

CONTROL OF COCOA POD BORER AND PHYTOPHTHORA POD ROT USING DEGRADABLE PLASTIC POD SLEEVES AND A NEMATODE, *Steinernema carpocapsae*

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ABSTRACT

Cocoa pod borer (CPB; *Conopomorpha cramerella*) and Phytophthora pod rot (PPR; *Phytophthora palmivora*) are serious pest and disease on cocoa plantations in Indonesia. Both pest and disease have been controlled with limited success using cultural practices such as pruning, frequent harvesting, sanitation, plastic sleeving, and chemical pesticides. An experiment was conducted on cocoa plantings in Pinrang Regency, South Sulawesi during the wet season of 2008/09 to test the effect of pod sleeving (with transparent degradable and non-degradable plastic bags) and nematode application on CPB and PPR infestation. The nematode, *Steinernema carpocapsae* (10,000 active juveniles per pod) was sprayed three times at intervals of 10 and 20 days. Pod damage by CPB was observed at harvest time, while PPR disease incidence was evaluated every week until harvest time. Results showed that all pods in the field were infested by CPB as indicated in control samples. Pod sleeving using both non-degradable and degradable plastics significantly reduced pod damage by CPB, from 62.3% in the control treatment compared to 8.4% in the CPB treatment. A combination of pod sleeving and nematode application had a synergistic reduction of pod damage by CPB resulting in totally healthy pods. Pod sleeving with degradable and non-degradable plastics also reduced pod damage by PPR significantly. Pod sleeving with non-degradable plastic suppressed the disease incidence almost zero until 6 weeks after sleeving and the rate of disease incidence was 3.6% per week. However, with degradable plastic, the disease suppression was even longer (7 weeks after sleeving), indicating that the degradable plastic is more effective. Combination of sleeving and nematode application slightly increased PPR infection. Sleeved pods in general had lower rates of PPR infection compared to pods treated with nematode or untreated pods (control). In these two applications, the rate of disease incidence was 7.8% and 8.3% per week respectively. The study implies that biological control using entomopathogenic *S. carpocapsae* and degradable plastic sleeves are effective and environmentally-friendly to control *C. cramerella* and *P. palmivora* on cocoa.

[**Keywords:** *Theobroma cacao*, *Conopomorpha cramerella*, *Steinernema carpocapsae*, *Phytophthora palmivora*, pest control, disease control]

INTRODUCTION

Conopomorpha cramerella Snellen (Lepidoptera: Gracillariidae) known as cocoa pod borer (CPB) is one of the most important limiting factors to cocoa production in Indonesia and Malaysia (Lim 1992; Wardjo 1996; van Grinsven 2003; Sulistyowaty and Wiryadiputra 2007; Shapiro *et al.* 2008). The newly-hatched larva tunnels through the floor of the eggshell and bores perpendicular to the pod surface. Larvae live and feed on the pulp and placenta until they reach maturity, then they emerge and pupate (Alba *et al.* 1985; Rauf 2008). Larval feeding results in pods that may ripen prematurely, with flat, small beans, that are often stuck together in a mass of dried mucilage (Mumford and Ho 1988).

Cultural practices such as pruning, frequent and complete harvesting, sanitation, and fertilization are aimed at reducing CPB infestations (Wood 1987; Mumford and Ho 1988; Kamaruddin 2000). Chemical control has largely been ineffective, but there is evidence that encouraging ants can provide some biological control (Wood 1987; Teh and Yeow 1995; See and Khoo 1996; Gassa *et al.* 2003; La Daha *et al.* 2003; Shapiro *et al.* 2008). Farmers have resorted to placing plastic sleeves over the young developing pods to prevent CPB from laying eggs on the pods (Moersamdono and Wardjo 1984; Mustafa 2006). This approach offers the best protection from CPB when there may still be 5-15% CPB infestation (Purung, personal comm.). However, the plastic sleeves often pollute the environment by entering streams and other areas. So far, no much information concerning the use of degradable plastic in pod sleeving for controlling pest both in fruits and in cocoa.

Biological control of CPB by using entomopathogenic nematodes is promising because nematodes are capable of seeking and killing their host rapidly (Gaugler *et al.* 1997; Adams and Nguyen 2002; Shapiro-Ilan *et al.* 2002). One potential entomopathogenic nematode species is *Steinernema carpocapsae* which can persist on the pod surface in both the dry and wet seasons and can penetrate the cocoa pods (Rosmana *et al.* 2009). Plastic sleeves would provide high humidity on pod surface and thus enable nematodes to persist for a longer period of time. However, higher humidity on the pod surface could increase the risk of the incidence of Phytophthora pod rot (PPR) caused by *Phytophthora palmivora*. The disease plays an important role in the reduction of cocoa production in Indonesia, not only in the wet season but also in the dry season when vectors such as ants exist in the field (McMahon and Purwantara 2004; Rosmana *et al.* 2006). Purung (personal comm.) found that in the wet season, the development of pod rot was relatively high in sleeved pods. On the other hand, Mustafa (2006) reported that pod sleeving can protect the pods from PPR infection. Nevertheless, the development of pod rot might occur in sleeved pods, because *P. palmivora* can infect pods before sleeving (Moersamdono and Wardoyo 1984).

Because of environmental issues associated with the use of non-degradable plastic, in this present work we evaluated the use of degradable plastic sleeves combined with the application of the nematode, *S. carpocapsae*, for controlling cocoa pod borer and observe its effect on the development of pods and PPR of cocoa.

MATERIALS AND METHODS

Design of Field Experiment

The research was carried out in Pinrang Regency, South Sulawesi, an endemic area of CPB and PPR, from November 2008 until February 2009 during the wet season. This season was correlated with a high infestation of CPB since it was the low season of cocoa pod production with relatively few pods on trees, and with a high intensity of PPR due to high humidity. The experiment used two kinds of plastic sleeves (non-degradable and biodegradable) combined with nematode application. Non-degradable plastic is a transparent polythene sleeve with a size of 17 cm x 30 cm x 0.02 mm, while biodegradable plastic is also a transparent polythene sleeve having a size of 17 cm x 30 cm x 0.01 mm that will degrade to carbon

dioxide and water in about 4 months, leaving no residue. Six treatments were included in the trial including control, biodegradable plastic sleeving, nematode application, biodegradable plastic sleeving plus nematode application, non-biodegradable plastic sleeving, and non-biodegradable plastic sleeving plus nematode application. The experiment was designed as a randomized block and repeated four times. Each treatment unit consisted of 16 trees, therefore, the total number of trees was 384. From these 16 trees in each replicate, 80 uniform pods of about 2-month old and 8-10 cm length were selected.

Pod Sleeving, Nematode Application and Assessment

To sleeve pods, a plastic sleeve (open at two ends) was placed over pods using a device made from a polyethylene pipe and bamboo stick, both 1 m length. The pipe, which is approximately 4 cm diameter, has a concave groove on its surface that facilitates pushing the plastic sleeves from the pipe by the bamboo stick onto the cocoa pod. Therefore, the transparent sleeve covered the young pod and remained open at the bottom for ventilation.

The nematode, *S. carpocapsae* was isolated from *Bombix mori* (ARN 1) in Sulawesi. Approximately 10,000 active juveniles were sprayed using small hand sprayer to the pod surface. Spraying was conducted three times. The first application was when pods were 2-month old (before sleeving) and the second and third applications were 10 and 20 days after the initial application, respectively. Nematode spraying on sleeved pods was done through the bottom of the sleeve.

The category of pod damage by CPB and fresh and dry weights of bean was assessed at harvest time. Effem method was used for categorization of pod damage (Purung, personal comm.). Damage intensity by CPB was analyzed using the formula of Lee *et al.* (1995) as follows:

$$\text{Damage intensity} = \frac{\{(\sum B \times 0.093) + (\sum C \times 0.297) + (\sum D)\}}{\sum (A + B + C + D)} \times 100\%$$

Notes:

- A = pods were free of CPB
- B = pod were attacked by CPB, but beans could be extracted by hand (low infestation)
- C = pods were attacked by CPB and the extraction of beans required a support device such as knife (moderate infestation)

D = beans in CPB-infested pods could not be extracted even by using a support device (severe infestation).

Categorization of pod damage and analysis of damage intensity by CPB per treatment unit were conducted on 50 pods completely free from *Phytophthora* infection. Fresh and dry weights of bean were also sampled from these pods, using 50 pods for each treatment.

The incidence of PPR was monitored weekly and its intensity was calculated as $P/Q \times 100\%$, where P was the number of pods attacked by PPR pathogen and Q was the total number of pods observed. The total pod observed per treatment unit for analysis of PPR intensity was 50 pods including those infested by CPB.

Analysis

The data of pod damage by CPB were analyzed after transformation to \arcsin . Duncan's multiple range test (DMRT) was then used for evaluating significant differences between the treatment means. The rates of PPR incidence in each treatment were analyzed by regression equation (PPR incidence in ordinate and time in abscissa). For evaluating significant differences between the regression coefficients (rates of PPR incidence) of each treatment, these regression coefficients were then analyzed by using T-test.

RESULTS AND DISCUSSION

Impact of Pod Sleeving and Nematode Application on Pod Damage

The degree of pod damage due to CPB depends upon the season of pod production. In the high season when the number of pods is high, only a small percentage of the pods is infested, while in the off (low) season, the CPB infestation is relatively high. The proportion of pods infested by CPB in November-February period (the low pod season), as indicated in the control, reached 100%. Pod sleeving, both with non-degradable and degradable plastics, reduced pod damage by CPB up to 50% (Table 1). These results indicated that pod sleeving did not totally protect the pods, because approximately 50% of pods were subjected to egg deposition before sleeving leading to larval invasion.

The egg oviposition pattern by female adult of CPB varies with pod age; and older pods of less than 7 weeks before ripening are preferred (Azhar and Long

1996). Fachrudin (2008) found that egg deposition on cocoa pod in Sulawesi was influenced by the cocoa clones. For example, on Bal 209 clone, a higher egg deposition occurred on 2-month-old pods than on 3-5 month-old pods. However, in Haris and some local clones, a higher egg deposition occurred on 3-month old pods, whereas in Aryadi and Baharuddin clones a higher rate of egg deposition occurred on 4-month-old pods (Fachrudin 2008). These data indicate that pods of all ages are subjected to egg laying by CPB and that younger pods must be sleeved for protecting their from egg laying. However, if pods of < 8 cm length or less than 2-month old are sleeved, wilt and PPR infection increased (Mustafa 2006). Purung (personal comm.) stated that sleeving on pods of 8-10 cm length still caused 5-15% of pods to be attacked by CPB, while Mustafa (2006) found that all pods sleeved at the size of 7-10 cm length were free from CPB infestation. Our trial was done in low season where the number of cocoa pods was very low, therefore egg deposition by female occurred on very young pods, less than 8-10 cm length.

Degradable plastic sleeves showed slightly more effective than non-degradable plastic in preventing pod damage by CPB. This is supported by the average number of healthy pods and B category pods, and fresh and dry bean weights (Table 1 and 2). A similar trial conducted in the dry season and in the transition of dry-wet season gave almost the same results (unpublished data). Studies on the use of plastics for pod sleeving suggest that the possible mechanism of reduced infestation of CPB larvae in the sleeved pods using degradable plastic is due to lack of oxygen. When pods are wet, the degradable plastic adheres to the pod surface, while the non-degradable plastic does not, therefore providing a space between the plastic and the pod surface.

Pod and bean damages were also reduced following the application of entomopathogenic nematodes. Effectiveness of this treatment was greater than that of the degradable and non-degradable plastic sleeving as shown by the higher healthy pod number, the low average pod damage, and the higher fresh bean weight (Table 2). However, the dry bean weight in the nematode treatment was comparable to that of the degradable plastic sleeving. These results indicate that entomopathogenic nematodes are capable of controlling high infestations of CPB. Possibly the nematodes persist longer on the pod surface in the wet season than that in the dry season, therefore killing more CPB larvae (Rosmana *et al.* 2009).

A combination of pod sleeving using degradable plastic and nematode application showed a syner-

Table 1. Impact of pod sleeving and *Steinernema carpocapsae* application on cocoa pod damage, Pinrang cocoa plantings, WS 2008/09.

Treatment	Pod damage (%)			
	A ¹⁾	B ²⁾	C ³⁾	D ⁴⁾
Control	0.00 ± 0.00a	26.50 ± 4.72d	19.50 ± 1.00d	54.00 ± 2.90b
Non-degradable plastic sleeving	46.50 ± 4.43b	36.50 ± 4.43e	17.00 ± 8.86e	0.00 ± 0.00a
Degradable plastic sleeving	56.00 ± 3.26c	26.00 ± 3.27d	18.00 ± 6.53d	0.00 ± 0.00a
Nematode	72.50 ± 5.51d	16.50 ± 2.52c	11.00 ± 5.03c	0.00 ± 0.00a
Degradable plastic sleeving + nematode	100.00 ± 0.00f	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Non-degradable plastic sleeving + nematode	79.00 ± 2.00e	10.50 ± 1.00b	10.50 ± 2.52b	0.00 ± 0.00a

¹⁾A = healthy pod free from CPB infestation; ²⁾B = pod with low infestation by CPB; ³⁾C = pods with moderate infestation of CPB; ⁴⁾D = pod with severe infestation of CPB.

Data were analyzed after transformation to $\text{arc}\sqrt{\sin}$.

Means in the same column followed by same letter are not significantly different according to 5% DMRT.

Table 2. Average pod damage by cacao pod borer, fresh bean weight, and dry bean weight after treatment with pod sleeving and *Steinernema carpocapsae* application, Pinrang cocoa plantings, WS 2008/09.

Treatment	Average pod damage ¹⁾ (%)	Fresh bean weight per 50 pods (kg)	Dry bean weight per 50 pods (kg)
Control	62.26 ± 6.11e	3.27 ± 0.19a	1.12 ± 0.17a
Non-degradable plastic sleeving	8.44 ± 2.22d	4.89 ± 0.13b	2.01 ± 0.14b
Degradable plastic sleeving	8.46 ± 2.15d	5.20 ± 0.07b	2.25 ± 0.09b
Nematode	4.88 ± 1.40c	5.53 ± 0.10bc	2.25 ± 0.03b
Degradable plastic sleeving + nematode	0.00 ± 0.00a	6.26 ± 0.20c	3.74 ± 0.16c
Non-degradable plastic sleeving + nematode	4.09 ± 0.69b	5.77 ± 0.26bc	2.52 ± 0.14b

¹⁾Data for average pod damage were analyzed after transformation to $\text{arc}\sqrt{\sin}$.

Means in the same column followed by same letter are not significantly different according to 5% DMRT.

gistic effect on reducing pod damage by CPB (Table 1 and 2). All pods treated with this combination were free from CPB and consequently gave the highest yield. As mentioned above, the degradable plastic sticks to the pod surface and tends to provide wet conditions in the pod's environment. This phenomenon apparently results in a dual impact. Firstly, the persistence and movement of nematodes is favored by wet conditions. Rosmana *et al.* (2009) showed that the persistence of nematodes on cocoa pod surface was longer in the wet season than that in the dry season. Secondly, as mentioned above, less oxygen might be available to larvae where the plastic sleeve adheres to the pod surface.

Practically, this combination can be used in off season, because this season is correlated with a high infestation of CPB and low production of pods. Therefore, it is easier to sleeve a small quantity of pods and to spray the nematode. In peak season, the CPB infestation is low and pod production is high, so the use of degradable plastic sleeving would take time and manpower; it is sufficient to apply nematode only.

Non-degradable plastics in particular are a significant contributor to many environmental problems and

until now they have been a virtually indestructible material. However, the availability of degradable plastic sleeves now provides an opportunity for cocoa stakeholder to encourage their use, thereby mitigating the adverse effects traditionally associated with plastics. The sleeves degrade irreversibly to carbon dioxide and water in about 4 months, leaving no residue (Rauf 2008). So far, no information concerning the use of degradable plastic sleeves for pest control both in cocoa and fruits. Therefore, our study is the first to assess the use of degradable plastic sleeve on cocoa.

Effect of Pod Sleeving and Nematode Application on Incidence of Phytophthora Pod Rot

Phytophthora pod rot (PPR) infection on cocoa pods varied between treatments. Earlier infections and higher incidence rates were observed on non-sleeved pods than that on the sleeved ones. In the non-sleeved pod treatments consisting of the control and nematode applications, the PPR infestation on pods was started in 4 and 3 weeks respectively after first

treatment, and the rate of incidence was 8.3% and 7.8% per week (Table 3). These mean that the PPR incidence was 60% and 55%, respectively. On the pods sleeved with non-degradable plastic, the disease incidence was almost zero for 6 weeks after sleeving and the rate of PPR incidence was 3.6% per week. However with the degradable plastic, the disease suppression was even longer, i.e. 7 weeks after sleeving and the rate of PPR incidence was 3.8% per week. When *S. carpocapsae* nematode was applied to the sleeved pods, PPR infection occurred earlier (4 days on the non-degradable plastic and 5 days on the degradable plastic treatments). PPR incidence was higher (26%) on the non-degradable sleeved pods

Table 3. Average *Phytophthora pod rot* incidence rate (regression coefficient) after treatment with pod sleeving and *Steinernema carpocapsae* application, Pinrang cocoa plantings, WS 2008/09.

Treatment	Rate of PPR incidence (%)
Control	8.3a
<i>S. carpocapsae</i> (nematode)	7.8a
Non-degradable plastic sleeving	3.6b
Degradable plastic sleeving	3.8b
Non-degradable plastic sleeving + nematode	3.9b
Degradable plastic sleeving + nematode	2.8c

Means in the same column followed by same letter are not significantly different according to T test ($P = 0.05$).

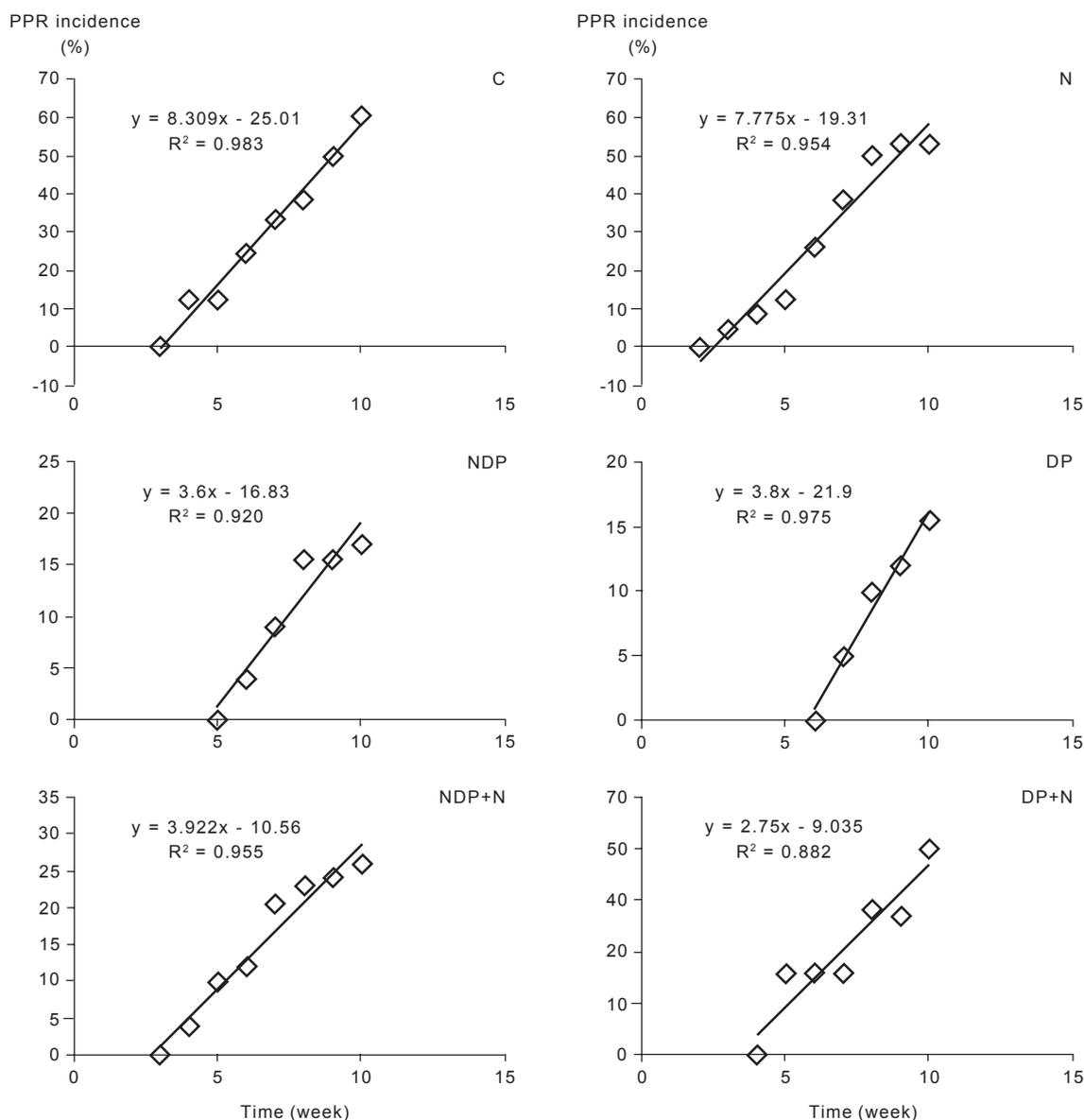


Fig. 1. Incidence of *Phytophthora pod rot* (PPR) following treatments by pod sleeving and *Steinernema carpocapsae* nematode, Pinrang cocoa plantings WS 2008/09; C = control; N = nematode; NDP = non-degradable plastic; DP = degradable plastic.

without nematodes (20%) than that on the degradable plastic sleeved pods. However, PPR infection rates were almost same with respectively sleeved pod and lower than those applied with nematodes alone (Fig. 1). Therefore, pod sleeving had a negative effect on PPR infection. The most effective method in reducing the PPR infection was sleeving the pods with degradable plastic. Even the rate of PPR incidence on the pods sleeved with degradable plastic was comparable to that on the pods sleeved with non-degradable plastic, its disease incidence delayed one week. It means that application of degradable plastic sleeve plays an important role in suppressing disease development on cocoa pods. In favorable conditions such as in wet season, small infection of PPR on pod can lead to rapid development of rot in few days (Rosmana *et al.* 2006).

The study suggests that pod sleeving with plastic protects the pods from the deposit of *P. palmivora* spores. The more closer/fix the plastic layer to the pod surface, the more effective on protecting the pods. *Phytophthora* mycelia or chlamydospores as primary inocula from soil or infection sites on the plant are transported to pods by termites and ants or by convection of rain-splashed drops as well as smaller aerosol-sized water droplets. Sporangia from infected pods become secondary inocula that can be dispersed horizontally by wind or water droplets as well as by flying insects and other fauna (Konam and Guest 2004; McMahon and Purwantara 2004). Therefore, when the degradable plastic sleeves become more adhered to the pod, especially in wet conditions, it gives more protection to the pods from vertical and horizontal distributions of *P. palmivora* spores. So far, it is known that high infection of PPR is not correlated with high infection of CPB. Moths of the CPB are most active at night, mating and laying eggs being carried out at this time on healthy pod surface, while during the day, adult moths normally rest underneath horizontal branches of the cocoa tree (Rauf 2008). Therefore, the possibility for moth to contact with *P. palmivora* spores is probably very small. As mentioned above, the sources of the fungus are the soil, infected pods, and main stems closer to the soil.

CONCLUSION

The use of degradable plastic for sleeving cocoa pods and its combination with entomopathogenic nematode (*S. carpocapsae*) application resulted in a synergistic reduction of pod damage by cocoa pod

borer to zero level, consequently giving highest bean yield. This combination of treatment can also reduce the rate of *Phytophthora* pod rot incidence. However, the most effective treatment for controlling the *Phytophthora* pod rot was sleeving the cocoa pods with degradable plastic without nematode application.

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