incidence (under 10%). The S strain may be a significant cause of aflatoxin contamination in South Texas.

Rapid detection of Ginseng root rot pathogen, *Cylindrocarpon destructans*, from soil samples using molecular biological techniques. C. S. Jang (1), J. B. Ra (2), S. J. Yoo (2), J. Y. Song (1), and H. G. KIM (1). (1) Dept. of Agricultural Biology, Chungnam National University, Daejeon, 305-764, Korea; (2) Bioshield Co., Ltd., Room 2221 HTVC KAIST 373-1 Gusung-Dong, Yusung-Gu, Daejeon, 305-701, Korea. Phytopathology 94:S45. Publication no. P-2004-0305-AMA.

Diagnostic method which utilizes molecular biological technique was developed for detection of *Cylindrocarpon destructans* causing root rot disease on ginseng. In experiment based on DNA, it is difficult to find optimal condition of DNA recovery and purification from soil samples include humic substances. Fortunately, we overcame the problem and could perform PCR. This method is not only specific but also sensitive, because based on Two Step Nested PCR amplification for species-specific fragments of the internal transcribed spacer(ITS). The species-specific primer set Dest 1 and Dest 4 were used in the second round of PCR to amplify 400bp fragment from 600bp fragment of first round. In this study, it was determined that DNA yields from a spore-cell are approximately 50 fentogram in optimal condition of soil DNA extraction method tested. And the nested PCR using ITS region detected DNA of *C. destructans* even a fentogram. It means PCR is possible even though more diluted samples from DNA of a spore-cell. In application to practice, we tested variable soil samples of ginseng fields and it was so sensitive that a few spore could be detected even fellow behind paddy-upland rotation. In view of the results so far achieved, we expect that our method should be useful as reliable and rapid tool for detecting and evaluating the population of *C. destructans* in soil effectively.

Control of blue mold of apple with *Metschnikowia pulcherrima* and sodium bicarbonate using a small scale bin drencher. W. J. JANISIEWICZ (1), D. L. Peterson (1), K. S. Yoder (2), and S. S. Miller (1). (1) USDA-ARS, AFRS, Kearneysville, WV; (2) VPI&SU, Winchester, VA. Phytopathology 94:S45. Publication no. P-2004-0306-AMA.

A portable drencher capable of drenching a single bin of fruit was built to simulate commercial application of chemicals to harvested apples in small orchard operations in the Central and Eastern United States. The drencher, as designed, required as little as 125 L of the treatment solution and permitted various bin travel speeds. The portable drencher was used to determine efficacy of the yeast biocontrol agent *M. pulcherrima* alone or in combination with sodium bicarbonate (SB) in controlling blue mold caused by *Penicillium expansum* on 'Golden Delicious' and 'Delicious' apples. Wounded apples were placed midway between the bottom and top of the bin in the center and near the four corners (20 fruit in each place) and covered with apples to fill the bin. The bins were drenched with a suspension containing *P. expansum* (3×10^3 conidia/ml) alone or in combination with 2% SB, antagonist (1.2×10^7 CFU/ml), or mixture of the two. Wounded fruit were evaluated for decay incidence after 3 mo. of storage at ~2°C. On 'Delicious' apples, the lowest incidence of the decay was on fruit treated with the antagonist with SB (8.8%), followed by the antagonist alone (26%), then SB (34%), and *P. expansum* alone (66%). On 'Golden Delicious', decay incidence was 31% for *P. expansum* alone, and less than 3.4% in all other treatments. The yeast *M. pulcherrima* appears to have good potential for control of apple decays in small orchard operations.

Use of a *luxgfp* bioreporter system to determine metabolic activity of an antagonist in fruit wounds. W. J. JANISIEWICZ (1), C. Bassett (1), M. E. Wisniewski (1), W. S. Conway (2), and D. P. Roberts (3). (1) USDA-ARS, AFRS, Kearneysville, WV; (2) USDA-ARS, PQSL, Beltsville, MD; (3) USDA-ARS, SASL, Beltsville, MD. Phytopathology 94:S46. Publication no. P-2004-0307-AMA.

Competition for nutrients has often been implicated as the main mechanism in biological control of postharvest diseases (BCPD) of fruits. High metabolic activity (MA) of the antagonists in fruit wounds (site of pathogen entry) should be essential for rapid removal of the limiting nutrients and for biocontrol to occur. To determine MA of the antagonist *Pseudomonas syringae* (L-59-66) in apple wounds, we constructed a derivative strain (L-GL-26) containing the *luxgfp* cassette. Bioluminescence due to expression of the *lux* cassette in L-GL-26 was proportional to MA of the cells and was easily quantified with a luminometer. Expression of *gfp* indicated the presence of the antagonist cells but not their MA. MA of the L-GL-26 cells in apple wounds declined greatly 48 h after application, but after the addition of 80 mM asparagine, increased 3-fold within 2 h at 24°C. L-GL-26 cells applied to apple wounds after starvation in phosphate buffer for 72 h increased MA more than 100-fold after 2 h at 24°C. Our results indicate that this bioreporter system can be used to optimize conditions to maximize MA of the antagonist in apple wounds.

Characterization of novel ubiquitination-related E3 rice genes in the defense response to fungal and bacterial pathogens. C. JANTASURIYARAT, B. Zhou, and G. L. Wang. The Ohio State University, Columbus, OH. Phytopathology 94:S46. Publication no. P-2004-0308-AMA.

Ubiquitination is emerging as a common regulatory mechanism that controls a range of cellular processes in plants. Recent exciting discoveries from several laboratories suggest that ubiquitination may also play an important role in plant disease resistance. In our lab, we have recently identified several rice ubiquitination-related E3 ligase genes, which were induced in the resistance to rice blast. The expression patterns of three E3 ligase genes were confirmed in Northern analyses and showed high correlation with the microarray experiments. These results suggest that the identified E3 ligase genes are most likely involved in plant defense signal transduction. To determine the general impact of ubiquitination on plant defense it will be necessary to identify and determine the key components of the ubiquitin machinery. We are isolating the full-length cDNAs of these three E3 ligase genes and screening for their interacting proteins using the yeast two-hybrid system. The functions of these genes will be revealed by analyzing insertion mutants or over-expressed and RNAi silenced transgenic lines. The results from this research will help us understand the role of protein degradation in the defense response and may open up a new way to engineer durably resistant rice cultivars.

A potential relation of starch hydrolysis of *Paenibacillus polymyxa* to rot of korean ginseng root and chili pepper fruit. Y. H. JEON, S. G. Kim, I. Hwang, and Y. H. Kim. School of Agricultural Biotechnology and Center for Molecular Genetics and Breeding Research, Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul 151-742, Korea. Phytopathology 94:S46. Publication no. P-2004-0309-AMA.

Several strains of *Paenibacillus polymyxa* induced a rot in Korean ginseng root and chili pepper fruit at high inoculum concentrations, only at which strong starch hydrolytic activity (SHA) was detected. Transposon mutants of a virulent *P. polymyxa* strain, which showed reduced virulence, also showed reduced or nullified SHA, and were differentiated in utilizing the Biolog system from those showing virulence. However, wild strains of *P. polymyxa* could be classified into two groups (virulent and avirulent groups) only according to the ERIC and BOX-PCR analyses and 16S rDNA sequencing, which were grouped depending on SHA also. Amylase activity was detected on starch medium only when the initial density was 10^8 CFU/ml, but not when it was 10^6 CFU/ml. The differences were not related to the population changes of the bacterium in culture media as the population densities became similar at hours after inoculation. Light and electron microscopy revealed the denser but thinner colony type from the lower initial inoculum than the higher inoculum, indicating that the total populations may be similar. The bacterial cells from the higher inoculum always formed endospores, but not those from the low inoculum. Similar beta-amylase gene expressions were detected inside the bacterial cells both from low and high inocula. Transposon mutants with low starch hydrolytic activity also lost the ability of endospore formation. These results suggest that endospore formation should be related to virulence and the enzyme activity of the bacterium.

Identification of secreted proteins from the rice blast fungus. J. S. JEONG and R. A. Dean. North Carolina State University. Phytopathology 94:S46. Publication no. P-2004-0310-AMA.

Secreted proteins (secretome) mediate a variety of roles in diverse organisms. In plant-microbe interactions, invading microbes deploy a set of secreted proteins to degrade the host cell wall and to damage the host cell. Some secreted proteins are recognized by host cell, and active defense mechanisms are activated to thwart the microbial challenge. Thus, the identification of secreted proteins from microbial pathogens may contribute to the identification of key secretomes for pathogenesis. Traditionally, secreted proteins are predicted by identification of N-terminal signal peptide using computational analysis tools, notably SignalP. However, there is relatively less development of experimental strategies for identification of secreted proteins. Thus, the establishment of experimental systems to identify secreted proteins in an efficient and high-throughput manner will contribute to the validation of bioinformatic analysis. It will also offer a better chance of finding secreted proteins that are not readily detectable by computer-based analysis. In this presentation, experimental methods to identify secreted proteins from the rice blast fungus will be discussed.

Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. P. JI (1), M. T. Momol (1), S. M. Olson (1), P. M. Pradhanang (1), and J. B. Jones (2). (1) North Florida Research and Education Center, University of Florida, Quincy, FL; (2) Plant Pathology Department, University of Florida, Gainesville, FL. Phytopathology 94:S46. Publication no. P-2004-0311-AMA.

Plant essential oils, thymol and palmarosa oil, were evaluated under field conditions for control of bacterial wilt of tomato caused by *Ralstonia solanacearum* (race 1, biovar 1). Field soils that were artificially infested with the pathogen were treated with the essential oils for 3 or 6 days under plastic mulch, and tomato seedlings were transplanted into the field 10 or 13 days after the field was infested. In fall of 2002, both thymol and palmarosa oil reduced bacterial wilt incidence and increased tomato yield significantly on the susceptible tomato cultivar Equinox when used at a concentration of 0.7%. Thymol was more effective than palmarosa oil since 33.1% and 48.1% of plants wilted in thymol and palmarosa oil treated plots, respectively. In 2003 field trial, thymol significantly reduced bacterial wilt incidence on the susceptible cultivar Solar Set. Disease incidence in untreated plots reached 65.5%, while in thymol treated plots, only 12% of plants wilted. Thymol treatment also increased tomato yield significantly. These results indicated that thymol provided effective control of bacterial wilt of tomato under field conditions when used as pre-transplanting treatment of soils. Because of its volatile property and broad-spectrum efficacy, thymol shows the potential to be used as a soil biofumigant in the integrated management of various plant pathogens.

Acibenzolar-S-methyl enhanced host resistance in tomato against *Ralstonia solanacearum*. P. JI (1), M. T. Momol (1), P. M. Pradhanang (1), S. M. Olson (1), J. L. Mayfield (1), and J. B. Jones (2). (1) North Florida Research and Education Center, University of Florida, Quincy, FL; (2) Plant Pathology Department, University of Florida, Gainesville, FL. Phytopathology 94:S46. Publication no. P-2004-0312-AMA.

Acibenzolar-S-methyl (ASM, Actigard 50 WG) was investigated to determine the effect on bacterial wilt of tomato caused by *Ralstonia solanacearum* (Rs, r1, bv1) on susceptible and moderately resistant cultivars under greenhouse and field conditions. ASM was applied as foliar spray and/or soil drench before and after transplanting at concentrations ranging from 3 to 30 microgram/ml. ASM was not effective in reducing bacterial wilt incidence on susceptible tomato cultivars when tomato plants were inoculated with high concentrations of Rs. However, greenhouse studies corroborated that ASM

significantly enhanced resistance in moderately bacterial wilt resistant cultivars. It appeared that ASM-mediated enhanced resistance was due to prevention of the spread of Rs toward upper stem tissues of tomato plants. The effect of ASM on moderately resistant cultivars was consistent in repeated field trials conducted in 2002 and 2003 in Quincy, FL, where bacterial wilt incidence was significantly reduced in ASM-treated BHN 466, FL 7514, and Neptune plants. ASM treated BHN and FL 7514 produced significantly higher tomato yield than the non-treated plants. This is the first report of ASM-mediated control of bacterial wilt under field conditions, which suggests that of this treatment for moderately resistant genotypes may be effective for control of bacterial wilt in warm sub-tropical and tropical areas of the world.

Molecular evolution of rice *Pi-ta* gene and fungal *Magnaporthe grisea AVR-Pita* gene. Y. JIA (1), P. Singh (2), E. M. Winston (1), Y. Wamishe (2), J. Correll (3), and B. Valent (4). (1) USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR 72160; (2) University of Arkansas, Rice Research and Extension Center, Stuttgart, AR 72160; (3) University of Arkansas, Fayetteville, AR 72701; (4) Kansas State University, Manhattan, KS 66506. Phytopathology 94:S47. Publication no. P-2004-0313-AMA.

Rapid changes in *Magnaporthe grisea* avirulence genes often defeat resistance gene mediated resistance. The *Pi-ta* resistance gene in rice is effective in preventing infections by *Magnaporthe grisea* isolates containing the corresponding avirulence gene *AVR-Pita*. Pi-ta is a putative cytoplasmic nucleotide binding site receptor that appears to interact with AVR-Pita directly in triggering the resistance. AVR-Pita is a predicted metalloprotease that may play roles in pathogenicity and fitness. In the Southern US, *Pi-ta* has been effective since its release in the 1980s. Fifteen *AVR-Pita* alleles from four MGR 586 DNA fingerprint groups A, B, C and D of the US field *M. grisea* isolates were sequenced. All 15 have an identical AVR-Pita amino acid sequence which has six amino acid alterations from published AVR-Pita protein. In contrast, most isolates regaining the ability to infect *Pi-ta* containing plants did not have an intact *AVR-Pita* allele. Analysis of coevolution of both *Pi-ta* and *AVR-Pita* and dual roles of *AVR-Pita* will be presented.

Development and characterization of rice mutant populations for functional genomics of host-pathogen interactions. Y. JIA (1), J. N. Rutger (1), Z. Wang (2), P. Singh (3), R. Martin (3), and R. M. Pinson (4). (1) USDA-ARS DB National Rice Research Center, Stuttgart, AR 72160; (2) Zhejiang Wanli University, P. R. China; (3) Oak Ridge National Laboratory, Oak Ridge, TN 37831; (4) USDA-ARS Rice Research Unit, Beaumont, TX. Phytopathology 94:S47. Publication no. P-2004-0314-AMA.

Understanding molecular mechanisms of *R* gene mediated defense responses should facilitate the development of both conventional and novel strategies for disease control. To dissect *R* gene-mediated signal transduction pathways the blast resistant US rice cultivar Katy was mutagenized by ethyl methane sulfonate (EMS) and fast neutrons. For EMS, seeds were soaked in 0.4%, 0.8% and 1.2% EMS solutions for 6 hours and a total of 7,199 M1 mutant lines was recovered. For fast neutron, seven dosages of fast neutron were applied and a total of 15,400 M1 mutant lines was recovered from treatments with 7.7 Gray, 26.3 Gray and 49.4 Gray. Preliminary analysis of M2 seedlings revealed defects in chlorophyll synthesis in approximately 4% of M1 derived lines. Katy containing the *Pi-ta* gene prevents infection of *Magnaporthe grisea* races in a gene for gene manner. *M. grisea* races containing the avirulence gene *AVR-Pita* are being used to identify blast susceptible mutants. A total of 42 blast susceptible mutants and 7 lesion mimic mutants were identified. Progress on characterization of these mutants will be reported.

Population structure of *Sclerotinia sclerotiorum* in a pea field in the Pacific Northwest. I. JIMENEZ-HIDALGO (1), N. J. Grunwald (1), M. Kohn (2), and W. Chen (3). (1) Washington State University and USDA-ARS, Prosser, WA; (2) University of Toronto; (3) Washington State University, Pullman, WA. Phytopathology 94:S47. Publication no. P-2004-0315-AMA.

The population structure of *Sclerotinia sclerotiorum* on canola and on soybean in central Canada is part of one population of the pathogen that is mainly clonal with some local subdivision. The objective of our research is to describe the population structure of *S. sclerotiorum* in an intensively sampled field of pea. Fifty isolates were obtained from sclerotia collected from inside diseased stems or from plant tissue excised from the margins of stem lesions. AFLP, microsatellite markers and mycelial compatibility grouping were used to describe genotypes of the pathogen population. Two AFLP primer combinations were used and in one, 45 different fingerprints were identified, while in the other 50 fingerprints were observed. No common AFLP fingerprint patterns were detected between isolates retrieved from the WA pea field and 10 Canadian isolates derived from canola and soybean, used as reference isolates. Twenty-six different MCGs were observed and 6 of these were represented by more than one DNA fingerprint (between 2 and 6 fingerprints). Four clones based on MCGs and AFLP fingerprints were identified. Some isolates were compatible with isolates in several MCGs. Non-transitivity of MCG testing was observed in 10 cases. Using four microsatellite primer sets, 9 microsatellite profiles were identified in pea isolates and they differed from the reference isolates. The population of *S. sclerotiorum* in pea has a higher genotypic diversity in the Pacific Northwest than expected from previous studies.

Pathogen phenotypes used to identify sources of resistance to rust from a specific common bean gene pool in southern Mozambique. C. Jochua (1), J. STEADMAN (1), M. Amane (2), and J. Fenton (1). (1) Plant Pathology Department, University of Nebraska-Lincoln, NE 68583; (2) Instituto Nacional de Investigacao Agronomica, Maputo, Mozambique. Phytopathology 94:S47. Publication no. P-2004-0316-AMA.

Rust caused by *Uromyces appendiculatus* is one of the major constraints to snap and dry edible bean production in southern Mozambique. Virulence patterns of 69 *U. appendiculatus* isolates collected from large and small fields located in southern Mozambique were derived from disease reactions on 12 bean rust differentials. These differentials are composed of common bean, *Phaseolus vulgaris*, lines with resistance genes from both Andean and Middle American common bean gene pool. The Andean gene pool sources of rust resistance were found to be generally susceptible to the 69 pathotypes of the bean rust pathogen. The Middle American common bean gene pool, e.g., genes Ur-3, Ur-5 and Ur-11, were resistant to 90, 93, and 100% of the isolates, respectively, and provided sources of both single and multiple genes conferring resistance to the 69 pathotypes found in this part of southern Africa.

Use of burley tobacco cultivars and fungicides to control blue mold in Virginia – 2001–2003. C. S. JOHNSON (1,2), D. R. Peek (2), and L. Wright (3). (1) Dept. of Plant Pathology, Physiology, and Weed Science, Virginia Tech; (2) So. Piedmont Agric. Res. & Ext. Ctr, Blackstone, VA 23824; (3) SW Virginia Agric. Res. & Ext. Ctr, Glade Spring, VA 24043. Phytopathology 94:S47. Publication no. P-2004-0317-AMA.

Peronospora tabacina (tobacco blue mold) remains one of the most serious disease problems of burley tobacco in Virginia. Although effective fungicides are available to minimize crop losses caused by blue mold, many producers are unable to achieve sufficient coverage of tobacco foliage to control the disease. Experiments were conducted in 2001 through 2003 to evaluate potential differences among burley cultivars in disease severity, yield, and economic value under various fungicide application regimes. Application of dimethomorph and mancozeb or acibenzolar-S-methyl generally improved blue mold control on all cultivars, but disease severity was also consistently lower on cultivars such as R 630, KT 200, and TN 90 compared with popular blue-mold susceptible cultivars Clay's 403 and NC BH129. Blue mold severity was significantly lower on NC 2000 than on most other cultivars in each of seven field experiments. Unfortunately, the yield and quality characteristics of the cultivars with reduced blue mold severity are often not competitive with the traits of cultivars more susceptible to the disease. Judicious selection of cultivars less susceptible to tobacco blue mold combined with appropriate fungicide use should enable farmers to reduce crop losses to this disease.

Factors influencing populations of plant parasitic nematodes on golf course greens in New England. K. S. JORDAN and N. A. Mitkowski. University of Rhode Island, Kingston, RI. Phytopathology 94:S47. Publication no. P-2004-0318-AMA.

Although generally considered a stress-related disease in the northeast, damage from nematodes has been observed on golf course greens throughout New England. The primary objective of this study is to identify and quantify populations of turf-parasitic nematodes in New England, and to correlate these levels with soil characteristics and management practices. Samples were collected in mid-spring, early summer, and early fall from 114 greens sampled from 38 golf courses in Connecticut, Rhode Island, and Massachusetts. Nematodes from 5 different genera were identified – the most common of which were *Criconemella* and *Tylenchorhynchus*. Data collected regarding soil characteristics and management practices included green age, soil pH, texture, bulk density, organic matter, nutrient levels, turfgrass height of cut, topdressing frequency, and chemical applications. Preliminary results from regression analysis indicate that green age was positively correlated with stunt and lance populations, and that bulk density was negatively correlated with spiral nematode populations. This study will be repeated during the 2004 season.

Complete nucleotide sequence and characterization of pBJS, a novel plasmid of the phytopathogen *Spiroplasma citri* BR3-3X. B. D. JOSHI, J. Rogers, J. Fletcher, and U. Melcher. Oklahoma State University, Stillwater, OK. Phytopathology 94:S47. Publication no. P-2004-0319-AMA.

The complete nucleotide sequence of a high copy number plasmid, pBJS, of the plant pathogenic mollicute *Spiroplasma citri* BR3-3X was determined. The sequence is 13 kb in length and encodes eight putative open reading frames (ORFs), two of which have no homologs in other bacteria. One of the ORFs with homologs (*arp1*), characterized previously from *S. citri* BR3-T, a derivative of BR3-3X, encodes the spiroplasma adhesion related protein 1 (SARP1), and another encodes the putative homolog of an adherence factor of the mouse pneumonitis agent, *Chlamydia muridarum*. SARP1 is a putative adhesin hypothesized to be necessary for the transmission of *S. citri* by its major U.S. vector, the beet leafhopper *Circulifer tenellus*. The rest of the ORFs (*soj*, *traE* and *mob*), except for the small spiroplasma viral pseudogene (*sv-orf4*) with an unknown function, are homologous to the genes involved in chromosome partitioning, ATP-dependent DNA transfer and mobilization, respectively, in other bacterial genera. The presence of genes such as *arp1*, *soj*, *traE* and *mob* as a cluster on pBJS may contribute to the acquisition and stability of the plasmid in spiroplasmas, in addition to influencing their leafhopper transmissibility and plant pathogenicity.

Identification and characterization of a *fas* operon on the *Streptomyces turgidiscabies* pathogenicity island. M. V. JOSHI, K. D. Cameron, J. A. Kers, and R. Loria. Department of Plant Pathology, Cornell University, Ithaca, NY 14853. Phytopathology 94:S48. Publication no. P-2004-0320-AMA.

Streptomyces turgidiscabies causes common scab of potato. DNA sequencing of a pathogenicity island (PAI) in *S. turgidiscabies* led us to discover a large operon carrying five ORFs with high similarity (67-84%) to five of the six ORFs in the *fas* operon of *Rhodococcus fascians*. The homologous ORFs are co-linear in the two species. The *fas* operon confers cytokinin production by *R. fascians* and is required for pathogenicity. The *fas* operon is absent in the scab pathogens *S. scabies* and *S. acidiscabies* and has a G+C content lower than *Streptomyces* DNA. These data suggest that the *fas* locus is a recent acquisition of the *S. turgidiscabies* PAI. The *fas* operon contains a gene that encodes an isopentenyltransferases; these proteins catalyze the first dedicated step in cytokinin biosynthesis. Tobacco plants inoculated with a thaxtomin-deficient mutant of *S. turgidiscabies* develop leafy galls at the point of inoculation. Additional characterization of the *S. turgidiscabies fas* operon is underway.

Gene expression and signal transduction during spore development in *Phytophthora infestans*. H. S. JUDELSON, A. Ah Fong, F. Blanco, K. S. Kim, and S. Tani. University of California, Riverside, CA. Phytopathology 94:S48. Publication no. P-2004-0321-AMA.

Spores represent the main infectious propagules of the oomycete plant pathogens, including *Phytophthora* spp. To understand the molecular and cellular events involved in their formation and germination, an integrated genetic, biochemical, and pharmacological approach is being used with *P. infestans*. Expression profiling experiments using cDNA arrays and micro-arrays identified several hundred genes that exhibit major changes in mRNA abundance during the formation and/or germination of spores. Distinct classes in their pattern of expression was revealed by studying the effects of chemical inhibitors and mutations, and by performing timecourses of development. Genes with potential regulatory roles, such as kinases and transcription factors, have been targeted for detailed analyses. To better understand their functions, transformants silenced in their expression have been generated, interacting proteins identified using the yeast two-hybrid approach, and cellular localizations determined using GFP fusions. Silencing of some genes, for example, has resulted in deficiencies in sporulation or zoospore behavior. In addition, transformants expressing promoter-GUS fusions have been generated, resulting in the identification of sporulation and zoosporogenesis-specific promoters. Deletion analyses of these promoters is identifying potential binding sites for transcription factors, and the study of these factors will help identify early events required for the formation or germination of the spores.

Fungi and mycotoxins associated with pearl millet from 1996 to 2002. Z. JURJEVIC (1), D. M. Wilson (1), J. P. Wilson (2), and M. Coulibaly (1). (1) University of Georgia, Tifton, GA; (2) USDA-ARS, Tifton, GA. Phytopathology 94:S48. Publication no. P-2004-0322-AMA.

Pearl millet (*Pennisetum glaucum*) samples collected from the 1996 to 2002 harvests were analyzed for fungi and mycotoxins. The most common fungi isolated were *Fusarium chlamydosporum*, *F. semitectum*, *Curvularia* spp., *Alternaria* spp., "*Helminthosporium*" spp., *Aspergillus flavus*, *F. verticilloides* and *F. pseudonygamai*. Less frequently found were *Aspergillus niger*, *A. ochraceus*, *A. tamari*, *A. candidus*, *A. fumigatus*, *Cladosporium herbarum*, *Cerebella andropogonis*, *Epicoccum* spp., *Gloeocercospora sorghi*, *Penicillium* spp., *Phyllosticta penicillariae*, *Rhizopus* spp., *Trichoderma* spp., and other fungi. Aflatoxins ranged from 0 to 87 ng/g in the field samples, and increased in storage up to 1750 ng/g depending on the year. Beauvericin was found in field samples from 23 to 1044 ng/g and during storage from 108 to 2438 ng/g. Moniliformin was found in the field samples from 0 to 175 ng/g and did not increase during storage. Negligible levels of deoxynivalenol, nivalenol, and zearalenone were detected. Fumonisins were not detected in either the field or storage.

A comparision of volatile metabolites produced by aflatoxin-tolerant and aflatoxin-susceptible peanut plants. Z. JURJEVIC (1), M. Tertuliano (1), G. C. Rains (1), D. M. Wilson (1), C. C. Holbrook (2), and W. J. Lewis (2). (1) University of Georgia, Tifton, GA; (2) USDA-ARS, Tifton, GA. Phytopathology 94:S48. Publication no. P-2004-0323-AMA.

Volatiles were characterized from aflatoxin-tolerant and aflatoxin-susceptible peanut plants. Volatiles were collected by trapping headspace volatiles using thermal desorption columns packed with Tenax TA and Carbotrap B. Columns were placed in a thermal desorption unit which injected the volatiles into a GC/MS for analysis and identification. All plants were inoculated with *Aspergillus flavus* NRRL 3357 (afla-toxigenic) or NRRL 1957 (non-afla-toxigenic). In a preliminary study predominant volatile metabolites common to all peanuts were 3-hexen-1-ol, (Z); 3-octanone; 3-hexen-1-ol, acetate (Z); 1,3-butadiene; 1-octen-3-on; 2-methyl; and 1,3-pentadiene. Volatiles associated with tolerant peanuts were cyclopropane, ethyidene; 2-butenal; and hexanal. Volatiles associated with susceptible peanut plants were cyclobutane, methylene; 1,3-butadiene, 2-methyl; 2-butanone; butanal, 2-methyl; 1,3-octadiene. The volatile profiles were different and may possibly be useful for early aflatoxin detection.

Genetic diversity of *Mycosphaerella graminicola*, the *Septoria tritici* blotch pathogen of wheat in Kansas. M. ABBAGE (1), K. A. Zeller (2), S. H. Hulbert (1), and W. W. Bockus (1). (1) Department of Plant Pathology, Kansas State University, Manhattan, KS; (2) USDA-APHIS, Laurel, MD. Phytopathology 94:S48. Publication no. P-2004-0324-AMA.

The genetic diversity of natural populations of *Mycosphaerella graminicola* has not been investigated in the central Great Plains of the USA, one of the most important wheat producing areas in the world. Studies of this fungus from other regions show regular recombination and significant gene flow. This study, in addition to investigating the statewide genetic diversity of this pathogen, focused on comparing fall versus spring populations. Genetic diversity was investigated at three levels, the entire state, an entire field, and micro-plot (~1 m2). For statewide diversity, isolates were obtained from 60 fields throughout Kansas. Micro-plot and field populations were examined at four different locations and at least 50 isolates were obtained from each site at each sample date. Pycnidiospores were isolated from single pycnidia on leaf lesions and grown on YG medium for DNA extraction. AFLP profiles of all isolates were generated to study each of the populations. Initial data show high diversity among Kansas isolates collected in the fall. This suggests that the sexual stage is important for initial infections. AFLP profiles from isolates obtained in the spring will be compared with those from the fall.

Characterization of a tobamovirus from tropical soda apple. I. KAMENOVA (1), E. N. Rosskopf (1), D. J. Lewandowski (2), and S. Adkins (1). (1) USDA-ARS-USHRL, Fort Pierce, FL 34945; (2) Univ. of Florida, CREC, Lake Alfred, FL 33850. Phytopathology 94:S48. Publication no. P-2004-0325-AMA.

Foliar symptoms suggestive of virus infection were recently observed on the noxious weed, tropical soda apple (*Solanum viarum*), in Florida. An agent was mechanically transmitted to *Nicotiana benthamiana* and virions were isolated from systemically infected leaves. Rod-shaped particles ~300 nm in length were observed in the purified preparations by electron microscopy. The host range determined by mechanical inoculation with purified virions included predominantly plants from the *Solanaceae*. Purified virions reacted both with IgG specific for *Tobacco mosaic virus* and also with IgG that recognizes TMV and related tobamoviruses (including *Tomato mosaic virus*) in indirect enzyme-linked immunosorbent assay. Sequence comparisons of reverse transcription-polymerase chain reaction products amplified from total RNA of infected *N. benthamiana* using primers specific for the coat protein (CP) of *Pepper mild mottle virus* (PMMoV) indicated that the CP sequence of the isolated virus was most closely related to PMMoV, with CP nucleotide and amino acid identities of 80 and 83%, respectively.

Monitoring *Phytophthora ramorum* in Oregon coastal forests. A. Kanaskie (1), M. McWilliams (1), J. Mair (1), J. Laine (1), J. Beeson (1), E. Goheen (2), R. Schroeter (2), E. Hansen (3), W. Sutton (3), N. OSTERBAUER (4), and L. Rehms (4). (1) Oregon Dept. of Forestry, Salem; (2) USDA Forest Service, Central Point, OR; (3) Oregon State University, Corvallis; (4) Oregon Dept. of Agriculture, Salem. Phytopathology 94:S48. Publication no. P-2004-0326-AMA.

Phytophthora ramorum was first detected in forests near Brookings (Curry County), Oregon in July 2001. By 2002, it was found infesting 21 sites (19.4 ha). In 2003, 12 new sites were detected by ground and aerial surveys. Infected trees were also found near eight previously known (old) sites. These discoveries add 4.9 ha to the total area infested. Most of the new sites occurred within 200-m of the old sites. However, three sites were found 2.9-, 1.3-, and 0.4-km from the nearest old site. As in 2002, new infections occurred either close to or in a northerly direction (along prevailing rain and wind patterns) from the previously known sites. *P. ramorum* was also detected in streams associated with infested sites and in a single rainwater trap located beneath an infected tan oak. As a result of the survey, the quarantined area in Oregon will increase from 14.5-km^2 to 17.7-km^2. Efforts to eradicate the pathogen will continue on the 24.3 infested hectares.

Developing a risk analysis strategy for *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) infestation of tomato seed. W. S. KANESHIRO and A. M. Alvarez. Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI 96822. Phytopathology 94:S49. Publication no. P-2004-0327-AMA.

Pathogen threshold levels required for plant infection and subsequent seed transmission are largely unknown. The goal of this study was to develop a risk analysis strategy for seedborne *Cmm* to predict plant infection based on pathogen concentrations in the initial seed lot. Seeds from three infested lots were assayed for two years to determine the change in *Cmm* populations over time. Germination percentage was determined for one lot, and resulting plants observed for symptoms. In two lots, *Cmm* populations decreased dramatically within two to six months post-harvest. In the third lot, *Cmm* titers were consistently high, indicating infestation is highly variable even between lots of the same tomato variety. Germination was reduced 40% with *Cmm* infestation. Of the emergent seedlings, 70% developed symptoms and one-third of these recovered and matured into asymptomatic plants. Population distributions, initial pathogen concentrations per seed, survival, seed infestation percentage, and the number of latent infections in surviving plants are the factors used to assess the risk of disease in second-generation plants.

Baseline sensitivities of *Penicillium digitatum* populations to azoxystrobin, fludioxonil, and pyrimethanil. L. KANETIS, A. Soto-Estrada, and J. E. Adaskaveg. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521. Phytopathology 94:S49. Publication no. P-2004-0328-AMA.

Green mold, caused by *Penicillium digitatum*, is the most important postharvest disease of citrus fruits in California. Currently, the fungicide component of an integrated management program is based mainly on use of the fungicides imazalil and thiabendazole. Because of the continuous use of these fungicides for more than twenty years, control has become problematic due to development of fungicide resistance in the pathogen. For the first time ever, three new fungicides (azoxystrobin, fludioxonil, and pyrimethanil) all belonging to different chemical classes, are planned for registration for postharvest use on citrus in the US. To establish baseline sensitivities of *P. digitatum* populations to these fungicides before their commercial use, 67 isolates of the pathogen were assayed using the spiral gradient dilution (SGD) method. Isolates were collected from groves and packinghouses throughout the citrus growing areas of California. Baseline EC_{50} values ranged from 0.008 to 0.025 (avg. = 0.014) mg/L for azoxystrobin, from 0.012 to 0.100 (avg. = 0.030) mg/L for fludioxonil and from 0.210 to 0.436 (avg. = 0.328) mg/L for pyrimethanil. No cross-resistance was detected between the old and new materials. EC_{50} values obtained using the SGD method were very similar to those obtained using the serial dilution method. This information is important for monitoring future shifts in population sensitivities and will aid in prolonging the efficient usage of these new materials.

Lipopolysacchride O-antigen of *Ralstonia solanacearum* contributes to virulence on tomato plants. Y. Kang, H. Liu, R. W. Carlson, and T. P. DENNY. The University of Georgia, Athens, GA. Phytopathology 94:S49. Publication no. P-2004-0329-AMA.

Lipopolysacchride (LPS) is a major constituent of the *Ralstonia solanacearum* outer membrane. The O-antigen sidechain of LPS projects outward from the membrane and is a primary interface between the bacterium and its environment. To evaluate the role of the O-antigen in pathogenesis, we attempted to eliminate this moiety without altering the remaining LPS molecule or the extracellular polysaccharide (EPS1) by site-specifically inactivating putative genes encoding either an undecaprenyl phosphate N-acetylglucosaminyl transferase (*wecA*; RS01594) or a lipid A core:O-antigen ligase (*waaL*; RS01395). Gel electrophoresis and composition analysis of purified LPS indicated that the *wecA* mutant produces at most a single O-antigen oligosaccharide repeat unit, whereas the *waaL* mutant completely lacks the O-antigen. Both of the mutants were resistant to an LPS-specific phage, but more sensitive to the antibiotics carbenicillin and novobiocin. Both mutants were comparable to the wild type in EPS1 production, growth rate in culture, short-term survival in potting soil, natural transformation, autoaggregation, biofilm formation, twitching motility, swimming motility, and elicitation of the hypersensitive reaction when infiltrated into tobacco leaves. In addition, both mutants were dramatically less virulent than the wild type on tomato plants when inoculated via wounded petioles or unwounded roots. The mechanism by which the O-antigen contributes to virulence is not known.

***AvrPto* suppresses nonhost pathogen induced cell death in *Nicotiana benthamiana* and tomato.** L. KANG (1), C. Ryu (1), X. Tang (2), and K. S. Mysore (1). (1) Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK; (2) Dept. of Plant Pathology, Kansas State University, Manhattan, KS. Phytopathology 94:S49. Publication no. P-2004-0330-AMA.

Many bacterial pathogens rely on a type III secretion system to deliver a number of effector proteins into host cell. Though many effectors have been shown to contribute to bacterial pathogenicity, function of bacterial effectors remains elusive. *Pseudomonas syringae* pv. *tomato* T1 causes a nonhost type hypersensitive response (HR) on *Nicotiana benthamiana* plants. Interestingly, nonhost HR-mediated rapid cell death was significantly delayed in plants inoculated with *P. s.* pv. *tomato* T1A, expressing the effector *avrPto*. In another nonhost interaction, *P. s.* pv. *tabaci* expressing *avrPto* also suppressed nonhost HR on tomato *prf3* and *ptoS* lines. Transient expression of *avrPto*, by *Agrobacterium*, in both *N. benthamiana* and tomato was able to suppress nonhost HRs. *AvrPto* failed to suppress cell death caused by *Erwinia amylovora* and chemicals. Experiments with *AvrPto* mutants revealed several amino acid residues important for the cell death suppression. G2A, G99V and a C-terminal deletion mutant partially lost suppression effects in both plant species while S94P and I96T mutants enhanced suppression ability in *N. benthamiana*. These results, together with others discovery, demonstrate that suppression of host programmed cell death is one of the common strategies bacterial pathogens use for its successful invasion.

***Tobacco mosaic virus* – A versatile tool for production of biomedicals in plants.** A. V. KARASEV and H. Koprowski. Thomas Jefferson University, Philadelphia, PA. Phytopathology 94:S49. Publication no. P-2004-0331-AMA.

Transient expression of medicinal proteins in plants is fast, efficient, and economical way to produce compounds for prophylactic and treatment of infectious diseases. *Tobacco mosaic virus* (TMV) was first to be used as a vector for production of enzymes, antibodies, and other value-added products in plants. At the moment, TMV has been developed for expression of various vaccine components and antibodies in several vector designs - gene addition, gene replacement, and different variants of homologous and heterologous capsid protein fusions. We present here several examples of transient production of vaccine components against HIV-1, hepatitis B, anthrax, and SARS virus, utilizing some of these vector designs. Main advantages of TMV as a vector are high production yield, rapid infection/expression cycle, and versatility. Some of the typical problems will also be discussed.

Induction of soil suppressiveness against *Rhizoctonia solani* by incorporation of specific dried plant residues into soil. M. Kasuya, A. R. Olivier, Y. Wada, H. Honjo, and R. FUKUI. Utsunomiya University, Tochigi, Japan. Phytopathology 94:S49. Publication no. P-2004-0332-AMA.

The effects of pathogen suppressiveness by dried residues of 12 cultivars of *Brassica rapa* on damping-off of sugar beet was evaluated in soils infested with *Rhizoctonia solani* AG2-2. Sugar beet seeds were sown in soils amended with individual residues of *B. rapa* (1% w/w); the residues of *Trifolium pratense* and/or *Arachis hypogaea* also were tested as the non-cruciferous control. Ten days after sowing, damping off was significantly suppressed in the soils treated with *B. rapa* 'Saori', clover, and peanut. The residues of the other 11 cultivars of *B. rapa* did not induce any disease suppression, or the effects were either minimal or inconsistent. The disease suppressiveness of the residue-amended soils was significantly diminished or totally nullified when antibacterial antibiotics were applied to the soils, suggesting that antagonistic bacteria were enriched in the residue-incorporated soils and thus were responsible for disease suppression. To determine the site of microbial interaction, the seeds that had been colonized by *R. solani* in the soil containing no residues were re-planted into the soils containing the residues. All soils with the residues were suppressive in this case. In contrast, when the seeds that had been colonized by microorganisms in the soils containing the individual residues were re-planted into the inoculated soil, damping-off was not suppressed in any cases. The results suggest that pathogen suppressiveness induced in the residue-amended soils is dependent on the kinds of residues (i.e., species and/or cultivars of the plants), and that the laimosphere is the site for microbial interaction, which is the chief principle of disease suppression.

Sensitivity of isolates of *Phytophthora capsici* from South Carolina to mefenoxam, dimethomorph, zoxamide and cymoxanil. A. P. KEINATH. Clemson University, Coastal REC, Charleston, SC 29414-5329. Phytopathology 94:S50. Publication no. P-2004-0333-AMA.

In fall 2003, *P. capsici* was isolated from fruit, crowns, and roots of pepper and squash plants exhibiting symptoms of Phytophthora blight. Isolates (120) were collected from six fields in two counties in South Carolina. All isolates were grown on V8 agar (V8A) amended with 0 or 100 mg/L mefenoxam. Based on relative colony diameters (RCD), 8 isolates were insensitive (RCD >90%), 60 isolates were sensitive (RCD <30%) and 52 isolates were moderately insensitive to mefenoxam. Fifty-four isolates were grown on V8A not amended or amended with three concentrations of dimethomorph or five concentrations of zoxamide. Fifteen isolates also were grown on V8A not amended or amended with six concentrations of cymoxanil. EC_{50} values for all isolates were between 0.1 and 0.3 mg/L dimethomorph and 0.03 and 3 mg/L zoxamide. EC_{50} for cymoxanil were >10 and >50 mg/L for 2 and 13 isolates, respectively. In 2003, isolates of *P. capsici* in South Carolina were sensitive to dimethomorph and zoxamide, moderately sensitive to cymoxanil, and sensitive or moderately insensitive to mefenoxam.

Effect of pesticides on recovery of *Didymella bryoniae* from cucurbit vines. A. P. KEINATH. Clemson Univ., Coastal REC, Charleston, SC 29414-5329. Phytopathology 94:S50. Publication no. P-2004-0334-AMA.

Didymella bryoniae, the fungus that causes gummy stem blight, survives between crops in cucurbit debris. A pesticide that eliminates the fungus from infested debris would reduce initial inoculum for subsequent crops planted in infested fields. Naturally infected, 5-cm muskmelon vine sections were sprayed with field-equivalent rates of three herbicides, four fungicides, six salts, three botanical extracts, or three organic pesticides. After 3 days, vine sections were cut into 1-cm pieces and cultured on 1/4 PDA plus antibiotics. Each pesticide was tested 2 to 4 times with 10 to 20 vine sections per treatment. Chlorothalonil, mancozeb, sodium bisulfite, and pyraclostrobin + boscalid (Pristine) consistently reduced recovery of *D. bryoniae* to an average of 63, 57, 41, and 8% of the vine pieces, respectively, compared to a water-treated control (99%). The other pesticides did not significantly reduce recovery of the fungus. Using Pristine to treat debris at the end of the season is not advisable, because of the risk of resistance to this fungicide. However, a non-specific material, such as a broad-spectrum fungicide or a salt, could be used to reduce the amount of surviving inoculum.

Hawaiian grown guava as a model for studying plant disease. L. M. KEITH and F. T. Zee. USDA-ARS, PBARC, Hilo, HI 96720. Phytopathology 94:S50. Publication no. P-2004-0335-AMA.

Guava is one of the most vigorous and widespread plants in the tropics and is associated with many fruit rot diseases. Guava is commonly processed into puree and juice so disease can decrease its marketability. In Hawaii, common guava (*Psidium guajava* L.) is found throughout the island at a variety of elevations and environmental conditions. Due to its widespread distribution, guava serves as an excellent model for studying disease. A survey was conducted at the Waiakea Agricultural Experiment Station where there are 50 accessions of guava grown. Disease symptoms were visible on leaves without fruit present and on the skin of young fruits which progressed as fruits matured. Disease ratings were developed to describe symptom severity. Samples of fruit and leaves were collected, photographed and aseptically cultured on potato dextrose agar. One of the main fungi consistently isolated from leaves and fruit was *Pestalotiopsis* sp. Morphology, colony characteristics, and pathogenicity of the isolates was examined and potential sources of host resistance were identified for germplasm characterization studies. The importance of *Pestalotiopsis* as a guava pathogen and its cross-infection potential will be discussed.

Antimicrobial and insecticidal properties of a protein isolated from seeds of the tropical forage legume *Clitoria ternatea* (L.). S. KELEMU, C. Cardona, and G. Segura. Centro Internacional de Agricultura Tropical (CIAT), A. A. 6713, Colombia, South America. Phytopathology 94:S50. Publication no. P-2004-0336-AMA.

Clitoria ternatea, a tropical forage legume, has important agronomic traits such as adaptation to a wide range of soil conditions, resistance to drought, pathogens and pests. Extracts from seeds of *C. ternatea* (L.) exhibited strong antifungal activity on the test fungus *Rhizoctonia solani in vitro*. Antimicrobial proteins and peptides are widely distributed in nature and are synthesized by plants, bacteria, insects, fungi, and mammals. Plant seeds use various strategies, including the synthesis of proteins and peptides that have antimicrobial and/or insecticidal activities, to germinate and survive in the soil that is densely inhabited with a wide range of micro- and macroorganisms. A highly basic protein was purified to homogeneity from seeds of *C. ternatea* by using ultrafiltration with Centricon-3 membrane tubes, and preparative granulated bed isoelectric focusing. The protein, designated Finotin, has a potent, nonspecific growth inhibitory effect on various important plant pathogenic fungi namely *Rhizoctonia solani*, *Fusarium solani*, *Colletotrichum lindemuthianum*, *Lasiodiplodia theobromae*, *Pyricularia grisea*, *Bipolaris oryzae* and *Colletotrichum gloeosporioides*, as well as the common bean bacterial blight pathogen *Xanthomonas axonopodis* pv. *phaseoli*. In addition, Finotin is strongly inhibitory to the bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus*. The potential use of this protein will be discussed.

Development of a fungal disease risk index for peanuts. R. C. KEMERAIT, Jr., T. B. Brenneman, A. K. Culbreath, and J. E. Woodward. Department of Plant Pathology, University of Georgia, Tifton, GA 31794. Phytopathology 94:S50. Publication no. P-2004-0337-AMA.

Fungal diseases of peanut cost growers millions of dollars each year in Georgia. Production practices, such as variety selection, tillage, row patterns (twin or single rows), irrigation, planting date, and crop rotation, have impact on potential for disease. A risk index has been developed to estimate the impact of these production practices on leaf spot diseases, southern stem rot, and Rhizoctonia limb rot in the field. Each practice is assigned a weighted point value. For example, variety selection and crop rotation have greater impact on disease risk than do tillage and row pattern and are assigned a greater number of points. Growers calculate a point total for each disease and determine if this value corresponds to a high, moderate, or low risk situation. In 2004, fungicide trials were conducted at several sites in Georgia. Where risk potential was calculated as low or moderate, reduced input fungicide programs were compared to full-season programs. Fungicide programs with reduced inputs were often more cost effective, considering yield and cost of production, than were full-season programs.

Survival of grape downy mildew (*Plasmopara viticola*) sporangia and lesions under field conditions. M. M. KENNELLY (1), R. C. Seem (1), D. M. Gadoury (1), W. F. Wilcox (1), and P. A. Magarey (2). (1) Dept. Plant Pathology, Cornell University, NY Agric. Exp. Stn., Geneva, NY 14456; (2) South Australian Research and Development Institute, Loxton SA 5333. Phytopathology 94:S50. Publication no. P-2004-0338-AMA.

Most forecasters for grape downy mildew use spore survival data from lab studies that may not reflect field conditions, and most models do not incorporate changes in lesion productivity. Our objective was to quantify spore viability and lesion longevity in the field. We exposed sporangia to field conditions for various lengths of time and measured ability of sporangia in water to release zoospores quickly (in 3-4 hr) and slowly (after >18 hr). After 8 hr exposure on warm days (high temp 29-30°C, RH 20-30%) quick germination of sporangia declined to ~0%. Some sporangia recovered with time: slow germination rates were 6-18%. After 8 hr exposure on cool, overcast days (17-24°C, RH >72), quick germination was 45-71% and slow germination was >80%. Studies of lesion viability mostly confirmed prior results in which we described a logarithmic decline in spore production with repeated sporulations. However, one day with extreme temperatures (>42°C) can permanently reduce sporulation to trace levels.

Translocation of metalaxyl to grape clusters from leaf and stem tissue controls grapevine downy mildew (*Plasmopara viticola*). M. M. KENNELLY, R. C. Seem, D. M. Gadoury, and W. F. Wilcox. Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456. Phytopathology 94:S50. Publication no. P-2004-0339-AMA.

Systemic movement of metalaxyl from roots to leaves has been reported to control a number of oomycete pathogens, but systemic movement from vegetative organs into fruit has been thought not to occur. We inoculated fruit clusters of Chardonnay, Riesling, Niagara, or Concord grapevines with *P. viticola* at prebloom, bloom, or 1 wk postbloom. We then used a paintbrush or small trigger bottle to apply metalaxyl (288 mg a.i./L) to the leaves and/or stem tissue 12 to 48 hours after inoculation. During application clusters were covered with plastic bags. No downy mildew developed on clusters when metalaxyl was applied to either leaf tissue, stem tissue, or both. Downy mildew symptoms were severe on the non-sprayed, inoculated control clusters. Parallel experiments using azoxystrobin indicated no comparable systemic movement from foliage to fruit. Systemic movement may enhance performance of metalaxyl against fruit infection, especially in dense canopies where spray penetration is reduced.

Contributions of oosporic inoculum to epidemics of grapevine downy mildew (*Plasmopara viticola*). M. M. KENNELLY (1), C. Eugster (2), D. M. Gadoury (1), C. D. Smart (1), R. C. Seem (1), D. Gobbin (2), and C. Gessler (2). (1) Dept. Plant Pathology, Cornell University NYSAES, Geneva, NY 14456; (2) Dept. Plant Pathology, Swiss Fed. Inst. Technol., CH-8092 ETH Zurich, Switzerland. Phytopathology 94:S50. Publication no. P-2004-0340-AMA.

In the eastern US, epidemics of grape downy mildew are putatively initiated by prebloom oosporic infections then driven by extensive secondary spread. Recent European studies suggest a longer period of oospore maturation and less dependence upon sporangial inoculum for disease increase. Conditions for primary infection differ from those for secondary spread, thus it is important to identify later season primaries. We used previously-developed (Eur. J. Plant Pathol. 109:153-164) microsatellite markers to analyze an epidemic in NY, USA. Results indicated that oospores were still causing ~50% of total lesions 40 days into the epidemic. Despite favorable weather, some genotypes did not increase in frequency, while others did so markedly. Certain aspects of the microsatellite results were difficult to reconcile with observed patterns of disease spread. Bioassay results for the forthcoming season will be reported, as will additional microsatellite data.

Mobilization of a pathogenicity island from a pathogen to a nonpathogen results in new pathogenic *Streptomyces* species. J. A. KERS, J. E. Morello, and R. Loria. Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA. Phytopathology 94:S51. Publication no. P-2004-0341-AMA.

Horizontal transfer of a pathogenicity island (PAI) has resulted in the evolution of new pathogenic *Streptomyces* species in agricultural systems. Plant pathogenic *Streptomyces* are Gram-positive, filamentous soilborne bacteria that are causal agents of potato scab disease. We have previously demonstrated conjugal transfer of a 660 kb PAI from the plant pathogen *S. turgidiscabies* to the non-pathogen *S. coelicolor*. The PAI integrates site-specifically into a putative membrane lipid kinase in the *S. coelicolor* chromosome. This site has not previously been described as a target for integration of PAIs or plasmids. Although *S. coelicolor* transconjugant strains are not pathogenic in a potato tuber slice assay, *S. diastatochromogenes* transconjugant strains that have acquired the PAI do have a pathogenic phenotype. Transconjugant strains are being used to study the genetic determinants encoded on the PAI that facilitate a pathogenic lifestyle.

Managing Rhizoctonia of sugarbeet with azoxystrobin, based on soil temperature. M. F. R. KHAN (1), J. Khan (2), C. A. Bradley (3), and R. Nelson (4). (1) Extension Sugarbeet Specialist, North Dakota State University, Fargo, ND and University of Minnesota, St. Paul, MN; (2) Graduate Research Assistant; (3) Extension Plant Pathologist; (4) Research Technician, North Dakota State University, Fargo, ND. Phytopathology 94:S51. Publication no. P-2004-0342-AMA.

Rhizoctonia root and crown rot, caused by *Rhizoctonia solani* AG-2-2, is becoming more widespread in the Red River Valley of North Dakota and Minnesota, USA. Growers estimate that about 74,000 acres are infected with varying levels of Rhizoctonia. The increase in disease severity and incidence is probably a combination of wet field conditions and close rotation of sugar-beet with soybean and edible bean, also hosts for *R. solani*. Azoxystrobin, registered in the USA for use on sugarbeet, controls *R. solani* when it is applied prior to infection. Field research was conducted to determine the soil temperature at which azoxystrobin should be applied to control *R. solani*. Azoxystrobin, applied between 50–79°F provided effective disease control and resulted in significantly higher recoverable sucrose per acre compared to azoxystrobin application at a higher temperature, and untreated plots. Greenhouse research was also conducted to determine the efficacy of Azoxystrobin applied before and after inoculation with *R. solani*. Results will be discussed.

Induced resistance against Fusarium head blight of wheat by autoclaved fungal biomass. N. I. KHAN (1) and B. Tisserat (2). (1) Biotechnology Research and Development Corporation, Peoria, IL; (2) Fermentation Biotechnology Research, NCAUR, USDA-ARS, Peoria, IL. Phytopathology 94:S51. Publication no. P-2004-0343-AMA.

Fusarium head blight (FHB) is a devastating disease throughout the wheat growing regions of the world. We studied the influence of autoclaved fungal biomass (AFB) for inducing resistance against FHB. In greenhouse trials, AFB were sprayed on the plants before or at flowering. After 7-10 days heads were sprayed with *Fusarium graminearum* conidia. Out of over 40 fungal isolates, significant reduction (~30 to 100%) in FHB disease severity was observed in plants sprayed with certain *Penicillium* sp., *Trichoderma* spp., and *Aspergillus* sp. isolates compared to untreated controls ($P = 0.05$). The stage of wheat plant development when sprayed with AFB influenced reduction of FHB disease severity. Minimum dosages of AFB required to initiate induced resistance are under study. Spraying AFB may have potential to be included in the integrated pest management programs against FHB.

A dual selection based, targeted gene knock-out method for fungi. C. H. KHANG (1), S.-Y. Park (1), Y.-H. Lee (2), and S. Kang (1). (1) Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA; (2) School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea. Phytopathology 94:S51. Publication no. P-2004-0344-AMA.

As the amount of available fungal genome sequences rapidly increases, determining the function of fungal genes will be an essential step in understanding fungal biology toward the goal of controlling pathogenic fungi while maximizing the value of beneficial fungi. The most widely applied approach to characterize gene function is to disrupt a target gene by gene knock-out (KO) and to assess the phenotype of a resulting mutant. Gene KO results from homologous recombination of a target gene by transformation. This approach in fungi, however, has been limited by the low efficiency of homologous recombination, resulting in a large number of transformants to be screened for isolating desired gene KO mutant. We have developed a novel tool to generate gene KO mutant efficiently, which is based on *Agrobacterium tumefaciens*-mediated transformation with a targeting gene followed by a dual selection to identify mutants. The efficacy of gene KO by this method was evaluated using *Magnaporthe grisea* and *Fusarium oxysporum*.

Risk assessment of biopesticides: importance of substrate and water in vertical transport of *Paecilomyces lilacinus* strain 251. S. KIEWNICK, C. Roumbos, A. Mendoza, and R. A. Sikora. University of Bonn, Dept. of Soil Ecosystem Phytopathology and Nematology, Germany. Phytopathology 94:S51. Publication no. P-2004-0345-AMA.

The biocontrol agent *Paecilomyces lilacinus* strain 251 (PL251), shows a great potential for the control of plant parasitic nematodes. The application of biological control products on a commercial scale requires investigations on the ecological safety. Information on the possible dissemination to non target sites is needed. The vertical transport of a biological nematicide, such as PL251 is important for two reasons: (i) the distribution along the root system or to nematode infested sites is crucial for its performance; (ii) the transport of the fungus could lead to ground water contamination. The role of substrate type and precipitation on vertical movement of commercially formulated conidia was investigated in columns containing homogenized substrates. Application of conidia as drench followed by simulated rainfall events (25, 50 and 100 mm per hour) showed only low vertical movement in field soil or sand supplemented with 2, 5, 10 and 25% organic matter, respectively. Between 90 and 100% of the applied conidia remained within the top first 10 cm of the column. However, field soil diluted with sand (1:1) showed higher vertical movement up to 30 cm deep. Simulated rainfall events played a minor role in dissemination of conidia. Additionally, PL251 was detected only rarely and in low numbers in the effluent. Consequently, contamination of ground water by this biopesticide is very unlikely.

Optimizing the biological control of plant parasitic nematodes with *Paecilomyces lilacinus* strain 251. S. KIEWNICK and R. A. Sikora. University of Bonn, Dept. of Soil Ecosystem Phytopathology and Nematology, Germany. Phytopathology 94:S51. Publication no. P-2004-0346-AMA.

The egg pathogenic fungus *P. aecilomyces lilacinus* strain 251 (PL251), shows a great potential as a biological nematicide in integrated approaches for the control of a broad spectrum of plant parasitic nematodes. In greenhouse experiments, PL251 was tested in a commercial water dispersible granule (WDG) formulation against the root-knot nematodes *Meloidogyne incognita* and *M. hapla* on tomato. The biocontrol fungus was incorporated into soil infested with root-knot nematode eggs six days prior to transplanting tomatoes. Soil application also was combined with a seedling treatment 24 hours before transplanting and a soil drench 14 days after transplanting. Alternatively, seedling and post planting treatment was combined with a soil application at planting. All treatments decreased root galling and the number of egg masses compared to the untreated control. However, combining seedling with a pre- or at-planting treatment of PL251 was necessary to achieve significantly higher levels of control. The efficacy of this modified application system was confirmed in a yield experiment with *M. hapla*. A combination of pre-planting plus seedling treatment supplemented with one post plant drench gave the best control and resulted in a significant increase in fruit yield and a decrease in the number of galls per root.

Weapons of mass destruction – genetic warfare between *Theobroma* and *Crinipellis*. A. KILARU (1), B. Bailey (2), and K. H. Hasenstein (1). (1) Department of Biology, UL Lafayette LA 70504; (2) USDA, Beltsville, MD 20705. Phytopathology 94:S51. Publication no. P-2004-0347-AMA.

Interaction of the fungus *Crinipellis perniciosa* with *Theobroma cacao* causes witches' broom disease. The pathogen initially causes excessive vegetative growth and necrosis that ultimately results in plant death. During this development the fungus undergoes phase transition from uninucleate to binucleate state. The phase transition depends on the developmental stage of the host tissue and occurs in 3-5 d on mature and 10-12 d on immature leaves of cacao. To understand the molecular changes during the development of the

disease, immature cocoa leaves were infected with the primary mycelium of *C. perniciosa*. Using mRNA differential display 53 differentially expressed genes were isolated 3 d after infection of leaves. Of these genes, 7 were down-regulated in the leaf and 15 in the fungus. Seventeen genes were upregulated in the leaf, one in the fungus and 13 in leaf/fungus interaction. Eleven of the upregulated genes were expressed *de novo* upon host-pathogen interaction. Thirty of these genes were cloned and sequenced. Their putative function was identified by comparison with data bases. The identified genes coded for pathogenesis/stress-related proteins in the host. Development and metabolism related proteins were down regulated in the pathogen. The upregulation of hormone, stress, and signal transduction related proteins in the host and down-regulation of the same in the pathogen, signify early and mutual recognition and response processes in the host and pathogen. The identification of the temporal changes of these genes 5, 10, 15 and 20 d post infection will improve our understanding of disease-related gene expression. Controlling expression of some of these genes may ameliorate pathogen-induced damage to the host.

Site-specific estimation of weather variables in mid-western U.S. using spatial interpolation. S. Kim (1), M. L. Gleason (1), and C. GÓNGORA-CANUL (2). (1) Iowa State University, Ames, IA; (2) Instituto de Fitosanidad, Colegio de Postgraduados, km. 36.5 Carr. Mex.– Texcoco, Montecillo, Mexico, 56230 Mex. Phytopathology 94:S52. Publication no. P-2004-0348-AMA.

Spatial interpolation was used to obtain estimates of air temperature, vapor pressure, and wind speed at sites in the Midwestern U.S. from May to September in 1998 and 1999. Hourly weather data for 60 randomly chosen days were obtained from High Plains Regional Climate Center (HPRCC) database (Lincoln, NE) for validation sites, interpolated using an inverse distance weight (IDW) method, and compared with measured data. In general, accuracy of air temperature estimates was better that that of other variables. Overall, the values of R2 were 0.85, 0.54 and 0.59 and for air temperature, vapor pressure, and wind speed. Accuracy of estimates also varied considerably by dates. In one test, for example, R2 for air temperature ranged from 0.71 to 0.99 for 51 days, but was <0.65 for the remaining 9 days. Hourly variation of spatial interpolation for each variable was also observed.

Durability of monogenic lines to Korean rice blast fungus using the greenhouse assay. B. R. Kim, J. H. Roh, S. H. Choi, and S. S. HAN. National Institute of Crop Science, RDA, Suwon, 441-857, Korea. Phytopathology 94:S52. Publication no. P-2004-0349-AMA.

Rice blast fungus *Magnaporthe grisea* exhibits high pathogenic variability, which enables blast pathogen to overcome host resistance in a short time. For a systematic resistant breeding to blast, improved techniques of screening methods are still needed. Present study was conducted to identify durable blast resistance in monogenic lines, harboring different major resistance gene for blast developed at International Rice Research Institute (from Dr. Fukuda), *Pi-b*, *Pi-9* and ten genes, by a simple greenhouse assay enforced with sequential planting. First planting of monogenic rice lines was inoculated by sprayer with mixture of 27 blast isolates consisted of genetically different lineages (23 races). There was a difference in time and quantity of susceptible response among cultivars. IRBL9-W harboring *Pi-9* produced very small and few lesions in the 1st inoculation. The size and number of susceptible lesions increased as planting set increased from 2nd to 7th. At the 3rd plant set, the IRBL9-W already showed abundant big size susceptible lesions and disease severity became severe at the 5th planting time. The isogenic line harboring *Pi-ta* and *Pi-b* etc showed higher than 40% of DLA at the 1st planting time and continuously showed high incidence of disease. Some commercial cultivars or isogenic lines including Palgongbyeo, IRBL5-M(*Pi-5(t)*) and IRBL3-M(*Pi-3*) showed less than 40% DLA during the whole time until 7th planting. The sequential planting method in this experiment used enough number of compatible isolates under favorable condition for disease development, suggesting that this method might be useful to evaluate durability of blast resistance.

Proteomic analysis of rice blast fungus, *Magnaporthe grisea*, during appressorium formation. S. T. Kim, Y. u, S. Y. Kang, H. J. Kim, D. H. Hwang, S. G. Kim, and K. Y. Kang. Division of Applied Life Science (BK21 program), Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Chinju, Korea. Phytopathology 94:S52. Publication no. P-2004-0350-AMA.

We used 2-DE to identify proteins induced in *Magnaporthe grisea* (*M. grisea*) during appressorium formation. Proteins were extracted either from germinated conidia on hydrophilic glass plates or from appressorium forming conidia on leaf wax coated hydrophobic glass plates at a time course of 4, 8, and 12 h after conidia germination. Five proteins induced during appressorium formation were identified by using internal amino acid sequencing. Two 20S proteasome alpha subunit (MgP 4 and 6), scytalone dehydratase (SCD1), short chain dehydrogenase/ reductase (SDR), and serine carboxypeptidase Y (MgCPY) were among those induced during appressorium formation; these have not been reported previously in rice blast fungus except for scytalone dehydratase. Based on Magnaporthe DataBase recently published genome sequence of *M. grisea*, three proteins (CPY1, MgP4, and MgP6) highly expressed on wax-coated plates were annotated with gene names from BLAST program. One of the identified proteins, alpha-subunit of 20S proteasome was further investigated to understand its involvement during appressorium formation. The elevated expression level of alpha-subunit of 20S proteasome at 8 h after incubation was identified by Western blot during appressorium formation. It was also highly induced by nutrient starvation; nitrogen starvation expedited its induction more than carbon starvation did. These results indicated that fungal proteome could provide important clues related to developmental changes such as special structure as an appressorium.

Increased leaf hardness and reticulate silcate architecture: A physical mechanism of silicon-induced rice resistance to blast disease. K. W. KIM (1), J. J. Park (2), Y. Kim (2), S. S. Han (3), and E. W. Park (1). (1) NICEM, Seoul National Univ., Seoul, Korea; (2) Dept. of Chemistry, Hankuk Univ. of Foreign Studies, Yongin, Korea; (3) National Inst. Crop Sci., RDA, Suwon, Korea. Phytopathology 94:S52. Publication no. P-2004-0351-AMA.

A physical mechanism of Si-induced rice resistance to blast disease was investigated in leaves by force modulation microscopy and ^{29}Si solid-state nuclear magnetic resonance (SS-NMR) spectroscopy. A susceptible cultivar Jinmi and a resistant cultivar Suwon 345 were grown under a hydroponic culture system with a nutrient solution containing 0 and 150 ppm of sodium silicate. FMM showed higher mean height values of elastic images in Si-treated plants (1.26 V) than in control plants (0.44 V), implying the increased leaf hardness by Si treatment. ^{29}Si SS-NMR spectroscopy exhibited typical spectra of amorphous silica and similar silicate architecture in two cultivars with slight difference in hydroxylation levels. It was found that functionalized silicate architecture in both cultivars would form a compact reticulation with cell wall components. The signal-to-noise ratios in the ^{29}Si SS-NMR spectra were higher in Jinmi than in Suwon 345, indicating that leaves of Jinmi contained greater amounts of Si than those of Suwon 345. These nanoscale studies suggest that Si increases leaf hardness by forming reticulate silicate architecture in cell wall, which may be closely associated with Si-induced disease resistance in rice by fortifying physical barrier protecting host cells from invasion of blast fungus.

Using empirical models to forecast site-specific leaf wetness duration for input to disease-warning systems. K. S. KIM, S. E. Taylor, and M. L. Gleason. Iowa State University, Ames, IA. Phytopathology 94:S52. Publication no. P-2004-0352-AMA.

Empirical models based on classification and regression tree analysis (CART model) and a fuzzy logic system (FL model) were utilized to forecast leaf wetness duration (LWD) 24 h in advance using site-specific weather forecasts at 15 sites in the Midwestern U.S. from 1998-1999. Forecasted LWD and air temperature were also used as inputs in simulation of the Melcast and TOM-CAST disease-warning systems. The CART and FL models under-predicted LWD by 2.3 and 3.9 h/day, respectively. The CFL model, which is a corrected version of the FL model using a weight value reduced mean error (ME) in LWD forecasts to –1.1 h/day. In Melcast and TOM-CAST simulation, the CART and CFL models predicted daily disease risk ratings similar to those derived from weather data measurements. Spray dates forecasted by both models were within 1 day of timing obtained from measurements for approximately 60% of periods.

Estimation of leaf wetness duration using empirical models in northwestern Costa Rica. K. S. KIM, S. E. Taylor (1), M. L. Gleason (1), R. Villalobos (2), and L. F. Arauz (3). (1) Iowa State University, Ames, IA; (2) Instituto Meteorológico Nacional. San José, Costa Rica; (3) Universidad de Costa Rica, San José, Costa Rica. Phytopathology 94:S52. Publication no. P-2004-0353-AMA.

Leaf wetness duration (LWD) estimation models developed in the Midwestern U.S. during May-September were assessed for portability to northwestern Costa Rica during wet and dry seasons. The CART/SLD/Wind (CART) model overestimated LWD by 5.1 h/day at five sites in northwestern Costa Rica during the 1999 wet season (May – September). The Fuzzy LWD (FL) model, in contrast, estimated LWD within 0.5 h/day at the same sites. In general, both models underestimated LWD at most sites during the 2000-2001 and 2002-2003 dry seasons, probably due to less humid conditions than the temperate climate prevailing in the Midwestern U.S. Accuracy of the FL model was substantially improved when a correction factor was utilized during the 2002-2003 dry season, however, indicating that this model could be adjusted to estimate LWD in a semi-arid climate with acceptable accuracy.

This result also suggested that the FL model could be adjusted to estimate LWD accurately in other regions with climate regimes similar to northwestern Costa Rica.

Development and validation of a leaf wetness duration model using a fuzzy logic system. K. S. KIM, S. E. Taylor, and M. L. Gleason. Iowa State University, Ames, IA. Phytopathology 94:S53. Publication no. P-2004-0354-AMA.

A model to estimate leaf wetness duration (LWD) based on fuzzy logic was developed and validated using hourly weather measurements at 15 sites in the Midwestern U.S. from 1997-1999. The Fuzzy LWD model was comparable to sensor measurements in overall accuracy of LWD estimation (<1 h / day) and used relatively few input variables and simplified calculations compared with physical models. The Fuzzy LWD model was also more portable within the study area than an empirical model, the CART/SLD/Wind model, even though both models required the same weather variables as inputs. Advantages of the Fuzzy LWD model over current physical and empirical LWD models were attributable to incorporation of energy balance principles within the empirical computation framework of a fuzzy logic system. Since the Fuzzy LWD model approximates wetness occurrence using a limited number of input variables, accuracy of this model can be improved using a correction factor when additional relevant inputs, e.g., solar radiation, become available.

Regulation and function of sporangia-specific genes in the oomycete *Phytophthora infestans*. K.-S. KIM and H. S. Judelson. University of California, Riverside, CA. Phytopathology 94:S53. Publication no. P-2004-0355-AMA.

Asexual sporangia play a key role in potato and tomato late blight. To help understand factors involved in spore biology, arrays of 5200 sequenced clones from a sporangia cDNA library were hybridized with total cDNA from sporangia, mycelia, germinated cysts, starved hyphae, and sporulation mutants. This identified 61 genes up-regulated >5-fold in sporangia versus hyphae. A subset was induced by starvation and in the non-sporulating mutant. Transcripts of some genes fell after cyst germination while others persisted. Genes encoding a NLI (nuclear LIM-interacting) transcription factor and a RAC protein kinase are now being studied in detail. Transformants of *P. infestans* containing their promoter regions fused to the beta-glucuronidase (GUS) reporter showed GUS expression only in sporangia, like the native gene. *Cis*-regulatory elements necessary for spore-specific transcription are being investigated through serial or internal deletions and mutations of the promoters. The roles of the genes in pathogenicity and/or spore development are being investigated through gene silencing or over-expression, yeast two-hybrid, and other methods.

Forecasting plant disease from local-scale, high-resolution weather data. K. R. KIM (1), R. C. Seem (1), J. Zack (2), and R. D. Magarey (3). (1) Cornell University, Geneva, NY; (2) MESO Inc., Troy, NY; (3) USDA/APHIS/CPHST, Raleigh, NC. Phytopathology 94:S53. Publication no. P-2004-0356-AMA.

Disease forecasts are usually generated from irregularly dispersed weather stations. We developed a system to simulate local-scale, high-resolution weather and plant disease which incorporates a simplified mesoscale boundary layer model for the estimation of local weather conditions such as air temperature and RH. The system also integrates models for surface wetness duration and disease, can incorporate current upper air conditions for true weather forecasts, or it can recreate weather forecasts utilizing a 55-yr database. Maximum horizontal resolution of 150m was achieved by running 5-step nested child grids inside coarse mother grids. The system simulated several growing seasons to drive the DMCast model, and thereby estimate the risk of grape downy mildew (*Plasmopara viticola*) with 333m resolution in two regions of NY. Outputs were represented as maps or as graphs for specific locations. While resolution is greatest over North America, the system is functional globally and may be useful for site selection and re-analysis of historical epidemics.

Identification and molecular characterizaton of rice blast fungus *M. grisea* induced proteins through proteomic analysis. S. G. [illegible] D. H. Hwang, S. T. Kim, S. Y. Kang, H. J. Kim, and K. Y. Kang. Division of Applied Life Science (BK21 program), Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, 900. Phytopathology 94:S53. Publication no. P-2004-0357-AMA.

To identify proteins involved in pathogen induced proteins, we extracted proteins from rice leaves inoculated with rice blast fungus at 48 and 72 h after inoculation with incompatible race (KJ401), compatible race (KJ301) or phytohormone (JA, SA, ABA) treatment. The protein profiles showing incompatible, compatible interaction and phytohormones treatment were analyzed by 2-DE. Eight proteins that were clearly induced or increased compared to those of control were two beta-1, 3-glucanase, receptor-like protein kinase, pathogenesis-related thaumatin-like protein (TLP), peroxidase (POX22.3), probenazole-induced protein (PBZ) and pathogenesis-related protein (OsPR-10), respectively. To confirm whether the expression profile is equal to the 2-DE data, we have isolated cDNA for the pathogen-induced proteins using PCR with gene specific primer sets we designed from GeneBank database (NCBI). The isolated seven cDNA clones were used as probes in Northern hybridization experiments using total RNA from leaf tissues inoculated with incompatible and compatible rice blast fungus races onto leaves of rice cultivar Jinheung. In the compatible interaction, all the six genes were expressed after 48 h post-inoculation. The genes encoding POX22.3, Glu1, Glu2, TLP, OsRLK, PBZ1, and OsPR-10 were activated in inoculated leaves, with TLP, OsRLK, PBZ1, and OsPR-10 being expressed more in the incompatible than compatible interaction. We also analysed OsRLK, Glu1, Glu2 and TLP transcript levels after treatment with various inhibitors.

***In planta* expressed genes of *Magnaporthe grisea*: Identification and analysis of their transcriptomes.** S. KIM and Y. H. Lee. Seoul National University, Seoul, Korea. Phytopathology 94:S53. Publication no. P-2004-0358-AMA.

Fungal genes involved in infectious growth within the host were identified through expressed sequence tag (EST) analysis of the *Magnaporthe grisea* KJ201 infected rice (cv. Hwacheong) cDNA libraries. Based on the similarity search results against *M. grisea* genome draft sequence and dbEST entries with BLASTN algorithm, and nr db with BLASTX, respectively, 708 uniESTs could be assigned as fungal genes out of 2,315 uniESTs. Eighty uniESTs have full ORFs and this information was used to identify sequence variation between *M. grisea* strains KJ201 and 70-15. Sequences in exon regions are highly conserved between the two *M. grisea* strains (less than 0.001%). Twenty five percent (20 out of 80) of the full length cDNA sequences does not match exactly to the corresponding loci to genome draft suggesting incorrect annotation or possibility of alternative splicing. Microarray analyses of 708 fungal cDNAs revealed that 106 exhibited differential expression patterns at the level of more than 2 folds induction or repression under nutrient depletions or during infection. These were grouped into 8 clusters according to their induction/repression profiles by principal component analysis. There are 27 members including *MHP1*, class II hydrophobin, whose expression was uniquely induced during invasive growth. Forty five genes (42%) were co-regulated by nitrogen starvation and growth in the plant cells, supporting the hypothesis that nitrogen starvation could serve as an environmental cue for pathogen to infect their host.

Characterization of *MST11* gene in the rice blast fungus. Y. KIM, X. Zhao, and J.-R. Xu. Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. Phytopathology 94:S53. Publication no. P-2004-0359-AMA.

MAP kinase cascades have been implicated in controlling cell growth and developmental processes in a variety of eukaryotic organisms. In the rice blast fungus *Magnaporthe grisea*, the *PMK1* MAP kinase is known to regulate appressorium formation and infectious growth. In this study, we functionally chactacterized the MAP kinase kinase kinase gene, *MST11*, that activates the *MST7*, MAP kinase kinase gene, which phosphorylates *PMK1* in *M. grisea*. The *mst11* deletion mutant, similar to the *pmk1* mutant, failed to form an appressorium and infect rice plants. It produced much less aerial hyphae and conidia, and often formed basal swollen bodies and developed germ tubes on hydrophobic surfaces. Expressing a constitutive *MST7* allele in the *mst11* mutant rescued appressorium formation but not plant infection. *MST11* is homologous to yeast *STE11* and has an N-terminal sterile alpha motif (SAM) that is involved in interacting with *STE50* during the pheromone response. *MST11* also contains a Ras associated domain (RA) that is unique to *STE11* homologs in filamentous fungi. Mutant alleles of *MST11* deleted in the SAM and RA domains and changed in two well-conserved phosphorylation sites have been generated. Phenotype characterizations of transformants expressing these mutant alleles of *MST11* will be presented.

Occurrence of *Sphaeropsis pyriputrescens* in apple in Washington State. Y. K. KIM (1), C. L. Xiao (1), and J. D. Rogers (2). Washington State University, (1) TFREC, Wenatchee, WA; (2) Dept. Plant Pathology, Pullman, WA. Phytopathology 94:S53. Publication no. P-2004-0360-AMA.

Sphaeropsis rot, caused by *S. pyriputrescens*, is a newly reported postharvest disease of apples and pears. A survey of postharvest diseases in apple was conducted in 2003 to determine the prevalence and incidence of Sphaeropsis rot and fruit rots caused by other pathogens. During June to August, 26 samples of approximately 50 decayed fruit each were collected during packing operations from commercial packinghouses. Sphaeropsis rot was observed in 25 of the 26 Red Delicious grower lots. Instances of severe

Sphaeropsis rot were observed on both thiabendazole (TBZ)-drenched fruit and nondrenched fruit, but incidence varied from lot to lot. Gray mold caused by *Botrytis cinerea*, blue mold caused by *Penicillium* spp., and Sphaeropsis rot were the three major postharvest diseases, accounting for an average of 46, 23 and 25% of the decay on nondrenched fruit, respectively, and 20, 50 and 22% of the decay on TBZ-drenched fruit, respectively. Sphaeropsis rot was also observed on apple c.v. Golden Delicious and Granny Smith. The results indicate that Sphaeropsis rot is an important component of storage decay of apples in the region.

Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. Y. K. KIM (1), C. L. Xiao (1), and J. D. Rogers (2). Washington State University, (1) TFREC, Wenatchee, WA; (2) Dept. Plant Pathology, Pullman, WA. Phytopathology 94:S54. Publication no. P-2004-0361-AMA.

S. pyriputrescens is the causal agent of Sphaeropsis rot, a newly reported postharvest fruit rot disease of pear and apple. In this study, effects of eight media and environmental factors on mycelial growth and pycnidial production of the fungus were evaluated. Apple juice agar and pear juice agar were most suitable for mycelial growth of all six isolates tested. Cornmeal agar was not suitable for either mycelial growth or pycnidial production. The fungus was able to grow from –3 to 25°C, with optimum growth at 20°C, little or no growth at 30°C and no growth at 35°C. The fungus was able to grow at water potential as low as –5.6 MPa but not at –7.3 MPa. The fungus grew at pH up to 11 with optimum growth at pH 3.3 to 4.2 but failed to grow at pH above 7.2. Regardless of medium tested, few or no pycnidia formed at 20°C in the dark. Pycnidial production was significantly enhanced by fluorescent light, but continuous light appeared to reduce pycnidial production, depending on the medium. Oatmeal agar at 12-h light/12-h dark seemed optimal for production of pycnidia and conidia.

Influence of temperature, wetness duration, and relative humidity on conidial germination of *Sphaeropsis pyriputrescens*. Y. K. KIM and C. L. Xiao. Washington State Univ., TFREC, Wenatchee, WA. Phytopathology 94:S54. Publication no. P-2004-0362-AMA.

S. pyriputrescens is the causal agent of Sphaeropsis rot in apples and pears. Infection of fruit occurs in the orchard and symptoms develop during storage. In this study, effects of temperature, wetness duration, and relative humidity (RH) on conidial germination of the fungus were evaluated. Conidial germination and germ tube elongation were observed at temperatures from 0 to 30°C. The optimum temperature for conidial germination was between 15 and 20°C, at which a minimum wetness period of 6 and 5 h was required for germination, respectively. Over 80% of conidia germinated after 16 h at 15 and 20°C. A minimum wetness period of 72, 24 and 12 h was required for conidial germination at 0, 5 and 10°C, respectively; 5 and 8 h was required for germination at 25 and 30°C, respectively. Optimum temperature for germ tube elongation was 20°C. Conidia germinated after 36 h at 20°C at 92% RH but not at 88.5% RH. Percent conidial germination and germ tube elongation decreased as RH was reduced from 100 to 95%. The results contribute to our understanding of conditions required for fungal infection of fruit leading to Sphaeropsis rot.

Potato late blight control incorporating host plant resistance and managed fungicide application. W. W. KIRK (1), J. B. Muhinyuza (1), D. S. Douches (2), R. Hammerschmidt (1), and F. M. Abu-El Samen (1). (1) Plant Pathology; (2) CSS, Michigan State University, E. Lansing, MI 48824. Phytopathology 94:S54. Publication no. P-2004-0363-AMA.

Potato late blight (*Phytophthora infestans*) remains a major threat to the production of high quality potatoes. The objective of this research was to determine if acceptable control of foliar late blight can be achieved by using increased fungicide spray intervals and reduced application rates of residual contact fungicides on potato germplasm with a range of susceptibility to late blight developed at each of the four potato breeding programs in Michigan, Minnesota, North Dakota and Wisconsin. The combination of cultivar/ABL resistance and managed application of protective fungicides reduced foliar late blight to acceptable levels in most situations. When conditions were moderately conducive to late blight development, reduced amounts of fluazinam were either fully or partially effective at all application rates tested on all cultivars/ABL compared to the non-treated controls. In some cultivar/ABL, 50% of the manufacturer recommended application rate (MRAR) was sufficient to achieve acceptable control, whereas other cultivars/ABL required 100% MRAR to control late blight. On late blight susceptible cultivars, applications of fluazinam at either 10 or 15-day intervals were partially effective for controlling late blight at the doses tested. However, in the resistant cultivars, Torridon and Jacqueline Lee the fungicides did not reduce the relative area under the disease progress curve (RAUDPC) in comparison with untreated plots of these cultivars.

Evaluation of three inbreds for resistance to Fusarium ear rot and fumonisin accumulation in grain. C. E. KLEINSCHMIDT, J. K. Pataky, and D. G. White. Dept. of Crop Sciences, University of Illinois, Urbana, IL 61801. Phytopathology 94:S54. Publication no. P-2004-0364-AMA.

Fusarium ear rot of corn caused by *Fusarium verticillioides* (syn. = *F. moniliforme*) and *F. proliferatum* is of concern due to the production of fumonisin mycotoxins. The objective of this study was to determine the heritability of resistance to fumonisin accumulation in grain and Fusarium ear rot and to estimate gain from selection in BC1S1 families (BC1S1 = ((Resistant Inbred × FR1064) × FR1064)(self)). The BC1S1 families of the resistant inbreds TBA76125, CG1 and CQ201 had heritability values of 59%, 65% and 54% for fumonisin accumulation in grain and 55%, 68% and 56% for Fusarium ear rot, respectively. Gain from selection was $\log_e$ 0.19 µg/g, 0.25 µg/g and 0.25 µg/g for fumonisin accumulation in grain and 0.13%, 0.20% and 0.21% for Fusarium ear rot for the three families, respectively. Pearson's correlation coefficient between fumonisin accumulation in grain and Fusarium ear rot were 0.48, 0.75 and 0.66 for the TBA76125, CG1 and CQ201 BC1S1 families, respectively.

Effect of salinity and cutting on symptom development of rapid blight of perennial rye. M. J. KOHOUT, D. M. Bigelow, and M. W. Olsen. Dept. of Plant Sciences, The University of Arizona, Tucson, AZ. Phytopathology 94:S54. Publication no. P-2004-0365-AMA.

Rapid blight is a new disease of cool season turfgrasses caused by a species of *Labyrinthula*. A water-soaked appearance is initially observed in affected turf. Disease often occurs on turf irrigated with high salinity water and in areas of frequent mowing. The effects of salinity of irrigation water and cutting on symptom development were studied in the laboratory. Two-week-old seedlings of perennial rye 'Brightstar SLT' were inoculated with a 2 × 10^5 cells/ml suspension of *Labyrinthula* isolated from diseased turf in Arizona. Irrigation water was adjusted to 0.8, 1.4, 1.8, 2.3, 2.8, 4.0, 6.0 and 8.0 dS/m by adding artificial seawater. Plants were infected, but not symptomatic, when irrigated with 0.5 dS/m water. At salinities from 0.8 to 8.0 dS/m, symptom development increased as salinity increased. Studies with plants that were cut or not cut prior to inoculation showed that cutting is not required for, but accelerates, symptom development. These findings substantiate field observations that rapid blight becomes increasingly more severe as salinity of irrigation water increases and that disease is exacerbated by mowing.

Assessment of interactions among important sweetpotato viruses using real-time quantitative PCR. C. D. KOKKINOS and C. A. Clark. Dept. Plant Pathology & Crop Physiology, Louisiana State University AgCenter, Baton Rouge, LA. Phytopathology 94:S54. Publication no. P-2004-0366-AMA.

Among the economically most important diseases of sweetpotato (*Ipomoea batatas*) are those caused by mixed infections of viruses. Understanding how the component viruses interact is of great importance in the effort to better understand their biology in relation to this crop. Virus-tested Beauregard plants, grown under standard greenhouse conditions, were graft-inoculated with SPFMV alone, SPCSV alone, SPLCV alone, SPFMV and SPCSV combined, and SPFMV and SPLCV. Real-Time PCR assays were developed for relative quantification of virus titers in singleplex reactions. Titers were assessed 3, 6, and 9 weeks after inoculation. SPFMV titers were significantly greater in plants co-inoculated with SPCSV compared to plants inoculated with SPFMV alone or SPFMV and SPLCV. This was true for all assessment dates with the second (6 weeks after inoculation) being significantly greater than the other two. No significant difference was observed when SPCSV titers were compared between treatments and assessment dates. SPLCV titers were significantly greater in plants co-inoculated with SPFMV compared to plants inoculated with SPLCV alone. The potyvirus SPFMV enhanced replication of SPLCV in dual infections, but the role was reversed in dual infections with SPCSV which enhanced replication of SPFMV.

Identification of *Pythium* species present in ornamental nursery irrigation systems. P. Kong, P. A. Richardson, and C. X. HONG. Virginia Tech, Virginia Beach, VA. Phytopathology 94:S54. Publication no. P-2004-0367-AMA.

Containing and recycling nursery effluent is a relatively simple method to avoid discharge of pollutants associated with fertilizer and pesticide applications; however, plant pathogens may be spread through irrigation water. *Pythium* species in recycled irrigation and effluent water were isolated at container nurseries in Virginia, using filtering and baiting with two selective media (PARP and PARPH). Representative isolates were subcultured on V8 agar and then identified using morphological characters and a DNA fingerprint. Over 20 species of *Pythium* have been identified in this study. Abundant *Pythium* species in Virginia nursery irrigation water included *P. dissotocum*, *P. torulosum*, *P. sulcatum*, and *P. porphyrae*. Results of this

study are essential for assessing disease risk associated with recycling irrigation and prioritizing respective research.

A molecular fingerprint for identification of multiple *Pythium* species. P. KONG (1), P. A. Richardson (1), G. W. Moorman (2), and C. X. Hong (1). (1) Virginia Tech; (2) Pennsylvania State University. Phytopathology 94:S55. Publication no. P-2004-0368-AMA.

Forty-six isolates representing 26 species of *Pythium* from five morphological groups of van der Plaats-Niterink were examined single strand-conformational polymorphism (SSCP) of PCR amplified internal transcribed spacer 1 (ITS-1) of ribosomal DNA. All species except for *P. deliense* and *P. aphanidermatum* produced distinct SSCP patterns. Intraspecific variations of ITS-1 SSCP were observed for *P. irregulare*, *P. cylindrosporum*, *P. spinosum* and *P. macrosporum*. One variant of *P. irregulare* produced a SSCP pattern similar to that of *P. cylindrosporum*. *P. spinosum* and *P. macrosporum* also produced similar patterns. This study provides a practical tool for rapid species identification and study of species complex within the genus *Pythium*. The results of this study will facilitate research on taxonomy and ecology of this important genus and diagnosis of Pythium diseases.

Towards the development of plant-based vaccine against HIV-1. H. Koprowski, N. Pogrebnyak, T. Pniewski, K. Bandurska, R. Egolf, and A. V. KARASEV. Biotechnology Foundation Laboratories, Thomas Jefferson University. Phytopathology 94:S55. Publication no. P-2004-0369-AMA.

A safe and effective vaccine represents the most promising strategy to control HIV infection. Our goal is to produce an inexpensive, efficient and safe vaccine in plants. Such a vaccine will not require refrigeration, it wil be easy to transport and administer. In this work we focused on two vaccine candidates, an envelope protein gp120 and an artificial fusion of gp120 with the cellular HIV-1 receptor CD4. Vaccine candidate genes gp120 and gp120-CD4 have been cloned into the plant binary vector pBI121. A strong constitutive plant promoter was used for expression of these genes in plants. Several plant species have been transformed by using Agrobacterium-mediated method. Transgenic lettuce (Lactuca sativa cv Aramir), tomato (Licopersicon esculentum cv Money Maker) and tobacco (Nicotiana tabacum cv Wisconsin) lines have been produced. Two systems have been tested for selection after transformation, the kanamycin selection showed a higher level of transformation efficiency than the glufosinate selection. Introduction of vaccine candidate genes into the genome of T0 and T1 plants have been confirmed by PCR analysis. Protein expression levels in T0 and T1 plants are currently under testing. All lettuce, tomato and tobacco transgenic plants will be tested by Western blot analysis and ELISA to find lines with the highest expression level. Homozygous lines will be produced for highest expressors and used in immunological experiments.

Detection of three plant viruses in nematode vectors by RT/PCR. J. Kraus, J. N. Pinkerton, and R. R. MARTIN. USDA-ARS-HCRL, Corvallis, OR 97330. Phytopathology 94:S55. Publication no. P-2004-0370-AMA.

Tomato ringspot virus (ToRSV) *Tobacco ringspot virus* (TRSV) and *Tobacco rattle virus* (TRV) are transmitted to healthy plants by viruliferous nematodes in the soil. These viruses cause diseases in economically important vegetable and fruit crops and have been controlled with mixed results. As of soil fumigants are now being phased out or banned due to environmental concerns, interest in alternatives, such as crop rotation to eliminate virus from nematodes, has increased. To examine the effectiveness of these alternatives in removing virus from the vector/virus/host system, traditional virus-transmission ass are being replaced by the faster and more sensitive PCR assays. We developed a method for extraction of viral RNA from nematodes and a sensitive nested RT/PCR detection assay. The procedure has been adapted to microscale for handling multiple samples. This assay is effective for detection of ToRSV or TRSV in *Xiphinema americanum* or TRV in *Paratrichodorus allius*. With this method, viruses can be detected in nematodes fed on infected plants or from field collected nematodes where the percentage of viruliferous nematodes is unknown.

Occurrence and phytotoxicity of fumonisin B_1 associated with cowpea seed. Q. KRITZINGER (1), T. A. S. Aveling (1), W. F. O. Ma is (2), J. P. Rheeder (2), and L. van der Westhuizen (2). (1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa; (2) PROMEC Unit, Medical Research Council, P.O. Box 19070, Tygerberg 7505, South Africa. Phytopathology 94:S55. Publication no. P-2004-0371-AMA.

Cowpea (*Vigna unguiculata*) plays an integral part in the diets of many rural people living in less developed countries. Mycotoxin contamination of the seeds can lead to adverse health effects in animals and humans. Cowpea seeds of seven cultivars were analysed for seed mycoflora and fumonisin contamination. A ngst other *Fusarium* spp., four isolates of *F. proliferatum* were isolated. The samples and the *F. proliferatum* isolates, cultured on maize patty media, were extracted with methanol/water and cleaned-up on strong anion exchange solid phase extraction cartridges. Fumonisins were quantified as o-phth delehyde pre-column derivatives by HPLC and fluorescence d tion. Fumonisin B_1 (FB_1) was present in four samples with a range of 0.12 - 0.61 mg/kg. All the *F. pro atum* isolates produced fumonisin B_1, B_2 and B_3. Total fumonisins ranged between 0.80 - 25.30 mg/kg and the highest FB_1 level was 16.86 mg/kg. Surface disinfected cowpea seeds were imbibed in sterile water amended with pure FB_1, to yield final concentrations of 10, 25, 50, 100 mg/kg. Percentage germination, determined according to the ISTA rules, was significantly decreased at all toxin concentrations. Embryonic axes and cotyledon tissues were removed and prepare r transmission electron microscopy. Ultrastructural deteriorations included compaction of the cytoplasm and separation of the plasmalemma from the cell wall.

Development of a liquid chromatographic m hod for determination of two classes of mycotoxins in maize silage. G. A. KUI AU (1), M. A. Mansfield (1), D. D. Archibald (2), and A. D. Jones (3). (1) Department of Plant Pathology; (2) Crop and Soil Sciences Department; (3) Department of Chemistry, The Pennsylvania State University, University Park, PA. Phytopathology 94:S55. Publication no. P-2004-0372-AMA.

Maize silage can be contaminated by several different mycotoxins produced by *Fusarium* and *Alternaria* species. *Fusarium* species, which produce fumonisins, are well known pathogens of corn, while *Alternaria*, which produce AAL-toxins, are epiphytes or weak pathogens. These toxins are not routinely tested for in maize silage and most methods primarily focus on a single toxin, m g it costly and laborious to attempt a multi-toxin study. Our objective is to develop a sensitive, multi-toxin method to quantify fumonisins and AAL-toxins. Silage collection was performed at dairies in Pennsylvania at harvest and after ensiling. In 2001 thirty-two dairies participated and in 2002 thirty-nine dairies were included in the study. Samples were dried, milled to a fine consistency, purified and concentrated using solid phase extraction columns packed with strong anion exchange media. Samples were derivatized with the fluorescent compound naphthalene-2,3-dicarboxaldehyde, which is more stable than the commonly used o-phthaldialdehyde reagent. The high performance liquid chromatographic analysis uses a C18 reverse phase column. This presentation will report our latest results in method development and maize silage analyses.

Identification of a SA receptor required for plant innate immun D. KUMAR, A. C. Vlot, H. G. Kang, F. Zhou, and D. F. Klessig. Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, NY 14853. Phytopathology 94:S55. Publication no. P-2004-0373-AMA.

Salicylic acid (SA) is a c hormone for signaling innate immunity in plants. While investigating how SA exerts its effects, several putative effector proteins have been identified including catalase, ascorbate peroxidase and carbonic anhydrase. All three bind SA with low to moderate affinity (K_d of 14-3.7 uM). Another SA-binding protein identified was SABP2. It is present in low abundance and binds SA with highest affinity (K_d = 90nM) of all the SA-binding proteins identified. It was purified 24,000 fold from tobacco leaves and its encoding gene was cloned. Recombinant SABP2 exhibits high affinity for SA, which can be competed by addition of active but not inactive analogs of SA. Sequence analysis predicted that SABP2 is a lipase belonging to the alpha/beta fold hydrolase super family. Confirming this prediction, recombinant SABP2 exhibited lipase activity against several synthetic substrates. Moreover, this activity was stimulated by SA-binding. Silencing of SABP2 expression suppressed local resistance to TMV, induction of PR-1 gene expression by SA and more importantly development of systemic acquired resistance. Together, these results suggest that SABP2 is an SA receptor that is required for the plant immune response.

Derivatives of the *Erwinia betavasculorum* type strain CFBP 2122 that differ by an order of m itude in relative root necrosis-inducing ability. L. D. KUYKENDALL. Molecular Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705. Phytopathology 94:S55. Publication no. P-2004-0374-AMA.

Erwinia betavasculorum, i.e., *Pectobacterium betavasculorum* type strain CFBP 2122 (ATCC 43762) obtained from Louis Gardan (Beaucouzé, France), is one of several subspecies of *E.* (*P.*) carotovora elevated to species rank in 2003. Plants of the root rot-susceptible sugar beet genotype L204, obtained from Bob Lewellen, ARS, USDA, Salinas, CA, were inoculated to determine the virulence of the bacterium. After several weeks, the infected roots were surface-sterilized, and bacteria were recovered by streaking onto King's B agar medium. Following re-isolation from plant roots, strain 2122 was resolved into two colony types: S (small) and L (large). Rather than a lab-attenuated strain, 2122S is probably a wild-type strain that carries avirulence

gene(s) since roots of sugar beet plants infected with this strain had limited areas of bacterial necrosis which correspond with the infectivity results obtained with the original culture and with the published description of Erwinia-induced root rot. The more "compatible" strain, 2122L produced considerably greater necrosis where the roots were rendered glutinous masses. Hyper-virulent derivatives like 2122L are evidently both selected and enriched by passage through a susceptible host plant. Either the loss of, a deletion in or a simple mutation in an avirulence gene(s) would explain the spontaneous appearance of plant-selected, hyper-virulent derivatives.

Genetic analysis of the Arabidopsis *avrRps4*-triggered disease resistance signaling pathway. S. I. KWON, J. M. Koczan, and W. Gassmann. University of Missouri, Columbia, MO. Phytopathology 94:S56. Publication no. P-2004-0375-AMA.

RPS4 specifies the Arabidopsis disease resistance response to *Pseudomonas syringae* pv. tomato (Pst) expressing *avrRps4*. *RPS4* was cloned based on the identification of RLD as a naturally occurring susceptible accession. To dissect the basis of disease resistance, we used a genetic approach to identify suppressor mutations that reactivate the *avrRps4*-triggered defense response in RLD. In this report, we describe two non-allelic *srfr* (suppressor of *rps4-RLD*) mutants, *srfr1* and *srfr3*, that were susceptible to virulent Pst strain DC3000, but resistant to DC3000(*avrRps4*). In quantitative bacterial growth assays, growth of DC3000 was similar in wild-type and mutant plants, indicating that basal resistance was not enhanced in *srfr1* and *srfr3*. Growth of DC3000(*avrRps4*) was approximately 30-fold lower in *srfr1* and *srfr3* than in RLD. However, growth was intermediate compared to fully resistant Col-0 and transgenic RLD containing *RPS4-Col*. The *srfr1* and *srfr3* mutants did not develop spontaneous lesions prior to inoculation, nor did they express the *PR-1* gene constitutively. Therefore, *srfr1* and *srfr3* are novel *avr*-specific mutants that differ from previously described Arabidopsis mutants with elevated disease resistance. The *srfr1* and *srfr3* mutations were recessive, and both mapped to the bottom of chromosome IV between markers CAT2 and nga1107. Genetic analysis indicated that resistance in *srfr1* and *srfr3* was independent of the *rps4-RLD* allele, but it was dependent on a second gene in RLD. We propose that SRFR1 and SRFR3 are negative regulators of *avrRps4*-triggered disease resistance.

PCR assay for *Methylobacterium mesophilicum* in sweet orange trees infected with *Xylella fastidiosa*. P. T. Lacava (1), J.-B. Li (2), W. L. Araujo (1), J. L. Azevedo (1), and J. S. HARTUNG (2). (1) University of São Paulo ESALQ, Piricicaba, SP, Brazil; (2) USDA ARS, Beltsville, MD. Phytopathology 94:S56. Publication no. P-2004-0376-AMA.

Xylella fastidiosa is a xylem-limited bacterium that causes citrus variegated chlorosis disease in sweet orange. There is evidence that *X. fastidiosa* interacts with endophytic bacteria resident in the xylem of sweet orange, and that these interactions, particularly with *Methylobacterium mesophilicum* may affect disease progress. We have previously developed quantitative PCR based assays specific for *X. fastidiosa* using the Lightcycler system, and now report the development of a quantitative PCR assay for *M. mesophilicum*. The assay is specific for *M. mesophilicum* and does not amplify DNA from other species of *Methylobacterium* or other bacteria commonly associated with citrus or plant tissue. Other bacteria tested include *Curtobacterium flaccumfaciens*, *Pantoea agglomerans*, *Enterobacter cloacae*, *Bacillis* sp., *Pseudomonas fluorescens*, *X. fastidiosa* and *Xanthomonas*. Our goal is to monitor population dynamics of both species of bacteria in sweet orange xylem during the course of disease development in both naturally and artificially infected plants.

Induction of jasmonic acid and salicylic acid by *Colletotrichum acutatum*, the cause of postbloom fruit drop of citrus. K. A. Lahey, J. K. Burns, L. W. Timmer, and K. R. CHUNG. Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850. Phytopathology 94:S56. Publication no. P-2004-0377-AMA.

Colletotrichum acutatum infects citrus flower petals, causing blossom blight, young fruit drop, formation of persistent calyces, and leaf distortion. In this study, the amounts of jasmonic acid (JA), 12-oxo-phytodienoic aid (PDA) (a precursor for JA biosynthesis), and salicylic acid (SA) were determined. After infection by *C. acutatum*, both *trans*- and *cis*-PDA increased significantly (by 8-10 fold) in citrus flower petals, but increased only slightly in pistils. In contrast, no significant difference of *trans*-JA was observed in citrus petals or pistils after fungal infection. However, a drastic increase of *cis*-JA was detected in affected petals (5-6 fold), but not in pistils. The amounts of SA were also elevated in the affected petals (increasing 2-fold), but not in pistils. A gene marker encoding a 12-oxo-phytodienoic acid reductase involved in JA biosynthesis was also highly up-regulated by infection with *C. acutatum*. Applications of clofibrate (a putative auxin inhibitor) and SA, but not TIBA (auxin transport inhibitor), significantly decreased its expression. Applying TIBA, clofibrate, and SA after *C. acutatum* infection increased fruit retention. The results are in consistent with the hypothesis that phytohormones are involved in the abscission of young fruit and persistence of the calyces associated with postbloom fruit drop of citrus.

Phytotoxicity of copper bactericides to peach and nectarine. N. LALANCETTE and K. A. Foster. Rutgers University, Agricultural Research and Extension Center, Bridgeton, NJ. Phytopathology 94:S56. Publication no. P-2004-0378-AMA.

Copper linoleate (CL) is important for post-bloom management of bacterial spot in NJ orchards. Rotation of CL with oxytetracycline reduces cost and provides for resistance management. However, because copper is also phytotoxic, higher rates and inorganic coppers have not been utilized. A study was conducted on 'Encore' peach and 'Redgold' nectarine to determine the quantitative relationship between copper concentration and phytotoxicity. Different rates of CL and copper hydroxide (CH) were applied 7 times post-bloom. Foliar injury and defoliation increased with number of applications and copper concentration. Maximum 'Encore' defoliation was 10-17%; 'Redgold' 30-35%. CH caused similar or less leaf injury and defoliation than CL. No injury was observed on fruit, even at 3 times the commercial copper rate. Fruit size and soluble solids were not influenced by copper. These results show that inorganic coppers may be viable alternatives to organometallic copper for post-bloom bacterial spot control; nectarine may be more sensitive to copper injury than peach; and higher rates of copper can be used to provide longer residual disease control.

Induced resistance and increased nutrient uptake in pepper associated with harpin protein treatment in the greenhouse. Z. LAN and Z. Wei. EDEN Bioscience Corp., Bothell, WA. Phytopathology 94:S56. Publication no. P-2004-0379-AMA.

Harpin proteins were studied for their ability to induce resistance in pepper to bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* and to improve nutrient uptake and plant development in the greenhouse. Peppers (cv. Encore) at bud break were sprayed with Messenger containing 3% active ingredient of harpin protein. Three days after the treatment, leaves were inoculated with *X. campestris* pv. *vesicatoria* at around 10^8 CFU/ml, incubated in a moist chamber, and then moved to a greenhouse with a 14-h photoperiod. Leaves treated with harpin had less disease severity compared with the water-sprayed control, and disease progressed significantly slower. In another experiment, pepper seeds were soaked in harpin-ab solutions at 0.5 to 3.0 ppm for 4 h and sown in pots in a growth chamber at 22 to 26°C with 14 h of light for 5 weeks. Seedlings were also sprayed with the corresponding treatments at 2 weeks after emergence. Relative to the controls, seedlings treated with harpin proteins had 8.9 to 13.1% more weight biomass and higher contents of total nutrients such as N, P, K, S, Ca, Mg, Mn, B, and Zn in the tissues.

Alternative strategies for onion Xanthomonas leaf blight management. J. M. LANG, H. F. Schwartz, and D. H. Gent. Dept. of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO. Phytopathology 94:S56. Publication no. P-2004-0380-AMA.

Xanthomonas leaf blight, caused by *Xanthomonas axonopodis* pv. *allii* is an economically devastating disease of onion. An alternative management strategy is critical to reduce excessive use of heavy metal bactericides. A biological control program using h-mutant bacteriophages has been evaluated. In 2003, phages were applied preventatively onto inoculated onions at two locations at 10^8 pfu/mL. Plants treated with phages and copper/mancozeb bactericide revealed a significant decrease in medium sized bulbs and an increase in jumbo sized bulbs as compared to the copper/macozeb treatment alone. Disease severity was reduced by the bacteriophage alone and with acibenzolar (plant defense stimulating compound). Phages were recovered from soils at two locations in eastern Colorado and characterized. Genetic resistance in *Allium cepa* germplasm can also be a valuable component in disease management. Over 150 onion cultivars and germplasm accessions were screened at four months old for resistance or tolerance *X. axonopodis* pv. *allii* by stabbing the two youngest, most fully extended leaves in three equidistant locations with a sterile needle bearing bacterial matrix of the pathogen. Plants were monitored for 12 days in a growth chamber at 28°C and 100% relative humidity while each stab wound was measured for lesion progression. Less susceptible cultivars were advanced to the field for further analysis.

Influence of biocontrol agents and other biological amendments on soil microbial communities. R. P. LARKIN. USDA, ARS, New England Plant, Soil, and Water Lab, University of Maine, Orono, ME. Phytopathology 94:S56. Publication no. P-2004-0381-AMA.

Biocontrol organisms added to soil for disease control affect and are affected by indigenous soil microbial communities. These interactions may determine

biocontrol capabilities, either in enhancing or disabling pathogen and disease suppression. However, little information exists on the fate and effects of biological amendments after application. In this research, the persistance and effects of commercially-available biocontrol products, including *Bacillus subtilis* GB03, *Burkholderia cepacia* J82, *Trichoderma harzianum* T-22, and *Trichoderma virens* Gl-21, on soil microbial communities in the field were evaluated. In addition, effects of other biological amendments, including mycorrhizae, microbial inoculants, biostimulants, compost, and compost tea, were evaluated in greenhouse tests. Biocontrol amendments significantly affected microbial community characteristics, based on changes in culturable population dynamics, substrate utilization, and fatty acid (FAME) profiles, and some effects were still evident 1 year after application. Implications and correlations with disease control will be discussed.

Identification of *Clover yellow vein virus* as the causal agent of pod necrosis ("chocolate pod") in snap bean. R. C. LARSEN (1) and K. C. Eastwell (2). (1) USDA-ARS, Prosser, WA; (2) Washington State University, IAREC, Prosser. Phytopathology 94:S57. Publication no. P-2004-0382-AMA.

Clover yellow vein virus (CYVV) and *Bean yellow mosaic virus* (BYMV) (Family: *Potyviridae*) have been important viruses of snap bean in the Great Lakes region. Serological differentiation of the two viruses has typically been difficult because of the similarity of their coat proteins. In addition, symptoms on bean are highly variable and this often results in erroneous identification of field isolates. Selected strains of both viruses are reported to cause pod distortion, vein necrosis, and apical or top necrosis resulting in plant death. During the 2000-2003 growing seasons, snap beans were observed showing extensive pod necrosis or "chocolate pod" in many fields. CYVV was suspected as the causal agent based on preliminary host range studies but identity of the pathogen was not conclusive. An RT-PCR assay was developed that can unambiguously distinguish between CYVV and BYMV in single and mixed infections of bean. The primers were also used to demonstrate that CYVV, but not BYMV, was directly associated with pod necrosis symptoms on snap bean collected from production fields in Wisconsin.

Safflower (*Carthamus tinctorius*): A potential alternate host of *Cercospora beticola*. R. T. LARTEY (1), T. C. Caesar-TonThat (1), A. J. Caesar (1), W. L. Shelver (2), and N. I. Sol (1). (1) USDA-ARS, Northern Plains Agricultural Research Lab, Sidney, MT; (2) USDA-ARS, Biosciences Research Lab, Fargo, ND. Phytopathology 94:S57. Publication no. P-2004-0383-AMA.

Safflower (*Carthamus tinctorius* L.) is an annual, broadleaf oilseed crop adapted essentially to the small-grain production areas of the western Great Plains. In the Northern Plains Area (NPA), safflower is being evaluated for potential rotation with sugar beet. Both safflower and sugar beet are susceptible to two species of *Cercospora*, *C. carthami* and *C. beticola* respectively. *C. carthami* has not been observed previously in the NPA but *C. beticola* is well established. Thus, the observance of unusual spots on safflower crops in the NPA prompted this study into the potential of safflower as an alternate host to *C. beticola*. All safflower plants, which were inoculated with each of the two isolates (C1, and S1) of *C. beticola*, produced leaf spot symptoms. PCR amplification of extracts from the lesion leaf tissues with *C. beticola* specific primers produced fragments which were comparable to fragments which were amplified from DNA from pure cultures of *C. beticola*. Antibody that was raised from C2 also bound to samples from the infected leaf tissues. Single spore isolate cultures from diseased tissues were successfully amplified with the *C. beticola* specific primers and bound to the *C. beticola* antibody. Spores from the single spore cultures infected both sugar beet and safflower and produced typical symptoms of leaf spot in sugar beet. We have for the first time, produced evidence that shows the potential of safflower to serve as alternate host of *C. beticola*.

Ascospore discharge and occurrence of *Togninia minima* (anamorph = *Phaeoacremonium aleophilum*) in California vineyards. S. R. LATHAM, A. Eskalen, and W. D. Gubler. University of California, Davis, Davis, CA. Phytopathology 94:S57. Publication no. P-2004-0384-AMA.

Species of *Phaeoacremonium* cause both Petri disease on young grapevines and esca (Black measles) on old grapevines. The most common species present in California vineyards is *P. aleophilum*. Recently, the teleomorph for this fungus was produced in-vitro and identified as *Togninia minima* (Calosphaeriales). As perithecia of *T. minima* and other species of *Togninia* mature in-vitro, sticky droplets containing masses of ascospores can be seen at the tips of perithecial necks. Therefore, it was speculated that ascal deliquescence occurs in the perithecium. Our findings, however, demonstrate that after dried *T. minima* perithecia are remoistened, ascospores are capable of being forcibly discharged from asci immediately after exiting the ostiole. Furthermore, spore release data collected from infected vineyards throughout California in 2002 and 2003 suggest that spore release is associated with rainfall. Although naturally occurring perithecia have not yet been found in nature, perithecia have been produced in the laboratory by moist incubation of naturally infected grapevine wood. These results represent the first report of how ascospores of *T. minima* are released and lead us to believe that ascospores serve as an important inoculum source in infected California vineyards.

Population dynamics of *Trichoderma hamatum* T382 on strawberry roots and in soil. L. F. LEANDRO (1), L. M. Ferguson (1), G. E. Fernandez (2), and F. J. Louws (1). (1) Dept. of Plant Pathology; (2) Dept. of Horticulture, North Carolina State University, Raleigh, NC 27695. Phytopathology 94:S57. Publication no. P-2004-0385-AMA.

Field trials were established to study the dynamics of the biocontrol agent *T. hamatum* strain T382 in strawberry production systems. Strawberry transplants were grown in a greenhouse in potting mix amended with compost + T382 or in untreated mix. After 4 weeks, the transplants were established in the field soil treated with compost, compost + T382, fumigant (Telone-C35), or untreated, using a split plot design. Plants and soil were sampled 6 times throughout the season (Oct 2002 – Jun 2003). Populations of *Trichoderma* spp. And *T. hamatum* were quantified by plating soil dilutions and root pieces. *Trichoderma* spp. Population in fumigated soil was morphologically homogeneous and 6- to 40-fold greater than in the other soil treatments, where diverse colony morphology was observed. *T. hamatum* populations were greater in the compost + T382 soil than in the other treatments and remained near 2×10^3 cfu/g soil throughout the season. *Trichoderma* spp. Were more frequently (25-45%) isolated from roots in fumigated soil than in other soil treatments (6-19%), while frequency of *T. hamatum* was similar among treatments (0-8%). Strain-specific primers were used to confirm the identity of T382. This study suggests that T382 can become established and survive in soil under field conditions, and illustrates methods to manipulate the soil environment to favor and support stable populations of beneficial organisms.

Control of powdery mildew of grapes in California. G. LEAVITT and T. Martin-Duvall. University of California Cooperative Extension, Madera, CA. Phytopathology 94:S57. Publication no. P-2004-0386-AMA.

Powdery mildew *Uncinula necator* is the most serious disease of grapes *Vitis vinifera* in California. It can reduce berry size, storage life, and promote bunch rots by causing berry crack. This study evaluated several fungicides with similar and different modes of action for the control of powdery mildew in commercial vineyards. A Carignane vineyard was divided into 24 treatments with four replications in a randomized complete block design. Treatments were applied at 7, 14, or 21 day intervals by manufacturer recommendation or the Gubler/Thomas mildew index. The index was monitored until veraison to determine need for applications at each interval. All treatments were applied with Stihl backpack mist blowers beginning on 6 May. The first two applications were at 100 GPA spray volume with the remaining at 150 GPA. Severity of mildew infection was evaluated on 30 June. The greatest control was achieved by Pristine on either treatment regime. Treatments containing sterol inhibitors – Elite, Procure, Rally, Rubigan, or spiroxamine provided good to excellent control except the rotation of Rally and Abound. Treatments including strobilurins provided good to excellent control. V10116 did not provide adequate control possibly because of the long (21 to 28 day) spray interval. Contact materials such as Nordox, Microthiol, and Kaligreen did not perform as well as in the past. This was probably due to inadequate coverage. Quintec, a new material with registration pending, has consistently provided good to excellent control. Most of the systemic materials tested will perform acceptably in a commercial vineyard. Contact materials need improved coverage in order to provide effective control.

An investigation of an outbreak of *Impatiens necrotic spot virus* in New Zealand. B. S. M. LEBAS, F. M. Ochoa-Corona, D. R. Elliott, Z. Tang, B. J. R. Alexander, and K. J. Froud. National Plant Pest Reference Laboratory, Ministry of Agriculture and Forestry of New Zealand, PO Box 2095, Auckland 1015, New Zealand. Phytopathology 94:S57. Publication no. P-2004-0387-AMA.

Impatiens necrotic spot tospovirus (INSV) causes significant losses in a wide variety of glasshouse ornamentals in Europe and the USA. INSV was detected for the first time in New Zealand (South Island) in August 2003 from freesia, a new host record. The virus was also found in impatiens, ranunculus, primula and lobelia from the same site. In a survey of 50 nurseries from the North and South Islands, a total of 637 samples were collected from more than 45 plant genera. INSV was detected on impatiens at two additional sites in the North Island. The infected impatiens were from both seed and tissue culture derived plants. It is unknown whether the imported plants or seeds became infected with INSV prior to arrival, or during the growing on stage in New Zealand.

INSV isolates shared 96-99% nucleotide identity (ca. 800-base sequence) with INSV isolates from USA, Japan and the Netherlands. The Ministry of Agriculture and Forestry and industry are discussing appropriate actions.

A surface loop of the potato leafroll virus coat protein is involved in virion stability and aphid transmission. L. Lee (1), I. K[illegible]AN (1), D. Ripoll (2), D. Liang (1), P. Palukaitis (3), and S. Gray (1). (1) USDA/ARS, Ithaca, NY; (2) Theory Center, Cornell University, Ithaca, NY; (3) Scottish Crop Research Institute, Invergowrie, UK. Phytopathology 94:S58. Publication no. P-2004-0388-AMA.

Two acidic domains of the potato leafroll virus (PLRV) coat protein separated by 55 amino acids that are predicted to be adjacent surface features on the virion, were the focus of a mutational analysis. A total of eleven, site-directed mutants were generated from a cloned infectious cDNA of PLRV. The cloned PLRV was delivered to plants using *Agrobacterium*-mediated mechanical inoculation. Alanine substitutions of any of the three amino acids of the sequence EWH (amino acids 171-173) affected the ability of the coat protein to assemble stable particles and the viral RNA to move systemically in four plant hosts. A similar phenotype was observed when alanine was substituted for D177. Alanine substitution of E109, D173 or E176 reduced the accumulation of virus in agroinfiltrated tissues and reduced the efficiency of systemic infection, but did not affect virion assembly. The efficiency of aphid transmission of these three mutants was also reduced relative to wild-type virus. A replacement of V169 with lysine did not affect virion assembly or plant infection, but did reduce the efficiency of aphid transmission. A structural model of the PLRV capsid predicts that the four amino acids critical for virion assembly are located in a depression located at the three-fold axis of symmetry, and the other amino acids affecting plant infection and/or aphid transmission are located around the perimeter of the depression.

Structure and dynamics of *Rhizoctonia* populations in creeping bentgrass in response to fungicide programs. D. J. LEE, L. P. Tredway, C. H. Peacock, and H. D. Shew. North Carolina State University, Raleigh, NC. Phytopathology 94:S58. Publication no. P-2004-0389-AMA.

Populations of *Rhizoctonia* spp. Within a creeping bentgrass (*Agrostis palustris*) putting green were monitored over time in response to fungicide applications. Fourteen fungicide programs were initiated in 1.52 m^2 plots of 'L-93' creeping bentgrass arranged in a randomized complete block with [illegible] replications. Twelve, 5 mm soil cores were taken from the top 1.27 cm from each plot prior to initiation of fungicide treatments and on 14 day intervals after initiation of treatments for a total of eight sampling periods. Soil cores were placed on alkaline water agar for selective isolation of *Rhizoctonia* spp. *R. zeae* isolates were identified morphologically. Isolates of *R. solani* (2-2IIIB) were identified to anastomosis group (AG) level by pairing with a known isolate of AG 2-2IIIB. Isolates were paired within and across sampling dates to identify isolates to the clonal level. Of 103 isolates collected during 2002, 74 were *R. solani* AG 2-2IIIB and 29 were *R. zeae*. Twenty-one distinct clones were characterized in the 2-2IIIB population. Only seven of the clones were recovered at multiple samplings. The efficiency of *Rhizoctonia* isolation and the number of clones collected decreased following the initiation of fungicide applications. All 21 clones responded similarly to fungicides *in vitro*. The population structure of these systems is diverse and shifts in these populations were not directly related to fungicide applications.

Relative role of minor and major resistance genes in flood mediated blast field resistance. F. N. LEE, M. P. Singh, and P. A. Counce. University of Arkansas. Phytopathology 94:S58. Publication no. P-2004-0390-AMA.

Arkansas rice growers produced record per acre rough rice yields when growing the blast, *Magnaporthe grisea*, susceptible cultivars Wells, LaGrue, CoCodrie during the blast conducive years 2000-2003. High field resistance induced by continuous ten to fifteen cm flood irrigation served as the primary rice blast control measure. The resistance mechanisms involved and the interactive relationships between inherent plant resistance genes and the resulting flood induced rice blast field resistance, as mediated by hypoxic soil conditions, are poorly understood. We investigated relative levels of flood induced resistance in susceptible cultivars with and without the *Pi-ta* resistance gene. Data summarized from seven tests where plants of susceptible cultivars growing upland were compared with those treated with a continuous fifteen cm flood for seven to ten days. The average blast index (BI) for the cultivars M-201, Newbonnet, LaGrue and Cypress was 86, 65, 70 and 54, respectively, under upland conditions. The BI's of these susceptible cultivars were reduced by 18, 44, 38 and 33 percent, respectively, under a continuous flood. Cultivars Katy, Drew, Kaybonnet and Ahrent, all having the *Pi-ta* resistance gene, were challenged with *M. grisea* race shift isolates IB-33, IE-1K, and MSG-19 when growing upland or with a continuous 15 cm flood. Overall, the blast index for these R gene cultivars inoculated with the race shift isolates was lower by 49% for plants growing in a continuous 15 cm flood.

Closely related but distinct phytoplasmas associated with potato purple top and potato witches'-broom diseases in the U.S. I.-M. LEE (1), K. D. Bottner (1), J. [illegible]nyaneza (2), W. L. Campbell (3), G. A. Secor (4), and N. C. Gudmestad (4). (1) USDA, ARS, MPPL, Beltsville, MD; (2) USDA, ARS, YARL, Wapato, WA; (3) Dept. of Natural Resources, Palmer, AK; (4) North Dakota State University, Fargo, ND. Phytopathology 94:S58. Publication no. P-2004-0391-AMA.

Phytoplasma strains belonging to the clover proliferation group (16SrVI), subgroup 16SrVI-A, have been reported to be associated with potato witches'-broom (PWB) disease in Canada and potato purple top (PPT) and similar diseases in Utah and the Columbia Basin region of Washington and Oregon. We found a potato disease similar to PWB in North Dakota and Alaska. A preliminary study indicated that this disease is also associated with 16SrVI-A phytoplasma strains. In nature, PPT and PWB phytoplasmas may represent two distinct ecological strain clusters that occur in different geographical regions and cause two different types of diseases. Based on RFLP analysis of highly conserved 16S rDNA sequences, PPT- and PWB-associated phytoplasmas could not be readily differentiated. In the present study, we employed ribosomal protein (rp) gene sequences (more variable than 16S rRNA gene) for differentiation. RFLP analyses of rp gene sequences with restriction enzymes, *Alu*I, *Mse*I, *Tsp509*I, and *Taq*I differentiated PPT and PWB phytoplasma strains into four rpVI-subgroups. These results support the concept that genetic heterogeneity exists among PWB and PPT phytoplasma strains.

The alignment between physical and genetic maps of *Gibberella zeae*. J. LE[illegible]), J. E. Jurgenson (2), J. F. Leslie (1), and R. L. Bowden (3). (1) Kansas State University, Manhattan, KS; (2) University of Northern Iowa, Cedar Falls, IA; (3) USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS. Phytopathology 94:S58. Publication no. P-2004-0392-AMA.

We previously published a genetic map of *Gibberella zeae* (*Fusarium graminearum*) based on a cross between Kansas strain Z-3639 (lineage 7) and Japanese strain R-5470 (lineage 6). The genetic map was based on 1048 AFLP markers and consisted of nine linkage groups. We aligned the genetic map with the first assembly of the genomic sequence of strain PH-1 (lineage 7) that was released by The Broad Institute (Cambridge, MA). We sequenced 126 AFLP markers from all nine linkage groups (LG) of the genetic map, 115 of which were associated with nine supercontigs (SC) of the genomic sequence, and 11 markers had no hits in the genomic sequence. LGs 1 and 9, LG 2, LG 3, LGs 4, 5, and 6, and LGs 7 and 8 were aligned with SC 2, SC 3, 8, and 9, SC 4 and 6, SC 1 and 7, and SC 5, respectively. Approximately 99% of the sequence was anchored to the genetic map, indicating the high quality of the sequence assembly. The physical alignments resulted in the reduction of genetic linkage groups from nine to five. Several apparent misalignments of the genetic and physical maps are being explored.

Comparison of transformation methods and development of gene replacement in *Monilinia fructicola*. M. H. LEE and R. M. Bostock. University of California, Davis, CA. Phytopathology 94:S58. Publication no. P-2004-0393-AMA.

Monilinia fructicola, the causal agent of brown rot disease of stone fruits, is a multinucleate fungus that until recently has been recalcitrant to molecular studies. We have transformed *M. fructicola* using either *Agrobacterium*-mediated transformation or protoplast transformation. A cutinase gene (*Mfcut1*) was used as a model gene to study the manner of gene replacement in *M. fructicola*. The ORF of *Mfcut1* and flanking regions were isolated by inverse PCR. A portion of the *Mfcut1* sequence was replaced with *NptII*. The resulting construct was placed in the binary vector pCAMBIA1300, and then used for transformation studies. About 150 transformants from *Agrobacterium*-mediated transformation were screened by PCR and DNA gel blot analyses, and 51% of these were confirmed to have a replacement of the wild-type *Mfcut1*. Results of protoplast transformation and progress towards purification of homokaryotic transformants will be presented.

Identification of microsatellite and STS markers for resistance genes *Rsp1*, *Rsp2*, and *Rsp3* to *Septoria passerinii* in barley. S. H. LEE and S. M. Neate. North Dakota State University, Dept. Plant Pathology, Fargo, ND 58105. Phytopathology 94:S58. Publication no. P-2004-0394-AMA.

Septoria speckled leaf blotch (SSLB) caused by the pathogen *Septoria passerinii* is a common leaf disease in cultivated barley in North Dakota. The disease causes economic losses in yield and malting quality and genetic resistance is the preferred method of control. Identification of molecular markers for SSLB resistance genes will allow marker-assisted selection (MAS) in breeding. To develop molecular markers for resistance, susceptible cultivars Robust and Foster, were crossed with three resistant lines, Ciho14300 containing the resistance gene *Rsp*1, Ciho4780 containing *Rsp*2,

and Ciho10644 containing *Rsp*3. RAPD markers that associated with the SSLB resistance genes *Rsp*1 (UBC253428), *Rsp*2 (OPBA20410), and *Rsp*3 (OPB11495) in the F_1 generations were identified. Sequence tagged site (STS) markers were developed from the RAPD markers. Microsatellite (SSR) markers were also tested for their association with the SSLB resistance genes. Seven SSR markers associated with *Rsp*1, four SSR markers associated with *Rsp*2, and five SSR markers associated with *Rsp*3 were identified. Validation in F_2 and subsequent generations in the greenhouse and field is underway.

Searching antimicrobial activities from plant rhizosphere metagenomic library. S.-W. LEE (1), H. K. Lim (1), E. J. Chung (1,2), K. S. Jang (1), J.-C. Kim (1), G. J. Choi (1), Y. R. Chung (2), and K. Y. Cho (1). (1) Biological Function Research Team, Korea Research Institute of Chemical Technology, Daejeon, Ko (2) Dept. Microbiology, Gyeongsang National University, Jinju, Korea. Phytopathology 94:S59. Publication no. P-2004-0395-AMA.

The construction and screening of metagenomic libraries constitute a valuable resource for obtaining novel antimicrobial activities. We constructed soil metagenomic library in *Escherichia coli* using DNA directly extracted from two different soils, pine tree rhizosphere soil and forest topsoil. Metagenomic libraries constructed from pine tree rhizosphere soil and forest topsoil consisted of approximately 33,700 clones and 112,000 clones with an average DNA insert size of 35-kb, respectively. Subsequently, we screened the libraries to select clones with antimicrobial activities against *Saccharomyces cerevisiae* and *Agrobacterium tumefaciens* using double agar layer method. We isolated a clone active against *S. cerevisiae* and a clone active against *A. tumefaciens* from the forest topsoil library. *In vitro* r thesis and DNA sequence analysis of the antifungal clone revealed the genes involved in the biosynthesis of antimicrobial secondary metabolite.

Identification of HrpA mutants that block type III secretion in *Pseudomonas syringae* pv. *tomato* DC3000. Y. H. LEE (1,3), O. O. Kolade (1,2), D. N. Arvidson (2), and S. Y. He (1). (1) Michigan University, Department of Energy Plant Research Laboratory; (2) Michigan State University, rtment of Microbiology and Molecular Genetics, East Lansing, MI; (3) National Institute of Agricu l Science and Technology, Rural Development Administration, Korea. Phytopathology 94:S59. Publication no. P-2004-0396-AMA.

Pseudomonas syringae pv. *tomato* DC3000 (*Pst*) infects susceptible tomato and Arabidopsis, causing chlorosis and n osis in the leaves. An essential virulence system in *Pst* DC3000 is the type III secretion system (TTSS), which delivers effector proteins into the host cell. A critical component of the TTSS in this bacterium is the Hrp pilus, which is made of the HrpA protein. We have previously shown that the Hrp pilus plays an essential role in the virulence of *Pst* DC3000 and functions as a conduit for trafficking effector proteins. Here, we identified several mutant HrpA proteins that exert a dominant-negative effect on type III secretion in wild-type *Pst* DC3000. These mutations can be grouped into two classes based on their locations in the HrpA protein. Class I affected in vitro type III secretion of AvrPto by *Pst* DC3000 as well as the ability of *Pst* DC3000 to elicit a hypersensitive response (HR) in the nonhost tobacco; whereas class II did not affect the ability of *Pst* DC3000 to secrete AvrPto in culture, but the ability of *Pst* DC3000 to elicit an HR in tobacco was inhibited. The effects of these dominant-negative mutants on Hrp pilus assembly are being examined.

The glassy-winged sharpshooter transmits *Xylella fastidiosa* between sycamore trees. T. D. LEININGER, N. M. Schiff, and K. C. bin. USDA, Forest Service, Southern Research Station, Stoneville, MS. Phytopathology 94:S59. Publication no. P-2004-0397-AMA.

American sycamore (*Platanus occidentalis* L.) has good commercial value because of its rapid growth and excellent pulping qualities. Widespread establishment of sycamore plantations in the southeastern United States has been hampered by bacterial leaf scorch (BLS), caused by *Xylella fastidiosa*, which typically occurs within five years after planting, especially on sites for which sycamore is not well suited. The glassy-winged sharpshooter (*Homalodisca coagulata*; GWSS) can transmit *X. f.* to grapes, causing Pierce's disease. GWSS were allowed to feed for 24 h on BLS-symptomatic sycamore branches that tested positive by ELISA for *X. f.*, after which they were collected. Six *X. f.*-infested GWSS were then caged for 24 h on each of thirty one-yr-old sycamore seedlings that tested negative by ELISA for *X. f.* Sycamore leaves were tested monthly by ELISA during the 2002 and 2003 growing seasons. Seventeen of 30 seedlings exposed to *X. f.*-infested GWSS tested positive for the bacterium, while no seedlings exposed to uninfected-GWSS tested positive. To our knowledge, this is the first report of GWSS transmitting *X. f.* to sycamore trees.

Calcium availability influences aggregation and adhesion of *Xylella fastidiosa*, the causal agent of the Pierce's disease in grapes. B. LEITE (1), M. L. Ishida (1), B. Brodbeck (1), L. Marques (2), M. E. Olson (2), M. R. Braga (3), and P. C. Andersen (1). (1) University of Florida, Quincy, FL; (2) University of Calgary, Calgary, Canada; (3) Institute of Botany, São Paulo, Brazil. Phytopathology 94:S59. Publication no. P-2004-0398-AMA.

Xylem fluid collected from the same grapevine cultivars growing in California and Florida showed significant effects of both cultivar and location on the ratio of calcium/phosphate (Ca/P) as well as pH. It has been reported that calcium bridging is critical for aggregation and adhesion of *X. fastidiosa*. Our results showed that under acidic pH (5.5), calcium remains in solution as free ions and aggregation is stimula In contrast, in basic pH (8) calcium phosphate precipitates. In plants from California, the ratio Ca/P is close to 1 for *Vitis rotundifolia* "Noble" (resistant) and 14.5 for *Vitis vinifera* "Chardonnay" (susceptible). Additionally, the content of acidic pectins, known to bind calcium, was found to be higher in the xylem walls of the resistant cultivar "Noble". These findings suggest that calcium availability may influence vessel plugging and symptom development in Pierce´s disease. Soil type (composition) may change xylem fluid pH, which in turn may influence calcium availability, which then impacts aggregation.

An overview of risk ratings that examined exotic plant pathogens in pest risk analyses. A. V. L Y and E. M. Sutker. USDA-APHIS-PPQ-CPHST-PERAL, Raleigh, NC. Phytopathology 94:S59. Publication no. P-2004-0399-AMA.

Pest Risk Analyses (PRAs) conducted by the United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine precede the regulatory authorization for the import of a commodity into the United States. Selected PRAs analyzed 329 quarantine pests likely to follow their respective commodity pathways. About 85% were arthropods and mollusks, while 15% were plant pathogens: 34 fungi, 9 nematodes, 5 viruses, 2 bacteria and 1 phytoplasma. The Pest Risk Potential (PRP) for pathogens generally rated lower than for arthropods, but almost all ratings exceeded the threshold to initiate consideration of mitigation measures. Generally, the PRP for genera containing saprophytes, moderately pathogenic or opportunistic organisms having a broad host range, such as *Pestalotia* and *Pestalotiospheria*, was not different from or exceeded the ratings for pathogens that severely affect a limited number of hosts, such as rust fungi. The influence of host range on various PRP criteria in the risk rating process is discussed.

Population dynamics of *Acidovorax avenae* subsp. *citrulli* on watermelon blossoms. J. T. LESSL and R. R. Walcott. University of Georgia. Phytopathology 94:S59. Publication no. P-2004-0400-AMA.

Acidovorax avenae subsp. *citrulli* (Aac), the causal agent of bacterial fruit blotch (BFB), can cause devastating losses in cucurbits in regions with high moisture and humidity. Primary inoculum is seedborne and watermelon blossom inoculation previously was shown to be one way of infesting seed, particularly within symptomless fruit. To better understand this process of seed infection, we investigated the temporal population dynamics of Aac in female watermelon blossoms. Aac populations were monitored in blossoms with and without pollination and their growth was compared to epiphytic colonization by *Pseudomonas syringae* pv. *syringae* (Pss) Cit7. Aac populations rapidly increased in watermelon blossoms from 2×10^2 colony-forming-units (CFU)/blossom to 5×10^8 CFU/blossom in 96h. Aac growth was not influenced by pollination and did not differ from that of Pss. We hypothosized that sti tic exudates in female blossoms can support significant growth of Aac. These data support the hypothesis that blossom colonization can be involved in seed infection in commercial seed production fields.

Induced resistance in plants treated with solarized soil or *Trichoderma*. N. O. LEVY (1,2), Y. Elad (1), and J. Katan (1,2). (1) Department of Plant Pathology, Volcani Center, Bet Daga rael; (2) Faculty of Agriculture, The Hebrew University, Rehovot, Israel. Phytopathology 94:S59. Publication no. P-2004-0401-AMA.

Both soil solarization and *Trichoderma* reduce incidence of many diseases due to their direct detrimental effect on pathogens. In the present work, we studied induced systemic resistance following solarization and/or *Trichoderma* treatments. In our system, only the roots were in contact with the solarized soil or *Trichoderma* preparation, while the foliage was inoculated with pathogens. Strawberry, cucumber and common bean grown on treated soil or growth coconut substrate showed significant reduction in disease after leaf infection with *Botrytis cinerea* or with *Sphaerotheca fuliginea*, hence indicating induced resistance. For example, the percentage disease coverage on foliage of cucumber caused by *S. fuliginea* was 25.0, 12.8, 11.4 and 12.7, in nontreated, solarization, *Trichoderma* and combined treatments, respectively. Attempts are being made to find the relationship between indigenous populations of microorganisms in the rhizosphere and disease control.

For this purpose we use a molecular approach based on 16S-rDNA and denaturating gradient gel electrophoresis (DGGE). The above-mentioned treatments resulted in changes in the DGGE patterns of soil and substrate populations. Induced resistance by soil solarization corresponds with previous studies demonstrating physiological changes in foliage of plants growing in solarized soil, and with higher population of fluorescent pseudomonads (known as resistance inducers) in the rhizosphere of the plants in solarized soils.

Root infection of rhododendron by *Phytophthora ramorum*. C. LEWIS (1), M. L. Roth (2), C. J. Choquette (2), and J. L. Parke (1,2). (1) Dept. of Botany and Plant Pathology, (2) Dept. of Crop and Soil Science, Oregon State University, Corvallis, OR. Phytopathology 94:S60. Publication no. P-2004-0402-AMA.

The foliar pathogen *Phytophthora ramorum* causes ramorum shoot dieback and leaf blight on many nursery species in Europe and N. America. Although it has been detected in soil, this has not been shown to be a source of inoculum for new infections, nor have roots of any species been found to be infected by *P. ramorum*. Three-year-old rhododendron 'Nova Zembla' plants grown from rooted cuttings were repotted in a 2:1 mixture of potting soil and V8-vermiculite inoculum of *P. ramorum* nursery isolate 03-74-N11A. Leaves never touched the potting media, and plants were watered from the bottom only. Leaves wilted and collapsed within 3-6 weeks and upwardly-expanding stem lesions became apparent within 4-6 weeks. *P. ramorum* was identified through both isolations and diagnostic PCR from symptomatic roots, stems and petioles. Control plants not inoculated with *P. ramorum* remained healthy and did not yield *P. ramorum*. Plant infection by soilborne inoculum of *P. ramorum* demonstrates a new potential means of disease spread, requiring reconsideration of appropriate disease management strategies.

An improved RT-PCR assay for the detection of two cherry foveaviruses in *Prunus* spp. R. LI and R. G. Mock. USDA-ARS, Fruit Laboratory/Plant Germplasm Quarantine Office, Beltsville, MD. Phytopathology 94:S60. Publication no. P-2004-0403-AMA.

A one-step reverse transcription-polymerase chain reaction (RT-PCR) procedure was developed to detect *Cherry green ring mottle virus* (CGRMV) and Cherry necrotic rusty mottle virus (CNRMV) in indicator and naturally-infected *Prunus* spp. Viral RNA suitable for RT-PCR was obtained by a simple method that did not require extraction of dsRNA and total RNA, availability of antibodies to virus or purification of viral particles. Consensus primers and degenerate primers, whose designs were based on alignments of published sequences of foveaviruses infecting cherry, were tested to amplify viral genomic fragments of five CGRMV isolates and one CNRMV isolate. RT-PCR using a pair of consensus primers allowed CGRMV detection in viral RNA and total RNA preparations equivalent to 80 ng and 400 mg of infected leaf tissue, respectively. CGRMV was detected in leaves, tender shoots, petioles, and young bark, whereas the strongest bands were obtained using young leaves and tender shoots. Detection was less consistent in summer when the temperature was elevated and plant tissues were old. The direct comparison of RT-PCR and bioassay indicates that the RT-PCR assay is sensitive, rapid and reliable. The method developed should allow for the rapid diagnosis of cherry foveaviruses in *Prunus* spp.

A national soybean pathogen collection system: The National Soybean Pathogen Collection Center. S. LI (1), G. L. Hartman (1,2), T. Niblack (1), and D. Phillips (3). (1) National Soybean Pathogen Collection Center, Dept. of Crop Sciences, Univ. of Illinois; (2) USDA-ARS; (3) University of Georgia. Phytopathology 94:S60. Publication no. P-2004-0404-AMA.

The National Soybean Pathogen Collection Center (NSPCC) was established in August 2002 and is located in the National Soybean Research Center at the University of Illinois. The goals of NSPCC are to: (i) assemble collections of living soybean pathogens representing a range of diversity, (ii) characterize, evaluate, maintain, and distribute isolates in the collections, and (iii) serve as a liaison among public and private researchers for information and training on germplasm screening, and to enhance the understanding of pathogen diversity. NSPCC maintains representative duplicates of individual state collections as well as maintains and seeks collections of its own. At the present time, the NSPCC has representative cultures of *Fusarium solani* f. sp. *glycines* (cause of sudden death syndrome), *Heterodera glycines* (soybean cyst nematode), *Meloidogyne* spp. (root-knot nematode), and *Phytophthora sojae* (cause of Phytophthora stem and root rot). Also, the NSPCC has in reserve minor collections of a number of other soybean pathogens. NSPCC has provided soybean pathogen cultures to over 50 national and international research labs and companies for their research. A centralized collection with a long-term strategy to address diversity and preservation of soybean pathogens will benefit soybean researchers and producers.

Real-time quantitative PCR detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots and soil. S. LI, X. Zeng, Y. Ge (1), and G. L. Hartman (1,2). (1) National Soybean Pathogen Collection Center, Dept. of Crop Sciences, Univ. of Illinois; (2) USDA-ARS, Urbana, IL 61801. Phytopathology 94:S60. Publication no. P-2004-0405-AMA.

Fusarium solani f. sp. *glycines* (*Fsg*) is a soilborne fungus that causes soybean sudden death syndrome (SDS). As part of a continuing effort to investigate the population structure of *Fsg* and develop disease control strategies, a real-time quantitative PCR (Q-PCR) method was developed to detect and quantify *Fsg* in soybean roots and soil. Specific primers or probes were designed based on our previous and new DNA sequence data from the mitochondrial small subunit ribosomal RNA gene and the elongation factor 1-alpha gene. *Fsg* DNA was detected and quantified in infected soybean roots and soil samples. Quantitative data obtained from both Q-PCR and traditional colony-forming units (CFU) was used to analyze colonization level of *Fsg* isolates from inoculated plants. Comparison of the Q-PCR, CFU, and pathogenicity data will be discussed. The specificity, sensitivity, accuracy, and reliability of the Q-PCR assay offer potential advantages in routine detection and quantification of *Fsg* in soybean roots and soil samples.

Green fluorescent protein-labeled strains of *Xylella fastidiosa* colonize citrus, grapevines and periwinkle. W. LI and J. S. Hartung. USDA-ARS, Fruit Laboratory, Beltsville, MD 20705. Phytopathology 94:S60. Publication no. P-2004-0406-AMA.

Grapevines, sweet orange and periwinkle are three natural symptomatic host plants of *Xylella fastidiosa*. Our previous studies showed that *X. fastidiosa* strains from citrus could also induced disease symptoms in grapevines and periwinkle. In our post-genomic research program, defined gfp-marked strains were constructed from a citrus strain of *X. fastidiosa* for studies in grapevines, citrus and periwinkle. We inoculated these three host plants mechanically with the gfp-labeled mutants in a greenhouse. These plants began to develop disease symptoms three months post-inoculation. The gfp-marked transposon inserted into the genomic DNA of the bacterium remained stable and the gfp gene expressed in the three different host plants. Histological studies of the gfp-marked strains in the three plants were conducted using a laser scanning confocal microscope. The population dynamics of the gfp-labeled strains in these plants were studied by using real-time quantitative PCR.

Disease changes associated with climate changes in China from 1952 to 2002. X. LI (1), X. B. Yang (1), B. H. Hu (2), and P. Sun (3). (1) Iowa State University, Ames, IA; (2) Ministry of Agriculture of China, Beijing, China; (3) University of Toronto, Canada. Phytopathology 94:S60. Publication no. P-2004-0407-AMA.

To examine the effects of climate changes on plant diseases in China, long-term data (1952 – 2002) for the occurrence of five major plant diseases, stripe rust, powdery mildew, scab on wheat, blast and sheath blight on rice, were analyzed with climatic data. Disease data consist of annual prevalence of each disease and a qualitative pandemic index of stripe rust collected systematically through a national disease monitoring system. Minimum, maximum, and mean temperatures as well as diurnal temperature range (DTR) were calculated for the growing season of rice or wheat. Regression analysis show that the prevalence of warm-temperature diseases (powdery mildew, scab, blast, and sheath blight) was positively correlated to increasing minimum and mean temperatures, indicating that increasing temperature was associated with disease increase since the 1970s. For cold-temperature disease (stripe rust), pandemic index was positively correlated to the decrease of DTR caused by the increase of minimum temperature, indicating that warmer temperature suppressed stripe rust.

Western Australian isolates of *Leptosphaeria maculans* show physiological adaptation to single dominant gene-based resistance in cultivars of *Brassica napus*. H. Li (1), M. J. Barbetti (2), L. Damour (3), and K. SIVASITHAMPARAM (1). (1) School of Earth and Geographical Sciences; (2) School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009, Australia; (3) ESMISAB, 30 rue Fregate La Boussole, 29200 Brest, France. Phytopathology 94:S60. Publication no. P-2004-0408-AMA.

Blackleg disease (*Leptosphaeria maculans*) causes severe yield loss in canola crops. New cultivars with single dominant gene-based resistance derived from *B. rapa* ssp. *sylvestris* were released in Australia-wide from 2000, initially showing outstanding resistance. In 2001, isolates of *L. maculans* obtained from such a cultivar, Surpass 400, in Western Australia were shown to overcome this single dominant gene-based resistance. These isolates, however, did not significantly affect the background polygenic resistance present in these cultivars. In contrast, isolates obtained from 2002 crops caused severe cotyledon and crown canker disease leading to lodgment and death of plants.

Against these isolates, the background polygenic resistance was inadequate to prevent severe disease. These results indicate that sole reliance upon this single dominant gene-based resistance is not sustainable.

Fusarium stem rot of vanilla in North Sulawesi. E. C. Y. LIEW (1), F. Rondonuwu (1), A. Pinaria (2), D. T. Sembel (2), B. A. Summerell (3), and L. W. Burgess (1). (1) Faculty of Agriculture, Food & Natural Resources, The University of Sydney, NSW 2006, Australia; (2) Department of Pests & Diseases, Faculty of Agriculture, Sam Ratulangi University, North Sulawesi, Indonesia; (3) Royal Botanic Gardens, Sydney, NSW 2000, Australia. Phytopathology 94:S61. Publication no. P-2004-0409-AMA.

Vanilla is an important and popular cash crop offering high economic returns to smallholder farmers in North Sulawesi. However, the production of vanilla in this region is greatly constrained by Fusarium stem rot. Although the disease is most severe on the stem of the vanilla vine, it is also found on the leaves and roots. On the stem internode, small brown water-soaked spots or lesions initially appear, which enlarge and become necrotic, eventually girdling and shriveling up the stem. The lesion often expands to adjacent internodes and develops along the vine. In a recent disease survey of vanilla farms on 12 sites throughout North Sulawesi, the disease was detected at incidences of 20-78% on each site. Several *Fusarium* species were isolated from various plant parts of diseased vines. Only *F. oxysporum* was shown to be pathogenic in greenhouse experiments. *Colletotrichum* was also isolated from diseased tissue but at a lower frequency.

***Ceratocystis* associated with clove decline in North Sulawesi.** E. C. Y. LIEW (1), B. Assa (2), M. J. Wingfield (3), D. T. Sembel (2), B. A. Summerell (4), and L. W. Burgess (1). (1) Faculty of Agriculture, Food & Natural Resources, The University of Sydney, NSW 2006, Australia; (2) Department of Pests & Diseases, Faculty of Agriculture, Sam Ratulangi University, North Sulawesi, Indonesia; (3) Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Republic of South Africa; (4) Royal Botanic Gardens, Sydney, NSW 2000, Australia. Phytopathology 94:S61. Publication no. P-2004-0410-AMA.

Clove decline is the most serious of clove diseases in North Sulawesi, Indonesia. Despite its devastating impact on one of the most important cash crops in this region, the aetiology of this disease has yet been established until recently. Infected trees show symptoms of wilt and defoliation, which often lead to the death of whole trees. In a recent disease survey of 17 sites and more than 100 farms throughout North Sulawesi, clove decline was observed on all sites, with disease incidence of greater than 90%. A fungus, closely resembling the wilt pathogen, *Ceratocystis fimbriata*, was consistently isolated from wood tissues of infected trees. This fungus appears to be associated with a wood-boring beetle, *Hexamitodera semivelutina*. The larvae bore into trees forming extensive galleries within the trunks. All isolates of this fungus were obtained from stained wood tissue adjacent to these wood-borer galleries. Multiple pathogenicity tests confirmed the pathogenicity of this fungus on both seedlings and mature clove trees. Molecular studies are being conducted on these isolates to confirm their identity.

Expression of *Soybean mosaic virus* (SMV) HC-Pro in transgenic soybean plants enhances SMV symptoms. H. S. LIM (1), T. S. Ko (2), L. L. Domier (1,3), H. G. Kinard (1), and G. L. Hartman (1,3). (1) University of Illinois Urbana, IL; (2) University of Minnesota, St. Paul, MN; (3) USDA-ARS Urbana, IL; (4) Chungnam National University, Daejeon, Korea. Phytopathology 94:S61. Publication no. P-2004-0411-AMA.

Transgenic soybean lines expressing the helper component-protease (HC-Pro) coding region of SMV G5 were produced by *Agrobacterium*-mediated transformation of immature soybean cotyledons. Homozygous transgenic lines were recovered with single copy insertions that expressed SMV HC-Pro mRNA at levels ranging from 5 to 34% of that found in SMV-infected plants. Ten days after inoculation with SMV, all HC-Pro transgenic lines had symptoms more severe than *uidA* transgenic plants. Symptom severity was related to HC-Pro expression levels in the transgenic lines, but SMV RNA titers did not differ among the lines. Similar levels of small interfering RNAs were detected in SMV-infected HC-Pro and *uidA* transgenic plants. Starting at 20 days after inoculation, new leaves of the transgenic line that expressed the highest level of HC-Pro mRNA no longer showed symptoms, and SMV RNA titers were drastically reduced. The titers of coat-protein and HC-Pro coding regions declined at similar rates suggesting that the HC-Pro coding region was not preferentially targeted. In soybean lines that expressed lower levels of HC-Pro, symptoms remained severe at all sampling dates and no reductions in SMV RNA titers were observed. These results show that HC-Pro enhances SMV symptom severity in transgenic plants and that even potyvirus HC-Pro transgenes are susceptible to virus-induced gene silencing when expressed at high levels.

Characterization of two new strains of grapevine rupestris stem pitting associated virus. M. F. LIMA (1), R. Alkowni (2), D. Golino (1), J. K. Uyemoto (1), and A. Rowhani (1). (1) Department of Plant Pathology, University of California, Davis, CA; (2) Arab American University, Jenin, Palestine. Phytopathology 94:S61. Publication no. P-2004-0412-AMA.

Grapevine rupestris stem pitting associated virus (GRSPaV) is graft-transmissible and associated with rupestris stem pitting disorder. GRSPaV is detected by indexing on *Vitis rupestris* cv. St. George, in which it induces pittings below the graft union. GRSPaV belongs to the genus *Foveavirus* in the family *Flexiviridae*. In field surveys in California, two new strains of GRSPaV from a *V. vinifera* cv. Syrah (SY-RSP) and cv. Pinot Noir (PN-RSP) were identified. CDNA libraries were made from dsRNA, using random primers. Selected cDNA clones were sequenced and specific primers were designed and used in RT-PCR to fill the gaps. Sequences were compared with the published sequences of GRSPaV. Both isolates showed similar genome organization, comprising six ORFs. Nucleotide and amino acid analysis showed 76-89% and 85-96% (SY-RSP), and 79-89% and 83-93% (PN-RSP), respectively, to published isolate. Specific detection primers for both isolates were designed for a survey to determine their rate of spread in the field.

Genotyping and assessing genetic diversity in the PD strains of *Xylella fastidiosa* by simple sequence repeat (SSR) DNA markers. H. LIN (1), M. Francis (2), S. Barros (2), R. Hu (2), E. L. Civerolo (1), and A. M. Walker (2). (1) ARS-USDA, 9611 S. Riverbend Avenue, Parlier, CA 93648; (2) Department of Viticulture & Enology, University of California, Davis, CA 95616. Phytopathology 94:S61. Publication no. P-2004-0413-AMA.

The insect-transmitted bacterium, *Xylella fastidiosa* (Xf), causes diseases in many economically important plants, including Pierce's disease in grape. Understanding the genetic diversity and population structure of the pathogen are critical steps in managing disease outbreaks. With the recently available genomic sequencing of four Xf strains, Pierce's disease of grapevine (PD), citrus variegated chlorosis (CVC), almond leaf scorch (ALS), and oleander leaf scorch (OLS), identification of repeated sequence loci is facilitated. A genome wide search was performed for identifying Simple Sequence Repeat (SSR) motifs among the all four strain sequence databases, and 60 SSR loci were selected for primer design. These simple repeat motifs consist of 2-8 bp repeat units and were distributed across the whole genome. We evaluated these SSR primers with 21 Xf isolates collected from Napa Valley, San Joaquin Valley and southern California vineyards. Seventy percent of these SSR primers were highly polymorphic and distinguished genetically close strains. This PCR-based SSR marker is a precise and repeatable marker system. The power of this polymorphic detection system makes it a useful tool for population structure, genetic diversity and epidemiological risk assessment analyses.

Formulating locally effective integrated management packages for tomato bacterial wilt. C.-H. Lin and J.-F. WANG. AVRDC-The World Vegetable Center, Tainan, Taiwan. Phytopathology 94:S61. Publication no. P-2004-0414-AMA.

Bacterial wilt, caused by *Ralstonia solanacearum*, is an endemic and important tomato disease worldwide. Efficacy of reported control methods, including tolerant cultivars, resistant rootstocks, and soil amendments, could be variable over strains, locations or soils. A case study was conducted on formulating effective management packages for a production area in Taiwan. First 52 local strains were profiled based on their aggressiveness. Representative strains were selected for evaluating commercial tolerant cultivars and resistant rootstocks. Eggplant and tomato varieties, EG203 (mean percent wilting 0%) and Hawaii7996 (mean percent wilting 21%), were selected as potential rootstocks. All tolerant cultivars tested were susceptible. Local soil samples were collected to evaluate the efficacy of a soil amendment consisting urea (825 kg/ha) and slaked lime (3993 kg/ha). The amendment could reduce the pathogen density from 7.0 to 4.7 log (cfu/g dry soil) and the disease incidence from 60% to 3% at 28°C in the greenhouse. A field trial was conducted in fall 2003 consisting of 6 treatments combining the soil amendment and 3 planting materials (ASVEG10, ASVEG10 grafted on EG203 or on Hawaii7996). At 105 days after transplanting, about 90% of ASVEG10 was wilted, while treatments with eggplant rootstock showed only 1 to 3% wilting. The soil amendment effect was only observed on the treatments with tomato rootstock, which provided additional 29% reduction of incidence. The results show that applying a pre-selection scheme could ensure the success of local integrated management package.

Rice bacterial blight resistance genes *Xa7*, *xa5*, and *Xa4* confer resistance during all developmental stages. K. M. LINHOLM (1,2), E. Garcia (2), C. M. Vera Cruz (2), and J. E. Leach (1). (1) Kansas State University, Manhattan, KS; (2) International Rice Research Institute, Manila, Philippines. Phytopathology 94:S61. Publication no. P-2004-0415-AMA.

Resistance to bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* follows a gene-for-gene model where a host resistance (*R*) gene confers recognition of a corresponding pathogen effector (*avr*) gene. Previous work indicated that resistance to bacterial blight conferred by the *Xa21* rice gene was developmentally regulated, i.e., resistance was not observed in seedling plants (less than 21 days post-emergence). We investigated the impact of plant development stage on the interaction of the rice R genes *Xa7*, *xa5*, and *Xa4* with *X. oryzae* pv. *oryzae* containing the corresponding *avr* genes. *X. oryzae* pv. *oryzae* isolates, PXO145 (containing *avrXa7*, *avrxa5*, and *avrXa4*) and PXO99 (with no relevant *avr* genes), were inoculated to rice cultivars with *Xa7*, *xa5*, *Xa4*, and the near-isogenic recurrent parent, IR24, at five developmental plant stages in the greenhouse. Lesion length and bacterial enumeration studies demonstrated that resistance controlled by *Xa7*, *xa5*, or *Xa4* is effective at all tested stages of development.

The effect of organic and synthetic fertility amendments and tillage on the resilience of soils to Phytophthora blight. B. LIU and J. B. Ristaino. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695. Phytopathology 94:S62. Publication no. P-2004-0416-AMA.

Organic soil fertility amendments including cotton gin trash, poultry manure, and a rye/vetch green manure or synthetic fertilizers were incorporated in subplots and either tillage or surface mulch was applied to main plots in a field. Soil samples were removed weekly for five weeks from each plot after incorporation of the amendments, brought to the greenhouse and infested with sporangia and mycelium of *Phytophthora capsici*. Four-week-old pepper seedlings were planted in the pots and disease incidence was measured. Final disease incidence was highest in plots with cotton gin trash, but declined over time from 52-39%. Disease incidence was lowest in plots amended with rye/vetchgreen manure and ranged from 25-16%. Disease also declined over time from 64-31% in plots amended with poultry manure. Disease incidence in plots amended with synthetic fertilizers ranged from 32-27%. Some organic amendments (green manures) increased the resilence of soil to disease, while others enhanced disease (cotton gin trash) compared to synthetic fertilizers.

Emergence of resistance-breaking isolates of *Beet necrotic yellow vein virus* in the Imperial Valley, California. H.-Y. LIU, J. L. Sears, and R. T. Lewellen. USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905. Phytopathology 94:S62. Publication no. P-2004-0417-AMA.

Beet necrotic yellow vein virus (BNYVV) is the causal agent of rhizomania disease of sugar beet. The virus is transmitted by the soil-borne fungus *Polymyxa betae*. The disease can only be controlled by the use of resistant cultivars. During 2002 and 2003 in the Imperial Valley of California partially resistant sugar beet cultivars with *Rz1* allele developed against this devastating disease seem to be compromised. Distinct BNYVV isolates were isolated from infected sugar beet roots (IV-BNYVV) by single local lesion isolation. These isolates do not contain RNA-5 as determined by RT-PCR. From the banding patterns of single-strand conformation polymorphism analyses we concluded that the resistance-breaking BNYVV isolates from Imperial Valley had likely evolved from the original existing A-type. The pathogenicity of IV-BNYVV isolates was studied. PCR products from coat protein (RNA-2) and 25-kDa protein (encoded by BNYVV-RNA-3, involved in symptom expression) of IV-BNYVV isolates were sequenced. Sequence alignments revealed only minor amino acid changes compared to the existing A-type of California BNYVV isolates.

Pyramiding unmarked mutations in *Ralstonia solanacearum* to assess the role of extracellular enzymes in pathogenesis. H. LIU, S. Zhang, C. Rodriguez, M. A. Schell, and T. P. Denny. Dept. of Plant Pathology, The University of Georgia, Athens, GA. Phytopathology 94:S62. Publication no. P-2004-0418-AMA.

Ralstonia solanacearum secretes six plant cell wall-degrading enzymes (PehA, PehB, PehC, Pme, Egl and CbhA) via a type II branch of the general secretion pathway. To evaluate in depth the contribution of these enzymes to virulence, mutants with one to six inactivated genes were created, each having at most one antibiotic resistance marker. To create unmarked deletions, genomic regions flanking the target gene were PCR amplified, fused using splice overlap extension (SOE)-PCR, cloned into pEX18Tc (a suicide plasmid harboring the *sacB* counter-selectable marker) and site-specifically integrated into the genome to create *cis* merodiploids. Selection for growth on minimal medium-sucrose plates and screening for tetracycline sensitivity resulted in 20-50% the colonies having an unmarked deletion of the target gene. Each round of mutagenesis required only 10 days. Six mutants of strain GMI1000 with individual deletions of either *pehA*, *pehB*, *pehC*, *pme*, *egl*, or a *cbhA::aphA3* inactive allele were constructed, and then various combinations of two to six mutations were combined to create a total of 15 different strains. The six-fold mutant, GMI-6, was comparable to the wild-type parent in every attribute tested in culture. Results of virulence assays comparing the mutants to the wild type and a *gspD* type II secretion mutant (defective in secretion of all six enzymes and many other extracellular proteins) will be reported.

Influence of nutrient and environmental factors on conidial germination of *Potebniamyces pyri*. Q. LIU and C. L. Xiao. Washington State Univ., TFREC, Wenatchee, WA. Phytopathology 94:S62. Publication no. P-2004-0419-AMA.

P. pyri is the causal agent of Phacidiopycnis rot in d'Anjou pears. Infection of fruit occurs in the orchard and symptoms develop during storage. In this study, conidial germination of *P. pyri* in response to various environmental factors was determined. Conidia germinated by either budding or emerging germ tubes in pear juice. The mode of conidial germination was nutrient-dependent. Low nutrient levels favored budding, whereas high nutrient levels favored germ tube development. Conidia germinated at 0 to 30°C, with optimum temperature between 20 and 25°C at which a wetness period of 4 to 5 and 6 to 8 h was required for budding and emerging germ tubes, respectively. Secondary conidia, produced by budding of conidia, initially increased their dimensions and later germinated in the same manner as mother conidia at 0 to 25°C, no germination at 30°C. Germ tubes elongated at 0 to 25°C, but not at 30°C. No germination occurred at RH 95%. At 20°C and RH 98%, conidia germinated only by producing germ tubes, while at RH 100%, germination was dominated by producing germ tubes. More than 80% of conidia germinated in 10% pear juice at pH 4 to 7 after 24 h at 20°C, while there was less than 50% germination at pH 3. The results are important to a better understanding of the biology of the fungus and environmental conditions required for fruit infection.

Genetics of virulence in the root-knot nematode, *Meloidogyne hapla*. Q. LIU and V. M. Williamson. Department of Nematology, Plant Pathology Graduate Program, University of California, Davis. Phytopathology 94:S62. Publication no. P-2004-0420-AMA.

Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasites whose genetics are poorly understood due to their complex reproductive modes. Although many agriculturally important species reproduce asexually, some strains of *M. hapla* undergo meiosis and sexual reproduction making them amenable to genetic crosses. We have five inbred strains of *M. hapla*: VC1R, HSL, UCR, HRO and HSMB. These strains have been inbred for 18 generations by single eggmass transfer on tomato. AFLP DNA fingerprinting revealed that DNA polymorphisms are very common among these strains. We have demonstrated that they represent the sexual form based on cytological studies. These strains also interact differently with the wild potato host *Solanum bulbocastanum*, in which VC1R and UCR are virulent while HSL, HSMB and HRO are avirulent. In this study, we crossed HSL (female parent) with VC1R (male parent). PCR-based markers revealed that the cross was successful. Currently, we are analyzing the segregation of the virulence marker in F2 populations. Upon completion, we will produce molecular markers by AFLP DNA fingerprinting and generate a genetic linkage map for *M. hapla*.

Effect of nitrogen, potassium and silicon on disease susceptibility of various herbaceous ornamental crop species. J. C. LOCKE (1), D. Pitchay (2), and J. M. Frantz (1). (1) USDA, ARS-ATRU, Greenhouse Production Research Group, Toledo, OH 43606; (2) University of Toledo, Plant Science Research Center, Toledo, OH 43606. Phytopathology 94:S62. Publication no. P-2004-0421-AMA.

These studies report the effect of two essential plant nutrients and a non-essential, beneficial nutrient on the susceptibility of a diversity of herbaceous ornamental plant species to the ubiquous fungal pathogen *Botrytis cinerea*. Both nitrogen and potassium are considered major essential elements to plant growth and are provided through fertigation using the appropriate fertilizer based on the N-P-K analysis. Over fertilization can often occur and the luxuriant plant growth that results is alleged to predispose plants to disease. Silicon is not considered an essential nutrient for plant growth, but with the advent of soilless culture media utilization in greenhouse production, the potential supply of silicon to the plant is eliminated. It has been reported that the presence of silicon can lead to enhanced plant resistance to fungal attack. Silicon is reported to be differentially taken up by plants based on their taxonomy. These studies demonstrate differential uptake and accumulation of these nutrients and the resulting differential susceptibility to attack by *B. cinerea*.

Resistance of B.9 apple rootstock to fire blight (*Erwinia amylovora*). N. LOGIUDICE (1), T. L. Robinson (2), G. Fazio (2,3), and H. S. Aldwinckle (1). Depts. of (1) Plant Pathology; (2) Horticultural Sciences, Cornell University; (3) USDA-ARS PGRU, Geneva, NY 14456. Phytopathology 94:S62. Publication no. P-2004-0422-AMA.

Widespread rises in orchard production costs due to destruction of apple rootstocks by *Erwinia amylovora* have made identification of blight-resistant rootstocks a high priority. While B.9 rootstock exhibited no blight in multiple field trials, ungrafted B.9 became severely infected in several greenhouse tests, necessitating further confirmation of its field resistance. Microsatellite markers failed to reveal significant genetic variation among B.9 rootstocks from different sources or among their parents. In field and greenhouse trials, movement of bacteria through asymptomatic tissue was characterized to determine how grafting to four scion cvs affected rootstock colonization. Inoculated trees were analyzed by PCR for presence of *E. amylovora*. B.9 liners from US and European sources displayed similar levels of susceptibility in the greenhouse and field. The pathogen was detected from above and below the graft union in all rootstocks tested. Collectively, these results indicate that field resistance of B.9 results from a suppressive effect on bacterial growth by the grafted rootstock.

Isolation and selection of antagonists to *Phytophthora infestans* on tomato. V. Lourenço, Jr., L. A. Maffia, R. S. Romeiro, and E. G. MIZUBUTI. Universidade Federal de Viçosa, 36570-000 Viçosa, MG, Brazil. Phytopathology 94:S63. Publication no. P-2004-0423-AMA.

As control of tomato late blight (LB) is heavily based on chemicals, reduction in fungicide usage is required for both conventional and organic production systems. Biological control is a potential alternative for LB management. Thus, 208 phylloplane microorganisms and 23 rhizobacteria were isolated from conventional or organic tomato fields and tested for antagonism to *P. infestans*. Based on inhibition of sporangia germination and detached leaflet bioassays, four phylloplane microorganisms *Aspergillus* sp., *Bacillus* sp., *Candida* sp., and *Cryptococcus* sp. Were selected. These microorganisms were either applied singly or combined on tomato plants treated or not with the rhizobacterium *Bacillus cereus*. On control plants, LB progress rate I, area under the disease progress curve (AUDPC), and final disease severity were 0.082, 272.5, and 65.2%, respectively. All antagonists reduced LB severity. Lowest values of severity (14.2%), r (0.0193), and AUDPC (65.6) were recorded on plants colonized with *B. cereus* and treated with *Aspergillus* sp. The mixture of all four antagonists plus root colonization with *B. cereus* was also effective in reducing LB severity. Colonization of roots with *B. cereus* improved LB control. Mixture of antagonists was more efficient than when each antagonist was applied singly. Integration of biological control with other measures can enhance LB management in both conventional and organic tomato production systems. Research funded by CNPq and FAPEMIG.

Methyl bromide alternatives in tomato production system F. J. LOUWS, L. M. Ferguson, N. P. Lynch, and P. B. Shoemaker. Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695. Phytopathology 94:S63. Publication no. P-2004-0424-AMA.

Verticillium wilt (Vw; *V. dahliae* race 2) is the primary reason tomato growers fumigate in Western North Carolina. No genetic resistance against race 2 is available. A three yr study was implemented to evaluate shank-applied and drip-applied fumigants as alternatives to methyl bromide (MB). Fumigant alternatives included 1, 3-dichloropropene (TC35; InLine), chloropicrin (Pic), metam-sodium (MS), iodomethane (MI), and fumigants in combination. In yr 1, terminal Vw incidence was low (mean range 0-19%) and fumigant effects on yield were minimal. In yrs 2 and 3, terminal incidence was higher (mean range 17-90%) and yield loss was associated with increasing weekly incidence values (R^2 up to 0.88). Shank-applied MI+Pic, TC35, Pic alone, and MS shank or drip applied, consistently provided similar control of Vw and generated statistically similar yields to MB+Pic (67:33). Results with drip applied InLine and other EC formulated products were variable. Additional research is needed to optimize drip application and more grower trials are needed to evaluate the general ability to commercially implement selective alternatives explored in this study.

Differentially expressed defense genes in the resistance reaction in wheat infected with *Tilletia tritici*, the common bunt pathogen. Z.-X. LU, D. A. Gaudet, B. Puchalski, M. Frick, B. Genswein, and A. Laroche. Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada. Phytopathology 94:S63. Publication no. P-2004-0425-AMA.

The wheat lines 'Neepawa' (compatible) and its sib 'BW553' (incompatible) that is essentially isogenic for the Bt-10 resistance gene and inoculated with the differentially virulent race T1 of *Tilletia tritici*, were employed to construct a suppression subtractive hybridization (SSH) cDNA library. The expression of several candidate genes from the library and wheat PR-proteins was evaluated using Real-Time PCR following 4 to 32 days post-inoculation in the incompatible and compatible interactions. Results confirmed the higher overall expression of a R-gene homologous Mla1, a lipase, two non-specific Lipid Transfer Proteins (ns-LTP) and the PR-proteins in inoculated, resistant BW553 compared to Neepawa. The differential upregulation of the lipase, PR-1.1, and chitinase 2 in the incompatible interaction 8 and 16 days post-inoculation compared with the compatible interaction was also observed. Salicylic acid (SA) or jasmonic acid (JA) were applied exogenously to wheat seedlings to study their effect on infection levels and on transcript accumulation of candidate genes. JA and SA reduced average infection levels by 41 and 58%, respectively. Upregulation of both ns-LTP genes was observed in SA treated plants. Conversely, the lipase was induced by JA. Expression of both PR-1.1 and PR-1.2 was induced by JA; expression of PR-1.1 was inhibited by SA whereas expression of PR1.2 was induced by SA. In general, chitinases were induced by JA and beta-1,3-glucanases were inhibited by both JA and SA. These results are the first to implicate defense-related genes in the expression of the incompatible interaction involving a Bt-resistance gene and provide insight into defence reactions for host-parasite interactions involving common bunt.

Oligonucleotide microarray analysis of the SalA regulon controlling phytotoxin production by *Pseudomonas syringae* pv. *syringae*. S.-E. LU, N. Wang, and D. C. Gross. Dept. Plant Pathology & Microbiology, Texas A&M Univ., College Station, TX 77843. Phytopathology 94:S63. Publication no. P-2004-0426-AMA.

The *salA* gene is a key regulatory element for syringomycin (SR) production and encodes a member of the LuxR regulatory protein family. Previous studies revealed that *salA*, a member of the GacS/GacA regulon, was required for bacterial virulence, SR production, and expression of the *syrB1* synthetase gene. To define the SalA regulon, a 70mer oligonucleotide microarray was constructed using gene-specific oligonucleotides of all the syringomycin (*syr*) and syringopeptin (*syp*) genes along with representative genes important to bacterial virulence, growth, and survival. Transcriptional profiling was conducted for both a *salA* mutant (B301DSL07) and the wild-type B301D by using the oligonucleotide microarray. Expression of 23 open-reading frames (ORFs) was observed to be over 2-fold higher in B301D as compared to the *salA* mutant; the maximum change ranged up to 15 fold for some toxin biosynthesis genes. Except for the *sylD* synthetase gene for syringolin production, all of the ORFs controlled by SalA were located in the *syr-syp* genomic island and were associated with biosynthesis, secretion, and regulation of SR and syringopeptin. The positive regulatory effect of SalA on transcription of *sypA*, *syrB1*, *syrC*, and *sylD* was verified by real-time PCR analysis. These results demonstrated that a subgenomic oligonucleotide microarray is a powerful tool for defining the SalA regulon and its relationship to other genes important to plant pathogenesis.

Comparison of two assay methods for evaluating the sensitivity of *Alternaria mali* to boscalid. Y. LU (1), J. Ma (1), T. B. Sutton (1), and H. Ypema (2). (1) NC State University, Dept. of Plant Pathology, Raleigh, NC 27695-7616; (2) BASF Corporation, Research Triangle Park, NC 27709. Phytopathology 94:S63. Publication no. P-2004-0427-AMA.

The ED_{50} value of *Alternaria mali* to boscalid was determined for 36 isolates of *A. mali* using two assay methods: inhibition of spore germination on fungicide amended water agar and mycelium growth inhibition on fungicide amended PDA. Isolates were collected from apple orchards in North Carolina from 1992 to 2003, prior the use of boscalid. ED_{50} values from spore germination test ranged from 0.003 to 0.742 mg/L (average 0.342 mg/L), and from mycelium growth inhibition test from 0.199 to 1.789 mg/L (average of 0.641 mg/L). The mean ED_{50} for the mycelium growth inhibition test had a smaller standard deviation and the test was less labor intensive to perform and rate. Both methods can be used to determine the sensitivity of *A. mali* to boscalid. The ED_{50} from either test can be considered as the baseline sensitivity for the method used.

Evaluation of *Brassica* crop residue effects on Verticillium wilt and Phytophthora root rot in chile peppers. G. C. LUDWIG (1), N. P. Goldberg (1), M. Remmenga (1), and L. Blackwell (1). (1) Cooperative Extension Plant Sciences Dept.; (2) University Statistics Center, New Mexico State University, Las Cruces, NM 88003. Phytopathology 94:S63. Publication no. P-2004-0428-AMA.

Phytophthora capsici and *Verticillium dahlia* are soil borne diseases which cause significant yield losses in chile in New Mexico. Currently chemical controls are not viable management options for either of these diseases. Good water management can help reduce losses from Phytophthora root rot; however, summer thunderstorms limit the overall effectiveness of this management practice. There are no effective cultural practices for managing Verticillium wilt in chile. This study investigates the effectiveness of *Brassica* crop residues as a management tool for both these diseases. The research was conducted in 2002 and 2003, in southern New Mexico, in a field with a history of disease caused by both organisms. Broccoli, cabbage, and oriental mustard were the *Brassica* crops selected for use in this study. The treatments included *Brassica* crop residues, *Brassica* crop residues plus Telone* C35, Telone*

C35, and control. Results indicated that plots treated with *Brassica* crop residues plus Telone* C35 had a significant reduction in disease, and a corresponding increase in yield. Results from both years demonstrated that *Brassica* crop residues, either in conjunction with Telone* C35 or alone, have the potential to reduce disease caused by *V. dahlia* and *P. capsici*, and potentially increase yield in pepper crops grown in infected fields in southern New Mexico.

Observations on why usable disease research goes unused by field level forest managers. J. E. LUNDQUIST. USDA Forest Service, Fort Collins, CO. Phytopathology 94:S64. Publication no. P-2004-0429-AMA.

The subject of communications between researchers and managers has received much attention recently, and the literature on causes and cures of communication barriers is growing. Most of this literature, however, deals with national policy issues and other administration factors, and only peripherally addresses field-level decision making. A recent survey of USDA Forest Service managers in Region 2 (Colorado, Wyoming, South Dakota, Nebraska) showed low usage rates of research results about diseases and other types of forest disturbances, despite the increasing emphasis on improving forest health. Subsequently, several field-level managers in Region 2 were asked about how they communicated research needs to researchers, how researchers communicated research results to them, and how researchers found out about the operational usefulness and relevance of their research. Results indicate that communications problems arise from personal and professional misperceptions, ineffective use of communication channels, and inefficiencies arising from a hierarchical organizational structure. Several specific suggestions on how to fix communication barriers were suggested by those interviewed, including: developing more systematic ways for managers to recognize and communicate research needs; establishing better means of feedback about products they use; emphasizing ways to ensure that research proposals are in-line with present or future management needs; instituting performance measures that reward management/research interactions; integrating boundary spanners; and minimizing cultural differences between segments of the organization.

Peanut functional genomics: Gene identification and marker development through EST-microarray analysis. M. Luo (1) and B. Z. GUO (2). (1) University of Georgia, Tifton, GA; (2) USDA-ARS, Tifton, GA. Phytopathology 94:S64. Publication no. P-2004-0430-AMA.

Expressed sequence tag (EST) libraries for cultivated peanut were developed from two cDNA libraries constructed using mRNA prepared from leaves of peanut line C34-24 (resistant to leaf spots and tomato spotted wilt virus) and immature pods of peanut line A13 (drought tolerance and lower preharvest aflatoxin contamination). Randomly selected cDNA clones were partially sequenced to generate a total of 1825 ESTs, 769 from C34-24 cDNA library and 1056 from A13 cDNA library, in which 536 and 769 unique ESTs were identified, respectively. The results of BLASTx search showed that 52.8% of the ESTs from leaf tissue and 78.6% of the ESTs from the pod tissue have homology to genes of known function. There are about 27.3% and 22.1% ESTs matching homologous sequences in dbEST of GenBank based on BLASTn algorithm which have unknown functions. The first batch of 1345 ESTs has been released to GenBank. Four hundred unigenes have been selected from these ESTs and arrayed on glass slides for gene expression analysis, and 44 EST-derived SSR markers have been characterized for cultivated peanut, in which over 20% produced polymorphic markers among 24 cultivated peanut genotypes. RT-PCR and real-time PCR have been used to validate the microarray data. This initiative could contribute considerable information to peanut functional genomics in novel gene discovery and marker development.

Best time period to determine latent infection by *Monilinia fructicola* in prune. Y. LUO and T. J. Michailides. Dept. of Plant Pathology, University of California-Davis, Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier 93648. Phytopathology 94:S64. Publication no. P-2004-0431-AMA.

Experiments using artificial periodic inoculations with the brown rot pathogen *Monilinia fructicola* and natural infections were conducted in prune orchards in California. Fruit were inoculated by injecting a spore suspension of *M. fructicola*. Incidence of fruit rot caused by inoculation and natural infection was recorded periodically during the season. A linear regression between the rate of fruit rot development and days after full bloom was obtained. The incidence of latent infection of blossoms did not correlate with the incidence of latent infection of fruit or fruit rot at any sampling time. The incidences of latent infection of fruit between any two of the fruit sampling times after mid season significantly correlated. The incidence of fruit rot significantly correlated with the incidence of latent infection of fruit at the early and late stages, but not at the mid stage. The best time to determine latent infection for predicting fruit rot would be from mid May to mid June. A decision support diagram was developed to guide growers when to determine latent infection.

Identification of proteins expressed during resistant and susceptible soybean rust interactions. D. G. LUSTER (1), M. B. McMahon (1), J. J. Choi (1), M. L. Carter (1), A. Nunez (2), and R. D. Frederick (1). (1) USDA ARS, FDWSRU, Ft. Detrick, MD; (2) USDA ARS, ERRC, Wyndmoor, PA. Phytopathology 94:S64. Publication no. P-2004-0432-AMA.

Soybean rust, caused by *Phakopsora pachyrhizi*, is a devastating disease that has spread from Asia to Africa and South America, but not yet to the continental United States. We focused on proteins involved in early responses between *P. pachyrhizi* and soybean (*Glycine max*). Proteins localized to the leaf apoplast were extracted from soybean cultivars following inoculation with isolates of soybean rust that resulted in susceptible and resistant interactions. Samples were taken at 0, 12, 24, 48, 72 hours and 6 days post inoculation. Trypsin-digested two-dimensional gel electrophoresis spots were peptide finger-printed and sequenced using Matrix-Assisted Laser Desorption/Ionization mass spectrometry with automated tandem time of flight fragmentation of selected ions (MALDI-TOF/TOF). Proteins identified from putative peptide sequences by database searches within a confidence interval greater than or equal to 95% were evaluated and placed into functional categories. Results show a large group of proteins involved in defense response and cell signaling.

Purification and characterization of a polygalacturonase from *Rhizoctonia solani* AG-3. M. MACHINANDIARENA (1), B. Latiño (2), G. Daleo (1), C. Vazquez (2), and M. T. Gonzalez-Jaén (2). (1) Universidad Nacional de Mar del Plata. Argentina; (2) Universidad Complutense de Madrid, España, E-mail: mfmachin@mdp.edu.ar. Phytopathology 94:S64. Publication no. P-2004-0433-AMA.

Rhizoctonia canker is considered a major important disease of potato. The disease is caused by *Rhizoctonia solani*. Most isolates of *R. solani* pathogenic to potato belong to anastomosis group 3 (AG-3). The mechanisms of pathogenicity and symptom induction by this fungus are poorly understood despite many ultrastructural, biochemical and genetics studies. We have studied the production of pectinolytic enzymes by this fungus during growth in vitro on pectin as carbon source, for the analysis of the role in pathogenicity of the major extracellular pectinolytic enzymes produced by *R. solani* AG-3. In the present work we report, for the first time, the purification of an extracellular endo-poligalacturonase (endoPG) from *Rhizoctonia solani* AG3. This enzime was purified by two anion-exchange chromatography steps. The molecular mass was estimated to be 40 kDa after SDS-PAGE and HPLC gel filtration and has an pI of 4.25. The optimum temperature for this enzyme was at 45°C. The activity remained stable up to 40°C and was rapidly inactivated after incubation at temperatures above 50°C. The optimum pH was 5.5. The endo PG was active on polygalacturonic acid and esterified pectin, but the activity on pectin decrease with an increase in esterification. The enzyme released a series of oligo-galacturonates from poligalacturonic acid with a degree of polymerization above trimer. The enzyme did not present activity on digalacturonic and trigalacturonic acid.

***Colletotrichum gloeosporioides* from non-cultivated hosts close to strawberry fields are more pathogenic to strawberry.** S. J. MACKENZIE (1), T. Freeijo (1), D. E. Legard (1), N. A. R. Peres (1), and L. W. Timmer (2). (1) Univ. of Florida, GCREC, Dover, FL 33527; (2) Univ. of Florida, CREC, Lake Alfred, FL 33850. Phytopathology 94:S64. Publication no. P-2004-0434-AMA.

Colletotrichum crown rot of strawberry is caused primarily by *Colletotrichum gloeosporioides* in Florida. In a previous study, *C. gloeosporioides* isolates from non-cultivated hosts growing close to strawberry fields appeared to be from the same population as those from strawberry. A relatively large proportion of these isolates were also pathogenic on strawberry. In the current study, *C. gloeosporioides* from wild grape and oak lesions were sampled from two sites close to strawberry fields and two sites distant from strawberry fields. PCR amplification using a *C. gloeosporioides*-specific primer and RAPD marker analyses were used to determine the species identity and population affinity of isolates. In a phylogenetic tree constructed from RAPD data, eighteen to twenty-three isolates from each of the four sites clustered along with those from diseased strawberry crowns. When these isolates were evaluated for pathogenicity on strawberry, the percent of pathogenic isolates collected from sites near strawberry fields was significantly higher than those collected from distant sites (46% vs. 15%). There was no significant difference in the percent of pathogenic isolates between the two sites located adjacent to strawberry fields (39% vs. 52%), nor between the two sites distant from strawberry production fields (17% vs. 13%), nor between isolates from the two hosts (29% vs. 32%).

Phenazines play a role in adhesion/biofilm formation in *Pseudomonas aureofaciens* strain 30-84. V. S. R. K. MADDULA, E. A. Pierson, and L. S. Pierson III. University of Arizona, Tucson, AZ. Phytopathology 94:S64. Publication no. P-2004-0435-AMA.

Pseudomonas aureofaciens strain 30-84 is a biocontrol agent against take all disease of wheat (*Gaumannomyces graminis* var. *tritici*). The PhzR/PhzI quorum sensing (QS) system regulates phenazine biosynthesis while the CsaR/CsaI QS system regulates aspects of the cell surface. We initiated studies on the effects of mutations in these two QS systems on cell adhesion/biofilm formation by strain 30-84. A static adhesion plate assay was used to quantify bacterial adhesion to polystyrene surfaces. Surprisingly, these experiments showed that *phzR* and *phzI* mutants and phenazine structural mutants *phzB* adhered less well than the wild type or *csaR* and *csaI* mutants. Addition of purified *N*-acyl homoserine lactones restored adhesion/biofilm formation to *phzI*, *csaI* and *phzI/csaI* double mutants but not to *phzB* mutants, supporting the role of QS in adhesion/biofilm formation in strain 30-84. Further, the addition of TLC-purified phenazines (2-OH-PCA, PCA) restored adhesion/biofilm formation to all phenazine mutants, including *phzB*. Addition of phenazines to the *phzR* mutant did not restore wild type adhesion levels, indicating this QS system controls other traits involved in adhesion. These results support a new hypothesis that phenazines play additional roles for the producing bacterium beyond antibiosis and competition.

A comparison of measurement methods using the sensitivity ratio: An application to screening for disease resistance in soybeans. G. Madisa (1), K. Eskridge (1), K. Powers (2), J. STEADMAN (2), G. Graef (3), R. Higgins (2), and C. Bellows (2). Depts. of (1) Statistics; (2) Plant Pathology; (3) Agronomy & Horticulture, University of Nebraska, Lincoln, NE 68583. Phytopathology 94:S65. Publication no. P-2004-0436-AMA.

More than a dozen different techniques are available to screen for resistance to *Sclerotinia sclerotiorum*. Many of these screening methods use different scales to measure resistance and are most commonly compared statistically by the coefficient of variation (CV), root mean square error (RMSE), standard deviation and confidence intervals. The sensitivity ratio (SR) is a scale independent statistical quantity that is useful for comparing the technical merit of different methods for measuring a physical, chemical or biological property. We used SR to compare two resistance screening methods with two different scales and to compare leaf lesion area measurement made by use of digital imaging and ruler. When compared to the RMSE or the CV, the SR ordered methods differently. We recommend the scale independent sensitivity ratio rather than standard precision statistics to compare different screening methods.

Comparison of two aerobiological approaches for predicting the aerial incursion of soybean rust into the United States. C. E. Main (1), T. Keever (1), S. A. Isard (2), R. D. MAGAREY (1), S. C. Redlin (3), and J. M. Russo (4). (1) North Carolina State University, Raleigh, NC; (2) University of Illinios, Urbana-Champaign, IL; (3) USDA-APHIS, Raleigh, NC; (4) ZedX Inc., Bellefonte, PA. Phytopathology 94:S65. Publication no. P-2004-0437-AMA.

Soybean rust (*Phakopsora pachyrhizi*) was first detected in Brazil in 2001 and is likely to be aerially transported into the U.S. The USDA-APHIS is funding a project to examine the threat of aerial incursion into the U.S. using two aerobiological models. The Integrated Aerobiology Modeling System (IAMS) uses the box-parcel method to predict spore movement and mortality. The HYSPLIT model uses a Lagrangian method to calculate air parcel trajectories and can provide complex dispersion and deposition simulations. Both models demonstrate that historical transoceanic movements of the fungus was feasible. The models are initialized with accurate source maps for South America and Africa. Both models showed that the aerial incursion of soybean rust is currently limited by a disconnect between when spores are produced in South American soybean fields and the availability of airflows to transport them across the intertropical convergence zone into the U.S. While the HYSPLIT model provides a useful method for case study analysis, the IAMS model had greater analytical capabilities. A qualitative risk assessment scheme using HYSPLIT has been used for 8 years to successfully forecast macro-scale downy mildew epidemics. Quantitative output from the IAMS model is being validated with the qualitative HYSPLIT modeling scheme using data from the 2004 downy mildew epidemic.

High diversity and evidence of recombination in South temperate samples of *Sclerotinia sclerotiorum*. G. MALVAREZ and L. M. Kohn. Dept. of Botany, University of Toronto, Mississauga, ON, Canada L5L 1C6. Phytopathology 94:S65. Publication no. P-2004-0438-AMA.

Previous samples from crops including canola, soybean and edible bean from North temperate areas and New Zealand have shown a mainly clonal structure with a few epidemic clones sampled frequently over wide geographic areas. We evaluated samples from lettuce, tomato, and edible bean from Uruguay and Argentina with the same markers, DNA fingerprinting and mycelial compatibility, finding high genetic diversity with almost every isolate representing a different MCG and fingerprint. Mycelial compatibility was intransitive (A compatible with B, B with C, but A incompatible with C) and fingerprints could be very different between compatible isolates. Compatibility reactions were not binary as for North temperate samples, but showed a range of reaction types consistent with segregation of multiple genetic determinants. We hypothesize that greater diversity and recombination are associated with extensive movement of agricultural materials, diversity of crops, and small spatial scales of fields in comparison to large-scale, intensive cropping that would result in clonality. We are investigating mating type frequency in both North and South.

Characteristics of *Phytophthora sojae* populations in Illinois and implications for management of Phytophthora rot of soybean. D. K. MALVICK and E. Grunden. Dept. of Crop Sciences, University of Illinois, Urbana, IL 61801. Phytopathology 94:S65. Publication no. P-2004-0439-AMA.

Phytophthora sojae causes Phytophthora rot of soybean, an important disease in Illinois that has become increasingly difficult to manage. Ongoing problems with this disease in Illinois suggested that an in-depth assessment of virulence pathotypes and other phenotypic traits in the pathogen population was warranted. Isolates of *P. sojae* (n = 121) were baited using susceptible soybean seedlings in field soils collected from 24 counties. Sensitivity to mefenoxam and metalaxyl was tested in-vitro with 60 isolates. Virulence was characterized via hypocotyl inoculation of 11 differential soybean cultivars with Rps genes 1a,1b,1c,1d, 1k, 2, 3a, 4, 5, 6, and 7. Twenty-two virulence pathotypes were identified based on eight Rps gene differentials (Rps 1a,1b,1c,1d, 1k, 3a, and 6), with the four most common races being 1, 4, 33, and 28. Thirty-one pathotypes were identified based on 11 differentials. The mean virulence complexity on the sets of 8 and 11 Rps gene differentials were 3.3 and 3.7, respectively. Populations of *P. sojae* in Illinois are diverse, and pathotypes varied in different crop reporting districts. The commercially available Rps genes 1a, 1c, 1k were susceptible to >38% of isolates, whereas Rps genes 3a, 4, and 6 were susceptible to <9% of isolates tested. All isolates were sensitive to 1.0 µg/ml AI mefenoxam and metalaxyl, suggesting these compounds remain effective for management of Phytophthora seedling rot.

Oak wilt: Research for future control. K. L. MANN and D. W. Fulbright. Michigan State University, East Lansing, MI. Phytopathology 94:S65. Publication no. P-2004-0440-AMA.

Oak wilt is a lethal disease of oak (*Quercus* spp.) caused by the fungus, *Ceratocystis fagacearum*. Limited applicability of current control methods necessitates research into additional control options. Three areas of research that could aid in the development of future control options were considered. The first objective was to determine whether the fungicide, propiconazole (Alamo® 14.3% a.i., Syngenta Speciality Products), could be used to inhibit proliferation of *C. fagacearum* through root grafts. Results from our research plot indicated that trees inoculated only with *C. fagacearum* died and trees treated with propiconazole did not develop symptoms of oak wilt. The second objective was to determine the efficacy of a laboratory-induced hypovirulent, *C. fagacearum* mutant, PM447, as a biocontrol agent. Results from greenhouse studies indicated that PM447 controlled oak wilt when inoculated into seedlings prior to inoculation with a wild-type isolate. Several factors appeared to influence disease expression and effectiveness of PM447, including isolate specificity and time of inoculation. The final objective was to use rep-PCR to determine the level of genetic diversity within the *C. fagacearum* population throughout the range of disease and to identify genetic markers to assist in population studies. Our rep-PCR protocol using the primer $(GTG)_5$ produces fingerprints showing several distinct bands, which suggests that $(GTG)_5$ may be a good primer candidate for this species. This work is still preliminary but results suggest that new control options may be available in the near future.

Isolation of bacteria identified in the rice genome sequence. P. MANOSALVA (1), S. Goff (2,3), G. Presting (2,4), and J. E. Leach (1). (1) Kansas State Univ., Manhattan, KS; (2) Torrey Mesa Research Inst., San Diego, CA; (3) Syngenta, Research Triangle Park, NC; (4) Univ. Hawaii, Honolulu. Phytopathology 94:S65. Publication no. P-2004-0441-AMA.

In 2002, Syngenta released a draft rice genome sequence. During assembly and analysis, 726 bacterial sequence contigs contaminating the rice sequence were found. The Syngenta bacterial sequences (SBS) were most closely related to sequences from *Xanthomonas* and *Xylella*. Our goal was to isolate and identify the corresponding bacteria from seed used in the sequencing project. Seedlings were ground and plated on nutrient agar and selective media for *Xanthomonas* and for *Xyllela*. Bacterial colonies were selected based on difference in colony appearance, tissue source and medium that supported growth. *Xyllela fastidiosa*-specifc antibodies did not detect any of the isolates in ELISA, suggesting that none were *X. fastidiosa*. PCR using primers that correspond to *RecA* genes in the Syngenta contigs amplified the expected sized fragment from a control *Xanthomonas* strain and nine isolates with similar colony morphology. The sequences of PCR products from three

strains were >99% similar to each other, and were more similar to the SBS (>94%) than any *Xanthomonas RecA* genes (>92%). Thus, these rice isolates are closely related to *Xanthomonas* and are candidates for the bacteria represented in the SBS. Identification of organisms that correspond to the sequence will help in understanding their association with the plant and their role in rice biology.

***Xylella fastidiosa* from grapevine shows decreased susceptibility to antibiotics as a biofilm compared to planktonic growth in vitro.** L. L. R. MARQUES (1), G. P. Manfio (2), E. Lee (1), H. Ceri (1), and M. E. Olson (1). (1) Biofilm Research Group, University of Calgary, Canada; (2) CPQBA/UNICAMP, Campinas (SP), Brazil. Phytopathology 94:S66. Publication no. P-2004-0442-AMA.

Xylella fastidiosa is a fastidious xylem-limited bacterium associated to a wide range of plant diseases in commercially important crops, including Pierce´s disease in grapevine, and citrus variegated chlorosis (CVC) in sweet orange, among others. *X. fastidiosa* forms highly developed biofilms in the plant xylem. Experimental data for biofilm-forming bacteria clearly demonstrate that this type of growth is less susceptible to antibiotics, biocides and other antimicrobial agents. These data are still not available for *X. fastidiosa*. In the current study, we compared the levels of efficacy of kanamycin and tetracycline in an in vitro assay comparing planktonic cells (PW liquid medium) and biofilms formed in a balsa wood support, a method developed by our study group. The data obtained clearly indicate that the *X. fastidiosa* grapevine isolate CCT 6752 (=ATCC 35870) is less susceptible to both antibiotics in the biofilm form. Differential susceptibility of biofilm and planktonic cells has important implications for designing future studies to identify disease control agents and in the application of these in the field.

Biofilms of *Erwinia carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* are less susceptible to antibiotics and biocides than planktonic populations. L. R. MARQUES (1), T. Bolduc (1), S. H. De Boer (2), H. Ceri (1), and M. E. Olson (1). (1) Univ. of Calgary, AB; (2) Centre for Animal and Plant Health, Charlottetown, PEI CANADA. Phytopathology 94:S66. Publication no. P-2004-0443-AMA.

Soft-rot of potato are caused by two closely related bacteria, *Erwinia carotovora* subsp. *atroseptica* (Eca) and *E. carotovora* subsp. *carotovora* (Ecc). Eca also causes the blackleg disease of potato, while Ecc causes soft rot of fleshy roots and leaves of many vegetable and ornamental crops. Contaminated surfaces of equipment, transport vehicles, and storages that come in contact with seed potatoes is known to be an important means of spreading the bacteria among tubers. Efficient disinfection of equipment and other contaminated surfaces may be compromised if the soft-rotting bacteria occur as microbial aggregates in the biofilm metabolic state. Such microbial cells are usually less susceptible to antibiotics and biocides than cells in the planktonic state. To evaluate biofilm formation and susceptibility of Eca and Ecc biofilms to various antimicrobials, we used two in vitro methods – a high throughput device consisting of plastic pegs in a 96-well format (MBEC) and wood strips. Ecc formed biofilms efficiently on both solid supports, whereas a strain of Eca did not form biofilms readily on the MBEC polystyrene surface. Ecc and Eca biofilms were less sensitive to antibiotics and biocides such as hydrogen peroxide and copper sulfate, than planktonic cells. Since recommendations for disinfection of agricultural implements is largely based on experiments using planktonic cell suspensions, our results suggest that the efficacy of disinfection may need to be re-evaluated.

Evaluation of fungicides for control of rapid blight on cool season grasses. S. B. MARTIN (1), M. W. Olsen (2), P. D. Peterson (1), and J. J. Camberato (1). (1) Clemson University, Pee Dee Research and Education Center, Florence, SC; (2) University of Arizona, Tucson, AZ. Phytopathology 94:S66. Publication no. P-2004-0444-AMA.

Rapid blight is a newly described highly destructive disease of many season turfgrasses caused by a species of *Labyrinthula*, a net slime mold. The purpose of this study was to evaluate fungicides, already labeled for other turfgrass diseases, for control of rapid blight. Fungicides were applied at label rates in 860 L ha^{-1} to 2-wk old perennial ryegrass (*Lolium perenne* L.) in greenhouse experiments. Ryegrass was irrigated twice daily with artificial seawater of 3.5 deciSiemens (dS) M^{-1}. Ryegrass leaves were inoculated with 1×10^5 cells placed arbitrarily in the canopy the day after fungicide application. Non-fungicide treated plants became completely blighted 3-4 wk post inoculation. Insignia (pyraclostrobin) at 0.055 grams (g) active ingredient (ai) M^{-2}, and Compass (trifloxystrobin) at 0.038 g ai M^{-2}, controlled the disease as single component sprays, with greatest efficacy from Insignia. Fore Rainshield (mancozeb) at 1.95 g ai M^{-2} and Fore + Insignia or Fore + Compass tank mixes were more effective than Fore alone. Daconil Ultrex (chlorothalonil) at 0.98 g ai M^{-2} controlled the disease up to 2 wk post inoculation. Field trials in SC and AZ showed effectiveness of Fore, Insignia, and Compass for control of rapid blight in rough bluegrass on golf greens. Azoxystrobin, fosetyl-Al, mefanoxam, propamocarb, iprodione, myclobutanil, thiophanate methyl, chloroneb, flutolanil, polyoxin, and fludioxonil did not control rapid blight in greenhouse or field trials.

Alternative fumigation strategies in California strawberry production and the use of remote sensing for evaluating their effect on crop production. F. N. MARTIN. USDA-ARS. Phytopathology 94:S66. Publication no. P-2004-0445-AMA.

Large-scale field trials with alternatives to methyl bromide have been undertaken in collaboration with commercial growers to evaluate their efficacy for management of soilborne diseases and their effect on subsequent fruit production cycles. Yield data was collected from replicated subplots within treatment blocks (market and cull) as well as from individual production blocks (commercial trays/acre). Data on plant growth parameters, leaf area, and canopy reflectance were also collected from the various fumigation treatments and examined to determine their relationship with crop yield. Calibrated, georeferenced aerial images (0.5 meter spatial resolution) of the production fields collected at 4-6 week intervals during the fruiting season were used to calculate vegetation indices and model how changes in a field over time related to crop productivity. The relationships between the ground based data, data from the aerial images and yield will be discussed.

The use of mitochondrial DNA for clarifying phylogenetic relationships and isolate identification with the genus *Phytophthora*. F. N. MARTIN (1) and P. Tooley (2). USDA-ARS, (1) Salinas, CA 93905; (2) Ft. Detrick, MD 21702. Phytopathology 94:S66. Publication no. P-2004-0446-AMA.

The mitochondrial genome in *Phytophthora* has provided additional tools for phylogenetic analysis (*cox* 1 and 2 genes) of the genus that allows for greater resolution among closely related species than the more commonly used rDNA-ITS region. In addition, a molecular marker system for detection of the pathogen in infected tissue was developed from mtDNA that uses a primer pair that is specific for *Phytophthora* spp. Multiplexed with a plant primer pair that serves as a positive control. A second round amplification with nested species-specific primers was used to detect specific species. This marker system was most widely tested with *P. ramorum*, but has been found to work on a range of other species as well. An alternative approach for isolate identification was developed using RFLP analysis of another PCR amplified region to accurately identify isolates to a species level. In evaluations with 153 isolates representing 31 species isolates were consistently identified to the correct species, including those not clearly differentiated by RFLP analysis of the rDNA-ITS region.

Managing pepper diseases with a new premix fungicide containing famoxadone and cymoxanil. M. MARTIN, R. Williams, S. Rick, D. Ganske, and S. Soehner. DuPont Ag and Nutrition, Stine-Haskell Research Center, Newark, DE. Phytopathology 94:S66. Publication no. P-2004-0447-AMA.

Tanos™, a new, broad-spectrum fungicide from DuPont, is registered for use on peppers, tomatoes, potatoes, cucurbits, and head lettuce for control or suppression of fungal and bacterial diseases. Tanos™ is a 50 WDG formulation comprised of a 1:1 ratio of Famoxate™ (famoxadone) and cymoxanil. In peppers, Tanos™ is labeled for suppression of the foliar and fruit phase of Phytophthora blight (*Phytophthora capsici*) with a use rate of 8-10 oz/ac. The utility of higher use rates against this disease as well as against bacterial spot (*Xanthomonas campestris* pv. *Vesicatoria*), bacterial soft rot (*Erwinia carotovora*), and anthracnose (*Colletotrichum* sp.) is under evaluation. Field research results-to-date with Famoxate™-based products demonstrate suppression of bacterial spot and soft rot, and control of anthracnose when used in programs with copper hydroxide and/or maneb.

A virus associated with blueberry fruit drop disease. R. R. MARTIN (1), I. E. Tzanetakis (2), M. Sweeney (3), and L. Wegener (4). (1) USDA-ARS-HCRL, Corvallis; (2) Bot & Plant Path OSU, Corvallis, OR, 97331; (3) BCMAFF, Abbotsford, B.C.; (4) Biol. Sciences, Simon Fraser Univ., Burnaby, B.C., Canada. Phytopathology 94:S66. Publication no. P-2004-0448-AMA.

During the past few years a fruit drop symptom has been observed in several blueberry fields in Oregon, Wash. And B.C. The plants flower normally, though the young leaves and flowers have a transient red coloration that is absent in healthy plants. The fruit develops to 3-5 mm in diameter and then aborts so that affected bushes mature virtually no fruit. The incidence within fields increases year to year suggesting that a pathogen is involved. Virus purification and mechanical transmissions to herbaceous hosts has been unsuccessful. Only one of more than 40 attempts at dsRNA purification was successful. Thus far, approximately 1700 nucleotides of the dsRNA template have been sequenced and three sets of primers for detection were developed. In RT-PCR assays, amplicons were obtained from symptomatic bushes with

all three sets of primers but not from asymptomatic bushes. The sequence obtained shows homology with fungal rather than plant viruses. The possibility of a systemic virus-infected fungal pathogen can not be ruled out at this time.

Epiphytic yeast's kinetics characterization and storage decay biocontrol on apple fruit. A. R. Martínez-Campos and V. M. GUERRERO-PRIETO. Center for Research on Food and Development. Phytopathology 94:S67. Publication no. P-2004-0449-AMA.

Fruit naturally occurring yeast have been targeted as potential antagonist to postharvest fungal diseases, since they exhibit several features that confer them greater potential for colonizing fruit surfaces and wound sites. Commercial use of these biocontrol agents is still very limited and accounts for only a very small fraction of their potential market. Understanding yeast's mode of action, is a must for developing successful biocontrol strategies. Three strains of *Candida oleophila*, isolated from apple fruit were tested for their control ability of storage decay on apples and their specific growth and consumption rate were determined. Parameters were established by batch growth in 2 l bioreactors. For inoculation experiment, yeast cells were cultured and harvested at exponential growth phase. Strain identified as L06, inhibited 62% of *Botrytis cinerea* growth, while L07, nonrugose (L) and rugose I strains only reduced 25% growth. Specific growth and consumption rates were; 0.25 and 0.19; 0.31 and 0.207; and 0.39, h^{-1} and g biomass/g glucose, for L06, L07L, and L07R, respectively. Results showed that lower specific growth rate increased yeast biocontrol activity, probably due to energy sources being used for lytic enzymes production.

Beneficial bacteria and acibenzolar-S-methyl plus imidacloprid as seedling treatments for the management of *Tomato spotted wilt virus* in flue-cured tobacco. N. MARTINEZ-OCHOA, S. W. Mullis, A. S. Csinos, M. Stephenson, and S. S. LaHue. University of Georgia, CPES, Tifton, GA 31793. Phytopathology 94:S67. Publication no. P-2004-0450-AMA.

Spotted wilt continues to be an overwhelming problem for tobacco growers in Georgia, and novel strategies to manage this disease are needed. Fourteen bacterial strains, which had shown growth promotion and induced systemic resistance to other viral diseases, were selected for testing *Tomato spotted wilt virus* (TSWV) reduction in K-326 tobacco. In the greenhouse three of these bacteria increased plant growth and reduced TSWV. In 2003, a field trial was initiated to evaluate bacterial strains as soil drenches, alone and in combination with an acibenzolar-S-methyl plus imidacloprid (ASM/I) pretransplant spray. Plants treated with ASM/I had reduced TSWV infection and stand loss but no effect on yield, while two of the bacteria had little or no effect on plant growth or TSWV. However, treatment with *Pseudomonas fluorescens* 89B-27 alone resulted in a significant increase in seedling height and yield but no decrease in TSWV. Further studies with beneficial bacteria are needed to confirm positive effects on tobacco growth and yield.

Characterization of mycoviruses infecting *Fusarium solani* f. sp. *glycines*. R. A. MARVELLI (1), L. L. Domier (2), and D. M. Eastburn (1). (1) University of Illinois at Urbana-Champaign; (2) USDA-ARS, Urbana, IL. Phytopathology 94:S67. Publication no. P-2004-0451-AMA.

There is potential to use mycoviruses that infect the fungal pathogen *Fusarium solani* f. sp. *glycines* in antagonist interactions for biological control of sudden death syndrome of soybean (*Glycine max* (L.) Merr). Mycoviruses are an alternative to other control methods that have proven to be problematic. We have cured isolates Fsg1063 and Fsg1066 of dsRNA mycoviruses, and shown preliminary evidence of suppression of virulence by the mycoviruses of 21 to 32% in greenhouse studies. Analysis based on fragment size and nucleic acid composition of known mycoviruses indicates that mycoviruses of *Fusarium solani* f. sp. *glycines* are likely to be from the virus families Hypoviridae and Partitiviridae. Additional molecular studies will be necessary to characterize these mycoviruses, including Northern blots to find sequence homology with representative mycoviruses from these virus families, and sequencing of cDNA from the *Fusarium solani* f. sp. *glycines* mycoviruses to compare with published sequences.

Evaluation of chemical and biological tools for management of Sclerotinia drop of lettuce. M. E. MATHERON and M. Porchas. Yuma Agricultural Center, University of Arizona, Yuma. Phytopathology 94:S67. Publication no. P-2004-0452-AMA.

Sclerotinia drop of lettuce in Arizona is caused by *Sclerotinia minor* and *S. sclerotiorum*. In field evaluation trials, lettuce was seeded on raised beds in double rows, 30 cm apart. After thinning to a 30-cm spacing within rows, approximately 2,100 sclerotia of *S. minor* or 800 sclerotia of *S. sclerotiorum* were distributed on the surface of each 7.6-m-long plot between the rows of lettuce, then incorporated into the top 5-cm layer of soil. After incorporation of sclerotia into soil within plots, each test material was applied to the soil surface and plants, with a second application 2 weeks later. Among the two pathogens, boscalid, *Bacillus subtilis*, fluazinam, boscalid + pyraclostrobin, dicloran and cyprodinil + fludioxonil were more effective in plots infested with *S. minor*, whereas vinclozolin and *Coniothyrium minitans* were more effective in plots infested with *S. sclerotiorum*. A reduction of 50% or higher in diseased lettuce plants was achieved in the presence of *S. minor* with fluazinam (66%), boscalid (55%), dicloran (53%) and cyprodinil + fludioxonil (50%) and in the presence of *S. sclerotiorum* with vinclozolin (58%) and fluazinam (53%).

Genetic diversity among bacteria associated with brown rot of potato in Russian Federation. E. V. MATVEEVA (1), E. V. Nikolaeva (1), E. Sh. Pekhtereva (1), V. K. Bobrova (2), I. A. Milyutina (2), A. V. Troitsky (2), A. N. Ignatov (1,3), and N. W. Schaad (4). (1) Russian Res. Inst. Phytopath., Moscow, Russia; (2) Moscow State Univ., Russia; (3) Centre Bioeng., Russian Acad. Sci., Moscow, Russia; (4) USDA-ARS, FD-WSRU, Ft. Detrick, MD. Phytopathology 94:S67. Publication no. P-2004-0453-AMA.

Within the Russian Federation (RF) there is an abundance of flora types and climats, however, little is known about the diversity of phytopathogenic bacteria associated with that flora. Brown rot of potato (BR), caused by *Ralstonia solanacearum* (Rs), has emerged as a major problem in RF. To identify Rs and other bacteria associated with BR, more than 100 different bacteria were isolated from diseased tubers collected from different regions of RF in 2003. Most had high GC contents and similar plasmids. Partial sequencing of the 16s rRNA gene of 14 strains showed 78% were Gram-negative alfa-(*Agrobacterium/Rhizobium* spp.), beta-(*Ralstonia/Alcaligenes* spp.) and gamma-(*Pseudomonas/Stenotrophamonas* spp.) proteobacteria. RFLP and AP- and rep-PCR fingerprinting showed that each group consisted of diverse strains. Several RS strains from regions of the RF were identified biochemically as Rs biovar 2.

Bacteriocin activity among 2,4-diacetylphloroglucinol (DAPG)-producing fluorescent *Pseudomonas* spp. D. MAVRODI (1), S. Validov (2), O. Mavrodi (1), L. De La Fuente (1), A. Boronin (2), D. Weller (1,3), and L. Thomashow (1,3). (1) Washington State University, Pullman, WA; (2) Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia; (3) USDA-ARS, Pullman, WA. Phytopathology 94:S67. Publication no. P-2004-0454-AMA.

Certain DAPG-producing *P. fluorescens* strains colonize roots and suppress soilborne pathogens more effectively than others from which they are almost indistinguishable. We recovered DNA fragments from the superior colonizer Q8r1-96 by suppression subtractive hybridization with DNA from the moderate colonizer Q2-87 in order to identify genetic differences that might account for superior colonization activity. One Q8r1-96 clone exhibited similarity to a pore-forming bacteriocin and resides in a 23-kb pyocin-like cluster that includes a functional two-gene lysis module and bacteriophage tail assembly. Treatment of Q8r1-96 with mitomycin C caused production of phage tail-like particles. Subsequent screening revealed that bacteriocin activity was common among DAPG-producing isolates representing 17 distinct genotypes and may contribute to strain competitiveness and persistence in vitro and in the rhizosphere.

Role of *sss* recombinase and *dsbA* in root colonization by *Pseudomonas fluorescens* Q8r1-96. O. V. MAVRODI (1), D. V. Mavrodi (1), D. M. Weller (1,2), and L. S. Thomashow (1,2). (1) Washington State University, Pullman, WA 99164; (2) USDA-ARS, Pullman, WA 99164. Phytopathology 94:S67. Publication no. P-2004-0455-AMA.

The 2,4-diacetylpholorglucinol (DAPG)-producing strain *Pseudomonas fluorescens* Q8r1-96 suppresses numerous soilborne pathogens and aggressively colonizes the roots of wheat and pea. Clones containing two genes, *sss* recombinase and *dsbA*, encoding a site-specific recombinase and a periplasmic disulfide bond-forming protein, respectively, were identified by Southern hybridization in a Q8r1-96 genomic library, sequenced, and used to construct gene replacement mutants in Q8r1-96. Colonies formed by the *sss* mutant did not differ from those of Q8r1-96, whereas those of the *dsbA* mutant exhibited altered colony morphology, reduced fluorescence and decreased motility. Both mutants produced DAPG and inhibited *Gaeumannomyces graminis* var. *tritici* in vitro. The mutants are being assessed in greenhouse studies for their ability to colonize and persist in the rhizosphere of wheat.

The use of quantitative real-time PCR in *Benyvirus* research. K. L. MAXSON-STEIN, M. E. Villanueva, J. M. Stein, and C. M. Rush. Texas Agricultural Experiment Station, Amarillo, TX 79106. Phytopathology 94:S67. Publication no. P-2004-0456-AMA.

Beet necrotic yellow vein virus (BNYVV) and *Beet soilborne mosaic virus* (BSBMV) of the genus *Benyvirus* are multi-partite, positive strand RNA

viruses that are vectored by the plasmodiophorid, *Polymyxa betae* and infect *Beta vulgaris* (sugar beet). BNYVV causes Rhizomania in sugar beets which results in low sugar and yield, and symptoms caused by BSBMV are variable. Real-time PCR assays were designed to specifically detect and quantify BNYVV and BSBMV. Sugar beet seedlings were inoculated with different strains of BNYVV, total RNA was extracted, reverse transcribed to cDNA, and real-time PCR was used to quantify differences in viral titer in the seedlings. CDNA was obtained as described above from either previously inoculated *Chenopodium quinoa* leaves or soil, and real-time PCR was used to detect and quantify viral inoculum in these sources. The BNYVV and BSBMV real-time PCR assays were effective for the quantification of resistance to BNYVV in various sugar beet cultivars, the comparison of virulence between BNYVV strains, and estimation of viral inoculum concentrations in the local lesion host, *C. quinoa*, and soil.

A major R-gene from *Solanum berthaultii* confers qualitative and quantitative resistance to late blight in potato tubers. H. MAYTON (1), I. Simko (2), G. Rauscher (1), B. Esposito (1), and W. E. Fry (1). (1) Cornell University, Ithaca, NY; (2) USDA-ARS-PSI, 10300 Baltimore Avenue, Beltsville, MD. Phytopathology 94:S68. Publication no. P-2004-0457-AMA.

The relationship between late blight resistance in the foliage and tuber can be ambiguous. In the present study we analyzed the influence of a major R-gene (R-12, from *S. berthaultii*) for resistance in tubers. The analyses were done in a backcross population to *Solanum tuberosum* (BCT) using a hybrid progeny obtained from a cross between *S. tuberosum* and *S. berthaulti*. Previous studies in our lab using this population have identified a major R-gene for resistance to foliar blight on chromosome X (R-12). Tuber resistance segregated whether the assays were conducted in the field, greenhouse, or laboratory. In the laboratory, two isolates of *Phytophthora infestans* were used. One isolate was a member of the US-8 clonal lineage (US940480) and produced an incompatible interaction when R-12 was present. These results were entirely consistent with results in the foliage. The other isolate was from Mexico (MX990005) and was used under quarantine conditions. This isolate was compatible with all of the BCT clones and revealed a residual effect of the defeated R-12 gene (75% tuber blight with the R-gene absent vs. 54% tuber blight with the R-gene present). This result is consistent with a previous demonstration of a residual effect of this defeated R-gene in assays of foliar resistance.

Impact of soybean and rapeseed seed meal on microbial populations and growth of apple in replant orchard soils. M. MAZZOLA (1), M. F. Cohen (1), and G. Fazio (2). USDA-ARS, (1) Wenatchee, WA; (2) Geneva, NY. Phytopathology 94:S68. Publication no. P-2004-0458-AMA.

Soybean meal (SBM) and rapeseed meal (RSM) amendments elicited differential effects on elements of orchard soil microbial communities. While application of either amendment resulted in increased total bacterial populations, SBM enhanced while RSM diminished fluorescent *Pseudomonas* spp. Populations. *Streptomyces* spp. Populations were significantly higher in RSM treated than in either SBM treated or non-treated soils. Both amendments suppressed lesion nematode root populations, and RSM but not SBM significantly depressed infection of apple roots by *Rhizoctonia solani*. In field trials, RSM amendment in conjunction with metalaxyl soil drench significantly increased growth and yield of Gala/M26 apple. The level of disease control and growth of all rootstocks achieved in response to RSM/metalaxyl treatment was superior to that obtained by pre-plant Telone C35 fumigation. However, relative increase in tree caliper varied among rootstocks, ranging from 263% (M7) to 561% (G16). Rootstocks supporting higher *Streptomyces* spp. Rhizosphere populations trended toward the higher range of growth promotion in response to RSM amendment.

Genetic variation in *Fusarium oxysporum* f. sp. *lactucae* causing wilt in lettuce. G. Y. MBOFUNG and B. M. Pryor. Univ. of Arizona, Tucson, AZ. Phytopathology 94:S68. Publication no. P-2004-0459-AMA.

Forty-seven isolates of *Fusarium oxysporum* f. sp. *lactucae* from Japan, Italy, Taiwan, California, and Arizona, including nonpathogenic field isolates of *F. oxysporum* were analyzed for genetic diversity using microsatellite-primed PCR. Cluster analyses of fingerprint patterns revealed 4 main groups with a similarity index of 58%. The pathogenic isolates formed lineages distinct from the nonpathogenic isolates. There was a correlation between fingerprint patterns and race. Race 1 isolates formed two clusters distinct from the cluster encompassing race 2 isolates, and similarity indices for these clusters were 73%, 77%, and 78%, respectively. Regarding race 1, there was an absence in correlation between geographical origin and fingerprint pattern. The clustering of Arizona isolates suggests that there have been at least two introductions of race 1 into Arizona. The nonpathogenic field isolates formed a single cluster with a similarity index of 74%. The importance of genetic variation within the *F. o.* f. sp. *lactucae* population with respect to breeding lettuce for resistance is discussed. The probable center of dispersal of the pathogen is also considered.

Synnema and sclerotium production in *Aspergillus caelatus* and the influence of substrate composition on their development in selected strains. C. E. MCALPIN. Mycotoxin Research Unit, National Center for Agricultural Utilization Research, USDA, ARS, Peoria, IL. Phytopathology 94:S68. Publication no. P-2004-0460-AMA.

The ability of *Aspergillus caelatus*, to produce synnemata and sclerotia was investigated. Forty-eight isolates of *A. caelatus* differed widely in their production of synnemata and sclerotia; 83% of the isolates produced varying numbers of synnemata and sclerotia and 17% produced neither sclerotia nor synnemata. Most strains produced synnemata and mostly sessile and few stipitate sclerotia on the same Czapek agar (CZA) plate. Two strains of *A. caelatus* were selected for further study because of the contrasting morphology of their synnemata and sclerotia: NRRL 25528, the type species which produced black sclerotia, and NRRL 26119, which formed tan sclerotia. CZA and CZA amended with dextrose, xylose, cellobiose, melibiose, and trehalose, as well as CZA amended with serine, threonine, KNO_3, and $NaNO_3$, produced abundant sclerotia and synnemata. The production of synnemata and stipitate/sessile sclerotia by several wild-type strains of *A. caelatus* further substantiates previous suggestions for an evolutionary link between the *Aspergillus* section *Flavi* and the synnematal species *A. togoensis*, which also produces stipitate sclerotia.

RNA interference in *Fusarium graminearum*. T. R. McDonald (1), D. W. Brown (2), T. M. HAMMOND (1), and N. P. Keller (1). (1) University of Wisconsin, Madison, WI; (2) USDA-ARS-NCAUR, Peoria, IL. Phytopathology 94:S68. Publication no. P-2004-0461-AMA.

Small grains like wheat are susceptible to scab, a disease caused primarily by the filamentous fungus *Fusarium graminearum*. Wheat head scab is characterized by the production of small, shriveled seeds often contaminated with the mycotoxin deoxynivalenol (DON or vomitoxin). DON and other trichothecenes cause a variety of animal diseases and are a significant agricultural concern. We are investigating RNA interference (RNAi) in an effort to identify novel ways to control trichothecene contamination of small grains by *F. graminearum*. We transformed *F. graminearum* with an inverted repeat (IVRT) construct targeting *tri6*, a transcription factor that positively regulates expression of trichothecene biosynthetic genes. Northern blots of *tri6* IVRT transformants but not wild-type showed the presence of degraded *tri6* transcript typical of RNAi. Wheat infection assays indicate that disease is significantly reduced in two independent *tri6* IVRT transformants as compared to wild-type and a transformant lacking the construct. We are now examining the *tri6* IVRT transformants for trichothecene biosynthetic gene expression and DON production.

Microbial suppression of *Phytophthora cinnamomi* in avocado soils. V. T. MCDONALD, J. A. Menge, E. Pond, M. Crowley, and B. McKee. Dept. of Plant Pathology, University of California, Riverside, CA 92521. Phytopathology 94:S68. Publication no. P-2004-0462-AMA.

In greenhouse studies, microbially suppressive avocado soil to *Phytophthora cinnamomi* was mixed with increasing concentrations of fumigated soil and the suppressive soil was transferable to the conducive soil at concentrations as low as 1:99. When the suppressive soil was treated with progressively higher temperatures, the suppressive effect decreased accordingly, confirming the microbial suppressive activity. In lab studies, hanging water columns were used to show the suppressive effect to be very sensitive to slight changes in soil matric potential. At 0 mbar there was no hyphal degradation of *P. cinnamomi* hyphal mats buried in the soils, yet at 10 mbar there was almost complete hyphal degradation. In four separate experiments done in 2001, infected root segments and hyphal mats of *P. cinnamomi* were buried in the suppressive soil and after eight days evaluated for chlamydospore formation and survival. The suppressive soil showed a seasonal inhibition of the pathogen, with chlamydospore inhibition low in January, higher in April, highest in July, and low again in October. However, a repeat of this experiment in 2002 did not show a similar seasonal inhibition, indicating that the suppressive effect fluctuated greatly over time but was not based on the seasons.

Genetic analysis of the ubiquitous plasmid pEA29 of *Erwinia amylovora* and determination of its role in virulence. G. C. McGhee, P. Teubig, and G. W. SUNDIN. Michigan State University, Dept. Plant Pathology, E. Lansing, MI 48824. Phytopathology 94:S68. Publication no. P-2004-0463-AMA.

All naturally-occurring strains of *Erwinia amylovora* contain a nontransmissible plasmid called pEA29 or pEA28 that significantly enhances virulence in an immature pear fruit inoculation assay. The complete sequence of pEA29 is

known. We used the PCR to clone the promoter regions of 10 genes from pEA29 (*aldD*, aldehyde dehydrogenase; *betT*, choline transporter; *ctpE*, methyl-accepting chemotaxis transducer; *msrA*, peptide methionine sulfoxide reductase; orf7, orf11, orf15, and orf20, unknown function; *thiO*, thiamine biosynthetic gene operon; *trlA*, transcriptional regulator) into a beta-glucoronidase reporter vector. Expression of several of these genes was elevated during pear fruit infection as compared to expression during growth in liquid culture. The expression of the chromosomal amylovoran biosynthetic operon promoter was also significantly elevated in the wild-type Ea110 strain compared to the Ea110/pEA29-cured strain. The effect of individual pEA29-encoded genes on virulence was studied using complementation and gene knockout experiments. We complemented the Ea110/pEA29-cured strain with *aldD*, *betT*, *msrA*, orf7, or the *thi* operon; each gene(s) was cloned in a low-copy vector with its native promoter. We also used a recombination approach to generate knockout mutations within *aldD*, *betT*, *msrA*, and *thiO*. Virulence was assessed using a pear fruit assay and shoot inoculation of susceptible ('Gala') and tolerant ('Red Delicious') apple.

Seasonal dynamics of resistance to QoI fungicides in *Podosphaera xanthii* and impact on control of cucurbit powdery mildew. M. T. MCGRATH. Cornell University. Phytopathology 94:S69. Publication no. P-2004-0464-AMA.

Resistance to QoI (strobilurin) fungicides in *Podosphaera xanthii* was first detected in US in 2002. A seedling bioassay was used in 2003 to monitor QoI resistance in cucurbit fields at 10 commercial farms and 1 research facility in Suffolk Co., NY. Not all sites were included in each bioassay. Squash seedlings were dipped in solutions of QoI fungicide (50 mg/L trifloxystrobin formulated as Flint), DMI fungicide (20 mg/L myclobutanil; Nova), or both. About 12 hrs later they were put with nontreated seedlings in fields for up to 1 day, then kept in a greenhouse until severity was rated. On 27 Jul, when mildew was first seen in the area, QoI resistant strains were found in 1 of 4 commercial fields with early planting summer squash not QoI-treated, but not in a research pumpkin field. On 19-21 Aug, mildew severity in 4 commercial pumpkin fields where QoIs were used averaged 1% on upper leaf surfaces and 11% on lower. On 31 Aug, QoI resistance was common in these fields, 2 other pumpkin fields, and a winter squash experiment (61 to 100% frequency). Nova but no QoIs was used in 1 field. Moderate DMI insensitivity occurred in all fields (12 to 56%). Severity then exceeded 50% on most lower leaf surfaces. On 25 Sep, QoI resistance was detected in 3 commercial pumpkin fields where no QoIs or DMIs were used (2, 38, and 56%) and in fields where QoIs and/or DMIs were used (88 to 97%). In summary, QoI resistant strains were present at mildew onset, their frequency increased greatly during the season, efficacy was affected, and they occurred in crops not treated with QoIs.

The impact of rainfall on leaf colonization by *Aureobasidium pullulans*. M. J. MCGRATH, R. N. Spear, and J. H. Andrews. University of Wisconsin, Madison, WI. Phytopathology 94:S69. Publication no. P-2004-0465-AMA.

In Spring 2003, we applied a GFP-tagged *Aureobasidium pullulans* population to 20 apple leaves on 17 trees in an experimental orchard at Madison, WI. About an hour before and after an intense September rain event, 10 leaves were sampled by using a semi-destructive sampling method that allowed for repeated sampling through time of individual leaves. Epifluorescence microscopy was used to determine the numbers of swollen cells and chlamydospores (SCC) and blastospores on mid-veins, smaller veins (secondary and tertiary), and non-veinal micro-sites (three transects/leaf sampled). The average number per 0.196 mm^2 microscope field (counts represent all fields viewed, not just fields containing feature of interest) of SCC on mid-veins, other veins, and non-veinal sites prior to the rain was 0.48, 3.14, and 0.14 respectively. Post-rain counts of SCC were 0.66, 3.48, and 0.38. The increase (or decrease) in SCC was not significant at any micro-site ($P > 0.05$). There were no blastospores on the mid-veins, other veins, or non-veinal sites before the rain, but following the rain the counts increased to 0.06, 0.97, and 0.97 respectively. This increase in blastospores was significant ($P < 0.05$) at all sites. Some blastospores budded from colonies of SCC, but most appeared as isolated cells. These results were supported by similar data from a second rain event. We conclude that rainfall can trigger immediate birth (growth) of blastospores, which potentially can develop into new colonies *in situ* if they are washed to favorable sites.

Microscopic localization of *Embellisia* in seed and seedlings of southern specklepod locoweed (*Astragalus lentiginosus*). J. MCLAIN-ROMERO, A. Padilla, and R. Creamer. New Mexico State University, Las Cruces, NM. Phytopathology 94:S69. Publication no. P-2004-0466-AMA.

Swainsonine-producing, *Embellisia* fungi have been correlated with the toxicity of some locoweeds (*Astragalus* and *Oxytropis* sp.). Most swainsonine-containing locoweed species contain a high percentage of fungus in their seeds. *Astragalus lentiginosus* seeds were prepared as whole mounts or sectioned and stained with trypan blue and sudan IV. By light microscopy, the *Embellisia* fungi were observed within the parenchyma and aleurone seed layers and not within embryonic tissue. Other fungi were rarely observed in seed tissue of *Astragalus crassicarpus* (control). Fungal identification was confirmed by culturing and PCR detection. The fungus was observed within plant tissues in 7-day-old seedlings germinated under sterile conditions with seedcoat adhering but not in 7-day-old seedlings germinated after seedcoat removal. The *Embellisia* fungi most likely enter the plant tissue during germination and events during germination may affect fungal intrusion.

Identification of proteins in *Phakopsora pachyrhizi* urediniospores using MALDI-TOF/TOF mass spectrometry. M. B. McMahon (1), J. J. Choi (1), M. L. Carter (1), A. Nunez (2), R. D. Frederick (1), and D. G. LUSTER (1). (1) USDA, ARS FDWSRU, Ft. Detrick, MD; (2) USDA, ARS ERRC, Wyndmoor, PA. Phytopathology 94:S69. Publication no. P-2004-0467-AMA.

Phakopsora pachyrhizi is the causal agent of soybean rust, a devastating foliar disease that occurs in most soybean growing regions throughout the world, except for Europe and North America. In order to understand the early events in the infection process, an initial study was conducted to characterize and identify proteins in *P. pachyrhizi* urediniospores before and during germination. Trypsin-digested two-dimensional gel electrophoresis spots were peptide finger-printed and sequenced using Matrix-Assisted Laser Desorption/Ionization mass spectrometry with automated tandem time of flight fragmentation of selected ions (MALDI-TOF/TOF). Proteins identified from putative peptide sequences by database searches within a confidence interval greater than or equal to 95% were evaluated and placed into functional categories, including transcription regulation, cell division and protein signaling.

The North Dakota IPM crop pest survey – using technology to improve information delivery. M. MCMULLEN and P. Glogoza. NDSU Extension Service, North Dakota State University, Fargo, ND. Phytopathology 94:S69. Publication no. P-2004-0468-AMA.

North Dakota's statewide IPM pest survey includes the use of global positioning systems (GPS) for location of survey fields, geographic information systems (GIS) for graphic interface of pest occurrence, and the Internet for frequent postings of pest development. Crop scouts surveyed six major crops for diseases and insect pests for a total of 1837 fields in 2002 and 2508 fields in 2003. Survey information was recorded on-site into spreadsheets using handheld computers with attached GPS units. These tools improved speed of delivery of information to producers and other stakeholders by decreasing the time to make data summaries, allowing easy illustration of distribution of sampled fields, and linking survey information to specific areas. Delivery of survey data was provided several times a week through: IPM and entomology update web sites; a weekly NDSU Crop and Pest Report; a web server; and news releases. The first occurrence, prevalence, severity and distribution of crop diseases such as wheat leaf rust and tan spot, wheat and barley head scab, canola blackleg, and pasmo of flax were provided to producers for timely management decisions.

Biocontrol capacities of *phlD+* pseudomonads that are prevalent in Ohio soils. B. B. SPADDEN GARDENER, R. Joshi, L. Gutierrez, and E. Lutton. The Ohio State University – OARDC, Wooster, OH. Phytopathology 94:S69. Publication no. P-2004-0469-AMA.

Despite great variation in soil composition, only a few genotypes of phlD-containing pseudomonads were observed to be common throughout Ohio. Multiple isolates of these genotypes were obtained from the roots of corn and soybean plants grown at different locations. When tested for their capacity to produce 2,4-diacetylphloroglucinol (DAPG) in vitro, a significant genotype by media interaction was observed. Notably, the A genotype produced more DAPG on media containing soybean extract than on other media. When applied as seed treatments, all three genotypes were capable of colonizing corn and soybean roots, but colonization by the D and S genotypes was most consistent. A field trial of three genotypes conducted in 2003 revealed that that the most significant increases in stand and yield were observed following applications of the D genotype.

Association of *Pantoea agglomerans* with seed rot of South Carolina cotton. E. G. MEDRANO (1), M. A. Jones (2), and A. Bell (1). (1) USDA-ARS, College Station, TX; (2) Pee Dee Research and Education Center, Florence, SC. Phytopathology 94:S69. Publication no. P-2004-0470-AMA.

In recent years, seed rot of cotton has occurred extensively in southeastern states causing considerable yield losses particularly in South Carolina. The causative agent(s) of the disease syndrome has not been identified. Symptoms of infected fruits include stunted fiber development and macerated locules.

Microorganisms were isolated on TSA plates from seeds that were excised from diseased bolls. Presumptive *P. agglomerans* isolates were recovered from 10 of 20 bolls tested based on colony morphology, Gram staining, and carbon utilization testing. Two randomly selected isolates were marked with resistance to rifampicin (Rif) by incremental exposure to the antibiotic up to 200 micrograms / ml. Pathogenicity testing was conducted by injecting the Rif resistant isolates into healthy cotton bolls. Rifampicin resistant mutants were recovered from diseased fruits harvested two weeks post inoculation whereas, mock inoculated controls demonstrated no disease symptoms. Thus, *P. agglomerans* was determined to be capable of producing symptoms analogous to those of the seed rot of cotton observed in South Carolina.

Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, *PR* gene expression and host resistance to fungal infection. C. M[illegible] (1), M. Qi (1), S. Zhang (2), G. Sheng (2), and Y. Yang (1). (1) Department of Plant Pathology; (2) Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701. Phytopathology 94:S70. Publication no. P-2004-0471-AMA.

Jasmonic acid (JA) is a lipid-derived signal molecule that plays an important role in biotic and abiotic stress responses in many plants. In this study, we characterized a rice allene oxide synthase (*OsAOS*) gene and generated transgenic rice with inducible overexpression of *OsAOS*. OsAOS contains four common domains of the P450 enzyme, but does not have the signal peptide for chloroplast targeting, which is generally present in AOS from dicotyledonous species. The basal expression of *OsAOS* is very low in leaves but relatively high in sheath, culm and flower. Paradoxically, the expression of *OsAOS* in leaves was hardly induced by infection of either virulent or avirulent strains of the blast fungus (*Magnaporthe grisea*). However, transgenic rice lines carrying the *OsAOS* transgene under the control of a pathogen-inducible *PR10* promoter did show abundant *OsAOS* transcripts and high JA levels, especially after the pathogen infection. These transgenic lines also exhibited significant induction of pathogenesis-related (*PR*) genes such as *PR1a*, [illegible], and *PR5* and enhanced resistance to *M. grisea* infection. Taken together, these results suggest that JA plays an important role in mediating *PR* gene induction and disease resistance against fungal infection.

Biological control of *Uncinula necator* by tydeid mites. H. M. MELIDOSSIAN, R. C. Seem, D. M. Gadoury, W. F. Wilcox, and G. M. English-Loeb. Cornell Univ., NYSAES, Geneva, NY 14456. Phytopathology 94:S70. Publication no. P-2004-0472-AMA.

Orthotydeus lambi reduced the severity of grape powdery mildew on fruit in a vineyard of *Vitis vinifera* 'Chardonnay' when released 2-3 wks prebloom in each of 3 years. Both protonymph and adult mites comparably reduced growth and sporulation of mildew colonies on leaf disks. Mites tore mycelium and conidia with their palps, leading to leakage, loss of turgor and collapse of hyphae. In a vineyard of several cultivars, mites reduced disease severity on fruit by >50% during 4 years of study, with the highest mite densities occurring on varieties with large aggregations of trichomes (domatia) in vein axils. Suppressive effects of mites and most fungicides were additive. However, sulfur, mancozeb, and the insecticide carbaryl were toxic to mites in bioassays, reduced mite densities in field trials, and may be responsible for low densities of *O. lambi* in many commercial vineyards. Use of alternative materials in vineyard trials both conserved mite populations and provided disease suppression comparable to conventional fungicides. Thus, conserving natural populations of *O. lambi* in vineyards may enhance powdery mildew control.

Use of population genetics for evaluation of inoculum sources of carrot leaf blights caused by *Alternaria dauci* and *Xanthomonas campestris* pv. *carotae* (Xcc). X. MENG, J. Nunez, R. M. Davis, and R. L. Gilbertson. Dept. Plant Pathology, University of California, Davis, CA 95616. Phytopathology 94:S70. Publication no. P-2004-0473-AMA.

Genetic diversity in *A. dauci* was revealed by restriction fragment length polymorphism analysis of the intergenic spacer (IGS) regions. Ten *A. dauci* IGS genotypes were identified. Genotype 2 isolates were predominant in California carrot fields in the 2001-2003 growing seasons, and the genotypic constitution of the *A. dauci* population in California was stable. Furthermore, *A. dauci* was not isolated from carrot seeds produced in Oregon and Washington and planted in California, suggesting that local inoculum sources of Alternaria leaf blight are important in disease epidemiology. Three genotypes (1a, 1b and 2) of Xcc were identified by repetitive element (rep)-PCR. Genotypes 1a and 2 strains were predominantly recovered from seeds and plants from California, Oregon and Washington; and distributions of Xcc genotypes recovered from California were similar to those from Oregon and Washington. These results suggest that contaminated seed is an inoculum source of bacterial leaf blight in California.

Influence of strawberry fruit age on susceptibility to *Colletotrichum acutatum*. J. C. MERTELY, T. E. Seijo, and N. A. Peres. Univ. of Florida, GCREC, Dover, FL 33527. Phytopathology 94:S70. Publication no. P-2004-0474-AMA.

Intact strawberry flowers and fruit were inoculated in the field to determine variations in susceptibility to the anthracnose fungus *Colletotrichum acutatum*. Newly-opened flowers in a commercial block of 'Camarosa' strawberry were tagged at regular intervals to produce sets of fruit of known ages. Half of the experimental flowers and fruit were inoculated with a 1×10^5 spore suspension of an aggressive isolate of *C. acutatum*. The remaining flowers and fruit served as controls. Fruit were examined for symptoms of anthracnose fruit rot 6 days after inoculation and every 2 days thereafter. Disease incidences and incubation periods were determined for each set of fruit. In replicate experiments done in 2002 and 2003, strawberry flowers were highly susceptible to infection with disease incidences exceeding 90%, and incubation periods ranging from 6 to 7 days. Disease incidences were lower for young green fruit, and incubation periods were longer (8 to 10 days). Older green fruit inoculated 18 days after anthesis had disease incidences similar to younger green fruit, but shorter incubation periods (6 to 7.5 days). Normal harvest of older inoculated fruit may not have allowed sufficient time for development of visible lesions on all fruit. Flowers are more susceptible to infection than young green fruit. Susceptibility may increase as the fruit age, but older fruit may be harvested before symptoms develop.

Screening for disease susceptibility genes to *Phytophthora sojae* in soybean line Ox20-8. S. X. MIDEROS[illegible], S. Costanzo (1), M. Nita (1), S. K. St. Martin (2), and A. E. Dorrance (1). (1) Dept. of Plant Pathology, The Ohio State University, Wooster, OH; (2) Dept. of Horticulture and Crop Science, The Ohio State University, Columbus, OH. Phytopathology 94:S70. Publication no. P-2004-0475-AMA.

Phytophthora root and stem rot of soybeans, caused by *P. sojae*, is a serious limitation to the production of soybeans in Ohio. This disease is managed through the deployment of single resistance genes (*Rps*), and in certain cases, in combination with partial resistance. Historically, soybean line Ox20-8 has shown high susceptibility to all races of *P. sojae*, even though it possesses *Rps1a*. Our objective in the present study was to test this line for the presence of a gene for susceptibility. A population of $F_{2:4}$ lines from the cross Ox20-8 x General was screened using the slant board assay to measure root lesion expansion. Ten plants from each of 100 lines from this cross were inoculated with a 7-day-old culture of *P. sojae* race 25. Seven days after the inoculation, lesion length was measured from the point of inoculation. The frequency distribution of the averaged lesion length observed among lines of this population was consistent with the inheritance of a quantitative trait. Moreover, the presence of transgressive segregation toward resistance indicated a positive contribution derived from the parental line Ox20-8, providing evidence in contrast to the hypothesis of a susceptibility gene present in this cultivar.

Temperature and maturity effects on latent period of *Botryosphaeria dothidea* on infected pistachio fruit. A. L. MILA and T. J. Michailides. Dept. of Plant Pathology, University of California-Davis, Kearney Ag. Center, Parlier 93648. Phytopathology 94:S70. Publication no. P-2004-0476-AMA.

Temperature is a major factor for infection, disease development, and sporulation of *B. dothidea* (BD). We examined the effect of temperature on length of latent period (time from inoculation to symptom development) in detached pistachio fruit inoculated with BD and incubated at 10, 15, 20, 25, and 30°C. After 3, 6, or 9 days, sets of fruit were transferred from the lower temperatures to 30°C, the optimum temperature for symptom development. One set of fruit was always at 10, 15, 20, 25, and 30°C. Experiments were repeated in 4 maturity stages (i.e., when kernel/cavity ratio was 0. 5, 0.6, 0.8, and 1.0). Length of latent period was 9-14 days at 10 and 15, 5-6 days at 20 and 25, and 3 days at 30°C. Apparently healthy fruit became blighted 2 to 3 days after their transfer to 30°C from 10, 15, 20, and 25°C. The total percentage of blighted fruit increased from 60 when fruit were immature to 90 when fruit were mature. These results suggest that temperature and maturity are critical factors for the length of latent period of *B. dothidea* on pistachio.

Progress of panicle and shoot blight of pistachio in California. A. L. MILA (1), G. F. Driever (2), and T. J. Michailides (1). (1) Dept. of Plant Pathology; (2) Dept. of Pomology, University of California-Davis, Kearney Ag. Center, Parlier 93648. Phytopathology 94:S70. Publication no. P-2004-0477-AMA.

Panicle and shoot blight, caused by *Botryosphaeria dothidea*, is a destructive disease of pistachio in California. Based on observations collected between 1999 and 2001 the disease progress during a growing season was modeled using the number of continuous and discontinuous days of rain, type of

orchard irrigation, deviation from the normal temperature of April and May, cumulative temperature from June until harvest in September, and percent of latent infection. In 2003, progress of panicle and shoot blight was monitored in 7 locations in which the predicted disease progress was calculated with the developed model. Because precipitation in spring of 2003 was higher than in springs of 1999 to 2001, disease progress was overestimated in 5 of the 7 locations. At the end of 2003, the disease progress model was updated with Bayesian methods using the 2003 data. Although the model is currently under further validation it appears that panicle and shoot blight progress can be sufficiently described based on environmental conditions, irrigation, and level of infection.

Global BYDV/CYDV sequencing project. W. A. Miller (1), S. Anderson (2), S. M. GRAY (3), X. Gai (1), and R. Beckett (1). (1) Iowa State University, Ames, IA; (2) USDA, ARS, West Lafayette, IN; (3) USDA, ARS, Ithaca, NY. Phytopathology 94:S71. Publication no. P-2004-0478-AMA.

The yellow dwarf viruses (YDVs), Barley yellow dwarf (BYDV) and Cereal yellow dwarf (CYDV), are, world wide, the most economically important viruses of wheat, barley and oats. Resistance genes are limited and many YDV isolates break resistance. YDVs have remarkable genetic and biological diversity. The handful of isolates that have been sequenced have resulted in the original BYDV being reclassified into at least three virus species in two different genera. Plant breeders and pathologists around the world attempting to breed resistant crops have discovered that YDV isolates within the same serotype can cause very different symptoms and respond differently to host resistance genes. This project will determine complete nucleotide sequences of up to 100 biologically important YDV isolates from around the world. We present methods for rapid full-length RT-PCR, sequencing, and construction and testing of full-length infectious clones. A large database of sequence information, detection methods and biological phenotypes will be posted on a web site to serve as a resource for epidemiological and molecular mechanistic studies. This will be useful for mapping vector specificity determinants, predicting key RNA secondary structures, and determining differential response to host resistance genes. We invite new collaborators to contribute isolates for sequencing. Funds are also available to train researchers in sequencing and molecular detection methods to facilitate their resistance and epidemiological studies.

Managing silver scurf on potatoes in storage with post-harvest fungicides and disinfestants. J. S. MILLER (1), P. B. Hamm (1), N. Olsen (3), B. D. Geary (4), and D. A. Johnson (5). (1) Univ. of Idaho, Aberdeen, ID; (2) Oregon State Univ., Hermiston, OR; (3) Univ. of Idaho, Twin Falls, ID; (4) Univ. of Idaho, Parma, ID; (5) Washington State Univ., Pullman, WA. Phytopathology 94:S71. Publication no. P-2004-0479-AMA.

Silver scurf (SS) of potato, caused by *Helminthosporium solani*, can cause rejection of tubers sold for table stock. Seed treatments are very effective in reducing SS on daughter tubers but long term storage of potatoes can still result in significant losses. Studies were conducted from 2001-2004 to examine the efficacy of various products on suppressing pathogen growth on naturally infected potato tubers after placement in storage. Daughter tubers from a seed lot with high levels of SS were grown and left in the ground for a month after vine kill. After harvest, tubers were treated with a low volume spray of test products and then stored at two locations. In 2001-2002, none of the products reduced SS at location 1. However, azoxystrobin reduced incidence and severity at both 2 and 6 months at location 2 and the biological control *Bacillus subtilis* reduced incidence at 6 months. In 2002-2003, potassium sorbate and *B. subtilis* reduced severity after 6 months in storage in location 1, while only azoxystrobin reduced incidence after 6 months in location 2. In 2003-2004 azoxystrobin reduced SS at both locations after 2 months of storage. Most products labeled for post-harvest silver scurf management were ineffective. While not currently registered, azoxystrobin use on tubers going into storage may be the last step needed by growers to control this disease.

Tissue-specific expression of a late blight R gene in wild and cultivated potato species. B. P. MILLETT and J. M. Bradeen. University of Minnesota, St. Paul. Phytopathology 94:S71. Publication no. P-2004-0480-AMA.

The NBS-LRR class of resistance I genes accounts for 75% of cloned plant R genes. These genes encode sentinels that are involved in pathogen detection and defense response initiation. As sentinels, R genes are presumed to be constitutively expressed in all plant tissues, even in the absence of the pathogen. The NBS-LRR R gene, *RB*, recently cloned from the wild potato species *Solanum bulbocastanum* imparts resistance to *Phytophthora infestans*, the causal organism of Late Blight, a disease responsible for multibillion dollar losses worldwide. Potato late blight tuber resistance is just as essential as foliar resistance since these two distinct tissues are both natural targets of *P. infestans*. Here, we examined *RB* expression using RT-PCR and compared expression in leaves and tubers. In *S. bulbocastanum*, we found that *RB* is constitutively expressed in leaves, but apparently not expressed at all in the tuber. We are currently examining *RB* expression in transgenic cultivated potato. The lack of expression of *RB* in the tuber could explain why tubers of foliar resistant potato plants are still susceptible to *P. infestans*.

Factors affecting bitter rot (*Greeneria uvicola*) fungus infection of wine-grape (*Vitis vinifera*). J. G. MIRANDA and T. B. Sutton. Department of Plant Pathology, North Carolina State University, Raleigh, NC. Phytopathology 94:S71. Publication no. P-2004-0481-AMA.

Bitter rot (*Greeneria uvicola*) is one of the most important fruit rot diseases of winegrapes (*Vitis vinifera*) in the Southeastern U.S. The period of fruit susceptibility was distinguished by inoculating intact clusters in Alamance Co. and Rockingham Co., NC, every 2 weeks from bloom until 2 weeks before harvest. Susceptibility of *V. vinifera* 'Merlot', 'Chardonnay', and 'Cabernet Franc' fruit increased from bloom until veraison (the stage when grapes ripen, soften, and change color). The relative susceptibility of 30 cultivars, including 19 *V. vinifera* cultivars and 5 French-American hybrids, was determined by inoculating and incubating detached fruit at 26°C. *V. vinifera* 'Rousanne' and 'Petite Sirah' were the most susceptible, while *V. aestivalis* 'Cynthiana Norton', *V. vinifera* 'Merlot' and 'Arkansas 1219' and French-American hybrid 'Traminette' were the most resistant. Growth chamber studies were also conducted to examine the influence of temperature and duration of wetness on infection. Detached fruit of *V. vinifera* 'Cabernet Sauvignon', 'Cabernet Franc', and 'Chardonnay' were inoculated and incubated at 14, 18, 22, 26, and 30°C for 6, 12, 18, and 24 hours of wetness. The optimal conditions for infection of fruit by *G. uvicola* were temperatures between 23.6-26.4°C with 7-9 hours of wetness.

Evaluation of postharvest treatment of table grapes with heated ethanol water to control gray mold. F. MLIKOTA GABLER and J. L. Smilanick. USDA, ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA. Phytopathology 94:S71. Publication no. P-2004-0482-AMA.

Postharvest gray mold, caused by *Botrytis cinerea*, Pers., develops during commercial cold storage at –0.5 to 1°C and during transport and marketing at warmer temperatures. The influence of a brief immersion of table grapes in water or ethanol at ambient or higher temperatures on the incidence of gray mold and grape quality was evaluated. Single Autumn Seedless berries with or without the pedicels attached were inoculated with *B. cinerea* conidia. Reliable control of gray mold was obtained by a 1 min immersion in 35% ethanol at 50°C, while ethanol at 25°C or water alone at 50°C were less effective. Heated ethanol controlled gray mold effectively even if applied up to 24 h after inoculation. Treatments were less effective when pedicels were removed from the berries before inoculation and treatment. Crimson Seedless clusters were inoculated, immersed for 1 min in 35% ethanol at 25 or 50°C, and stored for one month at 0.5°C, and 2 days at 25°C. Infected berries were 78.7, 26.2, and 3.4 per kg, respectively, among untreated grapes and those immersed in ethanol at 25 or 50°C. Rachis appearance, berry color (L* and hue), and berry shatter were not significantly changed by these treatments. Treatments that were longer in duration or with higher ethanol concentrations did not reduce epicuticular wax content, although treated grapes were more susceptible to subsequent infection compared to cool water-treated controls.

Toxicity of aqueous ethanol at various temperatures to spores of post-harvest pathogens. F. Mlikota Gabler (1), W. F. Mansour (1), J. L. SMILANICK (1), and B. E. Mackey (1). (1) USDA, ARS, San Joaquin Valley Agric. Sci. Center, Parlier, CA; (2) USDA, ARS, Western Regional Research Center, Albany, CA 94710-1105. Phytopathology 94:S71. Publication no. P-2004-0483-AMA.

Survival of spores of *Rhizopus stolonifer*, *Aspergillus niger*, *Botrytis cinerea*, and *Alternaria alternata* after brief exposure to heated, aqueous ethanol solutions was quantified. Spores were immersed in 0, 10, 20, or 30% ethanol at 25, 35, 40, 45, or 50°C for 30 s, then their survival was determined by germination on agar media. Logistic, second order, surface response models were prepared for each fungus. Ethanol and heat combinations were synergistic. Sub-inhibitory ethanol concentrations at ambient temperatures became inhibitory when heated at temperatures much lower than those that caused thermal destruction of the spores by water alone. Raising the temperature of the ethanol significantly reduced the subsequent germination of spores of all the fungi compared to the same concentrations at 25°C. Exposure to water alone at 50°C only slightly affected germination. At 40°C, estimated ethanol concentrations that inhibited the germination of 50% (LD50) of the spores of *B. cinerea*, *A. alternata*, *A. niger*, and *R. stolonifer* were 9.7, 13.5, 19.6, and 20.6%, respectively. *B. cinerea* and *A. alternata* were less resistant to the combination than *A. niger* or *R. stolonifer*. At 25°C, 30% ethanol reduced germination of *B. cinerea* and *A. alternata*, while it did not affect that of *R. stolonifer* or *A. niger*.

Identification of microorganism for biological control of powdery mildew in flowering dogwood. M. T. MMBAGA and F. A. Mrema. Tennessee State University. Phytopathology 94:S72. Publication no. P-2004-0484-AMA.

Leaves of flowering dogwood (*Cornus florida*) were collected from two uncultivated natural habitats and the microbial epiphytes were recovered by using leaf washings with a stomacher blender and dilution plating. Potato dextrose agar (PDA), nutrient agar (NA) and wort agar (WA) were used to isolate fungi, bacteria, and yeast respectively. The microorganisms were separated into recognizable morphological types and the different morphotypes were tested for potential in powdery mildew control. Out of 137 isolates evaluated, only 12% showed potential to reduce disease severity; these included 7 fungi, 6 bacteria and 2 yeast isolates. The fungi and yeast were identified using PCR-based DNA analysis using ITS1 and ITS4 primers and bacteria isolates were identified using PCR-based DNA analysis using universal primers FGPL132-38 and FGP56-63. The PCR products were sequenced and compared with information available in GenBank using Blast search. Among the isolated fungi that showed some potential as biocontrol agents for dogwood powdery mildew, two (*Acremonium* spp. and *Ampelomyces* spp.) have been previously used for the control powdery mildew on other plant species. *Cladosporium* spp., a mycoparasite fungus, was also identified in this study and it has been reported as antagonist in spore germination of *Cronartium flaccidum* and *Peridermium pini* and significantly reduced disease development in pine seedlings. Powdery mildew was not a significant problem in the wild locations sampled, it is thus possible that biological agents on plant foliage play a significant role in keeping the disease at insignificant levels.

How friendly are biorational fungicides to non-target organisms on plant foliage? M. T. MMBAGA and T. Amanyenu. Tennessee State University. Phytopathology 94:S72. Publication no. P-2004-0485-AMA.

Two biorational products, potassium bicarbonate (Armicarb™) and a household soap Ajax®, are effective in controlling powdery mildew on dogwood either alone or in rotation with conventional fungicides. A two year study was conducted to compare their effect on non-target organisms on *Cornus florida* foliage. Weekly applications of the products alone and in rotation with propiconazole were compared with semimonthly applications of thiophanate methyl, propiconazole and non treated control. Fungicide applications were initiated in late May and terminated at the end of August and dogwood leaves were collected monthly (May-September). Leaf washings and dilution plating were used to recover fungi on potato dextrose agar, bacteria on nutrient agar, and yeast on wort agar. Microbial diversity was assessed using colony morphological types. Data analysis showed an increase in the total microbial population over time in all treatments with highest number of colony forming units (CFUs) in August in the first year, and in September in the second year. Differences between treatments were significant in July and September in the first year and in July, August and September in the second year in treatments with Ajax/propiconazole rotations. The propiconazole treatment had the lowest CFUs; other differences between treatments were either insignificant or inconsistent in the two years. Bacteria and yeasts were dominant throughout the study, but the number of CFUs varied significantly over time without consistent treatment effects. Fungi had the smallest CFUs with the highest number in May/June (6-29% of the total population) with adecline to 0.2-3% in August/September. Leaves treated with biorational or conventional fungicides and non-treated had similar microbial diversity.

Suppression by abscisic acid of lignin production and monolignol pathway gene expression in interactions of Arabidopsis with oomycete and bacterial pathogens. P. G. Mohr and D. M. CAHILL. Deakin University, Geelong, VIC, Australia. Phytopathology 94:S72. Publication no. P-2004-0486-AMA.

The phytohormone, abscisic acid (ABA) has been shown to influence the outcome of the interactions between various hosts with biotrophic and hemibiotrophic pathogens. Susceptibility to avirulent isolates can be induced in plants by addition of low physiological concentrations of ABA. In contrast, addition of ABA biosynthesis inhibitors induced resistance following challenge of plants by virulent isolates. ABA deficient mutants of Arabidopsis, such as *aba1-1*, were resistant to virulent isolates of *Peronospora parasitica*. In interactions of Arabidopsis with avirulent isolates of *Pseudomonas syringae* pv. *tomato*, susceptibility was induced following addition of ABA or imposition of drought stress. These results indicate a pivotal, albiet undefined, role for ABA in determining either susceptibility or resistance to pathogen attack. We have found that the production of the cell wall strengthening compound, lignin, is increased during resistant interactions of *aba1-1* but suppressed in ABA-induced susceptible interactions. Using RT-PCR and microarray analysis we have found down-regulation by ABA of key genes of the phenylpropanoid pathway especially of those genes involved directly in lignin biosynthesis. ABA also down-regulates a number of genes in other functional classes including those involved in defence and cell signalling.

Comparison of forecasting methods for Fusarium head blight. J. E. MOLINEROS (1), L. Madden (2), P. Lipps (2), G. Shaner (3), L. Osborne (4), L. Francl (1), and E. D. De Wolf (1). (1) The Pennsylvania State University, University Park, PA; (2) The Ohio State University/OARDC, Wooster, OH; (3) Purdue University, West Lafayette, IN; (4) South Dakota State University, Brookings, SD. Phytopathology 94:S72. Publication no. P-2004-0487-AMA.

Fusarium head blight (FHB) is an important disease of wheat and barley east of the Rocky Mountains. A forecasting system with 70% accuracy has been deployed; however, models with improved accuracy could help producers manage the disease and associated mycotoxins. Prediction models were developed using 124 cases that included hourly weather, crop growth stage, disease level, and a corn residue variable. The cases were divided into data sets used for model development (n = 86) and validation (n = 38). Logistic regression, non-parametric discriminant analysis, and neural networks were compared for accuracy and other diagnostic criteria using both model development and validation data. Identified models used temperature, relative humidity, rain, and corn residue to distinguish epidemics from non-epidemics (<10% severity). Accuracy of all three modeling approaches was near 80% for both model development and validation data sets.

Effect of ascospore density, leaf age, leaf wetness, and fungicide application on epiphytic growth and development of symptoms of greasy spot caused by *Mycosphaerella citri*. S. N. MONDAL and L. W. Timmer. Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850. Phytopathology 94:S72. Publication no. P-2004-0488-AMA.

Greasy spot, caused by *Mycosphaerella citri*, produces lesions on leaves that result in premature defoliation, poor plant vigor, and reduced yield. Ascospores produced in the decomposing leaf litter are deposited on the leaf surface where they germinate, grow epiphytically, penetrate through stomates and produce greasy spot symptoms. In greenhouse studies, epiphytic growth reached a maximum in 30-45 days and symptoms were severe after 120 days following inoculation with 10^4 ascospores/ml. Lower densities of ascospores (10^1 to 10^2 ascospores/ml) produced symptoms of comparatively low severity after 180 days. Greasy spot was equally severe on leaves that were 10 to 60 days old at the time of inoculation. Leaf wetness durations of 24 h maximized epiphytic growth, while interrupted wetting periods did not diminish growth. Application of fenbuconazole up to 45 days before inoculation reduced disease severity, but post-inoculation applications were relatively ineffective. Modification of cultural practices to reduce inoculum might be the most effective approach to lessen disease severity.

Effects of nocturnal soil temperatures and *Meloidogyne incognita* densities on cotton seedling growth and the interaction with *Thielaviopsis basicola*. W. S. MONFORT (1), T. L. Kirkpatrick (2), and C. S. Rothrock (1). (1) University of Arkansas, Fayetteville, AR; (2) University of Arkansas, Hope, AR. Phytopathology 94:S72. Publication no. P-2004-0489-AMA.

Controlled environment experiments were conducted to evaluate the effects of *Meloidogyne incognita* densities and soil temperatures (temp) typical of environments in Arkansas on the interaction between *M. incognita* and *Thielaviopsis basicola* on cotton. Soil temp regimes were based on temp recorded at Rohwer, AR where nocturnal temp ranged from 15-19, 17-21, and 17-19°C for 2001, 2002, and 2003, respectively, for the first six weeks of the growing season and represented years with different levels of seedling damage from the interaction. Cottonseed were planted in soil infested with *T. basicola* at 100 propagules/g and *M. incognita* at 0, 4, 8, or 16 eggs/g of soil. Plant growth represented by height, nodes, fresh top weight, and fresh root weight was greater in 17-21 compared to 15-19 and 17-19 temp regimes. Percent root discoloration, incidence, and colonization by *T. basicola* were higher in the 15-19 and 17-19 than in the 17-21 temp regimes for most *M. incognita* levels, with the 17-19 temp regime having the lowest levels of damage, especially, when no *M. incognita* was present. These studies suggest that fluctuations in nocturnal soil temp and *M. incognita* densities influenced the damage due to the interaction between *T. basicola* and *M. incognita*, with *M. incognita* densities becoming more important when nocturnal temp were higher than optimum for *T. basicola* colonization.

Spatio-temporal analysis of Stewart's wilt of corn caused by *Pantoea stewartii* in Mexico. G. MORALES (1), H. V. Silva (1), D. L. Ochoa (2), A. Carballo (1), and L. Cordova (1). (1) Instituto de Recursos Geneticos y Productividad; (2) Instituto de Fitosanidad, Colegio de Postgraduados, Montecillo, Mexico 56230. Phytopathology 94:S72. Publication no. P-2004-0490-AMA.

The temporal and spatial patterns of Stewart's wilt of corn, caused by *Pantoea stewartii*, were studied on two dent corn hybrids (Triunfo and 9Bx52) naturally infected during the 2003 season crop in Montecillo, Mexico. Disease incidences were made once a week from 10 June to 02 September,

data sets were transformed and fitted to logistic, monomolecular and Gompertz models. The area under disease progress curves (AUDPC) were calculated with incidence data sets of the two hybrids. Data of temperature and relative humidity were obtained from the meteorological station at Montecillo. The BBD program was used to calculate the index of dispersion and spatio-temporal autocorrelations were studied using the STAUTO program. Disease incidences were 26.13% and 26.87% for Triunfo and 9Bx52, respectively. Temperature was significantly related to disease incidence. The best model that describes disease progress in both Triunfo and 9Bx52 hybrids was monomolecular. AUDPC was not significantly different between two hybrids. Tests of the index of dispersion indicated that the spatial pattern of Stewart's wilt of corn was aggregated on Triunfo and random on 9Bx52. The spatio-temporal autocorrelations decreased more in space that in time.

Extraction efficiency of cysts of soybean cyst nematode from soils. A. J. A. MOREIRA, C. C. Marett, F. W. Nutter, Jr., and G. L. Tylka. Iowa State University, Ames, IA. Phytopathology 94:S73. Publication no. P-2004-0491-AMA.

There are several extraction methods used to assess soybean cyst nematode, *Heterodera glycines*, population densities in soil, but there is a lack of information about how efficient and comparable the methods are. By extracting cysts from the same soil sample multiple times, the extraction efficiency of two methods was compared for four naturally infested soils. The total numbers of cysts extracted from the samples and proportions of the total numbers of cysts obtained in the first extraction were determined. Wet sieving was more efficient than a semi-automatic elutriator for extracting *H. glycines* cysts from the loamy sand soil. However, the differences in the total number of cysts extracted by the two extraction methods were less in soils with clay contents greater than the loamy sand soil. When differences in extraction efficiency occurred, the proportions of cysts recovered in the first extraction were greater for the elutriator than for the wet-sieving extraction method. Soil moisture levels did not affect the extraction efficiency of cysts.

Effect of light on germination of urediniospores of rusts on daylily, geranium, iris, native azalea, spearmint, and sweet corn. D. S. MUELLER (1) and J. W. Buck (2). (1) Iowa State University, Ames, IA; (2) University of Georgia, Griffin, GA. Phytopathology 94:S73. Publication no. P-2004-0492-AMA.

The objective of this study was to determine the effect of cool-white fluorescent light (FL) or incandescent light (IL) on germination of urediniospores of six rust species. Urediniospores of each species were incubated in the dark (control) or were exposed to continuous FL or IL on potato dextrose agar (PDA) for 24 hours (h) at 22°C. Urediniospore germination was reduced for all rusts by exposure to FL. Exposure to IL reduced germination of all rusts except *P. hemerocallidis* and *P. sorghi*. Urediniospores were allowed to initiate germination for 2 h on PDA in the dark and were then exposed to either FL or IL. Further germination and germ tube elongation of all species were halted by exposure to either light source. Spores were exposed to increasing hours of FL (1 to 10 h) followed by 24 h of dark. For all six rusts, significant decreases in germination and germ tube growth were observed with 1 h of FL. Exposure to 18 hours of FL followed by increasing hours of dark (2 to 24 h) resulted in no additional germination or germ tube growth for all six rusts. IL had a fungistatic effect on two species; germination and germ tube growth of urediniospores of *P. pelargonii-zonalis* and *Puccinia strum vaccinii* significantly increased the longer the dark incubation period after exposure to 18 h of IL.

Phylogenetic analysis of Kentucky *Xylella fastidiosa* strains. J. N. MUNDELL, J. R. Hartman, and C. L. Schardl. Univ. of Kentucky, Dept. of Plant Pathology, Lexington, KY 40546. Phytopathology 94:S73. Publication no. P-2004-0493-AMA.

In Kentucky (KY), *Xylella fastidiosa* (Xf) is associated with bacterial leaf scorch (BLS) of red, pin, scarlet, bur, swamp white and white oak. It is also associated with BLS of hackberry, mulberry, sycamore and sugar, red, and silver maple. Pierce's disease of grape, caused by Xf, was diagnosed in KY in 2001. Xf is also found in asymptomatic landscape plants such as osmanthus, Kentucky bluegrass, perennial ryegrass and vinca. The goal of this research is to differentiate between the strains of Xf found in KY. Sequence of the 16S-23S intergenic spacer region is commonly used to distinguish between strains of Xf. Phylogenetic analysis of this region is expected to reveal relationships among the KY Xf strains. Due to the fastidious nature of Xf, DNA isolation for most strains was done directly from plant tissue. Thus, Xf-specific primers will be used to amplify the 16S-23S region. Sequence data from these specifically amplified products will be assembled using Phrap, aligned with ClustalW, then analyzed with Paup 4.0. Comparisons with strains outside of KY will also be done, primarily with Xf sequences obtained from NCBI.

Adherence-deficient *Spiroplasma citri*: Optimization of transposome mutagenesis and variant enrichment. K. Mutaqin (1), A. Wayadande (1), U. Melcher (2), and J. FLETCHER (1). Dept. (1) Ento. & Pl. Path.; (2) Bioch. & Mol. Biol., Okla. State Univ., Stillwater, OK 74078. Phytopathology 94:S73. Publication no. P-2004-0494-AMA.

Insect transmission of phytopathogenic spiroplasmas involves adherence to cells within the vectors. We sought adherence-deficient spiroplasmas by mutagenesis and selection. Transformation of *S. citri* BR3-3X with the EZ::TN™<DHFR-1>Tnp Transposome (Epicentre) averaged 28.8 CFUs/ng transposome. PCR using primers for the dihydrofolate reductase (DHFR) gene confirmed the transposome's presence in selected transformants, and Southern blots showed its insertion was random. Natural binding-deficient BR3-3X variants were enriched by sequential passages on cultured cells (CT-1) of the *S. citri* vector, and unbound (putative adherence-defective) spiroplasmas were collected. Many of the selected mutants and some putative natural variants were growth-impaired in broth. Altering broth ingredients, adding cell culture enhancement factors, or conditioning with CT-1 cells did not improve growth, but using a reduced culture volume (50 µl) yielded robust growth. Mutants and natural variants are being tested for adherence capability.

Identification of races of *Xanthomonas campestris* pv. *phaseoli* and establishment of the first common bacterial blight differential *Phaseolus vulgaris* lines. N. Mutlu (1), A. K. VIDAVER (2), D. P. Coyne, J. R. Steadman (2), J. Reiser (1), and P. A. Lambrecht (1). (1) Department of Agronomy and Horticulture; (2) Department of Plant Pathology, University of Nebraska-Lincoln. Phytopathology 94:S73. Publication no. P-2004-0495-AMA.

There is a lack of information about the specificity between *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) and its variant *Xcp fuscans* (*Xcpf*) isolates and different quantitative trait loci and/or major genes conferring resistance in common bean. We investigated common bean genotypes to differentiate races of *Xcp* based on compatible vs. incompatible reactions and elucidate pathogenic variation between and within pathovars *Xcp* vs. *Xcpf*. From 34 common bean resistance sources, 12 lines were selected for screening with 70 *Xcp* and 14 *Xcpf* isolates that originated from different parts of the world. Pathogenic variation was greater within *Xcp* than *Xcpf* isolates. Four groups of isolates differentiated sources of resistance to *Xcp* that originated from the Middle American common bean gene pool, as opposed to the Andean gene pool. The set of 12 differential lines was established based on compatible vs. incompatible reactions and true physiological races of the *Xcp* were identified.

Effect of calcium concentration in nutrient solution on bacterial wilt development of tomato plants. I.-S. MYUNG, Y.-K. Lee, and K.-W. Nam. Plant Pathology Div., NIAST, Suwon 441-707, Korea. Phytopathology 94:S73. Publication no. P-2004-0496-AMA.

Effect of Ca nutrition on bacterial wilt development of tomato plants was examined. After tomato seedlings (cv. 'Seokwang 102') were transplanted, nutrient solutions containing with Ca at 2, 4, 8, 12 and 20 mM were provided. One week after Ca treatment, tomato seedlings were inoculated by wounding roots with knife and poured into the roots with a suspension of *Ralstonia solanacearum*. Disease incidence was recorded for a period of 26 days. The plants furnished with nutrient solution containing Ca at both of 2 and 4mM, and 8 mM were susceptible and moderately resistant to bacterial wilt, respectively, whereas the plants with 12 and 20 mM were resistant. With increasing Ca concentration in the nutrient solution, uptake of Ca tended to increase. These results were suggested that resistance to bacterial wilt and uptake of Ca in tomato induce with increasing Ca concentration furnished. Varietal differences among 20 cultivars in the resistance to bacterial wilt and nutrient uptake of tomato seedlings were investigated in hydroponic culture containing 10 mM of Ca. The tested cultivars were classified into two groups, moderately resistance and resistance based on the degree of resistance to the wilt. This result indicated that when 10 mM of Ca furnishes to tomato plants, the disease resistance to bacterial wilt differs in tomato varieties. However, the differences in resistance to bacterial wilt between moderate resistant and resistant cultivars were not characterized by Ca uptakes.

Corn smut induced maize genes. M. NADAL, M. D. Garcia-Pedrajas, and S. E. Gold. University of Georgia, Athens, GA. Phytopathology 94:S73. Publication no. P-2004-0497-AMA.

Ustilago maydis the causal agent of corn smut disease induces the formation of enlarged galls (tumors) on any above ground portion of its host, maize. We have identified fungal mutants that are unable to cause gall formation but are able to cause symptoms that typically lead up to gall formation, for example chlorosis and anthocyanin production. Employing a comparative approach between wild-type infections (galls) and mutant infections (no galls) we are identifying maize genes up- or downregulated during gall formation. The

method we have primarily used involves suppression subtractive hybridization PCR (SHP). Additionally, collaborative microarray experiments are underway. Because of the induction of highly modified structures (galls) in the plant, it is likely that some of the genes identified in this screening will represent genes positionally or temporally misexpressed in specific tissues or developmental stages. To date we have identified several genes upregulated during gall formation and we are in the process of more fully characterizing the temporal expression of a few select genes. One gene that will be presented as an example encodes a putative ETA subunit of the CCT chaperonin. This chaperonin subunit has been implicated in cell cycle progression. This suggests that corn smut infection induces changes in plant gene expression commensurate with typical phenotypic outcomes.

Use of directed molecular evolution for *Fusarium verticillioides* control by chitinases. M. NADIGA (1), H. Challberg (1), A. Boyce (1), N. Yalp (2), and M. L. Müller (1). (1) Verdia Inc., Redwood City, CA 94063; (2) Pioneer Hi-Bred International Inc., Johnston, IA 50131. Phytopathology 94:S74. Publication no. P-2004-0498-AMA.

Fusarium verticillioides causes ear and seed rot in corn. Approximately 80 to 100% of the corn harvested in the U.S. contains some level of Fusarium or Fusarium toxins (fumonisins). Fumonisins, which are usually present at low levels in most field-grown maize, can rise to high levels under certain environmental conditions, in which case they present acute toxicity to livestock, as well as potential carcinogenicity. The plant's natural defense mechanisms are insufficient to rid the crop of the pathogen. One of the approaches we have taken is to improve, through DNA shuffling, the antifungal potency of corn chitinases against *Fusarium verticillioides*. Four rounds of shuffling class IV chitinases yielded variants with greater than 30 fold improvement in antifungal activity in vitro. The shuffled chitinases exhibited resistance against Fusarium proteases. The expression of improved chitinase variants in planta represents a strategy geared towards achieving enhanced ear mold resistance under field conditions.

Seedling resistance to *Rhizoctonia solani* in sugar beet. S. NAGENDRAN (1) and J. M. McGrath (2). (1) Michigan State University, MI; (2) USDA-ARS, E. Lansing, MI. Phytopathology 94:S74. Publication no. P-2004-0499-AMA.

Rhizoctonia solani in sugar beet (*Beta vulgaris* L.) causes damping-off at the seedling stage and crown and root rot in the adult plant. Managing damping-off by azoxystrobin fungicides is promising but genetic resistance is being sought. Phenotypic and microscopic analysis of disease progress patterns in compatible and incompatible interactions showed that virulent (AG2-2 R1) and hypo-virulent (AG2-2 W2) strains of *R. solani* produced infection structures that penetrated hypocotyls of growth-chamber, soil-grown, 2-week-old seedlings. Seedlings infected with AG2-2 W2 initially showed damping-off symptoms but survived, unlike those infected with AG2-2 R1 which uniformly died. Pre-inoculation with AG2-2 W2 did not protect seedlings from subsequent infection by AG2-2 R1. Similar results were obtained in the greenhouse and field disease nursery settings. In the field, a recent USDA-ARS germplasm release EL51, bred for resistance to crown and root rot, showed putative resistance to Rhizoctonia seedling damping-off. CDNA AFLP analyses of compatible and incompatible interactions are being used to further define disease progress towards understanding molecular events associated with host-pathogen interaction.

Characterization of vector non-transmissible isolate of *Tomato spotted wilt virus*. R. A. NAIDU, J. L. Sherwood, and C. M. Deom. Department of Plant Pathology, The University of Georgia, Athens, GA 30602. Phytopathology 94:S74. Publication no. P-2004-0500-AMA.

We have obtained an isolate of *Tomato spotted wilt virus* (TSWV, Genus *Tospovirus*, Family *Bunyaviridae*) that is not transmissible by *Frankliniella occidentalis*. A range of host plants showed distinct symptoms when mechanically inoculated with the thrips non-transmissible isolate compared to a thrips transmissible isolate of TSWV. Electron microscopy revealed pleomorphic enveloped virus particles in purified preparations from *Emilia sonchifolia* plants infected with the thrips non-transmissible isolate. Western blot analysis of SDS-PAGE separated viral proteins with antibodies to glycoproteins (GP) confirmed that virus particles of the non-transmissible isolate contain both GPs of expected sizes. In preliminary experiments, the viral GPs failed to show binding to lectins previously shown to bind to the TSWV GPs indicating the possible absence of N-linked oligosaccharides on the viral particles. Sequencing of M-RNA of this isolate is being conducted to determine if mutations in the GP precursor protein are contributing to the lack of thrips transmission.

Diseases associated with plantation forest trees in Uganda. G. NAKABONGE, J. Roux, T. A. Coutinho, and M. J. Wingfield. Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, Republic of South Africa. Phytopathology 94:S74. Publication no. P-2004-0501-AMA.

Plantation forestry is important to Uganda as it represents a future resource of timber for fuel and structural purposes. Diseases, however, pose a serious threat to the productivity and sustainability of this emerging industry. Several surveys have been conducted in recent years in South Western Uganda to identify disease problems in plantations and woodlots. The most common disease of *Eucalyptus* spp. Is canker and die-back caused by *Botryosphaeria* spp. Bacterial wilt, caused by *Ralstonia solanacearum*, bacterial blight caused by *Pantoea ananatis*, canker and wilt caused by *Ceratocystis fimbriata* and leaf spot/blight caused by a *Cylindrocladium* sp. Also effect these trees. Ceratocystis wilt of *Acacia mearnsii*, caused by *Ceratocystis albofundus* is common on trees in woodlots where wounds on branches harvested for fuel and construction, provide sites for infection. The results obtained from these surveys will help to develop management strategies to reduce the impact of diseases affecting plantation forestry in Uganda. A major thrust of the future plantation health programe for Uganda will include field days and training of foresters, focused on disease diagnosis and management. Breeding for resistance to disease and selection of species matched to sites as well as silvicultural and cultural practices that reduce disease incidence, will become important components of forestry operations in the future.

Cryphonectria canker in South and Eastern Africa. G. NAKABONGE (1), R. N. Heath (2), M. Gryzenhout (1), M. J. Wingfield (1), and J. Roux (1). (1) Dept. of Microbiology and Plant Pathology, FABI, University of Pretoria, Pretoria 0002, Rep of South Africa; (2) Dept of Genetics, FABI, University of Pretoria, Pretoria 0002, Rep. of South Africa. Phytopathology 94:S74. Publication no. P-2004-0502-AMA.

Cryphonectria canker, caused by *Cryphonectria cubensis*, is an important disease of *Eucalyptus* spp. In the tropics and southern hemisphere, including South Africa. Phylogenetic studies conducted on South African, Asian and South American isolates have revealed that the South African fungus is different to that occurring in other areas and is currently being described as a discrete taxon. The teleomorph of the South African form of the fungus is rare on exotic *Eucalyptus* and *Tibouchina* spp., but is found more commonly on native *Syzygium* spp. This, together with a high population diversity, suggest that it may be native to Africa. The aim of this study was to determine the distribution of *C. cubensis* in South and East African countries. Isolates were collected from as many trees as possible and characterised based on their morphology and DNA sequence data for three gene regions. Results show, for the first time, that *C. cubensis* occurs in Mozambique, Zambia and Kenya both on native *Syzygium* and exotic *Eucalyptus* spp. Phylogenetic characterisation of isolates to determine whether they represent the South African form of the fungus, or that from other regions, is currently underway. Further studies on the diversity and structure of *C. cubensis* populations from Southern and Eastern Africa will follow.

***Bacillus* spp. with potential as biological control agents.** C. NAVA-DIAZ (2), M. Reinhenz (1), D. J. Doohan (1), M. L. Lewis (2), and S. A. Miller (2). (1) Horticulture and Crop Science; (2) Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Ave., Wooster, OH 44691. Phytopathology 94:S74. Publication no. P-2004-0503-AMA.

Three *Bacillus* spp. Strains with potential as biological control agents were characterized based on molecular and physiological attributes and evaluated for beneficial effects on seedlings of four tomato varieties challenged with *Xanthomonas campestris* pv. *vesicatoria* in the greenhouse during 2002-2003. Strains of *Bacillus subtilis* [MBI600 Ag Bio and GBO3 Gustafson Inc.] and *Bacillus* *oliquefaciens* [IN937 Auburn University] were readily differentiated using enterobacterial repetitive intergenic consensus sequence (ERIC) primers in rep-PCR. In both years, no significant increase in biomass was recorded on tomato plants root-inoculated with these bacteria. *Bacillus* spp. Were recovered from 33 – 75% (2002) or 6-50% (2003) of the root samples 49-56 days after planting with IN937 the most abundant. In 2002, plants treated with GBO3 exhibited lower density of bacterial spot lesions than the untreated control at levels achieved using the plant activator Acibenzolar-S-methyl (Syngenta: Actigard 50WG).

Study on penetration by *Fusarium solani* f. s*glycines*, causal agent of SDS on soybean. S. S. NAVI and X. B. Yang. Iowa State University, Dept. of Plant Pathology, Ames, IA 50011. Phytopathology 94:S74. Publication no. P-2004-0504-AMA.

Sudden death syndrome (SDS) is an important soybean disease caused by *Fusarium solani* f. sp. *glycines* (FSG). Our previous study indicated that infection in early seed germination stage associated with xylem colonization

is critical to foliar symptom expression. To understand the infection of the fungus at seed germination stage, a study was carried using Ripley (putative susceptible) and P 3981 (putative resistant). Germinated seeds with various radicle lengths were spray-inoculated with conidial suspension of FSG (1×10^6 conidia ml^{-1}) until the radicles were immersed in the suspension. Radicles and cotyledons were separated at the intersection prior to fixing the radicles in fixatives 1, 6, 12 and 24 h after inoculation. The samples were then examined using Scanning Electron Microscope. Preliminary observations indicated more penetrations occurred near root cap zone (where minimal or no root hairs) of a radicle. There appeared higher frequency of spore germination and fungal penetration in Ripley than in P 3981. Experiments are carried to quantify the differences between the two cultivars on germination and penetration variables of FSG.

Role of maize lipoxygenase genes in defenses against phytopathogenic fungi: A genomics approach. A. M. NEMCHENKO (1), C. Simmons (2), N. Yalpani (2), R. Meeley (2), and M. [illegible]omiets (1). (1) Texas A&M Univ., Dept. of Plant Pathol. & Microbiol., College Station, TX 77843; (2) Pioneer Hi-Bred Intl. Inc., Johnstown, IA. Phytopathology 94:S75. Publication no. P-2004-0505-AMA.

Plant lipoxygenases (LOXs) are group of enzymes that have been proposed to an important role in plant resistance to diverse pathogens. LOXs catalyze hydroperoxidation of polyunsaturated fatty acids such as linoleic and linolenic acids. Resultant hydroperoxides are further transformed through seven different pathways into an array of oxylipin metabolites many of which serve as signaling molecules in plant-microbe interactions as well as in various basic physiological processes. Here we report on identification, cloning and sequencing of 12 members of the LOX gene family in corn. In this study we discuss genomic structure and phylogenetic analysis of maize LOX genes and biochemical characterization of corresponding proteins. The focus of this presentation is the functional analysis of three LOX genes *ZmLOX10*, *ZmLOX11* and *ZmLOX12*. To elucidate physiological function of *ZmLOX10*, *ZmLOX11* and *ZmLOX12* we used reverse genetics approach and identified mutant alleles for these genes. Northern blot analysis and transcript profiling on expression patterns of these genes in response to treatment with defense-associated signaling molecules (JA, SA and ethylene) and to infection with selected fungal pathogens and infestation with herbivorous insects will be discussed.

Cell-cell signal interference in *Xylella fastidiosa* and *Xanthomonas campestris*. K. L. NEWMAN and S. E. Lindow. University of California, Berkeley. Phytopathology 94:S75. Publication no. P-2004-0506-AMA.

Xylella fastidiosa (Xf) is a Gram-negative bacterium that causes Pierce's disease of grapevine and other plant dise Xf is restricted to the xylem, where it forms an aggregated biofilm. In other systems, biofilms are characterized by community behavior under the control of cell density-dependent gene expression, which requires cell-cell signaling. Xf has homologs of the rpf cell-cell signaling genes found in the plant pathogen *Xanthomonas campestris* pv. *campestris* (Xcc) and probably shares a similar means of coordinating gene expression in a community. In Xcc, cell-cell signaling controls the expression of gen equired for virulence in plants (*Mol. Micro.* 24, 555-66). We investigated cell-cell signaling in Xf with the aim of developing cell-cell signaling disruption as a means of control Pierce's disease. We found that Xf produces a cell-cell signal (DSF) and that the *rpfF* gene is necessary and sufficient for synthesis of this signal. Xf *rpfF* mutants are hypervirulent suggesting t cell-cell signaling attenuates virulence in Xf (*PNAS* 101, 1737-1742). We screened collections of bacterial strains and identified strains that interfere with DSF-mediated signaling. We are testing how these strains interact with Xf and Xcc in infected plants, identifying to which species they belong and isolating the genes responsible for DSF-interference activity. DSF-interference genes can then be introduced into plants for potential disease control. However, since DSF appears to attenuate disease symptoms in plants infected by Xf, expressing DSF synthases in plants may also reduce disease. Therefore we are constructing transgenic plants that synthesize DSF to evaluate pathogenicity of Xf and Xcc in these plants.

Efficacy of two strobilurin fungicides in controlling frogeye leaf spot of soybean. M. A. NEWMAN. University of Tennessee. Phytopathology 94:S75. Publication no. P-2004-0507-AMA.

In 2003 Pyraclostrobin (Headline) and Azoxystro (Quadris) were compared to thiophate-methyl (Topsin-M) for control of Frogeye Leaf Spot (FLS) caused by *Cercospora sojina*. Under heavy FLS disease pressure all fungicide treatments increased yield and seed size. Disease ratings for FL d defoliation were significantly reduced with the Strobilurin fungicides. There was no significant difference in yield among any of the fungicide rates or number of applications. However, FLS ratings were significantly lower for higher rates of fungicides and for multiple applications of the Strobilurin fungicides. Seed weights were also higher for multiple applications and higher fungicide rates.

Quantitative and molecular features determining whitefly transmission of an engineered *Lettuce infectious yellows virus* defective RNA and LIYV mutants. J. NG, T. Tian, and B. Falk. Dept. Plant Pathology, University of California, Davis, CA. Phytopathology 94:S75. Publication no. P-2004-0508-AMA.

Lettuce infectious yellows virus (LIYV) is obligately transmitted by whiteflies. We used an *in vitro* acquisition and whole plant inoculation system to assess transmission of an engineered LIYV RNA 2 defective RNA (M5gfp D-RNA) and LIYV mutants. M5gfp D-RNA was transmitted to plants along with LIYV. However, unlike LIYV, it was not subsequently transmitted by whiteflies from initially-infected plants. When high concentrations of virions from plants infected by LIYV and M5gfp D-RNA were used for *in vitro* acquisition, both were transmitted. Although M5gfp D-RNA replicated within infected plants along with LIYV, compared to LIYV RNA 2, it was not as abundant in plants or resulting virions, suggesting that concentration of encapsidated RNAs is an important transmission parameter. We also analyzed the transmissibility of specific LIYV RNA 2 mutants. Synonymous and non-synonymous changes were seen in the major and minor capsid protein genes among the mutants. One mutant had a stop codon mutation in the CPm gene and, although capable of forming VLPs, no transmission has been observed.

Occurrence and distribution of complex diseases between root-knot nematode (*Meloidogyne incognita*) and fungal/bacterial wilt on Vietnamese tomato production. X. T. NGO (1) and S. D. Van Gundy (2). (1) Hanoi Agricultural University, Hanoi, Vietnam; (2) University of California, Riverside, CA. Phytopathology 94:S75. Publication no. P-2004-0509-AMA.

Root-knot nematodes (*Meloidogyne incognita*) and soil borne diseases caused by *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Ralstonia solanacearum* have recently been recognized as tropical complex diseases constraining tomato production in Vietnam. A survey in 2003 showed that yield losses were 30-80% throughout the year, unfortunately, identification and interaction between the fungal and bacterial diseases is still not clearly understood. A total of 155 isolates were collected throughout 21 counties and divided into two groups, 1) nematode/fungal wilt diseases and 2) nematode/bacterial wilt diseases. The complex disease of *M. incognita-Fusarium oxysporum*, *M. incognita-Rhizoctonia solani*, *M. incognita-Sclerotium rolfsii* and *M. incognita-Ralstonia solanacearum* were recovered from 84% of the sites. High root-knot nematode population densities (mean of 3,652 eggs and juveniles per 100 cc soil) were most frequently associated with two tomato crop rotations. *Meloidogyne incognita* is an important tropical nematode pest in the tomato-growing regions in Vietnam.

Use of three essential oils as seed treatments against seed-borne fungi of rice (*Oryza sativa* L.) r laboratory and field conditions. J. NG ACK (2), V. Leth (1), P. H. Amvam Zollo (2), and S. B. Mathur (1). (1) Danish Government I e of Seed Pathology for Developing Countries, Frederiksberg, Denmark; (2) Department of Biochemistry, University of Yaounde-I, Cameroon. Phytopathology 94:S75. Publication no. P-2004-0510-AMA.

The potential use of three essential oils (Eos) extracted from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* was investigated for their ability to control seed-borne infection and seed to seedling transmission of *Alternaria padwickii*, *Bipolaris oryzae* and *Fusarium moniliforme* in naturally infected seeds. Seven rice cultivars were tested and the Eos applied as slurry controlled the seed infection at a range of 48% to 100%. The treatment also reduced the seed to seedling transmission of the referred fungi at a range of 76 to 95%. The vigor of rice seedlings raised from the treated seeds was better compared to that of seedlings from the untreated seeds. The three Eos also increased the germination capacity of the treated seeds with 5 to 13%. On-farm evaluation conducted in 2002 in Cameroon indicated that these treatments have shown harmless effects on the different parameters evaluated. Positive effects have been recorded with statistical significance at 95% level of confidence. Reduction of disease inci have been recorded as a result of seed treatment with essential oils when compared to the non-treated control. Important increases have been noted in the yield (up to 35%) though these were not statistically significant compared to the non-treated seeds. It was noted that researcher managed plot where seed quality and seed health have been addressed produced enough rice meanwhile little or no rice production was found in neighboring farmers fields. The second field trial is presently in progress in Cameroon to consolidate the results from 2002 field trial. *Additional Keywords*: essential oils, rice, field trial, seed-borne fungi, seed treatment.

Does flower application of *Bacillus subtil* pose a risk to pollination-related variables in rabbiteye blueberry? H. K. NGUGI and H. Scherm. University of Georgia, Athens, GA. Phytopathology 94:S75. Publication no. P-2004-0511-AMA.

We have previously documented suppression of blueberry flower infection by *Monilinia vaccinii-corymbosi*, the mummy berry fungus, with a commercially

formulated *Bacillus subtilis* product (Serenade) applied directly to the stigma. The deliberate targeting of the stigma with biocontrol product in this pathosystem necessitated an evaluation of potential negative impacts on pollination and associated fruit characteristics. Application of Serenade to stigmas of detached flowers had no effect ($P > 0.05$) on the number and growth rates of pollen tubes entering the style. There also was no reciprocal effect of pollen on population dynamics of *B. subtilis*. In the greenhouse, application of Serenade did not impact fruit set or seed numbers but marginally ($P = 0.048$) affected fruit weight in one of two experiments. In field experiments utilizing honey bees to vector Serenade to open flowers, application of the product did not affect fruit weight but reduced fruit set ($P = 0.0382$) and seed numbers ($P = 0.0109$) in conditions where pollination was poor overall. Implications of these findings for different blueberry production situations will be discussed.

Differentiation of *Hemicriconemoides mangeriferae* and *Hemicriconemoides litchi* based on morphological and molecular characteristics. H. F. NI (1), Y. H. Cheng (1), D. Y. Chen (2), and R. S. Chen (3). (1) Department of Plant Protection, Chiayi Agricultural Experimental Station, Chiayi 600, Taiwan; (2) Department of Plant Protection, Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan; (3) Graduate Institute of Biotechnology, National Chiayi University, Chiayi 600, Taiwan. Phytopathology 94:S76. Publication no. P-2004-0512-AMA.

The taxonomic status of *Hemicriconemoides mangeriferae* and *Hemicriconemoides litchi* is still a question until now. In order to determine whether *H. mangeriferae* and *H. litchi* represent a single species, ten populations of *Hemicriconemoides* spp. were collected from mango, litchi, acrea nut, coconut, longon in Taiwan, and were differentiated by morphological and molecular data. Based on the comparisons of morphometrics data from adult females, five populations collected from mango, acrea nut and coconut were recognized as *H. mangeriferae*. Another five isolates collected from litchi and longon were recognized as *H. litchi*. We further compared the internal transcribed spacer (ITS) sequences of ribosomal DNA from different populations. The results revealed that only 94 percents similarity of the ITS sequences between *H. mangeriferae* populations and *H. litchi* populations. According to obtained morphological and molecular data, we proposed that *H. litchi* should be classed as an independent species differed from *H. mangeriferae*.

Cyclic production of sporangia and zoospores by *Phytophthora capsici* on pepper roots in hydroponic culture. C. J. NIELSEN, D. M. Ferrin, and M. E. Stanghellini. Department of Plant Pathology, University of California, Riverside, Riverside, CA 92521. Phytopathology 94:S76. Publication no. P-2004-0513-AMA.

Production of sporangia and zoospores by *Phytophthora capsici* on roots of hydroponically-grown peppers was shown to be cyclic in nature. Production of sporangia on peppers grown with a 12-hr photoperiod was diurnal; the greatest number of full sporangia were present at hours 8-10 of the light cycle, while empty sporangia were most abundant at hours 4-6 of the dark cycle. Zoospore production was primarily nocturnal; populations began to increase at the start of the dark cycle and, in general, peaked sharply at hour 4 of the dark cycle. Zoospore populations at this time ranged from 9-65 times (mean of 33) greater than when populations were at their lowest (hours 6-8 of the light cycle). Altogether, 83% of the total zoospore population, sampled every two hours over a 24-hr period, were isolated from the hydroponic solution during the dark cycle. When peppers were grown under continuous light, a cyclic pattern of sporangium and zoospore production did not occur. These findings could impact the implementation of detection methods and practices for managing *Phytophthora* in irrigation reservoirs, thus leading to an enhancement of the results obtained by customary assay procedures.

Evaluation of the efficacy of dormant applications of lime sulfur and fixed copper to control Phomopsis cane and leaf spot of grape. M. NITA, M. A. Ellis, and L. V. Madden. Ohio State University, OARDC, Wooster. Phytopathology 94:S76. Publication no. P-2004-0514-AMA.

Effects of dormant applications of liquid lime sulfur or fixed copper on control of Phomopsis cane and leaf spot of grape (causal agent: *Phomopsis viticola*) was studied under field conditions. Lime sulfur or fixed copper was applied in: I) fall, after formation of periderm tissues of canes; ii) spring, after bud swell, but before bud break; or iii) both fall and spring. During the early growing season (growth stage 1-7) conidia from the main trunk were collected using a spore trap consisting of a 20-cm funnel and 2-L plastic container. At 1 wk after bloom, disease incidence and severity were assessed for both internodes and leaves. At the end of the season, nine sections of canes from treatments were randomly sampled, placed in a wet chamber, and then the potential amount of sporulation was estimated by counting number of pycnidia per square centimeter. Preliminary results indicate significantly lower disease incidence and severity with spring, or both spring and fall, application of lime sulfur or fixed copper. Significantly less sporulation in the spring and at the end of the growing season was observed following a lime sulfur application. However, disease symptoms did not differ significantly between lime sulfur and fixed copper treatments.

Evaluation of fungicides and fungicide-adjuvant mixtures for post-infection control of Phomopsis cane and leaf spot of grape. M. NITA, M. A. Ellis, L. L. Wilson, and L. V. Madden. Ohio State University, Dept. of Plant Pathology, Wooster, OH 44691. Phytopathology 94:S76. Publication no. P-2004-0515-AMA.

Efficacy of post-infection (curative) applications of various fungicides alone and in combination with adjuvants for control of Phomopsis cane and leaf spot of grape, caused by *Phomopsis viticola* was studied. Post-infection applications included mancozeb, thiophanate-methyl, azoxystrobin, and myclobutanil combined with Regulaid (2-butoxyethanol, poloxalene, and monopropylen glycol) or JMS stylet oil, as well as mancozeb or liquid lime sulfur applied alone. Also, mancozeb or lime sulfur alone was applied as pre-infection (protectant) treatments. Grapes were grown in the greenhouse and leaves (< 1 wk old) were tagged for identification. Pre-infection applications were made 3 h prior to inoculation. A conidial suspension of *P. viticola* was applied to plants with an electric atomizer. Inoculated grapes were then placed in a moist chamber in order to achieve favorable infection periods, which ranged from 12 to 48 h including drying periods. Assessment of disease severity on internodes and leaves was made 4 wk after inoculation. Pre-infection applications of fungicide provided a high degree of control. All post-infection treatments were not effective for control of *P. viticola*.

Effect of inoculum source and azoxystrobin on potato black dot. N. NITZAN, T. Cummings, and D. A. Johnson. Washington State University, Pullman, WA. Phytopathology 94:S76. Publication no. P-2004-0516-AMA.

Colletotrichum coccodes, cause of potato black dot, reduces tuber yields up to 30%. Soil- and seed-borne inocula are main sources for infection; however, their relative roles have not been evaluated. Contribution of seed- and soil-borne inocula and effect of azoxystrobin on black dot development were evaluated using generation 1 (G1) and 3 (G3), certified seed of cultivars Russet Burbank (RB) and Norkotah Russet (NK) in 2003. Initial disease incidence on seed-tubers was 2% on G1/NK, 16% on G1/RB, 14% on G3/NK, and 49% on G3/RB. Azoxystrobin was applied in furrow at planting, and three times to foliage. In treated plots, yields were increased 11-23%, disease severity was reduced by 60-80%, and incidences of infected plants and progeny tuber were reduced 50-75% and 50-100%, respectively. Disease severity on stems from G3 seeds was 44%, and 26% higher on RB and NK, respectively compared to stems from G1 seeds. Tuber progeny had 86% higher incidence on G1/NK compared to G3/NK, and 20% higher on G3/RB compared to G1/RB. Results indicate that azoxystrobin reduces disease development, and that both inoculum sources contribute to disease development.

Evaluation of two models for lettuce downy mildew infection periods in Norway. B. NORDSKOG (1), A. Hermansen (1), R. C. Seem (2), and D. J. Gadoury (2). (1) Norwegian Crop Research Institute, 1432 Ås, Norway; (2) Dept. Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456. Phytopathology 94:S76. Publication no. P-2004-0517-AMA.

Light inhibits sporulation of *Bremia lactucae*, and is a component of models for lettuce downy mildew infection periods, but specific intensities and wavelengths involved are poorly understood. We evaluated environmental data and model performance in Norway for two systems, PlantPlus (PP) and Modell-Analys (MA), which are based on a model developed in California. Disease was detected following forecasts issued by both models in 5 of 8 fields. However, forecasted infection periods were not always followed by disease occurrence. Both PP and MA usually called for fewer fungicide applications than a calendar schedule of sprays at 10-day intervals, with no difference in disease suppression among treatments. Date of initial disease varied widely among sites, and may be related, in part, to light intensity and quality at Nordic latitudes, which differ substantially from those of California. Results of subsequent studies on light effects on *B. lactucae* will be reported.

Controlling fire blight in young apple trees with prohexadione-calcium. J. L. NORELLI and S. L. Miller. USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV. Phytopathology 94:S76. Publication no. P-2004-0518-AMA.

Prohexadione-calcium (Apogee) (Phd-Ca) is a plant growth regulator that suppresses shoot growth in apple. In mature orchards Phd-Ca is effective in managing fire blight caused by *Erwinia amylovora*. However, in young apple orchards there is a need to both control fire blight and allow sufficient tree growth for tree establishment, and the utility of Phd-Ca in young orchards

was unclear. When Phd-Ca was applied to orchard-grown apple trees ranging in age from newly planted to fifth season of growth (4-year-old orchards) it was found that two applications of 125 mg liter^{-1} Phd-Ca provided a better balance between fire blight control and growth in young orchards than three or more applications of 63 or 30 mg liter^{-1}. Although the high rate of Phd-Ca suppressed early season shoot growth more than the lower rates, trees that received the high rate of Phd-Ca tended to grow more in the latter part of the season resulting in little or no difference in total seasonal growth between trees that received two high or three low rate applications of Phd-Ca. Fire blight control with Phd-Ca required shoot growth suppression early in the growing season and 125 mg liter^{-1} Phd-Ca often provided significantly better fire blight control than treatment at lower rates. Poor fire blight control occurred when the rate of Phd-Ca was lowered sufficiently to allow greater early season growth. The results indicate that one to two Phd-Ca applications at 125 mg liter^{-1} can be used to manage fire blight in the fourth to sixth season of growth when there is a high risk of shoot blight.

Screening of chemicals for the control of bacterial wilt of geranium. NORMAN, J. Chen, J. M. F. Yuen, R. J. Donahoo, and R. Resendiz. Mid-Florida Research and Education Center, University of Florida, Apopka, FL 32703-8504. Phytopathology 94:S77. Publication no. P-2004-0519-AMA.

Ralstonia solanacearum Race 1 was used as a model system to screen for effective bactericides. The following chemicals at various application rates and intervals were tested: aluminum tris, benzothiadiazole, copper sulfate, copper pentahydrate, copper salts, hydrogen dioxide, oxolinic acid, and potassium salts of phosphorous acid. Aluminum tris, copper sulfate, copper pentahydrate, copper salts, and hydrogen dioxide slowed disease progression in geranium, but did not protect plants at high inoculum rates (2.9×10^8 cfu/gram soil). Benzothiadiazole appeared to protect the plants but caused leaf abscission. Both oxolinic acid and potassium salts of phosphorous acid were effective in protecting plants from infection when applied as a drench on a 7 to 14 day interval. Three phosphorus containing compounds were also tested with H_3PO_3 being more effective in controlling bacterial wilt than H_3PO_4 or P_2O_5.

Developing forensic protocols for the post-introduction attribution of threatening plant pathogens. F. W. NUTTER, JR. Department of Plant Pathology, Iowa State University, Ames, IA. Phytopathology 94:S77. Publication no. P-2004-0520-AMA.

Once a threatening plant pathogen has been detected and diagnosed in the United States, forensic protocols are needed for geospatially sampling and assessing disease intensity before invasive management tactics are deployed that might destroy or diminish spatial patterns of disease/pathogen occurrence. First, aerial and satellite images should be obtained before conducting any ground-based disease/pathogen assessments. After this, disease intensity assessments (incidence and/or severity) within the affected field, as well as among neighboring fields, should be geospatially-referenced using global positioning system (GPS) or by recording row/plant position coordinates. These data can then be coupled with geographic information system (GIS) to map disease intensity data. Obvious disease/pathogen patterns that are typical or atypical of a natural event can be analyzed using spatial statistics. Sampling pathogen isolates for subsequent genetic analyses should be obtained both from within and among disease foci by following appropriate chain of custody procedures. The combination of these approaches should help to improve both the speed and validity of conclusions concerning the deliberate, accidental, or natural introduction of newly detected threatening plant pathogens.

Field evaluation of nonchemical alternatives for control of *Mesocriconema xenoplax* on peach. A. P. NYCZEPIR. SE Fruit and Tree Nut Research Laboratory, USDA-ARS, Byron, GA 31008. Phytopathology 94:S77. Publication no. P-2004-0521-AMA.

The effects of 6 preplant rotation systems in conjunction with Guardian peach rootstock as alternatives to chemical control of *Mesocriconema xenoplax* (Mx) were investigated from 1996-2003. Rotation establishment was initiated in 1996 in a site known to be infested with Mx and having a history of peach tree short life (PTSL). Preplant crop rotation systems included (i) 1-year wheat-sorghum, (ii) 3-continuous years wheat-sorghum, (iii) 3-continuous years fallow-sorghum, (iv) 1-year canola-sorghum, (v) 3-continuous years canola-sorghum, and (vi) 3-continuous years peach. In 1999, all trees were removed from the continuous peach plots and half of these plots were then fumigated with methyl bromide. In 2000, all plots were planted to either Nemaguard or Guardian rootstock. Three-year rotations suppressed Mx populations for up to 21 months following orchard establishment. Three-year rotations were comparable to preplant methyl bromide fumigation in prolonging tree survival. Integrating selected rotations with Guardian rootstock provides an alternative to chemical control of Mx and PTSL.

Identification of rust resistance genes in four spring wheats. L. M. OELKE and J. A. Kolmer. USDA-ARS-Cereal Disease Laboratory, University of Minnesota, St. Paul, MN. Phytopathology 94:S77. Publication no. P-2004-0522-AMA.

Leaf rust of wheat (*Puccinia triticina* Eriks.) is the most common and wide spread pathogen of wheat worldwide. Four important varieties of spring wheat in the upper Midwest, 'Alsen', 'Norm', 'Ivan' and 'Knudson', were characterized for seedling and adult plant resistance to leaf rust using BCF_2 families in both field and greenhouse conditions. All four varieties have good field resistance to leaf rust. 'Alsen' is widely grown in Minnesota, South Dakota and North Dakota because of it has scab resistance. 'Alsen' has *Lr2a*, *Lr10* and is likely heterogeneous for *Lr16*. 'Norm' has *Lr1*, *Lr10* and *Lr16*. 'Norm' may also have *Lr23*. Both 'Alsen' and 'Norm' have *Lr13* and *Lr34* adult plant resistance genes. 'Knudson' has *Lr16* and 'Ivan' has *Lr16* and *Lr24*, plus additional seedling resistance genes. 'Knudson' has *Lr13* and *Lr34* adult plant resistance genes. 'Ivan' has *Lr34* adult plant resistance gene.

Survival analysis of time to defoliation of blueberry leaves affected by Septoria leaf spot. P. S. OJIAMBO and H. Scherm. University of Georgia, Athens, GA. Phytopathology 94:S77. Publication no. P-2004-0523-AMA.

Septoria leaf spot, caused by *Septoria albopunctata*, can result in premature defoliation of blueberry bushes during summer and fall, thereby reducing yield potential for the following year. The effects of disease severity and plant attributes on the dynamics (timing and extent) of defoliation were quantified in field plots of 'Premier' rabbiteye blueberry during 2002 and 2003. In each year, 50 shoots were selected for assessment in early spring, and all leaves on these shoots were monitored for disease progress and defoliation throughout the season. Survival analysis revealed that older leaves (located on the lower half of shoots) and leaves with high levels of disease (>30 spots/leaf in late August) defoliated significantly ($P < 0.0001$) earlier than younger leaves and leaves with a lower disease severity. Mean times to defoliation were about 1 month shorter in the leaf group afflicted by high levels of disease, and each additional leaf spot present in late August accelerated time to defoliation (expressed using late August as a starting point) by about 1%. Leaf position in upper or lower portions of the canopy had no significant effect on time to defoliation ($P > 0.05$).

Simultaneous identification and quantification of *Rhizoctonia solani* and *R. oryzae* from root samples using real-time PCR. P. A. OKUBARA and T. C. Paulitz. USDA ARS, Roots Disease & Biological Control Research Unit, Washington State University, Pullman, WA 99164-6430. Phytopathology 94:S77. Publication no. P-2004-0524-AMA.

In the Pacific Northwest (PNW), Rhizoctonia damping off and root rot of small grain cereals are attributed to *Rhizoctonia solani* AG-8 and *R. oryzae*. The former also associated with bare patch disease of wheat. Nucleotide sequence comparisons of the internal transcribed spacer (ITS) regions of *Rhizoctonia* indicate that PNW populations of *R. oryzae* and *R. solani* are comprised of at least three and four groups, respectively. PNW *R. solani* isolates, originally thought be of anastomosis group (AG) 8, shared significant ITS sequence identity to members of AG-2-1 and AG-10 in Genbank searches. AG has been correlated with ITS sequence for *R. solani* associated with bare batch of sugar beets in France. Our data indicate that PNW isolates associated with root rot and bare patch are more diverse than originally thought. We have developed an approach for designing real-time (quantitative) PCR primers that are specific to the ITS DNA of each lineage. The approach involves extensive scrutiny of primer and target sequences for potential PCR artifacts using a battery of sequence analysis software, and optimization of amplification conditions using a 96-sample Q-PCR thermocycler. A high throughput method for extraction of *Rhizoctonia* DNA from pure culture and plant material is being developed. Results of AG testing and primer performance will also be discussed. Our strategy for primer design and analysis can be applied to ITS sequences from any fungal pathogen, and can be extended to DNA sequences from bacterial and viral pathogens.

Comparison of late blight suppression by natural products and the impact of genotype diversity on disease development and control. O. M. OLANYA, R. P. Larkin, and C. W. Honeycutt. USDA-ARS, New England Plant, Soil and Water Lab, Orono, ME. Phytopathology 94:S77. Publication no. P-2004-0525-AMA.

The potential use of natural products for late blight management can promote reduced inputs and enhance sustainability of potato production. The objective of this research was to determine late blight control by non-conventional products on *Phytophthora infestans* isolates with diverse genotypes and quantify epidemic components under controlled conditions. Growth of isolates of different genotypes was evaluated in petri-dish assays when Rye B media was amended with essential oils (Lavender, Thyme, Thyme Borneal,

Oregano); and Serenade (*Bacillus subtilis* strain QST 713). The percent reduction of in-vitro growth differed significantly among fungal genotypes. Inhibition of in-vitro growth was highest on the 100/111/122 genotype. The reduction of fungal growth was best achieved when media was amended with Oregano and Serenade. In growth chamber studies, components of epidemic development (incubation & latent periods, lesion size, sporulation capacity, disease severity) incited by diverse fungal genotypes were evaluated on the variety Shepody. Incubation and latent periods ranged from 2-7 and 5-9 days, respectively. Average disease severity, lesion size and numbers were significantly impacted by fungal genotypes and isolates with the 100/111/122 genotype being most virulent. Foliar application of natural products prior to inoculation with isolates of diverse genotypes resulted in variable disease suppression. These results suggest that genotype diversity affect disease development; however, disease suppression was not genotype specific.

An approach for identifying microorganisms involved in soil suppressiveness: Utilization for identifying microorganisms that suppress the plant-parasitic nematode, *Heterodera schachtii*. R. O. OLATINWO, B. Yin, J. O. Becker, and J. Borneman. University of California, Riverside, CA 92521. Phytopathology 94:S78. Publication no. P-2004-0526-AMA.

An approach was developed for identifying microorganisms involved in soil suppressiveness. Phase one of the approach employed an rRNA gene (rDNA) method to identify bacteria and fungi that positively correlated with soil suppressiveness against the plant-parasitic nematode, *Heterodera schachtii*. Five soil treatments, generated by mixing different amounts of suppressive and fumigation-induced non-suppressive soil, were infested with *H. schachtii* juveniles and cropped with Swiss chard. After two nematode generations, the most abundant fungal and bacteria rDNA sequences found in *H. schachtii* cysts isolated from the highly suppressive soils had high identity to *Dactylella oviparasitica* and *Zoogloea* sp. rDNA, respectively. The most abundant fungal rDNA sequences found in *H. schachtii* cysts isolated from the minimally to moderately suppressive soils had high identity to *Fusarium oxysporum* rDNA. Phase two confirmed these population trends using sequence-selective quantitative PCR. In phase three, strains of *D. oviparasitica*, *F. oxysporum* and the *Zoogloea* sp. were isolated from *H. schachtii* cysts and reintroduced into non-suppressive soil; *F. oxysporum* and the *Zoogloea* sp. did not significantly decrease *H. schachtii* populations, while *D. oviparasitica* decreased *H. schachtii* populations to levels equivalent to that produced by the suppressive soil. This experimental approach should be useful for investigations of other suppressive soils and for identifying microorganisms involved in other functions.

***Trichoderma stromaticum*: A potential biological control agent for black root rot in strawberries.** R. O. OLATINWO (1), S. Sabaratnam (2), and A. M. C. Schilder (2). (1) Department of Plant Pathology, University of California, Riverside, CA 92521; (2) Department of Plant Pathology, Michigan State University, East Lansing, MI 48824. Phytopathology 94:S78. Publication no. P-2004-0527-AMA.

Trichoderma stromaticum (T-10), isolated from healthy strawberry roots, was previously shown to reduce black root rot (BRR) in 'Honeoye' strawberries planted in pots with naturally infested or inoculated soil. To determine the optimal concentration, aqueous spore suspensions of T-10 (10^6, 10^7, and 10^8 conidia/ml) were applied as a root dip to 'Allstar' transplants planted in soil inoculated with *Pythium* sp., *Rhizoctonia fragariae*, or both. At all three concentrations, T-10 significantly reduced severity of root lesions compared to the untreated control. However, T-10 was more effective at controlling lesions caused by *R. fragariae* than by *Pythium* sp. or both pathogens together. In 2002, T-10 was applied as a root dip to 'Honeoye' transplants at two field sites with a BRR history in Michigan. At one site, the total fruit weight and number of berries the following season was significantly greater in T-10 treated plants than in untreated control plants or plants treated with PlantShield (*T. harzianum*). Yields did not differ significantly at the other site. The results suggest that T-10 may be beneficial to strawberries as a pre-plant treatment, and may be better adapted to the strawberry rhizosphere than *T. harzianum*.

Sensitivity of *Monilinia fructicola* isolates collected from stone fruits to fludioxonil. G. OLAYA (1) and A. Tally (2). (1) Syngenta Crop Protection, Vero Beach Research Center, Vero Beach, FL 32967; (2) Syngenta Crop Protection Greensboro, NC 27419. Phytopathology 94:S78. Publication no. P-2004-0528-AMA.

The distribution of sensitivities to fludioxonil of monoconidial isolates of *Monilinia fructicola* was determined *in-vitro*. Isolates were obtained from infected peach and cherry fruit not previously exposed to fludioxonil applications. A total of eighty-one isolates from NY, NJ, CA, OR, PA, GA, NC and SC were tested. Sensitivities (ED50 values) of *M. fructicola* isolates to fludioxonil ranged from 0.0016 to 0.0191 mg/L with a mean value of 0.0078 mg/L. Fludioxonil (SCHOLAR®), a phenylpyrrole fungicide is a "reduced risk" fungicide registered by EPA. Fludioxonil is labelled for use as post harvest fungicide for the control of *Monilinia*, *Botrytis*, *Rhizopus*, and *Gilbertella* pathogens of stone fruit crops. Baseline information will be used as a reference in future resistance monitoring studies.

Baseline sensitivity distribution of *Penicillium digitatum* isolates collected from citrus to fludioxonil. G. OLAYA (1) and A. Tally (2). (1) Syngenta Crop Protection, Vero Beach Research Center, Vero Beach, FL 32967; (2) Syngenta Crop Protection, Greensboro, NC 27419. Phytopathology 94:S78. Publication no. P-2004-0529-AMA.

The baseline sensitivity distribution of *Penicillium digitatum* (green mold) to fludioxonil is being established. The sensitivity to fludioxonil of 47 isolates collected in California ranged from 0.002 to 0.020 mg/L with a mean value of 0.007 mg/L. The sensitivity to fludioxonil of *P. digitatum* isolates obtained from citrus in Florida and Texas are being determined. The sensitivity distribution of *P. italicum* (blue mold) is also under development. Fludioxonil (SCHOLAR®) is a reduced risk fungicide registered by EPA. Fludioxonil is being developed for use as post harvest fungicide for the control of *Penicillium* and *Diplodia* pathogens of citrus. Development of baseline sensitivity distributions and periodic monitoring of plant pathogenic fungi are key components of Syngenta's anti-resistance strategy for fludioxonil.

Inoculation and evaluation methods used for field efficacy trials of pink rot (*Phytophthora erythroseptica*) in potato. B. D. OLSON (1), W. Kirk (1), G. Kemmitt (1), A. McFadden (1), B. Oemichen (1), H. Yoshida (1), and D. Ouimette (1). (1) Dow AgroSciences LLC, Indianapolis, IN; (2) Michigan State University, E. Lansing. Phytopathology 94:S78. Publication no. P-2004-0530-AMA.

Field efficacy trials with fungicides for the control of pink rot of potatoes caused by *Phytophthora erythroseptica* are difficult to conduct because little is known about the epidemiology of the disease. In 2003, field trials were conducted in five locations in the United States and Canada to evaluate the efficacy of different in-furrow and foliar fungicide treatments for the control of pink rot of potato. Two different methods were used to inoculate potato fields with *P. erythroseptica*. The first method used inoculum grown in petri dishes containing potato dextrose agar. Each treatment was applied in-furrow over the potato seed pieces, then 3 – 6 cm of soil was placed over the seed pieces and the potato dextrose inoculum slurry was placed on top of the covering soil prior to closing the furrows. The second method used inoculum grown on sterilized cracked corn media. The inoculum was spread over the entire trial site twice, once prior to planting and application of the in-furrow treatments and again a month after planting prior to hilling. At harvest pink rot symptoms ranged from 0 to 10% with a grand mean of 3% within the in-furrow inoculated trials. The whole-field inoculated trials had a range of 0 to 20% incidence of pink rot at harvest with a grand mean of 5% infection. In one location potato plants were harvested four times during the growing season to determine the incidence of tubers infected with pink rot throughout the growing season.

Use of the MBEC technology to study the role of biofilms on microbial pathogenesis and determine the anti-biofilm activity of biocides used on plants and agriculture. M. E. OLSON (1,2), L. L. R. Marques (1,2), and H. Ceri (1,2). (1) Biofilm Research Group, University of Calgary, Calgary, AB; (2) MBEC BioProducts Inc., Calgary, AB. Phytopathology 94:S78. Publication no. P-2004-0531-AMA.

Bacterial and fungal biofilms have been shown to be important in pathogenesis of plant diseases and in post harvest losses. MBEC BioProducts Inc. has developed technologies to address a number of biofilm related issues in agriculture and horticulture. We have used a high throughput screening device and methodology to identify existing and novel compounds that have activity against biofilms involved in plant disease and post harvest problems. We have observed that the concentration of biocides like copper sulfate, bleach, silver nitrate, hydrogen peroxide and antibiotics such as tetracycline to kill biofilm pathogens was up to 100 fold greater than the planktonic forms. We have developed novel chemicals and protocols for biofilm control in plants and plant products, employing MBEC technology to understand the role of biofilms in microbial pathogenesis such as *Xylella* and *Pseudomonas* infection of plants. We have identified conditions (e.g. surfaces, ions, and quorum sensing molecules) where biofilm formation is promoted, as well as genes involved in biofilm formation (e.g., Gac A/GacS). The technology may be used in academic and industrial applications.

Ability of strains of *Pratylenchus penetrans* and influence of co-infection with *Verticillium dahliae* to cause potato early dying. M. OMER (1), R. Rowe (1), and A. MacGuidwin (2). (1) OSU-OARDC, Wooster, OH; (2) University of Wisconsin, Madison, WI. Phytopathology 94:S78. Publication no. P-2004-0532-AMA.

The individual and joint effects of eight strains of the root lesion nematode (*P. penetrans*)–obtained from regions with diverse soil type, and cultural practices–with two isolates of *V. dahliae*. belonging to VCG 4A and VCG 4B, were evaluated in a microplot study over 4 years. Compared to the control treatment, strains of *P. penetrans* increased disease severity by 37-70% in 2001, 9-31% in 2002, and 64-110% in 2003. The increase in disease severity was higher with *P. penetrans* plus VCG 4B and was the highest with Pp plus VCG 4A. Although there was a significant difference in aggressiveness among *P. penetrans* strains, when tested in the absence of *V. dahliae*, the difference was not consistent when the same strains were tested in the presence of VCG 4A or VCG 4B. For example, in the absence of *V. dahliae*, *P. penetrans* strain from Oregon caused significantly higher disease rating than the strain from Minnesota. Disease severity was similar for the two strains when tested with either VCG 4A or VCG 4B.

Virulence specificities of worldwide collections of *Puccinia triticina* from durum wheat. M. E. ORDONEZ (1), J. A. Kolmer (1,2), and J. V. Groth (1). (1) Department of Plant Pathology, University of Minnesota, St. Paul, MN; (2) USDA-ARS, Cereal Disease Laboratory, St. Paul, MN. Phytopathology 94:S79. Publication no. P-2004-0533-AMA.

Leaf rust caused by *Puccinia triticina* is an important disease of wheat. Resistance in durum wheat has been durable compared to the short-lived resistance of bread wheat cultivars. Recently, durum leaf rust has become more prevalent in several parts of the world. To determine race composition of the rust, collections of *P. triticina* on durum wheat from six countries were analyzed for virulence specificities on 36 isogenic lines of Thatcher wheat, that differ for single leaf rust resistance genes, and 24 cultivars of durum wheat. Collections from Argentina, France, Mexico, and California had high infection types on all durum cultivars and on *Lr10*, *Lr14b Lr23*, *Lr33* and *Lr41*. These genes are likely derived from the AABB wheats, except *Lr41*. Isolates from Chile and Northern USA had virulence specificities similar to leaf rust races collected from bread wheats, and had lower infection types on the durum cultivars. Collections from Ethiopia showed unique virulence specificities on the durum and bread wheat differential sets.

Evolutionary relationship between sympatric microcyclic and macrocyclic forms of *Tranzschelia* spp. M. E. ORDONEZ (1), L. J. Szabo (1,2), and J. V. Groth (1). (1) Dept. of Plant Pathology, University of Minnesota, St. Paul, MN; (2) USDA-ARS, Cereal Disease Laboratory, St. Paul, MN. Phytopathology 94:S79. Publication no. P-2004-0534-AMA.

Speculations have been made regarding the position of microcyclic life cycles in the evolution of rust fungi. It is believed that the change from a macrocyclic to a microcyclic form is unidirectional and permanent, serving as a basis for speciation. To test this we compared the microcyclic autoecious *Tranzschelia fusca* and the macrocyclic heteroecious, *T arthurii*. Paired collections of the two rusts occurring in close proximity in the Northern forests of Minnesota were made on *Anemone quinquefolia* as well as telial collections of *T. arthurii* from *Prunus* spp. Phylogenetic analysis of DNA sequence data from the nuclear rDNA ITS region revealed the separation of the two species into three well supported groups, rather than two: two macrocyclic, and one microcyclic. However, there were few exceptions. Physical proximity of pairs did not correlate with DNA sequence type. These results indicate that the primary separation of the macrocyclic *T. arthurii* and microcyclic *T. fusca* occurred long ago and suggests that switching between microcyclic and macrocyclic life cycles may occur occasionally.

Proliferation of *Trichoderma harzianum* quantified in non-sterile soil using GFP and image analysis. K. A. Orr and G. R. KNUDSEN. University of Idaho, Moscow, ID. Phytopathology 94:S79. Publication no. P-2004-0535-AMA.

Most methods for fungal biomass measurement are unable to distinguish biomass of an introduced fungus from that of the indigenous fungal community. We quantified biomass of a specific fungal biological control agent in non-sterile soil, using epifluorescence microscopy and image analysis of GFP-expressing *Trichoderma harzianum* (ThzID1-M3). With appropriate optical filters, ThzID1-M3 could easily be distinguished from other microbes and background fluorescence. Colony counts on a selective medium were compared to biomass estimates from image analysis, after ThzID1-M3 was incubated in soil that either remained moist for 14-21 days, or remained moist for approximately 5 days and then was allowed to dry. Reductions in both colony counts and biomass were observed over time when soil was allowed to dry. In soil that remained moist, colony counts increased over a 14-21 day period even though biomass declined after approximately 3-5 days. GFP, along with epifluorescence microscopy, was a useful tool to distinguish active hyphal biomass, which is functional in biological control, from inactive propagules such as conidia or chlamydospores.

***Fusarium graminearum* mycotoxin levels in spring wheat with varying amounts of corn residue.** L. E. Osborne. South Dakota State University, Brookings, SD. Phytopathology 94:S79. Publication no. P-2004-0536-AMA.

Field plots established in 2003 were designed to monitor *Fusarium* head blight (FHB) of spring wheat (*Triticum aestivum*) under low, medium and high inoculum loads. Corn-stalk residue was utilized in plots as a means of increasing inoculum potential, while propagule development remained environment-dependant. The objective of this study was to assess the impact of three levels of corn-stalk residue on mycotoxin contamination of grain grown on and adjacent to the monitoring plots, and to determine if cross-contamination was likely between plots. Whole plots (6m by 15.2m) were treated at the jointing stage by spreading corn-stalk pieces to achieve 0%, 30%, and 80% soil cover. The plots were isolated by 9.1m wide, residue-free, spring wheat buffer zones. At harvest both buffer zones and plots were sampled to determine the levels of three *Fusarium*-derived mycotoxins including deoxynivalenol (DON), 15-acetyldeoxynivalenol (15ADON) and nivalenol (NIV) in grain. The samples were collected from bulk grain harvested from 1.5m × 3.0m areas within plots and buffer zones. The FHB levels in plots were low (<5%, incidence*severity) throughout the field, resulting in low mycotoxin levels. Neither 15ADON nor NIV were detected in any of the samples. Levels of DON in samples ranged from below detection threshold (0.5ppm) to 3.1ppm (moderately low). There was no correlation between residue level and DON in grain for this experiment and no discernable gradient in DON concentration over distance from, or between any plots. It was concluded that cross contamination was not an issue for the 2003 experiment.

Autonomous lateral movement and root infection by sporangial and cyst derived zoospores of *Phytophthora* spp. in water. O. D. OLAYE and S. L. von Broembsen. Oklahoma State University, Stillwater, OK. Phytopathology 94:S79. Publication no. P-2004-0537-AMA.

Phytophthora spp. can produce successive generations of zoospores in water by repeated emergence of zoospores from cysts. Despite disease management implications posed by this phenomenon, little is known regarding the role of cyst derived zoospores (CDZs) as infective propagules of waterborne *Phytophthora* spp. The ability of CDZs and sporangial derived zoospores (SDZs) to move autonomously in water in model mazes or linear troughs and to infect roots of vinca (*Catharanthus roseus*) seedlings at fixed distances from a point of introduction were assessed after 4 h for three isolates, *P. parasitica* (GLN 9-3), *P. cryptogea* (FDM51) and *P. cryptogea* (FWDM4). Isolations showed that both CDZs and SDZs infected roots at distances up to 215 mm (model mazes) and 320 mm (linear troughs) and were also able to move the same distances in controls without seedlings. The results indicate that CDZs of *Phytophthora* spp. can serve as effective dispersal and infection units in water and suggests that zoospores may be able to move autonomously to greater distances in a non-restraining medium than previously thought.

Development of black-stain root disease on artificially inoculated ponderosa pine. W. J. OTROSINA (1), C. S. Sung (1), J. T. Kliejunas (2), S. Smith (2), and D. R. Cluck (2). (1) USDA Forest Service, Southern Research Station, Athens, GA 30677; (2) USDA Forest Service, Region 5, Susanville, CA. Phytopathology 94:S79. Publication no. P-2004-0538-AMA.

We inoculated woody ponderosa pine roots greater than 5 cm in diameter with an isolate of *Leptographium wageneri* recovered from the study site. Conidial spore suspensions in sterile distilled water were dispensed into roots during the summer of 2003. Spore concentrations of 30,500, 500, 50, and 5 spores/µl were dispensed in a 100 µl volume into 4.0 mm wide × 2.0 cm deep holes punched through the xylem using an increment hammer. Roots were re-excavated 10 weeks later and lesions were excised and measured. All spore concentrations produced lesions at least 1.5 times to four times greater than the sterile control wounds. *L. wageneri* was re-isolated from all lesions sampled. Lesions areas from plots that had prescribed burning were consistently less than those of unburned control plots. *L. wageneri* lesions produced at the 5 spores/µl concentration are significant and may relate to the hypothesized insect vector relationships in this disease.

Control of Asian soybean rust (*Phakopsora pachyrhizi*) in South America with myclobutanil. D. G. OUIMETTE, G. M. Kemmitt, M. T. Iamauti, and B. R. Sheppard. Dow AgroSciences LLC, Indianapolis, IN 46268. Phytopathology 94:S79. Publication no. P-2004-0539-AMA.

Asian soybean rust initially infested South America (Paraguay) in 2001 and within a short period of time had spread extensively throughout the main soybean production areas, especially Brazil. Due to factors such as the widespread cultivation of susceptible soybean varieties, an aggressive pathogen and ideal environmental conditions, severe epidemics quickly developed. Under conditions conducive for disease development, almost total defoliation

of infected plants can occur resulting in significant yield losses. Currently, the use of resistant varieties is not a viable option so fungicides are the only available means to control the disease. During the 2002-2003 and 2003-2004 growing seasons, field research trials with myclobutanil (Laredo™ fungicide by Dow AgroSciences) alongside current and emerging commercial standards were conducted in Brazil and Bolivia to evaluate the efficacy towards Asian soybean rust. Myclobutanil was shown to be highly effective in controlling the disease, especially when used in a protectant fashion. The effectiveness is due largely to the strong intrinsic activity of myclobutanil towards the pathogen as well as excellent mobility and rain fastness after application to the plant. Although myclobutanil possesses excellent curative activity towards a wide range of diseases, it is recommended that the product is used preventatively for maximum effectiveness against Asian soybean rust and that the first application be made at flower initiation with a second fungicide application approximately 20-25 days later. ™Trademark of Dow AgroSciences LLC

Enhancement of the fluorescent pseudomonad population after amending the recirculating nutrient solution of hydroponically-grown plants with a nitrogen stabilizer. D. PAGLIACCIA (1), D. Merhaut (2), and M. E. Stanghellini (3). (1) Dipartimento di Produzione delle Piante, Università degli Studi Della Tuscia, Viterbo, Italy 01100; (2) Departments of Botany and Plant Sciences; (3) Plant Pathology, University of California, Riverside 92521. Phytopathology 94:S80. Publication no. P-2004-0540-AMA.

Fluorescent *Pseudomonas* spp. have historically been associated, via diverse mechanisms, with suppression of root diseases caused by numerous fungal and fungal-like pathogens. However, inconsistency in performance in disease abatement following their application has been a problem. This has been attributed, in part, to the inability of the biocontrol bacterium to maintain a critical threshold population necessary for sustained biocontrol activity. Numerous methods, which include the addition of various food sources, have been used to selectively enhance and maintain desired populations of the biological control bacterium. Recently, we discovered that a nitrogen stabilizer (N-Serve, Dow Agrosciences) exhibited in vitro fungicidal activity towards zoospores of *Phytophthora* and *Pythium* spp. and selectively enhanced the resident population of fluorescent pseudomonads in the amended and recycled nutrient solution used in the cultivation of hydroponically-grown gerbera and pepper plants. Additionally, the chemical had a positive effect on plant growth, flower productivity (gerbera), and a stabilizing effect on the pH and electrical conductivity of the nutrient solution.

Inheritance of and molecular mapping of wheat and barley genes for resistance to inappropriate formae speciales of *Puccinia striiformis*. V. PAHALAWATTA and X. M. Chen. USDA-ARS and Dept. of Plant Pathology, Washington State Univ., Pullman, WA. Phytopathology 94:S80. Publication no. P-2004-0541-AMA.

Most wheat genotypes are resistant to barley stripe rust caused by *Puccinia striiformis* f. sp. *hordei* (PSH) and most barley genotypes are resistant to wheat stripe rust caused by *P. striiformis* f. sp. *tritici* (PST). To determine genetics of wheat resistance to PSH and barley resistance to PST, crosses were made between wheat genotypes Lemhi (resistant to PSH) and PI 478214 (susceptible to PSH) and between barley genotypes Steptoe (resistant PST) and Russell (susceptible to PST). Seedlings of F_1, BC_1, F_2, and F_3 progeny from the wheat cross were tested with races PSH-14, PSH-48, and PST-21, and those from the barley cross were tested with races PST-41 and PST-45 under controlled greenhouse conditions. Based on genetic analyses of infection type data, Lemhi has a dominant gene for resistance to the PSH races and the gene is closely linked (0.3 cM) to *Yr21* for resistance to PST-21; and Steptoe has a dominant and a recessive gene for resistance to races PST-41 and PST-45. Using the resistance gene analog polymorphism (RGAP) technique, the Lemhi gene for resistance to PSH and *Yr21* were mapped on a linkage group on chromosome 1B with 11 RGAP markers. A linkage group for the dominant gene in Steptoe for resistance to PST was constructed with 12 RGAP markers. The results show that resistance in wheat and barley to inappropriate stripe rust pathogens is qualitatively inherited.

***FvABC1*, a *Fusarium verticillioides* gene encoding an ATP-binding cassette protein, may be required for tolerance of phytoanticipins produced by corn (*Zea mays*).** E. R. LENCIA (1) and A. E. Glenn (2). (1) Department of Plant Pathology, The University of Georgia, Athens, GA; (2) USDA-ARS, Russell Research Center, Toxicology and Mycotoxin Research Unit, Athens, GA. Phytopathology 94:S80. Publication no. P-2004-0542-AMA.

The ascomycete *Fusarium verticillioides*, a common pathogen of corn (*Zea mays*) throughout the world, often causes significant concern due to production of mycotoxins such as fumonisins. Corn produces the phytoanticipins 6-methoxy-2-benzoxazolinone (MBOA) and 2-benzoxazolinone (BOA) as a general chemical defense mechanism, yet *F. verticillioides* can actively metabolize these compounds. The present study was initiated to characterize an ATP-binding-cassette (ABC) transporter gene which may be required for tolerance of both MBOA and BOA. ESTs, having homology to fungal ABC transporters, were previously identified in a subtracted cDNA library enriched for genes expressed in response to BOA. We have sequenced a putative ABC transporter gene (*FvABC1*) from a genomic cosmid clone. Characterization of *FvABC1* is underway, including creation of a gene knock-out mutant. Future plans include assessing the role of this ABC-transporter gene in *F. verticillioides* tolerance to antimicrobial compounds and whether such tolerance impacts *in planta* associations with corn or the ecological fitness of *F. verticillioides* in general.

Studies on natural spread of citrus psorosis disease. S. R. PALLE, H. Miao, M. Seyran, J. V. daGraca, and M. Skaria. Texas A&M University-Kingsville Citrus Center, Weslaco, TX. Phytopathology 94:S80. Publication no. P-2004-0543-AMA.

The incidence of citrus psorosis disease in Texas was significantly reduced during the past 50 years by four tree-killing freezes and the widespread use of a new cultivar of grapefruit, the Rio Red, which was psorosis-free. In recent years there has been an increase in the incidence of the disease in trees which were propagated from psorosis-free sources. Surveys based on symptoms, disease confirmation by biological indexing, serology, and PCR confirmed the psorosis etiology. Circumstantial evidence indicated a natural spread, and the pattern of adjacent infected trees along flood irrigation lines suggests the possible involvement of a soil inhabiting vector. An *Olpidium*-like fungus was found on feeder roots of symptomatic trees. The presence of the virus in roots was confirmed by PCR, and attempts to detect it in the fungus are underway.

Interaction of *Rotylenchulus reniformis* with seedling disease pathogens of cotton. A. J. PALMATEER (1), K. S. Lawrence (2), E. van Santen (3), and G. Morgan-Jones (2). (1) Tropical Research and Education Center, University of FL, Homestead, FL 33031; (2) Department of Entomology & Plant Pathology, Auburn University, Auburn, AL 36849; (3) Department of Agronomy and Soils, Auburn University, Auburn, AL 36849. Phytopathology 94:S80. Publication no. P-2004-0544-AMA.

Impact of ten *Fusarium* spp. in association with *Rotylenchulus reniformis* on cotton seedling disease was examined. Fungal treatments were *Fusarium chlamydosporum*, *F. equiseti*, *F. lateritium*, *F. moniliforme*, *F. oxysporum*, *F. oxysporum* f. sp. *vasinfectum*, *F. proliferatum*, *F. semitectum*, *F. solani*, and *F. sporotrichioides*, *Rhizoctonia solani*, and *Thielaviopsis basicola*. The experimental design was a 2 × 14 factorial in the presence or absence of *R. reniformis* and the 12 fungi plus two controls in autoclaved soil. In experiment 2, the design was a 2 × 2 × 14 factorial with the same treatments in autoclaved and non-autoclaved soil. *Fusarium oxysporum* f. sp. *vasinfectum*, *F. solani*, *R. solani*, and *T. basicola* displayed extensive root and hypocotyl necrosis that was more severe in the presence of *R. reniformis*. Soil treatment influenced the impact of *Fusarium* species on disease which was more severe in autoclaved soil. *Rotylenchulus reniformis* reproduction was greater in non-autoclaved soil compared to autoclaved soil ($P < 0.05$). This study suggests the importance of *Fusarium* spp. and *R. reniformis* in cotton seedling disease.

Simulation of airborne dispersal potentials of soybean rust from Africa to South America and from South America to North America. Z. PAN (1), S. Pivonia (2), R. Pasken (1), J. Pietrowicz (1), and X. B. Yang (2). (1) St. Louis University, St. Louis, MO; (2) Iowa State University, Ames, IA. Phytopathology 94:S80. Publication no. P-2004-0545-AMA.

Recent global spread of soybean rust (*Phakopsora pachyrhizi*) suggests a possibility of between-continents airborne movement of the disease. Meteorological model (MM5) coupled with dispersal model (HYSPLIT) was used to study the potential continental dispersal of soybean rust and the possibilities of rust movements 1) from Africa to South America, and 2) from South America to North America during the soybean growing season of each source continent. In the source continents, rust spore production was assumed to be proportional to the soybean acreage for the months during the peak rust epidemics and survivability of spores was affected by accumulated solar radiation intensity and/or cold temperatures. Our preliminary results indicate that major air parcel pathways can explain the disease occurrence locations when the rust was first reported in South America. When spores were released from Zimbabwe, air parcels moved in warm temperature zone (low levels) along the inter-tropical convergence zone (ITCZ) westward to South America, then turned south after being blocked by the Andes, and finally reached southern Brazil and Paraguay. The movement took about two weeks. For the spread from rust infected regions in South America to North America, however, northerly wind was blocked by ITCZ at lower levels. To reach

North America, the air parcels traveled at higher altitudes in cold temperatures (0 to –30°C) for about three weeks or longer.

A survey for the incidence of *Dahlia mosaic virus* in dahlias in the U.S. H. R. PAPPU and K. Druffel. Department of Plant Pathology, Washington State University, Pullman WA 99164-6430. Phytopathology 94:S81. Publication no. P-2004-0546-AMA.

Dahlia mosaic virus (DMV) has a double-stranded DNA genome and shares many genomic features with members of *Caulimoviridae* and is an important pathogen of dahlias. DMV is reported to be transmitted by several species of aphids and through vegetative cuttings and bulbs. Dahlia cuttings and bulbs were collected from several states in the US. The genome was cloned and sequenced and a sensitive and reliable PCR-based detection was used to screen for the incidence of DMV. DMV was found to be widely distributed as over 80% of the samples tested were positive. Use of real-time PCR allowed the detection of DMV in dilutions of total plant DNA of up to 1:1000. Findings suggest a relatively wide distribution of DMV in dahlias in the US and the need to grow virus-tested planting material to minimize its further spread.

A novel pathosystem: *Arabidopsis thaliana*–*Cochliobolus miyabeanus* interactions. J. Y. PARK, S. Kim, and Y. H. Lee. Seoul National University, Seoul, Korea. Phytopathology 94:S81. Publication no. P-2004-0547-AMA.

Cochliobolus miyabeanus is a necrotrophic fungal pathogen that causes brown leaf spot in rice. To understand molecular mechanisms of pathogenesis by *C. miyabeanus*, we established an experimental pathosystem between *C. miyabeanus* and the model plant *Arabidopsis thaliana*. Sixteen accessions of *A. thaliana* were inoculated with *C. miyabeanus* and they exhibited different levels of susceptibility/resistance. Small brownish necrotic lesions with marginal yellowing appeared on infected leaves of *A. thaliana* Col-0 at 3 days after inoculation and expanded throughout the entire leaves as time increases. Infected leaves withered and eventually died at 9 days after inoculation. The fungus recovered from the necrotic lesions induced the same lesions on healthy Arabidopsis plants. Expression of both *PR-1* and *PDF1.2* was induced by *C. miyabeanus* infection. Pretreatment of signaling molecules on plants had different effects on disease development. Ethylene increased susceptibility, but salicylic acid increased resistance. These results suggest that the establishment of resistance in *A. thaliana* against *C. miyabeanus* might be dependent on SA-dependent pathway. This is the first demonstration that Arabidopsis is host of the rice fungal pathogen. This pathosystem would help to dissect defense mechanisms in monocotyledonous and dicotyledonous plants against the same pathogen.

Morphological and molecular analysis of *Trichoderma* isolates associated with the green mold epidemic of oyster mushroom in K[illegible]. M. S. PARK (1), S. B. Kim (1), K. S. Bae (1), and S. H. Yu (2). (1) Korea Research Institute of Bioscience and Biotechnology, 52 Oun-dong, Yusong-gu, Daejon, 305-333, Korea; (2) Division of Applied Biology, Chemistry and Food Science, Chungnam National University, Daejeon, 305-764, Korea. Phytopathology 94:S81. Publication no. P-2004-0548-AMA.

Green mould disease caused by *Trichoderma* species is one of the most serious disease of oyster mushroom in Korea. A total of 179 isolates of *Trichoderma* species were isolated from oyster mushroom farms in Korea. Based on cultural and morphological characteristics, and molecular characteristics including analysis of RFLP and sequence of the ITS region, the *Trichoderma* species were identified as *T. harzianum*, *T. virens*, *T. atroviride*, *T. citrinoviride*, *T. longibrachiatum*, and two unrecorded species. The two unrecorded species were designated as Korean type 1 and Korean type 2, respectively. The predominant species was the Korean type 2 (86 isolates) follow by Korean type 1 (52 isolates) and *T. atroviride* (17 isolates). Colonization assessment confirmed that the Korean type 2 was found to be the main cause of the green mold epidermic in oyster mushroom substrate. *T. aggressivum* f. *europaeum* (Th 2) and *T. aggressivum* f. *aggressivum* (Th 4) were not isolated from oyster mushroom farms in Korea. Phylogenetic analysis of the ITS sequence data revealed that two Korean types were phylogenetically distinct from the previously reported *Trichoderma* species. Korean type 1 and Korean type 2 are also morphologically distinct from the other group by growth rate at 35°C, colony characteristics, shape of conidia and phialide, and phialide branching at the tips of branches. Based on the results of molecular and morphological characteristics, we propose the two Korean types as the new species.

Relative virulence of *Phytophthora ramorum* isolates in Oregon. J. L. PARKE and M. L. Reed. Dept. of Crop and Soil Science, Oregon State University, Corvallis, OR. Phytopathology 94:S81. Publication no. P-2004-0549-AMA.

P. ramorum has been detected in Oregon forests and nurseries. Three forest isolates and three nursery isolates were compared for virulence on *Vaccinium ovatum*, *Lithocarpus densiflorus*, *Pieris japonica*, *Viburnum plicatum*, and *Rhododendron* 'Nova Zembla'. Foliage on whole, intact plants was dipped in a zoospore suspension from each of the six isolates. After 3 weeks, plants were rated for disease severity, and the proportion of symptomatic tissue determined. Nursery isolates were generally more virulent than the forest isolates. On rhododendron and viburnum, the nursery isolates were much more virulent than the forest isolates. On tanoak, differences between forest and nursery isolates were less pronounced. The nursery isolates were of the European genotype, mating type A1 (E-A1), and the forest isolates were the N. American genotype, mating type A2 (NA-A2). In a subsequent test on rhododendron, a E-A1 nursery isolate, a NA-A2 nursery isolate, and a NA-A2 forest isolate were compared. The E-A1 nursery isolate was the most virulent. E-A1 isolates appear to pose a greater threat than the NA-A2 isolates, especially for the nursery plants tested.

Citrus canker symptom assessment using image analysis. P. E. PARKER (1), C. H. Bock (2), and T. R. Gottwald (2). (1) USDA-APHIS, Moore Air Base, Edinburg, TX 78539; (2) USDA-ARS-USHRL, 2001 S. Rock Rd., Ft. Pierce, FL 34945. Phytopathology 94:S81. Publication no. P-2004-0550-AMA.

Images of 214 citrus leaves with symptoms of citrus canker caused by *Xanthomonas axonopodis* pv. *citri*, were analyzed using image analysis (IA). In addition, three plant pathologists visually assessed (VA) each image for number of lesions, % area necrotic, and the % area necrotic + chlorotic. There was a consistent positive correlation ($r = 0.683$-0.916, $P < 0.0001$) between these three visual assessments of symptoms and the IA estimates. Although mean number of lesions estimated per leaf was similar (16.2-18.7), there was some discrepancy between the IA estimate and among assessors: for example, the number of leaves with no symptoms was 17 using IA, and 18-24 when visually assessed. The mean % area necrotic was 3.4 (IA) and 3.5-5.4 (VA), respectively, and mean area necrotic + chlorotic was 10.7 (IA) and 8.9-11.0 (VA), respectively. Minor discrepancy was found within other lesion number categories, and % area diseased estimates though frequency counts in each category were not dramatically different. IA appears to provide a reliable way to assess canker infected leaves for disease severity.

Identification and pathogenicity of *Pythium* spp. associated with roots of hybrid bermudagrass in overseeded golf course putting greens. S. R. PARKER, S. B. Martin, S. N. Jeffers, and J. J. Camberato. Clemson University, Clemson, SC. Phytopathology 94:S81. Publication no. P-2004-0551-AMA.

Pythium spp. were isolated from hybrid bermudagrass roots from putting greens overseeded with rough bluegrass (*Poa trivialis* L.) from March to November 2002. Rough bluegrass 'Winterplay' and common bermudagrass (*Cynodon dactylon* L.) were grown at 25°C and 32°C in growth chambers on a 12 hour day/night cycle and inoculated with *Pythium* spp. grown on V8-Vermiculite media. *P. myriotylum* Drechsler caused the greatest foliar blight and root rot (20-100% of root system) and lowest plant dry weight in both hosts (10-22% of control). Rough bluegrass roots were abundantly colonized by hyphae and oospores of *P. myriotylum*, but oospores were not found in common bermudagrass roots. Some isolates of *P. arrhenomanes* caused chlorosis and decreased plant density and dry weight (27-49% of control) in both grasses. Rough bluegrass was more susceptible at 32°C while common bermudagrass was more susceptible at 25°C to *Pythium* spp. These results suggest *Pythium* spp. are potentially significant pathogens of rough bluegrass-overseeded bermudagrass putting greens.

Effects of spring-applied fungicide applications on disease and quality of rough bluegrass overseeded bermudagrass putting greens. S. R. PARKER, S. B. Martin, J. J. Camberato, and S. N. Jeffers. Clemson University, Clemson, SC. Phytopathology 94:S81. Publication no. P-2004-0552-AMA.

Plots of rough bluegrass (*Poa trivialis* L.) overseeded 'TifDwarf' and 'TifEagle' bermudagrass (*Cynodon transvaalensis* Burtt-Davy × *C. dactylon* (L.) Pers.) greens were sprayed with fungicides during spring transition in 2002 and 2003. Fungicides were applied three times at labeled rates on a 21 day schedule beginning at the first signs of bermudagrass green up. Plots were visually rated every 7 days for turf quality, density and disease severity until summer. Significant improvements in turf quality and turf density were noted but with no obvious disease control with several broad spectrum fungicides and fungicide combinations. The tank mix Aliette Signature (fosetyl-Al) + Fore Rainshield (mancozeb) at 0.98 g and 1.95 g a. i. M-2, respectively, caused the greatest, earliest and longest lasting improvements in turf quality and density followed closely by Fore Rainshield + 3336F (thiophanate methyl) at 1.95 g and 0.84 g a. i. M-2, respectively. *Pythium*-specific fungicides Subdue Maxx (metalaxyl), Aliette (fosetyl-Al), and Vital (phosphonate)

caused infrequent, minor and ephemeral improvements in turf quality and density. These results suggest fungicides may have a considerable effect on improvement of turf density common to overseeded bermudagrass putting greens during spring transition, but the role of *Pythium* spp. is minor.

Biological control of *Sclerotinia minor* on peanut with *Coniothyrium minitans*. J. PARTRIDGE (1), T. B. Sutton (1), D. L. Jordan (2), and V. L. Curtis (1). North Carolina State University (1) Dept. of Plant Pathology; (2) Dept. of Crop Science, Raleigh 27695. Phytopathology 94:S82. Publication no. P-2004-0553-AMA.

Coniothyrium minitans, marketed as Contans WG, is a mycoparasite capable of colonizing sclerotia of *Sclerotinia* spp. A field experiment was conducted to determine if repeated soil applications of *C. minitans* would control of sclerotinia blight (*Sclerotinia minor*) on peanut. *C. minitans* was applied in the fall of 1999 and each subsequent fall for 2 years in a field with a history of peanut production and disease. In 2001, *C. minitans* application at 4 kg/ha reduced disease only in the moderately resistant cultivar Perry. In 2002 and 2003 both the 2 and 4 kg/ha rates of Contans WG reduced disease. Multiple years of soil application resulted in the lowest amount of disease and also reduced sclerotia numbers in the soil when compared to the untreated control. A management program integrating moderately resistant cultivars, applications in consecutive years of *C. minitans*, and fluazinam may provide the best control of sclerotinia blight.

Differential cultivars and molecular markers segregate isolates of *Uromyces appendiculatus* into two distinct groups that correspond to the gene pools of their common bean hosts. M. AUSTASTOR-CORRALES (1) and M. C. Aime (2). (1) Vegetable Laboratory; (2) Systematic Botany & Mycology Laboratory, USDA-ARS, Beltsville, MD. Phytopathology 94:S82. Publication no. P-2004-0554-AMA.

We explored virulence and molecular diversity in a collection of isolates of the bean rust pathogen (*U. appendiculatus*) from different parts of the world. For virulence analysis we used a set of 12 common bean (*Phaseolus vulgaris* L.) differential cultivars, six each from the Andean and the Middle American bean gene pools. We also examined the genotypic diversity of these isolates using the elongation factor 1alpha. Phenotypic and genotypic results were congruent and revealed the existence of two different groups of isolates of *U. appendiculatus* that corresponded to the Andean and Middle American gene pools of their common bean hosts respectively. These results also revealed that the Andean group of isolates is less diverse and has greater host-specificity compared to the Middle American group. A molecular assay has been developed to rapidly identify to which group isolates of *U. appendiculatus* belong.

Identification of putative parasitism genes of the beet cyst nematode (BCN), *Heterodera schachtii*. N. PATEL (1), H. Diab (1), B. Gao (2), X. Wang (1), R. S. Hussey (2), T. J. Baum (2), and E. L. Davis (1). (1) North Carolina State University, Raleigh, NC; (2) University of Georgia, Athens, GA; (3) Iowa State University, Ames, IA. Phytopathology 94:S82. Publication no. P-2004-0555-AMA.

The use of BCN and *Arabidopsis thaliana* as a model for understanding plant-nematode interactions can provide information to develop crop cultivars that are resistant to other cyst nematode species such as the soybean cyst nematode, *Heterodera glycines*. The close phylogeny and similarity in the infection process between SCN and BCN suggests that, use of available SCN genetic information may be useful in identifying putative parasitism genes of BCN. Greater than fifty candidate parasitism genes have been identified in SCN that encode products that may be secreted from the esophageal gland cells into plant tissues. Initial studies using BCN genomic DNA as template and primers designed to short regions of the SCN parasitism cDNA clones, 4EO2, 4FO1, 3BO5, 30DO8, 4DO9, and SYV46 have obtained PCR products, which further indicate similar parasitism strategies between the two nematode species. In addition, polyclonal antibodies to SCN parasitism gene proteins (4GO6 and 29FO7) bind within BCN esophageal gland cells, suggesting that BCN is an ideal model for functional analyses of SCN.

Influence of drop size, fall height, and target type on splash dispersal of *Gibberella zeae* and the amount of water splashed. P. A. PAUL, P. E. Lipps, and L. V. Madden. Dept. of Plant Pathology, The Ohio State University/OARDC, Wooster, OH 44691. Phytopathology 94:S82. Publication no. P-2004-0556-AMA.

A single drop generator was used to study the effect of different drop sizes and fall heights on the dispersal of spores of *Gibberella. zeae* and the mass of water splashed from different surfaces. Hypodermic needles were used to generate drops 2.7, 3.3, and 3.7 mm in diameter, falling from heights of 50, 75, 100, and 125 cm. Splashed droplets resulting from the impact of incident drops on spore-bearing wheat leaves and corn stalks, and a spore suspension of *G. zeae* were collected on petri plates containing Komada's selective media and sheets of water sensitive paper (WSP). Plates were incubated for 48 to 72 h and the number of colony forming units (CFU) counted. An image analysis system was used to determine the size of droplet traces on WSP. Fall height, drop size, and target type significantly affected the mass of water and number of CFUs splashed from the point of impact. Larger drops falling from 125 and 100 cm resulted in greater spore dispersal and mass of water splashed than smaller drops falling from 75 and 50 cm. More water was splashed from plant surfaces than from the spore suspension.

Relationship between the environment and the amount of *Gibberella zeae* inoculum recovered from wheat heads in Ohio. P. A. PAUL, P. E. Lipps, and L. V. Madden. Dept. of Plant Pathology, The Ohio State University/OARDC, Wooster, OH 44691. Phytopathology 94:S82. Publication no. P-2004-0557-AMA.

Correlation and regression analyses were used to determine the relationship between weather-related factors and the number of propagules of *Gibberella zeae*, primary causal agent of Fusarium head blight (FHB), recovered from wheat heads. Between 2000 and 2003, from Feekes growth stage 10 through 11.2, head samples were collected and assayed for propagules. Heads were placed in sterile distilled water and shaken to dislodge propagules. An aliquot of the wash was plated onto Komada's selective medium, and colonies were counted. Weather data were collected and summarized for different periods relative to the dates heads were sampled. Hours of daily air temperature between 15 and 30°C, hours of nightly relative humidity greater than 95%, rain intensity, and maximum wind speed showed strong correlation with CFUs/head. Multiple regression models developed using these variables explained between 55 and 77% of the variation in CFUs/head. Further exploration of these relationships could provide valuable information for the prediction of FHB.

A method for the quantification of *Rhizoctonia solani* and *Rhizoctonia oryzae* from soil using toothpicks. T. C. PAULITZ and K. L. Schroeder. USDA-ARS, Washington State University, Pullman, 99164-6430. Phytopathology 94:S82. Publication no. P-2004-0558-AMA.

Rhizoctonia solani AG-8 and *R. oryzae* are important root pathogens on wheat and barley in the dryland production areas of the inland Pacific Northwest. *R. solani* AG-8 is difficult to isolate from root systems and quantify in soil because of slow growth and low population densities. However, both pathogens form extensive hyphal networks in the soil and can grow a considerable distance from a food base. We developed a quantitative assay using toothpicks as baits inserted into sample soils. After 2 days in soil, toothpicks were placed on a selective medium, and the numbers of colonies that grew after 24 h were counted under a dissecting microscope. *R. solani* and *R. oryzae* could be distinguished from other fungi based on hyphal morphology. This method was tested in natural soils amended with known densities of *R. solani* AG-8 and *R. oryzae*. Regressions were used to compare the inoculum density/toothpick colonization curves to a predicted curve based on the volume of the toothpicks. The slopes and Y-intercept of log-log transformed regressions did not differ from the predicted curve in most cases. This technique was used to assess the hyphal activity of *R. solani* AG-8 and *R. oryzae* from soil cores taken from various positions in and around Rhizoctonia bare patches at two locations. Activity of *R. solani* was highest in the center and inside edge of the patch, but there was no effect of patch position on *R. oryzae*. This simple and inexpensive technique can be used for detection and diagnosis in grower fields and to study the ecology and epidemiology of Rhizoctonia.

Turfgrass disease characterizations in the state of Georgia before and after commercial disease management field trainings in 2002 and 2003. M. J. PEARCE, A. D. Martinez-Espinoza, and L. Burpee. University of Georgia, Griffin, GA. Phytopathology 94:S82. Publication no. P-2004-0559-AMA.

The turfgrass industries in Georgia are rapidly growing in number, volume, and types of services. There are approximately 1.6 million acres of turf in Georgia with a maintenance value of $1.56 billion. Using data collected in 2002 and 2003, the diagnosis, characterization, and dynamics of turfgrass disease was evaluated. In an attempt to reduce the number of disease cases and therefore limiting their economic impact, intensive educational programs were initiated to train turfgrass care professionals in disease identification and management issues. Clientele included landscape industry professionals, lawn care professionals, golf course personnel, the Georgia state agricultural inspectors and agricultural county agents. In 2003, turfgrass samples accounted for 18% of the total number of samples submitted to the disease clinic, compared to 30% in 2002. Despite the favorable conditions for disease development observed in 2003, the total commercial turfgrass samples fell from 54%

in 2002 to 39% in 2003. Specifically for golf courses, bentgrass disease dropped from 17% of the total turf samples in 2002 to only 5% in 2003. Meanwhile, homeowner samples accounted for 44% in 2002 and 61% of the turf samples in 2003. From these analyses, it can be concluded that the trainings provided in 2003 played a significant role in the reduction of turfgrass samples submitted to the disease clinic.

Commercial soybean variety response to *Tobacco ringspot virus* inoculation at different growth stages. R. A. PEKAREK, L. J. Giesler, L. C. Lane, and A. D. Ziems. Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0772. Phytopathology 94:S83. Publication no. P-2004-0560-AMA.

Soybeans are an important oilseed crop in the United States. *Tobacco ringspot virus* (TRSV) causes bud blight disease of soybeans that occurs throughout the Midwest. A study was conducted under greenhouse conditions to evaluate the response of 10 commercial soybean varieties inoculated with TRSV at three plant maturities (V3, V5, and R1). A non-inoculated control of each variety was included in every experimental unit. Qualitative data on occurrence of shoot-tip curving and necrosis, bud proliferation and necrosis, brown streaking on petioles, and dwarfed leaves was analyzed by generating percents of study time that a symptom was not observed. Height data was collected for analysis. Shoot-tip curving symptoms were exhibited by 8 of the 10 varieties tested. All varieties displayed terminal necrosis, bud proliferation, and brown streaks on petioles due to TRSV infection. There also was an effect of plant maturity at time of inoculation with these symptoms. There was significant plant inoculation timing by variety interaction ($P < 0.01$) for the development of dwarfed leaves. Significant inoculation timing by variety interaction ($P = 0.01$) was exhibited for overall plant height. These results indicate that plant maturity at the time of inoculation and soybean variety both influence the severity of bud blight disease symptoms.

Primers specific for detection of *Myxococcus* spp. by polymerase chain reaction. A. F. PEPPER (1), K. J. Martin (2), and C. T. Bull (1). (1) USDA/ARS, Salinas, CA; (2) University of West Florida, Pensacola, FL. Phytopathology 94:S83. Publication no. P-2004-0561-AMA.

Myxobacteria are ubiquitous in the soil and rhizosphere, produce many diverse secondary metabolites, and prey on bacteria and fungi, including plant pathogens. Our research has recently shown that myxobacteria can mitigate soilborne plant diseases *in situ*. In order to understand the interactions between plant pathogens and myxobacteria *in situ*, culture-independent methods of detection and enumeration of myxobacteria are needed. To this end, we designed primers based on published myxobacterial 16S rDNA sequences and evaluated them for their specificity. Twenty-six primer sets were evaluated for specific amplification of DNA from myxobacteria using PCR. Of these, three primer sets produced amplicons from myxobacterial DNA but not from DNA of bacteria used as negative controls. Reactions for five additional primer sets are being optimized to obtain the same level of specificity. In addition to these eight, three additional primer sets may be useful in that they produce amplicons from some, but not all myxobacterial species.

Molecular identification of *Guignardia citricarpa*, the causal agent of citrus black spot, and the development of a new protocol for detection in fruit tissue. N. A. R. PERES (1), R. Harakava (2), G. Carroll (3), L. Korsten (4), J. E. Adaskaveg (5), and L. W. Timmer (6). (1) Univ. Florida, GCREC, Dover, FL; (2) Inst. Biologico, Sao Paulo, Brazil; (3) Univ. Oregon, Eugene, OR; (4) Univ. Pretoria, South Africa; (5) Univ. California, Riverside, CA; (6) Univ. Florida, CREC, Lake Alfred, FL. Phytopathology 94:S83. Publication no. P-2004-0562-AMA.

Guignardia citricarpa, the cause of citrus black spot, infects fruit in the field and may remain as quiescent infections after harvest. Identification of the pathogen is difficult based on morphological characteristics because of its similarity to the non-pathogenic species *G. mangiferae*. This disease does not occur in the United States, thus, a rapid detection system is important to prevent the introduction of the pathogen. Several molecular diagnostic primers have been developed for identification of black spot and differentiation of the pathogenic from the non-pathogenic species based on ITS, ETS (internal and external transcribed spacer, respectively), chitin synthase, and other DNA regions. All primers proved to be effective for identification of the fungus from pure culture, but did not consistently detect the fungus in fruit tissues. Thus, new primers were developed from the ITS region of rDNA that were effective for differentiation of the *Guignardia* species and detection of *Guignardia citricarpa* directly from fruit tissue. A quick protocol for DNA extraction also has been adapted for the detection of the fungus in a range of black spot symptoms, but needs to be evaluated for detection of quiescent infections on asymptomatic fruit.

Olive disease surveys in Argentina. B. A. PEREZ (1), E. Oriolani (2), D. Docampo (3), L. Otero (3), C. Matías (4), and D. Barreto (1). (1) INTA-IMYZA, CC 25 (1712) Castelar, Argentina, bperez@cnia.inta.gov.ar; (2) INTA-EEA Mendoza; (3) INTA-IFFIVE, Córdoba; (4) INTA-EEA Catamarca. Phytopathology 94:S83. Publication no. P-2004-0563-AMA.

Olive is grown in Catamarca, Mendoza, La Rioja, and San Juan. In 2003, disease surveys were conducted in fall and spring. Two kinds of orchards were included: 70 year-old, and less than 10 year-old. The germplasm collections in Junín, Mendoza, and Pocito, San Juan, were evaluated. The fungi *Cercospora cladosporioides*, *Spilocaea oleagina*, *Capnodium elaeophilum*, and *Limacinia* sp. were observed with high severity in old abandoned orchards in Mendoza, and San Juan. The new plantations showed low foliar disease levels in the four provinces. *Fusarium solani* was frequently isolated from plants with drying syndrome in La Rioja. *Verticillium dahliae* was seldom cultured. High incidence of *Pseudomonas syringae* pv. *savastanoi* was recorded in old orchards in Mendoza. In Catamarca, we noted a low incidence of two new symptoms, trunk cankers in Arbequina, Carolea, and Picual, and trunk knots in Manzanilla. The germplasm collections showed low level of diseases. The surveys identified the main problems and provided a basis for future management strategies.

Assessing the role of kudzu *Pueraria lobata* in potential epidemics of soybean rust in the United States. O. PEREZ-HERNANDEZ, X. B. Yang, S. Pivonia, and J. Guan. Iowa State University, Dept. of Plant Pathology, Ames, IA 50011. Phytopathology 94:S83. Publication no. P-2004-0564-AMA.

Phakopsora pachyrhizi, causal agent of soybean rust (SBR), has a wide host range with more than 90 known alternative hosts, many of which are found in the US. Among the alternative hosts is kudzu *Pueraria lobata*, a very suitable host for SBR in South America and widely distributed in the US, could play an important role in the overwintering and northward movement of the pathogen in the US. Our work attempts to quantify the importance of kudzu in potential SBR epidemics, especially for year-round survival and seasonal spore dispersal of SBR. Foliar area of kudzu was monitored in southern US sites along a south-to-north potential SBR pathway by assessing plant sprouting time, leaf area index (LAI), and vegetative growth rate. Preliminary results indicate that the estimated LAI from southern Louisiana to southern Kentucky increased dramatically from April to May, with lower values as we move northward. In southern Mississippi, the FAI increased from ~zero in early April to 2.09 in early May. Foliage availability of kudzu in spring precedes that of soybean by about a month at the different locations studied. Calculated with these data, we found that prior to soybean foliage emergences, SBR might be able to complete two reproduction cycles on kudzu. Our survey results also indicated that the distribution and density of kudzu in the southern and middle states constitutes a continuous spatial distribution of host availability, which might facilitate the northward movement of rust early in the season.

Phylogenetic and genomic perspectives on plant-associated enterobacteria. N. T. PERNA and J. D. Glasner. University of Wisconsin-Madison. Phytopathology 94:S83. Publication no. P-2004-0565-AMA.

Genome sequencing projects are complete or underway for at least 39 members of the family Enterobacteriaceae. Until recently, no plant-associated enterobacteria were among this list. Now, projects for *Erwinia chrysanthemi* strain 3937 and *Erwinia carotovora* subspecies *atroseptica* are drawing to a close and projects for *Erwinia amylovora* and *Pantoea stewartii* subspecies *stewartii* are in progress. These four organisms represent two independent lineages of plant-associated bacteria within the clade that also includes the animal-associated genera *Yersinia*, *Salmonella*, and *Escherichia*, for which multiple genome sequences are available. Comparative analyses of enterobacterial genomes allow construction of a robust phylogeny and inference of the core set of genes present in the ancestor of both plant and animal-associated enterobacteria. Comparisons of more distantly related genomes of plant-associated, animal-associated and environmental bacteria are used to explore the origin of genes in plant-associated enterobacteria that were not present in the common ancestor.

Determining the turfgrass host range of *Labyrinthula* sp., the causal agent of rapid blight. P. D. PETERSON, S. B. Martin, and J. J. Camberato. Clemson University, Pee Dee Research and Education Center, Florence, SC. Phytopathology 94:S83. Publication no. P-2004-0566-AMA.

Forty-eight cool-season grasses in eight different genera were tested for their tolerance to the causal agent of rapid blight, *Labyrinthula* sp. Seeds of each test grass were sown in 7.6-cm pots and placed in a growth chamber for approximately three weeks at 23.8°C with 12 hours daylight and 12 hours darkness. Plants were watered until saturation every other day with deionized water for the first two weeks of growth, then with artificial sea water at a concentration of 3.5 deciSiemens per meter (dS/m). Inoculum was prepared by growing six isolates on 1% horse serum agar for four days, adding

colonized agar plugs to 1% horse serum broth and vortexing. Cell counts of 1.0-1.5 × 10^5 per ml were used each time for inoculations. Experimental design was a split plot with four reps where each pot was an experimental unit and the experiment was repeated. Plants in each pot were wounded by trimming with scissors prior to the application of 1ml of inoculum placed on leaf tissue in the form of droplets from a syringe. Disease severity was evaluated at 14 and 21 days post-inoculation. Results indicate a high degree of susceptibility in cultivars of rough bluegrass, colonial and velvet bentgrasses, and perennial ryegrass while certain cultivars of alkaligrass, creeping red fescue, and creeping bentgrass appear to have levels of tolerance.

Population structure of *Phyllosticta telopeae*, a foliar pathogen of Waratah (*Telopea speciosissima*) in production and natural ecosystems in NSW. S. A. PETERSON (1,2), B. A. Summerell (1), and L. W. Burgess (2). (1) The Botanic Gardens Trust; (2) The University of Sydney. Phytopathology 94:S84. Publication no. P-2004-0567-AMA.

Phyllosticta telopeae causes brown to grey circular lesions on the foliage of waratah (*Telopea speciosissima*) in production and natural ecosystems in NSW. The blemishes on commercially grown blooms caused by the fungus lead to a reduction in quality and market price. Production areas are within the natural range of the host. Broad spectrum fungicides are recommended for control of the disease but efficacy is quite variable. This is not surprising as little is known of population structure if the pathogen, its biology or epidemiology. Isolates were obtained using a hierarchical method for a study of the population structure of the pathogen on micro (within a plant) and macro (within a site) levels. The population structure and importance of sexual reproduction of the pathogen was determined using amplified fragment length polymorphisms (AFLP) techniques. Results indicate that there is little genetic variability within an infected plant or site. This finding suggests that sexual reproduction of the pathogen is not important as a dispersal mechanism unlike many other *Phyllosticta* spp. With a better understanding of the pathogen's genetic diversity more specific control measures may be able to be developed.

Detection and phylogenetic analysis of *Blueberry scorch virus*. N. PETROVIC (1), D. Linder-Basso (2), P. V. Oudemans (2), and B. I. Hillman (2). (1) Natl. Inst. of Biol., Dept. of Plant Physiol. and Biotech., Ljubljana, Slovenia; (2) Rutgers Univ., Dept. of Plant Biol. and Pathol., New Brunswick, NJ 08901. Phytopathology 94:S84. Publication no. P-2004-0568-AMA.

Monoclonal (MAb) and polyclonal (PAb) antibodies directed against the NJ 2 strain of *Blueberry scorch virus* (BlScV), a carlavirus, were used to develop ELISA and western blot systems for detection of virus from heterogeneous blueberry populations. The system was highly sensitive, able to detect purified virus in concentrations of 1 ng/ml, and BlScV in plant extracts diluted up to 200 times. The virus was detected in different cultivars and different tissues throughout the growing season. Electron microscopic analysis revealed that only the PAb and not the MAb decorated whole particles, suggesting that the MAb binds to an internal site of the coat protein (CP). The ELISA procedure was adapted for large-scale laboratory testing and was used for screening of 30,000 blueberry plants in the nursery stock by the New Jersey Dept. of Ag. during the summer of 2003. The variability within the BlScV population in New Jersey was evaluated through comparison of the CP sequence from 45 isolates representing different regions and blueberry cultivars. A new strain, NJ-3, was found to be distinct from the previously described strains, NJ-1 and 2 and WA-2 from Washington. Amino acid sequence identity of NJ-3 with NJ-1 and 2 was only 75%, with greatest variability around a proline-rich domain at the N-terminus of the CP.

A model for fungicide effects on within-plant spread of stem rust in ryegrass grown for seed. W. [illegible]ENDER (1), D. Upper (2), and C. Lipp (2). (1) USDA-ARS NFSPRC; (2) Oregon State University, Dept. Botany and Plant Pathology, Corvallis, OR 97331. Phytopathology 94:S84. Publication no. P-2004-0569-AMA.

Stem rust (caused by *Puccinia graminis* subsp. *graminicola*) is the major disease constraint on grass seed production in the Pacific Northwest. In stem rust of grasses, there is a unique form of within-plant disease spread related to host phenology. Rust pustules sporulating on the inner surface of leaf sheaths deposit spores on the enclosed stem as it extends past the pustule site, resulting in elongated stem lesions that can comprise 80% of final disease severity. We determined that the major fungicides used in grass seed production (propiconazole and azoxystrobilurin) differ in persistence and "kick-back" activity, transport through sheaths, and effects on viability of existing sheath pustules. We measured elongation rates of grass internodes to model within-plant spread of stem rust as a function of physiological time (heat units), and produced a simulation model combining plant phenology with fungicide activity. The relative efficacy of the two fungicides in controlling within-plant disease increase, and the importance of host stage in fungicide effectiveness, were demonstrated.

First report of *Soybean dwarf virus* on soybean in Wisconsin. A. PHIBBS (1), A. Barta (1), and L. L. Domier (2). (1) Wisconsin Department of Agriculture, [illegible]de and Consumer Protection, 4702 University Ave, Madison 53707; (2) USDA-ARS, University of Illinois, Urbana, IL 61801. Phytopathology 94:S84. Publication no. P-2004-0570-AMA.

Soybean dwarf virus (SbDV) causes widespread economic losses on soybeans (*Glycine max* (L.) Merr.) in Japan and has been reported on soybeans in Virginia. In 2003, 286 soybean fields in the R2-R4 growth stage were surveyed for SbDV in Wisconsin. Plants from five fields in four Wisconsin [illegible]nties (Columbia, Lafayette, Sauk and Waushara) tested positive for SbDV by double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA). [illegible]e presence of SbDV was confirmed and strain identity was inferred by reverse transcriptase polymerase chain reaction (RT PCR). The size of the RT-PCR products (110 bp) was consistent with the dwarfing strain of SbDV. All locations that tested positive for SbDV had soybean aphids (*Aphis glycines* Matsumura) on 100% of soybean plants. Although aphids were [illegible]ent, vector relations in the Wisconsin infections are unclear at this time. This is the first report of SbDV infecting soybean in Wisconsin.

A new technique for monitoring apple scab fungicide resistance in individual orchards. V. PHILION. IRDA, St-Hyacinthe, Qc J2S 7B8. Phytopathology 94:S84. Publication no. P-2004-0571-AMA.

In regions where apple scab is a problem, growers routinely use fungicides prone to the development of resistance. Unfortunately, the resistance status of individual orchards against the different chemistries is largely unknown. Current information is derived from regional surveys, which are inapplicable for individual sites a[illegible]o expensive for routine monitoring. We developed a reliable in vitro test to evaluate the population EC50 of *Venturia inaequalis* for cyprodinil, flusilazole, dodine, thiophanate-methyl, and kresoxim-methyl based on ascospore growth. Mycelial growth for each fungicide concentration was evaluated using a modif[illegible]ine-intercept method after incubating for 48 hours in a liquid suspension. Initial results showed that in certain commercial or[illegible]ls, resistance is confirmed only for flusilazole and thiophanate-methyl. In orchards with flusilazole resistance, a small shift in EC50 was recorded for both kresoxim-methyl and cyprodinil before these products were used. The method was also used with conidia to establish the distribution of sensitivity among clonal isolates and the results confirm the population EC50 assay. Although the threshold EC50 for practical field resistance still needs to be established, the method can already be used for routine monitoring. This technique is patent pending in the USA.

The effect of insect resistance on *Bean pod mottle virus* transmission in soybeans. D. PINO DEL CARPIO (1), M. G. Redinbaugh (1,2), J. L. Vacha ([illegible]S. A. Berry (1), R. Hammond (3), L. Madden (1), and A. E. Dorrance (1). (1) Dept. of Plant Pathology; (2) USDA-ARS; (3) Dept. of Entomology, The Ohio State University, Wooster, OH. Phytopathology 94:S84. Publication no. P-2004-0572-AMA.

Bean pod mottle virus (BPMV), a member of the genus Comovirus, was reported for the first time in Ohio in 1999. To determine whether insect resistance in soybeans influence the movement of BPMV, five soybean lines, three susceptible and two resistant to insect feeding, were evaluated in field trials in the presence of its vector, the bean leaf beetle. An Ohio BPMV isolate was purified from soybean leaves and used for inoculation of 2 or 20 plants per plot at the unifoliate stage. Plants were sampled at the R2 and R7 development stages to measure the incidence of BPMV within the plots. Preliminary results from the early sampling indicated that BPMV incidence was lower in HC94-24, an insect resistant line, than in plots with varieties without insect resistance. HC94-24, had 16.45 and 20.45% BPMV incidence in the plots for the two inoculation treatments, while Resnik, a susceptible line, had 25 and 37.6% incidence for the same treatments.

Possible link between El Nino events and introductions of airborne fungal pathogens from Sout[illegible]nerica to North America. S. PIVONIA (1), Z. Pan (2), and X. B. Yang (1). (1) Iowa State University, Ames, IA; (2) St. Louis University, St. Louis, MO. Phytopathology 94:S84. Publication no. P-2004-0573-AMA.

The concern for a potential introduction of soybean rust (*Phakopsora pachyrhizi*) from South to North America led us to examine the role of wind patterns in northward movement of this pathogen. In general, prevailing winds along the inter-tropical convergence zone (ITCZ) are easterly. However, during El Nino years, the easterly winds are somewhat disrupted, increasing the likelihood of southerly winds between the South and North Americas. In the last 30 years, two airborne diseases were believed to be i[illegible]duced from South to North Americas by winds. In 1987 barely stripe rust (*Puccinia striiformis* f. sp. *hordei*) was introduced into Mexico and in 1997 sorghum ergot (*Claviceps aficana*) entered North America; both events were

in El Nino years. The spread of sorghum ergot in South America and North America was much faster than barely stripe rust, perhaps due to combined effects of airborne nature and transport of sclerotia in seed-bags. Our analysis implies greater possibility for soybean rust natural movement from South America to North America in El Nino years than other years.

Development of Phytophthora root rot-resistant avocado rootstocks. R. C. PLOETZ (1), R. J. Schnell (2), and J. L. Haynes (1). (1) University of Florida, Homestead, FL; (2) USDA-ARS, Miami, FL. Phytopathology 94:S85. Publication no. P-2004-0574-AMA.

Phytophthora root rot, caused by *Phytophthora cinnamomi*, is the most important disease of avocado, *Persea americana*. To develop root rot-resistant rootstocks, we screened open-pollinated progeny from the National Germplasm Repository for avocado in Miami (NGR). 2,355 seedlings from 51 accessions were examined in potting mix infested with *P. cinnamomi*. Most seedlings developed severe root rot, but tolerance was observed among progeny of some accessions (families). Seedlings of the West Indian (WI) race, *P. americana* var. *americana*, and hybrids between it and the Guatemalan (G) race, *P. americana* var. *guatemalensis*, were more tolerant than those with other backgrounds ($P < 0.05$). Twelve families accounted for 82% (188 of 229) of the tolerant seedlings (<50% root necrosis), only two of which did not have a WI or G × WI pedigree. Broad sense heritability (H) for tolerance was 0.45. In ongoing work, we are screening additional open-pollinated progeny from the NGR and have begun to produce full-sib progeny with the previously identified superior parents.

Development of edible vaccine against SARS coronavirus. N. POGREBNYAK, V. Andrianov, A. V. Karasev, S. Spitsin, R. Egolf, and H. Koprowski. Biotechnology Foundation Laboratories, Thomas Jefferson University. Phytopathology 94:S85. Publication no. P-2004-0575-AMA.

There is a strong possibility that SARS-Coronavirus (CoV), carried by different animal species in nature may cause disease epidemics in humans for many years to come. Thus, there is a necessity to develop a vaccine which will be safe, easily administered to man for mass prevention and that can be produced at low cost. The goal of our research is to produce such a vaccine in edible plants. Virus envelope spike glycoprotein (S) is known to induce protective immunity to many coronaviruses. The human SARS-coronavirus S protein gene was kindly provided by Dr. I. Lipkin (Columbia University, NY). A major antigen polypeptide of the S protein (S1 fragment) was cloned in a plant expression vector. For Agrobacterium-mediated transformation experiments two constructs have been used. Both constructs contained the 2.1-kb truncated S gene (S1) under super promoter and NPTII gene under nos promoter. Signals for recombinant vaccine secretion and accumulation in endoplasmic reticulum were added in one vector construct. Tomato, cauliflower and tobacco plants have been used for Agrobacterium-mediated transformation experiments. Transgenic lines have been selected on medium with kanamycin. Transgenic tobacco and tomato lines were tested by Real-time quantitative PCR. Several transgenic lines express significant levels of S-specific mRNAs. Western blot analysis indicated that protein band of expected size occurs in transgenic tobacco plants. All transgenic plants will be tested to find the highest S-protein expressing lines. Immunogenicity of recombinant S-protein produced in plants will be evaluated in mice.

Dubious optical density readings in ELISA testing for *Citrus tristeza virus* in mandarin varieties of citrus. M. POLEK, P. D. Metheney, and C. Wallen. Central California Tristeza Eradication Agency, Tulare, CA. Phytopathology 94:S85. Publication no. P-2004-0576-AMA.

The California citrus industry is one of primarily fresh fruit including many specialty varieties. In recent years mandarin varieties have increased in consumer preference and hence, in hectares planted. *Citrus tristeza virus* is a regulated pathogen in California and the Central California Tristeza Eradication Agency (CCTEA) analyzes by ELISA nearly 500,000 samples for this virus each year. Often, tissue samples collected from field-grown mandarin varieties result in atypically high optical density readings that are considered either high negative or low positive results. This poses a serious dilemma to the diagnostician on whether to call a tree positive because a positive diagnosis leads to the removal of the tree. Budwood collected from trees demonstrating this phenomenon and grafted onto a greenhouse-grown rootstock then maintained under controlled greenhouse conditions lose this tendency. Because ELISA is a serological test, it is thought that a stress-related protein is involved in producing these high readings. A method is needed to evaluate borderline ELISA results to confidently distinguish between virus infection and assay reactions caused by environmental stress. Experiments in progress will compare stressed and non-stressed trees. Both field-grown trees and trees maintained in a greenhouse are subject to either induced high temperature or water stress events. Known healthy control trees maintained in controlled greenhouse conditions as well as field-grown trees from a research plot are monitored weekly. Leaf petiole tissue is analyzed by ELISA.

Identification of a *Xanthomonas* pathogen of coriander from Oregon USA. A. R. Poplawsky (1), L. Robles (1), W. Chun (1), L. DERIE (2), L. J. du Toit (2), X. Q. Meng (3), and R. L. Gilbertson (3). (1) University of Idaho, Moscow, ID; (2) Washington State University, Mount Vernon, WA; (3) University of California, Davis, CA. Phytopathology 94:S85. Publication no. P-2004-0577-AMA.

A coriander seed lot grown in Oregon yielded Xanthomonas-like colonies on MD5A agar, at 4.6×10^5 CFU/g seed. Colonies were mucoid, convex and yellow on YDC agar. Koch's postulates were completed on coriander. Water-soaked lesions developed on inoculated coriander leaves and turned necrotic in 1-2 weeks, with the growing point killed on some plants. The bacterium also was pathogenic on fennel, lovage and parsnip; but not on dill, celery or parsley. On carrot, isolates occasionally produced very mild symptoms 3-4 weeks after inoculation. The bacteria were motile, Gram-negative, aerobic rods, positive for production of amylase, catalase, xanthomonadins and H_2S from cysteine, but negative for quinate metabolism, oxidase and nitrate reductase. A 3S primer pair for detection of *X. campestris* pv. *carotae* (Xcc) amplified a 350 bp fragment of genomic DNA from the coriander isolates and Xcc, but rep-PCR fingerprints distinguished the coriander isolates from Xcc. This pathogen has not previously been reported in the USA.

Occurrence and fitness of mefenoxam resistant isolates of *Phytophthora erythroseptica* in Idaho. L. D. PORTER (1), J. S. Miller (1), and P. Nolte (2). (1) Univ. of Idaho, Aberdeen, ID; (2) Univ. of Idaho, Idaho Falls, ID. Phytopathology 94:S85. Publication no. P-2004-0578-AMA.

Pink rot of potato is a major late season and post-harvest problem in southeastern Idaho. Resistance to mefenoxam, used since 1987 for pink rot management, was first detected in Idaho in 1998. The incidence of mefenoxam resistance and the relative fitness of resistant isolates compared to sensitive isolates were assessed in this study. A total of 438 isolates of *Phytophthora erythroseptica* collected from 2001-2003 in 9 counties were tested for resistance to mefenoxam on amended agar at 0, 0.01, 0.1, 1.0, 10, 100 and 250 ppm. Resistant isolates were identified in seven of the nine counties. Of 220 isolates collected in 2001-2002, 163 were resistant and 57 sensitive with mean EC-50 values of 182 and 0.48 ppm of mefenoxam, respectively. Growth and oospore production on V8 agar were assessed for 20 sensitive and 20 resistant isolates at 10, 15, 20, 25 and 30°C. Colony diameter was measured after 4 days of growth and oospore production after 15 days. Oospore production was determined by removing 1.57 mm3 of colonized agar and counting oospores under a microscope. Optimal temperatures for growth and oospore production were 25°C and 20°C, respectively, for both resistant and sensitive isolates. Growth of resistant isolates was 2.5-3.1 times greater, and oospore production 6.8-20.5 times greater ($P < 0.0001$) for resistant isolates than sensitive isolates at temperatures from 10-25°C. Isolates resistant to mefenoxam may be more fit than sensitive isolates based on the fitness parameters studied.

Analysis of expressed sequence tags in the soybean rust pathogen *Phakopsora pachyrhizi* at different stages of the infection process. M. L. POSADA-BUITRAGO (1), J. L. Boore (1), and R. D. Frederick (2). (1) DOE-JGI-LBNL, Walnut Creek, CA; (2) USDA-ARS-FDWSRU, Ft. Detrick, MD. Phytopathology 94:S85. Publication no. P-2004-0579-AMA.

Phakopsora pachyrhizi causes soybean rust, which is the most devastating foliar disease in many soybean production areas around the world. The infection process was examined by comparing approximately 12,000 expressed sequences tags (ESTs) generated from three stage-specific unidirectional cDNA libraries from *P. pachyrhizi*. The EST cDNA libraries were constructed using mRNA isolated from urediniospores germinating on a water surface for 16 h and from infected soybean leaf tissue at 6-8 and 13-15 days post inoculation. Intercellular hyphal growth and the peak of sporulation occur at 6-8 and 13-15 days, respectively. The ESTs were analyzed for redundancy within and among the three libraries, and they were classified according to putative function based on similarity to known proteins using the Blast sequence algorithms. These ESTs will provide insight into the biochemical and physiological processes that are necessary for infection, survival and reproduction.

Identification of *Rathayibacter rathayi* in Oregon and Maryland. E. POSTNIKOVA (1), I. Agarkova (1), I. T. Riley (2), S. Alderman (3), C. M. Ocamb (4), A. K. Vidaver (1), and N. W. Schaad (5). (1) University of Nebraska, Lincoln, NE; (2) University of Adelaide, Australia; (3) USDA/ARS, Corvallis, OR; (4) Oregon State University, Corvallis, OR; (5) USDA/ARS, Ft. Detrick, MD. Phytopathology 94:S85. Publication no. P-2004-0580-AMA.

Most bacteria in the genus *Rathayibacter*, are carried by *Anguina* nematodes and cause gummosis in grasses and cereals. *R. toxicus* produces lethal animal

toxins in fodder grasses in Australia and Africa. *R. tritici* is officially regulated in the USA and some other wheat-growing countries. Bacterial gummosis in orchardgrass (*Dactylis glomerata*) is problematic for seed production in Oregon, with up to 5% heads affected in 2003. The causal organism was identified only as *Rathayibacter* sp. Several slow-growing yellow-pigmented, Gram-positive bacteria were isolated from affected plants from Oregon and Maryland. DNA was extracted for reassociation studies, AFLP analysis and partial ITS sequencing. The new strains shared more than 70% DNA relatedness with *R. rathayi* but less than 70% with *R. tritici* or *R. toxicus*. The AFLP pattern closely matched that of *R. rathayi* and the ITS sequence was identical. This is the first confirmation of *R. rathayi* in Oregon and Maryland.

Natural field spread of decline and non-decline inducing isolates of Citrus tristeza virus in Florida after the introduction of the brown citrus aphid. C. A. POWELL. University of Florida, Fort Pierce, FL. Phytopathology 94:S86. Publication no. P-2004-0581-AMA.

The effectiveness of seven different aphid control regimens in delaying movement of decline (DI) and non-decline (NDI introducing isolates of citrus tristeza virus (CTV) into a CTV free sweet orange on sour orange rootstock block was monitored annually for five years beginning in 1999, two years after the introduction of the brown citrus aphid (BrCA) into the region. After five years, the mean percentages of infection with DI CTV were 19, 19, 17, 29, 23, 19, and 14 for trees treated annually with imidacloprid, every six months with imidacloprid, every three months with imidaclorid, every two months with imidacloprid, annually with Temik, annually with Meta Systox-R, or untreated, respectively. The mean percentages of infection with only NDI isolates of CTV for the 7 treatments were 28, 22, 23, 27, 27, 27, and 23. There was no significant difference among either the DI or NDI CTV treatment means. The overall five year infection percentage for DI CTV (20%) was somewhat lower than that reported prior to the introduction of the BrCA (27%). Aphid densities (*T. citricidus* and *Aphis spiraecola*) varied considerably from year-to-year. Good aphid control was achieved with all 4 imidacloprid treatments, but not with Temik or Meta Systox-R. The level of aphid control did not influence overall CTV infection percentages.

Comparative survival of conidia of eight species of dematiaceous hyphomycetes in soil. R. G. PRATT. USDA, ARS, WM&FRU, Mississippi State, MS. Phytopathology 94:S86. Publication no. P-2004-0582-AMA.

Conidia of eight species of *Bipolaris*, *Curvularia*, and *Exserohilum*, produced on pieces of cellulose substrate (index card, filter paper), were incubated on substrates in nylon-mesh pouches in moist soil. At biweekly intervals up to 12 weeks, substrates were retrieved from soil and appressed to the surface of cornmeal agar. Conidia deposited on the agar were assayed for viability based on germination after 6-8 h. Significant differences in frequency of germination were attributed to species, incubation time, and species × time interactions. Species differences in survival of conidia were observed after 2 weeks and at all subsequent assay times. Mean survival of conidia was 0-68% for all species after 12 weeks. Conidia of *C. lunata*, *B. sorokiniana*, and *B. stenospila* usually survived longer or at higher frequencies in soil than did conidia of *B. cynodontis*, *B. hawaiiensis*, *C. geniculata*, and *E. rostratum*. *B. spicifera* gave variable results. *C. lunata*, *B. sorokiniana*, and *B. stenospila* may be preferred species for studies on survival of conidia in soil due to more prolonged and consistent survival of their conidia.

Characterization of genes correlated with aflatoxin production using a targeted cDNA microarray. M. S. Price and G. A. PAYNE. Center for Integrated Fungal Research, Box 7567, North Carolina State University, Raleigh, NC. Phytopathology 94:S86. Publication no. P-2004-0583-AMA.

The pathogens *Aspergillus flavus* and *A. parasiticus*, which cause disease on corn, peanuts, tree nuts, and cotton, are also capable of contaminating food and feed with aflatoxin (AF). Aflatoxin B1 is the most potent naturally occurring carcinogen and is regulated in food commodities at 20 ppb. Most of the biosynthetic pathway has been characterized, but much less is known about the regulatory circuits governing production of this potent mycotoxin. We are using a targeted cDNA microarray of 753 unique cDNA sequences shown to be correlated with secondary metabolism to uncover parts of this regulatory machinery. RNA preparations from *A. parasiticus* grown under conducive and non-conducive treatments for the four major variables known to affect aflatoxin production (nitrogen source, carbon source, culture temperature, and culture pH) were probed using our array. The expression data from these experiments were used to correlate consistent up- or down-regulated expression with aflatoxin production. Of the 768 genes on the array (including controls), 9 were found to be consistently up-regulated with toxin production, and 1 was consistently down-regulated with toxin production. The list of up-regulated genes included structural genes from the aflatoxin pathway cluster, as well as genes of unknown function. The lone down-regulated gene is of unknown function. Additionally, clustering of the expression data from all treatments revealed three distinct expression profile clusters for AF pathway genes. Other genes of putative and unknown function shared these expression profiles. Further functional characterization of these genes is underway.

Reduced sensitivity to sterol demethylation-inhibiting fungicides in populations of *Blumeriella jaapii* from Michigan. T. J. PROFFER (1,2), G. R. Ehret (1), and G. W. Sundin (1). (1) Michigan State University, East Lansing; (2) Kent State University-Salem, Salem, OH. Phytopathology 94:S86. Publication no. P-2004-0584-AMA.

In recent years grower observations and field trials have suggested the reduced efficacy of sterol demethylation-inhibiting fungicides (DMIs) for controlling cherry leaf spot. Monoconidial isolates of *Blumeriella jaapii* were collected in 2003 from naturally infected cherry trees (*Prunus cerasus* and *P. avium*) at sites with no history of DMI use, fungicide trial orchards with a mixed DMI history, and from an orchard where the DMI, Fenbuconazole, was used exclusively for eight years. Over 40 isolates from each group were grown on modified malt extract agar amended at 11 different rates of DMI (0.2 – 20 micrograms/milliliter) for three weeks. Four DMIs were tested: fenbuconzole, tebuconazole, fenarimol, and myclobutanil. Compared to the trees with no DMI history, the average minimal inhibitory concentrations for preventing colony growth were 12-67 times higher for isolates from the fungicide trial orchards and 32-88+ times higher for isolates from the orchard with exclusive DMI use. This is the first known report of resistance to DMIs for populations of *B. jaapii*.

Biochemical analyses of pomaceous stigma exudates and relevance to biological control of fire blight. P. L. PUSEY. USDA-ARS, Tree Fruit Research Laboratory, Wenatchee, WA. Phytopathology 94:S86. Publication no. P-2004-0585-AMA.

Microbial antagonism toward *Erwinia amylovora* on stigmas of pear and apple flowers has been widely demonstrated. Knowing the chemistry of stigma exudates could advance the selection and use of antagonists. Stigmas of different pomaceous cultivars and ages were analyzed for sugars and amino acids. Flowers collected in groups of 50 were pooled for analysis. Anthers were removed and stigma tips submerged and sonicated in 600 µl water. Pollen and macromolecules were removed by centrifugation and filtration. Sugars were converted to trimethylsilyl derivatives and analyzed by GC. Amino acids were converted to fluorescent derivatives using o-phthaldialdehyde and separated by HPLC. Predominant sugars were glucose and fructose. Predominant amino acids were asparagine, glutamine, proline and serine. Solid and liquid media developed with these compounds were used to evaluate antagonists based on competitive and antibiotic inhibition of *E. amylovora*. Preliminary results were consistent with in vivo flower assays. Stigma-based media are now being modified to understand modes of antagonism and their relation to chemical constituents on stigmas.

Genetic variation among *Verticillium dahliae* isolates. Q. M. QIN, G. E. Vallad, B. M. Wu, and K. V. Subbarao. Department of Plant Pathology, University of California, Davis, 1636 East Alisal Street, Salinas, CA 93905. Phytopathology 94:S86. Publication no. P-2004-0586-AMA.

Verticillium dahliae causes vascular wilt in a large number of economically important crops. In recent years, it has also appeared in crops once considered non-hosts. Little is known about the genetic relationships between isolates from new and established hosts. Sixty-six *V. dahliae* and three phytopathogenic *V. albo-atrum* isolates from 17 host species and several geographic locations were tested for genetic variation. The complete intergenic spacer (IGS) region of rRNA gene from each isolate was amplified, cloned and sequenced. The isolates showed significant genetic variation in the IGS region with the amplicons ranging from 1.7 kb to 2.2 kb. Isolates from artichoke and crucifer hosts were distinct from other isolates based on *Eco*RI digestion of the IGS amplicons, and 4 out 25 lettuce isolates had digestion patterns similar to artichoke isolates. IGS sequence analysis, amplified fragment length polymorphisms (AFLP) analysis and pathogenicity tests are being conducted to further clarify relationships among *V. dahliae* isolates.

Role of iron in development of cylindrocarpon root rot on ginseng roots. M. Rahman and Z. K. PUNJA. Simon Fraser University, BC, Canada. Phytopathology 94:S86. Publication no. P-2004-0587-AMA.

Cylindrocarpon root rot, caused by Cylindrocarpon destructans, is a major problem on American ginseng (Panax quinquefolius L.) grown in Canada. We studied the effects of iron (Fe) at 56 and 112 µg/ml on the disease severity. Hoagland's solution amended with these Fe concentrations significantly ($P < 0.0001$) enhanced disease initiation and severity on hydroponically-grown roots compared to zero (0) Fe. To elucidate the mechanisms by which Fe may

have increased disease, the effects of these levels of Fe on conidial germ tube length and secondary branching of hyphae were examined. After 8 and 16 hr of incubation, both parameters were significantly enhanced by Fe compared to 0 Fe. There was no effect of increased Fe levels on colony growth of C. destructans but significantly higher spore production was observed in the presence of Fe compared to 0 Fe in the medium. These levels of Fe had no effect on pectinase enzyme activity, but polyphenoloxidase activity was significantly ($P < 0.0001$) enhanced by the presence of Fe in the growth medium compared to 0 Fe. Foliar applications of Fe (as FeNaEDTA) to ginseng plants thrice during the 2002 growing season increased the Fe concentration in roots, and these roots had larger lesions following Cylindrocarpon inoculation in vitro. Wounding of roots followed by drenching the rhizosphere with a 0.05% solution of FeNaEDTA in the field during the 2003 growing season significantly enhanced root rotting (90% plant mortality) compared to water control. Higher levels of Fe in soil and in root tissues may enhance root rot through effects on the pathogen and the host.

Cloning and expression of phage holin and endolysin genes for potential control of diseases caused by Ralstonia and Xanthomonas. C. RAMADUGU and D. W. Gabriel. Integrated Plant Genetics, 12085 Research Drive, Alachua, FL 32615. Phytopathology 94:S87. Publication no. P-2004-0588-AMA.

Attempts to use bacteriophages to manage plant diseases has had only limited success, primarily because 1) bacteria in infected plants are often inaccessible to applied phage, and 2) bacteriophages attack only a narrow range of bacteria. We isolated and fully sequenced a double-stranded DNA phage that can infect and lyse *Xanthomonas campestris*. On plate overlay assays, infected *X. campestris* also caused lysis of heterologous bacteria that were not infected by the phage, including *Ralstonia solanacearum* and *X. citri*. The phage genome was cut with endonucleases and most fragments cloned. Some of the fragments, later determined to carry three holin and one endolysin genes, could not be cloned. The endolysin and the three holin genes were cloned without their native promoters in low copy, stringently regulated expression vectors. Upon induction of expression, all three holins and the endolysin caused lysis of several species of phytopathogenic bacteria, including *X. citri*, *X. pelargonii*, *X. vesicatoria*, *R. solanacearum* and *Xylella fastidiosa*. We are in the process of transforming citrus, tomato and *Pelargonium* plants with these lytic genes in order to evaluate their potential to control disease.

Interactions between *Puccinia psidii* and *Oxyops vitiosa*: The biological control agents of *Melaleuca quinquenervia* in Florida. M. B. RAYAMAJHI, T. K. Van, P. D. Pratt, and T. D. Center. USDA-ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL 33314. Phytopathology 94:S87. Publication no. P-2004-0589-AMA.

Puccinia psidii (rust fungus) and *Oxyops vitiosa* (weevil) compete for expanding foliage and cause damage to branch tips of the invasive tree *Melaleuca quinquenervia* (melaleuca) in Florida. Interactions between these biocontrol agents are unknown. Herein, we report results documenting their influence on each other's lifecycle. Adult weevils consumed less rust infected (2.4 mg/day) compared to healthy (3.3 mg/day) fresh-leaf tissues. Female weevils oviposit higher proportions (3:1) of eggs on healthy than on rust-diseased foliage. When fed only on diseased tissues, larval survival decreased with increased pustule coverage on foliage. Weevils feeding on rust infected leaves carried ca 20,000 spores on their body surface, which initiated infection on new plants they visited. Weevil damaged plants had more off-season foliar production that supported rust population year-around. Overall, rust-weevil association appears to result in more damage of new growth on melaleuca through fuller niche utilization.

Role of type I secretion in Pierce's disease. J. D. REDD (1), D. L. Hopkins (2), and D. W. Gabriel (1). (1) Plant Pathology Dept., University of Florida, Gainesville, FL; (2) Mid-Florida Research and Education Center, University of Florida, Apopka, FL. Phytopathology 94:S87. Publication no. P-2004-0590-AMA.

Xylella fastidiosa is a fastidious, xylem-inhabiting, Gram-negative bacterium that causes Pierce's disease (PD) of grape and Citrus Variegated Chlorosis (CVC). Both CVC and PD strains have been fully sequenced, revealing no type III, but two type I secretion systems, both dependent on a single *tolC* homologue. In an effort to generate knockout mutants of *tolC*, both marker interruption and marker exchange mutagenesis were used; only marker-exchange appeared to work, using pGEM-T as delivery vector and *nptII* as marker. PCR and Southern blot analyses confirmed marker exchange at the *tolC* locus. Grape (var. Carignane) plants inoculated with mutant (*tolC*::*nptII*) strains exhibited no symptoms of PD, indicating that pathogenic ability of PD strains may be dependant on *tolC* and type I secretion. Complementation assays using *tolC* in the mutant strains are in progress to help confirm this hypothesis.

Telomere-association of pathogenicity genes in *Magnaporthe grisea*–A novel mechanism for pathogenic adaptation? C. J. REHMEYER (1), W. Li (2), C. Staben (2), C. L. Schardl (1), and M. L. Farman (1). (1) Department of Plant Pathology; (2) Department of Biological Sciences, University of Kentucky, Lexington, KY. Phytopathology 94:S87. Publication no. P-2004-0591-AMA.

In many eukaryotic microbes, telomere regions harbor genes with important roles in niche exploitation. Frequent recombination in these locations is believed to produce the variation that fuels adaptation. Subtelomeric regions in *Magnaporthe grisea* are known to contain avirulence genes, suggesting that telomere-associated genes may contribute to pathogenic adaptation in this fungus. The *M. grisea* genome has been sequenced and assembled in a collaborative project between North Carolina State University and The Whitehead Institute. However, the telomeres and their associated sequences are largely absent from the assembly. We have used bioinformatic approaches to identify all fourteen *M. grisea* telomeres among sequence reads that were excluded from the NCSU-WI assembly. To extend the assembled DNA sequences out to the chromosome tips, we have sequenced fosmid clones representative of each telomere. We present an analysis of genes found in these regions, particularly those that may be involved with host/pathogen interactions. Finally, we are investigating the mutational mechanisms underlying telomere variation that may alter expression of pathogenicity genes, providing a means of rapid pathogenic adaptation in this fungus.

Analysis of *Pseudomonas syringae* populations associated with a bacterial canker epidemic in Michigan. L. J. RENICK, A. G. Cogal, and G. W. Sundin. Michigan State University, Dept. Plant Pathology, East Lansing, MI 48824. Phytopathology 94:S87. Publication no. P-2004-0592-AMA.

A severe bacterial canker epidemic occurred on several sweet cherry varieties in Michigan in 2002. Blossom infection and subsequent canker formation was observed following a prolonged freeze event during bloom. We recovered blossom and canker isolates of *P. syringae* from 42 orchards from the three major cherry-growing areas [Southwest (SW), West Central (WC) and Northwest (NW)] of Michigan in 2003. Average *P. syringae* populations were log 3.9, 5.6, and 5.1 cfu/g blossom tissue from the SW, WC, and NW areas, respectively. Copper-resistant *P. syringae* comprised 47.4%, 21.1%, and 3.1%, of the total populations from the SW, WC, and NW areas, respectively. Ten isolates were randomly chosen for analysis from each orchard population. Identification of these isolates using PCR assays confirmed that 66% and 1% of the isolates were *P. syringae* pv. *syringae* (Pss) and *P. syringae* pv. *morsprunorum*, respectively; we were unable to determine the pathovar status of 33% of the isolates. Pathogenicity of all isolates on immature cherry fruit was confirmed. The frequency of ice nucleation of individual Pss strains varied from log –4.20 to –1.05. Genetic fingerprinting of Pss strains using AP-PCR indicated a high level of diversity, and diversity was assessed at the level of blossom, orchard, and growing region.

Understanding the potato – *Phytophthora infestans* compatible interaction. S. Restrepo (1), W. E. Fry (2), and C. D. SMART (1). Department of Plant Pathology, Cornell University, (1) NY State Agricultural Experiment Station, Geneva NY 14456; (2) Ithaca, NY 14850. Phytopathology 94:S87. Publication no. P-2004-0593-AMA.

Potato cDNA microarrays were exploited in a search for genes that are differentially expressed during a compatible interaction with *Phytophthora infestans*. Gene expression was followed over time from 0 to 72 hours after inoculation of a susceptible potato cultivar with the pathogen. Array data were subjected to rigorous statistical analyses. A significant differential expression was observed for 13% of the genes. The transcripts for many proteins involved in photosynthesis were repressed at all time points. Genes involved in the biosynthesis of ethylene were strongly down-regulated 48h after the inoculation following an initial induction at 6h. In addition, an early and transient induction was observed for a group of genes encoding DNA binding proteins and proteinase inhibitors. Interestingly, a salicylic acid-binding protein, carbonic anhydrase, was repressed during the time course of the experiment. Preliminary data show that *Nicotiana benthamiana* plants with carbonic anhydrase silenced are more susceptible to *P. infestans* than empty vector controls.

Analysis of mating-type distribution and RAPD diversity of *Didymella rabiei* isolates from Tunisia. A. RHAIEM (1), M. Chérif (1), and P. S. Dyer (2). (1) Institut Natinal Agronomique de Tunisie, 43 Avenue Charles Nicolle, 1082 Cite Mahrajene, Tunis, TUNISIA; (2) School of Biological Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK. Phytopathology 94:S87. Publication no. P-2004-0594-AMA.

A total of 124 isolates of *Didymella rabiei* (anamorph *Ascochyta rabiei*) were obtained from chickpea affected with *Ascochyta* blight collected from different fields in the five main crop regions in Tunisia. The mating type

(MAT1-1 or MAT1-2) of isolates was determined using a recently developed molecular mating-type diagnostic PCR test, involving the use of a MAT1-1 specific primer (Sp21), a MAT1-2-specific primer (Tail 5) and a MAT-region common flanking primer (Com1). Among the 124 isolates tested for mating type, overall 81% were found to belong to MAT1-1 and 19% to MAT1-2. However, significant differences in the frequency of the two mating types were found at different sample locations. All isolates obtained from the two regions of Beja and Bizerte were of MAT1-1 genotype. The respective percentages of MAT1-1 and MAT1-2 in the other locations were 62% and 38% in Nabeul, 59 and 41% in Kef, 75% and 25% in Jendouba. Forty isolates representative of the 5 locations were analysed further by randomly amplified polymorphic DNA (RAPD) analysis (including primers OPW06, OPW15, OPW18 and OPW19) in order to evaluate genetic diversity within the populations of *D. rabiei*, and to assess possible correlations between RAPD groupings, geographic origin and mating type distribution. Key words: *Didymella rabiei*, Mating type, RAPD.

Quinoxyfen performance on powdery mildew in low desert melons, 1999-2003. J. M. RICHARDSON (1), M. E. Matheron (2), T. A. Turini (3), and J. P. Mueller (4). (1) Dow AgroSciences LLC, 9330 10th Ave., Hesperia, CA 92345, (2) University of Arizona, 6425 W. 8th St., Yuma, AZ 85364; (3) UC Cooperative Extension, 1050 E. Holton Rd., Holtville, CA 92250; (4) Dow AgroSciences LLC, 175 Mesquite Ct. Brentwood, CA 94513. Phytopathology 94:S88. Publication no. P-2004-0595-AMA.

Powdery mildew is a destructive disease of melons in the low desert production region of southern California and Arizona. Left uncontrolled, *Podosphaera xanthii* infestations can result in the collapse of commercial melon fields before the crop reaches maturity. Quinoxyfen (Quintec™), now registered on grapes, cherries and hops in the USA, has a unique mode of action for mildew control. Field studies were conducted with quinoxyfen to evaluate powdery mildew control in Yuma, AZ and Holtville, CA. Competitive standards and potential new products were compared to this member of the quinoline fungicide class. The various fungicides were applied repeatedly by ground application at 10-day intervals. Disease ratings were taken for upper and lower leaf surfaces. The results demonstrate that quinoxyfen will be among the best performing products for control of *Podosphaera xanthii* in melons when U.S. registration on this crop group is obtained.

New association of a toxigenic *Rathayibacter* sp. and *Anguina woodi* in *Ehrharta villosa* var. *villosa* in South Africa. I. T. Riley (1), A. Swart (2), E. Postnikova (3), I. Agarkova (3), A. K. Vidaver (3), and N. W. SCHAAD (4). (1) University of Adelaide, Australia; (2) Plant Protection Research Institute, South Africa; (3) University of Nebraska, Lincoln, NE; (4) USDA/ARS, Ft. Detrick, MD. Phytopathology 94:S88. Publication no. P-2004-0596-AMA.

A dry translucent yellow bacterial gummosis associated with *A. woodi*, a leaf gall nematode of *E. villosa* var. *villosa* in South Africa, was examined. Some affected galls contained bacteria only and some contained both bacteria and dormant nematodes. The bacterium was readily cultured and identified phenotypically as a *Rathayibacter* sp. The bacterium was Gram-positive, non-acid fast, catalase positive, hydrolyzed esculin, failed to produce acid from sorbitol, and oxidase negative. Colonies were round, non-mucoid, yellow, and 1-2 mm in diameter after 7-8 days at 25°C on NBY agar. Bacteria from galls and cultures adhered to *A. woodi* juveniles but not those of *A. funesta* or *A. tritici*. As with *R. toxicus*, the agent of ryegrass toxicity, bioassays indicated *in planta* production of toxin. DNA from the bacterium had only 70% homology with other *Rathayibacter* species and gave a distinct AFLP pattern. These data are indicative of a new *Rathayibacter* species.

Fungi associated with wood decomposition in forest soils of the northwestern U.S.A. R. C. RIPPY (1,2), N. B. Klopfenstein (1), M.-S. Kim (1), D. S. Page-Dumroese (1), L. M. Carris (2), J. D. Rogers (2), and M. F. Jurgensen (3). (1) USDA Forest Service – RMRS, Moscow, ID; (2) Washington State University, Pullman, WA; (3) Michigan Technological University, Houghton, MI. Phytopathology 94:S88. Publication no. P-2004-0597-AMA.

Fungi associated with wood decomposition have diverse roles that contribute to the chemical and physical components involved in long-term productivity of forest soils. As part of a two-year study, standard substrate wood stakes were buried to a depth of 30 cm in the mineral soil and at the forest floor/mineral soil interface on six forested sites in Idaho and Washington to examine soil temperature and moisture influences on fungal distribution and wood decomposition rates. Fungi are isolated from these stakes at six-month intervals. Morphological characterization and ITS rDNA sequencing are being used for fungal identification. At the midpoint of this study, 22 genera of basidiomycetes, 25 genera of ascomycetes, and 3 genera of zygomycetes have been identified. Determining the impacts of forest management on belowground productivity and fungal communities is essential to develop appropriate management practices.

Metabolic activity of *Enterobacter cloacae* in cucumber and pea spermosphere. D. P. ROBERTS (1), J. S. Buyer (1), and C. J. Baker (2). (1) USDA-ARS, SASL; (2) USDA-ARS, MPPL. Phytopathology 94:S88. Publication no. P-2004-0598-AMA.

In situ bioluminescence experiments and *in vitro* oxygen consumption experiments with *Enterobacter cloacae* 501R3 on cucumber and pea seed were conducted to determine the influence of host seed on metabolic activity by this bacterium in the spermosphere. Total metabolic activity per population of 501R3 and average metabolic activity per cell of 501R3 was significantly greater in pea spermosphere than in cucumber spermosphere. Pea seed releases orders of magnitude more carbohydrate and amino acid compounds than cucumber seed during the first few days of germination. These compounds supported high rates of metabolic activity by 501R3 in *in vitro* experiments. *In vitro* oxygen consumption experiments on cucumber and pea seed exudate and *in situ* bioluminescence experiments with metabolic mutants of 501R3, and with exogenous addition of reduced carbon compounds, confirmed that qualitative and quantitative differences in exudation by the host seed influence metabolic activity by *E. cloacae* in the spermosphere.

Phylogenetic analysis of Alaskan isolates of *Barley yellow dwarf virus*-PAV and *Cereal yellow dwarf virus*-RPV. N. L. ROBERTSON (1) and R. French (2). (1) USDA-ARS, Palmer, AK; (2) USDA-ARS, Lincoln, NE. Phytopathology 94:S88. Publication no. P-2004-0599-AMA.

Barley yellow dwarf is the most destructive viral disease in small grains worldwide. In 2002 and 2003, Alaskan isolates of *Barley yellow dwarf virus* (BYDV)-PAV and *Cereal yellow dwarf virus* (CYDV)-RPV were detected in barley (*Hordeum vulgare*) and oats (*Avena sativa*) by RT-PCR and ELISA. PCR products spanning the viral coat protein gene of 73 isolates were cloned and sequenced. Most (44/49) PAV isolates were similar to PAV-129 with the remainder related to PAV-NY. The RPV isolates clustered in three groups: 13 resembled an RPV isolate from Mexico, 5 were closely related to RPV-NY, and 6 were distinct from any CYDV sequence currently in GenBank. The mean within-group sequence diversities were less than 1% for each of the 2 Alaskan PAV and 3 Alaskan RPV genotype clusters.

Cytological and molecular aspects of silicon-mediated resistance to rice blast. F. A. RODRIGUES, J. A. Rollins, L. E. Datnoff, W. M. Jurick II, and J. B. Jones. Department of Plant Pathology, University of Florida, Gainesville, FL 32611-0680. Phytopathology 94:S88. Publication no. P-2004-0600-AMA.

Cytological and molecular aspects of an incompatible and a compatible rice-*Magnaporthe grisea* interaction with a silicon (Si) soil amendment were investigated. Katy, a resistant cultivar, responded to an avirulent race of *M. grisea* through the development of a hypersensitive reaction (intense granulation of the cytoplasm and bright autofluorescence of epidermal cells), strong induction of PR-1 and peroxidase transcripts, and accumulation of high levels of phenolics and lignin which dramatically reduced hyphal growth within the invaded epidermal cell. These defense responses were similar in both Si^- and Si^+ treatments. On the other hand, in Si^+ plants of the susceptible cultivar M201, a differential accumulation of glucanase, peroxidase and PR-1 transcripts and the production of phenolics and lignin restricted hyphal growth in the epidermal cells. Conversely, in Si^- plants, *M. grisea* successfully grew and formed an extensive branched mycelium in the first-invaded epidermal cell and colonized many neighboring cells despite strong induction of chitinases, glucanase, chalcone synthase, phenylalanine ammonia-lyase, peroxidase, and PR-1 transcripts and high levels of phenolics and lignin. Autofluorescence of epidermal cells was quenched as the lesions expanded when compared to Si^+ plants. In conclusion, the results of this study strongly suggest that Si plays an active role in rice blast resistance rather than just the formation of a physical barrier to penetration by *M. grisea*.

IPM strategies for control of foliar diseases in commercial carrot fields in Wisconsin. P. M. ROGERS and W. R. Stevenson. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706. Phytopathology 94:S88. Publication no. P-2004-0601-AMA.

Integrated Pest Management (IPM) practices in processing carrot production are important for managing foliar diseases with fewer pesticide inputs. Development of novel pest control strategies is crucial to offset industry dependence upon repeated chemical applications. The objective of this research was to compare current grower practices to reduced-risk weather-based spray programs and to assist in grower adoption of IPM. Components of IPM included in 2003 field trials were resistant or field tolerant cultivars, foliar disease monitoring (scouting), weather-timed sprays and reduced-risk chemical alternation. Fungicide programs were selected based upon previous field results and susceptibility of cultivars grown. Weather-based application intervals were determined by on-site microclimate data collected by in-canopy weather stations located at each farm. Results confirmed similar

yields, root quality and crop value were possible with 2 to 3 fewer fungicide applications. On resistant cultivars such as Bolero, Carson and Enterprise, fungicide inputs may be reduced by 50% (2 versus 4 sprays). For susceptible cultivars, similar control was observed with 4 sprays using the Tom-Cast scheduling program vs. 6 (Heritage) or 7 (Indiana) sprays using standard grower programs. The use of multiple IPM tactics has demonstrated real potential to reduce pesticide use in WI carrot production.

Comparison of weather-based fungicide programs on two carrot cultivars differing in susceptibility to *Alternaria dauci* and *Cercospora carotae*. P. M. ROGERS and W. R. Stevenson. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706. Phytopathology 94:S89. Publication no. P-2004-0602-AMA.

Carrot foliar diseases caused by *A. dauci* and *C. carotae* are recurrent problems for carrot production worldwide. Wisconsin's commercial carrot growers rely exclusively on 4-12 fungicide applications to manage these two diseases. New fungicide chemistry coupled with increased host resistance offers reduced-risk alternatives to current disease management programs. Field experiments in 2002 and 2003 evaluated fungicide application schedules triggered by pathogen-favorable environmental conditions on two carrot cultivars with different levels of disease resistance. Disease Severity Value (DSV) treatments were based on in-canopy leaf wetness and temperature data. These treatments were applied by using 15 and 20 DSV thresholds. Cultivar susceptibility played a key role in the performance of spray programs evaluated. Fungicide programs determined by pathogen favorable environmental conditions reduced fungicide inputs by stretching spray intervals, without sacrificing disease control or yield. By integrating host resistance, scouting and weather timed sprays, growers can sustain carrot production using fewer chemical inputs without sacrificing disease control, yield or quality.

Use of boron for the control of Eutypa dieback of grapevines. P. E. ROLSHAUSEN and W. D. Gubler. Dept. Plant Pathology, UC Davis, CA 95616. Phytopathology 94:S89. Publication no. P-2004-0603-AMA.

Eutypa dieback is a chronic disease that affects grape production worldwide. The causal agent, *Eutypa lata*, infects grapevines through pruning wounds by ascospores released after rain. Control of the disease is done by applying fungicides on the surface of pruning wounds. Boron in a boric acid formulation (17.5% Boron) was effective against *E. lata*. The EC_{50} value for ascospore germination and mycelial growth were determined to be 83 and 22 part per million of Boron, respectively. Two products were formulated with 5% boric acid (8750 part per million of Boron); biopaste (boric acid plus chemical paste) and bioshield (boric acid plus a spore suspension of *Cladosporium herbarum*) provided total protection in laboratory assays and gave long term protection of pruning wounds in several field trials. Other Boron formulations worked as well as boric acid *in vitro* when adjusted for Boron concentration. Systemic redistribution of Boron in green tissues was not detected. However, phytotoxicity was observed in one trial when the bud located at the first node below the treated wound failed to push in about 45% of the spurs.

Construction and characterization of a polygalacturonase mutant of *Xylella fastidiosa*. M. C. ROPER (1), L. Carl Greve (2), J. Labavitch (2), and B. Kirkpatrick (1). (1) Dept. Plant Pathology; (2) Dept. Pomology, University of California, Davis, 95616. Phytopathology 94:S89. Publication no. P-2004-0604-AMA.

Xylella fastidiosa (*Xf*), the causal agent of Pierce's disease, poses a major threat to the California grape industry. *Xf* colonizes the xylem elements of grapevines and is able to breach the pit pore "membranes" separating xylem vessels and, thus, develop systemic infections. The mechanism by which the pit membranes are disrupted is not known. It is likely that this bacterium utilizes cell wall degrading enzymes to break down these pit "membranes" because genes involved in plant cell wall metabolism have been identified in the *Xf* genome. These genes include several beta-1,4 endoglucanases, several xylanases, and one polygalacturonase. In this study we constructed mutants in the single copy polygalacturonase gene by marker exchange mutagenesis. Double crossover mutants were obtained and these mutants are currently being evaluated for potential differences in *Xf* movement and/or virulence in *Vitis vinifera* grapevines.

Value of environmental factors in predicting cotton seedling disease severity. C. S. Rothrock (1), M. L. SCHULZ (1), P. D. Colyer (2), E. E. Gbur (1), and T. L. Kirkpatrick (1). (1) University of Arkansas, Fayetteville, AR; (2) Louisiana State University Agricultural Center, Red River Research Station, Bossier City, LA. Phytopathology 94:S89. Publication no. P-2004-0605-AMA.

Seedling diseases of cotton reduce stand and early season vigor of plants, causing losses in yield, and in some cases replanting costs. The primary seedling disease pathogens of cotton are *Rhizoctonia solani*, *Thielaviopsis basicola*, *Pythium* spp., and *Fusarium* spp. These pathogens are ubiquitous in cotton soils, with the exception of *T. basicola*. Thus, soil environment at and shortly after planting appears to be the major factor for seedling disease severity and stand establishment. Growers must decide whether to invest in fungicides at planting to insure stands. In-furrow and hopper-box studies from Arkansas and Louisiana in growers' fields and at experiment stations were used to collect stand response, disease pressure, stand uniformity, and yield. Weather data including soil temperatures and rainfall also were collected. The studies were used to examine the effect of environmental factors on seedling diseases and develop a decision-aid for growers. The decision-aid will allow producers to make planting decisions to increase the likelihood of a uniform stand and economic decisions on the use of in-furrow and hopper-box fungicides. Models indicate that temperature and moisture affected seedling disease severity and stand, reinforcing the practicality of a decision-aid. The relative importance of stand response, disease indices, pathogen isolation, and product efficacy are also being examined from the data.

Characterization of a new foveavirus associated with cherry rusty mottle disease. M. E. ROTT, V. L. Johnson, and M. P. Belton. Centre for Plant Health, Canadian Food Inspection Agency. Phytopathology 94:S89. Publication no. P-2004-0606-AMA.

Cherry rusty mottle disease (CRMD) was first identified in North American and Europe in the 1940-50's. In Europe, CRMD covers a number of different diseases with common characteristics and symptoms. Symptoms range form rusty mottling of the leaf surface, to a yellowish-green leaf pattern, to no obvious symptoms, depending on the isolate and the host variety infected. It is widely distributed in England and other parts of Europe affecting many cultivars and is graft transmissible. Previous work identified an associated viral agent closely related to the foveavirus Cherry green ring mottle (CGRMV) with several European isolates of CRM. It was suggested that the disease was caused by a strain(s) of CGRMV. In North America CRMD is wide spread in Washington, Idaho, Montana, and Oregon. Symptoms of the disease vary depending on cherry cultivar, disease strain and environmental conditions. Infected trees lose up to 30-70% of leaves on ripening and fruits remain smaller and tasteless. We have identified a new foveavirus associated with a North American isolate of CRMD, Cherry rusty mottle virus (CRMV). Data will be presented on the complete genomic sequence of CRMV and comparisons will be made between the closely related cherry infecting foveaviruses, CRMV, CGRMV and Cherry necrotic rusty mottle virus.

Fungi associated with bark wounds on indigenous African trees. J. ROUX (1), R. N. Heath (1), G. Meke (2), C. Nguvulu (3), F. Mlambo (4), C. J. Geldenhuys (5), and M. J. Wingfield (1). (1) Dept of Genetics, TPCP, FABI, Univ. of Pretoria, Pretoria, South Africa; (2) Forestry Research Institute, PO Box 270, Zomba, Malawi; (3) Forest Research Div., PO Box 22099, Kitwe, Zambia; (4) Copperbelt Univ., Kitwe, Zambia; (5) Forestwood cc., PO Box 228, La Montagne 0184, Pretoria, South Africa. Phytopathology 94:S89. Publication no. P-2004-0607-AMA.

Wounds provide ideal infection sites for many fungal tree pathogens. In Africa, tree bark is commonly harvested for medicinal purposes. In some cases, these practices are not sustainable due to habitat destruction and unsustainable harvesting practices. As part of a larger study to develop sustainable bark harvesting practices, the possible role of fungal pathogens infecting wounds made during bark harvesting was investigated. Fungi infecting bark wounds on several indigenous tree species were identified and considered in terms of their probable roles as pathogens. Several tree species were artificially wounded in Malawi, South Africa and Zambia. Strips of bark, 1 metre long and 20 centimetres wide were removed from each tree to expose the cambium. Wounds were evaluated 6-10 weeks after wounding. Both wood and bark samples were collected. Isolations were made from the surfaces of wounds as well as from underlying wood. All isolates were identified based on morphological characteristics and some identifications were confirmed using DNA sequence data. Several fungi were isolated from wound surfaces, including species of *Penicillium*, *Aspergillus* and *Pestalotiopis*. Several species belonging to genera containing tree pathogens were also isolated. They included species of *Fusarium*, *Pesotum* and *Ceratocystis*. The latter fungi are currently being studied in greater detail. All indications are that fungal pathogens play an important role in the decline of trees from which bark has been harvested.

The occurrence of the wilt pathogen, *Ceratocystis albofundus*, on native South African trees. J. ROUX (1), L. Labuschagne (2), R. N. Heath (1), G. Kamgan Nkuekam (1), and M. J. Wingfield (1). (1) Department of Genetics, Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; (2) Gauteng Directorate of Nature Conservation, Private Bag 15163, Lynn East, 0039, South Africa. Phytopathology 94:S89. Publication no. P-2004-0608-AMA.

Ceratocystis albofundus causes a serious wilt disease of exotic plantation grown *Acacia mearnsii* trees in South Africa. The fungus was recently also reported from *A. mearnsii* trees in Uganda and Kenya. It has previously been suggested that *C. albofundus* is native to Southern Africa. This hypothesis was based on the fact that the fungus has been reported only from Africa and that herbarium specimens from a native South African *Protea* sp. contains a fungus resembling *C. albofundus*. Population genetic studies using molecular markers have shown that the diversity of *C. albofundus* in South Africa and Uganda is high. The aim of this study was to survey native South African plants, particularly *Protea* spp. for the presence of *C. albofundus*. Surveys of wounds on trees were conducted in a nature reserve near Pretoria. Fungi were identified based on morphology and DNA sequence data. *C. albofundus* was found on eight native South African tree genera including *Protea gaguedii*, *Burkea africana*, *Combretum molle*, *C. apiculatum* and *C. zeyherii*, *Terminalia sericea*, *Ochna pulchra*, *Ozoroa paniculata*, *Acacia caffra* and *Faurea saligna*. Trials to determine the pathogenicity of *C. albofundus* on native tree species are currently underway, but no tree deaths linked to the presence of the fungus has been noted. This, and the common occurrence of *C. albofundus* on a wide range of native trees provide further evidence suggesting that it is native to Africa and has adapted to infect exotic *A. mearnsii*.

Survival of *Aphanomyces cochlioides* oospores in soil following preconditioning at different relative humidities. J. M. ROWNTREE and C. E. Windels. University of Minnesota, Northwest Research & Outreach Center, Crookston, MN 56716. Phytopathology 94:S90. Publication no. P-2004-0609-AMA.

Long-term persistence of *A. cochlioides* (A.c.) in soil is attributed to survival of oospores. Our objective was to determine if survival of oospores in diseased sugar beet (*Beta vulgaris*) tissue was affected by preconditioning at various relative humidities (RH) before burial in soil. Two-week-old hypocotyls (2-cm length) of two cultivars were placed in sterile water, inoculated with A.c. and incubated for 5 weeks. Then, hypocotyls containing oospores were exposed to <1, 50 or 100% RH for 1 week. Hypocotyls (with an average of 4,885 viable oospores each) were placed in nylon mesh bags (10 micron pores, one hypocotyl/bag) and immediately buried at 2.5 or 15 cm in a sugar beet field in early June. Samples were removed weekly (June – July), biweekly in August, and once in September and rated decomposition of hypocotyls and number of living and dead oospores. There were no significant differences in oospore survival among treatments (preconditioning RH, variety, burial depth) or interactions. Numbers of oospores decreased linearly until 37 days after burial and then began to stabilize; by 109 days after burial, an average of 160 oospores/hypocotyl were alive. Decrease in viable oospores was correlated with loss of intact hypocotyl tissue (R^2 = 0.9012). Overall, survival of oospores of *A. cochlioides* was dependent on intact plant tissue and was unaffected by preconditioning of oospores at different relative humidities.

Changes in the population dynamics of a Florida *Citrus tristeza virus* isolate upon aphid transmissi A. ROY (1), K. L. Manjunath (2), and R. H. Brlansky (1). (1) Univ. of Florida, CREC, Lake Alfred, FL 33850; (2) Univ. of Florida, Plant Path. Dept., Gainesville, FL 32611. Phytopathology 94:S90. Publication no. P-2004-0610-AMA.

Citrus tristeza virus (CTV) usually occurs as a mixture of different genotypes in nature; with one of them often dominating the population. A CTV isolate from Florida, FS627, was selected for the present study since previous work indicated the presence of mixed populations and changed phenotypes upon aphid transmission. CTV has a monopartite, positive sense RNA genome of about 20 kb and exhibits over 30% diversity in the 5′ half and below 10% in the 3′ half among different genotypes. A 785 bp region in the 5′ (696-1480 nt) and a 672 bp coat protein gene (p25) in the 3′ end of the CTV genome were selected for analysis of FS627. Analysis of single strand conformational polymorphisms and RNA sequences of RT-PCR amplified products of the above regions were used to determine the genotype/s present in both parent and aphid transmitted isolates of FS627. While the parent isolate had T36 as the major genotype, T30 was the main genotype in most of the aphid-transmitted (AT) subisolates. Some intermediate genotypes were detected that differed from the original FS627 and AT subisolates.

Post-transcriptional gene silencing in transgenic *Nicotiana benthamiana* plants induced by self-complementary constructs containing chimeric cDNA sequences from *Citrus tristeza virus*. G. ROY (1), M. R. Su hana (1), A. M. Dandekar (2), S.-W. Ding (3), and B. W. Falk (1). (1) Dept. of Plant Pathology, University of California, Davis, CA 95616; (2) Dept. of Pomology, University of California, Davis, CA 95616; (3) Dept. of Plant Pathology, University of California, Reverside, CA 92521. Phytopathology 94:S90. Publication no. P-2004-0611-AMA.

Several transgenic *Nicotiana benthamiana* lines containing chimeric, self-complementary constructs of *Citrus tristeza virus* (CTV) sequences were generated and analyzed for post-transcriptional gene silencing (PTGS) mediated resistance. Recombinant *Potato virus X* (PVX) constructs containing corresponding CTV sequences to the transgene constructs were used to inoculate plants, and plants were analyzed by symptom phenotype, RT-PCR and northern blot hybridization. Most inoculated plants developed symptoms typical of PVX, although some lines showed a good proportion of non-symptomatic plants. RT-PCR analyses indicated that some symptomatic transgenic plants contained wild type PVX, but not the recombinant used for inoculation. Small interfering RNAs (siRNAs; hallmarks of PTGS) also were detected by northern hybridization in several plants. These data suggest that PTGS-mediated resistance to CTV sequences can be generated by this approach.

Detection of deletion mutants in spotted rays. M. RYBA-WHITE (1), P. Manosalva (1), S. Hulbert (1), H. Leung (2), and J. E. Leach (1). (1) Kansas State University, Manhattan, KS; (2) IRRI, Los Banos, Philippines. Phytopathology 94:S90. Publication no. P-2004-0612-AMA.

Mutants are essential tools for functional analysis of plant genes. We produced a large collection of chemical- and irradiation-induced mutants in IR64, the most widely grown indica variety of rice in Asia. Over 30,000 independent mutant lines have been advanced to the M4 generation (http://www.iris.irri.org/cgi-bin/MutantHome.pl). While these deletion mutants are easy and inexpensive to generate, the mutations are not tagged, making it difficult to identify them within a target gene. We developed a novel high throughput strategy to technically simplify and increase the efficiency in detection of deletions in target genes. The technique involves spotting of DNA from individual M4 mutant lines (homozygous at most deletion loci) onto membranes and hybridization with overlapping oligonucleotide (overgo) probes designed to a target gene, for example, a defense response gene. The overgo probes are short, but intensely labeled and, thus, provide a sufficient signal to detect the gene in wild type plants. Lack of hybridization of the overgo to the spotted genomic DNA indicates a deletion. Proof-of-concept and optimization studies were performed using DNA from the spotted leaf 11 lesion mimic mutant, which contains a 95 bp deletion in the (*spl11*) gene and the wild type parent IR64. This strategy will not only be useful for rice, but will likely be applicable to other cereal or crop species.

Fungus diseases on certified seed potato tubers. A. SAAD (1), L. Hanna (1), R. Abi Ghanem (1), and E. Choueiri (2). (1) American University of Beirut, Beirut, 1107 2020 Lebanon; (2) LARI, Rayak, Lebanon. Phytopathology 94:S90. Publication no. P-2004-0613-AMA.

The rapid development of tuber and soilborne diseases in areas that had never been cropped with potatoes before suggested that such diseases were being introduced on seed potatoes. Samples of potato tubers from thirty imported commercial seed stocks of different cultivars and classes, received in 2001 and 2003, were examined macroscopically, microscopically, and by ELISA for fungus diseases. Although all shipments of certified seed potato tubers were accompanied by phytosanitary certificates, none of the seed stocks sampled was free of fungal diseases. The pathogens and their percent incidence range were as follows: *Fusarium* spp. (1-54%), *Helminthosporium solani* (6-100%), *Polyscutalum pustulans* (3-24%), *Rhizoctonia solani* (1-64%), *Streptomyces scabies* (3-67%), and *Verticillium albo-atrum* (5-20%). Drastic changes in the potato certification schemes and inspection procedures, at international levels, need to be undertaken to improve the health status of seed potato tubers and avoid dissemination of diseases or new exotic strains of pathogens that may result in serious disease outbreaks and high economic losses.

Detection and identification of a new marafivirus from grapevine. S. SABANADZO C, N. Abou Ghanem-Sabanadzovic, and A. Rowhani. Department of Plant Pathology, University of California, Davis, CA 95616. Phytopathology 94:S90. Publication no. P-2004-0614-AMA.

In the process of developing a multiplex PCR detection (mPCR) for Grapevine fleck virus-like viruses, a new virus was identified and partially characterized from a Kober5BB accession. The genomic organization of the 3′ end of the virus genomic RNA resembled that of marafiviruses, encoding a putative polyprotein containing the conserved "marafibox" sequence that precedes the coat protein cistron. The sequence analysis shows also a high GC content (c. 60%), a hallmark of viruses belonging to the family *Tymoviridae*. In phylogenetic analyses this virus always clustered within the genus *Marafivirus*. The putative viral coat protein shared highest similarities with *Grapevine rupestris vein feathering virus* (c. 57%). The impact of infection by this virus on different grapevine species and/or cultivars is under study. Additional cDNA cloning and sequencing has been initiated to completely characterize this virus.

New data on cherry viruses in Northern California. S. Sabanadzovic, N. Abou Ghanem-Sabanadzovic, A. Rowhani, and J. K. UYEMOTO. USDA-ARS and Department of Plant Pathology, University of California, Davis, CA 95616. Phytopathology 94:S91. Publication no. P-2004-0615-AMA.

Bing and Kwanzan cherry trees exhibiting leaf symptoms ranging from necrotic veins, local lesions or necrotic tissues were sampled in 2003. Each collection was analyzed for dsRNA content and all of them produced bands of high molecular weights after electrophoresis in acrylamide gels. The dsRNA preparations were reverse transcribed with random primers and submitted to PCR assays with degenerate primer sets specific to members of the fam. *Flexiviridae* (F-PCR) and fam. *Closteroviridae* (HSP-PCR). All collections were positive with one or both primer sets. The PCR products were cloned, sequenced and analyzed. Comparisons of the sequence data identified presence of *Cherry virus A* (CVA), *Cherry necrotic rusty mottle virus* (CNRMV) or *Cherry green ring mottle virus* (CGRMV) in F-PCR assays. In HSP-PCR assays, one collection also contained a Closterovirus, which shared 77% (nt) and 85% (aa) homology with *Little cherry virus 1* (LChV-1). The presences of CVA, CNRMV and putative LChV-1 were identified for the first time in California.

Blueberry fruit surface microflora: Search for potential biological control agents for fruit rot pathogens. S. SABARATNAM, J. Dickman, and A. M. C. Schilder. Department of Plant Pathology, Michigan State University, East Lansing, MI 48824. Phytopathology 94:S91. Publication no. P-2004-0616-AMA.

Fruit rots reduce yield and quality of blueberries for the fresh and process market. The indigenous microflora on the fruit surface of blueberries was studied in search of potential biological control agents for fruit rot pathogens. Samples of healthy green and ripe (first and second harvest) berries were collected from 12 'Jersey' fields in Michigan in 2002 and 2003. Berries were placed in plastic bags with phosphate buffer and shaken for 30 min. The washings were plated on PDA and KB or TSA. Colony-forming units were counted after 2-5 days. Yeasts, fungi, and bacteria were commonly isolated and generally increased in number as the fruit ripened. *Colletotrichum acutatum* predominated among the fungi, and *Aureobasidium pullulans* and *Cryptococcus* spp. among the yeasts. Three yeast isolates (*A. pullulans*, *Cryptococcus magnus*, and *Rhizosphaera* sp.) were antagonistic to *C. acutatum* and *Phomopsis vaccinii* in culture. Further studies are required to determine the biocontrol potential of these microorganisms under field conditions.

Fungal chitosan extracts are as effective in reducing decay caused by *Botrytis cinerea*, *Penicillium expansum*, and *Penicillium solitum* as commercial seashell chitosan extract. C. E. SAMS (1), S. Zivanovic (1), W. S. Conway (3), C. S. Charron (1), T. Wu (2), and A. Blodgett (3). (1) Dept. of Plant Science; (2) Dept. of Food Science and Technology, The University of Tennessee, Knoxville 37996; (3) Produce Quality and Safety Lab, USDA ARS, Beltsville, MD 20705. Phytopathology 94:S91. Publication no. P-2004-0617-AMA.

'Golden Delicious' apples were surface disinfested with 70% ethanol and wounded once towards the top of each apple. Two % chitosan solutions in sterile de-ionized water were adjusted to pH 6 with sodium hydroxide or acetic acid. Five chitosan extract solutions were utilized as the following treatments: 1) a commercial seashell chitosan extract, 2) a crude extract from *P. expansum*, 3) a purified extract from *P. expansum*, 4) a crude extract from *P. solitum*, and 5) a purified extract from *P. solitum*. These were compared to a water control. Each chitosan solution was pipetted into 3mm × 3mm wounds on 30 apples (25 microL per wound) and allowed to air dry. Apples were placed in boxes at 25°C for 3 days. After the three days, an identical wound was made one inch below the original wound on each fruit and all wounds were inoculated with 25microL of a 1×10^4 spore suspension of either *Botrytis cinerea*, *P. expansum* or *P. solitum*. Apples were placed in boxes at 25°C and lesions were measured at 2-3 day intervals over 17 days. The area of decay caused by the three pathogens was reduced by all of the chitosan treatments. The rate of growth of *P. solitum* was significantly slower than the other pathogens. Chitosan from all three pathogens was effective at eliciting resistance to decay as was chitosan from commercial sources. Implications of this to host pathogen interactions and pathogen growth rate will be discussed.

Allelic mining for late blight resistance in wild *Solanum* species. M. J. SANCHEZ and J. M. Bradeen. University of Minnesota, St. Paul. Phytopathology 94:S91. Publication no. P-2004-0618-AMA.

The *RB* gene, a Late Blight (LB) resistance gene imparting broad-spectrum protection to LB pathogen races, is a valuable resource for the development of resistant potato cultivars. This work aims to mine the *RB* locus for the exploration of allelic diversity in geographically distant populations from the wild potato, *Solanum bulbocastanum* using Long Range-PCR (LR-PCR). Here, we improved three potentially limiting technical factors associated with LR-PCR: template DNA quality, amplification fidelity, and primer design. Our optimized LR-PCR method may isolate unique and putatively functional alleles useful in evolutionary analyses and eventual deployment. Our *RB* allelic mining efforts will test 2 proposed evolutionary hypotheses: i) *RB* flanking regions are conserved across genotypes within *S. bulbocastanum* and ii) specific *RB*-nucleotide sites undergo purifying/balancing selection. Moreover, the methods described and improved here may assist allelic mining research at other loci and in other plant species.

Phyllosphere-mediated removal of airborne pollutants. A. SANDHU and G. A. Beattie. Department of Plant Pathology, Iowa State University, Ames, IA. Phytopathology 94:S91. Publication no. P-2004-0619-AMA.

Although leaves can take up and accumulate airborne pollutants, little is known about the utilization of these pollutants by leaf-associated microorganisms. Using a green fluorescent protein (GFP)-based biosensor that responds to the model pollutant phenol, we have found that airborne phenol taken up by leaves is accessible to leaf-associated bacteria. We have demonstrated further that phyllosphere bacteria can augment the removal of airborne phenol by leaves in a closed system. Specifically, bean leaves inoculated with a phenol-degrading *Pseudomonas putida* strain removed a greater amount of airborne phenol (181 ± 22 microgram per gram (mg g^{-1} leaf)) within 2 h than the uninoculated leaves (134 ± 11) as analyzed by gas chromatography. Also, bacteria that were pre-exposed to phenol on leaves further enhanced the removal of airborne phenol (136 ± 14 mg g^{-1} removed within 1 h compared to the bacteria that were not pre-exposed (87 ± 15). These results illustrate that the phyllosphere and its associated microbiota offer great potential for removing airborne pollutants.

Soil water saturation and infection of chile by *Phytophthora capsici*. S. SANOGO (1), J. Carpenter (1), and S. Ghoshroy (2). New Mexico State University, (1) Dept. of Entomology, Plant Pathology, and Weed Science, (2) Dept. of Biology, Las Cruces, NM 88003. Phytopathology 94:S91. Publication no. P-2004-0620-AMA.

Soil water saturation has been shown to predispose crops such as alfalfa to Phytophthora root rot. *Phytophthora capsici* causes a serious root rot disease in chile pepper under high soil moisture conditions. Little is known about the predisposition effects of soil water saturation on infection of chile pepper by *P. capsici*. Greenhouse experiments were conducted to determine the effect of water saturation on interaction of chile by *P. capsici*. Potted chile pepper plants were subjected to non-saturated and saturated conditions for 3 and 6 days prior to inoculation with *P. capsici*. To determine the effect of soil water on plants in the absence of the pathogen, a set of non-inoculated plants were included in the experiment. Disease symptoms observed in inoculated plants consisted of stem necrosis, leaf drop, wilting, and plant death. In general, disease severity was similar in magnitude under saturated and non-saturated conditions. Results suggest that there is no significant predispositional effect of soil water saturation on the interaction of chile pepper by *P. capsici*.

Study on cane dieback of olive trees and shoot in Taroom (Zanjan province) and other parts (Gorgan, Mashhad) of Iran. H. Saremi and F. FARROKHI. Department of Plant Pathology, Faculty of Agric, Zanjan University, Iran. Phytopathology 94:S91. Publication no. P-2004-0621-AMA.

The olive *olea europea* is an indigenous tree in eastern Mediterranean regions especially in Iran. Dieback and wilting of one branch of olive trees or shoot is one of main important disease in most parts of Iran. Recent years (from 2000 to 2002) various areas including Zanjan, Gorgan and Mashhad provinces were surveyed. Wilting branch, leaves, roots and soils around the roots have been collected from the studied areas. The samples were cultured in PDA and SDA media after surface sterilization with sodium hypochlorite. Soil samples were diluted in water agar for isolation of pathogen. Morphological characteristics especially phialide and spore showed that the causal agent of the disease was *Verticillum dahlia* which is soilborne fungi and isolated from all samples and soil. The disease was severe in all studied areas but it was higher in temperate and relative humidity regions such as Gorgan. Investigation showed that some olive variety were resistant to the pathogen. On the other hand field crops used for newly established orchards may have high concentration inoculums of fungus as causal agent of disease. These two factors must be studied in the future and can be useful for reduction the disease. Keywords: Olive, Wilting, Branch, Verticillum, Variety.

Incidence of crown rot disease on wheat by new species, Fusarium. H. SAREMI and F. Farrokhi. Department of Plant Pathology, Faculty of Agric, Zanjan University, Iran. Phytopathology 94:S91. Publication no. P-2004-0622-AMA.

Crown rot disease of wheat was studied during 1999-2000 in four provinces of North West of Iran including Qazvin, Zanjan, east Azarbyjan and Ardabil provinces. Different wheat fields in studied areas were visited and suspected plant samples showing symptoms including clorosis, withering, whrting of spikes, growth reduction and white heads collected and transferred to laboratory. Totally 155 fungal isolates belonging to five genus were identified, however the most often isolated pathogen was *Fusarium pseudograminearum* (formerly known as *F. graminearum* Group1). This species normally caused crown rot and had severe damage in locations following dry conditions in spring season. Results showed the disease caused losses of 40% - 50% in crops where the season and rotation have allowed the disease to build up. Environmental conditions and susceptible cultivars were two main factors affecting the incidence of diseases. Keywords: Wheat, crown rot, *Fusarium pseuogramineanum*, Iran.

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Study on root and crown rot of rice in Gilan and Zanjan provinces. H. SAREMI and F. Farrokhi. Department of Plant Pathology, Faculty of Agric, Zanjan University, Iran. Phytopathology 94:S92. Publication no. P-2004-0623-AMA.

Root rot and crown rot of rice is one of important disease in Gilan and Zanjan provinces. Recent years from 1999 to 2002 different regions of rice grown in two provinces were studied. Plants infested symples and soils around the roots have been collected from studied areas. Roots and crowns of infected plants were cultured in PDA, PPA and CLA after surface sterilization with sodium hypochlorite. Suspensions of collected soils were provided in water agar liquid to isolate casual agent from soils around the roots. The casual agent of disease was a species of fungi namely *Fusarium moniliforme* which isolated from crown and root of infected symples and soils. The colonies initially were white then became violet to grey. Macroconidia were chain and macroconidia were cylindrical and long with 3-5 septums. The disease was severing in some studied areas especially in Zanjan province around the Ghazel Owzen river which had shown 70% to 80% infection in some places. There was not any interchange in rice cultivation in this area and just traditional race such as Champa or Gerdeh have been cultured. Binam race was the best in resistant and yield production in studied area. Most area in Zanjan province was used sowing seeds rather that using seedling which infected plants can be eliminated. Keyword: Rice rot, Fusarium, crown, root, Gilan, Zanjan, Iran.

Etiology of damping off or decline on bean in Zanjan province, eastern north of Iran. H. SAREMI and F. Farrokhi. Department of Plant Pathology, Faculty of Agric, Zanjan University, Iran. Phytopathology 94:S92. Publication no. P-2004-0624-AMA.

Field bean is a major crop in different parts of Zanjan province especially Abhar and Khoramdareh regions. Recently the bean plants were severely subjected to damping off or decline diseases. A regional research was done from 2001 to 2002 to get general information on causal agents of the diseases. Infected plants were collected from different studied areas and transferred to laboratory. Crown and plant roots were cultured in PDA as common media and PPA as selective media after surface sterilization with sodium hypochlorite. Plates were incubated in standard culture room then isolated fungi were identified. Different species were isolated from *Fusarium*, *Rhizoctinia* and *Pythium* fungal genus. But main species which isolated frequently from plant samples and soil around the roots in all locations was *F. sambucinum*. The pathogen city test has also confirmed that *F. sambucinum* is the main causal agent of the disease in bean growing areas. Keywords: Damping off, bean, Fusarium sambucinum, Zanjan, Iran.

Genetic analysis of *Citrus tristeza virus*: Effects of deletion of p33, p18 and p13 genes on host range. T. 93TYANARAYANA, C. J. Robertson, S. M. Garnsey, and W. O. Dawson. Dept. of Plant Pathology, CREC, University of Florida, Lake Alfred, FL 33850. Phytopathology 94:S92. Publication no. P-2004-0625-AMA.

10 *Citrus tristeza virus* (CTV), a member of the *Closteroviridae*, is the ca 13 agent of one of the most economically important diseases of citrus. The 19.3 kb positive sense RNA genome of CTV is organized into 12 ORFs. The 5′ half of the genome encodes replication-associated proteins and the ten internal genes are not required for replication. The closterovirus hallmark genes – p6, HSP70h, p61, CPm and CP – are required for virion assembly and/or virus translocation. CTV contains five additional genes – p33, p18, p13, p20, and p23 – in the 3′ half of the genome, which were examined for the requirement to infect citrus plants. The progeny virions from deletion mutants were amplified in *Nicotiana benthamiana* protoplasts and inoculated to small citrus plants by stem slashings. Mutants containing a single gene deletion of either p33, p18 or p13; a double gene deletion of p33 and p18; or a triple gene deletion of p33, p18, and p13 were able systemically to infect *Citrus macrophylla*, Mexican lime, and sweet orange plants. However, mutants with deletion of the p33 gene failed to infect sour orange plants and mutant with the deletion of all three g 224 ailed to infect grapefruit trees. Yet, deletion of either p18 or p13 did not have a significant effect on the ability of mutants to infect grapefruit and sour orange plants.

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Genetic variability and mycelial compatibility groups of *Sclerotium rolfsii*. C. Sau 65 1), H. A. MELOUK (2), and K. D. Chenault (2). (1) Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078; (2) USDA-ARS, Plant Science Research Laboratory, Stillwater, OK 74075. Phytopathology 94:S92. Publication no. P-2004-0626-AMA.

The genetic variability among 17 isolates of *Sclerotium rolfsii* from Oklahoma and other locations was investigated using mycelial compatibility groups (MCGs) and Random Amplification Pol 39 rphism DNA PCR (RAPD-PCR). Pairings among isolates of *S. rolfsii* on potato dextrose agar containing 100 µg/ml of streptomycin sulfate resulted in 14 different MCGs. Isolates from Oklahoma were placed in three different MCGs, and the remaining isolates were placed in individual MCGs. RAPD-PCR analyses revealed similarities in banding patterns of isolates belonging to the same MCG with primer 335. Four distinct groups of isolates were identified based on DNA polymorphisms identified by RAPD-PCR using 21 primers. Isolates were clustered together according to their ho 11 rigin rather than by their geographic location or year of recovery. These results indicate that MCG and RAPD-PCR analysis are valuable methods for differentiating the genetic variability of *S. rolfsii* isolates.

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Effects of simulated rainfall events on spore dispersal and spore stocks in three tropical pathosystems. S. SAVARY (1), J. L. Janeau (2), D. Allorent (3), M. Escalante (3), J. Avelino (4), and L. Willocquet (1). (1) BiO3P ENSAR-INRA, BP 35327, 35653 Le Rheu cedex, France; (2) IWMI, PO Box 1025, Jatujak, Bangkok, Thailand; (3) CATIE, 7170 Turrialba, Costa Rica; (4) IICA/ICAFE/PROMECAFE/CIRAD/CBGP, A.P. 552200, Coronado, San José, Costa Rica. Phytopathology 94:S92. Publication no. P-2004-0627-AMA.

64 Tap and puff followed by dry spore transport (TP), spore dislodging from lesion and drip (DR) along plant tissues, and splash dispersal (SP) were monitored under simulated rain conditions in coffee rust, peanut rust and bean angular leaf spot. All three processes were triggered by rainfall events in the three diseases. Results suggested that TP predominates in low intensity and short rainfalls, whereas SP remains stable in importance under high intensity, long rainfalls. Any rainfall event strongly depleted (20-40%) stocks of spores in a diseased canopy rendering the event of major epidemiological importance when repletion is slow. Medium-duration and intensity rainfalls (e.g., 5-10 mn, 30 $mm.h^{-1}$) generated large flows of spores to the soil (10-40% of stocks). For biotrophic pathogens, such events are thus strong epidemiological penalties if they do not lead to efficient disease spread.

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A rapid and reliable PCR method for detecting *Burkholderia glumae*, the causal agent of pan 9 e blight on rice. R. J. SAYLER (1), R. D. Cartwright (2), and Y. Yang (1). (1) Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701; (2) Department of Plant Pathology, University of Arkansas, Little Rock, AR 72204. Phytopathology 94:S92. Publication no. P-2004-0628-AMA.

41 235
Panicle blight of rice, caused by the bacterium *Burkhold 41 glumae*, is one of the most important new diseases in the southeastern 41 rice production areas. The pathogen is likely seed born, but until now, no practica 41 ethod existed for testing large numbers of seed lots for the presence of the pathogen 93 thereby prevent the planting of infected seed. In this study, we report the development of conventional and real- 41 PCR assays that are effective for the detection and quantification of the pathogen in seed lots and plants. This method is highly sensitive, rapid, reliable, and has great potential for analyzing large numbers of samples without the need for DNA extraction. The genetic diversity of 21 isolates from Arkansas was analyzed by rep-PCR fingerp 41 ing and by 16 S rDNA sequence analysis of representative isolates. The fact that the genetic make-up of these *B. glumae* isolates is highly similar reinforces the robustness of this method for detecting different isolates of the pathogen.

Effect of temperature on ascospore discharge by 66 *nilinia vaccinii-corymbosi*. A. M. C. Schilder and P. S. WHARTON. Department of Plant Pathology, Michigan State University, East Lansing, MI 48824. Phytopathology 94:S92. Publication no. P-2004-0629-AMA.

The effect of temperature on ascospore discharge by *Monilinia vaccinii-corymbosi*, the causal agent of mummy berry in blueberry, was studied. Pseudosclerotia with apothecial initials were collected from blueberry fields in Grand Junction, MI, in April of 2002 and 2003, and were incubated on moist sand at 15°C and 100% RH until apothecia developed at which time they were moved to incubators set at 10, 15, 20 or 25°C. Ascospores were

collected daily on glass cover slips placed a few mm above the apothecia and were counted. The number of ascospores discharged per day was greater at 20 and 25°C. However, the total number of ascospores discharged was greater at 10 and 15°C due to increased longevity of apothecia. Exposure of apothecia incubated at 15°C to freezing temperatures (–2, –4, –6, or –8°C) for 1 h resulted in a significant reduction in the number of ascospores discharged after freezing. However, all apothecia, except those exposed to –8°C, partially recovered their ability to discharge spores 2 d after freezing. Viability of discharged ascospores was significantly reduced in apothecia exposed to –4, –6 and –8°C compared to those exposed to –2°C and before freezing. The results suggest that severe frosts may reduce the risk of ascospore release and infection by *M. vaccinii-corymbosi*.

Oxalate regulation by two brown-rot fungi decaying oxalate-amended and non-amended wood. J. S. SCHILLING and J. Jellison. Dept. of Biological Sciences, University of Maine, Orono, ME 04469. Phytopathology 94:S93. Publication no. P-2004-0630-AMA.

Brown-rot wood degrading fungi may regulate oxalate concentration in wood in order to control extracellular pH and to enhance quinone-derived hydroxyl radical production by mobilizing ferric iron. We used an agar-block experiment to compare oxalate and pH regulation over 12 weeks of spruce heartwood degradation by *Fomitopsis pinicola* and *Meruliporia incrassata*. We used ion-exchange HPLC to quantify soluble and acid-extractable oxalate both in agar and in milled wood at weeks 1, 2, 4, and 12. In wood degraded by both fungi, oxalate and pH were stable by week 4, as weight-loss continued; however, oxalate concentrations and partitioning between agar and wood were significantly different between the two fungi. The experiment was repeated using wood pre-treated with 0, 1, 10, and 100 mM sodium oxalate, with a single harvest at week 12. Pre-treatment with oxalate had no effect on wood weight-loss, pH, or oxalate concentration in wood degraded by either fungus. Our results demonstrate the importance as well as the species-specificity of oxalate and pH control during brown rot wood decay.

Selection and field evaluation of choline-utilizing microbial strains as potential biocontrol agents of Fusarium head blight. A. SCHISLER (1), N. I. Khan (2), M. J. Boehm (3), S. Zhang (1,3), and P. J. Slininger (1). (1) USDA-ARS, NCAUR, Peoria, IL; (2) BRDC, Peoria, IL; (3) The Ohio State University, Columbus, OH. Phytopathology 94:S93. Publication no. P-2004-0631-AMA.

The importance of choline in Fusarium head blight (FHB) development is debatable (Plant Dis. 88:175-180). Choline in wheat anthers can stimulate growth of conidial germ tubes of *Gibberella zeae* suggesting that choline-utilizing strains (CUS) may be effective competitors of the pathogen. Because combinations of strains can potentially increase the efficacy and consistency of biocontrol, CUS were sought to combine with efficacious FHB biocontrol strain *Cryptococcus nodaensis* OH 182.9 (NRRL Y-30216). CUS were identified from a microbial collection obtained from wheat anthers. When choline chloride was supplied as a sole carbon source in liquid culture, 122 CUS were identified out of 738 (16.5%) strains assayed using a colorimetric, choline oxidase-based bioassay. Twelve CUS with FHB biocontrol activity in greenhouse assays were selected for field testing. Four of 12 CUS reduced FHB symptoms at 2 sites on 2 wheat varieties. A Gram-positive bacterial CUS AS 54.6 reduced disease severity by 17-60% on Pioneer 2545.

Airborne propagules of *Gibberella zeae*: Techniques for monitoring spore release and viability. D. G. SCHMALE III and G. C. Bergstrom. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14850. Phytopathology 94:S93. Publication no. P-2004-0632-AMA.

We report a series of techniques for monitoring spore release and viability of propagules of *Gibberella zeae*. A series of five identical wind tunnels were constructed to monitor the spore release of *G. zeae* under a variety of environmental conditions. The tunnels were operated at temperatures within a range of 10 to 30°C, and at varying degrees of air pressure. In preliminary experiments, ascospore discharge and distant movement in turbulent air currents occurred between 10 and 30°C, with peak discharge and movement at 25°C. To estimate propagule survival in air, viable propagules of *G. zeae* (ascospores and macroconidia) were applied to natural and artificial (plastic) wheat heads and placed in natural environments for varying durations of time. Spores were washed off treated heads, and quantified by plating out washes on selective medium and observing the number of resultant colony forming units. We documented significant ascospore and macroconidia viability on both natural and artificial wheat heads following up to three days of exposure to natural environments.

Spatial patterns of spore deposition of *Gibberella zeae* in corn and wheat fields. D. G. SCHMALE III (1), D. A. Shah (2), and G. C. Bergstrom (1). (1). Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853; (2) Dept. of Plant Pathology, NYSAES, Geneva, NY 14456. Phytopathology 94:S93. Publication no. P-2004-0633-AMA.

Knowledge of the spatial and temporal distribution of viable, airborne spores of *Gibberella zeae* in and around wheat and corn canopies is critical for an increased understanding of the epidemiology of Fusarium head blight and Gibberella ear rot. We examined spatial patterns of spore deposition of *G. zeae* over two years (2002 and 2003) in corn and wheat fields in New York. Viable, airborne spores (ascospores and macroconidia) of *G. zeae* were collected on Petri plates containing a selective medium. In 2002, spores of *G. zeae* were collected over 40 day and night sampling periods on Petri plates placed above wheat spikes. In 2003, spores of *G. zeae* were collected over 13 day and night sampling periods with Petri plates placed at the level of corn silks and tassels. Contour plots of spore counts over entire fields were created using the Windows program Surfer. Unique spatial patterns of spore deposition were revealed for each day and night sampling period. Variance-to-mean ratios indicated the spatial distribution of spores was not uniform in any of the sampling periods, but was aggregated in 43 periods and random in 21 periods. Spore deposition patterns during individual sampling periods were classified by Spatial Analysis by Distance IndicEs (SADIE) statistics. Of the aggregated distributions, 78% (14/18) were observed at night, and 56% (23/41) of the random distributions were observed during the day. Diurnal fluctuations in the magnitude of spore deposition and the lack of uniform distribution during any one period of spore deposition should be considered when assessing the cumulative exposure of wheat and corn to inocula of *G. zeae*.

Identification and characterization of Armillaria species from South Carolina. G. SCHNABEL, J. S. Ash, C. R. Simpson, and K. K. Bryson. Clemson University, Clemson, SC. Phytopathology 94:S93. Publication no. P-2004-0634-AMA.

Little is known about the occurrence, species composition, and genetic diversity of *Armillaria* pathogens causing Armillaria root rot in the southeastern United States. Fourty one isolates were collected from dead or dying peach trees and various ornamentals from South Carolina. Analysis of the ribosomal ITS1 region as well as compatibility studies with haploid tester isolates identified *A. tabescens* as the most predominant species. *A. mellea* was found in only one of thirty samples from peach and in none of the ornamental samples. Sequence analysis of the ITS1 region from 49 *A. tabescens* isolates from South Carolina and other states revealed 23 different genotypes due to nucleotide mutations and the presence of divergent ITS1 copies in the genome of individual isolates. Similarly, RFLP analysis of the IGS1 region of 52 *A. tabescens* isolates revealed 20 different restriction patterns. Representatives of each ITS1 genotype were co-cultured in diploid-diploid pairings to determine intraspecific compatibility. In most cases the pairings were incompatible, indicating the presence of different genets in South Carolina. A PCR-based molecular technique was developed that distinguished *A. tabescens* from *A. mellea* isolates and that recognized hybrids of the two species.

Detection and quantification of Plum pox virus double-stranded RNA with real-time PCR. W. L. SCHNEIDER, A. L. Stone, D. J. Sherman, and V. D. Damsteegt. USDA, ARS, NAA, FDWSR. Phytopathology 94:S93. Publication no. P-2004-0635-AMA.

Double-stranded RNA (dsRNA) is commonly associated with RNA virus replication. A two step reverse transcription-real time PCR assay was developed to detect and quantify plum pox potyvirus (PPV) dsRNA. The reverse transcription step uses plus and minus strand specific primers to synthesize strand-specific cDNAs as templates for real-time PCR reactions. The relative quantities of plus and minus strands were determined by comparing the cycle threshold (CT) value from the plus strand reaction to the minus strand reaction. Plus and minus strand PPV transcripts were used to test and quantify the system. The plus and minus strand assay CT values were equal when the transcripts were equimolar. When one of the transcripts was in excess the CT values for the high concentration transcript were significantly lower. The assay was also tested on purified PPV dsRNA and total RNA from infected plants. The minus and plus strand CT values for the purified dsRNA were equal. However in the infected plant assay the CT value for the plus strand assay was 9-10 cycles lower than the minus strand assay CT value, suggesting that the ratio of plus to minus strands was roughly 1000 to one.

Analysis of the *Sinorhizobium meliloti* 1021 ORFeome in a Functional Genomics Platform. B. K. SCHROEDER, B. L. House, M. W. Mortimer, K. L. Warren, S. C. Maloney, H. T. Ziemkiewicz, J. J. Bovitz, S. Yurgel, and M. L. Kahn. Washington State Univ., Inst. of Biol. Chem., Pullman, WA 99164-6340. Phytopathology 94:S93. Publication no. P-2004-0636-AMA.

Sinorhizobium meliloti, a Gram negative soil bacterium, forms a nitrogen-fixing symbiotic relationship with alfalfa (*Medicago sativa*) and *Medicago truncatula*. The DNA sequence of *S. meliloti* 1021 was recently completed

and 99.9% of the predicted open reading frames (ORFs) located on the chromosome and two "megaplasmids"; pSymA and pSymB, were cloned into a modified entry plasmid for use with the GATEWAY integrase-mediated recombination cloning system. The ORFeome is now being transferred from the entry vector into various destination vectors via a penta-parental mating. This technique utilizes a five parent mating mix to directionally transfer the cloned ORF from the entry vector to a destination vector without DNA preparation or the expense of additional enzymes. The resultant constructs enable the over-expression of predicted proteins, mutation of predicted ORFs and the ability to monitor gene expression using reporter gene fusions in *Escherichia coli* and *S. meliloti*. These new techniques are useful in large- and small-scale analysis of *S. meliloti*.

Real-time PCR identification of *Pythium* spp. from cereals in eastern Washington. K. L. SCHROEDER and T. C. Paulitz. USDA-ARS and Washington State University, Pullman, WA 99164-6430. Phytopathology 94:S94. Publication no. P-2004-0637-AMA.

A number of *Pythium* spp. cause root rots and yield losses in wheat and barley. These species possess varying degrees of virulence and many different species can be found in a single soil sample. Determining the species composition within a soil sample by classical methods is time-consuming and difficult. In this study, variable regions of the ITS rDNA were used to design species-specific primer pairs for *P. appressorium*, *P. debaryanum*, *P.* aff. *Echinulatum*, *P. heterothallicum*, *P. intermedium*, *P. irregulare*, *P. paroecandrum*, *P. rostratum*, and *P. ultimum*. The SYBR Green I fluorescent dye was used in conjunction with capillary PCR to develop standard curves for each primer pair and to demonstrate specificity with 5 to 11 isolates from each species. Soils inoculated with each *Pythium* spp. were used to correlate DNA amplification directly from soil with pathogen populations. Real-time PCR will prove invaluable to study the population dynamics of these pathogens in soil and to determine how *Pythium* spp. respond to different disease management strategies.

Documentation of a severe anthracnose epidemic in strawberry plug-plant production in North Carolina and evaluation of *Colletotrichum acutatum* isolates for sensitivity to strobilurin fungicides. R. SCHWEGLER (1), Y. Lu (1), H. Ypema (2), L. F. S. Leandro (1), and F. J. Louws (1). (1) Dept. Plant Pathology, North Carolina State University, Raleigh, NC 27695; (2) BASF, Research Triangle Park, NC 27709. Phytopathology 94:S94. Publication no. P-2004-0638-AMA.

Strawberry tips contaminated with *Colletotrichum acutatum* were rooted in NC in the fall of 2003 leading to a severe epidemic in strawberry plug-plant production operations. Rooted strawberry transplants displayed petiole lesions, wilting of emerging leaves, severe plant stunting, root necrosis and plant death. The distribution of symptomatic plug-plants was documented at two farms among a population of 60,000 strawberry plants at each site. Five non-symptomatic petioles were collected in 4 directions within a 1 m^2 area from each of 21 sampling points. Petioles were frozen, surface sterilized, incubated and scored for presence of *C. acutatum* acervuli. Petiole infection was 7% and 15% at each farm, respectively. Isolates from the petiole samples and from specimens submitted to the North Carolina State University Plant Disease and Insect Clinic were tested for resistance to the fungicide pyraclostrobin. Baseline EC_{50} and EC_{90} values using 23 isolates collected from strawberries (1999-2002) were 0.011+ 0.001 and 0.072+ 0.009 mg/L, respectively. Isolates analyzed in the 2003 were discovered to be sensitive and supported the extension recommendation that the strobilurins would be an effective tool in fruit production fields.

Suppression of gummy stem blight on watermelon and cantaloupe transplants with seed-applied fungicides. K. W. SEEBOLD. Department of Plant Pathology, University of Georgia, Tifton, GA. Phytopathology 94:S94. Publication no. P-2004-0639-AMA.

Gummy stem blight (GSB), caused by *Didymella bryoniae*, is the most destructive disease of watermelon and cantaloupe in the southeastern U.S. Epidemics of GSB in transplant production houses have become severe in recent years. Inoculum may be seedborne, or can enter transplant houses from external sources. Boscalid and tebuconazole were applied to untreated seed of watermelon (diploid and triploid) and cantaloupe at rates of 25 and 2.5 g of active ingredient per 100 kg of seed, respectively, to determine effects on the development of GSB on transplants. Seedlings were inoculated with a mycelial suspension of *D. bryoniae* (50,000 CFU per ml) at the appearance of the first true leaf; trays were covered with a plastic-tarped frame to maintain high humidity. At 2 weeks after inoculation, boscalid reduced the severity of GSB on inoculated plants by 60% and secondary spread was reduced by 80% compared to the untreated control. Tebuconazole did not affect GSB on inoculated plants; however, secondary spread was cut by 27%. Seed treatment may be an effective way to manage outbreaks of GSB on watermelon and cantaloupe transplants.

Biological control of gummy stem blight on watermelon and Phytophthora crown rot on summer squash with bacteria. K. W. SEEBOLD, R. D. Gitaitis, and F. H. Sanders. Department of Plant Pathology, University of Georgia, Tifton, GA. Phytopathology 94:S94. Publication no. P-2004-0640-AMA.

Gummy stem blight (GSB), caused by *Didymella bryoniae*, and Phytophthora crown and fruit rot (PCFR), caused by *Phytophthora capsici* are important diseases of watermelon (*Citrullus lanatus*) and squash (*Cucurbita pepo*), respectively, in the southeastern U.S. and are difficult to control with fungicides. Nonpathogenic bacteria isolated from sycamore (*Platanus occidentalis*) were screened for the type III secretion system (T3SS), hypothetically indicative of the ability to translocate resistance inducing proteins into plant cells. Strains also were screened for their ability to inhibit growth of *Pythium irregulare* and *D. bryoniae in vitro*. Watermelon and squash seedlings were inoculated with bacteria with the T3SS, with ability to inhibit fungal growth, or with both characteristics. Seedlings were challenged with *D. bryoniae* or *P. capsici* 5-7 days after inoculation and incubated. Some bacteria suppressed GSB and PCFR and were comparable to acibenzolar-s-methyl, which induces resistance in certain plants. Biological treatments could be used as part of an integrated system to manage GSB and PCFR in cucurbit transplant production.

Phylloid strawberry fruit associated with undetermined non-phytoplasmal cause in Florida. T. E. SEIJO, J. R. Duval, and N. A. Peres. Univ. of Florida, GCREC, Dover, FL 33527. Phytopathology 94:S94. Publication no. P-2004-0641-AMA.

During the 2003/04 season in west-central Florida, a phyllody of the achenes of strawberry fruit of an unknown origin was observed. The phylloid fruit were typically elongated and had enlarged sepals. The incidence of phyllody was recorded in seven cultivars, 'Strawberry Festival' (SF), 'Sweet Charlie' (SC), 'Carmine' (Car), 'Camarosa' (Cam), 'Camino Real' (CR), 'Ventana' (V) and 'Treasure' (T). SF had the highest incidence, 4.5 to 7.2% of marketable harvest was symptomatic during a period of peak disease in January. SC, Car, Cam, and V had symptomatic berries, but at a much lower incidence than SF. No symptomatic fruit were detected in CR or T. The only reported causes of phyllody of strawberry fruit in the US are phytoplasmal diseases such as aster yellows or strawberry green petal. The plants did not have additional signs of phytoplasmal infection, such as red coloration of the older leaves or stunting, cupping, or marginal chlorosis of the younger leaves, and usually produced only 1 or 2 phylloid fruit along side numerous healthy fruit. Phytoplasmas could not be detected in leaf petioles or phylloid fruit by PCR using universal phytoplasma primers fU5/rU3, P1/P7 and R16F2n/R2 alone and nested with 16MF2/R1. A new chelex based quick DNA extraction procedure was developed and shown to be effective for detecting phytoplasmas in strawberry plants with typical symptoms when used in conjunction with the primer set fU5/rU3.

Wave-like development of bacterial communities and a biocontrol agent introduced in the wheat rhisosphere. A. M. SEMENOV (1), A. H. C. van Bruggen (2), A. D. van Diepeningen (2), R. J. Sayler (3), V. V. Zelenev (1), and O. De Vos (2). (1) Microbiol. Dept., Moscow State Univ., Russia; (2) Biol. Farming Systems, Wageningen Univ., Netherlands; (3) Dept. Plant Pathol., Univ. Arkansas, Fayetteville, AR. Phytopathology 94:S94. Publication no. P-2004-0642-AMA.

For successful application of biocontrol agents of root pathogens we need to know the ecology of the agent and the native microbial community. We studied in detail the spatial patterns of the bacterial community in the rhizosphere of wheat and, in separate experiments, the distribution along wheat roots of *Pseudomonas fluorescens* 32 *gfp* introduced into soil. Rhizospere and bulk soil samples were collected every 1.5 cm along wheat roots, amounting to 25 or more pairs of samples per root. CFU of copiotrophic (CB) and oligotrophic bacteria (OB) were counted on rich and poor agar media. Microbial communities were analyzed by DGGE on 16S rDNA directly isolated from soil and from mixed colonies. CFU of CB, OB, and *gfp P. fluorescens*, and microscopic counts of *gfp* cells exhibited significant wave-like oscillations, revealed by harmonic analysis. Distributions of DGGE fragments suggested a succession of amplicons along the root. The most intense amplicons fluctuated in phase with CFU harmonics; less intense ones fluctuated out of phase. This suggests that individual species like *P. fluorescens* fluctuate in tandem with the oscillations observed for total CFUs, and probably run counter to infections by some plant pathogens as shown earlier.

Infection of onion seedlings by soilborne inoculum of *Aspergillus niger* expressing green fluorescent protein. A. M. SEYB, D. P. LoParco, and J. W. Lorbeer. Cornell University, Ithaca, NY 14853. Phytopathology 94:S94. Publication no. P-2004-0643-AMA.

Several isolates of *Aspergillus niger* pathogenic to onion were transformed with a gene that encodes green fluorescent protein (GFP). The GFP-ex-

pressing isolates were tested for virulence on onion seedlings. In a typical experiment, conidia of the transformed isolate S2 were added to steam-sterilized organic soil contained in small plastic pots. Twelve onion seeds (cultivar Norstar) were planted in the infested soil in each pot. One week after emergence, *A. niger* isolate S2 was detected in internal tissues of the onion seedlings in one of the pots by culture techniques and fluorescent microscopy. Two weeks after emergence, isolate S2 was detected in the roots, basal plates, and cotyledons of 58, 58, and 26% of the seedlings, respectively, growing in a second pot. At later growth stages, isolate S2 subsequently was detected internally in the plants growing in other pots.

Isolate by temperature interaction for aggressiveness among isolates of *Puccinia striiformis* f. sp. *tritici*. E. SEYRAN and E. Milus. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR. Phytopathology 94:S95. Publication no. P-2004-0644-AMA.

Since 2000, wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* has been more severe than in previous years, and "new" isolates (collected in 2000 and later) were suspected to be more aggressive at higher temperatures than "old" isolates (collected before 2000). Four old and 11 new isolates were evaluated at 12 and 18°C for latent period on susceptible seedlings and for urediospore germination rate on Nobel agar. There was a significant isolate by temperature interaction ($P < 0.0001$) for both latent period and germination rate. New isolates had significantly shorter latent periods at 18°C than at 12°C. Old isolates had similar latent periods at both temperatures and significantly longer latent periods than some new isolates. Eight isolates germinated faster at 18°C, one isolate germinated faster at 12°C, and six isolates had similar germination rates at both temperatures; however, there was no clear distinction between old and new isolates. The possible role of increased aggressiveness in recent epidemics will be discussed.

Possible sources of *Puccinia sorghi* inoculum initiating rust epidemics on sweet corn in western New York, 2002. D. A. SHAH and M. R. Dillard. Dept. of Plant Pathology, NYSAES, Geneva, NY 14456. Phytopathology 94:S95. Publication no. P-2004-0645-AMA.

Puccinia sorghi urediniospores immigrate annually into western New York (WNY) by passive atmospheric transport. The time of arrival of initial inoculum, as well as the identification of potential source regions, could facilitate the development of predictive risk models for rust establishment in WNY. To identify times of urediniospore first arrival, trap seedlings of the susceptible sweet corn hybrid *Snow White* were exposed for periods of about 48 hr at each of three sites in WNY, beginning late April. Rust was first detected on trap plants between July 3rd and 4th. Back trajectories, using the NOAA ARL's HYSPLIT model, were run hourly over positive trap periods to determine possible rust transport pathways to the WNY trap sites. Results were consistent with the model of initial long distance transport of urediniospores from the upper midwest region, followed by more localized immigration from sources, possibly in Michigan and southern Ontario, after establishment in the latter mentioned regions. None of the trajectories supported immigration into WNY via the eastern Atlantic *Puccinia* pathway.

Controlling fungal and bacterial diseases of vegetables with a new fungicide containing famoxadone and cymoxanil. C. Shepherd, R. Williams, M. Martin, S. Soehner, and R. GEDDEN. DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE. Phytopathology 94:S95. Publication no. P-2004-0646-AMA.

Effectively controlling both the damaging bacterial diseases as well as the numerous fungal diseases that affect tomatoes, peppers, cucurbits and other vegetables is a difficult challenge for growers. Tanos™, a new fungicide from DuPont, currently is registered in U.S. for use on tomatoes, peppers, cucurbits and head lettuce. Tanos™ offers broad-spectrum disease control and strong residual properties with high resistance to washoff. In addition to control of fungal diseases, Tanos™ complements the activity of copper hydroxide and EBDC fungicides like mancozeb and maneb in suppressing bacterial diseases in tomatoes, peppers and cucurbits. A vegetable disease management program combining Tanos™ with other fungicides with diverse modes of action broadens the spectrum of fungal and bacterial disease control, increases efficacy and consistency of performance as well as reduces the risk of fungicide resistance development.

Molecular analysis of powdery mildew pathogens in dogwood. A. Shi, M. T. MMBAGA, and S. Zhou. Tennessee State University. Phytopathology 94:S95. Publication no. P-2004-0647-AMA.

Two pathogens, *Erysiphe* (Sect. *Microsphaera*) *pulchra* and *Phyllactinia guttata*, of powdery mildew disease in dogwood (*Cornus* sp.) were analyzed by molecular approaches: PCR and PCR-RFLP of Internal Transcribed Spacer (ITS) region, microsatellite-primed PCR and AFLP of genomic DNA. The universal primer pair, ITS1 and ITS4, showed polymorphisms among *E. pulchra*, *P. guttata* and their host dogwood. The polymorphic bands were about 710bp for dogwood, 680bp for *P. guttata*, and 650bp for *E. pulchra*. PCR-RFLP from ITS region showed different patterns between *E. pulcha* and *P. guttata* after digestion by enzymes Sau3A I, Rsa I, Alu I, Dde I, Hae III or Hinf. The SSR primer $(GTG)_5$ showed polymorphisms between *E. pulchra* and *P. guttata* and the polymorphic bands were about 820bp and 750bp specific for *P. guttata* and the polymorphic bands 700bp, 600bp and 500bp specific for *E. pulchra*. 16 EcoR I/Mse I primer pairs were used for AFLP analysis and all of them showed prominent polymorphisms between *E. pulchra* and *P. guttata* ranging from 5 to 18 polymorphic fragments, and different isolates of *E. pulchra* showed similar polymorphisms. The PCR products from ITS region were sequenced and five pairs of specific primers were designed to distinguish among *E. pulchra*, *P. guttata* and their host dogwood. These results provide useful tools in the diagnosis of powdery mildew pathogens at conidial stage when the disease is prominent.

Identification of hrp up-regulated virulence genes in *Erwinia chrysanthemi* 3937 by hrp functional cloning. X. SHI (1), N. T. Perna (2), N. T. Keen (1), A. Cooksey (1), and C.-H. Yang (3). (1) Dept. of Plant Pathology and Center for Plant Cell Biology, Univ. of California, Riverside, CA 92521; (2) Dept. of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI 53706; (3) Dept. of Biological Sciences, University of Wisconsin, Milwaukee, WI 53211. Phytopathology 94:S95. Publication no. P-2004-0648-AMA.

Erwinia chrysanthemi causes soft-rot diseases, which are economically important in a wide range of crops. In our previous report, we described hrp mutants of *E. chrysanthemi* 3937 that are reduced in their ability to cause infection in plant hosts. To further identify genes of *E. chrysanthemi* 3937 that are up-regulated by the hrp cluster, we constructed a *E. chrysanthemi* library with a pPROBE-AT vector. The constructed library was electroporated into an *E. coli* strain carrying the *E. chrysanthemi* hrp cluster (pCPP3042). The *E. chrysanthemi*/pPROBE-AT clones that were specifically up-regulated by the hrp cluster were identified and their role in host virulence further investigated. From ca. 20,000 clones thus far screened, nineteen were found to be up-regulated by the hrp cluster of *E. chrysanthemi*. These hrp cluster up-regulated genes include 1) type III virulence factors, 2) regulatory genes, 3) genes involved in peptide secretion and 4) genes with unknown function. The complementary experiments undering *E. coli* and *E. chrysanthemi* genetic background confirmed these genes were actually up-regulated by hrp cluster of *E. chrysanthemi*. Mutants of several type III virulence genes, regulatory genes homologues and unknown function genes were reduced in their virulence on African violet (Rosalie cultivar). This phenomenon demonstrates that the pathogenicity of *E. chrysanthemi* may be controlled by a complex regulatory network among different virulence factors, metabolism genes and regulatory genes. The potential functions of these hrp up-regulated genes will be further analyzed by biochemical, genetic and biological methods in the future. This presentation is in memory of Dr. Noel T. Keen who passed away on April 18, 2002.

Persistence of *Phytophthora ramorum* in nursery plants and soil. N. SHISHKOFF and P. Tooley. USDA-ARS Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Fort Detrick, MD 21702. Phytopathology 94:S95. Publication no. P-2004-0649-AMA.

Phytophthora ramorum causes cankers, dieback, and foliar symptoms on a number of hosts, but its behavior in soil has not been well examined. When Rhododendron 'Cunningham's White' were inoculated with a sporangial suspension (approx. 2,500 sporangia/mL) on foliage, incubated in a dew chamber for 3 days, then kept in the greenhouse at 20-24°C, tissue taken weekly from the edge of lesions gave rise to colonies of *P. ramorum* on selective medium for at least 90 days. When leaf tissue containing chlamydospores was buried in mesh bags in pots containing nursery stock, incubated in the greenhouse and sampled monthly, chlamydospores formed colonies on selective media for at least 155 days after burial. When mycelium that included chlamydospores (grown in rye seed/water culture) was buried in a similar fashion and sampled monthly, chlamydospores also formed colonies for at least 155 days. When roots of rhododendron were dipped in a sporangial suspension (approx. 2,500 sporangia/mL) and then planted in coarse soilless medium, *P. ramorum* could be isolated from washed or 0.5% sodium hypochlorite surface-sterilized roots 10-66 days later. These results indicate that *P. ramorum* may persist in nursery container media.

Two putative new species of *Pythiogeton* associated with Cypress and English Ivy in North Carolina. H. V. SILVA-ROJAS (3,4), T. C. Creswell (2,3), J. L. Phillips (1,3), J. A. Abad (3), and Z. G. Abad (1,3). (1) Plant Pathogen Identification Laboratory; (2) Plant Disease and Insect Clinic; (3) NCSU, Raleigh, NC; (4) IREGEP. Colegio de Postgraduados, Montecillo, México. Phytopathology 94:S95. Publication no. P-2004-0650-AMA.

Pythiogeton in the Kingdom Straminipila with it's eight species is a not widely studied genus. In the USA, only *P. ramosum* and *P. autosytum* have been reported and that occurred in 1932. *P. ramosum* was isolated from twigs of an unknown plant in Cambridge, MA. *P. autosytum* has been associated with root rot of *Typha latifolia* L. in Port Clinton, OH. During the summer 2003 in NC, Cypress and English Ivy showed symptoms of root rot and samples of *Pythiogeton* were isolated from these hosts. Isolates from both hosts produced variable shapes of sporangia including: bursiform, cylindrical, pyriform, globose, ellipsoid, and irregular forms. *Pythiogeton* 1, the isolate from Cypress, produces individual sporangia. *Pythiogeton* 2, from English Ivy, produces occasional clusters of two or three sporangia and short chains of intercalary sporangia. Morphologically the closest species to both isolates is the unculturable *P. ramosum* a heterothallic specie. However, these heterothallic isolates from NC grow readily at different media including: PARPH, V8, CMA, PDA 30 and CA. Both species produce hyphal rings, a characteristic that has never been reported in the genus *Pythiogeton*. Pathogenicity tests are in progress. Sequences of the ITS rDNA region of both species are different and have been deposited at the GenBank. According to our observations these are two putative new species of *Pythiogeton*.

Toxicity of explosives contaminated soil to plants. M. SIMINI, R. T. Checkai, J. L. Kuperman, C. T. Phillips, J. E. Kolakowski, and C. W. Kurnas. U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. Phytopathology 94:S96. Publication no. P-2004-0651-AMA.

Military sites associated with munitions manufacturing, disposal, testing, and training frequently contain elevated levels of explosives and related materials in soil. Their effects on plants have not been sufficiently investigated. To address this problem, the U.S. Environmental Protection Agency, together with stakeholders, is developing Ecological Soil Screening Level (Eco-SSL) values for use in Ecological Risk Assessment of chemicals often found at Superfund sites, including explosives. We performed plant toxicity tests to develop Eco-SSL values for two energetic materials (EMs), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT) using adapted ASTM and U.S. EPA guidelines. Alfalfa, Japanese millet, and perennial ryegrass were subjected to various levels of the two EMs in 5 soil types having different levels of clay (12% to 37%), organic matter (1% to 5%), and pH (4.4 to 7.4). Growth chamber tests were conducted at 22+2°C and 16h light/ 8h dark cycle with >70% relative humidity. Percent seed germination and shoot wet and dry mass were measured after a two-week growth period. Preliminary results showed TNT toxicity to all species tested. Lowest Observed Adverse Effect Concentration (LOAEC) of TNT ranged from 100 mg/kg to 400 mg/kg. Soil type had no effect on TNT toxicity. RDX was not toxic to any of the species tested in all soils tested up to 10,000 mg/kg. Results of ongoing studies with TNT amended soils will be used to determine the effective concentration producing 20% reduction in biomass (EC_{20}) for Eco-SSL development.

An anatomical and physiological basis for flood-mediated rice blast field resistance. M. P. SINGH, P. A. Counce, and F. N. Lee. University of Arkansas. Phytopathology 94:S96. Publication no. P-2004-0652-AMA.

The mechanism of flood induced rice blast, *Magnaporthe grisea*, field resistance is poorly understood. Recently, we linked flood induced hypoxia mediated ethylene signaled plant defense responses with rice blast field resistance. To better understand this environmental activated phenomenon, a comparative anatomical study of inoculated and un-inoculated leaf tissue from upland, flooded, and upland-50 ppm ethephon treated plants was undertaken. With lesions of the very susceptible 'M-201' and susceptible 'Cypress' upland-treated cultivars, the upper epidermis, mesophyll, vascular bundles, bundle sheath, bulliforms cells and lower epidermis were thoroughly colonized with profuse external growth and sporulation. Typical spindle shaped blast lesions resulted from rapid and complete mycelia colonization along major and minor parallel veins and through transverse veins to adjacent veins. In contrast, degree and rate of mycelia growth was reduced in leaves of flood-treated plants where linear and lateral mycelial colonization appeared restricted by highly developed vascular bundles of enlarged major and minor vertical veins and by well developed transverse veins. Lesion progress in upland-ethephon treated plants were similar to that of flood-treated plants. These observations provide insight to the anatomical and physiological mechanisms of flood induced blast field resistance.

Utilization of field resistance to control rice blast. M. P. SINGH, F. N. Lee, and P. A. Counce. University of Arkansas Rice Research and Extension Center, 2900 Hwy 130E, Stuttgart, AR 72160. Phytopathology 94:S96. Publication no. P-2004-0653-AMA.

Flood induced field resistance provides a key rice blast, *Magnaporthe grisea*, control method in susceptible cultivars grown in Arkansas rice production areas. Induced field resistance is an hypoxia mediated response wherein susceptible cultivars growing in upland conditions become increasingly resistant with increased intensity and duration of hypoxic conditions. This host response has been observed in all blast susceptible cultivars tested to date. Also, the response was duplicated in greenhouse tests by soil-drench applications of ethephon which generates ethylene, indicating a role for root produced hormones. The nature of induced field resistance is being studied to identify and utilize useful rice germplasm which exhibits very high levels of blast field resistance. Historically, field resistant cultivars have provided a durable and sustainable strategy for use in integrated rice blast management programs.

Development of molecular strategies to control rice sheath blight disease. P. SINGH (1), Y. Wamishe (1), G. C. Eizenga (2), F. N. Lee (1), and Y. Jia (2). (1) University of Arkansas, Rice Research and Extension Center, Stuttgart, AR; (2) USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR. Phytopathology 94:S96. Publication no. P-2004-0654-AMA.

Sheath blight caused by *Rhizoctonia solani* is a serious disease of rice worldwide. No complete genetic source of resistance is known among rice cultivars. Rice cultivar, Jasmine 85, shows considerable resistance to sheath blight and this resistance appears to be under polygenic control. The objective of this study was to characterize these minor resistance genes for developing molecular strategies to control sheath blight disease. First, sheath blight isolates were collected from Arkansas rice fields. These isolates were subsequently characterized based on anastomosis and sequences of the ribosomal DNA internal transcribed spacer. A detached leaf inoculation method was developed to assess lesions caused by the pathogen and discern differential cultivar responses. A subtractive cDNA library was constructed by the PCR-s cDNA subtraction method using mRNAs from Jasmine 85 sixteen hours after inoculation with a highly virulent isolate. Out of the 200 genes sequenced, 10%, 9%, 5% and 3% genes were predicted to have roles in defense, signal transduction, stress response and fungal-host association, respectively. The remaining included 24% regulatory, 21% unknown, 16% metabolism and 12% from fungal origin. Molecular interaction mechanisms of rice with *R. solani* will be discussed.

Biological control of postharvest late blight of potatoes in storage. P. J. SLININGER (1), D. A. Sisler (1), and G. Kleinkopf (2). (1) USDA-ARS, NCAUR, Peoria, IL; (2) University of Idaho Kimberly Research and Extension Center, Kimberly, ID. Phytopathology 94:S96. Publication no. P-2004-0655-AMA.

Introduction of US-8 genotypes of *Phytophthora infestans* has coincided with an increase in severity of potato late blight in North America. As alternatives to chemical fungicides, 18 bacterial strains patented as biological control agents (BCA) of both sprouting and Fusarium dry rot were cultivated in liquid media and screened in wounded potato bioassays for their ability to suppress late blight incited by *P. infestans* (US-8, mating type A2). Stationary-phase bacteria were mixed with fungal zoospores to inoculate potato wounds with 5 microL containing ~10^8 bacterial CFU/mL and 2 × 10^4 zoospore count/mL. Disease suppressiveness was evaluated after tubers were stored one week at 15°C and 90% relative humidity. Several of the BCA treatments reduced late blight by 25 to 60% (including three strains of *Pseudomonas fluorescens* and one of *Enterobacter cloacae*). Larger-scale pilot testing of these four strains, alone and in combination, was conducted by spraying 0.8 mL of 4 × 10^4 *P. infestans* sporangia/mL followed by 0.8 mL of bacteria treatment at ~10^9 CFU/mL per each of 90 unwounded potatoes. Three replicate boxes per treatment (30 tubers per box) were randomized in storage and maintained at 7.2°C and 95% relative humidity for 4 weeks. All BCA treatments reduced disease. Disease suppression ranged from 35% (worst treatment) to 86% (best) the first test year and from 35 to 91% the second year. Unwashed bacteria outperformed those washed free of culture broth.

Characterization of a new *Phytophthora infestans* isolate from tomato. G. Smith, C. Enderle, J. Gilpert, T. Davis, S. Foor, and L. E. HOFFMAN. DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE 19714. Phytopathology 94:S96. Publication no. P-2004-0656-AMA.

A new isolate of *P. infestans* from tomato, PI4, was compared to two isolates of the common clonal lineages US1 and US8. The gpi (glucose-6-phosphate isomerase) allozyme genotype of PI4 (122/122) is rare in the US, shared by only one other isolate from a tomato field in NJ. PI4 grew poorly on V8 agar and stopped growing after 1 serial transfer. Hyphal growth of PI4 was similar to that of US8 and US1 on rye agar. US8 produced more sporangia than US1 on rye agar; PI4 produced the least. PI4 inoculum was harvested from plants for subsequent assays because zoospore release was faster and more consistent than in sporangia produced on rye agar plates incubated for 1 week. On 5-week old potato plants, the incubation period was ca. 3 days for all 3 isolates at 20°C. One week after inoculation, lesion size differed significantly

among the isolates, averaging 17.5, 12.3, and 8.3 sq. cm for US8, PI4 and US1, respectively. Mefenoxam applied at 200g ai/ha provided 99% control of US1, but only 32% of both PI4 and US8, indicating that PI4, like US8, is mefenoxam-insensitive.

Host range of *Corynespora cassiicola* and its occurrence on weeds, ornamentals and crops of Guam. L. J. SMITH and R. L. Schlub. University of Guam, Mangilao, GU. Phytopathology 94:S97. Publication no. P-2004-0657-AMA.

Corynespora cassiicola (historically classified under the genera *Helminthosporium* and *Cercospora*) is a plant pathogenic fungus with a wide host range most frequently reported from tropical and subtropical areas on cucumber, papaya, rubber, tomato, and soybean. Results from a nationwide plant disease clinic survey, observations on Guam, and a literature search indicate that this fungus can cause disease on 53 plant families and 141 genera. The diseases caused by *C. cassiicola* are highly variable and are often sporadic and severe. Little research has been conducted on the fungus, and little is known of its host range beyond economically important crops. A survey was conducted to determine its host range on plants of Guam. *C. cassiicola* was found to grow pathogenically on several weedy genera including *Asystasia*, *Desmodium*, *Ipomoea*, *Sida*, *Spathodea*, *Stachytarpheta*, and *Vitex*. It was also found to grow saprophytically on many different species when plant tissue was incubated in a moisture chamber. Preliminary cross inoculation studies indicate that there are many host specific isolates of the fungus on Guam.

Searching for Plum pox virus: A survey of stone fruit in New York State. K. L. SNOVER-CLIFT and S. L. Jensen-Tracy. Cornell University, Ithaca, NY. Phytopathology 94:S97. Publication no. P-2004-0658-AMA.

Plum Pox, also known as Sharka, is a viral disease of stone fruit trees such as plum, peach, and apricot. It has been a devastating disease in Europe since the early 1900s, when it was first reported in Bulgaria: it then spread throughout Europe. In recent years the disease has spread to the Americas, first being found in Canada in 1992, in the orchards of Adams County, Pennsylvania, in 1999, and in Ontario and Nova Scotia, Canada in 2000. The disease appears to remain localized with hopes that it has been contained and/or eradicated before it had a chance to spread to other parts of the Northeastern United States. Surveys have been conducted in NYS since 2000 with no virus detected. Even though testing has resulted in negative results, there is fear that the virus may have escaped the introduction area and is present elsewhere in low, non-detectable levels. In 2002, Plum Pox Virus was included on the Agricultural Bioterrorism Act because an established introduction of this exotic pathogen is capable of causing devastating damage to the Stone fruit industry. Because of these concerns, New York State Agriculture & Markets and the Plant Disease Diagnostic Clinic of the Cornell University, Department of Plant Pathology have continued to survey N.Y.S. orchards. During the 2003 and 2004 growing seasons, approximately 15,000 samples were tested for the presence of the virus. Additionally during 2004, real-time PCR technology was added to the current ELISA based testing methods. The results provided no evidence of the virus using ELISA and/or real-time PCR technologies.

The effect of *Potato virus Y* (PVY) infection on siRNA accumulation and transcription of a transgene targeted by RNA silencing. M. C. SOLOFOHARIVELO, Z. Weng, and Z. Xiong. Dept. of Plant Sciences, Div. Plant Pathology, Univ. of Arizona, Tucson, AZ. Phytopathology 94:S97. Publication no. P-2004-0659-AMA.

A transgenic line (D2) of Nicotiana benthamiana expressing the 5′ 1.2 kb sequence from *Red clover necrotic mosaic virus* (RCNMV) RNA-1 exhibited an RNA silencing-based immune response to RCNMV infection. The immune line contained multiple copies of the transgene and accumulated small interfering RNA (siRNA) homologous to the transgene. To further characterize the transgenic plants, the effect of RNA silencing suppressor on the transgenic line was tested using PVY. Interestingly, PVY was not able to reverse the silencing established in the D2 immune line. Furthermore, siRNA in the infected plants was produced at a much higher level. This result is consistent with the effect of HC-Pro suppression observed in transcription gene silencing as opposed to what was observed in post transcriptional gene silencing where HC-pro eliminates the accumulation of siRNA. In addition, PVY infection activated expression of different species of transgene mRNAs which were not expressed in the non-infected plants. The results suggest that PVY might activate the expression of transgenic mRNA by relieving some silencing mechanism such as transgene methylation at different loci.

A new air-sampling method for monitoring fungicide sensitivity and visualizing known types of resistance in *Penicillium digitatum* populations in citrus packinghouses. A. SOTO-ESTRADA, L. Kanetis, H. Förster, and J. E. Adaskaveg. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521. Phytopathology 94:S97. Publication no. P-2004-0660-AMA.

Continuous usage of thiabendazole or imazalil to control postharvest green mold of citrus has led to the development of resistant populations of *Penicillium digitatum*. With the planned registration of new fungicides, the development of monitoring practices and anti-resistance strategies is imperative. A spiral gradient dilution (SGD)-air sampling method for monitoring fungicide sensitivity in fungal populations was developed. SGD plates with exponential fungicide concentration gradients were prepared with EC_{95} concentrations positioned 15 mm from the edge of 150-mm diameter plates. After plates were exposed in a packinghouse and incubated, distinctive fungal growth patterns were observed. Fungal colonies evenly covered non-amended control plates. With imazalil, there was a gradual decrease in colony density from the edge to the center of the plate. With thiabendazole, an outer ring with a high colony density and an inner ring with a lower density were observed. These growth patterns allowed visualization of quantitative, polygenic and qualitative, monogenic resistance, respectively. For the new fungicides pyrimethanil and fludioxonil, a peripheral ring with a high colony concentration and a center core with no colonies were observed, indicating that no resistant isolates were present. Thus, using the SGD-air sampling method, the two known types of resistance can be visualized and large pathogen populations can be monitored for changes in fungicide sensitivity.

Regulation of coronatine in *P. syringae* pv. *tomato* DC3000. A. SREEDHARAN and C. L. Bender. Oklahoma State University, Stillwater, OK. Phytopathology 94:S97. Publication no. P-2004-0661-AMA.

Coronatine (COR) is produced by various pathovars of *P. syringae*, including *P. syringae* pv. *glycinea* PG4180 and *P. syringae* pv. *tomato* DC3000. COR is composed of coronafacic acid (CFA) and coronamic acid (CMA), which function as intermediates in the biosynthetic pathway. COR production in PG4180 is controlled by a two-component regulatory system consisting of *corS* (environmental sensor), *corR* and *corP* (response regulators). Both PG4180 and DC3000 synthesize COR; however, COR production is differentially regulated in these two strains. Because DC3000 is a model organism for studying *P. syringae* – plant interactions, it is important to understand the regulation of COR in this strain. In this study, *corR* and *corS* mutants of DC3000 failed to produce COR, CFA or CMA in multiple experiments. The multiplication and pathogenicity of the *corR* and the *corS* mutants were also reduced in tomato plants. Furthermore, studies were designed to investigate whether cross-talk exists between the different systems that are required for pathogenicity and virulence. For example, *hrpL* encodes an alternate sigma factor required for the expression of various transcripts in the *hrp* gene cluster, which encodes the type III secretion system (TTSS). A *hrpL* mutant of DC3000 was defective in COR production, suggesting that mutations in the TTSS may have regulatory effects on the production of virulence factors like COR. The study suggests that *corS* and *hrpL* control the expression of *cfl* and *cma* genes via the *corR* gene, thereby indicating that *corR* may function as a key regulator connecting the COR system and the TTSS.

Wheat leaf rust in a durum disomic chromosome substitution series. R. W. STACK, J. B. Rasmussen, and J. J. Jordahl. Plant Pathology Dept., North Dakota State Univ., Fargo, ND. Phytopathology 94:S97. Publication no. P-2004-0662-AMA.

Durum wheat is grown for production of pasta products. In general, durum is highly resistant to prevailing races of wheat leaf rust (LR), *Puccinia triticina*, but the genetic basis of leaf rust resistance in durum is not well studied. The durum cultivar Langdon is the background parent for a series of disomic substitution lines in which chromosomes from wild emmer (*Triticum dicoccoides*) replace the corresponding ones in Langdon durum. These lines were produced in the 1980s by USDA cytogeneticist L. R. Joppa. Langdon durum itself shows a necrotic fleck reaction (HR) when inoculated with either of two races of leaf rust, ("THBL", "MCDL"), both common in ND. We inoculated the set of 13 durum disomic substitution lines with these two leaf rust races in the greenhouse using using standard methods. Four lines were resistant (HR), similar to Langdon, and six lines were of intermediate or mixed reaction. Three of the lines showed a susceptible to highly susceptible (3-4) reaction to both races of leaf rust. The durum chromosomes (3A, 5A, 6A) which were replaced in those substitution lines may be sites of LR resistance genes in durum wheat.

A new book on field crop diseases as a teaching resource. R. W. STACK (1) and J. E. Kurle (2). (1) North Dakota State Univ., Fargo, ND; (2) Univ. of Minnesota, St. Paul, MN. Phytopathology 94:S97. Publication no. P-2004-0663-AMA.

The new book *Diseases of Field Crops in Canada* by K. L. Bailey *et al.*, published in 2003 by the Canadian Phytopathological Society is a valuable resource for students and teachers alike. The numerous high-quality color illustrations are especially useful in the classroom since fresh samples of many crop diseases are not available during the normal school year. The crop

diseases included are not limited to Canada; most are also found in the northern part of the United States, and many occur in temperate zone countries around the world. This book will not supplant the APS *Compendia of Plant Disease* as comprehensive references, it but provides a well-illustrated summary in a single package. The initial chapters give an excellent background and review of plant pathology concepts. The chapter on seed problems is especially useful, and the illustrations of seed discolorations are some of the best available anywhere. The very reasonable price by today's standards is an additional advantage for students. This book will also be useful for extension agents and crop consultants.

Assessing type 1 resistance to Fusarium head blight in high disease pressure screening nurseries. R. W. STACK and J. M. Hansen. Plant Pathology Dept., North Dakota State Univ., Fargo, ND. Phytopathology 94:S98. Publication no. P-2004-0664-AMA.

Expression of resistance in wheat to Fusarium head blight (FHB) is of two main types: "type 1" resistance reduces initial infection and is measured by disease incidence; "type 2" resistance reduces lesion expansion. Inoculated, irrigated screening nurseries are widely used in breeding for resistance to FHB, but these often give very high levels of disease incidence, making comparisons for type 1 resistance difficult. We propose that counting individual primary infection sites ("hits") per infected spike will allow estimation of type 1 resistance in a high disease pressure nursery. The relationship between the number of FHB hits per spike and the incidence of infected spikes is mathematical, and is expressed by the multiple infection transformation which was first applied to plant disease some 60 years ago by P. H. Gregory. Hits can be estimated by counting symtomatic spikelets per spike after the latent period is past. In spring wheat, this can readily be done at 10 days postanthesis, even in susceptible lines. Using this procedure we have identified lines with reduced incidence of FHB, indicating the presence of type 1 resistance.

Evaluation of real time quantitative PCR for quantifying *Bacillus cereus* populations in the rhizosphere of *Capsicum annum*. R. A. STAMLER, E. Morales, D. Sutton, and S. F. Hanson. Dept. of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM. Phytopathology 94:S98. Publication no. P-2004-0665-AMA.

Antibiotic producing microflora in the rhizosphere of crop plants are potentially useful for suppressing disease causing organisms. *Bacillus cereus* strains that produce the antibiotic zwittermicin have previously been shown to protect tomato and alfalfa plants from various soil born fungi including *Phytophthora* and *Pythium* sp. Preliminary experiments showed that several previously characterized strains of *B. cereus* weakly protected chile peppers (*Capsicum annum*) from chile wilt, a disease caused by *Phytophthora capsici*. We are working to identify or develop new strains of *B. cereus* that are better colonizers of pepper in order to develop more robust biocontrol agents for protecting chile peppers from soil-borne fungal diseases. The work presented here describes the development of a quantitative PCR assay as a rapid alternative to dilution plating for measuring rhizosphere populations of *B. cereus*. Primer sets specific for zwittermycin synthesis genes were evaluated for the ability to quantify *B. cereus* populations in rhizosphere samples.

Efficacy of *Beauveria bassiana* on colonized millet seed as a biopesticide for the control of shore flies in greenhouses. M. E. STANGHELLINI and Z. A. El-Hamalawi. Department of Plant Pathology, University of California, Riverside, CA 92521. Phytopathology 94:S98. Publication no. P-2004-0666-AMA.

Shore flies (*Scatella stagnalis*), in addition to their recognition as a nuisance (i.e., deposition of excrement on foliage), have been implicated as aerial vectors of several soilborne plant pathogens in greenhouse production of ornamental, floricultural and vegetable crops. A strain of *Beauveria bassiana* (Bb), isolated from an adult shore fly cadaver, was evaluated as a biopesticide for the control of shore flies. The fungus was grown on autoclaved millet seed for 2 weeks, air-dried at 24°C for 7 days and stored in paper bags. Dried Bb-colonized millet seeds were then broadcast (20 seeds/pot) on the surface of five, 15-cm-diam. pots containing potting medium naturally infested with larvae and pupae of shoreflies. Controls consisted of an equal number of insect-infested pots amended with autoclaved millet seeds. Pots of the same treatment were then placed in separate insect-proof screen cages in a greenhouse. At daily intervals over the 14 days subsequent to treatment application, adult fly populations were recorded in each cage. Adult fly populations in the control (no Bb) treatment were first recorded on day 3, increased progressively over the duration of the study and reached a final population of 70 adults on day 15. In contrast, one adult fly was first observed in the Bb treatment on day 8, variable populations (0-2 adults) between days 9 to 14, and a final population of 2 adults on day 15. These results, in repeated experiments, demonstrate the potential efficacy of *B. bassiana* on colonized millet seed as a biopesticide for the control of shore flies in greenhouses.

Survey for asymptomatic persistence of *Sphaeropsis sapinea* on or in stems of red pine seedlings from seven Great Lakes region nurseries. G. R. STANOSZ and D. R. Smith. Dept. Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 94:S98. Publication no. P-2004-0667-AMA.

Sphaeropsis sapinea persists on or in stems of red pine (*Pinus resinosa*) nursery seedlings, and proliferates to cause collar rot and mortality after planting. In spring 2002, seven nurseries were surveyed to determine the potential range in frequency of asymptomatic persistence: three operated by WI Dept. Natural Resources (DNR), two by MN DNR, one by MI DNR, and one by USDA Forest Service (in MI). At each nursery five groups of 20 asymptomatic red pine seedlings were collected near an inoculum source (red pine windbreak), if present, and five groups of 20 asymptomatic seedlings were collected away from such a source. A segment of the lower stem/root collar from each seedling was surface-disinfested and incubated on tannic acid agar. Transfers were made from resulting colonies and the pathogen identified from pycnidia and conidia produced in culture. A subset of isolates was characterized using ISSR-PCR analysis. The pathogen commonly was identified from asymptomatic seedlings collected in all WI and MN nurseries, but was never detected from seedlings from the MI DNR or USDA nurseries (which lacked red pine windbreaks). Frequency of detection was greater (as high as 88%) from seedlings near windbreaks than from others. Most isolates were the A group of *S. sapinea*, but a few from the MN nursery were B group, now referred to as *Diplodia scrobiculata*. Persistence of *S. sapinea* on or in asymptomatic seedlings continues to be problematic, in terms of subsequent mortality and distribution of the pathogen.

Influence of rhizomania and soil factors on sugarbeet yield. K. STEDDOM (1), M. W. Bredehoeft (2), J. A. Lamb (3), and C. M. Rush (1). (1) Texas Exp. Sta., Bushland, TX; (2) South. Minn. Beet Sugar Coop., Renville, MN; (3) University of Minnesota, St. Paul, MN. Phytopathology 94:S98. Publication no. P-2004-0668-AMA.

Rhizomania is an important disease of sugarbeet, affecting root weight, and extractable sucrose. In addition to rhizomania, numerous soil factors interact to affect yield. Three fields with highly variable soil factors and patchy distributions of rhizomania were sampled to determine factors affecting yield. Regression models were developed to predict root weight, sucrose concentration, and sucrose purity, with adj. R^2 of 0.53, 0.20, and 0.24, respectively. These models included rhizomania severity, residual soil nitrate at two depths to 1 m, pre-season applied nitrogen, organic matter, pH, and potassium. Surface nitrate had the greatest impact on root weight and purity, while rhizomania had the greatest impact on sucrose concentration and was the second greatest factor influencing purity. Predicted sucrose yield per hectare, calculated from these models, explained 50% of the variation in actual yield. The best prediction was observed at the site with the least severe rhizomania.

The quantification of *Tilletia indica* teliospores from regulated fields in Texas. J. M. STEIN, H. W. Maples, and C. M. Rush. Texas Agricultural Experiment Station. Phytopathology 94:S98. Publication no. P-2004-0669-AMA.

Karnal bunt (KB) of wheat, caused by the fungus *Tilletia indica*, is a USDA-APHIS quarantined organism. Little is known about the survival of teliospores in soil, and such information could assist in the deregulation process. We examined soil for the presence of *T. indica* teliospores from fields within the KB regulated regions of Texas that were found bunted kernel positive in 1997, 2001, both years, and neighboring fields that had never tested positive. In 2002, soil samples were collected in a grid pattern from nine fields in Central and six in North Texas. As a preliminary step in determining spore viability and distribution, soil was taken from each grid point and pooled to represent the entire field. Teliospores were extracted by sieve-washing and sucrose centrifugation and then enumerated microscopically. The maximum number of teliospores recovered from a single field was 307 teliospores/kg of soil. Teliospores were recovered from fields that had tested positive in all years as well as those that have never tested positive. No significant correlation was found between the number of teliospores, soil type, or the year(s) the field tested positive.

The optimization of real-time PCR to quantify the number of *Tilletia indica* teliospores in soil. J. M. STEIN, K. L. Maxson-Stein, and C. M. Rush. Texas Agricultural Experiment Station. Phytopathology 94:S98. Publication no. P-2004-0670-AMA.

Little is known about the survival and spatial distribution of *Tilletia indica* (Karnal bunt of wheat) teliospores in naturally infested wheat fields within the United States. Studying these topics is difficult because the conventional extraction process is inefficient and tedious; requiring microscopic examination of each sample. We examined the use of real-time PCR to detect and

quantify teliospore number in soil samples. Primer pairs and probes specific to *T. indica* and *T. tritici* were designed using published Internal Transcribed Spacer (ITS) sequences. Teliospores were spiked into i) whole soil samples for extraction using multiple techniques and ii) three partitions from the standard extraction technique in order to identify the most efficient method. Teliospore number from each extraction was quantified microscopically and with real-time PCR. Real-time PCR regularly detected 100 teliospores per sample and it was possible to estimate the number of teliospores in an unknown soil sample. We believe that real-time PCR has potential to greatly simplify detection and quantification of *T. indica* teliospores in soil.

Differential transmission of *Soybean mosaic virus* isolates through seed. T. A. STEINLAGE (1), J. S. Haudenshield (1,2), and L. L. Domier (1,2). University of Illinois, Urbana, IL; (2) USDA-ARS, Urbana, IL. Phytopathology 94:S99. Publication no. P-2004-0671-AMA.

Field and greenhouse experiments were conducted to compare the rates at which isolates of *Soybean mosaic virus* (SMV) were transmitted through seed. In the field experiment, six soybean plant introductions (PIs) were inoculated with eight SMV isolates, and six soybean PIs were inoculated with ten SMV isolates in the greenhouse. Transmission of SMV through seed ranged from 0 to 43% and 0% to 63% in seed from field- and greenhouse-grown plants, respectively. Differential transmission of SMV strains was observed. In addition, SMV isolate-by-cultivar interactions were seen in transmission rates. For example, SMV 88799 was transmitted from 43% of field-grown seed of PI 229324, but was not transmitted through seed of PIs 68522, 68671 or 86449. In contrast, SMV 413 was transmitted through seed from all PIs. SMV 1083 was highly transmitted (22 - 23%) in both field and greenhouse experiments. SMV G2 had very low seed transmission (0.0 - 0.4%) in both trials. Poorly-aphid transmissible SMV isolates that were maintained by mechanical inoculation were transmitted poorly through seed (2% from field seed and 6% from greenhouse seed). SMV isolates maintained by transmission by *Aphis glycines* were transmitted through seed at much higher rates (19% from field seed and 14% from greenhouse seed). This association between seed and aphid transmissibility suggests that the same regions of the SMV genome are involved in seed and aphid transmission.

Systematic replacement of *Wheat streak mosaic virus* HC-Pro. D. C. STENGER and R. French. USDA-ARS, Lincoln, NE. Phytopathology 94:S99. Publication no. P-2004-0672-AMA.

HC-Pro of *Wheat streak mosaic virus* strain Sidney 81 (WSMV-S81) was replaced with the corresponding cistron derived from four strains of WSMV (Type, TK1, CZ, and EB3), the tritimovirus *Oat necrotic mottle virus* (ONMV), the rymoviruses *Agropyron mosaic virus* and *Hordeum mosaic virus*, or the potyviruses *Tobacco etch virus* and *Turnip mosaic virus*. These HC-Pro proteins varied in amino acid sequence identity shared with HC-Pro of WSMV-S81 from high (WSMV strains at ~86-99%), to moderate (ONMV at 70%) to low (rymoviruses and potyviruses at ~15-17%). All chimeric viral genomes systemically infected wheat upon inoculation with RNA transcripts. HC-Pro replacements derived from tritimoviruses did not alter host range relative to WSMV-S81, and were able to systemically infect wheat, oat, and corn line SDp2. These results indicate that differences in host range among tritimoviruses, including the inability of ONMV to infect wheat or the inability of WSMV strains Type and EB3 to infect SDp2 corn, are not determined by HC-Pro. In contrast, chimeric viruses bearing HC-Pro replacements derived from rymoviruses or potyviruses were unable to infect oat or SDp2 corn. Collectively, these results indicate that HC-Pro from distantly related virus species of the family *Potyviridae* are competent to provide WSMV with all functions necessary for infection of a permissive host (wheat) and that virus-host interactions required for systemic infection of oat or SDp2 corn are more stringent. Changes in symptom severity or mechanical transmission efficiency observed for some chimeric viruses further suggests that HC-Pro affects virulence in WSMV.

Postinfection sprays with cyprodinil + fludioxinil against gray mold in Norwegian strawberry fields based on forecasting model from Ohio. A. Stensvand and G. M. STRØMENG. The Norwegian Crop Research Institute, Plant Protection Centre, Department of Plant Pathology, Høgskoleveien 7, 1432 Ås, Norway. Phytopathology 94:S99. Publication no. P-2004-0673-AMA.

In Norway, fungicides are applied up to 5-6 times per season against gray mold (*Botrytis cinerea*) in June bearing strawberries (*Fragaria* × *ananassa*) during the flowering period. In Ohio, a logistic model was developed to forecast infections of *B. cinerea* in strawberry flowers (1). A warning system based on the Ohio model for 25% flower infection was tested in strawberry fields in southern Norway over three years (cultivars Korona, Senga Sengana and Polka). Weather stations were stationed in each field. Standard fungicide treatments (calender spraying with 5-7 days spray intervals) were carried out with cyprodinil + fludioxinil (product: Switch), fenhexamid (product: Teldor), or tolylfluanid (Euparen M). If infection periods occurred during flowering, cyprodinil + fludioxinil were applied within 48 h. A mean of 2.6 (1 to 4) sprays were carried out based on infection predictions vs. 3.8 (2 to 4) in the standard spray schedule. Unsprayed, standard application, and spraying after infection resulted in the following percentage of fruits with gray mold (minimum and maximum in parentheses): 29.4 (6.6 to 69.9), 5.1 (0.4 to 18.3), and 9.2 (0.3 to 27.3), respectively. Only in one of eight instances did spraying after infection give significantly more ($P = 0.0001$) fruits with gray mold than the standard spray schedule.

Evidence for reduced sensitivity to propiconazole in the pecan scab fungus in Georgia. K. L. STEVENSON (1), P. F. Bertrand (2), and T. B. Brenneman (2). Dept. of Plant Pathology, University of Georgia, (1) Athens, GA; (2) Tifton, GA. Phytopathology 94:S99. Publication no. P-2004-0674-AMA.

Since 1988, demethylation-inhibiting (DMI) fungicides have been widely used in commercial pecan orchards in the Southeastern US for control of scab, caused by *Fusicladosporium effusum* (= *Cladosporium caryigenum*). Until recently, resistance management strategies have been successful in maintaining their effectiveness. In 2002, several growers reported unsatisfactory control of scab with DMIs. To monitor DMI sensitivity over time, monoconidial isolates of the fungus were collected in 1995 and 2003 from orchards with or without a history of DMI use. Sensitivity was determined based on relative mycelial growth (RG) of each isolate on medium amended with a discriminatory propiconazole concentration of 0.2 mg/liter. Comparison of RG values revealed that sensitivity declined significantly between 1995 and 2003 in sampled populations from 6 of 7 orchards with DMI use histories. No significant shift in sensitivity was detected in non-DMI-exposed populations. The effectiveness of DMIs for scab control in orchards with less sensitive pathogen populations is currently under investigation.

Molecular characterization of *Fusarium oxysporum* from tree nurseries: Tools for early detection of pathogens. J. E. STEWART (1), M.-S. Kim (1), R. L. James (2), R. K. Dumroese (3), and N. B. Klopfenstein (1). (1) USDA Forest Service-RMRS, Moscow, ID; (2) USDA Forest Service-R1, Coeur d'Alene, ID; (3) USDA Forest Service-SRS, Moscow, ID. Phytopathology 94:S99. Publication no. P-2004-0675-AMA.

Root-rot disease caused by *Fusarium oxysporum* can cause severe losses in conifer nurseries. This fungus is commonly found in most container and bareroot nurseries on healthy and diseased seedlings, in nursery soils, and on conifer seeds. Nursery managers attempt to manage the disease through soil fumigation, seed treatments, container sterilization, and fungicide applications; but early detection of pathogens is difficult. Although isolates of the fungus differ in virulence, studies have shown that pathogenicity and culture morphology are not correlated. For effective disease management, it is critical to easily and quickly distinguish between pathogenic and non-pathogenic isolates. Isolates of *F. oxysporum* collected from nurseries (soils, healthy and diseased seedlings) were selected for molecular characterization based on *in vitro* pathogenicity tests. We are currently performing Amplified Fragment Length Polymorphism (AFLP) and DNA sequencing in an attempt to identify genetic markers related to pathogenicity. We plan to develop molecular probes to differentiate pathogenic from non-pathogenic isolates of *F. oxysporum* to enable nursery managers to implement timely and appropriate disease management practices.

Influence of tillage on yield and root diseases in direct-seeded dryland wheat and barley in southeastern Idaho. C. A. STRAUSBAUGH and A. C. Koehn. Univ. of Idaho, Kimberly, ID. Phytopathology 94:S99. Publication no. P-2004-0676-AMA.

The influence of shallow subsoiling (8-10 inches with rigid shanks and narrow points) was compared with no tillage in direct-seeded dryland wheat and barley at two locations in southeastern Idaho to identify yield limiting parameters. The influence of tillage and crop was assessed using the following parameters: root disease, root lesion isolations, root number, root length, tiller number, nematode populations, stand, soil moisture, and soil nutrient analyses. In 2002 and 2003, diffuse-brown type lesions were predominant on the roots which was indicative of infection by *Fusarium* spp. and *Bipolaris sorokiniana*. In 2003, the most frequently isolated *Fusarium* spp. was *F. culmorum* at the Ririe field while *F. reticulatum*, *F. equiseti*, *F. acuminatum*, and *F. semitectum* were predominant in the Arbon Valley field. In 2002, subsoil tillage increased wheat yields by 9 and 39% at the Ririe and Arbon Valley locations, respectively. In 2003, with severe drought conditions and low yields, barley yield at Ririe did not differ but wheat yield in Arbon Valley was 32% greater with no tillage. Tillage treatments were found to differ for the following parameters: root disease, root number, tiller number, nematode populations, stand, and soil moisture. However these differences were not

always present each year at each location. These studies combined with future research will hopefully identify the most important yield limiting parameters in dryland cereal production so they can be managed to optimize yield.

Effects of tomato spotted wilt tospovirus (TSWV) on tobacco thrips are influenced by interaction among TSWV isolate, host plant and temperature. C. F. STUMPF and G. G. Kennedy. North Carolina State University, Raleigh, NC. Phytopathology 94:S100. Publication no. P-2004-0677-AMA.

TSWV replicates within its thrips vectors. In this study, we investigated the effects of different host plants and isolates of TSWV on its most important vector in North Carolina, the thrips species *Frankliniella fusca*. Thrips were reared until adult eclosion in the laboratory on excised leaves of the TSWV host plants *Datura stramonium* and *Emilia sonchifolia* under 3 temperature regimes (18.3, 23.9 and 29.4°C). Plants were either infected with the TSWV isolates CFL or RG2 or not infected (control). Measured variables included TSWV transmission to *Petunia* leaf disks, thrips development time to adult, adult size, and thrips infection with TSWV. Analyses revealed thrips infection by TSWV, as indicated by a positive ELISA test for the NSs protein, was associated with smaller adult size and prolonged development time to adult. Percentage of the initial cohort of *F. fusca* that transmitted TSWV was influenced by a host plant by TSWV isolate interaction, while percentage of thrips reaching adulthood was not. The greatest adverse effect on thrips size was observed on infected *D. stramonium*, a poor quality host plant for thrips only, under the highest temperature regime. Because metabolic demands are greatest under elevated temperatures, these results suggest that TSWV infection of thrips may lead to a reduced capacity to deal with metabolic stress such as that imposed by plants of marginal host quality.

Chemically-regulated *Cucumber mosaic virus* amplicon for the expression of foreign genes. M. R. SUDARSHANA (1), S. Uratsu (2), A. M. Dandekar (2), D. M. Tricoli (2), A. Jackman (3), K. McDonald (3), and B. W. Falk (1). (1) Dept. of Plant Pathology; (2) Dept. of Pomology; (3) Dept. of Chemical Engineering, Univ. of California, Davis, CA 95616. Phytopathology 94:S100. Publication no. P-2004-0678-AMA.

We have constructed a *Cucumber mosaic virus* (CMV) based amplicon, utilizing infectious cDNA clones of RNA-1, RNA-2, and RNA-3, for the expression of foreign genes/proteins in plants. The cDNA of RNA-1, sans the first 58 nt of the 5′-end, was fused to an operator sequence, for expression mediated by a glucocorticoid receptor based transactivator. A binary vector, carrying transactivator-tagged RNA-1 and cDNA copies of 35S promoter driven cDNA copies of RNA-2 and RNA-3 was agroinfiltrated into *Nicotiana benthamiana* leaves. Estradiol treatment of agroinfiltrated leaves expressed coat protein (CP) to levels comparable to those found in CMV-infected pumpkin. Agroinfiltration with another binary vector containing the beta-glucuronidase (GUS) gene in place of CP in RNA-3, followed by estradiol application allowed GUS expression. We are currently transforming *N. benthamiana* plants to generate cell lines for the analysis of protein expression levels.

Towards functional analysis of putative parasitism genes in *Heterodera glycines* using RNA interference. S. SUKNO (1), J. Cuiston (1), X. Wang (1), R. Hussey (2), T. Baum (3), and E. Davis. (1) North Carolina State University, Dept. of Plant Pathology, Box 7903, Raleigh, NC 27695; (2) Dept. Plant Pathology, University of Georgia, Athens, GA; (3) Dept. Plant Pathology, Iowa State University, Ames, IA. Phytopathology 94:S100. Publication no. P-2004-0679-AMA.

The soybean cyst nematode (SCN) *Heterodera glycines*, is a sedentary endoparasitic nematode of soybean that has become one of the major limiting factors in soybean production. Parasitism genes expressed in the esophageal gland cells of phytonematodes encode proteins that are secreted into the host cells that control the complex process of plant parasitism. Double stranded RNA interference (RNAi) of target gene expression has emerged as a powerful tool to inactivate specific genes for functional analysis. RNAi has been widely used in *C. elegans*, but transferring the technology to plant-parasitic nematodes has been challenging because of their obligate parasitic life cycle and lack of a transformation system. We are employing the RNAi soaking method of Urwin et al. (2002) for functional analysis of secreted proteins in *H. glycines*. For initial experiments and protocol optimization, we selected three parasitism genes of *H. glycines* as models for RNAi studies. The RNAi effects of double-stranded RNA ingestion on target SCN parasitism gene expression are being monitored by quantitative Real-Time PCR. If significant RNAi of target parasitism genes can be confirmed, subsequent analyses will assess these effects on nematode infection of plant roots.

Fitness of races O and 1 of the tobacco black shank pathogen. M. J. SULLIVAN, T. A. Melton, and H. D. Shew. Dept. of Plant Pathology, NC State University, Raleigh, NC 27695. Phytopathology 94:S100. Publication no. P-2004-0680-AMA.

Deployment of tobacco varieties with complete resistance to race O of *Phytophthora parasitica* var. *nicotianae* has increased field populations of race 1 in NC. In variety rotation studies, populations of race 1 decreased relative to race O when varieties with partial resistance to both races were planted, suggesting that race 1 isolates were not as fit as race O isolates. Experiments were conducted to determine if differences in fitness were due to differences in aggressiveness, survival, or both. Tobacco varieties with low, moderate, and high levels of partial resistance were inoculated with sixty pathogen isolates and symptom development was monitored for three weeks. Race O isolates were more aggressive than race 1 isolates on varieties with moderate or high levels of partial resistance under greenhouse conditions; symptoms developed sooner and were more severe with race 0 isolates. Isolates of race 1, however, caused greater stunting of plants than race O isolates. Field microplots were infested with either a single race or an equal mixture of each race. Soil samples were collected at the end of each growing season and again the following spring and compared. There were no differences in survival among plots infested with a single race. In the mixed race plots, however, race O increased relative to race 1 on varieties with partial resistance. Race 1 increased relative to race O in plots containing varieties with complete resistance. Shifts in relative population of races appear to be due to differences in pathogenic and not ecological fitness of the races.

Comparison of fungi in sooty blotch and flyspeck apple disease complex in Shaanxi Province China and U.S. based on ribosomal DNA. G. Y. Sun (1), J. C. Batzer (1), R. Zhang (1), Y. M. Zhang (1), and M. L. GLEASON (2). (1) College of Plant Protection, Northwest Sci-Tech University of Agriculture and Forestry, Yangling, Shaanxi, China; (2) Iowa State University, Ames, IA. Phytopathology 94:S100. Publication no. P-2004-0681-AMA.

The sooty blotch and flyspeck (SBFS) complex colonizes the cuticle of apple fruits in moist production regions worldwide, inciting cosmetic damage and causing significant economic losses. Large subunit (LSU) and internal transcriber spacer (ITS) regions of rDNA were used to characterize 38 SBFS isolates obtained from blemished apples grown in four orchards in the Shaanxi Province, China. Sequences from China and the Midwest and Southeast U.S. were compared using parsimony analysis. Two isolates from China were placed within the Chaetothyriales; LSU sequences formed a monophyletic grouping with U.S. SBFS member *Leptodontium elatius*. The other 36 isolates from China were Dothidiales. Four sequences clustered with a *Peltaster* sp. found in the Midwest U.S. One isolate grouped with flyspeck pathogen *Zygophiala jamaicensis* found throughout the U.S. Remaining sequences clustered with sequences of known *Mycosphaerella* spp. Obtained from GenBank. ITS sequences of China isolates clustered with midwestern SBFS *Xenostigmina* sp., *Dissoconium* spp., and *Pseudocercosporella* spp. Sequences of the 22 *Mycosphaerella* isolates from China did not group closely with any previously identified species of SBFS. These findings expand knowledge of the diversity of the SBFS complex and suggest that most of the SBFS fungi are within the same *Mycosphaerella* genus.

Specific PCR primers to identify sooty blotch and flyspeck fungi on apple in the Midwest U.S. G. Y. Sun (1), L. F. Leandro (1), J. C. Batzer (2), T. C. Harrington (2), and M. L. GLEASON (2). (1) College of Plant Protection, Northwest Sci-Tech University of Agriculture and Forestry, Yangling, Shaanxi, China; (2) Iowa State University, Ames, IA. Phytopathology 94:S100. Publication no. P-2004-0682-AMA.

The sooty blotch and flyspeck (SBFS) fungal complex damages the appearance of apple fruits in moist production regions worldwide. The ecology of SBFS is poorly understood because these fungi are difficult to culture and rarely produce fruiting bodies on either the apple surface or culture media. Therefore a set of PCR primers were designed to facilitate the identification of members of the SBFS complex. Target members of the SBFS complex included 28 recently discovered species isolated from infested apples grown in the midwestern U.S. and four previously documented species on apples grown in the southeastern U.S. A general Dothidiales primer was designed based on the sequence data of the internal transcribed spacer (ITS) and a portion of the large subunit (LSU) of ribosomal DNA. The Dothidiales primers amplified all the in-group isolates resulting in single band PCR products ranging from 0.8 to 1.5 kb. Further strategies are to use restriction digests of the resulting PCR products to identify fungi to the genus or species level based on restriction fragment length polymorphism patterns. In addition to the general Dothidiales primer, internal species-specific primers for the ITS regions are being designed for commonly occurring SBFS species, some of which are in other families of the Dothidiomycetes. Use of these primers will facilitate further studies on the ecology of the SBFS complex.

Accumulated rainfall thresholds for applying protectant fungicides on potato cultivars resistant to *Phytophthora infestans* en Ecuador. J. A. TAIPE (1), G. A. Forbes (2), and M. Taipe (2). (1) Programa Nacional de Raíces y Tubérculos-Papa, INAP, Quito, Ecuador; (2) Centro Internacional de la Papa, Quito, Ecuador. Phytopathology 94:S101. Publication no. P-2004-0683-AMA.

In Ecuador, susceptible potato cultivars must be protected from *P. infestans* with frequent fungicide applications, but resistant cultivars require much less fungicide. Ecuadorian farmers do not have access to equipment that would allow for sophisticated decision support systems, however, farmers can easily measure accumulated rainfall. In this experiment 5 resistant and one susceptible cultivar were grown without fungicide protection, sprayed with protectant fungicide weekly and after each 30 and 50 mm of rainfall. A minimum spray interval of 10 days was used with rainfall thresholds. Disease severity was high on all cultivars grown without fungicides, and no fungicide treatment gave ample control for the susceptible cultivar. All fungicide treatments controlled disease on the 5 resistant cultivars, but the 50 mm threshold resulted in the lowest number of applications. This threshold appears to be a useful indicator for fungicide timing on resistant cultivars under these conditions.

Incidence and prevalence of *Maize streak virus* (MSV), genus *Mastrevirus* and *Maize mottle/chlorotic stunt virus* in Lagos, Nigeria. M. A. TAIWO (1), J. d'A. Hughes (2), and K. E. Oke (1). (1) Department of Botany and Microbiology, University of Lagos, Akoka, Lagos; (2) International Institute of Tropical Agriculture, Ibadan, Nigeria. Phytopathology 94:S101. Publication no. P-2004-0684-AMA.

Despite the development and deployment of maize streak resistant (SR) germplasm, virus induced symptoms were still commonly observed on maize in Lagos, Nigeria. Surveys were therefore conducted between April 2001 and February 2002 to determine the identity, prevalence and incidence of maize viruses in 18 local government areas (LGAs) in and around Lagos. All the fields surveyed during the dry season (112) and late dry season (18) had plants infected by *Maize streak virus* (MSV), genus *Mastrevirus* while 96.5% of the 170 fields surveyed during the wet season had plants infected by MSV. *Maize mottle/chlorotic stunt virus* (MMCSV) was prevalent in 99.1%, 88.9% and 67.4% of the fields surveyed during the dry, late dry and wet seasons respectively. The incidences of the viruses were higher during the dry season. The incidence of MSV was higher than MMSCV in 16 of the LGAs with 18.9% and 7.4% as the highest incidences recorded for both viruses respectively. Serodiagnostic screening of 1475 of 1210 leaf samples suspected to be infected by MSV and MMCSV using antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) indicated that 1192 (80.8%) and 949 (78.4%) reacted positively to their corresponding antisera respectively. Vector transmission and host range studies confirmed the identity of the viruses. The result confirms the presence of MSV and MMSCV in Lagos and suggests that the use of MSV susceptible varieties is still widespread. Methods of ensuring effective utilization of existing SR germplasm and controlling maize viruses in general are discussed.

Natural infection of *Datura stramonium* L. by a unusual strain of *Pepper veinal mottle virus* genus *Potyvirus* in Nigeria. M. A. TAIWO (1), J. d'A. Hughes (2), and A. R. Akomah (1). (1) Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria; (2) International Institute of Tropical Agriculture, Ibadan, Nigeria. Phytopathology 94:S101. Publication no. P-2004-0685-AMA.

A disease causing mosaic and leaf distortion was observed in 80% of *Datura stramonium* L. plants in a garden in Ibadan, Nigeria. The causal agent had flexuous rod-shaped particles of about 750 – 800nm length. The virus was readily mechanically transmissible but was not seed-borne in the natural host. The virus was not transmissible to any other host genera, and was restricted to *D. stramonium* and *D. metel*. In serological tests using protein A-sandwich enzyme-linked immunosorbent assay (PAS-ELISA) and antisera to ten members of the family *Potyviridae*, the virus from *D. stramonium* reacted only with the antiserum raised against *Pepper veinal mottle virus* (PVMV). Optical density readings of 3.564 and 0.466 at 405 nm were obtained in reactions between PVMV antiserum and virus infected and healthy *Datura* spp. samples respectively. No serological relationship was detected between the virus from *D. stramonium* and *Bean common mosaic virus* genus *Potyvirus* (Blackeye strain), *Cowpea aphid-borne mosaic virus* genus *Potyvirus* and *Yam mosaic virus* genus *Potyvirus* in double antibody-sandwich ELISA and immunosorbent electron microscopy tests. Although some of the data on symptomatology, host range and serological relatedness could implicate *Datura shoestring virus* (DSV), genus *Potyvirus*, the virus may actually be an unusual strain of PVMV.

Differential gene expression during sclerotium formation in *Sclerotium rolfsii*. J. E. TAKACH and S. E. Gold. Department of Plant Pathology, University of Georgia, Athens, GA. Phytopathology 94:S101. Publication no. P-2004-0686-AMA.

Sclerotium rolfsii, the causal agent of southern blight or white mold of peanut, survives poor environmental conditions by differentiating into sclerotia. Resistant sclerotia are critical to the persistence of disease potential in infested fields. Environmental factors, including variation in light, temperature, and oxygen are known to trigger sclerotium development, yet little is known about the genetic basis of this differentiation. To further understand this process, we have begun to identify and characterize genes upregulated during sclerotium formation. We have identified a *S. rolfsii* isolate that consistently produces sclerotia in a highly programmed manner. With this strain we are using suppressive subtractive hybridization PCR (SSHP) to identify genes specifically upregulated during sclerotium formation. Characterization of these genes will yield insight into the process and regulation of sclerotium differentiation. Preliminary results of this study will be presented.

Control of soilborne pathogens using allelopathic plants to lower redox potential of soil. T. TAKEHARA, S. Hanzawa (2), M. Funabara (3), K. Nakaho (1), and A. Nakagawa (1). (1) National Agricultural Research Center, Tsukuba, Japan; (2) Miyagi Prefectural Agriculture and Horticulture Research Center, Japan; (3) Tottori Horticultural Experiment Station, Japan. Phytopathology 94:S101. Publication no. P-2004-0687-AMA.

Shinmura et al. (1999, Ann. Phytopathol. Soc. Jpn. 65:352) watered soil amended with readily decomposable organic matter to lower soil redox potential (Eh) to disinfest soil. Here, Indian mustard (*Brassica juncea*) or hairy vetch (*Vicia villosa*), which release antifungal compounds allyl isothiocyanate and cyanamide, respectively, was used to control diseases caused by *Fusarium oxysporum*. When soil infested with *F. o.* f. sp. *spinaciae* (5×10^3 CFU/g dry soil) was amended with shredded leaves of these plants at 30 g fresh weight/kg of air-dried soil and kept for 18 d at 35°C with 45% soil moisture in sealed bottles, the soil Eh dropped notably and the pathogen was undetectable. In a field test against *F. o.* f. sp. *lycopersici*, adding 13 kg/m^2 of *B. juncea* shoots in summer and then watering (over 150 L/m^2) under a transparent plastic sheet lowered Eh and gave better control than *B. juncea* alone. Lowering Eh using allelopathic plants may be an effective control measure.

Mechanisms of temperature-dependent gene expression during zoosporogenesis in *Phytophthora infestans*. S. TANI and H. Judelson. University of California, Riverside. Phytopathology 94:S101. Publication no. P-2004-0688-AMA.

Microbial pathogenesis is often associated with temperature-dependent dimorphism. In *Phytophthora infestans*, cool water (usually below 14°C) induces sporangia to form zoospores while warmer temperatures favor direct germination. To better understand these important parts of the disease cycle, zoosporogenesis-induced genes were identified using cDNA arrays. Sixty-nine genes showing at least 4-fold increases in mRNA levels were identified and their expression was characterized in time courses, other developmental stages, temperature regimes, and tissue treated with signaling inhibitors. The results indicated that the genes were regulated by multiple pathways (MPMI 17:330-7 [2004]). Detailed studies were initiated of several genes, such as a family of transcriptional coactivators (Nuclear LIM interactors) named *Pi-NLIC1*, *C2*, and *C3*. Using the beta-glucuronidase reporter, promoter mutation studies revealed that the region between 155 and 241 nt upstream of the start codon regulated cold-induction. Directed mutations are now defining the important *cis*-element(s), and studies of the function of the NLI proteins have been initiated.

Identification and prevalence of bean pod rot in Arkansas and Missouri. J. H. TAYLOR (1) and C. S. Rothrock (2). University of Arkansas. Phytopathology 94:S101. Publication no. P-2004-0689-AMA.

Snap beans (*Phaseolus vulgaris*) are an important crop grown for processing in both Arkansas and Missouri averaging 4856 to 6070 hectares per year. Snap bean crops are harvested mechanically with non-selective picking fingers and are shipped in bulk to processing plants at various locations throughout the mid-south. To maintain maximum profit, growers must produce crops that are virtually free of blemishes caused by insects and diseases. Pod rots consistently affect Arkansas' and Missouri's snap beans. Two of the causal agents of pod rot that have been reported, are *Pythium aphanidermatum* and a *Phytophthora* sp. Soil samples (at planting), pod samples (at harvest), and corresponding environmental data were taken from 15 grower locations. A baiting technique was developed to examine Pythium inoculum in the fields for soil collected at planting. At harvest, 600 pods were examined from 15 sites per field. Pods collected with symptoms of pod rot were either in direct contact with the soil, dead leaf tissue, or with other diseased pods. Pathogens associated with pod rot were *Pythium* spp. (14

sites), *Rhizoctonia solani* (5 sites), *Sclerotinia scleotiorum* (3 sites), and a *Phytophthora* sp. (1 site). Disease incidence on pods averaged 1.4% and ranged from 0 to 7.3% across all fields sampled for pod rot. No associations with the environmental data collected on cultural practices were observed with the increase in incidence of pod rot.

A putative oxidoreductase is necessary for cercosporin degradation by *Xanthomonas campestris* pv. *zinniae*. T. V. TAYLOR and M. E. Daub. North Carolina State University, Raleigh, NC. Phytopathology 94:S102. Publication no. P-2004-0690-AMA.

The bacterium *Xanthomonas campestris* pv. *zinniae* rapidly degrades the fungal toxin cercosporin, which plays a key role in pathogenesis by *Cercospora* spp. Non cercosporin-degrading mutants were generated using EMS mutagenesis, and complemented with a wild type library. Sequencing of the complementary region in all three mutants revealed that a mutation exists in a putative oxidoreductase gene in each mutant. RT-qPCR showed that expression of the oxidoreductase gene in the wild type strain is upregulated after exposure to cercosporin. However, the gene was not sufficient to provide degradation ability to other non cercosporin-degrading bacteria, suggesting that another gene(s) is involved. To identify mutants in additional genes, four new non-degrading mutants were generated by EMS mutagenesis in the wild type strain expressing a multicopy plasmid carrying the oxidoreductase gene. These mutants are being transformed with the genomic library to identify complementing gene(s). Once found, these gene(s) may allow for engineering of *Cercospora*-resistant plants.

Plant cell response to thaxtomin A and amelioration of toxic effect by auxin. R. S. TEGG, S. N. Shabala, L. Melian, A. Eyles, and C. R. Wilson. Tasmanian Institute of Agricultural Research, Australia. Phytopathology 94:S102. Publication no. P-2004-0691-AMA.

Thaxtomin A is a key phytotoxin produced by plant pathogenic *Streptomyces* sp. and is implicit in common scab disease expression in potato. The target and mode of action of thaxtomin A toxicity in plant cells is not well understood, although it is believed to act at the plant cell wall inhibiting cellulose deposition or synthesis. To further our understanding of thaxtomin A toxicity we examined early cell signalling activity of *Arabidopsis thaliana* to toxin exposure using the ion-selective microelectrode ion flux estimation (MIFE™) technique. Thaxtomin A altered the fluxes of the key ions hydrogen and calcium by interfering with specific ion channels. Immediate responses showed calcium was excluded from the cell wall in exchange for hydrogen, consistent with a Donnan type exchange system. Moreover, increased hydrogen efflux was consistent with thaxtomin activation of the proton pump. A tomato pollen tube elongation assay was also conducted to quantify the effect of the auxin (NAA) on the efficacy of the toxins; thaxtomin A, alternariol and fusaric acid. NAA was able to rescue pollen from thaxtomin A induced inhibition but not from inhibition following alternariol or fusaric acid treatment. These studies show initial cellular responses to thaxtomin A and demonstrate amelioration of thaxtomin A toxic effects with NAA application.

Histopathology of *Rhamnus alaternus* knots induced by *Pseudomonas savastanoi*. M. Temsah, L. Hanna, and A. T. SAAD. American University of Beirut, Beirut, 1107 2020 Lebanon. Phytopathology 94:S102. Publication no. P-2004-0692-AMA.

Buckthorn (*Rhamnus alaternus*) was recently reported as a new host of *Pseudomonas savastanoi*. The main symptom on buckthorn is the formation of knots on stems, branches, and occasionally on leaves. Anatomical changes in host tissues induced by *P. savastanoi* were studied by light microscopy and compared to those caused by the same pathogen on other hosts. Inoculations of buckthorn shoots by *P. savastanoi* initiated small green swellings that developed into fully-grown knots. The pathogen invades intercellular spaces of parenchyma tissues degrading cell walls and resulting in extensive cavities filled with bacterial cells. Many host cells contiguous to bacterial cavities were plasmolysed and necrotic. Parenchyma, cambium, and ray cells around bacterial cavities enlarge and proliferate to form the knots. Proliferating cells become multinucleate and then form cell walls around each nucleus. At later stages of tumor development, the cambium, within the tumor, appears dispersed and forms disoriented vascular tissues. The cells of layers adjacent to bacterial cavities and at surface of knots become suberized.

Conidial germination of three fungi in the sooty blotch and flyspeck complex. K. D. TENTINGER, J. C. Batzer, and M. L. Gleason. Iowa State University, Ames, IA. Phytopathology 94:S102. Publication no. P-2004-0693-AMA.

Fungi in the sooty blotch and flyspeck (SBFS) complex blemish apple fruit in humid growing regions worldwide. Environmental factors, such as leaf wetness duration and nutrient availability on apple fruit surface, may influence the temporal and geographic distribution of the fungi. Two isolates each of three SBFS fungi, *Peltaster fructicola*, *Peltaster* sp. P2 and *Peltaster* sp. P6, were compared. Conidia from 4-day-old cultures on PDA were suspended in sterile 0.05% apple juice (AJ) and sterile deionized water (SDW). Two 2-microliter drops, each containing approximately 200 conidia, were placed on parafilm mounted on glass slides. After drops were dried in a transfer hood, slides were incubated in a dew chamber at 24°C in the dark. Germination was determined for 100 conidia of each isolate at 0, 4, 8, 12 and 24 h. Conidial germination of *Peltaster* sp. P2 was higher in AJ than SDW, but germination in AJ did not differ from germination in SDW for *Peltaster fructicola* or *Peltaster* sp. P6. Rates of conidial germination differed among species.

Host resistance and metam sodium for managing root-knot nematodes in a pepper-cucumber rotation. J. A. THIES (1), R. F. Davis (2), J. D. Mueller (3), R. L. Fery (1), D. B. Langston (4), and G. Miller (3). (1) USDA-ARS, Charleston, SC; (2) USDA-ARS, Tifton, GA; (3) Clemson Univ., Blackville, SC; (4) Univ. of Georgia, Tifton, GA. Phytopathology 94:S102. Publication no. P-2004-0694-AMA.

A root-knot nematode resistant bell pepper cultivar, Charleston Belle (*Capsicum annuum*), and metam sodium were evaluated for managing southern root-knot nematode (*Meloidogyne incognita*) in fall-cropped cucumber (*Cucumis sativus*). 'Charleston Belle' and its susceptible recurrent parent, 'Keystone Resistant Giant', were planted as spring crops at Blackville, SC and Tifton, GA. 'Charleston Belle' exhibited high resistance and 'Keystone Resistant Giant' was susceptible. After termination of the bell pepper crop, one-half of the plots were treated with metam sodium delivered through the drip irrigation system. Cucumber grown after 'Charleston Belle' × metam sodium had the lowest root gall severity and lowest nematode reproduction. Root gall severity and nematode reproduction were highest for cucumber grown in plots following 'Keystone Resistant Giant' × control. Cucumber yields and numbers were highest for cucumber grown following metam sodium with either cultivar.

Use of morphological, molecular, and pathogenic characteristics to identify *Gaeumannomyces graminis* varieties. S. L. Thomas (1), P. E. Lipps (2), and M. J. BOEHM (1). (1) The Ohio State University, Columbus; (2) The Ohio State University, Wooster. Phytopathology 94:S102. Publication no. P-2004-0695-AMA.

Take-all, caused by *Gaeumannomyces graminis*, is a devastating root disease on cereal crops and turfgrasses worldwide. Three varieties of the fungus have been described and are distinguished from one another based on hyphopodium type and ascospore length. *G. graminis* var. *graminis* is readily differentiated from *G. graminis* var. *avenae* (Gga) and *G. graminis* var. *tritici* (Ggt) based on the production of lobed hyphopodia. Gga and Ggt produce simple hyphopodia and can only be distinguished morphologically from one another based on ascospore length. Although Gga has generally longer ascospores, most published ascospore length ranges contain a 5-10 µm overlap making it impossible to classify isolates with intermediate ascospore lengths. Pathogenicity on oats and variety-specific PCR primers can also be used to distinguish Gga from Ggt. The goal of this study was to compare the utility of using morphological, molecular and infectivity assays to classify isolates of *G. graminis* to the variety level. Seventeen of 38 isolates (45%) could be identified as either Gga or Ggt based solely on ascospore length. Molecular characterization using previously described variety-specific avenacinase or *G. graminis*-specific 18S rDNA primer sets allowed for Gga to be differentiated from Ggt 92% and 89% of the time, respectively. Pathogenicity on oats provided consistent and absolute (100%) differentiation of Gga and Ggt.

Pathogenicity of *Gaeumannomyces graminis* var. *tritici* on creeping bentgrass. S. L. THOMAS, P. Bonello, and M. J. Boehm. The Ohio State University, Columbus. Phytopathology 94:S102. Publication no. P-2004-0696-AMA.

The avenacin-avenacinase interaction has been shown to mediate the host-pathogen interaction of oats-*Gaeumannomyces graminis*. Avenacin is a fungitoxic saponin produced in oat roots which is detoxified through avenacinase activity. *G. graminis* var. *avenae* (Gga) produces avenacinase and is able to infect oats whereas *G. graminis* var. *tritici* (Ggt) does not produce avenacinase and cannot infect oats. Gga also causes take-all patch on creeping bentgrass, however, it is not known if the avenacin-avenacinase interaction plays a role in the creeping bentgrass-Gga interaction. The goals of this study were to determine the production of avenacin in creeping bentgrass and the ability of Ggt isolates to infect bentgrass. Fluorimetric, TLC, and HPLC analyses of root tip extracts revealed that avenacin was not produced by creeping bentgrass. Infectivity assays showed both Gga and Ggt were able to infect wheat and creeping bentgrass but only Gga incited disease on oats indicating that avenacinase production by *G. graminis* is not required for infection of creeping bentgrass.

IR-4 fungicide registration update. D. C. THOMPSON, H. Chen, V. R. Starner, W. P. Barney, and D. L. Kunkel. IR-4 Project, Rutgers University, North Brunswick, NJ. Phytopathology 94:S103. Publication no. P-2004-0697-AMA.

The IR-4 Project with the U.S. EPA, California's Department of Pesticide Regulation and Canada's Pest Management Regulatory Agency obtained a record 687 new chemical uses, 2 new biopesticide uses, 104 Section 18 uses, and 814 new ornamental uses. Nearly 40% of these uses are identified as "Reduced Risk" by EPA. Over the past 5 years, nearly 80% of the IR-4 field program has been conducted with reduced risk or lower risk chemistries. The Triazole Task Force is completing studies in 2004 and expects the U.S. EPA to be able to make decisions in late 2004 or early 2005. New registrations will hopefully follow. Post-harvest uses of fenhexamid and fludioxonil on pome fruit, stone fruit, kiwifruit, pomegranate and yam are in EPA's 2004 workplan. Efficacy work is being coordinated with BASF and Valent on their new fungicides. IR-4 is conducting a program with iodomethane to obtain uses on all crops. Quinoxyfen received first uses on hops, grapes and cherries. Boscalid received registration on a large number of crops. Spiroxamine registration on hops and grapes should be finalized by EPA early in 2004. We continue to work with Canada in their development of a minor-use program. Coordination on residue and efficacy projects of common interest has increased in 2004.

Gene deletion of manganese peroxidase-like genes in *Magnaporthe gris*
I. A. THOMPSON (1), J. R. Xu (1), D. M. Huber (1), and D. G. Schulze (2). (1) Dept. Botany and Plant Pathology; (2) Dept. of Agronomy, Purdue University, West Lafayette, IN. Phytopathology 94:S103. Publication no. P-2004-0698-AMA.

Manganese peroxidase (MnP) and versatile peroxidase (VPS) genes are found primarily in white-rot fungi. The enzyme products of these genes catalyze lignin depolymerization partially through the oxidation of Mn(II) to Mn(III), an active redox agent capable of reacting with phenolic compounds. Manganese oxidation is proposed to influence fungal virulence in two pathosystems, take all of wheat and blast of rice. Two genes with homology to MnP and VPS, designated *MnP1* and *MnP2*, were identified in the *M. grisea* genome. These genes do not appear to be common in other ascomycetes. Double knockout mutants for *MnP1* and *MnP2* were created through gene deletion and sexual crossing. Though still capable of Mn oxidation, double mutants and a single mutant for *mnp1* exhibited a unique Mn oxidation phenotype. The effects of these mutations on pathogen virulence and aggressiveness are also presented.

An airflow system for management of *Botrytis cinerea* on lisianthus. S. A. Tjosvold (1), S. N. WEGULO (2), J. F. Thompson (3), D. Chandler (1), and M. Vilchez (2). (1) UCCE Watsonville, CA 95076; (2) University of California, Riverside, CA 92521; (3) University of California, Davis, CA 95616. Phytopathology 94:S103. Publication no. P-2004-0699-AMA.

Diseases caused by *Botrytis cinerea* significantly reduce lisianthus (*Eustoma grandiflorum*) yields. A novel airflow system was engineered for management of these diseases. The system consisted of a 15-cm-diameter polyethylene tube shaped like an inverted T. The tube was installed in a lisianthus bed with the vertical portion at the bed center and attached to an electrically powered fan 1.5 m above the bed surface. Circular holes on each side of the horizontal portion of the tube allowed air to exit the tube into the canopy. Both ends of the horizontal portion of the tube were tightly secured to restrict air circulation to the canopy. Leaf wetness duration was shorter in air circulation beds than in non-treated controls. Following sprinkler irrigation, foliage in treated beds dried faster than in controls. Plant mortality due to *Botrytis* was 44% lower in treated beds than in controls. The airflow system can reduce use of fungicides and increase plant productivity through carbon dioxide enrichment of the canopy.

Development of specific primers for each variety of *Rhizoctonia circinata*. T. TODA (1), J. M. Mghalu (1), T. Hayakawa (2), and M. Hyakumachi (1). (1) Gifu University, Gifu, Japan; (2) Riken Green Co. Ltd., Iwata, Japan. Phytopathology 94:S103. Publication no. P-2004-0700-AMA.

Four varieties of *Rhizoctonia circinata*; var. *agrostis*, var. *circinata*, var. *oryzae* and var. *zeae*, are the causal agents of turfgrass diseases during warm seasons. As their symptoms are quite similar, it is difficult to distinguish among these four varieties. Conventional identification technique based on the cultural morphology of the isolated pathogen is laborious and time-consuming. For rapid detection of each variety of *R. circinata*, specific primers were designed from the sequence data of internal transcribed spacers of ribosomal DNA (rDNA-ITS region). The primers were Agro-1F for *R. c.* var. *agrostis*, Cir-2F for *R. c.* var. *circinata*, Ory-1F for *R. c.* var. *oryzae* and Zea-2F for *R. c.* var. *zeae*. Each primer pair of Agro-1F/ITS 4, Cir-1F/ITS 4, Ory-1F/ITS 4 and Zea-2F/ITS 4 could respectively produce a single PCR product from pure fungal DNA for each variety of *R. circinata*. When the primers were tested on other fungal pathogens of turfgrass, they showed no PCR products. The primer pairs of Agro-1F/ITS 4, Cir-2F/ITS 4, Ory-1F/ITS 4 and Zea-2F/ITS 4 are specific and useful for the rapid detection of respective *R. circinata* varieties.

Endomycorrhizal colonization of transgenic potato cultivars. J. A. TRAQUAIR and B. Singh. Southern Crop Protection and Food Research Center, Agriculture and Agri-Food Canada, 1391 Sandford Street, London ON, Canada N5V 4T3. Phytopathology 94:S103. Publication no. P-2004-0701-AMA.

High-value and intensively-managed horticultural crops are favourite candidates for investigations into the agronomic potential of arbuscular mycorrhizal (AM) symbioses for sustaining soil quality and plant protection against disease. Impacts of physical and chemical factors in the environment on mycorrhizal colonization are well-known as are biological factors such as host preferences and differences in mycorrhizal dependency between species and even cultivars of the same crop. Cultivars genetically engineered for insect pest resistance are rapidly gaining popularity with farmers in North America. For example, corn varieties containing *Bacillus thuringiensis* (Bt) genes for production of Cry protein toxin account for about 35% of total corn planted in Canada and 25% in USA. However, the non-target impacts of transgenes such as Bt toxin genes on mycorrhizal colonization are not always clear. Arbuscular mycorrhizal colonization of stained, randomly collected roots was monitored in the field and greenhouse for Bt-containing lines of the potato cultivars Shepody and Superior. There was no significant Bt effect on AM colonization of greenhouse-grown plants inoculated with *Glomus intraradices*. The AM colonization of Shepody by indigenous inoculum, was greater than that of Superior but there was no consistent Bt effect over a 3-year period.

Summer patch of creeping bentgrass caused by *Magnaporthe poae* in North Carolina. L. P. TREDWAY. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC. Phytopathology 94:S103. Publication no. P-2004-0702-AMA.

An unknown patch disease was observed on creeping bentgrass putting greens at Landfall Country Club in Wilmington, NC in summer 2002 and 2003. Affected greens were established in 2001 with a blend of cultivars 'A-1' and 'A-4'. Soil pH ranges from 7 to 8 at this location due to poor quality irrigation water. Circular patches of turf initially exhibited signs of wilt, followed by chlorosis and orange necrosis. Roots and crowns were necrotic and colonized with dark, ectotrophic hyphae. A fungus resembling *Magnaporthe* was consistently isolated from necrotic roots and crowns. Unknown isolates mated in vitro with *M. poae* 73-15 and are therefore *M. poae* mating type a. Pots of A-4 creeping bentgrass were inoculated with *M. poae* and placed in growth chambers with 12 hr day/night cycles at 30/25°C, 35/25°C, or 40/25°C. Foliar chlorosis and necrosis was observed 28 days after inoculation at 30°C and 35°C, but not at 40°C. Roots and crowns of inoculated plants were necrotic and colonized with ectotrophic hyphae, and *M. poae* was readily isolated from necrotic tissue. This is the first report of summer patch of creeping bentgrass within the zone of adaptation for this turfgrass species.

Sequencing and functional analysis of the maize fine streak virus (MFSV) genome. C. W. TSAI (1), M. G. Redinbaugh (1,2), K. Willie (2), S. Reed (1), M. Goodin (3), and S. A. Hogenhout (1). (1) Ohio State University; (2) USDA-ARS, Wooster, OH; (3) University of Kentucky, Lexington, KY. Phytopathology 94:S103. Publication no. P-2004-0703-AMA.

We sequenced the genome of maize fine streak rhabdovirus (MFSV). The genome has seven open reading frames (ORFs) on the viral complementary strand. Northern blot analysis showed that all seven genes are expressed during infection of maize. ORF1, ORF6, and ORF7 likely encode the nucleocapsid protein (N), glycoprotein (G), and polymerase (L), respectively. A putative nuclear localization signal (NLS) was identified in the N gene, and a transmembrane domain was identified in the G gene. ORF4 and ORF5 also contain putative NLS. We employed *in planta* localization of fluorescent protein fusions in agroinfiltrated tobacco leaves to determine which ORFs encode the phosphoprotein (P) and matrix (M) proteins. Results show that ORF5 likely encodes M because it targets the nuclei of plant cells. Further, ORF2 apparently encodes P because it is localized to the nuclei when co-expressed with N. The functions of ORF3 and ORF4 remain to be determined. These results suggest that the MFSV genome organization is 3′-N-P-3-4-M-G-L-5′.

Secretome analysis of *Pantoea stewartii* sbsp. *stewartii*. D. S. TSALTAS and S. V. Bodman. Plant Science Department, University of Connecticut, Storrs, CT. Phytopathology 94:S103. Publication no. P-2004-0704-AMA.

The phytopathogenic bacterium *Pantoea stewartii* subsp. *stewartii* (*Pss*) is the causal agent of the Stewart's wilt disease in maize. A beetle vector, *Chaetocnema pulicaria* transmits the disease during periods of feeding directly into the xylem of maize plants. The wilting symptomatology is largely the result of the xylem blockage due to the bacterial secretion of an extracellular polysaccharide (EPS) virulence factor. The EsaI/EsaR quorum sensing (QS) regulatory proteins control EPS synthesis in *Pss*. A mutation in the *esaI* gene causes parallel loss of the diffusible inducer N-(3-oxo-hexanoyl)-L-homoserine lactone (AHL), EPS production and virulence. Strains mutated in *esaR* or *esaR* and *esaI* are hypermucoid due to constitutive expression of the EPS biosynthetic pathway. Interestingly the hypermucoid mutant strains are less virulent even though they produce significantly higher amounts of EPS than wild strain DC283. We are analysing the wild and mutant quorum sensing strains for potential differences in their respective secretomes. The rational for this study is that secreted proteins or proteins loosely associated with the membrane surface play a critical role in the infection process, and that some of these proteins are controlled by quorum sensing.

Invasion, transmission and adaptation of new race strains of *Ralstonia solanacearum*, causal pathogens of bacterial wilt of Zingiberaceae plants in Japan. K. TSUCHIYA, H. Sawada, T. Yoshida, and M. Takahashi. National Institute for Agro-Environmental Sciences, 3-1-3, Kannondai, Tsukuba, Japan. Phytopathology 94:S104. Publication no. P-2004-0705-AMA.

A bacterial wilt disease of three Zingiberaceae plants of curcuma, ginger and mioga have occurred and been spreading successively since 1995 in Kochi Prefecture, Japan. All bacterial isolates were identified as *R. solanacearum* race 4 biovar 4 based on pathological and physiological properties. 16S rDNA sequencing revealed all strains belonged to Division 1. Based on rep-PCR analysis using BOX, REP and ERIC primers, two types of DNA fingerprints were obtained, neither of which was identical to those of indigeneous or representing races and biovars so far reported in the country. The DNA pattern of group 1 strains was identical to that of Thai strains, whereas that of group 2 was to Australian and Chinese strains, respectively. It is, therefore, presumable that these two exotic pathogenic strains might invade separately through contaminated seed materials and have been spreading in epidemic proportins through separate routes.

Characterization of Pseudomonads isolated from dormant sweet cherry budsticks in New York. W. W. TURECHEK, R. M. Edwards, and N. A. Werner. Cornell University, NYSAES, Geneva, NY. Phytopathology 94:S104. Publication no. P-2004-0706-AMA.

Bacterial canker of sweet cherry is caused by the bacteria *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *morsprunorum*. The bacteria surviving in vegetative buds are particularly important because they may be transmitted during propagation and serve as a source of inoculum in young orchards. In 2002 and 2003, we collected 1000 'Royalton' budsticks from each of 4 orchards in western NY in mid-January. The budsticks were subjected to various hot water treatments to investigate heat treatment as means of eliminating resident bacteria. Vegetative buds from heat treated and non-heat treated budsticks were excised, sterilized, and homogenized, and the homogeneate plated on KB media. Characteristic bacteria were isolated and identified via PCR and their sensitivities to copper and streptomycin were tested. In this poster, we report on the frequency that each species was found, their sensitivities to copper and streptomycin, and whether heat treatment favored the selection of resistant isolates. The results of this screening will help us refine spray programs and decide on the feasibility of heat treatment prior to propagation.

Complete nucleotide sequence, phylogenetic analysis and geographic distribution of *Fragaria chiloensis latent virus*, genus *Ilarvirus*. I. E. TZANETAKIS (1) and R. R. Martin (1,2). Dept. of Bot. and Plant Path., OSU, Corvallis 97331; (2) USDA-ARS-HCRL, Corvallis, OR 97333. Phytopathology 94:S104. Publication no. P-2004-0707-AMA.

Fragaria chiloensis latent virus (FClLV) is an *Ilarvirus* first identified in strawberry in 1993 in *F. chiloensis*, plants from Chile. At that time, tt was not detected in plants from the Pacific Coast of North America. We have now cloned dsRNA from a plant infected with FClLV. The complete nucleotide sequence of the virus has been determined. RNA 1 encodes for the virus replicase, RNA 2 for the polymerase and RNA 3 the movement and coat protein of the virus. Several smaller ORFs are also found in RNA 3 but their expression has not been examined. Phylogenetic analysis reveals that the virus is, contrary to previously reported, closely related to *Prune dwarf*, *Prunus necrotic ringspot*, *Apple mosaic* and *Alfalfa mosaic* viruses. Using RT-PCR, the virus has now been detected in strawberry samples from the western United States and Canada and testing is underway to determine the virus distribution in other areas. It is not clear if the detection with RT-PCR in North America is due to increased sensitivity over ELISA or that the McAb used in the 1993 study was strain specific.

Identification, detection and phylogenetic analysis of new viruses infecting mint. I. E. TZANETAKIS (1), J. D. Postman (2), and R. R. Martin (1,3). (1) Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, 97331; (2) USDA-ARS NCGR, Corvallis, OR 97333; (3) USDA-ARS HCRL, Corvallis, OR 97330. Phytopathology 94:S104. Publication no. P-2004-0708-AMA.

Many mint clones at the USDA-ARS National Clonal Germplasm Repository at Corvallis, Oregon have exhibited symptoms similar to those associated with virus infection. For example, *Mentha* × *gentiles* L. 'Variegata' (NCGR MEN-454) has shown dramatic vein-banding symptoms. Virus purification from infected plants of this cultivar revealed the presence of a spherical virus which was identified by RT-PCR as *Strawberry latent ringspot virus*. The dramatic symptoms of this clone led us to believe that more than one virus might be involved in symptom development. Cloning and sequencing of dsRNA revealed two previously undescribed viruses are also present in this host, a potex-and a closterovirus. The potexvirus, but not the closterovirus could be transmitted mechanically to a number of herbaceous hosts. Phylogenetic analysis revealed that the closterovirus had characteristics of all three genera of the family *Closteroviridae*, placing it in the center of the Closteroviridae universe.

A simplified cDNA cloning method for recalcitrant double-stranded RNA viral templates using reverse transcriptase. I. E. TZANETAKIS (1), K. E. Keller (2), and R. R. Martin (1). (1) Dept. Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, (2) USDA-ARS-HCRL, Corvallis. Phytopathology 94:S104. Publication no. P-2004-0709-AMA.

An unknown virus of pea, which had proven unclonable by conventional methods was characterized after the development of a novel cloning method. Using dsRNA viral templates and random primers, reverse transcriptase was used to generate first-strand cDNA. Second-strand synthesis was performed using a combination of reverse transcriptase and RNaseH with restriction enzyme(s) added to give shorter fragments that are more readily cloned. Taq DNA polymerase was used to A-tail the cDNA which was then cloned into a T-tailed cloning vector. Using this method, sequence data has been obtained from five separate viruses isolated from three different host plants. This cloning method resulted in a high number of cloned fragments, with clone sizes up to 1.5 kbp. Unlike traditional cDNA cloning methods, this procedure does not use DNA polymerase I and DNA ligase, making it much more efficient and economical. Finally and most important, it allows cloning of cDNA derived from dsRNA templates, whose cloning by conventional methods had not been successful.

Identification of viruses in declining strawberries along the west coast of North America. I. TZANETAKIS (1), M. Bolda (2), and R. Martin (1,3). (1) Dept. of Bot. and Plant Path., Oregon State University, Corvallis, 97331; (2) UC-Davis, Watsonville, CA 95076, (3) USDA-ARS-HCRL, Corvallis, OR 97330. Phytopathology 94:S104. Publication no. P-2004-0710-AMA.

In the 2002 and 2003 growing seasons, strawberry plants in coastal California and British Columbia developed symptoms of leaf reddening, stunting and plant death. Up to five viruses were identified in declining plants in these areas. In CA, the majority of the plants (75%) were infected with *Strawberry pallidosis associated* and *Beet pseudo-yellows* viruses, the two criniviruses that infect strawberry while the aphid-borne viruses, *Strawberry mild yellow edge* (SMYEV), *Strawberry mottle* (SMoV) and *Strawberry crinkle* (SCV) viruses were found in 20-25% of the plants. The presence of two viruses thought not to occur in the United States and Canada, *Strawberry latent ringspot* and *Fragaria chiloensis latent* viruses were identified in CA and B.C. In B.C., the majority of the plants (close to 100%) were found to be infected with SMYEV and SMoV, about 25% with SCV and very few with the whitefly transmitted viruses. Further investigations are under way to determine whether or not the individual viruses play a role in the decline problem.

Sustainable agricultural practices to aid taro, *Colocasia esculenta*, growers in Hawaii. J. Y. UCHIDA (1), C. Kadooka (1), J. Silva (2), and J. Deenick (2). (1) Dept. of Pl. and Env. Prot. Sci.; (2) Dept. of Trop. Pl. and Soil Sci. University of Hawaii, Honolulu, HI. Phytopathology 94:S104. Publication no. P-2004-0711-AMA.

With the increasing pressures to prevent nutrient run-off into the surrounding coastal ecosystem and the possible relationship between high fertility, succulent tissue, and higher disease levels, taro growers are re-evaluating traditional methods of cultivation. A major disease problem severely hampering production of taro corms is the formation of numerous pockets or cavities on the corm. An undescribed species of *Phytophthora* has been isolated from early stages of corm rots and reproduction of corm cavities has been accomplished. The species is homothallic, forms numerous oospores,

which are likely to survive in the paddy. To decrease inoculum levels and increase organic matter in the paddy, the paddy soil was dried after harvest and planted with Sunn Hemp as the cover crop. After 8 weeks the Sunn Hemp was cut, worked into the soil, and allowed to decompose for 6 weeks. The paddy was reflooded and planted with young taro plants. Adjacent to this paddy, was another paddy that was harvested, left undisturbed for 4 weeks and then replanted at the same time as the fallowed/cover cropped paddy. After 12 months, the crop was harvested with 80% survival of plants in the fallowed plot and 32% survival in the control lot. The total biomass for the treated or fallowed plot was 370 kg while that of the control plot was 80 kg. These practices reduce the addition of chemical fertilizers and associated loss into the ecosystem, and increased plant health and growth.

Glyceraldehyde-3-phosphate dehydrogenase (*gpd*) genes in *Phaeosphaeria nodorum* and *P. avenaria* species. P. P. ULLOA (1), E. Reszka (2), and K. R. Chung (3). (1) Mol. Plant Pathology Lab, USDA-ARS, Beltsville, MD; (2) Dept. of Plant Pathology, PBAI, Radzikow, Poland; (3) Citrus Research & Education Ctr, Univ. of Florida, Lake Alfred, FL. Phytopathology 94:S105. Publication no. P-2004-0712-AMA.

Stagonospora leaf blotch is a complex cereal disease caused by several *Phaeosphaeria* fungal species. Genetic diversity among glyceraldehyde-3-phosphate dehydrogenase (*gpd*) genes of *P. nodorum* (Pn), *P. avenaria* f. sp. *avenaria* (Paa) and *P. avenaria* f. sp. *triticea* (Pat 1, 2 and 3), three causal agents of stagonospora leaf blotch diseases, was evaluated in this study. The PCR-amplified *gpd* gene from these cereal *Phaeosphaeria* species contained 4 introns. The *gpd* gene in wheat-biotype Pn isolates ranged in size from 1,253-1,255bp. Sequence variations in intron 4 were used to divide the wheat-biotype Pn isolates into two subgroups. These two subgroups were equally represented in a survey of field wheat-biotype Pn isolates. Except Pat2, the *gpd* genes in Pat1, Pat3, Paa and the barley-biotype Pn were 1,251bp in length and phylogenetically closely related. As the nucleotide changes in *gpd* genes occurred mostly in intron regions and the third codons of amino acid coding triplets, the deduced GPD protein sequences in three *Phaeosphaeria* species were identical, except for Pat2. The *gpd*-encoded protein in Pat2 isolates contained two amino acid substitutions. The deduced GPD proteins of three cereal *Phaeosphaeria* species are closely related to the GPD protein that has been described in a maize pathogen, *Cochliobolus heterostrophus*.

Maintenance of fungal isolates in culture on the aquatic fern, *Ceratopteris richardii*. R. R. URS (1), P. D. Roberts (1), and S. Lalla (2). (1) SWFREC, University of Florida, Immokalee, FL; (2) P.L. Dunbar Middle School, Fort Myers, FL. Phytopathology 94:S105. Publication no. P-2004-0713-AMA.

Epiphytic survival of fungal plant pathogens on non-host plant *Ceratopteris richardi* [C-Fern] occurred when they grew in presence of fern gametophytes on basic C-fern medium. The fungi and their original hosts were: *Phytophthora capsici* from pepper; *Colletotrichum gloeospoorioides* from citrus; *Didymella bryoniae* from watermelon; *Corynespora cassiicola* from tomato; *Pestalotia* sp. from Indian Hawthorne; *Fusarium* sp. from citrus bark; and *Pythium myriotylum* from pepper. Reproductive structures such as conidia, sporangia, oospore and pycnidia, were observed 7 days after inoculating, 11-day-old culture dishes seeded with spores of fern gametophytes. Sex of the fern did not influence fungal growth or reproduction. Fungi did not penetrate the cells of fern gametophytes and were ectotropic when observed by microscopic examination of fixed (acetic acid-ethanol) and stained (fuchin-lactoglycerol-aniline blue) fern tissue. After 72 days of incubation at 24-C with 10-hour photoperiod, viability of fungi was confirmed by transferring the isolates onto fungal selective medium. During this incubation period no bacterial contamintion was detected with species of *Pythium* and *Phytophthora* grown in the presence of C-fern, which typically occurred, when these fungal like organisms were maintained on selective medium. Two fungal isolates *Sclerotium rolfsii* and *Scleortinia sclerotiarum*, which have wide host range, were pathogenic and evidently produced toxin on C-fern gametophytes, resulting in loss of chlorophyll, necrosis and tissue collapse within six days. This technique allows for long storage intervals of fungal isolates and ability to study interactions under gnotobiotic condition.

***Sweet potato leaf curl virus*: Detection by molecular hybridization.** R. A. VALVERDE, C. D. Kokkinos, and C. A. Clark. Dept. of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA. Phytopathology 94:S105. Publication no. P-2004-0714-AMA.

Sweet potato leaf curl is caused by the begomovirus *Sweet potato leaf curl virus* (SPLCV). Under experimental conditions, single infections of sweet potato (*Ipomoea batatas*) with this virus cause, occasionally, transient leaf curl symptoms. It is therefore difficult to assess the presence of this virus by visual inspections. Polymerase chain reaction (PCR) detection has been used successfully to detect SPLCV from plants in tissue culture, but in our hands, has not yielded consistent detection from field and greenhouse grown plants. A method using a SPLCV-specific probe direct-labeled with horseradish peroxidase and detected with chemiluminescence, has been tested with DNA samples from *I. batatas* and other *Ipomoea* species. Strong signals were obtained when tests were conducted using DNA from field (sweet potato) and greenhouse (*Ipomoea* species) grown plants that were infected with SPLCV. However, weak signals were obtained with DNA from greenhouse-grown *I. batatas* plants singly infected with SPLCV. Detection of SPLCV was successful with membranes previously spotted with DNA from *I. setosa* and mailed from Spain. These results suggest that molecular hybridization is a practical and sensitive method for the detection of SPLCV from field-collected samples. Furthermore, samples can be placed on membranes and mailed for testing to a centralized location.

Suppression of take-all disease in soils from organic versus conventional farms in relation to native and introduced *Pseudomonas fluorescens*. A. H. C. VAN BRUGGEN (1), G. A. Hiddink (1), A. V. Semenov (2), and A. M. Semenov (2). (1) Biol. Farming Systems, Wageningen Univ., NL; (2) Microbiol. Dept., Moscow State Univ., Russia. Phytopathology 94:S105. Publication no. P-2004-0715-AMA.

In 3 experiments with soils from organic and conventional farms, take-all disease on barley, wheat or triticale, caused by *Gaeumannomyces graminis* var. *tritici* (Ggt), was generally more suppressed in organic than in conventional soils (with crops in rotation or monoculture). This was true for soils with naturally occurring Ggt and inoculated with Ggt strain R3-111a-1. Take-all severity was negatively correlated with bacterial diversity as determined by DGGE analysis of 16S rDNA amplified from DNA extracted from soil. Natural populations of 2,4-diacetylphloroglucinol (dapg)-producing *Peudomonas* species were abundant in soil from a monoculture wheat field, less abundant in conventional soil in transition to organic, and almost absent in soil from a certified organic farm. Populations of an introduced *Gfp*-tagged, dapg-producing strain of *P. fluorescens* declined faster in organic than in conventional soils, and did not contribute as much to take-all suppression in the former than in the latter soils. The natural mechanism of take-all suppression in organically managed fields may be different from that in conventional fields with monoculture wheat or triticale.

Cell envelope constituents of *Pseudomonas putida* contributing to growth and survival in low-water-content habitats. M. van de Mortel and L. J. HALVERSON. Iowa State University, Ames, IA. Phytopathology 94:S105. Publication no. P-2004-0716-AMA.

Mechanisms for growth and survival in low-water-content habitats (matric stress) are likely to be different from those required for high osmolarity (solute stress) habitats. To better understand the molecular events underlying matric stress adaptation, we used mini-Tn*5::phoA* to identify genes of *P. putida* that are matric stress-regulated. We identified 20 matric stress-induced genes that were not induced by a thermodynamically equivalent solute stress and 11 genes that were induced by both matric and solute stress. Twenty-six genes were homologous to sequences present in the completed *P. putida KT2440* genome or pWWO plasmid sequences. Predicted functions include nutrient/solute transport, energy generation, protein fate, cell envelope structure and alginate synthesis; no function was predicted for 6 of the genes. Mutations in most of these genes conferred increased sensitivity to desiccation. These results provide insight into how bacteria perceive water-limiting conditions and suggest that the adaptative mechanisms for growth in low-water-content habitats are very different from those for growth in high osmolarity habitats.

Flow cytometry for multiplex detection of plant pathogenic bacteria, direct viable counting and cell sorting. J. M. VAN DER WOLF, J. Peters, P. S. van der Zouwen, R. W. van den Bulk, N. D. Tebaldi, and J. H. W. Bergervoet. Plant Research International, P.O. Box 16, NL-6700 AA Wageningen. Phytopathology 94:S105. Publication no. P-2004-0717-AMA.

Flow cytometry (FCM) allows multiparameter analysis and quantification of particles based on size, granularity and on emission of fluorescent light, after staining with a fluorescent dye. We developed a simple FCM immunodetection procedures for different plant pathogenic bacteria, including *Ralstonia solanacearum* (Rsol), *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) and *Xanthomonas axonopodis* pv. *phaseoli*. Plant extracts were filtered and stained with fluorochrome conjugated antibodies. The entire procedure was completed within 1 h and allowed detection of >10.000 cells per ml of plant extract. Detection levels were improved 10 times by enrichment of bacteria in semi-selective media prior to FCM. Staining of Cms with Alexa488 conjugated antibodies (green) and of Rsol with R-phycoerythrins conjugated antibodies (red) permitted detection of both pathogens simultaneously. FCM was also evaluated for direct viable counting of bacterial cells using combinations of propidium iodide (PI, red), SYTO9 (green) and Calcein-AM (green after conversion). SYTO9 penetrates all cells, PI only

(dead) cells with damaged membranes, whereas calceinAM is only converted by viable cells. Combination of PI and Alexa488-labelled antibodies allowed detection of the viable cells of a specific pathogen in mixtures of bacteria. FCM-based cell sorting offers possibilities for verification through isolation by dilution plating and PCR-amplification.

Quantifying symptom severity in wheat infected with *Wheat streak mosaic virus* using both visual and digital means of assessment. D. H. VAN WINKLE, R. French, and D. C. Stenger. USDA-ARS, University of Nebraska, Lincoln, NE. Phytopathology 94:S106. Publication no. P-2004-0718-AMA.

Five symptom attributes (mean latent period, leaf area, relative chlorosis, relative necrosis, and mean chlorotic density) of *Wheat streak mosaic virus* (WSMV) infection of wheat were evaluated. Seedlings of four wheat cultivars were mechanically inoculated with WSMV strains Sidney 81 (S81) or El Batán 3 (EB3). Mock-inoculated plants served as negative controls. For all cultivars, mosaic symptoms were first apparent in plants inoculated with S81. Mean latent period (time to onset of observable mosaic) differed among cultivars for both strains, with Arapahoe < Bobwhite < Michigan Amber < Tomahawk. At 14 days post inoculation, the two youngest, fully-expanded leaves (nodes 3 & 4 or 4 & 5) of each plant were digitally imaged and analyzed using Image-Pro Plus (version 4.5, Media Cybernetics™) software configured in accordance with user-defined parameters. Mean chlorotic density, relative chlorosis, and relative necrosis were significantly greater for plants infected with EB3 compared to S81. Bobwhite and Tomahawk plants infected with either strain exhibited more necrosis and chlorosis than did infected Arapahoe or Michigan Amber plants. Mean leaf area was lower for WSMV-infected plants compared to control but there was no significant difference between the two virus strains. Collectively, these results indicate that measurements of virulence based on timing of disease symptom onset may not be correlated with other measures of disease severity.

Utilization of the osmolyte melezitose and its effect on the growth and freeze-drying tolerance of *Cryptococcus nodaensis* OH 182.9. J. E. VANCAUWENBERGE, D. A. Schisler, and P. J. Slininger. NCAUR, USDA-ARS, Peoria, IL. Phytopathology 94:S106. Publication no. P-2004-0719-AMA.

Gibberella zeae is the primary causal agent of Fusarium head blight (FHB). Biocontrol agent *Cryptococcus nodaensis* OH 182.9 (NRRL Y-30216) reduces FHB severity. The trisaccharide melezitose enhances the survival of freeze-dried cells of yeast OH 182.9 stored at room temperature. To optimize the protective effect of melezitose and determine the extent of its utilization, melezitose (50mM final concentration) was added to a semi-defined liquid medium (C:N 11, 14g/L C) prior to, 24h, 48h, or 72h after inoculation or at the time of culture harvest (96h). Cell counts peaked at approximately 10^9 CFU/ml no later than 48h after inoculation and generally were not influenced by the timing of the melezitose amendment. Freeze drying reduced cell counts from 10^9 CFU/ml to 10^5 CFU/ml for cultures without melezitose and 10^8 CFU/ml for cultures with melezitose, regardless of the timing of addition. By 22 days, cell counts of cultures freeze-dried without melezitose had dropped further, while cell viability for cells freeze-dried in the presence of melezitose remained unchanged. Results of melezitose utilization by OH 182.9 will be presented.

California's San Joaquin Valley grape growers reduce fungicide applications. S. J. VASQUEZ (1) and G. M. Leavitt (2). (1) University of California Cooperative Extension, Fresno, CA; (2) University of California Cooperative Extension, Madera, CA. Phytopathology 94:S106. Publication no. P-2004-0720-AMA.

Powdery mildew (*Uncinula necator*) is the number one disease in California vineyards. *Vitis vinifera* cultivars grown for raisin, table or wine grape products have varying degrees of susceptibility. As a result, growers typically apply fungicides on a calendar schedule of seven, ten, or fourteen days to control the fungus. Sulfur is the most common fungicide used to control grapevine powdery mildew. Since the early 80's new powdery mildew fungicides have been developed. Fungal resistance to triadimefon in the mid 80's taught us that new chemistries also bring new challenges. Within three years of its introduction, triadimefon had lost its efficacy against grapevine powdery mildew. Growers reverted back to using sulfur exclusively or in combination with new chemistries on a calendar schedule. Growers rely heavily on sulfur for powdery mildew control due to its low cost, ease of application, and lack of powdery mildew resistance. But, its exclusive use also has downsides such as shorter application intervals, phytotoxicity, increased mite populations, eye and throat irritation, and drift and air quality issues. Most of the problems associated with sulfur use can be remedied by implementing the UC Davis Powdery Mildew Risk Index (PMI). The PMI, based on optimal temperatures for powdery mildew growth, assist growers in determining the best time to apply fungicides. Its validation and extensive use throughout most California grape growing areas has helped reduce worker exposure to fungicides, reduce drift incidences and particulate matter, and save growers money by eliminating unnecessary applications. San Joaquin Valley grape growers are also now benefiting from its implementation into their integrated pest management programs.

Disruption of mannitol biosynthetic genes in *Alternaria alternata*. H. VÉLEZ and M. E. Daub. Dept. of Plant Pathology, NC State University, Raleigh, NC. Phytopathology 94:S106. Publication no. P-2004-0721-AMA.

Mannitol is thought to be involved in plant-pathogen interactions by allowing fungi to counteract reactive oxygen species-mediated plant defenses. To test this hypothesis, a fungal strain of *Alternaria alternata* unable to synthesize mannitol is being created by disrupting the genes encoding mannitol dehydrogenase (MtDH), and mannitol 1-phosphate dehydrogenase (MPDH), the enzymes responsible for mannitol synthesis in this fungus. Both genes have been cloned and disruption constructs were made using hygromycin as a selectable maker. *A. alternata* protoplasts were transformed using the constructs, and hygromycin-resistant transformants were recovered. Single and double (MtDH, MPDH) disruptants will be analyzed for mannitol production, and will be assayed for pathogenicity on tobacco.

Progress in developing real-time PCR capabilities at the University of Kentucky Plant Disease Diagnostic Laboratory. P. VINCELLI and B. Amsden. University of Kentucky, Lexington, KY. Phytopathology 94:S106. Publication no. P-2004-0722-AMA.

Real-time PCR offers advantages for pathogen detection in plant disease diagnosis. When protocols are properly optimized and executed, real-time PCR is highly sensitive, very rapid and, compared to conventional PCR, more convenient and less subject to cross-contamination. With the purchase of a SmartCycler® in 2002, we began to develop our capability to use this technology for diagnostic purposes. At this stage we are principally adapting conventional PCR protocols to real-time PCR using SYBR® Green I; we also have adapted one published TaqMan® protocol. All of our protocols are experimental and currently are being evaluated. Protocols being tested include those for *Serratia marcescens*, *Ralstonia solanacearum* (all races), xanthomonads from tomato and pepper, *Erwinia tracheiphila*, and three species of *Ophiosphaerella*. We also plan to develop real-time PCR protocols for *Pseudomonas syringae* pv. *tomato*, *Agrobacterium vitis*, and *Verticillium dahliae*. Before this technology can be used for routine diagnosis, a PCR Service License is necessary.

Surveys of Oklahoma ornamental nurseries for *Phytophthora ramorum*, the cause of sudden oak death. S. L. VON BROEMBSEN (1), B. R. Olson (1), and M. A. Schnelle (2) (1) Dept. Entomology & Plant Pathology; (2) Dept. Horticulture & L.A., Oklahoma State University, Stillwater, OK 74078. Phytopathology 94:S106. Publication no. P-2004-0723-AMA.

Oklahoma has a number of very large ornamental nurseries which distribute diverse nursery stock to destinations throughout the USA. Many Oklahoma nurseries import plant materials from West Coast states and some carry large numbers of known hosts of *P. ramorum*. Surveys of known hosts occurring in Oklahoma nurseries were conducted in July 2002 and in May-June 2003. A preliminary survey was conducted in 2002 on rhododendron and viburnum at the two largest Oklahoma nurseries. This survey was used to establish field sampling and assay protocols. The survey process was expanded to all wholesale nurseries and key retail nurseries during 2003. Pieris and honeysuckle were added to the hosts surveyed in 2003. *Phytophthora ramorum* was not detected in any samples in 2002 or 2003 by either PCR analysis or by culturing. Other *Phytophthora* spp. (*P. cinnamomi*, *P. citricola*, *P. citrophthora* and *P. parasitica*) were isolated from samples.

Nitric oxide's role in common scab of potato: Phytotoxin biosynthesis and what else? M. Wach (1), E. G. JOHNSON (2), D. Gibson (3), and R. Loria (2). (1) USDA, APHIS, Riverdale, MD; (2) Department of Plant Pathology, Cornell University, Ithaca, NY 14853; (3) USDA Agricultural Research Service, US Plant, Soil, and Nutrition Laboratory, Ithaca, NY 14853. Phytopathology 94:S106. Publication no. P-2004-0724-AMA.

The thaxtomin family of phytotoxins are required by pathogenic *Streptomyces* species to cause common scab of potato. A gene encoding a nitric oxide synthase (NOS) is essential for normal levels of thaxtomin production. Evidence for the involvement of the *S. turgidiscabies* NOS (stNOS) in the biosynthesis of thaxtomin A (ThxA) supports a direct role for nitric oxide (NO) in the formation of the nitroindole moiety of ThxA. *Streptomyces turgidiscabies* was grown in the presence of a NO scavenger (CPTIO) to determine its effect on ThxA production. CPTIO reduced ThxA production without affecting bacterial growth. A strain of *S. turgidiscabies* from which

the st*nos* has been deleted was grown in the presence of three NO donors (DEANO, SIN, and SNAP), and all three partially restored ThxA production. These results indicate an unexpected uncoupling of NO production and ThxA biosynthesis and suggest that the highly reactive and diffusible NO radical is present intra- and extracellularly. If so, this raises questions about whether NO derived from stNOS affects host-pathogen interactions. The presence and function of free NO in pathogenic streptomycetes are under investigation.

The influence of temperature on the colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. N. R. WALKER (1), A. N. Morton (1), and T. K. Mitchell (2). (1) Dept. Entomology and Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078; (2) Fungal Genomics Laboratory, North Carolina State Univ., Raleigh, NC 27695. Phytopathology 94:S107. Publication no. P-2004-0725-AMA.

Spring dead spot, caused by *O. herpotricha*, is the most damaging disease of bermudagrass (*Cynodon dactylon* L.) in Oklahoma. The objective of this study was to evaluate the influence of temperature on the colonization of bermudagrass seedling roots by *O. herpotricha*. One root from each of four bermudagrass cv. Jackpot seedlings was inoculated with mycelium of *O. herpotricha*. Seedlings were maintained in a growth chamber at 12, 16, 17, 19, 21, 25, or 30°C with a 12 h photoperiod. Root lesions were measured twice after inoculation over a 7-10 day period. Each study was replicated three times for all temperatures. After 9-10 days, plants grown at 21°C or below contained root lesions which ranged from 8.6 to13.1 mm in length with the largest lesions occurring on plants grown at 17°C. At 25 and 30°C, root lesions were smaller (<3 mm). The results indicate that colonization of bermudagrass roots can occur over a wide range of cool soil temperatures and that lesion length is similar over this range.

Necrosis of resin ducts in roots of loblolly pines in decline. C. H. WALKINSHAW (1), N. J. Hess (1), and W. J. Otrosina (2). (1) U.S. Forest Service, Pineville, LA; (2) U.S. Forest Service, Athens, GA. Phytopathology 94:S107. Publication no. P-2004-0726-AMA.

Loblolly pine decline is affecting maturing pine stands on an increasing number of upland sites in central Alabama. Symptoms of decline suggest a role for root injury in the disease complex. We previously reported a relationship between wounding of roots and the reduction of five-year radial stem growth. The current study evaluates resin duct abnormalities and deteriation of fine roots of declining loblolly pines compared to roots of non-symptomatic loblolly trees within the study area. Fine roots of 33 symptomatic trees and 15 non-symptomatic trees were sampled and used in a histological study to determine the presence of resin duct necrosis. The proportion of roots with necrotic resin ducts ranged from 0 to 0.58 in roots of decline trees and 0 to 0.19 in the non-symptomatic trees. Empirically, the loblolly decline trees were consistently found to have reduced numbers of fine roots compared to the non-symptomatic trees. The reduction of feeder roots and high numbers of necrotic resin ducts appear to be significant factors in loblolly decline.

Anatomical responses of pine roots to starvation, fire, or pathogen infection. C. H. WALKINSHAW. U.S. Forest Service, Pineville, LA. Phytopathology 94:S107. Publication no. P-2004-0727-AMA.

Pine feeder roots were sectioned to examine their response to starvation, fire or pathogen treatment. The objective was to describe patterns of mortality as standards for classifying roots as dead or alive. Intact roots that were starved lacked starch grains and viable nuclei. Roots from heat treatment had excess tannin and dead cambium. Roots that were invaded by *Pythium* were colonized by fungal hyphae and had excess tannin. Effects of starvation were evident within 5 months in roots of felled pines. In contrast, high temperatures in prescribed burning appear to cause immediate changes in morphology but starch grains were retained and often were embedded in tannin. Hydrolysis of tissues occurred in *Pythium* treated tissues. Death of treated tissues followed distinct patterns that occurred in replicated specimens. Microscopic classification of tissue injury was easy with low-magnification. Empirical observations of root samples were unsatisfactory to define root mortality.

Characterization of *Rhizoctonia solani* isolates from rice in Arkansas. Y. A. WAMISHE (1), Y. Jia (2), P. Singh (1), and R. D. Cartwright (3). (1) University of Arkansas, Rice Research and Extension Center, Stuttgart, AR 72160; (2) USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR 72160; (3) University of Arkansas, Cooperative Extension Service, Little Rock, AR 72204. Phytopathology 94:S107. Publication no. P-2004-0728-AMA.

Rhizoctonia solani causes sheath blight, one of the most serious diseases of rice. Over 200 isolates of Rhizoctonia-like fungi were obtained from infected rice samples in Arkansas, of which103 isolates were identified as *R. solani* using a rDNA-ITS marker. The remaining isolates were either *R. sativa oryzae* or other sclerotia-forming fungi. To determine growth rates of *R. solani* isolates *in vitro*, the isolates were grown on potato dextrose agar in a completely randomized design at 30°C with three replications and hyphal growth after 24 + 3 h was recorded. There were significant differences among isolates for hyphal growth rate. Three fast, two intermediate, and three slow growing isolates were selected for a pathogenicity test, and all eight produced necrotic lesions on detached rice leaves. The selected isolates produced typical sheath blight symptoms on whole plants when leaves were inoculated with hyphal agar disks (pressed with thumb). Preliminary greenhouse inoculations showed positive correlation of hyphal growth rate with lesion length in most cases. Further characterization of the selected isolates and development of an inoculation technique for screening rice cultivars for resistance are underway and will be reported.

Characterization of the regulatory mechanism involved in co-regulation of the syringopeptin and syringomycin synthetase genes of *Pseudomonas syringae* pv. *syringae*. N. WANG, S.-E. Lu, and D. C. Gross. Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843. Phytopathology 94:S107. Publication no. P-2004-0729-AMA.

Syringopeptin and syringomycin are necrosis-inducing lipopeptide phytotoxins that are major virulence determinants of *P. syringae* pv. *syringae*. The syringomycin (*syr*) and syringopeptin (*syp*) gene clusters lie adjacent to one another on a 145-kb *syr-syp* genomic island and are distinguished by the presence of genes encoding nonribosomal peptide synthetases. Previous studies demonstrated that production of the two toxins was controlled by the *salA* and *syrF* regulatory genes. Recently, a DNA region upstream of the *sypA* and *syrB1* synthetase genes was identified that contained a potential *cis*-acting upstream activation sequence (UAS) element. Deletion analysis revealed that the UAS region (<50 bp in size) was located approximately 3 kb upstream of the translational start codon (TSC) of the *sypA* gene and about 260 bp upstream of the TSC of the *syrB1* gene. This UAS element promoted GUS expression of both the *sypA::uidA* and *syrB1::uidA* fusions. In addition, a SyrF-6xHis fusion protein was overexpressed and demonstrated to be functionally active because it restored toxigenesis to a *syrF* mutant. However, the overexpressed SyrF proteins failed to restore toxigenesis to *salA* mutant B301DSL7. The results show that SalA and SyrF are key regulators that control transcription of both *syr* and *syp* genes by an unique mechanism.

Rust resistance in the black bean Compuesto Negro Chimaltenango. X. K. WANG (1), C. M. Tandeski (1), J. J. Jordahl (1), P. L. Gross (1), K. F. Grafton (2), and J. B. Rasmussen (1). (1) Dept. of Plant Pathology; (2) Dept. of Plant Science, North Dakota State University, Fargo, ND. Phytopathology 94:S107. Publication no. P-2004-0730-AMA.

Compuesto Negro Chimaltenango (CNC) is one of the 19 dry bean *Phaseolus vulgaris* differentials used to distinguish races of the bean rust fungus, *Uromyces appendiculatus*. CNC is resistant against all bean rust races in the northern Great Plains of North America, so CNC may be useful for breeding programs. However, the genetics of CNC rust resistance has not been investigated. We crossed CNC to the adapted but rust susceptible pinto bean cv. "Othello". An F2-derived F6 recombinant inbred (RI) population of 100 lines was developed in the greenhouse by single seed descent. RI lines that comprise this population are being evaluated for reaction to multiple races of the pathogen. Preliminary data have identified AFLP markers linked to the resistance.

Molecular genetic dissection of the RPP7 resistance pathway. X.-J. WANG (1), J. Hoff (1), A. Cuzick (1,2), E. B. Holub (2), and J. M. McDowell (1). (1) Virginia Tech, Blacksburg, VA; (2) Horticulture Research International, Wellesbourne, Warwickshire, UK. Phytopathology 94:S107. Publication no. P-2004-0731-AMA.

The RPP7 gene activates race-specific resistance to the downy mildew pathogen Peronospora parasitica. Previously published genetic epistasis tests have established that RPP7 activates defense response through a signaling mechanism that does not require accumulation of salicylic acid (SA), or components of the ethylene and jasmonic acid response pathways encoded by EIN2, JAR1, or COI1. Furthermore, RPP7 is not suppressed by mutations in a variety of putative signal transducers that are required by various NBS-LRR resistance genes (e.g. pad4-1, ndr1-1, npr1-1, pbs2-1). In an effort to better understand the genetic requirements for signal transduction from RPP7, we have constructed a series of double mutants to test for additive or functionally redundant contributions by known defense signaling components. Most of these combinations support an enhanced level of asexual sporulation. Staining with DAB and Trypan Blue revealed that all of the double mutants are capable of inducing the oxidative burst and the hypersensitive response, but both responses are delayed by hours. These results reveal "cryptic" roles for PAD4, PBS2 (a.k.a. RAR1), NDR1, and NPR1 in RPP7 signaling, and

suggest that RPP7 activates resistance through multiple signaling pathways that collectively regulate the kinetics of the HR. We have also cloned the RPP7 gene, using map-based methods. This gene belongs to a cluster of eight related CC-NBS-LRR genes on Chr 49 which share significant similarity with RPP8. The RPP7 gene is at least 19 Kb long. Most of the gene is comprised of long introns within short 5′ and 3′ UTRs. The RPP7 transcript is alternatively spliced. The functional significance of this alternative splicing is currently unknown.

Evaluation of DNA extraction techniques for *Tilletia* spp. X. WANG and L. Carris. Dept. Plant Pathology, Washington State University, Pullman, WA 99164. Phytopathology 94:S108. Publication no. P-2004-0732-AMA.

Difficulty in culturing *Tilletia* spp. and extracting DNA directly from teliospores has hindered genetic studies on this important group of pathogens. Three methods of DNA extraction were compared: 1) extraction from 30-60mg of fresh mycelium using a phenol/chloroform protocol; 2) extraction from 1-2 mg of teliospores (~1 million teliospores) using a phenol/chloroform protocol; and 3) extraction directly from 2000-4000 teliospores crushed in water. Extracted DNA was quantified by fluorometry using calf thymus DNA as a standard. DNA yield using the phenol/chloroform extraction protocol was 32-181 µg/g of mycelium and 372-1044 µg/g of teliospores. DNA yield from teliospores using water was under limits of detection by fluorometry. However, PCR amplification of single-copy microsatellite loci was successful using DNA extracted by all three methods. This is the first report of amplification of single copy loci using DNA extracted directly from teliospores. This method will facilitate population-level studies of *Tilletia* spp. that have been difficult to germinate and grow in culture.

Development of a high-throughput screening method for *Pseudomonas syringae* pv. tomato DC3000 using tomato seedlings. T. WANGDI, S. R. Uppalapati, and C. L. Bender. Oklahoma State University, Stillwater, OK. Phytopathology 94:S108. Publication no. P-2004-0733-AMA.

P. syringae pv. tomato DC3000 (*Pst* DC3000) is a gram negative plant pathogen that causes bacterial speck of tomato. *Pst* DC3000 has become a model strain for investigating plant–microbe interactions, largely because of its genetic tractability, pathogenicity on Arabidopsis, and the availability of its genomic sequence. The current study addresses the use of a seedling assay for *Pst* DC3000 that can be used in a mutant screen. The seedling assay using defined *Pst* DC3000 mutants agreed well with results obtained by foliar inoculation of tomato plants. Furthermore, tomato seedlings infected with *Pst* DC3000 carrying transcriptional fusion of selected genes to the GUS reporter gene yielded expression results that are consistent with those obtained from foliar inoculation of 3-4 week old tomato plants. The use of a seedling assay for virulence and gene expression has several advantages over whole plant assays including a shorter growth and incubation period, ease of inoculation and handling, more replications and larger samples per assay.

***Streptomyces* strain variation and differences in inoculum density contribute to the severity of potato common scab.** L. A. WANNER. USDA ARS Vegetable Lab, 10300 Baltimore Ave, Beltsville, MD 20705. Phytopathology 94:S108. Publication no. P-2004-0734-AMA.

Common scab, caused by a complex of *Streptomyces* species, is an important potato disease that also affects other root and tuber crops worldwide. The basis for differences in scab severity observed in different years or different fields is poorly understood. Severity of scab lesions and other disease phenotypes caused by isolates of *Streptomyces* were examined in radish and potato. Disease severity differed when plants were grown in soil inoculated at initial inoculum densities over a range of 5 logs. Pathogen inoculum density was a primary determinant of disease severity on radish, including both lesions type and phytotoxicity to seedlings and older plants. Individual isolates differed in the inoculum concentration range at which disease symptoms became more severe, indicating that isolates differ in aggressiveness. Isolates differing in aggressiveness on radish also produced differences in disease symptoms on two potato cultivars. The ranking of aggressiveness was not always the same between radish and potato, or between potato cultivars. Disease symptoms were not limited to scabby lesions on potato tubers but also included plant stunting, wilting, necrosis and death, reduction in root biomass, reddish-brown lesions on underground portions of the stem, elongating stolons, and roots. Differences in densities of the population of scab-causing streptomycetes in the soil as well as differences in aggressiveness seem likely to contribute to differences in incidence and severity of scab seen in the field.

Effects of fungicides, plant density, and cultivars on powdery mildew of *Delphinium*. S. N. Wegulo (1), M. VILCHEZ (1), and F. Laemmlen (2). (1) University of California, Riverside, CA 92521; (2) UCCE, Santa Maria, CA 93455. Phytopathology 94:S108. Publication no. P-2004-0735-AMA.

Powdery mildew caused by *Golovinomyces cichoracearum*, *Erysiphe polygoni*, and *Podosphaera xanthii*, significantly reduces *Delphinium* yields. From 2002 to 2004, the effects of 11 fungicides, 2 plant densities, and 9 cultivars on disease severity were investigated. Propiconazole, chlorothalonil, myclobutanil, potassium bicarbonate, and sulfur treatments resulted in lower (P less than or equal to 0.05) disease severity than the control in 3 trials and in single (4 plants/m) and double (8 plants/m) rows. Jojoba oil and fenarimol performed better than the control in 2 of the 3 trials. *Pseudozyma flocculosa*, thiophanate-methyl, trifloxystrobin, and azoxystrobin did not perform better than the control. The overall effect of plant density was variable among the 3 trials. The hybrid cultivars Blue Bird, Cameliard, Galahad, and King Arthur were highly resistant in 4 trials. Bellamosum, Blue Shadow, Belladonna, and Casa Blanca were the most susceptible cultivars. Oriental Blue was moderately resistant. Growers can use these results to make informed decisions regarding choice of fungicides and cultivars.

Responses of *Botrytis cinerea* from lisianthus to fungicides in *in vitro* assays. S. N. WEGULO, N. Hyder, and M. Vilchez. University of California, Riverside, CA 92521. Phytopathology 94:S108. Publication no. P-2004-0736-AMA.

Blights caused by *Botrytis cinerea* significantly lower lisianthus (*Eustoma grandiflorum*) yields. In *in vitro* assays, responses of *B. cinerea* from lisiathus to 10 fungicides at label rates were investigated. Chlorothalonil (Chl), fludioxonil (Flu), potassium bicarbonate (Pbc), iprodione, (Ipr), mancozeb (Man), and vinclozolin effectively inhibited growth of *B. cinerea* on half-strength PDA (PDA). Sporulation and sclerotia formation on fenhexamid (Fen)- and thiophanate-methyl (Thm)-amended PDA were greater than the control. Spores from all treatments were pathogenic on detached lisianthus leaves with percent necrosis 6 days after inoculation lowest for azoxystrobin, pyraclostrobin, and Flu. Sporulation on these leaves did not differ from the control for Fen and Pbc. On stem pieces immersed in fungicides and inoculated with spores, percent necrosis 7 days after inoculation was lowest for Flu, Chl, and Man; sporulation for Thm, Pbc, and Ipr did not differ from the control 11 days after inoculation. Results from these studies can be used to identify efficacious fungicides as well as fungicides against which *B. cinerea* has potential to develop resistance.

A test of the validity of screening poplar clones for long-term canker disease damage by responses to inoculation with *Septoria musiva*. J. E. Weiland, J. C. Stanosz, and G. R. STANOSZ. Dept. Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 94:S108. Publication no. P-2004-0737-AMA.

The potential to predict long-term canker disease damage to poplars by responses to inoculation with *S. musiva* has been demonstrated, but screening is not yet routine. To test validity of screening procedures, 15 clones of commercial interest to a forest industry cooperator (including resistant and susceptible standards) were inoculated during their first season of growth in the field. On each stem, the fourth or fifth fully expanded leaf was removed and an agar plug colonized by an aggressive isolate of *S. musiva* was placed over the resulting wound. Four months after inoculation, canker incidence, canker length, and percent of stem circumference affected (girdle) were recorded. Resulting cankers resembled those attributed to *S. musiva* and responses of standards were consistent with those previously reported. Clones varied greatly in canker incidence (17-96%), mean canker length (5-55 mm), and mean girdle (10-91%). Logistic regression analysis was used to compare responses with canker disease damage categories assigned on the basis of information from longer-term field studies. Incidence, canker length, and girdle data all were informative, and for most clones there was a high probability that responses to inoculation correctly predicted assigned canker disease damage categories. These results validate previous work indicating the feasibility and benefit of screening juvenile poplar clones for responses to inoculation with *S. musiva* before extensive field trials and release to growers.

Induced systemic resistance (ISR) in *Arabidopsis* against *Pseudomonas syringae* pv. *tomato* by 2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas fluorescens*. D. M. WELLER (1), J. A. van Pelt (3), D. V. Murphy (2), C. M. J. Pieterse (3), P. A. H. M. Bakker (3), and L. C. van Loon (3). (1) USDA-ARS, Pullman, WA; (2) Washington State University, Pullman, WA; (3) Universiteit Utrecht, Utrecht, The Netherlands. Phytopathology 94:S108. Publication no. P-2004-0738-AMA.

Two-week-old *Arabidopsis thaliana* ecotype Col-0 seedlings were transferred into an autoclaved sand-soil mixture colonized by DAPG-producing *P. fluorescens* strain Pf-5 (rep-PCR genotype A), Q2-87 (B), Q8r1-96 (D) or HT5-1 (N) and the plants were challenge-inoculated with *Pseudomonas syringae* three weeks later. DAPG producers were not detected on the leaves. All DAPG producers induced resistance against *P. syringae* equivalent to the

well studied Dutch strain *P. fluorescens* 417r. *phlD* or *phlBC* mutants of Q2-87 (DAPG minus) were significantly reduced in ISR activity. A *phlF* regulatory mutant (over-producer) had ISR activity equivalent to Q2-87. Introduction of DAPG into soil at concentrations of 10 to 250 µM four days before challenge inoculation induced resistance equivalent to or better than the bacteria. These results suggest that DAPG is a major mechanism of ISR by DAPG producers.

Border cell specific gene expression. F. WEN, F. C. Gong, R. Celoy, S. Gardais, D. W. Galbraith, and M. C. Hawes. University of Arizona, Tucson, AZ. Phytopathology 94:S109. Publication no. P-2004-0739-AMA.

Plants of agronomically important species produce thousands of healthy 'border' cells programmed to separate from the root tip into the rhizosphere as roots penetrate through soil. Border cells constitute a uniquely differentiated component of root systems with distinct patterns of protein expression; upon differentiation into border cells, a switch in gene expression occurs with rapid export of a diverse array of extracellular enzymes and other proteins. Species- and genotype-specific border cell gene expression has been shown to convey a capacity to regulate behavior, growth, and gene expression in populations of soilborne microorganisms. In order to test the hypothesis that border cells protect plant health by controlling the ecology of root systems, gene expression in border cells was compared with that of progenitor root cap cells to identify border cell specific genes. Transcriptional profiling and proteomic analysis was used to define specific patterns of gene expression that underlie border cell specific properties, especially with respect to their influence on root infection by soilborne bacteria, fungi, and nematodes.

Identification of *Saguaro cactus virus* (SCV) coat protein as a suppressor of RNA silencing. Z. WENG and Z. Xiong. Dept. of Plant Sciences, Div. Plant Pathology, Univ. of Arizona, Tucson, AZ. Phytopathology 94:S109. Publication no. P-2004-0740-AMA.

The 37 kd capsid protein (CP) of SCV, a member of *Carmovirus* genus, is predicted to be a viral suppressor of RNA silencing based on the property of the homologous *Turnip crinkle carmovirus* CP. To test its suppressor activity, SCV CP was co-expressed with green fluorescent protein (GFP) in *Nicotiana benthamiana* leaves by agroinfiltration. Fluorescence in leaves expressing both GFP and SCV CP was more intense and persisted several days longer than in leaves expressing GFP alone. Small interfering RNA homologous to GFP was also reduced significantly in these leaves. These results demonstrated that SCV CP was a strong suppressor of RNA silencing. To localize the region of SCV CP responsible for the suppressor activity, a series of C-terminal frameshifting and N-terminal deletion mutants were analyzed. The suppressor activity was lost in all of these mutants, including a mutant missing only 8 C-terminal amino acids and another missing 43 N-terminal amino acids. These results suggested that the structure of SCV CP might be important for the suppressor activity. Additional SCV CP mutants are being studied further.

The N-terminal 14 amino acids of *Red clover necrotic mosaic virus* (RCNMV) p27 protein are essential for both ER-targeting and viral infection. Z. WENG and Z. Xiong. Dept. of Plant Sciences, Div. Plant Pathology, Univ. of Arizona, Tucson, AZ. Phytopathology 94:S109. Publication no. P-2004-0741-AMA.

Two viral proteins, p27 and p88, encoded in the RNA-1 of RCNMV are required for viral replication. p27 is encoded from the ORF1 of the RNA-1 while p88 RNA polymerase is a fusion protein containing p27 at the N-terminus and p57 encoded in the ORF2 at the C-terminus. An RCNMV mutant missing the N-terminal 14 amino acids of p27 and p88 was poorly infectious and incompetent in replication. Sequence analysis suggests that these 14 amino acids may contain a membrane-targeting signal. The wild type and mutant p27 with deletion of the N-terminal 14 amino acids were fused in-frame with GFP. These fusion proteins were then cloned into tomato bushy stunt virus (TBSV) expression vector pHST2 under the control of TBSV CP subgenomic promoter and into a Ti-plasmid under the control of 35S promoter. Leaves of *Nicotiana benthamiana* expressing these fusion proteins were examined by confocal microscopy. The confocal images revealed that the GFP fused to the wild type p27 localized to endoplasmic reticulum (ER). In contrast, GFP fused to the mutant p27 failed to localize in ER and distributed evenly in the whole cell. These results suggest that the N-terminal 14 amino acids contain an ER membrane targeting signal that is essential for viral replication.

Field testing of *Bacillus licheniformis* SB3086 biofungicide efficacy on *Colletotrichum graminicola*, *Rhizoctonia solani*, and *Sclerotinia homeocarpa*. L. WEST, S. Inman, and S. Semones. Novozymes Biologicals Inc., Salem, VA 24153. Phytopathology 94:S109. Publication no. P-2004-0742-AMA.

Novozymes Biologicals received EPA registration for *Bacillus licheniformis* SB 3086 in February 2003. This strain is the active ingredient of EcoGuard and was tested on 3 key turf diseases at various university sites in 2003. EcoGuard has been positioned as a product to be used as part of a disease management program with the primary target disease being dollar spot (*Sclerotinia homeocarpa*). Stand alone control with EcoGuard controlled low-moderate dollar spot pressure in the 80-90% range at 7 day application interval. In 2003 testing extended to anthracnose (*Colletotrichum graminicola*) and brown patch (*Rhizoctonia solani*). EcoGuard also showed significant control (up to 70-80%) of anthracnose and brown patch. These results reinforce current use recommendations of using this product at low-moderate disease pressure, but not at high disease pressures. 2004 trials will focus on rotations with EcoGuard and various other chemistries on these and possibly several other diseases.

Genetic and biochemical analysis of a tomato PCD-responsive gene. P. D. WEYMAN, D. G. Gilchrist, and R. M. Bostock. University of California, Davis, CA. Phytopathology 94:S109. Publication no. P-2004-0743-AMA.

Dea1 is a programmed cell death (PCD)-responsive gene cloned from tomato (*Lycopersicon esculentum*). The PCD-inducing elicitors, arachidonic acid, AAL-TA-toxin and fumonisin, induce *Dea1* transcript levels within 15 minutes of treatment. Transcript is also induced by tomato pathogens, *Phytophthora infestans* and *Pseudomonas syringae* pv. *tomato*, although at a slower rate. The notable speed at which the gene responds transcriptionally to death stimuli prompted us to examine the promoter region of *Dea1*. Characterization of this region is underway. This single copy gene does not possess any introns and has close relatives in potato (*Solanum tuberosum*) and tobacco (*Nicotiana tabacum*) that are 95% and 88% similar at the nucleotide level, respectively. This suggests that the gene is highly conserved among members of the family Solanaceae. The encoded protein is predicted to be structurally similar to a group of proteinase inhibitor, seed storage, and lipid transfer proteins. We present data on the expression of the protein and its biochemical properties.

Developing prediction and management systems for the control of *Rhizoctonia* diseases in potato and sugarbeet. P. S. WHARTON, D. Berry, and W. W. Kirk. Department of Plant Pathology, Michigan State University, East Lansing, MI 48824. Phytopathology 94:S109. Publication no. P-2004-0744-AMA.

In Michigan, *Rhizoctonia solani*, anastomosis group (AG) 3 causes stem canker and black scurf in potatoes, AG4 seedling damping-off, and AG2-2 root and crown rot in sugarbeet. Soil temperature is a critical factor in the initiation of disease. Disease severity is positively correlated with the soil temperature that is most favorable for pathogen growth. By monitoring soil temperatures it may be possible to predict when the greatest risk of disease occurs and vary the application of fungicides accordingly. Such predictive models predicated by/on fungicide optimization can reduce and accurately target fungicide usage without increasing disease, but may fail if inaccurate thresholds for disease initiation and development are utilized. The objectives of this study were to determine optimal conditions for pathogen growth and disease initiation, and to study the systematics of fungicide × temperature interactions in controlled environment experiments. Preliminary results suggest that the optimum temperature for pathogen growth is 25°C regardless of AG group. These results and the systematics of fungicide × temperature interactions will be presented.

Rhizosphere colonization by the fungus *Nectria haematococca* MPVI: Do conditionally dispensable chromosomes play a role? G. J. WHITE and H. D. VanEtten. University of Arizona, Tucson, AZ. Phytopathology 94:S109. Publication no. P-2004-0745-AMA.

Nectria haematococca Berk. & Br. mating population (MP) VI (anamorph *Fusarium solani* Mart.) is an ascomycetous filamentous fungus that exists in various plant associations including those of plant pathogen as well as rhizosphere colonizer. *N. haematococca* MPVI possesses "conditionally dispensable" (CD) chromosomes that are not needed for axenic growth, but carry genes that appear to affect the habitats which individual isolates can occupy. Previous results in our lab show that *N. haematococca* isolates highly virulent on pea (*Pisum sativum* L.) are able to utilize homoserine, an amino acid found in pea root exudates, as a sole carbon and nitrogen source whereas isolates that are not pathogenic on pea are rarely able to utilize this compound. The gene(s) for homoserine utilization (HUT) is located on a CD chromosome of this root rot pathogen. We used a replacement series approach to determine if the CD chromosome increases the ability of *N. haematococca* to colonize plant rhizospheres. Plants were grown in soil inoculated with pairs of *N. haematococca* isolates that were isogenic except for CD chromosomes. The rhizospheres were evaluated for the degree of colonization by each isolate using real-time PCR. Preliminary results indicate that rhizosphere competence is increased in isolates with the CD chromosome.

War between kingdoms: Induction of programmed cell death in filamentous fungi by *Pseudomonas syringae*. G. WICHMANN and S. E. Lindow. Dept. of Plant and Microbial Biology, University of California, Berkeley. Phytopathology 94:S110. Publication no. P-2004-0746-AMA.

Aerial plant surfaces constitute a major habitat for microbial colonization. Bacteria and fungi are common on healthy leaf surfaces, but are growth limited by the availability of nutrients. Fungi growing on a leaf surface represent a large potential source of nutrients for epiphytic bacteria. We present evidence of a novel, antagonistic interaction between the epiphytic bacterium *Pseudomonas syringae* and filamentous fungi, mediated by a previously uncharacterized *P. syringae* protein. This bacterial protein, termed Phc, has high similarity to Het-c from *Neurospora crassa*, a protein known to control fungal heterokaryon incompatibility (HI). HI is a programmed cell death pathway utilized by filamentous fungi during vegetative growth. Homologues of Het-c are also found in a wide variety of plant-associated fungi. We hypothesize that by co-opting HI in filamentous fungi, *P. syringae* can kill fungal cells in order to obtain nutrients on leaves. Transformation of *N. crassa* with *phc* yields transformants with HI phenotypes indistinguishable from HI phenotypes seen after transformation with the appropriate fungal *het-c* control. Further examination of the bacterial-fungal interaction has found that *P. syringae* can extensively colonize *N. crassa* hyphae, as well as use *N. crassa* as a nutrient source under nutrient limited conditions. In addition, the upstream regulatory region of *phc* contains a putative type III promoter element. The fitness advantage conferred by *phc* will be determined by quantifying wild type and *phc* mutant strains of *P. syringae* on leaves with and without various fungal associates. This work represents a new form of prokaryotic-eukaryotic interaction.

Introgression of virus resistance genes from *N. glutinosa* to *N. clevelandii*. B. E. WIGGINS and J. E. Schoelz. Department of Plant Microbiology and Pathology, University of Missouri, Columbia, MO. Phytopathology 94:S110. Publication no. P-2004-0747-AMA.

A new variety of *Nicotiana*, *N. edwardsonii* var. Columbia, was developed from crosses between *N. glutinosa* and *N. clevelandii*. Unlike the original *N. edwardsonii*, crosses between Columbia and either of its parents were fertile. Thus, the inheritance of virus resistance genes present in *N. glutinosa* could be characterized using Columbia. For example, over 70 viruses have been reported to infect *N. clevelandii* that are unable to infect *N. glutinosa*. To determine how virus resistance genes would segregate in interspecific crosses between Columbia and *N. clevelandii*, we followed the fate of the *N* gene, a single dominant gene that specifies resistance to *Tobacco mosaic virus*. We present evidence that the entire chromosome that contains the *N* gene was introgressed into *N. clevelandii* to create an addition line. The putative *N. clevelandii* addition line was homozygous for the *N* gene and contained 50 chromosomes instead of the 48 characteristic of *N. clevelandii*. These plants are being evaluated to identify other virus resistance genes that might reside on the *N* gene chromosome. For example, we have found that resistance to *Tomato bushy stunt virus* and *Cauliflower mosaic virus* must reside at another location. To develop molecular evidence that an *N. glutinosa* chromosome was introgressed into *N. clevelandii*, we utilized a PCR-based approach to adapt markers from the tomato genome map. Enough synteny exists between *Nicotiana* and tomato such that primer sequences developed against the tomato genome may be used for the creation of *Nicotiana* markers. These markers could be used to anchor the *N* gene and other virus resistance genes to the tomato genome map.

Physical modes of action of phosphites in control of grapevine downy mildew. W. F. WILCOX and J. A. Burr. Dept. Plant Pathology, Cornell University, NY State Agricultural Experiment Station, Geneva, NY. Phytopathology 94:S110. Publication no. P-2004-0748-AMA.

Field-grown grapevines were sprayed to runoff with a solution containing 1.6 gL^{-1} of potassium phosphite either 7 or 3 days before inoculation with *Plasmopara viticola* (protective assays) or 3 days after inoculation (curative assay). Control of disease severity ranged from 94-100% on the two youngest leaves at the time of inoculation but declined progressively with leaf age, to 25, 81, and 74% on the fifth-youngest leaf in the three respective assays. However, susceptibility of unsprayed leaves also declined with age. Following post-symptom incubation, the resulting sporulation was reduced by 85-100% (relative to the unsprayed check) on all treated leaves in all assays. In two additional trials, the same solution applied to visible infections (eradicant assay) reduced new sporulation by a mean of 76% relative to the check. Phosphites appear to control downy mildew through a combination of protective, curative, and antisporulant activities, but do not eradicate active infections. The ephemeral residual activity in older leaves may be compensated somewhat by their limited susceptibility to infection.

Controlling tomato diseases with a new fungicide containing famoxadone and cymoxanil. R. WILLIAMS, C. Shepherd, R. Geddens, and S. Soehner. DuPont Crop Protection, Stine-Haskell Research Center, Newark, DE. Phytopathology 94:S110. Publication no. P-2004-0749-AMA.

Tanos™, a mixture of Famoxate™ (famoxadone) and Curzate™ (cymoxanil), is a new fungicide from DuPont for U.S. tomato growers. Tanos™ offers broad-spectrum disease control, low use rates, and strong residual properties as well as post-infection control of late blight. Tanos™ controls a wide range of tomato diseases such as early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), anthracnose (*Colletotrichum coccodes*), target spot (*Corynespora cassicola*, a leaf spot (*Septoria lycopersici*). Tanos™ also suppresses bacterial spot (*X. campestris* pv. *vesicatoria*) and bacterial speck (*P. syringae* pv. *tomato*) when used with copper and EBDC fungicides. Tanos™ used in programs with complementary fungicides provides effective broad-spectrum disease control in tomatoes and reduces the risk of resistance development.

Modeling plant disease epidemics in a spatial hierarchy. L. WILLOCQUET (1) and S. Savary (2). (1) INRA-ENSAR BiO3P, BP 35327, 35653 Le Rheu cedex France; (2) ENSAR-INRA BiO3P, 65 rue de Saint-Brieuc CS 84215, 35042 Rennes cedex, France. Phytopathology 94:S110. Publication no. P-2004-0750-AMA.

An epidemiological model involving three nested hierarchy levels (e.g., site, leaf, plant) within a host population, and involving parameters of auto- and allo-deposition was developed. The model was used to assess the effects of patterns of propagule dispersal and of host structure on disease epidemics at the three organization levels. Epidemic speed was reduced as auto-deposition was increased, and when the leaf size was decreased. Interaction of the effects of host structure and spore dispersal pattern on simulated epidemics was detected from sensitivity analyses. When simulated outputs were plotted as incidence at higher hierarchical scale versus incidence at lower hierarchical scale over the course of the epidemics, the slope of curves increased with the allo-deposition parameter value, reflecting a lesser degree of disease aggregation. The model can be adapted to represent other ecological systems, where dissemination at different scales takes place.

Identification and discrimination of pesticide residues using electronic aroma detection. A. D. WILSON and C. S. Oberle. USDA Forest Service, Southern Hardwoods Laboratory, Stoneville, MS 38776. Phytopathology 94:S110. Publication no. P-2004-0751-AMA.

Pesticide residues in and on plant samples are often difficult to identify without elaborate chemical analyses. Electronic aroma detection (EAD) using conductive polymer analysis (CPA) was developed to rapidly detect and identify pesticides from many different categories and chemical classes. A diversity of pesticides, representing a cross section of pesticide types and chemical classes, were analyzed with CPA using an electronic nose device with a multisensor array, artificial neural network, digital pattern-recognition algorithms, and an application-specific recognition database developed for pesticides. A unique electronic aroma signature pattern (EASP) was produced for each pesticide from digital multisensor responses to headspace volatiles. Each EASP was specific to an individual pesticide and could be used as a diagnostic electronic fingerprint to identify and detect the presence of residues of individual pesticides. Using CPA for pesticide recognition offers numerous potential applications for real-time residue detections, crop protection or damage assessments, food safety, and crop biosecurity.

Incidence and severity of Botryosphaeria stem canker and dieback of pondberry (*Lindera melissifolia*) in Mississippi. A. D. WILSON, N. M. Schiff, M. S. Devall, K. F. Connor, P. B. Hamel, E. S. Gardiner, and T. D. Leininger. USDA Forest Service, Southern Hardwoods Laboratory, Stoneville, MS 38776. Phytopathology 94:S110. Publication no. P-2004-0752-AMA.

Pondberry is an endangered woody plant found in sporadic, often isolated locations within seasonally flooded wetlands of the southern United States. Stem canker and dieback are important symptoms of a newly recognized pondberry disease which has contributed significantly to stem mortality, reduced vigor, and erratic seed production. This disease was endemic to plants at all locations examined in Mississippi. Disease incidence and severity varied with location. Stem cankers were often highly elongated, producing necrotic tissue and abundant callus at canker margins. Tip dieback, associated with stem girdling by cankers and/or production of a mycotoxin, caused necrosis of apical meristematic tissue, precluding flowering, fruit set, and seed production in early infections; and promoted fruit rot in late infections. Pycnidia formed in black stromata within subepidermal canker tissues. The fungal pathogen responsible for this new pondberry disease was tentatively identified as *Botryosphaeria ribis* with *Fusicoccum* anamorph.

***In vitro* induction of resistance to common scab disease of potato in commercial cultivars.** C. R. WILSON, A. Eyles, and A. J. Wilson.

Tasmanian Institute of Agricultural Research, University of Tasmania, New Town Research Laboratories, 13 St. Johns Avenue, New Town, TAS 7008, Australia. Phytopathology 94:S111. Publication no. P-2004-0753-AMA.

Common scab disease is one of the greatest economic constraints facing the Australian potato industry and an important disease worldwide. The discovery of the thaxtomins, phytotoxins produced by pathogenic *Streptomyces* sp., and the demonstration of their implicit role in common scab disease expression in potato, has provided a unique target for plant resistance studies. Using thaxtomin A as a cell-screening tool, we have utilised plant-cell culture techniques to isolate and identify potato cell lines of important potato cultivars that show significantly greater thaxtomin A tolerance than parental lines. Pathogenicity trials of regenerated toxin-tolerant plants have shown promising expression of resistance to common scab disease. The extent of resistance appears correlated with expressed toxin tolerance. As the selection procedure is clonal we can retain the desirable agronomic traits of the parent cultivar whilst acquiring a disease resistance phenotype, a desirable attribute for commercial adoption.

Molecular characterization of *Magnaporthe grisea* avirulence *AVR-Pita*. E. M. WINSTON (1), P. Singh (2), Y. Jia (1), and J. Correll (3). (1) USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR 72160; (2) University of Arkansas Rice Research and Extension Center, Stuttgart, AR 72160; (3) University of Arkansas, Fayetteville, AR 72701. Phytopathology 94:S111. Publication no. P-2004-0754-AMA.

The *Pi-ta* gene in rice confers resistance to *Magnaporthe grisea* isolates containing the corresponding avirulence gene *AVR-Pita*. Maintaining durable *Pi-ta*-mediated resistance is challenging due to the high degree of pathogenic variability and genetic instability of *M. grisea*. Genetic mapping demonstrated that the *M. grisea AVR-Pita* gene is closely linked to the telometric region residing on chromosome 3. Evidence suggests that a blast race TM2 can delete allelic sequences from its genome, thereby infecting *Pi-ta* containing rice cultivars. The objective of this study was to determine the functionality of *AVR-Pita* alleles in isolates of *M. grisea* races found in the Southern United States. Utilizing gene specific primers, the native promoter and coding regions of the *AVR-Pita* gene were amplified from cDNA and genomic clones of the *M. grisea* wild-type isolates 0-137 and ZN61 (race IB-49), respectively. Fragments were directionally cloned into the vector pBluescript SK+. Clones were analyzed by restriction digestion and PCR amplification using *AVR-Pita* gene specific primers. Clones homologous to *AVR-Pita* genomic sequence were subsequently transformed into the race-shift isolate TM2. Complementation studies will be subsequently performed by inoculating the recombinant pathogen onto *Pi-ta* containing rice cultivars.

Sequence variability and host specificity among curtoviruses infecting weed and crop hosts in California. W. M. WINTERMANTEL. USDA-ARS, Salinas, CA. Phytopathology 94:S111. Publication no. P-2004-0755-AMA.

Curly top disease re-emerged in 2001 as a serious threat to agriculture in California's San Joaquin Valley. The disease is caused by *Beet curly top virus* (BCTV) and related curtoviruses, and is transmitted by the beet leafhopper (*Circulifer tenellus*). BCTV infects a broad range of crop hosts including sugar beet, pepper, and tomato, as well as numerous native weeds. Prior molecular characterization of a limited number of curtoviruses from broad hosts of the western US demonstrated that two related curtovirus species are responsible for most crop disease, but little information existed on curtovirus species distribution among weed hosts. Data collected over the past 2 years has focused on molecular characterization of large numbers of BCTV isolates from weed hosts present in the overwintering grounds of the beet leafhopper, as well as crop hosts in the San Joaquin Valley. Universal and specific primers were used to amplify viral DNA from infected plants. PCR, coupled with sequence and marker analysis, expands on previous studies and illustrates regional virus variability, host specificity of curtovirus species, and the continuum of isolates that exist in nature.

Genome organization and sequence of *Tomato chlorosis virus*. W. M. WINTERMANTEL (1), G. C. Wisler (2), A. V. Karasev (3), and H.-Y. Liu (1). (1) USDA-ARS, Salinas, CA; (2) University of Florida, Gainesville, FL; (3) Thomas Jefferson University, Doylestown, PA. Phytopathology 94:S111. Publication no. P-2004-0756-AMA.

The genome of *Tomato chlorosis virus* (ToCV) was cloned and the complete nucleotide sequence of the virus determined and compared with related crinivirus species. RNA1 is organized into 3 ORFs, and encodes genes involved in replication of viral RNA based on homology to other viral replication factors. RNA 2 is composed of 7 ORFs encoding a hsp70 homolog and two proteins involved in encapsidation of viral RNA, referred to as the coat protein and minor coat protein. Sequence homology between ToCV and other criniviruses varies throughout the viral genome. The ORF encoding the minor coat protein of ToCV, which forms part of the "rattlesnake tail" of virions and may be involved in determining the unique, broad vector transmissibility of ToCV, is larger than the comparable LIYV gene by 651 nucleotides. Among criniviruses sequenced, considerable variability exists in the size of some viral proteins. Analysis of these differences with respect to biological function may provide insights into the role crinivirus proteins play in virus infection and transmission.

Effect of timed post-infection fungicide sprays on urediniospore production and viability of *P. hemerocallidis*. K. A. WISE (1), D. S. Mueller (2), and J. W. Buck (1). (1) University of Georgia, Griffin, GA; (2) Iowa State University, Ames, IA. Phytopathology 94:S111. Publication no. P-2004-0757-AMA.

The repeated infection cycles of daylily rust, caused by *Puccinia hemerocallidis*, can make effective timing of fungicide applications difficult once disease is established. In a greenhouse study, 'Leebea Orange Crush' daylilies were inoculated over a two-week period to induce three stages of rust development: young lesions present but not erumpent (4 days after inoculation), lesions erumpent but not sporulating (7 days after inoculation), and lesions erumpent and sporulating (11 days after inoculation). Plants were treated with label rates of azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon, and urediniospores were vacuumed from plants 1, 3, and 7 days later. After seven days the average number of spores per lesion and percent germination for each fungicide treatment was lowest for plants without erumpent lesions present at the time of fungicide application. The average spores per lesion on plants treated with chlorothalonil and azoxystrobin were equal to or greater than the non-treated control for all rust stages; however, germination was almost eliminated (less than three percent) with these two fungicides.

Induction of pathogenesis-related proteins on potato sprouts by an alpha-(1,3)-glucan from cell wall of binucleate non-pathogenic *Rhizoctonia* sp. isolate. E. A. WOLSKI, G. R. Daleo, and A. B. Andreu. Mar del Plata National University, Box 1245, Buenos Aires, Argentina. Phytopathology 94:S111. Publication no. P-2004-0758-AMA.

Binucleate Rhizoctonia (BNR) isolate is an effective biocontrol agent for protection of potato from Rhizoctonia canker. This disease is caused by *Rhizoctonia solani*. Production of hydrolytic enzymes is one of the best known inducible defense responses in plants following microbial infection. In a previous work we have isolated and characterized an alpha-(1,3)-glucan from BNR that induce glucanase activities in potato sprouts, the primary site of *R. solani* infection. Here we report studies on the defense responses of potato sprouts treated with the alpha-(1,3)-glucan. A dose-response assay and a kinetic study of glucanase activities were performed. Maximal induction of beta-1,3-glucanase activity in potato sprouts was obtained with 250 micrograms of the alpha-(1,3)-glucan elicitor and after 6 days from inoculation. Both, BNR mycelium and the alpha-(1,3)-glucan induced a similar kinetic response of beta-1,3-glucanase activity. Immunological analysis of crude extracts from potato sprouts infiltrated with the alpha-(1,3)-glucan was performed using antibodies raised against pathogenesis-related proteins (PR). These experiments showed that the level of PR-2, PR-3 and PR-5 were increased when the sprouts were treated with the glucan. Maximal induction were obtained after 6 days from inoculation. These results show that this alpha-(1,3)-glucan may contribute to protection of the host plant against Rhizoctonia canker and may be utilized as a biocontrol factor.

Steps towards development of a site-specific sorghum ergot risk forecast system. F. [illegible]RKNEH (1), C. M. Rush (1), B. Narasimhan (2), and R. Srinivasan (2). (1) Texas Agri. Experiment Station, Bushland, TX 79012; (2) Texas A&M Univ., Dept. Forestry, Spatial Statistics Laboratory, College Station, TX 77843. Phytopathology 94:S111. Publication no. P-2004-0759-AMA.

Sorghum ergot, caused by *Claviceps africana*, has been causing periodic epidemics in hybrid-seed production fields in the Texas Panhandle since it was first introduced in 1997. Ergot is favored by high relative humidity; however, high relative humidity in the region is often associated with precipitation. To account for variability due to localized rain events, hourly rainfall data were obtained from a Doppler radar, which provided a resolution of 4 × 4 km. An ergot model based on temperature and relative humidity was integrated into a GIS system and interpolated to produce a continuous surface of weather data. The relative humidity in the model was entered as 90% whenever there was greater than 2.5 mm of rain. Temperature was interpolated from ground weather stations. This resulted in an output of a regional, site-specific ergot-risk map. There is a potential that the risk map can be developed into a web-based delivery system in which growers can determine ergot risks by providing the GPS locations of their individual fields.

Inoculum availability and seasonal survival of *Potebniamyces pyri* in pear orchards. C. L. XIAO and R. J. Boal. Washington State Univ., TFREC, Wenatchee, WA. Phytopathology 94:S112. Publication no. P-2004-0760-AMA.

P. pyri is the causal agent of Phacidiopycnis rot of pear fruit. The fungus is associated with bark necrosis and cankers of pear trees. To determine inoculum availability and seasonal survival of *P. pyri*, 10 dead bark and 10 dead fruit spurs were periodically collected from each of 10 selected trees in two orchards in 2002 and 2003. Samples were examined for presence and viability of fruiting bodies of *P. pyri*. In a research orchard, 2-year-old twigs were inoculated monthly with *P. pyri*. Inoculated twigs were sampled six months post-inoculation and pycnidia formation and reisolation of *P. pyri* were assessed. In both orchards, most trees were infected by *P. pyri*; pycnidia were observed on 42-78% of the sampled bark and 5-31% of the sampled spurs; and viable conidia were observed throughout the season. Apothecia of *P. pyri* were also observed but at a low frequency. *P. pyri* formed pycnidia on most freeze-treated, inoculated twigs. *P. pyri* was recovered from inoculated twigs throughout the year, but isolation frequency varied. The results indicate that viable inoculum of *P. pyri* is available throughout the pear fruit growing season and *P. pyri* can survive as mycelium in diseased twigs year around in the orchard.

Influence of substrate and environmental factors on growth and sporulation of *Potebniamyce pyri*. C. L. XIAO and J. W. Sitton. Washington State Univ., TFREC, Wenatchee, WA. Phytopathology 94:S112. Publication no. P-2004-0761-AMA.

P. pyri is the causal agent of Phacidiopycnis rot of pears. Little information is available on its basic biology. In this study, effects of culture media and environmental factors on mycelial growth and pycnidial production were evaluated. Prune juice agar, pear juice agar, apple juice agar, potato dextrose agar, and oatmeal agar (OMA) favored mycelial growth of the fungus. Czapek-Dox agar was not suitable for mycelial growth. The fungus was able to grow at temperatures from –3 to 25°C, with optimum growth at 15 to 20°C. The average radial growth rate on OMA was 4.4 mm/day at 15°C and 4.4 mm/day at 20°C. Mycelial growth was arrested at 30°C, but growth resumed at 20°C. The fungus failed to resume growth at 20°C after being incubated for 10 days at 35°C. The fungus was able to grow at water potential as low as –4 MPa but not at –7.3 MPa. Few or no pycnidia formed on the nine media tested at 20°C in the dark. Fluorescent light significantly stimulated formation of pycnidia. OMA was most efficient for production of pycnidia. Pycnidia on 8-wk-old OMA cultures at 20°C under 12-h dark/12-h light produced abundant conidia and the technique is recommended for inoculum production of conidia.

Underlying pathways in the *Mi-1*-mediated resistance to nematode and aphid. Q.-G. Xie, Q. Li, D. Sergeant, J. Smith-Becker, and I. KALOSHIAN. University of California, Riverside, CA. Phytopathology 94:S112. Publication no. P-2004-0762-AMA.

The tomato gene *Mi-1* confers resistance to three distinct animals, root-knot nematodes, potato aphid and whitefly. It is not clear whether the mechanism of resistance mediated by *Mi-1* to these organisms is identical. There are clear distinctions in the way *Mi-1*-mediates resistance to these organisms. The resistance mediated against root-knot nematodes is accompanied by hypersensitive response (HR) in roots near the head of the feeding nematode, however no HR is observed in leaves after aphid feeding. Similarly, resistance to aphids and whitefly is developmentally regulated with plants becoming resistant between the 4th and 6th week, while roots are resistant to nematodes very early in development. We are employing multi prong approaches to characterize the role of known plant defense genes in the *Mi-1*-mediated resistance and to identify underlying defense pathways. We are using tobacco rattle virus (TRV) based virus induced gene silencing (VIGS) to assess the role of a number of key regulatory genes, known to function in plant defense, in *Mi-1*-mediated resistance. We will present evidence that TRV-based VIGS is capable to silence genes in roots. We have also introduced *Mi-1* into tomato mutant backgrounds that are blocked in one of the major known defense pathways, salicylic acid, ethylene or jasmonic acid. Data from these approaches will be presented and the role of the 3 major defense pathways will be discussed.

Effect of soybean cyst nematode, soil moisture and infestation level on sudden death syndrome of soybean. L. J. XING and A. Westphal. Purdue University, West Lafayette, IN. Phytopathology 94:S112. Publication no. P-2004-0763-AMA.

Sudden death syndrome (SDS), caused by *Fusarium solani* f. sp. *glycines*, recently renamed *F. virguliforme*, is an important problem in soybean in the U.S. Midwest. We evaluated effects on SDS severity of *F. solani* f. sp. *glycines* infestation level and watering regime in natural or methyl bromide (MB)-fumigated soil. In a microplot trial at West Lafayette, IN, treatments in a 3-factor factorial design were: (A) non-treated soil or soil fumigated at 450 kg/ha MB, (B) natural precipitation or 15 L of water per plot per week added, and (C) four infestation levels. Treatments were arranged in a randomized complete block design with 4 replications. Two pathogen isolates (provided by S. Abney, USDA-ARS, Purdue University) were raised separately on sand-cornmeal at 25 ± 2°C for one month and then mixed with autoclaved infested sand-cornmeal to achieve 0, 6.8×10^3, 3.4×10^4 or 1.7×10^5 macrospores per ml of soil. There were 20 plants of soybean cv. Spencer per plot. At R6, SDS severity was rated on foliage and roots. At harvest, plant yields and population densities of *Heterodera glycines* were determined. SDS was more severe in the wetter regime in natural soil than in the other treatments. Only plots with high population densities of *H. glycines* had high levels of SDS indicating the importance of the nematode in SDS development under simulated field conditions. Fungal infestation level had limited effects on disease severity.

Jasmonic acid and ethylene are involved in the development of disease symptoms and defense responses in tomato plants infected with *Cucumber mosaic virus* D satellite RNA. P. XU, S. Rogers, and M. J. Roossinck. The Samuel Roberts Noble Foundation. Phytopathology 94:S112. Publication no. P-2004-0764-AMA.

Cucumber mosaic virus (CMV) D satellite RNA (satRNA) induces leaf epinasty and systemic necrosis in tomato. The systemic necrosis, where programmed cell death is involved, develops in a specific temporal and spatial pattern. The necrosis first appears as primary cell death in the developing phloem and cambium cells and then spreads to neighboring cells in the stem. Our previous work has shown that multiple defense responses are also induced during the symptom development. Recently, we found that the expression of lipoxygenase and ACC synthesase was increased during the disease development, indicating the up-regulation of the synthesis of jasmonic acid (JA) and ethylene. Furthermore, the systemic plant death was delayed when ethylene-insensitive (Nr) and JA synthesis-defective (def1) tomato mutants were inoculated at the 2-4 leaf stage, and leaf epinasty was attenuated in the infected Nr plants. When the Nr plants older than THE 5-leaf stage were inoculated, the symptoms of leaf epinasty and secondary cell death in the stem were suppressed and no expression of pathogenesis-related genes was detected during the processing of primary PCD in the infected vascular cells. The secondary cell death in def1 mutants infected with CMV/D satRNA was not affected although the primary cell death in the apical part of the plants was obviously delayed. This was correlated with a reduced accumulation of D satRNA. These results indicate that both JA and ethylene are involved in the development of systemic necrosis by different mechanisms and that ethylene also plays a role in the induced defense responses in the CMV/D satRNA infected wild type tomato plants.

Theoretical modelling of biological control of foliar plant disease. M. XU (1), M. Jeger (2), and P. Jeffries (3). (1) East Malling Research, UK; (2) Imperial College at Wye, UK; (3) University of Kent at Canterbury, UK. Phytopathology 94:S112. Publication no. P-2004-0765-AMA.

We have developed a general modelling framework to understand the dynamics of foliar pathogen-BCA (biocontrol agent) populations in order to predict the likelihood of successful biocontrol in relation to characteristics of biocontrol systems. The model considers biocontrol systems for foliar pathogens only and it does not address a particular biocontrol system but rather adopts a hypothetical 'generic' system where a fungal BCA is used to control fungal foliar pathogens system based on general biological knowledge. Four biocontrol mechanisms (competition, antibiosis, myco-parasitism and induced resistance) were incorporated into the model. Preliminary analysis of the model showed that the rate of a BCA colonising plant tissues and the duration that it remains effective are the two most important factors in determining the final outcome of a biocontrol system. In future, the model can be extended to include more factors such as spatio-temporal heterogeneity, fungicide effect, non-fungal BCAs, and strategies for BCA application.

Substrate-dependent isothiocyanate toxicity. L. E. YAKABE, J. D. MacDonald, and D. J. Kliebenstein. University of California, Davis, CA. Phytopathology 94:S112. Publication no. P-2004-0766-AMA.

The impending loss of methyl bromide as a fumigant has stimulated interest in isothiocyanates (ITCs), antimicrobial volatiles produced in Cruciferous tissues, for soil-borne pathogen control. We tested benzyl-, butyl-, ethyl-, and phenylethyl-ITC for relative efficacy against *Fusarium oxysporum* f. sp. *dianthi* in one sand and three soil substrates. Ethyl-ITC was consistently the most efficacious in all soil types. Benzyl-ITC was generally the least efficacious, and butyl- and phenylethyl-ITCs were variable, appearing relatively efficacious in some soils, and relatively non-efficacious in others. Lethal-dosage-50s (LD50s) for the tested ITCs were dependent on substrate.

ITC persistence in the different substrates varied as well. Persistence, however, did not correlate with LD50. Correlations between substrate characteristics, LD50s and ITC persistence suggests that substrate pH is related to phenylethyl-ITC LD50 and benzyl-, butyl- and ethyl-ITC persistence. This study demonstrates the importance of soil type in ITC toxicity to soil-borne pathogens.

Effect of biopesticides, microbial inoculants, and biorational products on *Phytophthora nicotianae* infection of periwinkle. C. B. YANDOC, J. Albano, and E. N. Rosskopf. USDA, ARS, U.S. Horticultural Research Laboratory. Phytopathology 94:S113. Publication no. P-2004-0767-AMA.

Phytophthora nicotianae is a serious pathogen of many crops and is highly problematic for ornamental plant production. Restrictions on the use of chemical soil fumigants and the increased prevalence of resistance to mefenoxam necessitate the use of other control measures for this pathogen. Experiments were performed in the greenhouse to determine the effect of various biopesticides, microbial inoculants, and biorational products combined with different fertilizer levels (0, 0.5, 1.0, 2.0 percent Hoagland solution) on the severity of *P. nicotianae* infection on periwinkle (*Catharanthus roseus*). Treatment and fertilizer level had significant effects on disease severity but there was no interaction effect between these two factors. Only the application of phosphonate-containing products resulted in significant disease reduction. Disease was less severe in plants that received 0 and 0.5% fertilizer. It is possible to manage greenhouse infections of *P. nicotianae* through the application of phosphonates and reduced fertilizer levels.

A new model of gene interaction between diploid host and haploid pathogen. R.-C. YANG (1), T. Z. Ye (2), and F. C. Yeh (1). (1) University of Alberta, Edmonton, AB; (2) Oregon State University, Corvallis, OR. Phytopathology 94:S113. Publication no. P-2004-0768-AMA.

A widely used population genetic model for studying interaction between diploid host and haploid pathogen has been the gene-for-gene coevolution model developed by K.J. Leonard when complete dominance of resistance is assumed. We have developed a new complementary model of gene interaction by allowing for arbitrary levels of dominance in the host. This model enables us to assess the effects of overdominance, incomplete dominance and underdominance on the equilibrium frequencies of resistance and virulence genes and on the stability of equilibria. Our model reduces to Leonard's model if there is complete dominance of resistance. Theoretical analysis and computer simulations show that our model has two new features. First, when there is overdominance or underdominance of resistance, the internal equilibrium points exist even when there is no cost of unnecessary virulence or when there is a cost of necessary virulence at the balance between cost of unnecessary virulence and effectiveness of resistance. Second, the occurrence of stable resistance and virulence polymorphism is strongly dependent on the level of dominance. These two features suggest the need for caution when using the gene-for-gene model, especially in the presence of overdominance or underdominance. Our model is particularly appropriate for studying the coevolutionary dynamics between plant populations and their pathogens in a natural pathosystem or a cropping system where genetic diversity of host plants is promoted.

Biological activity, biochemical mode of action, and physical properties of the natural picolinamides UK-2A and UK-3A. C. YAO, J. Owen, A. Meitl, G. DeBoer, S. Erhardt-Zabik, Y. Adelfinskaya, R. Holley, and C. Yerkes. Dow AgroSciences, Indianapolis, IN. Phytopathology 94:S113. Publication no. P-2004-0769-AMA.

The natural-occurring picolinamide UK-2A and its desmethoxy analog UK-3A, discovered and provided by Meiji Seika Kaisha, showed strong inhibition of spore germination and mycelial growth of various plant pathogenic fungi in in-vitro assays. Biochemical studies demonstrated that both compounds acted as potent inhibitors at the Qi site of the mitochondrial electron transport complex III, similar to antimycin A. In greenhouse evaluations, both compounds showed excellent disease control vs. key cereal diseases such as wheat brown rust, leaf blotch, and glume blotch when applied in a high-volume spray format. Wheat leaf mobility assays using brown rust as an indicator suggested that UK-2A had the ability to redistribute in wheat tissue via xylem, while UK-3A did not. This correlated very well with the poor performance of UK-3A in low-volume spray applications vs. the same diseases. The two natural products had similar log Kow values, but the pKa of UK-3A was about 1.5 units lower than UK-2A. UK-2A was slightly more water soluble, and had better membrane permeability relative to UK-3A. UK-2A was intrinsically unstable under UV radiation. Metabolism to inactive entities and binding of the active ingredient to cell components in wheat plants further limited biological availability of UK-2A to control fungal disease development.

Analysis of the *Erwinia chrysanthemi* Hrp regulon. M.-N. Yap and A. O. CHARKOWSKI. Dept. Plant Pathology, Univ. Wisconsin, Madison, WI 53706. Phytopathology 94:S113. Publication no. P-2004-0770-AMA.

The type III secretion system (TTSS) is important for virulence in many gram-negative bacterial pathogens, including *Erwinia chrysanthemi* (Ech). The *hrp* locus is composed of multiple divergently transcribed operons encoding TTSS structural and secreted proteins, and at least three regulatory systems, including a putative two-component system (TCS) consisting of the sensor kinase HrpX and the response regulator HrpY, a sigma-54 dependent enhancer protein HrpS, and an alternative sigma factor HrpL. Bacterial TCSs have been implicated in regulating diverse cellular processes, yet many aspects of Ech TCS are unclear. Preliminary results have shown that *hrpXY*, *hrpS* and *hrpL* mutants are defective in bacterial growth *in planta*, presumably because *hrp* genes are not expressed. Through the use of quantitative RT-PCR, we have determined that *hrpL* and *hrp* structural genes are expressed in minimal and rich medium. Plasmid-expressed *hrpL* recovered the phenotypes of a *hrpXhrpS* double mutant, indicating *hrpXY* and *hrpS* are epistatic to *hrpL*. The *hrpX*, *hrpY*, and *hrpS* mutants were also affected in cell aggregation and pigment formation, suggesting that these *hrp* regulators control expression of non-TTSS genes. Current studies are focused on elucidating the *hrp* regulatory cascade under various growth conditions and defining the binding sites of HrpY. The results will provide insights on how environmental signals are transmitted by the TCS to affect activities of TTSS during infection.

Migration of *Fusarium verticillioides* in corn ears. I. E. YATES (1) and D. Sparks (2). (1) Russell Agricultural Research Center, USDA/ARS, Athens, GA; (2) Dept. of Horticulture, University of Georgia, Athens, GA. Phytopathology 94:S113. Publication no. P-2004-0771-AMA.

Mycotoxins harmful to humans and animals are produced during colonization of corn kernels by *Fusarium verticillioides* creating a need to understand the host/pathogen interactions between the two organisms. Current methodology is limited by a consistent supply of host tissue as reproductively mature corn plants can be produced only once each year in temperate climates under field conditions and is limited under greenhouse conditions. *F. verticillioides* migration was assessed in corn ears obtained from a local grocery store, as well as from greenhouse and field grown plants. The *F. verticillioides* transformant used for inoculation was tagged with the selection gene for hygromycin resistance and the marker gene for beta-glucuronidase ensuring the identification of the source of subsequent mycelial growth. *F. verticillioides* colonized corn ears, regardless of source or incubation protocol. Migration of *F. verticillioides* proceeded both in an apical and a basal direction on the ear from the site of inoculation at the midpoint of total ear length, regardless of whether ears were attached or detached from the plant. Likewise, *F. verticillioides* migrated from the inoculation site both with shucks intact and removed. In summary, grocer ears can serve as a source of corn providing methodology for analyzing the relationship between corn and *F. verticillioides* to study this host/pathogen interaction regardless of the time of year.

Biocontrol of strawberry fusarium wilt by three antagonistic bacteria. S. J. YOO (1), M. H. Nam (2), J. B. Ra (1), I. S. You (1), D. W. Kwak (1), and H. G. Kim (3). (1) Bioshield Co., Ltd., Room 2221 HTVC KAIST 373-1, Yusung-Gu, Daejeon 305-701, Korea; (2) Strawberry Expt. Station, Chungnam ARES, Nonsan, Chungnam 320-862, Korea; (3) Dept. of Agricultural Biology, Chungnam National University, Daejeon, 305-764, Korea. Phytopathology 94:S113. Publication no. P-2004-0772-AMA.

This research selected three isolates of *Bacillus* sp. RK1, *Burkholderia pyrrocinia* BS36, and *Bacillus amyloliqeufaciens* BS87 from plants and rhizosphere soil in strawberry field, which showed the most antagonistic effects among antagonistic bacteria to biocontrol of Fusarium wilt of strawberry caused by *Fusarium oxysporum* f. sp. *fragariae*. Selected antagonistic bacteria showed similar antagonistic effect on *Phytophthora cactorum*, *Pythium* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, as well as *Rhizoctonia solani*, and excellent effect at the temperature range necessary for overall growth stage at strawberry cultivation in vitro. All three antagonistic bacteria secreted antifungal activity substance, and inhibition status of plant fungal pathogens showed diversity such as lysis of hypae cell wall, lysis of hypae cell membrane, hypae swelling, and inhibition of mycerial growth. From the field trials with 'Dochiodome' variety on artificially infested field around $\log_{10}^{5-6}$ CFU/g soil concentration, investigations were taken at 29 and 70 days after transplanting which were dipped into a bacterial suspension (2×10^7 CFU/ml) for 10 minutes, biocontrol efficacy of RK1, BS36, and BS87 was 88.7%, 77.8%, and 77.8%, respectively, compared to non-treatment and these biocontrol efficacy were similar to control of chemical fungicide (Copper hydroxide) of 88.7%. Viability of antagonistic bacteria right after treatment, meanwhile, was bacterial concentration of $\log_{10}^{5-6}$ CFU/g rhizosphere soil and showed relatively high viability of antagonistic bacteria concentration of $\log_{10}^{4-6}$ CFU/g rhizosphere soil at 14 weeks after treatment.

Phylogeny of *Gymnosporangium* spp. and evolution of morphological characteristics. H. Y. YUN (1), S. G. Hong (1), K. J. Lee (1), and K. S. Bae (2). (1) Department of Forest Resources, College of Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Korea; (2) Korea Research Institute of Bioscience and Biotechnology, 52 Oun-dong, Yusong-gu, Daejon, 305-333, Korea. Phytopathology 94:S114. Publication no. P-2004-0773-AMA.

Phylogenetic relationships of 41 specimens for four morphologically identified *Gymnosporangium* species, *G. asiaticum*, *G. cornutum*, *G. japonicum*, and *G. yamadae*, were analyzed by using 28S rDNA sequences. Eleven specimens of *G. japonicum* formed a well-supported monophyletic group, but *G. asiaticum* and *G. yamadae* did not form monophyletic groups. The main lineage of *G. yamadae* included one *G. asiaticum* specimen and the lineage of *G. asiaticum* included two *G. yamadae* specimens. Two specimens of *G. cornutum* included in this study formed a monophyletic group, but were classified into a different group from foreign species based on sequences from GenBank database. Long branches leading to each species lineage and short and unresolved internal branches implied rapid speciation event producing each species. Evolution of morphological characters were reconstructed on the 28S rDNA tree. Among ten morphological characters examined, telial host, telial shape, and telial location on the hosts were shown to be relatively well conserved.

Methods combining slow desiccation and slow freezing provide robust –80°C storage for agar-grown cultures of diverse fungi. P. J. ZAMBINO. USDA Forest Service-RMRS, Moscow, ID. Phytopathology 94:S114. Publication no. P-2004-0774-AMA.

Desiccation or freezing of fungal cultures grown on standard media has been unsuccessful for viable storage of *Armillaria*. Simple, successful methods are reported that combine both approaches. Cultures were grown to 2.5 cm diameter on agar medium (1.5% agar, 0.75% malt extract, 0.75% dextrose, 0.5% peptone) and cut into 4 × 4 mm pieces. These were coated with cryoprotectant (10% glycerol and powdered milk), placed on 4 × 6 mm pieces of filter paper, and positioned 8 mm from the edge within 60 × 15 mm plastic petri plates. Desiccation was 14 d at 4°C in a chamber that separated the covered petri plates from silica-gel desiccant with laboratory tissue on a plastic grid. Culture pieces were then placed in cryovials within a Styrofoam container (walls 3.8 cm) with 16-gauge sheet metal above and below the vials to provide uniform cooling from 4 to –80°C at ca. –0.4°C / min when placed in a –80°C freezer. Vials were thawed without heat shock, and pieces plated directly to fresh medium. Recovery from culture pieces after 10 additional cycles of freezing and thawing was 100% for 52/57 diploid and 15/17 haploid isolates of various *Armillaria* species and for 35 additional isolates representing Basidiomycetes (*Heterobasidion*, *Schizophyllum*, *Coprinus* and 14 other genera), Ascomycetes (*Byssochlamys* and *Fusarium*), and Zygomycetes (*Umbellopsis*). Recovery was ca. 100% and rapid for haploid and diploid *Armillaria* isolates with previously poor storage when culture pieces coated with cryoprotectant had been incubated 3 d at 20°C before starting desiccation.

In situ spatial distribution of chitinase IIB transcripts in attacked barley leaves differs between Mla1 and mlo5 powdery mildew R gene resistances. R. J. ZEYEN, T. A. Clark, and W. M. Kruger. University of Minnesota, St. Paul, MN. Phytopathology 94:S114. Publication no. P-2004-0775-AMA.

Plants produce the chitinase IIB enzyme to weaken walls of invading fungi. Northern blot analysis revealed no temporal or quantitative differences in accumulation of chitinase IIB mRNA transcripts purified from inoculated barley tissues containing Mla1 or mlo5 powdery mildew resistance genes. However, in situ hybridization with radioactive cDNA probes revealed striking spatial pattern differences in transcript location between Mla1 and mlo5 barley isolines. The Mla1 R gene encodes a NBS-LRR intercellular receptor protein and triggers reprogramming of epidermal and subtending mesophyll cells leading to rapid, hypersensitive cell death. In Mla1 inoculated leaves chitinase IIB transcripts were localized to attacked epidermal cells and subtending mesophyll, while unattacked regions on inoculated leaves had no accumulation. The Mlo gene encodes a transmembrane protein, and lack of this protein function in mlo recessive barley confers epidermal cell penetration resistance without accompanying cell death. In mlo5 inoculated leaves chitinase IIB transcripts were highly localized only to epidermal cells, and by 15 hai all epidermal cells whether on the inoculated surface or not showed chitinase IIB transcript accumulation. Thus Mla1 and mlo5 either directly or indirectly control spatial but not temporal transcription and transcript accumulation of chitinase IIB.

Molecular and genetic variability of *Diaporthe phaseolorum* var. *caulivora* isolates from South Dakota. D. Zhang and T. E. CHASE. South Dakota State University, Brookings, SD. Phytopathology 94:S114. Publication no. P-2004-0776-AMA.

Nineteen isolates of *Diaporthe phaseolorum* f. sp. *caulivora* (DPC), causal pathogen of Northern stem canker of soybean were assessed for variability by RAPD and vegetative compatibility. The isolates were collected from infected fields in South Dakota during the 1999-2001 growing seasons. A total of 277 fragments were generated from 48 of 71 primers tested. Of these, 87 fragments were polymorphic. A phenetic analysis utilizing the NTSYS - UPGMA program demonstrated a high degree of genetic variability among the DPC isolates. Previously identified DPC A and DPC B groups could not be distinguished from each other by RAPD. The highest coefficient of similarity between any two isolates was 84.2%; the majority of the isolates shared a similarity coefficient lower than 65%. Sixteen isolates were inoculated to a susceptible soybean variety in the greenhouse to assess relative virulence. Two isolates killed 100% of inoculated plants, and twelve isolates killed at least 66%. Two unidentified isolates from the collection, as well as two *Phomopsis longicolla* isolates had RAPD profiles that were completely different from DPC isolates. Vegetative compatibility groups (VCGs) were identified using pairing tests on potato dextrose agar. Many different VCGs were identified, and no single group was widespread or dominant within the collection. Isolates originating from different cankers on the same plant often belonged to the same VCG. These results have important implications for confirming that Northern stem canker is primarily a seed-borne disease.

Development of *Bean pod mottle virus* as vector for stable foreign gene expression in soybean. C. Zhang and S. A. Ghabrial. University of Kentucky, Dept. of Plant Pathology, Lexington, KY 40546. Phytopathology 94:S114. Publication no. P-2004-0777-AMA.

The comovirus *Bean pod mottle virus* (BPMV) has a bipartite positive-strand RNA genome consisting of RNA-1 and RNA-2. We developed BPMV as an expression vector by inserting a gene of interest into the RNA2-encoded polyprotein ORF and creating additional proteinase cleavage sites to flank the foreign protein. Green fluorescent protein (GFP)-expressing recombinant BPMV was constructed and demonstrated to be stable and to express GFP at high levels. The GFP gene was introduced at the cleavage site between the movement protein and the large coat protein coding sequences. To facilitate the utilization of BPMV as a vector for foreign gene expression, additional restriction sites were introduced for inserting the gene of interest. Recombinant BPMV constructs expressing antifungal and herbicide-resistance genes were generated and are being evaluated. In another study, BPMV constructs containing the helper componenent–proteinase (HC-Pro) coding sequences from *Soybean mosaic virus* (SMV) and *Tobacco etch virus* (TEV) were also produced. Interestingly, plants inoculated with transcripts containing TEV or SMV HC-Pro coding sequences developed severe systemic veinal necrosis symptoms reminiscent of those induced by the synergistic interaction between BPMV and SMV in doubly infected plants. The finding that HC-Pro from TEV, a virus that does not infect soybean, may function as a synergism-inducing sequence in a non-host when infected with a compatible virus has implications in risk assessment considerations of transgenic plants expressing viral sequences.

Genomic analysis of the maize 12-oxo-phytodienoic acid reductases and their involvement in plant-pathogen interactions. J. ZHANG, C. Simmons (2), R. Meeley (2), N. Yalpani (2), and M. Kolomiets (1). (1) Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX; (2) Pioneer Hi-Bred Intl. Inc., Johnstown, IA. Phytopathology 94:S114. Publication no. P-2004-0778-AMA.

12-oxo-phytodienoic acid reductases (OPRs) control the flow of metabolites from the 18-carbon group of alpha-linolenic acid derivative, 12-oxophytodienoic acid (OPDA) to the precursor of the natural jasmonic acid (JA). The precise role of JA in induction of defense genes is still elusive. Using full insert sequencing and 5′RACE cloning we identified seven OPR genes in the maize genome. The number of genes was confirmed by Southern blotting analysis and by using PCR with genomic DNA from oat-maize chromosome addition lines in order to identify the chromosome location of each OPR gene. Genomic structures were predicted for each of the maize OPR genes based on the available public GSS genomic database. Interestingly, promoters of several maize OPRs contain conserved transcription factor binding elements that have been shown to mediate stress-induced expression of many defense-related genes. MPSS data revealed the different tissue-specific expression pattern of OPR genes in stress-related conditions. Results of RNA profiling by methods including Northern blotting and RT-PCR indicate that expression of the OPR genes is differentially regulated in non-treated maize organs and in response to infection with fungal pathogens or treatment with defense-associated signaling molecules such as JA, ethylene and SA. This suggests OPRs/JA involvement in pathogen-induced defense responses. By using a reverse genetics strategy, we have identified maize mutants in which function

of one of the SA and *Fusarium verticillioides* inducible *ZmOPR* genes is interrupted by insertions of *Mutator* transposable elements in its coding sequence. A study is underway to test these mutants for their disease resistance to define this gene's role in interactions against pathogens.

The genetic structure of populations of *Apiosporina morbosa* in Canada. J. X. ZHANG, W. G. D. Fernando, and W. R. Remphrey. Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada. Phytopathology 94:S115. Publication no. P-2004-0779-AMA.

Black knot of *Prunus* spp. is an important disease caused by the fungal pathogen *Apiosporina morbosa*. The study was carried out to compare the genetic structures of the pathogen populations from different locations and host genotypes of *P. virginiana*. Genomic DNA from 129 single-spore isolates of the pathogen was collected from 17 locations in Canada comprised of two types of hosts, wild chokecherry and cv Shubert. The DNA was analyzed for the genetic structure of the pathogen using sequence-related amplified polymorphism (SRAP) and internal transcribed spacer (ITS) sequence data. Approximately 60% of single isolates were distinct genotypes, even the isolates from the same host genotype located in the same location. However, the degree of genetic similarity was greater among the *A. morbosa* isolates from the same host located in the same geographical area, than among those isolates from a different location. A significant subgroup with higher genetic similarity was detected from isolates collected from wild chokecherry trees in Riding Mountain National Park in Manitoba and Waterton National Park in Alberta. In short, high genetic variation was observed among all isolates of the pathogen populations regardless of host genotypes and geographical origins.

The potential of curing for green mold control of Florida citrus fruit. J. X. ZHANG and P. Swingle. Florida Dept. of Citrus, 700 Expt. Station Rd., Lake Alfred, FL 33850. Phytopathology 94:S115. Publication no. P-2004-0780-AMA.

Green mold caused by *Penicillium digitatum* is one of the leading postharvest diseases on Florida citrus. Curing was investigated as a potential method to control green mold on Florida citrus. When oranges were inoculated with *P. digitatum* or wounded and incubated at 21, 30 and 35°C for 48 hrs, the green mold incidence on the fruit cured at 30 and 35°C was reduced by 65.9 and 100%, respectively, for inoculated fruit, and by 39.4 and 100%, respectively, for wounded fruit compared to those kept at 21°C. However, a significant increase in stem-end rot caused by *Diplodia natalensis* occurred at 35°C when compared to 21°C. The minimum curing time to achieve at least 90% green mold control was determined to be 48 hrs at 35°C. Curing (35°C for 48 hrs) of inoculated oranges showed similar efficacy for green mold control to Imazalil (500 to 1,000 ppm) applied as a dip, and better efficacy when applied by packingline drip. Using wounded oranges and inoculated grapefruit, thiabendazole (500 ppm) drench followed by curing (35°C for 48 hrs) resulted in reductions of both green mold and Diplodia stem-end rot by more than 93%. All curing treatments were conducted at 95-100% relative humidity.

A genome island in plant pathogenic strains of *Serratia marcescens*, causal agent of cucurbit yellow vine disease, encodes fimbrial proteins. Q. ZHANG (1), U. Melcher (2), L. Zhou (3), B. A. Roe (3), and J. Fletcher (1). Depts. of (1) Ento. and Pl. Path., and (2) Bioch. and Mol. Biol., Okla. St. Univ., Stillwater, OK; (3) Dept. of Chem. and Bioch., Univ. of Okla., Norman, OK. Phytopathology 94:S115. Publication no. P-2004-0781-AMA.

Subtractive hybridization of the genome of an endophytic strain (R02) from that of a plant pathogenic strain (Z01) of *Serratia marcescens*, the causal agent of cucurbit yellow vine disease, revealed 32 genes specific to Z01. To investigate the function of these genes and studied their organization on the chromosome. We constructed a Z01 fosmid library using the CopyControl Fosmid Library Production kit (Epicentre). To identify individual fosmid clones containing Z01-unique sequences, the library was hybridized with a probe made from a pool of Z01 unique genes. Positive clones were then hybridized individually to a macro-array of Z01-unique genes. A 35kb genome island designated FOSU1 and containing sixteen Z01 unique sequences, was sequenced and annotated. This island is composed predominantly of fimbrial and transposase genes. Regulatory and unknown genes also were identified. A cluster of fimbrial genes is targeted for gene inactivation and functional analysis.

Cold shock increases air-drying survival of *Cryptococcus nodaensis* OH 182.9. S. ZHANG (1,2), D. A. Schisler (2), M. A. Jackson (2), M. J. Boehm (1), and P. J. Slininger (2). (1) The Ohio State University, Columbus, OH; (2) NCAUR, USDA-ARS, Peoria, IL. Phytopathology 94:S115. Publication no. P-2004-0782-AMA.

Fresh biomass or frozen concentrates of *Cryptococcus nodaensis* OH 182.9 (NRRL Y-30216) significantly decreased Fusarium head blight in greenhouse and field trials. Fermentation, formulation and drying studies are necessary and important in order to simplify transportation, storage and application of this agent. Air-drying is a convenient and economical drying method for developing microbial products. In experiments designed to test the effect of temperature shock during liquid cultivation on survival of OH 182.9 after air-drying, OH 182.9 was grown at various temperatures in semidefined complete liquid media, with cells grown at 25°C for 48 h serving as a control. Harvested cultures were mixed with 5 or 10% diatomaceous earth, vacuum filtered, and air dried for 15-20 h at 60-70% RH. Cell survival at 4°C for 14 weeks after air-drying was evaluated. In general, cells grown at 25°C for 20 h and then cultivated at 15°C for 28 h survived air-drying better than control cells. The survival of cells subjected to heat shock at 31°C generally did not differ from control cells regardless of whether heat shock was applied at the late exponential or early stationary stage of growth. In current studies, preliminary data indicate that prolonged (28 h) cold shock at 10 and 15°C after incubation at 25°C for 20 h increased desiccation tolerance during the formulation process.

Role of SGT1 genes in regulating plant defense in ornamental plants. S. ZHANG, S. E. Nada, R. V. Sairam, and S. L. Goldman. Plant Science Research Center, The University of Toledo, Toledo, OH 43606. Phytopathology 94:S115. Publication no. P-2004-0783-AMA.

Geraniums and begonias are among economically the most important ornamental species in floriculture worldwide. However, production of such plants can be severely affected or destructed owing to the presence of various diseases such as the bacterial leaf spot disease in geraniums caused by *Xanthomonas campestris* pv. *Pelargonii*. There have been no effective conventional ways to prevent from such a disastrous disease. We are interested in isolating and manipulating genes for resistance against pathogens in these ornamental species. Recent evidence showed that *Arabidopsis* SGT1 is required for a broad spectrum of resistance conferred by multiple R genes. Based on the sequence conservation of known SGT1 proteins, we designed degenerate primers and used the RT-PCR technique to isolate SGT1 homologs from ornamental plants. We have so far obtained SGT1-encoding DNA fragments from both geranium and begonia plants. Preliminary sequence analysis indicated that the geranium SGT1 proteins are over 93% similar to each other and to begonia SGT1 proteins. Further characterization of these SGT1 genes is underway, and role of the SGT1 genes in regulating plant defense will be presented.

Functional characterization of the Arabidopsis RPS4 gene. X.-C. ZHANG and W. Gassmann. Univ. of Missouri-Columbia, Columbia, MO. Phytopathology 94:S115. Publication no. P-2004-0784-AMA.

Arabidopsis *RPS4* belongs to the large family of nucleotide binding site (NBS)-leucine-rich-repeat (LRR) resistance (*R*) genes, and has an N-terminal Toll/interleukin-1 receptor (TIR) domain. Like other TIR-NBS-LRR *R* genes, *RPS4* produces alternative transcripts with truncated open reading frames. In the case of *RPS4*, introns 2 and 3 that contain in-frame stop codons are frequently retained in the alternative transcripts. To investigate the biological significance of these alternative transcripts, we removed introns 2 and 3, either individually or in combination, from a functional *RPS4-Ler* gene, and generated stable transformants with these intron-deficient transgenes. Removal of one or both introns abolished the function of the *RPS4* transgene, whereas expression was not affected. A C-terminally truncated *RPS4* transgene that mimicked the open reading frame present in naturally occurring alternative transcripts was not sufficient to confer resistance, suggesting that the combined presence of regular and alternative *RPS4* transcripts is required for function. This hypothesis was further confirmed by the reconstitution of *RPS4* function in double transgenic plants containing both intron-deficient and C-terminally truncated *RPS4* transgenes. Together with results from other gene family members, our data suggest that the generation of alternative TIR-NBS-LRR *R* gene transcripts is of general biological importance across plant species. Furthermore, these alternative transcripts could be potentially translated into C-terminally truncated proteins. Functional characterization of these putative truncated proteins using biochemical approaches is ongoing.

Combination of mechanisms in *Pseudomonas chlororaphis* strain PA-23 results in control of multiple pathogens. Y. ZHANG (1), W. D. Fernando (1), K. Kavitha (2), S. Nakkeeran (2), and R. Ramarathnam (1). (1) Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2; (2) Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, 641003, India. Phytopathology 94:S115. Publication no. P-2004-0785-AMA.

Phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol are two of the most potent antibiotics against fungal plant pathogens in the world. *Pseudomonas chlororaphis* strain PA-23 is the first bacterial strain identified to carry biosynthetic genes for both these antibiotics, identified through PCR and

subsequent purification and sequencing of the PCR products. Strain PA-23 was first shown to control ascospore germination, and stem rot of canola caused by *Sclerotinia sclerotiorum* in greenhouse and field studies. Bacterial organic volatiles (2-ethyl-1-hexanol, benzothiazole, and nonanal) produced by strain PA-23 inhibited sclerotinia ascospores, mycelial growth, and sclerotial germination. PA-23 induces the production and increased levels of phenyl ammonia lyase, polyphenol oxidase and peroxidase isoforms, and PGPR action in a variety of host plants. Antibiotic 2,4-DAPG extracted from PA-23 caused inhibition of sclerotial and spore germination, hyphal lysis, vacuolation, and protoplast leakage in a number of plant pathogens, including *S. sclerotiorum*, *Pythium aphanidermatum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotium rolfsii Fusarium oxysporum*, *Alternaria solani* and *Botryodiplodia theobromae*. Identification of genes important in biocontrol will be investigated through Tn-mutagenesis for increased or decreased antifungal activity.

Zwittermicin A detection in *Bacillus* spp. controlling *Sclerotinia sclerotiorum* on canola. Y. ZHANG and W. G. D. Fernando. Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2. Phytopathology 94:S116. Publication no. P-2004-0786-AMA.

Five Gram positive *Bacillus* species isolated from canola and wheat plant parts have been shown to inhibit *Sclerotinia sclerotiorum*, the causal agent of stem rot of canola (*Brassica napus* L.). It was hypothesized that antibiotic production was involved in biological control of this pathogen. Zwittermicin A-producing *Bacillus* spp. are known to be ubiquitous in soil, and involved in the inhibition of several plant pathogenic fungi. Strains *Bacillus amyloliquefaciens* BS6, *B. cereus* BS8, *B. subtilis* H, *B. cereus* L and *B. mycoides* S were tested for the production of zwittermicin A using a PCR-based method. Primers 678 (ATG TGC ACT TGT ATG GGC AG) and 677 (TAA AGC TCG TCC CTC TTC AG), which are specific for the zwittermicin A-resistance gene, were used in the PCR analysis. *Bacillus cereus* UW85 was used as the positive control. Strains BS8, H, L and S yielded the 950bp band for the zwittermicin A-resistant gene which correlates to the production of zwittermicin A, the only known aminopolyol antibiotic. Sequence analysis of the PCR-products and a BLAST search confirmed the presence of the zwittermicin A-resistance gene in the four bacterial strains. Strain BS6, the most consistent of the five strains in biological control of *S. sclerotiorum* in the lab, greenhouse and field studies, failed to amplify the desired PCR product. Southern hybridization will be employed as a complementary approach to search for the possible homologs between strains BS6 and UW85.

Reaching destinations: Secreted proteins and protein translocation pathways in *Spiroplasma kunkelii*. Y. ZHAO, Q. Liu, R. Jomantiene, R. W. Hammond, and R. E. Davis. Molecular Plant Pathology Laboratory, ARS-USDA, Beltsville, MD 20705. Phytopathology 94:S116. Publication no. P-2004-0787-AMA.

Spiroplasmas are cell wall-less, helical, and motile bacteria that parasitize insects, ticks, and plants. Under experimental conditions, certain spiroplasmas induce pathology in vertebrate animals. Spiroplasmas are thought to have evolved progressively from low G+C gram-positive bacteria and therefore to possess a compact genome with a gene set approaching the minimal complement necessary for cellular life and pathogenesis. The present study focused on the secreted and membrane-localized proteins and protein translocation systems encoded on the genome of *Spiroplasma kunkelii*, the causative agent of corn stunt disease and the first discovered spiroplasma. A total of 258 predicted proteins contained N-terminal signal peptides and/or membrane sorting signals. Genes encoding these proteins occupy 18% of the coding capacity of the entire genome. Based on the presence of genes encoding components of protein transport machineries and/or of signature motifs embedded in the signal peptides of transported preproteins, we predict the existence of multiple protein translocation pathways in *S. kunkelii*. These include a Sec-dependent secretion pathway, a type IV secretion pathway, a twin-arginine pathway, a signal recognition particle-dependent pathway, and an ABC transporter-mediated pathway. Since protein translocation is a major factor in pathogenesis, the discoveries from this study open new opportunities for understanding molecular mechanisms of spiroplasma interaction with their eukaryotic host(s).

Phylogenetic analysis based on multiple sets of concatenated proteins suggests earlier ancestors for phytoplasmas. Y. ZHAO, R. E. Davis, and I.-M. Lee. Molecular Plant Pathology Laboratory, ARS-USDA, Beltsville, MD 20705. Phytopathology 94:S116. Publication no. P-2004-0788-AMA.

Phytoplasmas are cell wall-less, nonculturable bacteria that cause diseases in several hundred plant species. Phytoplasmas are classified as Mollicutes and have been thought to be evolved retrogressively from ancestral low G+C Gram-positive bacteria of the *Bacillus/Clostridium* group. Since phytoplasmas parasitize both plant hosts and insect vectors, knowledge of phytoplasma evolution is important to understand their parasitic life style and pathogenicity. In this study, the evolutionary relationship between phytoplasmas and other micro-organisms were investigated using a comparative genomics approach. More than 50 evolutionarily conserved proteins that are ubiquitous to all bacteria were selected to build a consensus tree. These proteins were concatenated into five superproteins and phylogenetic trees were reconstructed based on the five superproteins using both Maximum likelihood and parsimony methods. A total of 40 bacterial species were used for the analysis. The topologies of the five trees are congruent in that phytoplasmas are clustered together with other Mollicutes, forming a monophyletic group. In all five trees, Bacillaceae, along with Lactobacillales, is paraphyletic to Mollicutes, rather than being ancestral. It appears that an unknown common ancestor gave rise to two sister clades, Bacillus/Mollicutes and Clostridia. This suggests that phytoplasmas may have evolved earlier than previously thought. Our results also revealed possible events of horizontal gene transfer in early stages of mollicute evolution.

Identification of *Erwinia amylovora* genes induced during infection. Y. F. ZHAO, S. E. BLUMER, and G. W. Sundin. Michigan State University, Dept. Plant Pathology, E. Lansing, MI 48824. Phytopathology 94:S116. Publication no. P-2004-0789-AMA.

The enterobacterium *Erwinia amylovora* is a devastating plant pathogen causing necrotrophic fire blight disease of apple, pear, and other rosaceous plants. The bacterium infects blossoms, leaves, succulent shoots, and immature fruits of susceptible hosts. Immature pears have been routinely used to test pathogenicity of the fire blight bacterium. In this study, we used a modified *in vivo* expression technology system to identify genes that are activated during infection of immature pear tissue. Identification of such genes will allow us to uncover potential new virulence factors and to understand the adaptive response of the pathogen to the environment. A library of 20,000 clones, each containing a random fragment of genomic DNA (0.8 - 2.0 kb) fused to a promoterless *uidA* gene was screened individually for GUS activity on slices of immature Bartlett pear tissue and on LB plates. More than 500 clones were collected that showed strong GUS activity on pear slices but no GUS activity on LB plates. Sequence comparison revealed that known and potential virulence genes, including *hrp/hrc*, *avr*, type II secretion, levansucrase and amylovoran biosynthetic genes are induced in pear tissue. Other pear-induced genes are involved in adaptation to environment, chemotaxis, DNA repair, regulation, membrane maintenance, nutrient acquisition and metabolism. Experiments to further characterize new potential virulence factors are in progress.

Effect of temperature on the expression of necrosis in soybean infected with *Soybean mosaic virus*. C. Zheng (1), P. Chen (1), and R. GERGERICH (2). (1) Department of Crop, Soil, and Environmental Sciences; (2) Department of Plant Pathology, University of Arkansas, Fayetteville, AR. Phytopathology 94:S116. Publication no. P-2004-0790-AMA.

Soybean mosaic virus (SMV) causes mosaic symptoms in susceptible soybean cultivars and stem-tip necrosis in some cultivars carrying resistance genes. The objective of this study was to investigate the effect of different temperature regimes on the expression of SMV-induced stem-tip necrosis. Three soybean isolines, 'Essex' (susceptible to SMV strains G1 and G7), V94-3971 (resistant to G1 and necrotic to G7), and V262 (necrotic to G1 and susceptible to G7) were inoculated with SMV strains G1 and G7. The inoculated plants were maintained in growth chambers with constant temperatures of 10, 15, 20, 25, 30, and 35°C. 'Essex' soybean exhibited mosaic symptoms when infected with G1 and G7 at all temperatures tested, and the severity of mosaic symptoms was not influenced by temperature. Symptoms on all soybean isolines developed sooner in plants at 25 to 32°C than at 10 to 20°C and 33 to 35°C. The necrosis in V262 induced by G1 and in V94-3971 by G7 developed at 10, 15, 20, 25, and 30°C, on 44, 41, 14, 8, and 8 days after inoculation, respectively. No necrotic reaction was induced by either strain at 35°C, and further studies demonstrated that necrosis did not develop in both genotypes at 33°C. The results of this study provide the framework for analysis of gene expression associated with the necrotic response to SMV infection in isolines carrying SMV resistance genes.

Characterization of a regional population of *Fusarium oxysporum* sp. *niveum* by cross pathogenicity, race, and vegetative compatibility. X. G. ZHOU (1) and K. L. Everts (1,2). (1) University of Maryland, Salisbury, 21801; (2) University of Delaware, Georgetown, 19947. Phytopathology 94:S116. Publication no. P-2004-0791-AMA.

Eighty-eight isolates of *Fusarium oxysporum* f. sp. *niveum* (FON) from wilted watermelons in Maryland and Delaware were characterized by cross pathogenicity to muskmelon, race, and vegetative compatibility group (VCG). Four isolates (4.5%) were moderately pathogenic to two or more of 18 muskmelon

cultivars in a greenhouse test, and one representative isolate also was slightly pathogenic in field microplots. The four isolate all were in VCG 0082, and were identified as race 2. Of the 74 FON isolates to which a VCG could be assigned, 41 were in VCG 0080, and the VCG distributed most widely; 27 were in VCG 0082; and six were in the newly described VCG 0083, and restricted to three fields. Among the isolates in VCG 0080, eight belonged to race 0, 21 to race 1, and 12 to race 2. Of the isolates in VCG 0082, six belonged to race 0, 11 to race 1, and 10 to race 2. All isolates in VCG 0083 were race 2. Isolates from more than one race within the same VCG or isolates from more than one VCG were recovered from single plants and fields. No differences in aggressiveness on differential watermelon cultivars were observed among isolates from different VCGs of the same race. A diverse association between virulence, VCG, and field of origin suggests that the pathotypes of FON may be of local origin or at least long existent in the region.

Investigation of the role of the *Bean dwarf mosaic virus* (BDMV) BV1 gene in pathogenicity and host resistance using recombinant viruses and viral proteins. Y. C. ZHOU (1), M. R. Sudarshana (1), W. J. Lucas (2), and R. L. Gilbertson (1). (1) Department of Plant Pathology; (2) Section of Plant Biology, University of California, Davis, CA 95616. Phytopathology 94:S117. Publication no. P-2004-0792-AMA.

The BV1 gene of the bipartite begomovirus BDMV encodes a nuclear shuttle protein and a determinant of avirulence and hypersensitive response. BDMV BV1 function was investigated using GFP reporters and recombinant viruses and proteins. Replacement of the *Bean golden yellow mosaic virus* (BGYMV) capsid protein (CP) gene with BDMV BV1 resulted in increased pathogenicity in the highly susceptible cv. Topcrop (Andean gene pool), but reduced or no infectivity in BGYMV-susceptible cvs. UI 114, Othello and Alpine (Middle American gene pool); mutation of the BGYMV BV1 gene in this virus reduced infectivity and pathogenicity in Topcrop and abolished infectivity in UI 114, Alpine or Othello. Viruses with BGYMV/BDMV BV1 hybrid proteins ranged from highly pathogenic to not infectious in Topcrop and were not infectious in UI 114 or Alpine. Thus, the BDMV BV1 is a viral pathogenicity factor, but infectivity is also influenced by other viral factors (*e.g.* CP) and the host gene pool.

Nematicidal effects of gas-producing *Muscodor* sp. on *Meloidogyne incognita* in *in vitro* assays. N. K. ZIDACK (1), R. A. Sikora (2), G. A. Strobel (1), and B. J. Jacobsen (1). (1) Montana State University, Bozeman, MT; (2) Institut für Pflanzenkrankheiten der Universität Bonn, Germany. Phytopathology 94:S117. Publication no. P-2004-0793-AMA.

Muscodor sp. are gas-producing, endophtyic fungi that have been isolated from a number of woody plants in both tropical and desert settings. These fungi produce gases which are toxic to a range of microorganisms including fungi and bacteria, and show potential for control of both soilborne and storage plant pathogens, an application named mycofumigation. In this study, nematicidal effects of 4 *Muscodor* sp. isolates on *Meloidogne incognita* were tested in *in vitro* assays using Petri plates divided into 4 sections. *Muscodor* isolates were inoculated onto 2 PDA filled quadrants and the 2 other quadrants were filled with 5ml sterile tap water. Plates were parafilmed, and incubated for 5 days after which ~40 *M. incognita* larvae were added to the water-filled quadrants. Larvae were visually monitored for movement and activity was scored as (+) or (-) at 1, 2, 3 and 7 days. Two *M. albus* isolates, and *M. roseus* all significantly reduced nematode activity after one day, and after 1 week, ~75% of larvae were inactive for all three isolates compared to 12% in the untreated control. When resuspended in fresh water, activity was not restored in the three inhibitory isolates, while 100% of untreated control nematodes were active. An isolate of *Muscodor vitigines* that produces naphthalene did not inactivate the nematodes at any observation time.

Effect of foliar insecticide timing on the incidence of *Bean pod mottle virus* in soybean. A. D. ZIEMS (1), L. J. Giesler (1), and T. E. Hunt (2). (1) Dept. of Plant Pathology; (2) Entomology Dept., University of Nebraska, Lincoln, NE 68583-0772. Phytopathology 94:S117. Publication no. P-2004-0794-AMA.

Bean pod mottle is an important disease of soybeans the U.S. The primary vector for *Bean pod mottle virus* (BPMV) is the bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster). A study was conducted in 2002 and 2003 to determine optimal application timing of the foliar insecticide lambda-cyhalothrin (Warrior at 0.32 lb ai/A) to control BLB and to manage BPMV. Foliar insecticide was applied at growth stages VC, V2, and targeting the F1 BLB population. BLB populations were determined every five days from mid-April through September. Plant samples were collected at growth stage V2, V5, V9, R1, and R3 to determine BPMV incidence. In 2002, insecticide applied for F1 BLB was the only to reduce the BLB populations ($P < 0.01$), but there was no difference in BPMV incidence between treatments. The application targeting F1 BLB in 2003 provided the greatest reduction in BLB ($P < 0.01$). In 2003, BPMV incidence was significantly lower ($P < 0.1$) at growth stage R1 and R4 compared to control plots. Applications applied at growth stage VC, V2, and targeted F1 BLB provided significant reduction in incidence. Foliar insecticide application can significantly reduce BLB populations and thereby reduce the incidence of BPMV, but results are variable and are affected by BLB population dynamics.

Induced systemic resistance in cucumber to *Glomerella cingulata* var. *orbiculare* and *Pseudomonas syringae* pv. *lachrymans* by *Bacillus mycoides*, isolate BacJ and *Bacillus pumilus*, isolate MSU 203-7. O. T. ZIETLOW, M. R. Johnston, N. K. Zidack, and B. J. Jacobsen. Montana State University, Bozeman, MT. Phytopathology 94:S117. Publication no. P-2004-0795-AMA.

The anthracnose and angular leaf spot-cucumber-pathosystems were used to compare disease control using induced systemic resistance by *Bacillus mycoides*, isolate BacJ and *Bacillus pumilus*, isolate MSU 203-7. Bacilli were applied as a first true leaf treatment at 10^9 colony forming units (cfu)/ml 5 days before challenge inoculation with *G. cingulata* = 10^5 conidia/ml and *P. syringae* = 10^4 cfu/ml. Five days (seven days for the angular leaf spot-pathosystem) after challenge inoculation, disease development was compared to water treated and pathogen-induced controls. *Bacillus* treatments significantly lengthened the latent period by approximately 1 day and decreased both total spore production by approximately 64% and the percentage of viable spores by approximately 54% in the anthracnose experiment. The percent of infected leaf area was significantly reduced by approximately 37% and 48% by *B. mycoides* and *B. pumilus*. Both *Bacillus* treatments also reduced the systemic movement by approximately 27% and 36% in the angular leaf spot experiment.

HPLC-MS analysis of type-A trichothecene-producing Fusaria for correspondence between toxin profiles and molecular phylogenetic groups. N. C. ZITOMER (1), D. M. Geiser (1), D. D. Archibald (2), T. J. Ward (3), K. O'Donnell (3), A. D. Jones (4), M. M. Jimenez-Gasco (1), and G. A. Kuldau (1). (1) Department of Plant Pathology, (2) Department of Crop and Soil Sciences, and (4) Department of Chemistry, Pennsylvania State University, University Park, PA; (3) Microbial Genomics and Bioprocessing Research Unit, NCAUR, USDA ARS, Peoria, IL. Phytopathology 94:S117. Publication no. P-2004-0796-AMA.

Trichothecenes are mycotoxins produced by *Fusarium* and other genera of fungi. These toxins can contaminate many grain-based foods and feeds. Type-A trichothecenes including T-2 toxin and diacetoxyscirpenol are among the most potent mycotoxins produced by Fusaria, causing gastrointestinal disturbances and immunosuppresion. Until recently *Fusarium* taxonomy has been difficult due to the limitations of morphological identification techniques. Molecular phylogenetics, however, has overcome this issue and also revealed greater species diversity within the genus. Utilizing the *Fusarium* collections of the Penn State *Fusarium* Research Center and the USDA NRRL at Peoria, we are using multilocus phylogenetic approaches to define species boundaries within this group. This analysis has revealed a high level of diversity among type-A producers, motivating renewed interest in toxin profiles for representative species and newly identified species within this group. We have developed a high-pressure liquid chromatographic-mass spectrometric (HPLC-MS) method that detects ten trichothecenes from fungal cultures and are using the method to create trichothecene profiles for representative isolates used in the phylogenetic analysis.

Effects of grape berry nitrogen content and high relative humidity on the activation and spread of latent *Botrytis cinerea* infections. S. M. ZITTER and W. F. Wilcox. Dept. of Plant Path., Cornell Univ., Geneva, NY 14456. Phytopathology 94:S117. Publication no. P-2004-0797-AMA.

Clusters of cvs. Chardonnay and Riesling were either sprayed with conidial suspensions of *B. cinerea* at late bloom (LB) to establish latent berry infections, or individual berries were injected with the inoculum suspension at veraison (Ve) to establish point sources of disease for additional spread. Four weekly sprays of urea (17 kg/ha) were applied to selected vines beginning 1 wk before Ve, to increase berry nitrogen (N) content without affecting canopy growth. This treatment increased both the activation of latent infections and preharvest disease spread. In another experiment, clusters of potted Chardonnay vines were inoculated at LB, maintained outdoors until Ve, then transferred to a screenhouse protected from rain. Plants were then split into group and incubated at 98% relative humidity (RH) for 0, 1, 2, or 4 days beginning either (i) immediately; or (ii) 10 days before harvest. Disease development was proportional to the length of the preharvest high-RH period, but was unaffected by this treatment at Ve. Both berry N and RH appear to be involved in Botrytis bunch rot development.

Incidence of *Phytophthora* spp. in a hardwood forest site in the southern Appalachian Mountains. D. C. ZWART (1), S. N. Jeffers (1), and T. A. Waldrop (2). (1) Clemson University; (2) USDA Forest Service, Clemson, SC. Phytopathology 94:S118. Publication no. P-2004-0798-AMA.

The effect of fire and fire surrogate (FFS) fuel reduction treatments (prescribed burn, thin, thin and prescribed burn, control) on the incidence of naturally occurring species of *Phytophthora* in a hardwood forest is under investigation in the southern Appalachian Mountains. There is one 12-ha replicate of each treatment in each of three geographically distinct blocks; each replicate contains ten 50 × 20-m plots. Twenty soil cores were collected from each plot and then combined and mixed to produce a composite sample. *Phytophthora* spp. were detected using a bioassay with camellia leaf disks and hemlock needles as baits. Baits were plated on PARPH-V8 selective medium, and colonies of *Phytophthora* spp. were counted, subcultured, and identified. Before treatments were applied, *Phytophthora* spp. occurred uniformly throughout the study area. *P. cinnamomi* was recovered from 100% of the 12 replicates and 34.2% of the 120 plots; *P. heveae* was recovered from 25% of the replicates and in just 2.5% of the plots. FFS treatments have been completed, and their effect on the incidence of *Phytophthora* spp. now is being determined.

Errata

Vol. 94, No. 6 (Suppl.), 2004

The following abstracts were submitted for presentation at the APS 2004 Annual Meeting in Anaheim, California, July 31–August 4, 2004.

Niche overlap index as an indicator for competition between endophytic colonization of maize by *Bacillus mojavensis* and *Fusarium verticillioides*. C. W. BACON and D. M. Hinton. USDA, ARS, Russell Research Center, Athens, GA. Publication no. P-2004-0799-AMA.

The use of endophytic organisms to control other endophytic organisms is complicated, but nevertheless viewed as being unique and an important strategy. There are endophytic species of fungi that produce mycotoxins such as *Fusarium verticillioides* that produces the fumonisins and other toxins, which are potentially harmful to humans, livestock, and poultry. The use of an endophytic biocontrol organism for prevention of mycotoxins during the endophytic state of *F. verticillioides* depends on the degree of dominance one organism has over the other. The use of the bacterial endophyte *Bacillus mojavensis* has some promise but predicting success of strains is difficult. A study of niche overlap indices was conducted to obtain a measure of competition between several strains of *B. mojavensis* and *F. verticillioides,* its ecological homologue in maize. The in vitro growth of these two organisms on several carbohydrates as sole carbon sources that are known to exist in maize apoplasm was measured with the Microbiology Reader Bioscreen C and niche overlap indices determined. The results indicated that while *F. verticillioides* is the more competitive of the two, other factors are responsible for the success of this fungus. Thus, niche overlap indices indicated that nutrient competition is perhaps not as important as the production of other competing factors, e.g., microbial inhibitors, by *F. verticillioides* for growth within the intercellular spaces of maize.

Evaluation of pearl millet germplasm accessions for resistance to grain mold. S. S. NAVI (1) and V. A. Tonapi (2). (1) Iowa State University, Dept. of Plant Pathology, Ames, IA 50011; (2) National Research Center for Sorghum, Rajendranagar, Hyderabad 500030, A.P., India. Publication no. P-2004-0800-AMA.

Little information is available on the molds in Indian pearl millet (*Pennisetum glaucum*) production areas. Since there is an increased concern of food and feed grain quality and mycotoxins in human and poultry health, studies were conducted at ICRISAT, India to evaluate (a) 447 germplasm accessions originated from 32 countries for mold reaction in field under natural environment during the rainy season, (b) threshed grain mold rating (TGMR) and mycoflora on grains sampled from 10 panicles of each accession, and (c) scores in field and *in vitro*. Post physiological maturity evaluation in field shown no accessions free from mold, and 16% of the accessions scored 2, while in TGMR, 18% were mold free and 57% were scored 2 on a 1–9 scale. Twenty representative accessions were assessed *in vitro* against individual and mixed conidial suspensions (1×10^6 conidia ml^{-1}) of *F. moniliforme* (FM), *F. pallidoroseum* (FP) and *Curvularia pennisetti* (CP). There was significant correlation ($r = 0.97$) between overall field scores and *in vitro* scores of mixed spores inoculations. After the TGMR, mycoflora in blotter test revealed, FM, FP, CP, *Helminthosporium* spp., *Alternaria* spp. and *Colletotrichum* spp. as major fungi affecting pearl millet grain.

Errata

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The following abstracts were submitted for presentation at the APS 2004 Annual Meeting in Anaheim, California, July 31–August 4, 2004.

Live cell imaging of plant rhabdovirus movement. Sharon Yelton (1), Debasish Ghosh (2), Stephanie Mathews (1), Judith Lesnaw (2), and Michael Goodin (1). (1) Department of Plant Pathology; (2) Department of Biology, University of Kentucky, Lexington, KY 40546. Phytopathology 94:912. Publication no. P-2004-0801-AMA.

Sonchus Yellow Net Virus (SYNV) is a plant rhabdovirus that undergoes replication and morphogenesis in the nuclei of host cells. Production of a recombinant virus expressing green fluorescent protein (GFP) is currently not possible for any rhabdovirus, thereby hindering studies aimed at systemic movement of these important plant pathogens. Using a novel assay, the systemic movement of SYNV in *Nicotiana benthamiana* was determined. Symptomatic tissues were characterized by the marked appearance of nuclear-localized GFP aggregates. Results indicate systemic spread of SYNV from the inoculated leaves to systemic leaf tissue, followed by movement into the lower portion of the stem and into the roots. In systemic leaves, we observed a manner of movement consistent with proximal to distal, midvein to minor veins and surrounding leaf tissue. Nuclei in the apical, root, and lateral meristematic tissues did not develop inclusions at any time in the study. This is the first study to characterize systemic movement of SYNV.

Mutation and recombination may limit the use of *Cucumber mosaic virus* as a virus-based gene expression vector. R. W. HAMMOND, L. G. Nemchinov, and Y. Zhao. USDA, ARS, Beltsville Agricultural Research Center, Molecular Plant Pathology Laboratory, Beltsville, MD 20705. Phytopathology 94:912. Publication no. P-2004-0802-AMA.

Cucumber mosaic virus (CMV), type member of the genus *Cucumovirus* in the family *Bromoviridae*, has among the widest host ranges among plant viruses, making it an attractive candidate as a viral-based transient expression vector for plants. A presentation system based on the coat protein (CP) of CMV as a display carrier was designed to express linear epitopes of the fusion and hemagglutinin-neuraminidase proteins of *Newcastle Disease Virus* (NDV), an avian paramyxovirus, as protein fusions in different regions of the CP. Recombinant CMV CP was placed either under the transcriptional control of the duplicated sub genomic promoter of *Potato Virus X*, or in infectious cDNA constructs of CMV. In the PVX constructs, CMV virus-like particles containing the F epitope were purified and reacted with anti-NDV sera. When the engineered CP was expressed in CMV, in some cases the modified CMV virus was infectious in plants, and NDV epitopes expressed on the surface of the CMV CP were recognized by anti-NDV sera. In other cases, insertions were either partially or wholly deleted. These findings have implications for the utility of CMV-based chimeras as antigen presentation systems as well as for the study of mechanisms controlling RNA recombination and genome stability in CMV.

Errata

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The following abstracts were submitted for presentation at the APS 2004 Annual Meeting in Anaheim, California, July 31–August 4, 2004.

Diversity of Pineapple mealybug wilt associated viruses in pineapple. D. M. Sether (1), M. J. Melzer (1), J. L. Busto (1), F. Zee (2), and J. S. HU. (1) University of Hawaii, Plant and Environmental Protection Science, Honolulu, HI; (2) USDA-ARS PBRC, National Clonal Germplasm Repository, Hilo, HI. Phytopathology 94:1031. Publication no. P-2004-0803-AMA.

Pineapple mealybug wilt associated virus-1 (PMWaV-1) and PMWaV-2 are mealybug transmitted closteroviruses that infect pineapple plants. PMWaV-2 infection and mealybug exposure are involved in the etiology of mealybug wilt of pineapple (MWP), whereas PMWaV-1 is not a necessary component in MWP but correlates with reduced yield. Degenerate primers derived from conserved sequence regions of the heat shock protein-70 (HSP-70) homolog gene of various ampeloviruses were used in reverse transcription-polymerase chain reaction (RT-PCR) assays to identify three additional viruses with closterovirus-like characteristics in pineapple. Sequence homology between the virus tentatively designated PMWaV-3, and PMWaV-1 and PMWaV-2 decreases toward the 5′ terminal region across three open reading frames (ORF). A virus tentatively designated PMWaV-4 shares less than 40% sequence homology with PMWaV-1 and PMWaV-2. Monoclonal antibodies (MAbs) 35-6-5 and 63-1-2 specific for PMWaV-1, and MAb 63-2-2 specific for PMWaV-2, do not cross-react with PMWaV-3 or PMWaV-4. PMWaV-3 is mealybug transmissible but does not appear to be a necessary component of MWP etiology. A potential PMWaV-2 mutant was identified, which shares over 95% amino acid identity but contains a 210 base pair deletion in the HSP70-like gene of PMWaV-2. Specific RT-PCR assays were developed for detection of PMWaV-3, PMWaV-4, and the deletion mutant.

Characterization and analysis of potato inhibitor proteins and their effect on *Phytophthora infestans* infection. R. W. JONES. USDA-ARS, Vegetable Laboratory, Beltsville, MD 20705. Phytopathology 94:1031. Publication no. P-2004-0804-AMA.

Late blight lesion expansion rates can vary significantly between individual potato cultivars, suggesting an effect of multigenic, non-R gene components. Inhibitor proteins represent one class of gene products that could exert a limiting effect on lesion expansion. Inhibitor proteins act principally by binding to cell-wall modifying enzymes. Higher levels of inhibitor proteins may lead to slower rates of lesion expansion. Specific inhibitors of interest in potato foliage and tubers are serine protease inhibitors, polygalacturonase inhibitors and the xyloglucan-specific endoglucanase inhibitor (AY321357). Genes for each inhibitor protein class are being cloned and introduced into a PVX vector and into Agrobacterium for functional determination of their effect on lesion expansion in different potato cultivar backgrounds and in tomato. Additionally, a family 12 xyloglucan-specific endoglucanase has been cloned from *Phytophthora sojae* (AY645943) and characterized, providing the first report of family 12 endoglucanases in *Phytophthora*. Family 12 endoglucanases are the only ones known to be inhibited by the xyloglucan specific endoglucanase inhibitor protein, and the only ones with extensin-like activity. The *P. infestans* homolog is currently being cloned.

Mutagenesis and sequencing of a biofilm-defective mutant of *Xylella fastidiosa*. Yaxin Li, Guixia Hao, Yizhi Meng, Cheryl D. Galvani, Harvey C. Hoch, and Thomas Burr. Department of Plant Pathology, Cornell University-NYSAES, Geneva, NY. Phytopathology 94:1031. Publication no. P-2004-0805-AMA.

Xylella fastidiosa (*Xf*) causes Pierce's disease (PD), which is a limiting factor for production of *Vitis vinifera* grape cultivars in certain regions of the U. S. *Xf* can form biofilms on wood and glass as well as plastic surfaces. It has been suggested that biofilm formation may be related to the plugging of xylem vessels by *Xf*. We developed a biofilm-defective mutants D50 using EZ::TN™ Transposome™ system. Further quantification tests showed a 90% reduction in biofilm formation by D50 as compared to the wild-type strain. The growth of D50 over seven days (the biofilm formation period) was much greater than that of the wild-type strain, indicating that reduced biofilm formation was not a result of impaired growth. The region flanking the Tn5 insertion was cloned and sequenced revealing that the reduced biofilm phenotype resulted in a gene that is responsible for producing a fimbrial subunit precursor. The mutation likely disrupted the production of fimbrins which make up the core of the fimbrial rod and the fibrillar tip of the fimbriae, thereby affecting the biofilm formation. Both the wild-type strain and the D50 mutant were examined by SEM for differences in fimbriae appearance. In addition, cell migration on growth chamber surfaces were examined for both cell types.

Effect of various *Trichoderma* strains on nutrients uptake and nitrogen use efficiency of sweet corn. Chaur-Tsuen Lo (1), Li-Rong Chiu (1,2), Chien-Yan Hsieh (2), and Chong-Ho Wang (3). (1) National Huwei University of Technology; (2) Dayeh University; (3) National Pitong University of Technology. Phytopathology 94:1031. Publication no. P-2004-0806-AMA.

The potential of the biocontrol agents, *Trichoderma harzianum* strain T22 and R16, and *T. virens* strain R42 to induce a growth response and nutrient uptake in corn plants was studied in greenhouse and fields. For field trials, this study is to investigate the effect of *Trichoderma* strain on the growth and nutrients uptake of sweet corn in different ways of fertilizer management. The results indicated that dry matter accumulation, nitrogen use efficiency, and nutrient absorption i.e. phosphorus, potassium, calcium, magnesium, manganese and copper of sweet corn increased significantly in chemical fertilizer plots treated with various *Trichoderma* strains as compared with untreated plots during the early growth stage (knee-high stage). However, there are no differences during the same stage in organic fertilizer plots and combination of chemical and organic fertilizer plots. For silking stage of sweet corn, dry matter accumulation, nitrogen uptake, and nutrient absorption increased significantly in three-types above of the fertilizer management plots treated with the *Trichoderma* strains. Similarly, the N % of leaves, leaf dry-weight per unit area, and chlorophyll content of sweet corn in the plots treated with *Trichoderma* were also higher than that of untreated plots during the silking stage. For harvesting stage, nevertheless, there was no difference among all treatments. Treatments by *Trichoderma* strains only slightly increased the yield of ears and silage of sweet corn, when fertilizers were supplied enough for growth of sweet corn. However, all *Trichoderma* strains especially for strain T-22 seemed to increase the efficiency of nitrogen utilization of sweet corn. There were significant effects on yield in a range level of supplied nitrogen fertilizer (90–180 kg/ka). For greenhouse trials, there also were the similar results for nutrient uptake and yield of ears of sweet corn during different stages. Taken together, the results indicated that *Trichoderma* strains might be applied to increase the ear production and nitrogen use efficiency of sweet corn in lower fertility capacity of soils and lower rate of nitrogen fertilizer application in field.

Studies of *Muscodor albus* based biofumigant in controlling soil-borne disease caused by *Rhizoctonia solani* on vegetables under greenhouse conditions. Caiyao Yuan and Sharon Stuart. AgraQuest Inc., Davis, CA. Phytopathology 94:1031. Publication no. P-2004-0807-AMA.

A biofumigant based on *M. albus* under development by AgraQuest, Inc., was evaluated for efficacy against soil-borne disease caused by *R. solani* on various vegetable crops under greenhouse conditions. Variables include two rates of *M. albus* (15 and 30 g *M. albus* rye seed formulation per gallon of greenhouse potting mix), and four post-fumigation periods (0, 3, 5 and 7 days) tested on strawberry, tomato and pepper. Seedlings, 2-3-leaf stage, were transplanted into one-gallon pots filled with potting mix with *M. albus* only, *M. albus* plus *R. solani*, and *R. solani* alone. Plant height and above-ground fresh shoot were collected and analyzed one month after transplanting

when the plants in the pathogen only treatment showed visible disease symptoms. Preliminary results indicate that both rates of *M. albus* provided significant control of *R. solani* on all three hosts at the 5 and 7 days post-fumigation period.

Gene expression profiling of *Magnaporthe grisea* during appressorium formation. Y. OH, N. Donofrio, H. Pan, T. Mitchell, and R. A. Dean. Fungal Genomics Lab, North Carolina State University, Raleigh, NC. Phytopathology 94:1032. Publication no. P-2004-0808-AMA.

The fungus *Magnaporthe grisea* causes blast disease on a wide variety of domesticated and wild grasses, but is most noted worldwide for devastating cultivated rice. *M. grisea* develops a specialized infection structure called an appressorium to gain access to its host. To find the genes crucial for appressorium formation, we have performed extensive gene expression profiling. Fungal RNA was extracted from cells elaborating appressoria at different time points induced by different physical and chemical cues. Using microarray technology, labeled RNA was hybridized with the *M. grisea* 22K element oligonucleotide chip, made in collaboration with Agilent Technologies. This chip contains both 13,666 *M. grisea* and 7,124 rice elements. Expression data has been analyzed using the commercial software package Genespring and will be presented.

Phylogenetic analysis of cytochrome P450s in pathogenic and non-pathogenic filamentous fungi. J. DENG (1), M. R. Thon (1), D. Nelson (2), and R. A. Dean (1). (1) North Carolina State University, Raleigh, NC. (2) University of Memphis, Memphis, TN. Phytopathology 94:1032. Publication no. P-2004-0809-AMA.

Cytochrome P450s form a large superfamily with diverse biological functions. P450 enzymes mediate oxidative transformation of a broad array of metabolic and catabolic pathways, often in response to abiotic and biotic stress. In fungi, P450s play a key role in detoxifying plant defense secondary metabolites as well as in the biosynthesis of virulence factors. Therefore, comparative studies of P450s in pathogenic and non-pathogenic fungi may provide novel insight into the evolution of plant pathogenesis. In this study, we are comparing the P450s of four filamentous ascomycetes including two plant pathogenic fungi (*M. grisea*, *F. graminearum*) and two non-pathogens (*A. nidulans*, *N. crassa*) for which complete genome sequence is available. P450s were identified using Interpro and have been classified into families and subfamilies according to standard nomenclature. The numbers of predicted P450s identified in the fungi range from 38 in *N. crassa* to 123 in *M. grisea*. Using a combination of Blast and other clustering tools, the phylogenetic relationships of these proteins has been established. The result of these analyses will be presented.

Fusarium stem rot of vanilla in north Sulawesi

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