Biological Activity of the Synthesized Sex Pheromone and Its Geometrical Isomers of *Spodoptera litura* (F.) (Lepidoptera : Noctuidae)

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Biological activities of the synthesized female sex pheromone and its geometrical isomers of *Spodoptera litura* (F.) were examined by a laboratory bioassay. A mixture of *cis*-9, *trans*-11-tetradecadien-1-ol acetate (*cis*-9, *trans*-11-TDDA) and *cis*-9, *trans*-12-tetradecadien-1-ol acetate (*cis*-9, *trans*-12-TDDA), the natural pheromonal components, was much more active than any other combinations between geometrical isomers of 9,11-TDDA and 9,12-TDDA. *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA evoked a weak response of the male moths at the levels higher than 10^{-2} μ g when the compounds were presented individually. Biological activities of the compounds were synergistically enhanced by mixing the two compounds (BR_{50} : 10^{-5} μ g). The optimum ratio of the two compounds in the mixture was 9 parts of *cis*-9, *trans*-11-TDDA and 1 part of *cis*-9, *trans*-12-TDDA.

Biological importance of quantitative relationships among multiple components of an insect sex pheromone was discussed.

INTRODUCTION

TAMAKI et al. (1973b) have isolated *cis*-9, *trans*-11-tetradecadien-1-ol acetate and *cis*-9, *trans*-12-tetradecadien-1-ol acetate and identified them to be the active components of the female sex pheromone of *Spodoptera litura* (F.), a serious pest of vegetable crops in Japan. Preliminary experiment on mixtures of *cis*-9, *trans*-11- and *cis*-9, *trans*-12-tetradecadien-1-ol acetates showed that the ratio of the two compounds in the mixture is an important factor of the activity of the sex pheromone. This paper is an account of the biological activity of synthesized pheromonal compounds and their geometrical isomers to the male moths of *Spodoptera litura* (F.) with special attention to synergism of the active compounds recognized in a laboratory bioassay.

MATERIALS AND METHODS

Compounds. *cis*-9, *trans*-11-Tetradecadien-1-ol acetate (*cis*-9, *trans*-11-TDDA) and its geometrical isomers were synthesized in this laboratory and purified with gas chromatography (TAMAKI et al., 1973b). *cis*-9, *trans*-12-Tetradecadien-1-ol acetate (*cis*-9, *trans*-12-TDDA) and its geometrical isomers were provided from the Pesticide Research Institute, Kyoto University, and purified with argentation thin-layer chromatography and gas chromatography.

Each of the eight compounds showed a single peak on a gas chromatogram (15

% PEGA, 2.5 m, 180°C).

Insects. Male moths of *Spodoptera litura* (F.) used in a biological assay were obtained from a laboratory stock culture of the insect on an artificial diet (YUSHIMA et al., 1973).

Bioassay. Biological activities of the synthesized compounds were examined according to a laboratory bioassaying procedure after TAMAKI et al. (1973a). Male moths were kept under continuous light at 20°C for 2 to 3 days after emergence. On the third to fourth day, five male moths were placed in a stainless-steel net cage 10 cm height and 9 cm in diameter, and then kept in darkness for 2 hr. On the inner wall of a glass-pipette (medicine-dropper) with a rubber bulb, 10 μ l of hexane solution containing a definite amount of synthesized compound(s) was applied, and the solvent was allowed to evaporate. The rubber bulb was squeezed and the tip of the pipette was directed toward the male moths in the cage under red dim light from a flash-light. A copulatory attempt (extension of clasper) was interpreted as a positive response of sexual behaviour of the male moths. Other reactions, such as wing vibration and erratic flight, were not adopted as positive response. As a minimum three replications were used for each test.

RESULTS

All the synthesized compounds, four each isomer of 9,11-TDDA and of 9,12-TDDA, evoked no response of the male moths when they were presented individually at a level lower than 10^{-3} μ g. However, both *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA presented individually elicited copulatory attempt from some male moths at a level higher than 10^{-2} μ g (Table 1). Other three geometrical isomers of 9,11-TDDA also evoked 7 to 13 percent response at a level of 10^{-2} μ g. The results clearly indicated that *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA, the pheromonal compounds of *S. litura*, were biologically active even when they were presented individually.

Results of combination experiments on the eight synthesized compounds are shown in Table 2. The ratio of 9,11-TDDA to 9,12-TDDA was adjusted from 20 : 1 to 7 : 1 by referring to the natural ratio of *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA isolated from the virgin female moths (TAMAKI et al., 1973a). Of 16 combinations on four each isomer between 9,11- and 9,12-TDDA, only three combinations evoked prominent male response higher than 50% at 10^{-2} μ g level. These three were *cis*-9, *trans*-12-TDDA combined with either one of *cis,cis*-, *cis,trans*-, or *trans,cis*-isomers

Table 1. BIOLOGICAL ACTIVITY OF *cis*-9, *trans*-11- AND *cis*-9, *trans*-12- TETRADECADIEN-1-OL ACETATES INDIVIDUALLY PRESENTED TO THE MALE MOTHS OF *Spodoptera litura*.

Amount presented (μ g)	Percent response to :	
	<i>cis</i> -9, <i>trans</i> -11-	<i>cis</i> -9, <i>trans</i> -12-
5	—	60
5×10^{-1}	46	—
2×10^{-1}	—	27
2×10^{-2}	44	20
2×10^{-3}	0	0
2×10^{-4}	0	0

Table 2. BIOLOGICAL ACTIVITY OF 16 COMBINATIONS OF FOUR EACH GEOMETRICAL ISOMER BETWEEN TETRADECA-9, 11-DIEN-1-OL ACETATE AND TETRADECA-9, 12-DIEN-1-OL ACETATE PRESENTED TO THE MALE MOTHS OF *Spodoptera litura*.

Tetradeca-9, 12-dien-1-ol acetate	Tetradeca-9, 11-dien-1-ol acetate ($2 \times 10^{-2} \mu\text{g}$)			
	<i>cis, cis</i>	<i>cis, trans</i>	<i>trans, cis</i>	<i>trans, trans</i>
<i>cis, cis</i> ($1 \times 10^{-3} \mu\text{g}$)	40%	27%	30%	33%
<i>cis, trans</i> ($2 \times 10^{-3} \mu\text{g}$)	66	90	83	30
<i>trans, cis</i> ($2 \times 10^{-3} \mu\text{g}$)	43	30	20	43
<i>trans, trans</i> ($3 \times 10^{-3} \mu\text{g}$)	33	30	23	15

Fig. 1. Relationships of the amount and the male response on the three combinations of geometrical isomers of 9,11-TDDA and 9,12-TDDA. A, 9 : 1 mixture of *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA; B, 9 : 1 mixture of *trans*-9, *cis*-11-TDDA and *cis*-9, *trans*-12-TDDA; C, 9 : 1 mixture of *cis*-9, *cis*-11-TDDA and *cis*-9, *trans*-12-TDDA.

of 9,11-TDDA. Relationships between the amount and the male response on these three combinations are shown in Fig. 1. The ratio of 9,11-TDDA to 9,12-TDDA in this experiment was 9 : 1. The most active combination was the mixture of *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA, which evoked 50% response of male moths at $10^{-5} \mu\text{g}$ level. The mixture of *cis*-9, *cis*-11-TDDA and *cis*-9, *trans*-12-TDDA as well as of *trans*-9, *cis*-11-TDDA and *cis*-9, *trans*-12-TDDA elicited 50% male response at $10^{-3} \mu\text{g}$ level. *cis*-9, *trans*-11-TDDA combined with *cis*-9, *trans*-12-TDDA, the active components of the natural sex pheromone, showed most potent biological activity, and the other two combinations were 100 times less active than the pheromonal combination at BR_{50} (50% behavioural response) level.

Various mixtures of *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA were examined to find an optimum ratio of these two compounds. Amounts of the mixture presented to the male moths were 2×10^{-4} and $2 \times 10^{-5} \mu\text{g}$. The results indicated that the optimum ratio of the two compounds was 9 parts of *cis*-9, *trans*-11-TDDA and 1 part of *cis*-9, *trans*-12-TDDA (Fig. 2). Neither *cis*-9, *trans*-11-TDDA nor *cis*-9, *trans*-12-TDDA

Fig. 2. Biological activity of *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA mixed at different ratios. The amounts of the mixtures presented were $2 \times 10^{-4} \mu\text{g}$ (A) and $2 \times 10^{-5} \mu\text{g}$ (B).

alone evoked any response of the male moths at the amount of 10^{-4} and $10^{-5} \mu\text{g}$ level.

DISCUSSION

Geometrical isomers of sex pheromones possess various degrees of biological activity; one acts as an inhibitor of pheromone perception (ROELOFS and COMEAU, 1968; JACOBSON, 1969) and the other possesses a weak activity as sex pheromone (BUTENANDT et al., 1962; BERGER and CANERDAY, 1968; JACOBSON et al., 1968; GASTON et al., 1972; SARMIENTO et al., 1972). A geometrical isomer of a sex pheromone is generally much less active than the sex pheromone itself. A representative case is found in *Bombyx mori* (BUTENANDT et al., 1962; TRUSCHEIT und EITER, 1962); the best isomer (*cis*-10, *trans*-12-hexadecadien-1-ol) was 10^{10} to 10^8 times less active than the sex pheromone itself (*trans*-10, *cis*-12-hexadecadien-1-ol). In the case of *Trichoplusia ni* the *trans*-isomer was 10^2 to 10^9 times less active than *cis*-7-dodecen-1-ol acetate, the sex pheromone of this insect (BERGER and CANERDAY, 1968; JACOBSON et al., 1968; TOBA et al., 1970; GASTON et al., 1972). Field examinations on various compounds structurally relating to dispalure, the sex pheromone of the gypsy moth (*cis*-7,8-epoxy-2-methyl octadecane), showed that the *trans*-isomer was 40 to 100 times less active than the *cis*-isomer (SARMIENTO et al., 1972). Present results on *Spodoptera litura* also indicated that the best combination of the geometrical isomers of the sex pheromone was about 100 times less active than the pheromonal combination in the laboratory bioassay.

Synthesized components of the sex pheromone of *Spodoptera litura*, *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA, were much more active when the two compounds were mixed in a definite ratio than the two were individually presented. Similar synergistic phenomena have also been reported on *Ips confusus* (WOOD et al., 1967), *Dendroctonus brevicornis* (BEDARD et al., 1969), *Anthonomus grandis* (TUMLINSON et al., 1969, 1971), and *Musca domestica* (MANSINGH et al., 1972). ROELOFS and his colleagues (ROELOFS and COMEAU, 1968, 1971; ROELOFS et al., 1973) found that an

artificial synergist such as dodecyl acetate or dodecyl alcohol enhanced the attractancy of synthesized sex pheromone of *Argyrotaenia velutinana* and *Grapholita molesta*. However, it has not been verified that these synergistic chemicals actually play important roles in the natural mating behaviour of these moths.

Adoxophyes fasciata and *A. orana* utilize two compounds, *cis*-9- and *cis*-11-tetradecen-1-ol acetates, as their sex pheromone (TAMAKI et al., 1971a, b; MEIJER et al., 1972). Male moths of *A. fasciata* and *A. orana* do not respond to the individual pheromonal components even when unusually large amount was presented. Simultaneous presentation of the two compounds is essential to release an overt sexual response of males of these two tortricid species. In the case of *Spodoptera litura*, however, the male moths apparently respond to the individual pheromonal components at the amount higher than 10^{-2} μ g level. Thus, there is an apparent difference between the two tortricid species and *S. litura* in the fashion of sex pheromone perception by males.

JACOBSON et al. (1970) isolated and identified two active components, *cis*-9-tetradecen-1-ol acetate (I) and *cis*-9, *trans*-12-tetradecadien-1-ol acetate (II), of the sex pheromone of *Spodoptera (Prodenia) eridania*; and they suggested that the compound I sexually excites the male when the sexes are in close proximity while compound II serves as a distant attractant for the male. In this context it is interesting to examine the different function, if any, of the two pheromonal components of *S. litura* under field conditions. The present experiments undertaken in the laboratory could not elucidate any differentiation in the function of the two active components of the sex pheromone of *S. litura*.

As were the case of *Adoxophyes fasciata* and *A. orana* (TAMAKI et al., 1971a,b) relative amount of the two pheromonal components of *S. litura* is a very important factor of biological activity of the sex pheromone. Nine to one mixture of *cis*-9, *trans*-11-TDDA and *cis*-9, *trans*-12-TDDA evoked an optimum response of the male moths. It should be noted that the reversed ratio of the two compounds, i.e., 1 : 9 mixture, did not elicit any response from the male moths at 10^{-4} to 10^{-5} μ g level. Difference in the responsiveness of the male moths of the two *Adoxophyes* spp. to a mixture of the pheromonal compounds, *cis*-9- and *cis*-11-tetradecen-1-ol acetates, was suggested to be an important base of the sexual isolation between the two sympatric sibling species (TAMAKI et al., 1971b, TAMAKI, 1972). Results of recent studies on insect sex pheromones apparently indicate that several insect species possess same compound(s) as their sex pheromones (JACOBSON, 1972; TAMAKI, 1972) and many insect sex pheromones are composed of multiple components (BAYER, 1966; BRADY and NORDLUND, 1971; JACOBSON, 1972; WALLIS et al., 1972; GEORGE and McDONOUGH, 1972; BARTELL and ROELOFS, 1973; JACOBSON et al., 1973). These facts support the suggestion that not only qualitative differences in pheromonal compounds but also quantitative relationships between components of a sex pheromone are important basis of the mate recognition and the sexual isolation in sympatric species (TAMAKI, 1972).

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