

## Response of the male bagworm moth (*Metisa plana* Walker, Lepidoptera: Psychidae) towards female bagworm pheromone lure in wind tunnel bioassays

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A study on the response of winged adult males of the bagworm moth *Metisa plana* towards sex pheromones of the sessile female bagworms was conducted using a dual choice olfactometer and a wind tunnel, between January 2009 and December 2010. Dual choice bioassays showed that male moths significantly frequently (Chi-square;  $p < 0.001$ ) approached pheromone lures made from case of calling female (CCF) and also from calling female (CF), within a time period of 2.7 min. In the wind tunnel, male moths were tested against 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 5.0, 8.0 and 10 female equivalents (FE) of extracts of CCF, CF, adult female (AF), case adult female (CAF), adult male (AM) and a control, from a distance of 100 cm. Males flew in an eight shape then landed on the pheromone spot. The activation threshold for male moths was 0.4FE for CCF and CF in the presence of wind at a distance of 100 cm. The optimal positive response of males toward both CCF and CF was shown at a concentration of 0.8FE. For groups of 10 males tested at the same time, the activation threshold was also at 0.4 FE for CCF under the same conditions. The results on the activation threshold and optimal responses of males towards the sex pheromone are important for the formulation of synthetic pheromone for integrated pest management of the bagworm.

**Key words:** *Metisa plana*, bagworm moth, Psychidae, Lepidoptera, pheromone, case of calling female, activation threshold, behavior, African oil palm, *Elaeis guineensis*, wind tunnel bioassay

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## INTRODUCTION

The bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) is an important pest of the introduced African oil palm, *Elaeis guineensis* Jacq. (Wood 1979) in Malaysia (Wood et al. 1973, Kamarudin & Arshad 2006). A damage of 50% will cause yield decline of around 43% over the next two years (Wood et al. 1973; Kamarudin & Arshad 2006). Defoliation by bagworms will reduce the aesthetic quality of the hosts, and may even kill them (Johnson & Lyon 1988, Ellis et al. 2005). Due to the immobility of the wingless female (Borror & White 1970) after hatching, the male plays a decisive role in mate searching, caused by sex pheromone release of the female.

In the Paulownia bagworm, *Clania variegata*, females release a sex pheromone from a gland in the dorsal mesothorax and expel pheromone-impregnated scales (hairs) out of the pupal case into the lower part of the case to attract males (Zhao 1981, 1984; Gries et al. 2006). Conspecific males, attracted by the pheromone, alight on the case, insert their extensible abdomen through the case into the female pupal case, and finally mate with the adult female (Gries et al. 2006). Rhainds et al. (2009) reviewed the bionomics of 18 species of bagworms, including *Metisa plana*. Kamarudin and Arshad (2006) reported 73.9 mean catches of males per night using four receptive females in vane trap as pheromone sources. Kamarudin et al. (2010) showed that mass trapping using four female live bagworms in sticky traps resulted in a reduction of 35-45% of fronds damage and 20-27% of fronds damage in control plots without any significant difference statistically.

Since the sex pheromone of the bagworm has not yet been synthetically produced, this study aimed to determine (i) which part of the bagworm would elicit the strongest male attracting response in a dual choice olfactometer and (ii) the activation threshold and optimal concentration of female pheromone sources at selected distances and different wind conditions in wind tunnel bioassays.

## MATERIALS AND METHODS

**Study sites.** The study was conducted from January 2009 until December 2010 in an oil palm plantation area at Besout (latitude 3° 53' 59" N, longitude 101° 19' 58" E) and Trolak (latitude 3° 52' 58" N, longitude 101° 22' 58" E), Perak, Malaysia (**Figure 1**). The study sites located 115 km (1 hr 16 min) from Kuala Lumpur are accessible by taking the North-South Highway with the exit at Sungkai.

### Insect collection

**Male bagworm moth.** Males were obtained using light traps set up following the methods described by Abdullah (2005) and Abdullah et al. (2008). Abdullah et al. (2005, 2008) set up light traps from 1700 to 2300h. However in this study, light traps were set up early in the morning, between 0400 to 0700h, because more male adult *M. plana* were visiting the light traps compared to the light traps set up between 1700 to 2300 h. *Metisa plana* males were active when average temperatures were around 22.5°C±1.291, with an average relative humidity of 93.13%±2.416. Males attracted to the light traps were manually collected and individually placed in pill bottles and

## Pheromone lure in bagworm moth, *Metisa plana*

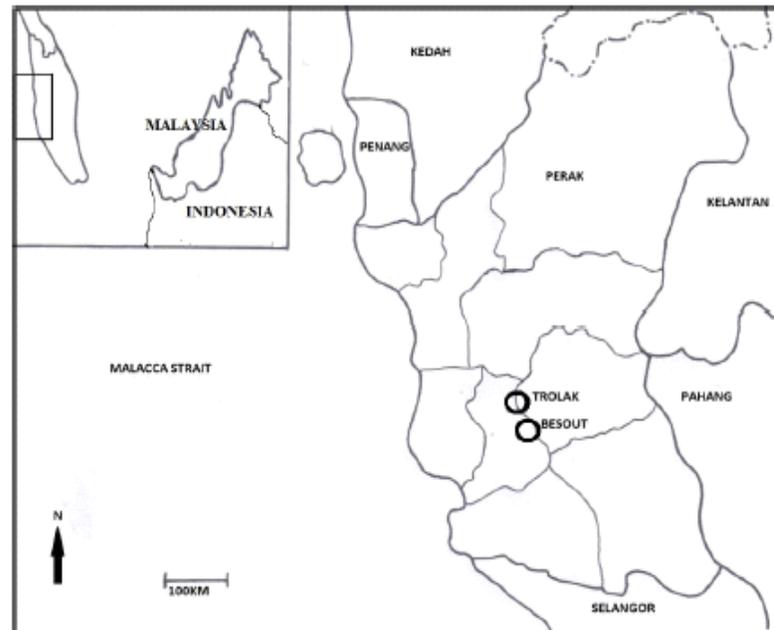


Figure 1. Location of study sites at Besout and Trolak, Perak, Malaysia.

immediately used for the bioassays. To avoid using the same male in different studies, all males already used in the bioassay were subsequently killed. Throughout the study, a total of 17,310 males were used.

**Female bagworm moth.** Female bagworms were handpicked from infected oil palm fronds during the day. To prevent contamination, gloves were used during collection. Several thousands of non-calling and calling females were used to make different pheromone lures.

**Determination of calling female bagworm moth.** A piece of masking tape was hung between two oil palm trees, the distance between one tree to another was 2.5 m and setting up between 0400 and 0700h, at a different location far away from the light traps. Two hundred and fifty virgin female bagworms enclosed within their pupal cases were placed on the masking tape hung between two palm trees, individually attached to the tape at a distance of 1 cm from each other. If a free flying adult male moth approached the receptive female bagworm, the female was removed from the tape before the copulation could occur, and the female collected was marked as a calling female.

**Mating behavior.** In order to observe mating behavior between approaching males and receptive females, some of the calling female bagworms glued on the tape were not removed for extraction. In these cases ( $n = 20$ ), the behavior of the approaching male towards the female on the tape was observed. The peak mating time was determined to be between 0400h and 0800h, and the behavioral bioassays were conducted during this peak period.

**Dissecting bagworm according to development stages.** Bagworms collected from the fronds of the oil palm trees were separated according to male and female cases. The female cases are pointed at the end while the male cases are not pointed in shape. The bagworms were dissected using a sharp scissor under microscope and gently using a feather forcep, the female was pulled from the case, sorted according to their stages (pupa, prepupa, calling, non calling female) and placed separately in petri dish. All glassware and tools used during dissection were cleaned using acetone before and after used to avoid contamination. Similar ways were used for calling female (CF), case adult female (CAF), case calling female (CCF), adult female (AF), and adult male (AM) until reached 1000 for all the development stages.

**Pheromone source.** A total of 1000 CFs without their cases were immersed for an hour in chloroform for extraction. The extract was filtered with sodium sulphate to remove any remaining water before use. Similarly, 1000 each of CCF, BAF, AF and AM were also extracted. Each extract was purified using flash chromatography. The pheromone concentration prepared were 0.1 female equivalents (FE) of CF, 0.1FE CCF, 0.1FE AF, 0.1FE CAF and 0.1FE AM. Other concentrations of female equivalents at 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 3.0, 5.0 and 10FE for all development stages were also prepared. Each fraction of the purified extract was concentrated under low pressure and kept in the refrigerator for further use in the bioassays.

**Male preference towards female pheromone in dual choice olfactometers.** A dual choice olfactometer was constructed using a T-shaped glass tube (3.0 cm diameter x 10.0 cm length; arms A, B and C) covered with a wire net at both ends of the horizontal arm. The pheromone source was placed inside the right horizontal arm (B), while the control was put inside the left horizontal arm (C). The male moth to be tested was released into the vertical arm (A). To prevent contamination, the glass tube was wiped with ethanol before and after each experiment. A pump (Gilman) pulled air from both ends towards the male at arm C and the speed of airflow through the tunnel was kept at a flow rate of  $0.2 \text{ ms}^{-1}$ .

A No. 1 Whatman filter paper cut into 4 x 4 cm was spotted with 1.0FE of CF using a Hamilton syringe. The spot was left to dry for one to two min and then placed inside the arm B. The control arm (C) was equipped with a filter paper spotted with solvent only. A test moth was released at the vertical arm (A) and the choice made by the male moth, either to approach the pheromone source or the control, was recorded. The time taken to reach either arm B or C was recorded. All tests were replicated 25 times, using a new male each time. CCF, BAF, AF and AM were each tested.

**Wind tunnel.** A wind tunnel (30.0 cm diameter x 100 cm length) was constructed for the behavioral bioassay studies using a transparent cylinder made of acrylic resin. One end was permanently covered with a plastic net and the other end closed using

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**Wind tunnel.** A wind tunnel (30.0 cm diameter x 100 cm length) was constructed for the behavioral bioassay studies using a transparent cylinder made of acrylic resin. One end was permanently covered with a plastic net and the other end closed using a cover made from similar netting. A fan was used to blow air through the tunnel. The speed of airflow in the wind tunnel was maintained at 0.2 m/s. Activated charcoal was placed in front of the fan to ensure that only clean air passed through the wind tunnel. The tunnel was marked in 10 cm segments using masking tape.

**Behavioral bioassay in wind tunnel.** The pheromone source to be tested was spotted on a 4 x 4 cm No. 1 Whatman filter paper and then left to dry for one to two min before being hung in the center at the upwind end of the wind tunnel. One male *M. plana* was released at the downwind end at a distance of 100 cm away from the bait.

Male movements, behavior and duration of each behavior in response to the pheromone source were recorded for a period of 15 min. This test was carried out in 20 replicates with a new *M. plana* adult male tested each time.

The pheromone sources tested were 0.1 female equivalent (FE) of CF, 0.1FE CCF, 0.1FE AF, 0.1FE CAF and 0.1FE AM. All treatments were tested 20 times using a new male each time. In order to determine which concentration evokes the optimal response, the pheromone dosage was increased to 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 3.0, 5.0 and 10FE for all the treatments. The male responses were scored as 1, 2, 3, 4, or 5 according to the conversion number system in **Table 1**.

Table 1. The conversion number system for scoring the male response in wind tunnel.

Score	Description
5	Strong positive (+++): less than 3 min until reaching bait target; movements of both wings and antennae; flying upwind; resting on or near bait no more than 30 cm away from bait.
4	Medium positive (++) : more than 3 but less than 5 min until reaching target, movements both wings and antennae; flying upwind; resting on or near bait no more than 30 cm away.
3	Slight positive (+): more than 5 min but less than 10 min until reaching target; movement of either wing or antennae; resting near bait no more than 30 cm away.
2	Negative (-): no response after 10 min toward target; no movement for either wings or antennae; resting at or more than 50 cm away from bait.
1	Strong Negative (—): no response after more than 15 min toward the target; no movement at all for either wings or antennae; resting down wind.

**Group male bioassay.** The above experiments were tested with groups of 10 males released at the downwind end.

**Statistical analysis.** Comparison of the means of the score from 20 replicates or each treatment and between treatments were tested using chi square test and, where appropriate, Tukey Test.

## RESULTS

**Mating behavior of a pair of *M. plana*.** In the field, one male *M. plana* moth was observed to fly directly to a hanging calling female bagworm, between 0600 and 0700 h, at the oil palm plantation. No other male was seen to approach the female that was approached by an earlier male. After approach, the male turned its body upside down to insert its aedeagus into the pupal case to reach the female inside the case, and mating occurred immediately. No courtship was observed. About 20 cases were observed in order to conclude the optimal mating period for this study. During these observation periods, the mean recorded temperature was  $22.5^{\circ}\text{C}\pm 1.29$  and the relative humidity was  $93.1\%\pm 2.42$  (Table 2). Similar to Rhainds et al. (2009) this study observed that upon being attracted by the pheromone plume of a virgin female and landing on her bag, the male inserted his extensible abdomen through the posterior opening of the female's bag all along her body inside her pupal case to reach the caudal genitalia.

Table 2. The mean temperature ( $^{\circ}\text{C}$ ) and humidity (%) during mating behavior observations in January 2009.

Parameter	Hour								Total	S.D*	Mean
	0400	0500	0600	0700	0800	0900	1000	1100			
Temperature ( $^{\circ}\text{C}$ )	21.0	21.2	21.6	22.3	22.3	23.5	23.7	24.6	180.2	1.29	22.53
Humidity (%)	90	90	92	92	95	95	95	96	745	2.42	93.13

\*S.D = Standard Deviation

### **Male preference towards female pheromone source in dual choice olfactometer.**

The results of the bioassays using a dual-choice T-shaped olfactometer to determine male *M. plana* preference for different pheromone sources offered are summarized in Table 3. The test males released at the vertical end (A) immediately flew (in less than 3 sec) toward the pheromone source at the end of the olfactometer arm (B). The test was replicated 25 times using a new male each time. The table shows that males significantly preferred the case calling females ( $X^2$ ;  $p<0.001$ ) and calling females ( $X^2$ ;  $p<0.001$ ) to controls. Meanwhile, other pheromone extracts of CAF, AF and AM were not significantly different from the controls.

### **Male response towards different concentrations of extracts in wind or no wind conditions for single adult male *M. plana* in wind tunnel.**

In the control (chloroform treated filter paper) experiments, the males, upon release, flew randomly, without showing any specific flying pattern. The male moths, including replicates (N=20), stayed at the downwind end near the point of release until the end of observation (mean score: 1.00) (Table 4). The antennae usually were held in a V-shaped pattern while resting. When using the pheromone lure, the males that showed a positive response to the lure would fly in an 8-shaped pattern twice before flying straight, and then flew straight and landed on the pheromone spot on the filter paper (Figure 2).

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Table 3. Response of male *Metisa plana* towards female *M. plana* pheromone source in dual choice bioassay tested using Chi-square test ( $p < 0.001$ ).

Sources		Males	$X^2$ value	
B	Empty	12	0.052	NS*
C	Empty	13		
B	Case Calling Female	21	9.754	$p < 0.001$
C	Control	4		
B	Adult Female	15	0.275	NS
C	Case Adult Female	10		
B	Calling Female	20	9.182	$p < 0.001$
C	Control	5		
B	Male Adult	11	0.371	NS
C	Control	14		
B	Adult Female	7	8.271	$p < 0.001$
C	Calling Female	18		
B	Empty	13	0.052	NS
C	Control	12		

\*NS = Not significant

**Figure 3** presents the percentage of different behavior components exhibited by 350 males tested, using fourteen different concentrations of pheromone lure of CCF, released from a distance of 100 cm. Males either (i) stayed downwind; (ii) flew upwind and lingered around the pheromone source; (iii) stayed at the upwind end within 30 cm from lure or (iv) landed on the pheromone source. In a positive response, males flew towards the pheromone source at the upwind end, and landed on the filter paper extract at 0.4 female equivalents (FE) of CCF. The intensity of the positive response increased as the concentration of the lure rose to 0.8FE. As the concentration of the lure increased, the percentage of landing males also increased, as shown in Figure 3. With 64% of males landing, 24% of males flying around the lure and 4% staying upwind, thus a total of 100% males were attracted to 0.8FE of CCF. When attracted to the bait, the antennae were opened up wide and the ends of the antennae touched the pheromone spot on the filter paper.

Table 4. Mean response of 20 adult male *M. pinnus* moths towards different treatments under wind conditions, at a distance of 100 cm, in the wind tunnel, using Tukey Test ( $p < 0.05$ ).

Wind Conditions	Treatment	Female Equivalent (FE)													
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	5.0	8.0	10.0
Case Calling Female	Case	1.70 <sup>a</sup>	1.75 <sup>a</sup>	2.50 <sup>b</sup>	3.50 <sup>c</sup>	4.20 <sup>d</sup>	4.35 <sup>d</sup>	4.55 <sup>e</sup>	4.60 <sup>e</sup>	4.10 <sup>e</sup>	3.90 <sup>e</sup>	3.70 <sup>e</sup>	3.60 <sup>e</sup>	2.95 <sup>bc</sup>	2.85 <sup>b</sup>
	Calling Female	2.20 <sup>ab</sup>	2.60 <sup>ab</sup>	3.20 <sup>bc</sup>	3.60 <sup>c</sup>	4.00 <sup>cd</sup>	4.1 <sup>cd</sup>	4.45 <sup>d</sup>	4.05 <sup>cd</sup>	3.90 <sup>cd</sup>	4.00 <sup>cd</sup>	3.95 <sup>cd</sup>	3.75 <sup>c</sup>	3.35 <sup>bc</sup>	3.3 <sup>b</sup>
(Distance 100 cm)	Adult Female	1.65 <sup>a</sup>	1.95 <sup>a</sup>	2.05 <sup>a</sup>	2.45 <sup>ab</sup>	3.20 <sup>bc</sup>	3.40 <sup>bc</sup>	3.55 <sup>c</sup>	3.90 <sup>c</sup>	3.75 <sup>c</sup>	3.50 <sup>c</sup>	3.40 <sup>c</sup>	3.25 <sup>bc</sup>	3.25 <sup>bc</sup>	2.55 <sup>a</sup>
	Case Adult Female	1.70 <sup>a</sup>	1.90 <sup>a</sup>	2.05 <sup>a</sup>	2.35 <sup>ab</sup>	3.05 <sup>b</sup>	3.25 <sup>b</sup>	3.55 <sup>c</sup>	3.8 <sup>c</sup>	3.95 <sup>c</sup>	3.85 <sup>c</sup>	3.10 <sup>b</sup>	2.85 <sup>b</sup>	2.60 <sup>ab</sup>	2.60 <sup>ab</sup>
Male Adult	Male Adult	1.55 <sup>a</sup>	2.05 <sup>ab</sup>	2.15 <sup>ab</sup>	2.30 <sup>ab</sup>	2.30 <sup>ab</sup>	2.00 <sup>ab</sup>	2.15 <sup>ab</sup>	1.90 <sup>a</sup>	1.90 <sup>a</sup>	1.80 <sup>a</sup>	1.80 <sup>a</sup>	1.55 <sup>a</sup>	1.70 <sup>a</sup>	1.70 <sup>a</sup>
	Control	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>

**Pheromone lure in bagworm moth, *Metisa plana***

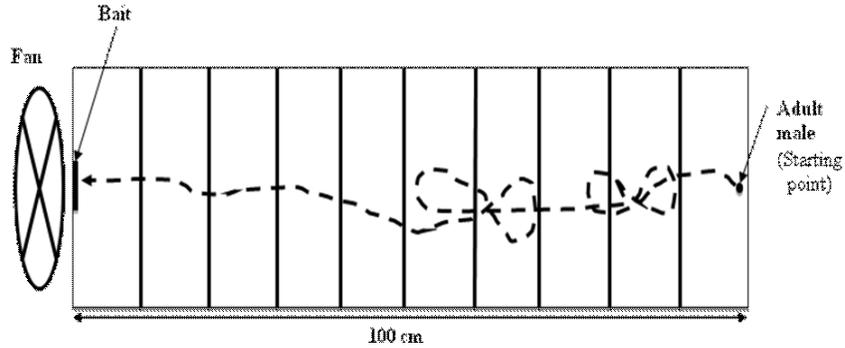


Figure 2. Positive response of adult male *Metisa plana* towards pheromone source on filter paper in a wind tunnel.

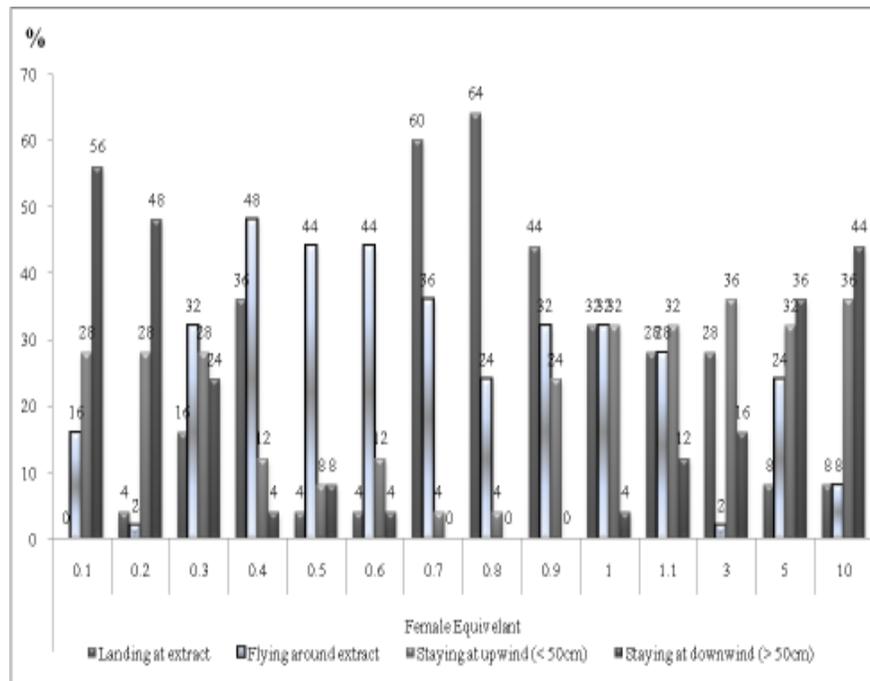


Figure 3. Percentage of behavior components exhibited by male moths (N=20) towards the concentration of CCF at 100 cm distance, under wind conditions.

**Table 4** shows the male responses towards different pheromone sources under wind conditions upon release from a 100 cm distance. The activation threshold (mean score: 3.50) for a positive male response to the CCF was determined at a concentration of 0.4FE. Males showed a maximum response towards CCF at concentrations of 0.7FE (mean score: 4.55) ( $p \leq 0.05$ ). Similarly, the activation threshold for males to show a positive response to CF was at 0.4FE, with the optimal response being observed at 0.7FE (mean score: 4.45) ( $p \leq 0.05$ ).

However, as the concentration of the pheromone lure was raised, the male response became negative towards both CCF (mean score: 2.95) and CF (mean score: 3.35), at a concentration of 8.0FE. For the pheromone lure made from AF and BAF, the activation threshold of the positive male moth response when the dosage was 0.7FE of AF (mean score: 3.55) and 0.7FE BAF (mean score: 3.55). The optimal male response was shown towards 0.8FE for AF (mean response: 3.90) and 0.9FE (mean response: 3.95) for BAF. The lures of AF and BAF began to elicit a negative response from the test males at 1.1FE (mean score:  $< 2.30$ ).

**Figure 4** shows the response of *M. plana* males from a distance of 100 cm and under wind conditions, using confidence intervals (CI) in a simple linear regression. *Metisa plana* regression model fit the 95% confidence interval ( $\hat{\alpha} = 0.05$ ). The confidence interval of CF was higher than that of CCF, followed by those of AF, CAF and AM.

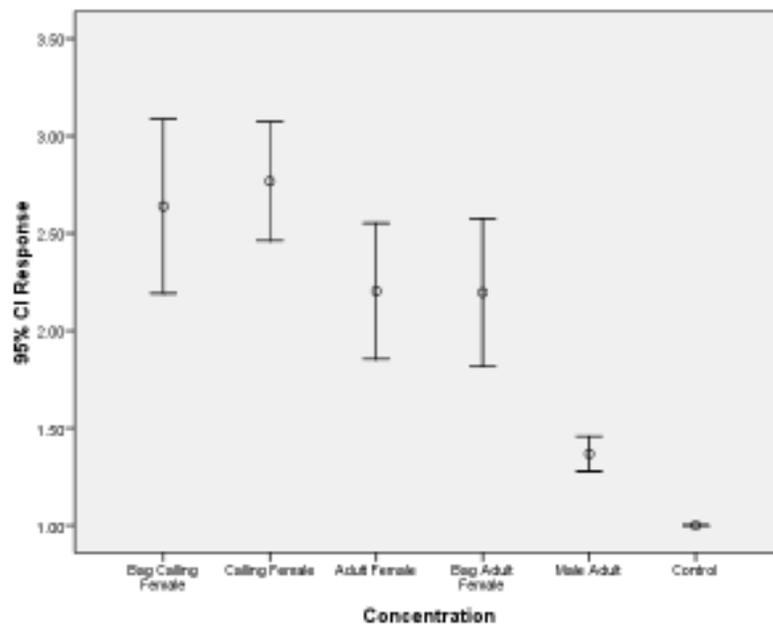


Figure 4. Response of *Metisa plana* upon release from a distance of 100 cm under wind conditions.

## **Pheromone lure in bagworm moth, *Metisa plana***

**Response of a group of *M. plana* males.** For the group bioassay of 10 males released at a time, the activation threshold for CCF was determined at 0.4FE (**Table 5**). When 10 males were simultaneously released into the wind tunnel at the downwind site, all males flew upwind in an 8-shaped pattern twice and then landed at the upwind end. Only one male was observed to have landed on the filter paper spotted with the female lure. Meanwhile, other males landed around the lure within 10 cm away from the filter paper. However, one or two of the males, after initiating flight, would land at the downwind end and remained resting there until the end of the experiment.

## **DISCUSSION**

This study has significantly contributed to the understanding of the role of the bagworm pheromone in Malaysia. This is the first study on the behavior of *M. plana* in a dual olfactometer and wind tunnel, in which the female pheromone extracted from CCF and CF, evoked the strongest male attractive response. This study has experimentally provided evidence that the highest amount of pheromone was obtained from the calling female. Extract of adult male moth was unattractive to tested males, showing that similar pheromone components are not found in the male. In this study, the mating behavior between an approaching male and a calling female hung on the tape in the field showed a highly modified mating behavior of females that remained within their case similar to observation made by Rhainds et al. (2009).

**Dual choice bioassay.** Svatoš et al. (1999) reported that many species of Lepidoptera have developed a remarkably sensitive communication system between sexes, whereby males can respond to minute amounts of a pheromone produced by virgin females. Similarly in this study, a minute amount of pheromone of 0.4FE of CCF elicited male upwind flight and landing behavior response. The attractiveness and responsiveness of adult male moths were investigated through their behavior, such as wing, antennae, head response and flying movement and pattern when exposed to the different dosages of and distances from the pheromone bait of female extracts. Kamarudin and Arshad (2006) and Kamarudin et al. (2010) reported that males of *M. plana* were attracted to a lure of four live females during mass trappings in smallholders. In contrast in this study, males of *M. plana* were attracted to the low amounts of 0.4FE of CCF extract. This is being supported by the finding of Rhainds et al. (2009) who reported that females periodically protrude their thorax outside of the lower section of the case, to further dissemination of pheromone.

This is in agreement with studies on the sex pheromone from another bagworm, *Clania variegata*, which has been reported to expel pheromone-impregnated scales (hairs) out of the pupal case into the lower part of the case to attract males (Zhao 1981, 1984; Gries et al. 2006).

Hagaman and Carde (1984) and McNeil et al. (1997) showed that virgin females produce more pheromones than mated females, and male moths may therefore respond better to higher pheromone concentrations as a way to increase their reproductive fitness. McNeil et al. (1997) reported that young and non-mated female moths release larger amounts of pheromones. The pheromone extract from this study from

non-mated calling females and non-mated non-calling females thus agreed with findings from previous researches.

***Effects of distance, concentrations and wind condition on male response, inside the wind tunnel.*** Adult male *M. plana* immediately responded to the female bait from a distance of 100 cm, under wind condition, by flying in an eight-shaped pattern twice and then flying straight forward towards the target and landing on it. Upon landing, the males' heads were raised up and the parabola antennae straightened, with the ends of the antennae drooping and touching the extract on the treated filtered paper. The wings were opened widely. Such concentrations of pheromone in the air depend in part on the initial rates of evaporation at the source, on the wind speed, and on patterns of diffusion, all of which are affected by environmental conditions (Flint et al. 1990, Zeoli et al. 1982, Bierl-Leonhardt 1982). In this study, male upwind flight towards the pheromone source was facilitated when wind was present, whereby males flew upwind faster, indicating that wind carried the pheromone chemicals in the air to the perceiving males.

Bossert and Wilson (1963) reported that the active space of the sex pheromone or the distance within which males are attracted to the pheromone source is essentially determined by the degree of diffusion of the pheromone in the atmosphere. Mafra-Neto and Carde (1994) reported that after a short delay, the males of the lepidopteran *Cadra cauttela*, in response to a pheromone source, would turn upwind and thus engage briefly in a faster and straighter flight along the wind line. Mafra-Neto (1993) stated that when the level of pheromone concentration is above the threshold, upwind flight behavior would occur. Pheromone concentrations below the threshold promote males to turn more perpendicular to the wind line.

In the Least-Squares Model, the best fitting line for the observed data is calculated by minimizing the sum of the squares of the vertical deviations from each data point to the line. Because the deviations are first squared, there are no cancellations between positive and negative values. A confidence interval describes the range of interval values, to show a point estimate as an estimate of parameter in a population. They are expressed by the following equations:

$$b_1 = \frac{\sum xy - 1/n(\sum x)(\sum y)}{\sum x^2 - 1/n(\sum x)^2}$$

$$b_2 = y - b_1x \quad (\text{Kiefer 1977})$$

The behavior of the adult male moths, flying towards the female sex pheromone, was influenced by the concentration of the pheromone. As the pheromone dosage increased from 0.1FE to 0.8FE, males flew upwind at a progressively higher speed. Some males landed on the bait, flew around the bait or landed near the bait (30 cm away from the bait). Kamm et al. (1989) reported that the overall pattern of response in *C. rosaceana* was an S-shaped curve, where males responded to increasing

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pheromone concentrations up to a maximum level, after which response remained stable. Similar patterns of response to a range of pheromone doses or concentrations have been observed with other Lepidoptera (Bellas & Bartell 1983). Similarly in this study, the orientation of the upwind moth follows an “8”-shaped pattern twice, before flying straight forward towards the bait and landing on it. Inside the wind tunnel, experiments with *Pandemis pyrusana* showed that the pheromone concentration was linearly related to the response (Curkovic et al. 2009). Exposure to the higher concentration caused a decreased response (Mazomenos et al. 2002). This study found that the inhibitory threshold was reached for both CCF and CF, where there was a reduction in response at the higher pheromone concentration tested. Males increase their fitness if they follow plumes that originate from sources with larger amounts of pheromone (Curkovic et al. 2009).

The findings on the activation threshold, optimal response dosage and inhibitory threshold, where the dosage gave a negative response, are important factors when formulating pheromone vials for managing bagworms in the field. Gries et al. (2006) reported (1S)-1-Ethyl-2-Methylpropyl 3,13-Dimethylpentadecanoate as a major sex pheromone component of the Paulownia bagworm, *Clania variegata*. Other bagworm pheromones reported include 1-Methylethyl octanoate of *Megalophanes viciella* (Subchev et al. 2002). Chiral esters were isolated from the sex pheromone of the bagworm *Oiketicus kirbyi*, including (2R)-Pentyl octanoate, (2R)-Pentyl nonanoate, (2R)-Pentyl decanoate, (2R)-Hexyl decanoate and (2R)-Pentyl dodecanoate (Rhains et al. 1994). Warthen et al. (1996) reported (2R)-Pentyl decanoate as structure-activity relationship observations for the bagworm moth pheromone.

A study is currently in progress with the aim to identify the active pheromone components from female *M. plana*. This would be of great economic importance for Malaysia and other African oil palm producing countries.

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