Field Longevity and Attractiveness of Trimedlure Plugs to Male Ceratitis capitata in Florida and Hawaii

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Field longevity and attractiveness of trimedlure plugs to male Ceratitis capitata in Florida and Hawaii

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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 that reduce volatilization and increase the effective field longevity of the lure. Many of the previous bioassays that measured trimedlure longevity were conducted in Hawaii and adopted a simple “50% rule” for trimedlure plug replacement, i.e., trimedlure plugs should be changed out when their attractiveness (trap captures of male medflies) drops below 50% that observed for fresh trimedlure liquid. The goal of the present study was to assess the field effectiveness of trimedlure plugs in Florida and apply standard statistical analyses to the trapping data. Sterile marked male medflies were released in a citrus orchard with traps containing 3 trimedlure treatments: 2 ml fresh liquid, 2 g fresh plugs, or 2 g plugs aged for intervals of 1, 2, 4, 6, 8, 10, or 12 wk. A single release of approximately 40,000 males was performed for each of the weathering intervals. Weathered trimedlure plugs were as effective as fresh lures when aged 6 wk or less, but catch was significantly reduced for plugs weathered 8 or more wk. At 8 to 12 wk, remaining trimedlure content per plug was ≤ 0.4 g. Ancillary field trials in Hawaii compared male medfly captures in traps baited with fresh 2 or 3 g trimedlure plugs, or 2 or 3 g plugs weathered for 6, 8, or 10 wk. Two and 3 g plugs weathered for 6 wk were as attractive as fresh lures, but 3 g plugs were more effective than 2 g plugs at the longer weathering intervals. Results are compared with prior studies, and implications for medfly management strategies are discussed.

Key Words: Tephritidae; Mediterranean fruit fly; medfly detection; fruit fly surveillance; attractant

The Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), is a notorious worldwide pest of fruits and vegetables, with females known to oviposit in more than 300 host plant species (Liquido et al. 1990). Detection and control of medfly rely heavily on traps baited with the male-specific attractant trimedlure (Tan et al. 1984) and King & Landolt (1984) reported that cotton wicks containing 2 mL liquid trimedlure lost their attractiveness within 2 to 4 wk. Various studies (e.g., Leonhardt et al. 1984; Dominguez-Ruiz et al. 2008) documented the direct relationship between temperature and trapping programs since the 1960s and initially was applied in liquid form to cotton wicks placed inside traps (Burditt 1975; Nakagawa et al. 1979).

Although fairly attractive, liquid trimedlure has high volatility and thus relatively short field longevity as an effective lure. Rice et al. (1984) and King & Landolt (1984) reported that cotton wicks containing 2 mL liquid trimedlure lost their attractiveness within 2 to 4 wk. Various studies (e.g., Leonhardt et al. 1984; Dominguez-Ruiz et al. 2008) documented the direct relationship between temperature and

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Materials and Methods

STUDY SITE-I

Field trapping of released medflies was conducted in a citrus (Citrus sinensis (L.) Osbeck) (Rutaceae) orchard in Duette, Florida, USA (elevation 24 masl), during Jun to Aug 2016. Within the 20 ha orchard, rows of trees were separated by 4 m, and trees within a row were 8 m apart. Average daily temperatures varied between 26.1 to 31.7 °C during the study period. Local rain showers were common and often intense (about 2.5 cm per d).

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. Flies were derived from a genetic sexing strain in which a temperature sensitive lethal(tsl) mutation allows for selective culling of female embryos (via thermal shock) and exclusive production of males (Caceres 2002). Following standard protocol (FAO/IAEA 2007), pupae were coated before irradiation and shipment with a fluorescent dye (neon red, DayGlo Corporation, Cleveland, Ohio, USA). Upon adult emergence, dye particles are retained on the face and under the wings and visible with a dissecting microscope under ultraviolet (black) light. Pupae and newly emerged males were maintained in Worley Eclosion Towers and provided sugar agar blocks for food and water (FAO/IAEA 2007). Flies were held at 25 to 27 °C, 50 to 80% RH, and a 12:12 (L:D) photoperiod.

TRAPS, TRIMEDLURE LURES, AND AGING PROTOCOL

Flies were captured using Jackson traps (Scentry Biologicals, Inc., Billings, Montana, USA) (FAO/IAEA 2013) that were white ‘delta’ traps made of thick waxed paper (12.7 cm L × 9.5 cm W × 8.4 cm H). A removable insert (also supplied by Scentry Biologicals, Inc.), made of the same waxed paper as the trap body and coated with an adhesive, was placed on the bottom of the trap to catch insects. Traps were suspended from tree branches using a metal hangar 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 hangar.

Three trimedlure treatments were compared during each trapping period: fresh (i.e., not weathered) liquid, fresh 2 g plugs, and weathered 2 g plugs (all lures supplied by Farma Tech Intl. Corp., North Bend, Washington, USA). The liquid was applied at a dose of 5 mL per cotton wick following the protocol of the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA. For weathering, plugs were placed in Jackson traps, which were kept outdoors beneath a tree canopy in Palmetto, Florida, USA, under similar climatic conditions as the Duette orchard. Sets of 12 trimedlure plugs were weathered for intervals of 1, 2, 4, 6, 8, 10, and 12 wk, respectively, prior to testing. As described below, 1 release of sterile male medflies and 1 subsequent trapping period were conducted for each of these 7 weathering intervals.

TRAPPING PROTOCOL

Sterile males (7 to 9 d old) were released 1 d prior to trap placement to allow adequate time for dispersal throughout the area. For release, flies were chilled (4 °C for 1 to 2 h) as part of the routine procedure for aerial release. Immobilized flies then were weighed collectively and divided equally into 6 screen cages (30 cm cubes) (BioQuip Products, Inc., Rancho Dominguez Hills, California, USA) for recovery and transport to the field. Sugar agar blocks and water-soaked cotton...
wicks were supplied in each cage. In the field, cages were suspended 1 to 3 m above ground from tree branches to avoid ant predation and left open to allow flies to exit freely. Releases were made at 6 trees located in 2 parallel rows (3 release trees per row) near the center of the orchard and evenly spaced to facilitate uniform dispersal. Based on standard quality control estimates (FAO/IAEA 2013) of fly weight and flight ability, approximately 40,000 flight-capable males were released per test. All releases were made between 7:00 AM and 10:00 AM.

A total of 36 traps were operated following each release (n = 7 releases, 1 for each weathering interval), i.e., 12 traps each for the 3 trimedlure treatments. Traps within each orchard were arranged in a grid of 10 rows with 3 to 4 traps per row separated by 25 to 50 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 and placed in the field between 7:00 AM and 10:00 AM, then collected 24 h later. Sticky inserts were examined for male medflies in the laboratory.

Captures of male medflies were compared among the 3 treatments for individual weathering intervals using 1-way ANOVA, as raw data or \log_{10} transformed data met the parametric assumptions of normality and equal variances in all cases. Where significant variation was detected, the Holm-Šídák test was used to identify pair wise differences.

TRIMEDLURE CONTENT OF DISPENSERS

Following each release, and subsequent trapping period, random subsamples of 6 lures from each treatment were collected for analysis of trimedlure content. Lures were immediately placed in sealed plastic bags and express mailed overnight to the Agricultural Quarantine Inspection (AQI) chemistry laboratory in Miami, Florida, USA, where the samples were stored at 20 °C until analysis.

To extract trimedlure, plugs and wicks were placed in 100 mL of tetrahydrofuran then shaken for 4 h at low speed on a mechanical shaker. A 2 mL aliquot of each sample then was transferred to a 100 mL volumetric flask, spiked with 1 mL of the internal standard tetradecane, and filled to the mark with hexane. A 2 mL aliquot was transferred to a GC vial and analyzed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector. The column used was a 30.0 m × 0.32 mm × 0.25μm film thickness HP-5 (Agilent Technologies, Santa Clara, California, USA) fused-silica capillary column. Injector and detector temperatures were 200 °C and 300 °C, respectively. Oven temperature was held at 50 °C for 30 s, raised to 100 °C at 25 °C per min and held for 1 min, then raised to 220 °C at 10 °C per min and held for 3 min. Carrier gas (helium) flow rate was 1.0 mL per min. The internal standard method was used to quantify trimedlure.

STUDY SITE-II

Field trapping of wild medflies was conducted in a coffee (Coffea arabica L.) (Rubiacae) field 10 km southeast of Haleiwa, Oahu, Hawaii, during three separate intervals in 2016 (May, Aug, and Oct to Nov). The field covers approximately 65 ha of a north-facing slope (elevation 90–100 masl). Rows, and plants within rows, were separated by 3 m. During trapping, average daily temperatures varied between 25.0 to 26.7 °C, and rainfall was slight (< 2 cm total rain per period). No fly releases were performed at this study site.

TRAPS, TRIMEDLURE LURES, AND AGING PROTOCOL

As in Florida, Jackson traps were used to capture medflies in Hawaii. Four trimedlure treatments were compared during each trapping period using: fresh and weathered 2 and 3 g trimedlure plugs (Farma Tech Intl. Corp., North Bend, Washington, USA). The 3 g trimedlure plugs were larger than the standard 2 g plugs but fit within the perforated basket inside the trap. For weathering, plugs were placed in Jackson traps on a shaded, outdoor porch at our laboratory in Halawa, Oahu, Hawaii, under similar conditions as the coffee field. Sets of 15 trimedlure plugs were weathered for intervals of 8 or 10 wk for the first (May) trapping period and 6, 8, or 10 wk for the other periods (Aug and Oct to Nov, respectively).

TRAPPING PROTOCOL

During the 3 sampling periods, 15 traps were operated for each of the 4 trimedlure treatments (i.e., 60 traps total). Traps were randomly assigned positions in a 10 × 6 grid, and were separated by 25 m within and between rows. The same trap sites were used over the entire study. As in Florida, traps were suspended from branches in shaded locations 1 to 2 m above ground. For all test periods, traps were placed in the field between 8:00 AM and 9:00 AM then removed 48 h later.

Captures of wild male medflies were compared among the 4 treatments for individual weathering intervals using the Kruskal-Wallis test, because, in most cases, neither raw nor transformed data met the parametric assumptions of normality and equal variances. Where significant variation was detected, the Student-Newman-Keuls test was used to identify pair wise differences (P < 0.05).

TRIMEDLURE CONTENT OF DISPENSERS

After field trapping for the 10-wk weathering interval, random samples (n = 5) of fresh and weathered 2 and 3 g plugs were immediately wrapped individually in aluminum foil, placed in sealed plastic bags, and stored in a freezer until express mailed to the aforementioned AQI laboratory in Miami, Florida, USA. Thus, for each replicate, 20 plugs (5 plugs for each of the 4 treatments) were analyzed for trimedlure content following the same protocol described earlier.

Results

CAPTURES OF STERILE MALE MEDFLIES

Most likely reflecting variation in environmental conditions and the vigor of the released flies in the Florida study, captures of sterile medflies varied greatly among the different trapping intervals. Summed over all 3 trimedlure treatments, the total number of captures ranged between 250 (8 wk) and 1,486 (10 wk) over the 7 trapping periods, with an average of 899 males, or 2.2% of males released, captured per period.

There was no significant variation detected in trap captures among the 3 lure treatments for the 1, 2, 4, or 6 wk weathering intervals (Fig. 1 – associated values: wk 1: F = 0.24, P = 0.78; wk 2: F = 0.93, P = 0.40; wk 4: F = 2.40, P = 0.11; wk 6: F = 1.27, P = 0.29). However, significant differences were detected in the 8, 10, and 12 wk weathering intervals (Fig. 1 – associated F, and P values: wk 8: F = 4.43, P = 0.02; wk 10: F = 3.57, P = 0.04; wk 12: F = 5.81, P = 0.01). In each of these wk, similar numbers of males were captured in traps containing fresh liquid trimedlure on wicks or fresh trimedlure plugs. Both treatments captured significantly more males than the weathered trimedlure plugs. Trimeledlure plugs weathered for 8, 10, or 12 wk captured, on average, 30 to 40% as many males as fresh treatments.
TRIMEDLURE CONTENT AND RELEASE RATE

Fresh liquid trimedlure wicks, in Florida, contained an average of 4.3 to 4.6 g of material during the study, with the exception of wk 1 where an average of 5.3 g of trimedlure was present per wick. This value most likely reflected an error in the amount of trimedlure (i.e., > 5 mL) initially applied to 1 or more of the wicks. The average trimedlure content of fresh plugs was relatively constant over the different weathering intervals and varied only between 1.9 to 2.1 g per plug. For weathered plugs, trimedlure content declined rapidly over the first 4 wk then relatively slowly thereafter (Fig. 2). As noted above, relative to the fresh lures, medfly captures were significantly lower in traps baited with plugs weathered for 8, 10, or 12 wk, whereas trimedlure content was < 0.4 g over these intervals. Release rates ranged between 2 to 4 mg per h during the first 4 wk of weathering and were 1 to 2 mg per h over wk 6 to 12 (Fig. 2).

CAPTURES OF WILD MALE MEDFLIES

In the Hawaii study, no significant differences among the 4 treatments occurred for 2 of the 3 replicates from the 6-wk weathering trials (Fig. 3). All replicates of the 8-wk weathering assays and 2 replicates of the 2 g aged plugs captured significantly fewer males compared with traps with fresh plugs or traps with 3 g aged plugs. At 8 wk, the aged 3 g plugs were as effective as fresh 2 or 3 g plugs in 2 of the 3 replicates. For 10-wk weathering assays, traps containing fresh plugs captured significantly more medflies than aged plugs in all 3 replicates. However, as in the 8-wk tests, the 3 g aged plugs were significantly more attractive than the 2 g aged plugs in 2 of the 3 replicates.

TRIMEDLURE CONTENT AND RELEASE RATE

Pooling the Hawaii data across the 3 replicates, we found that 2 g trimedlure plugs aged for 10 wk retained an average of 0.34 g of trimedlure, representing an average loss of 83% of the original content (measured as 1.96 g and not 2.0 g; Table 1). In comparison, the 3 g trimedlure plugs contained an average of 0.59 g of trimedlure, representing an average loss of 82% of the original content (measured as 3.19 g and not 3.0 g; Table 1). Thus, after 10 wk of weathering, 3 g plugs contained an average of 75% more trimedlure than 2 g plugs. This difference was presumably responsible for the significantly higher fly captures observed for 3 g plugs after 10 wk of weathering. Trimedlure release rates were not calculated for these data given the extended time interval (10 wk) between measurements of fresh and weathered plugs.
Discussion

Trapping data from Florida and Hawaii showed a similar effect of weathering on the attractiveness of 2 g trimedlure plugs to *C. capitata* males. At both locations, 2 g trimedlure plugs weathered up to 6 wk resulted in trap captures similar to those observed for fresh trimedlure baits but when weathered for ≥ 8 wk were always (Florida) or usually (Hawaii) less effective than fresh trimedlure baits. Thus, data from both locations reinforce and support the presently used servicing interval of 6 wk for 2 g trimedlure plugs.

Based on the Florida study, the trimedlure content in 2 g plugs at 6 wk of age was approximately 0.4 g, which was identified as an amount below which *C. capitata* males showed significantly reduced attraction. This threshold is very similar to that reported by Leonhardt et al. (1989) who suggested replacement of 2 g trimedlure plugs when captures dropped below 50% of those observed for fresh trimedlure liquid. This relative decline in capture effectiveness was observed when residual trimedlure content in 2 g plugs was 0.5 g (Leonhardt et al. 1987), 0.4 g (Leonhardt et al. 1989), and 0.35 g (Warthen et al. 1999). In addition, and consistent with earlier studies, our data show that the trimedlure content (and corresponding release rate) of plugs did not decrease uniformly over time but declined most rapidly soon after deployment, then gradually over longer weathering intervals. In the present study, trimedlure content decreased 34% (1–1.32 g per 2.0 g) after the first week of weathering, but to only 13% (1–0.41 g per 0.47 g) between wk 4 and 6. For example, Warthen et al. (1999) reported a similar pattern with trimedlure content in 2 g plugs decreasing about 50% after 1 wk of aging and about 20% between wk 4 and 6. It should be noted that the present study and those of Warthen et al. (1999) were conducted under similar temperature regimes, because the loss rate of trimedlure from polymeric plugs is directly related to ambient temperature (Leonhardt et al. 1989; Domínguez-Ruiz et al. 2008).

Aside from the use of extenders, whose effectiveness in field tests has been inconsistent (Baker et al. 1988; Shelly 2013), a possible solution...
to increasing field longevity of trimedlure polymeric plugs is to use plugs containing greater amounts of trimedlure. Leonhardt et al. (1989) tested 4 g trimedlure plugs and found they performed well up to 12 wk. Here, we assessed 3 g trimedlure plugs because they are only slightly larger than the standard 2 g plugs and fit in the perforated baskets currently used with Jackson traps. The results were encouraging. After 8 wk of weathering, traps baited with 3 g plugs captured (i) as many medflies as traps baited with fresh 2 or 3 g plugs in 2 of 3 replicates, and (ii) significantly more medflies compared with traps baited with 8-wk-old 2 g plugs in 2 of 3 replicates. After 10 wk of weathering, traps baited with 3 g plugs did not perform as well as fresh plugs but had significantly higher fly captures than traps baited with 10-wk-old 2 g plugs in 2 of 3 replicates.

While the use of 3 g trimedlure plugs would potentially allow extension of the trap servicing interval to 8 wk, increasing this interval to 10 wk is even more desirable as this would permit synchronization of lure replacement for male-specific traps targeting a variety of other major pest taxa, i.e., Ceratitis, Bactrocera spp., and Zeugodacus spp. (Diptera: Tephritidae). Shelly et al. (2017) weathered solid lures containing methyl eugenol or cue-lure in Florida and Arizona, and tested their field effectiveness in Hawaii. Wafers containing methyl eugenol were weathered up to 10 wk resulted in similar numbers of captured male Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) as fresh methyl eugenol (presented as a liquid or in wafers). Plugs containing cue-lure weathered for as long as 20 wk generated similar numbers of captures of male Zeugodacus cucurbitae (Coequillet) (Diptera: Tephritidae) as fresh cue-lure (presented as a liquid or in plugs). Thus, increasing the servicing interval of trimedlure-baited traps to 10 wk would synchronize replacement of trimedlure and methyl eugenol in the traps; cue-lure could be changed every 10 wk as well to maintain uniformity in the trap servicing schedule or every other 10 wk to further reduce costs of labor and materials.

Thus, in practical terms, the question becomes: what trimedlure delivery protocol could be adopted that (i) assures an effective field life of 10 wk, and (ii) is usable in Jackson traps (the trap type deployed by large-scale detection programs in the USA) (IPRFSP 2006). One approach involves deployment of plugs containing 4 g (or more) of trimedlure, although lure dispensers might necessitate use of larger baskets than those currently in use. An alternative suggestion is to replace plug dispensers (and associated baskets) entirely with larger wafers capable of holding larger amounts of trimedlure. Such wafers may be capable of holding as much as 5 g of trimedlure (P. Cook, personal communication), and field tests of this dispenser are planned in Hawaii. If effective, costs associated with adopting a new trimedlure dispenser and holder may be offset by cost reductions generated via reduced frequency of trap servicing.

**Table 1.** Measurements of trimedlure content for fresh and 10-wk weathered trimedlure plugs for the 3 trapping periods in Hawaii and their associated release rates. Presumed values refer to the categorization of plugs as containing either 2 or 3 g trimedlure. Actual values represent means (± SE); n = 5. Calculated release rates were based on mean values.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Presumed trimedlure content, fresh (g)</th>
<th>Weathering status</th>
<th>Actual trimedlure content (g)</th>
</tr>
</thead>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Fresh</td>
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<tr>
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<td>3</td>
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<td>Fresh</td>
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<td>10 weeks</td>
<td>0.67 (0.02)</td>
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<td>2</td>
<td>Fresh</td>
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<td>3</td>
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<td>Fresh</td>
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<tr>
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<td>10 weeks</td>
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References Cited


