

PEST MANAGEMENT

The Effects of Mortality and Influence of Pheromone Trapping on the Infestation of *Oryctes rhinoceros* in an Oil Palm Plantation

Norman Hj Kamarudin*, Mohd Basri Wahid, Ramle Moslim and Siti Ramlah Ahmad Ali

Malaysian Palm Oil Board, P.O Box 10620, 50720 Kuala Lumpur, Malaysia

Abstract The infestation levels of *O. rhinoceros* were investigated in the oil palm trunk heaps in three replanting blocks (Blocks A, B and C) at Sepang, Selangor, Malaysia. These blocks had different densities of pheromone trapping: high (11 traps per ha, Block B), normal (1 trap per 2 ha, Block C), while no pheromone trapping was conducted in (Block A). Without pheromone trapping, the infestation levels of *O. rhinoceros* can reach a maximum density of between 25 to 50 individuals per m² (IPMS). The *O. rhinoceros* population was still detected in the heaps up to more than two years (at 26 months after replanting). With high density trapping, infestation levels were maintained below 10 IPMS and was not detected in the heaps after a period of 16 months of replanting. In the normal trapping density, the population was also maintained below 10 IPMS but had sustained itself in the heaps for up to 24 months. The mortality factors associated with the larval stages sampled within these replanting blocks was determined by visual symptoms with subsequent verification with microbial analysis. The analysis was narrowed down to detect only bacteria (*Bacillus thuringiensis* and *B. popilliae*), virus (*Oryctes* virus) and fungus (*Metarrhizium*). In Block A, the population of *O. rhinoceros* in the trunks was reduced drastically at 14 months after replanting (MAR), which could be due to the high mortality (88%) of the third instar larvae, 3 months earlier. In Block B, the percentage mortality for the larval stage was always highest at the third instars (between 21-82%). In Block C however, the mortality of the third instars was low, never exceeding 35%. In these sampling blocks, the infection of *Metarrhizium* seem to be less dominant compared to virus or bacteria. The k factor analysis indicates density dependence among the third instar larvae had contributed to the change in its population density in Block A. Therefore, with proper manipulation of pheromone traps and targeted application of disease agents (i.e virus,

fungus and bacteria), the population of *O. rhinoceros* can be managed more effectively in an oil palm replanting.

Key words *Oryctes rhinoceros*, Population density, Oil palm trunk heaps, Pheromone trapping, Mortality factors

Introduction

Within an oil palm replanting ecosystem, the study of population and infestation levels of *O. rhinoceros* are fundamentally important in order to understand its relationship with the environmental factors and its natural enemies. With this approach, we would be more equipped to control the pest more effectively. The importance of ecological studies of the pest has been stressed by Olivin (1980). In general, information on the ecological or pest population are important in the management and control of pests in Malaysia (Wood, 1970). Without this information, control techniques can be rendered less effective, hence increasing the overall management costs.

Various ecologically inclined approaches to assess the population of *O. rhinoceros* have been conducted on coconut (Hinckley, 1973; Bedford, 1975; Zelazny and Alfiler, 1991). Ecological studies of *O. rhinoceros* in Malaysia were rather limited. Among those earlier studies were the population studies of *O. rhinoceros* in oil palm, planted ex-rubber (Barlow and Chew, 1970), in a burned replanting technique (Liau and Ahmad, 1991; Samsudin *et al.*, 1993) and underplanting (Samsudin *et al.*, 1993). A study on the *O. rhinoceros* population in a zero burned area was once conducted by Liew and Sulaiman (1993).

A more recent study has suggested that in certain areas, *O. rhinoceros* can readily migrate to infest an area as soon as replanting is conducted, i.e. when there is an abundance of oil palm trunk chips (Norman *et al.*, 2004).

*Corresponding author.

Email: norman@mpob.gov.my

Tel: +603-87694477; Fax: +603-89258215

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There are some pathogenic microbes inflicting the larval stages of *O. rhinoceros* in the field. Among the common ones are virus (Zelazny *et al.*, 1992), fungus (Latch, 1976; Sundara-Babu *et al.*, 1983) and bacteria (Krishnamurthy and Sultana, 1977; Kannan *et al.*, 1980). Usually, the infected larva will propagate the pathogen to the colony via ingestion and contact. In the case of virus transmission, the infected adult spreads the virus to the larval stages at the breeding sites (Zelazny *et al.*, 1992). In Malaysia, the use of biocontrol agents (virus and fungus) for *O. rhinoceros* control seems promising, relying on the adult beetle as vectors for transmission of the disease (Ho, 1996).

Symptoms of mortality at the larval stages include soft bodied cadavers which were accompanied with a colour change (brown, blue-black, green, black) (Lacey and Brooks, 1997). However, there are also cases where infected larvae become hardened, followed by sporulations of the pathogenic fungus (Poinar and Thomas, 1984).

The following study will focus on the determination of population trends, infestation levels and life stages of *O. rhinoceros* in the oil palm trunk heaps, in three zero-burned replanting blocks, of which some were incorporated with pheromone traps for capturing adult beetles. Some pathogens may be responsible for the fluctuation of pest populations in the field. The causes of mortality in the larval stages could be separated according to the colour change and physical symptoms.

Materials and Methods

Location

The sampling of *O. rhinoceros* population was conducted in three different blocks (Blocks A, B and C) in the same plantation management. The plantation is located at Sepang, Selangor. These blocks were replanted in different periods, with different trap densities for the control of *O. rhinoceros* in two of the blocks (Block B and C).

Block A:

The area of the whole block was 21.6 hectares. Replanting was conducted in October 1994. Three one-hectare plots (Plots 1-3) were laid from the edge towards the centre of the block. Sampling was conducted from the oil palm trunk heaps, with the first sampling at approximately one-year after replanting. No pheromone traps were placed in the block. Occasionally, workers will apply carbofuran and scout for adults by winking.

Block B:

The area of the whole block was only 4.5 hectares. Replanting was conducted in August 1996. The block was divided into three plots (Plots 1-3) of 1.5 hectares each. Sampling from the palm heaps was initiated at about 4 months after replanting. Pheromone traps -plastic pail trap fixed with a crossed vane made of zinc plates, with a pheromone satchet (containing ethyl 4-methyloctanoate) placed at the interlocking space, were placed at an initial density of 11 traps per hectare for the first 6 months, followed by 5 traps per hectare.

Block C:

The area for this block was 18.5 hectares. Replanting was conducted in December 1996. Sampling from the palm heaps was carried out at about 3 months after replanting. Similarly, 3 one-hectare plots (Plots 1-3) were arranged from the edge towards the centre of the block. Pheromone traps were placed at a density of 1 in 2 hectares.

Sampling procedures

Samples of the *O. rhinoceros* population were taken from a subplot measuring approximately 1 metre square, in the heaps. Samples were taken systematically, at every 3 palms. Sampling was predetermined every 2-3 months and was stopped when the mean population for each subplot was less than 1. A total of between 27-36 samples (at 9-12 samples per plot) were gathered from each block for each consecutive sampling.

Biological parameters recorded

All the stages of *O. rhinoceros* development (egg, larva, pupa and adult) present in each subplots were recorded and brought back to the laboratory for rearing. Each stage of development were reared individually in a 250 ml plastic container, half-filled with moist, shredded chips of the palm trunk. Each container was checked weekly for recording mortality, colour change and succession of development.

Construction of life tables for key mortality factors and determination of density dependence

Based from the rearing exercise conducted above, a life table was constructed, to calculate and tabulate

the key mortality factors (k) for each developmental stages and later to get the total generational mortality (K) (Dent, 2000) where,

$$k_n = \log (\text{stage } n) - \log (\text{stage } n+1)$$

$$K = k_1 + k_2 + k_3 + k_4 + k_5$$

Each of these k values can later be plotted against the generation (of each successive sampling periods) to determine which particular stage has caused higher mortality as the density increases (direct density dependence).

Classification of dead cadavers

The dead cadavers were then classified visually by its external symptoms (i.e. shrunken, soft or hardened) and colour change (brown, black or blue black) (Figure 1). For hardened cadavers, the occurrence of white mycelium or green spores indicate the infection of *Metarhizium* (Mohan *et al.*, 1982; Sivapragasam and Tey, 1995) (Figure 1). In addition, *Metarhizium* often caused brown, blister-like streaks on the cuticle of the larva before its death (Latch, 1976; Sivapragasam and Tey, 1994). According to Monty (1974) a virus infection can be assumed for adults that emerged with abnormal or wrinkled wings. Similarly, a larva with a prolapsed rectum can also be classified as having infected with virus (Bedford, 1976).

A subsample was then taken from each classification for the determination of mortality factors. Each individual larva was then dissected to remove its midgut and separated evenly to determine the occurrence of bacteria (Thiery and Frachon, 1997; Klein, 1997), virus (Richards *et al.*, 1999) and *Metarhizium* (Tulloch, 1968; Rombach *et al.*, 1987). The occurrence of *Bacillus thuringiensis* (Bt) and *Bacillus popilliae* (Bp) was differentiated by gram staining. Bt is gram positive while Bp is gram negative. Bt can also be differentiated by the presence of protein crystals.

Results

Determination of mortality based from External Symptoms

The colour and physical condition of the cadavers (Fig. 1) were paired with the results of the microbial analysis of the midgut (Table 1). Determination of microbial presence was narrowed down to two species of pathogenic bacteria (*Bacillus thuringiensis* and *B. popilliae*), fungus (*Metarhizium*) and virus (*Oryctes*

virus). Results of the microbial analysis indicated that the soft and black cadavers recorded 32% occurrence of both pathogenic bacteria (*Bacillus thuringiensis* and *B. popilliae*), with no occurrence of either virus or fungus (Table 1). Similarly, there were no occurrence of either virus or fungus on the shrunken and brown cadavers. This may suggest that the two bacteria may be solely responsible for those symptoms. However, in soft and brown cadavers, the percentage of bacterial occurrence was higher than virus or *Metarhizium* ($\chi^2 = 4.6$, df = 1, $p < 0.05$) (Table 1). This may also suggest that bacteria was likely to be responsible to express the symptom. On soft and pale cadavers, the percentage of having virus infection was higher than either bacteria or *Metarhizium* ($\chi^2 = 9$, df = 1, $p < 0.05$) (Table 1). There was also no detection of *B. thuringiensis*. Finally, the hard and green cadavers were definitely infested with *Metarhizium* (100%) but with some occurrence of *B. thuringiensis* as well (Table 1).

However, based from these microbial analysis, there was a higher probability of the larvae dying from unknown causes (could be due to low nutritional conditions or inability of the larva to accumulate enough fat reserves for pupation). Except for *Metarhizium*, the probability of determining other microbial pathogens based from visual symptoms was rather low. The highest percentage was 45% occurrence of bacterial pathogen, for cadavers with black and soft symptoms (Table 1).

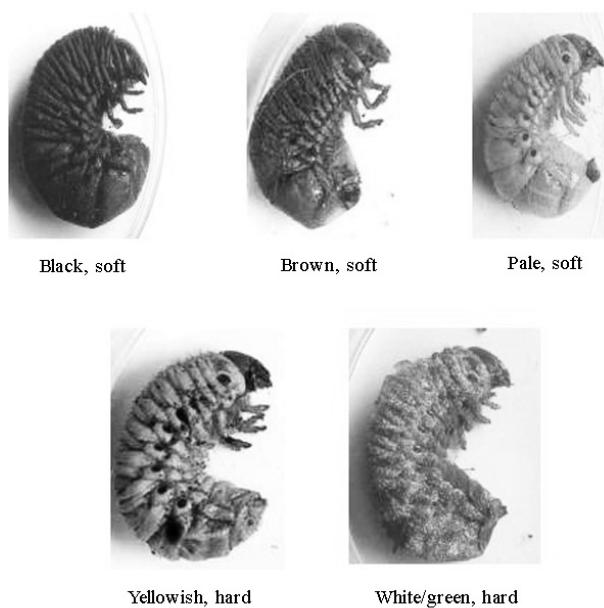


Fig. 1. Visual symptoms and coloration of dead *Oryctes* larva.

Table 1. Visual symptoms and percentage occurrence of microbial pathogens

Visual symptoms	N	Percentage of occurrence					Microbial pathogen (pooled)
		<i>Metarhizium</i>	<i>Bacillus thuringiensis</i>	<i>Bacillus popillae</i>	<i>Oryctes</i> Virus	Unknown	
Soft (black/blue black)	22	0	32	32	0	54	45
Soft (brown)	39	3	15	10	3	77	23
Soft (clear/transparent)	11	9	0	9	27	64	36
Hard (green/black/white)	15	100	26	0	0	0	100
Shrunken/soft (brown)	23	0	17	9	0	78	22

Note: N refers to number of dead larvae screened for the pathogens. Some of the larvae may carry two or more microbes.

Population trends and percent mortality of field populations

Block A

With occasional control measures, the initial sampling at 11 months after replanting (MAR) revealed a high density of population within the heaps, at a mean of 16 individuals per square metres (ISM) (Figure 2a). However, there was a significant drop to only 4 ISM, at 14 MAR, three months later (Figure 2a). At 17 MAR, it was observed that some beetles may have come to the heaps for oviposition or mating. The occurrence of the first instar and the increase in the percentage of the second instar larvae at 20 MAR confirmed that oviposition had taken place earlier (Figure 3). However, the population failed to build up further, likely due to the rapidly decomposed trunks coupled with the dense growth of the cover crop. After that period, the population density fluctuated at low levels (<5 ISM) until it is virtually absent by 26 MAR. However, it was interesting to note that adult beetles can still search for breeding sites even under heavy ground cover (>90 cm), at 20 and 23 MAR (Figure 3). However, the number of early instars found is almost negligible.

Between 11 to 26 MAR, percent mortality of the larvae was always higher in the third instars (between 11-74%) compared to other stages (Figure 4). The first instar recorded the lowest mortality, at less than 2%. The second instar mainly recorded mortality of below 20% (Figure 4). Generally, the prepupa and pupa only recorded less than 10% mortality (Figure 5).

Based from the external symptoms and microbial verifications, the majority of the larval stages may have died due to bacterial and virus infections (Figure 4). The first and second instars may have died mainly due to virus. The third instars, prepupal and pupal stages may have been infected by bacteria (Figures

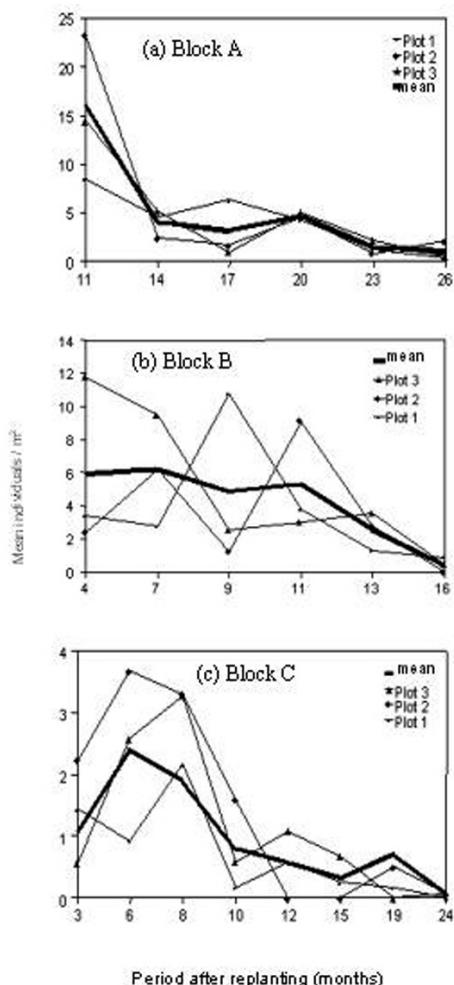


Fig. 2. Population density of *O. rhinoceros* in oil palm heaps.

4 and 5). Infection of *Metarhizium* at the larval stages was very low (less than 2%) (Figure 4). However, it was noted that infection of *Metarhizium* was quite prominent at the prepupa stage at 14 MAR (Figure 5).

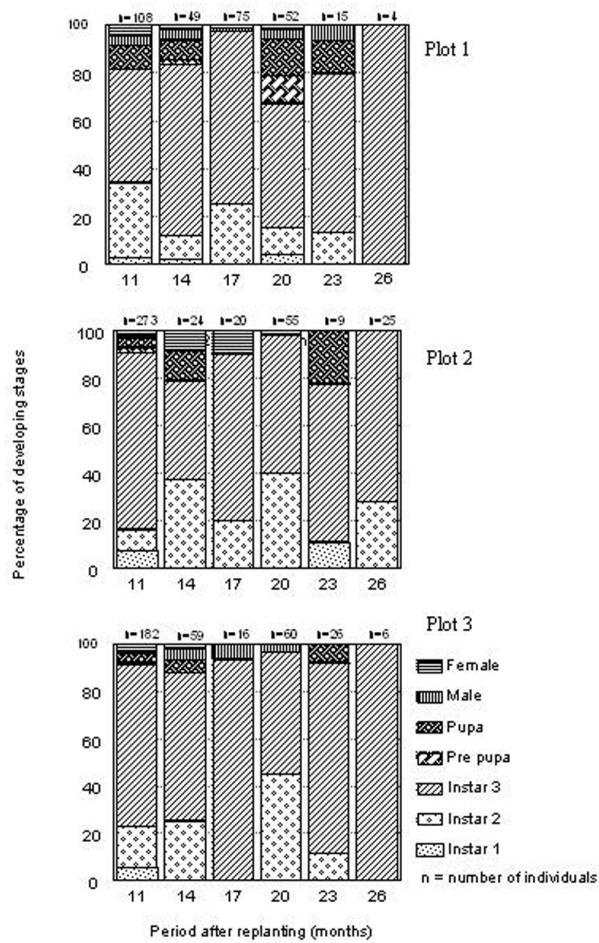
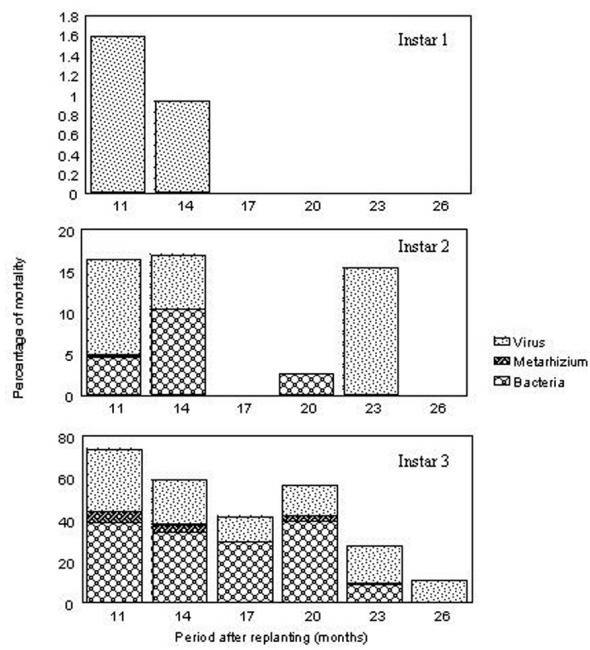


Fig. 3. Developing stages of *O. rhinoceros* in oil palm trunk heaps at Block A.

Block B

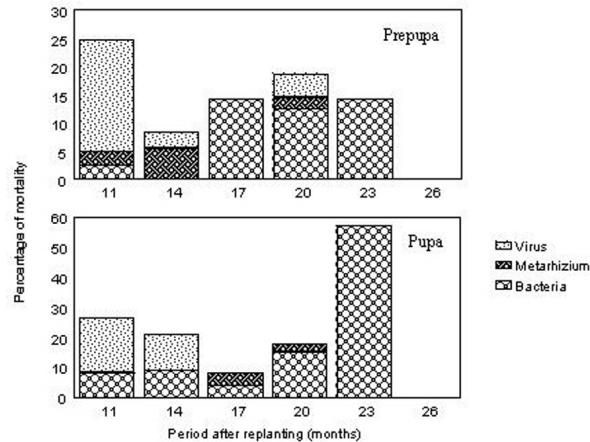
For this block, sampling was initiated much earlier, at 4 MAR. The population density at this time was quite low, only 6 ISM (Figure 2b). However, during the course of sampling, the mean population density did not increase further, but fluctuated below 6 ISM (Figure 2b). With the incorporation of pheromone traps, it was indicated that the low initial population had declined to below 10 ISM at 11 MAR, much earlier than without trapping, at Block A. The capture of beetles was always higher at the fringes of the planting block (between 0.05-0.30 adults/trap/night) compared to the inner part of the block (0.02-0.16 adults/trap/night). There was evidence that the beetles had started breeding there at the onset of replanting (Norman, 2004). The use of pheromone traps was also instrumental as it captured most of the gravid females looking for breeding sites (Norman, 2004).

By 16 MAR, the density dropped further to below 1 ISM and sampling was terminated (Figure 2b). No population could be detected by 18 MAR. The



Note* Assumptions of infections based from external symptoms:
Virus: pale, soft-bodied cadavers
Metarhizium: green/white/black hardened cadavers
Bacteria: black, soft bodied cadavers

Fig. 4. Percentage of mortality* for *O. rhinoceros* larvae in Block A.



Note* Assumptions of infections based from external symptoms:
Virus: pale, soft-bodied cadavers
Metarhizium: green/white/black hardened cadavers
Bacteria: black, soft bodied cadavers

Fig. 5. Percentage of mortality* for the prepupal and pupal stages of *O. rhinoceros* in Block A.

dominant stage of development occurred was the third instar larvae (Figure 6). The first and second instar was rarely encountered, and numbers never exceeded

2 ISM. This suggest the short duration of these life stages in the field.

At 13 MAR, there was a higher percentage of beetles in Plot 1 and 3, including some first instar larvae in Plot 3 (Figure 6). This may suggest some breeding activity in those plots. However by 16 MAR, there were no apparent buildup of population, with the mean population density remained less than 1 ISM (Figure 2b). Population was evidently absent in Plot 2 by that time (Figure 2b).

Between 4 to 16 MAR, the percentage mortality for the larval stage was always highest at the third instar (between 21-82%) compared to other instars (less than 20%) (Figure 7). The highest mortality (82%) occurred to the third larval instar at 16 MAR. The highest survivorship was recorded at 9 MAR (65%) and lowest at 11 MAR (26%).

Based from the external symptoms, most larvae may have died due to bacterial and viral infections (Figure 7). Low infection of *Metarrhizium* (less than 1%) only occurred at the third instar and pupal stages (Figures 7 and 8).

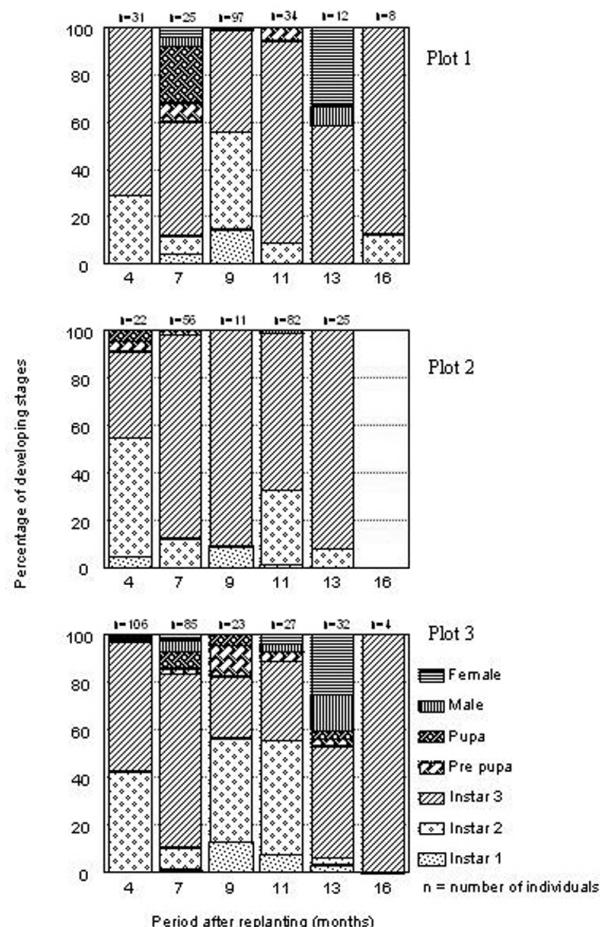


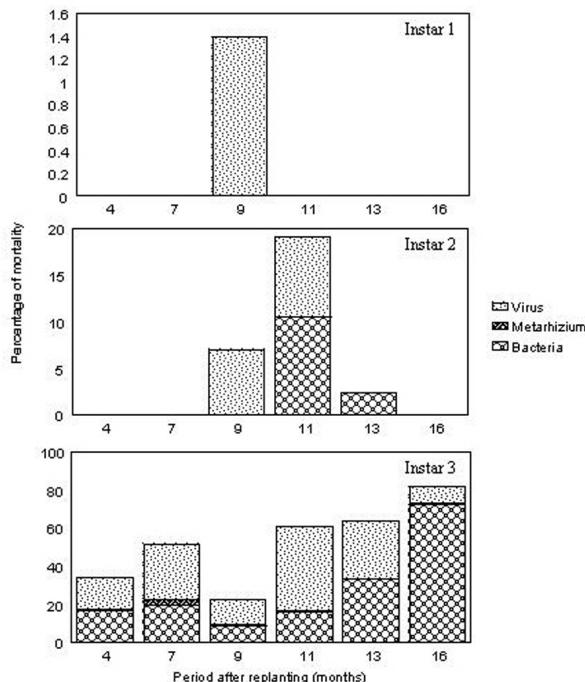
Fig. 6. Developing stages of *O. rhinoceros* in oil palm trunk heaps at Block B.

Block C

In Block C, the population density was initially quite low, at 0.5-3.5 ISM (from 3-10 MAR) (Figure 2c). Pheromone traps were also placed, but at a density of 1 trap per 2 hectares. Similarly, the population had dropped to below 1 ISM at 10 MAR (Figure 2c). This further suggest that the initially low population density of the pest (less than 5 ISM) could be quickly reduced with the incorporation of pheromone trapping in the area.

For this block, sampling was also initiated early, at 3 MAR. During this period, there were no presence of adult beetles in the heaps, indicating the population is still in the first generation (Figure 9). The mean population density for this block remained low, never exceeding 2 ISM until 24 MAR when it dropped to less than 1 ISM, when sampling was then terminated (Figure 2c).

At 6 MAR, there were adult beetles coming into Plot 3 for breeding. This was presumably so, based from the non-existence presence of the pupal stages (Figure 9). At 8 MAR, the adult beetles present in Plot 3 may be those which had newly emerged (Figure 9). It was also interesting to note that in Plot 2, there



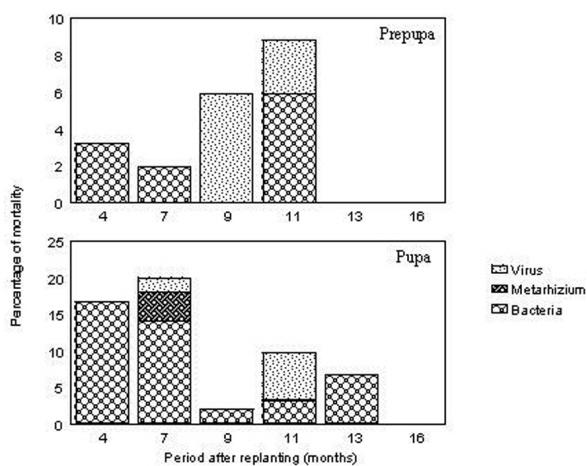
Note* Assumptions of infections based from external symptoms:

Virus: pale, soft-bodied cadavers

Metarrhizium: green/white/black hardened cadavers

Bacteria: black, soft-bodied cadavers

Fig. 7. Percentage of mortality* for *O. rhinoceros* larvae in Block B.



Note* Assumptions of infections based from external symptoms:
 Virus: pale, soft-bodied cadavers
 Metarhizium: green/white/black hardened cadavers
 Bacteria: black, soft bodied cadavers

Fig. 8. Percentage of mortality* for the prepupal and pupal stages of *O. rhinoceros* in Block B.

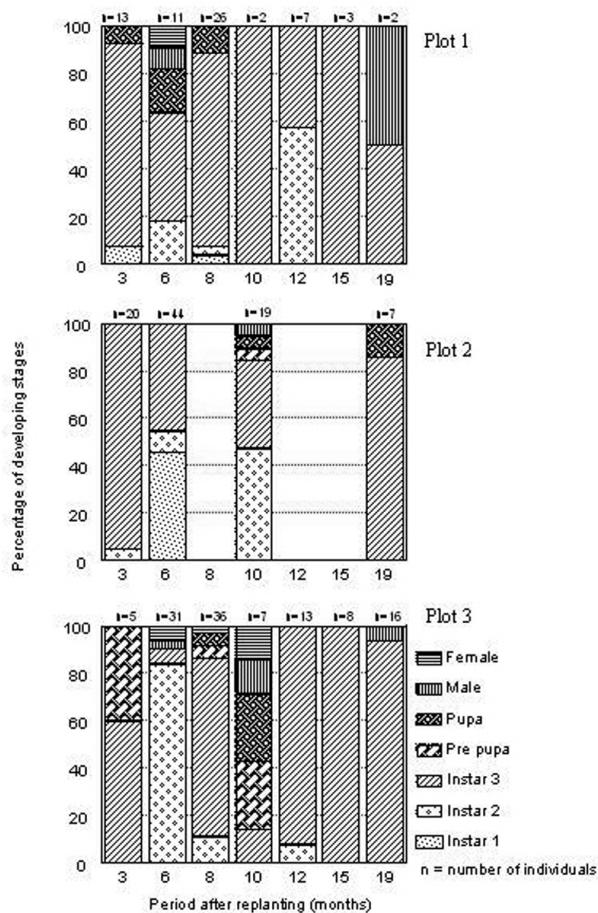


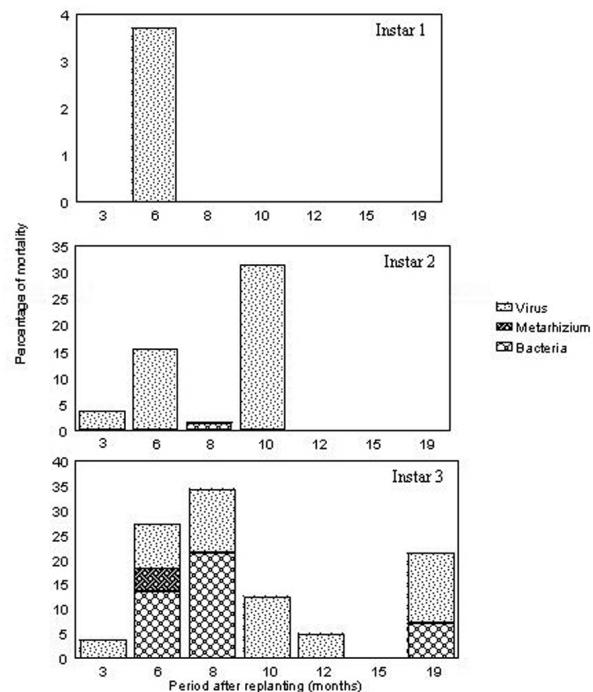
Fig. 9. Developing stages of *O. rhinoceros* in oil palm trunk heaps at Block C.

was a population crash at 8 MAR and between 12 and 15 MAR (Figure 9). It was also assumed that the population which were present at 10 MAR in Plot 2 had possibly came from the migration of breeding adults from the adjacent plots (Plots 1 and 3) (Figure 9).

A new generation was detected in Plot 1 and 3 at 12 MAR. This was presumably so, as only second and third instar larvae were present at 12 MAR (Figure 9). Prior to this, the plots recorded mostly late 3rd instars, pupa and presumably newly emerged adults, most evident in Plot 3 at 10 MAR. (Figure 9). Even at 19 MAR, there were still some adults coming to oviposit, presumably so, as there were no prepupal or pupal stages present (Figure 9). However, by that period, the heaps were no longer suitable as they were at the later stages of decomposition. The population was virtually absent at 24 MAR.

Throughout the course of sampling, most of the larvae were in the third instar stage (between 0.3-1 ISM) (Figure 9). The first and second instar were rarely encountered, indicating less activity of breeding and probably had ceased after 12 MAR (Figure 9).

Based from the external symptoms, most of the larvae may have died likely due to bacterial and viral infections (Figure 10). All of the first and second



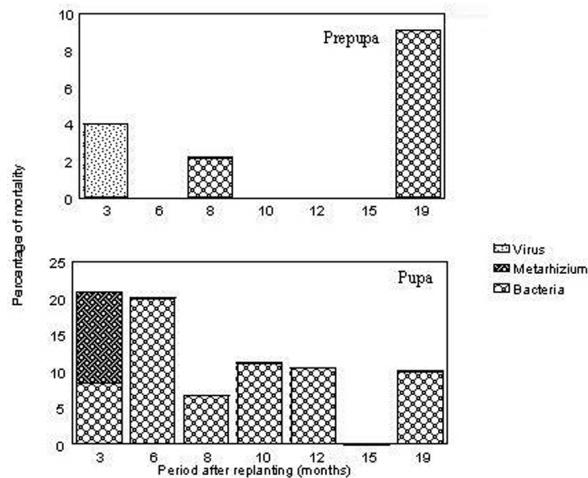
Note* Assumptions of infections based from external symptoms:
 Virus: pale, soft-bodied cadavers
 Metarhizium: green/white/black hardened cadavers
 Bacteria: black, soft bodied cadavers

Fig. 10. Percentage of mortality* for *O. rhinoceros* larvae in Block C.

instar larvae had presumably died due to virus, at 6 and 10 MAR. Similar to the earlier two blocks, the percentage mortality was highest for the third larval instars, with highest mortality (35%) observed at 8 MAR. This could possibly explain the population which crashed out at 8 MAR and 12 to 15 MAR, in one of the plots. The infection by *Metarhizium* on the third instar larvae was still very low (2%) (Figure 10) compared to the pupae which was quite high (12%), at 3 MAR (Figure 11).

Analysis of key factors (k) and density dependence of *O. rhinoceros* population

The key factor (k) was derived based from the life table made for blocks A, B and C, (Table 2). A graph was then plotted to determine which trends of the various k values follow the trend of K (summation of all k values). Figure 12 shows that only k_3 (k value for third instar larvae) seem to follow the trend of K. This indicates that for all three blocks, the mortality factors affecting the third larval instar were most important in changing the population density of *O. rhinoceros* in the field.



Note*: Assumptions of infections based from external symptoms:

Virus: pale, soft-bodied cadavers

Metarhizium: green/white/black hardened cadavers
Bacteria: black, soft bodied cadavers

Fig. 11. Percentage of mortality* for the prepupal and pupal stages of *O. rhinoceros* in Block C.

A significant correlation ($p < 0.01$) was found between the k values for all stages and the \log_{10} number of

Table 2. Key Mortality Factor (k) and Generational Mortality Factor (K) values derived from densities of different developmental stages of the rhinoceros beetle, in different blocks over different periods after replanting.

Blocks	MAR	Instar 1	k_1	Instar 2	k_2	Instar 3	k_3	Prepupa	k_4	Pupa	k_5	Adult	K
A	11	378	0.01	372	0.08	311	0.58	81	0.13	60	0.13	44	0.93
	14	107	0.00	106	0.08	88	0.39	36	0.04	33	0.10	26	0.61
	17	48	0.00	48	0.00	48	0.23	28	0.07	24	0.04	22	0.34
	20	113	0.00	113	0.01	110	0.36	48	0.09	39	0.09	32	0.55
	23	13	0.00	13	0.07	11	0.14	8	0.06	7	0.37	3	0.64
	26	28	0.00	28	0.00	28	0.05	25	0.00	25	0.02	24	0.07
B	4	94	0.00	94	0.00	94	0.18	62	0.01	60	0.08	50	0.27
	7	106	0.00	106	0.00	106	0.32	51	0.01	50	0.10	40	0.42
	9	72	0.01	71	0.03	66	0.11	51	0.03	48	0.01	47	0.19
	11	106	0.00	106	0.09	86	0.40	34	0.04	31	0.04	28	0.58
	13	41	0.00	41	0.01	40	0.43	15	0.00	15	0.03	14	0.47
	16	11	0.00	11	0.00	11	0.26	6	0.00	6	0.00	6	0.26
C	3	27	0.00	27	0.02	26	0.02	25	0.02	24	0.10	19	0.15
	6	27	0.02	26	0.07	22	0.17	15	0.00	15	0.10	12	0.35
	8	71	0.00	71	0.01	70	0.18	46	0.01	45	0.03	42	0.23
	10	16	0.00	16	0.00	16	0.25	9	0.00	9	0.05	8	0.30
	12	20	0.00	20	0.00	20	0.02	19	0.00	19	0.05	17	0.07
	19	14	0.00	14	0.00	14	0.10	11	0.04	10	0.05	9	0.19

Note: $k_n = \log(\text{stage } n) - \log(\text{stage } n+1)$

$K = k_1 + k_2 + k_3 + k_4 + k_5$

MAR: months after replanting

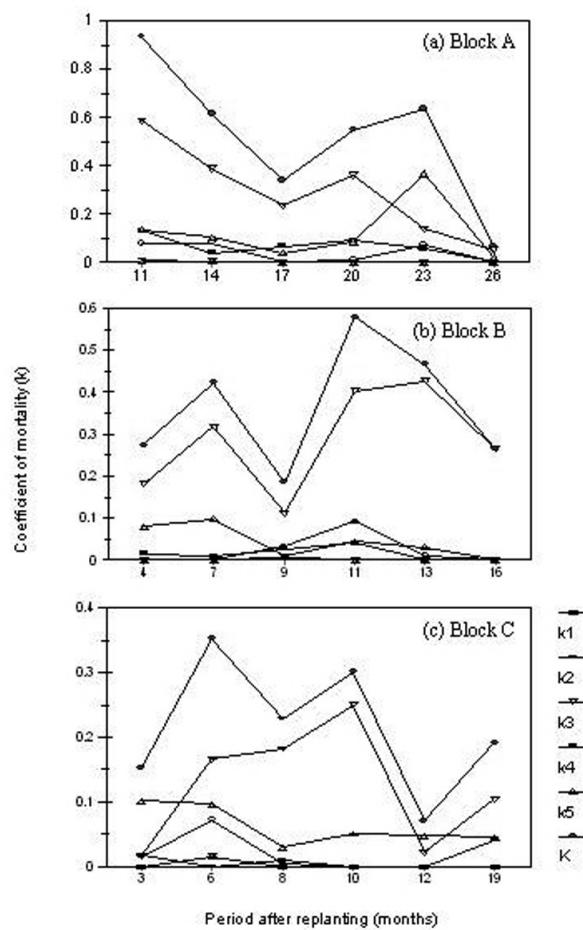


Fig. 12. Changes in the k values against time.

each stage, for the third instar larvae in Block A (Figure 13). This indicates that there was density dependence among the third instar larvae which had contributed to the change in its population density in Block A.

Discussion

The decaying logs stacked as heaps was suitable as habitat for *O. rhinoceros*. However, its population density may depend on the location and trapping situations. In this study, Block A seems to have a higher population density, likely due to the absence of trapping.

The population density was still high (16 ISM) even at 11 MAR. Breeding was still active, evident by the presence of the second larval instars, due to the suitability of the breeding site. A shredded palm trunk chip may take a long time to be fully decomposed. Samsudin *et al.* (1993) reported that an unburned oil palm trunk may take as long as 24 months to decom-

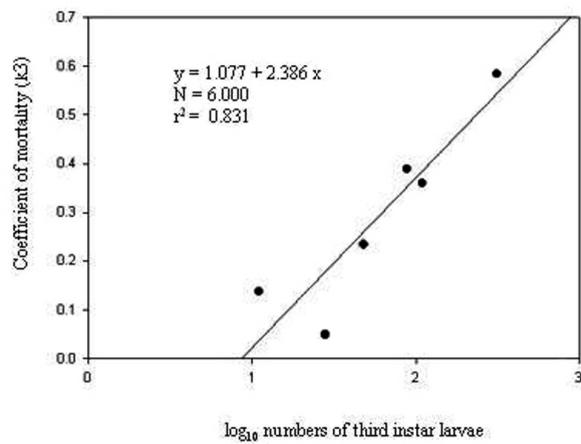


Fig. 13. A linear regression showing the density dependence of the third instar larvae in Block A.

pose. Burning normally hasten decomposition. Liau and Ahmad (1991) have shown that the population density can reach up to 8 ISM in unshredded and partially burnt trunks, but only up to 3 ISM in the shredded and partially burnt ones. Even when the trunks were partially burnt, the population can still be detected in the trunks until after 10 to 17 MAR (Liau and Ahmad, 1991; Samsudin *et al.*, 1993). In a zero-burned situation as this current study, *O. rhinoceros* were still breeding at 12 MAR (Blok C, Plots 1 and 3), 16 MAR (Block B, Plot 1) and even 26 MAR (Block A, Plot 2). The potential of *O. rhinoceros* to generate subsequent generations might depend on environmental factors such as the decomposition stages of the trunk and thickness of the cover crop. In this study, the breeding potential of *O. rhinoceros* reduces, after about 10-12 MAR.

In Block A, the population of *O. rhinoceros* in the trunks was drastically reduced at 14 MAR. This could be due to the high percentage of mortality for the third instar larvae at 11 MAR (88%) (Figure 4), which caused a low survivorship (12%) for that period. For the next sampling period, percentage survivorship had increased 2 folds to 24% (Figure 4). Even though the percentage survivorship had increased to 86% at 26 MAR (Figure 4), the population failed to persist further due to the overall low density (less than 1 ISM) and insuitability of the trunk, of which most of the material had already decomposed. An analysis of density dependence (Figure 13) had indicated that the third instar larvae had contributed to the change in the population density in Block A. The higher density of the third instar larvae might have caused easier spread of diseases (fungus, bacteria or virus) therefore affecting the overall population density. Field experiments have shown that *Metarrhizium* mostly affected the third instar larvae (Ramle *et al.*,

1999). The third larval instar had the longest developmental period (between 60-165 days) (Bedford, 1980), which exposed the larvae to various diseases. Therefore, the timing of application of any biocontrol agent can be targeted to the third larval instars for an effective control.

In Block B, there was a higher and simultaneous occurrence of adult beetles in plots 1 and 3 (Figure 6). This suggests the immigration of adults from adjacent mature blocks, as both plots (1 and 3) were located at the edge of the replanting block. Similarly, both blocks also recorded higher occurrence of first and second larval instars and somewhat overlapping generations (Figure 6). This also consolidates that adults were initially breeding in areas close to the edge of the block. The immigrating adults might also have brought and spread microbial agents to the developing stages at the edge of the block. Adults beetles have been reported as to be vectors of virus and fungus (Monty, 1974; Zelazny *et al.*, 1992; Ho, 1996). Based from external symptoms, the developing stages could have been infected by virus and bacteria.

It was suggested that the high density of pheromone trapping (around 11 traps per hectare) in block B, have caused the low population density of *O. rhinoceros* (below 4 ISM) after 13 MAR, which finally ceased to exist after 18 MAR. Mass trapping had been reported to reduce palm damage in several areas (Chung, 1997). However, the population density of *O. rhinoceros* in the breeding sites was not reported.

In Block C, the initial population was rather low compared to Blocks A and B (Figure 2c). The use of pheromone traps at the recommended density (1 trap per 2 hectares) was suggested to keep the population of *O. rhinoceros* at low levels. This was quite evident as the highest population density recorded in the area was only 2 ISM (Figure 2c). Similarly as in Block B, the population had remained below 1 ISM after 10 MAR and finally ceased to exist by 24 MAR (Figure 2c). This suggest that at low population densities, the pheromone traps were able to control the breeding of *O. rhinoceros* in the trunk heaps.

The mortality of the third instar larvae in this block was low, less than 35% (Figure 10). This caused the overall percentage survivorship in this block seems to be high, between 44-85%. However, the overall population density of *O. rhinoceros* in this block seems to be lower than blocks A or B (Figure 2c). Therefore, there may have been an equilibrium of the microbial agents and its host in maintaining a low population density in the area.

All replanting blocks in this study have indicated low mortality of the first (between 2-4%) and second larval instars (up to 30%) which might be due to viral or bacterial infections. Besides *Oryctes* virus, there

may be other groups of viruses involved which were not able to be detected by the PCR technique. Other mortality factors involved could be naturally induced, either due to nutrition or physiological stress. Hinckley (1973) reported that percentage mortality of the larval stage was high at the first instars, when they start feeding on hard substrates, and at the third instars, when they could not accumulate enough fat reserves to enter the pupal stage.

The infection of *Metarhizium* was only prevalent in the third instar larvae (Figures 4, 7 and 10). Generally, *Metarhizium* only killed the third instar larvae within 7-16 days after infection (Latch, 1976). In these blocks however, the infection of this fungus seems to be less dominant compared to virus or bacteria. This could be due to the strain of the fungus being less virulent (Ramle *et al.*, 1999). Furthermore, in the field, natural infections of *O. rhinoceros* by *Metarhizium* was extremely low, generally less than 2 percent (Tey, 1993). This phenomenon was proven true in all the three blocks examined. Although the influence of disease agents seems prominent, other environmental factors such as rainfall, cover crops and trunk hardness may also play a role in determining the fluctuations of the population.

In general, the population of *O. rhinoceros* was noted to be clumped (aggregated) and not normally distributed. The coefficient of distribution (CD) was defined as the variance divided with the value of the mean (Brown & Downhower, 1988). An aggregated population will have a CD of more than 1 (Brown & Downhower, 1988). The CD calculated for all plots were between 3.5 to 38.6, indicating the clumped distribution of the population. This phenomena is true because the female beetle normally oviposits in batches (Hinckley, 1973), which caused the larvae to be aggregatedly distributed. There was also indications of overlapping generations (Figures 3, 6 and 9). In most sampling periods, almost all developing stages had occurred simultaneously.

Conclusion

Without any control measures, *Oryctes* population has been found to establish itself in the oil palm trunk heaps, up to a period of about 2 years. Pheromone traps can be placed early, during the onset of replanting as a useful tool to reduce chances of breeding by removing adult beetles. Constant trapping during the early replanting stage reduces the population density in the trunk heaps and therefore prevents the population from building up. For a medium sized population, trapping can reduce the population density to below 10 ISM, in less than a year after replanting. With proper manipulation of pheromone traps and targeted

application of disease agents (i.e. virus, fungus and bacteria), the population of *Oryctes rhinoceros* can be managed more effectively in oil palm replanting.

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