Effects of Ratio and Amount of the Two Sex-Pheromonal Components of the Diamondback Moth on Male Behavioral Response

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The responsiveness of male diamondback moth to its sex pheromone was examined in the laboratory. The responses were observed in a flask after chemicals adsorbed on a piece of filter paper were provided. Male response showed a peak at a dose of 1×10^{-3} μg on the filter paper with 1:1 mixture of (Z)-11-hexadecenyl acetate and (Z)-11-hexadecenal. Response decreased when the chemicals were increased to more than 1×10^{-3} μg and finally was completely inhibited by 1 μg of chemicals; the optimum concentration inducing male sexual behavior was determined. Optimum ratio of the mixture ranged from 8:2 to 5:5 (acetate:aldehyde) when the amount was fixed at 1×10^{-3} μg and no sharp peak was observed.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), is one of the most serious pests of cruciferous vegetables in the world. The sex pheromone of this species was identified as a mixture of two compounds, (Z)-11-hexadecenyl acetate (Z11-16Ac) and (Z)-11-hexadecenal (Z11-16Ald) (TAMAKI et al., 1977). High attractiveness to males with an 8:2 to 4:6 mixture of Z11-16Ac and Z11-16Ald was observed in the field and 1 μg of a 1:1 mixture of these compounds absorbed in a cotton wick was equivalent to 5 virgin females (KOSHIHARA et al., 1978).

The amount of chemicals adsorbed on a dispenser affected the attractiveness for males and the range of optimum amount for attraction was reported with a 1:1 mixture of Z11-16Ac and Z11-16Ald (KOSHIHARA et al., 1978; KOSHIHARA and YAMADA, 1980), the optimum concentration for the male mating dance, however, was not reported by ASHIHARA (1977). Existence of an optimum amount of a pheromonal mixture in a dispenser for field trapping has been reported for several species (ROELOFS et al., 1973; SMITH et al., 1974; MAITLEN et al., 1976; MILLER et al., 1976). However, the behavioral responses observed in the laboratory usually show sigmoidal type curve (ROELOFS and FENG, 1967; TAMAKI and YUSHIMA, 1974; COFFELT et al., 1979; NOGUCHI, 1979) and the optimum dose has been observed in only a few species (BAKER, et al., 1981; CASTROVILLO and CARDÉ, 1979).

Existence of the optimum amount in a trap catch means that a large quantity of the chemical affected some behavioral sequence in the attracted males. In this experi-

ment, the effect of amount and mixture ratio of Z-11-Ac and Z11-16Ald on male behavior was examined in the laboratory.

MATERIALS AND METHODS

Diamondback moths were reared by a mass-rearing method (KOSHIHARA and YAMADA, 1976) under the 16 light–8 dark photoperiodic condition at 23°C. Pupae were segregated by sex and kept separately under continuous light at 20°C. After emergence, 8 males were kept in each 200 ml flask with a cotton stopper and a moist cotton ball under continuous light. Behavioral observations were made 2 to 4 days after emergence under the light according to ASHIHARA (1977).

Both chemicals were purified with TLC on silicic acid impregnated with AgNO₃. Gas chromatographic analysis of test chemicals showed the purity of Z11-16Ac and Z11-16Ald to be at least 99 and 98.3 %, respectively. Each chemical was used singly or mixed in a 1:1 ratio as a serially diluted hexane solution. To examine the effect of mixture ratio on male response, the two chemicals were mixed in various ratios as 1×10^{-3} µg/ml solutions.

Females which had been kept 2 or 3 days after emergence under continuous light, were dipped into ethyl ether for 1 night. After filtration and dehydration with Na₂SO₄, the extract was concentrated and serially diluted with ether. Concentration of the extract was expressed as female equivalent (F.E.) units.

Ten microliters of the solution was applied on a small piece of filter paper (ca. 0.6 cm²). The paper was thrown into the bottom of the flask after the solvent had evaporated and male behavior was observed for 60 sec. Male responses noted were walking, flying and mating dance. In this paper, mating dance is used to describe wing fanning while walking, and this behavior was typical of sexually excited males. The maximum number of responding males was scored between 10–30 sec after the treatment except for the experiment with a single component, because male response was largely stabilized within this time interval. As a blank experiment, a filter paper without chemicals was thrown into the flask. At least 4 replications of the experiment were made and male response ratio was calculated as an average of these replications.

RESULTS

Response of males to various amounts of 1:1 mixture of the two components

Figure 1 shows the response of males to various amounts of a 1:1 mixture of the two chemicals on a filter paper. Activated individuals showing walking and flying response increased for a time with a larger amount of chemicals. The peak of male response was observed at 1×10^{-3} µg and it then decreased with further increase in chemical amount. The same tendency was seen in the male mating dance, which was completely suppressed with 1 µg of the chemicals on a filter paper. These results indicated the presence of an optimum aerial concentration of sex pheromone inducing sexual behavior.

Response of males to the female extract

As there was a possibility that the decrease in the male response to the mixture of Z11-16Ac and Z11-16Ald at high concentrations resulted from the lack of some other

Fig. 1. Response of males to various amounts of a 1:1 mixture of Z11-16Ac and Z11-16Ald. Hollow and solid circles represent walking and flying individuals and those who did the mating dance, respectively.

Fig. 2. Response of males to various amounts of female crude extract. Symbols are the same as in Fig. 1.

Fig. 3. Response of males to various mixture ratios of Z11-16Ac and Z11-16Ald. Total amount of chemicals was fixed at $1 \times 10^{-3} \mu\text{g}$. Symbols are the same as in Fig. 1.

Fig. 4. Response of males to various amounts of Z11-16Ald. A: percentage of walking and flying individuals. B: percentage of males which did the mating dance. Triangles, squares, and circles show the maximum percentage of individuals responding between 0 to 10, 10 to 30, and 30 to 60 sec after treatment, respectively.

components emitted by the female, male response to female extract was also examined (Fig. 2). The result showed an optimum concentration at 1×10^{-3} F.E. Male responses were not high, but the tendency was the same as in the previous experiment.

Response of males to various ratios of the two chemicals

As the optimal amount of the sex pheromone was $1 \times 10^{-3} \mu\text{g}$ with a 1:1 mixture (Fig. 1), total amount of the two chemicals was fixed at $1 \times 10^{-3} \mu\text{g}$ and the ratio was varied (Fig. 3). The mating dance was observed at every ratio except when the two components were offered singly (10:0 or 0:10). Males responded well to the ratios

Fig. 5. Response of males to various amounts of Z11-16Ac. Symbols are the same as in Fig. 4.

between 8:2 to 5:5 (Z11-16Ac: Z11-16Ald), where over 70 % of them were activated and over 40 % performed the mating dance. This means that a high percentage of response was obtained when the amount of Z11-16Ac exceeded that of Z11-16Ald.

Response of males to various amounts of each component

When the components were used singly, male response was low. Most mating dances were observed just after the treatment and, after that, only sporadically. Observation was continued for 60 sec and the male behavior was scored at 0–10, 10–30, and 30–60 sec after the treatment.

The male mating dance was scarcely induced by Z11-16Ald irrespective of the amount used (Fig. 4). The percentage of activated individuals was slightly increased with amount, but the relationship was not clear. On the contrary, about 15 % of the males did the mating dance constantly with an amount of more than $1 \times 10^{-4} \mu\text{g}$ of Z11-16Ac (Fig. 5). These dances took place immediately after treatment and were observed momentarily, but no definite relationship between the amount of chemical and the percentage of activated individuals was observed.

DISCUSSION

Field tests with a 1:1 mixture of Z11-16Ac and Z11-16Ald showed the existence of an optimum concentration range in a trap catch (KOSHIHARA et al., 1978; KOSHIHARA and YAMADA, 1980). Many behavioral steps are involved in attracting and catching a moth in a trap, and it is difficult to know which step is suppressed by high aerial concentration of the sex pheromone only by a field trap catch. In this experiment, the existence of an optimum aerial concentration in behavioral response was clearly confirmed in the laboratory. Although no optimum chemical concentration for the mating dance was reported by ASHIHARA (1977), the amount he used on a filter paper ranged between 3×10^{-5} to 3×10^{-3} F.E. and higher amounts were not tested.

The mating dance and other activities decreased with the increase in amount of pheromone and males were gradually immobilized above the optimum range. FUJII-YOSHI et al. (1979) observed the same phenomenon in a mating inhibition experiment with 0.1 and 1 μg of a 1:1 mixture of Z11-16Ac and Z11-16Ald in the laboratory.

These results show that male activity is suppressed by exposure to higher aerial concentration of their sex pheromone.

This type of response is basically different from the sigmoidal type observed in the laboratory in which males did not show the decreased response (ROELOFS and FENG, 1967; TAMAKI and YUSHIMA, 1974; COFFELT et al., 1979; NOGUCHI, 1979). *P. xylostella* male behavior is finely controlled by a concentration of the 1:1 mixture of the two components, since males have an optimum concentration for behavioral response. The same type of response has also been reported in *Grapholitha molesta* (BAKER et al., 1981), *Spodoptera littoralis* (TAMAKI and YUSHIMA, 1974), and *Laspeyresia pomonella* (CASTROVILLO and CARDÉ 1979). In *S. littoralis*, an optimum amount was more clearly observed when the major component of the two component system was used alone (TAMAKI and YUSHIMA, 1974). In field trappings, there are also two types: the first type show no optimum concentration and no decrease in trap catch is observed with a large amount of chemicals within the tested range (HOWLAND et al., 1969; YONGE et al., 1976; SUGIE et al., 1977; STECK et al., 1980). The other type shows an optimum amount or trap catch decreases with a large chemical quantity (KAAE et al., 1973; ROELOFS et al., 1973; SMITH et al., 1974; MILLER et al., 1976; MAITLEN et al., 1976).

One possible reason for there being no reported decrease in response or trap catch is that experiments were not conducted with a great enough quantity of chemicals to suppress the behavior or catch. The male behavioral response of *Epiphyas postvittana* showed a sigmoidal curve which was followed by a decrease in response ratio to the highest amount (BARTELL and SHOREY, 1969) and a decrease in trap catch under this condition has also been observed in some species (KAAE et al., 1973; ROELOFS et al., 1973; SMITH et al., 1974; MILLER et al., 1976; MAITLEN et al., 1976). These results support the possibility that the same phenomenon takes place in other species. Even if higher aerial concentration generally suppresses male behavior, his response may be classified into two types which correspond to the sigmoidal and the optimum. The former has broad optimum range and responds to a relatively higher chemical concentration than the other type, while the latter has a narrow optimum range at lower concentrations. If we adopt these two classifications, *P. xylostella* belongs to the narrow optimum range type.

An optimum mixture ratio showed a broad range (Fig. 3). This result coincides with that of field tests (KOSHIHARA et al., 1978; KOSHIHARA and YAMADA, 1980). A high response of males was observed to ratios between 8:2 and 5:5 when the quantity of Z11-16Ac exceeded that of Z11-16Ald. In experiments with a single component, Z11-16Ac induced the male mating dance (Fig. 5) while Z11-16Ald seldom did. These results suggest that Z11-16Ac is a key component in inducing the mating dance when mixed with Z11-16Ald. However, further experiments are necessary to determine the function of each component in the mating behavior.

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