SHORT COMMUNICATION

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TRAP FOR THE AUTO DISSEMINATION OF *Metarhizium anisopliae* IN THE MANAGEMENT OF RHINOCEROS BEETLE, *Oryctes rhinoceros*

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ABSTRACT

An inoculation trap for the auto dissemination of spores of Metarhizium anisopliae *for the management of* Oryctes rhinoceros *in the field was designed and tested. The efficiency of the inoculation trap in capturing the adult rhinoceros beetles was found to be as good as the commercial pail type trap. The trap capture rate was 2.5 adults per trap per night (a/t/n), no different (at P>0.05) from the capture rate by the pail type trap (2.4 a/t/n). A performance test showed that 66.7% of the trapped adults that escaped from the inoculation trap were subsequently confirmed dead due to infection by the fungus. Laboratory tests also found that the infected adults had disseminated the spores to the breeding site, killing 91.7% of the larvae by fungal infection. The mortalities of the released inoculated adults were between 63% and 69%, due to infection by* M. anisopliae. *A field test showed that the percentage of trapped adults leaving the trap was between 85% and 95%. Both rates of spore solution (at 2 and 4 g litre^{-1}) caused high mortality to adults within the period of 15-30 days after trapping (DAT), and complete mortality was recorded at 45 DAT. Some 75% to 90% of the dead adults were confirmed to be infected by* M. anisopliae. *The density of viable spores collected from the soil in the trapping region showed an increase, suggesting that the* M. anisopliae *had been established in the breeding sites of the beetle.*

Keywords: *Metarhizium anisopliae*, *Oryctes rhinoceros*, oil palm pest, inoculation trap, biological control.

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INTRODUCTION

The rhinoceros beetle, *Oryctes rhinoceros* (L.), is a major pest of the oil palm, especially in Southeast Asia (Bedford, 1980; Norman and Basri, 1997). Adult beetle attacks the crown region of the palm by feeding on the spear tissues (Bedford, 1980). Severely attacked young palms are delayed in reaching maturity, while repeated attacks can kill the palms. Commonly practiced methods to control the pest include the use of a trapping method (Turner, 1973). In the early development of the trapping method, the trap was made using the plant itself as the attractant. These types of traps include the use of plant compost, wood, coconut and palm logs, or empty fruit bunches laid on the ground (Turner, 1973; Bedford, 1980). Rapid development of an attractant pheromone started from the use of ethyl dihydro-chrysanthemumate, then of ethyl chrysanthemumate (Maddisson *et al.,* 1973), and more lately to the highly effective attractant, ethyl 4-methyloctanoate (Hallet *et al.,* 1995). The use of pheromones has led to the development of new types of traps. Initially from bucket traps with metal vanes, several designs made from cheaper materials such as polyvinyl plastic have been developed (Ho, 1996; Desmier *et al.,* 2001). Currently, mass trapping of adult beetles using pheromone trap is commonly practiced by planters as it can reduce the level of palm damage (Chung, 1997; Norman *et al.,* 2001).

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The entomopathogenic fungus *Metarhizium anisopliae* var. *major* has proven effective in the management of the *O. rhinoceros* beetle, especially in reducing the immature stages of the pest. Field studies show that the adult beetles can carry and disseminate the spores to their breeding habitat (Ramle *et al.,* 1999; 2007). Taking advantage of this behaviour, an attempt to use the pheromone trap for disseminating the spores of *M. anisopliae* was investigated. This study reports the potential use of a specifically designed inoculation trap to disseminate the spores of *M. anisopliae,* thus creating an epizootic in the natural breeding habitats of the pest.

MATERIALS AND METHODS

Trap Design and Trapping Concept

The inoculation trap consists of four compartments (*Figure 1*). Each of these compartments has a specific function (*Figure 2*). The inoculation trap uses the aggregation pheromone, ethyl 4-methyloctanoate, to attract the adult beetles. Flying attracted adults will collide with the vanes and fall into the collecting chamber where a specially designed disc containing a spore solution of the fungus *M. anisopliae* var. *major* is placed. The fallen adult beetles will be exposed to and infected by the fungus in this inoculation disc. The design of the chamber and the disc allows the infected adults to escape from the trap, carrying the spores of the pathogen, so that they can disseminate these spores to the pest's natural habitat. The fungus then spreads to other healthy beetles which come into contact with the contaminated materials.

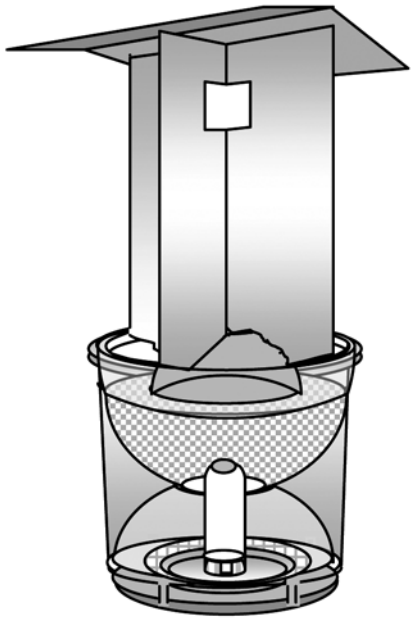

Figure 1. Perspective view of the trap for auto dissemination of spores of M. anisopliae *into the field.*

The efficiency of the inoculation traps in capturing the adults of *O. rhinoceros* was compared with the commercial pail type traps. The experiment was conducted in Johor and Selangor in Malaysia over a total of 10 days. The number of traps tested was between four and six traps per treatment. At both sites, the traps were placed side by side, over a distance of about 30 m. The capture was monitored daily and the mean number of adults captured per trap per night (a/t/n) was calculated. The treatment means were analyzed using t-test at P=0.05 (SAS, 1997).

Performance of Trap in Infecting Trapped Adults and Disseminating the Fungus to Larvae

The number of adults which escaped from the inoculation trap and were infected with the pathogen was determined in the field. The test was conducted by placing two inoculation traps in the field over four consecutive days. An inoculation disc filled with a spore solution of *M. anisopliae* (at 2 g spores litre^{-1}) formulated in water plus 0.02% wetting agent, Tween 80, was placed in the inoculation trap. Spores were produced following the method by Ramle *et al.,* (2006). The trapped adults were collected daily and kept in groups of five adults in plastic boxes, filled with rotting oil palm trunk tissues. The boxes were also supplied with 10-15 cm long pieces of sugar-cane stems as a food source for the beetles. All the boxes were kept in the laboratory at 25°C-28°C for 30 days. The numbers of dead and infected adults were recorded every alternate day. As a control, two traps were used where the inoculation disc was only filled with water plus 0.02% wetting agent.

The performance of the infected adults in disseminating the pathogen to healthy third instar larvae (L3) of *O. rhinoceros* was evaluated in the laboratory. Field-trapped adults were inoculated by soaking them in spore solutions (4 g spores litre^{-1}) for 30 s. The adults were given a resting time by placing them in an empty container for 5 min. They were then transferred into a plastic box (40 cm long × 30 cm wide × 25 cm high) that was already half-filled with oil palm rotting materials. To the box was added 12 field-collected third instar larvae (L3) of *O. rhinoceros* that were confirmed to be free from infection by *M. anisopliae*. Two rates of inoculated adults were tested, 2 and 4 inoculated adults per box (ia/b). For the treatment 2 ia/b, the experiment was replicated twice, while for the 4 ia/b treatment it was replicated four times. For the control, a box containing four non-inoculated adults was used, and this was replicated four times. Data on mortality and infection by *M. anisopliae* of both

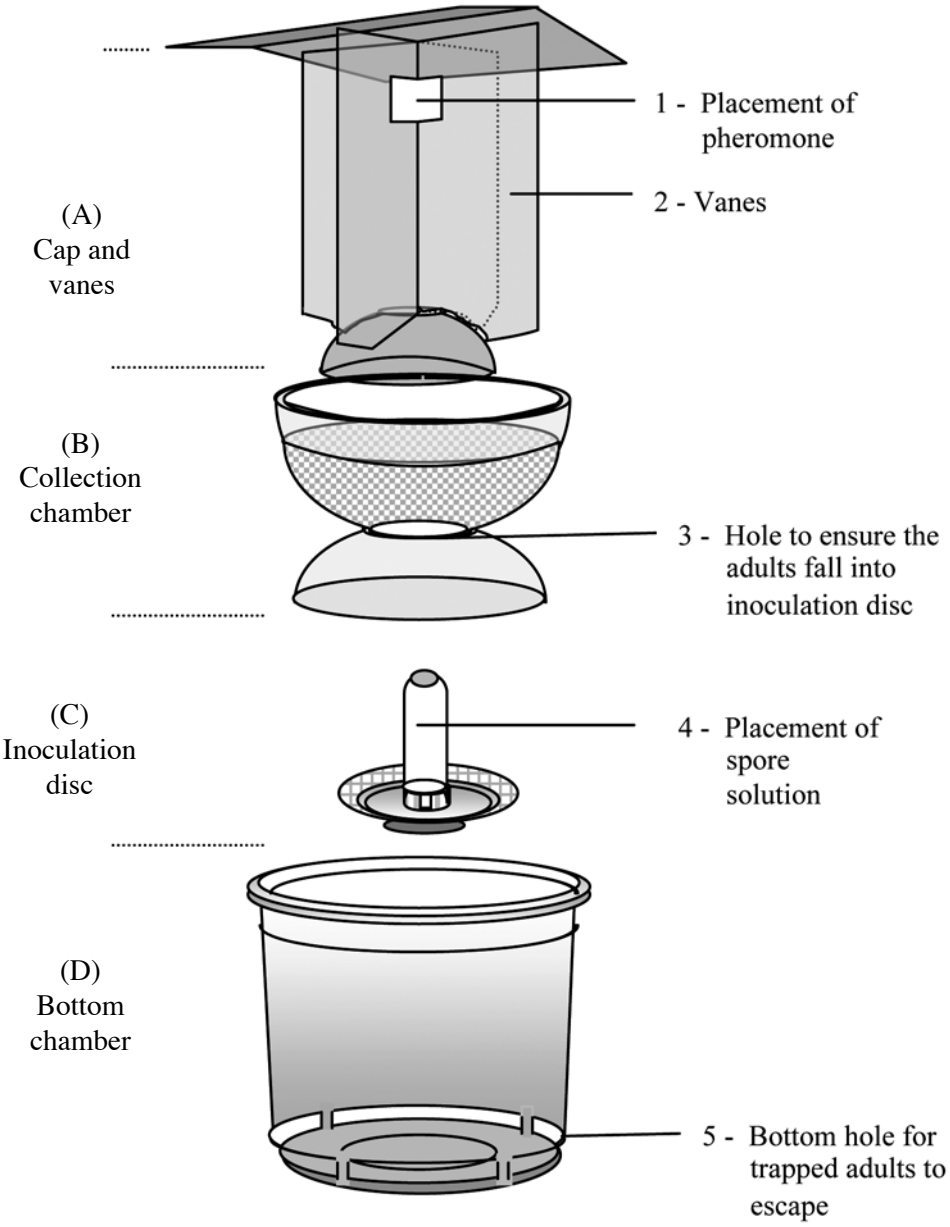

Figure 2. Perspective view of the inoculation trap showing each compartment (A-D).

the inoculated adults and larvae were recorded at two weeks after treatment (WAT). An analysis of variance was performed using PROC GLM of the Statistical Analysis System software (SAS, 1997) at P=0.05, based on angular-transformed percent mortality and infection data.

Performance of Trap in Disseminating of Spores in the Field

The study was conducted in a randomized block design at the Malaysia Palm Oil Board (MPOB) Research Station in Kluang, Johor, Malaysia. The area is hilly, totalling about 150 ha. The area was divided into 20 trapping regions, each region being about 5-10 ha in size. One inoculation trap was placed in each region (*Figure 3*). The traps were marked by region number, from 1 to 20. Inoculation traps were placed in regions 1 to 10. The traps carried two rates of spore solutions, 2 and 4 g litre^{-1}, prepared by using distilled water plus 0.02% Tween 80. Five inoculation traps were used for each rate, and were randomly placed in the trapping region.

Results from regions 1 to 10 were compared with those of the pail type traps in regions 11 to 20. Ten pail type traps were used. Five pail traps were treated as controls, while the remaining five pail traps were used as treatment traps where trapped adults were soaked for 1 min in spore solutions prepared from 4 g spores litre^{-1} in 0.02% Tween 80. These adults were released back into the field to disseminate the spores. Trapping activity was conducted for 15 consecutive days a

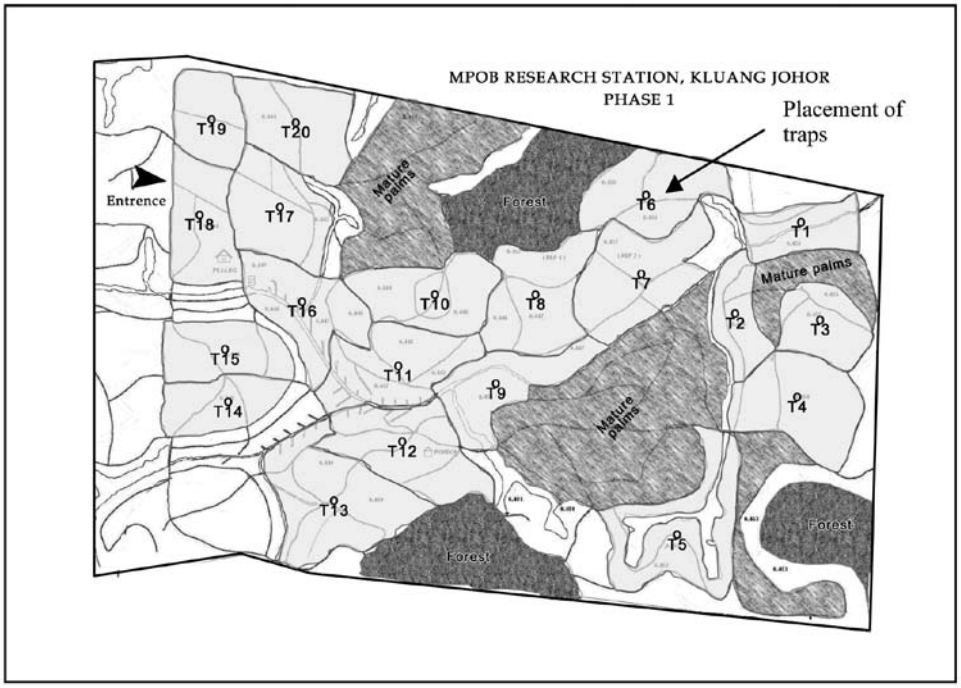

Figure 3. Map showing the experimental area and trapping regions. Trapping regions 1-10 were placed with inoculation traps supplied with spore solutions at 2 g litre^{-1} (traps 1-5) and 4 g litre^{-1} (traps 5-10). Trapping regions 11-20 were placed with only commercial pail type traps. Traps 11-15 were controls. Traps 16-20 were treatment traps where the trapped adults were inoculated by dipping them for 10 s in a spore solution at 4 g litre^{-1}.

month for every alternate month. The experiment was conducted over a period of 10 months after trapping (MAT). The trapped adults were collected every morning.

The density of viable spores in the soil and in the rotting materials in the beetle breeding sites was determined at six months from the first trapping activity. Soil samples (about 100 g) containing rotting materials were collected from six points in each trapping region (two samples each at a distance of 10, 20 and 30 m from the trap). After the soil was homogenously mixed, a sample of 1 g of the soil mixture was placed in a universal bottle. Ten millilitres of sterilized distilled water plus 0.02% Tween 80 were added, and the contents were then thoroughly shaken. The soil solution was diluted 10 times. The number of colony-forming units (cfu) of *M. anisopliae* was determined on *Metarhizium* selective media (MSM) (Ramle *et al.*, 1999). Analysis of variance (ANOVA) was performed using PROC ANOVA at P=0.05 (SAS, 1997). Means were compared using the least significant difference (LSD) test at P=0.05.

RESULTS AND DISCUSSION

The average number of adults captured by the inoculation traps was 2.5 a/t/n, and this was not significantly different (P>0.05) from the capture by the commercial pail traps (2.4 a/t/n) (*Table 1*). Ho (1996) reported that projection of vanes into the bucket, whether they are straight or bent, is superior in retaining the catch of beetles. Furthermore, a study by Desmier *et al.* (2001) demonstrated that the vanes fixed to the pail type trap and parabolic type trap captured about the same number of beetles, which was significantly higher than those captured by other traps without the vanes. An initial field test showed that out of 15 trapped adults, 10 of them or 66.7% were subsequently confirmed dead with infection by *M. anisopliae* (*Table 2*). This study indicated that water plus 0.02% wetting agent Tween 80 can be used to formulate the spores of *M. anisopliae*.

The capability of the adults in carrying sufficient numbers of spores, and subsequently distributing them into the habitat, was demonstrated in this study. The fungus killed 63%-69% of the adults that escaped from the traps (*Table 3*). Introduction of the escaped inoculated adults from the inoculation trap at both 2 and 4 adults per box caused as high as 91.7% larval mortality by cross-infection.

The field test found the same trends of capture by the inoculation traps and pail traps. The number of captured adults increased gradually from the first month after trapping and peaked at 4 MAT before gradually declining by 10 MAT. The performance of the inoculation trap in trapping and inoculating the adults was also monitored. It was determined

TABLE 1. EFFICACY OF INOCULATION TRAPS IN CAPTURING ADULTS OF *O. rhinoceros* COMPARED TO PAIL TYPE TRAPS

Locality	No. of trapping (days)	Inoculation trap			Commercial pail trap		
		No. of traps tested	Total capture	Mean of capture (adults/trap/ night)	No. of traps tested	Total capture	Mean of capture (adults/trap/ night)
Johor	1	6	10	1.67	4	14	3.50
	2	6	17	2.83	4	6	1.50
	3	6	20	3.50	4	16	4.00
	4	4	12	3.00	4	2	0.50
	5	4	8	2.00	4	8	2.00
	6	4	12	3.00	4	12	3.00
Selangor	1	6	16	2.67	4	16	4.00
	2	6	15	2.50	4	4	1.00
	3	6	14	2.33	4	12	3.00
	4	6	10	1.50	4	4	1.00
Average	-	-	-	2.50	-	-	2.35 ^{ns}

Note: ^{ns} Means were not significantly different by t-test at P=0.05.

TABLE 2. MORTALITY OF 15 *O. rhinoceros* ADULTS AFTER ENTERING THE INOCULATION TRAPS CONTAINING SPORE SOLUTIONS OF *M. anisopliae*

No. of captured adults	No. of dead adults (days after trapping)					No. of dead adults	No. of infected adults
	6	12	18	24	30		
15	0	9*	1*	1	1	12	10*

Note: * Dead adults confirmed to be infected by *M. anisopliae.*

TABLE 3. PERFORMANCE IN DISSEMINATING SPORES OF *M. anisopliae* BY ADULTS CAUSING MORTALITY OF *O. rhinoceros* LARVAE

Treatment (inoculated adults/box)	Adults/box	Adults			Larvae		
		Tested (N)	Mortality (%)	Infected (%)	Tested (N)	Mortality (%)	Infected (%)
T1 (2)	2	8	100.0a	62.5a	48	100.0a	91.7a
T2 (4)	4	16	87.5a	68.5a	48	100.0a	91.7a
Control (0)	4	12	16.7b	0.0b	48	45.6b	0.0b

Note: Means within a column bearing the same letter are not significantly different by LSD at P=0.05.

that 85%-95% of the trapped adults escaped from the inoculation trap and disseminated the spores into the breeding sites. The density of viable spores collected from the soil in each trapping region increased over time, suggesting that the disease had established in the breeding sites (*Table 4*). Spores were also detected in the control trapping region, which suggests that they were carried over by the infected adults that escaped from any of the inoculation traps placed in the surrounding regions. Data on mortality showed that the time taken for the infected adults to die was between 15 and 30 days after trapping (DAT). Both rates of 2 and 4 g litre^{-1} spore solution caused high mortality in the adults in the period of 15-30 DAT, and complete mortality was recorded at 45 DAT. Such a long period gave ample time for the infected adults to contaminate the habitat with the spores. Between 75% and 90% of the dead adults were confirmed to be infected by *M. anisopliae*. Growing cover crops on the rotting heaps will protect the spores from detrimental abiotic factors and could prolong the life span and survival of the spores (Moore *et al.,* 1993; Ekesi *et al.,* 2003). Therefore only a low number of viable spores is required to make a significant impact on the larvae of *O. rhinoceros* (Ramle *et al.,* 2007).

TABLE 4. DENSITY OF VIABLE SPORES OF *M. anisopliae* IN TRAPPING REGIONS OVER TIME

Type of trap	Rate (spores per litre)	Density of viable spores (× 10^3 cfu)* at various months after trapping (MAT)			
		Before trapping	6	8	10
Pail type	Control	0.0	0.0564a	0.0500a	0.1000a
	4 g	0.0	0.3000a	0.2000a	0.3840a
Inoculation	2 g	0.0	0.2000a	0.2500a	2.0500a
	4 g	0.0	0.2680a	0.0320a	0.4840a

Note: * Means of density were recorded from six plates of *Metarhizium* selective media.
Means within column bearing the same letter are not significantly different by LSD at P=0.05.

In the field study, the impact of the inoculation traps in reducing the population of adults was not observed, probably because of low trap density and the migrating behaviour of *O. rhinoceros* adults. A longer period of study is required in order to measure any significant impact of the inoculation traps in reducing the overall population of *O. rhinoceros*.

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