A Functional Difference of the Individual Components of *Spodoptera litura* (F.) (Lepidoptera : Noctuidae) Sex Pheromone in the Attraction of Flying Male Moths

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(Received August 30, 1980)

The functional difference of the two components of sex pheromone of *Spodoptera litura*, (Z)-9, (E)-11-tetradecaadienyl acetate (compound A) and (Z)-9, (E)-12-tetradecaadienyl acetate (compound B), in the attraction of flying male moths was analysed in a large field cage. Various dispensers containing compounds A, or B, or a mixture of both were used as lure sources and put in a continuous air stream generated by a fan. The flight courses of released male moths around these lure dispensers were recorded by a video recorder through a TV camera equipped with a night scope. Analysis of flight course traces indicated that the mixing state of compounds A and B in the air is an essential factor in inducing the upwind flight and searching flight of male moths around it. It was also ascertained that the concentration of compound A is an important signal for male moths to locate the pheromone source.

INTRODUCTION

Recent studies on sex pheromones of lepidopterous insects have revealed that many of them use multi-component systems in pheromonal communication between male and female (Tamaki, 1977). In addition, it has frequently been pointed out that in many lepidopterous insects, a blend of two or more components of their sex pheromones is a very important factor in attracting male moths. However, the role of individual pheromone components in communication between sexes still remains unknown except for a few species.

In the Oriental fruit moth, Grapholitha molesta, (Z)-8-dodecyl alcohol has the effect of inducing landing behavior of the male moths flying to a pheromone source (Cardé et al., 1975, Baker and Cardé, 1979). Baker et al. (1976) also reported that in the red-banded leaf roller moth, Argyrotaenia velutinana, dodecyl acetate has a similar function as (Z)-8-dodecyl alcohol in the Oriental fruit moth.

The sex pheromone of *Spodoptera litura* was identified by Tamaki et al. (1973) as a mixture of (Z)-9, (E)-11-tetradecaadienyl acetate (compound A) and (Z)-9, (E)-12-tetradecaadienyl acetate (Compound B). In a laboratory bioassay, males responded most actively to a blend of compounds A and B at a ratio of 9:1 (Tamaki and Yushima, 1974). Field experiments also showed that a mixture of 8:2 to 39:1 was the most attractive lure to the male moths (Yushima et al., 1974). Nakamura and Kawasaki, (1977) analysed the trapping process of the males by the sex phe-
romone and reported that the effective range of the sex pheromone was probably determined by compound A. NAKAMURA (1976, 1979) made a model to explain the orientation process of male to female, and in that model, he assumed that male moths fly upwind when they perceive compound A and then they fly directly to a lure source after they detect the mixture of both compounds. But that assumption was not based on direct observation of flight behavior of male moths. Hidaka (1976) observed the flight behavior of S. litura males attracted to a lure source containing a mixture of compounds A and B at a ratio of 10:1, and concluded that they switched their behavior from undirected rapid flight to a slow zigzag near the pheromone source. But the function of individual components was not elucidated.

In this experiment, the flight course of male S. litura around a pheromone source was analysed by direct observation in order to detect the difference in the function of two components in male attraction.

**MATERIALS AND METHODS**

The moths used for this experiment were obtained from the larvae reared on an artificial diet (Fujiie and Miyashita 1973) at 25°C with LD 16:8. The moths emerged from the pupae were kept for 2 to 5 days under natural conditions providing 10% honey solution as food.

Compounds A and B were purified using TLC or preparative GLC. Purities of compounds A and B were 93.6 and 95.6%, respectively. Known amounts of each compound dissolved in hexane was absorbed in various rubber dispensers. Dispensors containing a mixture of 1 mg and 10 mg of compound A, and 0.1 mg and 1 mg of compound B were prepared. In the same manner, the dispenser containing a mixture of 1 mg of compound A and 0.1 mg of compound B was also prepared as a lure source. These dispensers will be called A 1, A 10, B 0.1, B 1, and A + B, respectively.

Fig. 1 shows the arrangement of apparatus. The flight behavior of male moths was observed in the experimental field cage (5×10×2 m). This apparatus consisted of a fan 40 cm in diameter, a TV camera equipped with a night scope, a TV monitor, and a video recorder. A continuous air stream was generated by the fan throughout each experiment. Three rods were set at 60 cm intervals in the air stream and various pheromone dispensers were attached to them 1 m above the ground. Wind velocity around these three rods was 0.8 to 1.5 m/sec. The observed horizontally range by the TV camera was about 1.5 m.

Male moths were released just before dusk at the downwind position of the air stream in the cage. Number of males released in each observation was 100 to 200. When male moths began to fly, one or two of the prepared dis-
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pensors containing compound A, B, or a mixture of the two compounds in different amounts were attached to the rods in various combinations. The flight courses of the male moths around the dispensors were continuously watched by the TV monitor and recorded by video recorder at the same time. Flight behavior was observed within 1 hour from dusk. Composition of the compounds in the air stream was easily changed by adding or removing dispensors even on a way of observation.

In order to analyse the flight pattern of male moths around lure sources, the TV image recorded in the video recorder was played back repeatedly and the flight courses of many individuals were superimposed on the screen.

RESULTS

1. Flight patterns of male moths around the lure dispenser containing compounds A or B.

Fig. 2 shows flight traces of male moths around a lure source containing either compound A or B. In this experiment, only one lure source was attached to the center rod. Fig. 2-a shows a basic flight pattern of male moths. There was no lure source in the cage, and we cannot detect any characteristic pattern from the flight traces. The flight of males was slightly changed by a dispenser A 1 placed on the center rod (Fig. 2-b). They flew actively in the cage, but no upwind flight of male moths toward the lure source was observed. And no clear change in flight was caused by increasing the amount of compound A from 1 mg (A 1) to 10 mg (A 10) (Fig. 2-c).

Fig. 2. Flight course traces of male moths around a pheromone dispenser containing compound A or B. Solid circle shows the position of pheromone dispenser. Wind direction was from right to left.

a: no dispenser  b: 1 mg of compound A  c: 10 mg of compound A  d: 0.1 mg of compound B
In the experiment with lure source B 0.1, there no difference was observed in the flight pattern between this and experiments without the lure source, except that the number of males observed was somewhat smaller.

2. Effect of mixing compounds A and B on the flight patterns of male moths

Fig. 3 shows the male flight pattern when lure dispensers A 1 and B 0.1 were attached together on the same rod or on two separate rods. When both lure sources were attached to the same rod located upwind (Fig. 3-a), the flight traces showed were attached to the same rod located farthest upwind (Fig. 3-a), the flight traces showed a clear difference from those in the previous experiment (Fig. 2). The male flight traces concentrated on the downwind side of the lure source. This resulted from the males moths' always approaching the lure source from downwind and when they reached about 60 cm downwind of the lure source, they reduced their flight speed and flew back and forth repeatedly. These flights, which will be called "searching flight," were observed within the range between the rod with lure sources and the next downwind one. The searching flight was restricted to within 60 cm downwind from the lure source.

When only dispenser A 1 was removed from the original rod on the right with dispenser B 0.1 and attached on the center one, the searching flight of males was confined to within the range between the center rod with the lure dispenser A 1 and the next 60 cm downwind rod, where both compounds A and B mixed together in the air (Fig. 3-b). Some moths passed through to the side of dispenser A 1 and flew upwind to dispenser B 0.1, where only compound B was present. However, they did

Fig. 3. Flight course traces of male moths around pheromone dispensers containing 1 mg of compound A and 0.1 mg of compound B.

a: both dispensers were placed on the same rod  b, c: each was placed separately on two rods.
not show typical searching flight or upwind flight any more. They changed flight course and then flew away. As a result, the range of searching flight moved about 60 cm downwind compared with the case in Fig. 2-a. When the positions of the dispenser A 1 and B 0.1 were exchanged (Fig. 3-c), the flight course trace obtained was almost the same as the previous case (Fig. 3-b). Typical searching flight was observed between the center rod and the left one, where compound A and B drifted together in the air. Most of the moths which flew into the range between the right and center rods, where only compound A was present, stopped searching and upwind flight and then changed their flight course.

These results lead to the conclusion that the mixing state of compounds A and B in the air is an essential factor for male moths in inducing direct orientation and searching flight to the lure source, since this behavior was observed only in the range where the flying male moths could detect both compounds mixing in the air.

3. Effect of individual components on the orientation of males to the mixture of both compounds.

When two dispensers containing compounds A and B were placed at the same position on the right rod, searching flight of male moths was observed only between the rod with the dispensers and next one (Fig. 3-a). Instead of the two dispensers, a single dispenser containing the mixture of 1 mg compound A and 0.1 mg compound B (dispenser A+B) was placed on the right rod, and the same result was obtained as expected (Fig. 4-a). In the next step, leaving dispenser A+B on the right rod, another dispenser containing either compound A or B was placed on the next downwind center rod and the flight courses of male moths were observed. When dispenser A 1 was added on the center rod, the range of searching flight was expanded to the left rod (Fig. 4-b). This means that dispenser A 1 expanded the range of searching flight 60 cm downwind from it. Next, dispenser A 1 was replaced by A 10. Then, most of the searching flight of male moths concentrated on the downwind side of the dispenser A 10 (Fig. 4-c). In this case, most of the searching flight that was previously observed in the range 60 cm downwind of dispenser A + B (Fig. 4-b, 4-c) disappeared.

When dispenser B 0.1 was added in the air stream instead of dispenser A 1 or A 10, we could not detect any change in the flight courses compared with the case without dispenser B 0.1 (Fig. 4-d, 4-a). Change in the amount of compound B in rubber dispenser from 0.1 mg (B 0.1) to 1 mg (B 1) resulted in no change in the male flight courses, except some moths changed flight courses in front of dispenser B 1.

The results mentioned above show that it is essential for the male moth's orientation to the lure source and searching flight near the lure source that both compounds A and B exist together in the air. These flights are strongly affected by the addition of compound A in the air stream containing the mixture of compounds A and B. This fact indicate that the aerial concentration of compound A plays an important role in locating the position of the lure source. However, compound B seems to have no such function.

DISCUSSION

The results obtained here show that the two components of the sex pheromone of S. litura have different functions in attraction of male moths to a pheromone source. Male moths showed searching and upwind flight to the lure source when it contained
Fig. 4. Flight course traces of male moths around pheromone dispenser containing a mixture of 1 mg of compound A and 0.1 mg of compound B and an additional dispenser containing various amounts of compound A or B added on the another rod.

a: dispenser A+B only  b: dispenser A+B and dispenser A 1  c: dispenser A+B and dispenser A 10  d: dispenser A+B and dispenser B 0.1  e: dispenser A+B and dispenser B 1

a mixture of compounds A and B. Under this condition, they were able to locate the position of the lure source by detecting the higher aerial concentration of compound A (Fig. 4-b, 4-c). But compound A alone did not induce the orientation of male moths to the lure dispenser (Fig. 2-b, 2-c). On the other hand, the presence of a dispenser of compound B had no effect on the male's orientation to the lure source containing the mixture of two compounds (Fig. 4-d, 4-e). These results indicate that the function of compound A is to guide male moths to the position of the lure source containing both compounds A and B. This function of compound A acts only under the condition that compounds A and B are mixed in the air.

The inhibitory effect of compounds A or B on the mating of tethered females were ascertained in the field (YUSHIMA et al., 1975; KAWASAKI and MIYASHITA, 1976;
Oyama, 1977; Oyama and Wakamura, 1977). These results were obtained by using large amounts of compounds A or B absorbed in cotton wicks. Thus the amounts of compounds A or B released into the air would be very high. In this experiment, the addition of dispensers containing compounds A or B did not have such an inhibitory effect on the orientation of males to the lure source (Fig. 4). The mixing ratio of compounds A and B was expected to range from 110:1 (Fig. 4-c) to 1:1.1 (Fig. 4-d) in this experiment. This range is not always the best mixing ratio to attract males but can attract a considerable number (Yushima et al., 1974).

Hidaka (1976) observed the flight of S. litura males attracted by a rubber dispenser containing 1 or 5 mg of the 10:1 mixture of compounds A and B. He observed swarms of male moths, in which they flew in a zigzag pattern, in the range from a meter from the lure source to 1.5 m downwind. This flight pattern is the same as the searching flight observed in the present experiment. Outside this range, he observed no the searching flight. But within the range of 1.5 m to 3.0 m from the lure source, he observed that male moths did not fly so rapidly as at farther than 3 m downwind from it. Such a change in the manner of flight was also observed in Hyphantria cunea male attracted by a virgin female (Hidaka, 1972).

Murlis and Bettany (1977) observed the flight paths of male Spodoptera littoralis attracted by a pheromone source in the field, and identified three different phases of flight pattern. These experimental results indicate that the flight behavior of male moths follows several steps. Bartell and Shorey (1969) reported that in laboratory experiment on the light-brown apple moth, Eupholus postvittana, the mating behavior of male moths also followed a sequence of several different steps corresponding to the difference in pheromone concentrations. In S. litura, this experiment shows that the aerial concentration of compound A is a cue to find out the female position when diffused with compound B. Then the aerial concentration of the mixture of the two compounds, particular that of compound A, possibly switches the behavior pattern of the sexually activated male moth.

Nakamura and Kawasaki (1977) suggested from the results of release and recapture experiments with males in the field that the effective range of the sex pheromone of S. litura was probably determined by the amount of compound A diffusing downwind from the pheromone source. Nakamura (1976, 1979) simulated the trapping processes of male moths with a model assuming a double active space mechanism: male moths fly upwind when they enter the effective range determined by compound A in which they detect only compound A and then they orient directly to the pheromone source when they enter the second active space consisting of the mixture of compounds A and B.

However, Nakamura's assumption seems to be inconsistent with the present conclusion that the upwind flight to a lure source would not be induced by compound A alone, but only by the mixture of compounds A and B in the air stream. The reason is not clear but in this experiment, the observed range was about 1.5 m downwind from the lure source whereas in Nakamura's model, the range in which males begin upwind flight is much greater, as much as 60 m from the lure source. Activation of male flight was observed around the compound A lure source of in this experiment. This behavior must be caused by a rather high concentration of compound A around the lure source. But what changes in behavior will be caused when males come in contact with low concentration of compound A is not yet known.
ACKNOWLEDGEMENT

I am greatful to Prof. K. MIYASHITA, Tokyo Metropolitan University, Drs. Y. TAMAKI, K. NAKAMURA, and K. KIRITANI, National Institute of Agricultural Sciences for their valuable discussion and critical reading of the manuscript.

REFERENCES


