Journal of Chemical Ecology, Vol. 9, No. 6, 1983

BEHAVIORAL RESPONSES OF MALE *Heliothis virescens*[1] IN A SUSTAINED-FLIGHT TUNNEL TO COMBINATIONS OF SEVEN COMPOUNDS IDENTIFIED FROM FEMALE SEX PHEROMONE GLANDS

RICHARD S. VETTER and THOMAS C. BAKER

Division of Toxicology and Physiology, Department of Entomology University of California, Riverside, California 92521-0137

(Received July 6, 1982; revised October 29, 1982)

Abstract—Each of the seven compounds that have been identified from female *Heliothis virescens* sex pheromone glands was examined for its ability to elicit sexual responses from male moths in a flight tunnel. The two compounds initially described as pheromone components, (*Z*)-11-hexadecenal and (*Z*)-9-tetradecenal, were necessary for behavioral activity to occur. Of the remaining five compounds, hexadecanal was most consistent in elevating behavioral activity of males when it was added to treatments. Live, calling females elicited greater sexual activity from males than did the 7-compound mixture on rubber septa.

Key Words—Tobacco budworm, *Heliothis virescens*, Lepidoptera, Noctuidae, flight tunnel, sex pheromone, moth behavior, rubber septa.

INTRODUCTION

The sex pheromone of the female tobacco budworm moth, *Heliothis virescens* (F.) was first identified as a 2-component mixture, (*Z*)-11-hexadecenal (*Z*11–16:ALD) and (*Z*)-9-tetradecenal (*Z*9–14:ALD) (Roelofs et al., 1974; Tumlinson et al., 1975). Klun et al., (1979, 1980) isolated and identified five additional compounds from washes of female sex pheromone glands: hexadecanal (16:ALD), tetradecanal (14:ALD), (*Z*)-7-hexadecenal (*Z*7–16:ALD), (*Z*)-9-hexadecenal (*Z*9–16:ALD), and (*Z*)-11-hexadecenol (*Z*11–16:OH). In tests with cotton wicks, the 7-compound mixture outperformed the 2-

[1] Lepidoptera:Noctuidae.

component mixture in field-trapping experiments (Sparks et al., 1979; Hartstack et al., 1980). However, in other tests using rubber septa or plastic laminates, the two treatments were equally attractive or the 2-component mixture was superior (Hartstack et al., 1980). Due to the large number of possible permutations of compounds, only a limited number of combinations have been tested in the field. The purpose of this study was to systematically determine the role, if any, that each of the seven compounds plays in *H. virescens* sexual communication, using upwind flight and precopulatory behaviors of the male moths in a sustained-flight tunnel as the criteria for differentiation of the treatments.

METHODS AND MATERIALS

Rearing

Tobacco budworm larvae were raised on a modified pinto bean diet (Shorey and Hale, 1965). Pupae were segregated by sex, moths were aged, and the sexes kept in separate chambers after emergence. Larvae and adults were maintained at $25 \pm 2°C$ on a 14:10 light–dark photoperiod. Adults always had access to a 8% sucrose solution.

Chemicals

All compounds were obtained from the Controlled Release Division of Albany International Corporation. Purity was determined by gas–liquid chromatography, using 10% XF-1150 (50% cyanoethyl, methyl silicone) on Chromosorb W, AW DMCS, 100/120 mesh (2.5 m × 2 mm). Purities for the compounds were as follows: 14:ALD (100%), *Z*11–16:OH (99%), *Z*9–14:ALD, *Z*7–16:ALD, and *Z*11–16:ALD (98%), *Z*9–16:ALD (96%), and 16:ALD (95%). The stock solutions were made prior to each series of tests and were stored at below −20°C. Rubber septa (A.H. Thomas Co. No 8753-D22, sleeve type, 5 × 9 mm) were loaded on the small end with 10 μl of solution in hexane such that the emission rates of the seven compounds, as measured with an airborne collection device (Baker et al., 1981), approximated those of the same compounds emitted from the forcibly extruded gland of a *H. virescens* female (Table 1). *Z*11–16:OH was not detectable in emissions from female glands and, therefore, it was loaded on the septum at 1 μg which was 1% of the total blend as found in extracts of gland tissues (Klun et al., 1979, 1980).

Behavioral Observations

Compound Mixtures on Septa. Male moths were tested during the 5th through 8th hr of their 4th or 5th scotophase in a clear plastic flight tunnel (1 m wide at the floor, 0.9 m high, 3.65 m long) modified after Miller and

TABLE 1. LOADING AND EMISSION RATES OF COMPOUNDS ON RUBBER SEPTA AND EMISSION RATES OF FORCIBLY EXTRUDED FEMALE SEX PHEROMONE GLANDS (DATA FROM POPE ET AL., 1982) AT 22°C

Compound	Loading on rubber septa (μg)	Emission rates ng/min (% of total)	
		Rubber septa	Female
Z11-16:ALD	100	1.14 (58)	2.19 (73)
Z9-14:ALD	2.5	0.18 (9)	0.16 (5)
16:ALD	50	0.36 (19)	0.39 (13)
14:ALD	5	0.25 (13)	0.22 (7)
Z7-16:ALD	1	0.02 (1)	0.02 (1)
Z9-16:ALD	1	0.03 (1.5)	
Z11-16:OH	1	ND (0)	ND (0)

Roelofs (1978). Further details of construction are found in Kuenen and Baker (1982). The males were transferred to the flight-tunnel room ca. 1.5 hr prior to the beginning of observations in order to acclimate the moths to wind-tunnel conditions (0.5 m/sec wind velocity, 24 ± 3°C, 0.3 lux light level). They were then transferred from the holding cage to individual release cages [6 cm long × 6 cm diam (3.15 wires/cm) galvanized mesh] at least 30 min prior to testing.

Septa were loaded daily with solutions ca. 30 min before testing. They were placed in an exhaust hood for 15 min, and then transferred to individual, capped, holding vials. Treatments within an experimental series were drawn in random order for each daily testing in a complete-block design. At the beginning of a trial, an impregnated septum was placed at the center of a piece of sheet metal (15 × 15 × 0.05 cm) situated 15 cm above the tunnel floor on a sheet-metal platform that was 50 cm from the upwind end of the tunnel. Each male was released ca. 3 m downwind of the source by placing the individual cage, open end up, on a metal platform, held by a ringstand positioned in the middle of the pheromone plume. The location of the plume was predetermined by a $TiCl_4$ smoke source. Cages and platforms were washed with acetone daily after use.

The attractiveness of a mixture was determined by the intensity of the males' responses upon leaving the release cage. Those males capable of sustained flight were scored for the following behaviors: *UpW*, moth flies upwind in the pheromone plume from the release point; *Pl*, moth flies within 10 cm of platform edge; *S*, moth lands on source; *Hp*, after landing on the source, moth everts his hairpencils; and *C*, after landing on the source, moth exhibits the copulatory response (i.e., full hairpencil eversion, curling of the abdomen). For each behavioral category, no male contributed more than 1 data point and the number reported in the table is the percentage of males that exhibited at least that level of behavior (i.e., a male that exhibited a *Hp* response added one positive response to each of the first four categories but not the last). Observations of hairpencil extrusions near the source were aided with a flashlight that had several layers of red cellophane placed over the lens.

Males making multiple approaches were observed until they flew up and out of the plume toward the top of the flight tunnel. If there were uncertainty of the intensity of the male's response (e.g., septum blocking the observer's view), the most intense response observed was recorded. Males were used once and then discarded from further testing; no more than 8 males were flown to one treatment per day. Each trial (i.e., the amount of time the septum was in the flight tunnel airstream) lasted ca. 15 min. All rubber septa were used within 30 min to 4 hr of initial loading and discarded after use.

All statistical analysis was performed using a χ^2 2×2 test of independence with Yates' correction at the 0.05 level of significance.

Female vs. Septa. Rubber septa impregnated with the 7-compound mixture were tested against live, calling *H. virescens* females. Females were used during the 4th through 7th hr of their 4th scotophase. They were transferred to the flight tunnel room ca. 1 hr prior to the beginning of the bioassay and placed in individual cages in the rear of the flight tunnel, upwind of the exhaust hood. When a female was observed calling (i.e., pheromone gland exposed), she was gently transferred, in her cage, to the upwind end of the flight tunnel and placed in the center of the sheet-metal platform. When the rubber septum was tested, an identical cage was placed over it to mimic the conditions under which the female was contained. Data were recorded from males that flew upwind in the plume. If a male initiated flight but did not fly upwind in the plume, it was not known if he had not responded to the pheromone or if the female had stopped calling and no pheromone was present; observations on these males were discarded. Upon landing on the cage containing the female or rubber septum, the male was observed for 5 sec and then was removed. This action was necessary since the presence of the male occasionally disturbed the female, whereupon she would move about the cage and sometimes cease calling. [This should have caused no biasing of the data since in other series of experiments with synthetic mixtures to which there were 505 multiple approaches by males, the most intense response was exhibited on the first approach (91%); we should have retained discrimination regardless of the male removal procedure.] No more than eight males were flown to one female and most trials with a female lasted 15 min; none lasted more than 30 min.

Rubber Septa vs. Cotton Wicks. Two types of dispensers were compared: rubber septa and cotton wicks. Cotton dental wicks, 36 mm long × 15 mm diam., were cut in half (to approximate the height of a septum) and 10 μl

TABLE 2. RESPONSE OF MALE MOTHS TO 2-COMPOUND MIXTURES

Treatments	% Behavioral Response[a] 16 flights/treatment				
	UpW[b]	*Pl*	*S*	*Hp*	*C*
Z11-16:ALD + Z9-14:ALD	100a	81a	62a	38a	6a
All other possible permutations of two compounds (20 treatments)	0b	0b	0b	0b	0a

[a]*UpW*, moth flies upwind in plume from release point; *Pl*, moth flies <10 cm from platform edge; *S*, moth lands on source; *Hp*, after landing, moth everts hairpencils; *C*, after landing, moth exhibits copulatory response.
[b]Percentages in the same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yates' correction ($P < 0.05$).

of the 7-compound mixture in hexane was impregnated on the uncut end of the wick. The wick was placed in an exhaust hood 15 min prior to initiation of the bioassay. Rubber septa were prepared as before and males were flown to both dispensers.

RESULTS

Two-Compound Mixtures. All 21 possible combinations of binary mixtures from seven compounds were tested, but only one elicited upwind flight from the males (Table 2). This was the combination of Z11-16:ALD and Z9-14:ALD which was initially described as the pheromone for *H. virescens* (Roelofs et al., 1974; Tumlinson et al., 1975) and hereafter will be referred to as the 2-component mixture.

Three-Compound Mixtures. Using the information obtained from the first experiment, a series of 3-compound mixtures was run, admixing one of each of the additional five compounds to the 2-component mixture. These were then compared to the 2-component and 7-compound mixtures.

The 7-compound mixture evoked significantly more *S* and *Hp* behaviors than the 2-component mixture (Table 3). Of the five 3-compound treatments, only addition of 16:ALD elicited significantly more *Hp* responses than the 2-component mixture but not significantly greater *S* or *Hp* responses than the 7-compound mixture. The treatments containing 14:ALD, Z11-16:OH, Z7-16:ALD, and Z9-16:ALD did not elicit significantly greater responses than the 2-component mixture.

Six-Compound Mixtures. One of each of the seven compounds was deleted from the 7-compound mixture to determine which one(s), if any,

TABLE 3. RESPONSE OF MALE MOTHS TO 3-COMPOUND MIXTURES

Treatments	Additional compound	% Behavioral Response[a] 100 flights/treatment				
		UpW[b]	*Pl*	*S*	*Hp*	*C*
*Z*11–16:ALD + *Z*9–14:ALD	16:ALD	90a	83a	64ab	38ab	6a
	14:ALD	80ab	71ab	58ab	24c	1a
	*Z*7–16:ALD	85ab	74ab	58ab	32abc	3a
	*Z*9–16:ALD	86ab	75ab	57ab	34abc	5a
	*Z*11–16:OH	74b	63b	54b	27bc	2a
2-Component mixture		82ab	72ab	53b	24c	1a
7-Compound mixture		84ab	79a	69a	45a	4a

[a]Abbreviations for the behavioral responses are described in Table 2 and in Methods and Materials.
[b]Percentages in the same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yates' correction ($P < 0.05$).

would cause a significant reduction of behavioral response when compared to the blend of seven compounds. Since the first two components (*Z*11–16:ALD and *Z*9–14:ALD) together were crucial for eliciting upwind flight from males (Table 2), the first part of this experiment consisted of three treatments: the 2-component mixture and two 6-compound mixtures (one of the two initial components was deleted from the 7-compound mixture to form the appropriate 6-compound mixture). Again, it was obvious that both *Z*11–16:ALD and *Z*9–14:ALD were essential for upwind flight to occur (Table 4, Part A).

In the second set of experiments, each of the five additional compounds was deleted, one at a time, from a 7-compound mixture and compared to the 2-compound and 7-compound mixtures. The blend of seven compounds again elicited a significantly greater number of *S* and *Hp* behaviors than did the 2-component mixture (Table 4, Part B). Of the five 6-compound mixtures, the treatment lacking 16:ALD caused the greatest decrease at the *S* and *Hp* levels of behavioral activity and both levels were significantly lower than the 7-compound blend. Responses to this treatment were not significantly different from those of the 2-component mixture. The only other 6-compound mixture to which there was reduced response compared to the 7-compound blend was the one lacking 14:ALD; it was significantly weaker at the *Pl* and *S* levels.

Four-Compound Mixtures. Because the greatest change in activity from the last two series of experiments occurred with the addition of 16:ALD to the 2-component mixture or with the omission of 16:ALD from the 7-compound mixture, the basis for this series became a 3-compound blend comprised of the original 2-component mixture plus 16:ALD, henceforth referred to as the 3-component mixture. The 4-compound mixtures tested

TABLE 4. RESPONSE OF MALE MOTHS TO 6-COMPOUND MIXTURES

Treatments	Missing compound	% Behavioral response[a] 24 flights/treatment				
		UpW[b]	*Pl*	*S*	*Hp*	*C*
Part A						
6-Compound mixtures	*Z*11–16:ALD	0b	0b	0b	0b	0a
	*Z*9–14:ALD	0b	0b	0b	0b	0a
2-Component mixture		79a	79a	62a	46a	0a
		100 flights/treatment				
Part B						
6-Compound mixtures	16:ALD	85a	68ab	51c	37c	4a
	14:ALD	78a	62b	55bc	41bc	6a
	*Z*7–16:ALD	82a	73ab	68ab	57a	3a
	*Z*9–16:ALD	82a	74ab	67ab	54ab	9a
	*Z*11–16:OH	85a	77a	66ab	44abc	7a
2-Component mixture		83a	67ab	57bc	34c	6a
7-Compound mixture		85a	78a	72a	55ab	11a

[a]Abbreviations for the behavioral responses are found in Table 2 and in Methods and Materials.
[b]For each part, percentages in the same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with a Yate's correction ($P < 0.05$).

in this series consisted of the 3-component mixture admixed with one of the remaining compounds (14:ALD, *Z*7–16:ALD, *Z*9–16:ALD, or *Z*11–16:OH (Table 5)). The reference treatments were the 2- and 3-component mixtures and the 7-compound mixture.

After 100 moth flights/treatment, no clear-cut differences were ap-

TABLE 5. RESPONSE OF MALE MOTHS TO 4-COMPOUND MIXTURES

Treatments	Additional compound	% Behavioral response[a] 100 flights/treatment				
		UpW[b]	*Pl*	*S*	*Hp*	*C*
*Z*11–16:ALD	14:ALD	86ab	73a	66a	37b	7a
+ *Z*9–14:ALD	*Z*7–16:ALD	92ab	78a	72a	36b	10a
+ 16:ALD	*Z*9–16:ALD	88ab	78a	72a	42ab	3a
	*Z*11–16:OH	86ab	83a	72a	55a	7a
2-Component mixture		93a	83a	71a	41ab	5a
3-Component mixture		91a	72a	61a	40a	5a
7-Compound mixture		82b	78a	73a	57a	4a

[a]Abbreviations for the behavioral responses are described in Table 2 and in Methods and Materials.
[b]Percentages in the same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yate's correction ($P < 0.05$).

parent due to the higher elicitation of activity by the two components, in contrast to the first sets of experiments. We could not discriminate among treatments containing compounds added to the 2 components, and here 16:ALD, when added to the 2-component mixture, did not elevate the males' responses above their already high levels. We include these results to show that the behavioral effects of adding 16:ALD could not always be observed in every experiment; this was the only experiment in which the effects of 16:ALD could not be seen.

Mixtures Containing Saturated Aldehydes and the Alcohol. A series of treatments was tested involving selected combinations of the saturated aldehydes and the alcohol with the 2-component blend which might provide further information on the effects of additional compounds. The treatments tested were two 3-compound blends containing the two components plus either 16:ALD or 14:ALD, two 4-compound mixes containing the 3-component mixture plus 14:ALD or *Z*11–16:OH, and a 5-compound mixture containing the 3-component blend plus 14:ALD and *Z*11–16:OH. The 2-component and 7-compound mixtures were included as the reference treatments.

The 7-compound mixture elicited significantly greater *S* and *Hp* behavioral responses than did the 2-component mixture, which was consistent with the first two experiments (Table 6). Of the remaining five treatments, those which contained 16:ALD caused behavioral activity similar to that of the 7-compound mixture. The only treatment lacking 16:ALD was the 3-compound blend which contained 14:ALD; this blend performed only as well as the 2-component mixture.

TABLE 6. RESPONSE OF MALE MOTHS TO TREATMENTS COMPRISED OF SELECTED COMBINATIONS OF 16:ALD, 14:ALD, AND *Z*11–16:OH[a]

	Additional compounds			% Behavioral response[b] 100 flights/treatment				
Treatments	16:ALD	14:ALD	*Z*11–16:OH	*UpW*[c]	*Pl*	*S*	*Hp*	*C*
*Z*11–16:ALD	+			89a	81a	68ab	48a	3a
plus		+		83a	73a	50c	28b	2a
*Z*9–14:ALD	+	+		84a	76a	67ab	51a	7a
	+		+	85a	75a	67ab	56a	5a
	+	+	+	89a	82a	72a	54a	7a
2-Component mixture				84a	80a	56bc	28b	1a
7-Compound mixture				88a	76a	71a	54a	3a

[a] A "+" indicates the presence of this compound in the treatment
[b] Abbreviations for the behavioral responses are described in Table 2 and in Methods and Materials.
[c] Percentages in the same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yates' correction ($P < 0.05$)

TABLE 7. RESPONSE OF MALE MOTHS TO 7-COMPOUND MIXTURE EVAPORATED FROM RUBBER SEPTA AND TO LIVE, CALLING FEMALES

	% Behavioral response[a] 100 flights/treatment				
Treatments	*UpW*[b]	*Pl*	*S*	*Hp*	*C*
Calling female	99a	91a	91a	81a	15a
7-Compound mixture	96a	83a	70b	40b	0b

[a] Abbreviations for the behavioral responses are described in Table 2 and in Methods and Materials.
[b] Percentages in the same column with no letters in common are significantly different according to a χ^2 2 × 2 test of independence with a Yates' correction ($P < 0.05$).

Septum vs. Live Female. Live, calling females elicited a significantly greater number of *S*, *Hp*, and *C* responses than did the 7-compound mixture on rubber septa (Table 7). No treatment in any of the series was able to elicit such high levels of activity from the males in the *S* and *Hp* categories.

Septum vs. Cotton Wick. The dental wick loaded with the 7-compound mixture elicited significantly more *UpW* and *Pl* behaviors from the males than did the septum, but there was no difference between the dispensers in the percentage of males landing on the source. However, the 7-compound mixture on the rubber septum caused a significantly greater number of *Hp* behaviors in the males than did the cotton wick (Table 8). Cotton wicks emit the compounds at rates two to three times greater than do rubber septa at the loading used in this study.

DISCUSSION

Two components, *Z*11–16:ALD and *Z*9–14:ALD, are the major mediators of chemical communication in *H. virescens* (Roelofs et al., 1974; Tumlinson et al., 1975; Klun et al., 1980; this study). In our flight tunnel, behaviors associated with mate-finding and close-range sexual activity (i.e., upwind flight to the source and hairpencil eversion) were not exhibited by the male moths when either of these two components was missing from a pheromone test blend (Tables 2 and 4, Part A). The five additional compounds identified by Klun et al. (1980) were nonessential for the attraction of a male to the source since the omission of any of these compounds from the 7-compound mixture did not prevent upwind flight and source location (Table 4, Part B).

However, in three of four of our series where the 2-component and the 7-compound mixtures were compared, the blend of seven compounds elic-

TABLE 8. RESPONSE OF MALE MOTHS TO 7-COMPOUND MIXTURE FORMULATED AT A DOSAGE OF 100 μg Z11-16:ALD ON TWO DISPENSERS: COTTON WICK AND RUBBER SEPTA

	% Behavioral response[a] 100 flights/treatment				
Treatments	*UpW*[b]	*Pl*	*S*	*Hp*	*C*
Rubber septum	78b	67b	63a	48a	2a
Cotton wick	92a	82a	59a	34b	2a

[a] Abbreviations for the behavioral responses are described in Table 2 and in Methods and Materials.
[b] Percentages in the same column having no letters in common are significantly different according to a χ^2 2 × 2 test of independence with Yates' correction ($P < 0.05$).

ited significantly more responses in at least one of the close-range behavioral categories than did the two components (Tables 3, 4, Part B and 6). Therefore, the elevated behavioral response must have been due to one of the additional five compounds or to a combination of two or more compounds.

Of the five compounds tested in our flight tunnel, the saturated 16:ALD was the most important additional compound to the 2-component blend, accounting for all of the increased close-range behavior observed in the 7-compound mixture (Tables 3 and 6). Also, its omission from the 7-compound blend caused a significant decrease in close-range behaviors of males, making it only as effective as the 2-component mixture (Table 4, Part B). Of the five compounds, the presence or absence of 16:ALD was the most consistent in predictability of behavioral effects, although it was not absolute (Table 5). When the remaining compounds (14:ALD, Z7-16:ALD, Z9-16:ALD, and Z11-16:OH) were considered as singular additives, singular omissions, or in combination with other compounds, there was no consistent effect on attraction or close-range behaviors (Tables 4, Part B, 5, and 6).

We feel that there is now enough evidence that 16:ALD is a pheromone component of *H. virescens* since it usually increased close-range sexual behaviors of the male moths when added to blends containing Z11-16:ALD and Z9-14:ALD. None of the other four compounds identified by Klun et al. (1980) were consistent enough in our tests to warrant the classification of "pheromone component," although this does not preclude them from being such. Our test may not have been sensitive enough to distinguish any further function of these compounds. Nonetheless, we feel that 16:ALD is the most important of the five additional compounds, and its effects probably overshadowed any slight enhancement by the other four compounds.

Consistent with this, Pope et al. (1982) found that 16:ALD was not only present in the gland extracts but it was emitted by 98% of the females in their peak emission period. Other than *Z*11-16:ALD, which was detectable 100% of the time, no compound was as abundant or detected as often as 16:ALD in volatile collections.

In some previous studies (Sparks et al., 1979; Hartstack et al., 1980), *Z*11-16:OH significantly enhanced trap catch when added to treatments formulated on cotton wicks. However, in our experiments, the presence of *Z*11-16:OH with the 2-component mixture on rubber septa caused no increase in male response (Table 3). This is in agreement with Hartstack et al. (1980) who found the presence of *Z*11-16:OH as part of the 7-compound mixture in Hercon®-laminated flakes, Albany International® hollow fibers, or on rubber septa did not increase trap catch of males and sometimes significantly reduced captures. In the wind tunnel, our formulation of the seven compounds on a rubber septum attracted as many males to the source as did a cotton wick loaded with the same quantity of compounds (Table 8), and hence, there was nothing obviously inferior about septa as a release substrate that would account for the above-mentioned differences in field experiments. In a blend ratio series, males in the flight tunnel were attracted equally well to lower levels of the alcohol added to the two components, but exhibited significantly reduced levels of upwind flight to 3% or greater levels of the alcohol; 79%, 82%, 78%, 54%, 43%, and 30% of the males flew upwind to 0, 0.3, 1, 3, 10, and 30% of the alcohol relative to the *Z*11-16:ALD ($N = 29$, 22, 27, 28, 23, and 27, respectively). In airborne pheromone collection studies, no detectable amounts of *Z*11-16:OH were emitted by females, in contrast to the measurable quantities of the other six compounds that were collected (Pope et al., 1982). This information, plus our flight tunnel observations, indicate that *Z*11-16:OH may not contribute to sexual communication in this species.

The observations from the flight tunnel may be instructive in explaining the difficulty of discriminating subtle, close-range effects of purported pheromone compounds in field-trapping experiments using large traps. Most of the differences among treatments in the flight tunnel from which we made our conclusions occurred at the *S* and *Hp* levels of behavioral activity. If similar proportions of moths exhibiting *Pl* behaviors were trapped, then, from our results, it is easy to see why there might be no differences in trap catch among different treatments in the field; for example, between treatments containing and lacking 16:ALD. A *Pl* observation required that the male moth fly within 10 cm of the pheromone source. When no longer attracted to the source, these males would fly upward and most likely would be captured in the *Heliothis* cone trap which has an opening at the underside of 50 cm (Hartstack et al., 1979). On the other hand, flight tunnel studies may lack discrimination for compounds functioning mainly at longer range, and here trapping experiments would be superior. Flight tunnels normally cannot duplicate the plume dimensions and concentrations that occur at great distances from a pheromone source and therefore cannot elicit from males the types of movements required to orient from those distances in a shifting wind field.

Possibly the most enlightening experiment was the comparison of the 7-compound mixture on a rubber septum to a live, calling female. When a female was tested against a rubber septum in the flight tunnel, there were no differences between *UpW* or *Pl* behavior elicitation. However, there was statistical significance at the remaining three levels. The greater number of *Hp* and *C* behaviors elicited by females could be due to better chemical as well as nonchemical (tactile, visual, and auditory) cues. However, the significantly greater number of *S* behaviors implies that the difference between septa and females may be explained best by chemical cues. Tactile cues are not possible since the male is still airborne just prior to landing on the source. Vision seems to play a minor role since males land on a pheromone-loaded cotton wick preferentially to a dead female pinned to an unloaded wick even when separated by only 2 cm (unpublished data) and females are relatively still when calling. Auditory cues cannot be ruled out and were not controlled in the experiment. Inasmuch as other cues may play a minor role in attracting males to a source, the factor more likely responsible for the elevated responses to live females in our study was chemical, due to components that are as yet unidentified or to blend quantity or quality differences that, despite our attempts to mimic female emissions, made the septa inferior at close range.

Acknowledgments—We would like to thank Drs. J. Weatherston and J. Greenblatt of the Controlled Release Division of the Albany International Corporation for their support and assistance during this project, Dr. L.K. Gaston and M.M. Pope for assistance with the chemical aspects of the study, the insect behavior seminar class for making critical comments on drafts of the manuscript, L.P.S. Kuenen for assistance at various points in the study, and for aiding in the rearing of the animals used, we thank our many lab helpers especially C.F. Kennedy, F. Kim, R.W. Simmons, Jr., and J. Norman III.

REFERENCES

BAKER, T.C., GASTON, L.K., POPE, M.M., KUENEN, L.P.S., and VETTER, R.S. 1981. A high-efficiency collection device for quantifying sex pheromone volatilized from female glands and synthetic sources. *J. Chem. Ecol.* 7:961–968.

HARTSTACK, A.W., WITZ, J.A., and BUCK, D.R. 1979. Moth traps for the tobacco budworm. *J. Econ. Entomol.* 72:519–522.

HARTSTACK, A.W., JR., LOPEZ, J.D., KLUN, J.A., WITZ, J.A., SHAVER, T.N., and PLIMMER, J.R. 1980. New trap designs and pheromone bait formulation for *Heliothis*. *Proc. Belt. Cotton Prod. Res. Conf.* 132–135.

KLUN, J.A., PLIMMER, J.R., BIERL-LEONHARDT, B.A., SPARKS, A.N., and CHAPMAN, O.L. 1979. Trace chemicals: The essence of sexual communication systems in *Heliothis* species. *Science* 204:1328–1330.

KLUN, J.A., BIERL-LEONHARDT, B.A., PLIMMER, J.R., SPARKS, A.N., PRIMIANI, M., CHAPMAN, O.L., LEPONE, G., and LEE, G.H. 1980. Sex pheromone chemistry of the female tobacco budworm moth, *Heliothis virescens*. *J. Chem. Ecol.* 6:177–183.

KUENEN, L.P.S., and BAKER, T.C. 1982. Optomotor regulation of ground velocity in moths during flight to sex pheromone at different heights. *Physiol. Entomol.* 7:193–202.

MILLER, J.R., and ROELOFS, W.L. 1978. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. *J. Chem. Ecol.* 4:187–198.

POPE, M.M., GASTON, L.K., and BAKER, T.C. 1982. Composition, quantification, and periodicity of sex pheromone gland volatiles from individual *Heliothis virescens* females. *J. Chem. Ecol.* 8:1043–1055.

ROELOFS, W.L., HILL, A.S., CARDÉ, R.T., and BAKER, T.C. 1974. Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. *Life Sci.* 14:1555–1562.

SHOREY, H.H., and HALE, R.L. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *J. Econ. Entomol.* 58:522–524.

SPARKS, A.N., RAULSTON, J.R., LINGREN, P.D., CARPENTER, J.E., KLUN, J.A., and MULLINIX, B.G. 1979. Field response of male *Heliothis virescens* to pheromonal stimuli and traps. *Bull. Entomol. Soc. Am.* 25:268–274.

TUMLINSON, J.H., HENDRICKS, D.E., MITCHELL, E.R., DOOLITTLE, R.E., and BRENNAN, M.M. 1975. Isolation, identification, and synthesis of the sex pheromone of the tobacco budworm. *J. Chem. Ecol.* 1:203–214.