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Increasing the resilience of cacao to major pest and disease threats in the 21st century

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Increasing the resilience of cacao to major pest and disease threats in the 21st century

Proceedings of an Asia-Pacific Regional Cocoa IPM symposium

Jerome Niogret, Vanessa Sanchez and Jean-Philippe Marelli (Editors)

ACIAR

2020

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Cover: Cacao is one of the top 10 commodities traded in the world. There is potential to increase production through improved pest and disease management. Photo: ACIAR, Connor Ashleigh 2019

Foreword

Cocoa is an important agricultural crop for millions of smallholder farmers in the Asia-Pacific region and an important agricultural export for many countries. The ongoing increase in demand and refinement of consumer tastes provide incentives and opportunities for smallholder farmers to increase both quantity and quality of production.

Pests and diseases present a significant threat to the cocoa supply chain and industry, and of course the livelihoods of cocoa growers. Globally, plant health, including the management of pests and diseases, is challenged by climate change, land-use changes, and international trade and travel. Plant protection practices that integrate physical, chemical and biological management approaches are required for the sustainability and profitability of smallholder cocoa producers.

The Australian Centre for International Agricultural Research (ACIAR) was mandated, as set out in the ACIAR Act (1982), to work with partners across the Indo-Pacific region to generate the knowledge and technologies that underpin improvements in agricultural productivity, sustainability and food systems resilience. We do this by funding, brokering and managing research partnerships for the benefit of partner countries and Australia.

ACIAR has invested in cocoa research since 1983, in Indonesia, Papua New Guinea, Fiji, Samoa, Solomon Islands and Vanuatu. Mars Wrigley has been a significant research partner and co-investor over this period, and has helped to scale out innovations from research projects.

The motivation to host a symposium on increasing the resilience of cacao to pest and disease threats was the opportunity to share and capture knowledge from diverse experts from Asia-Pacific cocoa-producing countries. Participants in the symposium included pathologists, entomologists and cocoa breeders from the Asia-Pacific region, as well as international integrated pest management (IPM) experts. Scientists examined IPM in the region and the development of practical approaches through blending regional knowledge with local expertise.

These proceedings capture the research papers and abstracts of work presented at the symposium, and summarise the latest in cocoa pest and disease management research across the Asia-Pacific region. ACIAR will continue to invest in cocoa and plant health research in partner countries to ensure that smallholder farmers have a suite of tools for plant protection to buttress food security and improve farmer and rural livelihoods.

Andrew Campbell
Chief Executive Officer, ACIAR

Contents

Units

Unit	Definition
cm	centimetre
g	gram
ha	hectare
kg	kilogram
µm	microgram
mL	millilitre
mm^2	square millimetre
ppm	parts per million
rpm	revolutions per minute
t	tonne

Welcome message

Dear participants to the Mars IPM Asia Pacific Symposium!

It is with great excitement that I welcome you to this event, bringing together cacao scientists from the Asia-Pacific region, as well as international scientists working on cacao and other crops.

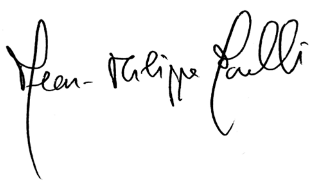
As Integrated Pest Management Director at Mars Wrigley Confectionery, it is my job to understand and mitigate risks to the cacao crop and its supply chain. It is astounding that up to 38% of the cacao crop is lost to fungal, viral and pest problems each year, and yet cacao is still massively understudied.

Although it is one of the top 10 commodities traded, cacao has not received the same scientific attention paid to other crops such as rice, corn and wheat. Over the past few decades, yields for other crops have dramatically improved, but cacao yield has plateaued. In theory, cacao farming can produce more than 4 tonnes of crop per hectare. However, real yields are only one-fifth to one-half of this, with the average current yields at a low of 400 kg per hectare.

This event, whose inspiration is to 'increase the resilience of cacao to major pest and disease threats in the 21st century', aims at breaking this cycle of decline, in the context of the additional challenges we face, including climate change, competition with other crops and scarcity of labour.

I invite everyone to come to the sessions with a lot of energy and motivation for changing the status quo.

I also would like to thank the contributions of the Australian Centre for International Agricultural Research and all the people within Mars who have made this event possible.



Jean-Philippe Marelli

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Integrated Pest Management
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Session 1

Cocoa pod borer ecology and behaviour

1 The influence of plant anatomy (trichome and secretory duct) on selection behaviour of female cocoa pod borer, *Conopomorpha cramerella*, for oviposition

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Abstract

Cocoa pod trichome and secretory ducts were thought to play a defensive role against cocoa pod borer (CPB) by influencing the selection behaviour of female CPB for oviposition. The objectives of this study were to investigate the density of cocoa pod trichome and secretory ducts at various pod developmental stages in relation to oviposition behaviour. The result showed that trichome density varied significantly among clones. A clone with a rough pod surface always had a higher density of trichomes than a clone with a smooth pod surface. Clones with a high density of trichomes received more CPB eggs than those with a lower density of trichomes in an enclosed system under natural field conditions. This may indicate that the presence of cocoa pod trichomes on the pod surface did not provide a significant barrier to CPB oviposition because CPB moths and eggs are larger than the trichomes. In another study, an image of each pod wall sample was examined with an AVerMedia EZ Capture light microscope and then analysed using ImageJ software. Results showed that the distribution of secretory ducts was dense in the immature pods and began to decrease as pods developed. Visual observation indicates the presence of a sticky substance secreted from secretory ducts. A high density of secretory ducts in the epicarp layer of immature pods could affect the path of entry of the first larval instars because the tunnels bored by the first instar larvae are slightly smaller than the ducts. However, it is not known whether the first larval instars will die when feeding through the ducts. In conclusion, both studies suggested that cocoa pod trichomes and secretory ducts have some influence on selection behaviour of female CPB for oviposition. However, the entire sequence of the host selection behaviour involves a combination of visual, mechanical and chemical processes. CPB is a nocturnal insect. Selecting favourable pods for oviposition requires skill because pod age is crucial for the successful development of CPB offspring.

Keywords: cocoa pod, cocoa pod borer, epicarp, secretory duct, cocoa pod trichome

Introduction

Many insects use shape, colour, plant volatiles or a combination of these characteristics as guiding cues to locate suitable host plants for oviposition. The selection process usually involves a series of sequential behaviours, host finding and host recognition. The lack of a significant effect of the external characteristics of the cocoa pod on the ovipositional behaviour of cocoa pod borer (CPB), *Conopomorpha cramerella*, suggests that orientation and navigation do not solely depend on visual cues, particularly for insects that are active during the night such as CPB. Most likely, a combination of sensory cues (visual, mechanical and chemical) is used, which involves the entire sequence of the host selection behaviour (Renwick 1989). Although many reports of CPB attack under field conditions have been published, the mechanism involved in host finding and host recognition for oviposition is unclear. It is not known whether the presence of cocoa pod trichomes and secretory ducts influences the selection behaviour of female CPB for oviposition or limits the ability of the first larval instars to penetrate the mesocarp layer. Therefore, the objectives of the study are to investigate the density of cocoa pod trichome and secretory ducts at various pod developmental stages.

Materials and methods

Cocoa pod trichomes

Six cocoa clones with different hardness in the sclerotic layer were selected for investigation. Clones EET 353, ICS 1 and SIC 1 have a hard sclerotic layer, whereas clones EET 308, CC 10 and ICS 16 have a soft sclerotic layer. Samples of pod walls were extracted from the middle portion of the pod, because this is where CPB moths prefer to lay their eggs (Azhar 1990; Lim et al. 1982). One sample was taken from each primary furrow, making a total sample of five for each pod. The pod walls, approximately 2 cm long by 2 cm wide, were cut down to the endocarp layer and were examined with an AVerMedia EZ Capture light microscope fitted with 'icamscope' (Sometech Inc., Seoul, Korea) linked to a computer at a magnification of 300×. The numbers of trichomes per image (equivalent to 1.04 mm^2 per image) were recorded and compared between clones.

Secretory ducts

The pod walls of the six selected clones were extracted from cocoa pods for histological analysis. The pod wall samples were fixed into several series of alcohol and Histo-Clear before being embedded in paraffin. The embedded tissues were cut into 12 μm sections using a microtome, and then placed onto glass slides for staining with safranin O and counterstaining with alcian blue. The slides were examined with a Zeiss-Axioskop Plan 2 light microscope (Zeiss, Oberkochen, Germany) fitted with an 'AxioCam' camera linked to a computer. Images of secretory ducts in the epicarp layer were examined at a magnification of 50× at a standard size of 1.34 mm^2. The numbers of secretory ducts found in the epicarp layer of various pod ages and clones were counted. This was followed by measuring the size of each secretory duct using the same ImageJ software program. The mean size of secretory ducts was calculated by dividing the total area of secretory ducts by the number of ducts for each clone at four pod ages.

Results and discussion

Cocoa pod trichomes

Considerable variation was observed in pod trichome density among the six selected cocoa clones. The density of pod trichomes varied not only among clones but also among clonal groups (Figure 1.1). A lower trichome density was observed in the clones with a hard pod than in the soft pod group of clones. Such variations were also closely associated with the texture of the pod surface. Clones with a smooth pod surface had a lower density of cocoa pod trichomes, as shown in clone SIC 1. These clones normally suffer less damage from CPB attack than clones with a rough pod surface, but clones with a smooth pod surface could incur severe losses during low pod production. Visual observation also suggested that the presence of trichomes on the pod surface did not provide a physical barrier to CPB oviposition because the trichome is smaller than the CPB egg.

The study postulates that cocoa pod trichomes secrete volatile chemicals, which stimulate CPB females to lay eggs on the pod. The moth is nocturnal, with mating and egg laying occurring at night (Lim et al. 1982). In the dark, female CPB are more likely to use volatile chemicals as oviposition cues for finding favourable pods; the external characteristics of the pod (e.g. pod shape and colour) are less important for ovipositional preference (Azhar & Long 1993). The result is consistent with reports by Renwick and Chew (1994), Fatzinger and Merkel (1985) and Hughes, Gailey and Knapp (2003), who stated that females lay more eggs in response to volatile compounds. A rough pod surface clone always had a higher density of trichomes, and received more CPB eggs, than a clone with a lower density of trichomes.

Secretory ducts

During the early stages of pod development, secretory ducts appear to be dense and scattered in a random pattern. As the pod matures, the number of secretory ducts progressively decreases, and they are further apart (Figure 1.2). The number of secretory ducts is negatively correlated with the mean area

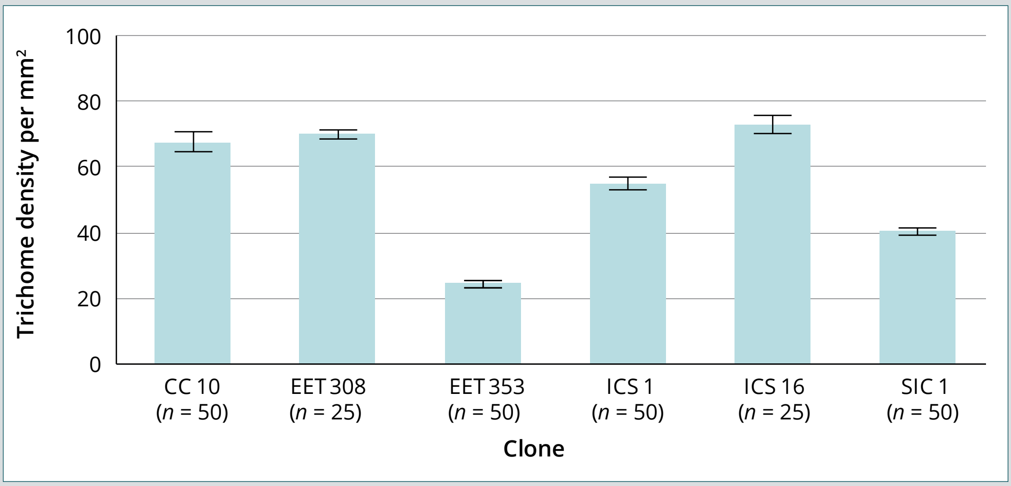

Figure 1.1 Mean density of cocoa pod trichomes on a 4-month-old cocoa pod of the six selected clones

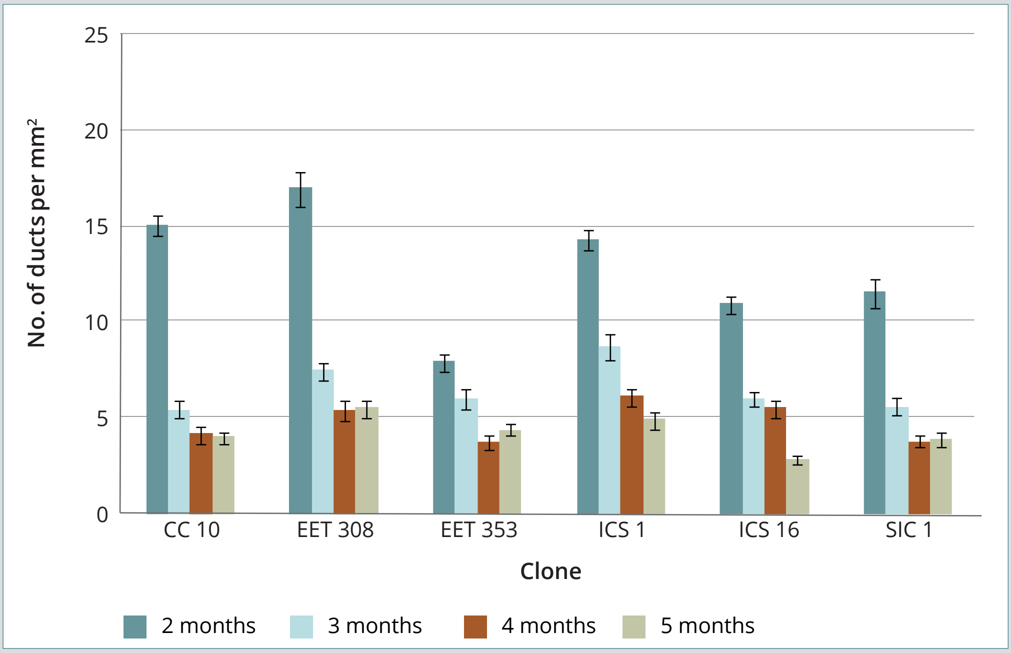

Figure 1.2 Number of secretory ducts per mm^2 at four different pod ages

of secretory ducts. The role of secretory ducts in the development of the cocoa pod is not known; however, they contain polysaccharides such as D-galacturonic acid, D-galactose, L-rhamnose, L-arabinose, D-mannose and D-xylose (Blakemore, Dewar & Hodge 1996).

However, it is not clear how the development of secretory ducts and their distribution in the pod walls directly influence the ovipositional behaviour of CPB. Immature pods generally have a higher number of secretory ducts. Therefore, if CPB females deposit eggs on immature pods, the newly hatched larvae may drown or become immobilised in polysaccharides while boring inside the pod. As a result, females may stay away from immature pods for oviposition unless they do not have a choice. This may explain why female moths prefer laying eggs on mature pods rather than immature pods.

In conclusion, variations in trichome and secretory duct densities have been noted among cocoa clones. Clones with a rough pod surface tend to have a higher density of cocoa pod trichomes than clones with a smooth pod surface, and receive a higher number of CPB eggs under a controlled environment. Volatile chemicals might be released from the cocoa pod trichomes and stimulate female CPB to select suitable pods for egg laying. The presence of a secretory duct in the presclerotic layer may affect the path of entry of the first larval instars because the tunnel is slightly smaller than the ducts. However, it is not known whether the first larval instars will succumb to death when feeding through the ducts, and this warrants further study. The pods respond to injury from CPB attack by development of secondary cell walls or lignification of cell walls surrounding the wounded cells to prevent further damage.

References

Azhar, I 1990, 'Studies on sclerotic layer hardness of cocoa pods', *MARDI Research Journal*, vol. 18, pp. 63–9.

—— & Long, GE 1993, 'Role of pod shape, colour and spatial distribution on egg distribution and egg parasitism of the cocoa pod borer, *Conopomorpha cramerella* (Snellen) (Lepidoptera: Gracilariidae)', *MARDI Research Journal*, vol. 21, pp. 59–70.

Blakemore, WR, Dewar, ET & Hodge, RA 1966, 'Polysaccharides of the cocoa pod husk', *Journal of Science and Food Agriculture*, vol. 17, pp. 558–600.

Fatzinger, CW & Merkel, EP 1985, 'Oviposition and feeding preferences of the southern pine coneworm (Lepidoptera: Pyralidae) for different host-plant materials and observations on monoterpenes as an oviposition stimulant', *Journal of Chemical Ecology*, vol. 11, pp. 943–52.

Hughes, WOH, Gailey, D & Knapp, JJ 2003, 'Host location by adult and larval codling moth and the potential for its disruption by application of kairomones', *Entomologia Experimentalis et Applicata*, vol. 106, pp. 147–53.

Lim, GT, Tay, EB, Pang, TC & Pan, KY 1982, 'The biology of cocoa pod borer *Acrocercops cramerella* Snellen and its control in Sabah, Malaysia', in KL Heong, BS Lee, TM Lim, CH Teoh & I Yusof (eds), *Proceeding of International Conference of Plant Protection in Tropics*, Malaysian Plant Protection Society, Kuala Lumpur, pp. 275–87.

Renwick, JAA 1989, 'Chemical ecology of oviposition in phytophagous insect', *Experientia*, vol. 45, pp. 223–38.

—— & Chew, FS 1994, 'Oviposition behaviour in Lepidoptera', *Annual Review of Entomology*, vol. 39, pp. 377–400.

2 Biological activity evaluation of the sex pheromone of cocoa pod borer, *Conopomorpha cramerella* Snellen

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Abstract

Cacao is a promising high-value crop in the Philippines and considered by the government as a priority crop. Before the Philippines can become a major producer of cacao, production constraints such as pest management need to be addressed. One of the major insect pests of cacao in the Philippines is cocoa pod borer (CPB), *Conopomorpha cramerella*, which can cause up to 50% annual loss, or even higher, if not properly managed. Management of CPB relies heavily on chemical control, but this is very expensive and not safe for humans or the environment. The use of sex pheromone for monitoring and controlling CPB is gaining attention as a major component of integrated pest management (IPM) for cacao. A new blend of the synthetic sex pheromone of CPB has shown potential in monitoring and mass trapping activities. Field bioassay of a CPB sex pheromone trap showed efficient trap catches when the trap was installed 1.0 m above the canopy. Determining the correct height of CPB traps is valuable for maximising their efficiency for inclusion in an IPM program for CPB.

Keywords: cocoa pod borer, sex pheromone, integrated pest management

Introduction

Amid a global shortfall in cacao supply, the Philippines is poised to contribute to the estimated 1 million tonne global deficit in 2020 (Cerda 2013). Acknowledging this opportunity, the Cocoa Foundation of the Philippines Inc. (CocoaPhil), in coordination with government agencies and other private sector organisations, crafted the Philippine

Cacao Road Map, which envisages cultivation of 50 million cacao trees on at least 100,000 hectares of land to generate an annual production of 100,000 tonnes of cocoa beans by 2020 (CocoaPhil 2014).

Although the Philippines has good agroclimatic conditions for cacao growing and vast available areas for intercropping with coconuts, the cacao industry suffers inadequate research, development and extension services, and, most importantly, a high prevalence of pests and diseases that affect the quality and yield of cacao beans on smallholder farms (COCAFM 2011). Unfortunately, most smallholder farmers do not have access to the capital needed to purchase the materials and equipment used in modern commercial production.

This study evaluated the potential of semiochemicals as a biological system to monitor and manage the population of cocoa pod borer (CPB) (*Conopomorpha cramerella* Snellen). The main objective was to evaluate the biological activity of the purified sex pheromone of CPB in the field with the participation of cacao farmers.

Materials and methods

The preliminary field bioassay was conducted on one of the cacao farms in Talandang, Tugbok, Davao City. The farm was selected because of the pest pressure from CPB and an absence of pesticide application. This study was conducted to evaluate the effect of pheromone trap height on insect catches. Determination of the correct height of CPB traps is valuable for maximising the efficiency of the traps for inclusion in the integrated pest management program for CPB in the Philippines. The trap used in the experiment was the delta trap with sticky liners. CPB lures containing 100 μg loading of pheromone blends in polyethylene vials were installed in each delta trap, except in the control treatment trap. The lures were obtained from Dr Aijun Zhang (Agricultural Research Service, United States Department of Agriculture, Maryland). Four treatments were used:

- treatment A—trap installed 1.0 m above the canopy
- treatment B—trap installed 0.5 m above the canopy
- treatment C—trap installed 0.0 m above the canopy (along the canopy)
- treatment D—trap installed 0.5 m below the canopy.

A control trap was installed 1.0 m above the canopy but had no CPB lure.

The experiment used a randomised complete block design with three replications. The sticky liners were collected and replaced every month for 12 months. The numbers of CPB trapped in the collected sticky liners were counted. Delta traps were rotated every 3 months to avoid bias in the position of the traps. Average counts of CPB trap catches per month were obtained and compared among treatments.

Results and discussion

Average recovered CPB captures per treatment per month are shown in Figure 2.1. The highest recovered CPB capture was recorded in the trap installed 0.5 m above the canopy during the October 2018 sampling period. Average trap catches for the different treatments were recorded in the following order (highest to lowest): 0.5 m above the canopy, 1.0 m above the canopy, 0.5 m below the canopy, and 0.0 m above the canopy (along the canopy). No CPB were recovered in the control treatment except in February, August and September 2018, when only one individual was found in one of the traps. This suggests that the CPB female sex pheromone was effective in attracting male CPB. The relatively high count of CPB in traps 1.0 m and 0.5 m above the canopy suggests that mating activity of CPB occurs in an open area. Raising the traps at least 1.0 m above the canopy was based on the method of Vanhove et. al (2015).

Table 2.1 shows the overall average CPB trap catches for the different treatments. The highest count was recorded in the trap raised 0.5 m above the canopy, followed by 1.0 m above the canopy. Our results suggest that 0.5 m above the canopy could be the most appropriate placement of the trap. A follow-up study is underway to verify this result and to determine the optimum number of traps for 1 hectare of cacao farm. We will also evaluate the efficiency of the lure with regard to the length of time of exposure in the field. Vanhove et al. (2015) reported that the CPB lure with 100 µg can remain effective for 6–8 weeks and recommended replacing the lure after 2 months.

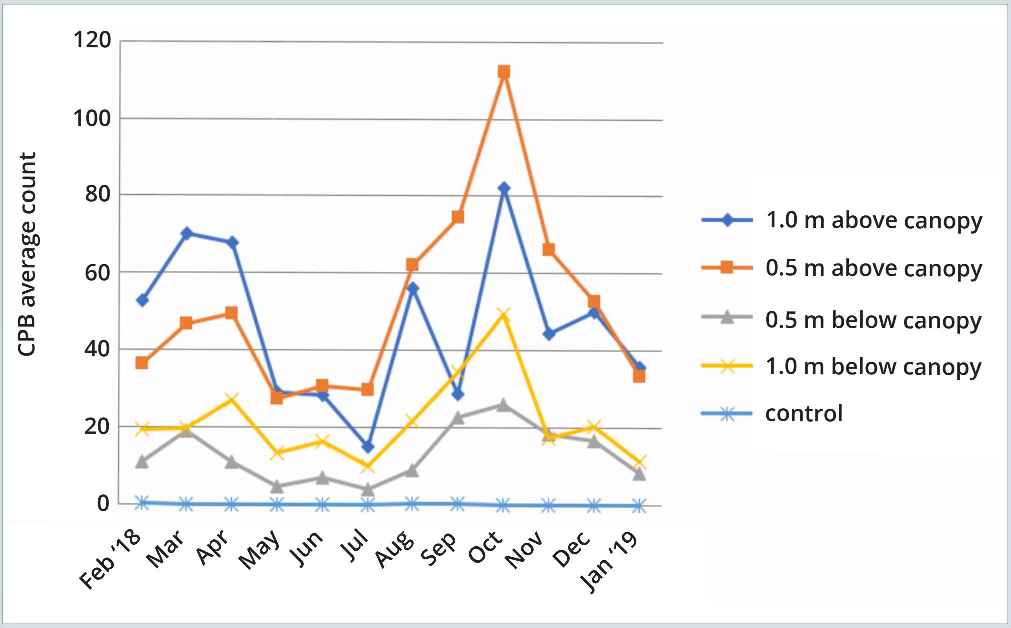

Figure 2.1 Cocoa pod borer average count per month for each treatment

Table 2.1 Overall average CPB trap catches for different treatments

Treatment	Average monthly CPB count (± standard error)
1.0 m above canopy	46.61 ± 14.44
0.5 m above canopy	51.72 ± 18.12
0.0 m above canopy (along canopy)	13.14 ± 6.16
0.5 m below canopy	21.67 ± 7.61
Control: 1.0 m above canopy; no lure	0.12 ± 0.08

CPB = cocoa pod borer

References

Cerda, J 2013, 'Philippines urged to increase cacao production', *Philippine Star*, 24 August.

COCAFM (Congressional Oversight Committee on Agriculture and Fisheries Modernization) 2011, *Republic Act 10068 (Organic Agriculture Act of 2010) and its implementing rules and regulations (IRR)*, Agriculture and Fisheries Information Service, Philippines Department of Agriculture, http://ati.da.gov.ph/rtc8/sites/default/files/RA10068_IRR.pdf.

CocoaPhil (Cocoa Foundation of the Philippines) 2014, Foundation website, viewed 8 December 2014, https://www.devex.com/organizations/cocoa-foundation-of-the-philippines-cocoaphil-51839.

Vanhove, W, Vanhoudt, N, Bhanu, KRM, Abubeker, S, Feng, Y, Yu, M, Van Damme, P & Zhang, A 2015, 'Geometric isomers of sex pheromone components do not affect attractancy of *Conopomorpha cramerella* in cocoa plantations', *Journal of Applied Entomology*, vol. 139, pp. 660–8.

Abstracts

Cocoa pod borer, *Conopomorpha cramerella* Snellen, bioecology and control in Indonesia

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Cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae) was first recorded in Indonesia in North Sulawesi in the 1860s. Before 1991, the occurrence of CPB in Indonesia was limited to East Kalimantan (Pulau Sebatik), North Sulawesi and North Moluccas. Since CPB was found in Central Sulawesi in 1991, infestation has spread rapidly. Now, CPB has infested all areas where cocoa is planted. CPB is also a serious pest of cocoa in Malaysia, the Philippines and Papua New Guinea. *C. cramerella* causes losses to cocoa by boring into the placental tissues and the wall of the pod, disrupting the development of the beans. This results in pods that may ripen prematurely, with small, flat beans, which often stick together in a mass of dried mucilage. Yield loss due to CPB is worth more than US$300 million per year. Controlling CPB using integrated pest management involves using resistant/ high-yield planting materials, using cultural practices (pruning, frequent harvesting, fertilisation and sanitation), preventing CPB attack through pod sleeving and spraying of biocoater, chopping and composting cocoa husk, and using sex pheromone. Biological control has been recommended in cocoa areas with light infestation; this includes use of entomopathogenic fungi (*Paecilomyces fumosoroseus*, *Beauveria bassiana*) and black ants (*Dolichoderus thoracicus*), and application of botanical insecticides. Rational use of chemical insecticides is still needed, especially when infestation levels reach 40%, causing heavy damage.

Ecology and behaviour of cocoa pod borer: updates and perspectives

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Despite the economic importance of cocoa pod borer (CPB), its basic ecology is poorly understood, indirectly resulting in poor efficiency of current pest management methods. Our study confirmed and updated knowledge of the natural distribution of CPB within trees in managed cocoa orchards and movement of adults after they were disturbed. Females demonstrated a greater capacity for movement after disturbance and differed from males in the position of their resting sites within a tree. Bioassays results show preferences for specific cocoa clones over others. Our observations provide new ecological knowledge and new perspectives on potential integrated pest management methods for CPB populations.

Phytochemical approach, using plant extracts, to control cocoa pod borer

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Plant extracts have the potential to be developed as a pest control material because of their various secondary metabolites. Some secondary metabolites function as repellents, whereas others attract insects. Chlorogenic acid is an example of a secondary metabolite attractive to insects. This compound, belonging to the phenol group, is present in almost all plants, including cacao. It is suspected that chlorogenic acid in cacao fruit is attractive to cocoa pod borer (CPB) because this compound is an oviposition stimulant. Extracting chlorogenic acid from cacao pod is considered ineffective because the beans are harvested by farmers, but analogues of this compound can also be found in other plants. In this study, chorogenic acid was extracted from coffee leaves, carrot leaves and purple sweetpotatoes using 70% methanol, and these extracts were tested on CPB in bioassays. Twenty CPB adults (10 males and 10 females) were inserted into a testing cabinet set consisting of several boxes; each box contained a specific concentration of plant extracts. The numbers of CPB adults visiting each treatment were recorded for 24 hours. Our results suggested that the extracts were attractive at different concentrations depending on the plant origin. Coffee leaf extract was attractive at 5% concentration, whereas both carrot leaf and purple sweetpotato extracts were attractive at 7%.

Basic research on cocoa pod borer in Papua New Guinea to permit effective pest management

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Cocoa pod borer (CPB), *Conopomorpha cramerella* (Lepidoptera: Gracillariidae), is widely distributed and indigenous to South-East Asia and the western Pacific. It is a pest of cocoa and other tropical fruits, including rambutan. In Papua New Guinea (PNG), CPB was not considered a pest until 2006, following an outbreak in East New Britain Province. CPB is now one of the main concerns for PNG cocoa farmers, who are mainly smallholder farmers, and some plantations. The Cocoa Board of PNG lists mitigation of this pest as a priority area for the cocoa industry. In PNG, cocoa production represents 17% of agricultural revenue, or approximately US$95–114 million per year. PNG produces up to 9% of the world's fine-flavoured cocoa.

A range of management techniques have been developed to combat this pest moth. However, the biology, behaviour and ecology of the pest are still not well understood. Here we demonstrate progress in addressing several gaps in knowledge about this pest, including resolving whether CPB is part of a species complex or if there are several biotypes/races. We also used a novel technique to develop an artificial diet that will enable the insect to be cultured, offering the ability to study this pest in depth, as well as the potential for development of the sterile insect technique to manage the pest.

Ultimately, this project will produce evidence-based results, including a diagnostic for CPB and the development of an artificial diet that will underpin future studies of this pest.

Potential of *Calotropis gigantea* formulation as an alternative pesticide to control cocoa pod borer and *Helopeltis*

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Calotropis gigantea L. is a weed plant commonly known as giant milkweed. It has been reported as an insecticide that is effective in controlling pests because it contains secondary metabolites that can be toxic, deter feeding and inhibit growth, as well as having repellent, ovicidal, larvicidal and sterility properties towards pests. *C. gigantea* extract formulation is an option as an environmentally friendly agent to control insect pests.

The current research aimed to investigate the liquid formulation of *C. gigantea* leaf against cocoa pod borer (CPB) and *Helopeltis* in a cacao field. The formulation was applied every 10 days in five sample trees. Five fruits on each sample tree were observed. The study showed that the liquid formulation of *C. gigantea* reduced the incidence of CPB and *Helopeltis* compared with the control.

IPM approach and cocoa ecosystem re-engineering for cocoa pod borer control

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Cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae), is an insect pest of cocoa that has affected Malaysia's cocoa industry. An integrated pest management (IPM) approach to managing CPB has existed for a long time in Malaysia. The Malaysian Cocoa Board has conducted an IPM project and found it to be viable for managing CPB. Trials for CPB management consisted of three treatments—control, pesticide spraying every 2 weeks, and an IPM plot (pod sleeving and pesticide spraying when necessary)—on four blocks in Sabah. The study showed that the cacao pod should be treated when the pod diameter is 40–60 mm or the pod length is 80–120 mm. The best yield was obtained from the IPM plot (pod sleeving and pesticide spraying when necessary). In a large area of cocoa farm, CPB control through IPM could be enhanced further by ecosystem re-engineering. Cocoa farms are normally simple ecosystems that are dominated by a single crop species. Ecosystem re-engineering in cocoa farms could be done by revising the current planting pattern to allow another species of plant to be introduced, thereby creating a more complex ecosystem. Beneficial plants that produce flowers could be planted in cocoa farms. The planted area should not be treated with any chemical pesticide. This area should be able to provide a food source, breeding site and shelter for the natural enemies of CPB. Therefore, ecosystem re-engineering in cocoa farms should enhance the success of IPM by encouraging the build-up of natural enemies and further reducing use of insecticide to manage CPB.

Session 2

Updates on *Helopeltis* and *Apogonia* beetle research

3 Damage by *Helopeltis theivora* to cocoa trees, cash crops and fruit trees in Vietnam, and its management

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Abstract

Mirids (*Helopeltis theivora*) are serious pests on cocoa trees. They are currently spreading and causing epidemics in several valuable cash crops and fruit trees in Vietnam, such as pepper, coffee and citrus. This is the main factor affecting the productivity, yield and quality of cocoa pods, and limiting the development of cocoa trees.

Recommendati from ons research on prevention of mirids on cocoa trees in major growing areas of Vietnam include:

- planting high-yielding cocoa varieties recognised by the Ministry of Agriculture and Rural Development, such as TD3, TD6, TD7, TD8, TD9, TD10, TC21, TD28 and TD31
- practising appropriate canopy pruning, and maintaining appropriate shade trees and windbreak trees for cocoa trees at the beginning of the rainy season
- applying sufficient and balanced organic, inorganic and micro-organic fertiliser
- regularly monitoring cocoa trees, especially at the stage of young shoots and young fruits; buds and fruits seriously affected by mirids should be frequently cut off, collected and disposed of
- breeding and maintaining one of two types of black ants (*Dolichoderus thoracicus* Smith) or weaver ants (*Oecophylla smaragdina* Fabricius) to better control mirids
- applying insecticides when the rate of fruit affected by mirids >5%; this involves two sprays at an interval of 10–12 days with alpha-cypermethrin, lambda-cyhalothrin + thiamethoxam, or thiamethoxam.

These recommendations have been transferred to farmers via training, workshops, leaflets, books, and so on, contributing to the development of sustainable cocoa production in Vietnam.

Keywords: *Helopeltis*, mirid, crop damage, integrated crop management

Introduction

Mirids are pests that cause harm in different parts of cocoa trees, such as young shoots, leaves, pods and young twigs. They affect the productivity, yield and quality of cocoa pods, and limit the development of cocoa trees.

Mirids are also spreading and causing epidemics in several valuable cash crops and fruit trees in Vietnam, such as pepper, coffee and citrus.

Helopeltis theivora (*H. theobromae*) is one of six species of mirids that are harmful in cocoa-growing areas in South-East Asia. Other species are *H. antonii*, *H. bakeri*, *H. clavifer*, *H. sulawesi* and *H. sumatranus* (Bateman 2007).

The Western Highlands Agriculture and Forestry Science Institute began studying mirids on cocoa trees in 2011. Results of research on prevention of mirids on cocoa trees have been published and transferred to production. However, in practice in some regions, mirids still cause serious harm, because management measures have not been applied properly.

Materials and methods

Evaluating damage by mirids

For cocoa trees, damage caused by mirids in the Central Highlands and Southeast regions was investigated using a survey form, combined with field survey and assessment.

For cash crops and fruit trees, damage caused by mirids was investigated using primary data from units under the Ministry of Agriculture and Rural Development.

Integrated mirid management on cocoa trees

Selecting varieties

We assessed the status of mirid bugs on cocoa varieties recognised by the Ministry of Agriculture and Rural Development, including TD3, TD6, TD7, TD8, TD9, TD10, TC21, TD28 and TD31, in the main growing areas of Vietnam.

Pruning

The pruning experiments ran for 4 years (2011–14) on business cocoa gardens 7–10 years old at three locations: Dak Lak, Binh Phuoc and Ben Tre. The areas were 0.5 ha of monoculture and 1.0 ha of intercropping. A randomised complete block design was used, with four treatments: no pruning (control), pruning of branches to allow 20% of light penetration, pruning of branches to allow 40% of light penetration, and pruning of branches to allow 60% of light penetration. There were three replicate plots, each with 30 plants. Monitoring indicators were fruiting rate, pod drop rate, yield, and damage level by mirids.

Sanitation

The fields were regularly checked and cleaned to limit damage by mirids.

Pod wrapping

When the pods were 7–8 cm long (at 60–70 days old), they were sprayed with a mirid repellent before being wrapped in white plastic bags (30 cm × 14 cm, with two open ends) for 10–15 days.

Biological control

Using biological control products

Biological control products were used in 2012–13 at Dak Lak, Binh Phuoc and Ben Tre. The experimental design was a randomised complete block design with six treatments, three replicates and 20 plants per plot. The experimental treatments were *Bacillus thuringiensis* (1.0%), *Beauveria bassiana* (1.0%), *Metarhizium anisopliae* (1.0%), *Paecilomyces* (1.0%), and *Beauveria* + *Metarhizium* + *Entomophthorales* (1.0%)

Observations were made of the rate and index scale of mirids on cocoa pods (percentage) and yield (tonnes of dried nuts per hectare).

*Control using black ants (*Dolichoderus thoracicus*)*

Control using black ants was conducted at village 10, Hoa Thang commune, Buon Ma Thuot city, Dak Lak province in 2012–14. The experiment was large scale, with no replication, involving two treatments (0.5 ha/treatment). The treatments were predation by black ants, and no predation by black ants (control).

Chemical control

The experimental time, layout, treatment methods and monitoring indicators for chemical control experiments were similar to those used for biological control experiments. Treatments were Fidur 220 EC (0.3%) (chlorpyrifos ethyl + imidacloprid), Actara 25 WG (0.025%) (thiamethoxam), Angun 5 WG (0.35%) (emamectin benzoate), Pertox 5 EC (0.3%) (alpha-cypermethrin), Movento 150 OD (0.125%) (spirotetramat), and control (no pesticide).

Results and discussion

Damage by mirids

Cocoa trees

Mirids are common in cocoa trees in Vietnam. Mirids harm cocoa parts such as young shoots, young leaves, young twigs and pods. They cause serious damage to pods. First symptoms are grey lesions, which then darken. In the Southeast and Central Highlands regions, the area affected by mirids was large (35.2–42.9%), compared with the Southwest region (10.7%) (Table 3.1). Mirids are multi-food insects, attacking other crops including cash crops (tea, cashew, coffee, pepper) and fruit trees (green skin pomelo, custard apple, avocado).

Cashew trees

The area affected by mirids by the end of April 2017 was 57,795 ha, reducing productivity by 15–20%. Severely affected provinces that grow cashew were Lam Dong, Binh Phuoc, Dong Nai, Binh Thuan and Dak Lak. In 2018, 20,974 ha was affected, with a rate of harm of 5–46%.

Arabica coffee trees

The area affected was 4,710 ha (an increase of 2,615 ha compared with 2017), concentrated in Lam Dong (Plant Protection Department 2018). The rate of damage was 25–75%.

Table 3.1 Proportion of cocoa-growing areas of Vietnam affected by *Helopeltis theivora*

Region	Light (%)	Medium (%)	Heavy (%)
Southeast	44.8	20.0	35.2
Southwest	75.6	13.7	10.7
Central Highlands	46.0	11.1	42.9
Average	55.4	14.9	29.7

Source: Truong Hong et al. (2017)

Tea trees

The area affected was 2,759 ha (a decrease of 1,334 ha compared with 2017), in Lam Dong, Phu Tho, Thai Nguyen, Yen Bai and Gia Lai provinces (Plant Protection Department 2018).

Fruit trees (avocado, mango, durian, plum, guava, orange, tangerine, passionfruit)

The current area of harmful mirids is increasing and spreading on a large scale, severely affecting productivity.

Integrated mirid management on cocoa trees

Selecting varieties

Cocoa is quite susceptible to pests and diseases. Currently in Vietnam, cocoa varieties are harmed by mirids at different levels. Cocoa varieties that have been approved by the Ministry of Agriculture and Rural Development for planting in Vietnam, such as TD3, TD6, TD7, TD8, TD9, TD10, TC21, TD28 and TD31, were less susceptible to mirids than other cocoa varieties.

Pruning

Shade trees are pruned at the beginning of the rainy season, creating a canopy for cocoa trees. Through analysis and evaluation of productivity from experiments at three study sites, we founded that proper pruning would reduce the rate of fallen pods and reduce damage by mirids, increasing the productivity of the cocoa. Pruning to allow light penetration of 40% led to the highest yield in Dak Lak and Binh Phuoc. In Ben Tre, pruning to allow light penetration of 40–60% was most successful (Truong Hong et al. 2016).

Sanitation

Sanitation involves regularly monitoring cocoa trees, especially at the stage of young shoots and young pods. Buds and pods seriously affected by mirids should be frequently cut off, collected and disposed of.

Pod wrapping

Cocoa pod wrapping in plastic bags not only helps pods grow and develop normally, but also helps to protect pods from mirids and black pod.

Wrapping also limits the number of sprays and pesticide residues, leading to safer products; reduces input costs, resulting in increased economic efficiency; and avoids negative impacts on the environment. Results of cocoa pod wrapping experiments showed that productivity increased by 21.1% compared with the control (Dao Thi Lam Huong & Bui Thi Phong Lan 2015, 2016).

Biological control

Using bioproducts

In Binh Phuoc, treatment using *Beauveria* parasitic fungi led to the highest harvested yield (0.87 tonnes of seeds/ha), followed by treatment with *Metarhizium anisopliae* and *Bacillus thuringiensis*. The control only reached 0.50 tonnes of seeds/ha. In Ben Tre, the average yield of dried cocoa beans over 2 years (2012–13) was highest for treatment with *Beauveria*, followed by treatment with *Metarhizum anisopliae* and *Paecilomyces*. Productivity using these treatments was significantly higher than those using other inoculants and control treatments (no treatment).

*Control using black ants (*Dolichoderus thoracicus*)*

In year 3, using black ants controlled mirids better than the control treatment. In November 2015, the index of damage decreased by 6.97% for the treatment using black ants compared with the control.

Using black ants limited the ratio of pods damaged by mirids and contributed to increased productivity.

Productivity in the third year of the experiment was 7.78% higher using black ants than in the control.

Chemical control

Table 3.2 shows the results of using different chemical control methods on profits from cocoa crops.

Crops were observed regularly and sprayed when >5% of pods were affected by mirids. This involved spraying twice, at an interval of 10–12 days. Young pod and buds were sprayed thoroughly. All pesticides used in the experiment were effective against mirids; the most effective was alpha-cypermethrin (Pertox 5 EC, 0.3%).

Conclusions and recommendations

Conclusions

Mirids are pests that damage cocoa trees and industrial crops (e.g. pepper, coffee) and fruit trees (e.g. orange, avocado) in Vietnam.

Practices of integrated crop management had positive impacts in controlling mirids. They included:

- planting high-yielding cocoa varieties, such as TD3, TD6, TD7, TD8, TD9, TD10, TC21, TD28 and TD31
- pruning the canopy properly, and maintaining appropriate shade trees and windbreak trees
- using regular sanitation at the beginning of the rainy season
- applying balanced organic, inorganic and micro-organic fertiliser
- feeding and maintaining black ants to prevent *H. theivora* damage to cocoa pods
- applying biological control (using one of the two bioproducts 1% *Beauveria bassiana* or 1% *Metarhizium anisopliae*)
- using chemical control when the rate of pods affected by mirid bugs is >5%; this involves spraying twice at an interval of 10–12 days using one of alpha-cypermethrin (Pertox 5 EC, 0.3%), thiamethoxam (Actara 25 WG, 0.025%), or chlorpyrifos ethyl + imidacloprid (Fidur 220 EC, 0.3%).

Table 3.2 Economic efficiency in the prevention of mirids using chemical control

		Average profit for 2 years (2012–13) (US$/ha)		
TT	**Treatment**	**Dak Lak**	**Binh Phuoc**	**Ben Tre**
1	Fidur 220 EC (0.3%)	307.62	339.52	307.62
2	Actara 25 WG (0.025%)	260.95	234.29	260.95
3	Angun 5 WG (0.35%)	277.62	103.81	277.62
4	Pertox 5 EC (0.3%)	670.95	531.43	670.95
5	Movento 150 OD (0.125%)	301.43	205.24	301.43
6	Control	419.05	80.48	419.05

Note: 1 US$ = 21,000 VN$
Source: Truong Hong et al. (2017)

Recommendations

These research results should be transferred and disseminated to cocoa production through training, workshops, leaflets, books and so on, to develop cocoa in a sustainable way.

References

Bateman, R 2007, *ICCO annual report 2007–2008*, International Cocoa Organization, Abidjan, Côte d'Ivoire.

Dao Thi Lam Huong & Bui Thi Phong Lan 2015, 'Some technical advances in cocoa production in Vietnam', Scientific and Technical Information, Dak Lak Union of Science and Technology Associations, No. 31.

——& Bui Thi Phong Lan 2016, 'Introducing some technical advances in cocoa production in Vietnam', in *Conference to review activities in 2016 and directions for 2017*, Vietnam Cocoa Coordination Committee, Ministry of Agriculture and Rural Development, Ho Chi Minh City, 23 December 2016, pp. 36–9.

Plant Protection Department 2018, 'Announcing the situation of major pests 7 days in some plants (from October 5, 2018 until October 11, 2018)', Notice No. 41/TBSB-BVTV, 12 October 2018.

Truong Hong, Nguyen Thi Ngoc Ha, Vo Thi Thu Van & Hoang Hai Long 2016, 'Study watering and pruning techniques suitable for cocoa trees', in *Conference to review activities in 2016 and directions for 2017*, Vietnam Cocoa Coordination Committee, Ministry of Agriculture and Rural Development, Ho Chi Minh City, 23 December 2016, pp. 40–51.

——, Dao Thi Lam Huong, Dao Thi Lan Hoa, Phan Thanh Binh, Nguyen Thi Ngoc Ha, Nguyen Tang Ton, Doan Thi Hong Cam, Le Van Bon, Bui Thi Phong Lan, Pham Hong Duc Phuoc & Phan Thanh Hai 2017, 'Synthesis report on scientific and technological results of the topic "Research and development of sustainable cocoa production in Vietnam"', Code 35/2011/HD-DTĐL, Agency for Project Management, Ministry of Science and Technology, Vietnam Academy of Agricultural Sciences, Ministry of Agriculture and Rural Development. Responsible agency: Tay Nguyen Agriculture and Forestry Science Institute.

4 A review of biopesticide research for controlling *Helopeltis* spp. on cocoa in Indonesia

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Abstract

Chemical pesticides are still used intensively to control *Helopeltis* spp. in Indonesia. The use of biopesticides is safer for the environment, humans and other nontarget organisms. This review discusses the research on biopesticides that has been carried out in Indonesia to control *Helopeltis* spp. pests on cocoa. Research has used plant extracts, entomopathogenic fungi and predatory insects. Research on the use of plant extracts is still at the scale of laboratory efficacy testing and limited field trials. Plant extract materials used in control are formulated in water solvents. They act as contact toxins and repellents against *Helopeltis* spp. Entomopathogens that have been studied to control *Helopeltis* spp. are *Paecilomyces fumosoroseus*, *Aspergillus* spp., *Beauveria bassiana*, *Lecanicillium lecanii* and *Metarhizium anisopliae*. The biopesticide formulations used are mostly in powder and solution formulations. *B. bassiana* has also been studied as an endophytic fungus. Products containing *B. bassiana* are already sold commercially. Research related to the optimisation of biopesticide formulations needs to be developed further so that the active ingredients of biopesticides can be stored for long periods and still be effective when applied.

Keywords: entomopathogen, formulation, plant extract

Introduction

Helopeltis spp. (mirids) are important pests in cocoa plants. They can damage cocoa pods by injecting a toxin through their stylet into the pod surface, which causes the internal tissue of the pods to die (Vos, Ritchie & Flood 2003). Currently, control of *Helopeltis* spp. in cocoa plantations is dominated by synthetic pesticides. Synthetic pesticides are known to harm the environment and humans, decreasing ecosystem services, so their use must be minimised.

Environmentally friendly control techniques such as biopesticides are useful. Biopesticides include beneficial microbes (viruses, bacteria and fungi); entomophagous nematodes; plant-extract pesticides (botanicals); secondary metabolites from micro-organisms (antibiotics); insect pheromones for mating distortion, monitoring or lure-and-kill strategies; and genetic techniques allowing the crops to become more resistant to target insects

(Copping & Menn 2000). Plant extracts and entomopathogens are widely studied to control *Helopeltis* spp. in Indonesia. This paper addresses development of biopesticide research to control *Helopeltis* spp. on cocoa in Indonesia.

Plant extracts for controlling *Helopeltis* spp. in Indonesia

The active ingredients of synthetic pesticides are often obtained from plant extracts exploration. Plant extracts that have been tested on *Helopeltis* spp. are neem oil, seed extract of custard apple, tobacco, red ginger, nutmeg, ocimum oil, citronella, Piperaceae extract, lemongrass, garlic, marigold, galangal rhizome and babadotan leaves (Table 4.1). Research for plant extracts has been done at the laboratory scale and in the field. Some publications suggest that plant extracts are specifically active against *Helopeltis*. For example, *Piper retrofractum* extract can inhibit the cytochrome P450 enzyme in *Helopeltis* spp. Some plant extracts act as a repellent against *Helopeltis* spp., including galangal rhizome, citronella, lemongrass, garlic and marigold.

Entomopathogens for controlling *Helopeltis* spp. in Indonesia

Entomopathogens are an effective and selective biopesticide for controlling *Helopeltis* spp. Entomopathogens that have been tested to control *Helopeltis* spp. in Indonesia are *Paecilomyces fumosoroseus*, *Metarhizium anisopliae*, *Aspergillus* sp. and *Beauveria bassiana* (Table 4.2). *M. anisopliae* is basically an entomopathogen in the

Table 4.1 Plant-extract biopesticides to control *Helopeltis* spp.

Plant extracts	Formulation	Mode of action	Scale	References
Neem oil and seed extract of custard apple	Water soluble	Insecticide (unspecific)	Laboratory	Wiryadiputra (1998)
Tobacco extract	Water soluble	Insecticide (unspecific)	Laboratory and field	Wiryadiputra (2003)
Red ginger, nutmeg and ocimum oil	Water soluble	Contact insecticide	Laboratory	Atmadja (2008)
Citronella	Water soluble	Repellent and contact insecticide	Laboratory	Nurmansyah (2011)
Piperaceae extract	Water soluble	Piperin extract from *Piper retrofractum* suggested as a nerve toxin (cytochrome P450 enzyme inhibitor)	Laboratory	Indriati & Samsudin (2014)
Lemongrass, garlic, marigold	Water soluble	Repellent and contact insecticide	Field	Sulistyowati et al. (2014)
Galangal rhizome, lemongrass, and babadotan leaves	Water soluble	Galangal rhizome, lemongrass as repellents; babadotan as an attractant	Field	Hastuti & Rusmana (2015)

Table 4.2 Entomopathogens used to control *Helopeltis* spp.

Entomopathogen	Formulation	Mode of action	Scale	References
Paecilomyces fumosoroseus	Water soluble	Insecticide (unspecific)	Laboratory and field	Sulistyowati, Mufriati & Andayani (2006)
Metarhizium anisopliae	Wettable powder (on rice)	Contact insecticide	Laboratory	Saputra et al. (2013)
Metarhizium anisopliae and *Beauveria bassiana*	Wettable powder (on rice)	Insecticide (unspecific)	Laboratory and field	Irawan et al. (2015)
Aspergillus sp.	Water soluble	Insecticide (unspecific)	Laboratory	Pasaru, Panggeso & Khasanah (2017)
Aspergillus sp.	PDA and corn formulation	Insecticide (unspecific)	Laboratory and field	Supriyadi, Pasaru & Lakani (2017)
Beauveria bassiana	Kaoline powder		Field	Indriyanti, Faizah & Slamet (2017)

PDA = potato dextrose agar

rhizosphere. This is not effective in controlling pests that have all their life stages on the surface, and attack plants on the surface of pods or leaves. *B. bassiana* and *Aspergillus* sp. are entomopathogens that are on the plant surface. *B. bassiana* is commonly used to control *Helopeltis* spp., which occurs on the surface of the plant.

Further research opportunities

Plant extracts and entomopathogens are potential biopesticides to control *Helopeltis* spp. Plant extracts have high potential as biopesticides because they can be stored and formulated easily. But production is sometimes insufficient to produce large amounts. Research related to organic compounds from plant extracts that function as biopesticides is needed to show the type of compounds that are effective and their mode of action. Entomopathogens have a weakness in that their viability in biopesticide formulations is easily reduced. However, the results of the study show that some entomopathogens act as endophytic fungi in plants. *B. bassiana* can be an endophytic fungus in cocoa plants. *B. bassiana* inoculated in cocoa seedlings can be isolated for up to 6 weeks (Sulistyowati & Aini 2015). The dry formulation of *B. bassiana* is effective in controlling *Helopeltis* spp. for 1–6 months (Irawan et al. 2015). *B. bassiana* might be the best entomopathogen for sustainably controlling *Helopeltis* spp. on cocoa.

Future studies relating to use of biopesticides to control *Helopeltis* spp. in cocoa need to:

- find new beneficial micro-organisms, such as dark septate endophytes or endophytic fungi
- look for effective methods for multiplying *Helopeltis*
- explore active compounds in plant extracts as botanical pesticides
- improve mass production technology and formulations
- explore plant extracts or chemical compounds that act as semiochemicals, especially kairomone compounds.

References

Atmadja, WR 2008, ‘Pengaruh minyak jahe merah, pala dan selasih terhadap *Helopeltis antonii* sign pada inang alternatif’, *Buletin Penelitian Tanaman Rempah dan Obat*, vol. 19, no. 2, pp. 154–63.

Copping, LG & Menn, JJ 2000, ‘Biopesticides: a review of their action, applications and efficacy’, *Pest Management Science*, vol. 56, no. 8, pp. 651–76.

Hastuti, D & Rusmana, HP 2015, ‘Uji efektifitas larutan pestisida nabati rimpang lengkuas, daun serai, dan daun babadotan pada pengendalian hama penghisap buah (*Helopeltis* sp.) tanaman kakao’, *Jurnal Agroekoteknologi*, vol. 7, no. 2, pp. 97–105.

Indriati, G & Samsudin, S 2014, ‘Aktivitas insektisida ekstrak piperaceae terhadap *Helopeltis antonii* pada kakao’, *Jurnal Tanaman Industri dan Penyegar*, vol. 1, no. 1, pp. 7–14.

Indriyanti, DR, Faizah, SN & Slamet, M 2017, ‘Efficacy of *Beauveria bassiana* against *Helopeltis* sp. on cacao (*Theobroma cacao*)’, *International Journal of Scientific & Technology Research*, vol. 6, no. 10, pp. 14–17.

Irawan, N, Purnomo, P, Indriyati, I & Wibowo, L 2015, ‘Pengujian formulasi kering *Metarhizium anisopliae* isolat UGM dan tegineneng serta *Beauveria bassiana* isolat tegineneng untuk mematikan *Helopeltis* spp. di laboratorium dan di lapangan’, *Jurnal Agrotek Tropika*, vol. 3, no. 1, pp. 138–43.

Nurmansyah, N 2011, ‘Efektivitas serai wangi terhadap hama pengisap buah kakao *Helopeltis antonii*’, *Buletin Penelitian Tanaman Rempah dan Obat*, vol. 22, no. 2, pp. 205–13.

Pasaru, RB, Panggeso, J & Khasanah, N 2017, ‘Pengaruh umur biakan cendawan *Aspergillus* sp. Isolat lokal palolo terhadap mortalitas *Helopeltis* spp. (Hemiptera: Miridae)’, *AGROTEKBIS*, vol. 5, no. 2, pp. 183–9.

Saputra, Z, Purnomo, P, Yasin, N & Wibowo, L 2013, ‘Pengaruh aplikasi beberapa konsentrasi formulasi kering *Metarhizium anisopliae* (Metsch.) sorokin isolat tegineneng terhadap mortalitas hama pengisap buah kakao (*Helopeltis* spp.)’, *Jurnal Agrotek Tropika*, vol. 1, no. 3, pp. 309–14.

Sulistyowati, E & Nur Aini, F 2015, ‘Existence of entomopathogen fungi, *Beauveria bassiana* as an endophyte in cacao seedlings’, *Pelita Perkebunan* (a Coffee and Cocoa Research Journal), vol. 31, no. 3, pp. 186–95.

——, Mufriati, E & Andayani, B 2006, ‘Pengaruh samping aplikasi *Paecilomyces fumosoroseus* terhadap semut hitam, *Dolichoderus thoracicus*, predator *Helopeltis antonii* dan penggerek buah kakao’, *Pelita Perkebunan*, vol. 22, no. 2, pp. 91–100.

——, Ghorir, M, Wardani, S & Purwoko, S 2014, ‘Keefektifan serai, bawang putih, dan bunga paitan sebagai insektisida nabati terhadap penghisap buah kakao, *Helopeltis antonii*’, *Pelita Perkebunan*, vol. 30, no. 1, pp. 35–46.

Supriyadi, D, Pasaru, F & Lakani, I 2017, ‘Efikasi cendawan *Aspergillus* sp. terhadap hama penghisap buah kakao *Helopeltis* sp. (Hemiptera: Miridae) pada tanaman kakao’, *AGROTEKBIS*, vol. 5, no. 3, pp. 300–7.

Vos, JGM, Ritchie, BJ & Flood, J 2003, *Discovery learning about cocoa: an inspirational guide for training facilitators*, CABI Bioscience, Bakeham, UK.

Wiryadiputra, S 1998, ‘Percobaan pendahuluan pengaruh minyak mimba dan ekstrak biji srikaya terhadap mortalitas *Helopeltis* sp. (Heteroptera: Miridae)’, *Journal Perlindungan Tanaman Indonesia*, vol. 4, no. 2, p. 9.

——2003, ‘Keefektifan limbah tembakau sebagai insektisida nabati untuk mengendalikan hama *Helopeltis* sp. pada kakao’, *Jurnal Perlindungan Tanaman Indonesia*, vol. 9, no. 1, pp. 35–45.

Abstracts

Controlling *Apogonia* beetle on cocoa in Vietnam

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In Vietnam, *Apogonia* beetle is the main insect on cocoa and some other crops such as acacia, durian and longan during the establishment stage. The insects eat the leaf blades and leave the veins and midribs. This reduces the leaf area and thus photosynthesis. Less leaf area also means more direct sunlight on the young cacao plant, leading to sunburn and algal rust disease. In serious cases, the whole tree dries out and dies.

To control this insect, cocoa farmers in Vietnam usually spray leaves with chemicals such as imidacloprid, diazinon, thiamethoxam and cypermethrin. However, the effect is low because the insect hides in the soil during the day, and rain at night washes away the chemical. Chemical sprays are also harmful to the environment. Other farmers use fertiliser bags to cover young cocoa trees to prevent contact with the insect. Although this technique is very effective, it is laborious and difficult to scale up.

In 2018, a new cocoa crop was planted on a farm that was highly eroded. To improve the soil, regenerative farming was practised. Wild grasses were allowed to develop between cocoa rows. Among grasses species, fountain grass (*Pennisetum setaceum*) and cogon grass (*Imperata cylindrica*) dominated. These two grasses can reach 1.5–2 m in height. No pesticide, fungicide or herbicide was used in the first 8 months after planting. Data showed that the percentage of damaged trees and the damaged trees index were much lower than for the control (no grasses between cocoa rows). The same result was recorded on a cocoa farm in the highlands using only fountain grass between rows.

Preliminary study of the relationship between *Helopeltis* sp. and pod rot disease on cacao

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Helopeltis sp. is one of the most important pests in cacao plants in Indonesia. This pest attacks the cacao pod by piercing and sucking from the young pod stage until the pod ripens. The most significant attack occurs in the small pod phase, when it can cause death of the pod. Field observations have shown that cacao plantations with a high intensity of *Phytophthora palmivora* attack, causing pod rot disease, also have a high population of *Helopeltis* sp. Based on this observation, research was carried out to prove the relationship. The research was conducted in three stages:

- studying the relationship between the intensity of *Helopeltis* sp. and pod rot disease
- isolating fungi from *Helopeltis* sp.
- testing *Helopeltis* sp. as a vector of *P. palmivora*.

The study showed that the intensity of *Helopeltis* sp. infestation and pod rot disease increased every week. After 4 weeks, 39.36% of pods were attacked by *Helopeltis* sp., while 28% of pods exhibited pod rot disease. Regression analysis showed that r = 0.987, indicating a very strong relationship between occurrence of *Helopeltis* sp. and pod rot disease.

The pathogen *P. palmivora* was not found attached to the body parts of *Helopeltis* sp. (head, legs or whole body) but *Botryodiplodia theobromae* and *Trichoderma* spp. were found.

Test results did not show *Helopeltis* sp. as a vector of *P. palmivora*.

Helopeltis sp. and pod rot disease are associated with causing damage through a mechanism whereby insects make wounds on plant parts, which can be infected by pathogens. Other environmental factors can contribute to this damage.

Session 3

Management of coca pod borer in South-East Asia (latest updates by country)

5 Incidence and control of cocoa pod borer and *Helopeltis* in Indonesia

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Abstract

A survey was conducted of 120 farms in eight provinces in Indonesia (Central, South, South-East and West Sulawesi; West Sumatra; Lampung; East Java; and West Papua) of cocoa pod borer (CPB) and *Helopeltis* incidence and control. The data were collected over a 3-year period (2014–17) as part of a broader survey of farmer practices and productivity. Farmers were interviewed each year, and farms were monitored every 6 weeks for productivity, and pest and disease incidence. The presence of pests was identified as a key challenge by a high proportion of farmers (98%, 89.8% and 77.6% of farmers in 2014, 2015 and 2016, respectively).

CPB was recorded in all provinces; the highest incidence of severe infestation was in Central Sulawesi, and the lowest incidence was in Lampung. Most farmers conducted some sort of control against CPB; the largest proportion used pesticides (72.5%, 73.7% and 69.8% of farmers in 2014, 2015 and 2016, respectively). *Helopeltis* incidence varied significantly between provinces ($P < 0.001$); the highest number of infested pods per tree was recorded in East Java. The proportion of farmers using pesticides against *Helopeltis* was 60.8% in 2014 and fell to 49.1% in 2016. A number of farmers in East Java used biological control in the form of black ants against both pests. On average, 10% of on-farm expenditure was on pesticides.

Overall, the survey illustrated the wide-ranging occurrence of both pests in Indonesia, with infestation being higher in some provinces than others. Although many farmers were employing control measures, there was a high level of dependency on pesticides, suggesting that more targeted control strategies may be needed.

Keywords: cocoa pod borer, *Helopeltis*, pest control

Introduction

Cocoa pod borer (CPB, *Conopomorpha cramerella*) and *Helopeltis theobromae* are both widespread pests of cocoa in South-East Asia. The larvae of *C. cramerella* burrow through the pod husk, resulting in clumping of the beans (Awung & Lamin 2017). *H. theobromae* feeds on the parenchymous husk tissue of cocoa pods, which often induces cherelle wilt in young pods. *Helopeltis* also causes damage to the canopy by feeding on young shoots, particularly when few pods are developing on the tree (Bakar, Awang & Ismail 2017).

To develop control strategies for these pests, it is important to have baseline data on their prevalence and methods used by farmers for their control. This study surveyed farms across key growing areas in Indonesia over a 3-year period. It was part of larger project surveying productivity and practices on cocoa farms ('Mapping Cocoa Productivity', www.mappingcocoaproductivity.org; Daymond et al. 2018).

Materials and methods

Farms were selected in eight provinces that reflected both current important areas of production and anticipated future key areas of production. These were Western Sumatra, Lampung, West Sulawesi, Central Sulawesi, South-East Sulawesi, South Sulawesi, East Java and West Papua. Within each province, 15 farms were selected from three districts, except for West Papua, where two districts were chosen. Farms were purposefully sampled to account for different levels of farmer management. Approximately one-third of farms in each province fell into each of the following categories: highly managed, moderately managed and little managed.

Farmers were interviewed between April and May 2014 using a structured questionnaire about farm management practices in place and challenges faced. This interview was repeated in 2015 and 2016.

On each farm, 16 trees were tagged at the beginning of the project for subsequent detailed observations of productivity, and pest and disease incidence, which took place every 6 weeks between May 2014 and May 2017. In the case of *Helopeltis*, this was recorded as the number of pods showing lesions on each of the tagged trees. To assess CPB incidence, on each monitored farm, at least 20 pods were opened, and the percentage of pods that fell into the following categories were recorded: free, light, medium and severe. These categories were defined as follows: free = no symptoms; light = 1–10% clumped beans; medium = 11–50% clumped beans; and severe = >50% clumped beans.

Differences between regions in intensity of CPB and *Helopeltis* were tested using analysis of variance.

Results and discussion

When farmers were asked to list key challenges faced, incidence of pests was the most widely cited problem, being cited by 98%, 89.8% and 77.6% of respondents in 2014, 2015 and 2016, respectively. This illustrates the economic importance of the species under study.

CPB was present to a greater or lesser extent in all the provinces surveyed. Differences in infestation levels between provinces were significant ($P < 0.001$). The highest levels of infestation were observed in Central Sulawesi, where 58–93% of sampled pods showed some level of infestation over the 3 years of sampling; severe levels of infestation in this province varied from 12% to 80% at different times. Assuming that all severely infested pods were unusable, this would represent a loss of at least 31% of potential production over the 3 years (higher if some of the 'medium' infested pods were also unusable). The lowest levels of infestation were observed in Lampung, where there were times when the proportion of severely infested pods fell to zero (Figure 5.1).

Overall, clear links between the cropping pattern and CPB incidence were not observed, suggesting that a variety of factors may contribute to spatial and temporal differences in infestation levels. These might include a combination of microclimate, control measures and background population levels.

The majority of farmers stated that they were conducting some sort of control against CPB, with the largest proportion using pesticides (72.5%, 73.7% and 69.8% of farmers in 2014, 2015 and 2016, respectively; Table 5.1). Frequent harvesting was the second most cited control method. It should be noted that some farmers who did not cite this method were nevertheless harvesting at frequent intervals (2 weeks or less). Pod sleeving was used by a relatively small number of farmers, presumably due to the high level of labour involved in this procedure. A small proportion of farmers used biological control (typically black ants, *Dolichoderus thoracicus*). This practice was

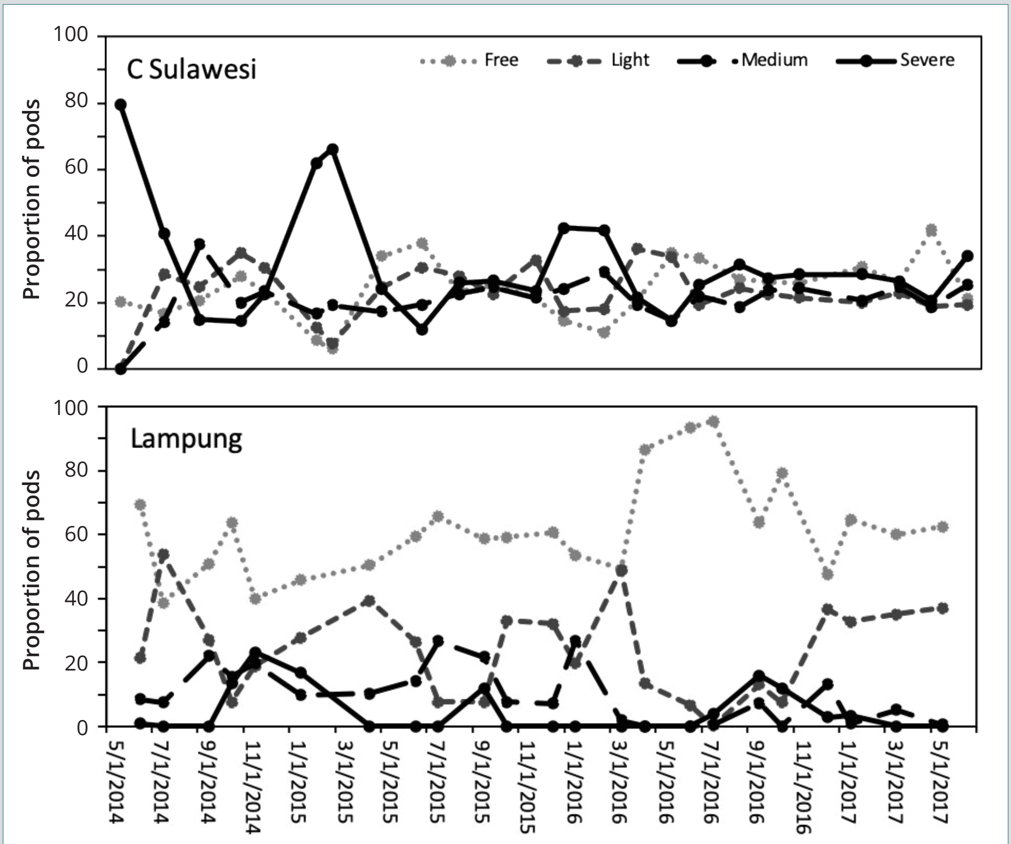

Figure 5.1 Proportion of pods infested with cocoa pod borer in Central Sulawesi and Lampung, May 2014 to May 2017

Table 5.1 Control methods used by farmers against cocoa pod borer

Control method	Percentage of farmers		
	2014	2015	2016
Pesticide	72.5	73.7	69.8
Frequent harvesting	30.8	58.5	48.3
Pod sleeving	13.3	14.4	11.2
No control	7.5	16.1	12.9
Natural enemy	6.7	4.2	14.7
Pruning	5.0	0	1.7
Pheromone trap	0	0	3.4
Botanical pesticide	0	0	3.4

Note: Respondents could provide more than one answer.

mainly observed in East Java and may reflect proximity to extension agents from the Indonesian Coffee and Cocoa Research Institute (ICCRI) in this province. The use of pheromone traps was cited by four farmers in the 2016 survey (three in South Sulawesi and one in East Java). Overall, the proportion of farmers who used two or more control strategies was 30% in 2014. This rose to 57% in 2015 and fell back to 42% in 2016.

Although no relationship was observed between pesticide use and CPB infestation, a general trend was observed of lower incidences of severe CPB infestation on more highly managed farms.

Although *Helopeltis* was observed in all areas sampled, there were significant differences between provinces ($P < 0.001$). The highest levels were observed in East Java and Lampung. Generally, levels of infestation were lower in Sulawesi (Figure 5.2).

Overall, the proportion of farmers using some sort of control against *Helopeltis* was lower than for CPB (63.8% in 2014, falling to 50.9% in 2016), probably reflecting large differences in severity of *Helopeltis* infestation between farms. The most widely cited control method for *Helopeltis* was use of pesticides (Table 5.2). The use of ants as a biological control method was mainly cited by farmers in East Java, who were also using this method against CPB.

To conclude, results of the survey presented here illustrate the widespread nature of both CPB and *Helopeltis* in Indonesia, although with varying intensity of incidence across different provinces. The importance of both pests is illustrated both by a large proportion of farmers citing pests as a challenge faced and by many farmers employing control methods. Overall, a high dependence on pesticides was apparent. Some farmers were spraying at a high (excessive) frequency, suggesting that more targeted approaches may be needed for effective pest control.

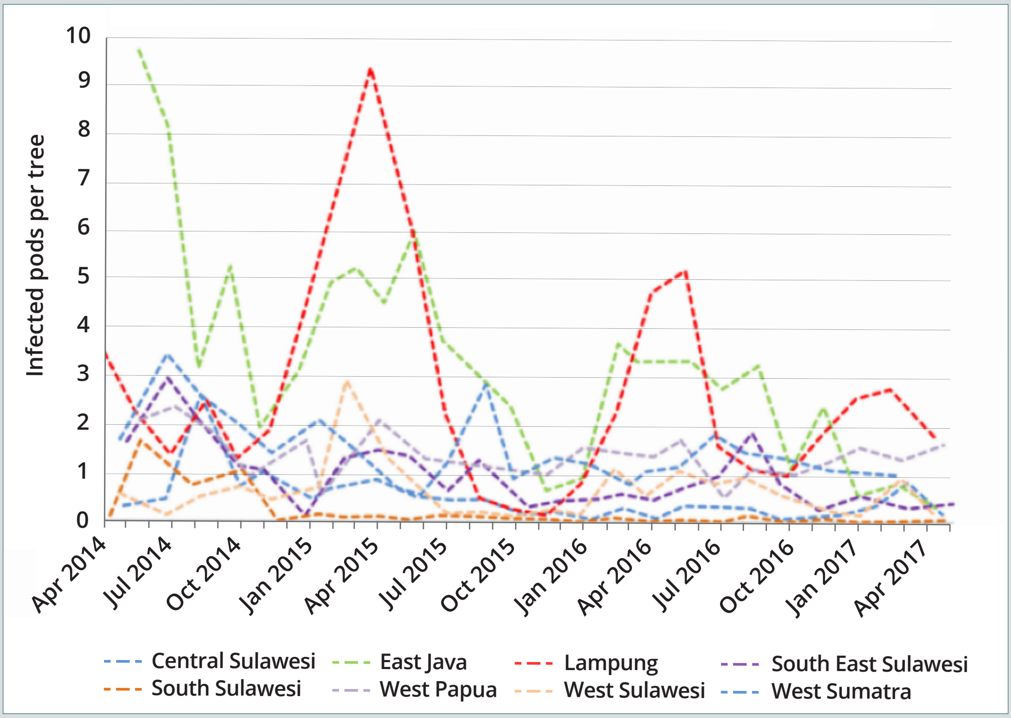

Figure 5.2 Differences in *Helopeltis* infestation between provinces, 2014–17

Table 5.2 Summary of control methods used by farmers against *Helopeltis*

Control method	Percentage of farmers		
	2014	2015	2016
No control	36.2	39.1	49.1
Biocontrol (ants)	8.3	5.2	14.7
Pesticides	60.8	55.7	46.6
Botanical insecticide	0	0	5.2

Note: Respondents could provide more than one answer.

Acknowledgments

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References

Awang, A & Lamin, K 2017, 'Cocoa pod borer', in MJ End, AJ Daymond & P Hadley (eds), *Technical guidelines for the safe movement of cacao germplasm*, Global Cacao Genetic Resources Network (CacaoNet), Bioversity International, Rome, pp. 66–9.

Bakar, S, Awang, A & Ismail, A 2017, 'Mowquito bug', in MJ End, AJ Daymond & P Hadley (eds), *Technical guidelines for the safe movement of cacao germplasm*, Global Cacao Genetic Resources Network (CacaoNet), Bioversity International, Rome, pp. 73–6.

Daymond, AJ, Acheampong, K, Prawoto, A, Abdoellah, A, Addo, G, Adu-Yeboah, P, Arthur, A, Cryer, NC, Dankwa, YN, Lahive, F, Konlan, S, Susilo, A, Turnbull, CJ & Hadley, P 2018, 'Mapping cocoa productivity in Ghana, Indonesia and Côte d'Ivoire', in *Proceedings of the International Symposium on Cocoa Research (ISCR)*, Lima, 13–17 November 2017.

6 Zingiberaceae essential oils as a potential biopesticide for cocoa pod borer, *Conopomorpha cramerella* Snellen

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Abstract

Cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae), has become a major threat to the cocoa-growing countries of Malaysia, Indonesia and Papua New Guinea. Various control efforts have been studied, introduced and implemented in Malaysia. However, less focus has been given to plant-derived products, such as those from the Zingiberaceae family. Members of the Zingiberaceae (order Zingiberales) are the most important herbaceous species in the tropics, and several species have shown potential for managing some lepidopteran and dipteran pests. Therefore, a study was undertaken to evaluate the effect of Zingiberaceae essential oils on the *C. cramerella* life stages. Three essential oils (EOs) were selected for this study, and tested for their effect on pupation ability and adult emergence: *Zingiber officinale* (ginger), *Curcuma longa* (turmeric) and *Alpinia galanga* (galangal). The results showed that *C. cramerella* pupation on cocoa pods was lower than on cocoa leaves, regardless of different treatments, through 10 days of observation. Pupation on the cocoa leaves was significantly different ($P < 0.05$) on day 2 (1.653) from days 6, 8, 9 and 10. Pupa emergence on cocoa pods was significantly different ($P < 0.05$) between the control treatment (0.324), *Z. officinale treatment* (0.556), *C. longa* treatment (0.580) and *A. galanga* treatment (0.641). No significant difference was seen among EOs on pupation on cocoa pods. In the other study, the percentage of unhatched pupae was highest for *C. longa* treatment (49.75%), which was significantly different ($P < 0.05$) from *A. galanga* (48.00%), *Z. officinale* (40.98%) and control (13.59%) treatments. Healthy adult emergence was highest under the control treatment (75.78%), and significantly different with all Zingiberaceae EOs.

The overall results showed that most body and wing deformities occurred after pupae were treated with the Zingiberaceae EOs at high concentrations (400 and 800 ppm). The potential of Zingiberaceae as an adult emergence inhibitor and pupation deterrent can possibly be used for development of Zingiberaceae-based botanical pesticides in the future.

Keywords: cocoa, *Theobroma cacao*, cocoa pod borer, *Conopomorpha cramerella*, Zingiberaceae

Introduction

The family Zingiberaceae (Zingiberales) is important because of its wide distribution in the tropics. With 52 genera and more than 1,000 species (Wohlmuth 2008), Zingiberaceae species were commercially planted, and used in cooking as food preservatives, flavouring agents and spices (Bakar et al. 2017). Species in the Zingiberaceae also have a positive impact in managing insect pests, especially lepidopteran and dipteran species. Monoterpenoids, diterpenoids and sesquiterpenoids obtained from Zingiberaceae extracts are important essential components for controlling insect pests (Isman 2008; Ukeh et al. 2009). The potential use of botanical extracts derived from plants for controlling insect pests appeared promising based on numerous studies conducted in different countries and continents. Botanical insecticides are often relatively target-specific and biodegradable, and can be used in insecticide resistance management programs.

Currently, little information is available on the use of Zingiberaceae in managing insect pests in Malaysia. Therefore, the objective of this study was to evaluate the potential use of some Zingiberaceae species against the pests of cocoa, *Theobroma cacao* L. (Malvales: Sterculiaceae). Like other economic crops, cocoa is exposed to a wide range of insect pests, from early development in the nursery to the mature tree. Lee et al. (2013) listed 15 species of insect pests. Among them is cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen (Lepidoptera: Gracillariidae). The pest has become a significant threat to cocoa-growing countries in South-East Asia. This tiny moth may cause severe crop loss to farmers if left untreated, and continuous infestations may cause an unacceptable level of damage. Since pest outbreaks began, actions have been taken to suppress pest populations, as well as to reduce damage to pods. Control tactics implemented include rampasan, pod sleeving, frequent harvesting of ripe pods, use of natural enemies, quarantine measures, management practices, mechanical control, biological approaches (Saripah 2014a) and chemical spraying (Saripah 2014b). Among the control methods, use of chemical insecticides is the most common practice used by cocoa growers (Azhar 1992; Saripah 2014b).

Prolonged and excessive use of chemical insecticides may increase the risk of insecticide resistance, and may pose environmental problems and health risks to growers. Alternatives to currently used insecticides are needed. One of the approaches is the use of natural products synthesised by plant species. Three Zingiberaceae species—*Zingiber officinale* (ginger), *Curcuma longa* (turmeric) and *Alpinia galanga* (galanga)—were selected for this study. These species were selected because of the presence of chemical components such as camphene, camphor, 1-8-cineole and α-humulene in their rhizome; these chemicals are effective against *Sitophilus zeamais* and *Tribolium castaneum* (Suthisut, Fields & Chandrapatya 2011). The potential of Zingiberaceae essential oils (EOs) was tested based on their effect on the pupation ability of *C. cramerella*, as well as observations of the adult emergence of *C. cramerella*.

Materials and methods

Laboratory observation on the pupation and adult emergence of *C. cramerella* was undertaken at the Malaysian Cocoa Board, Cocoa Research and Development Center, Bagan Datuk, Perak (3.906 N, 100.866 E). Commercial EOs of *Z. officinale*, *C. longa* and *A. galanga* were obtained from the local authorised dealer. For observation of pupation behaviour, EOs were added with polyoxyethylene (20) sorbitan monooleate (Tween 80), and the solution was diluted to 100 mL with water to obtain a concentration of 400 ppm. A 100 mL amount of control solution was prepared by combining Tween 80 and 100 mL of water. All prepared solutions were vortexed (2 minutes at 1,800 rpm), then stored in an amber dark glass bottle at 4 °C. Solutions were applied to *C. cramerella* after 24 hours. Cocoa pods at the age of 4–5 months were obtained from an untreated field and divided into four different treatments (10 pods/treatment). Pods were hung, sprayed accordingly to the treatment using a hand sprayer at a range of 15 cm and then left to air-dry for 1–2 hours. Pods were then placed as a single layer in a container and covered with dry cocoa leaves. Pupae on the pod surface or dry cocoa leaves were recorded daily until day 10. Bioassays were repeated five times for each treatment.

For observation of *C. cramerella* adult emergence, experiments were performed at four concentrations of EOs (100, 200, 400 and 800 ppm). Mature cocoa pods with symptoms of *C. cramerella* infestation were harvested for pupae collection. Pupae on the leaves were sprayed with EOs using the hand sprayer and then air-dried for 2 hours before being kept in a transparent container. The numbers of successful emerging adults, deformed adults and pupae were observed from 96 hours onwards. The experiment was repeated four times for each EO.

Experimental data on pupation and adult emergence were recorded based on the treatments and replicates in Microsoft® Excel 2007. Data were subjected to statistical analysis using analysis of variance (ANOVA) and PROC GLM, SAS® Software version 8.

Results and discussion

C. cramerella pupation was lower on cocoa pods than on cocoa leaves, regardless of treatment, through 10 days of observation. The emergence of *C. cramerella* pupae was the highest at day 8 (2.07 ± 3.06) during the 10 days of observation. Pupation on the cocoa pod surface (Figure 6.1) was the highest at day 4 for *Z. officinale* EO (0.35 ± 0.48). The highest pupal emergence for *C. longa* EO (0.30 ± 1.13) was on day 6, and the highest emergence for *A. galanga* EO (0.10 ± 0.45) was at days 7 and 8. Pupation was greater for the control than for all EOs throughout the observation period. The highest emergence of pupae on the dry cocoa leaves was on day 2 (Figure 6.2) for Zingiberaceae treatments (*Z. officinale* [1.05 ± 1.14], *C. longa* [2.50 ± 2.98] and *A. galanga* [1.30 ± 1.49]). This was different from the results of the

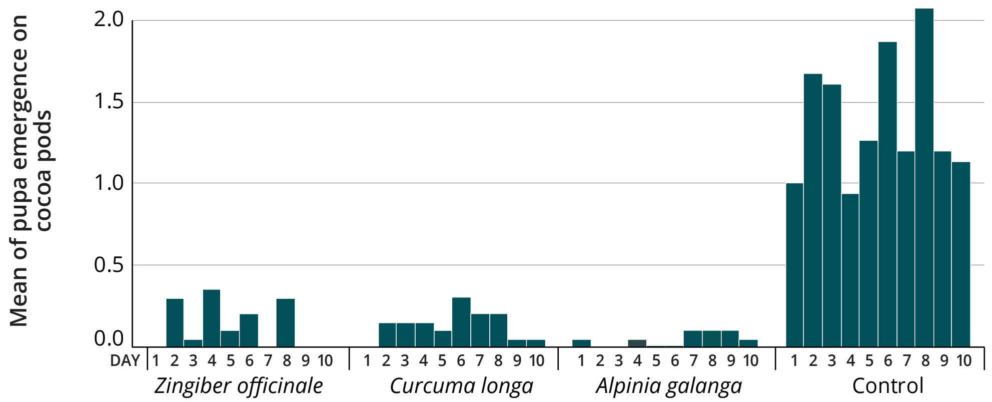

Figure 6.1 Pupation of *C. cramerella* on the cocoa pod surface

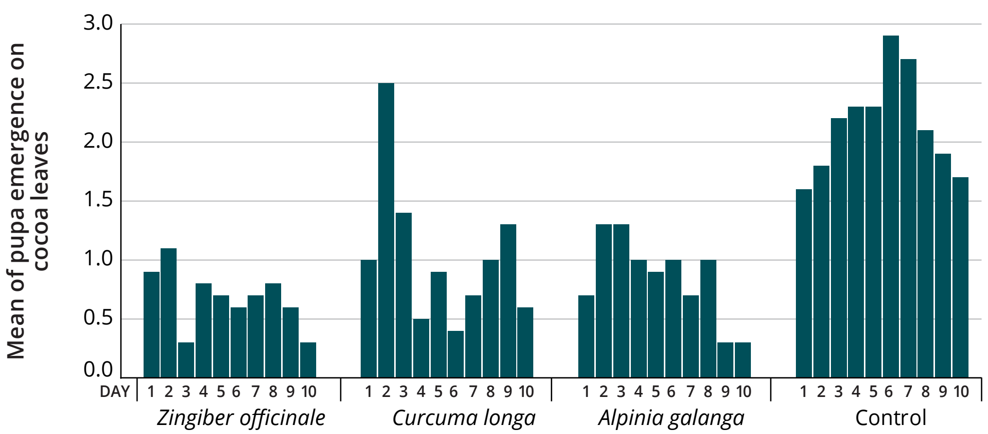

Figure 6.2 Pupation of *C. cramerella* on cocoa leaves

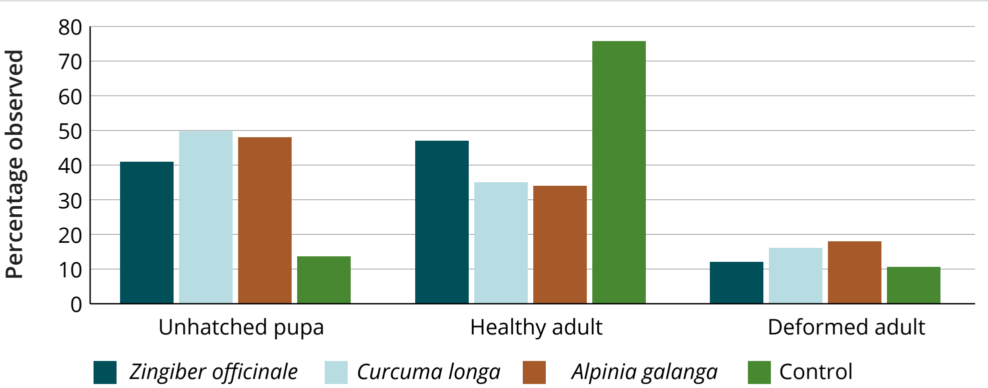

Figure 6.3 Percentage of adult emergence of *C. cramerella*

control, where the highest number of pupae was obtained at day 6 (2.93 ± 2.96).

In the other study, the percentage of unhatched pupae was the highest for *C. longa* EO (49.75%), and significantly different ($P < 0.05$) for *A. galanga* EO (48.00%), *Z. officinale* EO (40.98%) and the control treatment (13.59%) (Figure 6.3). The highest healthy adult emergence was recorded under the control treatment (75.78%), which was significantly different from all Zingiberaceae EOs (*Z. officinale* [46.98%], *C. longa* [35.08%] and *A. galanga* [34.00%]). Observations of deformed adults were highest after treating with *A. galanga* EO (18.00%), which was significantly different from *C. longa* EO (15.17%), *Z. officinale* EO (12.04%) and the control (10.63%). The overall results showed that most body and wing deformities occurred after pupae were treated with the Zingiberaceae EOs at high concentrations (400 and 800 ppm).

The presence of a suitable substrate is critical to completion of the pupal–adult metamorphosis of insects (Wang et al. 2018). In this study, *C. cramerella* pupae preferred cocoa leaves rather than pods because pods were sprayed directly with the different treatments being tested; therefore, this action may provide an unfavourable surface to pupate. The presence of Zingiberaceae EO residue on the treated pods may reduce pupation on pods—that is, the oily coating on the pod surfaces is an abiotic factor that influences the low emergence of *C. cramerella*. Between treatments, *Z. officinale* EO might have more influence on site selection, where the pre-pupae tend to wander away from the treated pods, compared with EOs of *C. longa* and *A. galanga*. For adult emergence, the proportion of healthy *C. cramerella* adults was the lowest for *A. galanga* EO and highest for the control treatment. *A. galanga* was the best for producing the highest percentage of deformed adults.

In conclusion, the potential use of Zingiberaceae as botanical insecticides for controlling *C. cramerella* has been demonstrated, either as an adult emergence inhibitor or a pupation deterrent. This warrants further study, including field tests.

References

Azhar, I 1992, 'Progress and development of cocoa pod borer research in MARDI', *MARDI Occasional Papers*, vol. 5, pp. 1–6.

Bakar, S, Latip, SNHM, Awang, A & Zhang, A 2017, 'Effects of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* essential oils on the morphological characteristic of cocoa pod borer, *Conopomorpha cramerella*', *Journal of Fundamental and Applied Sciences*, vol. 9, no. 6S, pp. 25–38.

Isman, MB 2008, 'Perspective botanical insecticides: for richer, poorer', *Journal of Pest Management Science*, vol. 64, pp. 8–11.

Lee, CH, Kelvin, L, Haya, R, Navies, M & Saripah, B (eds) 2013, *Cocoa planting manual: sustainable cocoa*, Malaysian Cocoa Board, Kota Kinabalu, Sabah, Malaysia.

Saripah, B 2014a, 'Sustaining cocoa black ants *Dolichoderus thoracicus* Smith using artificial nest in the cocoa ecosystem', *Malaysian Cocoa Journal*, vol. 8, pp. 23–30.

——2014b, 'Control of cocoa pod borer using insecticides and cocoa black ants', *Malaysian Cocoa Journal*, vol. 8, pp. 14–22.

Suthisut, D, Fields, PG & Chandrapatya, A 2011, 'Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids', *Journal of Stored Products Research*, vol. 47, pp. 222–30.

Ukeh, DA, Birkett, MA, Pickett, JA, Bowman, AS & Luntz, AJ 2009, 'Repellent activity of alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale* against the maize weevil, *Sitophilus zeamais*', *Phytochemistry,* vol. 70, pp. 751–8.

Wang, H, Liang, S, Ma, T, Xiao, Q, Cao, P, Chen, X, Qin, W, Xiong, H, Sun, Z, Wen, X & Wang, C 2018, 'No-substrate and low-moisture conditions during pupating adversely affect *Ectropis grisescens* (Lepidoptera: Geometridae) adults', *Journal of Asia-Pacific Entomology*, vol. 21, pp. 657–62.

Wohlmuth, H 2008, 'Phytochemistry and pharmacology of plants from the ginger family Zingiberaceae', PhD thesis, Southern Cross University, Lismore, New South Wales.

7 Evaluation of Asia-Pacific cocoa varieties for cocoa pod borer tolerance in Malaysia

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Abstract

A study to test cocoa varieties from the Asia-Pacific region was conducted in Malaysia. The objectives were to produce new hybrid populations with a high degree of tolerance to cocoa pod borer (CPB), and good yield and bean characteristics; and to evaluate their performance under Malaysian agroclimatic conditions. KEE43 X K72 was the variety with the highest yield. Agronomic characteristation showed that more than 50 individual trees had large beans and a high number of beans per pod, and fewer than 20 individual trees had lower pod value. It was found that 21 individual trees of 11 varieties (PBC 123 X LAFI 7, QH 22 X NA 33, MCBC 3 X KKM 22, KEE 42 X K72-124, KEE 42 X NAB II, PA 138 X SCA 9, TD 62, TD 64, TD 17, TD 19 and TD 36) had the best pod and bean characteristics (high bean number per pod, large beans and low pod value). The varieties were assessed for CPB entry holes, exit holes and damage category. TD 35 was found to be the variety with the lowest number of entry and exit holes per pod compared with the other 15 varieties. From the result obtained, more than 100 individual trees have been selected for high yield potential, with good agronomic traits, combined with an acceptable degree of CPB tolerance.

Keywords: hybrid population, agronomic characteristics, cocoa pod borer, infestation, varieties

Introduction

The cacao breeding program of the Malaysian Cocoa Board (MCB) has several objectives, including breeding for high yield, tolerance to common pests (cocoa pod borer—CPB) and diseases (vascular streak dieback, black pod), superior bean quality, high butter and powder content, and suitability to a wide range of agroclimatic conditions (Haya et al. 2007). As CPB remains a serious threat to the cocoa industry, especially in South-East Asia (Saripah et al. 2007), research is in progress on this problem. Cacao growers manage CPB mostly by using chemical control and integrated pest management. Although several cacao planting materials are available, there is a lack of host plant resistance to CPB.

In overcoming the CPB problem and to fulfil the objectives above, MCB has established and developed several progeny and clonal trials throughout Malaysia. One of the trials established a field trial plot to evaluate progenies and clones from countries in the Asia-Pacific region such as Malaysia, Papua New Guinea, the Philippines and Vietnam. The objective of the trial is to produce new hybrid populations that have a high degree of tolerance to CPB, good yield, and good bean characteristics.

Materials and methods

Eleven progenies from the Philippines, Malaysia and Papua New Guinea (PBC 123 X QH 22, QH 22 X NA 33, MCB C3 X NA 33, MCB C3 X KKM 22, KKM 22 X LAFI 7, BR 25 X S5, BR 25 X K2, PBC 123 X LAFI 7, KEE 43 X K 72, KEE 42 X K 72-124 and KEE 42 X NAB 11) and six clones from Vietnam (TD 19, TD 36, TD 62, TD 35, TD 17 and TD 64) were planted in unreplicated plots with 3 m × 3 m planting distance. They were evaluated against UIT1 X NA33 and PA138 X SCA9 (control progenies). *Gliricidia* was used as a shade tree. Standard field maintenance and data recording were applied.

Evaluation of agronomic traits

The data collected for the trial plot were the number of mature pods, and results from pod and bean analysis, which determine bean parameters such as average dried bean weight, pod value and bean number per pod (Haya et al. 2007).

Assessment of CPB category

Data on CPB infestation were collected by harvesting ripe pods. The pods were split and categorised according to their Average Damage Severity Index (ADSI) values (Azhar 1995). ADSI indicates the degree of infestation caused by CPB. It is calculated using the information in Table 7.1, Figure 7.1 and the formula below.

ADSI values were calculated based on the following formula (Azhar 1995):

$$[(0 \times n1) + (1 \times n2) + (2 \times n3) + (3 \times n4) + (4 \times n5)]/N,$$

where

n1 is the number of pods in category 0 (healthy)

n2 is the number of pods in category 1 (slight)

n3 is the number of pods in category 2 (light)

n4 is the number of pods in category 3 (medium)

n5 is the number of pods in category 4 (heavy)

N is the total number of pods examined.

Results and discussion

The yield components of the trial plot include pod yield per tree, bean size, bean

Table 7.1 Category of cocoa pod borer infestation

Score	Category	Description
0	Healthy	Healthy: no larvae have penetrated the sclerotic layer, and all beans are extricable
1	Slight	Slight damage: larvae have penetrated the sclerotic layer, with signs of infestation inside the pod, such as the frass and cell growth on the inner endodermis, but all beans are extricable
2	Light	Light damage: <20% of the beans are inextricable
3	Medium	Moderate damage: 21–50% of the beans are inextricable
4	Heavy	Heavy damage: more than 50% of the beans are inextricable
5	Heavy spoon	All the beans are inextricable

Figure 7.1 Healthy, slight, light, medium, heavy infested pods (from left)

number per pod and pod value. Figures 7.2 and 7.3 show pod yield per tree of the progenies and clones, respectively, over the 10-year period. The mean pod yield per tree was 10.30 pods for the progenies and 6.88 pods for the clones. Four progenies (KEE43 X K72, KEE42 X K72-124, MCBC 3 X NA 33 and PBC 123 X QH 22) had pod yield per tree above the mean level. KEE43 X K72 had the highest pod yield per tree (31.33 pods) over the 10-year period, followed by KEE42 X K72-124 (13.53 pods). For the clones, three clones (TD 62, TD 64 and TD 17) produced pod yield per tree above the mean and control progenies. TD 62 showed the highest pod yield per tree (7.80 pods), followed by TD 64 (7.70 pods).

Figure 7.4 shows pod and bean analysis of the trial at the MCB trial plot. Sixty-six individual trees of 13 progeny populations and six clones were collected for pod and bean analysis. The first bar chart of Figure 7.4 shows that KEE 42 X NAB 11 (NP 14), where NP is the nombor pokok or tree number, had the highest number of beans per pod (51.50 beans), followed by MCBC3 X KKM22 (NP 9) and QH 22 X NA 33 (NP 51), with 48.75 and 48 beans, respectively. TD 17 had the most beans among clones (44.33 beans).

Forty-nine individual trees of progenies and six clones had average dry bean weight exceeding 1 g. Clone TD 17 had the biggest bean size among the clones, at 1.84 g, followed by clone TD 64 at 1.62 g. MCBC3 X KKM22 (NP 116) had the biggest beans in the progeny category, at 1.56 g, followed by PBC 138 XSCA 9 (NP 113) at 1.52 g.

Results for pod value are related to the number of cocoa beans; TD 17 was the best clone, having 12 pods to produce 1 kg dry beans. KEE 42 X K 72 (NP 16) was the best progeny tree, with pod value of 14. From the results of the pod and bean analysis, 21 individual trees had a high bean number per pod, large bean size and low pod value, including PBC 123 X LAFI 7, QH 22 X NA 33, MCBC 3 X KKM 22, KEE 42 X K72-12, KEE 42 X NAB II, PA 138 X SCA 9 and five clones (TD 62, TD 64, TD 17, TD 19 and TD 36).

CPB infestation was assessed based on CPB entry holes, exit holes and damage category. Ten trees were selected for

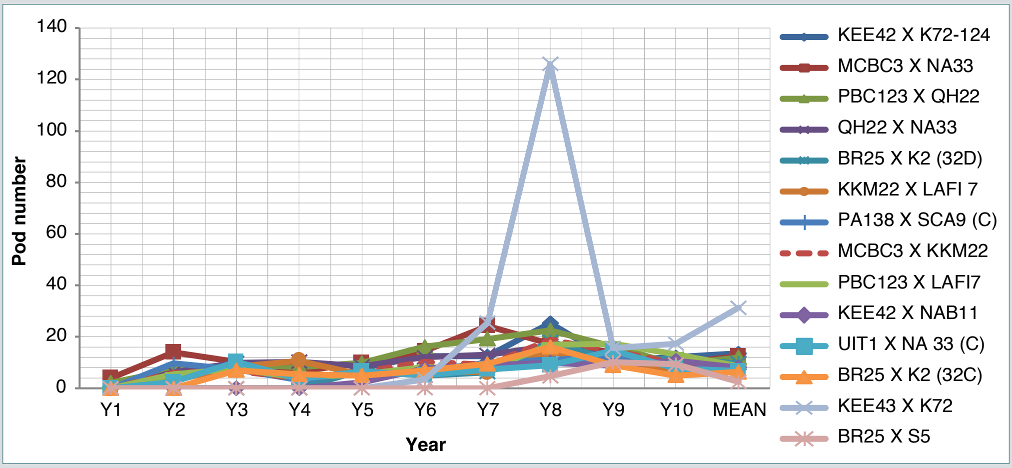

Figure 7.2 Yield performance of the progeny trial plot

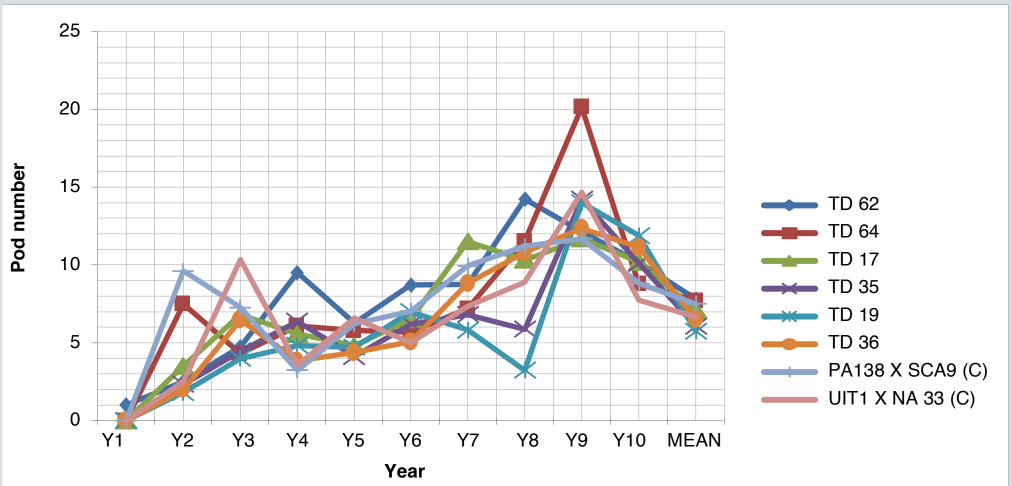

Figure 7.3 Yield performance of the clone trial plot

each clone, and all trees were examined individually for each progeny. About 325 trees produced 3,772 pods for evaluation of CPB infestation. PBC 123 x QH 22 recorded the highest number of pods (26%; 968 pods). This was followed by QH 22 x NA 33 (19%; 710 pods). Other progenies that produced a high number of pods were MCB C3 x KKM 22 (15%) and PA 138 x SCA 9 (9%). The data showed that 98 pods were free from CPB attacks (no sign of entry holes). A total of 336 pods had 1–4 entry holes per pod, 1,826 pods had more than 20 holes, and 14 pods had more than 100 holes. For the exit holes, 226 pods had no sign of exit holes, and 401 pods had one hole. A total of 111 pods were healthy, 11 pods had slight damage, 1,035 pods had light damage, 510 pods had medium damage, and 2,105 pods had heavy damage.

Ten trees were obtained from each progeny and TD clones with the lowest exit holes.

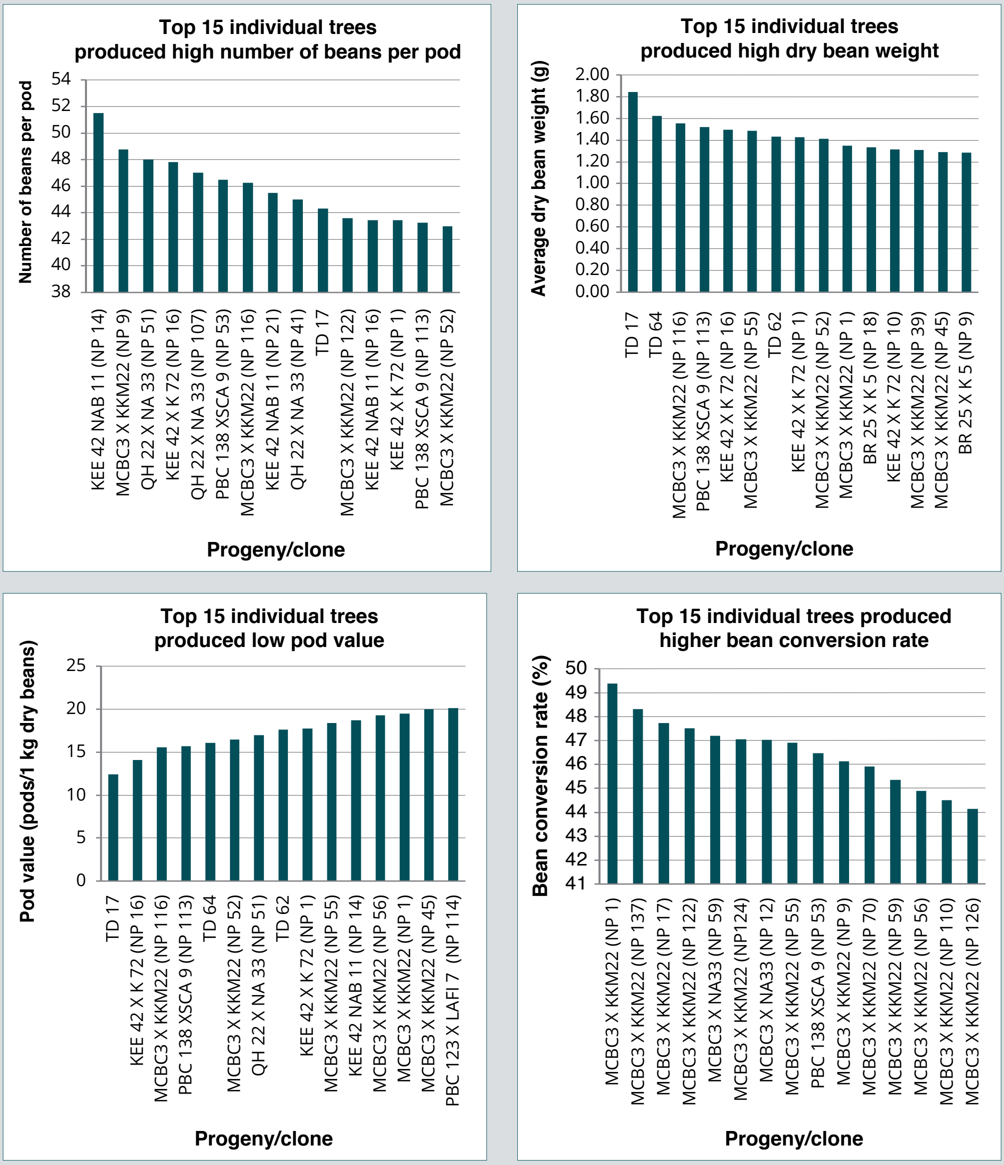

Figure 7.4 Pod and bean analysis on individual trees of the trial plot

Each tree was evaluated individually for CPB infestation. Tree numbers 8, 38 and 51 of PA 138 x SCA 9 had the lowest number of exit holes. The other trees that produced good results were tree numbers 53, 2, 57, 55, 5, 65 and 52. For progeny QH 22 x NA 33, tree numbers 73, 23 and 113 showed the lowest number of exit holes, followed by tree numbers 53, 126, 72, 13, 127, 48 and 1.

In MCB C3 x NA 33, tree numbers 98, 59 and 127 had the lowest number of exit holes, followed by tree numbers 16, 12, 84, 121, 8, 79 and 35. For progeny MCB C3 x KKM 22, tree numbers 111, 40 and 74 recorded the lowest number of exit holes. The other trees that produced good results were tree numbers 72, 31, 62, 121, 90, 75 and 3. Progeny PBC 123 x LAFI 7 had the lowest number of exit holes in tree numbers 13, 39 and 37. For PBC 123 x QH 22, tree numbers 52, 59 and 35 had the lowest number of exit holes, followed by tree numbers 50, 55, 1, 75, 51, 103 and 38.

The lowest number of exit holes was recorded for tree number 42 of BR 25 x K2, followed by tree numbers 22 and 53 of BR 25 x K2 and KEE 42 x NAB II, respectively. All TD clones were combined for the analyses, and tree number 1 of TD19 recorded the lowest number of exit holes, followed by tree numbers 8 and 7 of TD 64 and TD 17, respectively.

Throughout the observations, 111 pods were recorded as healthy, with no sign of exit holes and no or few entry holes. Out of 19 assessed clones and progenies, 11 had successfully produced healthy pods. Among the individual trees, tree number 121 of MCB C3 x KKM 22 was the most promising tree: nine pods were categorised as healthy. This was followed by tree number 98 of MCB C3 x NA 33 and tree number 4 of KKM 22 x LAFI 7. The best 10 clones or progenies, based on the lowest number of entry and exit holes, are listed in Tables 7.2 and 7.3, respectively. Clone TD 35 recorded the lowest number of entry and exit holes per pod, compared with 18 other assessed clones and progenies.

The results of this study may help breeders to select trees that have the highest number of healthy pods, as well as the lowest number of entry and exit holes for future breeding programs.

Conclusions

KEE43 X K72 was the variety with the highest yield compared with others. The agronomic characterisation revealed that more than 50 individual trees had large bean size and high number of beans per pod. Results also showed that fewer than 20 individual trees had low pod value. The trial suggested that 21 individual trees of 11 varieties (PBC 123 X LAFI 7, QH 22 X NA 33, MCBC 3 X KKM 22, KEE 42 X K72-124, KEE 42 X NAB II, PA 138 X SCA 9, TD 62, TD 64,

Table 7.2 Best 10 clones and progenies, entry holes

Rank	Progeny or clone	Number of pods	Mean of entry holes
1	TD 35	32	11.938
2	KKM22 X LAFI7	82	15.024
3	MCBC3 X NA33	325	16.745[a]
4	MCBC3 X KKM22	570	17.774[a]
5	TD 62	74	18.946[b]
6	TD 36	49	19.490[b]
7	PBC123 X LAFI7	280	19.671[b]
8	KEE42 X NAB11	30	21.433[c]
9	QH22 X NA33	710	21.810[c]
10	BR25 X S5	7	22.143[c]

Note: Means labelled with the same letter (a, b, c) were not significantly different.

Table 7.3 Best 10 clones and progenies, exit holes

Rank	Progeny or clone	Number of pods	Mean of exit holes
1	TD 35	32	3.188[a]
2	TD 19	9	3.333[a]
3	MCBC3 X NA33	325	3.711
4	BR25 X K2 (32D)	32	3.906[b]
5	KKM22 X LAFI7	82	3.915[b]
6	MCBC3 X KKM22	570	4.209[b]
7	TD 62	74	4.243[b]
8	KEE42 X NAB11	30	4.600[c]
9	PBC123 X LAFI7	280	4.918[c]
10	TD 36	49	4.980[c]

Note: Means labelled with the same letter (a, b, c) were not significantly different.

TD 17, TD 19 and TD 36) exhibited the best pod and bean characteristics, such as high bean number per pod, large bean size and low pod value. Evaluation of CPB infestation showed that TD 35 was the variety with the lowest number of entry and exit holes per pod, compared with 19 other varieties. From the results obtained, more than 100 individual trees have been selected for high yield potential with good agronomic traits and with CPB tolerance.

Acknowledgments

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References

Azhar, I 1995, 'An overview on the management of key insect pests of cocoa with major emphasis on the cocoa pod borer, *Conopomorpha cramerella*', *The Planter*, vol. 71, no. 835, pp. 469–80.

Haya, R, Kelvin, L, Francis, A, Nuraziawati, MY & Mohd. Jaafar, H 2007, *Manual on cocoa breeding scheme, trial designs and data recordings*, Malaysian Cocoa Board, Kota Kinabalu.

Saripah, B, Azhar, I, Roslan, S & Abdul Shukor, M 2007, 'Implementation of cocoa pod sleeving in controlling cocoa pod borer infestation', in *Proceedings of the 2007 Conference on Plant Plantation Commodities*, Kuala Lumpur, Malaysia, 3–4 July 2007, pp. 221–7.

8 Application of biodegradable bioplastic sleeves to control *Helopeltis* spp. in cocoa plantations

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Abstract

Helopeltis spp. and *Conopomorpha cramerella* are important pests in Indonesian cocoa plantations. *Helopeltis* spp. cause fruit destruction and loss of seed production, by up to 82.2%. Sleeving and bagging of cocoa pods is the most effective control method for the pest, and could reduce pest attacks by 83%. Sleeving of the pods using conventional plastic generates plastic waste that is environmentally unfriendly. The aim of this research was to test the effectiveness on *Helopeltis* spp. infestation of pod sleeving using biodegradable plastic. The test was conducted in the Cibodas Experimental Plantation Station, Bogor district, West Java, which was formerly categorised as a high-infestation block, with more than 80% of the cocoa pods attacked by the pest. Two different thickness of bioplastics (30 µm and 80 µm) were tested on clone TSH 858 and Sulawesi clone (SUL 1). The control cacao pods could not be harvested because of heavy damage.

Keywords: pest control, bioplastic, biodegradable, cocoa pod borer

Introduction

Indonesian cocoa productivity has declined since 2000. It was 724 kg/ha in 2000 and decreased by 52% to 383 kg/ha in 2015 (Dirjenbun 2016). The decline in cocoa production can be accounted for by a number of factors: decreasing soil fertility and organic matter content, and an increased incidence of pests and diseases, such as cocoa pod borer (CPB) caused by *Conopomorpha cramerella*, *Helopeltis* spp. and *Phytophthora* pod rot (McMahon et al. 2015). Attack by CPB caused damage to 62.3% of the cocoa beans (Afrizon & Rosmana 2002) and loss of production of up to 82.20% (Depparaba 2002). An effective control approach for pests is cocoa pod sleeving using plastic or biodegradable plastic (Joseph et al. 2017; Rosmana et al. 2013; Saripah et al. 2007). This method is more effective than other methods. Sleeving of the cocoa pod can suppress CPB attacks by up to 85–95%. However, the use of plastic that is not biodegradable leads to plastic waste (Jambeck et al. 2015). Use of biodegradable plastic could be a potential technique to control pests in cocoa plantations and save the environment. The aim of this research was to test the effect on *Helopeltis* spp. infestation of pod sleeving using biodegradable plastic. The test was conducted in a cocoa plantation that was formerly categorised as a highly infested block, with more than 80% of the cocoa pods attacked by the pest.

Materials and methods

Starch-based bioplastic bags were obtained from PT Inter Aneka Lestari Kimia with two different thickness: 30 µm and 80 µm. They were 15 cm × 20 cm in size. Sleeving was conducted at the Cibodas Experimental Plantation Station with two cocoa clones: TSH 858 and Sulawesi (SUL 1) (Figure 8.1). The treatments were control (without sleeving), sleeving with 30 µm thick plastic and sleeving with 80 µm thick plastic for each clone in a randomised block design. Fifty very young pods (approximately 10 cm long) with no visual pest infestation were selected for every treatment (Figure 8.2). Damage to the bioplastic sleeves was assessed in weeks 0, 8 and 14 after application. The cocoa pods then were harvested, evaluated and processed to obtain cocoa bean. Criteria for damage to the bioplastic bags were normal, lightly damaged, moderately damaged and heavily damaged. Criteria for pod damage were good, moderate, damaged and heavily damaged, or dead.

Figure 8.1 Cocoa pods from clones used in the experiment: clones TSH 858 (left) and SUL 1 (right)

Results and discussion

Starch-based bioplastic is biodegradable; it can be degraded by micro-organisms to produce carbon dioxide (Isroi et al. 2018). Starch-base bioplastic is also not resistant to water. When exposed to the open environment, the bioplastic is gradually damaged. Damage to the bioplastic during this experiment was observed visually, at the beginning of the sleeve application and every 4 weeks. Levels of damage are shown in Figure 8.3 (normal, lightly damaged, moderately damaged and heavily damaged).

Figure 8.2 Cocoa pod (approximatively 10 cm long) used in the initial trial. Cocoa pod for control experiment was marked by bioplastic rope on the pod stalk (left). Pod for treatment was sleeved with bioplastic (right).

Averages of the percentage data are shown in Figure 8.4. Thin bioplastic (30 μm) was easier to damage than thick bioplastic (80 μm). Thin bioplastic has lower physical strength than thick bioplastic. The bioplastic absorbs water from a humid environment, which affects its properties. Eight weeks after application, levels of heavily damaged bioplastic were 9.26% (TSH—30 μm) and 0% (SUL—30 μm), compared with 4% (TSH—80 μm) and 0% (SUL—80 μm). The percentage of the heavily damaged bioplastic was significantly increased after 14 weeks, at 48.15% (TSH—30 μm) and 41.51% (SUL—30 μm). When the cocoa pods were growing, the pod would press and tear the bioplastic. The exposed pod surface could then be infested by pests or diseases (Figure 8.3D).

The percentage of harvestable fruit from the treatment using 30 μm bioplastic sleeves was 74% (TSH) and 92% (SUL); for the 80 μm bioplastic, it was 58% (TSH) and 80% (SUL). Bioplastic bags were gradually damaged during the test. Damage of the bioplastic sleeve 14 weeks after application was 44.88% (30 μm) and 37.60% (80 μm).

Bioplastic sleeves act as a physical barrier to *Helopeltis* spp., like a plastic sleeve (Paper et al. 2007). They protect the pod and prevent adults from laying an egg, thereby reducing the degree of infestation. Cocoa pod sleeving ensures that the pods are protected from egg-laying, and is practical in smallholdings with trees of reasonable height (Azhar, Alias & Meriam 2000). Most cocoa pods with sleeves in good condition had no pest infestation.

Levels of cocoa pod damage are shown in Figure 8.5. Only pods with damage levels 1–3 are harvested. Heavily damaged pods cannot be harvested. This block did not receive an insecticide application. *Helopeltis* spp. feed on pods of all ages and young shoots of cocoa (Wielgoss et al. 2012). All control experiments for TSH 858 and SUL 1 were heavily damaged and not harvested.

The percentages of each level of damage to the cocoa pods are shown in Figure 8.6. Pods under all control treatments (i.e. not sleeved) were heavily damaged or dead. Thick bioplastic (80 μm) tended to give a lower percentage of good condition pods than thin bioplastic (30 μm) for both cocoa clones. Some of the moderate and damaged cocoa pods with thick bioplastic (80 μm) were infected by fungi. Thick bioplastic

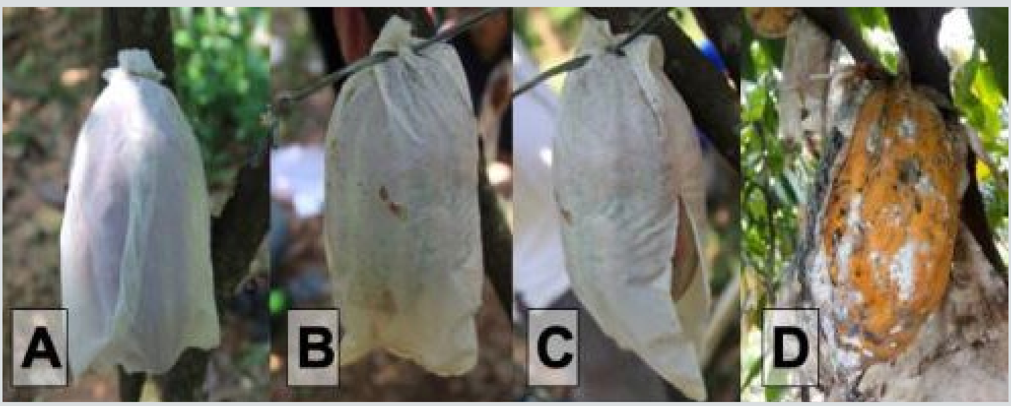

Figure 8.3 Criteria for bioplastic damage: (A) normal, (B) lightly damaged, (C) moderately damaged and (D) heavily damaged

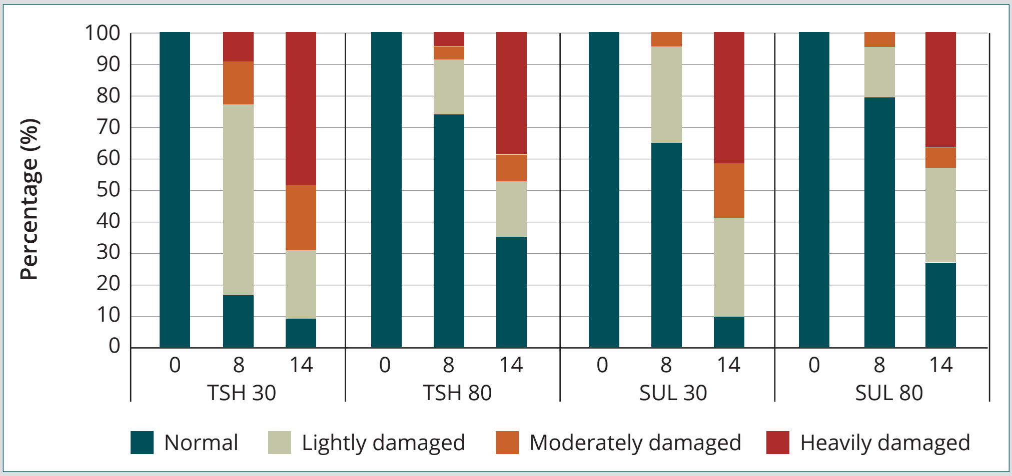

Figure 8.4 Percentage of bioplastic damage at 0, 8 and 14 weeks after application

Figure 8.5 Criteria for cocoa pod damage: good, moderate, damaged and heavily damaged, or dead

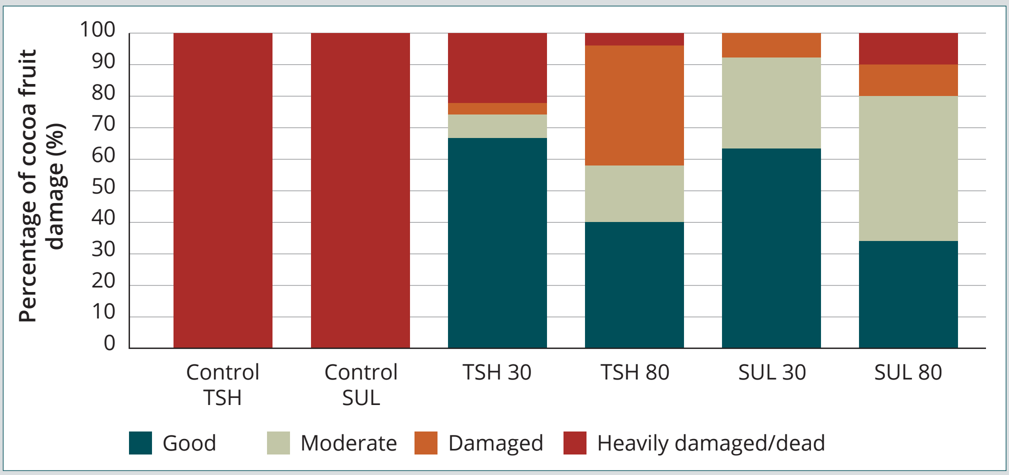

Figure 8.6 Percentage of cocoa pod damage

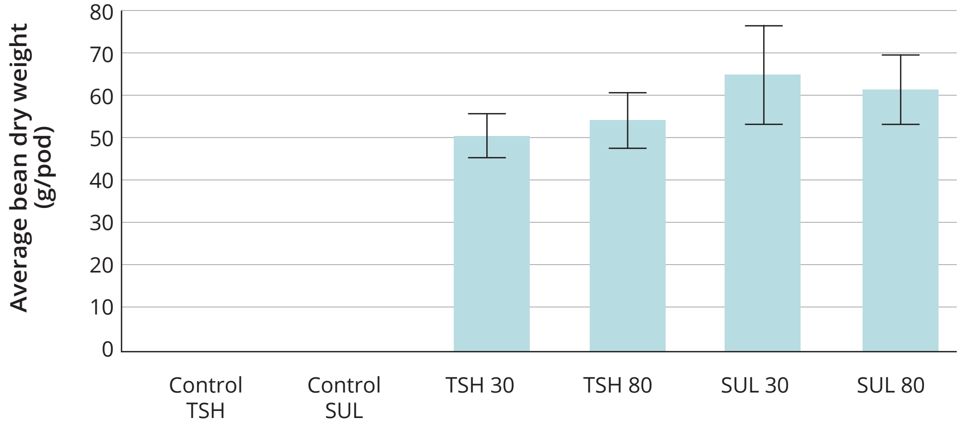

Figure 8.7 Bean dry weight/pod of the harvested cocoa

sleeve. This humid environment was good for fungal growth.

Bean dry weight of every pod is shown in Figure 8.7. The average of the bean dry weight was 57.99 g/pod. There was no significant difference between treatments, except for the control. The highest dry weight was 65.07 g/pod for SUL (30 μm).

Acknowledgments

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References

Afrizon & Rosmana, S 2002, 'Efektivitas komponen pengendalian hama penggerek buah kakao (PBK) terhadap tingkat serangan PBK di kabupaten kepahiang', *Jurnal Litbang Pertanian*, vol. 21, no. 2, pp. 69–74.

Azhar, I, Alias, A & Meriam, M 2000, 'Research on the cocoa pod borer in Malaysia', in *Proceedings Incoped 3rd International Seminar*, Kota Kinabalu, Sabah, Malaysia, 16–17 October, pp. 105–13.

Depparaba, F 2002, 'Penggerek buah kakao (*Conopomorpha cramerella* Snellen) dan penanggulangannya', *Jurnal Litbang Pertanian*, vol. 21, no. 2, pp. 69–74.

Dirjenbun 2016, *Statistik perkebunan Indonesia: kakao 2014–2016*, Jakarta.

Isroi, I, Supeni, G, Eris, DD & Cahyaningtyas, AA 2018, 'Biodegradability of cassava edible bioplastics in landfill soil and plantation soil', *Jurnal Kimia Dan Kemasan*, vol. 40, no. 2, pp. 129–40.

Jambeck, JR, Geyer, R, Wilcox, C, Siegler, TR, Perryman, M, Andrady, A, Narayan, R & Law, KL 2015, 'Plastic waste inputs from land into the ocean', *Science*, vol. 347, no. 6223, pp. 768–71.

Joseph, KC, Montepio, RC, Abenoja, RM & Tuyogon, RF 2017, 'Design, development and evaluation of a mechanical cacao pod bagger/sleever', *International Journal of Scientific & Engineering Research*, vol. 8, no. 5, pp. 585–9.

McMahon, P, bin Purung, H, Lambert, S, Mulia, S, Susilo, AW, Sulistyowati, E, Sukamto, S, Israel, M, Saftar, A, Amir, A, Purwantara, A, Iswanto, A, Guest, D & Keane, P 2015, 'Testing local cocoa selections in three provinces in Sulawesi: (i) Productivity and resistance to cocoa pod borer and *Phytophthora* pod rot (black pod)', *Crop Protection*, vol. 70, pp. 28–39.

to cocoa pod borer and *Phytophthora* pod rot (black pod)', *Crop Protection*, vol. 70, pp. 28–39.

Paper, C, Bakar, S, Cocoa, M, Ismail, A & Cocoa, M 2007, 'Implementation of cocoa pod sleeving in controlling cocoa pod borer', in *Proceedings 2007 Conference on Plant Plantation Commodities*, Kuala Lumpur, Malaysia, 3–4 July 2007.

Rosmana, A, Shepard, M, Hebbar, P & Mustari, A 2013, 'Control of cocoa pod borer and *Phytophthora* pod rot using degradable plastic pod sleeves and a nematode, *Steinernema carpocapsae*', *Indonesian Journal of Agricultural Science*, vol. 11, no. 2, pp. 41–7.

Saripah, B, Azhar, I, Board, MC & Floor, WS 2007, 'Implementation of cocoa pod sleeving in controlling cocoa pod borer infestation', in *Proceedings 2007 Conference on Plant Plantation Commodities*, Kuala Lumpur, Malaysia, 3–4 July 2007.

Wielgoss, A, Clough, Y, Fiala, B, Rumede, A & Tscharntke, T 2012, 'A minor pest reduces yield losses by a major pest: plant-mediated herbivore interactions in Indonesian cacao, *Journal of Applied Ecology*, vol. 49, no. 2, pp. 465–73.

Abstracts

Application of sex pheromone to manage cocoa pod borer pests

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Cocoa pod borer (CPB) is a major insect threat to cocoa-growing countries in South-East Asia. Crop loss to farmers can be up to 50%. Until now, management of CPB has heavily relied on pesticide applications, which are neither environmentally sustainable nor economically effective. Although sex attractants of CPB were identified in the 1980s, their application to control CPB was halted in the early 1990s for economic reasons and because commercial quantities were not available. In collaboration with many international collaborators, we confirmed the activity of identified CPB sex attractants in the Indo-Malayan archipelago in 2004. We modified a synthetic method for more economical production of CPB sex attractant. Field tests conducted in Malaysia during 2013–14 indicated that other nontarget compounds and impurities generated during this scale-up modification did not reduce the attractant's ability to attract CPB in the cocoa plantation. The effectiveness of an attract-and-kill strategy based on these impure attractants was tested during 2015–16, using high and low sex attractant loadings in attract-and-kill stations. Our results demonstrated that the attract-and-kill stations with lower dose loadings significantly reduced the number of CPB-infested cocoa pods. This indicated that the attract-and-kill strategy based on sex attractants has great potential to be an efficient, safe and environmentally friendly CPB control alternative in an integrated pest management program.

Low-toxicity insecticides to control cocoa pod borer (*Conopomorpha cramerella*)

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In Sulawesi, more than 90% of cocoa farmers rely on insecticide use to control cocoa pod borer (CPB) infestation. Our survey results showed that farmers are using many highly toxic insecticides. Many attempts have been made to identify low-toxicity insecticides that are effective in controlling CPB. A large replicated field experiment tested 10 different insecticides available to Indonesian cocoa farmers. Each tested insecticide had a different mode of action. Sleeving was used as a positive control treatment and water as a negative control treatment.

The experiment was implemented in an old cocoa orchard with high CPB infestation. Six

insecticide applications were made twice a year for 2 years in both of two annual harvest cycles. Fortnightly harvested cocoa pods were categorised by the level of CPB infestation (A, B, C, D). The criterion for determining the efficiency of tested insecticides was the total number of healthy pods (A) and pods with light infestation (B), versus the number of highly infested pods (C + D).

Two low-toxicity insecticides with high efficiency in controlling CPB were identified. Chlorantraniliprole (Prevathon; toxicity level IV) and thiamethoxam (Actara; toxicity level III) showed good CPB control. They gave proportions of A + B pods of 74.75% and 71.43%, respectively, which are close to the level for the positive control (87.03%). Results also showed that two of the most popular insecticides used by cocoa farmers from our survey—Nurelle (chlorpyrifos + cypermethrin) and chloride (chlorpyrifos alone; toxicity level I)—which are highly toxic, showed very poor performance in controlling CPB infestation. They gave levels of A + B pods of 45.76%, which is close to, or even lower than, the negative control. Farmers should be discouraged from using these insecticides and encouraged to use less toxic and more efficient insecticides to control CPB infestation in their cocoa crops.

Session 4

Cocoa pod borer rearing, and feasibility of the sterile insect technique

Abstracts

Area-wide integrated pest management and the sterile insect technique: principles and practice

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Most insect pests have, in the past, been managed by the liberal use of broad-spectrum insecticides that have a negative impact on the environment, leave residues on food commodities and are a severe threat to the health of the general public. In addition, many insect pests have developed resistance to the most commonly used insecticides. Therefore, new, innovative pest control tactics and strategies are needed that are both effective and not detrimental to the environment. As part of an arsenal of environmentally friendly control tactics, the sterile insect technique (SIT) has proved to be a very effective tool against selected insect pests when used as part of an area-wide integrated pest management (AW-IPM) approach. The SIT requires mass-rearing of the target insect in large facilities, sterilisation of the male insects, and sustained release of the sterile males in the target area. Mating of a sterile male with a virgin wild female results in no offspring.

Ionising radiation can be readily employed to effectively and safely induce sexual sterility in insect populations. The SIT has very often been associated with an eradication strategy, but major advances in rearing efficiency, and improved handling and release methods have made the use of sterile insects economically feasible for insect pest suppression, prevention or containment.

The action of sterile insects is unique and is inversely dependent on the density of the target population. In addition, sterile insects have the intrinsic capacity to actively search for and mate with the last individuals of a pest population. AW-IPM programs that integrate the use of sterile insects are often complex and management-intensive. However, past and current programs have shown the enormous benefit:cost ratios that these programs can generate, and their importance for enhanced agriculture is increasing.

Effect of antifungal agent on the performance of the cocoa pod borer artificial diet: preliminary study

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Cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen, is the most devastating insect pest for cocoa throughout South-East

Asia. An efficient CPB laboratory rearing system is greatly needed to facilitate many studies on CPB, including a sterile insect technique (SIT) program. An artificial diet that could sustain the CPB complete life cycle is being developed. Currently, successful moth emergence from the initial eggs transferred onto the artificial diet averages less than 20%. Efforts to improve the performance of the diet are ongoing. The main limitations remain fungal and bacterial contamination of the diet. A preliminary study was undertaken on the effect of sorbic acid as an antifungal agent to control or inhibit mould growth, and its effect on development and survival of CPB larvae. A single dose of 0.05% sorbic acid was tested. The effects of this antifungal agent were evaluated on CPB egg hatching, larval survival, adult moth emergence and mould growth. The experiment was done in three replicates with a sample size of 24 eggs per replicate. The results reported here were obtained 3 weeks after placement of eggs onto the artificial diet.

Artificial diet for enhancing research and implementation of cocoa pod borer management: learning from the Asian corn borer

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Cocoa pod borer (CPB), *Conopomorpha cramerella*, is one of the major pests in several cocoa-producing countries in South and South-East Asia. This insect damages the pods, causing significant economic loss. After hatching, the larvae tunnel into the husk and live inside the pod; this leaves a narrow biological window for managing the insect. Research to develop a technology for insect pest management is often hampered by the limited number of insects available for experiments. The role of artificial diets in supporting laboratory and field research has been long realised, and many lepidopteran insect diets have been successfully developed. However, not every attempt to develop an artificial diet for a certain insect has resulted in an established laboratory mass-rearing procedure. From the published research and our experience to establish an artificial diet for Asian corn borer (ACB), *Ostrinia nubilalis*, four major components contribute to success: the microenvironment for rearing, the nutritional contents of the diet, the physical characteristics of the diet, and understanding of the biology of the insect. Using an artificial diet, we have produced hundreds of thousands of newly emerged larvae of ACB per day, to allow us to test the resistance of corn to this insect in the field. Progress and challenges in developing an artificial diet for CPB, and the relevance to the artificial diet for ACB will be discussed during the presentation.

Sex pheromone lures as a monitoring tool for cocoa pod borer, *Conopomorpha cramerella* Snellen

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Cocoa pod borer (CPB), *Conopomorpha cramerella* Snellen, is the most devastating

pest of cocoa in the South-East Asia and Pacific archipelagos. It is currently responsible for an average of 40–60% loss of cocoa production. Pesticides have not been a satisfactory solution to control this insect pest. Development and implementation of pest control technology based on behaviour-controlling chemicals, or semiochemicals, offer a unique opportunity to move in a new direction. Semiochemicals are chemical messages that organisms use to communicate with each other. Among the semiochemicals, insect sex pheromones have probably received the most attention from the scientific, regulatory and agricultural communities. Sex pheromones show promise as monitoring tools for CPB. By their nature, sex pheromones are highly specific and would not disrupt other biological interactions within a cropping system. To understand the potential of sex pheromone lures of CPB, and how we can improve our monitoring capability for research, we have compared pheromone sources, various formulations, doses and longevity in the cocoa field.

Session 5

Updates on *Phytophthora* research

9 Biological control of cocoa pod rot, *Phytophthora palmivora*, using *Trichoderma harzianum* as an endophyte

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Abstract

Phytophthora pod rot (PPR) is the most dangerous disease of cocoa, and reduces cocoa yield in Indonesia. Controlling pod rot is usually based on integrated pest management practices that include biological control using fungal species in the genus *Trichoderma*. Use of endophytic fungi on perennial plants is known to increase plant growth, and induce plant resistance and tolerance to abiotic stress, including drought. To improve the effectiveness of antagonism of fungi for controlling PPR, the possible use of *T. harzianum* as an endophyte was studied in three stages. The first stage was in vitro assessment of the antagonistic activity of *T. harzianum* against *Phytophthora palmivora*, the second was establishment of *T. harzianum* as an endophyte, and the third was application of various concentrations of *T. harzianum* as an endophyte on cocoa pods and seedlings. The trial used a completely randomised block design with four replications. The results showed that *T. harzianum* isolates Jember and Banyuwangi were able to inhibit the growth of *P. palmivora* in vitro by antibiosis and competition, and that these isolates in concentrations of 108, 109 and 1010 spore/mL became established as endophytes in cocoa seedlings. Colony growth of endophytic *T. harzianum* was found in leaves and roots of cocoa seedlings. Application of *T. harzianum* isolates as endophytes in concentrations of 10^8 to 10^{10} spore/mL did not induce cocoa seedlings with resistance against *P. palmivora*.

Keywords: cocoa, *Phytophthora palmivora*, biological control, *Trichoderma harzianum*, endophytes

Introduction

Phytophthora palmivora is one of the main pathogens on cocoa and causes significant production losses in Indonesia. *Phytophthora* is a genus of the Oomycota belonging to the order Pythiales and the family Pythiaceae. This genus is known as a plant destroyer (Luz & Silva 2001). *P. palmivora* causes pod rot, seedling blight, stem canker, leaf blight, root rot and chupon wilt on cocoa (Drenth & Guest 2004).

Cultural practices, the use of resistant material and the use of chemicals have been the traditional methods for controlling this disease (Thevenin et al. 2005). Environmental problems associated with the use of chemicals mean that a new approach is needed to pest and disease management. Hence, a range of other management strategies are being developed. Biological control is one such new approach. Biological control offers a potential alternative control measure for plant diseases and is a promising tool for agricultural production (Kaur, Kaur & Singh 2010).

The genus *Trichoderma* comprises a large number of fungal strains that act as biological control agents. *Trichoderma* species are free-living fungi that occur in nearly all soils and other natural habitats (Sharma & Gothalwal 2017). *Trichoderma* species are typically considered soilborne organisms associated with plant roots. They have been considered for their potential to control plant disease because of their close association with plants, with many aspects typical of endophytic associations (Bailey et al. 2008; Harman et al. 2004). Modes of action of *Trichoderma* include competition and antagonism with pathogens, production of certain compounds against pathogenic fungi, parasitism on pathogenic fungi, and induction of the self-defence mechanism of plants. *Trichoderma* improves plant health and vigour, and may perhaps stimulate nutritional uptake when abundant populations are established in the root zone. *Trichoderma* has a protective and immunising effect on the host plant. It can survive long term within the host plant without causing any damage to the plant (Nederhoff 2001).

In this study, we evaluated the ability of the biological agent *T. harzianum* as an endophyte and as a biological agent to control *P. palmivora* on ICCRI 03 and TSH 858 cocoa clones.

Materials and methods

Fungal isolates

A culture of *P. palmivora* was isolated from a cocoa pod that was infected by pod rot. Cultures of *T. harzianum* isolates Banyuwangi (ThB) and Jember (ThJ) were obtained from a previous experiment (McMahon et al. 2015). The fungi were transferred onto potato dextrose agar (PDA) slants and maintained as stock cultures for this experiment.

Cocoa clones ICCRI 03 and TSH 858

Cocoa clones ICCRI 03 (moderately resistant to *Phytophthora* pod rot) and TSH 858 (susceptible to *Phytophthora* pod rot) were obtained from the experimental garden of the Indonesian Coffee and Cocoa Research Institute.

In vitro assessment of the antagonistic activity of *T. harzianum* against *P. palmivora*

T. harzianum isolates ThB and ThJ were assessed for their in vitro antagonistic activity against *P. palmivora* by a dual-culture method (Dennis & Webster 1971) using PDA. A mycelial disc (5 mm) obtained from the peripheral region of the colony of a 7-day-old culture of targeted *P. palmivora* was placed on PDA at the edge of each plate, 3 cm from the periphery. Then a disc of mycelium 5 mm in diameter cut from the growing edge of a 7-day-old culture of *T. harzianum* was placed on each plate, opposite the *P. palmivora* inoculum, 3 cm from the periphery. A completely randomised design (CRD) was used, with 10 replicates for each isolate. In the control plate, a sterile agar disc was inoculated on the side opposite *P. palmivora*. All the innoculated plates were incubated at room temperature (25 ± 2 °C) for 9 days until the control plate was full. During the incubation period, growth of *P. palmivora* was measured daily, and the percentage growth inhibition (GI) was calculated relative to the control, as follows:

$$GI = ((D1 - D2)/D1) \times 100\%,$$

where

D1 is diameter of pathogen colony in control

D2 is diameter of pathogen colony in the treatment.

Establishment of *T. harzianum* as an endophyte

Seeds with a radicle 3 cm long were inoculated with *T. harzianum* (ThJ and ThB) by dipping into spore suspension (1×10^8 spore/mL, 1×10^9 spore/mL, 1×10^{10} spore/mL) for 2 hours. Seeds were planted into sterile soil in a polybag, consisting of soil and organic fertiliser (2:1 ratio). The establishment of *T. harzianum* as an endophyte in the plant tissue was evaluated by dissecting root and leaf tissue every 4 weeks for 3 months. Then the root and leaf tissue were isolated on PDA media. *T. harzianum* that emerged, and colonised root and leaf tissue was identified as endophytic fungi.

Evaluation of *T. harzianum* for controlling *Phytophthora* pod rot of two cocoa clones (ICCRI 03 and TSH 858) in the laboratory

In a moisture chamber, where temperature and humidity were maintained, five treatments were arranged in CRD with five replications of five pods. Cocoa pods of ICCRI 03 and TSH 858 were inoculated with *T. harzianum* (ThJ and ThB) by dipping into spore suspension (1×10^8 spore/mL, 1×10^9 spore/mL, 1×10^{10} spore/mL). Then ICCRI 03 and TSH 858 cocoa clones were inoculated with *P. palmivora* and incubated for 7 days at 25 °C in the moisture chamber until the control pods were fully infected with pod rot. During the incubation period, black spot symptoms on the cocoa pod were measured daily. Data were analysed using analysis of variance (ANOVA). Treatment means were compared using Duncan's multiple range test (DMRT).

Evaluation of *T. harzianum* for controlling seedling blight *Phytophthora* of two cocoa clones (ICCRI 03 and TSH 858)

Within each cocoa clone, seven treatments were arranged in CRD with four replications of five seedlings. Seedlings were inoculated with *T. harzianum* (ThJ and ThB) by dipping into spore suspension (1×10^8 spore/mL, 1×10^9 spore/mL, 1×10^{10} spore/mL) for 30 minutes. Seedlings were planted into sterile soil in a polybag, consisting of soil and organic fertiliser (2:1 ratio). *T. harzianum* was evaluated as a biocontrol agent by placing leaves from the cocoa seedlings into a moisture chamber in the laboratory and inoculating them with *P. palmivora*. The inoculated leaves were incubated for 9 days until the control leaves were fully infected with *P. palmivora*. During the incubation period, the spot area of *P. palmivora* was measured daily. Data were analysed using ANOVA. Treatment means were compared using DMRT.

Results and discussion

In vitro assessment of the antagonistic activity of *T. harzianum* against *P. palmivora*

The inhibition ability of *T. harzianum* against *P. palmivora* is shown in Table 9.1.

Table 9.1 Assessment of antagonistic activity of *T. harzianum* on colony growth of *P. palmivora* using a dual-culture antagonistic test

Biocontrol agent	Growth inhibition (%)			Mechanism		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
T. harzianum Banyuwangi (ThB)	33.95	68.75	75.54	A	C	C
T. harzianum Jember (ThJ)	43.46	65.97	76.6	A	C	C

A = antibiosis; C = competition; DAI = days after inoculation

T. harzianum significantly reduced the colony growth of *P. palmivora* at 3 days after inoculation. At 7 days after inoculation, inhibition of *P. palmivora* by ThB and ThJ was 75.54% and 76.6%, respectively. The main mechanism of inhibition in vitro was attributed to antibiosis and competition. Antibiosis of nonvolatile metabolites was more pronounced at the early stage of incubation and was followed by competition, which could be involved in the antagonistic process against *P. palmivora* (Figure 9.1).

The ability of endophytic *Trichoderma* to inhibit the growth of *P. palmivora* was shown by the inhibition zones where the *T. harzianum* colony met the *P. palmivora* colony. Colony growth of *T. harzianum* clearly suppress *P. palmivora*.

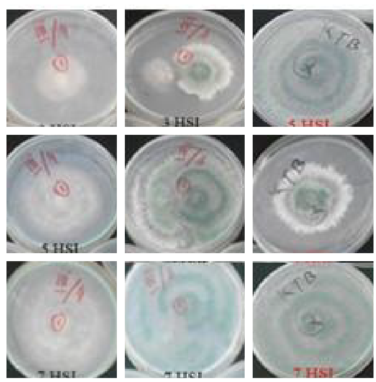

Figure 9.1 Mono and dual culture of *P. palmivora* and *T. harzianum* on potato dextrose agar

Establishment of *T. harzianum* as an endophyte on cocoa plant tissue

Observations of ICCRI 03 and TSH 858 clones showed that the fungus still colonised root and leaf tissue 1 month after the inoculation of *T. harzianum* into the seeds. It showed that *T. harzianum* could successfully penetrate into healthy tissue. This showed the ability of *T. harzianum* to act as an endophyte (Figures 9.2–9.5).

Evaluation of *T. harzianum* for controlling *Phytophthora* pod rot of two cocoa clones (ICCRI 03 and TSH 858) in the laboratory

Pod rot symptoms on clone TSH 858 were more severe than on clone ICCRI 03. This indicates that the type of clone affected the incidence of cocoa pod rot disease. Use of *T. harzianum* as a biocontrol agent will therefore be more effective when combined with the use of resistant planting materials.

Figure 9.5 shows that ThB concentrations of 10^8, 10^9 and 10^{10} spore/mL were effective in suppressing the development of *P. palmivora* on ICCRI 03. In the susceptible clone TSH 858, ThB isolates were less effective in suppressing *P. palmivora* pod rot.

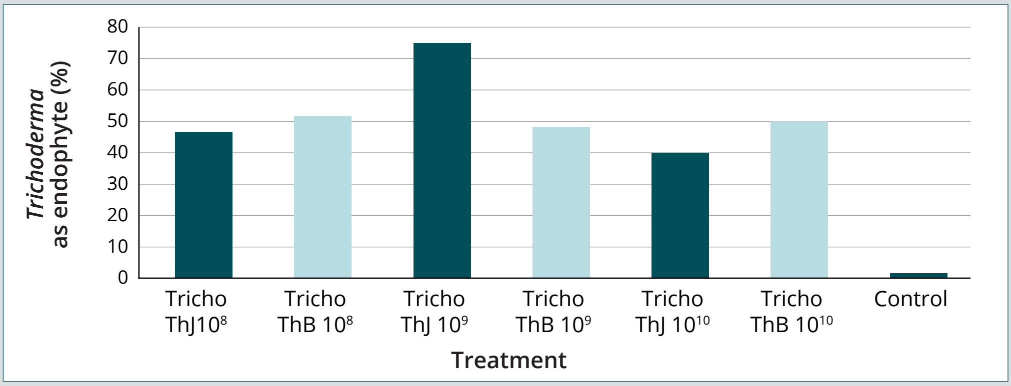

Figure 9.2 Percentage colonisation by *T. harzianum* as an endophyte on root tissue of cocoa clone ICCRI 03

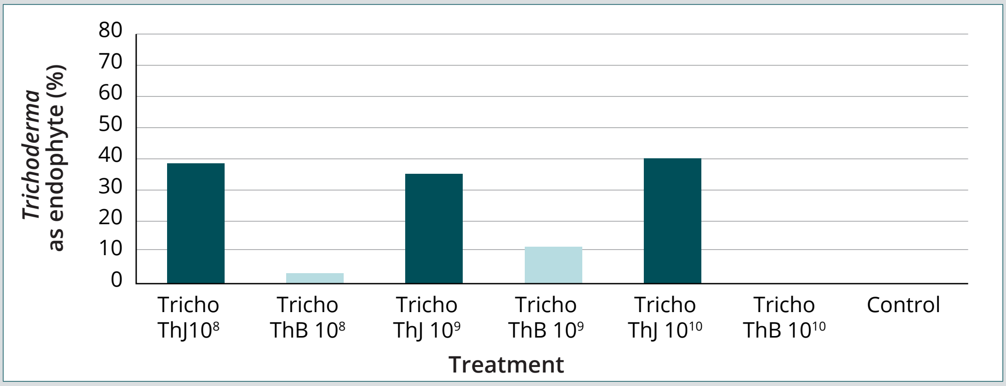

Figure 9.3 Percentage colonisation by *T. harzianum* as an endophyte on leaf tissue of cocoa clone ICCRI 03

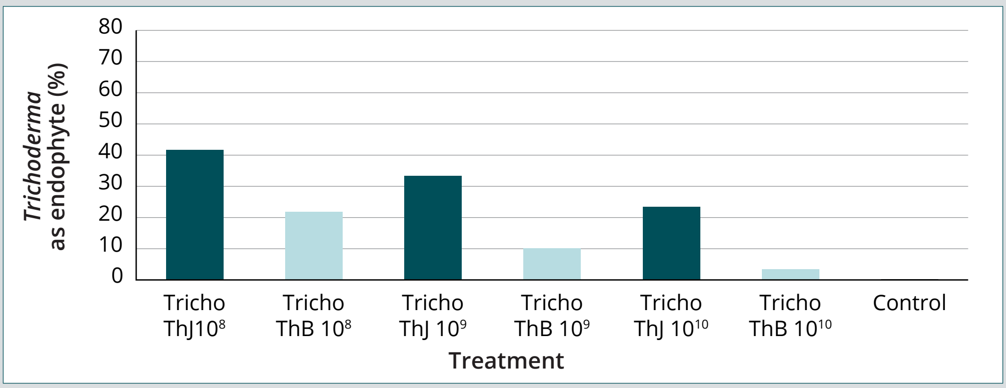

Figure 9.4 Percentage colonisation by *T. harzianum* as an endophyte on root tissue of cocoa clone TSH 858

Evaluation of *T. harzianum* for controlling seedling blight *Phytophthora* of two cocoa clones (TSH 858 and ICCRI 03) in the laboratory

Leaves of clones TSH 858 and ICCRI 03 that were inoculated with *P. palmivora* alone were severely rotten, with black spot areas of 83.7 and 77.12 cm^2. The treatment with ThJ at a concentration of 10^{10} spore/mL on TSH 858 and ICCRI 03 showed the smallest black spot areas of 59.48 and 55.05 cm^2. All treatments showed disease suppression below 30% (Tables 9.2 and 9.3). This indicates that *T. harzianum* ThJ and ThB as endophytes did not induce resistance of cocoa seedlings to *P. palmivora*.

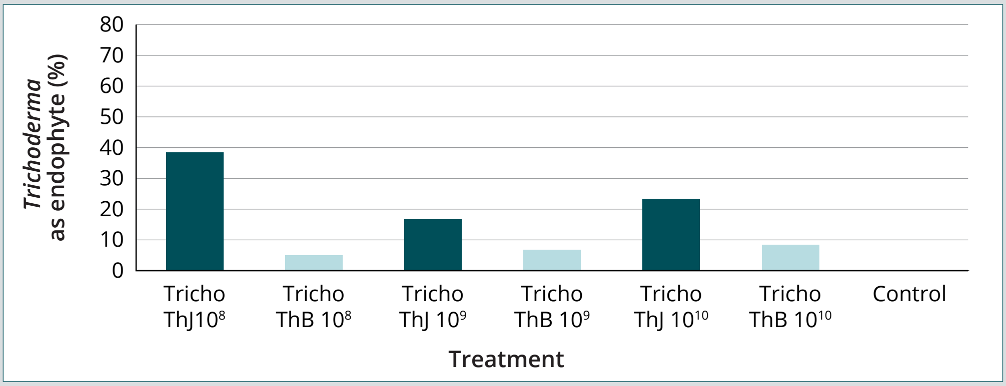

Figure 9.5 Percentage colonisation by *T. harzianum* as an endophyte on leaf tissue of cocoa clone TSH 858

Table 9.2 Black spot area on leaves of clone ICCRI 03 induced endophytically with *T. harzianum*

Isolate	Spore concentration (spore/mL)	Spot area (cm^2)	Effectivity (%)
T. harzianum Jember (ThJ)	10^8	62.80[a]	18.57
	10^9	60.58	21.45
	10^{10}	55.05	28.62
T. harzianum Banyuwangi (ThB)	10^8	73.54	4.64
	10^9	66.57[a]	13.68
	10^{10}	62.95[a]	18.37
Control: *P. palmivora*	na	77.12	na

na = not applicable
Notes:
1. Means labelled with the same letter (a) were not significantly different.
2. Data are shown for 6 days after inoculation.

Table 9.3 Black spot area on leaves of clone TSH 858 induced endophytically with *T. harzianum*

Isolate	Spore concentration (spore/mL)	Spot area (cm²)	Effectivity (%)
T. harzianum Jember (ThJ)	10^8	67.63[a]	19.20
	10^9	67.61[a]	19.22
	10^{10}	59.48	28.94
T. harzianum Banyuwangi (ThB)	10^8	81.85[b]	2.21
	10^9	80.71	3.57
	10^{10}	71.31[a]	14.80
Control: *P. palmivora*	na	83.70[b]	na

na = not applicable
Notes:
1. Means labelled with the same letter (a, b) were not significantly different.
2. Data are shown for 6 days after inoculation.

References

Bailey, BA, Bae, H, Stream, MD, Crozier, J, Thomas, SE, Samuel, GJ, Vinyard, BT & Holmes, KA 2008, 'Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*', *Biological Control*, vol. 46, pp. 24–35.

Dennis, C & Webster, J 1971, 'Antagonistic properties of species-groups of *Trichoderma*: II. Production of volatile antibiotics', *Transactions of the British Mycological Society*, vol. 57, pp. 363–9.

Drenth, A & Guest, DI 2004, *Diversity and management of* Phytophthora *in Southeast Asia*, ACIAR monograph 14, Australian Centre for International Agricultural Research, Canberra.

Harman, GE, Howell, CR, Viterbo, A, Chet, I & Lorito, M 2004, '*Trichoderma* species: opportunistic, avirulent plant symbionts', *Nature Reviews Microbiology*, vol. 2, pp. 43–56.

Kaur, R, Kaur, J & Singh, RS 2010, 'Nonpathogenic *Fusarium* as a biological control agent', *Plant Pathology*, vol. 9, pp. 79–91.

Luz, EDMN & Silva, SDVM 2001, 'Podridão-parda dos frutos, cancro e outras doenças causadas por *Phytophthora* no cacaueiro', in EDMN Luz, AF dos Santos, K Matsuoka & JL Bezerra (eds), Doenças causadas por Phytophthora no Brasil, Editora Rural, São Paulo, Brazil, pp. 175–265.

McMahon, P, bin Purung, H, Lambert, S, Mulia, S, Nurlaila, Susilo, AW, Sulistyowati, E, Sukamto, S, Israel, M, Saftar, A, Amir, A, Purwantara, A, Iswanto, A, Guest, D & Keane, P 2015, 'Testing local cocoa selections in three provinces in Sulawesi: (i) Productivity and resistance to cocoa pod borer and *Phytophthora* pod rot (black pod)', *Crop Protection*, vol. 70, pp. 28–39.

Nederhoff, E 2001, 'Biological control of root disease especially with *Trichoderma*', *Grower*, vol. 56, pp. 24–5.

Sharma, PK & Gothalwal, R 2017, '*Trichoderma*: a potent fungus as biological control agent', in JS Singh & G Seneviratne (eds), *Agro-environmental sustainability*, Springer, pp. 113–25.

Thevenin, JM, Umaharan, R, Surujdeo-Maharaj, S, Latchman, B, Cilas, C & Butler, DR 2005, 'Relationship between black pod and witches'-broom diseases in *Theobroma cacao*', *Phytopathology*, vol. 95, pp. 1301–7.

10 Use of fungicide and soluble silicon for control of vascular streak dieback

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Abstract

Vascular streak dieback (VSD) is one of the limiting factors in cocoa production in Malaysia. The pathogen *Ceratobasidium theobromae* can kill branches of mature cocoa trees, and serious damage may occur if the pathogen infects cocoa seedlings less than 10 months old. Common practices for controlling VSD are using fungicides, resistant planting materials and cultural practices. A promising alternative method for managing crop diseases is the application of silicon as a physical barrier to pathogen penetration. Little is currently known about the effectiveness of silicon for controlling *C. theobromae*. Our study on screening of fungicides demonstrated that difenoconazole is the most effective for controlling VSD. In vitro evaluation of silicon shows good potential for suppressing the growth of *C. theobromae*. We hope that the effectiveness of difenoconazole can be improved by incorporating silicon.

Keywords: vascular streak dieback, *Ceratobasidium theobromae*, soluble silicon

Introduction

Vascular streak dieback (VSD) is a major systemic disease of cocoa (*Theobroma cacao* L.). It occurs in the west Pacific and South-East Asian regions (Guest & Keane 2007; Keane & Prior 1991). The pathogen *Ceratobasidium theobromae* (syn. *Thanatephorus theobromae*, *Oncobasidium theobromae*) can kill branches of mature cocoa trees, and serious damage may occur if the pathogen infests cocoa seedlings less than 10 months old (Guest & Keane 2018). Several practices have been applied to manage this disease, including fungicides, biological control, resistant planting materials, quarantine and cultural practices. Currently, the used of silicon (Si) to manage crop diseases is a promising alternative. Plants supplied with Si can produce chitinases, peroxidases, phenolics, phytoalexins and polyphenoloxydases that can enhance their defence mechanisms in response to fungal infection (Chérif, Asselin & Bélanger 1994; Remus-Borel, Menzies & Bélanger 2005). Our study on screening of fungicides demonstrated that difenoconazole is effective in controlling VSD under in vitro conditions and at the seedling stage. However, it is not commercially viable in established field plantations. Since Si shows good potential for suppressing the growth of *C. theobromae* in vitro, we hope that the effectiveness of difenoconazole can be improved by incorporating Si.

Materials and methods

In vitro screening of fungicides

Fungicides were first screened under in vitro conditions. Four different fungicides, with the active ingredients difenoconazole, hexaconazole, propiconazole and tebuconazole, were tested to evaluate their effect on colony growth of the pathogen using the poison food technique. The required quantity of each fungicide was added to coconut water agar plates to obtain final concentrations of 1, 2, 5, 10 and 20 ppm. A disc of *C. theobromae* culture was then centrally inoculated onto these media. Untreated medium was used as a control. Each treatment was replicated 10 times. The colony diameter of the pathogen was recorded daily for 7 days.

Screening of fungicides on cocoa seedlings

Fungicides were further evaluated on cocoa seedlings by selecting the three best fungicides from the in vitro study: difenoconazole, tebuconazole and propiconazole. Healthy cocoa seedlings at 3 months of age were naturally infected with VSD by placing them under infected cocoa trees in a cocoa field. Fungicide solutions were prepared as recommended by the manufacturers and sprayed at 2-week intervals. Disease severity based on the intensity of natural infection was scored monthly for 12 months.

Screening of fungicides on mature trees

Tebuconazole and difenoconazole were selected because of their effectiveness in controlling VSD at the seedling stage. Eight-year-old mature cocoa trees were used, and all infected branches were removed before evaluation. Fungicides were applied by spraying over tree branches every 2 weeks following the manufacturers' recommended rates. Data were recorded using a disease scoring index.

In vitro screening of soluble silicon

The effectiveness of soluble Si in inhibiting the mycelial growth of *C. theobromae* was evaluated in vitro using the poison food technique, as described above. Five different concentration of Si were tested: 0.5%, 1.0%, 1.5%, 2.0% and 2.5 % (v/v). Potassium silicate was used as the positive control. Each treatment was replicated 10 times. The diameter of the pathogen colony was recorded daily for 7 days.

Results and discussion

Evaluation of fungicides in controlling *C. theobromae*

Systemic fungicides belonging to the triazole group had shown promise in reducing the incidence of VSD in cocoa nurseries and field plantations (Holderness 1990). Preliminary screening of fungicides (difenoconazole, hexaconazole, propiconazole and tebuconazole) was conducted in vitro. Figure 10.1 presents the colony diameter of *C. theobromae* after 7 days of incubation on media impregnated with each fungicide. It was found that tebuconazole is the most effective fungicide, as no pathogen could grow at any concentration tested. This was followed by difenoconazole (5, 10 and 20 ppm) and propiconazole (10 and 20 ppm). This result is supported by a previous study in Papua New Guinea that found that foliar spray of tebuconazole gave effective protection against VSD (Holderness 1990).

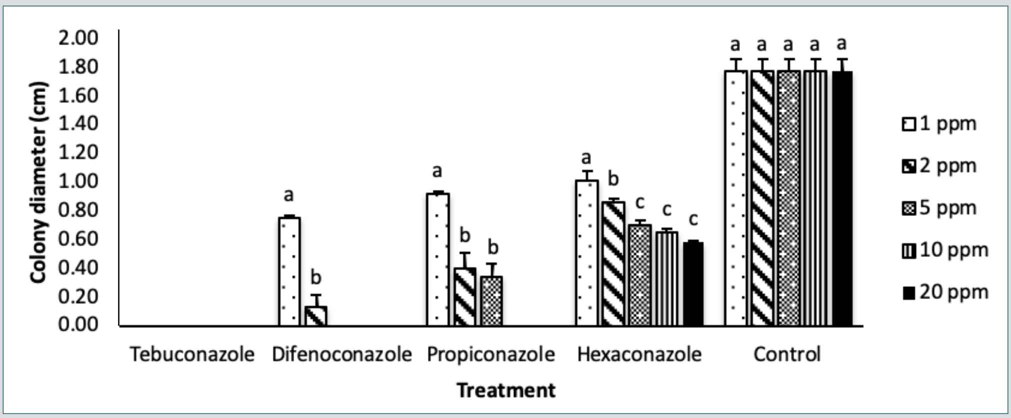
The effectiveness of three fungicides was further tested on seedlings. Based on disease severity, difenoconazole showed the best effect, followed by tebuconazole and propiconazole (Figure 10.2). On exposure to disease pressure in the cocoa field, seedlings treated with difenoconazole and tebuconazole remained without any disease symptoms for up to 5 months. All other seedlings started showing disease symptoms after 3 months. The severity of disease in seedlings treated with difenoconazole and tebuconazole was also significantly lower than for other treatments. It is suggested that difenozonazole and tebuconazole have a longer protective effect and can suppress disease for up to 1 year.

The effect of difenoconazole and tebuconazole on mature cocoa trees is shown in Figure 10.3. These fungicides were effective in reducing VSD symptoms for 8–11 months after application. However, in the following months, disease was not suppressed, giving negative disease reduction values. This shows that difenoconazole and tebuconazole are not really viable for use in established cacao plantations in the long term. Guest and Keane (2018) reported that flutriafol, hexaconazole, propiconazole, tebuconazole and triadimenol have been effectively used to control VSD under experimental and nursery conditions in Malaysia. However, none of these fungicides is successful in the cacao field. Therefore, different methods might need to be investigated to enhance the effectiveness of these chemicals in controlling VSD in the cocoa field.

Evaluation of soluble silicon in controlling *C. theobromae*

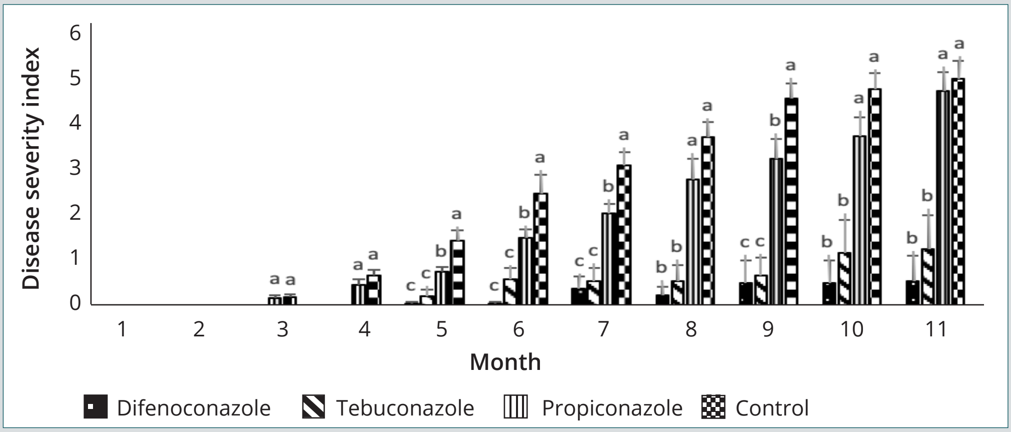
A preliminary result on evaluation of Si is shown in Figure 10.4. Si almost completely suppressed the growth of *C. theobromae,* with a colony diameter of 0.10 cm at a concentration of 0.25%, compared with 2.01 cm in control plates. Colony diameters of 0.31 cm and 0.77 cm were recorded by plates impregnated with 0.20% and 0.15% of Si, respectively. Potassium silicate did not show a significant effect on colony growth compared with the control.

Inhibition of the colony diameter of the pathogen clearly indicated that Si has a



Note: Means of colony diameter in the same treatment followed by the same letter are not significantly different according to the Duncan test ($P \geq 0.05$).

Figure 10.1 Laboratory evaluation of effect of fungicides on colony diameter of *C. theobromae*



Note: Means of disease severity index in the same month followed by the same letter are not significantly different according to the Duncan test ($P \geq 0.05$).

Figure 10.2 Disease severity index in cocoa seedlings treated with difenoconazole, tebuconazole and propiconazole

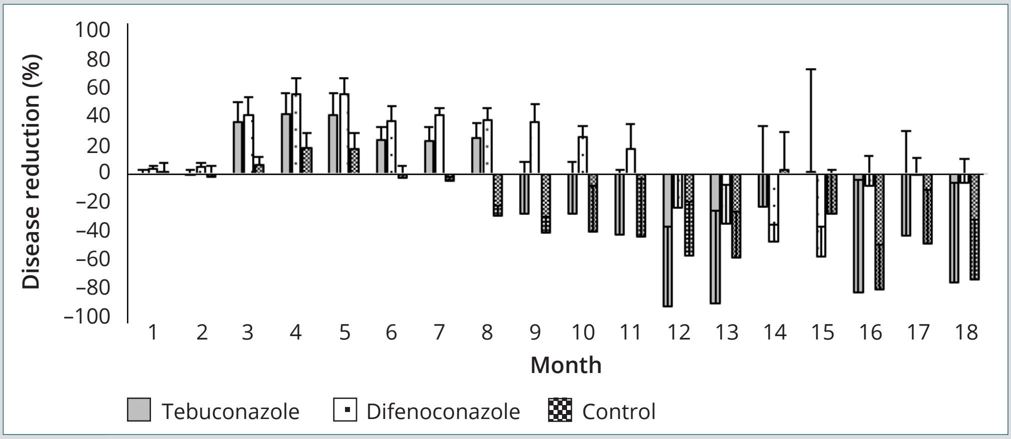
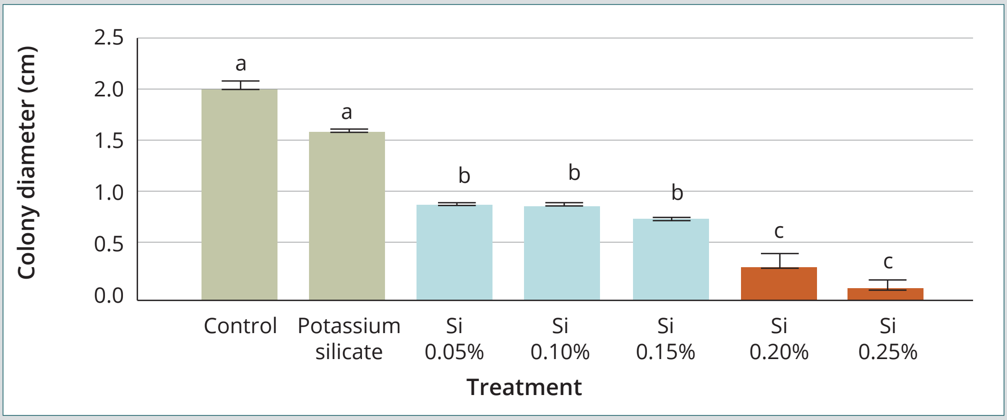


Figure 10.3 Disease severity index in mature cocoa trees treated with difenoconazole and tebuconazole

fungicidal activity that suppresses the growth of *C. theobromae*. Several authors have reported promising results of Si application for control of diseases such as rice blast (*Pyricularia grisea*), brown spot of rice (*Bipolaris oryzae*), anthracnose of sorghum (*Colletotrichum sublineolum*) and sugarcane rust (*Puccinia melanocephala*) (Naidoo et al. 2009; Prabhu et al. 2012; Resende et al. 2013). Evaluation of the effect of Si on cocoa seedlings and mature trees is ongoing in this study. Since Si had shown good potential to control *C. theobromae* under in vitro conditions, incorporation of Si with previously tested fungicides (difenoconazole and tebuconazole) might be effective in suppressing VSD in established cocoa plantations. Further study on this combination is needed to come up with a better approach for controlling VSD.



Si = silicon
Note: Means of colony diameter in the same treatment followed by the same letter are not significantly different according to the Duncan test ($P \geq 0.05$).

Figure 10.4 Laboratory evaluation of silicon on colony diameter of *C. theobromae*

References

Chérif, M, Asselin, A & Bélanger, RR 1994, 'Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp.', *Phytopathology*, vol. 84, pp. 236–42.

Guest, DI & Keane, PJ 2007, 'Vascular-streak dieback: a new encounter disease of cacao in Papua New Guinea and Southeast Asia caused by the obligate basidiomycete *Oncobasidium theobromae*', *Phytopathology*, vol. 97, pp. 1654–7.

—— & Keane, PJ 2018, 'Cacao diseases: vascular-streak dieback', in P Umaharan (ed.), *Achieving sustainable cultivation of cocoa*, Burleigh Dodds Science Publishing, Cambridge, UK.

Holderness, M 1990, 'Control of vascular-streak dieback of cocoa with triazole fungicides and the problem of phytotoxicity', *Plant Pathology*, vol. 39, no. 2, pp. 286–93.

Keane, PJ & Prior, C 1991, 'Vascular-streak dieback of cocoa', Phytopathological Papers No. 33, CAB International, Mycological Institute IV Series, p. 40.

Naidoo, PV, McFarlane, S, Keeping, MG & Caldwell, PM 2009, 'Deposition of silicon in leaves of sugarcane (*Saccharum* spp. hybrids) and its effect on the severity of brown rust caused by *Puccinia melanocephala*', *Proceedings of South Africa Sugar Technology*, vol. 82, pp. 542–6.

Prabhu, AS, Barbosa Filho, MP, Datnoff, LE, Snyder, GH, Berni RF, Rodrigues, FA & Dallagnol, LJ 2012, 'Silicon reduces brown spot severity and grain discoloration on several rice genotypes', *Tropical Plant Pathology*, vol. 37, pp. 409–14.

Resende, RS, Rodrigues, FA, Gomes, RJ & Nascimento, KJT 2013, 'Microscopic and biochemical aspects of sorghum resistance to anthracnose mediated by silicon', *Annals of Applied Biology*, vol. 163, pp. 114–23.

Remus-Borel, W, Menzies, JG & Bélanger, RR 2005, 'Silicon induces antifungal compounds in powdery mildew-infected wheat', *Physiological and Molecular Plant Pathology*, vol. 66, pp. 108–15.

11 Observations on cocoa planting, and pest and disease incidence in the highlands of Papua New Guinea

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Abstract

Cocoa, the third major cash crop of Papua New Guinea (PNG), is grown in the 14 maritime provinces of PNG. Production during the past 44 years has been around 40,000 tonnes, and the national government has set a target to increase production by more than 500% by 2030. In early 2008, 10 crosses of the commercially recommended SG2 hybrids were introduced on 32 ha of state land on Karimui station. Eighteen recommended cocoa clones were introduced in November 2017 to interested farmers.

Farmers are trialling these materials at varying altitudes, from 900 m to more than 1,300 m. Anecdotal reports indicate that cocoa can perform at these altitudes at a level comparable to that in traditional coastal provinces. Trees were slow to grow and required better shade to thrive well; exposure to direct sunlight caused stunted tree growth. To date, major and serious cocoa diseases commonly found in maritime growing areas have not been observed at significantly damaging levels. Negligible *Phytophthora* seedling blight has been observed. Incidences of *Phytophthora* pod rot, soft rot and pink disease have also been relatively low. To date, no vascular streak dieback has been observed. The major insect pest problems observed are caused by mirids damaging cherelles and developing pods. Grey weevil attacks are commonly observed at the early planting stage and subside as young plants mature. The pest and disease survey is continuing; however, fully replicated trials are needed to determine cocoa performance against the major PNG pests and diseases of cocoa in the highlands.

It appears that it may be possible to select superior types of cocoa, adapted to producing well at higher altitudes, from among the great genetic diversity of types initially established through the introduction of SG2 hybrid seedlings. The superior types are being selected and propagated by farmers. Nonconventional methods and propagation strategies are applied to spread plantings. Farmers are empowered with vegetative propagation skills and knowledge to field graft on their own farms when location-specific materials from trials and farmers' own selections become available. Four neighbouring highland provinces are now interested in trialling cocoa as an alternative cash crop following the recent incursion of the coffee pest coffee berry borer.

Keywords: Cocoa Model Farm, pest and disease incidence

Introduction

Cocoa, the third most important cash crop of Papua New Guinea (PNG), is grown traditionally in the 14 lowland provinces of PNG, contributing 17% of the nation's agricultural commodity revenue. Annual production over the past 44 years has averaged around 40,000 t of dry beans. The national government and the cocoa industry have set an ambitious target to increase production by more than 500% by 2030.

Phytophthora pod rot (*Phytophthora palmivora*) commonly has an incidence of 10% in the lowland provinces and up to 30% in wetter areas. Seedling blight caused by *P. palmivora* in the nurseries is common during the wet season. Vascular streak dieback (VSD) caused by *Oncobasidium* (*Ceratobasidium*) *theobromae* is common throughout the country, except in New Ireland and Bougainville. Pink disease (*Corticium salmonicolor*) and *Botryodiplodia* soft rot of pods are also common. Pests of common concern in these traditional growing areas are mirids, attacking pods, and longicorns, attacking stems, associated with stem and branch canker caused by *P. palmivora*.

Yinil et al. (2017) documented where cocoa can be grown successfully in PNG, including a preliminary report of successful testing of a cocoa planting at Karimui at an altitude of 1,200 m in Simbu province. In 2009, following earlier discussions with Simbu Department of Primary Industry (DPI) officers (J Konam & P Epaina, pers. comm.), staff of the PNG Cocoa Coconut Institute (CCI) set up a cocoa trial in Karimui, an area with a high population and excellent agricultural potential but lacking road access. Internationally, cocoa has been grown up to an altitude of only about 600 m (Wood & Lass 1985), and so trialling cocoa at twice that altitude was ambitious.

Seedlings from 10 crosses between two locally adapted Trinitario male parents (K82, KA2-106) and six Upper Amazonian female parents (KEE5, KEE12, KEE23, KEE42, KEE43, KEE47), known as SG2 hybrids, were planted in April–May 2009 at four planting densities (4 m × 2.5, 3.0, 3.5 or 4.0 m) under *Gliricidia sepium* shade over 32 ha of land formerly used to grow coffee and vegetables. Five of these crosses produced offspring with vigorous growth (SG2-Big) and five with less vigorous growth (SG2-Small). The area has highly fertile young volcanic soils derived from basalt. The average annual rainfall is about 3,000 mm, and mean insolation is 5.6 h/day. Temperatures range from 20 °C at night to 28.5 °C during the day.

The first flowering of the cocoa was recorded in June 2011, about 2 years after field planting. In 2012, 3 years after field planting, most of the trees were bearing pods. The vegetative growth of the trees was lower, but the yield performances of both SG2-S and SG2-B were very similar to cocoa grown in the coastal provinces. The growth of *Gliricidia* shade trees was also reduced compared with that in coastal areas. The cocoa trees, especially the SG2-Small trees, had more compact growth, shorter internodes, reduced tree height and shorter stems to the jorquette. However, the number of pods per tree, number of beans per pod, bean size and quality attributes of both crosses were not reduced compared with those expected in the lowlands.

This paper provides a brief overview of the progress of the hybrid cocoa now growing in Karimui, and the incidence of pests and diseases.

ACIAR project work

On the basis of this early success of cocoa in the test planting, Simbu was included in an ACIAR project, HORT/2014/096, 'Enterprise-driven transformation of family cocoa production in East Sepik, Madang, New Ireland and Simbu provinces of Papua New Guinea'. The project strategy was extended to build on the early success and extend the work more widely to farmers in Karimui. Before the ACIAR project commenced in early 2016, self-motivated Karimui farmers obtained open-pollinated seeds from the test planting of hybrid seedlings on the DPI station, and planted the seeds before good technical advice became available.

Most farmers copied the planting spacing they observed at the DPI station, commonly either 4 m × 4 m or 4 m × 3 m, under already established *Casuarina* shade trees in coffee gardens or in old food gardens where banana and cassava provided shade. *Gliricidia* shade trees were advocated after the ACIAR project started and technical advice was provided.

An interested farmer from each of seven wards in Karimui district was recruited and trained by project staff on all aspects of cocoa husbandry. These farmers, referred to as 'Cocoa Model Farmers' (CMFs), then trained initially 25 interested farmers in their own communities. The CMFs were identified to represent altitudes from CMF 1 at 900 m to CMF 7 at 1,360 m. Since then, the number of interested farmers has increased to cover the entire community, making it hard to keep accurate records of identified farmers as community jealousy and infights for technical attention and involvement increased.

Because of the cost of nursery construction and polybags, and the difficulties of transporting plants in polybags to widely scattered and inaccessible field sites, the project in Karimui modified the nursery-based approach commonly advocated in the coastal provinces. Open-pollinated seeds from the SG2 trees on the DPI station were germinated in prepared seedling beds. Upon germination, seedlings were transplanted directly into the field. Seedlings were monitoring regularly for pest and disease attacks.

For comparison with the seedlings being planted by farmers, and to test the best cocoa from the lowlands, 18 of the latest released CCI cocoa clones were also introduced, beginning in November 2017, following strict quarantine procedures. Clones were brought in in two lots. The first lot was held in quarantine in polybags at CCI, Tavilo, for 8 months and cleared free from pests and diseases before being bare-rooted and flown into Karimui, where they were again held in quarantine for 8 months before being distributed in the field. During transportation, 1,260 of the seedlings died, and so another 1,260 were brought in from New Ireland as replacements. New Ireland is free from VSD, and so there was no need for quarantine at point of export. In Karimui, the bare-rooted seedlings were repacked into polybags and grown in a temporary quarantine plastic house for 8 months before they were declared free from pests and diseases for planting. The CMFs were issued with the 18 CCI released clones and trained to plant at either 4 m × 4 m or 4 m × 3 m spacing for big clones and small clones, respectively.

During visits by the resident field-based technician to the trial plots of the CMFs, observations were made on yield, agronomy, and incidence of pest and diseases for the hybrids and the introduced clones.

Incidence of pests and diseases

Mirid attack was noted as serious if more than 20% of the pods on a tree and 20 trees or more were observed infested. Pods suspected of infestation with cocoa pod borer (CPB) were harvested and broken to confirm if they were free from CPB.

To date, major and serious cocoa diseases commonly found in maritime growing areas have not been observed at significantly damaging incidence levels. Grey weevils have been observed commonly attacking young seedlings at the time of 1st to 10th leaf flush, although the seedlings commonly recover and the weevil attack subsides as the plants mature. The major insect pest problem observed is caused by mirids damaging cherelles and developing pods. *Phytophthora* pod rot and seedling blight are serious cocoa diseases, causing significant losses in PNG coastal provinces. However, to date, neither have been reported in Karimui. No VSD has been observed. During drier periods, some trees showed signs of dieback associated with anthracnose, but upon closer examination no internal damage was evident. Incidence of soft rot of pods and pink disease has also been very low. Pest and disease surveys are continuing, but the overall impression is that, apart from mirids, pests and diseases are much less a problem than in coastal provinces.

Discussion

The model of using a CMF from the area to provide leadership to spread skills and knowledge within the community has proved a useful extension strategy. It provides good contact with a wide group of farmers. Observations continue to provide evidence that trees are slower growing than at lower altitudes, but many individual trees produce a similar number of pods as coastal trees. Pods are often larger than is common on the coast. Exposure to direct sunlight caused stunted tree growth, although these trees recovered and perform well. It is likely that the lower average temperatures, especially at night, suppressed vegetative growth, and may have promoted flowering and increased pod production. Because of the reduced vegetative growth at higher altitude, it will be possible to increase planting densities, and the requirement for pruning to reduce plant height will be reduced. Although the results are preliminary, they indicate great potential for growing cocoa in certain highland areas of PNG. Replicate field trials are required to fully compare growth and production of cocoa in the highlands and lowlands.

We have observed many farmers harvesting significant numbers of healthy, ripe pods from their open-pollinated SG2 hybrid seedling trees. It appears that it may be possible to select superior types of cocoa, adapted to producing well at higher altitudes, from among the great genetic diversity of types initially established through the introduction of SG2 hybrid seedlings. The SG2 hybrids released by CCI are a segregating F1 generation as they are produced from heterozygous parents. The trees derived from seeds of open-pollinated pods of the SG2 hybrids planted by farmers in Karimui are the F2 generation. It will be interesting to note the segregation and variability for traits under a fully replicated trial. No known studies have been done in PNG with the F2 type progeny population. The large F2 plantings in Karimui are the first known undertaking; thus interesting genetic and useful breeding information is likely to be generated. It may be possible to identify superior genotypes that are high yielding, with pest and disease resistance, desirable agronomic traits, and adaptation to higher altitudes than the SG2 hybrids or the released clonal varieties. We observed that many farmers are harvesting more healthy, ripe pods per tree, with many big beans, from the segregating F2 progeny trees. Our farmers are identifying F2 elite trees to propagate in their farms, creating a basis for farmer-assisted selection for development of location-specific planting materials.

The nonconventional planting methods, where seeds are germinated and sown directly without the need for a nursery or polybags, are proving to be a successful approach in enabling farmers with few resources to rapidly establish cocoa groves. These methods eliminated the usual cost associated with production of planting materials via nursery methods. Although there may be risk of losses due to pest and disease attacks, if meticulously planned, it is likely that huge areas of cocoa can be planted cheaply using this method. Individual trees that prove to be unproductive can serve as rootstock for grafting of selected superior types. Again, a careful study could help to confirm that large areas could be cultivated with cocoa cheaply to stimulate cocoa cultivation in PNG. Farmers could be empowered with vegetative propagation skills and knowledge in preparation to field-graft their own farms when location-specific plant materials from trials and farmers' own selections become available. In Karimui, this approach is seen as the most suitable for planting cocoa in inaccessible areas while awaiting cocoa roads. The approach is gaining traction in the other four neighbouring highland provinces, where there is growing interest in trialling cocoa as an alternative cash crop following the recent incursion of the pest coffee berry borer. This approach is now being advocated in coastal provinces to save costs for farmers and speed up the spread of good planting material on farms.

References

Wood, GAR & Lass, RA 1985, *Cocoa*, 4th edn, Tropical Agriculture Series, Longmans, London.

Yinil, D, Tade, E, Bapiwai, P & Fidelis, C 2017, 'Where can cocoa be grown successfully in Papua New Guinea', in *PNG cocoa extension manual*, PNG Cocoa and Coconut Institute, Tavilo, East New Britain province, Papua New Guinea, pp. 14–23.

Abstract

Research updates on cacao pod rot disease and management in the Philippines

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Cacao (*Theobroma cacao*) is one of the most important crops, as a potential source of income, in the Philippines. So far, the country is a net importer of cocoa. This deficit in production has given rise to the Philippine Cacao Challenge, which commits the Philippines to producing 100,000 tonnes of cocoa by 2020 and onwards. However, cocoa production is confronted by destructive diseases such as *Phytophthora* pod rot. The *Phytophthora* diseases, most notably black pod rot and stem canker, are ubiquitous wherever cacao is grown. Understanding the diversity of the pathogen is essential for effective disease management. This paper describes molecular characterisation and identification of *Phytophthora* isolates from cacao collected from major cacao-producing provinces in Southern Mindanao, Philippines, through sequencing of the internal transcribed spacer in ribosomal DNA and construction of a phylogenetic tree. Recommended cacao clones were also screened for resistance to the *Phytophthora* pathogen.

Session 6

Updates on research on vascular streak dieback and other cocoa diseases

12 *Lasiodiplodia theobromae:* an emerging threat to cocoa causes dieback and canker disease in Sulawesi

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Abstract

Lasiodiplodia, a member of the Botryosphaeriaceae family, is becoming a significant constraint to the cocoa production area, including the major cocoa-growing areas in Sulawesi. In both mature and young trees, the pathogen causes dieback and stem cankers. The disease symptoms also appear on seedlings, particularly on grafted seedlings that use infected scion. The fungus can act as a secondary or synergistic pathogen, and the disease scenario is worse under biotic stress. Very limited reports have been made of *Lasiodiplodia* species on cocoa in Sulawesi as causal agents of dieback and stem cankers.

The pathogen causes typical dieback symptoms: affected trees show sudden yellowing and browning of leaves, followed by rapid dieback of branches. Branches and twigs of diseased trees show internal discolouration, with strong brown streaks in the vascular tissue. Dry leaves and pods remain attached to declining trees for several weeks.

Two important *Lasiodiplodia* species that have been isolated from infected tissue are *L. theobromae* and *L. pseudotheobromae*. They are identified based on morphological characters, PCR and sequencing of the internal transcribed spacer region. Pathogenicity tests revealed that the pathogens induced typical disease symptoms on seedlings after inoculation, such as brown streaking, and also caused declines in seedlings. *L. theobromae* remains inside the seedling tissue, and was reisolated 8 months after inoculation. With poor management of trees, this pathogen is a potential threat to future cocoa production in Sulawesi.

Keywords: *Lasiodiplodia theobromae*, dieback, stem canker

Introduction

Cacao is a popular perennial crop that is cultivated by smallholder farmers in Sulawesi, Indonesia. The plant has contributed to an improved quality of life for farmers through increasing income. Market demand for this commodity is always high. In the past 5 years, productivity of cacao has decreased on smallholder farms because of limiting factors such as nutritional deficiencies, insect pests and diseases. Dieback disease is one of the significant diseases causing yield loss, including dieback caused by *Lasiodiplodia* species.

Lasiodiplodia theobromae (Pat.) Griff. & Maubl. (syn. *Botryodiplodia theobromae* Pat.), the anamorph stage of *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx, a member of the Botryosphaeriaceae, is a cosmopolitan fungus occurring predominantly throughout tropical and subtropical regions (Burgess et al. 2006; Punithalingam 1980). Recently, dieback disease on cacao branches with new symptoms was observed in Sulawesi, Indonesia.

Materials and methods

Sampling of dieback tissues

Infested cacao trees were on a cacao plantation area belonging to a farmer in South Sulawesi. The disease was observed on both hybrid and grafted trees. Samples of the diseased tissue were cut with pruning scissors, placed in bags and sent to the laboratory overnight.

Fungus characterisation

Morphology characterisation, to examine conidial characteristics, was done on two *Lasiodiplodia* species using a pathogenicity test from Barnet and Hunter (1972) and Dugan (2006). To induce sporulation, isolates were grown on 2% water agar (20 g agar per litre of water), with a sterilised cacao branch placed on the surface of the medium (Smith et al. 1996). Plates were incubated at 27–30 °C for 2 weeks. Pycnidia and mycelia with conidia were mounted and crushed in one drop of sterile water on glass slides under a microscope. Another method used to induce sporulation was putting isolates above young cacao leaves, beside the leaf midrib. The isolates were also identified by molecular characterisation.

Pathogenicity characterisation

Pathogenicity tests were performed on seedlings of cacao. The treatment seedlings were 2 and 4 months old. Four plants of each isolate and controls were used. The epidermis of the stem was disinfected with 70% ethanol and left to dry. A 7 mm triangle cut was made into the epidermis, between two nodes and below the apex of the stem. A 7 mm diameter mycelial potato dextrose agar (PDA) triangle plug was removed from the edge of actively growing cultures and placed onto the stem wounds, with the mycelium facing the cambium. The inoculated wounds were wrapped with plastic wrap to prevent desiccation and contamination. Control plants were inoculated with sterile PDA plugs and without agar media. Two and 4 months after inoculation, the length of cambium discolouration was measured to assess the pathogenicity of the tested isolates, and the length of streaking on wood was measured.

Statistical analysis

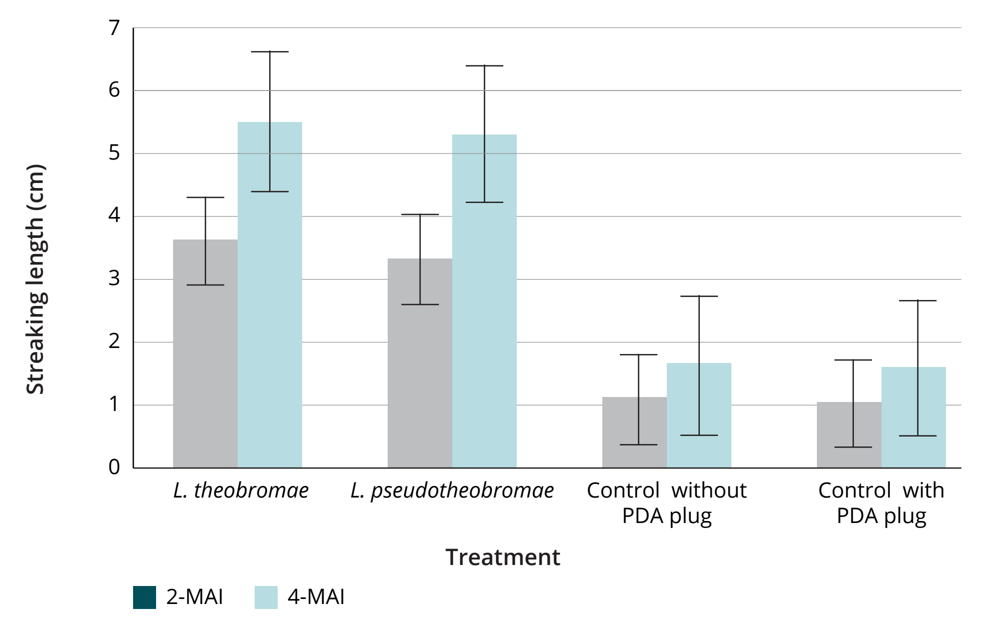
Aggressivity of fungi was determined using analysis of variance (ANOVA) and standard error. Means separated by a 5% probability level were considered significantly different.

Results

Diseased trees showed sudden yellowing and browning of leaves, followed by rapid dieback of branches (Figure 12.1). Branches and twigs of diseased trees showed internal discolouration, with brown streaks in the vascular tissue. Dry leaves and pods remained attached to declining trees for several weeks. The disease symptoms also appeared on seedlings, particularly on grafted seedlings that used infected scion; 16 months after grafting, the upper part of the seedling was in decline. The scion showed decline—leaves were brown and dry, and the wood tissue showed brown to dark streaking before and after decline.

The pathogenicity tests showed that *L. theobromae* and *L. pseudotheobromae* are not significantly different from each other. They caused streaking on wood faster than the control without agar and with PDA; streaking developed to 3.63 and 3.33 cm, respectively, 2 months after inoculation. There was a significant difference ($P < 0.05$) from all controls (Figure 12.2). Four months after inoculation, both *Lasiodiplodia* species remained aggressive, causing more streaking than on controls; streaking

Figure 12.1 Typical symptoms of *Lasiodiplodia* dieback disease on mature tree: sudden browning of leaves (left), brown to dark streaks in the vascular tissue of the branch (right)



MAI = months after inoculation; PDA = potato dextrose agar
Note: Bars above columns are the standard error of the mean.

Figure 12.2 Average lengths of streaking on wood of seedlings at 2 and 4 months after inoculation with fungi (*Lasiodiplodia* spp.) associated with dieback disease

developed to 5.50 and 5.30 cm for *L. theobromae* and *L. pseudotheobromae*, respectively (Figure 12.2). Moreover, the results obtained with *L. pseudotheobromae* on the pathogenicity test of 2-month-old seedlings was that 50% of the inoculated tested seedlings declined 2–3 months after inoculation.

L. theobromae conidia were subovoid to ellipsoid–ovoid, with rounded apex and base rounded to tapering. They were widest in the middle and thick walled. Young conidia were hyaline, ovoid and aseptate, with granular contents. Mature conidia were dark walled, ovoid to ellipsoidal, one-septate and striate.

Discussion

L. theobromae is a prevalent pathogen throughout trees, causing several diseases including dieback of trees. The fungus has been reported on cocoa, causing dieback diseases, in other parts in the world, including Cameroon, India and the Philippines (Adu-Acheampong, Archer & Leather 2012; Alvindia & Gallema 2017; Kannan, Karthik & Priya 2010; Mbenoun et al. 2008). There has been no previous report of dieback on cacao caused by *Lasiodiplodia* species in Indonesia. Pathogenicity results indicated that both *L. theobromae* and *L. pseutheobromae* were able to cause dieback. The pathogen caused internal symptoms above and below points of inoculation, resulting in brown to black discolouration of vascular tissues. The fungi can colonise and spread rapidly through the vascular tissues. Because it is highly aggressive, the fungus can induce severe lesions on cacao branches.

Conclusion

L. theobromae may cause dieback symptoms on cacao. The fungus is a potential threat to cocoa production in the future.

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References

Adu-Acheampong, R, Archer, S & Leather, S 2012, 'Resistance to dieback disease caused by *Fusarium* and *Lasiodiplodia* species in cacao (*Theobroma cacao* L.) genotypes', *Experimental Agriculture*, vol. 48, no. 1, pp. 85–98.

Alvindia, DG & Gallema, FLM 2017, '*Lasiodiplodia theobromae* causes vascular streak dieback (VSD)–like symptoms of cacao in Davao Region, Philippines', *Australasian Plant Disease Notes*, vol. 12, p. 54.

Barnet, HL & Hunter, BB 1972, *Illustrated genera of imperfect fungi*, 3rd edn, Burgess Publishing Company, Minnesota.

Burgess, TI, Barber, A, Mohali, S, Pegg, G, De Beer, W & Wingfield, MJ 2006, 'Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology', *Mycologia*, vol. 98, no. 2, pp. 423–43.

Dugan, MF 2006, *The identification of fungi: an illustrated introduction with keys, glossary, and guide to literature*, American Phytopathological Society, St Paul, Minnesota.

Kannan, C, Karthik, M & Priya, K 2010, '*Lasiodiplodia theobromae* causes a damaging dieback of cocoa in India', *Plant Pathology*, vol. 59, p. 410.

Mbenoun, M, Momo Zeutsa, EH, Samuels, G, Nsouga Amougou, F & Nyasse, S 2008, 'Dieback due to *Lasiodiplodia theobromae*, a new constraint to cocoa production in Cameroon', *Plant Pathology*, vol. 57, p. 381.

Punithalingam, E 1980, *Plant diseases attributed to* Botryodiplodia theobromae *Pat.*, J Cramer, Vaduz, Germany.

Smith, H, Wingfield, MJ, Crous, PW & Coutinho, TA 1996, '*Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa', *South African Journal of Botany*, vol. 62, pp. 86–88.

Abstracts

A complete system for field diagnosis of cocoa diseases

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Early disease diagnosis is vital for management and containment. Unfortunately, cocoa plantations are often located in isolated sites, far from equipped laboratories. We have developed a combination of technologies that allow disease diagnostics to be performed by nonspecialised personnel in the field. These technologies include the following:

- Sample processing and DNA purification is completed in 30 seconds using our patented DNA dipstick, without need for scientific equipment.
- Individual assay reactions have been stabilised to withstand storage and transport to the field at room temperature and reduce contamination risks.
- We have designed and built the 'Diagnostic Droid', a simplified and miniaturised version of a real-time loop-mediated isothermal amplification (LAMP) machine. We have conceived it with portability and robustness in mind, and it can be powered up by a car lighter or a battery. The Diagnostic Droid contains all the electronics and detection optics, and connects with android phones using bluetooth to provide communication. A microchip in the Diagnostic Droid performs calculations using a newly developed mathematical algorithm to interpret the results of the tests. Two prototypes have been built with capacity for 6 and 12 simultaneous reactions. The technology has been successfully tested in the field.
- An Android phone app allows control of the Diagnostic Droid and communicates with it to retrieve the results of the assay.

The combination of these technologies allows us to perform diagnostic assays in approximately 1 hour in rural and remote environments.

Control of vascular streak dieback disease on cacao using composted plant residues in combination with *Trichoderma asperellum*

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Vascular streak dieback (VSD) can be controlled using the fungus *Trichoderma asperellum*. For greater suppression of this disease, we applied composted plant residues in combination with the fungus. Laboratory trials indicated that the combination reduced VSD incidence, compared with the initial incidence, by 37.5% in 3 months post-application through the soil, and by 24.9%, 11.0% and 4.0% for *T. asperellum* alone, compost alone, and positive control, respectively. In the field on the susceptible Jalani cacao clone, compared with the control, the combination reduced disease incidence by 31.0% after the first application through soil in the first year and 44.4% after the second application in the second year. On moderately resistant

MCC 01 and MCC 02 cacao clones, the combination decreased VSD incidence by 72.8% after the first application in the first year and by 77.0% after the second application in the second year. These data showed the effectiveness of the combination of composted plant residues and *T. asperellum* applied through soil in controlling VSD in the field. The treatment could potentially be used on a large scale for suppressing this disease.

Symptom diversity of vascular streak dieback disease of cocoa in Sulawesi: a co-infection phenomenon

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Vascular streak dieback (VSD) symptoms caused by *Ceratobasidium theobromae* have changed from green-spotted chlorosis to a new symptom, necrosis. Either symptom or both symptoms can be present on a tree at the same time. Symptom diversity has been associated with *Lasiodiplodia theobromae,* an endophytic fungus, and therefore a hypothesis of co-infection has been proposed. This paper examines 1,500 infected tissues with VSD disease symptoms in different altitudes across Sulawesi, and tests for the presence of both *C. theobromae* and other fungi (endophytic community) using PCR analysis, confirmed using specific primers of Than_ITS1&2 and universal primers of ITS 1&4.

New symptoms and management of vascular streak dieback of cocoa under climate change

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Vascular streak dieback (VSD), which is caused by a fungus, causes serious problems in cocoa throughout the Asia-Pacific region, including Indonesia. This study analysed changes in symptoms to improve management of the disease. The results showed that symptoms change after the first signs of chlorosis appear on the leaf lamina. These changes usually involve darkening of the lamina and necrosis of the vascular tissue. The symptoms can cause 80% damage.

Microscopy of hyphae in twigs, leaves and leaf laminae, and sporocarps of infected stems showed that the fungus associated with these new symptoms is *Oncobasidium theobromae* Talbot & Keane, now renamed *Ceratobasidium theobromae* (Talbot & Keane) Samuels & Keane. Isolation of fungi from infected xylem confirmed that growth is slow, and the fungus cannot easily be subcultured.

The results of this study did not find evidence that new symptoms of VSD are caused by a new cocoa fungus or a combination of fungi. Rather, the symptoms are due to *C. theobromae*, as described for the original disease in Papua New Guinea. It is possible that the recently observed symptoms of VSD are due to changes that affect the host response to fungi, such as climate change and soil fertility. Microscopic observations of the hyphae in the xylem tissue emphasise that proper pruning protocols must be followed to prevent transmission of the pathogen.

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