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BIONOMICS OF THE TEAK BEEHOLE BORER, *XYLEUTES CERAMICUS*, IN NORTHERN THAILAND : MATING BEHAVIOR

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บทคัดย่อ

การศึกษาพฤติกรรมการจับคู่ของผีเสื้อมอดป่าเจาะต้นสัก ได้ทำการทดลองที่สวนผลิตแมลงพันธุ์ไม้ป่าแม่กา อ.เมือง จ.พะเยา โดยการติดกับดักแบบลวดตาข่ายครอบไว้ที่ปากรูของมอดป่าเพื่อดักจับผีเสื้อของมอดป่าแล้วนำไปศึกษาพฤติกรรมในกรงเลี้ยงแมลงและในสภาพแปลงสัก พบว่าผีเสื้อเริ่มแสดงพฤติกรรมต่าง ๆ ตั้งแต่เวลา 19.00 น. ตัวเมียจะแสดงอาการเรียกตัวผู้ให้เข้ามาจับคู่ผสมพันธุ์ในช่วงเวลา 20.00-23.00 น. ตัวผู้ตอบสนองต่อการเรียกของตัวเมียโดยการกระพือปีกแล้วเดินเข้าหาตัวเมียเพื่อจับคู่ผสมพันธุ์ ตัวเมียสามารถรับการผสมพันธุ์ได้เพียงครั้งเดียวในขณะที่ตัวผู้สามารถผสมพันธุ์ได้ 2 ครั้ง

การทดสอบการตอบสนองต่อสารกลิ่นเพศ (sex pheromone) ในแปลงสัก ในปี ค.ศ. 1989 และ 1990 พบตัวผู้ 4 ตัวบินไปยังกับดักที่มีตัวเมียคลุกภายใน แต่ไม่พบตัวผู้บินไปยังกับดักที่ใส่สารสกัดจากตัวเมีย อย่างไรก็ตามเมื่อได้แยกสารสกัดออกเป็นส่วน ๆ พบว่าตัวผู้มีการตอบสนองต่อสารสกัดบางส่วนที่แยกได้ เมื่อเป่าลมผ่านสารสกัดที่ทำให้แห้งบนก้อนสำลีและกระดาษกรอง

ABSTRACT

Female displayed calling behavior from 20:00 to 23:00 and males responded to the calling of females with wing-fluttering. Males approached females with fluttering wings and copulated them. Females copulated only once but males copulated twice.

Males walked up wind and approached females when virgin females were placed windward on an olfactometer. In the field, four males were captured on the traps baited with virgin female in both 1989 and 1990, although no male was captured on the traps baited with crude extract of sex pheromone. Some of the chromatographically separated fractions, however, elicited wing-fluttering of male moths when exposed to air-puffing.

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INTRODUCTION

The teak beehole borer (TBB), *Xyleutes ceramicus* Walker, is the most serious insect pest of the teak (*Tectona grandis* Linn. f.) in northern Thailand (Hutacharern, 1983), and, therefore, effective control strategies are urgently requested (Eungwijarnpanya *et* al., In preparation).

To monitor the TBB population or to control it, control tactics utilizing sex pheromone are suggested to be effective as other lepidopterous insects infesting trees (Speight and Wainhouse, 1989). We, therefore, observed the mating behavior of the TBB and attempted to extract sex pheromone released from female moths during the emergence season both in 1989 and 1990.

This paper describes the mating behavior of the TBB and reports behavioral assays in the field and in the laboratory.

METERIALS AND METHODS

Observations on Mating Behavior

The moths captured in the emergence traps (Eungwijarnpanya *et al*, submitted) were released into a screen cage (50 × 50 × 50 cm, with 2×2 mm screen net) at 18:00. Behavior of one to four couples of virgin males and females with 0-3 days old after emergence were observed every 30 min or continuously from 19:00 to midnight until moths stopped their movements.

Frequency of copulation and time elapsed for copulations were recorded with several combinations of couples.

Females were also collected from fields outside the emergence traps and from a light trap and were released into an insect cage to observe their fertility.

Preparation of Crude Extract and Its Separation on Column-chromatography

For the preliminary pheromone work, fifty virgin females were collected from emergence traps between 16:00 and 17:00. One cm off the tip of the abdomen was cut on either the same day or the next day around 21:00 after being kept in a screen cage. A solution of pheromone was obtained by extracting the abdominal tips for three days. The crude extract was filtered through cotton wicks and concentrated under the air flow to 50 female equivalent (FE)/1 ml, one tenth of which was used for behavioral assays on an olfactometer and in the field to estimate the appropriate height of traps to hang on trees in 1989.

The crude extract used for the traps in the field and the behavioral assays in a screen cage was 6.2 FE or 10.2 FE, which were prepared separately.

Chromatographical separation of the crude extract was done for behavioral assays. To prepare crude extract of the female abdominal tips for the column-chromatography, virgin female captured in the emergence traps were collected between 15:00 and 17:00 and one cm off the tip of the abdomen was cut on the same day around 19:30. Abdominal tips were immersed into distilled *n*-hexane. After standing for ca. 24 h, the solution was filtered through cotton wicks, evaporated by the air flow and chromatographed on Silica^{R} gel. Each ten ml of 20 fractions were taken by eluting 0.5%-, 1%-, 3%-, 5%- and 10%- ether-*n*-hexane (four fractions for each). They were con-

centrated to 1 ml and one tenth of the solution which corresponds to 5.7 females (5.7 FE) was used for both the sticky paper traps in the field and the behavioral assays with air puffing in a screen cage.

Behavioral Assay on an Olfactometer

An olfactometer was constructed as shown in Figure 1. The side and bottom was constructed of a styrenes foam, and a transparent vinyl chloride was pinned to the top. The width and length of the corridor where moths can move was 18 cm and 120 cm, respectively. A screen cage which contain the lure (a virgin female moth, the crude extract or a male moth) was placed on the upwind end of the corridor, and moths tested were placed on the downwind end at 19:00. The movement of the moths in the corridor was continuously observed until 22:00. The attractiveness was marked when either one of the two test moths moved towards or came very close to the screen cage, where the lure was located.

Behavioral Assay by Air–puffing

To compare the activity of 20 chromatographically–separated fractions as a sex pheromone component, we puffed the air containing either crude extract (6.2 FE to 10.2 FE) or one of 20 chromatographed fractions to males in a screen cage (50×50×50 cm) and counted the males displaying wing fluttering at 21:00 and 23:00.

Behavioral Assay in the Field

For the behavioral assays in the field in 1989 we devised four kinds of traps; A : a bowl water trap (80 cm in dia.×20 cm height), B : a horizontal sticky plate (60×60 cm), C : a cylinder shape sticky trap (20 cm in dia.×30 cm height) and D : a strip of sticky paper (20×40 cm) wrapped around the tree trunk at 4 m high. The traps of B and C were made of commercially available sticky plate (Takeda Chemical Industries Co. Ltd., Tokyo, Japan). Each trap was baited with one or two virgin females.

Five traps of type A were placed on the ground at over 20 m apart from each other in the teak plantation area. Traps B

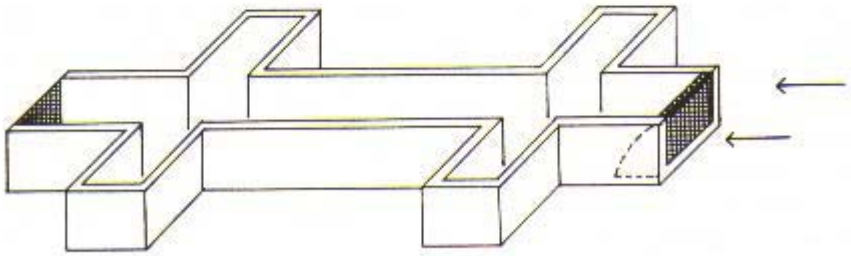

Fig. 1. Diagram of an olfactometer. The arrows indicate the direction of the wind and the checkered area represents the screen cage and net.

were hung on the branch of teak trees at different heights from 2 – 10 m in another nearby area. The twelve traps of type B, which were chained vertically at 2, 4, 6, 8 and 10 m high were hung up to know the relationship between the height of traps and the attractiveness of the lure to flying males. Two traps of type C and seven of type D were set up on other sites nearby. Numbers of moths captured on each trap were recorded every day for 5 days after the placement of the traps.

Thirteen horizontal sticky plates and thirteen delta sticky traps (trapping area: 24×29 cm, Takeda Chemical Industries Co. Ltd.) were placed in the field on March 10, 1990 at 4 m height baited with either a crude extract of virgin female, two virgin females, or one of the chromatographically – separated fractions of an extract. One – hundred microlitters of pheromone solution were applied to a paper disk (8 cm in dia., Toyo Roshi, Tokyo, Japan) and the solvent was allowed to evaporate. The behavioral assays with horizontal sticky plates were repeated on March 12, 1990. Numbers of moths captured on each trap were recorded every day for one week after the placement of the traps.

RESULTS AND DISCUSSION

Mating Behavior

The moths, both male and female, started to crawl on a floor or on a screen soon after sunset around 19:00. Males periodically fluttered their wings with vibrating antennae during 19:00 – 23:00. Females also fluttered their wings at the beginning of dark, then became calm and started calling behavior between 20:00 and 23:00. The peak of calling behaviors was at 21:00, when the abdominal tip was raised and lowered slightly and the ovipositor pulsated. When a female is in the calling position, a male came closer to the female with fluttering wings and aimed himself to the female such that they are parallel. A male sometime aligned in the reverse position with the head of the male alongside the abdomen of the female, then aligned himself with the female head to head, followed by the completion of mating. From coming closer to female up to mating, it took only one minute or less. These observation suggest that females release sex pheromone and males responded to it and approached females and then copulated.

Table 1 shows that a female copulated only once but a male copulated twice. Copulation lasted 7 – 15 hours, depending on couples. A couple of virgin female and male copulated in a paper box (25×50×3 cm) and the female laid eggs on a paper surface. Females collected from both fields, outside the emergence trap and a light trap, were mostly virgin. Only one female each out of an observed eleven and four females, respectively, laid fertilized eggs, resulting in hatched larvae.

Behavioral Assays

Bioassay on an olfactometer was conducted mainly to know if the crude extract obtained from the abdominal tips of virgin females show attractiveness corresponding to virgin females. However, the crude extract equivalent to fifteen females did not show any attractiveness, despite one virgin female

Table 1. Frequency of Copulation of *Xyleutes ceramicus* Walker

Age in days (No. of copulations experienced before)		No. of couples examined	No. of copulation observed
Male	**Female**		
0–1 (0)	0–1 (0)	5	4
0–1 (0)	0–1 (1)	4	0
1–2 (1)	0–1 (0)	5	4
1–2 (1)	1–2 (1)	3	0
2–3 (2)	0–1 (0)	7	0
2–3 (2)	1–2 (1)	3	0

Table 2. Response of Adult Moths to Several Lures on An Olfactometer

Lure	Adult moth tested	Replicates	No. of moths responding to the lure
One virgin female	2 males	6	4
Crude extract	2 males	2	0
None	2 males	2	0
One male	2 females	1	0

Table 3. Number of Males Caught by the Traps Baited with Virgin Females or the Crude Extract in 1989

Type of trap	No. of trap	Lure	Period tested (day)	Height of trap (m)	No. of male caught
A	5	virgin female*	6	0.5	1
B	2	virgin female*	5	2.0	2
	4	virgin female*	5	4.0	1
	3	virgin female*	5	6.0	0
	2	virgin female*	5	8.0	0
	1	virgin female*	5	10.0	0
C	2	crude extract (15 FE)	2	4.0	0
D	7	virgin female**	2	1.5 – 3.0	0

* Number of females used as a lure were one or two depending on the day.

** Number of females used as a lure were two

Table 4. Number of Moths Caught by the Sticky Traps in the Field and Percentage of Males Responding to Air–puffing in the Screen Cage.

	Number of males caught by the traps in Experiment			% (n) responding to air puffing			
	1	2	3	at 21:00		at 23:00	
crude extract (6.2 FE)	0	0	0	19.8%	(34)	23.2%	(34)
crude extract (10.2 FE)	0	0	0	4.2	(32)	12.6	(31)
virgin female	1	3	0	–		–	
fraction 1	–	–	–	14.7	(29)	5.6	(26)
2	0	0	0	0	(18)	0	(19)
3	0	0	0	9.2	(49)	4.2	(40)
4	–	–	–	8.8	(29)	4.2	(28)
5	–	–	–	0	(20)	0	(16)
6	0	0	0	0	(19)	0	(17)
7	0	0	0	9.0	(47)	4.8	(45)
8	–	–	–	5.3	(19)	0	(20)
9	–	–	–	9.4	(29)	20	(33)
10	0	0	0	0	(16)	5.9	(17)
11	0	0	0	4.8	(35)	5.2	(19)
12	–	–	–	5.6	(18)	7.1	(14)
13	–	–	–	0	(18)	0	(13)
14	0	0	0	9.4	(28)	0	(28)
15	0	0	0	14.7	(42)	8.6	(34)
16	–	–	–	0	(14)	0	(14)
17	–	–	–	0	(17)	7.1	(14)
18	0	0	0	0	(17)	0	(13)
19	0	0	0	6.3	(35)	0	(21)
20	–	–	–	0	(15)	0	(11)

(Table 2). The same results were given in the field test using sticky traps (Table 3 and 4). On the air-puffing bioassay, however, the crude extract showed a positive attractiveness to the male moth, although it is not strong enough (23% at the most in Table 4). Several chromatographically separated fractions also showed no stronger attractiveness than the crude extract.

These results suggest that the pheromonous ingredients were not extracted enough to exhibit attractiveness on these three behavioral assays employed. As the olfactometer and the horizontal sricky traps in the field we devised worked at certain extent when virgin females were employed as a lure, further devices to obtain much more amounts of pheromone is necessary.

The behavioral assay conducted in the field in 1989 with traps chained vertically from 2 to 10 m in height (Talbe 3) indicates that males are likely to fly 2–4 m high to orientate to virgin females.

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