Field Capture of Male Mediterranean Fruit Flies (Diptera: Tephritidae) in Traps Baited with Varying Amounts of Trimedlure

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Field capture of male Mediterranean fruit flies (Diptera: Tephritidae) in traps baited with varying amounts of trimedlure

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Abstract

Detection of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), relies heavily on traps baited with the male attractant trimedlure. This lure is dispensed from solid polymeric plugs (2 g a.i.) that are changed out every 6 wk, because beyond this interval their attractiveness drops significantly below that of fresh lure. Existing data suggest that plugs loaded with 4 g of trimedlure may have longer attractancy, which would allow for longer servicing intervals with associated reductions in material and labor costs. The objective of the present study was to gather additional data on the effectiveness of plugs having trimedlure loadings greater than 2 g. In 4 field experiments conducted in Hawaii, the capture of male medflies was compared over 10 or 12 wk periods between control traps baited with 2 mL fresh liquid trimedlure on a cotton wick and treatment traps baited with aged plugs containing 2 to 4 g of the lure. In all experiments, 6-wk-aged plugs, regardless of the specific loading, attracted similar numbers of males as the fresh liquid lure. Conversely, in 3 of the 4 experiments, plugs aged for 10 or 12 wk attracted significantly fewer males than the fresh lure. The performance of plugs aged 8 wk was inconsistent, showing similar attractiveness as fresh lure in certain cases but not others. Based on these findings, we conclude that, counter to our expectations, the use of 4 g trimedlure plugs would not allow effective trapping over longer inter-servicing intervals.

Key Words: *Ceratitis capitata*; detection; trapping program; invasive species; male lure

Resumen

La detección de la mosca mediterránea de la fruta (moscamed), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), depende en gran medida de trampas cebadas con trimedlure, un señuelo de machos. Este señuelo se dispensa de tapones poliméricos sólidos (2 g a.i.) que se cambian cada 6 semanas, porque después de este intervalo su atractivo baja significativamente con respecto a un señuelo fresco. Los datos existentes sugieren que los tapones cargados con 4 g de señuelo trimedlure pueden tener una atracción más larga, lo que permitiría intervalos de servicio más largos con reducciones asociadas en los costos de material y mano de obra. El objetivo del presente estudio fue reunir datos adicionales sobre la efectividad de los tapones que tienen la cantidad de señuelo trimedlure mayor de 2 g. En 4 experimentos de campo realizados en Hawai, se comparó la captura de moscas moscas macho durante 10 o 12 semanas entre trampas de control cebadas con 2 ml de trimedlure líquido fresco en una mecha de algodón y trampas de tratamiento cebadas con tapones viejos que contienen de 2 a 4 g de señuelo. En todos los experimentos, los tapones de 6 semanas de edad, independientemente de la cantidad de trimedlure específica, atrajeron un número similar de machos como las trampas con señuelo de líquido fresco. Por el contrario, en 3 de los 4 experimentos, los tapones usados para 10 o 12 semanas atrajeron significativamente menos machos que el señuelo fresco. El rendimiento de los tapones de 8 semanas de edad fue inconsistente, mostrando un atractivo similar al señuelo fresco en ciertos casos, pero no en otros. Con base en estos hallazgos, llegamos a la conclusión de que, en contra de nuestras expectativas, el uso de tapones trimedlure de 4 g no permitiría la captura efectiva durante intervalos más largos entre servicios.

Palabras Clave: *Ceratitis capitata*; detección; programa de trampeo; especies invasivas; señuelo de machos

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most serious agricultural pests in the world. Females lay their eggs, and larvae subsequently consume and develop, in over 300 species of fruits and vegetables, including such important crops as oranges, loquats, plums, guava, and papaya (USDA-APHIS 2017). In addition to the direct damage caused by larval feeding, the occurrence of medfly may trigger the establishment of quarantine areas, strict constraints on commercial movement of crops both nationally and internationally, and the demand for post-harvest treatment of crops to ensure safe (medfly-free) trade outside the infested region (Papadopoulos 2014; Karsten et al. 2015). The economic costs of medfly invasion and establishment are potentially enormous. For example, Siebert and Cooper (1995) estimated that medfly establishment in California and the resulting trade embargos by Asian countries would cost $1.2 billion (approximately $2 billion in inflation-adjusted 2018 dollars) in the state gross product, plus the elimination of over 10,000 jobs.

Early detection of a medfly infestation is crucial for successful control because it allows prompt implementation of management activities, such as delimitation, fruit stripping, protein bait spraying, and potentially the Sterile Insect Technique, which are most effective against small populations (Hendrichs et al. 1995; Dowell et al. 1999). In addition, suppression of incipient infestations entails much lower costs than control of invasive populations that have grown in size and

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become widely distributed (Lodge et al. 2006; Suckling et al. 2016). Medfly detection currently relies on 2 types of traps. Food-based traps, which are considered only weakly attractive, capture both sexes but females primarily (Epsky et al. 2014). Male-lure traps, as the name indicates, are sex-specific and capture males exclusively (Jang & Light 1996; but see Nakagawa et al. 1970 for an exception). The lure most commonly used in these traps is the synthetic compound trimedlure (*tert*-butyl 4(and 5)-chloro-*trans*-2methylcyclohexane-1-carboxylate) (Beroza et al. 1961), which replaced earlier attractants, such as angelica seed oil (Steiner et al. 1957) and siglure (Steiner et al. 1961).

Whereas a strong attractant, liquid trimedlure is highly volatile, which limits its effective longevity under field conditions. Cotton wicks containing 2 mL of trimedlure lose their attractiveness within 2 to 4 wk (King and Landolt 1984; Rice et al. 1984). This rapid decline in attractiveness necessitates frequent replacement, which increases associated labor and lure costs (Leonhardt et al. 1987). Thus, the high evaporative rate of trimedlure results in opposing trends: high volatility enhances lure attractiveness but simultaneously shortens the interval over which the lure is attractive. Most medfly detection methods have balanced these trends through the development of solid dispensers as an alternative to the use of liquid trimedlure. Various types of dispensers have been investigated (Leonhardt et al. 1984, 1987; Rice et al. 1984; Warthen et al. 1997; Domínguez-Ruiz et al. 2008), but a polymeric plug containing 2 g trimedlure is currently the most commonly used dispenser (FAO/IAEA 2013). This device was shown to have equivalent attractiveness, but greater longevity, than 2 mL of liquid trimedlure applied to a cotton wick (Leonhardt et al. 1987, 1989). Leonhardt et al. (1989) suggested replacing aged plugs when captures of male medflies with aged plugs dropped below 50% of captures with 2 mL fresh trimedlure on cotton wicks. Based on field trials conducted in Hawaii, Leonhardt et al. (1987) proposed a plug replacement interval of 6 wk, and this practice is still followed in medfly surveillance programs in the US (IPRFFSP 2006). More recently, using statistical analyses rather than the 50% rule, Dean et al. (2018) confirmed that traps baited with 2 g trimedlure plugs captured similar numbers of male medflies as traps baited with fresh lures up to 6 wk of age, but had significantly lower catch when plugs were weathered for longer intervals.

Interestingly, Leonhardt et al. (1989) also tested plugs containing 4 g of trimedlure and found that traps baited with these larger plugs captured > 50% as many male medflies as 2 mL fresh trimedlure on a cotton wick for at least 12 wk. Despite this prolonged effectiveness, the 4 g plugs were not adopted as the standard dispenser, apparently because of their higher cost and a perceived need to confirm their field longevity through additional testing (Leonhardt et al. 1989). Dean et al. (2018) examined the performance of 3 g trimedlure plugs and, consistent with Leonhardt et al. (1989), found that traps baited with 3 g plugs aged for 8 wk generally (2 of 3 trials) captured similar numbers of male medflies as traps baited with fresh 2 g plugs, whereas traps baited with 2 g plugs aged 8 wk generally (2 of 3 trials) captured significantly fewer males than traps baited with fresh 2 g plugs.

The goal of the present study was to conduct additional tests assessing the effective field longevity of trimedlure plugs containing different amounts (or loadings) of the attractant. Results of 4 experiments are described, with the first 3 involving capture of wild male medflies in a coffee (*Coffea arabica* L.; Rubiaceae) field, and the final one utilizing mass-reared males released in an orange tree (*Citrus sinensis* (L.) Osbeck; Rutaceae) orchard. As described below, control traps were baited with 2 mL fresh trimedlure liquid in all experiments, and their catch was compared to traps baited with plugs that contained higher amounts of trimedlure and that were aged up to 12 wk. Implications of the present results for surveillance and monitoring programs for *C. capitata* are discussed.

Materials and Methods

The methods relating to experiments involving capture of wild male medflies in a coffee field are described initially, since (i) the protocol was very similar among them, and (ii) this protocol differed substantially from the release-recapture technique used in the citrus grove, which is described separately.

COFFEE FIELD EXPERIMENTS

Study Site

The coffee field, which covers approximately 65 ha of a gentle, north-facing slope, was 10 km southeast of Haleiwa (elevation 90–100 masl). Plants were 2 to 4 m tall, and were grown in parallel rows spaced 2 to 3 m apart. Within a row, trunks of individual plants were separated by 1 to 2 m, but foliage generally was contiguous between neighboring plants. Trapping for the different experiments was conducted at varying times of the yr, and temperatures during the different sampling intervals are presented in Table 1. Rainfall generally was slight and was less than 1 cm over all trapping periods in most experiments. Weather data were based on measurements taken in Haleiwa (weather.com).

Traps and Trimedlure Lures

Flies were captured using Jackson traps (Scentry Biologicals, Inc., Billings, Montana, USA) (FAO/IAEA 2013), the standard type used in fruit fly surveillance programs in the US (IPRFFSP 2006). Jackson traps were white, 'delta' traps made of thick waxed paper (12.7 cm L × 9.5 cm

Table 1. Basic features of the 4 trapping experiments. Experiments 1 to 3 were conducted in a coffee field, and Experiment 4 was conducted in an orange tree orchard. For each experiment, control traps were deployed that were baited with 2 mL of fresh trimedlure on a wick. Temperatures represent average daily maximum and minimum readings during trapping periods (to the nearest °C) based on data collected in Haleiwa (coffee field) or Kapolei (citrus grove), Oahu, Hawaii, USA (weather.com). Treatments refer to the different trimedlure loadings (g) per plug tested in each experiment.

Experiment	Replicates	Timing (mo/yr)	Min/max temp (range) °C	Treatments[1] (trimedlure per plug, g)	Traps per treatment	Weathering intervals (wk)
1	1	Dec 2017 to Mar 2018	66–80 (59–83)	4	12	0, 6, 8, 10, 12
2	1	Mar 2018 to May 2018	69–82 (66–85)	2, 4	12	0, 6, 8, 10
	2	May 2018 to Jul 2018	73–86 (67–89)			
3	1	Jul 2018 to Sep 2018	74–88 (71–90)	3, 3.5, 4	12	0, 6, 8, 10
	2	Oct 2018 to Dec 2018	69–83 (65–87)			
4	1	Feb 2018 to May 2018	69–82 (61–84)	2, 4	12	0, 6, 8, 10, 12

[1]All trimedlure lures, including both liquid control and solid plugs, were obtained from Farma Tech Intl. Corporation, North Bend, Washington, USA, except that Experiment 3 also included 4 g plugs produced by Scentry Biologicals, Inc., Billings, Montana, USA.

W × 8.4 cm H). A removable insert, made of the same waxed paper as the trap body and coated with "stickum," was placed on the bottom of the trap to catch insects. Traps were suspended from tree branches using a metal hanger, with a straight rod positioned under the roof along the apex of the trap. In the trap, the lure was held in a perforated plastic basket suspended above the sticky insert from the metal hanger.

Control traps were baited with 2 mL of liquid trimedlure placed on a cotton wick (2.5 cm long and 2.0 cm diam) immediately before placement in the field (i.e., the lure was considered "fresh"). Regular quality control testing of the trimedlure liquid and plugs used in USDA-APHIS surveillance programs showed that, when trimedlure dispensers were weathered over short intervals (< 7 d), captures of *C. capitata* males were similar among traps baited with 2 g plugs or 2 mL liquid aliquots applied to wicks (T. E. Shelly, unpublished data; the specific gravity of trimedlure is approximately 1.0). Thus, the control used here was considered equivalent to the standard 2 g plugs used in monitoring programs in southern states of the USA (IPRFFSP 2006).

Captures in control traps were compared with traps baited with aged 4 g plugs (Experiment 1), aged 2 and 4 g plugs (Experiment 2), or aged 3, 3.5, and 4 g plugs (Experiment 3). All trimedlure lures, including both liquid and plugs, were obtained from Farma Tech Intl. Corp., North Bend, Washington, USA, except that Experiment 3 also included 4 g plugs provided by Scentry Biologicals, Inc., Billings, Montana, USA. All plugs fitted easily within the plastic baskets held within Jackson traps and therefore could, if desired, be incorporated in the current trapping protocol.

Trapping Protocol and Experiments

Jackson traps were placed on wind-break trees (Norfolk pines, *Araucaria heterophylla* (Salisb.) Franco; Araucariaceae) planted along the edge of the coffee field. This protocol was adopted because of our uncertainty regarding the timing and location of harvesting, which is performed by a large, mobile machine that straddles a row and violently rattles the coffee plants beneath to loosen and collect ripe berries. This procedure completely destroys any traps placed in harvested rows. Traps, which were approximately 10 m from the nearest row of coffee, were placed 1.5 to 2.5 m aboveground in shaded locations between 7:00 AM to 10:00 AM. Traps were placed approximately 25 m apart, with treatments positioned in repeating sequences along the windbreak. Twelve or 15 traps were deployed per treatment (see below); sampling intervals ranged from 3 to 7 d. Collected traps were returned to the laboratory, where sticky inserts were removed, and captured flies were counted. Plugs were left in the trap bodies, and these were hung 2 to 2.5 m aboveground in a shaded location outside the laboratory for weathering (in similar environmental conditions as the coffee field).

The basic features of the 3 experiments conducted in the coffee field are presented in Table 1.

ORANGE TREE ORCHARD EXPERIMENT

Insects

As noted above, one experiment involved release of mass-reared male medflies. The flies were obtained as pupae from the California Department of Food and Agriculture, Fruit Fly Rearing Facility, Waimanalo, Hawaii, USA. The flies were derived from a genetic sexing strain in which a temperature sensitive lethal (*tsl*) mutation allowed for selective culling of female embryos (via thermal shock) and exclusive production of males (Caceres 2002). Although dyed and irradiated for the Sterile Insect Technique, the pupae used in the present study were undyed and non-irradiated. Preliminary trapping showed that wild medflies were rare at the study site, and we assume all males trapped in the present study were released flies. Pupae and newly emerged males were held in screen mesh, cubical cages (30 cm per side). Sugar-yeast hydrolysate mixture (3:1 v:v) held within a Petri dish was placed on the floor of the holding cages for food, and a water cup with an emerging cotton wick also was placed inside each holding cage. For each release, 62.5 mL of pupae (about 3,750 flies, 1 mL pupae about 60 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 to 6 d old.

Study Site

The release-recapture experiment was performed in an orange tree orchard (approximately 10 ha) within a large vegetable and fruit farm 4 km north of Kapolei (20–30 masl). Within a row, trunks of individual trees, which were 4 to 5 m tall, were separated by 6 to 7 m, with inter-canopy distances of 2 to 4 m. Trunks of adjacent trees were separated by 8 to 9 m between parallel rows, and rows were separated by 3 to 5 m of mowed grass. Daily maximum and minimum temperatures during trapping periods were similar to those recorded for the coffee field (Table 1). Rainfall was slight and ranged from 0 to 0.5 cm during trap operation.

Traps and Lures

As in the coffee field, Jackson traps were used exclusively, and male captures in control traps (2 mL fresh trimedlure) were compared with traps baited with aged plugs containing either 2 or 4 g of trimedlure. Traps were placed in shaded locations within the canopy of the orange trees at 1.5 to 2 m aboveground.

Release and Trapping Protocol

A grid (about 0.8 ha) was established at the center of the orange tree orchard in which trap locations were separated by approximately 20 m within and between rows. Two traps of each treatment type (methyl eugenol liquid, 2 g plug, and 4 g plug) were placed in each of 6 rows for a total of 36 traps (2 traps per treatment per row × 6 rows × 3 treatments = 36 total traps). Within a row, treatments were placed in an alternating sequence (e.g., ABCABC), and the "initial" treatment was alternated between rows (ABCABC, BCABCA, CABCAB, and so on).

On a release d, the 4 fly-holding cages were transported to the field, 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 AM to 10:00 AM under full or partial sun, and never during rainy conditions. After 15 to 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 the d after release. Traps operated for 24 h and were then collected and returned to the laboratory to count captured males.

Summary information for the single experiment conducted in the citrus orchard is presented in Table 1.

DATA ANALYSIS

Data for all 4 experiments were analyzed using a 2-way ANOVA, with weathering intervals (wk) and lure type as the main effects. Log_{10} transformed data normally were distributed in half of the cases (3 of 6 analyses), and the equal variance assumption was met in all but 1 case. For those instances where data were non-normal, ANOVA using ranked data (Conover & Iman 1981) generated results identical to those obtained using the raw data, indicating that the parametric analyses of raw data were sufficiently robust to accommodate the degree of non-normality present in some cases. As shown below, interaction terms generally were significant, probably owing to temporal variation in the abundance of wild flies (Experiments 1–3) or in the flight activity and lure response of released flies (Experiment 4). Accordingly, captures in traps having different lures were compared within weathering periods (wk) using the Holm-Šidák multiple comparisons test with a significance level of 0.05. All analyses were performed using SigmaPlot vers. 11 (Systat Software, San Jose, California, USA).

Results

COFFEE FIELD EXPERIMENT

With only 1 exception (interaction term, Experiment 1), the main effects and their interaction had a statistically significant influence on trap captures in all 3 experiments conducted in the coffee field (Figs. 1–3; Table 2). Comparisons done wk-by-wk among lures (Table 3) revealed no significant differences among lures for tests performed at 0 (fresh) or 6 wk of weathering, with one exception where 2 g plugs weathered for 6 wk attracted fewer males than the fresh liquid or the 6-wk-aged 4 g plugs (Experiment 2, trial 2). Thus, traps baited with plugs having ≥ 2 g of trimedlure usually captured similar numbers of male medflies as traps baited with 2 mL of fresh trimedlure on a wick when the plugs were fresh or aged 6 wk. Conversely, control traps invariably had significantly higher trap captures than traps baited with plugs having ≥ 2 g trimedlure when the plugs were weathered for 10 or 12 wk. For plugs aged 10 wk, traps baited with 4 g plugs captured significantly more males than traps baited with 2 g plugs (Experiment 2, both trials), but captured similar numbers of males as 3 or 3.5 g plugs in Experiment 3, trial 2. In this latter experiment, the 4 g plugs from Scentry attracted significantly more males than the 3 or 3.5 g plugs in trial 2, whereas male numbers did not differ between the 4 g plugs from Farma Tech and the plugs with lower trimedlure loadings.

In contrast to the uniformity in relative performance among plugs weathered 0 or 6 wk on one hand (similar to control lure) and 10 or 12 wk on the other (fewer captures than control), results after 8 wk of weathering revealed variable attractiveness among plugs with different trimedlure loadings. At this aging interval, traps baited with plugs containing 2 g (Experiment 2) or 3 or 3.5 g (Experiment 3, both trials) of trimedlure always captured fewer male medflies than the control traps (Table 3). However, plugs with 4 g of trimedlure that were aged 8 wk performed as well as wicks with 2 mL of fresh trimedlure in several instances, namely Experiment 1; Experiment 2, trial 2; and Experiment 3, trials 1 and 2 for the Scentry-produced 4 g plugs. In these instances, traps baited with the 4 g plugs also captured significantly more males than traps baited with 2 g (Experiment 2), or 3 or 3.5 g (Experiment 3) plugs.

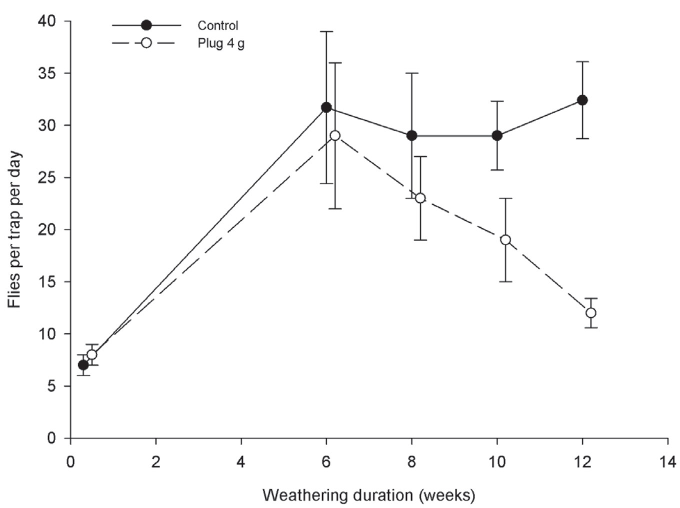

Fig. 1. In the single trial of Experiment 1, captures of wild male medflies per trap per d in a Hawaiian coffee field in Jackson traps baited with 2 mL of fresh trimedlure on a cotton wick (control), or 4 g trimedlure plugs weathered for varying durations. Symbols represent means ± 1 SE; n = 12 in all cases. Results of Holm-Šidák multiple comparisons tests are given in Table 3.

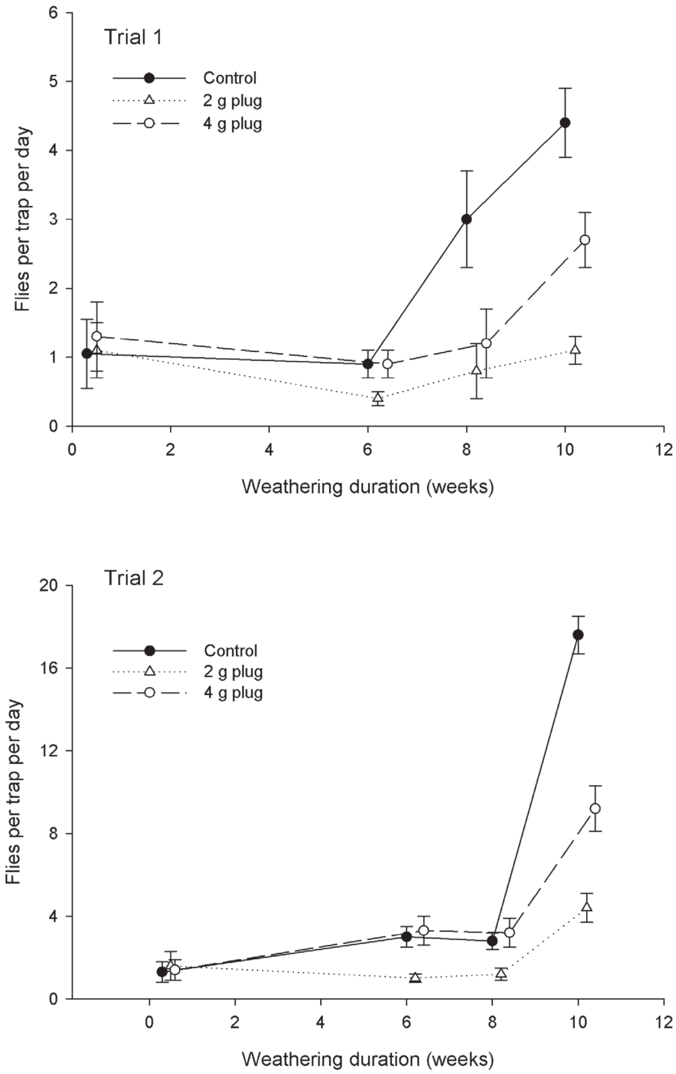

Fig. 2. In the 2 trials of Experiment 2, captures of wild male medflies per trap per d in a Hawaiian coffee field in Jackson traps baited with 2 mL of fresh trimedlure on a cotton wick (control), or 2 or 4 g trimedlure plugs weathered for varying durations. Symbols represent means ± 1 SE; n = 12 in all cases. Results of Holm-Šidák multiple comparisons tests are given in Table 3.

ORANGE TREE ORCHARD EXPERIMENT

As in the coffee field, the main effects and their interaction had a statistically significant influence on trap captures in the experiment conducted in the citrus orchard (Fig. 4; Table 2). Comparisons don wk-by-wk among lures (Table 3) revealed no significant differences among lures for tests performed at 0 (fresh) or 6 wk of weathering. Sampling at 8, 10, and 12 wk of weathering yielded uniform results: there were

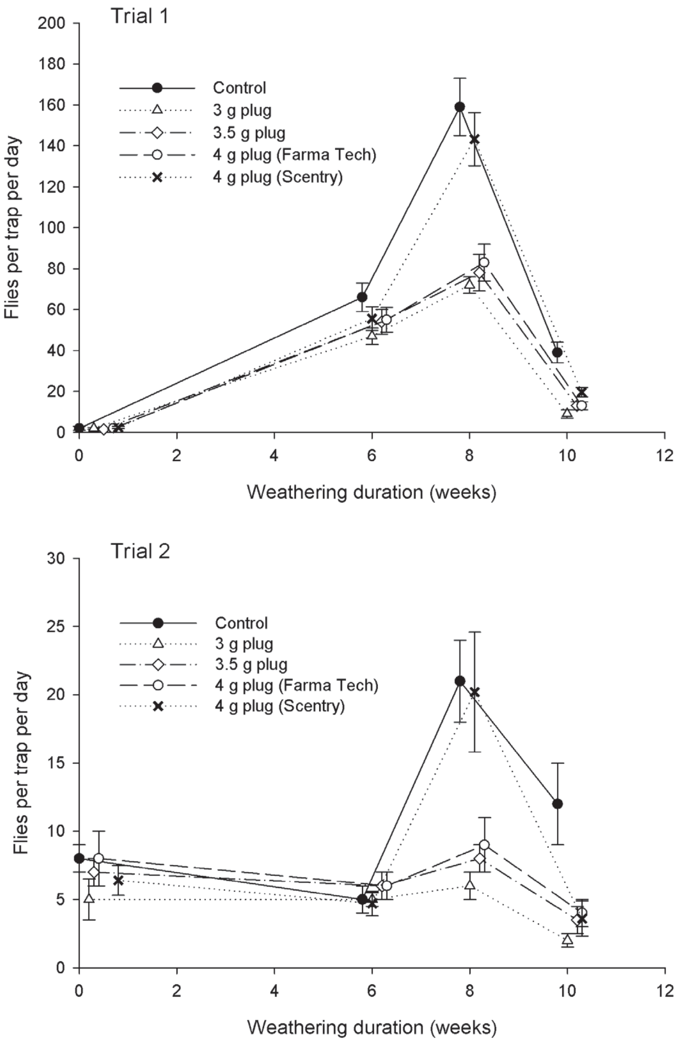

Fig. 3. In the 2 trials of Experiment 3, captures of wild male medflies per trap per d in a Hawaiian coffee field in Jackson traps baited with 2 mL of fresh trimedlure on a cotton wick (control), or 3, 3.5, or 4 g trimedlure plugs weathered for varying durations. Symbols represent means ± 1 SE; n = 15 in all cases. Results of Holm-Šidák multiple comparisons tests are given in Table 3.

no significant differences in male captures between control traps and traps baited with 4 g plugs, and both of these trap types captured significantly more males than traps baited with 2 g plugs.

Discussion

Identifying lure/dispenser combinations that extend the effective longevity of traps in the field is of paramount importance to fruit fly monitoring programs, because such combinations potentially lead to substantial cost savings through reduced material (i.e., fewer traps and lures) and labor needs. In practical terms, the task involves identifying the length of time weathered lure/dispenser combinations generate trap catches that are equivalent to fresh combinations. Replacement should occur at the weathering interval at which aged devices become less attractive than fresh ones. Earlier replacement is an inefficient use of supplies and labor, while later replacement results in field-operating traps that are less effective than fresh traps. Historically, replacement of lures/dispensers was recommended when trap captures for weathered devices dropped below 50% of captures for fresh ones (Leonhardt et al. 1989). Although this guideline was straightforward and thus easily implemented, it was not based on a statistical framework. As a result, weathered traps may have exceeded 50% captures in fresh traps, but statistically may have captured significantly fewer insects than fresh traps. The present study assumes that maximally effective fruit fly surveillance programs strive for replacement intervals that result in no statistical decline in trap performance.

Consistent with Dean et al. (2018), the present study showed that the 6-wk replacement interval for 2 g trimedlure plugs is justified based on their lower attractiveness beyond this weathering duration (Experiments 2 and 4). Among fresh or 6-wk aged plugs, we found no evidence that higher loadings of trimedlure led to higher captures of male medflies (Experiments 1–4). This finding confirms previous work (T. E. Shelly, unpublished data) that found no difference in male captures between traps baited with 2 or 5 mL of trimedlure on a cotton wick, and compared within d of deployment. Thus, within the 2 to 5 mL (or g) range examined, more trimedlure per dispenser does not result in higher captures among newly deployed traps. Thus, the chief potential benefit associated with increased trimedlure loading is probably not increased attractiveness relative to the standard loading (at least, among newly deployed traps) but lengthening of the effective period of attractiveness.

The 3 experiments conducted in the coffee field uniformly showed that 4 g trimedlure plugs weathered for 10 or 12 wk were significantly less attractive than fresh lures. Thus, when placed within a known, wild population of medfly, it is evident that traps baited with 10- or 12-wk-aged 4 g plugs were not as effective as traps baited with fresh lure. However, results for 8-wk-aged 4 g plugs were less consistent. Regarding the 4 g plugs produced by Farma Tech, which were used in Experiments 1 to 3, 8-wk-aged 4 g plugs were compared with fresh lure in 5 cases. In 3 of these, the fresh lure attracted significantly more males than the plugs, but in 2 instances there was no significant difference between aged 4 g plugs and fresh lure. Experiment 3 also included 4 g plugs produced by Scentry, and in both trials of this experiment the 8-wk-aged plugs from Scentry performed as well as fresh lure.

Data from the experiment conducted in the citrus grove further confounds interpretation regarding the performance of 4 g plugs aged for extended intervals. In this case, captures were not statistically different between traps baited with fresh lure or 4 g plugs that had been weathered for 8 wk or even 10 or 12 wk. It is not known why 4 g plugs exhibited such prolonged attractiveness in this experiment. The attractiveness of plugs depends on the release rate of trimedlure, which is highly temperature dependent (Leonhardt et al. 1984; Domínguez-Ruiz et al. 2008). As shown, however, temperatures at the orange tree orchard were similar to those at the coffee field, thus release rates of trimedlure, and correspondingly residual amounts of trimedlure, most likely followed similar temporal trends at the 2 locations.

Alternatively, the use of released, mass-reared males in the citrus orchard suggests, at first glance, that the release protocol or the laboratory origin of the males affected the results, but straightforward explanations are not apparent. As noted, the traps were placed in the field 24 h after release, which allowed the males to move about the habitat in the absence of trimedlure sources. Also, the traps with fresh or aged baits were evenly spaced in the study plot to avoid spatial bias of trap captures. Thus, the release method itself did not appear to influence trap captures in any obvious direction. Moreover, males from the same mass-reared strain used here were found to be less, not more, responsive to trimedlure than wild males, presumably as a consequence of genetic changes resulting from domestication (Shelly & Edu 2009). Thus, mass-reared males possibly were less able to detect and locate well-weathered plugs having lower trimedlure release rates than fresh baits.

Table 2. Results of 2-way ANOVA for trap capture data (flies per trap per d) for experiments carried out in coffee field (Experiments 1 to 3) or the orange tree orchard (Experiment 4). Weathering durations and sample sizes are given in Table 1.

		Factor					
		Lure		Week		Lure × week	
Experiment	Trial	*F*	*P*	*F*	*P*	*F*	*P*
1	1	5.3	0.020	47.0	< 0.001	2.2	0.080
2	1	12.7	< 0.001	15.1	< 0.001	3.4	< 0.010
	2	24.4	< 0.001	74.3	< 0.001	5.0	< 0.001
3	1	20.4	< 0.001	538.2	< 0.001	3.4	< 0.001
	2	9.9	0.001	32.6	< 0.001	3.6	< 0.001
4	1	11.8	< 0.001	3.3	0.010	3.3	0.002

A final conjecture is that a difference in the olfactory environment of the coffee and orange tree sites affected the ability of male medflies to respond to the lower trimedlure emissions of plugs aged for 10 or 12 wk. Background odors are known to influence insect behavior (Schröder & Hilker 2008), and it is possible that (i) certain odors in the citrus grove enhanced male responsiveness to trimedlure volatiles (see, for example, Dickens et al. 1990; Said et al. 2005), and (ii) certain odors in the coffee field masked trimedlure volatiles and reduced male ability to detect and locate the lure (see, for example, Thiery and Visser 1986; Yamasaki et al. 1997). To our knowledge, no data exist regarding the relative attractiveness of trimedlure in habitats with differing hosts, and consequently the importance of background olfactory environments as an explanation in the present study is unknown.

Table 3. Results of Holm-Šidák multiple comparisons test among different lures for each weathering duration for Experiments 1 to 4, where Con = control (2 mL trimedlure on wick), and 2, 3, 3.5, and 4 g represent trimedlure loadings in plugs. Underlining presents results of significance testing (*P* = 0.05), where lure types having common underlining were not significantly different.

Experiment	Replicate	Wk	Results
1	1	0	Con 4g
		6	Con 4g
		8	Con 4g
		10	Con 4g
		12	Con 4g
2	1	0	Con 4g 2g
		6	Con 4g 2g
		8	Con 4g 2g
		10	Con 4g 2g
	2	0	Con 4g 2g
		6	Con 4g 2g
		8	Con 4g 2g
		10	Con 4g 2g
3	1	0	Con 4g-S 4g-F 3.5g 3g
		6	Con 4g-S 4g-F 3.5g 3g
		8	Con 4g-S 4g-F 3.5g 3g
		10	Con 4g-S 4g-F 3.5g 3g
	2	0	Con 4g-S 4g-F 3.5g 3g
		6	Con 4g-S 4g-F 3.5g 3g
		8	Con 4g-S 4g-F 3.5g 3g
		10	Con 4g-S 4g-F 3.5g 3g
4		0	Con 4g 2g
		6	Con 4g 2g
		8	Con 4g 2g
		10	Con 4g 2g
		12	Con 4g 2g

[1]All trimedlure lures, including both liquid control and solid plugs, were obtained from Farma Tech Intl. Corporation, North Bend, Washington, USA, except that Experiment 3 also included 4 g plugs produced by Scentry Biologicals, Inc., Billings, Montana, USA. The 2 types of 4 g plugs used in Experiment 3 are designated in the table as 4g-S and 4g-F for Scentry and Farma Tech, respectively.

In conclusion, the present study lends little support to the notion that the incorporation of 4 g trimedlure plugs in medfly detection programs would allow effective trapping over longer inter-servicing intervals. Aside from the results obtained in the citrus grove, 4 g plugs weathered for 10 or 12 wk were significantly less attractive than fresh baits. Results obtained after 8 wk of weathering were more promising, but the performance of 4 g plugs aged for this interval was not consistent relative to the fresh lure. Although included in Experiment 3 only, the 4 g plugs from Scentry were as attractive as fresh lure after 8 wk of weathering in both trials, suggesting these plugs should be tested more extensively.

The present findings were somewhat surprising given previous data (Dean et al. 2018) showing that 8-wk-aged 3 g trimedlure plugs were as effective as fresh lure in 2 of 3 trials conducted in the same coffee field used here. That study also showed that after 10 wk of weathering the 3 g plugs contained, on average, 0.59 g of residual trimedlure, an amount above the 0.4 to 0.5 g threshold associated with high (approximately fresh) attractiveness (Leonhardt et al. 1987, 1989; Warthen et al. 1997; Dean et al. 2018). Neither trimedlure release rates nor residual amounts were measured in the present study, but we expected

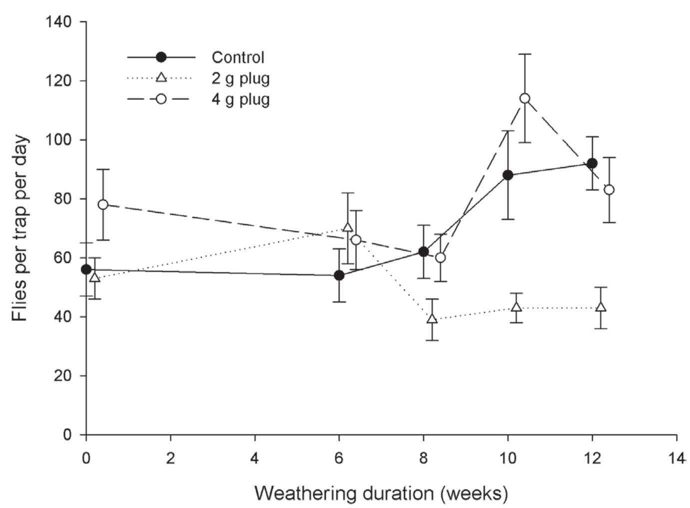

Fig. 4. In the trial replicate of Experiment 4, captures of released male medflies per trap per d in a Hawaiian orange tree orchard in Jackson traps baited with 2 mL of fresh trimedlure on a cotton wick (control), or 2 or 4 g trimedlure plugs weathered for varying durations. Symbols represent means ± 1 SE; n = 12 in all cases. Results of Holm-Šidák multiple comparisons tests are given in Table 3.

that 4 g plugs aged for 8 to 10 wk would contain more trimedlure and have higher release rates than similarly aged plugs containing less trimedlure, and hence be consistently more attractive to male medflies. Apparently, however, if such differences existed, they were not large enough to generate reliably significant differences in trap catch.

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