Relationship between Field Captures of Mediterranean Fruit Flies (Diptera: Tephritidae) and the Residual Amount and Release Rate of Trimedlure from Polymeric Plugs

Authors: Francis, Antonio, Levy, Andrew, Martin, Rodolfo Mesa, Mosser, Lisa, Bazelet, Corinna, et al.

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Relationship between field captures of Mediterranean fruit flies (Diptera: Tephritidae) and the residual amount and release rate of trimedlure from polymeric plugs

Antonio Francis[1], Andrew Levy[2], Rodolfo Mesa Martin[1], Lisa Mosser[1], Corinna Bazelet[3], and Todd Shelly[4,]*

Abstract

Detection of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), relies heavily on traps baited with trimedlure, a male-specific attractant. Originally used as a liquid, trimedlure is now dispensed from solid polymeric plugs (2 g active ingredient) that reduce volatilization and increase the effective longevity of the lure. Even so, plugs are attractive over a relatively short interval, and guidelines suggest that trimedlure plugs be replaced every 6 wk. The present study had 2 goals. First, at periodic intervals up to 10 or 12 wk, we evaluated the performance of plugs containing 4 g of trimedlure (i.e., twice the standard amount) to determine whether the increased loading resulted in an increased period of effective attractiveness. Second, we examined the relationship between trap captures and temporal changes in the residual amount and release rate of trimedlure. In 2 of the 3 field trials conducted in Hawaii and Florida, 4 g plugs weathered for as long as 8 wk, but not for 10 or 12 wk, performed as well as fresh liquid trimedlure applied to cotton wicks. In the other trial, plugs weathered for as long as 12 wk were as effective as fresh liquid. Chemical analyses revealed that the plugs were effective until the residual amount of trimedlure decreased below approximately 1.4 g and the release rate dropped below approximately 2.0 mg per h. The potential usefulness of 4 g trimedlure plugs in large-scale detection programs is discussed considering these findings.

Key Words: *Ceratitis capitata*; male lure; detection; trapping

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 atrayente específico para machos. Utilizado originalmente como líquido, el trimedlure ahora se dispensa a partir de tapones poliméricos sólidos (2 g de ingrediente activo) que reducen la volatilización y aumentan la longevidad efectiva del señuelo. Aun así, los tapones son atractivos durante un intervalo relativamente corto, y las pautas sugieren que los tapones trimedlure se reemplacen cada 6 semanas. El presente estudio tuvo 2 objetivos. Primero, a intervalos periódicos de hasta 10 o 12 semanas, evaluamos el desempeño de los tapones que contenían 4 g de trimedlure (es decir, el doble de la cantidad estándar) para determinar si el aumento de la carga resultó en un mayor período de atractivo efectivo. En segundo lugar, examinamos la relación entre las capturas de trampas y los cambios temporales en la cantidad residual y la tasa de liberación de trimedlure. En 2 de los 3 ensayos de campo realizados en Hawái y Florida, los tapones de 4 g resistidos durante 8 semanas, pero no durante 10 o 12 semanas, funcionaron tan bien como trimedlure líquido fresco aplicado a mechas de algodón. En el otro ensayo, los tapones expuestos a la intemperie durante 12 semanas fueron tan efectivos como los líquidos frescos. Los análisis químicos revelaron que los tapones fueron efectivos hasta que la cantidad residual de trimedlure disminuyó por debajo de aproximadamente 1,4 g y con la tasa de liberación cayendo por debajo de aproximadamente 2,0 mg por hora. La utilidad potencial de los tapones de trimedlure de 4 g en los programas de detección a gran escala se discute considerando estos hallazgos.

Palabras Clave: *Ceratitis capitata*; señuelo macho; detección; captura

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most serious agricultural pests in the world (Garcia et al. 2020). Females oviposit in over 300 species of fruits and vegetables, including such important crops as oranges, loquats, plums, guava, and papaya (USDA-APHIS 2017). Because of the threat posed, trapping systems frequently are operated in medfly-free regions to ensure early detection of infestations, which in turn allows prompt control actions, such as delimitation, fruit stripping, protein bait spraying, and the Sterile Insect Technique (Duarte et al. 2020, 2021). These management responses are both more effective and less costly when the outbreak involves small populations occurring in limited areas (Lodge et al. 2006; Suckling et al. 2016).

The primary baiting agent for medfly is the synthetic compound trimedlure (*tert*-butyl 4-chloro-2-methylcyclohexane-1-carboxylate)

[1]USDA-APHIS-PPQ, 13601 Old Cutler Road, Building 01, Coral Gables, Florida 33158, USA; E-mail: Antonio.Francis@usda.gov (A. F.), Rodolfo.MesaMartin@usda.gov (R. M. M.), Lisa.K.Mosser@usda.gov (L. K. M.)
[2]FDACS-DPI, 7525 Commerce Court, Sarasota, Florida 34243, USA; E-mail: Andrew.Levy@fdacs.gov (A. L.)
[3]USDA-APHIS-PPQ, 1730 Varsity Drive, Raleigh, North Carolina 27606, USA; E-mail: Corinna.S.Bazelet@usda.gov (C. S. B.)
[4]USDA-APHIS-PPQ, 41-650 Ahiki Street, Waimanalo, Hawaii 96795, USA; E-mail: Todd.E.Shelly@usda.gov (T. E. S.)
*Corresponding author; E-mail: todd.e.shelly@usda.gov

(Beroza et al. 1961). This lure is sex-specific and attracts males exclusively (Jang & Light 1996). Trimedlure usually is presented in solid, polymeric plugs that are suspended in open-ended, delta (Jackson) traps as described below. According to standard guidelines (FAO/IAEA 2018), the plugs contain 2 g of trimedlure, and the recommended replacement interval is 6 wk. This relatively short interval reflects the high volatility of the lure, which contributes to its attractiveness but also limits its effective field longevity (Leonhardt et al. 1989).

One approach to increase the field longevity of trimedlure plugs has involved the development of additives (termed extenders) to reduce the lure's volatility. Capilure® is the most commonly used trimedlure-plus-extender blend and has proven effective in certain instances (see McGovern et al. 1966; Nakagawa et al. 1981; Rice et al. 1984; Hill 1987). However, Baker et al. (1988) found that, whereas Capilure® retained its attractiveness longer than trimedlure, it was less attractive during the first 8 wk of deployment (see Shelly 2013). Alternatively, it appears that increasing the amount or 'loading' of trimedlure placed in dispensers might increase the time interval over which the plugs are attractive. In fact, Leonhardt et al. (1989) tested plugs containing 4 g of trimedlure and found that, even after 12 wk of weathering, traps baited with these plugs had high capture numbers relative to wicks containing 2 mL of fresh liquid trimedlure. Despite this prolonged effectiveness, the 4 g plugs were not adopted as the standard dispenser.

Note that, for Hawaii, trapping data for 4 g plugs have already been published (Shelly & Kurashima 2020). However, this earlier study did not include chemical data on the trimedlure plugs used in that study. Consequently, the relation between field performance and lure content and release is described here for the first time. In the already published study (Shelly & Kurashima 2020), captures in control traps were compared to traps containing 3.0, 3.5, or 4.0 g trimedlure plugs. Moreover, the 4 g plugs were provided by 2 suppliers (Scentry Biologicals Inc., Billings, Montana, USA, and Farma Tech Intl. Corp., North Bend, Washington, USA, respectively), and the comparative performance of these 2 sets of plugs was assessed. However, the present paper includes trapping and chemical data only for the 4 g Scentry plugs because these dispensers outperformed those from Farma Tech.

The present study had 2 main objectives. First, based on fieldwork conducted in Hawaii and Florida, captures of male medflies were compared between traps baited with fresh liquid trimedlure placed on wicks and 4 g trimedlure plugs that were weathered over periods of 10 to 12 wk. We selected 4 g plugs (and not the more traditional 2 g plugs), because 4 g trimedlure plugs have been adopted recently for use in Florida (A. Fox, personal communication). As described in the subsection on data analysis, the approach of comparing a fresh lure with a weathered lure is standard procedure for assessing the suitability of male lures in USDA-APHIS fruit fly detection programs. Second, the trimedlure content of weathered plugs was measured to determine the loss rate and derive the release rate of the compound. Measurements of these chemical parameters coincided with trapping periods, allowing assessment of the temporal relationship between the residual amount and release rate of trimedlure from plugs and the attractiveness of the plugs to male medflies.

Materials and Methods

Trapping was conducted in both Hawaii and Florida; however, the methods differed greatly between the 2 locations. Accordingly, the methods are presented separately for clarity. Trapping methods for Hawaii were presented in an earlier study (Shelly & Kurashima 2020), consequently an abbreviated account is given here.

HAWAII

Study Site

Trapping was conducted in a coffee (*Coffea arabica* L.; Rubiaceae) field (65 ha, 90 masl) located in central Oahu approximately 10 km southeast of Haleiwa, Hawaii, USA (21.527344°W, 58.037864°N). Plants were 2 to 4 m tall and were grown in parallel rows 2 to 3 m apart. Within a row, trunks of individual plants were separated by 1 to 2 m, and foliage generally was contiguous between neighboring plants. The trapping data were gathered from Oct to Dec 2018 and previously published in Shelly and Kurashima (2020). The average daily minimum/maximum temperatures during this period were 20.5 °C and 28.3 °C, respectively, with relative humidity generally between 60% and 85% (Wheeler Army Airfield, Wahiawa, Hawaii, USA).

Traps and Lures

Wild flies were captured using Jackson traps (Scentry Biologicals, Inc., Billings, Montana, USA) (photograph appears in FAO/IAEA 2018), the standard trap used in fruit fly surveillance programs in the USA (IPRFFSP 2006). Jackson traps were white, 'delta' traps made of thick waxed paper, with a removable insert, coated with 'stickum,' placed on the bottom of the trap to catch insects. Traps were suspended from tree branches using a metal hanger. In the trap, the lure dispensers (both wicks and plugs) were held in a perforated plastic basket suspended above the sticky insert from the metal hanger. Traps contained no insecticide.

Control traps were baited with 2 mL of liquid trimedlure (Farma Tech Intl. Corp., North Bend, Washington, USA) placed on a cotton wick immediately before placement in the field. Routine 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 or 5 mL liquid aliquots applied to wicks (T. E. Shelly, unpublished data). Thus, the control used in Hawaii was considered a valid standard representative of monitoring programs in US southern states (IPRFFSP 2006). The 4 g plugs were received directly from the vendor (Scentry Biologicals, Inc.) in individual, sealed packets and thus lost no (or very little) trimedlure before use.

Trapping and Weathering Protocol

Jackson traps were placed on wind-break trees (Norfolk Island 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 shakes the coffee plants beneath to loosen and collect ripe berries. This procedure 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 above ground in shaded locations between 7:00 AM and 10:00 AM. Traps were > 25 m apart, with treatment positions rotated between successive sampling intervals, and were operated 3 d per sampling interval. Twelve traps were deployed per treatment.

Collected traps were returned to the laboratory, where sticky inserts were removed, and captured flies counted. Plugs were left in the trap bodies, and these were hung 2 to 2.5 m above ground in a shaded location outside the laboratory for weathering under similar environmental conditions as the coffee field. The average daily minimum/maximum temperatures at the weathering site were 21.0 °C and 29.7 °C, respectively, and the relative humidity was typically between 55%

and 75%. Field sampling was conducted when plugs were weathered for 0 (fresh), 6, 8, and 10 wk.

Chemical Analysis for Residual Amount of Trimedlure

In addition to the plugs used in the field, additional plugs were weathered exclusively for analysis of trimedlure. Weathering of these additional plugs, which began on the first d of field trapping, was carried out in the area next to the laboratory noted above. After every trapping period, 5 plugs were wrapped individually in aluminum foil, placed in sealed, plastic bags, and placed in a freezer for storage. Thus, trap capture of male *C. capitata* as well as trimedlure content of plugs were measured for the same set of weathering intervals. In addition, 5 plugs were weathered for 3 d to obtain an estimate of the initial release rate. After the final trapping period, all plugs were placed in insulated boxes with coolant and express mailed to the Treatment and Inspection Methods Laboratory in Miami, Florida, USA, where the samples were analyzed upon receipt.

Trimedlure plugs were removed from the manufacturer packing or aluminum foil wrapping (if they had been weathered) and placed in 100 mL glass vials with screw caps for dissolution and extraction with 50 mL of tetrahydrofuran (Sigma Aldrich, Saint Louis, Missouri, USA; PN 186562, anhydrous, contains 250 ppm butylated hydroxytoluene as an inhibitor, ≥ 99.9%). The glass vials were securely capped and placed on a mechanical shaker for 2 h (low setting). After shaking they were allowed to rest for 5 min before being opened. A 56 µL aliquot of each extraction solution was mixed with 50 µL of 5.00 mg per mL tetradecane (internal standard) (Sigma Aldrich, PN 70129, analytical standard) and diluted to 1,000 µL with tetrahydrofuran. This solution was then diluted further with tetrahydrofuran (200 µL to 1,000 µL). The resulting solution was added to an Agilent 7890B gas chromatograph with flame ionization detection sequence that included injections for a triplicate injection 3-point calibration curve: 0.100, 0.500, and 1.000 mg per mL trimedlure (Sigma Aldrich, PN 04607, analytical standard), where each calibration solution contained 0.050 mg per mL tetradecane as the internal standard.

The gas chromatograph with flame ionization detection method consisted of 1 µL splitless injections at 200 °C (purged after 1 min). At a constant flow rate of 1 mL per min (helium carrier gas), the separation was achieved through an MXT®-WAX column (Restek, 70638, 30 m by 250 µm ID, 0.5 µm film thickness). The gas chromatograph oven started at 50 °C for 0.5 min and ramped to 100 °C at 25 °C per min (held for 1 min). The temperature was then ramped up to 185 °C at 5 °C per min and held for 5 min. The flame ionization detection was set at 300 °C, 450 mL per min air flow, 40 mL per min hydrogen flow, and 45 mL per min helium constant make-up flow. The data analysis section of the instrumental method calculated the concentration of trimedlure in the solution injected (mg per mL) to the internal standard adjusted mass (g) of trimedlure in each plug.

FLORIDA (2020)

Study Site

Trapping was conducted in a citrus (*Citrus sinensis* (L.) Osbeck; Rutaceae) orchard (6 ha) in Parrish, Florida, USA (27.595507°W, 82.481087°N) from Jul to Oct 2020. Rows of trees were separated by 8 m, and trees within a row were 4 m apart. The average daily minimum/maximum temperatures during this period were 22.6 °C and 32.3 °C, respectively (UF-IFAS Florida Automated Weather Network, Gainesville, Florida, USA). Afternoon to evening rain showers were frequent, brief, and often torrential.

Study Flies

Sterile male medflies were shipped as pupae from the USDA Medfly Production Facility, El Pino, Guatemala, to the USDA Sterile Insect Release Facility, Sarasota, Florida, USA, for emergence and release. Pupae and newly emerged males were maintained in Worley Eclosion Towers (Dowell et al. 2021) and provided sugar agar blocks for food and water. Flies were held at 25 to 27 °C, 50 to 80% RH, and a 12:12 h (L:D) photoperiod. At 7 to 9 d of age, adult flies were chilled (4 °C for 1 to 2 h) as part of the routine procedure for aerial release. Immobilized flies were then weighed collectively and divided equally into 8 screen cages (30 cm^3) (BioQuip Products Inc., Compton, California, USA) for recovery and transport to the field. Sugar agar blocks and water-soaked cotton wicks were supplied in each cage. The cages were transported from Sarasota to Parrish in an air-conditioned truck (approximately 45 min) and then released.

Releases occurred 1 d before trap placement to allow adequate time for flies to disperse throughout the experimental area. In the field, cages were suspended 1.5 to 2 m above ground from tree branches to avoid ant predation and left open to allow flies to exit freely. Releases were made at 8 trees located in 2 parallel rows (4 release trees per row) spaced 24 m apart near the center of the orchard to facilitate uniform dispersal. Approximately 40,000 flight-capable males were released per test. All releases were made from 9:30 AM to 10:00 AM. Release cages were removed 24 h later before deploying the trimedlure traps. A total of 7 releases were made corresponding to weathering intervals of 1, 2, 4, 6, 8, 10, and 12 wk for the plugs.

Traps and Lures

As in Hawaii, flies were captured using Jackson traps. Control traps were baited with 5 mL of liquid trimedlure placed on a cotton wick immediately before placement in the field. As noted above, when used fresh, this loading was found to attract similar numbers of male medflies as 2 mL liquid lure or 2 g plugs. The test traps each contained a 4 g plug (Scentry Biologicals Inc., Billings, Montana, USA).

Trapping and Weathering Protocol

A total of 24 traps (12 traps each for the 2 treatments) were deployed following each medfly release. Traps within the experimental area were arranged in a grid and were separated by approximately 28 m. The same trap sites were used for all releases, and treatments were rotated 1 position between successive releases. Traps were suspended from tree branches in shaded locations approximately 2 m above ground between 8:00 AM and 9:00 AM and collected 24 h later. Sticky inserts were examined for male medflies and counted in the laboratory. Plugs were kept in the Jackson traps for weathering until the next trapping period; traps were placed outdoors beneath a tree canopy in Sarasota, Florida, USA. At this site, the average daily minimum/maximum temperatures during the weathering period were 23 °C and 32 °C, respectively, and the average humidity was 80% (Sarasota/Bradenton International Airport, Florida, USA).

Chemical Analysis for Residual Amount of Trimedlure

As in Hawaii, 5 plugs were weathered for each interval and sent to the Treatment and Inspection Methods Laboratory, Miami, Florida, USA, for analysis. In addition, 5 plugs were weathered for 1 d and sent to the laboratory to obtain an estimate of initial release rate.

FLORIDA (2021)

Methods followed those described for Florida (2020) with the following exceptions. Trapping of released flies was conducted in a citrus orchard (7 ha) in Fort Pierce, Florida, USA (27.447869°W, 80.481819°N) from Jun to Sep 2021. Over the study period, daily minimum and maximum temperatures averaged 23.4 °C and 31.8 °C, respectively (UF-IFAS Florida Automated Weather Network, Gainesville, Florida, USA). Approximately 50,000 flight-capable males were released per test. All releases were made from 10:00 AM to 10:30 AM. The cages were transported from Sarasota to Fort Pierce in an air-conditioned truck (approximately 2.5 h) and then released. Traps were collected 2 d after deployment. Estimates of the initial release rate of trimedlure were based on analyses of plugs weathered for 1 wk.

DATA ANALYSIS

Two-way ANOVA, with wk and lure as the main effects, was used to analyze trap captures. Log_{10} transformed raw data met the parametric assumptions of normality and homoscedasticity for Hawaii and Florida (2020) data sets. Although transformed data were non-normal for Florida (2021), ANOVA was nonetheless employed, because a non-parametric equivalent of ANOVA using ranked data (Conover & Iman 1981) generated results identical to those using transformed data. Consequently, it appeared that the parametric-based analysis was sufficiently robust to accommodate the level of non-normality present in the data set. Release rates of trimedlure in weathered dispensers were calculated as the difference between the average residual mass of the chemical in time intervals x and x+1, respectively, divided by the h elapsed between these time intervals. Statistical analyses were performed using JMP 14 software (SAS Institute, Cary, North Carolina, USA) and SigmaPlot 11 (Systat Software, San Jose, California, USA).

In assessing the suitability of novel trimedlure dispensers in trapping programs, USDA-APHIS-PPQ uses the so-called '50% rule' (Leonhardt et al. 1989), which in the context of the present study holds that 4 g trimedlure plugs are an acceptable replacement for the standard, currently used 2 g plugs if, after weathering for 6 wk, traps baited with 4 g plugs capture > 50% as many male medflies as traps baited with fresh 2 g trimedlure plugs. Given its operational importance, we therefore evaluate the 50% rule for weathering periods ≥ 6 wk for each of the experiments.

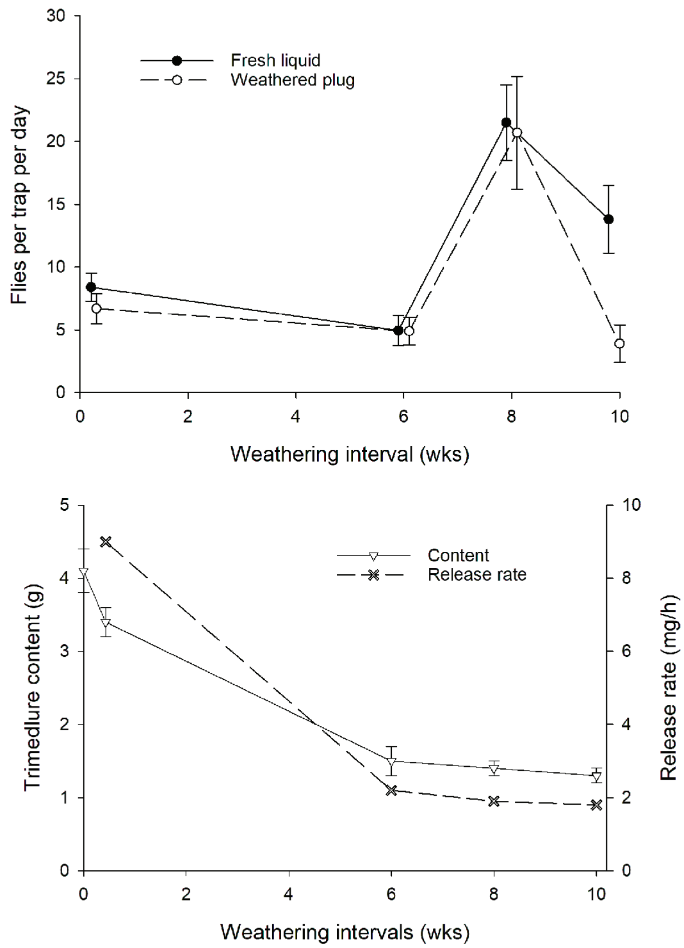

Fig. 1. Captures of male medflies, *Ceratitis capitata*, in traps baited with fresh liquid trimedlure or weathered trimedlure plugs over the 10-wk study period in Hawaii in 2018 (top; means ± SE, *N* = 12 per treatment) and trimedlure content (means ± SD) and release rate from plugs over the study period (bottom, *N* = 5 per parameter).

Results

HAWAII

Both wk ($F_{3, 88}$ = 12.5; P < 0.001) and lure ($F_{1, 88}$ = 8.8; P = 0.008) had significant effects on captures of male medflies (Fig. 1). The interaction term was significant as well ($F_{3, 88}$ = 4.2; P = 0.02). The temporal variation in captures likely reflected natural changes in the size of the wild population as well as possible differences in the environmental conditions during the sampling periods (e.g., wind speed and direction). Given the significant interaction, captures by lure type were analyzed weekly using the Tukey test (P = 0.05), which showed that captures were similar in liquid- and plug-baited traps in wk 0, 6, and 8 but that traps with fresh liquid bait captured significantly more male medflies than weathered plug-baited traps in wk 10. Consistent with this result, captures in traps baited with 10-wk weathered plugs were less than 50% of those observed in traps baited with fresh liquid.

Both the residual amount and release rate of trimedlure from the plugs declined most rapidly in the initial wk of weathering (Fig. 1). For example, 63% of the original amount of trimedlure was lost after 6 wk but only an additional 4% (67% total) was lost after 10 wk. Similarly, the release rate was approximately 9.0 mg per h over the first 3 d of weathering but was only 2.5 mg per h over the following 39 d (based on measurements at 6 wk) and < 2 mg per h between 6 and 10 wk.

FLORIDA (2020)

Both wk ($F_{6, 154}$ = 4.4; P < 0.001) and lure ($F_{1, 154}$ = 8.0; P = 0.005) had significant effects on captures of male medflies (Fig. 2). The interaction term was not significant ($F_{6, 154}$ = 0.2; P = 0.97). As the ANOVA identified an overall effect of lure, pairwise comparisons were made between fresh liquid vs. weathered plugs for specific weathering intervals using Student *t*-tests (with log_{10} transformed data). This analysis showed that the only significant, within-interval difference in captures between traps baited with fresh liquid or weathered plugs was found for wk 10 (t = 2.2; P = 0.04). Regarding the 50% rule, captures in traps baited with weathered plugs were < 50% of those in traps baited with fresh liquid for wk 1, 10, and 12.

Temporal variation in both the residual amount and release rate of trimedlure from the plugs was similar to that observed in Hawaii, with both parameters declining most rapidly in the initial wk of weathering (Fig. 2). After 6 wk of weathering, 67% of the original amount of trimedlure was lost, but only an additional 14% (81% to-

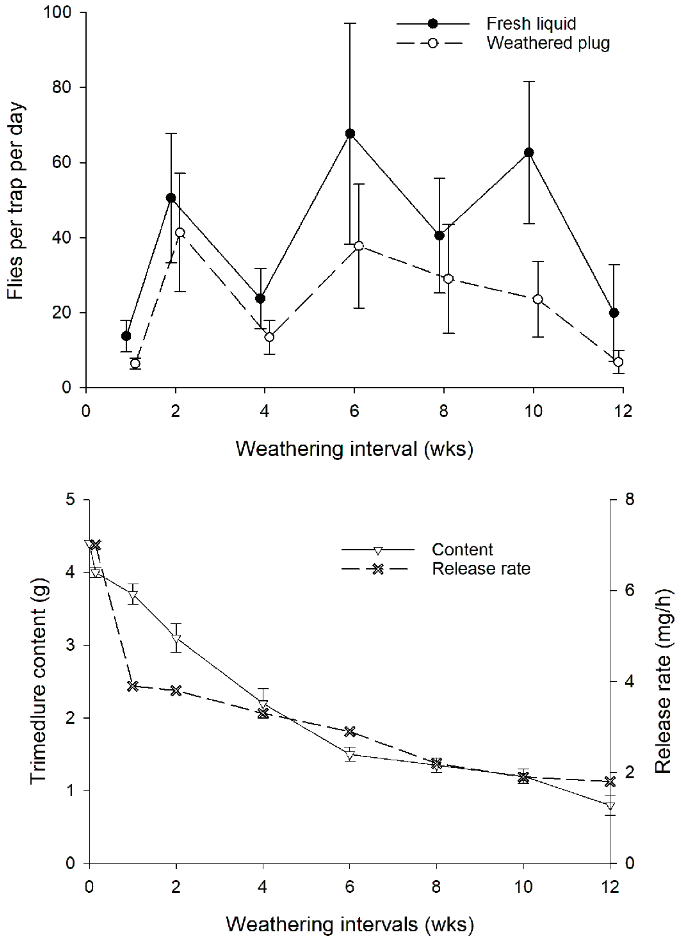

Fig 2. Captures of male medflies, *Ceratitis capitata*, in traps baited with fresh liquid trimedlure or weathered trimedlure plugs over the 12-wk study period in Florida in 2020 (top; means ± SE, N = 12 per treatment) and trimedlure content (means ± SD) and release rate from plugs over the study period (bottom, N = 5 per parameter).

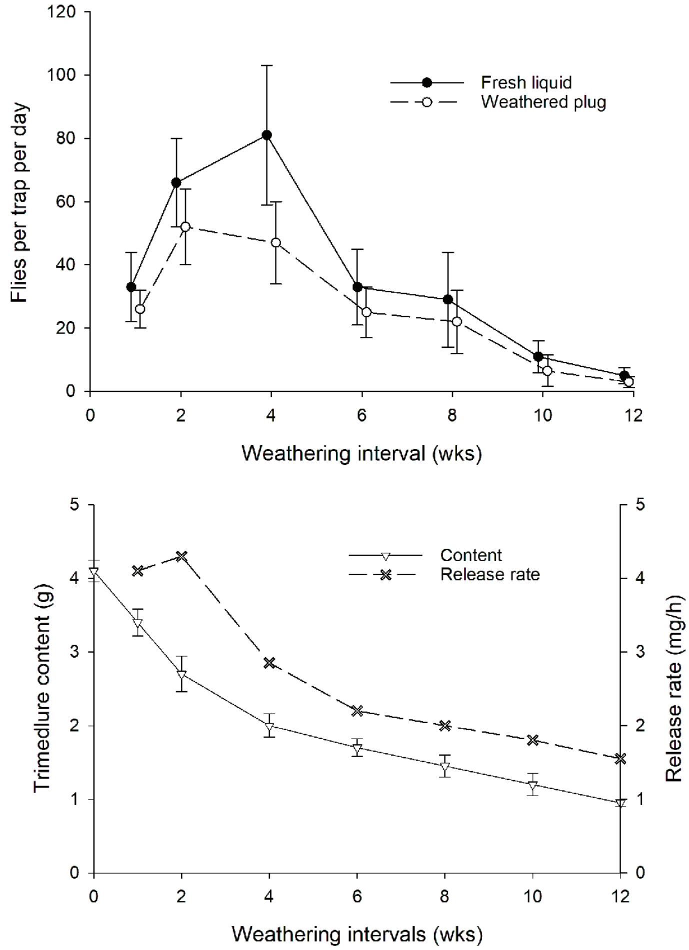

Fig 3. Captures of male medflies, *Ceratitis capitata*, in traps baited with fresh liquid trimedlure or weathered trimedlure plugs over the 12-wk study period in Florida in 2021 (top; means ± SE, N = 12 per treatment) and trimedlure content (means ± SD) and release rate from plugs over the study period (bottom, N = 5 per parameter).

tal) was lost after 12 wk. Similarly, the release rate was 7.1 mg per h over the first 1 d of weathering but was < 2.5 mg per h after ≥ 8 wk of weathering.

FLORIDA (2021)

Both wk ($F_{6, 154}$ = 22.9; P < 0.001) and lure ($F_{1, 154}$ = 4.1; P = 0.04) had significant effects on captures of male medflies (Fig. 3). The interaction term was not significant ($F_{6, 154}$ = 0.4; P = 0.89). As above, Student *t*-tests (with $\log_{10}$ transformed data) were conducted to make pairwise comparisons of captures between traps having fresh liquid or weathered plugs for specific weathering intervals. Despite lure having a significant effect overall, no significant differences were detected for the individual intervals. Regarding the 50% rule, captures in traps baited with weathered plugs were > 50% of those in traps baited with fresh liquid for all wk.

As in the data sets presented above, both the residual amount and release rate of trimedlure from the plugs declined most rapidly in the initial wk of weathering (Fig. 3). After 6 wk of weathering, 59% of the original amount of trimedlure was lost, but only an additional 16% (75% total) was lost after 12 wk. Similarly, the release rate was 4.2 mg per h over the first 1 wk of weathering but was ≤ 2.0 mg per h after ≥ 8 wk of weathering.

Discussion

In a seminal paper, Leonhardt et al. (1989) compared the capture efficacy of weathered 2 and 4 g trimedlure polymeric plugs against the then-standard dispenser, cotton wicks containing 2 mL of freshly applied liquid trimedlure. Their results clearly showed that the traps baited with 2 g plugs captured ≥ 50% as many male medflies as traps baited with the standard dispenser for approximately 8 wk, while the 4 g plugs met the 50% rule for as long as 12 wk (when tests were terminated). Based on these findings, Leonhardt et al. (1989) anticipated that 4 g trimedlure plugs would be adopted by large-scale detection programs. This did not occur, however, and 2 g trimedlure plugs replaced cotton wicks as the recommended dispensers in medfly trapping programs in the 1990s (FAO/IAEA 2018).

To our knowledge, the present study represents the first re-examination of 4 g trimedlure plugs since Leonhardt et al. (1989). In contrast to that earlier study, the present findings fail to show the prolonged effectiveness of 4 g plugs. Based on the collective outcome of the statistical analysis and 50% rule, the results from Hawaii and Florida (2020) showed that 4 g trimedlure plugs were as effective as fresh trimedlure liquid on wicks for up to 8 wk but were less effective when weathered longer than 8 wk. Only in the trial conducted in Florida in 2021 did the 4 g plugs attract statistically similar numbers of males as fresh liquid on wicks (when analyzed on a wk-by-wk basis), and meet the 50% criterion, for all weathering durations including 12 wk. As noted above, however, lure type had a significant overall effect on captures, and traps baited with fresh liquid had higher catch than traps with aged plugs on all sampling dates. A comparable study (Dean et al. 2018), also conducted in Hawaii and Florida, found that traps baited with 2 g trimedlure plugs weathered for more than 6 wk captured less than 50% as many medfly males as traps baited with fresh trimedlure liquid. Thus, comparing Dean et al. (2018) with the present study, we suggest that increasing the trimedlure loading to 4 g extends the effective life of the lure approximately only 2 wk beyond that exhibited by 2 g plugs (i.e., 8 vs. 6 wk, respectively). Although key environmental parameters (e.g., wind speed) were not recorded, it is important to note that Dean et al. (2018) and the present study were conducted under similar temperature and humidity regimes. As temperature has a direct effect on trimedlure loss from dispensers (Suckling et al. 2008; Flores et al. 2017), the uniformity across trials indicates that temperature differences were not responsible for the observed variation in the effective longevity of the plugs tested.

Although limited, available data regarding the relationship between trimedlure loss and release rate and capture of male medflies have been consistent for 2 g plugs. Three studies (Leonhardt et al. 1989; Warthen et al. 1999; Dean et al. 2018) have found that the 2 g trimedlure plugs become less attractive than freshly baited wicks when the residual content of trimedlure drops below 0.4 to 0.5 g and the release rate decreases below 1 mg per h. In contrast, data for 4 g trimedlure plugs have been less consistent. For example, in the present study, the 4 g plugs used in Florida in 2020 were ineffective at 12 wk when the residual amount and release rate of trimedlure were 1.3 g and 1.95 mg per h, respectively. Yet, in the 2021 trial in Florida, the 4 g plugs were attractive at 12 wk even with lower content and release rate (1.0 g and 1.5 mg per h, respectively). Even greater discrepancies are evident when comparing the present study with Leonhardt et al. (1989). This latter study found 4 g plugs to be attractive at 10 to 12 wk when the residual amount and release rate of trimedlure were 0.8 to 1.0 g and 0.1 to 0.3 mg per h, respectively. However, in the present study, 4 g plugs weathered for 10 wk had higher amounts of trimedlure (Hawaii: 2.2 g; Florida (2020): 1.2 g) and higher release rates (Hawaii: 1.8 mg per h; Florida (2020): 2.0 mg per h) but were less attractive than the control lures.

Comparisons between the present study and Leonhardt et al. (1989) are confounded by the differing environment conditions characteristic of the 2 studies. Although Leonhardt et al. (1989) conducted trapping in Hawaii (under conditions similar to those of the present study), the plugs were weathered near Parlier in the Central Valley of California, USA, where temperatures were much more extreme (minimum: 8–10 °C; maximum: 39–40 °C) and humidity was likely much lower (measurements not provided). Aside from this more obvious difference, it is possible also that differences in the olfactory environment in which the studies were conducted contributed to differing responsiveness of male medflies to trimedlure. In other insects, background odors have been shown to enhance (e.g., Said et al. 2005) or reduce (e.g., De Groot & MacDonald 1999) attraction to pheromones. Possibly, then, the fact that Leonhardt et al. (1989) performed bioassays in a macadamia nut orchard (*Macadamia integrifolia* Maiden & Betche; Proteaceae), whereas trapping in the present study took place in coffee and citrus fields may have influenced the responsiveness of male medflies to trimedlure. Other explanations appear less likely. For example, the flies released and trapped in Leonhardt et al. (1989) were from a bisexual strain (HI-Lab), whereas the flies trapped in the present study were either wild or from a genetic sexing strain (*tsl*). Thus, males of the different strains may vary in their sensitivity to trimedlure, which may have led to the difference noted between Leonhardt et al. (1989) and the present study.

By itself, the finding that 4 g trimedlure plugs extend the replacement interval for 2 wk does not promote the adoption of an 8-wk schedule for fruit fly detection programs. However, if the other male lures (methyl eugenol and cue-lure) also were shown to be effective for 8 wk, then the replacement interval could be lengthened from 6 to 8 wk for all male lures, which would reduce the number of lure replacements from approximately 8 per yr to 6 per yr and likely result in substantial cost reductions. Data on 6 g methyl eugenol plugs are encouraging and indicate an effective field longevity of at least 12 wk (Shelly et al. 2022), while data on cue-lure plugs are being gathered presently in Hawaii.

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