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BEHAVIORAL RESPONSES OF *Spodoptera littoralis* MALES TO SEX PHEROMONE COMPONENTS AND VIRGIN FEMALES IN WIND TUNNEL[1]

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Abstract—The major component of the sex pheromone of female *Spodoptera littoralis*, (*Z*,*E*)-9,11-tetradecadienyl acetate (**1**), elicited all steps of the male behavioral sequence, i.e., wing fanning and taking flight, oriented upwind flight and arrival to the middle of the tunnel, close approach and contact with the source. The activity was equivalent to that elicited by virgin females. In the range of doses tested, the dosage of **1** had no significant effect on the number of source contacts. Male response was significantly affected by light intensity, being optimum at 3 lux. Activity of the minor components (*Z*)-9-tetradecenyl acetate (**2**), (*E*)-11-tetradecenyl acetate (**3**), tetradecyl acetate (**4**), (*Z*)-11-tetradecenyl acetate (**5**), and (*Z*,*E*)-9,12-tetradecadienyl acetate (**6**) was significantly lower than that of the major component when assayed individually. In multicomponent blends compound **4** appeared to strongly decrease the number of males arrested at the source, the effect being particularly important when compound **5** was present in the blend. Results of single sensillum experiments confirmed the existence of two main physiologically distinct sensillar types. The most common type of sensilla contained a neuron that responded specifically to compound **1**. A second type of sensilla, located laterally on the ventral sensory surface, contained two receptor neurons responding to compound **6** and to (*Z*)-9-tetradecenol. Among short sensilla, one hair responded to compound **4** and could represent a minor sensillar type. No sensory neuron was found to detect the other minor pheromone compounds **2**, **3**, and **5**.

Key Words—Wind tunnel, behavior, single sensillum recording, *Spodoptera littoralis*, Lepidoptera, Noctuidae, Egyptian armyworm.

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[1]Dedicated to the memory of the late Prof. Félix Serratosa (1925–1995).

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INTRODUCTION

Wind tunnels have been widely used as a reliable technique to study the orientation mechanisms of flying insects to their sex pheromones (Miller and Roelofs, 1978; Cardé, 1984; Mafra-Neto and Cardé, 1994), as well as for evaluation of the mating disruption process (Sanders, 1982) and inhibition induced by specific synthetic compounds (Preiss and Priesner, 1988).

The sex pheromone of the Egyptian armyworm *Spodoptera littoralis*, an important pest of cotton and vegetable crops in Europe, Asia, and Africa, was first identified by Nesbitt et al. (1973) as a mixture of (*Z*,*E*)-9,11-tetradecadienyl acetate (**1**), (*Z*)-9-tetradecenyl acetate (**2**), (*E*)-11-tetradecenyl acetate (**3**), and tetradecyl acetate (**4**) (Figure 1). The pheromone composition, however, varies with the origin of the strain analyzed. Thus, while Dunkelblum et al. (1982) in Israel reported that the sex pheromone complex was a mixture of **1** (33%), **2** (46%), **3** (9%), (*Z*)-11-tetradecenyl acetate (**5**) (7%), (*Z*,*Z*)-9,11-tetradecadienyl acetate (4%), and (*Z*,*E*)-9,12-tetradecadienyl acetate (**6**) (0.5–1%), Tamaki and Yushima (1974) found that abdominal tip extracts from Kenyan insects contained a mixture of only **1** and **6**. Different compositions were also found by Campion et al. (1980) from calling virgin females of Crete, Israel, and Egypt. Thus, whereas volatiles of virgin females from Egypt contained much larger amounts of compounds **4** and **2** + **3** than **1**, the Israeli strain showed compounds **4** and **5** as the major components, and the Cretan stock produced only saturated acetate **4**. The small number of insects available, however, made the results of this study of limited value. In previous studies carried out on our laboratory strain, Martínez et al. (1990) found that the pheromone gland contained a mixture of compounds **1–5** in the ratio 66 : 12 : 11 : 2 : 9, respectively. The estimated amounts

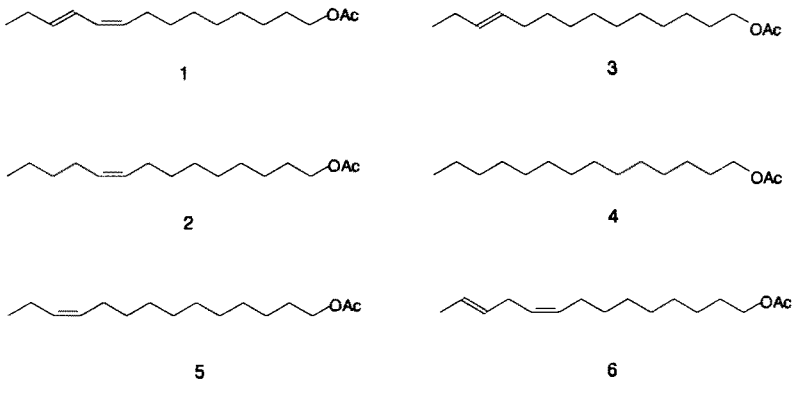


FIG. 1. Structures of compounds **1–6** tested.

of these compounds per female were ca. 40, 7, 7, 1, and 6 ng, respectively. The difference in composition of the pheromone of our strain relative to others led us to study the behavioral activity induced by each component found in the gland alone and in blends with the major compound **1.**

Although field tests are the definitive experiments to assess pheromonal activity (Ridgway et al., 1990), behavioral assays to determine male response to pheromone components in comparison with virgin females are also beneficial for gaining insight into the communication system of the species (Baker and Cardé, 1984; Löfstedt et al., 1985; Linn et al., 1987). In this regard, only one report describing minor component effects on male behavior of Egyptian and Cretan origins has been found in the literature (Haines, 1983). In this paper we report behavioral activity of components **1–5** found in the female gland of our strain, alone and in blends with the major compound **1.** Activity of diene **6,** absent in our population but present in others (see above), and comparison of the activity of the major component and the natural blend with that of virgin females is also presented. Single sensillum experiments (Kaissling, 1979) were also conducted in order to establish the existence of specific receptor cells for the pheromone components found in the gland.

METHODS AND MATERIALS

Insects. Insects were obtained from a laboratory colony taken from the Station de Zoologie (INRA, Montfavet, France) and reared in our laboratory on a slightly modified artificial diet from the one previously reported (Poitout et al., 1972). Modifications include replacement of aureomycin chlorohydrate by Wesson salt mixture (1%, Sigma) and addition of 0.08% of 35% aq. formaldehyde. Pupae were sexed, placed in groups of 20–25 in 20- × 20-cm plastic boxes, and maintained in a climatic chamber on a 16L:8D regime at 25 ± 1°C with 60–70% relative humidity until emergence. Adults were separated daily by age and provided with 10% sucrose solution.

Chemicals. Compounds **1–3** were obtained from Sigma Chem. Co. (St. Louis, Missouri) and their purity (>95%) was checked by GLC on a SPB-5 fused silica capillary column. Stereochemical purity of **1** was *Z,E*:*E,E* 96:4. Compounds **4** and **5** were prepared by acetylation of tetradecyl alcohol and (*Z*)-11-tetradecenol (Sigma), respectively, with acetyl chloride 99%+ (Aldrich Chemie, Steinheim, Germany) followed by purification by column chromatography to assess a >98% purity by GLC analysis. Just prior to the experiments, solutions were prepared by dissolving the compounds in 100 μl of nanograde hexane. The required volume to achieve the test doses (1–1000 μg) was applied to a 1.5- × 1-cm cotton wick and the solvent evaporated. The wicks were discarded after use.

Wind Tunnel. The tests were performed in a glass wind tunnel 180 cm long, 55 cm wide, and 50 cm high. The frame of the tunnel was made of aluminum and access was gained by two sliding doors, each one covering one half of the tunnel. The air was pushed through by a centrifugal fan and pulled out of the building with the aid of an exhaust blower. The fan and blower worked simultaneously and their action was carefully regulated by a potentiometer to avoid undesired turbulence in the moving air. Turbulence was controlled through a SO_3-containing airflow tester (Drägerwerk Aktiengesellschaft, Germany). The airstream was purified through a 2-cm-thick glass wool bed and conducted through two consecutive nylon screens to smooth the airflow and get a laminar regime through the tunnel. A 58-W red fluorescent light located 16 cm above the tunnel provided the required illumination. Light intensity was adjusted to 3–10 lux by covering the top of the tunnel with red cellophane paper strips. A white board containing irregular-shaped, dark brown spots was placed on the floor of the tunnel since striped patterns may induce unexpected effects on the orientation of flying insects (David, 1982). The airspeed was 45–55 cm/sec, the temperature of the experiment room was 22 $\pm$ 1°C, and the relative humidity 60 $\pm$ 5%. The wind tunnel was thoroughly cleansed with acetone before every change of formulation.

Bioassays. Newly emerged moths were separated from the remaining pupae twice a day and placed into 31- $\times$ 12- $\times$ 21-cm plastic boxes. Only insects on their first and second scotophase were tested since they had shown maximum EAG responses in comparison to older insects (Murgó and Guerrero, unpublished data). At the onset of the scotophase, males were distributed in groups of five individuals, placed in Petri dishes, and maintained in the dark for 3 hr. They were then allowed to acclimate to the tunnel conditions for 60 min and utilized only once. The males were taken from their containers, placed over a filter paper on a stainless steel jack at 130 cm downwind from the source, and allowed 5 min to respond. When virgin females were used as the pheromone source, five individuals (10–35 hr old) were placed in 6.5- $\times$ 4 $\times$ 3-cm stainless steel cages of 0.2- $\times$ 0.2-cm mesh and suspended about 18 cm from the top and 40 cm from the upwind end of the tunnel. Males were introduced into the tunnel only after the virgin females had adopted the calling position. To study the effect of synthetic chemicals, cotton wicks containing the compounds were hung on the same holders. The experiments were carried out 3–6 hr into the scotophase since female calling behavior reaches its maximum 3–9 hr into the dark period (Dunkelblum et al., 1987). Responses of 15–20 males were recorded per day of experiment with a minimum of two replicates per formulation.

Males were scored according to the following behavior: wing fanning and taking flight, arrival at the middle of the tunnel (65 cm), close approach to the lure (ca. 10 cm), and contact with the source. In the tests with individual compounds, latency, i.e., delay in time spent by males to leave the filter paper,

was also considered. Only those males arrested at the source for a minimum period of 5 sec were recorded as source contacts. The effect of a specific formulation was calculated as the number of males showing a particular behavior in comparison with the total number of insects released. The results were analyzed for significance using a chi-square 2 × 2 test of independence (Sokal and Rohlf, 1969).

Electrophysiology. Single sensillum recordings (SSRs) were performed on whole insect preparations according to the standard tip recording technique (Kaissling and Thorson, 1980). Glass electrodes were filled with sensillar saline for the recording electrode and with hemolymph saline for the reference electrode. After cutting the more distal segments, the tip of the antenna was covered with the hemolymph saline. The tips of several sensilla were cut off using sharpened forceps, and the recording electrode was slipped over the cut end of one hair. The SSR responses were filtered (150–5000 Hz) and amplified (×1000). The recordings were stored on a PC-AT microcomputer via a DASH 16 analog-to-digital conversion board. Acquisition and analysis of the recordings were performed by programs we developed in Asyst (McMillan Software Co.). Records were plotted with Awave, a software developed by F. Marion-Poll in Visual C++.

In addition to pheromone components **1–6,** other pheromone analogs were also tested on several trichoid sensilla, i.e., dodecyl acetate, (*Z*)-7-dodecenyl acetate, (*Z*)-9-dodecenyl acetate, and (*Z*)-9-tetradecenol, in a randomized order. The olfactory stimulations were achieved by blowing a puff of air (0.5 liters/min) of 1 sec duration through a Pasteur pipet containing a filter paper, on which 0.1, 0.5, or 5 μg of the test compound diluted in 1 μl of hexane had been deposited. The antenna was continuously flushed with humidified air (1.0 liter/min, 100% relative humidity). Receptor neurons tuned to compound **4** were searched for in olfactory hairs sampled among all different morphological classes and locations of sensilla trichodea. In addition, the activity of binary mixtures **1** + **4** were compared to that displayed by the individual compounds alone in order to check for a possible interaction of these two components at the receptor cell level.

RESULTS

Male Response to Major Component 1. The effect of dosage of major component **1** on male behavioral response is shown in Figure 2. In the range of doses tested (1–1000 μg), dosage had no significant effect on the number of males displaying every stage of the courtship sequence. In contrast, light intensity exerted a clear effect on male responses to major component **1,** particularly at moderate dose (Figure 3). Thus, when 1, 3, and 10 lux were tested, males

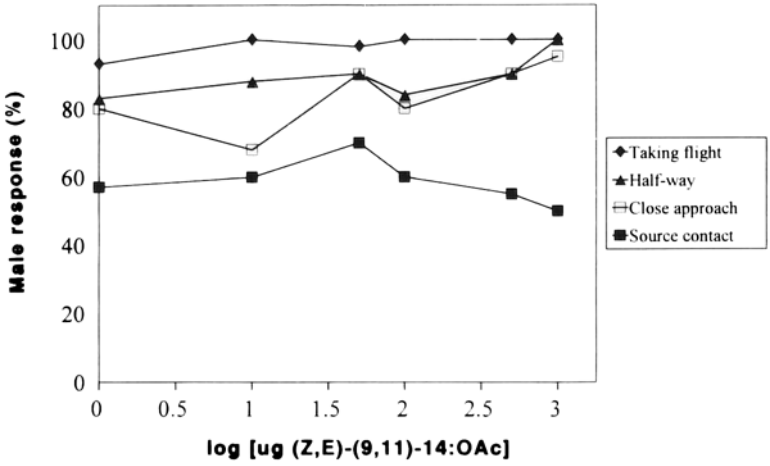


FIG. 2. Percentage of *Spodoptera littoralis* males exhibiting all types of behavior in response to various doses of (*Z*,*E*)-9,11-tetradecadienyl acetate (**1**) in wind tunnel at an airspeed of 45 cm/sec and 3 lux of light intensity.

displayed highest responses at 3 lux and significantly higher responses at 50- to 100-μg doses than those elicited with other illuminations. The effect was dose-dependent, the difference in number of males contacting the source under the three light intensities was higher at higher concentrations.

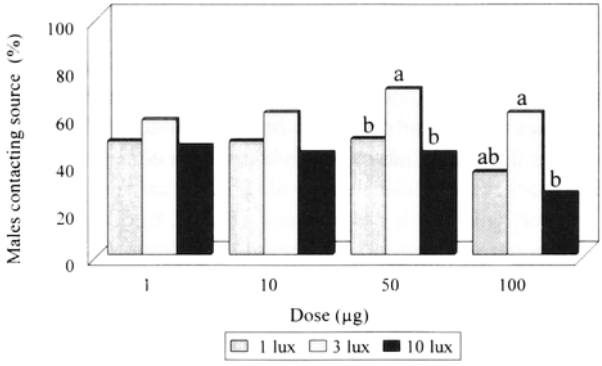


FIG. 3. Percentage of *Spodoptera littoralis* males contacting the source in response to several doses of (*Z*,*E*)-9,11-tetradecadienyl acetate (**1**) under different light intensities. Bars topped by different letters within a dose are significantly different at $P < 0.05$ (chi-square 2 × 2 test).

Male Response to the Minor Components 2–6. The minor components **2–5,** found in our strain, as well as diene **6** found in some strains, were assayed individually at several concentrations (100–1000 μg). Little activity was generally elicited at any of the doses tested, the activity being significantly lower than that displayed by the major component **1** ($P < 0.05$) (Figure 4). Lack of response was evident at the earliest stages of behavior, i.e., wing fanning and higher latency (> 120 sec) in comparison with insects responding to major component **1** (60–100 sec). In most cases the number of source contacts was negligible, so activity was evaluated in terms of number of males performing close approach. At the 100-μg dosage, compound **2** was the most active minor component ($P < 0.05$). It was able to induce landing of 35% of males at that concentration. Compound **3** was also active, the effect being higher at increasing concentrations, although activity was not significant relative to the other minor components. Compounds **4** and **5,** in turn, were only able to elicit close approach to the source in 20–30% of males, whereas compound **6** showed moderate attractivity only at 1000 μg.

Male Response to Binary Mixtures. The effects induced by binary mixtures of **1** with minor components **2–6** in a similar ratio to that found in the pheromone gland were also studied. Cotton wicks were loaded with previously prepared

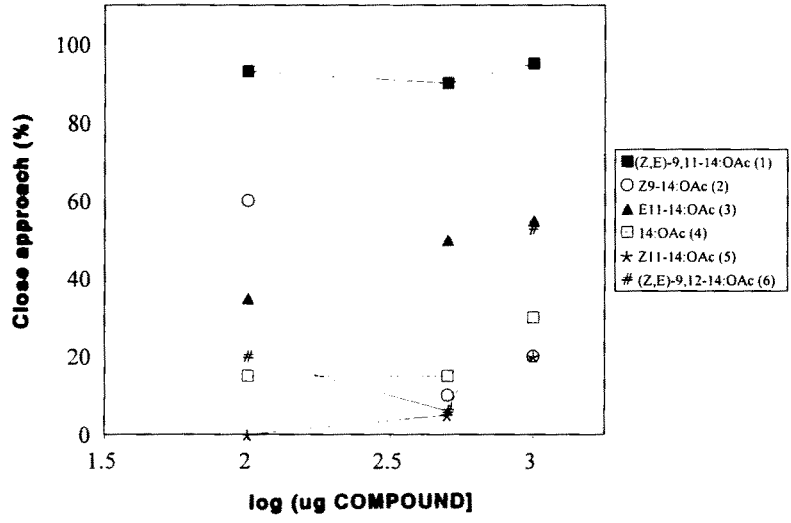


FIG. 4. Percentage of *Spodoptera littoralis* males performing close approach behavior in response to various doses of Z9–14:OAc (**2**), *E*11–14:OAc (**3**), 14:OAc (**4**), Z11–14:OAc (**5**), and (*Z,E*)-9,12–14:OAc (**6**) in wind tunnel. Responses to (*Z,E*)-9,11-tetradecadienyl acetate (**1**) are given for comparison.

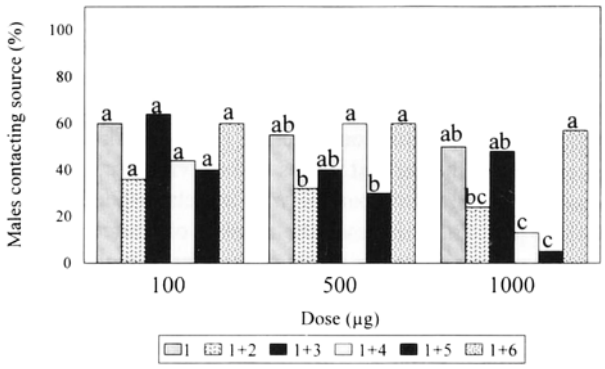


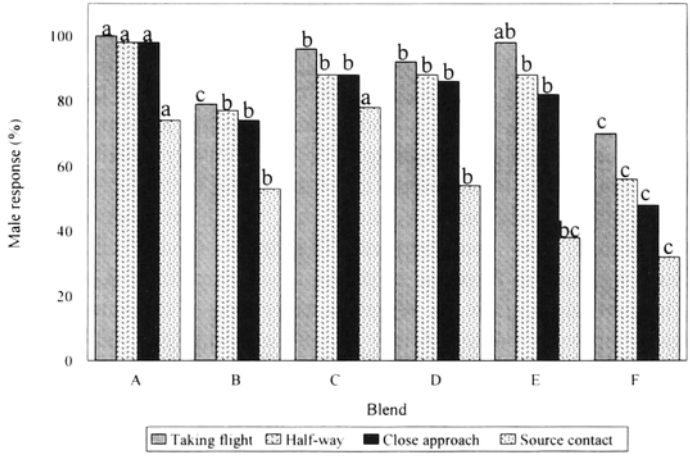
FIG. 5. Percentage of *Spodoptera littoralis* males contacting the source in response to binary mixtures of (*Z*,*E*)-9,11-tetradecadienyl acetate (**1**) with *Z*9-14:OAc (**2**), *E*11-14:OAc (**3**), 14:OAc (**4**), *Z*11-14:OAc (**5**), and (*Z*,*E*)-9,12-14:OAc (**6**) at different doses. Bars bearing different letters within a dose are significantly different at $P < 0.05$ (chi-square 2 × 2 test).

mixtures of 100, 500, and 1000 μg of **1** and the corresponding amount of the minor compounds (Figure 5).

In general, these binary blends did not increase the number of source contacts elicited by compound **1.** Differences in activity between the major component and mixtures increased with the concentration, being significant ($P < 0.05$) at the highest doses (500–1000 μg). Among the binary mixtures, formulation **1** + **3** was the most active, but the number of source contacts was very similar to that displayed by compound **1** alone. A slightly lower effect was elicited by mixture **1** + **2,** with activity being independent of the concentration assayed. The effect of the dosage was particularly evident when blends **1** + **4** and **1** + **5** were tested at 1000 μg, wherein both mixtures elicited a notable inhibition of landing response. The effect was significant ($P < 0.05$) relative to the one displayed by the major compound alone. Compound **6** was completely ineffective when mixed with **1** at every dosage tested, in comparison with the activity induced by the major component alone.

Male Response to Multicomponent Blends. In order to compare the response to synthetic pheromone blends with that elicited by virgin females, several multiple combinations of compounds **1–5** were assayed in similar ratios to the one found in the gland. Six blends, A–F, were prepared by adding the corresponding amounts of components **2–5** to 1000 μg of the major component **1.** The final formulations were the following: A: **1** + **2** + **3,** 75:13:12; B: **1** + **2** + **3** + **4,** 66:12:11:11; C: **1** + **2** + **3** + **5,** 67:12:11:9; D: **1** + **2** + **3** + **4** + **5,** 67:12:11:0.5:9; E: **1** + **2** + **3** + **4** + **5,** 66:12:11:2:9; F: **1** + **2** + **3** + **4** + **5,** 61:8:10:10:11.

Formulation E, which mimicked the natural pheromone blend, was, surprisingly, one of the less effective blends, with only 38% of males landing at the source (Figure 6). Formulation A, in contrast, was the most active, with 74% of males contacting the lure. Blend C was also very effective in eliciting complete flights to the source, and although the number of males taking flight and approaching the source was lower than with blend A ($P < 0.05$), there was no significant difference in the number of source contacts. Activity of formulation C was not significantly higher than that of virgin females, but induced a significantly higher number of male arrests at the source than compound **1** alone ($P < 0.05$, Figure 7). Addition of **4** to blend A (formulation B) significantly decreased male response. This effect was confirmed when the same compound was added to formulation C to make blend E. In this case, however, only the number of contacts with the source was significantly diminished. The same effect was noticed with formulation D, containing only 0.5% of compound **4.** The difference in behavior elicited relative to blend C was also significant ($P < 0.05$). Furthermore, formulation B, containing 11% of compound **4,** significantly reduced the number of males displaying all types of behavior in comparison with blend A. The inhibitory effect induced by **4** can be also noticed in



A: 1 + 2 + 3 (75:13:12)
B: 1 + 2 + 3 + 4 (66:12:11:11)
C: 1 + 2 + 3 + 5 (67:12:11:9)
D: 1 + 2 + 3 + 4 + 5 (67:12:11:0.5:9)
E: 1 + 2 + 3 + 4 + 5 (66:12:11:2:9)
F: 1 + 2 + 3 + 4 + 5 (61:8:10:10:11)

FIG. 6. Percentage of *Spodoptera littoralis* males exhibiting all types of behavior in response to multicomponent mixtures of (*Z,E*)-9,11-tetradecadienyl acetate (**1**) with *Z*9-14:OAc (**2**), *E*11-14:OAc (**3**), 14:OAc (**4**), and *Z*11-14:OAc (**5**). Values corresponding to the same type of behavior have been compared and bars topped by different letters are significantly different at $P < 0.05$ (chi-square 2 × 2 test).

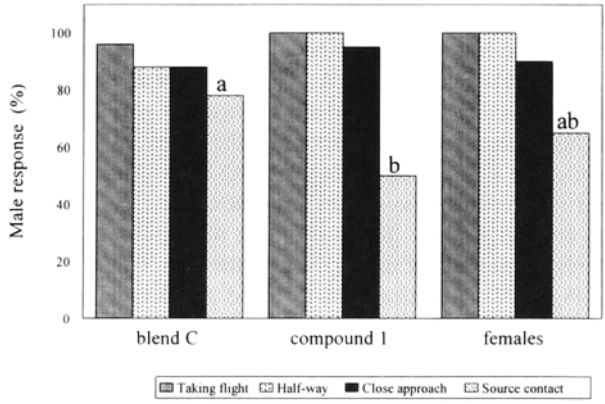


FIG. 7. Comparison of male responses to blend C, major compound **1**, and virgin females. Bars topped by different letters are significantly different at $P < 0.05$ (chi-square 2 × 2 test).

formulation F, wherein the number of males performing all behaviors was also significantly lower ($P < 0.05$) in comparison to those responding to blend C (Figure 6).

Single Sensillum Recordings. The activity of more than 100 long and 100 short sensilla trichodea chosen at random in the proximal half of the antenna was recorded. When the amplitude of their action potentials could be clearly distinguished from each other, A and B designate the sensory neurons firing action potentials of high and low amplitude, respectively. Most short and long trichoid sensilla housed a large-spike cell responding only to compound **1** (Figure 8a). In some of these hairs, a second cell, which fired action potentials of smaller amplitude, was also observed. The firing activity, however, did not significantly increase in response to pheromone compounds. Long sensilla trichodea, located laterally on the ventral sensory side of the antenna, housed A cells responding to compound **6** and B cells responding to (*Z*)-9-tetradecenol (Figure 8b,c). No response to **4,** even at 5 μg, was recorded from these two sensillar types. However, one short sensillum trichodeum showed a different cell response pattern. Its firing activity consisted of a single class of action potential amplitude. This sensillum neither responded to compounds **1, 6,** and (*Z*)-9-tetradecenol at 500 ng and 5 μg doses nor to compound **4** (500 ng), but stimulations with 5 μg of the latter component elicited significant responses (Figure 8d). Despite extensive efforts, no other hair displaying the same type of response could be found. Numerous short sensilla did not respond to any of the pheromone molecules assayed.

Sixteen sensilla from three males containing a receptor neuron tuned to

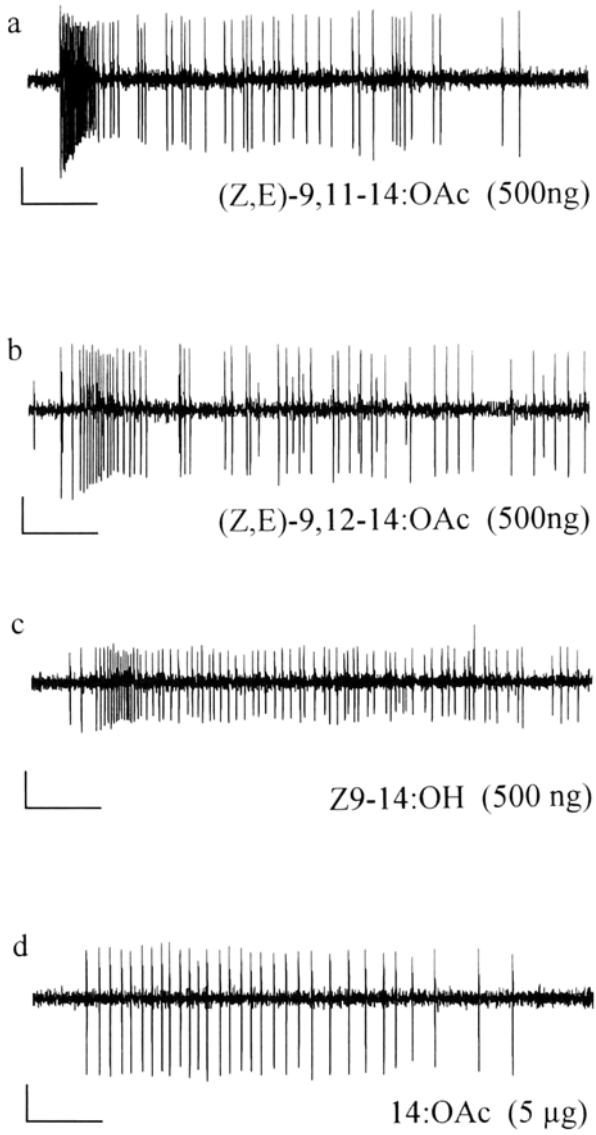


FIG. 8. Single sensillum recordings from the three types of sensilla trichodea: (a) from a sensillum responding to (*Z,E*)-9,11-tetradecadienyl acetate (**1**); (b) and (c) from a sensillum responding to (*Z,E*)-9,11-tetradecadienyl acetate (**1**) (A cell), and to *Z*9–14:OH (B cell); and (d) from a sensillum responding to 14:OAc (**4**). Scale bars: 200 msec, 0.5 mV. Stimulation duration: 1 sec.

compound **1** were subjected to compounds **1** (100 ng), **4** (10, 100 ng), and mixtures of **1** + **4** (100 + 10 ng, 100 + 100 ng). Compound **4** alone was inactive but when mixed with compound **1**, it slightly increased the firing frequency by 2–5 spikes/sec, but the difference was not significant (data not shown).

DISCUSSION

Our results show that in our strain, major compound **1**, whose presence in the gland accounts, on average, for 66% of the total amount of pheromone components, is able to produce alone all steps of the behavioral sequence in males. The activity is similar to that displayed by virgin females. The response level is clearly dependent on light intensity, particularly at 50 μg dosage and higher. Wind velocity had practically no significant effect at speeds ranging from 45 to 80 cm/sec, whereas at 40 cm/sec only 25% of males initiated upwind flight towards the source (data not shown). This is in agreement with the experiments of Haines (1983) on other strains.

Low loadings (1 μg) of the major component are sufficient to promote all behaviors of the mate-finding sequence, but higher doses do not impair the ability of males to orient. Many insects respond to low amounts of chemicals in their flight orientation behavior, i.e., *Mamestra suasa* (Frérot et al., 1989), *Grapholita molesta* (Linn and Roelofs, 1983; Linn et al., 1988), *Agrotis segetum* (Löfstedt et al., 1985), *Laspeyresia pomonella* (Preiss and Priesner, 1988), *Lymantria dispar* (Charlton et al., 1993), or *S. litura* (Kawasaki, 1986), among others. The high attractivity of **1** had been previously noticed in the field by Kehat et al. (1976), who found that 1 mg of the chemical was equivalent in effectiveness to a trap baited with virgin females.

In general, little activity was displayed by the minor components when assayed individually, the lack of response being evident at the earliest steps of the behavioral sequence. However, compounds **2** and **3** evoked source contacts in 20–30% of males at 100 μg and 500 or 1000 μg, respectively (data not shown). Compound **2** was found to be encoded by a specific receptor by Priesner (1979), but we did not find a specific receptor cell for **2.** In the field, compounds **2** and **5** behaved as inhibitors of the male activation response induced by compound **1** (Campion et al., 1980), although Kehat et al. (1976) found that addition of small amounts of **5** to the "pure" major component **1** in the Israeli strain markedly enhanced the activity of the diene. Acetate **6,** whose presence in other strains appeared essential for a maximum trap catch in the field (Kehat et al., 1976; Dunkelblum et al., 1982), elicited activation and orientation to the plume only at high doses but failed to induce close approach to the source.

Binary mixtures of the minor components with major compound **1** did not significantly increase the number of males responding in comparison with com-

pound **1** alone. There was, however, a significant inhibitory effect in the number of contacts elicited by compounds **4** and **5** at high dosage (1000 μg). The effect induced by **4** is in agreement with the results of Haines (1983) on a Cretan population, wherein addition of ≥ 10% of **4** significantly reduced the percentage of males responding. The same mixture, however, did not elicit any appreciable effect on the Egyptian strain. These results suggest that minor components **4** and **5** exert their arrestment activity at a close distance to the source and at relatively high atmospheric concentrations, in a similar way to the activity shown by dodecyl acetate in *Trichoplusia ni* (Linn and Gaston, 1981).

The effect exerted by compound **4** was confirmed when multiple component blends B–F were assayed. Comparison of formulations D and E with C clearly shows an inhibition effect in the number of males arrested at the source, the presence of only 0.5% of **4** being enough to induce a significant decrease of source contacts. The effect was dose-dependent, the highest inhibition of response being elicited by composition F containing 10% of the minor component. The effect was specially remarkable when compound **5** was also present in the pheromone composition (compare formulations B and F). Compound **4** appears, therefore, to be a close-range inhibitor, but depending on the presence and concentration of **5**, it may also inhibit other behaviors, such as wing fanning, orientation to the plume, and close approach to the source.

The most active formulations were A and C, the latter inducing slightly lower activation and orientation to the plume but with similar numbers of males arrested at the source. Compound **5**, present in formulation C, appears, therefore, to be a behaviorally neutral minor component. The high activity elicited by blends A and C, analogous to that of virgin females, and the inhibition shown by compound **4** suggest that perhaps females do not release this minor component. Despite extensive effort, we have been unable to detect saturated acetate **4** in aeration experiments of female volatiles.

The presence of compound **4** in the pheromone gland induced us to look for a specific receptor cell and to study its peripheral detection by male antennae. Single sensillum recordings confirmed the existence of two main physiological types of sensilla trichodea on the male antenna, as previously described by Ljungberg et al. (1993). Thus, despite differences in the composition of their sex pheromones, the two populations show the same sensillar organization. In addition, among short hairs, one different sensillum responded to saturated acetate **4**. No sensillum of the same type was, however, recorded in further experiments, which can be interpreted in two ways. Either hairs of this type are present in very low numbers on the antenna, as is the case for the (*Z*)-9-tetradecenyl acetate receptor cell in *Agrotis segetum* (Löfstedt et al., 1982), or the hairs responding to the inhibitor may have been undersampled due to their location or size. Considering the very weak EAG activity of compound **4** (Nes-

bitt et al., 1973; Ljungberg et al., 1993), the former possibility seems more likely. Responses from this sensillum were recorded only to 5 μg of compound **4**, ten times higher than the dose required for eliciting activity on the other two sensillar types. This high threshold may be due to the absence of tuning of this sensillum to the minor component, or it may originate from the experimental conditions, which include cutting the tip of the sensilla and covering its distal part. On a short hair, this may result in a considerable diminution of the sensory zone in contact with the stimulating pheromone molecules.

In summary, the major component **1** is a very effective attractant for the Spanish strain of *Spodoptera littoralis* males in behavioral studies, its activity being equivalent to that elicited by virgin females. Among the minor components present in the gland and in multicomponent blends, compound **5** is behaviorally neutral, while **4** appears to be a close-range inhibitor of arrestant behavior.

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