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First report of *Phytophthora* black pod disease of cocoa spread by *Iridomyrmex cordatus* in Sulawesi: A dilemma about predatory insect for cocoa pest control

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Abstract. While implementing biological control agent driven from predatory insect to retain an ecological population balance of main cocoa pest, has become popular in environment, the unforeseen consequence of this technique is due to uncontrolled insect behaviour, a potential spread of a major cocoa disease-*Phytophthora* black pod and stem cancer, caused by a superior oomycete *Phytophthora palmivora* species and this attempt poses a difficult dilemma. This paper will investigate potential risk of insect ant species and explain its behaviour in transmitting cocoa disease to new trees before suggestion way to diminish negative impact and to improve a specific and selective management of cocoa pests and diseases.

1. Introduction

Oomycete *Phytophthora* species is a superior living microbe. With its life cycle as crop and tree pathogens and with a wider host-range in different climates and landscapes the species has become a primary concern in agricultural and forest fields [1]. The species is one of the most global limiting food crops and forest trees. Once the pathogen attacks, its control is demanding, and it can cause significant loss of crop production and kill the tree. The main factor of successful pathogen biology in environment is due to being equipped with evolutionary genetics and reproductive mechanisms [2]. Unique mating type forms as the species generates sexual structure in both male and female at the same time. One of *Phytophthora* species attacks over thousand different hosts [3,4]. Of many important host-crops, cocoa (*Theobromae cocoa* L.) is an important host of *Phytophthora palmivora.* It causes *Phytophthora* black pod disease (PBPD) and stem cancer and the pathogen spreads wherever cocoa is grown [3-8]. The contribution to global cocoa production loss due to the disease itself is undeniable with 450,000 MT annually [5, 6].

In cocoa pests, insect cocoa pod borer, *Conopomorpha cramerella* is a major cocoa pest with a significant economic impact in developing cocoa industry [7]. *C. cramerella* is a tiny moth becomes a significant threat to the Southeast Asia cocoa growers, particularly in Indonesia and Malaysia. The quality of cocoa beans was reduced due to the feeding behavior the neonate larva, which resulted in damaged or clumped beans [8]. Due mainly to significantly reducing cocoa yield, many attempts to control cocoa pest have been recommended. One of actions is by using biological controlled agent

driven from predatory insect i.e. ant species [9, 10]. Study on the ant species for controlling cocoa pests particularly *C. cramerella* was vastly undertaken in Malaysia [8, 11]. Several ant species were observed to have potential and found in cocoa agro-ecosystems are cocoa black Ants (*Dolichoderus thoracicus)*, weaver ant (*Oecophylla smaragdina*), crazy ant (*Anoplolepis* spp) and Saint Valentine ant, (*Crematogaster* spp). Among the species, *D. thoracicus* was proven as a biological control. *D. thoracicus* is ubiquitous in cocoa-coconut ecosystems and may thrive well due to the availability of nesting sites on the coconut crown. Their population can be increased with the augmentation of artificial ant nest stuffed with dry cocoa leaves.

Figure 1. Symptom expression on pod surface. Pod infected by *Phytophthora palmivora* in the branch far from ground (red arrowhead) with necrotic lesion development.

In cocoa orchards, native ant species is not apart from cocoa life. The insects actively foraging and build their nest for colony and territory allowing their services to help the cocoa farmers from insect pests. Using ant colony as biological control agent is inexpensive cost and effective way since the colony can maintain their life for giving protection service in real time to cocoa pods. Its impact is that pesticide use is reduced and as consequence of their foraging behaviour which is actively to visit pods, adult female of insect cocoa pod borer (*C. cramerella*) is difficult to lay eggs unless preyed and therefore, optimizing its role to protect cocoa health from insect pests is important.

However, while a successful story of native ant species in controlling cocoa pests, unforeseen constraint behind uncontrolled foraging activities is unavoidable. It is known that insect association with cocoa diseases was reported. In PNG, a group of beetle was detected to spread PBPD disease [6] but the role of ant species associated with cocoa disease remains unclear. The study will examine native ant species and PBPD association and explain its role of disease transmission before a recommendation is put forward.

2. Methods

Two types of cocoa farms were chosen i.e. implemented good agricultural practices (GAPs) and lack of GAPs with 300-400 trees in each farm. The study was split into several steps; field observation of damage intensity of PBPD and ant behavior, isolation and identification ant species and testing the presence of *P. palmivora*. In the field observation, the focus was native ant species, ant behavior, nests and PBPD pathogen and to achieve this, a proper choice of number of sufficient trees in both farms being occupied with ant colony and at the same time infected pod exist was important. Observation of ant colony behavior including foraging and nest development and infected pods was undertaken for 5 weeks in the cocoa farms in Soppeng, South Sulawesi. Collecting all native ant species and nests found in the field were carried to the Lab before they were examined its species and checked its role of disease association. Testing the presence of PBPD pathogen obtained from all ant species and nests was conducted with attached method into fresh pods (mature pods) and once the lesion developed from the centrum of the body and nest attached, the assumption of disease association was

convincingly proven. Examining number of ant species and nests found in the trees were assessed under light microscope 400 x magnification and reference support.

3. Results and discussion

3.1. Ant species and spore identification and testing the presence of PBPD in ant bodies and nests

The research observation revealed that there were five native ant species colonizing trees in GAPs farm including *Iridomyrmex cordatus* (local named: fire ant) and *Anaplolepis longipes* (local: crazy ant), *Oecophylla smaragdina* (local: weaver ant), *Monomorium* sp. (local: a giant red ant) and *Crematogaster difformis* (local: fire ant) and in the farm with poor managing practice there were only two dominant species to colonize the trees; *I. cordatus* and *A. longipes* [12, 13, 14]**.** The observation of PBPD found that intensity of pod damages in unmanaged cocoa farm was likely to be much higher than in GAP farm. In unmanaged cocoa trees, there were lot of fallen leaves, organic matters covered land surface, high dense tree foliage and very rare sunlight exposure to land surface. The findings suggest that inoculum source of PBPD was sufficiently available. Unlikely, good managed farm seemed to be rare PBPD exist**.**

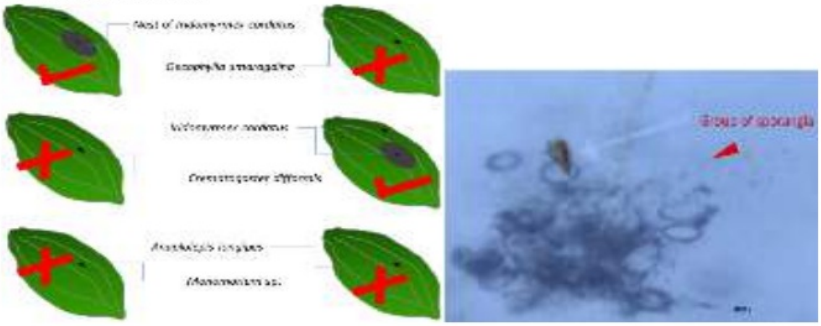

Figure 2. (Left) Testing the presence of *P. palmivora* obtained from different sources of ant species to assure the role of ant species. (Right) A group of sporangia was obtained from tunnel nest of *I. cordatus* and identified under microscope 400 x magnification. (×= absent lesion; ✓ = lesion developed).

Figure 2 depicts that all the bodies and nests collected from the field were attached into fresh pods and several days later the lesion only developed in the pod in the trial of *I. cordatus* and its nest. Observing sporangia under light microscope 400 x magnification obtained from tunnel was present and the sporangium shape is likely to be obturbinate and or ovoid which is very typical spore of *P. palmivora* [2]. No papillate performed since the sporangia were not grown in the culture. The finding suggests that transmission of PBPD to new pods and trees depends on the foraging behaviour of *I. cordatus* in the ground and tunnel nest built from organic matters. Consistently, testing the presence of *P. palmivora* in the fresh pod and detecting sporangia collected from tunnel nest of *I. cordatus* were relevant and therefore we believed that *I. cordatus* and its tunnel nest noticeably contribute to transmit PBPD to new host in nature.

3.2. Association between I. cordatus and PBPD distribution

In general, the association of active roles of ant species and disease transmission was detail described in the table 1.

Table 1. Native ant species associated with PBPD distribution

Species	Niche	Foraging behaviour	Role
Oecophylla smaragdina	Branch	From branch to branch, pods and stem	Nt
Crematogaster difformis	Stem and ground	Nesting from organic matter and cracking stem	Nt but potential risk
Monomorium sp.	Ground	Ground, organic matter, stem, fallen leaves	Nt
Anaplolepis longipes	Ground and organic matter	Ground, organic matter, fallen leaves	Nt
Iridomyrmex cordatus	Stem and ground	Nesting from organic matter, built tunnel to access stems, branches, and pods	t

Nt = No transmitted; t = transmitted

Table 1 shows that of five native ant species found in the different farm, two ant species built the nests from organic matters i.e. *C. difformis* and *I. cordatus*. The niches of ant species vary depending on territory-share. For instance, *O. smaragdina* only occupies branches and rare to hunt food in the ground while the habitat of *Monomorium* sp. is in the ground only. The other three native ant species i.e. *C. difformis*, *A. longipes* and *I. cordatus* foraging actively up and down the tree. Although most of ant species are active to seek food in different places, tunnel nest is important association with disease transmission since it is made from organic matters linking from the main nest to stem and pods. A bunch of organic matters and ground is a home of *Phytophthora palmivora* to complete its life cycle and produce generation. Similar with *I. cordatus*, ant species of *C. difformis* develops the nest from organic matters but the nest is unlikely shaped a tunnel. Therefore, it is assumed that the nest of *C. difformis* may contain the sporangia. The observation also revealed that very rare different ant species occupied in the same tree because a tree was usually dominated by a single species. Overall, the role of foraging was important factor to transmit the disease to new host i.e. disease spread from infected pod to new pod and from infected tree to new tree.

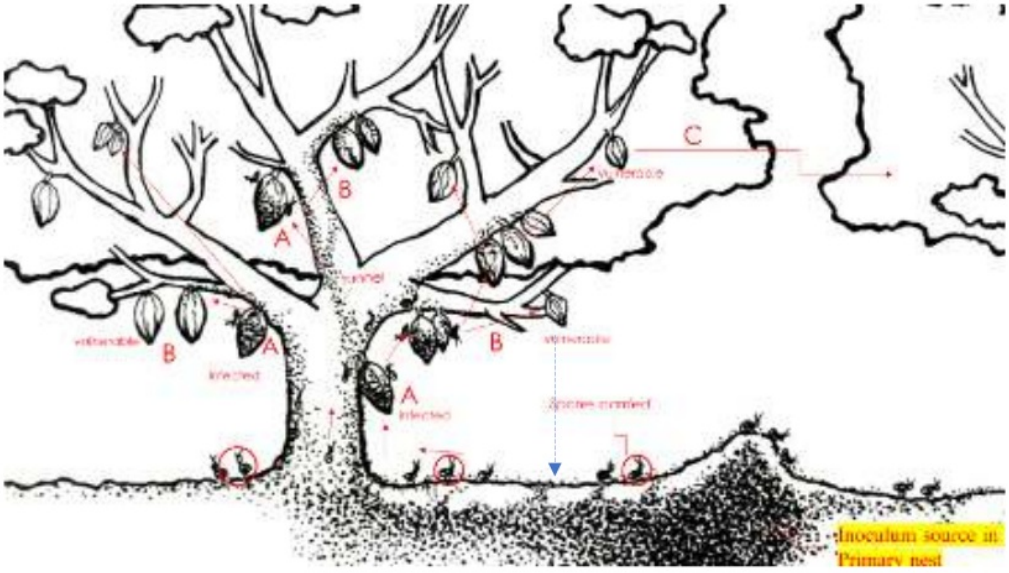

Figure 3. Mode of action of *Phytophthora* black pod disease transmitted by *I. cordatus*

Two scenarios of disease transmission by native ant species in nature are proposed. The first scenario is disease transmission from infected pod to pod. Ant species with foraging activities accidently takes the inoculum source of PBPD from primary nest (red circle and red arrow), carried it through tunnel to reach adjacent pod (A). Ant's tunnel built to link with host is utilized to protect their colony from its enemy interference and physical constraints. From this, initial infection commences to occur. Once the pod is successfully infected, symptomatic lesion develops until necrosis completes to cover pod surface. From this, a new generation is produced through pathogen sporulation stage. Subsequently, new spores developed on pod surface are accidently taken (B) and transmitted to above adjacent pods until infection cycle completes to generate sufficient new sporangia. Or, sporangia drop to the soil (blue arrow). The second scenario is disease transmission from infected tree to tree. A member of ant species successfully takes the sporangia or hyphae from infected pod and sporangia are then carried to new tree (C) for developing new infection and its generation. Infection circle process is the same with first scenario. The density of foliage facilitates an earlier infection to new host. The sporangia are carried because twigs and branches of trees are linked as a better bridge to move forward a new tree. Once this establishes, the ant colony claims to have their territory and infection process does the same in previous tree. *I. cordatus* is a typical ant species which builds their nest from organic matters in the soil surface connecting from main nest to the tree and foliage. The nest is unique shape as a tunnel. Due to making from organic matters and clay which is a better source for *P. palmivora*. The pathogen species enriches its population and subsequently attacks rhizosphere system due to a typical soil borne pathogen.

4. Conclusion and recommendation

Five ant species occupying a managed farm and only two species were in unmanaged farm. *I. cordatus* and its tunnel nest linking from main nest to host, contributed to disease spread in different way. Disease distribution was from pod to pod and from tree to tree. A group of sporangia was discovered in the tunnel nest. In one hand, presence of native ant species has an important role of helping the farmer in controlling insect pests especially main cocoa pest, cocoa pod borer (*Conopomorpha cramerella*) and on the other hand, its role contributes to major cocoa disease. This study highly recommends that the need for a selective ant species giving a service in controlling cocoa pests is

necessarily. *I. cordatus* and its tunnel nest should be eliminated from trees to avoid a high damage intensity of PBPD. Manipulating the role of ant is important to accommodate its benefit for pest control while reducing its negative impact of disease spread. Combination between bio control agent like *Trichoderma* spp. against cocoa pathogens and ant behaviour in reducing inoculum of PBPD is another better solution. For instance, the main nest of ant built in the soil surface is spread out with *Trichoderma* formulation such as powder or pellet and once the ants foraging in the tree, the colony lifts and spreads it to control pathogen of PBPD before the pathogen initiates to infect the pods and the tree.

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