

Sugar Diet Affects Odor Reception but Variation in Sugar Concentration Plays Minimal Role in the Response of the Parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae), to Host-Related Plant Volatiles

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Abstract

Parasitoids utilize various sugar resources in nature, and rely on odor cues from plants to locate their food and hosts. However, lack of sugar in the diet may negatively impact odor reception in parasitoids, thus affecting foraging efficiency. We used *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a larval endoparasitoid of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), as a model species to test the hypothesis that variation in sugar diet of parasitoids affects their olfactory response to host-related odors. *Heliothis virescens* is a major pest of cotton and other important crops. Response of female *M. croceipes* fed different diet treatments (i.e., 40%, 20%, 10%, or 0% sucrose/water solution [w/v]) to select cotton volatiles were tested in electroantennogram (EAG) and Y-tube olfactometer bioassays. The following cotton plant odors were tested: *cis*-3-hexenol, α -pinene, 50/50 v/v binary mixture of *cis*-3-hexenol and α -pinene, and *H. virescens*-infested cotton. Sucrose-fed parasitoids showed higher EAG response to the binary mixture and host-infested plant volatile extract, compared with sucrose-starved (0% sucrose) parasitoids. However, there was no significant difference in EAG response of parasitoids to odor treatments among individuals fed 40%, 20%, or 10% sucrose. In a Y-tube olfactometer, female *M. croceipes* fed 40% sucrose were significantly more attracted to host-infested cotton than to a control (no plant). However, parasitoids were not significantly attracted to other odor stimuli. These results suggest that the availability of sugar diet affects odor reception in *M. croceipes* but variation in sugar concentration probably plays a minimal role in olfactory response of *M. croceipes* to host-related odors.

Key words: sucrose, electroantennogram, olfactometer, cotton plant, *Heliothis virescens*

Several species of parasitoids rely on sugar resources as their primary sources of nourishment. Consequently, maximization of their fitness is dependent on availability of sugar. In the field, energetic demands can be met through utilization of nectar and homopteran honeydew (Jervis et al. 1993, Jervis and Kidd 1996, Olson et al. 2000, Wäckers et al. 2008), which typically contain between 10–50% sucrose (Van Handel et al. 1972, Fadamiro et al. 2005). Parasitoids are biological control agents whose foraging success relies on the use of plant volatile organic compounds (VOCs). Parasitoids use odor cues from plants to locate both food and host resources (Takasu and Lewis 1993, Wäckers 1994, Kugimaya et al. 2010). However, parasitoid olfactory response to such resources is greatly influenced by their physiological state, and in particular, their nutritional status (Browne 1993, Takasu and Lewis 1993,

Martel et al. 2009). The effect of nutrition on host-searching ability of the parasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), has been previously studied (Wäckers 1994, Takasu and Lewis 1995, Stapel et al. 1997, Lewis et al. 1998, Wäckers 2004, Heimpel and Jervis 2005). Stapel et al. (1997) investigated the effect of extrafloral nectar, honeydew, and sucrose feeding on detectability and searching time of *M. croceipes* for its caterpillar host, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). Access to extrafloral nectar and sucrose enhanced searching time and rate of parasitism in *M. croceipes* (Stapel et al. 1997). Other studies have investigated attraction of parasitoids to host-related odors versus food odors (Takasu and Lewis 1993, Wäckers 1994, Lewis et al. 1998, Kugimaya et al. 2010). Wäckers (1994) reported that sugar-fed parasitoids preferred odors associated with

rape seed leaf damage (host-related) while sugar-deprived parasitoids preferred flower odors (food-related).

In particular, it has been suggested that *M. croceipes* modulates their foraging behavior by decreasing sensitivity to host-related odors or increasing sensitivity to food-associated odors (Takasu and Lewis 1993). Optimal foraging and energetic considerations in female parasitoids often involve foraging tradeoff between food and hosts. The less time and energy parasitoids spend searching for sugar resources, the more time they can allocate toward host searching (Sirost and Berstein 1996, Tenhumberg et al. 2006). In the field, parasitoids obtain sugar needed to meet energetic demands of foraging through utilization of nectar and homopteran honeydew (Jervis et al. 1993, Jervis and Kidd 1996, Olson et al. 2000, Wäckers et al. 2008). Sucrose concentration of these sugar sources typically ranges between 10–50% (Van Handel et al. 1972, Fadamiro et al. 2005). It has been reported that lack of sugar can negatively impact searching efficiency and parasitism in parasitoids (Takasu and Lewis 1993, Wäckers 1994, Takasu and Lewis 1995, Stapel et al. 1997, Wäckers 2004), but it is not clear whether this is related to reduced perception and response to host-related odor.

In this study, we tested the hypothesis that variation in sugar diet of female *M. croceipes* affects their olfactory response to plant VOCs. *Microplitis croceipes* is a relatively specialized larval endoparasitoid of *Heliothis/Heliocoverpa* spp., which are important pests of cotton and several other crops. Previous studies have reported attraction of *M. croceipes* to VOCs released from undamaged and herbivore-damaged cotton. (McCall et al. 1993, Rose et al. 1998, Wei et al. 2007, Yu et al. 2010, Ngumbi and Fadamiro 2012, Morawo and Fadamiro 2014). Here, the effect of varying sucrose diet on olfactory response of *M. croceipes* to select cotton VOCs was investigated using electroantennogram (EAG) and Y-tube olfactometer bioassays. *cis*-3-Hexenol and α -pinene were chosen as test VOCs because they have been detected consistently in the headspace of cotton plants (Rose and Tumlinson 2004, Ngumbi et al. 2009, Magalhaes et al. 2012) and have elicited olfactory response in *M. croceipes* (Chen and Fadamiro 2007, Ngumbi et al. 2012, Morawo and Fadamiro 2014). *cis*-3-Hexenol is a host-induced green leaf volatile emitted by cotton. α -Pinene is a constitutive monoterpene that is passively released from cotton plant but also emitted in higher amounts in caterpillar-damaged cotton. In addition, a binary mixture of α -pinene and *cis*-3-hexenol, as well as the natural blend of headspace VOCs from *Heliothis virescens* (F.) (Lepidoptera: Noctuidae)-infested cotton were tested. Previous studies have shown that VOC mixtures may be more attractive than individual compounds because they are more representative of the natural context in which parasitoids encounter host-related odors (Mumm and Hilker 2005, van Wijk et al. 2011, Morawo and Fadamiro 2014). We are not aware of previous studies that have investigated effect of varying concentration of sucrose diet on olfactory response of parasitoids to plant VOCs. The physiological and ecological implications of the present results are discussed.

Materials and Methods

Insects

Microplitis croceipes were reared in our laboratory (Auburn University, AL) on second–third-instar larvae of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). The rearing procedures were similar to those described by Ngumbi et al. (2009) and Morawo and Fadamiro (2014). Upon emergence, adult wasps were transferred to aerated plastic cages (30 by 30 by 30 cm) and supplied with 10%

sucrose/water solution (w/v). Mated, naive (untrained) parasitoids (aged 1–3-d-old) were used in electroantennogram (EAG) and behavioral bioassays. Most behavioral studies have used mated parasitoids because mating is known to increase the probability of host-searching behavior in parasitoids (Chen and Fadamiro 2007). Larvae of *H. virescens* were reared on pinto bean artificial diet (Shorey and Hale 1965). The general rearing conditions for all insects were $25 \pm 1^\circ\text{C}$, $75 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h.

Plants

Cotton (*Gossypium hirsutum*, var. max 9) plants were grown in individual pots (9 cm high, 11 cm diameter) in a growth chamber (Auburn University) at 26.6°C day, 25.6°C night, 60% RH, and a photoperiod of 16:8 (L:D) h. Seeds were planted in a top soil and vermiculate mixture. Plants used for headspace volatile collections were 4–6 wk old.

Diet Treatments

Parasitoids used for EAG bioassays were transferred into 9-cm aerated petri dishes upon emergence. Petri dishes were aerated by cutting a 5-cm-diameter hole in the lid and covering the hole with a black mesh. Petri dishes were supplied with diet treatments: 40%, 20%, 10%, or 0% (water only) sucrose/water solution (w/v) on two cotton balls placed at the bottom of the petri dish and by smearing the sugar treatment on the underside of the lid. Diet treatments were re-applied to cotton balls and lids daily to limit effect of water evaporation. Parasitoids used for Y-tube olfactometer bioassays were transferred to aerated plastic cages (30 by 30 by 30 cm) upon emergence. A larger containment was used for parasitoids tested in the olfactometer bioassays in order to avoid crowding of insects, which may affect behavioral responses of parasitoids. Each cage was supplied with different diet treatments by using cotton balls soaked in similar volumes of water or sucrose solution, and by smearing the inner top of the cages. Likewise, water or sugar solution was re-applied each day. Female parasitoids used in all experiments were aged 1–3-d-old and assumed mated after interacting with males for at least 24 h within their designated treatment cages or petri dishes. It should be noted that female *M. croceipes* are sexually mature at age 1-d-old. Generally, close to 40% of the lifetime egg complement of *M. croceipes* are matured upon emergence (Jervis et al. 1996), and parasitoids demonstrate readiness to seek and parasitize suitable hosts within few hours of emergence. Parasitoids were allowed to feed *ad libitum* for at least 24 h before use in bioassays. Temperature and humidity were maintained at the general rearing conditions while parasitoids fed on different diet treatments.

Odor Stimuli

cis-3-Hexenol (green leaf volatile) and α -pinene (constitutive monoterpene) are two components detected in the headspace of *H. virescens*-infested cotton (Loughrin et al. 1994, DeMoraes et al. 1998, Rose and Tumlinson 2004, Ngumbi et al. 2009, Magalhaes et al. 2012, Ngumbi et al. 2012), and have been demonstrated to elicit olfactory response in *M. croceipes* (Rose et al. 1998, Chen and Fadamiro 2007, Ngumbi et al. 2012, Morawo and Fadamiro 2014). Thus, both compounds were tested in this study. Synthetic compounds (purity 95–99%) were purchased from Sigma Chemical Co. (St. Louis, MO). A binary mixture of both compounds (*cis*-3-hexenol + α -pinene) was also formulated at 50/50 v/v as the third odor stimulus. Synthetic compounds were formulated in hexane (HPLC-grade) at $1 \mu\text{g}/\mu\text{l}$ concentration and delivered as 10 μl samples (10 μg dose). In addition, *H. virescens*-infested cotton was tested in

Y-tube bioassays while headspace volatile extract of *H. virescens*-infested cotton was tested in EAG as the fourth odor stimulus.

Headspace Volatile Collection

Headspace volatiles were collected from *H. virescens*-infested cotton plants using the methodology and protocols reported by Ngumbi et al. (2009). To induce release of HIPVs from plants, 30 second-third-instar larvae of *H. virescens* were allowed to feed on a cotton plant for 24 h during volatile collection. Pots with soil were wrapped with aluminum foil to minimize contamination. The plant was then placed in a volatile collection chamber (Analytical Research Systems, Inc., Gainesville, FL) consisting of a 5-liter glass jar. A purified air stream of 500 ml/min was passed through the jar at room temperature for 24 h. Headspace volatiles were collected with a trap containing 50 mg of Super-Q (Alltech Associates, Deerfield, IL). Traps were eluted with 300 μ l of methylene chloride and the resulting extracts were stored in a freezer (at -20°C) until use.

EAG Recording

The EAG protocol was similar to that previously described by Chen and Fadmiro (2007) and Ngumbi et al. (2009). A reference electrode consisting of a glass capillary (1.1 mm ID) filled with Ringer solution was connected to the back of the head of a female *M. croceipes*. The recording electrode consisted of another glass capillary connected to the cut tip of the terminal segment of the antenna. Chlorinated silver-silver junctions maintained electrical conduct between the electrode and input of the preamplifier. The analog signal was detected through a probe (INR-II, Syntech, Hilversum, the Netherlands), and was captured and processed with a data acquisition controller (IDAC-4, Syntech, Hilversum, the Netherlands) and analyzed using EAG 2000 software (Syntech, Hilversum, the Netherlands) on a computer. *cis*-3-Hexenol, α -pinene, and a 50/50 binary mixture were delivered as 10- μ l samples on a filter paper strip (7 by 40 mm, Whatman1 No. 1). Headspace volatile extract of *H. virescens*-infested cotton was also delivered as 10- μ l samples on filter paper. A Pasteur pipette (14 cm in length, Fisher Scientific, Pittsburgh, PA) was used as an odor cartridge and a filter paper strip impregnated with the test odor was inserted. Hexane (control for synthetic stimuli) or methylene chloride (control for *H. virescens*-infested cotton extract) were introduced in the same manner. The tip of the pipette was placed about 3 mm into a small hole in the wall of a glass tube (13 cm long, 8 mm diameter) oriented toward the antennal preparation (0.5 cm away from the preparation). The stimuli were delivered as 0.2-s puffs of air into continuous humidified air stream at 1,000 ml/min generated by an air stimulus controller (CS-55, Syntech, Hilversum, the Netherlands). A time interval of 2 min was allowed between odor puffs in order to account for antennal recovery.

For each antennal preparation, the following stimuli were presented: hexane (control), methylene chloride (control), *cis*-3-hexenol, α -pinene, binary mixture, headspace volatile extract. Hexane and methylene chloride (controls) were applied at the beginning and end of each recording series while the position of other test stimuli was randomized across replicates. Experiments were performed in a randomized complete block design with equal number of insect replicates from each diet treatment tested per day. For analysis, EAG response to the solvent control was deducted from the EAG amplitudes elicited by test stimuli (Absolute EAG). EAG data met the key assumptions of parametric tests and were not transformed prior to analysis. EAG responses of parasitoids fed different diet treatments were compared using one-way analysis of variance (ANOVA) for each odor stimulus. EAG responses were recorded from 10 replicates (insects) per diet treatment. Means were

separated using Tukey-Kramer HSD multiple comparison test ($P < 0.05$; SAS 9.2; SAS Institute 2010).

Y-tube Olfactometer Bioassay

A Y-tube olfactometer (Analytical Research Systems, Inc.) was used to test attraction of female *M. croceipes* to the four odor stimuli. The setup and protocol was similar to that reported by Ngumbi and Fadmiro (2012). The inlet air was pushed at a constant rate of 250 ml/min into each arm of the olfactometer. Air was simultaneously removed by suction via a vacuum pump through the central arm of the olfactometer at the rate of 500 ml/min. Parasitoids were introduced individually into the central arm of the Y-tube olfactometer. The choice of a parasitoid was recorded after the insect walked into one of the arms of the Y-tube and remained in that arm for at least 15 s. If a parasitoid did not make a choice within 5 min of being introduced into the olfactometer, it was removed, scored as no choice and excluded from the analyses. The number of nonresponding parasitoids in the 16 sets of bioassays ranged from 0 to 6, with a mean of 2.3 insects per test. Experiments were performed in a randomized complete block design with equal number of insect replicates from each diet treatment tested per day. After three individual parasitoids had been tested, the olfactometer arms were rotated 180° to minimize positional effect. In-between diet treatments, the olfactometer was cleaned with soap, rinsed with water, dried and then rinsed with acetone. Each synthetic compound (1 μ g/ μ l *cis*-3-hexenol, α -pinene, or 50/50 binary mixture) was delivered as a 10- μ l sample placed on filter paper strips (7 by 25 mm, Whatman No. 1). After allowing 10 s for solvent evaporation, a filter paper strip was inserted into a designated arm of the olfactometer. Another filter paper strip containing 10 μ l of hexane (solvent control) was inserted into the other arm of the olfactometer. Filter paper strips were replaced and olfactometer arms were rotated after testing three insects. Equal replicates of parasitoids assigned to each diet treatment were represented each day bioassays were conducted.

Attraction of parasitoids to volatiles released from *H. virescens*-infested cotton was tested using a protocol similar to Balusu and Fadmiro (2011) and Chen et al. (2009). One arm of the olfactometer was connected to an air-tight glass jar (5 liter) containing *H. virescens*-infested cotton. The other arm was connected to a similar glass jar containing a pot of soil covered with aluminum foil, which served as a negative control. Cotton plant was infested with 30 second-third-instar larvae of *H. virescens* that were allowed to feed for 24 h. A new plant was used on different days in which bioassays were conducted (five to seven plant replicates). Caterpillars remained on the cotton plant while conducting the bioassay. Humidified and purified (charcoal filtered) air was let into the jars at a constant rate of 300 ml/min. Air was continuously pushed through the jars and into the arms of the olfactometer and simultaneously removed by a vacuum suction through the central arm of the olfactometer at the rate of 600 ml/min. Olfactometer data were recorded from 20 replicates per diet treatment per odor stimulus. The deviation of parasitoid response to stimulus versus control from a 50:50 ratio was analyzed using a Chi-square goodness-of-fit test ($P < 0.05$; SAS 9.2; SAS Institute 2010).

Results

Effect of Diet on EAG Response

In general, sucrose-fed (40%, 20%, or 10% sucrose/water solution) parasitoids showed higher EAG response to all test odors, compared with sucrose-starved (0% sucrose/water) parasitoids (Fig. 1).

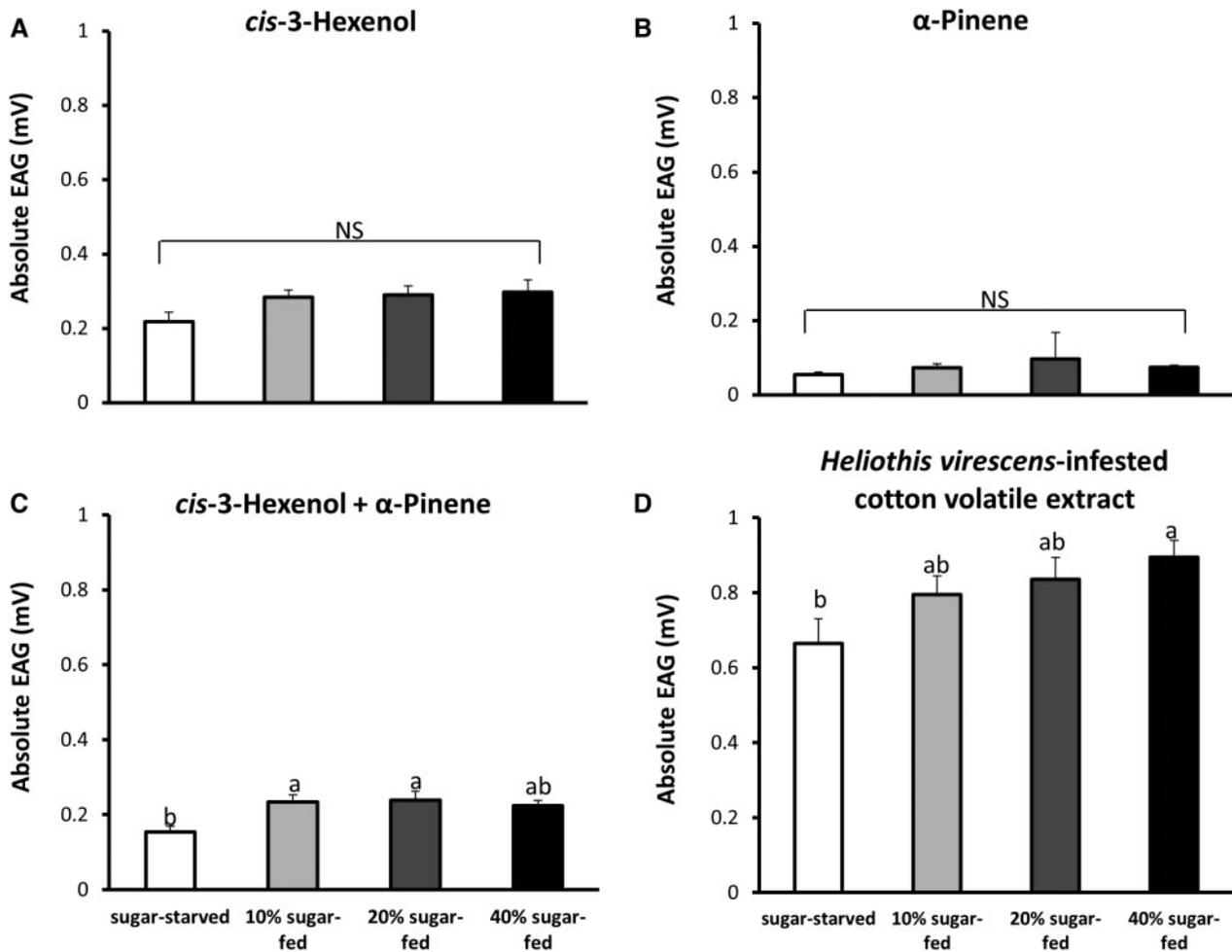


Fig. 1. Effect of diet on the EAG response of *M. croceipes* to various host-related odor treatments. Figure shows absolute EAG responses ($mV \pm SE$, $n = 10$) of *M. croceipes* females fed 40%, 20%, 10%, or 0% sucrose solution (w/v) to *cis*-3-hexenol (A), α -pinene (B), a 50/50 binary mixture of *cis*-3-hexenol and α -pinene (C), and a complex natural odor blend (*Heliothis virescens*-infested cotton headspace volatile extract) (D). All stimuli were presented at $10 \mu g$ dose. "NS" indicates no significant difference. Means for each odor stimulus having no letter in common are significantly different (ANOVA, Tukey HSD test, $P < 0.05$).

Specifically, parasitoids fed 20% or 10% sucrose/water solution showed significantly higher EAG response to the binary mixture (*cis*-3-hexenol + α -pinene) than sugar-starved parasitoids ($F = 4.58$, $df = 3$, $P = 0.0081$; Fig. 1C). Similarly, parasitoids fed 40% sucrose solution showed significantly higher EAG response to *H. virescens*-infested cotton volatile extract compared to the sugar-starved control ($F = 3.11$, $df = 3$, $P = 0.0383$; Fig. 1D). However, there was no significant difference in the EAG response of parasitoids to odor treatments among individuals fed 40%, 20%, or 10% sucrose/water solution. In addition, diet treatments had no significant effect on the EAG responses of parasitoids to the single compounds, *cis*-3-hexenol (Fig. 1A) or α -pinene (Fig. 1B).

Effect of Diet on Behavioral Response

Diet had a variable effect on the behavioral response of *M. croceipes* to the single compounds (*cis*-3-hexenol and α -pinene) and binary mixture (Fig. 2A–C). Compared to solvent control, sugar-fed parasitoids generally showed greater attraction to *H. virescens*-infested cotton than sugar-starved parasitoids. More parasitoids (65–80% of tested insects) fed with diets containing sucrose were attracted to *H. virescens*-infested cotton, compared to control. In contrast, only 55% of sugar-starved insects chose infested plant over control (Fig. 2D). However, only parasitoids fed 40% sucrose/water solution

were significantly ($\chi^2 = 6.06$, $df = 1$, $P = 0.0138$) more attracted to *H. virescens*-infested cotton than to control (Fig. 2D).

Discussion

The present results suggest that sugar feeding impacts olfactory response in *M. croceipes*. Sucrose-fed parasitoids showed higher EAG response to the binary odor mixture and host-infested plant volatile extract, compared with sucrose-starved parasitoids. In contrast, diet treatments had no significant effect on the EAG responses of parasitoids to the single compounds, *cis*-3-hexenol and α -pinene. Arguably, odor mixtures and natural blends convey better information as foraging cues to parasitoids than single compounds (Hoballah and Turlings 2005, Schnee et al. 2006, Riffell et al. 2009, Van Dam et al. 2010, van Wijk et al. 2011, Morawo and Fadamiro 2014). Although sucrose-fed parasitoids showed higher EAG response to the binary mixture and plant volatile extract than sucrose-starved parasitoids, there was no significant difference in the EAG response of parasitoids among individuals fed 40%, 20%, or 10% sucrose/water solution. Since EAG measures antennal sensitivity, these results suggest that availability of sugar diet affects odor reception in *M. croceipes* but variation in sugar concentration probably plays a

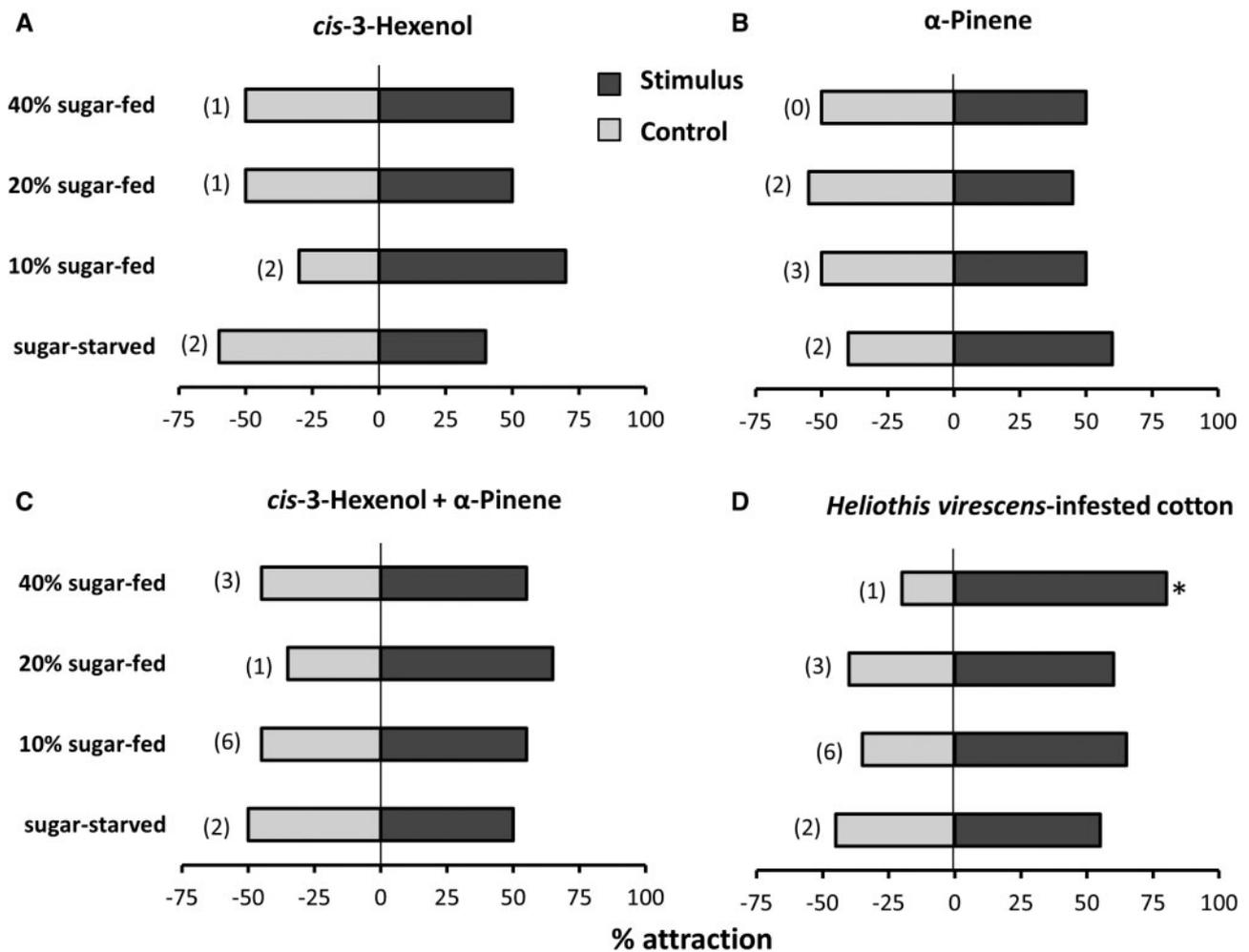


Fig. 2. Effect of diet on the behavioral response of *M. croceipes* to various host-related odor treatments. Figure shows response of *M. croceipes* females fed 40%, 20%, 10%, or 0% sucrose solution (w/v) in a Y-tube olfactometer when given a choice between hexane (solvent control) and *cis*-3-hexenol (A), α -pinene (B), a 50/50 binary mixture of *cis*-3-hexenol and α -pinene (C), and a choice between control jar and *Heliothis virescens*-infested cotton (D). Synthetic stimuli were presented at 10 μ g dose. $n = 20$ responding parasitoids per choice test. Values in parenthesis indicate number of nonresponding insects per choice test. Asterisk (*) indicates significant differences between stimulus and control (chi-square goodness-of-fit test, $P < 0.05$).

minimal role in olfactory response of parasitoids to host-related odors.

In Y-tube olfactometer bioassays, *M. croceipes* fed 40% sucrose/water solution showed significant attraction to host-infested cotton (natural odor blend) while sugar-starved parasitoids could not distinguish between the natural odor blend and solvent control. No significant difference was recorded in other Y-tube olfactometer bioassays testing parasitoid attraction to single compounds and binary mixtures. This may be due, in part to the fact that other sensory input are involved in behavioral responses of insects, compared to antennal responses. This is noteworthy given that EAG activity does not always translate to a behavioral response in insects (Blaney et al. 1986, Park et al. 2001, Ngumbi et al. 2010). While EAG is a useful tool for measuring the summed activity of olfactory receptor neurons, behavioral bioassays are necessary to determine the functional and ecological relevance of odor stimuli (Birch 1971, Ngumbi et al. 2010).

Furthermore, our results suggest that parasitoids that have access to sugar sources in the field are likely to show greater response to host-related odors than sugar-deprived individuals. In the field, parasitoids obtain sugar needed to meet energetic demands of foraging through utilization of nectar and homopteran honeydew (Jervis et al. 1993, Jervis and Kidd 1996, Olson et al. 2000, Wäckers et al. 2008).

Sucrose concentration of these sugar sources typically ranges between 10–50% (Van Handel et al. 1972, Fadamiro et al. 2005). This finding is in agreement with previous studies which showed a strong connection between sugar-feeding and host foraging in parasitoids (Takasu and Lewis 1993, Wäckers 1994, Takasu and Lewis 1995, Lewis et al. 1998, Kugimaya et al. 2010). Takasu and Lewis (1993) reported that sucrose-fed *M. croceipes* visited and spent more time on leaves containing *Helicoverpa zea* frass extract than leaves with sucrose and vanilla extract, while starved *M. croceipes* preferred food plants. In a related study, female parasitoids [*Apanteles aristoteliae* Viereck (Hymenoptera: Braconidae)] fed 2.5% or 10% sucrose preferred host-related odors over food-related odors, while starved (fed water only) parasitoids showed no preference (Lightle et al. 2010). Furthermore, Takasu and Lewis (1993) reported that sucrose-fed *M. croceipes* oviposit at a higher rate than unfed *M. croceipes* on its caterpillar host *Helicoverpa zea*. Similarly, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) oviposition on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) was suppressed when sugar was not available (Mitsunaga et al. 2004). These studies suggest a switch from host searching to food searching behavior in response to sugar deprivation.

Various optimal foraging models can lend support to the tradeoff between food foraging and host searching in parasitoids (Charnov

1976, Stephens and Krebs 1986, Sirot and Berstein 1996, Tenhumberg et al. 2006). For example, the TSK (Tenhumberg Siekmann Keller) model predicts that parasitoids will choose to search for either food or hosts, but not both concurrently (Tenhumberg et al. 2006). Host-searching increases the chance of locating hosts for oviposition; however, energy reserves are constantly depleted during the process. Food-foraging delays the initial opportunity to oviposit, but later maximizes the time and energy that a parasitoid may invest in host-searching. This theory may explain our findings in which sugar-starved *M. croceipes* generally showed lower response to host-related volatiles compared with sugar-fed individuals.

However, the present results also suggest that variation in sucrose concentration of different sugar sources in the field may have minimal impact on response of parasitoids to host-related odors, similar to the report of Lightle et al. (2010). This may be especially true for young female parasitoids. In the present study, relatively younger parasitoids (1–3-d-old) and four diets with concentrations of sucrose ranging from 0–40% were tested. It is possible that testing older parasitoids and other sucrose concentrations within these thresholds may result in differences in parasitoid responses. Also, parasitoids were allowed to feed *ad libitum* in the present study. Therefore, it was not possible to ascertain whether parasitoids provided with diet containing lower concentration of sucrose fed more (increased volume) to compensate for the amount of sucrose obtained. However, a previous study (Lightle et al. 2010) showed that the higher the concentration of sucrose in the diet of the parasitoid *A. aristoteliae*, the higher the volume of diet consumed, suggesting that parasitoids did not compensate for lower sucrose concentration in their diet by feeding more under laboratory conditions.

In summary, the present study showed that sugar diet is important for odor reception but variation in sugar concentration plays minimal role in the response of the parasitoid, *Microplitis croceipes*, to host-related plant volatiles. Availability of supplemental sugar sources in the field either in the form of habitat manipulation (i.e. farmscaping) or artificial sugar provisioning may increase parasitoid longevity and fecundity, as well as enhance their response to host-related plant volatiles. Subsequently, attractive odor cues can be manipulated to optimize efficiency of parasitoids in the field (Vet and Dicke 1992, Lewis et al. 1998, Heimpel and Jervis 2005). Future studies should investigate the neuronal and hormonal mechanisms by which sugar feeding can enhance olfactory response in parasitoids.

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