

Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to hostplant volatiles change with age and mating status

Authors: Mechaber, W. L., Capaldo, C. T., and Hildebrand, J. G.

Source: Journal of Insect Science, 2(5) : 1-8

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.002.0501>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



Behavioral responses of adult female tobacco hornworms, *Manduca sexta*, to hostplant volatiles change with age and mating status

W.L. Mechauber¹, C.T. Capaldo^{1,2}, J. G. Hildebrand¹

¹ ARL Division of Neurobiology, University of Arizona, Tucson AZ 85721-0077, USA

² Present address: Department of Pharmacology, University of Virginia, Charlottesville VA 22908, USA
mechauber@neurobio.arizona.edu

Received 23 October 2001, Accepted 9 January 2002, Published 18 March, 2002

Abstract

We present evidence for two behaviors influenced by intact, vegetative plant odor — upwind flight and abdomen curling — in female *Manduca sexta* and demonstrate the influence of the age and mating status of the moths on these behaviors. We compared the behavioral responses of laboratory-reared *M. sexta* of discrete ages and physiological states (2,3, and 4 day old for virgin; 2 and 3 day old for mated) as individual moths flew upwind in a flight tunnel to a source of hostplant volatiles. We monitored odor-modulated flight and abdomen curling in the presence of volatiles released by potted hostplants. Mated 3 day old females exhibited the highest incidence of odor-modulated flight and abdomen curling. Similarly, as virgin moths aged, a greater percentage of the individuals displayed odor-modulated flight patterns and abdomen curling. In contrast, younger virgin moths exhibited high levels of abdomen curling only after contact with the plant.

Keywords: *Manduca sexta*; moths; flight; oviposition; hostplant volatiles

Introduction

For most species of insects, and for moths in particular, olfactory cues provide information about biologically relevant resources such as food, mates, and oviposition sites. Pheromone-modulated behaviors, especially the responses of male moths to the sex pheromones released by conspecific females, have been studied in depth and are increasingly well understood in terms of both chemical ecology and neuroethology. By contrast, the role of olfactory information in a female moth's search for oviposition sites is less well understood. Volatile chemicals emanating from fruit have been identified as attractants for female moths (Cossé, et al., 1994), as have volatiles emanating from flowers (Haynes, et al., 1991; Heath, et al., 1992) and the volatile ensemble released from potted hostplants (Landolt, 1989). It is not known, however, which compounds emitted by the vegetation of intact plants are necessary and sufficient to elicit upwind flight and oviposition.

This report presents the first findings from our systematic examination of the behavioral responses of female adult tobacco hornworm moths, *Manduca sexta*, to volatiles released by intact, actively growing hostplants. In general, *M. sexta* females require hostplants both as oviposition sites and as floral nectar sources. This study employed cultivated tomato plants, *Lycopersicon esculentum* L. (Solanaceae), hostplants that are readily accepted by *M. sexta* for oviposition (Madden and Chamberlin, 1938; Yamamoto and Fraenkel, 1960; Yamamoto and Jenkins, 1972) but that do not serve as nectar sources because tomato flowers do not produce nectar

(Schneck, 1928; Symon, 1976). Thus the *M. sexta* - tomato plant relationship is a useful model system for investigations of mechanisms underlying the interactions of an ovipositing female moth with potential oviposition sites.

Earlier studies of female *M. sexta* documented flight of the moths in field cages (Yamamoto et al., 1969) and in a wind tunnel in a study of the mechanics of upwind flight (Willis and Arbas, 1991). Yamamoto et al. (1969) suggested that arrival of *M. sexta* at a hostplant requires two levels of attractants: those that stimulate the moth to fly to the hostplant and those that stimulate the moth to oviposit. Experiments using steam distillates of tobacco and tomato plants demonstrated their stimulatory properties in bioassays in which female moths contacted the distillates (Tichenor and Seigler, 1980; Tichenor, et al. 1981).

In the work reported here, we examined the effect of volatiles released by intact, growing hostplants on two behaviors of female *M. sexta* moths: (1) odor-modulated, upwind flight in the wind tunnel, and (2) abdomen curling when the moths are close to the hostplant, but in most cases have been prevented from contacting it. Abdomen curling is a precursor to egg deposition in lepidopteran females and occurs when the last two abdominal segments are extruded to permit egg placement on the plant (Matsuda, 1976; Eaton, 1986). Although in nature female moths contact hostplants with tarsi prior to egg deposition, we limited our focus to the effects of plant-released volatiles on female moth behaviors. We found that responses to hostplant odor depend on both the age and the

mating status of the moths. Based on the results of these experiments, we suggest that these responses of female *M. sexta* moths to plant odor are altered by mating and intensified with increasing age.

Materials and Methods

Experimental Insects

Manduca sexta L. (Lepidoptera: Sphingidae) came from our rearing facility, which was established in 1985 with *M. sexta* obtained from the U.S. Department of Agriculture in Beltsville, MD. Larvae were reared on artificial diet (modified from that of Bell and Joachim, 1976) under long-day photoperiod regimen (17:7 L:D) at 25–26°C and 40–50% RH (Prescott et al., 1977; Tolbert et al., 1983). Pupae were taken approximately one week after the onset of larval wandering, segregated by sex, and held in clear plastic containers in a rearing room under reverse photoperiod conditions (14:10 L:D) and with a superimposed temperature cycle: 26°:24°C (L:D). Two days before adult eclosion, pupae were transferred to fiberglass-screen emergence cages (31 x 31 x 32 cm), still segregated by sex, and maintained under the same environmental conditions in a small laboratory room adjacent to the wind tunnel. Eclosion typically occurred 4–5 h before the onset of scotophase, as documented by time-lapse video recordings (data not shown). After emergence, moths were maintained in groups of approximately 10 of the same age and mating status. In most cases, moths had access to 10% sucrose solution and plain water. Because we never observed feeding from these sources, in some populations water and sugar-water were omitted. At no time prior to experimentation were moths exposed to plant odor. Moths were tested in behavioral bioassays 1.5–3 h after the onset of scotophase.

Wind Tunnel

Behavioral experiments were conducted in a wind tunnel constructed of Plexiglas® (L x W x H = 2.5 x 1 x 1 m). At the upwind end of the tunnel, the air was filtered through a pleated carbon-cloth filter (Activfilter, Lewcote Corp., Millbury, MA), and a series of baffles to create approximately laminar flow through the tunnel, and exhausted at the downwind end through a duct vented into a laboratory fume hood. The wind speed, as measured with a hot-wire anemometer (Solomat, Norwalk, CT), was 54 cm/s and was constant at the two ends of the tunnel in the absence of a plant. With the introduction of a potted plant at the upwind end, wind speed fluctuated between 30 cm/sec in front of the densest foliage to 48 cm/sec at the periphery of the plant vegetation. At the downwind end of the tunnel, the wind speed was 45 cm/s in line with the densest part of the foliage and 54 cm/s in line with the periphery of the vegetation. Experiments were conducted under low-light conditions, with incandescent red light bulbs (General Electric, Cleveland, OH) as the only source of lighting. Light levels were measured at 1.5 lumens/sq ft by a portable light meter positioned in the tunnel (Onset Computer Corp., Pocasset, MA). To provide visual stimuli for flying moths, white cloth bearing an approximately random pattern of large, red dots (10 cm in diameter and covering approximately 25% of the floor area) was positioned under the clear floor of the tunnel. The tunnel was cleaned with 70% ethanol after each day's experiments. To estimate the shape of an odor plume flowing downwind from a small potted plant, a

burning unscented incense stick was positioned at the upwind end of the tunnel at the site used for plants and other odor sources. To fragment the smoke plume so that it more closely resembled the complex plume coming off the vegetation of a plant, we positioned a stainless steel baffle (W x H = 16 x 19 cm, welded onto supports 19 cm high) 6.5 cm downwind from the burning incense.

Hostplants

Tomato plants, *Lycopersicon esculentum* Mill. (Solanaceae) var. Money Maker, were used as sources of volatiles for these studies. Plants were grown in a greenhouse from seeds in a 3:2:1 (by volume) mixture of autoclaved Sunshine All-Purpose potting mix (Bellevue, WA), vermiculite, and silica sand and fertilized with Osmocote® 14-14-14 (Scotts-Sierra Horticultural Products Co., Marysville, OH). Plants in individual 6-inch pots were housed within cages constructed of antiviral insect screen (Humert Intl., Earth City, MO) to eliminate the need for insecticide. Plants were transferred from the greenhouse to the fume hood in the wind-tunnel laboratory shortly before testing, and a plant was positioned in the tunnel near its upwind end immediately before testing. All plants used in the bioassays were young, vegetative tomato plants, with approximate stem heights of 25 cm.

Behavioral Bioassays

A standard procedure was used to handle moths in preparation for behavioral testing. A moth was transferred from the small rearing room to the adjacent wind-tunnel laboratory 1 h after the beginning of the scotophase, and allowed to settle to a resting position in a cylindrical wire cage (14 cm in diameter and 30.5 cm in height, constructed of 0.32-cm mesh galvanized-steel screen with 14-cm plastic petri-dish bottoms on each end).

Before a moth was introduced into the wind tunnel, the hostplant was covered with an overturned, clean glass chromatography tank, and volatiles were exhausted from the tunnel for 5–7 min before commencement of behavioral testing. Then the wire cage housing an individual moth was placed on a stainless-steel release platform (L x W = 17.5 x 15.5 cm) held on a clamp 20 cm (plant-foliage height) above the floor of the tunnel at the downwind end. One minute later, the glass cover was removed from the plant and the moth was transferred from the wire cage onto the release platform. An investigator wearing a vinyl glove gently nudged the moth's tarsi with a finger extended into the cage, so that the moth grasped the glove. The moth was then positioned on the release platform. In most cases (90%), the moths showed no external display of arousal associated with transfer. If the moths appeared to be aroused by the transfer (e.g., commencing wing fanning), they were removed from the wind tunnel and not used until they returned to their quiescent state. In such cases, we repeated the process of introducing the moth into a clean tunnel. From the time that moths were placed on the release platform, they were allowed up to 4 min to activate (fan their wings) and fly in the tunnel. A moth that did not do so within the 4 min period was then gently prodded by brushing the tip of its abdomen with a gloved finger. If the moth activated and flew in response to the prod, it was included in the data set; if not, the moth was removed from the tunnel and not counted. For all classes of moths tested, the response rates averaged 80%.

Following activation, a moth flew upwind, and in all experiments that tested its responses to close-range exposure to plant volatiles, the moth was captured in flight 20 cm downwind from the plant in a wire cage (constructed of 0.64 cm mesh galvanized steel). The captured moth was then held in the wire cage 20 cm downwind from the plant, on a platform at a height similar to that of the one holding the potted plant. The moth was exposed for 1 min to plant volatiles following capture, and then the presence or absence of abdomen curling (the posture necessary for oviposition) was noted, but not egg deposition itself. Between uses, all cages were washed with hot soapy water and rinsed thoroughly, and the “upwind screen cages” were rinsed with acetone as well. We tested the responses of 2, 3 and 4 day old virgin females, and 2 and 3 day old mated females to potted hostplants. All moths were observed during mating and once a female paired with a male, the mating pair was separated from the other individuals. The younger, mated females were mated on day 1 and tested on day 2, versus the older mated females that were mated on day 2 and tested on day 3.

Three day old females are routinely mated and fully ready to oviposit; therefore we tested only females that had mated on day 1 or day 2 after eclosion, testing them the day following mating in order to look for differences in response between mated moths of these two ages. We tested 4 day old virgin female moths as well, because responsiveness of female moths to plants was still increasing. In addition, we tested the responses of 1 and 2 day old virgin females to tarsal contact with the plant in order to verify that young virgin females could curl their abdomens upon contact-stimulation from hostplant.

Collection and Analysis of Data

Behavioral observations were recorded at the downwind end of the wind tunnel with a video camera (Sony security camera, model SSC-M354; equipped with a Javelin lens model JL 12575, Ademco, Torrence, CA), and at the upwind end and overhead by two video cameras (Model TC351A, Burle Inc., Ireland, equipped with the same model Javelin lens). At the conclusion of the experiments, the overhead flight tracks were examined for evidence of oriented flight, as determined both by flight pattern and position within the wind tunnel. Overhead flight tracks were digitized to 2-dimensional images starting with the frame of the videotape in which the moth commenced flight, and digitized through to the frame containing the moth’s landing or capture point prior to contacting the plant. Therefore the point at which the moth began flight, rather than the release platform, is labeled on the flight track figure. Flight tracks were digitized with PEAK Motus Motion Measurement System (1996-8; Peak Performance Technologies, Inc., Englewood, CO).

Included in this report are representative flight tracks of moths from the different physiological states tested, as well as categorical analysis of flight patterns by the age and mating status of the moths. Statistical analyses were conducted with StatXact 3 for Windows (CYTEL Software Corp., 1995, Cambridge, MA), and are calculated and reported as exact p-values. Using the null hypothesis of no differences in distribution of moth flight patterns based on age or mating status of the moths, we tested for significance with the Likelihood Ratio test. In addition, we analyzed the frequency of individual females displaying abdomen curling, with

a null hypothesis of no differences in percentages of moths of different age or mating status curling their abdomens.

Results

Background and Control Observations

Mated 2 and 3 day old females were obtained by permitting the moths to mate during the scotophase prior to flight testing. There was no apparent shift in calling cycles between 1 and 2 day old females, so that the time of mating was similar for females of each age group.

We first examined the behavioral responses of individual, mated and virgin, 3 day old females when they were introduced into the wind tunnel and exposed only to flowing, filtered air. Moths of both descriptions responded to clean air by flying in the tunnel with high, circular flight along its longitudinal axis. In addition, we observed the flight patterns of female moths of the same age and mating status when they were placed in the wind tunnel with either the metal baffle alone or a greenhouse pot, containing moistened soil and a plant stake but no plant, at the upwind end of the tunnel. Responses of the moths to either object at the upwind end were similar to those responses observed when the wind tunnel was empty; there was no evidence of directed flight. As another behavioral control, we also ran tests in which female moths were allowed to fly in an empty tunnel and then were captured in a clean cage 20 cm from the upwind end. None of these moths responded to capture by abdomen curling in the empty cage.

In order to test whether female moths might respond to plant-derived compounds adsorbed to the wire mesh of the cylindrical cages used to trap the moths in the wind tunnel, we tested responses of female moths to cages possibly coated with plant volatiles. Clean cages were placed 20 cm downwind from tomato plants in the wind tunnel and allowed to “accumulate” volatiles for 2 minutes. The plant was then removed from the tunnel, and a mated 3 day old female was then placed in the cage and observed for signs of abdomen curling. None of the 10 females tested in this way displayed any abdomen curling in response to the “contaminated” cage mesh.

Flight response to host volatiles

Under the bioassay conditions used in this study, moths did not respond behaviorally to visual cues provided by a glass-enclosed hostplant at the upwind end of the wind tunnel. Moths initiated flight only after the glass cover was removed from the plant (in some cases they flew only after prodding on the release platform — see Methods). Visual analysis of videotaped flights revealed three distinct types of flight patterns in response to a potted hostplant: (1) complete odor-modulated flight; (2) incomplete odor-modulated flight; and (3) non-odor-modulated flight. Complete odor-modulated flight occurred primarily at mid-altitude in the tunnel as the moth maintained upwind movement, and often conducted a series of counterturns as it headed toward the plant. Incomplete odor-modulated flights were those in which the moth appeared to fly in and out of the odor plume with counterturns but did not orient to the plant exclusively and did not arrive at it. In these flight patterns, segments of odor-modulated flight were interspersed with non-odor-modulated flight. Non-odor-modulated flight often occurred in two

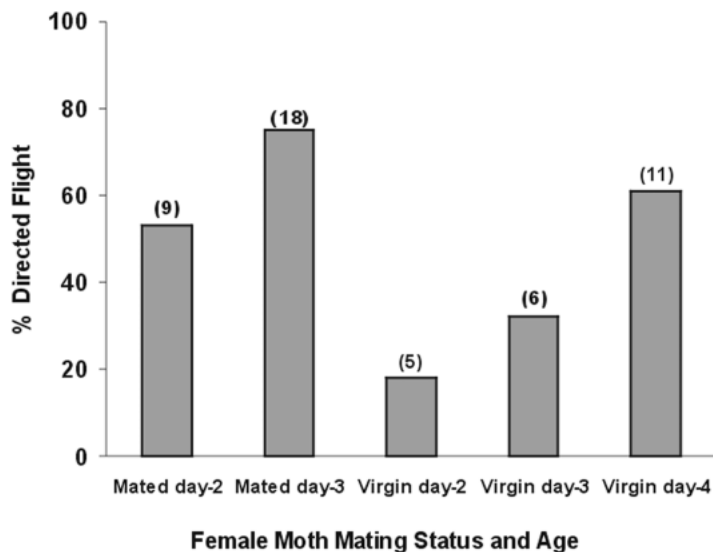


Figure 1. The percentage of female *Manduca sexta* that exhibited directed flight to the potted tomato plant as a function of the mating status and age of the moths. Numbers in parentheses indicate the number of individuals showing directed flight for that moth group.

ways: (1) at maximum altitudes in the tunnel primarily along its ceiling and often consisting of large circular flight paths with both upwind and downwind segments, and (2) along the edges of the tunnel, presumably not in contact with the odor plume.

Three day old mated females displayed the highest frequency (75%), and 2 day old virgin females the lowest frequency (18%), of complete odor-modulated flights (Fig.1). Fig. 2 shows the digitized 2-dimensional flight track of a female mated the day of eclosion and flown as a 2 day old female that engaged in non-odor-modulated flight upon first exposure to hostplant volatiles. This flight track contrasts with that shown in Fig. 3, in which another such female exhibited odor-modulated flight upon first exposure to hostplant volatiles. Fig. 4 shows the flight track of a 4 day old virgin female that responded with odor-modulated flight to hostplant volatiles.

Statistical analysis comparing the distribution of flight types by moth age and mating status (complete odor-modulated, directed flight vs. non-odor-modulated flight) revealed a significant difference

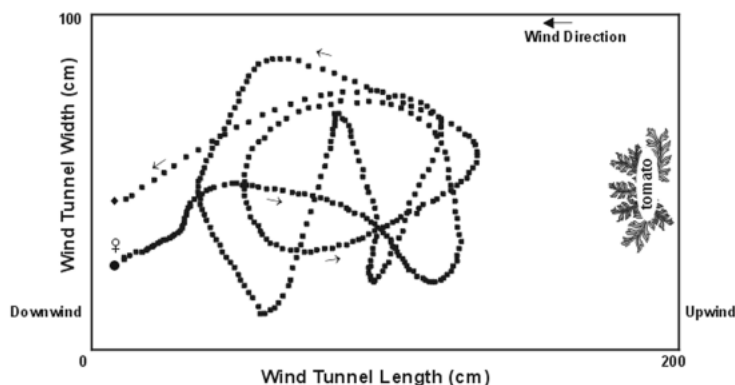


Figure 2. Digitized flight track of a 2 day old mated female *Manduca sexta* exposed to a potted tomato plant in the wind-tunnel bioassay. View is from the ceiling above the wind tunnel, looking down. Filled circle = start of digitized flight track; diamond = final landing spot; small arrow = direction of flight; large arrow = wind direction. This flight track is an example of non-directed flight by a mated female.

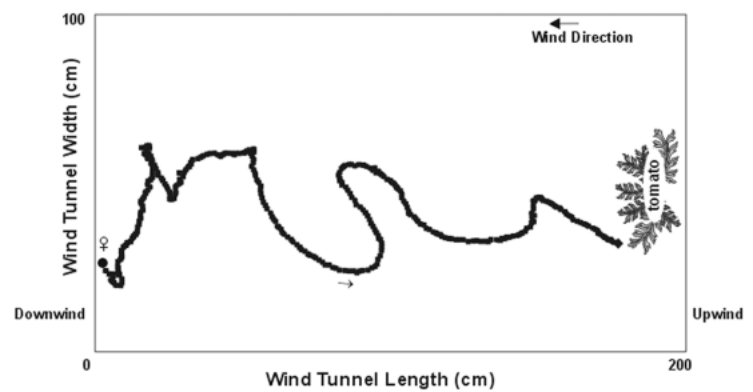


Figure 3. Digitized flight track of a 2 day old mated female *Manduca sexta* exposed to a potted tomato plant in the wind-tunnel bioassay. View is from the ceiling above the wind tunnel, looking down. Filled circle = start of digitized flight track; filled diamond = final landing spot; small arrow = direction of flight; large arrow = wind direction. This flight track is an example of directed flight by a mated female.

among the groups of moths (likelihood ratio test statistic = 21.0, exact p-value = 0.001). For moths of the same age, 2 day old mated females completed more directed flights (53%) to the plants than did 2 day old virgin females (18%) (likelihood ratio test statistic = 5.87, exact p-value = 0.02), as did 3 day old mated females when compared with 3 day old virgin females (likelihood ratio test statistic = 8.35, exact p-value = 0.03). Although a greater percentage of 3 day old mated females (75%) displayed complete, directed, odor-modulated flight than did 2 day old mated moths (53%), the difference was not statistically significant (likelihood ratio test statistic = 2.14, exact p-value = 0.19). For virgin female moths, however, there were significant differences in the response distributions across the three age classes tested (likelihood ratio test statistic = 10.6, exact p-value = 0.04), with increases in response occurring with increase in age.

In all bioassay groups, some individuals flew as if there were no hostplant at the upwind end of the tunnel, despite the presence of a potted hostplant. For mated females, this occurred in 12% of 2 day old and 8% of 3 day old females; for virgin females in 15% of 2 day old, 21% of 3 day old, and 17% of 4 day old females. A majority of individuals tested displayed one type of flight. A few

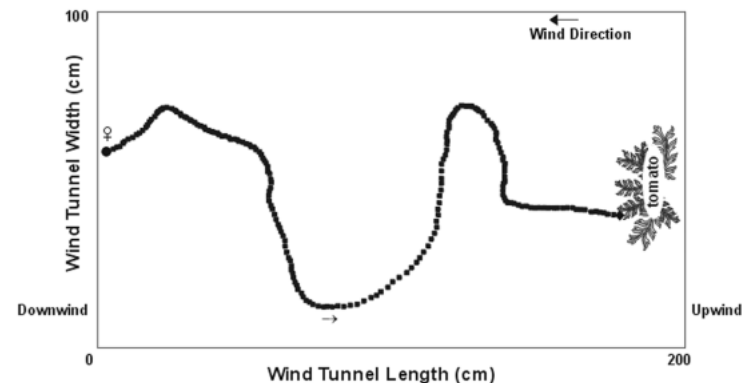


Figure 4. Digitized flight track of a 4 day old virgin female *Manduca sexta* offered a potted tomato plant in the wind-tunnel bioassay. View is from the ceiling above the wind tunnel, looking down. Filled circle = start of digitized flight track; filled diamond = final landing spot; small arrow = direction of flight; large arrow = wind direction. This flight track is an example of directed flight by an older, virgin female.

of the virgin moths, however, switched from odor-modulated, directed flight to non-directed flight, e.g., initially flying upwind in an odor-modulated pattern, and then changing to non-directed flight. This phenomenon was observed in 10% of 3 day old virgin, and 11% of 4 day old virgin females tested.

Abdomen Curling

Following close-range exposure to plant volatiles, mated females gave the highest percentages of abdomen curling (Fig. 5), with significantly more mated females than virgins of both age classes responding with abdomen curling (likelihood ratio test statistic = 5.55, exact p-value = 0.04). Females that had mated on day 1, and were tested on day 2 exhibited greater abdomen-curling responses than did 2 day old virgin moths (likelihood ratio test statistic = 11.2, exact p-value = 0.002). Females that had mated on day 2, and were tested on day 3 also showed greater abdomen-curling responses when compared with 3 day old virgin moths (likelihood ratio test statistic = 20.1, exact p-value < 0.0001).

Among mated females, a significantly greater percentage of moths mated on day 2 and tested on day 3 showed the abdomen-curling posture than did those mated on day 1 (likelihood ratio test statistic = 10.0, exact p-value = 0.01). The increase in abdomen curling by age was evident in virgin females as well. 81% of the 4 day old virgin females tested exhibited abdomen curling, as opposed to 0% of the day 1 old virgins (likelihood ratio test statistic = 41.4, exact p-value < 0.0001). When virgin females were allowed to contact the plant during their first exposure to plant volatiles, replicating the type of interaction that occurs for mated females in the field, abdomen curling rates increased significantly for 2 day old virgin females (likelihood ratio test statistic = 34.46, exact p-value < 0.0001), as well as by age when comparing 1 day old and 2 day old virgin females as shown in Fig. 6 (likelihood ratio test statistic = 20.7, exact p-value < 0.0001).

Discussion

Hostplant chemicals are primary stimuli for location of hostplants and selection of oviposition sites (Honda, 1995). We examined female moth behavioral responses to a potted hostplant

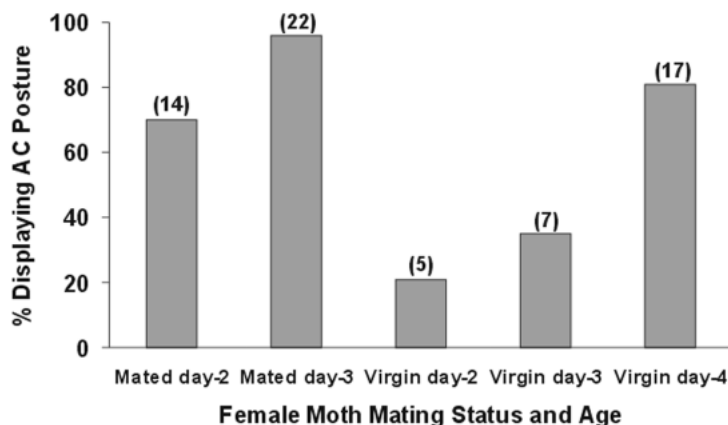


Figure 5. The percentage of female *Manduca sexta* that displayed abdomen curling (AC) when exposed to volatiles from a potted tomato plant, as a function of moth mating status and age. Numbers in parentheses indicate the number of individuals that exhibited AC for that moth group.

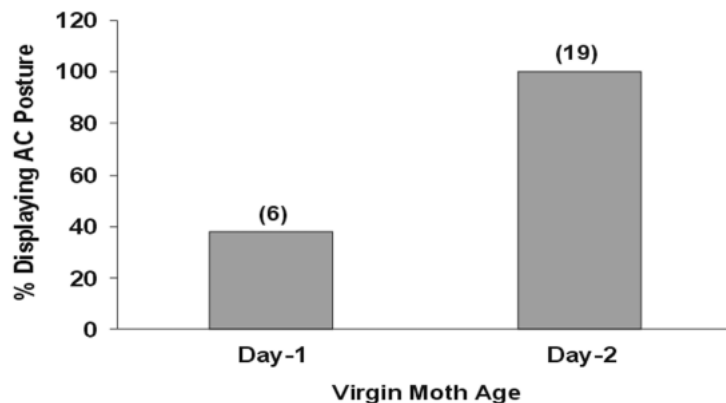


Figure 6. The percentage of virgin female *Manduca sexta* that displayed abdomen curling (AC) when allowed to contact potted tomato plant vegetation, as a function of moth age. Numbers in parentheses indicate the number of individuals that exhibited AC for that moth group.

in a laboratory model system in order to determine which components of behavior are truly mediated by olfaction. We found that volatiles that are released by an actively growing hostplant are important in eliciting upwind flight and the abdomen curling posture. We were able to demonstrate the role of plant volatiles by preventing female moths from contacting the plant throughout the bioassay.

By studying laboratory-reared moths in a wind tunnel, we could eliminate much of the variability inherent in field studies and manipulate moth age and mating status exclusively. These manipulations did not determine the extent to which individual sensory modalities are utilized by moths in the field, but rather demonstrated unequivocally that olfaction is a strong contributor to the behaviors necessary for successful oviposition. We were able to examine how moth age and mating status may contribute to short-range orientation flights to the hostplant and how the responses of female moths to plant volatiles change with mating status and/or age. Both upwind flight and the abdomen-curling response increased with the shift from virgin to mated status and also with the age of the moth.

Some of the moths caught in screen cages at the upwind end of the wind tunnel, near the hostplant, laid eggs on the wire mesh without having contacted the plant. We did not incorporate egg deposition as a parameter in this study, however, because in the field *M. sexta* lays eggs after tarsal contact with the plant and presumably seldom does so at a distance from a hostplant. In order to prevent learning or conditioning from contributing to the moth's behavioral responses, we tested female moths only on their first exposure to volatiles.

Upwind Flight and Reproductive Physiology

In *M. sexta*, eggs mature at an increased rate in mated females even when oviposition is prevented (Sasaki and Riddiford, 1984). The behavioral differences between mated moths tested as younger (day 2) or older (day 3) individuals, particularly for abdomen-curling, may be related to the total number of mature eggs present in the older females. The observed differences suggest that female physiology, rather than an effect of mating or the transfer of compounds from the male to the female during mating, is responsible for the altered behavior. As virgin females aged, they also exhibited increased odor-modulated flight and abdomen curling. In *M. sexta*,

maturation of eggs after eclosion depends on the presence of juvenile hormone (Sroka and Gilbert, 1971; Nijhout and Riddiford, 1974; Sasaki and Riddiford, 1984) and not mating (Nijhout and Riddiford, 1974). The first mature eggs are found in the ovaries 24 hours after eclosion (Nijhout and Riddiford, 1974). Virgin females of *M. sexta* lay only sterile eggs (Sasaki and Riddiford, 1984), and only *ca* 100 mature eggs are produced by four day old virgin females (Nijhout and Riddiford, 1974), the age corresponding to the oldest virgin females tested in our studies. Although the experiments of Nijhout and Riddiford (1974) were conducted with *M. sexta* from a different laboratory colony, those moths were also unfed and therefore similar in age and treatment to ours. Observations in other species of moths have revealed two additional female-specific behaviors that change with mating and aging: (1) calling, or release of sex pheromone, by female moths (Kingan et al., 1993; Del Socorro and Gregg, 1997), and (2) the pattern of egg deposition (Adler et al., 1991). In none of the virgin or mated females, however, did we observe an initiation of calling behavior upon exposure to hostplant volatiles in the wind tunnel, in agreement with observations from a different laboratory strain (Sasaki and Riddiford, 1984).

Attraction of virgin females to hostplant volatiles is a characteristic that varies by insect species. *Cydia pomonella* (Lepidoptera: Tortricidae) virgin females respond positively to hostplant volatiles (Hern and Dorn, 1999; Yan et al., 1999), as did *Trichoplusia ni* (Lepidoptera: Noctuidae) (Landolt, 1989). In other studies, attraction to host odors was noted only in mated individuals, e.g., *Pimpla ruficollis* (Hymenoptera: Ichneumonidae), a parasite of *Rhyacionia buoliana*, becomes attracted to the food-plant odors of the host only when ready to oviposit (Thorpe and Caudle, 1938). Similarly, in a study of upwind flight by female *Ameyeloides transitella* (Lepidoptera: Pyralidae), Phelan and Baker (1987) found that mated, but not virgin, females flew upwind in an odor plume of almond by-products in a wind tunnel. Rojas (1999) demonstrated that mated females of *Mamestra brassicae* (Lepidoptera: Noctuidae) flew upwind to allylisothiocyanate, as a hostplant extract, and landed at the odorant target, whereas virgin females did not.

Our findings of increasing responsiveness of *M. sexta* to host volatiles as moths increased in age parallels findings from a butterfly-plant system. Saxena and Goyal (1978) recorded behavioral responses by age of *Papilio demoleus* L. (Lepidoptera: Papilionidae, the citrus butterfly), to volatiles released by hostplant and non-hostplant cuttings and extracts. Orientation behavior, abdomen curling, and oviposition in response to volatiles alone increased with the increasing age of the butterflies. In a dipteran, Campan and Blanc (1970) demonstrated that the age of female *Calliphora vomitoria* determined their responsiveness to host odor (ammonia), with older females up to a particular age exhibiting greater attraction to host odor. Campan (1977) examined response to odor in combination with physiological state, and showed that female *C. vomitoria* that had been castrated upon emergence had low levels of responsiveness to host odor, even at an advanced age. Oviposition response to host volatiles alone appears to be species-specific. In mated female *Heliothis virescens* (Lepidoptera: Noctuidae), in the absence of tarsal contact, volatiles from host versus non-host are indistinguishable (Ramaswamy et al., 1987). In *Papilio polyxenes* (Lepidoptera: Papilionidae), hostplant volatiles increase landing rates, but not oviposition (Feeny et al., 1989);

whereas for *Eurytides marcellus* (Lepidoptera: Papilionidae) hostplant volatiles increase oviposition rates when butterflies are tested in the absence of contact stimulants (Haribal and Feeny, 1998). Barrer and Jay (1980), however, demonstrated that gravid *Ephestia cautella* (Lepidoptera: Phycitidae), a pest of stored grain, locate their host (kibbled wheat) and oviposit in response to volatiles alone.

Because of the differences in treatment of the many insect and plant variables among studies, it is difficult to make clear comparisons among studies examining female moth response to hostplant odor. Most notably, differences in the ages of moths and the sources of odors tested, e.g., steam-distilled or cut bouquets of vegetation rather than headspace volatiles from growing plants, prevent direct comparisons. The limitations in the present study result from our use of potted plants in the photophase of their light/dark cycle. These plants were grown in a greenhouse, and this prevented the reversal of the photoperiod regimen as we did with the moths. Nevertheless, we proceeded with greenhouse-grown plants because flight and abdomen curling occurred readily, and our preliminary studies of headspace volatiles sampled in the day and at night suggest that the differences may be quantitative rather than qualitative (Mechauber, in preparation).

Tarsal Contact and Oviposition

For many Lepidoptera, tarsal contact with an appropriate hostplant surface is the source of necessary and sufficient stimuli to initiate oviposition (as summarized by Renwick and Chew, 1994). Earlier studies of *M. sexta* evaluated the degree to which tarsal contact in the presence or absence of odor contributed to egg deposition (Sparks, 1973). The highest levels of egg deposition occurred in those bioassays in which the females were offered non-hostplant leaves that had been sprayed with hostplant extract. *M. sexta* females permitted free access to a hostplant contact the plant with their pro- and metathoracic foretarsi when laying eggs (Sparks, 1973).

In this study, we observed egg deposition by mated females on the metal screen cages upon close-range exposure to hostplant volatiles alone (approximately 10% of the moths tested). It is likely that the intensity of the oviposition response, reflected by the number of eggs laid, and the duration of an oviposition bout, is reduced in the absence of tarsal contact. Moreover, tarsal contact also appears to be a more effective stimulant in eliciting abdomen-curling responses from virgin females. Two day old virgin females that do not exhibit abdomen curling in the presence of plant volatiles alone, do so upon tarsal contact with the plant surface. It is possible that the increasing age of the female alters the dynamic relationship between the stimulus and the internal threshold for response.

In addition to chemosensory stimuli, visual cues often contribute to hostplant selection, particularly for oviposition site selection in day-flying insects (Prokopy and Owens, 1983). In our studies, by enclosing a hostplant under a glass cover at the start of the bioassay, we tested the effect on female moth behavior of seeing the hostplant without smelling it. Because female moths remained still in the presence of the visible plant, and did not begin activation (wing fanning) or flight until the glass cover had been removed from the hostplant, we suggest that for female *M. sexta* in a quiescent state, visual cues may not be important in eliciting activity. Nevertheless, vision may play a role in closer-range behavior (but see White et

al., 1994).

Our behavioral experiments also suggest that the distance between a female moth and a potted hostplant may be critical for the type of behavior that a female moth expresses. With tomato plants, we rarely observed female moths abdomen curling at the downwind end of the tunnel; instead, females initiated flight toward the plant, and abdomen curling began when the females were closer to the plant. Ongoing work seeks to determine the identities and relative abundances of the plant volatiles that elicit each of these discrete behaviors.

Acknowledgements

We thank Mark Willis for sharing his wind tunnel and motion-analysis system with us, Reg Chapman and Elizabeth Bernays for helpful discussions about the design of the bioassays, Chip Hedgcock for assistance with the figures and A.A. Osman for rearing *M. sexta*. This research was supported by the National Science Foundation (grant IBN 9604134), and CTC was supported by an REU supplement to that grant.

References

- Adler PH, Willey M B, Bowen MR. 1991. Temporal oviposition patterns of *Heliothis zea* and *Spodoptera ornithogalli*. *Entomologia Experimentalis et Applicata* 58: 159-164.
- Barrer PM, Jay E J. 1980. Laboratory observations on the ability of *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) to locate, and to oviposit in response to a source of grain odour. *Journal of Stored Products Research* 16: 1-7.
- Bell RA, Joachim FA. 1976. Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Annals of the Entomological Society of America* 69: 365-373.
- Campan M. 1977. Étude du comportement d'orientation des femelles de *Calliphora vomitoria* (Diptères) vers l'odeur du lieu de ponte. Mise en évidence du rôle de l'ovaire. *General and Comparative Endocrinology* 31: 442-450.
- Campan M, Blanc JM. 1970. Variations, en fonction de l'âge, de la réactivité des femelles de *Calliphora vomitoria*, à l'odeur de l'ammoniac. *Revue Comportement Animal* 4: 76-78.
- Cossé AA, Endris JJ, Millar JG, Baker TC. 1994. Identification of volatile compounds from fungus-infected date fruit that stimulate upwind flight in female *Ectomyelois ceratoniae*. *Entomologia Experimentalis et Applicata*. 72:233-238.
- Del Socorro AP, Gregg PC. 1997. Calling behavior in *M. convecta* females under different temperature and photoperiodic conditions. *Physiological Entomology* 22: 20-28.
- Eaton JL 1986. Morphology of abdominal segments eight and nine of the female tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae). *Annals of the Entomological Society of America* 79: 629- 635.
- Feeny P, Städler E, Åhman I, Carter M. 1989. Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). *Journal of Insect Behavior* 2: 803-827.
- Haribal M, Feeny P. 1998. Oviposition stimulant for the zebra swallowtail butterfly, *Eurytides marcellus*, from the foliage of pawpaw, *Asimina triloba*. *Chemoecology* 8:99-110.
- Haynes KF, Zhao JZ, Latif A 1991. Identification of floral compounds from *Abelia grandiflora* that stimulate upwind flight in cabbage looper moths. *Journal of Chemical Ecology* 17(3):637-646.
- Heath RR, Manukian A. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. *Journal of Chemical Ecology*.18(7): 1209-1226.
- Hern A, Dorn S. 1999. Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response. *Entomologia Experimentalis et Applicata*. 92: 63-72.
- Honda K. 1995. Chemical basis of differential oviposition by lepidopterous insects. *Archives of Insect Biochemistry and Physiology* 30: 1-23.
- Kingan TG, Thomas-Laemont PA, Raina AK. 1993. Male accessory gland factors elicit change from 'virgin' to 'mated' behavior in the female corn earworm moth *Helicoverpa zea*. *Journal of Experimental Biology* 183: 61-76.
- Landolt PJ. 1989. Attraction of the cabbage looper to host plants and host plant odor in the laboratory. *Entomologia Experimentalis et Applicata*. 53:117-124.
- Madden AH, Chamberlin FS. 1938. Biological studies of the tomato worm on tobacco in Florida, 1936 and 1937. *Journal of Economic Entomology* 31: 703-706.
- Matsuda R. 1976. Morphology and Evolution of the Insect Abdomen, Pergamon.
- Nijhout MM, Riddiford LM. 1974. The control of egg maturation by juvenile hormone in the tobacco hornworm moth, *Manduca sexta*. *Biological Bulletin* 146: 377-392.
- Phelan PL, Baker TC 1987. An attracticide for control of *Amyelois transitella* (Lepidoptera: Pyralidae) in almonds. *Journal of Economic Entomology* 80: 779-783.
- Prescott DJ, Hildebrand JG, Sanes JR, Jewett S. 1977. Biochemical and developmental studies of acetylcholine metabolism in the central nervous system of the moth, *Manduca sexta*. *Comparative Biochemistry and Physiology* 56C: 77-84.
- Prokopy RJ, Owens ED. 1983. Visual detection of plants by herbivorous insects. *Annual Review of Entomology* 28: 40-48.
- Ramaswamy SB, Ma WK, Baker G T. 1987. Sensory cues and receptors for oviposition by *Heliothis virescens*. *Entomologia Experimentalis et Applicata*. 43: 159-168.
- Renwick JAA, Chew FS. 1994. Oviposition behavior in Lepidoptera. *Annual Review of Entomology*. 39: 377-400.
- Rojas JC. 1999. Electrophysiological and behavioral responses of the cabbage moth to plant volatiles. *Journal of Chemical Ecology* 25: 1867-1883.
- Sasaki M, Riddiford LM. 1984. Regulation of reproductive behavior and egg maturation in the tobacco hawk moth, *Manduca sexta*. *Physiological Entomology* 9: 315-327.
- Saxena K N, Goyal S. 1978. Host-plant relations of the citrus butterfly *Papilio demoleus* L.: Orientational and ovipositional responses. *Entomologia Experimentalis et Applicata* 24: 1-10.
- Schneck HW. 1928. Pollination of greenhouse tomatoes. NY (Cornell) Agricultural Experiment Station Bulletin 470, 60 pp.
- Sparks MR. 1973. Physical and chemical stimuli affecting oviposition preference of *Manduca sexta* (Lepidoptera:

- Sphingidae). *Annals of the Entomological Society of America* 66: 571-573.
- Sroka P, Gilbert LI. 1971. Studies on the endocrine control of post-emergence ovarian maturation in *Manduca sexta*. *Journal of Insect Physiology* 17: 2409-2419.
- Symon DE. 1976. Sex forms in *Solanum* (Solanaceae) and the role of pollen collecting insects. In: Hawkes JG, Lester RN, Skelding, AG, editors. *The Biology and Taxonomy of the Solanaceae. Linnaean Society Symposium Series*, 7: 385-397. London: Academic Press.
- Thorpe WH, Caudle H B. 1938. A study of the olfactory responses of insect parasites to the food plant of their host. *Parasitology* 30: 523-528.
- Tichenor LH, Seigler DS. 1980. Electroantennogram and oviposition responses of *Manduca sexta* to volatile components of tobacco and tomato. *Journal of Insect Physiology* 26: 309-314.
- Tichenor LH, Seigler DS, Wei L. 1981. Oviposition responses of *Manduca sexta* to solanaceous volatile components. *Transactions of the Illinois State Academy of Science*. 74: 35-41.
- Tolbert LP, Matsumoto SG, Hildebrand JG. 1983. Development of synapses in the antennal lobes of the moth *Manduca sexta* during metamorphosis. *Journal of Neuroscience* 3: 1158-1175.
- White RH, Stevenson RD, Bennett RR, Cutler DE. 1994. Wavelength discrimination and the role of ultraviolet vision in the feeding behavior of hawkmoths. *Biotropica* 26(4):427-435.
- Willis MA, Arbas E A 1991. Odor-modulated upwind flight of the sphinx moth, *Manduca sexta* L. *Journal of Comparative Physiology A* 169: 427-440.
- Yamamoto RT, Fraenkel G. 1960. The physiological basis for the selection of plants for egg-laying in the tobacco hornworm, *Protoparce sexta* (Johan.). XI International Kongress of Entomology Wein 3: 127-133.
- Yamamoto RT, Jenkins RY, McClusky RK. 1969. Factors determining the selection of plants for oviposition by the tobacco hornworm *Manduca sexta*. *Entomologia Experimentalis et Applicata* 12: 504-508.
- Yamamoto RT, Jenkins RY 1972. Hostplant preferences of tobacco hornworm moths. In: Rodriguez JG, editor. *Insect and Mite Nutrition*, 567-574. Amsterdam: North Holland.
- Yan F, Bengtsson M, Witzgall P. 1999. Behavioral response of female codling moths, *Cydia pomonella*, to apple volatiles. *Journal of Chemical Ecology* 25: 1343-1351.