Fruit bait traps of vector insect of Phyhtophtora palmivora Butl in Cocoa Plants

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Fruit bait traps of vector insect of *Phyhtophtora palmivora* Butl in Cocoa Plants

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ABSTRACT

Phyhtopthora palmivora **is a fungus that causes serious losses in cocoa plantations as it attacks young unripened and ripe cocoa pods. The available control technology only controls the attacked cocoa pods, while the vector of *P. palmivora* remains uncontrolled. Insects are assumed to also spread *P. palmivora* among cacao fruits. Previous studies on the information of insects as a vector of *P. palmivora* were still limited. The present study examined the types of fruit that could be traps for insects in cocoa plantations and determined the insects carrying *P. palmivora* through inoculation of healthy cocoa pods. Three types of beetles were found in bananas, pineapples, melons, and cacao, namely *Carpophilus* sp., *Ambrosiophilus* sp., and *Ashverus* sp. The highest population of *Carpophilus* sp. was found in bananas. Meanwhile, the greatest number of ambrosia beetles *Ambrosiophilus* sp. was found in cacao pods. The diverse beetle species of was found in cacao pods, while only *Carpophilus* sp. was found in bananas, pineapple, and melon. The three beetles inoculated in the healthy cocoa pods showed blackish brown symptoms of *P. palmivora*. The infection of 3-5 imago was found when beetles inoculated in the healthy cocoa pods. Fungi that were isolated in *Potato Dextrose Agar* (*PDA*) showed the morphological characters of *P. palmivora*. Based on the relative abundance, *Ambrosiophilus* sp., and *Carpophilus* sp. carry *P. palmivora* among the cocoa fruit. Cocoa and banana are used to monitor beetles that carry fungus on cocoa plants.**

Key words : Beetle, carrier, cocoa pod rot fruit, trap.

INTRODUCTION

Cocoa plays a significant role in the Indonesian economy since it is exported to numerous countries in Europe, America, and Asia. Indonesia is the world's third-largest cocoa bean production after Côte d'Ivoire and Ghana. In Indonesia, Cocoa Commodity contributes the third largest foreign exchange after palm oil and rubber plantation sector (Anonymous, 2016; Wahyudi *et al.*, 2008). The main factors causing low yields of cocoa production are the cocoa pod borer, *Conopomorpha cramerella*, and cocoa pod rot disease, *P. palmivora* (Kandowangko *et al.*, 2015; Rubiyo and Siswanto, 2013). The species of *Phytophthora* that causes cocoa pod rot in Indonesia is *P. palmivora*. (Guerro-Aceboet *et al.*, 2012; Semangun, 2000; Sukamto and Pujiastuti, 2004). According to Sukamto and Pujiastuti (2004), pathogens can attack the fruit and cause the seeds to rot reducing their quality. Cocoa pod rot causes significant losses in wet climates.

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Cocoa pod rot disease has spread in almost all cocoa plantations in Indonesia. Some literature states that *P. palmivora* attacks cacao on the fruit, trunk, and branches. *P. palmivora* attack starts from young unripened fruit to the ripe one or in the harvest time. The attack on the young unripened fruit causes the fruit to stop developing and the attack on the ripe fruit lowers the quality of the harvest (Sastri, 2008). In North Sulawesi, the intensity of the damage caused by *P. palmivora* was 21.90 - 59.52%, the highest was found in Bolaang Mongondow Regency (Kandowangkao *et al.*, 2015). *P. palmivora* attacks the fruit tissue that is responsible for cocoa beans' wrinkle and color changing. When dried in the sun, the seeds will wrinkle and weigh less. Semangun (2000) reported that *P. palmivora* attack was directly proportional to the number of fruits and the rainfall intensity. The pod rot is more common in fruit trees. The yield loss caused by *P. palmivora* reaches 90%, especially in the rainy or dry season on land with a large ant population (Rosmana *et al.*, 2010). In

Indonesia, losses due to pod rot disease range from 25% - 50% per harvest season (Drenth and Guest, 2004). Dayati (2013) reported that the intensity of damage to cocoa pods by *P. palmivora* was 28.70 - 35.54% causing a harvest loss of 46.99 - 52.33%.

Evans (1973) pinpoints that insects can spread fungi to higher and farther places since they move easily. Kandowangko *et al*. (2015) reported that almost most of the cocoa pods that were horizontally adjacent were not attacked by *P. palmivora. Thus,* it was assumed that the carrier of *P. palmivora* were insects. The spread of *P. palmivora* is very likely to be rapid since the sporangia of this fungus are disseminated by rain, wind, ants, insects, rats, bats, contaminated harvesting and pruning implements, and contaminated soil (Guest, 2007). Agrios (2004) reported that *Phytophthora* sp. can be spread by insects, especially the family Nitidulidae family. These insects can spread *P. palmivora* between cocoa pods at a considerable distance. Stem canker in pine trees in Caliofornia caused by *Fusarium circinatum* and its spread by insects *Pityophthorus* sp. (Storer *et al.*, 2004).

The continuous and excessive use of synthetic chemical compounds will provide resistance to *P. palmivora* and negatively damage the environment (Erwin and Ribiero, 1996). Until now, there is no report on the insects that spread *P. palmivora* in the cocoa plantations. Information on the insect carrying *P. palmivora* is the basis for a strategy to control pod rot disease. Clyod (2011) reported that the insect vector of pathogenic fungi are Nitudulidae and Scolytidae insects. Scolytidae and Nitudulidae are attracted to fruits (Agrios, 2004).

MATERIALS AND METHODS

The research activity to find the insect vector of pod rot disease was carried out in Muntoi village of N 00078748 and Poigar of N00098701; E1240 24532, in Bolaang Mongondow Regency. Bolaang Mongondow Regency was selected since it is the center of cocoa production for North Sulawesi. The research activities were carried out in 2020. To obtain the insect vector *P. palmivora*, ripe/ rotten cocoa (*Theobroma cacao*), banana (*Musa paradisiaca*), pineapple (*Ananas comosus*), and melon (*Cucumis melo*) were prepared. The research location of one hectare had produced cocoa. Some of the pods have been attacked by pod rot disease, *P. palmivora*. The research location was divided into two parts of the cardinal directions, namely north and south. Each of the cardinal directions was given three treatments of bananas, pineapples, and melons. Thus, in the experimental location, there were six fruits placed as baits for the insect vector carrying *P. palmivora*.

The weight of each fruit for the treatment was 200 g; the fruit was cut in order to attract the vector insect carrying the *P. palmovora*. Especially, cocoa fruits were not weighed since the treatment was done in the cocoa plantation. The ripe bananas, pineapples, and melons were fresh and not fermented. They were put in a plastic container with a diameter of 20 cm and a length of 15 cm. The lid of the plastic container was covered with gauze and a hole of 1.5 mm in diameter for small insects that were trapped in the hole such as Nitidulidae and Curculionidae. The plastic container with the fruit was hung in the cocoa tree using a blue rope. The plastic container was hung 1.5 m from the ground. The bait of banana, pineapple, and melon was left for seven days in the cocoa plantation to be fermentated. After seven days, the fruit was taken back to explore the type and population of insects trapped in each fruit. Fruit samples taken from the field were placed separately in the laboratory to prevent the movement of insects among fruits. Then the species and population abundance of the insects were examined. After being separated based on the species, the insects were then inoculated on healthy cocoa pods. The ten cacao pods taken were from each direction of the north and south. The criteria for cocoa pods taken in this experiment were blackish-brown to black. The fruit traps as the bait was carried out two times at intervals of once a month. The first trap was placed in May 2020 and the second was in June 2020. The next activity was insect identification and insect inoculation on healthy cocoa pods.

One day the beetles taken from the field were still alive and inoculated on healthy cocoa pods. The cocoa pods taken were ± 20 cm long or about 3 months old to be inoculated with beetles. The experiment used four types of beetles which were inoculated with healthy cocoa pods. Each type of beetle was used three times, except for *Ashverus* sp. with only 2 replications as the population was low. The four beetles inoculated on the cocoa pods were based on the identification results. The number of insects inoculated on the cocoa pods were 2, 3, 4, and 5 individuals per hole. The site where the beetle was inoculated was wounded. In one cocoa pod, there were 2-3 wound sites with a varying number of beetles inoculated. The part of the healthy pod was perforated using a sterilized one creating a hole 5 mm deep with the width and length according to the size of the beetle.

The length of the beetle was 1.5 - 2.4 mm. The beetle was put into the hole and covered with the pulp that has been cut earlier. The beetles in the cocoa pods were observed for seven days to obtain the symptoms of *P. palmivora* on the cocoa pods. After the symptoms of *P. palmivora* appeared on the cocoa pods from the inoculation, the isolation was continued onto PDA. The blackish-brown pods infected with *P. palmivora* were cut into 1 cm. The fruit pieces were sterilized and soaked in distilled water, 70% ethanol, and distilled water again. Then, it was dried in a petri dish that had been coated with filter paper, then cultured on the growth medium. The fungus that grew on PDA were subcultured in order to obtain a pure culture. Then, it was observed microscopically. Each sampling of beetles was inoculated on healthy cocoa pods and cultured on the growth medium. The main factor of this study was to determine the vector of *P. palmivora*. After finding out the fungal-carrying beetle, the symptoms of fungus fungi-carrying beetle were isolated onto PDA growth media.

RESULTS AND DISCUSSION

Insects that are found in ecosystem of cocoa plantation by using traps of banana, pineapple, melon and cocoa fruit, consist of *Carpophilus* sp. (Nitidulidae), *Ambrosiophilus* sp. (Curculionidae) and *Ashverus* sp. (Silvanidae). Traps of banana, pineapple and melon fruit were only found by *Carpophilus* sp., while on cocoa pods were found three type of beetles namely *Carpophilus* sp., *Ambrosiophilus* sp. and *Ashverus* sp. There was a positive correlation between the relative abundance of *Carpophilus* sp. in several fruit traps. Analysis of population abundance using the chi-sguare test for *Carpophilus* sp. in Poigar village F = 100.59; $p < 0.000$ ($p = 0.05$), in the village of Muntoi F = 87,824; $p < 0.000$ (Fig. 1).

Most fungi do not depend on insects for resources or nutrition, whereas insects depend on fungi as part of their diet (Schigel, 2012). Based on observations on the second day after the installation of fruit traps of bananas, pineapples and melons, the *Carpophilus* sp. beetle was not found in fresh fruit, but the beetle population began to appear on the third day, because the fruit had undergone fermentation. Unlike the cocoa pods that are already available in the cocoa ecosystem and have undergone decay or fermentation, the *Carpophilus* sp. beetle population is easy to find.

Several researchers have documented interactions between insects and plants. *Colopterus truncatus* and *Carpophilus sayi* beetles are attracted to new wounds in oak plants (Noris, 1956). Nitidulidae beetles are attracted to fruit that has undergone fermentation or has begun to rot and the presence of fungus on the fruit (Downie and Arnett, 1996). Interest of beetles in plant wounds has been reported by Juzwik *et al.* (2004), Nitidulidae beetles were found in large numbers in wounds and contaminated with fungi. *Coleopterus truncatus* and *C. Carpophilus sayi* contributed > 95% of Nitidulidae collected from fresh wounds ≤ 5 days old on oak plants. Nitidulidae beetles were collected in new wounds, each location varies in population abundance. Species richness was present in ≤ 2 weeks old wounds on oak trees between April and June in northern Iowa (Hayslett *et al.*, 2008). Retrieval of banana, pineapple and melon traps is appropriate for 7 days to obtain *Carpophilus* sp. populations. Kandowangko *et al.* (2015) reported that bananas were used as traps on cocoa plants, it turned out that the population of *Carpophilus* sp. was very low and even the beetles

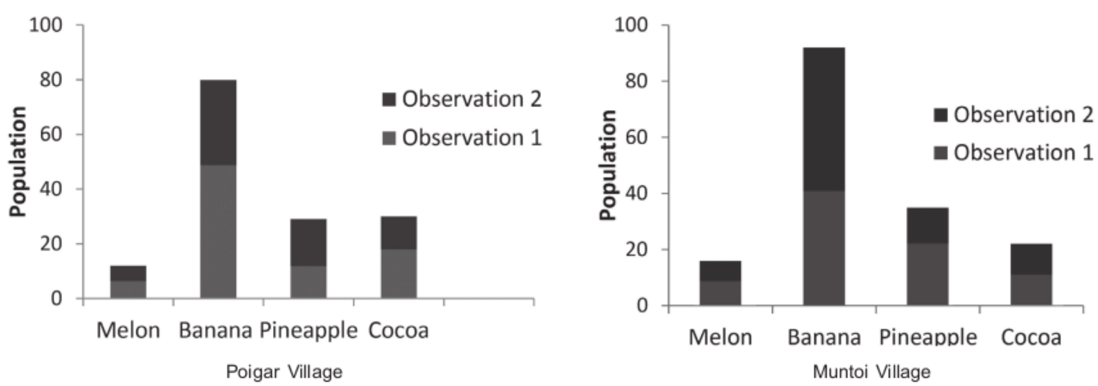

Fig. 1. Population of *Carpophilus* sp. beetle on fruit traps in Poigar and Muntoi villages.

were not found in some banana fruit trap treatments, due to observations on banana traps after 12 days.

Chi-sguare analysis of the relative abundance of beetles on cocoa pods had a significant effect in Poigar Village, $F = 73.724$; $p < 0.000$. Likewise, in Muntoi Village, F = 139,959 $p < 0.000$ (Fig. 2).

The diversity of beetle's species was found in cocoa pods, while in fruit of bananas, pineapples, melons, it was only limited to *Carpophilus* sp. In general, black pods disease are rarely found in beetles, but the abundance of population and types of beetles found in cocoa pods is blackish brown, because the flesh is soft and as food for beetles, while the pods are black, the flesh has hardened and is older. old. Only 0-3 adults were found in black cocoa pods. The dominant population in cocoa pods is the *Ambrosiophilus* sp. Beetle, which is an indication of the type and abundance of the beetle population on blackish brown cocoa pods. Pineapple, melon and banana fruit are not suitable to be used as trap for *Ambrosiophilus* sp. and *Ashverus* sp., because neither population was found. Assa (2014) using banana fruit bait in the clove plant ecosystem was only found by *Carpophilus* sp. in several locations in the province of North Sulawesi.*Carpophilus* sp. underlings of the fungus *Ceratocystis polychroma*, which causes clove leaf fall in North Sulawesi. Ambrosia *Ambrosiophilus* sp. is only found in rotting cocoa pods and no healthy fruit is found. Which has become rotten brownish black to black.

Beetles can be attracted to several types of fruit, possibly due to the volatile compounds released by the fruit, which are the attraction of the beetles. *Ambrosiophilus* sp. and *Ashverus* sp. beetles were only found in cocoa pods. In contrast to the *Carpophilus* sp. beetle found in all fruit traps,

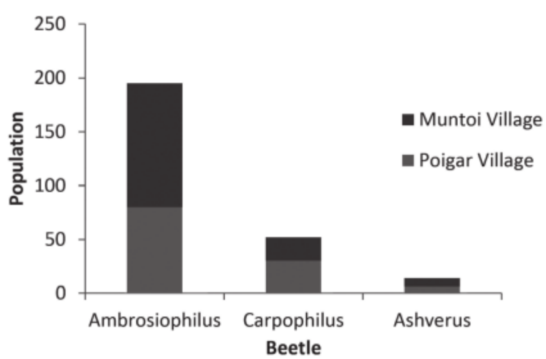

Fig. 2. Beetle population on cocoa traps in Poigar and Muntoi villages.

it is classified as a polyphagus. An increase in the population of *Carpophilus* sp. on bananas because there is a relationship with microbes that are closer to these beetles. Ambrosia *Ambrosiophilus* sp. and *Ashverus* sp. beetles are not attracted to melons, pineapples and bananas. This is probably due to the absence of *P. palmivora* fungus on the fruit. *Ambrosiophilus* sp. and *Ashverus* sp. beetles are attracted to cocoa pods due to the presence of *P. palmivora* fungus and fruit tissue as food. The interaction of ambrosia beetles with their coniferous hosts is mediated in part by the presence of microbes (Adams *et al*., 2013; Six, 2013; Therrien *et al*., 2015). Ophiostomatoid fungi emit various volatile organic compounds under laboratory conditions are fusel alcohol, terpenoids, aromatic compounds, and aliphatic alcohol. The presence of the compound has been shown to obtain a behavioral response to the Ambrosia beetle, functioning as an attractant or repellant (Kandasamy *et al*., 2016).

The results of this study found the *Ashverus* sp. beetle, this is very interesting because previously these beetles have never been reported to be associated with agricultural crops, *Ashverus* sp. is known as a storage pest on grains (Haines, 1991). This beetle can be categorized as a vector of fungi *P. palmivora*. Inoculation of these beetles on healthy cocoa pods can cause symptoms of *P. palmivora* infection *Carpophilus* sp. beetle is also found on cocoa pods with a low population, while the highest population abundance is in bananas fruit, so that bananas can be used for monitoring the population of *Carpophilus* sp. in the cocoa plant ecosystem. There are indications that the pods are rotting or that fermentation has occurred can be used as traps for other ambrosia beetles in agricultural and forest ecosystem.

The main factor in this experiment was to prove adult beetles as carriers of *P. palmivora* which are spread on healthy cocoa pods. The three types of *Carpophilus* sp., *Ashverus* sp. and *Ambrosiophilus* sp. beetles inoculated on healthy cocoa pods have caused symptoms of black pod disease caused by *P. palmivora*. Symptoms of *P. palmivora* on cocoa pods are blackish brown spots. Symptoms of *P. palmivora* resulting from beetle inoculation in healthy cocoa, then grown on PDA media show the morphological form of *P. palmivora* (Figs. 3-4).

The fungus *P. palmivora* is carried by insects. It is possible that the spores have attached to body surfaces such as the thoracic, elytra, abdomen and mouth parts. The three beetles have fine fur located on the body surface allowing *P. palmivora* spores to

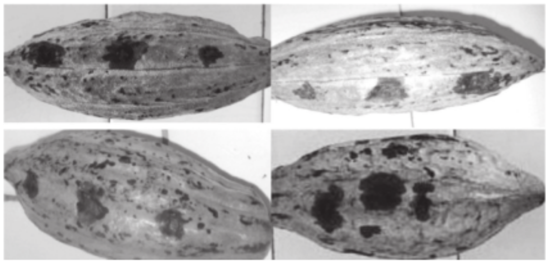

Fig. 3. Black pod disease, *P. palmivora* on cocoa pods after the inoculation of 3-5 beetles.

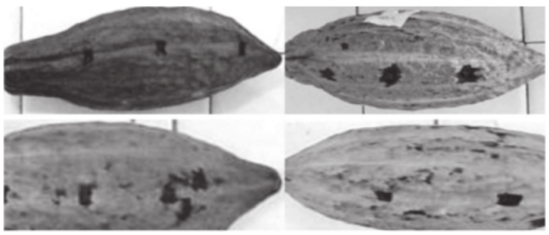

Fig. 4. The inoculation of 2 beetles showing no symptoms of *P. palmivora*.

attach to these hairs. Insects carrying fungi are located in the mouth, abdomen and elytra found in both females and males (Gebhardt *et al*., 2004). Spores can stick and stick to the surface of the insect body to be transmitted to healthy trees (Klepzig and Six, 2004; Harrington, 2005). Assa (2014) transmission of *C. polychroma* fungi to clove plants by *Carpophilus* sp. beetles and only occurs when trees are injured. *C. truncatus* and *C. sayi* in invading trees, if the tree has wounds from various families (Juzwik *et al*., 2004; Price, 2003). Dead wood-dwelling beetles contribute to the spread of fungi, which can affect fungal community assembly and ecosystem processes such as wood rot (Seibold *et al*., 2019).

Based on the population of beetles that were inoculated on healthy cocoa pods, it has affected the symptoms of *P. palmivora*. All adult beetles with a population of 2 individuals per hole inoculated on healthy cocoa pods did not show *P. palmivora* symptoms, although 2 individual beetles were unable to cause *P. palmivora* symptoms, this was not without spores, but perhaps the spore density on the beetle's body surface was relative. low. The mechanism of *P. palmivora* infection on cocoa pods by beetles as a fungal transmission may depend on the propagules available in the beetle's body and the virulence of *P. palmivora* to cause disease. *C. truncatus* and *C. sayi* found 95% of all Nitidulidae beetles attracted to new wounds, a further 78% were contaminated with *Ceratocystis fagacearum* (Juzwik *et al*., 2004).

Inoculation of beetles on healthy cocoa pods, the symptoms of *P. palmivora* disease were found in the beetle population of 3-5 individuals per hole. Symptoms of *P. palmivora* cacao rot disease begin to appear 3 days after inoculation. Symptoms of *P. palmivora* infection on cocoa pods are characterized by the presence of blackish brown spots covered with white mycelium sometimes not visible like white hairs on the pods. The pod rot will turn black rot and harden after approximately 13 days after the beetle is inoculated on healthy cocoa pods. Observations in the field of pod rot disease symptoms in cocoa are in the form of blackish brown spots on the base, middle and tip of cocoa pods. The same microscopic observations are conveyed in various literature that *P. palmivora* has hyphae that are not insulated and hyaline. The sporangium is shaped like a pear, at the end there is a papilla. In addition to the sporangium, there are also spherical chlamydospores with a diameter of approximately 30 μm.

Cocoa pods that have shown symptoms of dark brown spots can be isolated and grown on PDA media, resulting in non-insulated *P. palmivora* hyphae, the shape of the sporangium is like pear fruit with clear papillae and has somewhat transparent hyphae. The sporangium of the fungus *P. palmivora* is shaped like a pear fruit, has clear papillae and pedicels, round zoospores and terminal round chlamydospores (Semangun, 2000). Distribution of *P. palmivora* via *Carpophilus* sp., *Ambrosiophilus* sp. and *Ashverus* sp. The relation with the control of *P. palmivora* should be done in two ways, namely the cocoa pods that have been attacked by *P. palmivora* and the carrying insects of *P. palmivora.*

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