

Aromatherapy and Medfly SIT

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INTRODUCTION

Concern about the biological competence of mass-reared, sterile males of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.), arose simultaneously with the development and implementation of the sterile insect technique (SIT) against this pest. After several decades of SIT, the performance of sterile male medflies in sexual competition against wild males, is still a vital topic of conference symposia and research projects. Several years ago, at one such workshop held in Guatemala, I presented information on the potential application of 'aromatherapy' to improve the mating success of sterile medfly males. In general, the talk was well-received, but one skeptic described ginger root oil, the aromatizing agent, as snake oil, a reference to the phony, cure-all medicine offered to unsuspecting dupes by traveling salesmen in the Wild West of the US.

Undeterred by that comment, or perhaps motivated by it, our laboratory in Hawaii continued its investigation of the ginger root oil-medfly interaction to investigate more fully its possible usefulness and implementation in sterile insect release programs. The objective of this report is to summarize briefly the main findings of this research program and to wrest ginger root oil firmly away from the 'snake oil' label. The report assumes a loosely chronological framework as it documents progression along two experimental scales,

(i) the number of males simultaneously exposed to ginger root oil, starting with small groups of 25 males and ending with rooms with nearly 200 million males and (ii) the experimental arena used to test the effects of aromatherapy, progressing from standard field-cages to large field enclosures to the open field. In addition, brief comments are offered regarding the potential negative effects of GRO exposure, the mechanisms underlying GRO-mediated improvement in male mating success, and the financial costs of GRO aromatherapy.

This report makes no attempt to review the sexual behavior of medfly, the general operational and conceptual underpinnings of SIT, or the use, in general terms, of behavior-altering chemicals as a management tactic in insect control programs. Those interested in these topics should consult Yuval and Hendrichs (2000) and Eberhard (2000) for medfly mating behavior, Hendrichs et al. (2002), Robinson et al. (2002), and Caceres et al. (2004) for medfly SIT, and Ridgway et al. (1990), Jang and Light (1996) and Renou and Guerrero (2000) for use of behavior-modifying chemicals.

THE METHYL EUGENOL-ORIENTAL FRUIT FLY INTERACTION AND INITIAL STUDIES ON MEDFLY AROMATHERAPY

Research on medfly aromatherapy had its genesis in earlier studies that investigated the biological significance of methyl eugenol to males of the oriental fruit fly, *Bac-*

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trocera dorsalis (Hendel). Methyl eugenol was, of course, well known as a powerful attractant to *B. dorsalis* males (as well as males of over 50 congeneric species, Metcalf 1990), who feed voraciously on this chemical. This strong response led to the development and implementation of the male annihilation technique, where the distribution of fiber boards containing methyl eugenol plus a toxicant led to the successful eradication of island populations of this pest (Steiner et al. 1965, 1970; Koyama et al. 1984). While its value as a management tool was well-established, relatively little attention had been given to the underlying function of methyl eugenol in the biology of *Bactrocera* species. In other words, the question of why *B. dorsalis* males might be so strongly attracted to methyl eugenol was not itself the subject of much investigation or conjecture (but see Metcalf 1979, 1990; Metcalf and Metcalf 1992; Metcalf et al. 1979, 1983).

A seminal study by Nishida et al. (1988) showed that break-down products of methyl eugenol were recovered from the rectal gland of *B. dorsalis* males. However, several years lapsed before behavioral studies linking methyl eugenol consumption and male mating performance were conducted. Then, in the mid-1990s, several studies (Shelly and Dewire 1994; Shelly et al. 1996a; Shelly 2000a, 2002; Tan and Nishida 1996, 1998; Hee and Tan 1998) demonstrated that males of *B. dorsalis* (or other methyl-eugenol responding species) given either methyl eugeneol or methyl eugenol-containing flowers had a pronounced mating advantage over unfed, control males, owing apparently to increased signaling activity as well as production of a more attractive pheromonal signal. Additional studies (Shelly and Villalobos 1995; Shelly 2000b) confirmed, and expanded upon, these initial results, and a similar, albeit less pronounced phenomenon, was described for male melon flies, *B. cucurbitae*, and cue lure and raspberry ketone.

Based on these findings, two projects were conducted to determine what effect, if any, trimedlure might have upon mating success of male medflies. Like methyl eugenol, trimedlure was a known attractant of male medflies used widely in survey and detection programs, but the biological significance of this association was unknown and unstudied. The nature of this association was, if anything, more perplexing, because male medflies do not ingest trimedlure but simply rest near it in a quiescent state. The first project (Shelly et al. 1996b) used flies from a recently established colony and examined the effect of trimedlure in mating trials conducted in small cages in the laboratory. Treated males were exposed to trimedlure in small groups (75 males) held in small (5 liter volume) containers. Trials revealed that trimedlure increased male mating success only when testing occurred immediately after exposure; no effect of trimedlure was evident when males were tested even 1 day after exposure. Field tests of female attraction suggested that the short-lived boost in male mating success resulted from increased calling activity but not from the production of a more attractive signal. The second project (Shelly 1999) examined the effect of trimedlure on males mating competition from a long-established, mass-reared (bisexual) strain. The results, which derived from trials conducted on field-caged host trees (3 m diameter, 2.5 m in height), were fairly encouraging. In trials conducted 1 day after trimedlure exposure, the mass-reared (irradiated) males obtained approximately 2/3 of all matings, a result opposite of that observed for non-exposed males (who obtained about 1/3 of all matings). As before, trimedlure appeared to have a short-lived effect, but even in trials conducted 3 or 7 days after exposure, the treated, mass-reared males displayed a higher mating ability than non-exposed males and achieved about the same number of matings as the competing laboratory males. Unlike the previous study,

however, trimedlure had no obvious effect on male calling propensity, and consequently there was greater uncertainty regarding the factor(s) responsible for the trimedlure-induced increase in mating success.

GINGER ROOT OIL AROMATHERAPY: INCREASING THE SCALE OF EXPOSURE

The relatively short-lasting effect of trimedlure prompted a search for other chemicals that might improve the mating performance of male medflies. Two colleagues, T.W. Phillips and K.Y. Kaneshiro, independently suggested ginger root oil (*Zingiber officinale*) and angelica seed oil (*Angelica archangelica*), respectively, both of which contain the known attractant α -copaene (Flath et al. 1994a,b). Working with flies from a recently established colony, we exposed groups of 25 males held in plastic drinking cups (400 ml volume) to 20 μ l of angelica seed oil (0.9% α -copaene), or ginger root oil (0.4% α -copaene). Given limited amounts of material, a single exposure regime was investigated for α -copaene and angelica seed oil, respectively. Namely, mature males were exposed for 6 hours and tested against non-exposed males 2 days later. As with the trimedlure trials, mating performance was assessed using field-caged host trees. The effect of exposure was dramatic, and, in both cases, the treated males achieved approximately 70% of the matings (Shelly 2001).

The commercial availability of ginger root oil (GRO hereafter) allowed for additional tests, and these provided the first hint of the utility of GRO in medfly SIT (Shelly 2001). In trials again conducted in field-caged host trees, GRO-treated males displayed a mating advantage when subject to the same exposure and testing protocol as α -copaene- or angelica seed oil-exposed males, and a test in which males were prevented from contacting the GRO source confirmed that ex-

posure to GRO aroma alone was responsible for the enhanced mating ability. Additional experiments revealed that GRO-treated, mature males had heightened mating success for as long as 5 days after exposure and that even GRO-treated, immature males (1 day old) exhibited a mating advantage over non-exposed males when tested 8-10 days later. Thus, not only did GRO exposure have a long-lasting effect, but it conferred a mating advantage even when administered before sexual maturation, an important point since sterile males are often released at a relatively young age (e.g., 2 days old in the ongoing California Preventive Release Program). In contrast, exposing immature stages to GRO appears ineffective: neither exposing pupae to GRO aroma nor adding GRO to the larval diet (Shelly, unpublished data) influenced the mating success of the subsequently eclosed males.

As noted, these initial studies with GRO were performed using flies from a recently established laboratory colony, i.e., the experimental animals presumably retained many behavioral characteristics of wild flies. Accordingly, the next set of experiments, which still involved GRO exposure on a small-scale (25 males in 400 or 1,000 ml containers exposed to 20 μ l GRO for 3 - 6 hours) and still utilized standard single-tree field cages, compared the mating success between GRO-exposed and non-exposed (irradiated) males from mass-reared strains competing against wild males for copulations with wild females. In Hawaii, Shelly and McInnis (2001) found that GRO exposure boosted the mating ability of males from a 5-year-old bisexual strain dramatically. Whether exposed as immature or mature adults, mass-reared males achieved ~75% of all matings in tests conducted 2 or 4 days following exposure compared to only ~25% for non-exposed, mass-reared males. As found for semi-wild males, mass-reared males contacting the GRO source still displayed increased mating success, again implicating

GRO aroma as responsible for the observed behavioral response.

Subsequent studies investigated the effect of GRO exposure on the mating success of males from genetic sexing strains using temperature sensitive lethal (*ts/*) mutations to cull females from the sterile release population (Franz et al. 1996). Studies conducted in Austria (Shelly et al. 2002), Guatemala (Shelly et al. 2003), and Hawaii (Barry et al. 2003) utilized different combinations of *ts/* and wild strains yet all showed that GRO exposure boosted the mating competitiveness of *ts/* males in field-cage trials. Moreover, females first mated to wild males were more likely to remate with GRO-exposed *ts/* males than GRO-deprived *ts/* males (Shelly et al. 2004a), which, given last male sperm precedence (Saul and McCombs 1993), may constitute an additional benefit of GRO aromatherapy. Barry et al. (2003) expanded the focus in an interesting direction by comparing mating success of GRO-exposed and non-exposed *ts/* males under different overflooding ratios. Importantly, they reported that the proportion of matings obtained by GRO-exposed *ts/* males released in equal numbers as wild males was comparable to that observed for non-exposed *ts/* males released in numbers 10 times greater than the wild males. Given the boost in mating ability, Barry et al. (2003) suggested that pre-release GRO exposure might allow for substantial reductions in the numbers of sterile males released in SIT programs.

While the results obtained for mass-reared, sterile males were uniformly promising, GRO exposure in all of these studies was conducted using small groups of males held in small containers. Progressing toward GRO implementation at a programmatic scale, we next assessed the effectiveness of GRO exposure using individual PARC boxes (Shelly et al. 2004b). PARC (Plastic Adult Rearing Containers) boxes (0.48 by 0.60 by 0.33 m) are used in the ongoing SIT programs in California

and Guatemala. Typically, pupae are placed in paper bags (100 ml per bag, where 1 ml contains ~ 60 pupae), and six bags are placed in individual PARC boxes (~ 36 000 pupae per box). Emerged males feed on a sugar-agar block placed on a screened opening on top of the box prior to chilling and release. In one series of tests, GRO was applied to PARC boxes (by placing oil-laden blotter paper on the screened opening on the top of the box) after the day of peak adult emergence at doses of 0.0625, 0.25, 0.5, 1.0, and 2.0 ml GRO per box. As before, GRO-exposed and non-exposed, irradiated *ts/* males competed against wild males for matings with wild females on field-caged trees. GRO significantly increased the mating success of *ts/* males for all doses < 2 ml, and the effect of GRO was uniform over these doses. However, the 2.0 ml dose was ineffective, and the relative mating success of *ts/* males exposed to this high dose was similar to that of non-exposed *ts/* males. Thus, while we did not identify a 'minimum' dose below which GRO was ineffective, we discovered that GRO may have a diminished impact at high doses.

In a second series of tests involving PARC boxes, we applied GRO (0.25 or 1.0 ml) at the time of pupal placement and left it in place until the males were removed for testing (6 days later). GRO had no effect at the 0.25 ml dose but did increase male mating success at the 1.0 ml dose to a level similar to that observed in the aforementioned adult exposure tests. Thus, owing to volatilization before adult emergence, it seems that a pupal-adult exposure regime requires higher doses of GRO (than the adult-only exposure regime) to effect an increase in mating frequency. Coupled with the earlier data showing no effect of GRO exposure on the pupal stage, this result argues for GRO application during the adult stage exclusively.

The data obtained from PARC boxes represented the first solid evidence that GRO could influence the mating ability of large numbers

of sterile males. However, GRO exposure to individual PARC boxes was not deemed practical at a programmatic scale, where *ts/* males from hundreds or thousands of PARC boxes are released each day. Accordingly, we shifted our research efforts to the David Rumsey Sterile Fruit Fly Ecllosion Facility of the Medfly Preventative Release Program, Los Alamitos, CA. Started in 1996, this program makes aerial releases of sterile *ts/* males over an area of ca. 6 400 km² that includes the Los Angeles basin and surrounding areas. Approximately 40–45 million sterile males (from ca. 1 000 – 1 200 PARC boxes) are released daily. PARC boxes containing pupae, and subsequently eclosed adults, are stored in holding trailers prior to release, with each trailer holding ~ 360 PARC boxes or ~ 14 million sterile males. Our objective was straightforward - to run mating trials in field cages that permit comparison of the relative mating success of sterile *ts/* males from GRO-aromatized trailers versus non-aromatized trailers.

Although a variety of doses and spatial distributions of GRO sources were investigated, a logistically simple, and effective, protocol was identified (Shelly et al. 2007a) and subsequently implemented at the Los Alamitos facility (from January 2005 to present). The trailers, which are approximately 18 m long by 3 m wide by 2.5 m (volume ~ 135 m³), contain fans at 1/3 and 2/3 the total length; the fans are mounted on boards (~ 1 m high) and blow in the same lengthwise direction. Four GRO sources were set up per trailer, two at each fan 'bank'. At each source, we applied 3 ml of GRO to each of 3 cotton wicks resting in an aluminum foil-lined Petri dish, which, in turn, was placed on the wooden structure used to support the fans. Thus, two Petri dishes, each containing 9 ml of GRO, were present at each fan bank for a total dose of 36 ml of GRO per trailer (ml GRO/m³ room volume = 0.27). GRO exposure lasted 24 h and was initiated the day before chilling (and release) when most of the *ts/* males were 2 days old.

Because the study was conducted in California, we were unable to use wild flies in the mating trials and instead used (bisexual) laboratory strains that had been irradiated as pupae prior to shipment. This fact notwithstanding, we found that the aforementioned exposure regime enhanced the mating success of *ts/* males in mating trials involving two different strains. In competing against males from a Guatemalan strain for females from that same strain, GRO-exposed *ts/* males obtained 55% of the total matings, whereas non-exposed *ts/* males accounted for 36% of the total matings. Similarly, in comparable trials involving a Hawaiian strain, *ts/* males from the GRO treated trailers obtained 53% of the total matings compared to 38% for non-exposed *ts/* males. Against both strains, exposure to GRO resulted in a significant increase in the mating success of *ts/* males over that observed for non-exposed *ts/* males.

Although the results are preliminary, 'whole room' exposure has also been tested at the eclosion facility at Retalhuleu, Guatemala with promising results. In three different rooms holding 83–179 million *ts/* males, we placed 160–400 ml of GRO at 16–40 locations (ml GRO/m³ room volume = 0.37–0.50). As before, GRO exposure lasted 24 hours and was initiated the day before chilling. In mating trials using wild flies, we found that GRO-exposed *ts/* males achieved a significantly higher proportion of the total matings than non-exposed *ts/* males (38% versus 24% total matings over all tests). This result is remarkable, because it indicates that room aromatization with GRO can improve the mating success of more than 100 million medfly males. Additional tests involving substantially higher and lower doses (e.g., 0.80 and 0.20 ml GRO/m³ room volume) should be conducted to better characterize the dose-dependent response of the *ts/* males.

While aromatizing entire rooms containing PARC boxes appears feasible, eclosion towers may soon replace PARC boxes as the pre-

ferred storage unit for sterile male medflies (Salvato et al. 2004). A tower consists of interlocking, screen-paneled, aluminum frames or trays stacked on a wheeled base. Pupae are placed in a trough around the edge of a tray, and food is placed on the screen panel. This procedure is repeated for each of the 60-80 trays that comprise a completed tower. Upon emergence, the males move on to the screen panel to feed. As a small fan (blowing upwards) is fitted on the top of the tower for ventilation, the entire contents of a tower (approximately 1.25 million flies) is easily aromatized by placing a GRO source beneath the lowest tray. Studies of *ts/* males conducted in Florida and Hawaii have shown that placement of 1 ml of GRO below a tower for 24 hours resulted in increased mating performance relative to that observed for males held in non-aromatized towers (Shelly et al. 2006). While GRO application to individual towers appears effective, the effectiveness of aromatizing entire rooms holding multiple towers has not yet been assessed.

GINGER ROOT OIL AROMATHERAPY: INCREASING THE SCALE OF EVALUATION

In all the studies noted above, evaluation of GRO aromatherapy was conducted using standard, 'single-tree' field cages. While this experimental stage is far superior to small, laboratory cages, more rigorous assessment requires testing in large field enclosures (containing multiple host trees) or the open field. To date, we have conducted one study (Shelly et al. 2005) using large field enclosures (containing > 10 guava trees) and one study in a coffee field (Shelly et al. 2007b). In both cases, *ts/* males were exposed to GRO in PARC boxes.

In the large enclosures, we released wild flies and *ts/* males, either GRO-exposed or non-exposed, at variable overflooding (*ts/*:wild males) ratios (5:1, 10:1, 30:1, and

60:1), dissected eggs from apples suspended in the tree canopy as oviposition sinks, and then incubated the eggs to determine the incidence of egg sterility (a control cage containing wild flies exclusively was also run to determine the 'natural' frequency of egg sterility). At all four ratios tested, we found that the proportion of unhatched eggs was significantly greater in enclosures with GRO-treated *ts/* males than control, non-treated *ts/* males (Fig. 1). Interestingly, the level of egg sterility observed increased (from 67% to 89%) with increasing overflooding ratios for releases of non-exposed *ts/* males but was fairly constant (86% - 96%), and not significantly different, over the different overflooding ratios for releases of GRO-treated *ts/* males. As a result, the percent egg sterility observed for GRO-exposed *ts/* males at the 5:1 overflooding ratio was similar to that observed for non-exposed *ts/* males at all higher overflooding ratios, including even 60:1. Thus, like the aforementioned study by Barry et al. (2003), these results indicate that pre-release GRO exposure may allow a reduction in the numbers of sterile males released.

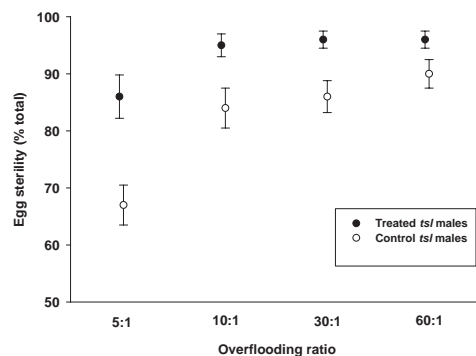


Figure 1. Relative number of sterile (unhatched) eggs collected (% total) for GRO-exposed (treated) and non-exposed (control) *ts/* males at the four overflooding ratios tested in large field enclosures in Hawaii. Symbols represent mean values (± 1 SE; $n = 7$).

Data from a Hawaiian coffee field also provided evidence for improved SIT through aromatherapy. At weekly intervals over a 13-week period, we released GRO-exposed or non-exposed *ts/* males in two small plots and monitored wild and sterile male populations as well as egg sterility levels (eggs were dissected from coffee fruits and then incubated). The wild fly population was very large (>1 000 wild males/trap/day), consequently overflooding was not achieved. Despite the fact that, in general, lower numbers of *ts/* males were trapped in the plot receiving GRO-exposed males, egg sterility levels were higher in that plot than the plot receiving non-exposed *ts/* males for most weeks (Fig. 2). Over the entire study, the average incidence of unhatched eggs was 17% for the plot with GRO-treated *ts/* males compared to 11% for non-exposed *ts/* males, a statistically significant difference. Correspondingly, the average value of Fried's competitiveness index (C) was significantly higher for the aromatized *ts/* males than the control *ts/* males (0.42 vs. 0.17, respectively, computed over entire study period without time lag; Fig. 3). While this study has two flaws – no SIT-induced reduction in wild fly numbers

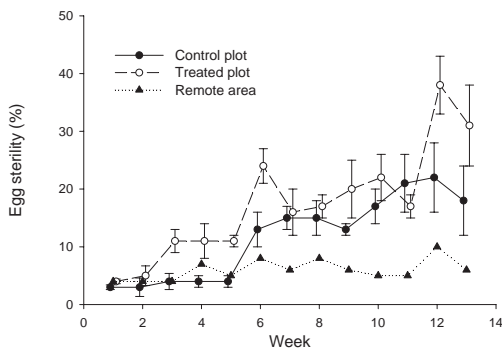


Figure 2. Levels of egg sterility observed in control (non-exposed *ts/* males) and treated (GRO-exposed *ts/* males) plots and the remote area (without releases of *ts/* males) in a Hawaiian coffee field. Values are weekly means (\pm SE) based on dissections of 20 fruits per sampling area ($n = 5$) per plot (i.e., 100 fruits total).

was observed and bad weather prevented replication the following year with treatments reversed in the two study plots – it probably presents the strongest case for the incorporation of GRO aromatherapy in medfly SIT as the data derive entirely from the open field.

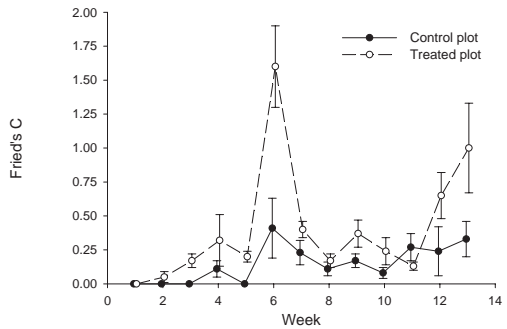


Figure 3. Values of Fried's C for control (non-exposed *ts/* males) and treated (GRO-exposed *ts/* males) released in a Hawaiian coffee field. Values are weekly means (\pm SE) based on trap captures and egg sterility estimates for five sampling areas per plot.

POTENTIAL NEGATIVE IMPACTS OF GRO EXPOSURE

The benefits of GRO aromatherapy regarding increased mating competitiveness must be weighed against potential negative effects on survivorship and dispersal. Fortunately, it appears that aromatization has no negative impact on either of these parameters. In Hawaii, GRO-exposed and non-exposed *ts/* males displayed similar survival over a two day period in field cages lacking food or water (Shelly et al. 2004b), and, in a laboratory study, GRO exposure had no effect on the ability of *ts/* males to withstand starvation following removal of the sugar-agar-gel diet (Levy et al. 2005). Similarly, after aerial release of sterile males in Florida, the decline in daily capture rate was nearly identical between GRO-exposed and non-exposed *ts/* males (Shelly et al. 2006). In this latter study, there was also evidence, based on presence/

absence data over all traps, that dispersal was independent of GRO treatment. Consistent with this field result, J. Zermeño (personal communication) measured travel distances of tethered males on a laboratory flight mill and found no difference between GRO-exposed and non-exposed males.

MECHANISM OF AROMATHERAPY

Our research has focused primarily on documenting the effects of GRO exposure on male mating success and not on elucidating the behavioral, physiological, or chemical factors responsible for the improved mating performance. However, studies conducted in a large field enclosure (Shelly 2001) and in a laboratory wind-tunnel (Papadopoulos et al. 2006) both indicate that GRO exposure does not affect the attractiveness of the male sex pheromone to females. Instead, four preliminary findings from ongoing work indicate that GRO aroma interacts with the male exoskeleton in some way to produce a scent attractive to females. First, medfly males exposed to GRO aroma for 30 s immediately before mating trials had a mating advantage over control males. Given the short interval between GRO exposure and testing, it appears unlikely that the increased success of treated males required incorporation and physiological processing of airborne chemicals. Second, in screen cages in the laboratory, females preferentially land on chilled (dead) males that had been exposed (while alive) to GRO the previous day compared to chilled (dead) non-exposed males. The same result was obtained whether the males were visible to females or covered by a cotton cloth (blocking visual but not olfactory stimuli). Third, rinsing aromatized males in hexane removed this female preference. Fourth, males whose antennae were surgically removed prior to GRO exposure still have a mating advantage over control (sham operated) males,

indicating that males do not need to smell the aroma to gain a mating advantage.

FINANCIAL COST OF AROMATHERAPY

Relative to other costs incurred in SIT programs, the use of GRO would represent a minor expense. For the California SIT program, the cost of GRO exposure is approximately \$0.20 per million *ts*/males (based on a price of \$67 per kg of GRO, a dose of 41 g (~ 36 ml) per trailer, and 14 million *ts*/males per trailer). As the California program pays approximately \$180 per million *ts*/pupae from Guatemala, the added cost of GRO exposure is negligible ($\$0.20/\$180 = 0.1\%$). Other supplies (cotton wicks, pipettes, etc.) plus labor would, of course, increase the total cost but only by a small amount.

GRO AROMATHERAPY: A TECHNIQUE ROOTED IN THE CHEMICAL ECOLOGY OF *CERATITIS CAPITATA*

The aroma of GRO increases the mating performance of male medflies, and, with the proper implementation, this fact can be used to improve the effectiveness of medfly SIT. The practical value of aromatherapy research should not obscure the emerging biological view upon which it relies, namely that plant chemistry has important effects on the sexual behavior of the medfly. Although not particularly well studied, this association has been identified in several studies. Working with wild flies, Papadopoulos et al. (2001) showed that males were highly attracted to and arrested on ripe, wounded oranges (cuts made in the peel) and that males given direct access to such fruits had a mating advantage over fruit-deprived males (see also Shelly et al. 2004c). Likewise, Shelly and Villalobos (2004) observed clusters of wild males at specific sites on the branches of guava trees and

found that males given access to these locations have a mating advantage over males exposed to guava branches lacking such sites. Additionally, exposure to ripe (unwounded) guava fruits conferred a mating advantage.

Given these results, it appears that, independent of GRO aromatherapy, *tsl* males may enjoy increased mating competitiveness through post-release contact with plants rich in α -copaene. Pre-release exposure to GRO, however, eliminates the 'need' for *tsl* males to locate chemical sources in the environment (thereby eliminating time and energy costs associated with searching) and guarantees that *tsl* males benefit fully from exposure to the performance-enhancing oil. Shelly and McInnis (2001) found that, when both wild and *tsl* males were exposed to GRO, wild males maintained a mating advantage over the *tsl* males. Importantly, however, the advantage observed was smaller than that observed when neither male type was exposed to GRO. Thus, while the effectiveness of GRO aromatherapy may vary with host tree availability in the target area (being lower where α -copaene-rich plants are prevalent), the procedure should improve medfly SIT over all areas.

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