Mating Suppression by Individual Components of the Sex Pheromone of *Spodoptera litura* F. (Lepidoptera: Noctuidae) under Field Conditions

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The effect of individual components of sex pheromone (*cis*-9, *trans*-11-tetradecadienyl acetate (compound A) and *cis*-9, *trans*-12-tetradecadienyl acetate (compound B) upon the mating of *Spodoptera litura* were examined by means of arrangement of tethered females in an experimental field and liberation of marked males in different sex ratios. The application of a large amount of compound A and B (7.2 mg/are) in a field had an effect to suppress matings: that is, the mating rate decreased below the level of 50 percent as compared with that of 75—95 percent in blank trials. *cis*-9-Tetradecenyl acetate (compound C), a related substance of the sex pheromone, was also examined, but the mating was not clearly suppressed. The mating suppression was most strongly appeared when the males were liberated around the field treated by compound B.

INTRODUCTION

In the past several years, many sex pheromones were identified in many insect species and their difference in attractiveness to the opposite sex was frequently reported by many authors. However, several workers have recently reported that in some Lepidopterous insects, a high concentration of sex pheromone often suppressed their matings or disrupted communications between males and females in laboratory and field conditions (SHOREY et al., 1967, 1972; STEVENS et al., 1972; HIRAI et al., 1974; TAMAKI et al., 1975).

TAMAKI et al. (1973) revealed that the sex pheromone of *Spodoptera litura* consisted of 2 different components, *cis*-9, *trans*-11-tetradecadienyl acetate (compound A) and *cis*-9, *trans*-12-tetradecadienyl acetate (compound B). YUSHIMA et al. (1974) confirmed that the optimum ratio of compound A and B with respect to attractiveness to the male fell into a range from 8 : 2 to 39 : 1. It has also known that under field conditions, the application of a large amount of either component or mixture in ratio of 10 : 1 brought about a suppression of matings of this insect (YUSHIMA et al. 1975).

In this experiment, the authors attempted to investigate the effect of individual components of the sex pheromone of *Spodoptera litura* to matings under field conditions.

MATERIALS AND METHODS

The moths used in the experiment were obtained from the larvae reared on artificial diet as described by FUJIE and MIYASHITA (1973) at 25°C with 16-hr lightening and 8-hr dark. However, the pupae obtained were kept under natural condition, and

the moths emerged from those pupae were also kept under natural condition for 1 or 2 days with 5 percent sucrose solution as their foods.

The field used was 50 are ($50\text{ m} \times 100\text{ m}$) in area locating in the campus of Chiba Prefectural Agricultural Experiment Station. In that field, orchard grass and alfalfa were growing together with various weeds.

In order to examine the effect of two respective components (compound A and B) of the sex pheromone to mating, 100 tethered females were artificially distributed within the experimental field and, just after setting of evaporation wicks of compound A or B in the field, the various numbers of males were liberated. *cis*-9-Tetradecenyl acetate (compound C), a related compound of sex pheromone of *S. litura*, was also tested as an additional material.

Here, the tethered female means the individual which have been tied by a cotton thread at their wing and attached to the bamboo rods standing in the field at intervals of $5\text{ m} \times 10\text{ m}$ by another end of the thread (see Fig. 1). The attached portion was 50–60 cm above the ground. Such females were known to have performed mating normally with the liberated or wild males in the field (OYAMA, 1974).

The settlement of evaporation wicks of each compound within the field was made by the method as follows: 36 bamboo rods were set up in the field at intervals of $10\text{ m} \times 20\text{ m}$ as shown in Fig. 1, and 10 mg of test compound absorbed in a cotton wick were attached on each bamboo rod at the height of 150 cm above the ground. Thus, the total amount of compound applied to the field was 360 mg in total per night (7.2 mg/are). The purity of compound was 67.4 percent in A, 88.4 percent in B, and 89.3 percent in C, respectively. The arrangements of tethered females and cotton wicks in the field were made in the evening and the liberation of males were also made just before dusk.

Fig. 1. The map showing arrangement of tethered females, evaporating wicks of test compounds, and 3 virgin female traps in the experimental field. The area used was $50\text{ m} \times 100\text{ m}$. The intervals of tethered females and that of evaporating wicks were $5\text{ m} \times 10\text{ m}$ and $10\text{ m} \times 20\text{ m}$, respectively.

Table 1. EXPERIMENTAL SCHEDULE AND THE WEATHER CONDITIONS AT EACH NIGHT

Date	No. of females		No. of males released	Place of release	Treatment by compound	Weather
	Arranged	Dead				
1974, June 13	100	1	137	inside	A	Fine
14	"	0	135	"	B	Light rain
26	"	2	161	outside	B	Fine
27	"	0	153	"	A	Light rain
28	"	0	165	"	none	"
July 18	"	3	130	inside	A	Cloudy
19	"	0	270	outside	B	"
20	"	4	122	"	A	"
21	"	3	143	"	none	Fine
Aug. 20	"	9	135	"	A	"
21	"	2	150	inside	A	"
22	"	0	172	"	B	"
23	"	0	142	"	none	"
1975, June 9	"	10	162	inside	none	Cloudy
10	"	8	163	"	B	"
11	"	7	216	outside	B	" ¹
12	"	7	187	inside	C	Light rain
24	"	4	251	"	B	Cloudy
25	"	2	223	"	C	Light rain
26	"	1	167	"	C	Cloudy
27	"	0	156	"	A	Fine
July 5	"	2	179	outside	B	Light rain
6	"	10	164	inside	C	Cloudy
7	"	5	225	outside	none	Fine ²

¹ Cool night (temp. dropped below 19°C by 9 p.m.).

² Warm night with strong wind.

The liberated males were previously marked by a quick drying ink on their hind wings. The number of males liberated at each time was shown in Table 1. As shown in Table 1, the liberation of marked males was made by two different ways. One is the case described as 'inside' where the males were randomly liberated within the experimental field. Another is 'outside' where the males were liberated along the 4 lines surrounding the field. (thick-dotted lines in Fig. 1)

The counts of matings in tethered females with liberated males were made 3 to 5 times from dusk to 11 p.m. at 1-hr interval. At the times of those observations, the couples in mating status were carefully collected, and brought into laboratory. In the case of males collected by such a way, the marks on their wings were examined. All tethered females were dissected in the morning for detecting spermatophores in the bursa copulatrix during the period from 11 p.m. to the morning.

For the purpose of monitoring the movement of liberated males, 3 traps baited with 10 virgin females were placed on the points far about 130 m North, 130 m East-South, and 100 m South-West from the center of the experimental field (see Fig. 1). The males trapped at each night were examined for the marks on their wings.

The mating occurred between tethered females and wild males was observed, but its occurrence was not so frequent. Therefore, we have omitted such a case from

the total number of matings.

RESULTS AND DISCUSSION

The effects of individual components of sex pheromone upon mating rate

Fig. 2 shows the relationship between sex ratio and mating rate of tethered females under the presence or absence of compound A, B or C. As shown in Table 1, the tethered female arranged on the field were fixed in number in each time and were completely collected by the morning. However the liberated males was differed in number from time to time and some of them were survived for one or more days in the field, although the individuals mated with the tethered females by the time from dusk to 11 p.m. were collected with females and killed. Therefore, the matings between these survivals from previous night(s) and newly arranged females were occurred, since the experiments were repeatedly carried out for 2–4 successive nights. From this reason, the sex ratios (σ/φ) in each experiment were not easily calculated from the number of liberated males and that of arranged females shown in Table 1. We could, however, easily recognize those males which survived from the previous night(s) and mated with newly arranged females by analysis of marks on their wings. From these records, it was calculated that the remaining rate of liberated males was, on the average, 10 percent for the 2nd night, 5 percent for the 3rd night, and negligible for the 4th night, since there was no large difference in the remaining rate between 'inside' liberation and 'outside' one. By using these remaining rates, we estimated the expected numbers of survivors for the 2nd and 3rd nights in each case, and the number of males in Table 1 were corrected by adding these expected numbers of survivors, respectively. The sex ratios shown in Figs 2 and 4 were calculated from these corrected number of males and the number of tethered females shown in Table 1.

Fig. 2. The mating rates of tethered females under the presence and absence of individual components of the sex pheromone, plotted against sex ratio. Figures attached on some symbols coincide with foot-notes in Table 1.

According to Fig. 2, it is clear that the application of compound A or B (7.2 mg/are) in the field had a suppressive effect upon the mating of this insect even when sex ratio was greatly differed: that is, the mating rates were dropped into the level lower than 50 per cent, except one case, in contrast to 75–95 percent in blank trials. There were no clear difference in the effect between compound A and B but, in the application of compound B, the effect appeared more strongly when the males were liberated outside the experimental field than when they were liberated inside.

According to M. OYAMA (personal communication), the treatment by compound C also suppressed mating in a considerable degree, but such a tendency was not observed in this experiment, although only one case out of 4 showed a decrease in mating rate lower than 50 percent.

Hourly change of mating rate in relation to the presence and absence of individual component of the sex pheromone

Fig. 3 shows the hourly changes of mating rates in relation to the presence and absence of individual components of the sex pheromone. In blank trials, mating rate showed a sharp increase from dusk to 1 or 2 hour after it, but a slow increase thereafter (Figs. 3-1 and 2). This pattern did not change even when the liberation method of males was differed.

In the application of compound A and B, the pattern of hourly change of mating rate was greatly changed when males were liberated outside the field; that is, the mating rate showed a gradual linear increase (Figs. 3-3 and 5). It seems to suggest that such an increase of mating rate was realized only by the sporadically occurring invasion of males from outside to inside of the field during the night. In other words, an abrupt invasion of males occurred just after dusk as in the blank trials was completely prevented by the application of compound A and B, and thus the mating rate was still remained at a low level. In contrast, when males were liberated inside the field, the increase of mating rate followed the same pattern as observed in blank

Fig. 3. Hourly changes of mating rates in relation to presence and absence of individual components of sex pheromone. Vertical bars show the standard deviations.

Fig. 4. The trapping rates of liberated males by 3 virgin female traps under persence and absence of individual components of the sex pheromone, plotted against sex ratio. The illustration of symbols is the same as in Fig. 2.

trials (Figs. 3–4 and 6), although the mating rate was quite low as compared with that in blank trials.

The flight of liberated males to the virgin female traps standing at a distance from the experimental field

Fig. 4 shows the relationship between the sex ratio and the trapping rate of liberated males by 3 virgin female traps standing at a distance from the experimental field where the compound A or B was applied. From the graph, it is very difficult to draw a clear conclusion. It can be said, however, that the rate of capture tended to become high when compound B was applied as compared with when compound A was applied. In blank trials, the rate was also low, except one case obtained in the warm night with strong wind. In the application of compound C, no clear tendency was detected. It is suggested from these results that the single component of the sex pheromone exerts quite different effects upon the mating behaviour, but the elucidation of this point will require more detailed examinations.

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