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TWO SEX PHEROMONE COMPONENTS OF THE TOBACCO BUDWORM MOTH, HELIOTHIS VIRESCENS

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Summary

Two compounds were isolated from female Heliothis virescens (Lepidoptera: Noctuidae) extracts and identified as cis-9-tetradecenal and cis-11-hexadecenal. Together they elicit intense male H. virescens response in laboratory tests and have attracted males in the field. Although cis-11-hexadecenal is an H. zea sex pheromone, no evidence was obtained for cis-9-tetradecenal in H. zea.

The tobacco budworm, Heliothis virescens, is a major economic pest in the United States. Resistance to insecticides previously effective against this species has increased the need for alternative methods of control. Identification of the sex pheromone of this species would stimulate research on the use of synthetic pheromones in control programs.

We have isolated two components of the tobacco budworm pheromone system from female moth extracts and identified them as cis-9-tetradecenal (c9-14:Ald) and cis-11-hexadecenal (c11-16:Ald).

Materials and Methods

H. virescens and H. zea employed in this study were obtained from laboratory cultures maintained on an artificial medium (1) with a 16 hr photophase regime at 25° C. Pupae were segregated by sex and held along with emergent adults in separate rearing chambers. The abdominal tips of 0.5 to 4 day old females were excised during the 3.5 to 5th hr of scotophase and extracted with methylene chloride.

Bioassays of male pheromone response were conducted with an apparatus similar to that described by Bartell and Shorey (2). The cylindrical excitation

chamber was 17 cm in height and 15 cm in diameter. Filtered air from an outside source was introduced continuously through the bottom of each cage at a rate of 5 liters per minute and the test stimulus was placed in the air flow on 2 cm filter paper circles. One hr before the initiation of scotophase, 0.5 to 5 day-old males were distributed randomly at 10 to 13 males per bioassay chamber utilizing equal numbers of males per chamber on each test day. All assays were conducted 3.5 to 4.5 hours after the initiation of scotophase at 0.35 lux. Flight was selected as the key behavioral response. Background activity was monitored for 1 min immediately prior to the 1 min test stimulus. Responses elicited by the pheromones are corrected for spontaneous activity according to the formula of Bartell and Shorey (2).

Field tests were conducted in a tobacco field near Raleigh, North Carolina, using Pherotrap® (Zoecon Corp., Palo Alto, Calif.) traps, with test chemicals placed on rubber septa (5 x 9 mm rubber-stoppers, sleeve-type, Arthur H. Thomas Co.). Each aldehyde was prepared by oxidation of the corresponding alcohol using CrO_3-pyridine in methylene chloride (3). Any remaining alcohol was removed by chromatography on Florisil® using redistilled benzene; the product still contained ca 5% trans aldehyde and <5% of other impurities. Traps were hung from the plants at 7 m intervals at a height of 1.5 m and were rerandomized at the time of sampling.

Electroantennograms (EAG) were run as previously described (4) for assaying gas chromatographic collections of female tip extracts and for plotting response profiles with long-chain acetates, alcohols and aldehydes. Gas chromatographic columns (glass, 2m x 4mm or 4m x 2mm) were packed with 3% OV-1 (methyl silicone) on Gas-Chrom Q or 3% PDEAS (phenyldiethanolamine succinate) on Chromosorb W-AW-DMCS. The mass spectrometer used for component B was a Hitachi RMU-6E interfaced with an Aerograph 1740-10 gas chromatograph.

Results and Discussion

Heliothis virescens: Female H. virescens abdomen tip extract was injected onto the nonpolar OV-1 column @ 170° and the effluent was collected in 1-min

fractions for 20 min. EAG analysis of the collected fractions using male H. virescens antennae showed major areas of activity at 7-8 min (component A) and at 12-13 min (component B). The retentions of n-dodecyl, n-tridecyl and n-tetradecyl acetates were 5.65, 8.55 and 13.0 min, respectively. Laboratory behavioral tests with gas chromatographic (GC) fractions suggested that both components are important for male H. virescens stimulation. In bioassays (10 replicates) of male activity responses to GC collected fractions, component A at 1 ng elicited 7.7% (±3.7 S.E.), and component B at 16 ng elicited 19.6% (±6.0 S.E.), whereas A and B combined elicited 96.7% (±3.4 S.E.). The ratios of component A to component B in female extracts varied from 1:16 to 1:3; these extracts contained ca. 34 ng per female of component B. GC-collected material exclusive of A and B elicited 13.9% (±4.3 S.E.) while spontaneous activity was 4.2% (±0.7 S.E.).

Component A: Additional material of the 7-8 min component was collected from OV-1. Treatment of component A with sodium borohydride produced a compound with a retention time identical to cis-9-tetradecen-1-ol (8.4 min @ 173°) on OV-1. Component A had the same retention time on a PDEAS column @ 173° as c9-14:Ald (6.0 min), but was dissimilar to trans-9-tetradecenal (5.8 min). No trans-isomer in component A was detectable on PDEAS (<1%). Ozonolysis of component A gave a product with a retention time identical to that of 9-oxononanal (16.4 min) on OV-1 using a temperature program from 40° to 140°. A mass spectrum of component A was identical to that of c9-14:Ald. The above data support the characterization of component A as c9-14:Ald.

Component B: The greatest EAG activity was found in the fraction with a retention time of 12-13 min. Additional samples were collected from OV-1 and tested for chemical functionality. Saponification did not eliminate EAG activity at the retention time of component B. Treatment with sodium borohydride produced a compound with the retention time of cis-11-hexadecen-1-ol on OV-1 (18.9 min @ 173°). On PDEAS @ 173° component B had a retention time (11.35 min) similar to that of c11-16:Ald (11.3 min), but dissimilar to that of

trans-11-hexadecenal (10.85 min). No detectable amount of trans was observed (<0.5%). Ozonolysis of component B produced a compound with retention times similar to 11-oxoundecanal on OV-1 (3.75 and 3.7 min @ 178°, respectively, and 21.6 and 21.7 min on a programmed run from 40-140°, respectively), and on PDEAS (16.6 and 16.5 min @ 178°, respectively). A mass spectrum of component B was identical to that of c11-16:Ald. The above data all support the characterization of component B as c11-16:Ald.

In addition, a second set of laboratory behavioral tests (Table 1) with male H. virescens showed moderate levels of activity with c9-14:Ald and c11-16:Ald alone and intense activity with combinations of the two in the ratios of 1:25, 1:10 and 1:5, respectively. Activity was diminished when c9-14:Ald was increased to a 1:1 ratio.

TABLE 1

Male Bioassay of H. virescens

Treatment	Mean % response ± S.E.*
Spontaneous activity	7.0 ± 0.7
25 ng c11-16:Ald	31.0 ± 3.3
25 ng c9-14:Ald	14.2 ± 3.8
25 ng c11-16:Ald + 1 ng c9-14:Ald	63.3 ± 9.8
25 ng c11-16:Ald + 2.5 ng c9-14:Ald	88.4 ± 5.0
25 ng c11-16:Ald + 5 ng c9-14:Ald	85.1 ± 6.8
25 ng c11-16:Ald + 25 ng c9-14:Ald	47.5 ± 8.7

*12 replicates

Heliothis zea: Female H. zea abdomen tip extract was collected from an OV-1 column @ 174° in 1-min fractions for 20 min. EAG analysis of the collected fractions using antennae from male H. zea or H. virescens showed only 1 area of activity at 7-8 min which corresponded to the retention time of c11-16:Ald (7.4 min). Collection of this fraction from a PDEAS column @ 174° gave EAG activity (10.25-12 min) at the retention time of c11-16:Ald (11.0

min). No trans isomer was detectable on PDEAS (<0.5%). These data support the report of Sparks (Paper No. 63, National Entomological Society of America Meetings, Dallas, Texas, 1973) that c11-16:Ald is part of the sex pheromone system of H. zea. We found no evidence for c9-14:Ald in H. zea extract. Although male H. zea and H. virescens antennae gave good EAG responses to the c9-14:Ald present in female H. virescens abdomen tip extracts collected from OV-1, neither type of antenna responded to female H. zea abdomen tip extracts collected from OV-1 at the retention time of c9-14:Ald.

Standard EAG: Male antennal responses to 124 compounds in the 12-, 14- and 16-carbon aldehyde, alcohol and acetate series showed that both H. zea and H. virescens give the greatest response to c11-16:Ald. Compounds giving the next largest responses with H. virescens were c9-14:Ald, cis-9-tetradecen-1-ol, and cis-9-tetradecenyl acetate, similar to H. zea with the exception that cis-11-tetradecenal was more active on H. zea antennae than was c9-14:Ald.

Bioassay: Laboratory behavioral responses were conducted with both species. A series of treatments utilizing c9-14:Ald and c11-16:Ald alone and then in a 1:3 mixture was used. The 1:3 ratio of c9-14:Ald : c11-16:Ald is within the range of ratios found in female H. virescens gland extracts. The data (Table 2) show that the mixture is very stimulatory for H. virescens males, but slightly inhibitory for H. zea males.

Field Tests: The two aldehydes were tested alone and in combination in the field for H. virescens and H. zea attractancy. Field tests were not successful in brief trials conducted in California and Texas, but some attractancy for H. virescens was shown with the combination in North Carolina. The results (Table 3) with various treatments show that each aldehyde alone is not attractive, but treatments containing ratios of c11-16:Ald : c9-14:Ald from 200:1 to 2:1 are attractive. The actual ratio in the air would be different from that of the compounds on a septum as the 14-carbon aldehyde evaporates at a faster rate than the 16-carbon aldehyde.

TABLE 2

Male Bioassay of H. virescens and H. zea

(mean percent response ± standard error)

Treatment	H. virescens*	H. zea†
(spontaneous activity)	3.2 ± 1.1	3.9 ± 1.6
100 ng c11-16:Ald	19.3 ± 3.0	66.5 ± 7.5
30 ng c9-14:Ald	7.5 ± 2.9	6.3 ± 4.3
100 ng c11-16:Ald + 30 ng c9-14:Ald	90.8 ± 4.9	32.2 ± 6.5

*8 replicates

†6 replicates

Ratios in the septa also would change with time due to the higher evaporation rate of the 14-carbon aldehyde. Preliminary tests with materials free of trans isomers showed no improvement in field attractancy.

Conclusions: Two aldehydes, c9-14:Ald and c11-16:Ald, are components of the sex pheromone system of H. virescens. In combination, these compounds are not only highly stimulatory to male H. virescens in laboratory tests but are also attractive to male H. virescens in the field in North Carolina. More field trials with various release rates, compounds of greater purity, and different ratios of compounds are needed to optimize male attractancy. Although the two compounds have not been shown to be extremely potent in field trapping to date, they are still potentially useful in mating disruption programs, possibly by permeation of the air with microencapsulated material. The sex pheromone system of H. zea includes c11-16:Ald, but our evidence suggests that c9-14:Ald is not involved. Thus, these two sympatric species utilize one common pheromone component, but separation can be effected by unique blends comprised of various additional components (5).

TABLE 3

Field Tests with Synthetic Compounds for H. virescens Attractancy

Treatments		No. males trapped		
c11-16:Ald	c9-14:Ald	9/10-9/20/73	9/20-9/26/73	Total
1000 µg	0 µg	0	0	0
"	2.5	0	0	0
"	5	10	0	10
"	7.5	6	0	6
"	15	1	2	3
"	30	3	4	7
"	40	16	10	26
"	50	0	1	1
"	75	2	4	6
"	100	11	6	17
"	150	5	0	5
"	200	17	8	25
"	300	7	4	11
"	500	0	2	2
500	500	0	0	0
250	"	0	0	0
100	"	0	0	0
50	"	0	0	0
0	"	0	0	0
Unbaited		0	0	0
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