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PEST MANAGEMENT

Natural Oil Lure Outperforms Trimedlure in Capturing Males of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae)

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Abstract

Females of certain tephritid fruit fly species (Diptera: Tephritidae) pose an enormous agricultural threat, as they oviposit in commercially important fruits and vegetables. Trapping networks are often operated in fruit fly-free areas to detect incipient infestations. Trapping relies largely on male attractants, so-called male lures, with trimedlure (TML) being used to detect invasive *Ceratitis* spp. Operating large-scale surveillance programs incurs substantial costs for both supplies and labor, and the problem is exacerbated by the fact that trimedlure (as well as other male lures) is effective for relatively short intervals in the field (6–8 weeks). Because frequent servicing increases costs, there is considerable interest in modifying existing lures or developing new formulations to extend their effective field longevity. Here, we present results of a field study in Hawaii on a wild population of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), that compared male captures in traps baited with (i) fresh liquid TML, (ii) TML plugs, (iii) a novel controlled-release TML sachet, and (iv) a novel natural oil blend dispensed from a sachet. Catch was recorded weekly for 12 weeks and then at 16 and 20 weeks, with 12 traps deployed per treatment. The natural oil formulation, which contains the natural plant product α-copaene, was as effective as the fresh liquid TML even after weathering for 20 weeks. Future work will focus on developing a dispenser for this formulation that is compatible with standard trap design and deployment.

Keywords Invasive species · Agriculture · Detection · Trapping · Male lures

Introduction

In southern areas of the USA, states with extensive horticultural industries, such as Florida, Texas, and California, operate year-round trapping networks to detect infestations of true fruit flies (Diptera: Tephritidae) (IPRFFSP 2006; Gilbert et al. 2013). Females of certain tephritid species pose an enormous agricultural threat, as they oviposit in many commercially important fruits and vegetables, causing direct losses as well as triggering potential quarantine restrictions that hinder trade (Enkerlin 2021). For example, the establishment of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) in California, would cost an estimated 1–2 billion US$ in losses due to control and trade embargos (Siebert and Cooper 1995; CDFA 2008).

Trapping relies largely on male attractants, so-called male lures, with the most important lures being trimedlure (TML; *Ceratitis* spp.), methyl eugenol (ME; *Bactrocera* spp.), and cue-lure (CL; *Bactrocera* and *Zeugodacus* spp.) (Tan et al. 2014). Operating large-scale surveillance programs incurs substantial costs for both supplies and labor required to maintain and service the traps. For example, at any one time, trapping networks in California and Florida operate approximately 10,000 and 15,000 TML-baited traps, respectively, for detection of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Vargas et al. 2013; A. Fox, pers. comm.).

Adding to the challenge of running such large programs, commercially available male lures are effective for relatively short periods of time. With respect to medfly, international guidelines (FAO/IAEA 2018) recommend replacing TML lures every 4–10 weeks, and both California and Florida programs typically re-bait traps every 6 weeks (M. Alzubaidy and A. Fox, pers. comm.). Because frequent

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servicing increases costs, there is considerable interest in modifying existing lures or developing new formulations to extend their effective field longevity. One approach is to increase the amount of TML placed in individual traps. The standard dispensers are polymeric plugs (2-g TML), which are effective for 6–8 weeks (Leonhardt et al. 1987, 1989; Dean et al. 2018). Several studies (Leonhardt et al. 1989; Shelly and Kurashima 2020; Francis et al. 2023) have investigated the effect of doubling TML content in the plugs from 2 to 4 g, but the results have been inconsistent.

Alternatively, TML can be combined with additives (termed extenders) that reduce the lure's volatility. Capilure® is the most commonly used TML-plus-extender blend, but studies have yielded inconsistent results. In some cases, Capilure was as attractive as TML and had much longer field effectiveness (Hill 1987; Nakagawa et al. 1981; Rice et al. 1984), but in other studies Capilure had lower potency and/or longevity than TML (Baker et al. 1988; Shelly 2013).

Finally, alternative substances might be identified that are equally, or even more, attractive than TML and have inherently lower volatility or can be presented in controlled, slow-release dispensers. The most attractive natural product known for male medflies is the sesquiterpene (+)-α-copaene (Teranishi et al. 1987; Flath et al. 1994a,b; Lull and Gil-Ortiz 2023). Angelica seed oil (*Angelica archangelica* L.), a source of (+)-α-copaene (Takeoka et al. 1990), was used in the 1956 state-wide medfly monitoring program of Florida (Steiner et al 1957). Even though angelica seed oil-baited traps captured approximately three times as many medfly males as TML-baited traps, the use of angelica seed oil was discontinued in favor of TML because of the restricted availability of angelica seed oil (Steiner et al 1957).

An alternative and inexpensive source of (+)-α-copaene is ginger root oil (*Zingiber officinalis* Roscoe), and fractional distillation can increase the concentration of the compound from 0.4% in commercially available oil to 8% in so-called enriched ginger oil (EGO; Shelly and Pahio 2002). Several studies have compared the attractiveness of TML to EGO, but the results have been inconsistent. Studies in southern Africa (Mwatawala et al. 2013, 2015; Manrakhan et al. 2017) reported that EGO was equally or even more attractive to *C. capitata* males than TML, whereas field tests from Tunisia (Hafsi et al. 2019) and Hawaii (Shelly and Pahio 2002; Shelly 2013) found that TML was significantly more attractive than EGO (although interpretation is confounded, the TML and EGO sources were not uniform across all these studies). Niogret et al. (2017, 2018) studied the attraction of *C. capitata* males to several other natural oils, including cubeb oil, ginger root oil, tea tree oil, manuka oil, and Valencia orange oil, and intriguingly found that attraction was not correlated with α-copaene content, suggesting that other volatiles interact synergistically with α-copaene to attract male medflies.

The present study describes a field test comparing captures of *C. capitata* males in Jackson traps baited with liquid TML or TML plugs with (i) TML presented in a controlled-release membranous sachet, which slows the release of the lure, such that its loss is linear rather than exponential (C. Oehlschlager, unpubl. data) and (ii) a novel lure composed of natural oils that include α-copaene and was dispensed from the same type of slow-release membranous sachet. As described below, trapping of wild medflies was conducted in a coffee field in Hawaii over a period of 20 weeks, with liquid TML serving as the standard (and being replaced weekly) and the other baits weathered over the course of the study. Our chief objective was to evaluate the potency and longevity of the TML plugs, the TML sachet, and the natural oil sachet relative to the standard TML liquid treatment.

Materials and methods

Study site

Fieldwork was performed in a commercial coffee field (*Coffea arabica* L.; 65 ha, 100-m elevation) in central Oahu approximately 10 km southeast of Haleiwa, HI, USA. Plant rows were spaced 3 m apart, and individual plants were maintained at a height of 2–3 m. Within a row, trunks of adjacent plants were separated by 1–2 m, but foliage was generally contiguous between neighboring plants. Trapping was conducted between June 29–November 16, 2022, during which average daily minimum and maximum temperatures were 20.0 °C (range: 17.8–22.8 °C) and 28.6 °C (range: 26.1–29.4 °C), respectively, with relative humidity generally between 55 and 85%. The study period was relatively dry, with rainfall of approximately 2.3 cm per month (weather data from Wheeler Army Airfield, Wahiawa, HI, approximately 10 km from the field site).

Traps and lures

Flies were captured using Jackson traps (Scentry Biologicals, Inc., Billings, MT), the standard trap used in fruit fly surveillance programs in the USA (IPRFFSP 2006). Jackson traps were white, "delta" traps made of thick polymer-coated paper (12.7 cm length × 9.5 cm width at base × 8.4 cm height at apex). A removable insert, made of the same polymer-coated paper as the trap body and coated with "stickem," was placed on the bottom of the trap to retain insects. Traps were suspended from branches using a metal hanger, with a straight rod positioned under the roof along the apex of the trap.

Four lures were compared:

(i) Liquid TML (2 mL; FarmaTech Intl., North Bend, WA) applied to a cotton dental wick. Within a trap, the treated wick was held in a perforated, plastic basket (2.5 cm length × 3.0-cm diameter at open end [covered by plastic flap once wick in place] × 2.0-cm diameter at closed end). This treatment was considered the control, and traps were baited with fresh TML-impregnated wicks at weekly intervals. Although the 2-mL dose was lower than the other treatments (see below), previous work showed that, over short intervals (≤ 1 week), this dose attracted as many *C. capitata* males as wicks containing 5 mL of TML (Shelly et al. 2016).

(ii) TML plugs (4 g). These devices (Scentry Biologicals Intl.) were polymeric plugs manufactured to fit snugly in the perforated baskets held in the Jackson traps. While 2-g TML plugs are still widely used, 4-g TML plugs are now used in Florida's trapping program (A. Fox, pers. comm.). Plugs were not replaced but were weathered over the 20-week course of the study (as described below).

(iii) TML (2.15 g) sachet. These dispensers (ChemTica Intl., Sto. Domingo, Costa Rica) were sachets (5.7 × 9 cm folded to make a device approximately 5.7 × 4.5 cm) prepared following Heath (2000) using a proprietary plastic film to release the TML. The sachet was suspended in the trap by from the hanger, which was inserted through two holes located along the top edge of the sachet. The TML sachets were not replaced but were weathered over the course of the study.

(iv) Natural oil formulation. These dispensers (ChemTica Intl.) were sachets of the same dimensions as above and contained 4.2 g proprietary blend of natural oils enriched with α-copaene (P388-B Lure, ChemTica Intl.). The natural oil sachets were not replaced but were weathered over the course of the study.

Trapping protocol

Jackson traps were placed on windbreak trees (Norfolk pines, *Araucaria heterophylla* (Salisb.) Franco; Araucariaceae) planted in a row through the coffee field. This protocol was adopted because of our uncertainty regarding the timing and location of harvesting, which is performed by a mobile machine that straddles a row and violently shakes the coffee plants beneath to loosen and collect ripe berries. This procedure destroys any traps placed in the harvested rows. Traps, which were 3–4 m from the nearest rows of coffee, were placed 1.5–2.5 m above ground in shaded locations, with adjacent traps separated by approximately 15 m. Traps were serviced (i.e., sticky inserts were replaced in all traps, and fresh TML-impregnated wicks were placed in control traps) at weekly intervals from week 0 (all lures fresh) to week 12. Following this, traps were left in the field (without sticky inserts) until week 15, when sticky inserts were placed in all traps, and fresh TML-impregnated wicks were placed in control traps. After a week of trapping (i.e., weeks 15–16), this procedure was repeated until week 19, which was the final week of trapping (i.e., week 19–20). Twelve traps per treatment were deployed (total = 48 traps), with treatments positioned in repeating sequences along the windbreak. Traps were advanced one position between weeks to minimize any locational bias in captures. Collected sticky inserts were taken to the laboratory to identify and count the flies.

Statistical analysis

Capture data were analyzed using generalized linear models (GLM) utilizing a Poisson distribution with a log link function, with week (weathering interval) and treatment as independent variables. The significance of the independent variables was tested using a likelihood ratio chi-square. An initial analysis included data over all sampling periods, but the results generated were not considered particularly relevant to the 6-week re-baiting schedule practiced in California and Florida. Consequently, data were analyzed for three distinct time categories: 1–6 weeks, 7–12 weeks, and 16 and 20 weeks. Within each of these three intervals, post hoc pair-wise comparisons were made using the nonparametric Wilcoxon rank sum test. Note that pair-wise comparisons were drawn and interpreted for treatments only and not weeks, as focus centered on the performance of the different treatments and not temporal fluctuations in captures, which likely reflected both natural fluctuations in medfly population size and variable environmental conditions (particularly, wind speed and direction) during trapping intervals. Analyses were performed using JMP (Version 12, SAS Institute Inc., Cary, NC, USA).

Results

Trap captures for all treatments over all trapping periods are presented in Fig. 1. Based on data from all sampling periods, both week ($\chi^2 = 831.3$, $P < 0.001$, df = 13) and treatment ($\chi^2 = 38{,}079.8$, $P < 0.001$, df = 3) were significant. The interaction was significant as well ($\chi^2 = 13{,}694.6$, $P < 0.001$, df = 39). Among treatments, every pair-wise comparison was significant ($P < 0.002$ in all cases).

As noted above, additional analyses considered weathering intervals more germane to the standard re-baiting schedule. For weeks 1–6, week ($\chi^2 = 543.3$, $P < 0.001$, df = 5)

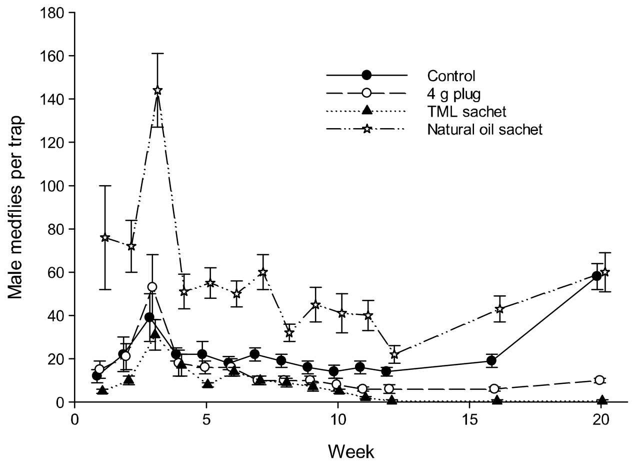

Fig. 1 Captures of *Ceratitis capitata* males over the 20-week study period in Jackson traps baited with four different treatments: control (2-mL liquid TML on a wick), 4-g TML plug, TML sachet (2.15-g TML), and natural oil formulation. Symbols represent averages (±1 SE; $N=12$ traps per treatment per week). The control treatment was refreshed weekly; all other treatments were weathered and not replaced over the study period

and treatment ($\chi^2=3796.6$, $P<0.001$, df = 3) were significant. The interaction was significant as well ($\chi^2=412.1$, $P<0.001$, df = 15). The Wilcoxon test revealed that, for weeks 1–6, traps baited with the natural oil lure captured significantly more flies than any of the other treatments (Table 1). Traps baited with the control or 4 g lures captured similar numbers of flies, and both of these treatments exceeded captures with the TML sachet.

Table 1 Captures of *Ceratitis capitata* males in three trapping intervals: weeks 1–6, weeks 7–12, and weeks 16 and 20. Values are averages (±1 SE); for each treatment, $N=72$ for weeks 1–6 and weeks 7–12, and $N=24$ for weeks 16 and 20. Within a particular temporal category, means followed by the same letter did not differ significantly (Wilcoxon rank sum test; $P>0.05$)

Trapping interval	Treatment	*C. capitata* males captured
1–6 wks	Control	22.4 (5.6) A
	4-g TML plugs	23.2 (6.2) A
	TML sachet	14.2 (3.1) B
	Natural oil sachet	74.6 (12.5) C
7–12 wks	Control	16.9 (2.7) A
	4-g TML plugs	8.4 (2.1) B
	TML sachet	5.5 (1.3) C
	Natural oil sachet	40.0 (6.6) D
16 + 20 wks	Control	38.5 (5.1) A
	4-g TML plugs	8.3 (1.6) B
	TML sachet	0.1 (0.1) C
	Natural oil sachet	51.2 (7.8) A

For weeks 7–12, week ($\chi^2=350.3$, $P<0.001$, df = 5) and treatment ($\chi^2=2613.7$, $P<0.001$, df = 3) were significant. The interaction was significant as well ($\chi^2=226.5$, $P<0.001$, df = 15). The Wilcoxon test showed that captures differed significantly among all treatments, with captures being greatest with natural oil followed by the control, 4 g, and TML sachet treatments, respectively (Table 1).

For weeks 16 and 20, week ($\chi^2=7.9$, $P<0.01$, df = 1) and treatment ($\chi^2=2040.3$, $P<0.001$, df = 3) were significant. The interaction was significant as well ($\chi^2=70.9$, $P<0.001$, df = 3). Captures did not differ significantly between the control and natural oil treatments, which both exceeded captures in the remaining two treatments (Table 1).

Plotting the performance of the three treatments relative to the control (2-mL liquid TML on wick) provides a visual summary of these statistical results (Fig. 2). Weekly ratios of captures by treatment:control lures quickly indicate the effectiveness of a given treatment, where a ratio of 1 indicates equal performance. Thus, the natural oil sachet captured 1.6–6.4 times as many flies as the control during weeks 1–12 and 1.03 times as many flies in week 20. In contrast, both the 4-g TML plug and the TML sachet captured fewer flies than the control (ratios < 1) from week 6 onward.

Discussion

The present study yielded several clear-cut results. First, consistent with other recent studies, 4-g TML plugs did not display prolonged effectiveness. Both Shelly and Kurashima (2020) and Francis et al. (2023) found that 4-g TML plugs attracted *C. capitata* males in similar numbers as traps baited

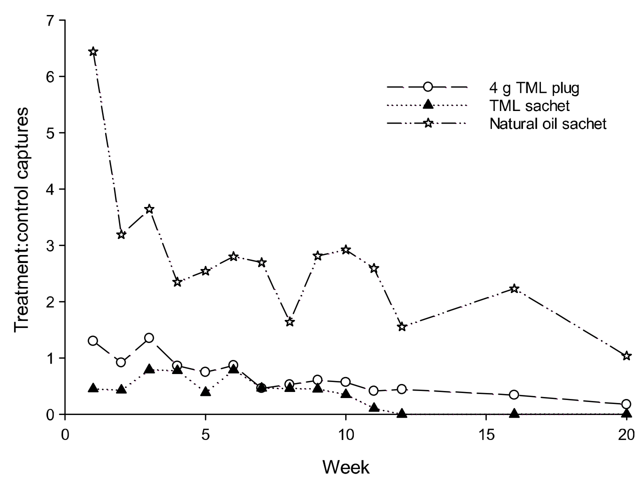

Fig. 2 Relative performance of 4-g TML plugs, TML sachets, and natural oil sachets to the control lure (2-mL liquid TML on wick) for individual weeks. Capture ratios (treatment:control captures of *Ceratitis capitata* males) were computed using average captures for each lure type for a given week

with 2-mL TML on a wick renewed weekly (control) during weeks 1–8 but attracted significantly fewer males than control traps after week 10. In the present study, captures were similar between fresh TML and 4-g plugs weathered 1–6 weeks, but fresh lure attracted significantly more male medflies than 4-g plugs weathered 7–12 weeks or 16–20 weeks. Second, traps baited with the TML sachet captured significantly fewer flies than traps baited with any of the other treatments. This was true even for weeks 1–6, when the TML sachets were (like all baits) relatively fresh. This result was surprising, as these same TML sachets were effective in field tests conducted in Costa Rica (C. Oehlschlager, unpub. data). Finally, the natural oil lure was both potent and long-lasting. The natural oil lure attracted more *C. capitata* males than the control lure after both 1–6 and 7–12 weeks of weathering and attracted similar numbers of males as fresh TML even after 16 and 20 weeks of weathering.

Comparisons of captures in traps baited with TML sachets or TML plugs (2 g) in Costa Rica revealed no significant difference in captures over several trials (Gonzalez et al. 2022). In addition, this study found that the release rate of TML from sachets was uniform over 6 weeks of weathering and did not display the steep decline in TML content often observed for newly deployed 2 g polymeric plugs (Warthen et al. 1999; Suckling et al. 2008; Flores et al. 2017; Dean et al. 2018). When tested in Hawaii, however, traps baited with the TML sachet (2.15-g load) captured fewer *C. capitata* males than traps baited with 4-g TML plugs, presumably because the release of TML from the sachets was lower than from the plugs. To our knowledge, only Leonhardt et al. (1989) have made simultaneous measurements of release rates and captures in traps baited with 2- or 4-g polymeric plugs, and their tests revealed both higher release rates and higher attractancy for the 4-g plugs. TML release rate is known to influence capture of *C. capitata* males, though this relationship appears to be nonlinear and variable (Alfaro et al. 2008; Domínguez-Ruiz et al. 2008; Navarro-Llopis et al. 2011). Leonhardt et al. (1987) found that a particular membrane/disk dispenser released TML at such a low rate that few male medflies were attracted, and the limited effectiveness of the TML sachet in the present study may have reflected low emission rates of TML at the Hawaiian study site.

The strong attractancy of the natural oil sachets mirrored their performance in a preliminary a field test in Costa Rica. In that trial, traps with the natural oil sachets captured significantly more *C. capitata* males over a 6-week period than did traps baited with 2-g TML plugs (weekly averages: 77 vs. 5 males per trap, respectively; C. Oehlschlager, unpubl. data). Here, natural oil sachets weathered for 24 weeks were found to be as effective as TML liquid freshly applied to a wick. Thus, it appears that the natural oil attractant may allow for increased replacement intervals of male medfly lures in traps. However, as noted above for panel dispensers, the sachet delivery system may be considered unwieldy and inefficient for large trapping programs involving thousands of medfly-targeted traps. Ongoing research is evaluating the effectiveness of natural oil-impregnated plugs that are designed to fit in the perforated baskets used in Jackson traps and hence compatible with domestic trapping protocol in the USA.

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Author Contributions T. S.: conceptualization; data curation and data analysis; writing—original draft; C. O.: conceptualization; methodology; writing—review and edit; R. K.: investigation; data curation.

Declarations

Conflict of Interest The authors declare no competing interests.

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