Honey Bees Attracted to the Semiochemical Methyl Eugenol, Used for Male Annihilation of the Oriental Fruit Fly (Diptera: Tephritidae)

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ABSTRACT. As part of a study to examine the effects of the male annihilation technique on the oriental fruit fly (*Bactrocera dorsalis* (Hendel)) in Hawai'i, honey bees in high-elevation native forest were found to be weakly attracted to traps baited with methyl eugenol. Analyses of pollen from a sample of captured bees showed that all bees had been foraging on plants in the family Myrtaceae and that 75% of the pollen sample were from plants in this family. Gas chromatography of the floral extracts indicated that methyl eugenol is not present in the flowers of the forage plants. It is therefore suggested that methyl eugenol, while naturally occurring in some plants, was in this study only similar to other phenyl propanoid compounds produced by the forage plant blossoms, explaining its weak attractiveness to honey bees. The facultative and weak attractance of methyl eugenol to honey bees suggests that its use in a male annihilation program in Hawai'i would probably not significantly impact honey bee populations.

The oriental fruit fly (*Bactrocera dorsalis* (Hendel)) became established in the Hawaiian Islands in 1946 (Fullaway 1948) and has since replaced the Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) as the prime pest tephritid in this area (Bess 1953, Keiser et al. 1965). For decades, research directed at developing and testing eradication techniques for the oriental fruit fly has been conducted primarily in Hawai'i (Yaninek & Geraud 1989). The results of this technology development have been applied successfully in the eradication of established oriental fruit fly populations in the Marianas Islands (Steiner et al. 1965, Steiner et al. 1970), the Amami Islands (Tanaka 1980, Ushio et al. 1982), the Okinawa Islands (Koyoma et al. 1984), and incipient populations in California (Chambers et al. 1974, Philips 1976).

The male annihilation technique is considered the *sine qua non* for eradication of this pest (Cunningham 1991). This technique involves the use of a plant-derived lure, methyl eugenol, which is strongly attractive to male oriental fruit flies (Steiner 1952), combined with a toxicant (malathion 9, or naled) (Steiner *et al.* 1965). The lure + toxicant is carried by fiberboard squares or cotton wicks which are hung from trees or as spot application of a thickened liquid in urban areas but distributed by aircraft over large agricultural or forested areas.

Because methyl eugenol has been thought to be strongly attractive to only a small group of dacine fruit flies (Drew 1974, Drew & Hooper 1981), and the amount of pesticide used in an eradication program is extremely small (e.g. 260 grams/mi²), male annihilation has been considered to be an environmentally benign technique (Chambers *et al.* 1974). Although Metcalf & Metcalf (1992) have warned against the use of male annihilation in areas with endemic, non-pest Tephritidae, and large numbers of green lace wings (Neuroptera: Chrysopidae) have been reported from methyl eugenol traps (Suda & Cunningham 1970, Umeya & Hirao 1974), the potential effects of male annihilation on nontarget insects has largely been ignored.

Preliminary studies have suggested that some species of endemic Hawaiian Drosophilidae may be attracted to methyl eugenol (Conant 1981, Loope & Medeiros 1992). With this in mind, we conducted an experiment to determine the attractiveness of methyl

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eugenol to native insects on the island of Kaua'i (Asquith & Kido 1994). In doing so, we discovered large numbers of honey bees in our methyl eugenol traps. Considering the active apiculture industry in Hawai'i (Messing 1991) and the importance of bees as pollinators of both agricultural crops and native plants, the potential susceptibility of honey bees to an oriental fruit fly male annihilation program is of concern. In this paper we report on the attractiveness of methyl eugenol to honey bees and the ecology of attraction, and discuss the significance of these findings to a Hawai'i eradication program.

MATERIALS AND METHODS

The island of Kaua'i is currently the focal point of United States Department of Agriculture, Agriculture Research Service (USDA-ARS) research on fruit fly control and eradication in Hawai'i. Under the current plan, Kaua'i would be the first island to receive full-scale eradication efforts if a program were initiated, thus this island was chosen to begin our examinations of the effects of male annihilation efforts on nontarget organisms.

Methyl eugenol attraction

The methyl eugenol attraction experiment was conducted in Koke'e State Park at ca. 1200 m elevation in the island's central Alaka'i massif. The experiment had 3 replicates in space. One replicate (Alaka'i) was located along the Alaka'i Swamp Trail, an area of wet, native forest dominated by *Metrosideros polymorpha* Gaud., *Cheirodendron trigynum* (Gaud.) Heller, *Melicope anisata* (H. Mann), *Syzygium sandwicense* (Gray) Ndz., and *Coprosma* sp. The second replicate was the Möhihi site located at the junction of Kumuwela and Möhihi roads, a mesic to wet area with vegetation similar to that at the Alaka'i site but with the addition of the invasive, alien strawberry guava (*Psidium cattleianum* Sabine). The third replicate was the Waininiua site located on a trail off Kumuwela road in mesic forest. Vegetation at this site was predominately *Metrosideros polymorpha*, *Acacia koa* Gray, *Nestigis sandwicensis* (Gray), *Allelicope* spp., and strawberry guava.

Each replicate consisted of 4 sets of 4 traps, with a set at ground level, 1 m, 2 m, and 4 m above ground. Each trap above ground level was suspended from a PVC pipe "tree," and consisted of a 120 ml NalgeneTM specimen cup with 5 1-cm dia. holes in the sides. A cotton wick soaked with the test compound was suspended from the cap inside the trap. The bottom of each trap was filled to a depth of 1.5 cm with ethylene glycol. This fluid was used as a preservative to remove the influence of decomposing flies as an additional source of a ttraction. Pitfall traps were used at ground level, consisting of 500 ml plastic cups buried to the lip, with a smaller 120 ml cup in the bottom filled with ethylene glycol. A funnel was also fitted inside the larger cup, rims flush with each other; a wire extended across the top end of the funnel from which the cotton wick was suspended. Finally, a round, 20 cm diameter plastic rain cover supported by nails was placed ca. 5 cm

Each set of traps consisted of 4 treatments: 1) methyl eugenol, 2) methyl eugenol + toxin (naled), 3) toxin, and 4) control (cotton wick only). Each test was run for ca. 14 days, after which the contents of the trap were emptied for scoring, the cottons wicks replaced, the above ground traps rotated to remove position effects, and the experiment repeated. To account for the possible seasonal phenology, tests were run continuously from March 1992 to April 1993.

Log transformed data (log x+1) were analyzed by four-way analysis of variance (ANOVA, GLM procedure) (SAS Institute 1985) evaluating the effects of treatment, trap height, site, and sampling date. Because we were primarily interested in the effects of the

Taxon	% of Pollen Sum	# of corbiculae
Myrtaceae, Total	73.4	8
Eugenia/Syzygium	44.1	8
Metrosideros/Eucalyptus	27.7	7
Psidium	1.6	3
Lamiaceae		
Ocimum	8.5	4
Araliaceae		
Cheirodendron	6.4	4
16 other types	< 2% each	1-3

Table 1. Percentages of major pollen types present in combined total pollen (n = 188) extracted from single corbiculae of honey bees captured with methyl eugenol-baited traps in Hawai'i.

treatments, we do not report the ANOVA results of site and date, although the variation caused by these 2 factors was included in the model. Means were separated by Duncan's test and untransformed means reported as #bees/trap/day.

Determination of Forage Plants

To determine the plants at which captured bees had been foraging, 8 individual bees that had conspicuously pollen-loaded corbiculae were removed from the traps and placed in individual vials with alcohol. Sampled bees derived from throughout the study period, from June to March and from all trap heights except ground level. Pollen samples were taken from a single leg, bearing an intact corbicula, from each of the 8 bees. Strong vortexing in distilled water was used to dissociate the pollen from the leg, followed by processing of the entire sample (including leg tissue) in acetolysis mixture and KOH as described in Faegri *et al.* (1989). To check for pollen loss or contamination in the collecting and preservation fluids, samples of these liquids were also processed and examined, but these samples contained little or no pollen.

The pollen residues from the bee legs were mounted in liquid glycerin slides and scanned at 100x and 400x on a Zeiss Axioplan light microscope. All pollen types were recorded.

Methyl Eugenol Assay

To assay for the presence of methyl eugenol in the forage plants, entire blossoms of *Metrosideros polymorpha, Cheirodendron trigynum, and Syzygium sandwicense* were collected from the study site and either frozen or immediately processed for analysis. 50-100 gm of flowers were finely ground in 1 L of water and then steam codistilled with 10 ml of hexane for ca. 2 h. One μ l of sample concentrate was analyzed by mass spectrometry and gas chromatography in a Hewlett Packard model 5970A mass spectrometer and Hewlett Packard model 5890 gas chromatograph. The selective ion monitoring mode was used at m/z 178. Coinjection of standards with samples showed a resolution of 0.1 nanograms. Spectral peaks of flower samples were compared to those obtained from a methyl eugenol standard.



Fig. 1. Capture rates (#Arap/day) of honey bees in traps baited with methyl eugenol (diagonal lines), methyl eugenol + toxin (vertical lines), toxin (dots) or control traps (solid), placed at different heights in the forest.

RESULTS

Methyl eugenol attraction

Traps with methyl eugenol caught significantly more honey bees than control traps or those with toxin only (n = 252, F = 45.85, P < 0.0001). There was apparently some slight repulsive effect of the toxin because twice as many bees were caught in methyl eugenol-only compared to methyl eugenol + toxin traps (Fig. 1). Fewer bees were caught in ground level-traps than traps above ground, but there were no differences in captures among the 1 m, 2 m, and 4 m traps (n = 252, F = 15.21, P < 0.0001, Fig. 1). There was distinct seasonal variation in the capture rate of honey bees. Peak capture rates occurred from late June to early August, whereas few bees were captured from late March through May (Fig. 2).

Determination of forage plants

Two groups of plants, Eugenia/Syzygium and Eucalyptus/Metrosideros, contributed 70% of the pollen recovered from captured honey bees and all corbiculae examined contained Eugenia/Syzygium pollen (Table 1). The only other significant contributors (> 5%) to pollen loads were Cheirodendron and Ocimum basilicum L. Sixteen other pollen types were represented only as traces (< 2% each) and probably did not indicate recent flower visitations.

Pollen is not readily distinguishable between Eugenia vs Syzygium, and Eucalyptus vs Metrosideros, but the samples probably represent Syzygium sandwicense and Metrosideros polymorpha, which along with Cheirodendron trigynum were among the most



Fig. 2. Seasonal capture (#/trap/day) of honey bees in methyl eugenol baited traps in Koke'e, Hawai'i.

common trees in the study area. All these trees produce abundant flowers and peak capture rates for bees corresponded with the flowering period of *Cheirodendron trigynum and Syzygium sandwicense. Ocimum basilicum* is a non-native ruderal species that has not been recorded from our study area but probably occurs along heavily used trails and roads.

Methyl eugenol assay

At a retention time of seven minutes, the methyl eugenol standard showed a distinct spectral peak at m/z 178, the molecular weight of methyl eugenol (Fig. 3 A). None of the floral extracts showed spectral peaks at m/z 178 (e.g. *Syzygium* in Fig. 3B) indicating methyl eugenol was not present. *Ocimum basilicum* L., from which half of the sampled bees had gathered pollen, was not assayed but is known to contain methyl eugenol at least in its leaves (Chen & Wu, 1970; Pushpangadan *et al.*, 1975).

DISCUSSION

Honey bees have not previously been reported as attracted to methyl eugenol. Only 2 other groups of Hymenoptera, a parasitic Proctotrupidae (Asquith & Kido 1994) and euglossine bees (Williams & Whitten 1983) are known to be attracted to this compound. Although there are no data demonstrating honey bees' ability to olfactorally detect methyl eugenol *per se*, they have been trained toward unmethylated eugenol (Schwarz 1955) and have placode sensillae in the antennae that respond only to terpenes such as methyl eugenol (Vareschi 1971).



Fig. 3. Spectrum (top) and ion chromatograms (bottom) of the methyl eugenol standard (3A), showing spectral peak at m/z 178, and floral extracts of Syzygium sandwicense (3B) showing no peak at m/z 178. Chromatograms of the extracts of other honey bee foraging plants were similar to that of Syzygium sandwicense in that there were no peaks at m/z 178.

Asquith & Burney: Effects of methyl eugenol on honey bees

Floral fragrances are important to both scout and forager honey bees in locating pollen and nectar sources (Frisch 1919). High concentrations of phenylpropanoid compounds are produced in plant blossoms during pollen maturation (Wierman 1970) and methyl eugenol itself occurs in the 10 blossoms of *Cassia fistula* L. (Kawano *et al.* 1968) and probably numerous other plants (Mitchell 1965). Because only bees with near-full corbiculae were collected for pollen analysis, it is likely that these individuals entered the traps because the odor was similar or identical to what they experienced at the blossoms of the forage plant. Because *Syzygium* was the only plant represented in all corbiculae samples and honey bees typically only forage on one species of plant each trip, it is likely that foraging at *Syzygium sandwicense* "trained" the bees toward methyl eugenol-like compounds and they were subsequently enticed into the traps. Because methyl eugenol was not detected in the blossoms of *Syzygium sandwicense* or the other main forage plants with the possible exception of *Ocimum*, methyl eugenol either mimics some other compound in the blossoms, or the bees are not responding to specific compounds but the general phenyl propanoids produced by the flowers (Wierman 1970).

There are at least 2 other hypotheses in addition to our interpretation. First it is possible that the blossoms do produce methyl eugenol and it either occurs in quantities too small for us to detect (< 0.1 ppm) or it is volatilized and lost between collection and analysis. Alternatively, the methyl eugenol in our traps may have mimicked similar compounds produced not by the blossoms but by the bees themselves. Terpenoid pheromones produced by the Nasonov gland are used by worker bees to mark the entrance to the colony (Ferguson & Free 1981), to mark water sources (Free & Williams 1970) or mark particularly rich nectar sources (Free & Williams 1972).

Whether the attractant is blossom produced, bee produced or both, it is clear that the ecological basis for honey bee attraction to methyl eugenol is very different than that identified for other species. In the oriental fruit fly and euglossine bees, only males are attracted to methyl eugenol, as they require it as a female attractant (Whitten *et al.* 1989) or as a precursor to sex pheromone production (Nishida *et al.* 1988). Both males and females of some species of Hawaiian Drosophilidae are apparently attracted to methyl eugenol because it orients them to the adult feeding and oviposition substrates (Asquith & Kido 1994).

The absence of methyl eugenol in the forage plant blossoms suggests that while still eliciting a response from bees, it is not the compound to which bees were naturally orienting in the environment. A similar result was found with 3 species of Hawaiian Drosophilidae, which are all clearly attracted to methyl eugenol (Asquith & Kido 1994), but whose feeding and oviposition/larval substrate of decaying *Cheirodendron* leaves does not contain this compound (A. Asquith & M. Kawate unpubl. data, Kircher 1969). With the caveat that individual workers foraging at blossoms that do produce methyl eugenol (e.g. *Cassia fistula*) may facultatively respond more strongly, in general methyl eugenol is probably not an important attractant to honey bees. This is supported by the fact that although there were active hives within 30 m of 2 of the trap sites only 336 bees were captured in all traps over an entire year. Thus while there would certainly be some mortality of individual workers, it is unlikely that a male annihilation program for the oriental fruit fly in Hawaii would have any significant impact on honey bee populations.

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