RESPONSES OF MULTIPLE SPECIES OF TEPHRITID (DIPTERA) FRUIT FLY PARASITOIDS (HYMENOPTERA: BRACONIDAE: OPIINAE) TO SYMPATRIC AND EXOTIC FRUIT VOLATILES

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Abstract

Opiine braconid parasitoids of tephritid pests have augmentative biological control potential, but there are no synthetic attractants to monitor their survival and dispersal following release. Adults feed on fruit juices and these could be sources of attractive compounds. While orange juice (*Citrus sinensis* L.) is nutritious, guava juice (*Psidium guajava* L.) is toxic. The behavioral responses to the two juices and the juices' volatiles were examined in two species ancestrally sympatric with guava (*Doryctobracon areolatus* [Szepligeti] and *Utetes anastrephae* [Viereck]) and a species exotic to guava's neotropical region of origin (*Diachasmimorpha longicaudata* [Ashmead]). It was predicted that males of the sympatric species, with an evolutionary opportunity to recognize and avoid guava, would neither feed on nor respond to guava volatiles. Females of the sympatric species were predicted to avoid feeding upon guava while responding to volatiles in the context of searching for oviposition sites. Males and females of the exotic species were predicted to feed upon and respond to both guava and orange. Orange was universally fed upon and its volatiles were found to be attractive. None of the males, including the exotic species', occupied guava feeding-stations in no-choice situations, but male *U. anastrephae* responded to guava volatiles. Against expectations, female *D. areolatus* occupied guava feeding stations. In general, responses to orange were stronger and more universal than those to guava. The preference differences the various species showed between guava and orange are a step forward in identifying the chemical basis of fruit-attractiveness. They provide 1) further evidence that not all uninfested fruit are equally attractive and 2) a relatively attractive and easily obtainable standard, orange, with which to compare the attractiveness of other fruit in the future.

Key Words: foraging, biological control, monitoring, *Diachasmimorpha, Doryctobracon, Utetes, Anastrepha*

Resumen

Los braconidos de la subfamilia Opiinae, parasitoides de plagas de la familia Tephritidae, tienen un potencial de control biológico aumentativo, pero no hay atrayentes sintéticos para monitorear su sobrevivencia y dispersión después de ser liberados. Los adultos se alimentan de los jugos de frutas y éstas podrían ser fuentes de compuestos atractivos. Mientras que el jugo de naranja (*Citrus sinensis* L.) es nutritivo, el jugo de guayaba (*Psidium guajava* L.) es tóxico. Se examinó la respuesta de comportamiento a las 2 clases de jugo y sus volátiles en 2 especies ancestralmente simpátricas con guayaba (*Doryctobracon areolatus* [Szepligeti] y *Utetes anastrephae* [Viereck]) y una especie exótica a la región neotropical del origen de guayaba (*Diachasmimorpha longicaudata* [Ashmead]). Se predijo que los machos de las especies simpátricas, con una oportunidad evolutiva para reconocer y evitar la guayaba, ni se alimentan de compuestos volátiles, ni responden a la guayaba. Se predijo que las hembras de las especies simpátricas iban a evitar el alimentarse sobre la guayaba, pero iban a responder a los volátiles en el contexto de la búsqueda de sitios de oviposición. Se predijo que machos y hembras de las especies exóticas iban a alimentarse y responder a la guayaba y la naranja. Se alimentaron universalmente sobre la naranja y se encontró que sus volátiles fueron atractivos. Ninguno de los machos, incluyendo los de la especie exótica, ocuparon las estaciones de alimentación de guayaba en situaciones de no-opción, pero los machos de *U. anastrephae* respondieron a los volátiles de guayaba. Contra lo esperado, las hembras de *D. areolatus* ocuparon las estaciones de alimentación de guayaba. En general, las respuestas a las naranjas eran más fuertes y más universal que las de guayaba. Las diferencias en las preferencias que mostraron las distintas especies entre la guayaba y la naranja son un paso adelante en la identificación de la base química de su atracción hacia las frutas. Estas preferencias proveen 1) más evidencia de que no todas las especies de frutas no-infestadas son igualmente atractivas y 2) una simple naranja

que es relativamente atractiva y fácil de obtener, con la cual se puede comparar lo atractivo de otras especies frutales en el futuro.

Palabras Clave: forrageo, control biológico, monitoreo, *Diachasmimorpha, Doryctobracon, Utetes, Anastrepha*

Chemical cues from parasitoid hosts and the substrates in and on which the hosts develop are important cues for orientation during foraging for oviposition opportunities, adult food and perhaps mating-convention-sites (Stuhl et al. 2011b; Vet & Dicke 1992; Godfray 1994). However, within any particular guild of parasitoids, which chemicals are attractive may vary between species or even the sexes of the same species. For example, females confronted with exotic hosts might not orient towards their chemical bouquets, even if they are otherwise suitable for their offspring. Even within a native guild exploiting the same host a specialist female might search for a cue that uniquely identifies the oviposition site (Vet & van Alphen 1985), while a generalist species might react to chemicals shared between various hosts (Godfray 1994). Thus the gender of an insect, its evolutionary history with a potential host and its degree of specialization could all influence its behavior in the presence of a particular host-associated odor.

Species of opiine braconids are often the most abundant and diverse elements in New World parasitoid guilds attacking *Anastrepha* spp. (López et al. 1999; Sivinski et al. 2000). In addition to pest population suppression performed by native species, various opiines have been introduced to attack invasive flies and these are sometimes mass-reared and inundatively released for area-wide control (Sivinski et al. 1996; Montoya et al. 2000; Rendon et al. 2006). All species examined feed as adults on a variety of foods including extrafloral nectar and hemipteran honeydew (Sivinski et al. 2006). Fruit or fruit juices are an obvious food source for a "fruit fly" parasitoid and several tephritid-attacking opiine braconids also thrive on juices seeping from injured fruits (Sivinski et al. 2006; Stuhl et al. 2011a). In addition to fruit juice being generally nutritious, feeding on fruit minimizes the costs and risks of separate foraging for food and oviposition/mating opportunities (Sivinski et al. 2006; Bernstein & Jervis 2008).

However, fruit juices vary in nutritional quality and sometimes contain compounds poisonous to certain Hymenoptera (Barker 1977). Two fruits, one toxic and the other nutritious, are commonly encountered by tephritid parasitoids throughout the Neotropics and subtropics. *Psidium guajava* L. (guava; Myrtaceae) originated in Mesoamerica (Popenoe 1974) and is a major host of *Anastrepha fraterculus* (Wiedemann)*, striata* Schiner*, suspensa* (Loew) and, in some environments, *obliqua* (Macquart). However, it is toxic to both sexes of adult braconid fruit fly parasitoids (Stuhl et al. 2011a). *Citrus sinensis* L., orange, comes from Asia (Scora 1975) and it and other citrus are infested in Mexico by *Anastrepha ludens* (Loew). Orange juice/pulp is a high quality food for adult fruit fly parasitoids (Sivinski et al. 2006; Stuhl et al. 2011a).

This dynamic, a toxic host-fruit, guava, and a non-toxic host-fruit, orange, allowed us to test the hypotheses that a toxic fruit might be discriminated against by historically sympatric parasitoid species on the basis of volatiles emitted by the juice. On the other hand, a historically allopatric species may not be able to identify a toxic fruit and both native and exotic female responses may be influenced by oviposition rather than feeding opportunities. Below we first introduce the tephritid parasitoids examined and then present in detail our hypotheses regarding their responses to purported feeding cues.

Parasitoids

In Florida, USA and Mexico exclusive of high altitudes (Sivinski et al. 2000), 2 native opiines and 1 introduced species are the most common parasitoids of pests such as *A. ludens* (Loew) (Mexican fruit fly), *A. obliqua* (Macquart) (West Indian fruit fly) and *A. striata* Schiner (guava fruit fly) (Ovruski et al. 2000). These species are: *Diachasmimorpha longicaudata* (Ashmead); *Doryctobracon areolatus* (Szepligeti) and *Utetes anastrephae* (Viereck).

Diachasmimorpha longicaudata was originally collected in the Indo-Philippine/Malaysian region attacking *Bactrocera* spp. and has been established in the New World for little more than half a century (Ovruski et al. 2000). They parasitize larvae in fruit on the tree and also forage in fallen fruit (Purcell et al. 1994; Garcia-Medel et al. 2007). *D. longicaudata* are attracted to by-products of fungal decomposition, such as ethanol and ethyl acetate (Greany et al. 1977), as well as the odors of both infested and uninfested fruit (Eben et al. 2000). Mating observed in nature occurred on and under host trees (J. Sivinski unpublished data). Males appear to emit a pheromone while resting on the upper surfaces of leaves, which they defend from rival males. Periodically they leave the foliage to investigate the ground from which virgin females might emerge.

Doryctobracon areolatus is a wide spread Neotropical native koinobiont parasitoid of late-instar *Anastrepha* spp., and rarely of *Rhagoletis* spp.,

larvae (Wharton et al. 1981; Aluja et al. 1990). *Doryctobracon areolatus*, unlike *D. longicaudata*, does not forage over fallen fruit but attacks larvae in fruit on the tree (Garcia-Medel et al. 2007). The few observations of sexual behavior in the field suggest that its mating system resembles that of *D. longicaudata* with aggregated males defending leaf-territories from which they emit a pheromone and periodically sally to the ground to search for emerging females (J. Sivinski unpublished data).

Utetes anastrephae is also a wide-spread neotropical-native koinobiont parasitoid of late-instar larvae, and one that often occurs in close sympatry with *D. areolatus* (Sivinski & Petersson 1997). *Utetes anastrephae* attacks larvae in hanging fruit, but because of a relatively short ovipositor is restricted to parasitizing hosts in smaller fruit (Sivinski et al. 2001). It appears to be able to coexist with *D. areolatus* because of its superiority in larval-larval intrinsic competition (Aluja et al. unpublished). Nothing is known about the mating system of *U. anastrephae*.

Hypotheses Regarding Adult Parasitoid Responses to Fruit-Juice Volatiles

While all the above are fruit-fly generalists, in the sense of multiple-hosts and multiple host-fruit, and all attack late instar larvae (Sivinski et al. 1996; Aluja et al. 2008), they differ in regions of origin relative to the fruit examined. Thus we predict there will be potential sexual and species-specific differences in their responses to fruits based in part on their value as an adult food (Stuhl et al. 2011a). In the specific predictions below "shared volatile components" refer to those present in both orange and guava and which are assumed for the purpose of formulating the hypotheses to be present in fruit in general. "Unique volatile components" are those found in only one fruit or the other and for the purpose of formulating the hypotheses serve to specifically identify that particular fruit. Two other assumptions are made in order to predict responses (flight bioassay) to fruit outside of the context of feeding. These are; 1) oviposition-seeking females of all species will respond to uninfested as well as infested fruit odors (Eben et al. 2000; Stuhl et al. 2011b), and 2) that males locate mate-encounter sites through fruit decomposition products since these will be closely associated with the emergence of virgin females from the soil (Greany et al. 1977)

Materials and Methods

Source of Parasitoids

Parasitoids were reared on larvae of *A. suspensa* that developed in an artificial diet obtained from the Florida Department of Agriculture and Consumer Services Division of Plant Industries, Gainesville, Florida, USA (FDACS 1995). *Diachasmimorpha longicaudata, D. areolatus* and *U. anastrephae*, were obtained from colonies at the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology (USDA-ARS-CMAVE), Gainesville, Florida, USA. *Diachasmimorpha*

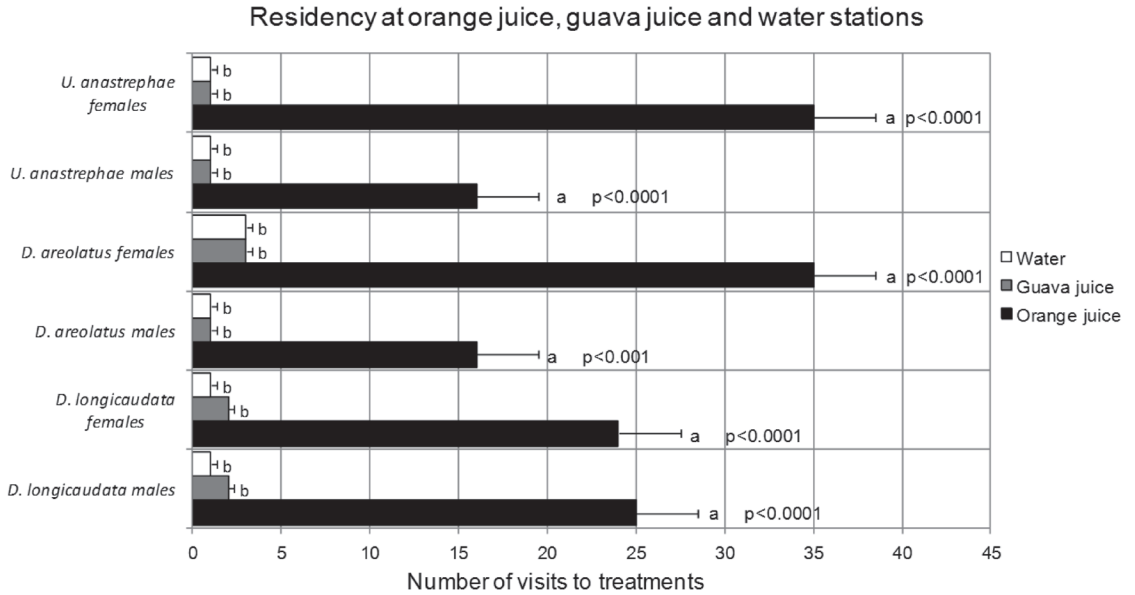


Fig. 1. Mean (±SE) parasitoid residency at orange juice, guava juice and water feeding stations. Means that share a letter are not significantly different.

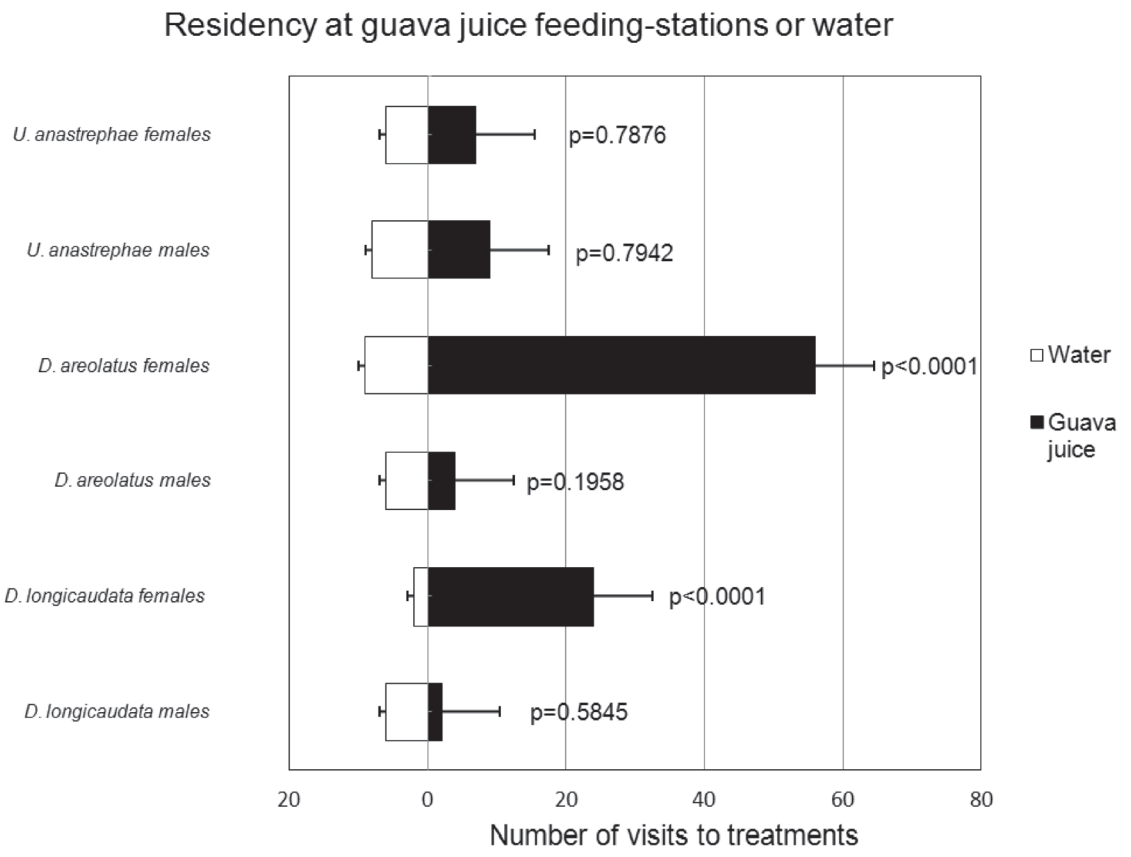


Fig. 2. Mean (±SE) numbers of parasitoids observed at either guava juice and water feeding-stations.

longicaudata had been in colony for ~10 years, *D. areolatus* ~2 years and *U. anastrephae* for ~1 year. Insects were reared in a temperature controlled chamber at 23 ± 5 °C, 60% RH, and 12:12 h L:D. Breeding colony adults were fed a diet of 10% sucrose solution.

Occupancy of Feeding Stations: Choice and No-Choice Tests

To determine the response of *D. areolatus, D. longicaudata* and *U. anastrephae* to various food sources, parasitoids were observed in a flight tunnel. Parasitoids had no prior exposure to the treatments. Ten males and 10 mated females 4-6-d old were starved 24 h prior to the experiment, then introduced into a flight tunnel. The flight tunnel was constructed of clear acrylic sheets 128 cm long × 31.8 cm × 31.8 cm and located inside a laboratory at CMAVE. Illumination was provided two 120 cm fluorescent bulbs suspended above the flight tunnel. The light source and the light emitted by the room lighting produced an illumination within the tunnel of ~1600 lux. The room temperature ranged from 28.7-28.8 ºC and humidity between 37.6-38.1% RH. The room air was pulled into the flight tunnel by using a Shaded Pole Blower (Dayton Electric Mfg. C., Niles, Illinois). This produced air flow within the tunnel through a charcoal filter and exhausted it into a hood. The exhaust was covered with screen to prevent insects from entering the tube. A baffle inside a tube that connected the downwind end of the tunnel with the exhaust system of the hood allowed for air flow adjustment. Air speed was maintained at 0.2 m/s. Previous studies performed by Messing et al. (1997) determined this speed to stimulate the best flight in *D. longicaudata*.

Three treatments consisting of guava juice, orange juice or water were utilized. Ripe guava was collected from a small orchard organically grown at USDA-ARS-CMAVE, Gainesville, Florida, USA. Guava juice was extracted from peeled ripe fruit by pressing the pulp through mesh to remove the seeds. The pulp and an equal amount of water were blended together. Juice was collected by filtering the blend through cloth to separate the solids from the juice. Orange pulp was prepared from organic fruit purchased from a local market then peeled, sectioned (~60 g) and sliced crosswise to expose pulp. Juice was manually extracted.

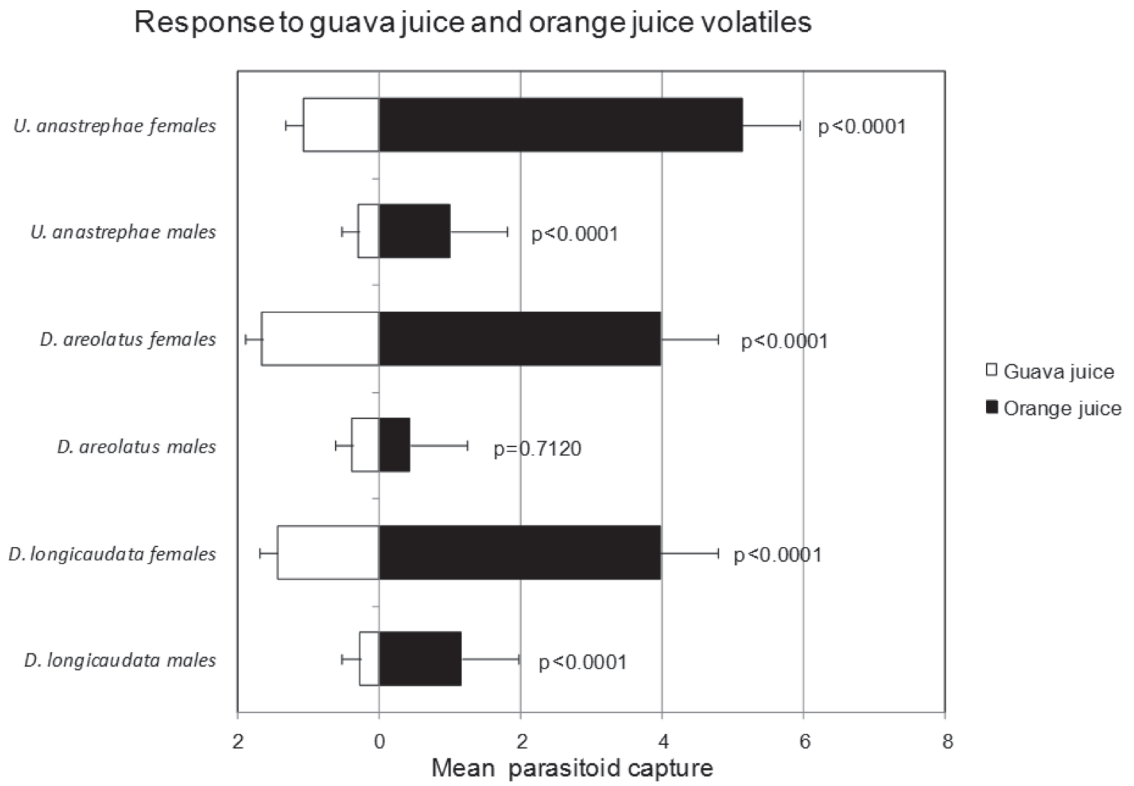


Fig. 3. Mean (±SE) numbers of parasitoids in flight-tunnels attracted to either orange juice volatiles or guava juice volatiles.

Saturated cotton balls containing 10 mL of liquid were suspended inside 60 mL soufflé cups (SoloHP47, Solo Cup Co., Lake Forest, Illinois) ~10 cm from the top of the flight tunnel using wire. There were 2 rows of 3 sites suspended and spaced 15 cm apart along the short axis and 30 cm apart along the long axis. The placement of the treatments was rotated clockwise over consecutive replicates and repetitions were rotated between the tunnels to account for positional effect. A total of 6 replicates were performed for each parasitoid species. Parasitoids were introduced into the flight tunnel through an opening in the top at the extreme downwind portion. Observations were made every half h beginning at 0900 h and ending at 1400 h. When an observation was made and an insect was noted on a treatment, it was removed and replaced with another of the same sex. Following this experiment, in which parasitoids had a choice of fruit juices, a similar set of experiments was conducted to compare responses to guava juice or water. Comparisons of guava juice, orange juice and water were made through Analysis of Variance with subsequent mean comparisons by Waller's test (SAS Inst. 2002). A paired t-test procedure was performed for the analysis of guava juice and water (SAS Inst. 2002).

Attraction to Fruit Juice Volatiles: Choice and No-Choice Tests

To distinguish between attraction and arresting, a flight tunnel bioassay was developed to determine the longer-distance (~1m) parasitoid responses. Ten males and 10 mated females 4-6 d were introduced into the flight tunnel as previously described. Treatments consisted guava and orange fruit juice and a water control. Two cylindrical stainless steel chambers (18.5 cm tall and 15 cm OD) with a stainless steel lid, a single union bulkhead inlet and a single outlet were used to contain our samples. To measure the response to a single fruit juice, one chamber contained the juice while the other held a blank control. For the comparison of fruit juices, each chamber contained a fruit juice. The treatments consisted of a saturated cotton ball containing 10 mL of liquid inside a 60 mL soufflé cup (Solo HP47). Clean air was separately passed over each of the two odor sources before emerging in the flight tunnel. Air flow into the fruit containers was controlled by adjustable flow meters (Aalborg Instruments, Monsey, NY) set at ~0.5 L/min. Treated air emerged into 2 insect traps located at the upwind end of the tunnel and placed midway between its ceil-

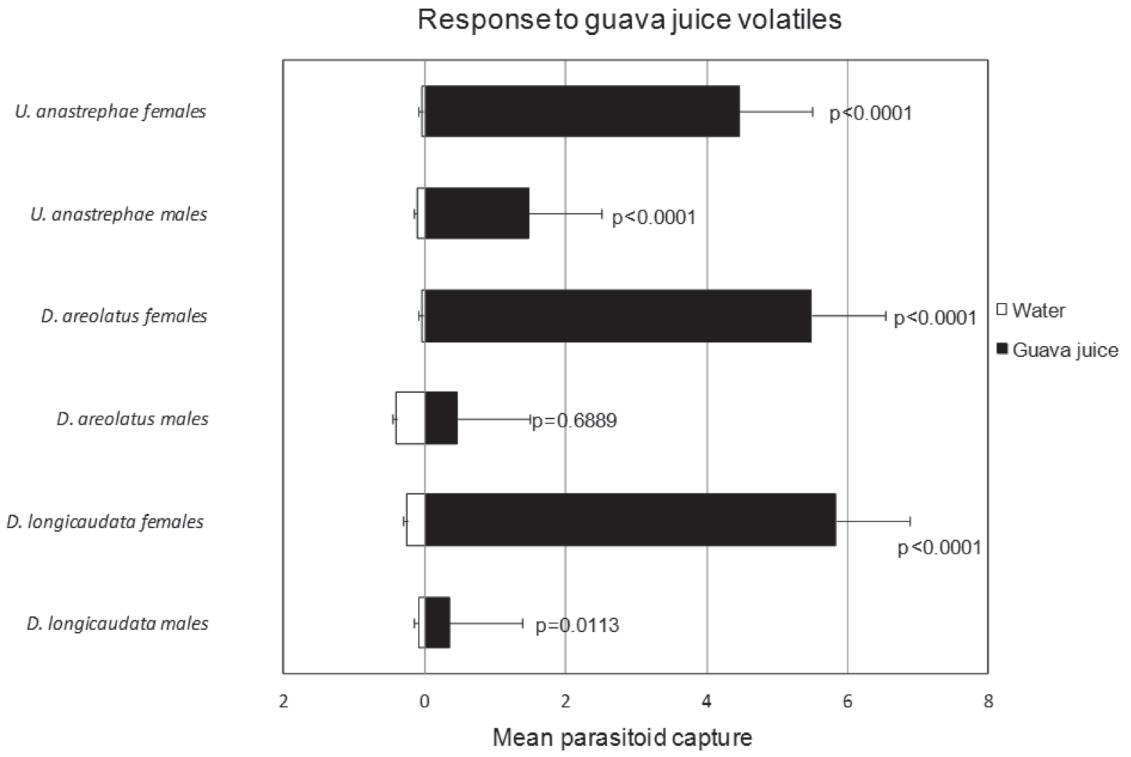


Fig. 4. Mean (±SE) numbers of parasitoids in flight-tunnels attracted to either guava juice volatiles or a water control.

ing and floor. These were constructed from two 64 dram clear polypropylene round-bottom Drosophila stock bottles (16004-066, VWR International, LLC, Radnor, Pennsylvania). A 10 mm hole was placed in the center of the cap to allow insects to enter the trap. The flight tunnel and its traps were checked every 30 min from 0900 to 1400 h. A positive response was recorded when there was a parasitoid inside the trap. The insect was removed from the trap and replaced with a naive insect (i.e., never exposed to fruit odors) from a stock cage where the original insects had been obtained. The position of the treatment and control were changed after each replication to prevent positional effects. There were 5 replicates each of type of fruit. Means were compared by a paired t-test procedure (SAS Inst. 2002).

Results

Occupancy of Feeding Stations Containing Fruit Juices or Water

When provided with feeding stations containing guava juice, orange juice or water, both sexes of all species were significantly more likely to be located at the orange stations than at those containing guava (Fig. 1). When given the more limited choice of water or guava juice, females of *D. longicaudata* and *D. areolatus* were observed more often at guava stations. However, female *U. anastrephae* and males of all 3 species had no preference for guava juice over water (Fig. 2).

Attraction to Fruit Juice Volatiles: Choice and No-Choice Tests

When presented the volatiles of orange juice or control volatiles collected from water, both sexes of all 3 species significantly selected those of orange juice (Fig. 3). When provided a choice of guava juice volatiles or water, all females were attracted to guava juice volatiles (Fig. 4). *Diachasmimorpha longicaudata* and *U. anastrephae* males also showed preference for guava juice while *D. areolatus* males chose equally between the treatments. Males and females of all 3 species were more attracted to the volatiles of orange juice than to those from guava juice (Fig. 5).

Discussion

Predicted residencies at food-stations and attractions to volatiles were often met but there were also several unfulfilled predictions. Both males and females of all 3 species chose to spend time feeding on orange juice when simultaneously presented a choice between feeding-stations with orange juice, guava juice or water. There were no

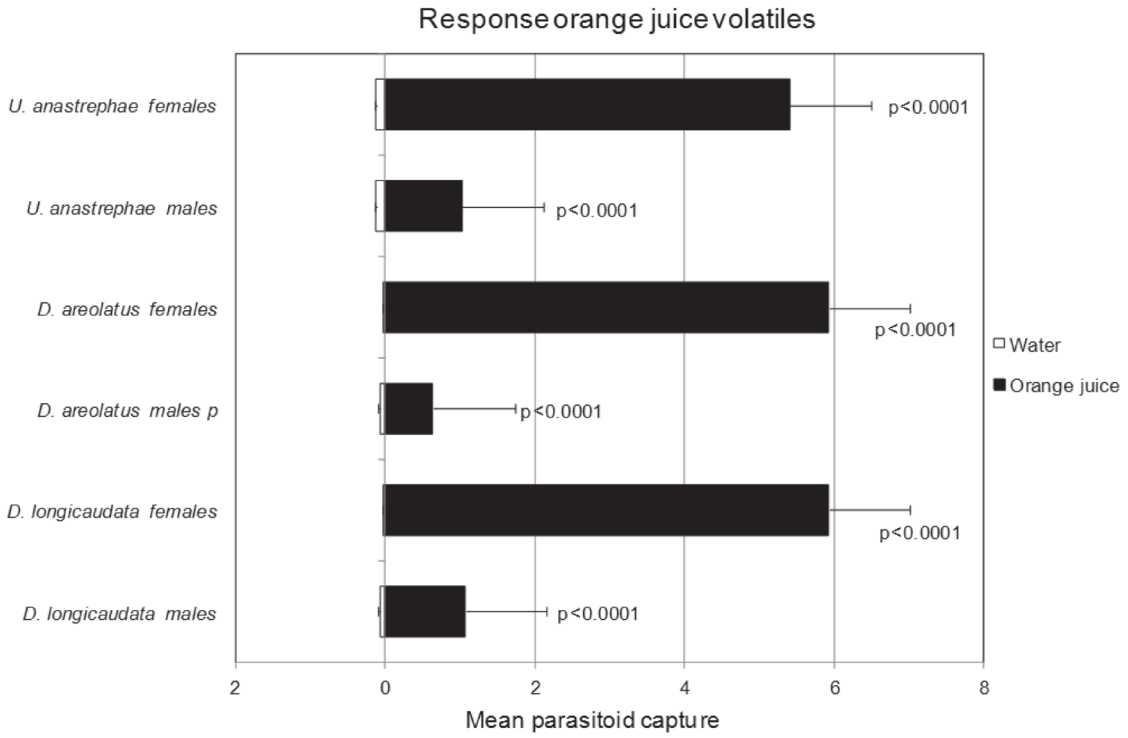


Fig. 5. Mean (±SE) numbers of parasitoids in flight-tunnels attracted to either orange juice or a water control.

differences between water and guava juice. However, when guava juice or water were the only choices, females of the originally Asian *D. longicaudata* and the Neotropical-native *D. areolatus* were significantly more likely to be located at the guava stations. Female *U. anastrephae* and males of all 3 species had no significant preference for guava juice over water. Because guava juice is toxic to *D. areolatus* and since guava and *D. areolatus* have presumably been sympatric over evolutionary time, female *D. areolatus* residency at guava even when no other food was available, was unexpected. Also unexpected was the rejection of guava juice by male *D. longicaudata.* This species has only recently become sympatric with guava and has had less opportunity to evolve distaste for the toxic fruit.

Males and females of all 3 species were significantly attracted to orange juice volatiles when presented with a choice of orange juice or water. As predicted, females of all 3 species responded positively to guava volatiles presumably because at least some of its constituents serve as cues to potential oviposition sites. However, males of *D. longicaudata* and *U. anastrephae* also showed positive responses to guava juice volatiles, albeit weak responses relative to those of females. Only *D. areolatus* males met the prediction that toxic guava would be recognized by its volatiles and rejected.

One of these failed predictions concerned the behavior of male *D. longicaudata.* Contrary to expectations males did not occupy guava feeding-stations but did respond, to a small but significant degree, to guava volatiles. It had been proposed that a lack of evolutionary history between *D. longicaudata* and guava would leave them unable to recognize a toxic food but this did not seem to be the case. Perhaps a yet unidentified toxin in guava might be found in Asian fruits as well. Earlier, Messing & Jang (1992) had captured female *D. longicaudata* in orange and guava baited traps hung in field cages but obtained no evidence of male attraction. Another failed prediction was that *D. areolatus,* having a history of sympatry with guava, would avoid feeding-stations with toxic food. This was the case for males, but not for females. It is possible that females seeking both oviposition and feeding sites might investigate feeding-stations not for nutrition but as possible host-locations. Finally, it was predicted that male *U. anastrephae,* like *D. areolatus*, would reject guava feeding-stations and not be attracted by guava volatiles. The first of these predictions was fulfilled, but males were attracted by guava volatiles. We have no ready explanation for this difference in responses but suggest that males might use guava volatiles to locate mates although they would not stay to feed if sexually active females

were not available. Nothing about the *U. anastrephae* mating system is known at this time.

Over all, it was clear that when given a choice between orange and guava both sexes of all 3 species were more likely to occupy orange feeding-stations and, with the exception of *D. areolatus,* more attracted to orange volatiles. Thus feeding and foraging behaviors are closely related to food quality. While the means, presumably chemical, by which food quality is recognized are not yet identified the present study reemphasizes the potential usefulness of fruit odors as components of an attractant. Augmentative parasitoid releases have promise as a resource for area-wide fruit fly control, particularly in urban environments and protected natural sites where insecticides cannot be repeatedly applied. However there are technical challenges associated with its use. One of these is the inability to easily and cheaply monitor the survival and dispersal of the released parasitoids. This need might be met by an effective trap baited with synthetic feeding and/or ovipositional cues.

There are at least 5 potential sources of opiine-attractive compounds: fruit, other adult foods such as honey dew or flower-nectar (e.g., acetophenone from *Lobularia maritima* L. in Rohrig et al. 2008; Sivinski et al. 2006; Sivinski et al. 2011; Wang et al. 2011), fruit fly larvae (e.g., para-ethylacetophenone in Stuhl et al. 2011b), the by-products of fruit fly infestation and decay (e.g., fungal-derived ethanol, acetaldehyde and acetic acid in Greany et al. 1977; 2-phenylethyl acetate unique to infested mango [*Mangifera indica* L.] in Carrasco et al. 2005), and adult-host semiochemicals (e.g., Prokopy & Webster 1978; Roitberg & Lalonde 1991). It seems possible that all of these, in one combination or another, might contribute to a useful monitoring system. The differences between the attractiveness of guava and orange are a step in the identification of the chemical basis of fruit-attractiveness. They provide 1) further evidence that not all uninfested fruit are equally attractive and 2) a relatively attractive and easily obtainable standard, orange, with which to compare the attractiveness of other fruit in the future. A hierarchy of opiine-attractiveness in fruit would substantially aid the identification of chemicals correlated to positive parasitoid responses (Messing & Jang 1992).

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