Exposure to Ginger Root Oil Decreases Capture of Male Mediterranean Fruit Flies (Diptera: Tephritidae) in Trimedlure-Baited Traps

Todd E. Shelly[1,2], **James Edu**[1], and **Elaine Pahio**[1]
[1]USDA-APHIS, 41-650 Ahiki Street, Waimanalo, HI 96795
[2]Center for Conservation Research and Training, University of Hawaii, Honolulu, HI 96822

Abstract. Detection programs for pestiferous tephritid fruit flies rely on traps baited with either natural or synthetic food substances, or so-called male lures. While studies on several tephritid species have demonstrated that protein feeding reduces subsequent attraction to protein food baits, comparable data for male lures are scant and largely restricted to the genus *Bactrocera*. Ginger root oil (GRO) is attractive to males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and males exposed to this oil's scent exhibit heightened mating competitiveness. Because of this increased mating success, several sterile male release programs against *C. capitata* now include pre-release, GRO exposure as part of their standard operating procedures. However, the impact of such exposure on subsequent trap capture has received little study. The purpose of the present study was to measure the effect of GRO exposure on subsequent capture of sterile male medflies in trimedlure-baited traps in two fruit orchards in Hawaii. At each study site, 600 control (non-exposed) and 600 treated (GRO-exposed) males from a mass-reared, genetic sexing strain were released per replicate from a central release point, and trap captures were scored 2 d post-release for eight trimedlure-baited Jackson traps placed in a circular array around the release point. At both orchards, control males were, on average, captured in significantly greater numbers than treated males. This result did not appear to reflect differential mortality between the male types: mortality in screen cages under field conditions was similar over a 48 h period for control and treated males. Implications of these findings for sterile release programs are discussed.

Introduction

Detection programs for economically important tephritid fruit flies rely on traps baited with either natural or synthetic food (or food-based) substances or with so-called male lures (parapheromones) (Jang and Light 1996). Among the factors affecting trap attractiveness, prior access to resources resembling the baits may reduce the response of flies to the traps. Studies on several tephritid species (Prokopy et al. 1992, Prokopy et al. 1994, Vargas and Prokopy 2006) have demonstrated that protein feeding reduces subsequent attraction to protein food baits. Although data are scant and largely restricted to the genus *Bactrocera*, previous exposure to male lures likewise appears to reduce the attractiveness of male lure-baited traps. For example, males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), that were given pre-release access to methyl eugenol, an extremely powerful sex-specific attractant (Steiner 1952), were captured in significantly lower numbers in methyl eugenol-baited traps than non-exposed males (Shelly 1994). Similar results were reported for methyl eugenol-responding males of *B. cacuminata* (Hering) (Brieze-Stegeman et al. 1978) and cue lure-responding males of the melon fly, *B. cucurbitae* (Coquillett) (Chambers et al. 1972).

Males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) are also attracted to parapheromones, most notably the synthetic compound trimedlure (Beroza et al.

1961). Male *C. capitata* are also attracted to certain plant-borne compounds, particularly the sesquiterpene hydrocarbon α-copaene (Flath et al. 1994a,b). Botanical oils containing this compound may be attractive to males (Shelly and Pahio 2002, Shelly et al. 2004a, 2007a), and in the case of ginger root oil (GRO hereafter), which contains 0.4% α-copaene, may enhance male mating success (females, by contrast, are not attracted to GRO, Shelly and Pahio 2002). For example, when competing against wild males for copulations with wild females in field cages, sterile males exposed to the aroma of GRO obtain approximately the same number of matings as wild males, whereas non-exposed sterile males achieve only 33–50% as many matings as wild males (Shelly et al. 2004b). Based on these results, Shelly et al. (2007b) have urged adoption of pre-release, GRO exposure in Sterile Insect Technique (SIT) programs against medfly, and programs in California and Florida have, in fact, implemented 'aromatherapy' as part of standard operating procedures.

For the medfly, few studies relate trap capture to previous exposure to parapheromones, and these provide inconsistent results. In a field experiment conducted in a Hawaiian guava orchard with males from a long-established laboratory strain, Shelly (1999) found no effect of pre-release exposure to trimedlure on subsequent trap capture. However, in a larger-scale project involving aerial release of mass-reared, sterile males over Tampa, Florida, males exposed to GRO prior to release were found in significantly higher numbers in trimedlure-baited traps than non-exposed sterile males (Shelly et al. 2006). From the perspective of the Sterile Insect Technique (SIT), implementation of pre-release exposure to GRO would be most effective if such exposure reduced, rather than increased, post-release capture in survey traps pre-release, since any trap-captured sterile males are, of course, removed from the potential mating population.

The purpose of the present study was to measure the effect of GRO exposure on subsequent capture of sterile male medflies in trimedlure-baited traps in two mixed fruit orchards in Hawaii. Relative to the aforementioned work conducted in Florida, this study monitored trap captures over a small spatial scale and a short time interval. In Florida, sterile males were released aerially along reference lines that were 1.58 km long, and traps (spaced 0.26 km apart along the release lines) were checked at weekly intervals. Here, releases were made at the center of a circular array of traps with a radius of 40 m, and traps were checked 48 h post-release. Thus, whereas trap captures in the Florida study likely reflected a combination of male responsiveness to traps as well as male survivorship and dispersion, the present study focused more narrowly on male responsiveness to trimedlure-baited traps.

Materials and Methods

Study sites. Field work was conducted during June-August, 2006, at two locations near Waimanalo, Oahu. At the University of Hawaii Agricultural Experiment Station, trapping was conducted in a mixed fruit orchard (≈ 2 ha) that contained mango (*Mangifera indica* L.), guava (*Psidium guajava* L.), orange (*Citrus sinensis* (L.) Osbeck), lime (*C. aurantiifolia* (Christm.) Swingle), and breadfruit (*Artocarpus altilis* (Parkins.) Fosb.) along with other non-host trees. Trapping was also performed at a nearby commercial orchard (Frankie's Nursery, ≈ 1 ha) containing mango and assorted citrus trees. Daily maximum and minimum temperatures ranged between 28–32°C and 22–25°C, respectively, during the study period.

Study insects. The flies used in this study were from a temperature sensitive lethal (*tsl*) strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo. The strain has been mass-reared at the USDA-APHIS facility in El Pino, Guatemala, since 1999, and in 2001 ≈ 1.25 million eggs from this facility were used to start the colony in Hawaii. Like other *tsl* strains, Vienna-7/Tol-99

possesses a sex-linked mutation, such that treating the eggs with high temperature kills the female zygotes, thereby allowing production of males exclusively (Franz et al. 1996). Pupae were received 2 d before emergence and divided into two groups (those assigned for adult GRO exposure [treated] and those assigned no GRO exposure [control]), each of which was mixed with a differently colored fluorescent dye (2 g dye per liter of pupae). Dye colors were alternated between treatments between successive replicates. After being dyed, pupae were irradiated in air at 150 Gy of gamma irradiation from a ^{137}Cs source. Adult males were held in screen-covered plastic buckets (5-liter volume; 100–150 males per bucket) with ample food (sugar-agar gel) and water at 23-27 °C and 60-90% RH under natural and artificial light with a photoperiod of 12:12 h (L:D).

GRO exposure. Treated males were exposed to GRO 1 d before release. The GRO was purchased from Citrus and Allied Essences Ltd., Lake Success, NY. We applied 100 µl of GRO to a cotton wick (1.0 cm long) held in an aluminum foil-lined Petri dish (5.4 cm diameter) and placed the dish on the bottom of a male-containing bucket. Males may rest near the GRO source but do not feed on it (Shelly 2001). GRO was placed in 5–6 buckets per replicate to obtain 600 treated males for release (see below). GRO exposure commenced between 0900 and 1000 hrs and lasted 3 h. To avoid inadvertent exposure of control males, GRO exposure was conducted in an isolated room, and, following exposure, the treated males were transferred to a room away from the control males. On the day of a trial, we transferred males to the 'release' buckets and then transported them to the field. In all replicates, both treated and control males were 5–6 d old when released.

Trapping protocol. The same trapping protocol was followed at the two study sites. Jackson traps, containing a sticky insert, were baited with 2 ml of trimedlure applied to a cotton wick (2.5 cm long), which was then placed in a perforated, plastic basket that was, in turn, suspended within the Jackson trap. Traps were prepared in the laboratory (1 km from the study sites) and then immediately transported to the field. Traps were suspended singly in the canopies of eight host trees (2 m above ground) arranged in a circle (radius 40 m) about a central tree, which served as the release point. The same test trees were used in all replicates.

For a given replicate, we set out the traps between 1000 and 1100 hrs and then released 600 GRO-exposed males and 600 non-exposed males at the central release point. Flies were released by placing four buckets (two buckets per male type, 300 males per bucket) on the ground beneath the release tree and gently removing the screen covers from the buckets. The buckets were not tapped or shaken, and the flies exited the buckets on their own volition. Traps were collected 48 h after release, and the flies were examined under a black (UV) light and identified by dye color. In general, successive releases were separated by an interval of 2–4 d to allow previously released flies time to disperse from the study area. Eleven replicates were conducted at each of the two study sites.

At each of the study sites, counts of treated and control males were compared using a paired t-test (df = 10); data from both sites met the assumptions of normality and equal variance. Means ± 1 SE are presented. Analyses were performed using SigmaStat Statistical Software (version 2.0).

Mortality measurements. To determine the potential effect of differential mortality on trap catch, we monitored the survivorship of treated and control males in screen cages held outdoors adjacent (≈ 1 km) to the orchard at the University of Hawaii facility. The cages rested on a table (the legs of which were coated with Tanglefoot to provide protection from ants) in a roofed, but open-sided, wooden structure; the cages were thus shaded and protected from rain. Thirty males (5 d old) of a given type along with food (sugar agar gel) and water were placed in each cage (30 cm cubes) between 0900 and 1000 hrs, and deaths were recorded 48 h later. Treated males were exposed to GRO 1 d before testing following

the procedure described above. Fourteen replicates were performed for control and treated males, respectively, with 7 cages established per treatment on two different days. Results were analyzed using a t-test, as the data met the assumptions of normality and equal variance.

Results

On average, control males were captured in significantly greater numbers than treated males at both sites. At the University of Hawaii facility, an average of 39.6 ± 6.1 (range: 14–82) control males were captured per replicate (totaled over all traps) compared to an average of only 10.6 ± 2.7 (range: 1–31) treated males ($t = 5.0$, $P < 0.001$). At Frankie's Nursery, an average of 127.1 ± 76.5 (range: 65–259) control males were captured per replicate (totaled over all traps) compared to an average of only 80.3 ± 10.8 (range: 39–148) treated males ($t = 2.4$, $P = 0.04$).

Mortality did not differ between control and treated males. On average, 3.3 ± 0.5 control males died per cage over the 48 h test interval compared to 3.0 ± 0.7 treated males ($t = 0.3$, $df = 26$, $P = 0.74$).

Discussion

Pre-release exposure to GRO reduced the recapture probability of mass-reared, sterile males of the Mediterranean fruit fly in field trials conducted at two locations in Hawaii. Differential recapture between GRO-exposed and non-exposed males did not appear to reflect differential mortality: survivorship was similar between the treatments in small cages held under field conditions for 2 d. This finding is consistent with earlier data, which showed no difference in the survival of GRO-exposed and non-exposed males on field-caged host trees over a 2 d interval (Shelly et al. 2004b).

The trapping results presented here were opposite to those reported in the aforementioned Florida study (Shelly et al. 2006), where GRO-exposed males were recaptured in larger numbers than non-exposed males in trimedlure-baited Jackson traps following aerial release. Despite this apparent discrepancy, we suggest that the two data sets may, in fact, not be contradictory but simply reflect the differing experimental designs used in the two studies. The present study involved small spatial and short temporal scales and consequently constituted a direct test of male attraction to trimedlure-baited lures. Correspondingly, the reduced trap capture of GRO-exposed males represents strong evidence of a lessened response to trimedlure. This outcome is advantageous from the standpoint of SIT, because it indicates that GRO exposure would, independent of any effect on mating ability, effectively increase the number of sterile males available for mating with wild females.

The Florida study, by contrast, involved a much larger spatial scale and longer sampling intervals, and consequently trap captures reflected, not only differential response to trimedlure, but also differential mortality and dispersion between GRO-exposed and non-exposed males. Assuming that, as in Hawaii, GRO exposure reduced trap attraction in Florida, the greater capture of GRO-exposed males may have indicated higher survival and/or dispersal ability of GRO-exposed males relative to non-exposed males. In other words, in Florida the greater longevity and dispersion of GRO-exposed males may have "overshadowed" their lessened responsiveness to trimedlure and resulted in higher trap captures for GRO-exposed males. If this interpretation is valid, GRO exposure may benefit SIT, not only through enhanced male mating ability and decreased trap capture, but also through increased longevity and dispersion of released sterile males.

Assessment of this interpretation clearly requires additional data on the effects of GRO

exposure on the longevity and flight ability of sterile *C. capitata* males. In Hawaii, we are currently conducting a field study that directly compares the movement of GRO-exposed and non-exposed males outward from a central release point in a relatively homogeneous habitat (coffee field). Additional studies on longevity under field conditions are also planned.

We further recommend that future studies investigate the potential effect of natural sources of α-copaene (e.g., peel of citrus fruits, MacLeod et al. 1988, Nishida et al. 2000) on the responsiveness of wild *C. capitata* males to trimedlure-baited traps. Such work might improve our understanding of the sensitivity of trimedlure-based survey and detection programs in identifying incipient infestations. Any naturally occurring decrease in male responsiveness would obviously represent a serious obstacle for detection programs.

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