CHEMICAL ECOSYSTEM

Pepper Weevil Attraction to Volatiles from Host and Nonhost Plants

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ABSTRACT The location of wild and cultivated host plants by pepper weevil (Anthonomus eugenii Cano) may be aided by visual cues, the male-produced aggregation pheromone, herbivore-induced, or constitutive host plant volatiles. The attractiveness of constitutive plant volatiles to pioneer weevils is important in understanding, and perhaps controlling, dispersal of this insect between wild and cultivated hosts. Ten-day-old male and 2- and 10-day-old female weevils were tested in short-range Y-tube assays. Ten-day-old male and female weevils were attracted to the volatiles released by whole plants of three known oviposition hosts, ‘Jalapeno’ pepper, American black nightshade, and eggplant, as well as tomato, a congener, which supports feeding but not oviposition. Two-day-old females were attracted to all plants tested, including lima bean, an unrelated, nonhost plant. Fruit volatiles from all three hosts and flower volatiles from nightshade and eggplant were also attractive. In choice tests, weevils showed different preferences for the oviposition hosts, depending on age and sex. Upwind response of 10-day-old male and female weevils to host plant volatiles was also tested in long-range wind tunnel assays. Weevils responded to pepper, nightshade, and eggplant volatiles by moving upwind. There was no difference in the observed upwind response of the weevils to the three host plants under no-choice conditions. Reproductively mature pepper weevils can detect, orient to, and discriminate between the volatile plumes of host plants in the absence of visual cues, conspecific feeding damage, or the presence of their aggregation pheromone.

KEY WORDS Anthonomus eugenii, host plant volatiles, Capsicum annuum, Solanum americanum, Solanum melongena

Phytophagous insects use a wide range of general and host-specific cues to locate their host plants within a heterogeneous environment (Schonhoven et al. 1998). An insect, moving randomly through space, will use long-range visual and/or olfactory cues to lead it to the vicinity of the plant. Once the insect makes contact with a potential host, short-range olfactory, mechanical and gustatory cues verify the plant’s suitability as a feeding or oviposition site. Other factors, such as species-specific pheromone signals, add to the complexity of host plant selection patterns as these signals may also work to draw an insect into a habitable patch, as well as aid in mate location.

Determining the sequence of events leading to host acceptance is particularly important for phytophagous insect pests. The pepper weevil, Anthonomus eugenii Cano, commonly infests cultivated pepper (Capsicum spp.) fields in the southern United States, Central America, and the Caribbean (Goff and Wilson 1937, O’Brien and Wibmer 1982, Abreu and Cruz 1985). In addition to feeding on Capsicum spp., the weevil is also capable of reproducing in the southern United States on a number of wild and cultivated plants in the genus Solanum, including American black nightshade, Solanum americanum Mill. (Patrock and Schuster 1987) and eggplant, Solanum melongena L.—a species of Asian origin (Diaz et al. 2004). The ability of the pepper weevil to survive the fallow season on wild nightshades makes this insect difficult to eradicate because nightshade-residing populations are able to reinfest pepper fields the following season. Although these generalized dispersal patterns of the weevil have been described by previous authors (Patrock and Schuster 1987), no one has yet addressed how pepper weevils initially locate their cultivated and wild host plants.

Although no research describing the pepper weevil’s response to host plant volatiles exists, there is a body of literature on the volatile attractants for three of its congeners, the boll weevil (Anthonomus grandis Boheman), the apple blossom weevil (Anthonomus pomorum L.), and the strawberry blossom weevil (Anthonomus rubi Herbst). Previous behavioral bioassays and electroantennogram studies have shown that these congeners can detect and orient to host plant volatiles. The boll weevil was attracted to cotton plant volatile oils and cotton square extracts in behavioral bioassays (Hardee et al. 1971, McKibben et al. 1977). Later, Dickens (1984, 1986, 1989, 1990) verified boll weevil detection of six-carbon “green leaf volatiles”
and host-specific volatiles using electroantennography (EAG) and single cell recording (SCR) techniques. An examination of apple blossom weevil response to volatile blends of various apple cultivars also suggests that this species uses volatiles as cues to locate its host plants (Kalinova et al. 2000). More recently, studies of the olfactory neurons of the strawberry blossom weevil identified 15 receptor types that detected a total of 54 host and nonhost plant volatiles (Bichão et al. 2005b), as well as five additional receptor types detecting volatiles from strawberry plants that are induced by weevil feeding (Bichão et al. 2005a).

Our goal is to better understand the complexities of pepper weevil host plant selection. We did this by first addressing the response of weevils to constitutive volatile cues released from host and nonhost plants in the absence of visual cues or pheromone signals. Removing these alternate sources of information will allow us to focus solely on the importance of plant volatiles to the weevil’s ability to locate a host plant. In addition, any differences between male and female response to plant volatiles can be identified without confounding differences in sensitivity to the male-produced aggregation pheromone (Eller et al. 1994). We were also able to identify changes in volatile discrimination by females at different ages. From a life-history perspective, newly emerged females should spend the majority of their time seeking mates and feeding locations, whereas older, previously mated females should be more motivated to search for oviposition sites. These changes in female behavior over time may lead to changes in volatile preferences if plant hosts sufficient for mating and adult feeding are not necessarily optimal sites for larval development.

We assayed five plant species (pepper, eggplant, American black nightshade, tomato, and lima bean) with varying degrees of suitability for adult feeding and oviposition—three species that support adult feeding and larval development, one species that can support adult feeding and one species that supports neither feeding nor oviposition. The three host plants differed in their level of acceptability and length of association with pepper weevil. Both pepper and American black nightshade are New World species that have historically served as hosts plants for the weevil, whereas eggplant is an Asian species with which the pepper weevil has only recently been in contact. Although the weevil has been observed feeding and ovipositing in eggplant flowers (Diaz et al. 2004), the weevil has not been a major source of economic injury to cultivated crops and eggplant does not appear to be a preferred host in the presence of pepper (P. A. Stansly, personal communication).

To begin unraveling the complexities of pepper weevil host plant selection we seek to answer the following questions: (1) will pepper weevils orient to host plant volatiles?; (2) will pepper weevils orient to general plant volatiles (i.e., emanating from a non-host)?; (3) will pepper weevils show a preference for the volatiles of particular host plants?; (4) will males and females respond differently to plant volatiles?; (5) will newly emerged females respond differently than reproductively mature females to host plant volatiles?; and (6) will pepper weevils respond to host plant volatiles in both short- and long-range orientation assays?

Materials and Methods

Insects and Plants. A pepper weevil colony was established at the University of Florida, Gainesville, FL, in the spring of 2004 from insects collected from pepper fields in Clewiston, FL. Additional wild insects from Immokalee, Bradenton, and Wimauma were introduced into the colony in the fall of 2005 and 2006 to maintain colony health. The colony was held under a 14:10 L:D light regimen at 27°C and 30% RH. Weevils were reared on greenhouse-grown ‘Jalapeño’ peppers (Capsicum annum L.), with water and honey supplements. Gravid females (>10 d old) were placed singly in oviposition cups with a single pepper fruit, which was replaced every 2 d. Oviposition cups with screened lids were made from waxed cardboard cans (250 ml, 8.5 cm diameter). Infested fruit were held in emergence containers with screen lids (1.5-liter Tupperware containers) until all weevils emerged. Newly emerged weevils were collected and transferred into a colony cage for use in assays.

Jalapeño pepper (Capsicum annum L.), ‘Ghost-buster’ eggplant, American black nightshade, ‘Better Boy’ tomato (Solanum lycopersicum L.), and ‘Fordhook 242’ bush lima bean (Phaseolus lunatus L.) plants (Illinois Foundation Seeds, Champagne, IL) were grown in a greenhouse. Plants were grown in 12-cm-diameter pots in 50:50 mixture of Metro-Mix 200 and 500 and fertilized using Osmocote 14-14-14 slow release pellets (The Scotts Company, Marysville, OH).

Y-tube Olfactometer Experimental Design. Bioassays were conducted in a glass Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) with Teflon tubing connections. Breathing quality compressed air was pushed through a charcoal filter and humidified with deionized water before splitting into two holding chambers. Three types of holding chambers were used in our assays, depending on the size of the plant part used: (1) Plexiglas chambers (60 cm tall, 15 cm internal diameter), (2) large glass chambers (21 cm length, 3 cm internal diameter), and (3) small glass chambers (6 cm length, 2 cm internal diameter). Airflow was maintained at 250 ml/min by two inline flow meters (Manostat, New York, NY). The glass Y-tube (12-cm common tube, 10-cm arms, 2.5-cm internal diameter) was held at a 30° angle above horizontal inside a three-walled cardboard enclosure (46 by 28 by 42 cm). Holding chambers were placed outside the enclosure to eliminate visual cues. The Y-tube assembly was illuminated by a fluorescent light fixture (four 85-W bulbs) suspended 70 cm above the table surface. The assay room was maintained at 25–27°C and 40% RH.

Weevils were sexed according to Eller (1995). Male and female weevils were starved overnight (~12 h) before assay without access to water. Two age classes of females (newly emerged and >10 days old) and one
male age class (>10 days old) were tested under each set of experimental conditions. Older females had been confined with males in the colony cage since their emergence and were presumed to have mated. Forty insects in each age/sex class were assayed individually or in groups of 5 and 10 per day on 4 separate days. Ten weevils in each of the three classes were assayed each day. Insects were given 15 min to make a choice of arms in the olfactometer. Weevils that passed halfway or further into one arm of the Y-tube were recorded as making a choice. If no choice was made in 15 min, the assay was concluded. After five assays, the airflow was reversed to the opposite side to control for right- or left-handed bias. After one sex/age class of weevils was assayed, the Y-tube was cleaned with soapy water, rinsed with ethanol, and dried on the bench before the next class was assayed. The starting class was randomly selected each day and assays were run within the previously established activity period for oviposition of 1000–1700 hours (Patrock and Schuster 1992).

**Y-tube Attraction to Host and Nonhost Plant Volatiles.** Three host plants (pepper, eggplant, and nightshade) and two nonhost plants, one solanaceous plant that supported feeding but not oviposition (tomato) and one nonsolanaceous species that was not acceptable as a feeding or oviposition host (lima bean) were evaluated in one-way choice tests against a purified air control. In a second study, three known pepper weevil host plants (pepper, eggplant, nightshade) were evaluated in pairwise choice tests to determine preference among the plant odors. Flowering plants used in both assays were between 2 and 3 months old and were presented in Plexiglas cylinders.

**Y-tube Attraction to Host Flower or Fruit Volatiles.** Pepper, eggplant, and nightshade flowers and fruit were assayed in one-way choice tests against a purified air control. Flowers and fruit were picked immediately before use. Flower stems were wrapped with moistened cotton balls to prevent wilting. In the flower assays, 5 pepper flowers, 2 eggplant flowers, or 40 nightshade flowers were placed in small glass (pepper) or large glass (eggplant and nightshade) chambers. In the fruit assays, 2 pepper fruit (<5 cm long), 2 eggplant fruit (<2 cm diameter), or 25 nightshade fruit (≈0.5 cm diameter) were placed in small glass (nightshade) or large glass (pepper and eggplant) chambers. Different numbers of flowers or fruits were presented to approximate equivalent mass.

**Wind Tunnel Design.** Bioassays were conducted in two sizes of Plexiglas wind tunnels at the USDA-ARS CMAVE facility in Gainesville, FL. The large wind tunnel (160 by 45 by 45 cm) was used for assays that required whole plants to be placed in the tunnel and weevils to be introduced in the center of the tunnel. The large wind tunnel was housed in a small greenhouse (approximate temperature 30°C, 75% RH). Airflow of 0.2 m/s was created by pulling air through the wind tunnel using a vacuum. Volatiles were released from a plant placed directly inside the tunnel, thus exposing insects to visual and volatile plant cues. Once weevils made contact with the plant source, they did not leave, and no traps were used in these assays.

Four small wind tunnels (120 by 30 by 30 cm) were housed in a separate greenhouse (approximate temperature 30°C, 66–75% RH). All remaining plant volatile assays were conducted in the small tunnels. In these assays, purified air passed through two Plexiglas cylinders (60 cm tall, 15 cm internal diameter) before entering the wind tunnels. Cylinders contained plants or were empty, depending on the experiment. Air from each cylinder was split with Teflon tubing into four streams, which were attached to odor ports in each of the four wind tunnels. Compressed air entered the wind tunnels at 0.6 liters/min, maintained by inline flow meters, and air in the tunnels was pulled by a vacuum at 0.2 m/s. All assays were carried out between 0900 and 1500 hours.

Traps were placed at the upwind end of the small wind tunnels to capture any weevils attempting to contact the odor port. Two vial traps were constructed from 25-dram plastic vials (5.0 cm diameter, 8.5 cm height; Bioquip, Gardena, CA) placed horizontally and fitted with a 1-ml plastic centrifuge tube to allow weevils to enter the trap but not escape. Traps were attached to the odor ports 25 cm above the floor. In preliminary assays, it was discovered that weevils preferred to walk rather than fly in the wind tunnel and a ramp leading up to the vial traps was constructed from white TOMCAT glue boards (10.0 by 24.0 cm; Motomco, Clearwater, FL) to trap the weevils as they attempted to walk up to the vial traps. Thus, weevils could be trapped at the odor source either in the vial traps (if they flew to the source) or on the sticky board (if they walked up to the source).

**Wind Tunnel Bioassays.** Male and mated female weevils (10–20 d old) were used in the following assays. Weevils were sexed and held separately in groups of 10 in 7-dram plastic vials with air holes. Weevils were held overnight (~15 h) with no food or water before assay. The same vials were placed into the downwind end of the wind tunnel, unless stated otherwise (120 or 60 cm from the odor source in the large or small wind tunnel, respectively). Weevils were released in groups of 10 by removing the vial lid at the start of each assay.

Weevil location was categorized and their upwind orientation to plant volatiles was recorded 15, 30, 60, and 300 min after their release, unless stated otherwise. Weevil locations recorded were (1) weevils that moved more than halfway up the tunnel (60 cm upwind in the large tunnel, 45 cm in the small tunnel; >50%) and (2) those that made contact with the odor source (SC). Source contact was defined as contacting the source plant, if present in the wind tunnel, or entering the vial trap or being caught on the sticky board ramp leading to the trap, if the host plant was not present. Total upwind response was calculated by summing the values in the >50% and SC categories. All experiments were repeated on four separate days for a total of 40 weevils per sex per treatment.
Wind Tunnel Orientation of Weevils in the Absence of Odors. This assay was conducted in the large wind tunnel to determine weevil orientation in a wind tunnel in the absence of plant odors. Weevils were placed in the center of the wind tunnel with no olfactory stimuli but with wind speed set at 0.2 m/s. Weevil orientation was observed 15 and 30 min after their introduction to determine whether the insects preferred to move upwind or downwind in the absence of odors. Weevils were recorded as having oriented in one direction or the other if they moved 60 cm or more from the center of the tunnel. This distance was chosen because it represented the halfway distance of the large tunnel (the distance used to define orientation in the volatile assays). This assay permitted us to justify the “orientation distance” for our plant volatile assays by determining whether weevils will walk 60 cm upwind in the absence of volatiles. Differences in upwind and downwind orientation were compared within and between sexes.

Wind Tunnel Orientation of Weevils to Constitutive Pepper Volatiles. Two experiments were conducted to quantify weevil orientation toward constitutive host plant volatiles. The first experiment was carried out in the large wind tunnel. In this assay, a 3-mo-old pepper plant was placed upwind inside the large wind tunnel to see if male and female weevils would move upwind in the presence of both visual and olfactory stimuli. Male or female weevils were released in groups of 10 at the downwind end of the tunnel. Their location in the wind tunnel was recorded 15, 30, and 60 min after their release. Male and female response to the visual and olfactory stimulus was compared.

In the second experiment, the small wind tunnels were used. To test weevil response to volatiles in the absence of visual stimuli, pepper plant volatiles were piped in through the odor port of each chamber. Ten males or females were released at the downwind end of the wind tunnel. Weevil location was recorded at 15, 30, 60 and 300 min and male and female response to plant volatiles in the absence of visual stimuli was compared.

Wind Tunnel Orientation of Weevils to American Black Nightshade and Eggplant. In separate assays, nightshade or eggplant volatiles were piped into small wind tunnels in no-choice tests. Ten male or female weevils were released at the downwind end of each wind tunnel. Weevil location was recorded at 300 min only and male and female response to the plant volatiles without visual stimuli was compared. Upwind movement to eggplant and nightshade volatiles was compared with orientation to pepper plant volatiles in no-choice tests run simultaneously.

Data Analysis. Y-tube and wind tunnel data were analyzed as total percent response to each source using \( \chi^2 \) analysis (SAS Institute 2006). For the Y-tube and wind tunnel data, the percentage of nonresponders (i.e., those weevils not making a choice [Y tube] or moving upwind [wind tunnel] in the allotted time period) in each sex/class assayed was also compared using \( \chi^2 \) analyses within and among the experiments.

Results

Y-tube Attraction to Host and Nonhost Plant Volatiles. In one-way choice tests, 10-d-old males and females preferred pepper, eggplant, nightshade, and tomato volatiles over purified air but showed no preference for lima bean (Fig. 1). Two-day-old females were attracted to volatiles from all plants, including the nonhost lima bean.

In pairwise choice tests, 10-d-old females preferred pepper volatiles over nightshade, whereas 10-d-old males showed no preference (Fig. 2). Ten-day-old males and females preferred nightshade volatiles to eggplant volatiles. Ten-day-old males preferred pepper over eggplant but 10-d-old females showed no preferences. Two-day-old females showed no preference between any of the three host plants.

Wind Tunnel Orientation of Weevils in the Absence of Odors. Pepper weevils occasionally made short distance flights from the release point and sometimes from floor to ceiling (or vice versa); however, the majority of movement was through walking. A significantly greater percentage of females moved downwind (27%) rather than upwind (5%) at 15 \( \chi^2 = 15.12, P < 0.0001 \) and 30 min (47.5% versus 5%; \( \chi^2 = 34.40, P < 0.0001 \)) after release. There was no difference in male location 15 min after release (12.5% downwind versus 7.5% upwind; \( \chi^2 = 1.25, P = 0.2636 \)), but after 30 min a larger percentage of males had moved downwind (25%) than had moved upwind (12.5%; \( \chi^2 = 4.17, P = 0.0412 \)).

Wind Tunnel Orientation of Weevils to Constitutive Pepper Volatiles. When a pepper plant was placed in the large wind tunnel, female total upwind response (movement >50% + SC) increased over time with significant differences observed among the 15-, 30-, and 60-min time intervals (\( \chi^2 = 9.13, P = 0.00104 \)). The total upwind response of males also increased with time (\( \chi^2 = 10.77, P = 0.0046 \)). There was no difference between male and female total upwind responses to pepper volatiles at any observation time (15 min: \( \chi^2 = 0.11, P = 0.7416; 30 \text{ min: } \chi^2 = 3.46, P = 0.0628; 60 \text{ min: } \chi^2 = 1.45, P = 0.2280 \), with 47.5% of females and 60.5% of males showing an upwind response by 60 min. Slightly more males than females were in contact...
with the volatile source at 60 min (SC; female = 25.0%, male = 40.5%; $\chi^2 = 3.67, P = 0.0555$).

In small wind tunnels, upwind response of female (H9273 = 53.06, $P < 0.0001$) and male weevils (H11005 = 58.11, $P < 0.0001$) to pepper volatiles increased with time. There was no difference in total response of males and females after 300 min (female = 77.5%, male = 77.4%; $\chi^2 = 0.03, P = 0.8651$). However, more females than males were in contact with the odor source at 300 min (female = 57.5%, male = 37.4%; $\chi^2 = 4.26, P = 0.0391$).

**Wind Tunnel Orientation of Weevils to American Black Nightshade and Eggplant.** Pepper weevil total upwind response did not differ by sex when insects were presented the control (pepper), nightshade or eggplant volatiles (Table 1). In no-choice comparisons, total upwind response to pepper and nightshade volatiles did not differ for males or females. There was also no difference in source contact in the two host plant treatments for either sex. In the second no-choice assay, there was no difference in total upwind response or source contact to pepper and eggplant volatiles for males or females.

**Nonresponder Data.** Some insects remained at the base of the Y-tube or did not make a choice in the 15 min allotted. Ten-day-old males and 2-d- and 10-d-old females all showed variation in nonresponders across assays. Male nonresponders ranged from a low of 5% in the pepper flower assay to a high of 33% in the nightshade whole plant assay ($\chi^2 = 33.36, P < 0.0001$). Ten-day-old females nonresponders ranged from a low of 10% in the pepper flower and fruit assays to a high of 42% in the eggplant fruit assay ($\chi^2 = 52.23, P < 0.0001$). Two-day-old female nonresponders ranged from 2.5% in the eggplant and pepper flower assays to 22% in the bean whole plant volatile assay ($\chi^2 = 42.32, P < 0.0001$). A significant difference was also observed among sex/classes, with 2-d-old females having the lowest percentage of weevils failing to make a choice (2-d-old females = 8.1 ± 1.7 [SE] SE; 10-d-old females = 26 ± 2.7%; 10-d-old males = 22.9 ± 2.1%; $\chi^2 = 135.1, P < 0.0001$). Sexually mature males and females did not differ in percentage of nonresponders; however, 2-d- and 10-d-old females did differ with younger females responding more often ($\chi^2 = 131.68, P < 0.0001$).

For the three wind tunnel assays, we analyzed nonresponding males and females across treatments at 300 min. Overall, slightly more females than males failed to respond (females = 30.4 ± 2.6%, males = 22.2 ± 1.5%; $\chi^2 = 3.86, P = 0.0493$). Neither males nor females differed in the percentage of nonresponders to pepper, eggplant, or nightshade whole plant volatiles.
Our bioassays showed that pepper weevils can orient to constitutive host plant volatiles in the absence of visual and pheromone stimuli. In the short-range Y-tube assays, males and females were attracted to the volatiles of known host plants but also responded to tomato, a nonhost plant within the genus *Solanum*. In a series of feeding and oviposition assays, Patrock and Schuster (1992) observed pepper weevil feeding on tomato plants and other species within the family Solanaceae. However, the weevils oviposited on only a small subset of the *Solanum* spp. (which did not include tomato) but oviposited on all *Capsicum* spp. tested. This suggests that there may be volatiles specific to plants in the family that can draw weevils in from a distance, whereas further contact or short-range cues determine the acceptability of the plant species for oviposition.

We also found differences between the response of newly emerged females <2 d old and responses of the reproductively mature females and males. In the single-choice plant Y-tube assays, 10-d-old males and females were not attracted to the general plant volatiles of lima bean but 2-d-old females did respond to this nonhost plant. The reasons for the difference in response may be caused by experience or developmental differences in neurological response. In addition to showing a positive response to a nonhost plant, 2-d-old females also failed to show the same discriminatory patterns as older females in pairwise comparisons. All weevils used in these assays were reared on 'Jalapenño' pepper fruit and none had previous experience with the alternative host and nonhost plants or pepper plant and flower volatiles. Therefore, naivety should not be a factor affecting the differences observed in the behavior of 2-d-old females because 10-d-old males and females were also unfamiliar with the alternative plant volatiles. It is therefore most likely that differences in central processing or development of olfactory neurons was responsible for the observed differences in response. Dickens and Mooreman (1990) came to the same conclusion when addressing the changing response of male and female boll weevils to host plant volatiles with age. Between
emerged pepper weevils can detect the presence of plant volatiles, their receptor neurons are not yet sensitive enough to differentiate between two complex odor plumes.

Ten-day-old males and females showed preferences for different host plants in the Y-tube olfactometer, and these preferences changed depending on sex and which two species were offered. It is important to note that, whereas 10-d-old females had a strong affinity for American black nightshade volatiles in the single-choice assays (Fig. 1b), when put in competition with pepper volatiles, the females overwhelmingly chose pepper (Fig. 2a). This occurred even though a small percentage of females walked to the air-laden arm in the single-choice assay with pepper in the alternative arm, whereas 100% of females walked to nightshade in the same series of assay. It is likely the difference in preference is caused by the larger size and presumably better quality of pepper fruit as a larval host source making it selectively advantageous for females to orient toward pepper volatiles. Females are rarely observed depositing more than one egg in nightshade fruit, whereas the weevils deposit many more in the larger ‘Jalapeno’ fruit (unpublished data). Females are therefore likely to have more egg laying opportunities in pepper than in nightshade. Also, our colony insects were reared on pepper fruit, and although they had no prior experience with whole pepper plant volatiles, the potential similarities in the volatile plumes of fruit and foliage may have biased the weevils toward pepper plants.

Because pepper weevils feed primarily and oviposit solely on flower buds and small fruits, we might have expected the weevils to be attracted to volatiles from host plant flowers and fruits. This does not seem to be true in all cases. Although 10-d-old male and female weevils oriented toward the immature fruit of pepper, nightshade, and eggplant, they only responded to flowers of nightshade and eggplant. No attraction to pepper flower volatiles was observed. It is possible that the attractive compounds present in the flowers of the two Solanum spp. are absent from C. annuum, alternatively, the amount of volatiles being released from the pepper flowers may have been below the behavioral threshold for response. Further analysis of flower volatile profiles, GC-EAD, and behavioral bioassays are being planned to test this hypothesis.

![Fig. 4. Pepper weevil attraction to host plant fruit volatiles in a Y-tube olfactometer. Data are represented as percent responding. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in χ² analysis.](image)

Table 1. Response of pepper weevil males and females to host plant volatiles in no-choice tests conducted in a wind tunnel

<table>
<thead>
<tr>
<th>Orientation class (percent response)</th>
<th>Control (pepper)</th>
<th>Nightshade</th>
<th>Eggplant</th>
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<tr>
<td></td>
<td>&gt;50%</td>
<td>SC</td>
<td>Total response</td>
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<td><strong>Female</strong></td>
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<td>300-min assays</td>
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<td>Trials to assess attraction to nightshade or eggplant were run simultaneously with pepper controls.</td>
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<td>50% = weevils walked &gt;45 cm upwind in small wind tunnel.</td>
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<td>SC = source contact, weevils contacted the sticky board or entered the vial trap.</td>
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The short-range volatile attraction exhibited in the Y-tube assay showed that the pepper weevil can discriminate between the volatiles of different host plants. Although short-range studies offer us some information about pepper weevil preferences, a wind tunnel gives us the opportunity to observe weevil behavior on a larger scale. One concern about insect behavior in a wind tunnel is whether the insects are cueing in on attractive volatiles or if they are merely positively anemotactic. We showed here that, in the absence of plant volatiles, both male and female weevils are more inclined to move downwind or remain stationary than to move upwind. Therefore, the upwind response observed in the wind tunnel assays with plants can be attributed to attraction to plant volatiles. In the first three pepper volatile assays, both males and females oriented upwind, regardless of whether the plant source was visible or not. There was no difference in the total numbers of males and females orienting to the plant volatiles. In addition, both male and female weevils showed similar responses to pepper, eggplant and nightshade plant volatiles in the wind tunnel under no-choice conditions. Ideally, we would like to repeat our choice tests (conducted in the Y-tube olfactometer) in the larger-scale wind tunnel as well; however we ran into problems when attempting to run choice tests. Although pepper weevil does fly, it prefers to walk in the wind tunnel as it does in the Y-tube. This makes trap captures unreliable because the weevils do not fly into the mouth of the vial trap; they prefer to walk along the walls of the tunnel and climb onto the trap.

The experiments described in this study were specifically designed to exclude alternative cues often used in host plant location. Other volatile attractants excluded include the male-produced pepper weevil aggregation pheromone (Coudriet and Kishaba 1988, Eller et al. 1994) as well as plant volatiles induced by weevil feeding and/or oviposition. While arguably, the very first weevil to arrive at an uninfested plant must rely solely on constitutive plant cues to locate the host, subsequent arrivals may be orienting to a combination of plant and insect-specific volatiles. If the first arrival is female, her feeding or oviposition will induce changes in the volatile plume of the plant, which may potentially increase the plant’s attractiveness to other weevils. If the first arrival is male, a combination of plant volatiles and male-produced pheromone will be available to aid other weevils in locating the plant.

With regard to the six questions posed at the beginning of this study, we found that (1) pepper weevils will orient to host plant volatiles, (2) that sexually mature pepper weevils will not orient to general plant volatiles emanating from a nonhost outside the Solanaceae, but may be attracted to other nonhost Solanaceae, (3) that pepper weevils show preferences for the volatiles of different host plants, but (4) that males and females respond differently to different plants, (5) that newly emerged females <2 d old respond differently than reproducitively mature females, showing none of the preferences observed in older females, and (6) that pepper weevils will respond to host plant volatiles in both short- and long-range orientation assays.

More work is needed to understand the intricacies of pepper weevil host plant location and, more specifically, the role plant volatiles play in this complex chain of events. More interesting is the relationship between alternative host plants and the volatile cues that cause male and female weevils to select one species over another and allow populations to survive eradication by migrating back and forth between cultivated and closely related wild species. Work is currently being conducted on the effect of feeding damage by male and female weevils on attraction of conspecifics in Y-tube and wind tunnel assays. We will also use GC-EAD and behavioral bioassays to identify the most attractive host-specific volatiles common to the pepper weevil’s host plants. Our two-fold purpose is to understand how an insect discriminates between the volatile plumes of multiple host species in close proximity and to use that knowledge to develop ecologically friendly trapping systems for monitoring and management of pepper weevil.

Acknowledgments

We thank R. Tanay and J. Meyer for assistance with colony maintenance and F. Eller and P. Stansly for comments on an earlier draft of the manuscript. We also thank the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology (Gainesville, FL) for the use of their wind tunnels. This research was supported by the Florida Agricultural Research Station.

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Received 10 June 2008; accepted 16 October 2008.