FEEDING ON METHYL EUGENOL AND FAGRAEA BERTERIANA FLOWERS INCREASES LONG-RANGE FEMALE ATTRACTION BY MALES OF THE ORIENTAL FRUIT FLY (DIPTERA: TEPHRITIDAE)

TODD E. SHELLY
USDA-APHIS, P.O. Box 1040, Waimanalo, HI 96795

ABSTRACT
Males of the oriental fruit fly, Bactrocera dorsalis (Hendel), are strongly attracted to methyl eugenol. Recent evidence indicates that treated males fed methyl eugenol have higher mating success and signaling (wing-fanning) activity than control (unfed) males. Chemical analyses have further shown that metabolites of methyl eugenol are incorporated into the male sex pheromone, and laboratory tests revealed that, at least over short distances (<2 m), the pheromonal signals of methyl eugenol-fed males are more attractive to females than those of unfed males. The main goal of the present study was to determine whether feeding on methyl eugenol or flowers of Fagraea berteriana A. Gray that contain a methyl eugenol-like compound increases the long-distance attractiveness of male B. dorsalis under field conditions. Male aggregations, composed of either treated or control males, were established on orange trees, females were released from a central point (12 m from the male groups), and male wing-fanning and female visitation were recorded. For both methyl eugenol and F. berteriana flowers, aggregations of treated males had higher wing-fanning levels and attracted more females on both an absolute (total female sightings per male group) and relative (female sightings per wing-fanning male per group) basis than aggregations of control males. In an additional laboratory experiment, males that fed upon F. berteriana flowers were found to be more attractive to females over short distances (<2 m) than control males, consistent with results from other methyl eugenol-containing plant species.

Key Words: Bactrocera dorsalis, methyl eugenol, pheromone, lek

RESUMEN
Los machos de la mosca oriental de la fruta Bactrocera dorsalis (Hendel), son atraídos fuertemente por el metil eugenol. Evidencias recientes indican que los machos tratados (alimentados) con metil eugenol tienen un alto éxito de apareamiento y actividad de comunicación (abanicado de alas) comparados con los machos control (no alimentados). Análisis químicos también han demostrado que los metabolitos del metil eugenol son incorporados en la feromon sexual del macho, y pruebas de laboratorio han revelado que, al menos en distancias cortas (<2 m), las señales feromonales de los machos alimentados con metil eugenol son más atractivas a las hembras que aquellas de machos no alimentados. La meta principal de este estudio fue determinar si la alimentación con metil eugenol ó flores de Fagraea berteriana A. Gray que contienen un compuesto similar al metil eugenol, incrementa la atracción a larga distancia de machos de B. dorsalis bajo condiciones de campo. Agregaciones de machos, tanto tratados como no tratados, fueron establecidas en árboles de naranja, las hembras fueron liberadas desde un punto central (a 12 m del grupo de machos), y se tomó nota del abanicado de alas por los machos y de la visita por las hembras. Tanto para metil eugenol como para las flores de F. berteriana, las agregaciones de machos tratados mostraron niveles más altos de abanicado de alas y atrajeron a más hembras, en base absoluta (observación del total de hembras por grupo de machos) y relativa (observación de hembras por macho abanicando sus alas por grupo), comparado con las agregaciones de machos-control. En un experimento de laboratorio adicional, se encontró que machos alimentados con flores de F. berteriana fueron más atractivos a las hembras en cortas distancias (<2 m) en comparación con los machos-control, siendo esto consistente con resultados de otras especies que contienen metil eugenol.

Males of many Bactrocera species are strongly attracted to methyl eugenol, a naturally occurring compound found in at least 10 plant families (Metcalf 1990). This attraction is so powerful that methyl eugenol in combination with insecticides has been used successfully in programs of “male annihilation” against the oriental fruit fly, Bactrocera dorsalis (Hendel) (Steiner et al. 1965, 1970). Until recently, however, little attention was given to the underlying biological basis of male attraction. Research completed in Asia (Tan & Nishida 1996; Hee & Tan 1998) and Hawaii (Shelly & Dewire 1994; Shelly et al. 1996; Shelly 2000a, 2001a) provides strong evidence that ingestion of methyl eugenol enhances male mating success in several Bactrocera species, including B. dorsalis. Although most of the cited studies used pure, synthetic methyl eugenol, additional experiments us-
ing natural sources of methyl eugenol or methyl eugenol-like compounds have also documented a large positive effect on the mating success of male *B. dorsalis* (Nishida et al. 1997; Shelly 2000a, 2001a).

The factor(s) underlying this increase in male mating competitiveness is not known. For *B. dorsalis*, chemical analysis of the rectal gland contents (the presumed storage reservoir of male pheromone) of males fed methyl eugenol revealed the presence of metabolites of methyl eugenol, suggesting they function in pheromonal synthesis (Nishida et al. 1988). Behavioral assays conducted in laboratory cages furnished supporting data: *B. dorsalis* males that fed on methyl eugenol (Shelly & Dewire 1994) or methyl eugenol-containing flowers (Shelly 2000a) were found to signal more frequently and to attract more females than did control males. Similar results were reported by Hee & Tan (1998). Although supportive, these behavioral studies were conducted in artificial environments and monitored female attraction over short distances (<2 m).

The main objective of the present study was to determine whether exposure of *B. dorsalis* males to pure methyl eugenol or flowers of the puakenikeni tree, *Pogarea berteriana* A. Gray, which contain a methyl eugenol-like compound (Nishida et al. 1997), enhances female attraction over long distances (>10 m) in the field. To supplement previous experiments with methyl eugenol and flowers of the golden shower tree, *Cassia fistula* L., a laboratory experiment was conducted using an indoor cage to test whether exposure to puakenikeni flowers affected short-range attractiveness of males.

*B. dorsalis* appears to display a lek mating system. Males aggregate on the foliage of host trees approximately 1 h before dusk and defend individual leaves as mating territories (Shelly & Kaneshiro 1991). While perching, males engage in vigorous wing-fanning, an activity that both produces an audible buzz and disperses a pheromone attractive to females (Fletcher 1987). Upon detecting a female in their territory, males immediately cease wing-fanning and jump on the female (Shelly & Kaneshiro 1991). The female then either decamps or copulation ensues. Mating couples remain paired through the night and separate at sunrise (Fletcher 1987).

**MATERIALS AND METHODS**

**Study Animals**

Because wild flies were reluctant to wing-fan in small cages, the flies used in this study were from a colony maintained by the USDA-ARS Tropical Fruit, Vegetable, and Ornamental Crop Laboratory (USDA-ARS), Honolulu, for about 70 generations (Tanaka et al. 1969). Non-irradiated pupae were obtained 2 d before eclosion, and adults were separated by sex within 5 d of emergence (sexual maturity in this stock is attained at about 10 d of age; Shelly, unpublished data). Adults were held in plastic buckets (5-liter volume; 60-80 individuals per bucket) covered with screen mesh and supplied with ample food (a mixture of honey and protein hydrolysate) and water. Room temperature was maintained at 23-26°C and relative humidity at 65-85%. When used in the experiments, males were 14-20 d old, and females were 13-19 d old.

**Field Experiments**

Field work was conducted in a grove of approximately 30 orange trees, *Citrus sinensis* (L.) Osbeck, at the Agricultural Experiment Station of the University of Hawaii in Waimanalo, Oahu, during September-October, 1994. Several trees in the orchard had fruit, but none of these was included in the set of test trees (see below). Air temperatures during the trials ranged from 26-29°C.

The same protocol was followed to examine the influence of methyl eugenol and puakenikeni flowers on female attraction to calling males. Groups of 5 treated (methyl eugenol- or floral-exposed) or control (unexposed) males were placed in transparent cups (400 ml) that were covered on both ends with wire mesh. Cups were transported to the field and hung, two per tree within 30 cm of one another, in the canopy of the test trees. Cups were suspended horizontally (i.e. with the long axis of the cup parallel to the ground) with wire at a height of approximately 1.5 m, and strips of green masking tape on the upper surface of the cups provided shaded, leaf-like perching sites for the males. Cups were placed in 8 different orange trees, with 4 trees containing 10 treated males (2 cups) exclusively and 4 trees containing 10 control males (2 cups) exclusively. The test trees were located in a circle (12 m radius) around a central tree that served as the female release point. For a given replicate, treated and control males were alternated between adjacent trees, and for a given tree, they were alternated between successive replicates.

Treated males exposed to methyl eugenol were given unrestricted access during a 1-h period to a cotton wick (5 cm long) to which 1.5 ml of methyl eugenol had been applied. The wick, held upright in a small plastic container, was placed in a cubical screen cage (30 cm on a side) during midday, and 50-60 males were introduced into the cage. At the end of the exposure period, the wick was removed, and males were provided with food and water and held 2 d before use in the experiment. Treated males exposed to puakenikeni flowers were given unrestricted access to 25-35 flowers during a 6-h period (0900-1500 hours). Fresh flowers were obtained from lei shops and placed...
in a screen cage the same day as purchase along with 50-60 males. Flowers were held in a plastic tray, and at the end of the exposure period the tray was removed. As with methyl eugenol, males were used 2 d after floral exposure. Although systematic observations were not made, groups of males were frequently observed dabbing the methyl eugenol-containing wick and the puakenikeni flowers with their mouth parts. For both treatments, exposure was performed outdoors to minimize exposure of volatiles to control males.

Males were placed in the trees between 1800-1815 hours, and 10 min later 300 females were released from the central tree. Numbers of wing-fanning males and female sightings at each aggregation (both cups combined) were recorded at 5 min intervals for the next 60 min (i.e. 13 observations per tree per replicate). Females were scored if they were perching directly on or within 15 cm of the cups. Because females were unmarked, the number of female sightings at a lek was a composite score that included both arrival and retention at a given site. Six and 7 replicates were performed for methyl eugenol and puakenikeni floral exposure, respectively. Replicates were conducted ≥2 d apart to guarantee dispersal of released females from the study area.

Laboratory Experiment

A laboratory experiment was performed to investigate the influence of puakenikeni flowers on male attractiveness over shorter distances, likely characteristic of intersexual communication within a tree canopy. Tests were conducted on groups of 8 males (4 treated, 4 control) in a screen cage (1.2 m by 0.6 m by 0.6 m) that contained 3 potted plants Ficus sp. Treated males were exposed to puakenikeni flowers in the manner described above and were used 2 or 7 d after exposure. Approximately 3 h before sunset, males were placed singly in smaller cages (wire screen cylinders 6 cm long and 3 cm diameter suspended from branches with a wire hook), which were placed at specific locations on the caged plants. The mean nearest-neighbor distance between the small cages was 20 cm (range: 16-22 cm). Small cages were placed at the same locations over all trials, but male type (treated or control) at a given location was assigned randomly at the start of a trial. Immediately after the males were in place, 40 females were released into the large cage containing the Ficus plants. The room lights were extinguished, and the cage, which was adjacent to a west-facing window, received only natural light. Starting 1 h before sunset, males were checked at 1-min intervals for the presence/absence of wing-fanning and for the number of females resting on the individual small cages. Observations were made on 8 d and 5 d for the 2-d and 7-d post-exposure intervals, respectively.

Statistical Analyses

In both field experiments, male wing-fanning activity for individual leks was expressed as the mean over all observations in a replicate. In the laboratory study, signaling activity for individual males was the number of 1-min checks during which males were wing-fanning. In both the field and laboratory experiments, female sightings were compared between treated and control males on an absolute basis (total female sightings per aggregation or per individual male) and a relative basis (female sightings per wing-fanning male or per min wing-fanning). Comparisons between aggregations of treated versus control males (field experiments) and between individual treated versus control males (laboratory experiment) were made using the Mann-Whitney test (test statistic T).

RESULTS

Field Experiments

Males exposed to methyl eugenol displayed higher levels of wing-fanning than control males (Fig. 1a). Among the individual leks, an average of 2.9 treated males were wing-fanning per observation compared with 2.1 control males (T = 727.0; P < 0.05; n₁ = n₂ = 24 for this experiment). Similarly, the total number of female sightings was greater for groups of treated males (x = 3.8) than control males (x = 0.4; T = 806.0; P < 0.001; Fig. 1b). When ratios of female sightings to wing-fanning males were calculated on a per observation basis, the leks consisting of treated males were characterized by significantly higher values (x = 0.05) than leks composed of control males (x = .01; T = 808.5; P < 0.001; Fig. 1c).

Similar results were obtained in the experiment testing puakenikeni flowers. Males exposed to flowers displayed higher levels of wing-fanning than control males (Fig. 2a). Among the individual leks, a mean of 2.6 treated males were wing-fanning per observation compared to 1.3 control males (T = 1106.5; P < 0.001; n₁ = n₂ = 28 for this experiment). Similarly, number of female sightings was greater for groups of treated males (x = 6.0) than control males (x = 1.9; T = 1039.0; P < 0.001; Fig. 2b). When ratios of female sightings to wing-fanning males were calculated on a per observation basis, the leks consisting of treated males were characterized by significantly higher values (x = 0.08) than leks composed of control males (x = .05; T = 927.5; P < 0.05; Fig. 2c).

Laboratory Experiment

Floral exposure had a significant effect on male wing-fanning and female sightings in tests run 2 d after exposure. On average, treated males wing-
fanned for 16.3 min compared to 10.5 min for control males (T = 1202.5; P < 0.05; n₁ = n₂ = 32; Fig. 3a). Female sightings were also greater for treated (x̄ = 22.4 sightings) than control (x̄ = 6.3 sightings) males (T = 1336.0; P < 0.001; n₁ = n₂ = 32; Fig. 3b). In addition to this difference in absolute numbers, female sightings per min of wing-fanning were also greater for treated (x̄ = 1.6 females/min wing-fanning) than control (x̄ = 0.6 females/min wing-fanning) males (T = 460.0; P < 0.001; n₁ = 26, n₂ = 29; Fig. 3c).
In tests conducted 7 d after exposure, floral exposure had no detectable effect on male wing-fanning level but had a significant effect on female sightings (Fig. 4). On average, treated males wing-fanned for 15.7 min compared to 12.4 min for control males (T = 443.0; NS; n₁ = n₂ = 20; Fig. 4a). Despite this similarity, female sightings were significantly greater for treated (x = 16.5 female sightings) than control (x = 3.8 sightings) males (T = 503.0; P < 0.05; n₁ = n₂ = 20; Fig. 4b). Likewise, when calculated on the basis of time spent wing-fanning, female sightings were greater for treated (x = 1.1 females/min wing-fanning) than control (x = 0.3 females/min wing-fanning) males (T = 159.5; P < 0.01; n₁ = 15, n₂ = 16; Fig. 4c).

**Discussion**

Coupled with previous findings (Shelly & Dewire 1994; Nishida et al. 1997; Shelly 2000a, 2001a), the results described here indicate that *B. dorsalis* males that feed on methyl eugenol or flowers containing methyl eugenol or methyl eugenol-like compounds have an advantage over unfed males at different spatial scales in sexual competition. In the aforementioned studies, treated males obtained 61-89% of all matings in tests conducted in small cages (30 cm³), where long-distance attraction had little or no role and close-range, courtship signals were presumably the prime determinant of male mating success. Likewise, prior studies along with the present findings showed that female sightings were approximately 2-5 times more frequent near treated males than control males in tests performed in large cages (1.2 by 0.6 by 0.6 m) that mimicked the environment in which “intra-tree” communication occurs between the sexes. Finally, the present field experiments demonstrated that treated males attracted more females than control males over a large distance (12 m), indicating an advantage in “inter-tree” signaling as well.

The increased female visitation observed for treated males appears to reflect both an increase in wing-fanning activity and the attractiveness of the pheromonal signal per se. This dual effect was noted following feeding on pure methyl eugenol (Shelly & Dewire 1994) or flowers containing methyl eugenol (Shelly 2001a) or a methyl eugenol-like compound (this study). The only exception involved tests with methyl eugenol-containing flowers of the golden shower tree where signal attractiveness was enhanced but the overall level of male signaling was not (Shelly 2000a). As noted above, the increase in signal attractiveness may result from the incorporation of breakdown products of methyl eugenol into the pheromone (Nishida et al. 1997). This scenario resembles that described for various Lepidoptera, where adult males acquire plant compounds (pyrrolizidine alkaloids) and use them as pheromone precursors.
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...the attractiveness of the signal produced (Shelly 2001b). Unlike the oriental fruit fly, however, C. capitata males do not feed on this attractant.

Although there is a strong female preference for methyl eugenol-fed males of B. dorsalis, its adaptive basis, if any, remains unknown. The preference does not appear to arise from any direct benefits of mating: egg production and hatch rate do not differ between females mated to methyl eugenol-fed or unfed males (Shelly 2000b). Alternatively, incorporation of metabolites of methyl eugenol in the sex pheromone may elicit a strong, pre-existing sensory bias that has evolved in a different context (e.g., food searching). This notion is supported by reports that male pheromones in other tephritid species contain certain compounds that mimic food and host odors (Baker et al. 1990; Robacker & Warfield 1993). Thus, the methyl eugenol-bearing pheromone may represent a sensory trap (West-Eberhard 1984) or a case of sensory exploitation (Ryan 1990). A final (and not mutually exclusive) hypothesis is that pheromone composition indicates male ability to locate natural sources of methyl eugenol in the environment. As such, by selecting males whose pheromone contains methyl eugenol metabolites, females may increase the probability that their sons will have a high ability to locate methyl eugenol sources and hence enjoy a high mating success. If true, this scenario represents a case of runaway selection (Andersson 1994), where female preference is based on a trait that is arbitrary with respect to offspring viability but does confer an advantage to male progeny in mating competition.

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