Presence of a Sex Pheromone in the
Pine Moth, *Dendrolimus spectabilis*
BUTLER (Lepidoptera: Noctuidae)¹

Although a variety of sex pheromones have been reported for many species of Lepidoptera (JACOBSON, 1972), no reference has yet been published with regard to the pine moth, *Dendrolimus spectabilis* BUTLER. The pine moth is an important defoliator occurring in Japan, which has caused extensive damage on members of the genus *Pinus*, particularly on red, black, and Chosen pines.

Moths of this species usually emerge once a year from the end of June until mid-August, and the females lay from 200 to 700 eggs primarily on the leaves of pines. Larvae hatch within 7 days and shortly thereafter, actively inflict damage to trees by devouring leaves.

In the present experiment, an olfactometer and a field trap were devised in order to (1) demonstrate the existence of a possible sex pheromone, (2) determine the age and nocturnal time of the mating, and (3) find a solvent suitable for extraction of an active principle.

Pupae were collected in the field and individually placed in small glass jars for emergence. An olfactometer was constructed based on modification of that described by McINDOO (1926) and is shown in Fig. 1. The apparatus essentially consists of a 5-liter, 3-necked, round bottom glass flask (A) with the flask portion utilized as a releasing chamber. Inserted within each of the side necks is a cone-shaped plastic net (c), and each neck in turn is extended with a glass tubing (B). A glass tubing, fixed to the central neck, was in turn connected *via* rubber tube to an aspirator. One of a pair of glass tubings (D and E) covered with plastic netting across both ends,

was used to accommodate either virgin females or males, or extract, while the other served as a control. Air flow rate in the apparatus was 500 ml per min. The apparatus was placed in a room where the following environmental conditions were maintained: temperature, $25 \pm 2^\circ\text{C}$; relative humidity, $65 \pm 5\%$; photoperiod, 16 hr light : 8 hr dark (16L : 8D), with the dark period commencing at 9 P.M.

The trap used in field tests was modification of that developed for the gypsy moth (BURGESS, 1950) and is seen in Fig. 2. It consists of a metal can having openings at both ends covered with a plastic net. Small holes (60) were drilled around the side of the can, and the outer surface was coated with a commercial adhesive (Fugitangle, Fuji Yakuhin, Co.). The interior was served to accommodate virgin females or extracts. The traps were baited and suspended under pine trees 3 to 5 m above the ground and spaced roughly 30 m apart. Field tests were conducted in the village of Ina, Ibaraki Prefecture, from July 15th to 28th, 1969.

Female attractancy to the males was tested with the olfactometer in the following manner: Five virgin female moths were confined in portion D and 15 one-day-old unmated male moths were released in the flask A. E was used as a control containing no females. Male moths known to be responsive to virgin females were placed in flask A 30 minutes to one hour prior to the test. The male moths in the traps D and E were counted at the end of a 8 hour period beginning at 9 P.M. The results in Table 1 show that a significant number of the male moths were attracted to the virgin female moths. In experiments where 15 virgin females were exposed to 5 caged unmated males for 8 hours, numbers of attracted females were insignificant from that of the control (Table 2).

Fig. 1. The olfactometer used for laboratory test

Fig. 2. The trap used for field test

¹ Appl. Ent. Zool. 8 (4): 248-250 (1973)

Table 1. NUMBERS OF MALE PINE MOTHS
ATTRACTED TO ONE-DAY-OLD
VIRGIN FEMALES IN THE OLFACTOMETER TESTS

Expt. No.	Number of attracted males	
	responsive to females	control
1	6	2
2	4	1
3	4	1
Average	4.66	1.33

Table 2. NUMBERS OF FEMALE PINE MOTHS
ATTRACTED TO ONE-DAY-OLD
UNMATED MALES IN THE OLFACTOMETER TESTS

Expt. No.	Number of attracted females	
	responsive to males	control
1	2	1
2	1	1
3	1	1
Average	1.33	1.00

Table 3. NUMBERS OF MALE PINE MOTHS CAUGHT IN TRAPS BAITED
WITH VIRGIN FEMALES OF DIFFERENT AGES

Number of virgin female moths per trap	Number of male moths caught on indicated day following emergence								Total
	1	2	3	4	5	6	7	8	
1	8	3	0	0	0	0	0	0	11
5	13	4	0	0	1	9	0	0	18
0 (control)	0	0	0	0	0	0	0	0	0

Table 4. NUMBERS OF MALE PINE MOTHS CAUGHT DURING INDICATED
TIME OF DAY BY 20 TRAPS, EACH BAITED WITH A VIRGIN FEMALE

	PM				AM		
	4-6	6-8	8-10	10-12	0-2	2-4	4-6
Number of captured male moths	0	11	30	34	11	4	0
Percentages of captured males(%)	0	12.2	33.3	37.8	12.2	4.4	0

Difference in female attractiveness with female age was determined in the field study. Ten traps baited with one or five virgin females soon after emergence were suspended under the trees. Numbers of moths caught by the traps were recorded daily. Table 3 shows the number of males caught in relation to female age up to 8 days, which represent the average life span of females. Young females less than 3-day old were especially attractive.

With a similar method, the peak daily time of mating was determined, by counting the numbers of males caught by the traps each of which was baited with single virgin female and distributed in the forest. It can be seen from Table 4 that about 71% of the total males caught occurred during the period between 8 to 12 P.M.

Several solvents were tested for extraction efficiency of the active principle from female

abdomens. The last 3 abdominal segments of 20 virgin females were immersed in 20 ml of any one of the following six solvents, crushed, and filtered. The extract was concentrated to 4 ml. Solvents tested were methylene chloride, acetone, benzene, *n*-hexane, ethyl alcohol and ether. One ml each of the extracts with these solvents was applied to a strip of filter paper (20×50 mm), and placed in either the D or E portion of the olfactometer. The remaining cage served as a control was provided with a filter paper replaced the old ones at 2 hr intervals from 9 P.M. to 5 A.M. 15 one-day old virgin males were released into the flask A and the numbers found in the both cages were counted and recorded at the termination of the exposure. The results showed methylene chloride to be the most efficient solvent (Table 5).

For the field tests, 60 abdominal segments were

Table 5. NUMBERS OF MALE PINE MOTHS TRAPPED BY THE SIX SOLVENT EXTRACTS OF ONE-DAY-OLD VIRGIN FEMALE ABDOMENS (LABORATORY TEST).

Solvent used	Number of male moths attracted ^a									
	Expt. No.						Total		Average	
	1		2		3					
	E	C	E	C	E	C	E	C	E	C
Aceton	3	0	3	1	3	0	9	1	3.0	0.3
Benzene	2	1	2	0	1	0	5	1	1.7	0.3
Ethyl alcohol	0	0	1	0	0	0	1	0	0.3	0
Ethyl ether	1	0	0	1	4	3	5	4	1.7	1.3
<i>n</i> -Hexane	1	0	0	1	4	3	5	4	1.7	1.3
Methylene chloride	5	2	6	2	4	0	15	4	5.0	1.3

^a E=extract, C=control.

Table 6. NUMBERS OF MALE PINE MOTHS CAUGHT BY TRAPS BAITED WITH THE SIX SOLVENT EXTRACTS OF ONE-DAY-OLD VIRGIN FEMALE ABDOMENS (FIELD TEST)

Solvent used	Number of male moths attracted						
	Expt. No.					Total	Average
	1	2	3	4	5		
Acetone	3	2	2	1	2	10	2.0
Benzene	1	1	0	1	2	5	1.0
Ethyl alcohol	0	0	0	0	0	0	0
Ethyl ether	0	0	0	0	0	0	0
<i>n</i> -Hexane	0	0	0	0	0	0	0
Methylene chloride	2	3	5	2	3	15	3.0

extracted with 60 ml of the same solvent and the extract was concentrated to 6 ml. One ml of the extract was applied to a strip of filter paper (80×25 mm). The traps were baited with the strip and exposed from 6 P.M. to 6 A.M., with the bait being renewed at 2 hr intervals. Control traps were similarly baited only with the respective solvent. Table 6 indicates methylene chloride to be most efficient, acetone somewhat less while the other solvents were much less effective. Control traps baited only with the solvents failed to catch any moths.

On the basis of these tests, the presence of a sex pheromone in the female moth is evident, and further bioassay examination is necessary for the isolation of the active principle. Typical mating behavior frequently observed in excited male moths was not apparent in our experiments.

REFERENCES

- BURGESS, E. M. (1950) *J. econ. Entomol.* **43**: 325–328.
- JACOBSON, M. (1972) *Insect Sex Pheromones*. Academic Press, London and New York, 382 pp.
- McINDOO, N. E. (1926) *J. Econ. Entomol.* **19**: 545–571.
- Kazuo SAKURAI¹
Kazuhiro MATSUDA²
Ryozo SUGAWARA
Toshio MUTO
- Laboratory of Pesticide Chemistry,
Faculty of Agriculture,
The Tokyo University of Education,
Komaba, Meguro-ku, Tokyo, 153 Japan
Received September 30, 1973*
- ¹ The authors wish to thank Mr. A. IWATA, Government Forest Experiment Station and Mr. H. KONDO, Ibaraki Prefectural Forest Experiment Station for their helpful advice in the collection of the pine moths.
- ² Present Address: Laboratory of Applied Entomology, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo, 113 Japan