Distribution and Abundance of Female Oriental Fruit Flies Near Methyl Eugenol-Baited Traps[1]

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Abstract Methyl eugenol is an extremely effective attractant for male oriental fruit flies, *Bactrocera dorsalis* (Hendel). Field experiments were conducted in a commercial guava orchard to determine whether the presence of methyl eugenol-baited traps affected the distribution and abundance of female oriental fruit flies near the traps. Captures of females on spheres did not increase within the vicinity of methyl eugenol-baited traps. Captures of males were significantly greater on spheres hung in trees containing methyl eugenol-baited traps than on spheres hung in other trees. An experiment was conducted to determine if methyl eugenol would influence the dispersal of unmated, sexually mature female oriental fruit flies. There were no significant differences in the numbers of marked or wild females captured on traps at different distances from the methyl eugenol lure. This study did not find any evidence that the presence of methyl eugenol-baited traps in orchards would affect female abundance in the vicinity of traps.

Key Words Methyl eugenol, male lure, *Bactrocera dorsalis,* attractant

The use of male lures is an important component of detection and control programs for tephritid fruit fly pests. Male oriental fruit flies, *Bactrocera dorsalis* (Hendel), are extremely attracted to methyl eugenol and will feed on toxic methyl eugenol baits. Because of this behavior, methyl eugenol-baited traps have been widely used for oriental fruit fly detection and control programs (Cunningham 1989). Methyl eugenol-baited traps have been used successfully to eradicate isolated populations in areas such as the Marianas Islands (Steiner et al. 1965), the Amami Islands (Ushio et al. 1982), and the Okinawa Islands (Koyama et al. 1984). Despite the success of male lures in large-scale eradication programs, they have not been proven effective for reducing fruit infestations on the farm level if oriental fruit flies are able to migrate into orchards from surrounding habitats (Koyama et al. 1984, Cunningham and Suda 1986).

Virgin females actively search for mates and are attracted to male pheromones. Virgin female Mediterranean fruit flies were more attracted to male pheromones than to host fruit odors (Jang 1995). In laboratory tests with three *Bactrocera* species, virgin females were attracted to methyl eugenol (Fitt 1981). In field experiments, methyl eugenol-baited traps captured a negligible number of female oriental fruit flies

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if males were present (Steiner 1952, Steiner et al. 1965). However, if the male population declined by 99%, virgin, sexually-mature females began to respond to methyl eugenol. When males reappeared, female captures declined again (Steiner et al. 1965).

Virgin females searching for mates may be attracted to areas with male lures because of the large numbers of males moving into those areas. If methyl eugenol-baited traps are attracting virgin females to the area without luring and killing them in the traps, and allowing them to find mates, methyl eugenol-baited traps could be having a negative impact on the level of fruit damage in an orchard. This study evaluated the response of female oriental fruit flies to the presence of methyl eugenol-baited traps in a commercial guava orchard. Also, an experiment was performed to determine whether the presence of methyl eugenol influenced the dispersal behavior of laboratory-cultured, virgin, sexually-mature females released in the field.

Materials and Methods

Experiments 1 and 2 were conducted in a 160-ha unsprayed commercial orchard of common guava, *Psidium guajava* L. (var. 'Beaumont'), located in Kilauea, Kauai. The methyl eugenol-baited traps consisted of white buckets (20 cm high, 20 cm diam) with 4 entry holes (2.5 cm diam) each. The effect of methyl eugenol-baited traps on the dispersal patterns of oriental fruit flies was examined by using plastic yellow spheres (7.0 cm diam) (Euro-matic LTD, Ashland, OH), coated with Tangletrap® (Tanglefoot, Grand Rapids, MI), and hung by wire within the canopy of a tree from 1 to 2 m above the ground and within 1 m from the edge of the tree.

In Experiment 1, there were 2 blocks. Blocks were located at least 500 m apart because male populations in a previous study were not affected by methyl eugenol traps in plots 600 m away (Cunningham and Suda 1986). There were 4 bucket traps per block. This experiment was designed to attract very large numbers of male oriental fruit flies into the center of the block. A cotton wick (8 cm in length) was soaked in a 10 ml solution of 5% malathion and 95% water and hung by wire from the lid in the center of each bucket. Buckets were hung in 4 trees (one bucket per tree) in the center of each block. There were 20 spheres per block placed in a grid around the buckets. A sphere was placed in the same tree as the bucket at a distance of 3 m from the bucket. Then, a sphere was placed in each tree in the next two rows directly behind the 4 trees containing a bucket, eight spheres/row (Fig. 1).

Spheres were replaced every 4 d for 12 d. After 12 d, the bucket traps were moved to a new experimental block in a different location in the orchard to control for any block effect and left in their new locations for 7 d to allow time for movement of flies in response to traps. After 7 d, spheres were placed in trees surrounding the traps as described above. Spheres were replaced every 4 d for 12 d. Fruit fly capture data were collected from 4 experimental blocks with 3 sampling dates for each block. This experiment was conducted from 5 September until 7 October 1996. Numbers of female and male oriental fruit flies captured on spheres at each distance from bucket traps were compared using analysis of variance (ANOVA). Treatment means were separated using the Tukey HSD test (Analytical Software 1996). Data were transformed by log (x + 1) to stabilize variances before analysis.

Experiment 2 was set up as described previously, except that there was only a single bucket trap in each block. In the first treatment block, the solution consisted of 95% methyl eugenol and 5% malathion and in the second treatment block, the so-

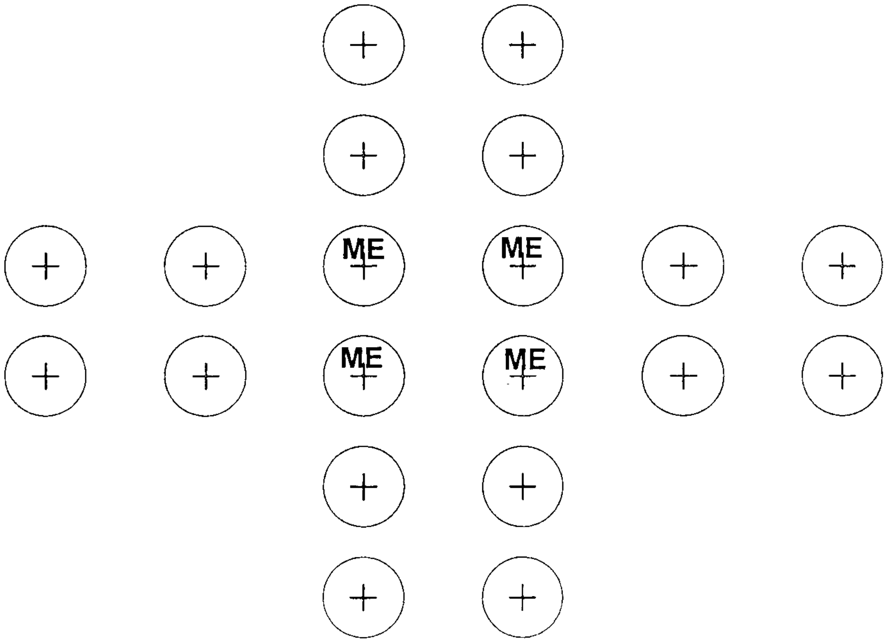

Fig. 1. In Experiment 1, there were 4 methyl eugenol-baited bucket traps in the center of each block. Buckets were hung in 4 trees in a square formation (one bucket per tree). There were 20 spheres per block placed in a grid around the buckets. A sphere was placed in the same tree as the bucket at a distance of 3 m from the bucket and then on trees (one sphere per tree) in the next two rows directly behind each tree containing a bucket, eight spheres/row.

lution consisted of 99% methyl eugenol and 1% malathion. There were 12 yellow spheres per block. Two spheres were hung within the same tree as the bucket at a distance of 2 m from the bucket. Then a sphere was hung on the next five trees in the same row on each side of the tree containing the bucket so that there were two spheres hung at each distance from the bucket (one sphere per tree) (Fig. 2). Distance

Fig. 2. In Experiment 2, there was 1 methyl eugenol-baited bucket trap in the center of each block. There were 12 yellow spheres per block. Two spheres were hung within the same tree as the bucket at a distance of 2 m from the bucket, and then a sphere was hung on the next 5 trees in the same row on each side of the tree containing the bucket so that there were two spheres hung at each distance from the bucket (one sphere per tree).

between trees within a row was 6 m. Spheres were hung at distances from the male lure of 2, 6, 12, 18, 24, and 30 m.

Cotton wicks and yellow spheres were replaced once a week, and captures of oriental fruit flies on spheres were recorded for 21 d. Bucket traps were emptied weekly. Weekly observations were made to determine if males were present on the foliage or trunks of trees containing bucket traps. After 21 d, treatment blocks were rotated to a different part of the orchard to control for any block effect and left in their new locations for 5 d to allow time for movement of flies in response to traps. After 5 d, spheres were placed in trees surrounding the bucket traps as described above and captures of oriental fruit flies on spheres were recorded once a week for 21 d. Thus, fruit fly capture data were collected from 4 experimental blocks, 2 blocks for each treatment (5% malathion vs 1% malathion), on 3 sampling dates for each block. This experiment was conducted from 1 April until 16 May 1997.

Weekly bucket trap captures of male oriental fruit flies were air-dried and weighed. All flies were counted and sexed from a 2 g sample of flies captured in methyl eugenol-baited bucket traps, resulting in 130 males and 0 females per gram of flies. Weekly dry weights of male oriental fruit flies for the two treatments were compared with a paired t-test. Fruit fly capture data were compared using a 2-way ANOVA by distance from bucket traps and treatment blocks (5% malathion vs 1% malathion in bucket traps). Means were separated using Tukey HSD test (Analytical Software 1996). Data were transformed by log $(x + 1)$ to stabilize variances before analysis.

Experiment 3 was performed to determine whether the presence of methyl eugenol influenced the dispersal behavior of laboratory-cultured, unmated, sexually mature females released in the field. Laboratory colonies of oriental fruit flies were obtained as larvae from the mass rearing facilities at the USDA Tropical Fruit, Vegetable, and Ornamental Crop Research Laboratory in Honolulu, HI. Pupae were coated with a fluorescent dye, which remained on the adult flies after eclosion. Females were separated from males within 48 h of emergence. Laboratory-cultured oriental fruit flies do not mate until at least 72 h after emergence (Wong et al. 1982). From the time of eclosion until flies were released, flies were maintained in $30 \times 30 \times 30$ cm screened cages supplied with water and a mixture of enzymatic yeast hydrolysate and sucrose.

This experiment was conducted in a grove of 75 non-fruiting grapefruit trees located on the grounds of the Kauai Agricultural Research Station in Kapaa, Kauai. Laboratory-cultured female flies were released from a tree in the center of the grove. A methyl eugenol lure was hung in the center of one of the trees adjacent to the release tree. The methyl eugenol lure consisted of a cotton wick (8 cm in length), soaked in 10 ml of methyl eugenol. To prevent ingestion of methyl eugenol, the cotton wick was placed in the bottom of a 5 dram polyethylene vial. The lid of the vial contained a 2-cm diam screen-covered hole. Ladd traps (Ladd Research Industries, Burlington, VT) were hung in a grid formation in the 4 trees adjacent to the release tree and in the 4 trees in the row directly behind these trees (Fig. 3). There were 2 traps hung in each tree. Traps were placed on the side of each tree facing the release tree. This design was used in order to capture females as they dispersed from the release tree and to determine whether or not females were moving in the direction of the male lure. Ladd traps consist of a polyethylene yellow panel (28×22 cm) and a 9-cm diam polyethylene red sphere attached to the center of the panel so that there is a hemisphere on each side of the panel.

Both sides of Ladd traps were coated with Tangletrap® (Tanglefoot, Grand Rapids,

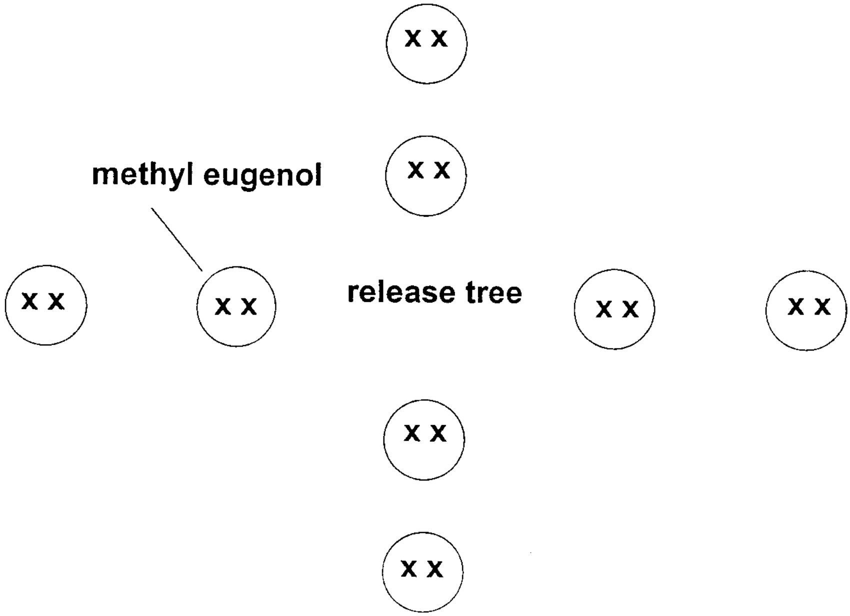

Fig. 3. In Experiment 3, laboratory-cultured female flies were released from a tree in the center of a nonfruiting grapefruit grove. A methyl eugenol lure was hung in the center of one of the trees adjacent to the release tree. Ladd traps were hung in a grid formation in the 4 trees adjacent to the release tree and in the 4 trees in the row directly behind these trees (2 traps per tree). Traps were placed on the side of each tree facing the release tree.

MI). There were 4 replicates and for each replicate, the location of the methyl eugenol lure was rotated among the 4 trees in the first row adjacent to the release tree. The lure was hung in the tree 1 h before females were released. Within 5 min, males began to arrive at the lure. Males were observed congregating near the lure throughout each replicate. For each replicate, 200 marked, unmated, 15 to 20 d old female flies were released from a cage placed in the center of the release tree with the cage opening facing upward. After 24 h, traps were collected. The numbers of marked and wild female oriental fruit flies captured in each trap were recorded. Captures of wild and released females on Ladd traps were compared for each trap location using a one-way ANOVA (Analytical Software 1996).

Results and Discussion

In Experiment 1, numbers of females captured on spheres hung in trees that were 14 m away from bucket traps were significantly greater than on spheres that were hung in the same trees as bucket traps or in trees adjacent to bucket traps ($F = 6.86$; df = 2; $P < 0.001$) (Table 1). It, thus, appears possible that the high concentration of methyl eugenol (4 traps per block) actually had a repellent effect on foraging females.

Table 1. Mean ± SEM number of female and male *B. dorsalis* captured per sphere at different distances from methyl eugenol-baited bucket traps

Distance from ME trap (m)*	Females	Males
3 (same tree as trap)	7.2 ± 1.0b	3.5 ± 0.6a
7	10.8 ± 1.3b	1.4 ± 0.2b
14	15.6 ± 1.9a	1.9 ± 0.3b

Means within a column followed by the same letter are not significantly different ($P < 0.05$; Tukey HSD test on log [x + 1] transformed data).
* Fruit fly capture data were collected from 4 experimental blocks with 3 sampling dates for each block.

In Experiment 2, there were no differences in the numbers of females captured on spheres at different distances from a single bucket trap ($F = 1.28$; df = 5, 5; $P = 0.28$) or differences in the numbers of females captured in different treatment blocks ($F = 0.40$; df = 1, 5; $P = 0.53$) (Table 2).

Numbers of males captured were significantly greater on spheres hung in the same trees as bucket traps than on other trees in both Experiment 1 ($F = 9.26$; df = 2; $P < 0.001$) and Experiment 2 ($F = 4.82$; df = 5, 5; $P < 0.001$). Spheres hung in the same trees as bucket traps probably intercepted males as they approached bucket traps. In Experiment 2, there were also differences in the numbers of males captured on spheres in the 2 treatment blocks ($F = 5.89$; df = 1, 5; $P < 0.02$), but there was no interaction between distance of spheres from traps and treatment blocks. Numbers of males captured on spheres in treatment blocks where bucket traps contained 5% malathion and 95% methyl eugenol were greater (1.69 ± 0.38 males per sphere (mean ± SEM)) than in treatment blocks where bucket traps contained 1% malathion and 99% methyl eugenol (0.93 ± 0.34 males per sphere). The lures with 99% methyl eugenol may have been more effective at drawing males directly into bucket traps so that fewer males were captured on yellow spheres. Mean dry weights of weekly

Table 2. Mean ± SEM number of female and male *B. dorsalis* captured per sphere at different distances from methyl eugenol-baited bucket traps

Distance from ME trap (m)*	Females	Males
2 (same tree as trap)	0.9 ± 0.3a	4.5 ± 1.3a
6	0.8 ± 0.2a	0.8 ± 0.2b
12	1.4 ± 0.3a	0.8 ± 0.3b
18	1.5 ± 0.4a	0.5 ± 0.2b
24	1.9 ± 0.4a	0.6 ± 0.2b
30	1.1 ± 0.4a	0.7 ± 0.2b

Means within a column followed by the same letter are not significantly different ($P < 0.05$; Tukey HSD test on log [x + 1] transformed data).
* Fruit fly capture data were collected from 4 experimental blocks, 2 blocks for each treatment (5% malathion versus 1% malathion), on 3 sampling dates for each block.

captures of male oriental fruit flies in bucket traps in the 2 treatment blocks were 15.3 ± 1.8 (mean ± SEM) g per week for traps with 5% malathion and 18.8 ± 1.5 g per week for traps with 1% malathion. Differences in dry weights of male oriental fruit flies captured in bucket traps were not significant (paired t-test: $t = 2.26$; df = 7; $P = 0.09$).

In Experiments 1 and 2, captures of females on spheres did not increase within the vicinity of methyl eugenol-baited traps. In another field test, trimedlure baits had no effect on the abundance of wild female Mediterranean fruit flies near baits (Shelly et al. 1993). Because methyl eugenol is such a strong attractant to male oriental fruit flies, virtually all males within 500 m of traps are killed (Cunningham and Suda 1986). On sampling days, there were no males observed on the foliage or trunks of trees containing bucket traps. Also, males feed on methyl eugenol baits immediately after their arrival at baits (Nishida and Vargas 1990). Therefore, it is not likely that virgin females would find mates in the vicinity of methyl eugenol-baited traps. However, virgin females might follow males into the orchard from surrounding habitats. Further studies need to be conducted to determine whether the pattern of migration of females into orchards is affected by the presence of methyl eugenol-baited traps.

In Experiment 3, there were no significant differences in the number of laboratory-cultured virgin females captured on Ladd traps hung at different distances from the methyl eugenol lure ($F = 1.61$; df = 7; $P = 0.15$). However, the number of released females that were recaptured was very low. Hence, effects of methyl eugenol on the dispersal of virgin females may have been detected if larger numbers of females were released. There were no differences in the numbers of wild females captured on Ladd traps hung at different distances from the methyl eugenol lure ($F = 0.73$; df = 7; $P = 0.65$) (Table 3). Only unmated females would be expected to respond to aggregations of males near methyl eugenol lures. Because wild female populations include both mated and unmated individuals, captures of wild females are less likely to be affected by methyl eugenol lures than captures of released, virgin females.

Methyl eugenol-baited traps are extremely effective for capturing male oriental fruit flies, but they do not capture female oriental fruit flies unless male populations are almost completely eliminated (Steiner et al. 1965). In the absence of a large-scale eradication program, an effective integrated pest management strategy for oriental fruit fly control will need to focus on reducing female populations in orchards. Traps that rely on a combination of visual and olfactory cues show the most promise for capturing tropical female tephritid fruit fly pests (Epsky and Heath 1998). The use of methyl eugenol traps in combination with traps designed to capture female fruit flies may prove to be an effective pest control strategy. When methyl eugenol-baited traps reduced male populations by over 99% in a papaya orchard, fruit infestation levels were reduced by 48% (Cunningham and Suda 1986). However, it is not known whether the level of fruit infestation in commercial orchards could be further reduced by combining the use of methyl eugenol-baited traps with a trapping system designed to capture females. Because mated female oriental fruit flies are able to migrate into orchards from surrounding habitats, it may not be cost-effective for farmers to include methyl eugenol-baited traps as a component of an integrated pest management strategy to reduce damage caused by oriental fruit flies. Although this study did not find any evidence that the presence of methyl eugenol-baited traps in orchards increased female abundance near traps, further studies need to be conducted to determine whether the use of methyl eugenol-baited traps, in combination with other trapping systems, could reduce fruit infestation levels at the farm level.

Table 3. Mean ± SEM number of released laboratory-cultured and wild female *B. dorsalis* captured on Ladd traps hung in trees (2 traps per tree) in a grid formation with a release tree in the center in a nonfruiting grapefruit orchard. One tree in the first row contained a methyl eugenol lure.

Ladd Trap Location*	Marked Females	Wild Females
First Row		
Methyl Eugenol (same tree as lure)	1.25 ± 0.25a	1.00 ± 0.60a
Adjacent (Left)	0.50 ± 0.27a	0.88 ± 0.44a
Adjacent (Right)	0.50 ± 0.4a	1.75 ± 0.37a
Opposite	0.63 ± 0.18a	1.00 ± 0.38a
Second Row		
Adjacent (behind tree with lure)	0.13 ± 0.13a	0.75 ± 0.25a
Left	0.50 ± 0.38a	0.63 ± 0.32a
Right	0.38 ± 0.26a	0.75 ± 0.16a
Opposite	0.25 ± 0.16a	1.00 ± 0.53a

Means within a column followed by the same letter are not significantly different ($P < 0.05$; ANOVA on log [x + 1] transformed data).

* For each trial, 200 marked, unmated, 15 to 20 d old female flies were released. There were 4 trials and for each trial, the location of the methyl eugenol lure was rotated among the 4 trees in the first row adjacent to the release tree.

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