Wafers in Saddle Bags: A Novel Dispensing System for Male Lures Used to Detect Invasive Fruit Flies (Diptera: Tephritidae)

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Abstract. Detection of the agricultural pests *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) relies heavily on traps baited with male-specific attractants. For *B. dorsalis*, traps are baited with liquid (6 ml) methyl eugenol (ME), while polymeric plugs containing trimedlure (TML; 2 g) are used in traps targeting *C. capitata*. In both cases, the attractant volatilizes rapidly, and lures are changed out every 6 weeks to insure high trap attractancy. Lures having greater longevity would be beneficial, because they would lengthen the trap servicing interval and thus reduce both supply and labor costs. Here, we tested the effectiveness of a saddle bag dispenser that (i) held two solid wafers impregnated with male lure, thus eliminating handling of liquid methyl eugenol (a potential carcinogen), (ii) was easy to place in traps, and (iii) allowed a high loading of male lure in trap (total loading of 6 g per trap for each lure). Field experiments, each lasting 12–14 weeks, were conducted on Hawaii island and Oahu, Hawaii, that compared captures of *B. dorsalis* and *C. capitata* males in traps baited in the standard manner versus traps baited with saddle bag dispensers. Traps baited with ME saddle bags weathered up to 12 or 14 weeks generally captured similar numbers of *B. dorsalis* males as traps baited with fresh ME liquid and significantly more males than traps baited with weathered ME liquid. Similar results were obtained for *C. capitata*: traps baited with TML saddle bags weathered up to 12 or 14 weeks captured similar numbers of *C. capitata* males as traps baited with fresh TML plugs and significantly more males than traps baited with weathered TML plugs.

Key words: *Bactrocera dorsalis*, *Ceratitis capitata*, male lures, detection

Invasive fruit flies (Diptera: Tephritidae) are among the most serious insect pests of fruits and vegetables worldwide (White and Elson-Harris 1992). Among these species, the oriental fruit fly *Bactrocera dorsalis* (Hendel) and the Mediterranean fruit fly (or medfly) *Ceratitis capitata* (Wiedemann) pose severe threats, because the adults of both species are relatively long-lived (Foote and Carey 1987, Carey 2011) and highly vagile (Froerer et al. 2010, Meats and Smallridge 2007), and females of both species have high fecundity (Huang and Chi 2014, Costa et al. 2011) and broad host range of several hundred plant species (USDA-APHIS 2018). To thwart post-invasion spread and establishment, many countries and several US states (most notably California, Florida, and Texas) operate area-wide trapping programs to detect incipient infestations of *B. dorsalis* and *C. capitata* (IPRFFSP 2006).

In addition to food-based traps, which are considered weakly attractive (Epsky et al. 2014), these programs rely on traps baited with male-specific lures. For *B. dorsalis* (and related species), the standard male lure is methyl eugenol (ME; 4-allyl-1, 2-dimethoxybenzene-carboxylate) (Vargas et al. 2010a), and for *C. capitata* (and related species), trimedlure (TML; tert-butyl 4(and 5)-chloro-cis- and trans-2-methylcyclohexane-1-carboxylate) is the most widely used male attractant (Tan et al. 2014).

The attractiveness of both ME and TML derives, in part, from their high volatility, which at the same time limits their effective longevity under field conditions. ME is typically dispensed as a liquid, with 5 or 6 ml applied to cotton wicks that are then placed in delta traps (described below). The effective field longevity for such ME-baited traps is considered 4–8 weeks (FAO/IAEA 2018), and ME replacement occurs at 6-week intervals in USA fruit fly detection programs (e.g., Gilbert et al. 2013). TML, on the other hand, is deployed in solid form, with 2 g of the attractant injected into polymeric plugs that are placed in the delta traps. Although these solid dispensers reduce volatilization and thus extend the effectiveness over liquid formulations (Leonhardt et al. 1987, 1989), the field longevity of 2 g TML plugs is considered only 6–8 weeks (FAO/IAEA 2018), and a recent study in Florida (Dean et al. 2018) showed that traps baited with TML plugs weathered > 6 weeks attracted significantly fewer *C. capitata* males than traps baited with fresh TML in either liquid or solid form. TML replacement occurs at 6-week intervals in USA fruit fly detection programs (e.g., Gilbert et al. 2013).

Several possible solutions to extending lure longevity are apparent. For ME, for example, the development of solid dispensers (with slower, controlled release rates) might prolong its attraction, and indeed several studies (e.g., Shelly 2010) have demonstrated this. Moreover, the adoption of solid dispensers for ME may reduce health risks associated with measuring and applying liquid, as ME has been shown to be carcinogenic in rodents (National Toxicity Program 2000). In addition, simply placing more ME or TML per trap might increase lure longevity. Jang (2011), for example, found that solid dispensers with 10 g of ME attracted *B. dorsalis* males for as long as 9 months. Similarly, Leonhardt et al. (1989) reported that plugs containing 4 g of TML were effective for as long as 12 weeks. While the use of controlled-release solid dispensers with increased lure loadings appears to be a straightforward solution, the approach is constrained by the method currently used to hold lure dispensers in traps. Trap design is described in detail below, but essentially the device (a perforated basket) used to hold the lure is not large enough to accommodate solid dispensers that contain large amounts (> 4 g) of male lure. Or, stated another way, it is not technically possible to produce polymeric plugs that both contain large amounts of lure and are small enough to fit inside the currently used holding device.

The objective of the present study was to describe a novel delivery system for solid dispensers containing 6 g of ME or 6.4 g of TML per trap and present the results of field experiments conducted in Hawaii that assessed the field longevity of these dispensers. The amounts of residual lure in weathered dispensers were also estimated. As documented below, traps baited with this new system, which relies on a "saddle bag" dispenser, and weathered for 12-14 weeks generally captured as many *B. dorsalis* or *C. capitata* males as traps containing fresh lure. This outcome suggests that the saddle bag method could extend the servicing interval of detec-

Figure 1. Standard method of baiting Jackson traps. Top row: Cotton wick containing ME in perforated basket and basket positioned inside Jackson trap. Bottom row: Polymeric plug containing TML in perforated basket and basket positioned inside Jackson trap.

tion traps beyond 6 weeks, resulting in significant reductions in both supply and labor costs. Note that a third important male lure (cue-lure), used in detection and trapping of certain *Bactrocera* spp., is not considered here, because this lure has a low volatilization rate and an effective field longevity of several months (Shelly et al. 2017).

Materials and Methods

Traps. Jackson traps (Scentry Biologicals, Inc., Billings, Montana) are the most commonly used traps for detecting invasive fruit fly species, including *B. dorsalis* and *C. capitata* (FAO/IAEA 2018), and were used in the present study as well. Jackson traps are white, triangular (or "delta") traps made of thick waxed paper (12.7 by 9.5 by 8.4 cm; L:W:H). A removable insert made of the same waxed paper as the trap body and coated with a clear adhesive is placed on the floor of the trap to catch the insects. Traps are generally suspended from tree branches using a metal hanger with a straight rod positioned under the roof along the apex of the trap (see below).

Standard baiting method. Jackson traps targeting *B. dorsalis* males are typically baited by applying 5 or 6 ml of liquid ME (plus 1% naled) to a cotton wick that is held in a perforated basket suspended above the sticky insert from the metal hanger (Fig. 1). Jackson traps targeting *C. capitata* males are typically baited by inserting a 2 g TML plug in the perforated basket (Fig. 1). These traps generally con-

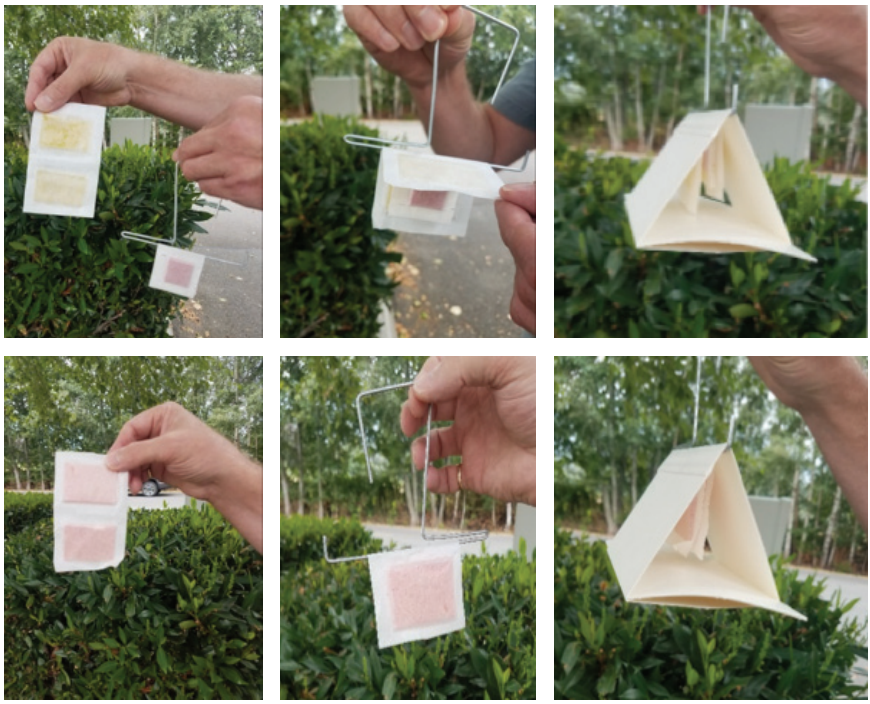

Figure 2. Saddle bag dispensers. Top row (l to r): ME saddle bag in hand, with DDVP saddle bag on hanger; placement of ME saddle bag over DDVP saddle bag; hanger positioned inside Jackson trap. Bottom row (l to r): TML saddle bag in hand; TML saddle bag placed on hanger; hanger positioned inside Jackson trap.

tain no insecticide.

Saddle bag method. The term "saddle bag" here refers to a rectangular piece of membranous, biodegradable material with a built-in pocket toward either end, each of which holds one lure-impregnated wafer (i.e., one saddle bag holds 2 wafers; U.S. Pat. App. Ser. No. 16/532,214). In targeting *B. dorsalis*, a given trap holds 2 saddle bags—one holding 2 ME wafers and one holding 2 DDVP strips. The ME wafers were 5 by 3.8 cm (4 mm thick), and each contained 3 g ME initially (i.e., 6 g ME total per trap); the DDVP strips were 2.54 cm squares (2 mm thickness), and each held 0.09 g active ingredient (i.e., 0.18 g DDVP total per trap). The saddle bags were draped over the trap's metal hanger with the wafers hanging down on either side of the hanger, with the ME saddle bag overlying the DDVP saddle bag (Fig. 2). In targeting *C. capitata*, a given trap holds only 1 saddle bag, which holds 2 TML wafers; no insecticidal agent was applied (Fig. 2; male medflies are considered weak fliers, and Jackson traps baited with TML typically contain no toxicant (FAO/IAEA 2018). The TML wafers were the same size as the ME wafers, and each contained 3.2 g TML initially (i.e., 6.4 g TML total per trap).

Field trapping: *B. dorsalis*. Captures of *B. dorsalis* males in standard wick-baited traps and saddle bag-baited traps were compared in two tests. On the island of Hawaii ("the Big Island"), trapping was conducted from September to December, 2018, at the edge of second-growth forest 10 km south of Hilo. Three treatments

were tested: (A) fresh liquid ME (6 ml, 1% naled) on a cotton wick, (B) aged liquid ME (6 ml, 1% naled) on a wick, and (C) aged saddle bags (6 g ME, 0.18 g DDVP). Treatments were alternated along the forest border, with neighboring traps separated by 50 m. Fifteen traps were deployed for each of the 3 treatments (45 total traps). On the island of Oahu, trapping was conducted from October, 2018, to January, 2019 in a citrus orchard (Aloun Farm) 4 km north of Kapolei. Two treatments were tested: (A) fresh liquid ME (6 ml, 1% naled) on a cotton wick and (B) aged saddle bags (6 g ME, 0.18 g DDVP). On Oahu, traps were placed in a 5 x 6 grid, with adjacent traps separated by 30 m. Fifteen traps were deployed for each of the 2 treatments (30 total traps). At both sites, traps were hung 1.5–2 m above ground on shaded branches between 8 and 10 am, collected after 24 h, and then returned to the laboratory where flies were counted. Traps containing aged treatments (but without sticky insert) were suspended 1–2 m above ground in a shaded area outside the laboratory under environmental conditions similar to the trapping location. On the Big Island, traps were placed in the field when treatments were aged 0 (fresh), 6, 8, 10, and 12 weeks. On Oahu, traps were placed in the field when treatments were aged 0 (fresh), 6, 8, 10, 12, and 14 weeks. On both islands, traps were placed at the same sites over the entire study, but treatments were rotated among sites between successive sampling intervals.

Field trapping: *C. capitata*. Captures of *C. capitata* males in standard plug-baited traps and saddle bag-baited traps were compared in two tests conducted on Oahu, the first involving wild flies and the second using mass-reared, released flies. The first experiment was conducted during January–May, 2019, in a commercial coffee (*Coffea arabica* L.) field 10 km southeast of Haleiwa. Three treatments were tested: (A) fresh 2 g TML plug, (B) aged 2 g TML plug, and (C) aged saddle bags (6.4 g TML). Traps were deployed in 7 plant rows, each of which contained 2 traps per treatment, plus one additional row that contained only 1 trap per treatment. Thus, 15 traps were deployed per treatment (45 traps total). Rows with traps were separated by 3–4 rows lacking traps; neighboring traps were separated by approximately 30 m both within and between rows. Traps were hung 1.5–2 m above ground on shaded branches. Traps were deployed between 8 and 10 a.m., collected after 3–4 d, and then returned to the laboratory where flies were counted. For analysis, captures were expressed on a daily basis (i.e., flies per trap per day). Lures were aged following the procedures given above. Traps were placed in the coffee field when treatments were aged 0 (fresh), 6, 8, 10, 12, and 14 weeks.

The second experiment was conducted during February–May, 2019, at the same citrus orchard used previously for *B. dorsalis* trapping. The same 3 treatments used in the coffee field were also used in the citrus orchard. Twelve traps per treatment (n = 36 total traps) were placed in a 6 x 6 grid with approximately 25 m separating adjacent traps. Wild flies were rare at the site, consequently trapping involved captures of released *C. capitata* males. The flies were obtained as pupae (non-irradiated, undyed) from a genetic sexing (male-only) strain reared by the California Department of Food and Agriculture, Fruit Fly Rearing Facility, Waimanalo, Oahu. Pupae and newly emerged males were held in screen mesh, cubical cages and were provided food and water. For each release, 62.5 ml of pupae (≈ 3,750 flies) were placed in each of 4 cages (i.e., 15,000 total males), which were transported to the field for release when males were 5–6 d old. On a release day, the 4 fly-holding cages were placed on the ground in the center of the

study plot and then opened to allow flies to exit on their own volition. Releases were made between 9:00 and 10:00 a.m. under full or partial sun and never during rainy conditions. After 15–20 min, the cages were gently tapped to promote flight by the remaining flies. Mortality was low, and <100 dead flies were counted in any cage. To allow fly movement through the habitat, traps were not deployed until 3 days after release. Traps operated for 24 h and were then collected and returned to the laboratory to count captured males. Lures were aged following the procedures given above. Traps were placed in the coffee field when treatments were aged 0 (fresh), 6, 8, 10, 12, and 14 weeks. In both experiments, traps were placed at the same sites over the entire study, but treatments were rotated among sites between successive sampling intervals.

Chemical analysis of weathered wafers. Immediately after the trapping intervals at 14 weeks, 4 ME wafers and 6 TML wafers weathered on Oahu were wrapped in aluminum foil, held overnight in a refrigerator, and then express mailed to Farma Tech for measurement of residual lure.

The following procedure was followed for both ME and TML wafers. Each wafer was placed in 100 ml of a 1:1 (v/v) mixture of acetone and n-heptane. After 48 h, 3 ml of the solution was further diluted in 10 ml of 1:1 (v/v) of acetone and n-heptane. Two µl of the diluted solution was injected into a Shimadzu GC-2010AF Gas Chromatograph with a RTX-1 column (30 m by 0.53 mm by 1.5 µm). Sample time was 1 min, with an injector temperature of 225°C and the FID of 275°C. The injection mode was split-less, with a purge flow of 3 ml/min and a split ratio of 0.4. The initial column temperature was 110°C for 1 min, then increased 15°C per s to a maximum temperature of 200°C and held there for 6 min. An external standard was used to calculate the amount of ME or TML in the wafers.

Data analysis. For all field tests, data were analyzed using $\log_{10}$ transformed values in a 2-way ANOVA, with weathering interval (weeks) and lure treatment as the two main effects. The Holm-Šídák multiple comparisons test was used to identify significant differences in pair wise comparisons. With one exception (ME test on Oahu), the ANOVA was performed using data from > 0 weeks of weathering (i.e., omitting week 0), because one treatment (aged plugs) was not included in week 0. In these cases, for week 0, captures between fresh wicks and fresh saddle bags were compared using a t test (with $\log_{10}$ transformed data). Note that transformed data did not meet the parametric assumptions of normality and equal variances in all cases. Therefore, to assess the robustness of our analyses, we performed a non-parametric equivalent of ANOVA using ranked data (following Conover and Iman 1981). This procedure generated results identical to those obtained using the raw data, indicating that the parametric analyses of raw data were sufficiently robust to accommodate the levels of non-normality and heteroscedasticity present in the data set. Similarly, for the week 0 pairwise comparisons, the non-parametric Mann-Whitney test was conducted, and in all instances the result obtained was the same as that given by the t-test.

Results

Field trapping: *B. dorsalis*. On the Big Island, traps baited with fresh ME saddle bags captured significantly more *B. dorsalis* males than traps baited with fresh liquid ME ($t = 3.8$, $P < 0.001$, $df = 28$; Fig. 3A, week 0). Over weeks 6–12, ANOVA revealed significant effects for both week ($F_{3, 168}=14.8$, $P < 0.001$) and lure ($F_{2, 168 =} 44.0$, $P < 0.001$), but a significant interaction term was evident as well ($F_{6,}$

$_{168}$ = 4.1, P < 0.001; Fig. 3A). Considering pair wise comparisons for the individual weeks, captures in fresh liquid ME- and ME saddle bag-baited traps did not differ significantly for any week with the exception of week 10 when traps with fresh liquid captured significantly more *B. dorsalis* males than traps with saddle bags. Traps baited with ME saddle bags had significantly higher captures than traps baited with aged ME wicks for weeks 6–12. Likewise, traps with fresh liquid ME captured significantly more *B. dorsalis* males than traps with aged ME wicks over all weeks with the exception of week 6 when no significant difference was detected.

On Oahu, where only fresh liquid ME and saddle bags were compared, ANOVA revealed a significant effect for week ($F_{5, 168}$ =26.6, P < 0.001) but no significant effect for lure ($F_{1, 168}$ = 1.6, P = 0.21; Fig. 3B). The interaction was also non-significant ($F_{5, 168}$ = 0.18, P = 0.97).

Field trapping: *C. capitata*. In the coffee field, there was no significant difference in captures of *C. capitata* males between traps baited with fresh plugs or fresh saddle bags (t = 1.81, P = 0.08, df = 28; Fig. 4A, week 0). Over weeks 6–14, ANOVA revealed significant effects for both week ($F_{4, 210}$ = 12.2, P < 0.001) and lure ($F_{2, 210}$ = 24.5, P < 0.001), but a significant interaction term was evident as well ($F_{8, 210}$ = 2.5, P = 0.01; Fig. 4A). There were no significant differences in captures among any of the lure treatments at 6 or 8 weeks of weathering. Captures in fresh TML plug- and TML saddle bag-baited traps likewise did not differ significantly for saddle bags aged 10, 12, or 14 weeks. However, traps baited with fresh TML plugs or aged TML saddle bags caught significantly higher numbers of *C. capitata* males than aged TML plugs for weathering intervals of 10, 12, or 14 weeks.

In the citrus orchard, where released *C. capitata* males were used, there was no significant difference in captures between traps baited with fresh plugs or fresh saddle bags (t = 0.65, P = 0.53, df = 22; Fig. 4B, week 0). Over weeks 6–14, ANOVA revealed significant effects for both week ($F_{4, 165}$ = 4.9, P < 0.001) and lure ($F_{2, 165}$ = 20.8, P < 0.001); the interaction term was not significant ($F_{8, 165}$ = 0.5, P = 0.84; Fig. 4B). For each of these weeks, the same trend was observed among lure treatments: traps baited with fresh TML plugs or aged TML saddle bags captured significantly more *C. capitata* males than traps with aged TML plugs.

Estimates of residual lure. After 14 weeks of weathering, an average of 1.33 g (range: 0.87–1.92 g, n = 4) of ME remained per saddle bag (i.e., total for 2 wafers per trap), representing 22.2% of the initial loading (1.33/6.0 g), and an average of 0.74 g (range: 0.51-0.94 g, n = 6) of TML remained per saddle bag (i.e., total for 2 wafers per trap), representing 11.6% of the initial loading (1.33/6.4 g).

Discussion

A series of studies published over the past decade (Jang 2011, Jang et al. 2013, Vargas et al. 2009, 2010b, Shelly et al. 2017) have compared captures of *B. dorsalis* males in traps baited with the standard 6 ml liquid ME versus traps baited with solid dispensers containing similar or greater loadings of the lure. In all cases, the solid dispensers were found to be as effective as the liquid ME for at least 6 weeks, and in several instances (Vargas et al. 2010b, Jang 2011) they were attractive for as long as 6–9 months. Perhaps because a polymeric plug is already widely used to dispense TML, there has been little work dedicated to assessing the efficacy of higher TML loadings. As noted above, Leonhardt et al. (1989) found that 4 g TML plugs were effective for 8 weeks, a result that has been corroborated recently

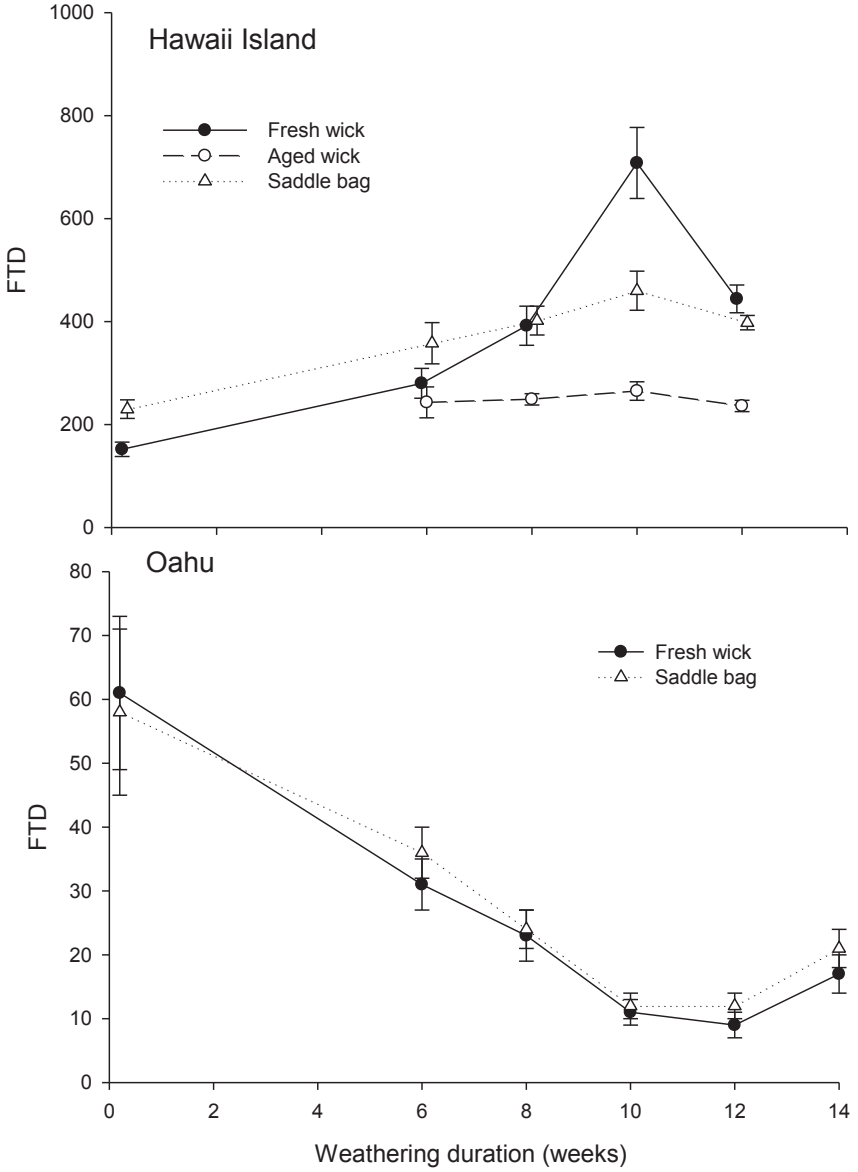

Figure 3. Captures of *Bactrocera dorsalis* males in Jackson traps baited with either a cotton wick containing 6 mL ME or a saddle-bag containing 6 g ME at study sites on Hawaii island or Oahu. Both fresh and aged wicks were deployed on Hawaii, but only fresh wicks were deployed on Oahu. Symbols represent means ± 1 SE, where n = 15 traps per treatment per weathering interval for both study sites. For a given weathering interval, means marked by different letters were significantly different ($P < 0.05$, Holm-Šídák test).

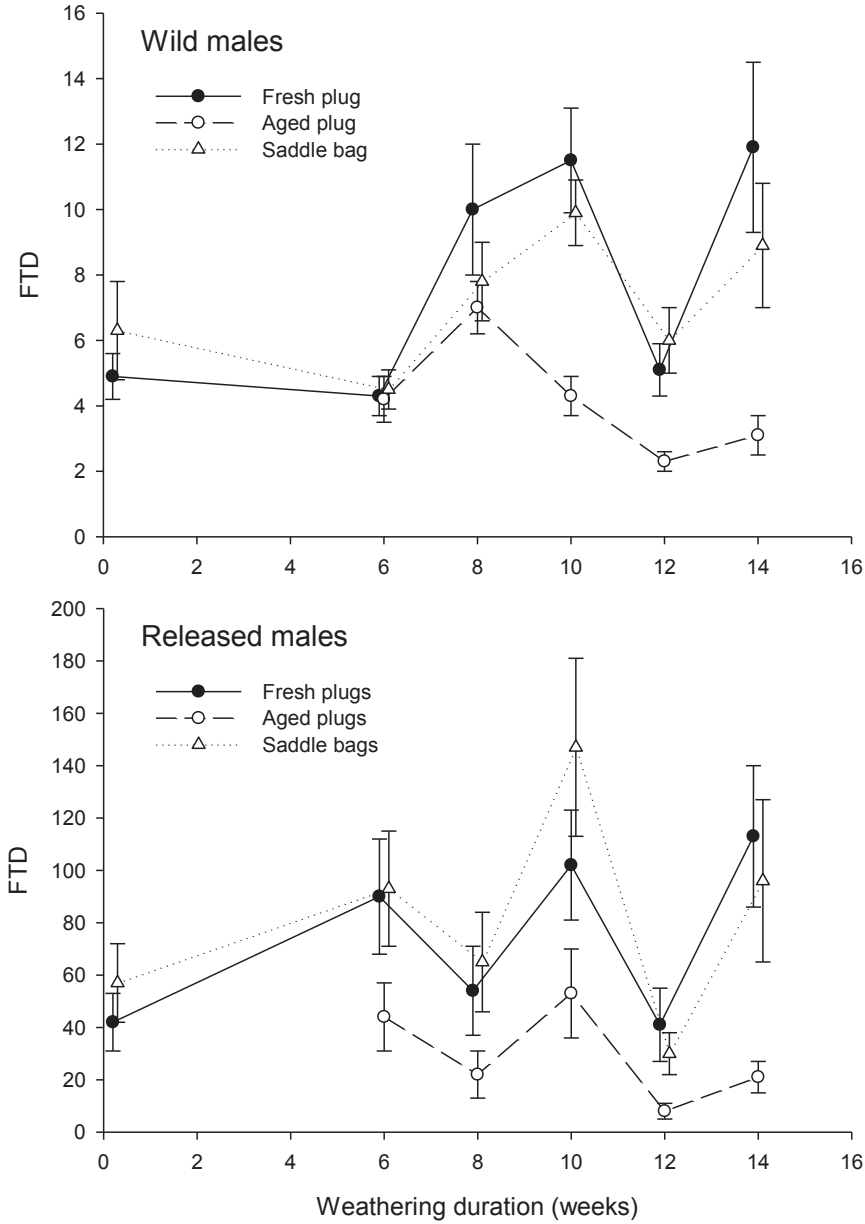

Figure 4. Captures of *Ceratitis capitata* males in Jackson traps baited with either a polymeric plug containing 2 g TML or a saddle-bag containing 6 g TML at an Oahu (A) coffee field (wild males) or (B) citrus orchard (released males). Symbols represent means ± 1 SE, where n = 15 traps per treatment per weathering interval at the coffee field and n = 12 at the citrus orchard. For a given weathering interval, means marked by different letters were significantly different ($P < 0.05$, Holm-Šídák test).

with a wild population of medfly in Hawaii (Shelly and Kurashima 2019).

Thus, the efficacy of heavily loaded, solid dispensers appears well established, and using such dispensers to extend trap servicing intervals awaits the development of a Jackson trap-dispenser combination that both accommodates the dispenser and allows easy handling and replacement of the dispenser. Previous combinations served experimental purposes but were not feasible for large-scale surveillance programs. For example, the ME wafers tested were relatively large (e.g., 7.5 by 5 cm; Shelly et al. 2017) and were attached to the trap hanger using twist ties, a time-consuming procedure not suited for large trapping programs. Likewise, heavily loaded plugs were held by two perforated baskets (with lids removed) positioned opening-to-opening and wired together to create a single larger holder (Shelly et al. 2017). Again, while suitable for experimentation, this procedure was not practical for area-wide detection efforts.

The saddle bag method both confirms the prolonged longevity of wafers containing high loadings of male lure and offers an improved method for holding such wafers inside Jackson traps. Importantly, the saddle bag can be inserted and removed easily from the trap. Aside from the saddle bag, no extra parts (i.e., clips, fasteners, etc.) are required for assembly and deployment of the Jackson traps. In addition, the saddle bags provide the opportunity to easily deploy multiple types of lures (e.g., a TML saddle bag and an ME saddle bag) in a single Jackson trap.

The relationships noted here between lure attractiveness and amounts of lure remaining after weathering are consistent with previous studies. Little data exist for ME, but Vargas et al. (2015) found that weathered ME wafers (containing 7.85 g of ME initially and not enclosed in a saddle bag) were significantly less attractive than fresh wafers when the amount of ME decreased below 0.95 g, which occurred at 12 weeks of weathering in their study. Here, two ME wafers (initial total of 6 g ME) held per saddle bag were as attractive as fresh liquid ME for 14 weeks at which time the average residual amount of ME was 1.33 g per trap. As these values suggest, Vargas et al. (2015) observed a greater loss rate of ME relative to the present study: only 12% ME (0.95/7.85 g) remained after 12 weeks of weathering in that earlier study compared to 22% residual ME (1.33/6 g) after 14 weeks in the present study. As the present study was conducted at a warmer and drier site than that used by Vargas et al. (2015), it does not appear that weather conditions were responsible for the differing loss rates. The most likely explanation is that encasing the wafer in the saddle bag reduced ME volatilization below that observed for exposed ME wafers (as in Vargas et al. 2015). It seems likely that the saddle bag material itself lowered the ME release rate simply by acting as a physical barrier that lessens the impact of wind on lure volatilization. If true, the reduced release rate of ME, and the concomitant increase in lure longevity, constitutes another benefit of the saddle bag method. Importantly, too, the reduced release rate was achieved without the addition of specific chemical extenders.

Similarly, two TML wafers in saddle bags (initial total of 6.4 g TML) were as attractive as fresh TML plugs even after 14 weeks of weathering when the average residual TML amount was 0.74 g per trap. Studies relating the attractiveness of TML plugs to their residual lure content have identified 0.35–0.50 g as the range below which weathered TML plugs are significantly less attractive than fresh TML plugs (Leonhardt et al. 1987, 1989, Warthen et al. 1999, Dean et al. 2018). The average residual level in the present study clearly exceeded this critical range, and,

consistent with this previous research, traps baited with weathered wafers captured as many flies as traps baited with fresh plugs.

In conclusion, the saddle bag–solid dispenser method prolonged the effective field longevity of both ME and TML under field conditions in Hawaii. Whether or not the same results would be found in the drier climate of Southern California, for example, remains unknown, and we encourage field work in the Los Angeles Basin comparing standard lures and saddle bag-held lures using mark-release-recapture of irradiated flies. Additionally, and as noted above, future work might examine whether saddle bags holding different lures (e.g., TML and ME) could be deployed effectively in a single Jackson trap.

Acknowledgments

We thank Island Princess Hawaii, Aloun Farm, and the Dole Food Company for permission to conduct studies on their respective properties. Saddle bag dispenser for fruit fly lures has Patent Pending 62/715,156.

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