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# ATTRACTION OF *Heliothis virescens* (F.) (LEPIDOPTERA: NOCTUIDAE) TO VOLATILES FROM EXTRACTS OF COTTON FLOWERS

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**Abstract**—Mated and virgin female and virgin male tobacco budworm moths, *Heliothis virescens* (F.), responded via directed upwind flight in wind-tunnel bioassays to volatiles from floral extracts of cotton, *Gossypium hirsutum* L. Significantly more male and female moths landed on cloth dispensers treated with a methylene chloride extract of debracted flowers than on control dispensers treated only with methylene chloride. Only mated females landed in significant numbers on the dispensers treated with extract of cotton squares, flowers (including bracts), or flower petals. After landing, most of the moths examined the dispensers by probing with their antennae, proboscis, and/or ovipositor.

**Key Words**—Tobacco budworm, *Heliothis virescens*, Lepidoptera, Noctuidae, cotton, *gossypium hirsutum*, oviposition, attractant, floral extracts.

## INTRODUCTION

Phytophagous insects often use volatile chemicals emitted from plants to locate suitable oviposition sites and food sources for themselves or their progeny. In studies on the behavioral effects of host and nonhost allelochemicals on *Heliothis* (Lepidoptera: Noctuidae) , we have observed attraction and oviposition stimulation of *H. virescens* (F.) and *H. subflexa* (Guenée) moths by host-plant extracts (Mitchell and Heath, 1987; Mitchell et al., 1990; Tingle et al., 1989, 1990). Volatiles from extracts of cotton leaves and squares (flower buds) and leaves

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from tobacco and *Desmodium tortuosum* (Swartz) de Candolle (a wild host) induced mated *H. virescens* females to exhibit a full array of behaviors (upwind flight, landing, surface examination with antennae, and oviposition on treated substrates) expected of gravid individuals seeking sites to oviposit. Virgin female and male moths were significantly less responsive to the attractant than were mated females. Mitchell et al. (1991) presented data suggesting that cotton squares have a greater concentration of flight- and orientation-stimulating compounds than do the leaves.

Wiesenborn and Baker (1990) found in wind tunnel bioassays that pink bollworm moths, *Pectinophora gossypiella* (Saunders), were attracted to a hexane extract of cotton flowers. They proposed that the flower volatiles act as a food lure, attracting moths to extra floral nectaries. Similarly, Haynes et al. (1991) demonstrated in wind-tunnel assays that olfactory stimuli played an important role in the cabbage looper's, *Trichoplusia ni* (Hübner), ability to locate nectar on honeysuckle flowers, *Abelia grandifora* (Andre). The study reported here describes the behavioral responses exhibited by *H. virescens* females (virgin and mated) and virgin males in wind-tunnel assays when exposed to extract from the flowering parts of cotton, *Gossypium hirsutum* L.

## METHODS AND MATERIALS

A Plexiglas wind tunnel (0.6 × 0.6 × 1.95 m) was used to observe flight and other behavioral responses by *H. virescens* to crude extracts of cotton squares (flower buds), whole blooms, blooms with bracts removed, and petals (including pistils and stamens) from blooms. The extracts were prepared from field-grown cotton (variety McNair 220) by washing ca. 400 g of fresh plant material for 30 sec in 1 liter of solvent (methylene chloride), which was filtered and stored in glass containers at 0°C until used (Mitchell and Heath, 1987). Before testing, the extracts were concentrated in a rotary evaporator to 2-g equivalents (2 gE) per 200 $\mu$l methylene chloride. Mitchell et al. (1991) determined previously, that 2 gE of cotton square extract attracted over 50% of the mated female *H. virescens* tested in wind-tunnel bioassays.

Test insects were reared in our laboratory on a modified pinto bean diet using the methods described by Guy et al. (1985) and Mitchell et al. (1988). Pupae were held in 5.5-liter plastic cages with screened tops until emergence. Upon eclosion, virgin females and males were held separately or combined for mating (21 females and 14 males/cage). The moths were fed a 10% honey-water solution and held under a reversed 14:10-hr light–dark cycle in a holding room maintained at 26 ± 1°C and 65 ± 5% relative humidity for two to four days prior to testing.

On the day of testing, the sex of each moth was verified by gently squeezing the abdomen to extrude the genitalia. Mated and virgin females and virgin males were confined separately in three 25 × 25 × 25-cm Plexiglas holding cages and placed in the flight tunnel room (3.0 × 2.6 × 2.1 m) ca. 1 hr before scotophase. An intake vent in the wall allowed a continuous flow of fresh unfiltered air from the outdoors into the room, which was maintained at the same environmental conditions as the holding room. An electric timer was used to control overhead fluorescent lights (two banks of two 40-W bulbs). The flight tunnel was the same as described by Tingle et al. (1989). Three incandescent 25-W red light bulbs equally spaced above the tunnel remained on continuously. The light level during scotophase was 2.4 lux inside the tunnel. Air was pulled through the tunnel at ca. 0.4 m/sec, when measured at the center of the tunnel, and exhausted ca. 3 m above the roof covering the flight-tunnel room via a 30-cm-diam. flexible pipe equipped with a fan.

Crude extract (2-gE) from either cotton squares, whole blooms, debracted blooms, or petals from blooms was applied to white cotton muslin cloth that was secured over the end of a glass cylinder dispenser (3.5-cm opening) with a rubber band. On a second cloth-covered dispenser, 200 $\mu$l of methylene chloride was applied as a control. After waiting 5 min for the solvent to evaporate, the two dispensers were placed 30-cm apart at the upwind end of the tunnel. Treated and control dispensers were rotated between positions after each replication. Air was blown through each cylinder with an aquarium pump at the rate of 1 liter/min to provide a continuous flow (plume) of the extract odor through the tunnel. The location and form of the plumes were verified by introducing smoke into the dispenser system and observing the smoke trails. The plume from each dispenser remained separate until merging ca. 65 cm from the opposite (downwind) end of the tunnel.

Each moth was removed from the holding cage and placed into a cylindrical 4.0 × 6.5-cm plastic release cage with screened ends. After placement of the release cage into the downwind end of the flight tunnel, the moth was released immediately and observed with the aid of a low-light video camera for 3 min. Behavioral responses, including random and oriented flight, and contacts, landings, and oviposition on extract-treated and control substrates were recorded. Five replicates each of 10 mated females, virgin females, or virgin males were tested individually for responses to each of the four extracts. The data were converted to arcsin $\sqrt{\text{percentage}}$ and analyzed using either the paired or unpaired *t*- test (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

Volatiles from extracts of whole (intact) cotton flowers, flowers without bracts, and the extract of petals, pistils, and stamens from flowers attracted significantly more (paired *t*-test) mated and virgin female and male *H. virescens* to extract-treated substrates than did the corresponding controls (Figure 1, top). The extract from cotton squares attracted significantly more virgin males and mated females (but not virgin females) than did the control. When response to each extract, as demonstrated by upwind flight toward the treated substrate, was compared with the response to each of the other extracts (unpaired *t*-test), the highest significant response was recorded for mated and virgin females to volatiles emitted from the extract of debracted flowers (Table 1). There were no significant differences among the extracts attracting virgin males toward the

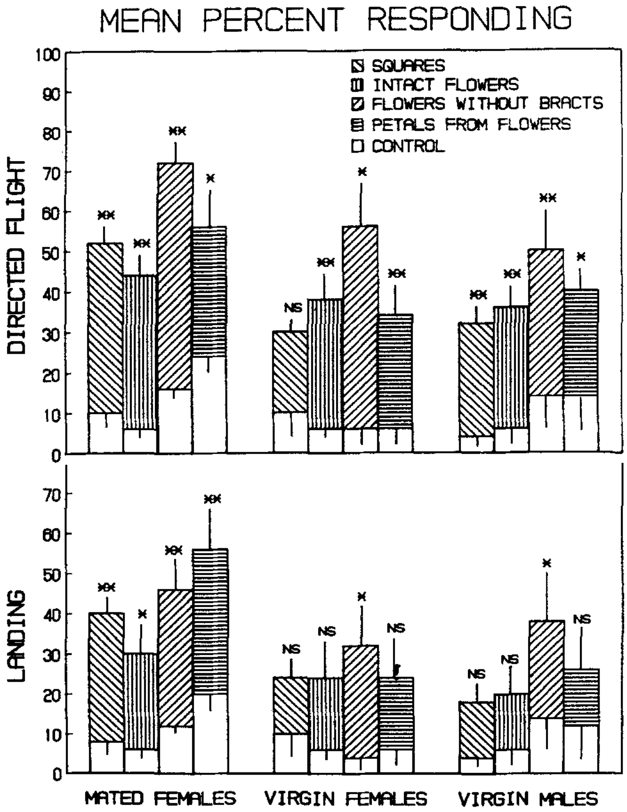

FIG. 1. Response of *Heliothis virescens* moths exposed in a wind tunnel to extracts from cotton. Mean percentages indicate positive anemotactic response by directed flight toward the odor source (top) or landing on extract-treated substrates (bottom). Thin lines above or below the tops of bars indicate standard error of the mean. Significance determined by Student's paired *t*-test from percentage of moths flying to or landing on treated and untreated (control) substrates in choice tests: NS = nonsignificant at $P < 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$.

TABLE 1. COMPARISON BETWEEN COTTON EXTRACTS FOR ATTRACTING *Heliothis virescens* IN A WIND TUNNEL

| | | Treatment[b] | | | |
|---|---|---|---|---|---|
| Treatment | Flight response (% ± SE)[a] | Square | Whole flower | Debracted flower | Petals only |
| Mated females | | | | | |
| Square | 53 (3.7) | | | | |
| Whole flower | 44 (5.1) | 1.269NS | | | |
| Debracted flower | 72 (4.9) | 3.039* | 3.738** | | |
| Petals only | 56 (9.3) | 0.453NS | 1.143NS | 1.506NS | |
| Virgin females | | | | | |
| Square | 30 (3.2) | | | | |
| Whole flower | 38 (6.3) | 1.054NS | | | |
| Debracted flower | 56(10.3) | 2.409* | 1.494NS | | |
| Petals only | 34 (7.5) | 0.440NS | 0.490NS | 1.763NS | |
| Males | | | | | |
| Square | 32 (3.7) | | | | |
| Whole flower | 36 (5.1) | 0.601NS | | | |
| Debracted flower | 50 (9.5) | 1.756NS | 1.054NS | | |
| Petals only | 40 (4.5) | 1.370NS | 0.606NS | 0.954NS | |

[a] Five replicates per treatment (10 moths tested individually per replicate).

[b] Response to each extract compared with response to each of the other extracts; $t$ values determined by unpaired $t$ test (Steel and Torrie, 1960): NS = nonsignificant, $P < 0.05$; * = significant, $P < 0.05$; ** = significant, $P < 0.01$.

treated substrates. The flight sequence was similar to that previously described by Tingle et al. (1989, 1990) and Mitchell et al. (1991). Most of the moths exited the release cage and began random flight in an upwind direction. Moths that detected the odor plume from the extract flew upwind in a zigzag pattern toward the dispenser. A few moths flew directly to the dispenser after exiting the release cage and landed on the cloth substrate.

Significantly more mated females landed on dispensers treated with each of the extracts (paired $t$-test) than on control dispensers (Figure 1, bottom). Virgin females and males landed in significant numbers only on the dispensers treated with the extract of debracted flowers. Most of the moths that landed examined the cloth substrates by probing with their antennae, proboscis, and/or ovipositor. Where 20% of the landings by mated females resulted in egg deposition on the treated substrates, only 4% of the landings by virgin females resulted in egg laying. There were no apparent differences in the effect of the various extracts on oviposition. Because it is unclear whether the chemicals that induced the moths to respond by anemotaxis in the flight tunnel are the same compounds that stimulated oviposition, the oviposition olfactometer developed by Mitchell and Heath (1987) is a better system than the flight tunnel for evaluating oviposition behavior per se.

During previous tests, we had not observed probing of substrates with the proboscis (Tingle et al., 1989, 1990; Mitchell et al., 1991; Tingle and Mitchell, 1991). However, by using a low-light video camera to record behavioral responses to the cotton extracts, we were able to observe extension of the proboscis by males and females (virgin and mated) in this study. Over 50% of the landings on treated substrates by mated females resulted in proboscis extension, which did not vary significantly among the various substrates ($P < 0.05$, unpaired $t$ test). All of the virgin females and males that landed on substrates treated with the extract from whole flowers exhibited proboscis extension, but significantly fewer ($P < 0.05$, unpaired $t$ test) of the moths (47 and 66%, respectively) that landed on the other extract-treated substrates showed this response. Proboscis extension was not surprising because Lepidoptera have contact chemoreceptors located on the proboscis, as well as on the antennae, tarsi, and ovipositor. In a review of contact chemoreception, Stadler (1984) stated that after phytophagous insects have located a potential host plant using olfactory or visual cues, contact with the plant surface appears to be essential before feeding or oviposition is initiated.

Information received via probing with the proboscis may be assessed in conjunction with that from the tarsi, as suggested by Blaney and Simmonds (1988). Their work indicated that feeding behavior by *H. virescens*, *H. armigera* (Hübner), and *Spodoptera littoralis* (Boisdval) adults was correlated with the electrophysiological response of sensilla on the proboscis. They reported that responses of the sensilla to sugar and amino acids were significantly correlated to feeding behavior in each of these species. Additionally, Stadler and Seabrook (1975) determined that sensilla on the proboscis of the eastern spruce budworm, *Choristoneura fumiferana* Chem., were sensitive to sugars, which are involved in feeding behavior, but not to host-specific chemicals that promote oviposition.

We have not yet determined the chemical composition of the cotton extracts that stimulated host-finding, oviposition, and feeding-like responses noted here for *H. virescens*. We also do not know if the same chemical components are present in leaves, squares, and flowers, or if all the components exist in varying ratios throughout the plant. Identification of the chemicals and blends thereof that attract tobacco budworm moths and that stimulate them to feed or oviposit may permit development of systems to regulate populations of this important insect pest.

Plant-produced semiochemicals provide a variety of opportunities to develop manipulative schemes for controlling insect pests on crops. Such schemes may involve: (1) the elimination or reduction of attractants or oviposition stimulants from otherwise desirable cultivars via plant breeding; (2) the development of baits using attractive substances from plants alone; or (3) the development of baits containing plant-attractive substances in combination with sex pheromones. Such baits could be used to detect or monitor pest insects or for direct control of pest populations using attractive baits containing a toxicant.

The vast majority of attractant chemicals identified have been sex-specific pheromones, primarily produced by females and attractive to males (Mayer and McLaughlin, 1991). Therefore, the use of sex attractant pheromones as predictive tools to forecast pest numbers or potential damage levels in specific crops has been significantly limited because attractants are usually directed at only one sex. Thus, identification of plant constituents that attract *H. virescens* adults, especially females, and that directly affect their feeding or reproductive behavior would greatly expand opportunities for manipulation of the biological and environmental events that control the establishment, development, and dispersal of this important crop pest.

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