

Upwind Flight to Cotton Flowers by *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)

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Environ. Entomol. 19(3): 490-493 (1990)

ABSTRACT Wind tunnel bioassays were performed to examine upwind flight by pink bollworm moths, *Pectinophora gossypiella* (Saunders), to different parts of the cotton plant (*Gossypium hirsutum* L.) More moths approached and landed on flowers than on flower buds, bolls, or leaves. Mated female moths were more readily attracted to flowers than were unmated female or male moths. Attraction of moths to flowers was virtually eliminated by washing flowers in pentane, suggesting that the attraction was mediated by olfactory cues. Moths also responded to a hexane extract of flowers and to flowers without petals. We propose that flower volatiles act as a food lure attracting moths to extrafloral nectaries.

KEY WORDS Insecta, *Pectinophora gossypiella*, *Gossypium*, attraction

THE PINK BOLLWORM, *Pectinophora gossypiella* (Saunders), is a key pest of cotton (*Gossypium* spp.) in Arizona and southern California (University of California 1984). Most economic losses caused by this pest are caused by larvae feeding within bolls. The host range of *P. gossypiella* is restricted mainly to members of the Malvaceae, with cotton as its preferred host (Shiller et al. 1962). The origin of Old World cotton in the Indus Valley of Pakistan (Hutchinson et al. 1947) and the initial description of *P. gossypiella* in India (Noble 1969) also indicate a close association between these species.

The affinity of *P. gossypiella* for cotton suggests that behavioral cues may be used by this insect to locate host plants. One type of behavioral cue, an attractant, is a chemical that causes net movement by an insect toward the source (Baker 1985). Following this reasoning, Pomonis et al. (1980) analyzed volatiles produced by host and nonhost plants of *P. gossypiella* but were unable to identify a potential attractant. Further searching for an attractant would benefit from knowing this insect is indeed attracted to the cotton plant. Herein, we describe upwind flight by *P. gossypiella* to cotton flowers, the first evidence that such an attraction exists.

Materials and Methods

Adult *P. gossypiella* were taken from a laboratory colony established with larvae collected from cotton plants in the Coachella Valley, Calif. Larvae from the field have not been introduced into the colony since 1979. Larvae were reared on a shredded wheat germ diet similar to that of Adkisson et al. (1960). Adults to be bioassayed were fed distilled water and maintained at $26 \pm 1^\circ\text{C}$ and 16:8 (L:D)

photoperiod. Plant material used in the bioassays was cut daily from *Gossypium hirsutum* L. 'Acala SJ2' plants grown in a glasshouse.

Moths were examined for upwind flight in a wind tunnel (0.6 by 0.6, 1.8 m long) constructed of 3-mm Plexiglas bolted to an aluminum frame. A 30-cm extension of sheet metal at the downwind end included a door for placing moths into the odor plume. The tunnel was enclosed at the upwind end with fine-mesh Teflon screen and at the downwind end with plastic screen. A blower fan produced 0.4 m/s^{-1} wind velocity. Ducting centered at, but not sealed to, the downwind end of the tunnel evacuated air into a fume hood. Alternating bands of 10-cm-wide black and white paper perpendicular to the length of the tunnel were laid beneath the tunnel floor. White cardboard covering the opposite wall of the room increased contrast between the moths and their background to enable observing behavior at very low light intensity. A single incandescent lamp illuminated the tunnel from above.

For observation in the wind tunnel, groups of 2-5 moths were placed into cylindrical wire-screen cages (5 cm diameter, 8 cm long) covered with plastic Petri dish lids. Light intensity was reduced at the beginning of scotophase every 20 min to 32, 5, 1.3, and 0.6 lx, and moths then were kept at 0.6 lx until used. In each trial, 1-3 cages of moths were used sequentially.

Plant material was placed in the center of the wind tunnel between two 17- by 17-cm vertical pieces of wire mesh (3 wires/cm) that were parallel to the airflow and atop a 23-cm stand. Each cage of moths was opened 1 m downwind within the odor plume (as observed with TiCl_4), and moths were observed for 5 min. Moths that flew upwind

Table 1. Attraction of *P. gossypiella* females to cotton flowers, pentane-washed flowers, and a hexane extract of flowers

Treatment	n	% Ap- proaching ^a	% Landing ^b
Flowers	76	26	26
Pentane-washed flowers	77	2.7	1.3
Hexane extract of flowers	83	17	11
Pentane-washed flowers combined with hexane extract	83	15	12
Filter paper alone	70	0.0	0.0

^a Total $\chi^2 = 28.27$ ($P < 0.005$)^b Total $\chi^2 = 32.52$ ($P < 0.005$)

within the odor plume to within 10 cm of the wire mesh but did not land were scored as an approach. Moths that flew the final 10 cm within the odor plume and landed on the wire mesh or plant material were scored as both an approach and a landing. Each treatment occurred once during each testing period and the same number of times at each position in the testing order. Light intensity was kept constant, temperature was maintained at $\pm 0.5^\circ\text{C}$, and relative humidity was maintained at $\pm 5\%$ during each testing period. Across all experiments, light intensity was 0.3–0.6 lx, temperature was 23–30°C, and relative humidity was 50–75%.

In the first experiment, the relative attraction of different parts of the cotton plant to 2–4-d-old mated female moths was compared. Two leaves 7.8–10.5 cm long, four flower buds (squares) with <3 mm of the developing corolla extending beyond the calyx, four flowers, or four bolls 1.4–2.6 cm in diameter were placed within the wire mesh with peduncles pointing upwind. Flowers were laterally compressed but not damaged by the wire mesh.

We compared the attraction of male moths, unmated female moths, and mated female moths to cotton flowers in the second experiment. Moths were segregated by sex after emerging, and half of the females were caged with males. Moths were bioassayed when they were 2–3 d old. Female moths captured after each trial were dissected and examined for spermatophores to determine if they had mated. In addition, we determined if adult diet influenced the attraction of moths to flowers. After emergence, mated female moths were fed either water or 8% sucrose solution and were bioassayed when 2–4 d old for attraction to four flowers.

In the third experiment, we determined if attraction of moths to flowers depended upon olfactory cues. We bioassayed the attraction of 2–4-d-old mated female moths to five different treatments: (1) flowers, (2) pentane-washed flowers, (3) a hexane extract of flowers, (4) pentane-washed flowers combined with a hexane extract of flowers, and (5) filter paper. Four flowers or pentane-washed flowers were placed within the wire mesh. Pentane-washed flowers were agitated individually for 1 min in 50 ml of pentane, and air then was blown over them to ensure complete evaporation of sol-

vent. Washed flowers differed visually from fresh flowers only by slight discoloration. The hexane extract of flowers was prepared by individually washing cotton flowers for 1 min in the same 50 ml of hexane. Aliquots of the wash were evaporated under nitrogen from 10 ml to 1 ml, and 0.3–0.6 ml (equivalent to 7.5–10.4 washed flowers) was added to 5.5-cm filter paper. The filter paper was attached parallel with the airflow to the upwind end of the wire mesh.

Different parts of the cotton flower were compared for attraction of moths in the fourth experiment. Flowers were cut through the ovary into two sections. One section consisted of the corolla, stamens, and pistil, and the other section consisted of the calyx and bracts. These two sections, cut from four flowers, were presented in the wind tunnel to 2–4-d-old female moths.

The number of moths that approached, did not approach, landed, or did not land was summed within each treatment in each experiment. Treatments were compared with χ^2 tests and considered significantly different if $P \leq 0.05$.

Results

All *P. gossypiella* adults that approached untreated material from cotton plants also landed. More moths were attracted to flowers (24%, $n = 85$) than to squares (4.7%, $n = 85$) and bolls (5.9%, $n = 85$) ($\chi^2 = 24.2$, $P < 0.005$), whereas the attraction to squares and bolls was not different ($\chi^2 = 0.07$, $P > 0.75$). Moths ($n = 85$) were not observed landing on leaves.

More mated females (29%, $n = 110$) than unmated females (19%, $n = 110$) and males (19%, $n = 130$) flew upwind and landed on flowers ($\chi^2 = 4.29$, $P < 0.05$). Of the moths classified as unmated that were dissected, 3.6% ($n = 55$) contained a spermatophore. One or more spermatophores were found in 84% ($n = 67$) of the sampled moths classified as mated. Adult diet did not influence the response by mated females to flowers; 36% ($n = 133$) of moths fed water landed, and 39% ($n = 123$) of moths fed 8% sucrose solution landed ($\chi^2 = 0.23$, $P > 0.5$).

The response by moths to flowers was greatly reduced by washing flowers in pentane (Table 1), suggesting that olfactory cues were the dominant stimuli. Cotton flowers washed with pentane elicited fewer approaches ($\chi^2 = 17.5$, $P < 0.005$) and landings ($\chi^2 = 20.2$, $P < 0.005$) than did unwashed flowers. When the hexane extract of flowers was combined with washed flowers, the number of approaches was not different from that observed when unwashed flowers were bioassayed ($\chi^2 = 3.5$, $P > 0.05$), but the number of landings was fewer ($\chi^2 = 5.3$, $P < 0.025$). Washed flowers, when added to the extract, did not influence behavior; the extract alone and combined with washed flowers elicited the same number of approaches ($\chi^2 = 0.18$, $P > 0.5$) and landings ($\chi^2 = 0.06$, $P > 0.75$). The visual

stimuli of washed flowers, therefore, did not significantly contribute to the observed attraction. The hexane extract of flowers elicited more approaches ($\chi^2 = 13.0$, $P < 0.005$) and landings ($\chi^2 = 8.1$, $P < 0.005$) than did filter paper alone.

The two sections of the cotton flower did not differ in the number of landings by moths ($\chi^2 = 0.55$, $P > 0.25$). The corolla, stamens, and pistil induced 29% ($n = 75$) of the moths to fly upwind and land, and the calyx and bracts elicited 24% ($n = 75$) of the moths. The source of olfactory cues responsible for attracting moths to flowers does not appear to be localized. Response of moths to flowers without petals substantiates the relative unimportance of visual cues in attracting *P. gossypiella* to cotton flowers.

Discussion

The attraction of *P. gossypiella* adults to flowers is more likely a response to food sources than to oviposition sites. This conclusion is supported by the attraction of unmated female and male moths as well as mated female moths to flowers. In addition, few eggs are placed by *P. gossypiella* on flowers. Lukefahr (1957) found that leaves, terminals, and bolls received 69.5% of eggs, whereas squares and flowers received only 4.5%. *P. gossypiella* adults are known to feed on nectar in the field (Noble 1969), and we have observed them feeding from subbracteal nectaries after flying to cotton flowers in the wind tunnel. Cotton nectar provides a rich source of sugars (Butler et al. 1972), amino acids (Hanny & Elmore 1974), and lipids (Stone et al. 1985), and dependence of *P. gossypiella* on nectar is indicated by the reduced populations of this insect that occur on nectariless cotton (Wilson & Wilson 1976). Interestingly, extrafloral nectaries also are found on cotton leaves, although leaves were not attractive to adult *P. gossypiella* in the present study.

Although adult *P. gossypiella* benefits from flying to cotton flowers in that they locate food sources, the cotton plant apparently does not benefit. Cotton is self-pollinated or cross-pollinated by diurnal insects such as Hymenoptera, because the large, radially symmetrical corolla is open only once and only during the day (Free 1970, McGregor 1976, Fryxell 1979). Asynchronous with flowering, the nocturnal flight of *P. gossypiella* (Lukefahr 1957) hinders its pollination of cotton. Subbracteal and circumbacteal extrafloral nectaries located outside of the corolla (McGregor 1976) continue to offer nectar to nocturnal moths after the flower has closed.

Our observation that volatile compounds rather than visual cues attracted *P. gossypiella* to flowers is consistent with this insect's nocturnal behavior and the diurnal flowering of cotton. Although flowers that bloom during the night may present visual cues to nocturnal moths (Kevan & Baker 1983), diminished visual cues would be expected from cotton flowers during the night because of their

closed corollas. Production of volatiles by flowers has been described by Fahn (1979) as diffusion of lipophilic substances from epidermal and adjacent mesophyll cells through the cell walls and cuticle to the outside. The lipophilic nature of at least some of the volatiles attracting *P. gossypiella* to flowers was demonstrated by the loss of attraction when flowers were washed with a nonpolar solvent. Similarly, Salama et al. (1984) found that a petroleum ether (nonpolar) extract of cotton flowers attracted more *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) adults than did extracts using other solvents. The lipophilic compound phenylacetaldehyde has been shown to attract a wide variety of moths to bladder flower (Cantelo & Jacobson 1979), but it reportedly repels *P. gossypiella* (Flint et al. 1977). Rather than a single compound attracting *P. gossypiella* to cotton flowers, a blend of compounds may be involved as exemplified by the attraction of diabroticite beetles to *Cucurbita* flowers (Metcalf 1987). Fewer landings by moths in the current study on washed flowers combined with the hexane extract than on unwashed flowers may have been due to loss or alteration of chemical attractants during preparation of the extract.

Attraction to flowers and the subsequent feeding on nectar may not be completely independent of host finding and selection. Adjei-Mafo & Wilson (1983) found that, in choice tests, oviposition by *Heliothis punctigera* Wallengren (Lepidoptera: Noctuidae) on cotton plants was correlated with extrafloral nectar production and suggested that the correlation was because of differences in host attractiveness. Indeed, emission of volatiles by cotton plants is highest during squaring and flowering (Hedin 1976). Our observation that mated female moths were attracted more readily to flowers than were unmated female and male moths emphasizes the importance of determining the influence of flower kairomones on host selection by *P. gossypiella*.

Identification of the volatiles that attract *P. gossypiella* females to cotton flowers would provide an important tool for managing this pest insect. Monitoring traps employing these compounds could be used to estimate the density of female moths. Estimates of female density likely would correlate more closely with subsequent levels of crop damage than would estimates of male density currently provided by pheromone traps. Trapping female moths also could provide an alternative monitoring strategy when sex pheromone applied for mating disruption reduces the effectiveness of pheromone traps. The use of pheromone traps to monitor insecticide resistance (Haynes et al. 1987) could be extended to female moths. Formulation of an attracticide could enable a more efficient use of insecticides. The efficacy of an attractant that mimics flower odor may be limited, however, by competition with the cotton crop and lack of specificity for a single insect species. The elucidation and exploitation of the chemical attraction between *P.*

gossypiella and cotton flowers present opportunities for future research.

Acknowledgment

We are indebted to W. M. Dugger and R. L. Palmer, Department of Botany and Plant Sciences, University of California, Riverside, for providing plant material from their cotton plants. We also thank R. Vetter for maintaining the insect colony. Support from the Cotton Pest Control Board made this study possible.

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Received for publication 26 June 1989; accepted 28 August 1989.