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A Compound Produced by Fruigivorous Tephritidae (Diptera) Larvae Promotes Oviposition Behavior by the Biological Control Agent *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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ABSTRACT Tephritid fruit fly parasitoids use fruit-derived chemical cues and the vibrations that result from larval movements to locate hosts sequestered inside fruit. However, compounds produced by the larvae themselves have not been previously described nor their significance to parasitoid foraging determined. We collected the volatiles from four species of tropical and subtropical Tephritidae: *Anastrepha suspensa* (Loew), *Bactrocera dorsalis* Hendel, *Bactrocera cucurbitae* Coquillett, and *Ceratitis capitata* (Wiedemann), representing two subfamilies (Dacinae and Trypetinae). Para-ethylacetophenone, an analog of a known tephritid parasitoid attractant, was a major constituent of all four, and was not associated with larvae of another acalypterate fly, *Drosophila melanogaster* Meigen, or with the calypterate *Musca domestica* L. It also was present in volatiles from whole, *A. suspensa* infested fruits of *Eugenia uniflora* (L.). Para-ethylacetophenone was not necessarily produced as a direct consequence of fruit consumption because it also was detected from larvae that developed in two artificial diets and in spent diets subsequent to larval development. Sensillae on both the antennae and ovipositor of the opiine braconid fruit fly parasitoid, *Diachasmimorpha longicaudata* (Ashmead) responded to the para-ethylacetophenone in larval volatiles and as a synthetic. Although a potential cue to foraging parasitoids, para-ethylacetophenone showed no long range (>1m) attractiveness to the adult female parasitoid, but did stimulate ovipositor-insertion and oviposition into both a natural (fruit) and an artificial (parafilm) substrate. Thus it may prove useful in colonizing and mass-rearing opine fruit fly parasitoids.

KEY WORDS *Diachasmimorpha longicaudata*, *Bactrocera*, *Anastrepha*, *Ceratitis*, para-ethylacetophenone

Host location in parasitoid Hymenoptera relies on a variety of visual, tactile, and chemical cues (Godfray 1994). The range at which these cues are perceived varies from many meters to actual contact (Vinson 1984), but hosts sequestered inside plant tissue, where they cannot be seen and chemical signatures might be blocked or masked, create a special set of foraging problems. However, parasitoids have evolved a number of tactics to deal with these difficulties, including searching visually for associated damage (Faeth 1990), sensing synomones (chemical signals produced by plants under attack) (Dutton et al. 2000), pinpointing infrared emissions from fruit (Richerson and Borden 1972), orienting to larval feeding sounds (Lawrence 1981), and even echolocate through antennal vibrations that place the inactive pupae of stem-boring Lepidoptera (Wäckers et al. 1998). Moreover, not all substrates are completely impervious to host-derived chemical cues, and in some cases host-refugia might be probed with the ovipositor for chemical evidence of occupancy (Vet and Van Alphen 1985).

Tephritid fruit fly larvae are hosts of the parasitoid, and the larvae are sequestered inside the fruit. While a variety of chalcidoids, diapriids, figitids, and ichneumonoids manage to parasitize Tephritidae (Lopez et al. 1999), braconids of the subfamily Opiinae typically are the most numerous and diverse members of the guilds attacking frugivorous species (Purcell 1998). Opiines are solitary, koinobiont, larvaland egg-prepupal, mostly endoparasitoids of Cyclorrhapha Diptera (Wharton and Marsh 1978). Several species are considered important regulators of fruit fly populations and have been introduced, and frequently established throughout the world. *Diachasmimorpha longicaudata* (Ashmead), the species used in our experiments, is one of the most widely employed (Ovruski et al. 2000). Adult females use their relatively long ovipositor to

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parasitize a number of second and third-instar larvae of various fruit fly species infesting a wide range of host fruits (Sivinski et al. 2001). The species originally was discovered in the Indo-Philippine region, where it attacks *Bactrocera* spp. (Wharton and Marsh 1978), and in 1947 was introduced into Hawaii for the control of oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Clancy et al. 1952). In 1972, *D. longicaudata* was established in Florida to control the Caribbean fruit fly, *Anastrepha suspensa* (Loew), and subsequently reduced populations by ≈40% in a 5-yr period of releases (Baranowski et al. 1993).

D. longicaudata and other fruit fly parasitoids use both chemical attractants (host-location cues) and arrestants (host-habitat cues). *D. longicaudata* is attracted to acetaldehyde, ethanol, and acetic acid released by a fungus that grows on decaying fruit (Greany et al. 1977a), to unidentified volatiles emitted by uninfested fruit (Eben et al. 2000) and acetophenone, a chemical originally identified from a floral volatile (Rohrig et al. 2008). Females of other opiines, such as *Fopius arisanus* (Sonan), are also attracted to fruit volatiles (Messing and Jang 1992, Eben et al. 2000, Altuzar et al. 2004), and *Psyttalia fletcheri* Silvestri, to decaying fruits and leaves of pumpkins and cucumbers (*Cucurbita* spp; Cucurbitaceae) (Messing et al. 1996). Earlier, Nishida (1956) had found that stem tissues of cucurbits are attractive to *P. fletcheri*, and Messing et al. (1996) suggested that the basis of the attraction was "green leaf volatiles", a suite of common leaf-derived compounds known to be attractive to other braconid species (Whitman and Eller 1990).

The opiine *Utetes canniculatus* (Gahan) (reported as *Opius lectus*), as well as the pteromalid fruit fly parasitoid *Halticoptera rosae* Burks, are arrested by the oviposition deterring pheromone of their *Rhagoletis* hosts and concentrate their searching on marked fruit (Prokopy and Webster 1978, Roitberg and Lalonde 1991). Other apparent arrestants are produced by uninfested fruit and can be used to stimulate oviposition in opiines such as *Doryctobracon areolatus* (Szepligeti) (Eitam et al. 2003).

It is apparent that fruit infested with host larvae are sources of semiochemicals important to parasitoid foraging. However, there is a source of kairomones not previously considered, tephritid larvae themselves. In the present paper we 1) described the composition of volatiles emitted by four species of tephritid larvae in three genera (*Anastrepha* [subfamily Trypetinae], *Bactrocera* and *Ceratitis* [subfamily Dacinae]) and compared them to nontephritid Diptera; 2) compared the above larval volatiles to those derived from two artificial diets before and after their use as developmental substrates; 3) analyzed the volatiles from Surinam cherry (*Eugenia uniflora* L.) fruit occupied by *A. suspensa* larvae to discover if larval volatile components escape the surface of infested fruit; 4) examined through electroantennograms and electroovipositorgrams the responses of sensillae on the antennae and ovipositor of *D. longicaudata* to para-ethylacetophenone, a major larval volatile component and chemical analog of the known floral-derived attractant acetophenone (see above); 5) determined, in a flight tunnel, if para-ethylacetophenone attracted either male or female *D. longicaudata;* 6) tested the capacity of para-ethylacetophenone to act as an *D. longicaudata* arrestant/oviposition stimulus, both by itself and in the presence of host fruit; and 7) examined if *D. longicaudata* was stimulated by para-ethylacetophenone to oviposit into a device used to mass-rear of opiine braconids destined for augmentative release.

Materials and Methods

Volatile Collection. *A. suspensa* larvae were obtained from a mass reared colony derived from wild stock several years previously and maintained at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS DPI 1995), Gainesville, FL.Volatiles were collected (see below) from 10 ml of third-instar larvae, 25 ml of artificial diet (FDACS DPI 1995) before use by larvae, and 25 ml of diet subsequent to larval development. In addition, volatiles from 0.75 liters of field-obtained host fruit, *E. uniflora* L., were collected and subsequently 5 ml of mature larvae were removed from the same fruit, in total, and their volatiles collected. Volatile collections also were taken from intact and uninfested individual *Psidium guava* L. and *E. uniflora* (Myrtaceae) host fruits. All *A. suspensa* related collections were performed at the USDA–ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL (CMAVE). *Bactrocera dorsalis* and *Ceratitis capitata* (Wiedemann) larvae also were taken from mass-rearing artificial diets (FDACS DPI 1995), and as above volatiles were collected from larvae outside of diet and then from diet both before and subsequent to its use as a rearing medium. In the case of *Bactrocera cucurbitae* (Coquillett) larval volatiles were collected from larvae, used artificial diet, and infested fruit (zucchini, *Cucurbita pepo* L.) gathered from the field in the vicinity of Honolulu, HI. Volatiles from *B. dorsalis*, *B. cucurbitae*, and *C. capitata* all were collected at the USDA–ARS, Tropical Plant Pests Research Unit, Honolulu, HI. Volatiles were collected onto a HayeSepQ filter (Hayes Separation Inc., Bandera, TX) and later analyzed using gas chromatography-mass spectrometry at CMAVE. In addition to Tephritidae, volatiles were collected from 5 ml of another acalypterate fly larvae, *Drosophila melanogaster* Meigen (Drosophilidae) and 10 ml of a calypterate fly larvae, *Musca domestica* L. (Muscidae) both obtained from long-maintained colonies at the CMAVE.

Volatiles were collected using a head space collection technique (Heath and Manukian 1992). This technique was used for volatiles collected from larvae and fruit. Samples were placed in a glass volatile collection chamber (34 cm long and 4 cm outside diameter) with a glass frit inlet and a glass joint outlet and a single port collector base. Dry charcoal filtered air was pushed into one end of the chamber and over the larvae and exited the chamber via a vacuum system. The air then passed through a volatile collection filter

containing 50 mg of HayeSepQ. Filters were eluted with 175-μl methylene dichloride to remove volatile components. Individual larvae were selected from the artificial diet fruit by using soft forceps until a volume of 10 ml was obtained. Larvae were kept moist using deionized water. There were five replicates for each species and rearing medium.

Identification of Larval Volatiles. Volatile analysis was performed by electron impact gas chromatography-mass spectrometry (EI GS-MS) by using an HP 6890 gas chromatograph coupled to an HP 5973 MS detector. One μl of the sample was injected using a splitless injector (injector purge at 0.5 min) onto an HP-5MS dimethylpolysiloxane column (30 m × 250 μm (i.d.) × 0.25-μm film, Agilent Technologies, Palo Alto, CA). The GC oven was programmed from 35°C (1.0 min hold) to 230°C at 10°C/min. Helium was used as a carrier gas at 1.2 ml/min. Volatiles were identified by comparison of mass spectra 1) with mass spectra libraries (NIST and Department of Chemical Ecology, Göteborg University, Sweden) and 2) with mass spectra and retention times of authentic standards.

Electroantennogram (EAG) and Electroovipositorgram (EOG) Measured Response in *D. longicaudata* to a Major Larval Volatile. *D. longicaudata* were obtained from consecutive generations reared in colonies at CMAVE, Gainesville, FL in conjunction with USDA–APHIS/PPQ, Gainesville, FL. The colony had been maintained for ≈10 yr with occasional (≈yearly) introductions of feral individuals collected throughout southern Florida. Parasitoids were reared on *A. suspensa* larvae obtained from FDACS DPI Gainesville, FL.

To determine if female *D. longicaudata* had a sensory response to para-ethyl acetophenone, 25 ng/μl of para-ethylacetophenone (Sigma-Aldrich) in 100% ethanol (USI Chemical Company, Tuscola, IL) and the natural compounds collected from late-instar *A. suspensa* larvae were exposed to both the parasitoids antennae and ovipositor by using an electroantennographic detector.

Extracts were analyzed with a GC interfaced to both flame ionization (FID) and electroantennograph detectors. In this manner, antennal and ovipositor responses were matched with FID signals for compounds eluting from the GC. Volatile extracts were prepared in the manner described above, and 1-μl aliquots were analyzed on a Hewlett–Packard (HP) 5890 Series II gas chromatograph equipped with an HP-5 column (30 m × 0.32 mm ID × 0.25 mm) (Agilent, Palo Alto, CA). The oven temperature was held at 40°C for 5 min, then programmed to increase to 10°C /min to 220°C and held at this temperature for 5 min. Helium was used as a carrier gas at a flow rate of 2.0 ml/min. A charcoal filtered humidified air stream was delivered over the antenna is at 1 ml/min.

Antennae from female wasps were excised by grasping the scape at its base with a jeweler's forceps (No. 5, Miltex Instrument Company Inc, Switzerland). The extreme distal and proximal ends of the antennae were held between electrodes (Syntech, Germany) in conductivity gel (Parker labs, Fairfield, NJ). Ovipositors were excised by grasping the base with the same jeweler's forceps. The valvulae were pulled away from the abdomen, leaving a nerve bundle exposed. Ovipositors were placed between the electrodes as described above, with the proximal portion placed on one fork but the distal tip protruded past the other. This prevented sensory structures from being encased in conductivity gel and allowed exposure to the volatile compounds. The electroantennal detector (EAD) and FID signals were concurrently recorded with a GC-EAD program (Syntech EAGPro, Germany) that analyzed the amplified signals on a personal computer.

Ovipositor Insertion Into Treated and Untreated Cattley Guava Fruit. *A. suspensa* pupae previously parasitized by *D. longicaudata* were placed in a 30.5 by 30.5 by 30.5-cm cage constructed of sheet acrylic and screen (13 by 13 lines per square cm). The newly eclosed parasitoids were provided with a 10% raw, unfiltered and uncooked honey and water solution presented in a 118-ml plastic cup. The insects obtained moisture on a cotton dental wick (braided roll), (Richmond Dental Company, Charlotte, NC) inserted through the lid. A 118-ml cup provided water by the same method. Each repetition consisted of one cage containing 100 male and 100 female parasitoids ≈4 d old with no prior exposure to fruit or larvae. Twenty-four h before fruit exposure the insects were transferred to 20- by 20- by 20-cm^2 cages constructed of sheet acrylic and screen (13 by 13 lines per square cm). Food and water were provided by the same method as previously described.

Ripe cattley guava (*Psidium cattleianum* Sabine), a principal host of *A. suspensa* in Florida and one in which *A. suspensa* is heavily parasitized by *D. longicaudata* (Sivinski 1991), was collected from trees grown at CMAVE, Gainesville, FL. Each repetition used two mature fruits. One fruit was treated with 25 ng/μl of para-ethylacetophenone (Sigma-Aldrich, St. Louis, MO), a major component of larval volatiles (see results), in 100% ethanol (USI Chemical Company, Tuscola, IL). This concentration was chosen on the basis of the positive response by *D. longicaudata* to volatile acetophenone, a floral compound with a similar chemical structure (Rohrig et al. 2008). The para-ethylacetophenone in ethanol was applied to the entire circumference of the fruit with fine painter's brush and allowed to dry before presentation. This allowed for the ethanol to evaporate. The second fruit was brushed with 100% ethanol applied in the same manner. Each fruit was presented to the parasitoids on top of an inverted 60-ml plastic cup placed 8 cm apart on the floor of the cage. Each repetition consisted of one fruit treated with para-ethylacetophenone in ethanol and another treated with ethanol alone. The insects were observed for a period of 10 min; complete insertion of the ovipositor into the fruit was noted as a positive response. Ten replicates were performed. Numbers of insertions in the two types of fruit were compared by Wilcoxon paired sample test (Zar 1974).

Oviposition Device Choice Test. Parasitoids were allowed to oviposit into one of five differently treated "oviposition devices" commonly used for *D. longicaudata* mass-rearing. The oviposition device contained

third-instar *A. suspensa* larvae with treated Parafilm (American National Can, Menasha, WI). The devices were based on a 7.5-cm-diameter plastic embroidery ring (1004-W025, Westex Corp., Union, NJ), with a 15- by 15-cm piece of organdy cloth placed on one half of the open ring. Five ml of third-instar *A. suspensa* larvae, thoroughly washed with water to remove diet particles, were placed in the center of the cloth. An additional piece of organdy cloth along with a 15- by 15-cm piece of Parafilm was placed over the larvae. The ring was then assembled, sandwiching the larvae inside.

When the treatment included exposure to compounds from the surface of a fruit, a ripe commercially produced 'Bartlett' pear, *Pyrus communis* L. subsp. *communis*, was used as the source. *Pyrus communis* is the standard fruit used to provide oviposition cues for rearing the opiine tephritid parasitoid *D. areolatus* in the laboratory. The fruit was wrapped with Parafilm 24 h before allow for absorption of odors (Eitam et al. 2003). Other treatments were applied directly to the Parafilm and the five treatments were as follows: no treatment; fruit compounds; 20 μl of para-ethylacetophenone solution (25 ng/μl of para-ethylacetophenone [Sigma-Aldrich] in 100% ethanol [USI Chemical Company, Tuscola, IL]); fruit compounds and 20 μl of para-ethylacetophenone + ethanol solution; and 20-μl 100% ethanol.

Ten female parasitoids ≈3–10 d old were placed in a 473-ml clear polypropylene container (RD16, Placon Products, Madison, WI). A 7.5-cm-diameter hole was placed in the center of the lid and replaced with Organdy cloth hot-glued into place. This allowed for the parasitoids to oviposit through the top of the container. An oviposition device with one of the Parafilm treatments was placed face down on the organdy lid. Observations of the number of insects on the underside of the lid and parasitoids probing with their ovipositors were recorded as separate data points at 1-min intervals for a period of 15 min.

After 15 min the larvae from each treatment were placed in moistened vermiculite inside a 15-mm plastic petri dish (3488-B28, Thomas Scientific, Swedesboro, NJ). After ≈12 d, 100 pupae were transferred to a 473-ml plastic container with an organdy lid to allow air circulation and held for 5 wk or until eclosion. Percent parasitism (number of parasitoids per number of *A. suspensa* pupae) was then calculated. There were 19 replicates. Percent data were transformed by taking the arcsines of their square roots. Statistical analysis was through Analysis of Variance with subsequent mean comparisons by Waller's test (SAS Institute 2002).

Flight Tunnel Bioassays. A flight tunnel bioassay was developed to determine the longer-distance response of *D. longicaudata* to fruit treated with either a para-ethylacetophenone solution or a fruit with ethanol alone. The flight tunnel was constructed of clear acrylic sheets and measured 128 by 31.8 by 31.8 cm and located inside a laboratory at the CMAVE, Gainesville, FL. Illumination was provided by 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 was 28.7 to 28.8°C and 37.6 −38.1% RH. Air flow within the tunnel was produced by a Shaded Pole Blower (Dayton, Niles, IL) that pulled outside air into the tunnel through a charcoal filter and exhausted into a hood. The exhaust end was screened to prevent insects from entering the tube. Airflow could be adjusted by the use of a baffle inside a tube that connected the downwind end of the tunnel with the exhaust system of the hood. Air speed was maintained at 0.2 m/s. Previous studies performed by Messing et al. (1997) determined this to be the speed that most stimulated flight in *D. longicaudata.*

Two 3.8-liter glass jars fitted with a metal lid containing two brass hose fittings contained the fruit and allowed air to pass over two odor sources and emerge separately in the flight tunnel. Air flow into the fruit containers was controlled by an adjustable flow meter (Aalborg Instruments, Monsey, NY) set at ≈0.5 liters/min. Treated air emerged into two insect traps located at the upwind end of the tunnel and placed midway between its ceiling and floor. These were constructed from 40-dram clear plastic snap cap vials (Thornton Plastics, Salt Lake City, UT). A 10-mm hole was placed in the center of the cap to allow insects to enter the chamber. The wind tunnel was checked every .5 h from the period of 0900–1400 hours. 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 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 five replicates each of two different para-ethylacetophenone concentrations (25 and 50 ng/μl of para-ethylacetophenone in 100% ethanol) applied to two different, but related, host fruits both heavily parasitized by *D. longicaudata* in the field (*P. cattleianum* and *P. guajava* L. [common guava]). Dilutions were chosen on the basis of concentrations used with positive results in previous oviposition experiments, (this paper) and experiments with a related compound, acetophenone (Rohrig et al. 2008). All were compared with species of fruit to which it was applied. Analysis of data began with multivariant analysis of variance (ANOVA) (SAS Institute 2002). When variables proved to have an insignificant effect on numbers of males and females captured, data were pooled and pair-wise comparisons of responses to treated and control fruit accomplished with the Wilcoxon paired-sample test (Zar 1974).

Results

Identification of Larval Volatiles. Para-ethylacetophenone was consistently a major volatile component of all tephritid larvae, regardless of species or larval diet (Fig. 1). The compound was not identified in either the larvae of a nontephritid acalypterate species (*D. melanogaster*) or a calypterate species (*M. domestica*). Para-ethylacetophenone was detected in both

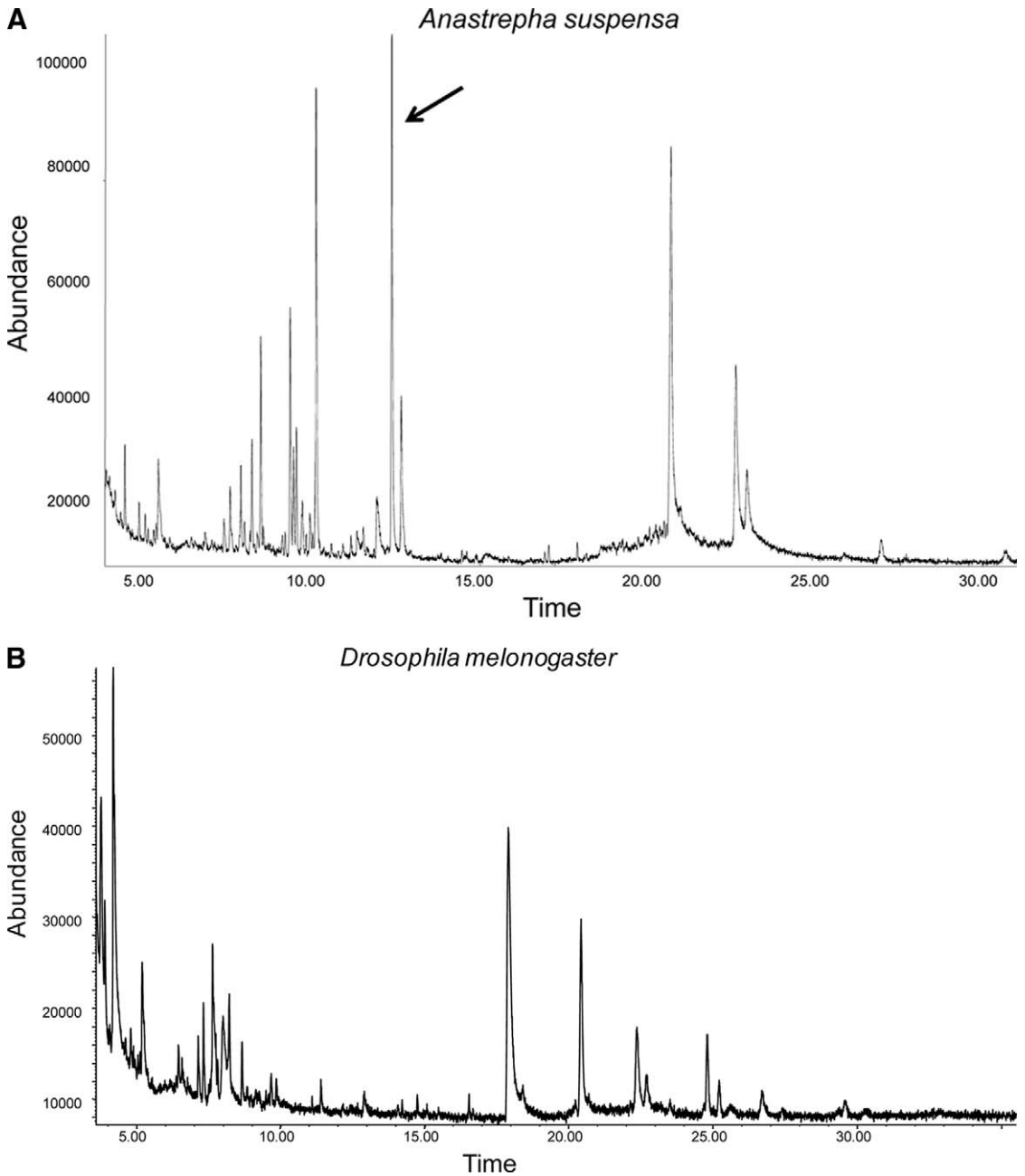

Fig. 1. A) Chromatogram showing the presence of para-ethylacetophenone (arrow and notation indicating the 12.1 min retention time) among the constituents of a volatile sample taken from *A. suspensa.* B), Para- ethylacetophenone is not present in *D. melanogaster* sample.

the used artificial diets and from *E. uniflora* fruit infested with *A. suspensa* larvae but with intact skin. It was not detected in either the unused artificial diets or the uninfested *P. guajava* or *E. uniflora.* In summary, all tephritid larvae and used larval developmental-substrates emitted para-ethylacetophenone, but neither of the other two flies or the unoccupied larva-substrates tested did so.

Electroantennogram and Electroovipositorgram Responses of Parasitoid Antennae and Ovipositor to a Major Larval Volatile Component. Sensillae on the antennae (Fig. 2) and ovipositor (Fig. 3) of *D. longicaudata* responded to synthetic para-ethylacetophenone.

Ovipositor Insertion in Treated and Untreated Fruit. Female *D. longicaudata* inserted their ovipositors significantly more frequently into *P. cattleianum*

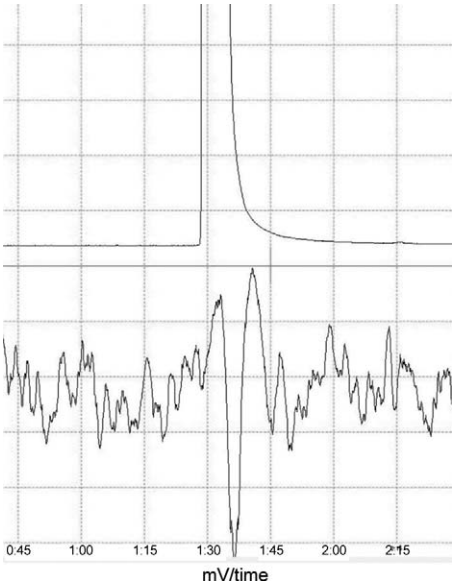

Fig. 2. The Gas Chromatography (GC) - Electroantennographic Detection (EAD) response of *D. longicaudata*'s to 25 ng/μl of synthetic para-ethylacetophenone. The top peak represents the flame ionization detection (FID) of para-ethylacetophenone; the bottom peak represents the EAD response of *D. longicaudata*'s antenna to the presence of para- ethylacetophenone.

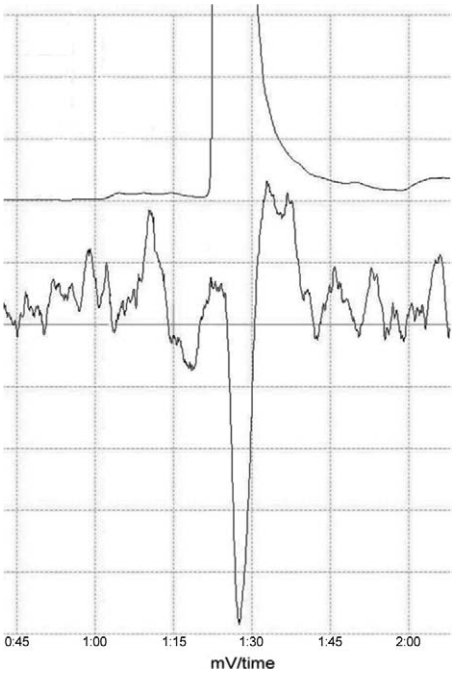

Fig. 3. The Gas Chromatography (GC) - Electroovipositorgram Detection (EOD) response of *D. longicaudata*'s ovipositor to 200 ng/μl of synthetic para-ethylacetophenone. The top peak represents the flame ionization detection (FID) of para-ethylacetophenone; the bottom peak represents the EOD response of *D. longicaudata*'s ovipositor to the presence of para- ethylacetophenone in 100% ethanol.

treated with a dilution of para-ethylacetophenone than controls brushed with ethanol alone (mean [treated] = 18.1 (SE = 2.3) versus mean [control] = 5.5 (SE = 1.7); (T = 7.64; df = 10; $P < 0.0001$) (Fig. 4).

Oviposition Device Choice Test. There were no significant differences in either the number of females observed landing on the variously treated oviposition devices ($F = 0.39$; df (model) = 4; df (error) = 70; $P = 0.81$). However, there were significant differences in the number of oviposition insertions per female landed ($F = 2.67$; df (model) = 4; df (error) = 70; $P < 0.04$; Fig. 5a), with para-ethylacetophenone + ethanol, para-ethylacetophenone + ethanol + fruit, and ethanol alone all having higher values than the control. "Fruit" (Parafilm wrapped for 24 h around a ripe pear) did not differ significantly from the control.

In terms of actual oviposition, as determined by the mean numbers of eclosing adult parasitoids in each treatment, only para-ethylacetophenone + ethanol + fruit was significantly different from the untreated control ($F = 1.74$; df (model) = 4; df (error) = 90; $P = 0.15$; Fig. 5b). Interestingly, we found that ethanol-treated Parafilm continued to emit volatiles for at least 18 h. This was confirmed by volatile collection and GC-MS analysis of the Parafilm after the 18 h period. (C. S., unpublished data). This long period of emission was unlikely to be the case in the previous experiment where 100% ethanol was applied directly to the fruit and quickly evaporated.

Flight Tunnel. Neither male nor female captures were significantly influenced by the dose-response of para-ethylacetophenone or the species of fruit it was applied to. Capture data were thus pooled and compared solely on the basis of whether or not para-ethylacetophenone was applied. In neither males nor females were there significantly different responses to treated or control fruit. Given the lack of significance, the response data were further pooled and male and female captures compared across fruit species, treatment, and dilution of para-ethylacetophenone (mean [male] = 3.5 [SE = 0.43] versus mean [female] = 11.0 [SE = 0.50]). Significantly more females than males were captured (T = 0, $n = 40$, $P < 0.001$).

Discussion

Para-ethylacetophenone is a chemical analog of acetophenone, a floral-derived compound that is attractive to female, but not male, *D. longicaudata.* In addition it was a major constituent of the volatiles produced by larvae of three genera from two subfamilies, Dacinae and Trypetinae, of tropical and subtropical Tephritidae and thus a chemical that might be expected to mediate female opiine behavior. It appeared to be particularly associated with fruit flies because it was not found in larvae of another acalypterate fly, *D. melanogaster*, or in the calypterate *M. domestica.* However, it was not produced as a consequence of fruit consumption, because it was detected from *A. suspensa* larvae that developed in both fruits

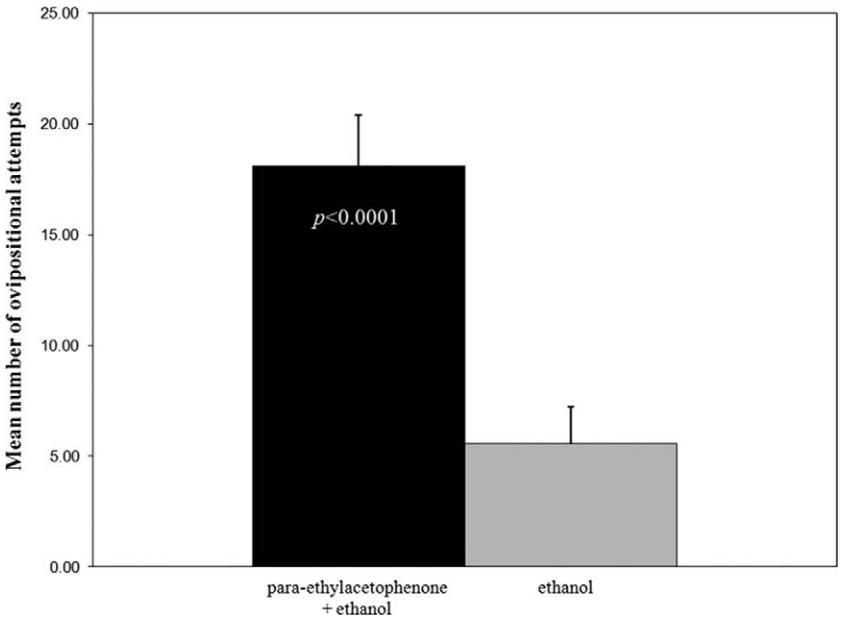

Fig. 4. Mean number of ovipositional attempts of *D. longicaudata* into treated *Psidium cattleianum* fruit. The fruit was treated with 25 ng/μl of synthetic para-ethylacetophenone + ethanol and ethanol alone.

and artificial diet and also from *B. dorsalis* and *C. capitata* reared on artificial diets or from guava fruit.

Although collected in the present work as a volatile, it is presumably also may have been present in the fluid surrounding larvae inside fruit and so could act as a cue to foraging parasitoids identifying a suitable fruit-microhabitat in which to search. If a chemical gradient occurs within infested fruit it could lead ovipositor-probing parasitoids to the larvae themselves. Because para-ethylacetophenone was emitted across the surface of intact but infested *E. uniflora*, it could in principle serve as a longer-distance attractant as well. There was no evidence that this was the case, but it did act as an oviposition stimulant when applied to *P. cattleianum* fruit.

In a yet smaller experimental arena, where the entire ceiling consisted of a treated artificial-oviposition device, there were no differences in the numbers of females present on the various devices but the numbers of oviposition insertions per female were greater in treatments that included both para-ethylacetophenone and an ethanol dilutant. Because ethanol is known to attract *D. longicaudata* to decaying fruit, (Greany et al. 1977a), it was not clear that para-ethylacetophenone played any important role in this particular result. When comparing the number of parasitoids emerging from the different cohorts of exposed larvae, ethanol alone did not differ significantly from the control, whereas ethanol + para-ethylacetophenone + fruit showed significance.

Taken together, these experiments suggest that para-ethylacetophenone is not a powerful attractant but might be characterized as a cue that stimulates ovipositor probing and oviposition, perhaps mediated in part by sensillae on the ovipositor.

Sensillae on both the antennae and ovipositor responded to para-ethylacetophenone. Although host location, determination of host suitability through ovipositor chemosensation, or both have been indirectly supported (Le Ralec et al. 1996), only recently has a gustatory response of an inserted ovipositor to host haemolymph been unequivocally demonstrated (van Lenteren et al. 2007). Two types of sensillae have been located on the ovipositor of *D. longicaudata* (Greany et al. 1977b). Lawrence (1981) examined oviposition behavior in *D. longicaudata* (then *Biosteres longicaudatus*) and concluded that dead or anesthetized larvae in the laboratory did not elicit ovipositor probing by foraging females. She suggested vibrations produced by larval activity were the principal means of host location. Dead or anesthetized larvae were approached even more frequently than active larvae and at the beginning of "nonrandom searching" after host encounter the ovipositor "quivers" from side to side. This movement might be performed to sample an extended airspace for volatiles. While vibration may play an important role in host location, we found that *D. longicaudata* probed fruit in the complete absence of larvae, particularly if para-ethylacetophenone was present.

Para-ethylacetophenone, other than that derived from host larvae, sporadically occurs in the chemical environment of some tephritid flies and their parasitoids. It has been identified as a component of the volatiles from intact oranges (*Citrus sinensis* L.) where it elicits an electroantennogram response from *C. capitata* (Hernandez et al. 1996). Its role, if any, in the biology of fruit fly larvae is unknown, although a number of ketones are components of insect defensive secretions and semiochemicals (Forney and Markovetz 1971).

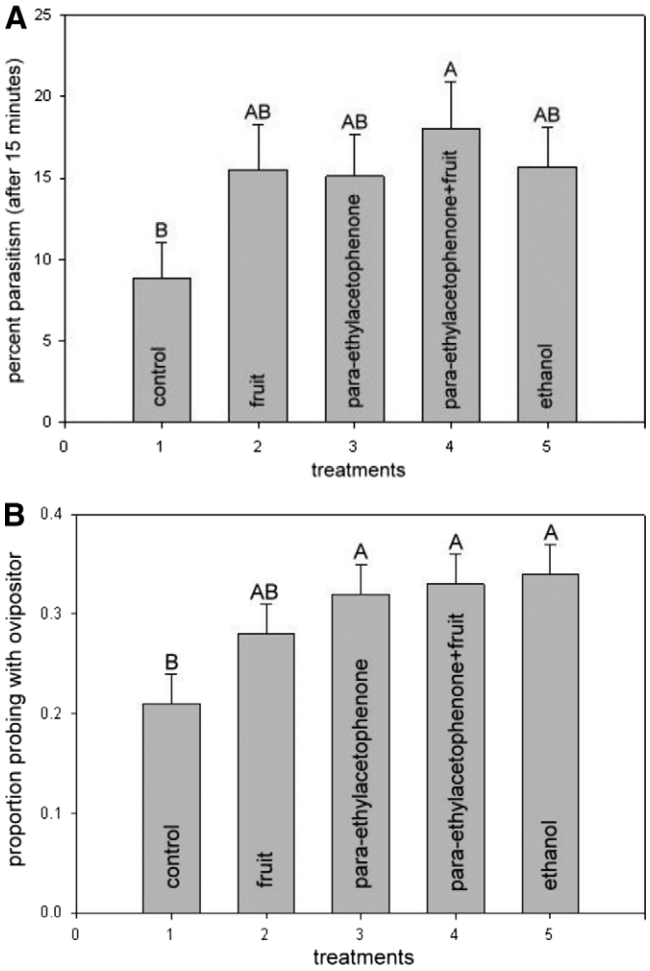

Fig. 5. A) Proportions of females present on artificial oviposition devices containing larvae of *A. suspensa* that were inserting their ovipositors. Parafilm sheets included in the devices were treated in the following manners: 1- untreated control; 2- exposure to pear fruit (*Pyrus communis*); 3- application of para-ethylacetophenone + ethanol; 4- application of para-ethylacetophenone + ethanol + exposure to *P. communis*; 5- application of ethanol alone. Means with shared letters are not significantly different. B) Percent parasitism of larvae included in the above described oviposition devices. Means with shared letters are not significantly different.

Although it is possible, perhaps likely, that chemosensillae on the ovipositor would be gustatory in function, sensing compounds present in fluids such as insect hemolymph or fruit juice; we cannot rule out that they sense volatiles escaping from the surface of infested fruit. We propose that a positive volatile response would be consistent with a positive gustatory response, but that a negative volatile response would require the additional experiment of exposing the ovipositor to para-ethylacetophenone in a fluid.

Compounds that arrest foraging parasitoids and stimulate oviposition have several potential uses in control of pest tephritids, particularly in terms of mass rearing. While *D. longicaudata* readily accepts host larvae presented in a number of substrates and enclosed under numerous types of coverings, not all opines are equally obliging. For example, *D. areolatus* is the most widespread, and typically the most abundant, endemic fruit fly parasitoid in Latin America (Ovruski et al. 2000). With the recent spread of the West Indian fruit fly, *Anastrepha obliqua* (Macquart), to new islands in the Caribbean there has been an effort to introduce natural enemies as part of an integrated management scheme (Palanchar et al. 2009). *D. areolatus* was a leading candidate because it develops well in *A. obliqua*, and in mainland habitats commonly co-occurs naturally with another opine, *U. anastrephae*, a species often already present in Caribbean environments (Lopez et al. 1999). This frequently encountered sympatry suggested that the new species would not displace the original and that *D. areolatus* would be suited to the new conditions (J. S., unpublished data). While *D. areolatus* was eventually introduced into Puerto Rico and the Dominican Republic the effort expended by USDA–APHIS to produce the parasitoids was relatively high (J. S., unpub-

lished data). If there were a synthetic suite of chemicals that could be quickly and economically applied to the rearing devices, much of the cost and labor required to rear *D. areolatus* could be eliminated. Perhaps such a treatment would include para-ethylacetophenone as well as fruit volatiles and be used for the rearing of still other species, such as some tephritid attacking Figitidae (Aluja et al. 2008), that attain higher rates of parasitism or are easier to colonize in the presence of chemical cues associated with fruit and larvae.

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