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IDENTIFICATION OF A MALE-PRODUCED PHEROMONE OF *Anticarsia gemmatalis* (HÜBNER) (LEPIDOPTERA; NOCTUIDAE) ATTRACTIVE TO CONSPECIFIC MALES[2]

R.R. HEATH, P.J. LANDOLT, N.C. LEPPLA, and B.D. DUEBEN

Insect Attractants, Behavior, and Basic Biology Research Laboratory
Agricultural Research Service, U.S. Department of Agriculture
Gainesville, Florida 32604

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Abstract—Observations in the laboratory and in the field indicated that male *Anticarsia gemmatalis* (Hübner), the velvetbean caterpillar (VBC), are attracted to conspecific courting males. Male VBC subsequently were found to be attracted to extracts of male abdominal tips including the extrudable hairpencils. The active chemical in these extracts was identified as (*Z,Z,Z*)-3,6,9-heneicosatriene, which is also one of the major components of the female VBC sex pheromone. Male VBC in a wind tunnel and in the field exhibited a bimodal response distribution to a range of ratios of the (*Z,Z,Z*)-3,6,9-heneicosatriene and (*Z,Z,Z*)-3,6,9-eicosatriene, with one maximum at the pure heneicosatriene alone and the other at the 60:40 female blend. This demonstrates that the male response to the male hairpencil component is distinct from that to the female sex pheromone.

Key Words—*Anticarsia gemmatalis*, velvetbean caterpillar, Lepidoptera, Noctuidae, attractant, pheromone, hairpencils, (*Z,Z,Z*)-3,6,9-heneicosatriene, male-produced pheromone.

INTRODUCTION

During field studies of the sex pheromone behavior of the male velvetbean caterpillar (VBC), *Anticarsia gemmatalis* (Hübner) (Heath et al., 1983), males

[1]This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by USDA.

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were observed orienting to other males that were responding at close range to synthetic female sex pheromone [40:60 ratio of (*Z*,*Z*,*Z*)-3,6,9-eicosatriene (C-20 triene) and (*Z*,*Z*,*Z*)-3,6,9-heneicosatriene) (C-21 triene)]. Similar behavior had been observed during studies of VBC sex pheromone behavior in a flight tunnel, involving calling females and multiple males (unpublished data). Whether these were visual responses of males to the shape of a moth or possibly a response to male-produced chemicals was not apparent.

Male-produced sex pheromones have been reported or suggested for a number of moth species, based on chemical analyses, behavioral studies of attraction and courtship, or hairpencil or brush morphology. Overt responses by females to male-produced pheromones have been demonstrated in few cases. Baker et al. (1981) demonstrated short-range attraction of female *Grapholitha molesta* (Busck) to a male sex pheromone released during courtship from abdominal hairpencils. *Ephestia elutella* (Hübner) females flex the abdomen ventrally in response to male sex pheromone (Krasnoff and Vick, 1984). Other studies suggest an aphrodisiac role for many male sex pheromones (Birch, 1974), and intrasexual responses to male pheromone have been reported in a few cases. Baker (1983) demonstrated that displaying *G. molesta* males attract other males at close range. Male attraction to males, resulting in aggregations in the field, is known for *Estigmene acrea* (Drury) (Willis and Birch, 1982). Also, male pheromone may reduce the attractiveness of calling females in *Heliothis virescens* (Boddie) (Hendricks and Shaver, 1975) and *Pseudaletia unipuncta* (Haworth) (Hirai et al., 1978).

Like many other species of Lepidoptera, male VBC have sexually dimorphic brushes and hairpencils. Greene (1974) described long scales on the prothoracic femorae and mesathoracic tibiae of VBC males. Male VBC also possess terminal abdominal hairpencils and scent brushes on the venter of the eighth abdominal segment. While it is presumed that these brushes and hairpencils are involved in courtship interactions possibly for the dispersal of pheromones affecting female behavior, their functions and the products of any associated exocrine glands are unknown.

We report here the results of experiments demonstrating attraction of male VBC to extracts of the male abdominal tips, including the hairpencils, and the identification of a chemical from those extracts that was attractive to other male VBC in a flight-tunnel bioassay and in field trapping tests.

METHODS AND MATERIALS

Insect Rearing and Maintenance. All insects were obtained as pupae from a colony maintained as described by Leppla (1985) at the USDA Insect Attrac-

tants, Behavior, and Basic Biology Research Laboratory, Gainesville, Florida. Pupae were sorted by sex, placed in 24 × 24 × 24-cm plastic screen and Plexiglas cages, and supplied with a sucrose solution on cotton. Males and females were held in separate environmental chambers on a 14:10 light–dark cycle at 24°C and 65% relative humidity. Pupae were transferred daily to new cages to provide adult moths of known age. Males were tested when 4–7 days old. Bioassays were conducted during third to fifth hours of the scotophase. Pheromone extracts from males were obtained during the first hour of the scotophase.

Bioassays of Male VBC Extracts. Preliminary bioassays of extracts of abdominal tips, whole insects, and leg scales indicated the attractant activity was confined to the abdominal tip. Male VBC abdominal tips were extruded by squeezing the abdomens between the thumb and index finger, extruding the terminal hairpencils, and exposing the ventral brushes. The tip was cut between the more anterior ventral brushes and hairpencils. Tips were placed in vials that contained 1 ml hexane in 50 or 25 tip batches. After several hours, the solvent extract was transferred to a new vial.

Tip extracts were tested for attractiveness to male VBC in a flight-tunnel bioassay in comparison to hexane blanks and female abdominal tip extracts [two female equivalents (FE)]. Extract dosages (as male equivalents) were placed on filter papers and, after 2 min of air drying under a fume hood, were hung on a stand near the center of the upwind end of the flight tunnel. Males were released singly at the middle of the length of the tunnel, ca. 2 m from the upwind end. Moths were scored during a 2-min period for upwind-oriented flight to the filter paper.

Abdominal tip extracts of male VBC were tested at dosages of 0.2, 1.0, and 5.0 male equivalents (ME). Either 5 or 10 males were tested (assayed) individually per treatment on each of seven different days to provide data sets composed of male responses to the female pheromone, hexane blanks, and the three dosages of male extracts.

Isolation and Identification. An initial purification of the male abdominal tip extracts was obtained on a gravity flow glass column (5 × 0.62 cm ID) prepared by slurry packing 60–100 mesh silica (J.T. Baker Chemical Co.) in hexane. Solvents used to elute the column were hexane, 10% ether–hexane, 50% ether–hexane, and 100% ether. The active fraction from the gravity flow was further purified using a Varian 1400 gas chromatograph (GC) equipped with a flame ionization detector. A glass column (2 m × 2.3 mm ID) packed with 4.4% OV-101 on 80–100 mesh Chromosorb G-HP was used, and 2% of the effluent was routed to the detector. The remaining 98% of the effluent was collected in a cooled 30-cm glass capillary tube according to the method described by Brownlee and Silverstein (1968). Helium was used as the carrier

gas (28 ml/min), and the column was temperature programmed from 80 to 240°C at 10°/min. Analyses of the active fraction eluted from the packed GC column and of the synthesized chemicals used in this study were obtained using a Varian 3700 GC equipped with splitless capillary injector and flame ionization detector. The output of the detector was interfaced with a Nelson 4000 data system. Helium was used as the carrier gas for all analyses at a linear flow velocity of 18 cm/sec. Columns used for analyses (from Quadrex Corp., New Haven, Connecticut) were a 50 m × 0.25mm ID fused silica column coated with a 0.25-μm film of methyl silicone and a 30 × 0.25 mm ID fused silica column coated with a 0.25-μm film of Carbowax 20 M. Columns were held at an initial temperature of 60°C for 2 min and then temperature programmed to 200°C at 32°/min. Additionally, a 30 m × 0.25 mm ID cholesterol para-chlorocinnamate liquid crystal capillary column was used isothermally at 160°C (Heath et al., 1979). Confirmation of the identity of a compound was made by cochromatography with a synthetic standard. Additionally, mass spectral data (methane chemical ionization and electron impact using a Nermag model R1010) were obtained on the active material from abdominal tip extracts. Mass spectrometry samples were introduced with an HP 5790 gas chromotograph equipped with a splitless injector and the 50-m methyl silicone column described previously.

Bioassays of Synthetic Pheromone. In a flight-tunnel bioassay, male VBC were tested for response to a range of ratios of the two components identified as the female sex pheromone and the active material obtained from male abdominal tip extracts and over a range of dosages. The pheromone was formulated in rubber septa at combined loads of 1, 2, 20, or 200 μg/septum dissolved in 100 μl of hexane and air dried for 2 days prior to use. Load ratios tested were 0:100, 2:98, 6:94, 10:90, 18:82, 45:55, 64:36, 85:15, and 100:0 of the C-20 triene–C-21 triene, respectively, with predicted release ratios (Heath et al., 1986) of 0:100, 6:94, 15:85, 22:78, 33:67, 66:34, 81:19, 90:10, and 100:0 of C-20 triene–C-21 triene. Release ratios were confirmed by analysis of volatiles collected from septa using the system described previously (Mitchell and Heath, 1986).

At each dosage, 40 male VBC were tested as four sets of 10 moths flown one at a time to each ratio. Males were scored for attraction to within 5 cm of the source and for reorientation to the source. All bioassay conditions were as described above.

Field Test of Synthetic Pheromone. The attractiveness of a range of ratios of the female sex pheromone and male pheromone component was evaluated in the field in a trapping test at 200-μg and 1-mg dosages on rubber septa. Screen cone traps (Hartstack et al., 1979) were mounted on steel poles (with the septa

positioned at the base of the cone) just above the vegetation. The test was conducted in a soybean field near Alachua, Alachua County, Florida, during September 1984. Two sets of nine traps were placed in rows perpendicular to the prevailing wind (north to south), with 10 m between traps and 90 m between rows of traps. The traps were checked daily and captured moths were identified to species, sexed, and counted. The positions of the traps were randomized daily. Daily trap catch data were converted to ranks by treatment for analysis of variance. Treatment rank means were separated using Duncan's new multiple-range test at the $P = 0.05$ level (Duncan, 1955).

RESULTS

Extracts of male abdominal tips were attractive to other males in the flight-tunnel bioassay at all dosages tested. At 0.2 ME, 36.7% of males tested responded (oriented flight upwind to the filter paper), 44.6% to the 1.0 ME samples, and 63.8% to the 5.0 ME samples. No males responded to the hexane blanks, and 94% of those tested responded to the 2 FE female abdominal tip extract standard. All of the activity from the tip extracts was recovered in the hexane fraction of the gravity-flow silica column. Male response was not increased by the addition of fractions obtained by further elution of the column using 10% and 50% ether–hexane and the 100% ether as the solvent. The active hexane fraction from the gravity-flow column was further purified by GC using the packed OV-101 column. All the activity was obtained in one peak that had a Kovats index of 2071. Further analysis of this peak on the 30-m polar Carbowax and 50-m apolar methyl silicone capillary columns resulted in a single peak.

Mass spectra obtained using electron impact and methane chemical ionization of the active peak from the packed column were identical to that obtained for the (*Z*,*Z*,*Z*)-3,6,9-heneicosatriene reported previously as one of the pheromone components obtained from female VBC (Heath et al., 1983). Further analysis on a cholesterol para-chlorocinnamate column capable of resolving all eight possible isomers confirmed that the geometry was *Z*,*Z*,*Z*.

Using GC analysis on both polar and apolar capillary columns, it was determined that 4- to 7-day-old males contained an average of 5.9 ng/male (SD = 5.09, $N = 24$) of the (*Z*,*Z*,*Z*)-3,6,9-heneicosatriene.

Bioassays of Synthetic Pheromone. At all dosages tested, C-21 triene was attractive to male VBC when tested alone, and at most ratios with C-20 triene (Figure 1). The greatest percentages were observed to load ratios which were

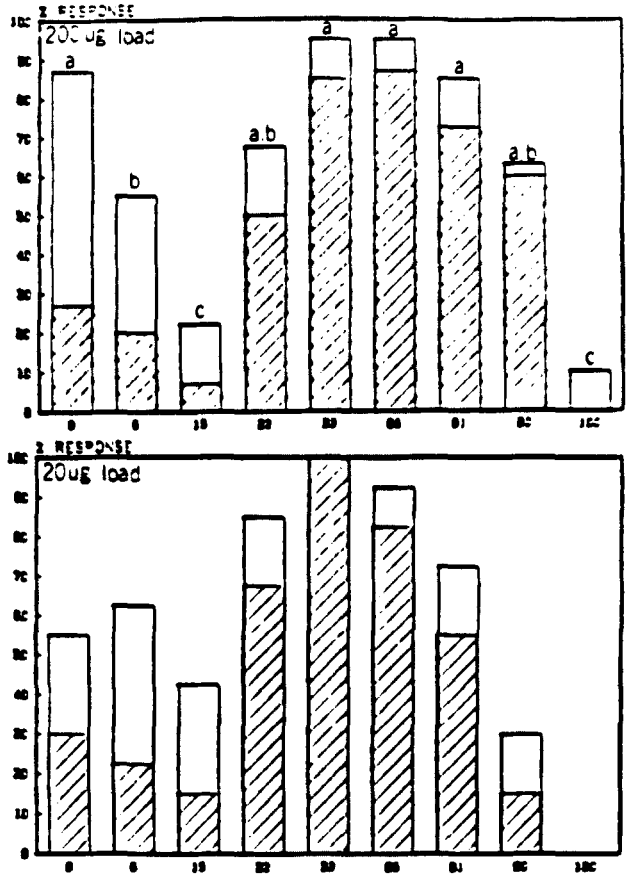

FIG. 1 Mean percentages of male *A. gemmatalis* attracted (open bar) and then reoriented (slash bar) to various release ratios of (Z,Z,Z)-3,6,9-eicosatriene and (Z,Z,Z)-3,6,9-heneicosatriene in a flight tunnel bioassay at 1-, 2-, 20-, and 200-μg loads on rubber septa. At the 200-μg load, columns having the same letter are not significantly different (Duncan, 1955).

similar to the 40:60 ratio (equivalent to a 60:40 release ratio) found in female VBC abdominal tips (Heath et al., 1983). At higher release rates, an unexpected bimodal distribution of male response was observed with minima at the 15:85% ratio of C-20–C-21 triene hydrocarbons and 100% C-20 triene hydrocarbon. At the 200-μg load, the mean percentages of males attracted to the source for the

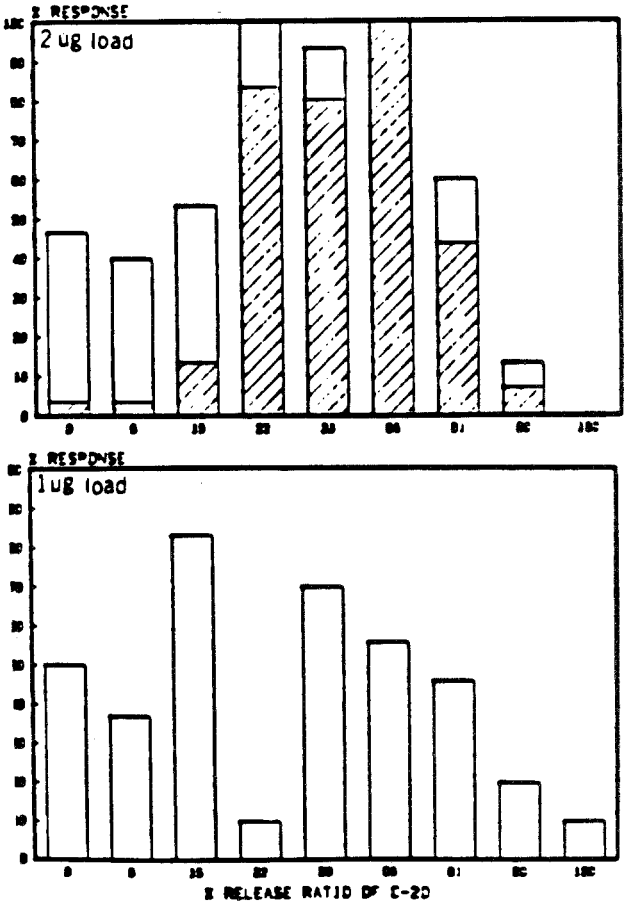

FIG. 1. Continued.

100% C-21 triene septa and the septa that released the 22, 33, 66, 81, and 90% ratios of the C-20–C-21 trienes were not significantly different.

Field Tests. At the 200-μg dose, a unimodal distribution of male response with a maximum at the female pheromone ratio (66% C-20 and 34% C-21 triene) occurred (Figure 2). However, at the 1-mg dose, a pattern similar to that observed in the wind tunnel using the 200-μg loaded septa was obtained in the field (Figure 2). That is, males were captured in a bimodal distribution, where the C-21 triene alone attracted significantly more males than septa releasing the 6:94 ratio of C-20–C21 triene.

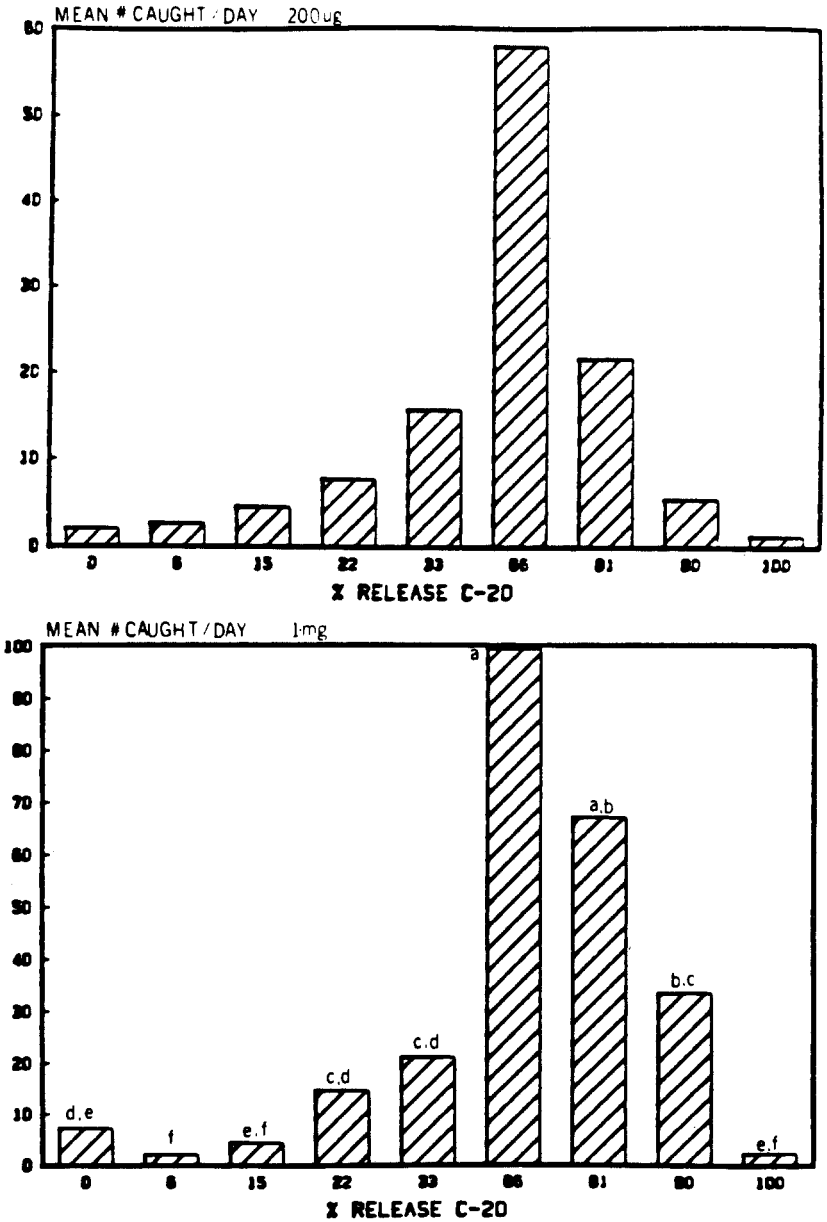

FIG. 2. Mean percentage of total trap catches of male *A. gemmatalis* in cone traps baited with ratios of (Z,Z,Z)-3,6,9-eicosatriene and (Z,Z,Z)-3,6,9-heneicosatriene at 200-μg and 1-mg loads on rubber septa. At the 1-mg load, columns having the same letter are not significantly different (Duncan, 1955).

DISCUSSION

The results of these studies demonstrate the presence of (Z,Z,Z)-3,6,9-heneicosatriene in abdominal tips of male VBC. This compound also is found as one of the sex pheromone components produced by female VBC. Male response to the male component alone and in combination with the C-20 triene produced by females resulted in a bimodal distribution.

It is doubtful that males release pheromone specifically as an attractant signal to conspecific males. According to Leppla et al. (1987), male VBC display the abdominal hairpencils and abdominal scent brushes when very near the female, as they approach, and during courtship interactions preceding mating. This suggests a courtship function for the male pheromone, directed at a calling female, possibly as an aphrodisiac. Male attraction to this signal possibly then is a secondary phenomenon resulting from male–male competition for females. Baker (1983) found that the mating success of courting *G. molesta* was reduced greatly by late-arriving competitors, which succeeded in mating 21% of the time. Behavior exhibited by late-arrivals was attraction to the first-arrivals' display, which caused the first male to misdirect a copulatory thrust, thereby disrupting normal courtship (Baker, 1983). Male VBC may be similarly able to interrupt courtship and successfully mate if they arrive before coupling. In other words, as in *G. molesta*, VBC males close by may be attracted to other courtship signals because they can sometimes interfere with an ongoing male–female courtship and gain opportunities for mating with that female.

The results from the field experiments supported in part the observations of the laboratory experiments. At the 200-μg load, no significant response (trap catch) was obtained with the male VBC pheromone. However, when septa were loaded with 1-mg amounts of pheromone, the trap catch pattern for the ratios of C-20 and C-21 triene tested resulted in a bimodal distribution similar to that observed in the wind-tunnel experiments: The male pheromone, a blend distinct from the female pheromone, was significantly more attractive than were other ratios differing from the female blend. One possible explanation of the trap catch data at the higher dose is that males release their component only during courtship and over a very short time period, perhaps at a relatively high release rate. Thus, only males in close proximity to males releasing the C-21 triene may be responsive to this male chemical cue.

We have presented in this study the identification of a compound produced by male VBC, which is also found as one of the components of the sex pheromone of female VBC. Additionally, we have demonstrated this male compound is attractive to other males in wind-tunnel and in field tests. We suspect that the primary function of this compound is as a courtship pheromone to evoke female acceptance, although this remains to be demonstrated.

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