Weathering of Methyl Eugenol Solid Dispensers: Effects on Residual Amount, Release Rate, and Field Capture of Bactrocera dorsalis Males (Diptera: Tephritidae)

Authors: Shelly, Todd, Kurashima, Rick, Mosser, Lisa, Martin, Rodolfo Mesa, and Bazelet, Corinna

Source: Florida Entomologist, 105(3) : 219-224

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.105.0307

Weathering of methyl eugenol solid dispensers: effects on residual amount, release rate, and field capture of *Bactrocera dorsalis* males (Diptera: Tephritidae)

Todd Shelly[1,], Rick Kurashima[1], Lisa Mosser[2], Rodolfo Mesa Martin[2], and Corinna Bazelet[3]*

Abstract

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a global agricultural pest that attacks many commercially important fruits and vegetables. Many countries maintain trapping programs to detect incursions of this pest, and trapping relies heavily on methyl eugenol, a powerful attractant to *B. dorsalis* males, which typically is applied as a liquid to a cotton wick contained inside Jackson traps. However, this method is time-consuming, incurs high volatility (i.e., loss) of the lure, and entails health risks. Existing data indicate that solid dispensers of methyl eugenol are a viable alternative to the use of liquid lure. Based on fieldwork in a Hawaiian coffee field, the present study shows non-significant differences in captures of wild *B. dorsalis* males over 12-wk intervals between traps baited with a wick containing 6 mL of freshly applied liquid methyl eugenol and traps baited with a polymeric plug or wafer that contained a similar amount of methyl eugenol. The residual content of methyl eugenol also was measured for solid dispensers over the weathering period, but their long-lasting attractancy (≥ 12 wk) precluded identification of the threshold level of the lure below which solid dispensers were ineffective. Implications of these findings for trapping programs are discussed.

Key Words: oriental fruit fly; invasive species; trapping; male lure

Resumen

La mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), es una plaga agrícola mundial que ataca una gran variedad de frutas y verduras de importancia comercial. Muchos países mantienen programas de trampeo para detectar incursiones de esta plaga, y el trampeo depende en gran medida del metil eugenol, un poderoso atrayente para los machos de *B. dorsalis*, que generalmente se aplica como líquido a una mecha de algodón contenida dentro de las trampas Jackson. Sin embargo, este método requiere mucho tiempo, incurre en una alta volatilidad (pérdida) del señuelo y conlleva riesgos para la salud. Los datos existentes indican que los dispensadores sólidos de metil eugenol son una alternativa viable en vez del uso de señuelos líquidos. Basado en el trabajo de campo en cafetal de Hawái, el presente estudio muestra diferencias no significativas en las capturas de machos de *B. dorsalis* silvestres en intervalos de 12 semanas entre trampas cebadas con una mecha que contiene 6 ml de metil eugenol líquido recién aplicado y trampas cebadas con una tapón polimérico u oblea que contenía una cantidad similar de metil eugenol. El contenido residual de metil eugenol también se midió para dispensadores sólidos durante el período de exposición a la intemperie, pero su atractivo duradero (≥ 12 semanas) impidió la identificación del nivel umbral del señuelo por debajo del cual los dispensadores sólidos eran ineficaces. Se discuten las implicaciones de estos hallazgos para los programas de trampeo.

Palabras Claves: mosca oriental de la fruta; especies invasores; captura; señuelo de macho

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a global agricultural pest, attacking many commercially important fruits and vegetables (White & Elson-Harris 1992). Native to tropical Asia, *B. dorsalis* is highly invasive, and resident populations now occur throughout Southeast Asia, China, Taiwan, Philippines, French Polynesia, Hawaii, and many countries in sub-Saharan Africa (Schutze et al. 2012; Leblanc et al. 2013; Vargas et al. 2015a; De Villiers et al. 2016). Incursions (and subsequent eradication) also have been reported from Australia, Japan, and the continental USA (Cantrell et al. 2002; Ohno et al. 2009; Steck et al. 2019). Because of the threat posed, many countries maintain continuous trapping programs to detect incursions and monitor the subsequent spread of the pest. Trapping relies on the plant compound methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate), which is a powerful attractant to *B. dorsalis* males (Vargas et al. 2010a). Traditionally, methyl eugenol (mixed with a small amount of naled killing agent) is applied as a liquid to a cotton wick contained inside Jackson traps (also referred to as Delta traps; FAO/IAEA 2018).

Despite its usefulness in detection and monitoring programs, liquid methyl eugenol has 3 major shortcomings. First, measuring and applying the liquid lure to wicks is an inherently slow procedure that requires considerable handling time. Second, the chemical is highly volatile and consequently attractive for relatively short periods. International guidelines (FAO/IAEA 2018) state that liquid

[1]USDA-APHIS-PPQ, 41-650 Ahiki Street, Waimanalo, Hawaii 96795, USA; E-mail: Todd.E.Shelly@usda.gov (T. E. S.), Rick.S.Kurashima@usda.gov (R. S. K.)
[2]USDA-APHIS-PPQ, 13601 Old Cutler Road, Coral Gables, Florida 33158, USA; E-mail: Lisa.K.Mosser@usda.gov (L. K. M.), Rodolfo.MesaMartin@usda.gov (R. M. M.)
[3]USDA-APHIS-PPQ, 1730 Varsity Drive, Raleigh, North Carolina 27606, USA; E-mail: Corinna.S.Bazelet@usda.gov (C. B.)
*Corresponding author; E-mail: Todd.E.Shelly@usda.gov

methyl eugenol has an effective field longevity of 4 to 8 wk, necessitating frequent replacement. Third, data derived from rodents indicate that methyl eugenol may be carcinogenic, as subjects administered high doses showed increased incidence of liver cancer and mortality (National Toxicology Program 2000). Thus, accidental contact or ingestion of the liquid lure while preparing the traps may pose health risks.

Various studies (Tan et al. 2014) have investigated polymeric methyl eugenol dispensers that would reduce the time required for trap preparation as well as health risks (dispensers are individually packaged and can be placed directly in traps without contact). Additionally, these solid dispensers appear to be equally, or even more, attractive than liquid-baited wicks over long intervals. For example, Vargas et al. (2010b) reported that traps baited with methyl eugenol- containing plastic wafers captured similar numbers of *B. dorsalis* males as traps baited with the lure-bearing wicks in 2 trials lasting 8 and 10 wk, respectively (see also Vargas et al. 2009; Shelly 2010; Leblanc et al. 2011).

Despite their promise, and unlike the situation for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), solid lure dispensers have not been adopted broadly in detection programs for *B. dorsalis*. Plugs containing the male lure trimedlure are now used worldwide to monitor the medfly (FAO/IAEA 2018), but application of liquid methyl eugenol to cotton wicks is used still in large-scale detection programs (e.g., California; Gilbert et al. 2013). We believe this reflects the establishment and evaluation of particular performance parameters in the case of trimedlure plugs and the lack of a similar framework for methyl eugenol solid dispensers. Specifically, following extensive field testing of trimedlure plugs, Leonhardt et al. (1989) proposed the so-called "50% rule" for accepting or rejecting an alternative to the standard use of liquid trimedlure on wicks, i.e., plugs (as an alternative) should be considered a viable replacement to liquid (the standard) if, after weathering for 6 wk, traps baited with plugs captured > 50% as many flies as traps baited with freshly applied liquid. This simple criterion has been adopted by USDA-APHIS as the benchmark for field performance of any proposed alternative male lure. Importantly, concurrent with field assays, Leonhardt et al. (1989) measured the loss of trimedlure from plugs weathered for different intervals, which allowed them to identify the residual amount of lure (0.4 g remaining in 2 g plugs) below which field captures of plug-baited traps did not satisfy the 50% rule. Thus, for medfly, there exist data-supported guidelines, based on both field performance and chemical analysis, for assessment of polymeric plugs as an alternative to the traditional liquid treatment.

As documented in more detail below, existing studies on solid methyl eugenol dispensers generally do not allow either a test of the 50% rule or identification of the minimum level of residual methyl eugenol necessary for adequate attraction and trap capture. The present study was undertaken to address these twin needs. First, trap captures were compared between Jackson traps baited with a wick containing 6 mL of freshly applied liquid methyl eugenol and Jackson traps baited with a polymeric plug or wafer that contained a similar amount of methyl eugenol (though not the same, see below) weathered over a 12-wk period. Second, the residual content of methyl eugenol was measured, and its release rate was estimated for plugs and wafers, thus allowing possible identification of the threshold level below which these solid dispensers were not sufficiently attractive to *B. dorsalis* males. Note that, whereas Shelly et al. (2020) also compared captures in traps containing the same 2 baits used here, that earlier study did not gather any chemical data on weathered solid dispensers.

Materials and Methods

STUDY SITE

Trapping was conducted in a coffee (*Coffea arabica* L.; Rubiaceae) field in central Oahu, Hawaii, USA, that covers approximately 65 ha of a gentle, north-facing slope about 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. Methyl eugenol-bearing wafers (wafers hereafter) were tested from 15 Apr to 8 Jul 2020, and methyl eugenol-bearing plugs (plugs hereafter) were tested from 15 Sep to 9 Dec 2020.

TRAPS AND LURES

Flies were captured using Jackson traps (Scentry Biologicals, Inc., Billings, Montana, USA) (FAO/IAEA 2018), the standard type used in fruit fly surveillance programs in the US (IPRFFSP 2006). Jackson traps are white, 'delta' traps made of thick waxed paper (12.7 L × 9.5 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 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 suspended above the sticky insert from the metal hanger.

Three types of methyl eugenol lures were used in the study: (1) In both sampling periods, control traps were baited with 6 mL (1% naled insecticide) of liquid methyl eugenol (Farma Tech International Corp., North Bend, Washington, USA) placed on a cotton wick (2.5 cm long × 2 cm diam) immediately before placement in the field (i.e., the lure was considered 'fresh'). Lure-bearing wicks were placed in a perforated plastic basket (Scentry Biologicals, Inc., Billings, Montana, USA) suspended inside the trap from the metal hanger. (2) In the initial study period (Apr–Jul 2020), traps with polymeric wafers (7.6 L × 5.1 cm W; 3 mm thick; Farma Tech International Corp., North Bend, Washington, USA) were compared with control traps. A 6 g loading was requested from the supplier, and wafers contained an average of 6.43 g of methyl eugenol (see below). The specific gravity of methyl eugenol is 1.035, thus wafers contained approximately 4% more methyl eugenol than the wicks (6.0 mL × 1.035 = 6.21 g; 6.43 g/6.21 g = 1.04). The wafers were suspended in the traps by inserting a 'twist tie' through a pre-made hole along one of the long sides of the dispenser and wrapping this around the hanger. Because the wafers contained no killing agent, an insecticidal DDVP square (2.54 cm per side, 0.09 g a.i.; Plato Industries, Houston, Texas, USA) was placed in a perforated plastic basket, suspended from the metal hanger adjacent to the wafer. (3) In the second study period (Sep–Dec 2020), traps with polymeric plugs (5 cm L × 2 cm diam; Scentry Biologicals, Inc., Billings, Montana, USA) were compared with control traps. Again, a 6 g loading was requested from the supplier, but plugs contained an average of 7.42 g of methyl eugenol (see below). Thus, plugs contained approximately 19% more methyl eugenol than the wicks (6.0 mL × 1.035 = 6.21g; 7.42 g/6.21 g = 1.19). Plugs were placed in the perforated plastic basket suspended from the metal hanger. As with the wafers, plugs did not contain a toxicant, and the efficacies of 2 types of 'kill-strips' were compared. Half of the plug-baited traps contained the same DDVP square noted above, whereas half had a DDVP 'cube' (so named by the manufacturer; 1.5 L × 1.5 cm W; 5 mm thick; 0.27 g a.i.; Biotrap Australia Pty. Ltd., Ocean Grove, Victoria, Australia). Both the Plato squares and Biotrap cubes were placed in a perforated plastic basket suspended next to the plug.

It is important to note that in USDA-APHIS programs, lures (and insecticides) are held within a perforated plastic basket(s) (Scentry Biologicals, Inc., Billings, Montana, USA) that is affixed easily to the trap's metal hanger. As such, wafers are impractical for large-scale monitoring programs, because their large size precludes easy placement within the body of a Jackson trap. Wafers were included in this study primarily to allow comparisons with previous studies (many of which used wafers, e.g., Vargas et al. 2009; Shelly 2010; Leblanc et al. 2011) as well as the plugs used here. Nonetheless, plugs represent a far more viable alternative to the standard liquid lure, and their performance relative to the control has more bearing on any possible transition from liquid to solid lures.

TRAPPING PROTOCOL

The same trapping protocol was employed in the 2 study periods. Jackson traps were placed on wind-break trees (Norfolk pines, *Araucaria heterophylla* (Salisb.) Franco; Araucariaceae) planted through and along the edge of the coffee field. This protocol was adopted because of our uncertainty regarding the timing and location of harvesting by a large, mobile machine that destroys all traps occurring in harvested rows. Traps located 5 to 10 m from the nearest coffee plants were placed 1.5 to 2.0 m aboveground in shaded locations between 8 AM to 9 AM and collected 24 h later. Traps were placed 25 to 30 m apart, with treatments positioned in repeating sequences along windbreaks. The same trees were used over the entire course of an experiment, but the identity of a treatment on a particular tree was alternated between sampling periods (i.e., traps were rotated to minimize any position effect).

Control traps (liquid methyl eugenol applied to cotton wicks) were prepared fresh just before field deployment at the start of each trapping period, whereas wafers and plugs were deployed after different intervals of weathering. Specifically, traps were baited with wafers that were weathered 0 (i.e., fresh), 6, 8, 10, or 12 wk, or plugs that had been weathered 0, 4, 6, 8, 10, or 12 wk. The 12-wk period was selected, because this represents a doubling over the current servicing interval (i.e., 6 wk) (Gilbert et al. 2013; USDA 2015), which could (if indeed the plugs proved effective over 12 wk) be incorporated easily into programmatic trap servicing schedules. Fifteen control traps and 15 traps with solid dispensers (wafers or plugs) were deployed per sampling period. Collected traps were returned to the laboratory, where sticky inserts were removed, and captured flies were counted. Plugs and DDVP devices were left in the trap bodies, and these were hung 2 to 2.5 m aboveground in a shaded location outside the laboratory for aging (in environmental conditions similar to the coffee field). For the first trapping period, trap processing and wafer aging occurred at the USDA-APHIS laboratory in Kapolei, Hawaii, USA, and daily minimum and maximum temperatures averaged 23.2 °C (range: 20.0 to 24.8 °C) and 31.3 °C (range: 27.2 to 32.2 °C), respectively (National Weather Service, Kalaeloa Airport, Kapolei, Hawaii, USA). For the second trapping period, trap processing and plug aging occurred at the USDA-APHIS in Aiea, Hawaii, USA, and daily minimum and maximum temperatures averaged 23.1 °C (range: 21.4 to 26.1 °C) and 29.9 °C (range: 28.9 to 32.2 °C), respectively (National Weather Service, Daniel K. Inouye International Airport, Honolulu, Hawaii, USA).

CHEMICAL ANALYSIS FOR RESIDUAL CONTENT OF METHYL EUGENOL

In addition to the dispensers used in the field, sets of wafers and plugs were weathered exclusively for analysis of methyl eugenol content. Weathering of these dispensers, which occurred at the aforementioned laboratories, was coincident with the respective trapping periods, and after every sampling interval 3 wafers and 5 plugs were wrapped in aluminum foil, placed in sealed plastic bags, and placed in a freezer for storage. After the final trapping period, all dispensers were placed in insulated boxes with coolant and express mailed to the Agricultural Quarantine Inspection (AQI) chemistry laboratory in Miami, Florida, USA, where the samples were stored at 4 °C until analysis.

Methyl eugenol plugs were removed from manufacturer packing (fresh plugs) 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, St. Louis, Missouri, USA, PN 186562, anhydrous, contains 250 ppm BHT as inhibitor, purity ≥ 99.9%). The glass vials were capped securely and placed on a mechanical shaker for 4 h (low setting). After shaking, the vials remained in place and were opened after 5 min. A 58 µL aliquot of each extraction solution was mixed with 50 µL of 10.00 mg per mL methyl myristate (internal standard) (Sigma Aldrich, PN 70129, analytical standard) and diluted to 1,000 µL with tetrahydrofuran. This solution was then diluted further with tetrahydrofuran (100 µL to 1,000 µL). The resulting solution was added to an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, California, USA) with Flame Ionization Detection sequence that started with a triplicate injection 3-point calibration curve: 0.100, 0.500, and 1.000 mg per mL methyl eugenol (Sigma Aldrich, PN 04607, analytical standard), where each calibration solution contained 0.050 mg per mL methyl myristate as the internal standard.

The Gas Chromatograph with Flame Ionization Detection method consisted of 1 µL splitless injection at 200 °C (purged after 1.00 min). At a constant flow rate of 1 mL per min (helium carrier gas), the compounds were separated through an HP-5 column (Agilent, 19091J-413, 30 m by 320 µm ID, 0.25 µm film thickness). The Gas Chromatograph oven equilibrated at 50 °C for 1 min and ramped to 200 °C at 10 °C per min (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, 45 mL per min helium constant make-up flow. The data analysis section of the instrumental method was set to include calculations that convert concentration of methyl eugenol in the solution injected (mg per mL) to internal standard adjusted mass (g) of methyl eugenol in each plug.

Methyl eugenol wafers were analyzed with the same methodology, except that the extraction volume was 100 mL of tetrahydrofuran (instead of 50 mL) and dilutions of the extracted solutions were adjusted accordingly.

DATA ANALYSIS

Data on field trapping were analyzed using 2-way ANOVA (with wk and lure type as the main effects) as $\log_{10}$ transformed raw data were both normally distributed and homoscedastic. Analysis involving wafers included 2 treatments (liquid methyl eugenol with naled and wafers with DDVP squares), whereas analysis involving plugs included 3 treatments (liquid methyl eugenol and naled, plugs with DDVP squares, and plugs with DDVP cubes). Residual amounts of methyl eugenol in weathered dispensers were presented as averages ± 1 SE. Release rates of methyl eugenol were calculated as 'sequential' or 'cumulative' values. Sequential rates were calculated as the difference between the average residual mass of methyl eugenol in time intervals × and × + 1, respectively, divided by the h elapsed between these time intervals. Cumulative rates were calculated as the difference between the average residual mass of methyl eugenol in wk 0 and time interval ×, respectively, divided by the h elapsed between these time intervals. Note that plugs weathered 12 wk were mishandled and were not judged suitable for analysis.

Results

TRAP CAPTURES

In the comparison between new liquid methyl eugenol and weathered methyl eugenol-bearing wafers, wk had a significant effect on captures of *B. dorsalis* males ($F_{4, 140}$ = 13.9; P < 0.001), but lure/killing agent did not ($F_{1, 140}$ = 1.6; P = 0.20) (Fig. 1). The interaction term was not significant ($F_{4, 140}$ = 0.4; P = 0.84). Temporal variation in trap catch presumably reflected both natural population dynamics as well as possible differences in environmental conditions (e.g., wind speed and direction) among sampling bouts.

In the comparison between liquid methyl eugenol and methyl eugenol-bearing plugs, wk had a significant effect on captures of *B. dorsalis* males ($F_{5, 252}$ = 128.1; P < 0.001), but lure/killing agent did not ($F_{2, 252}$ = 0.5; P = 0.61) (Fig. 2). The interaction term was not significant ($F_{10, 252}$ = 1.0; P = 0.48). As before, weekly variation in captures probably reflected natural variation in population size and differences in wind speed and direction during sampling.

CHEMICAL ANALYSIS

Comparisons between wafers and plugs were confounded by the difference in their initial methyl eugenol content (Fig. 3). On average, fresh wafers contained 6.43 g of methyl eugenol, and fresh plugs held 7.42 g of the compound. Despite this, it is apparent that more methyl eugenol was lost from wafers than plugs. For example, after 6 wk of weathering, approximately 60% of the methyl eugenol was lost from wafers compared to only 26% from plugs. Similarly, after 10 wk of weathering, 86% of the initial amount was lost from wafers compared to only 50% from plugs. As these values indicate, loss of methyl eugenol from wafers was greater during the first 6 wk of deployment than the following 6 wk. On average, the initial and final amounts of methyl eugenol in wafers were 6.43 and 0.4 g, respectively. Of the total amount lost (6.03 g), 64% (3.86/6.03 g) was lost during the first 6 wk and 36% was lost in the following 6 wk. In comparison, loss of methyl eugenol from plugs was more consistent over time. On average, the initial and final amounts of methyl eugenol in plugs were 7.42 and 3.70 g, respectively. Of the total amount lost (3.72 g), 51% (1.89/3.72 g) was lost during the first 6 wk and 49% was lost in the following 4 wk. These trends are clearly reflected in the best fit curves shown in Figure 3, namely a negative exponential for wafers but a linear regression for plugs.

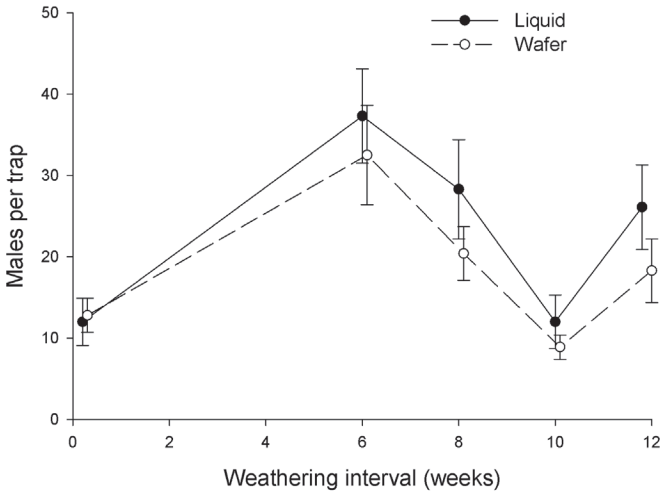

Fig. 1. Captures of *Bactrocera dorsalis* males in Jackson traps baited with liquid methyl eugenol (with 1% naled) on a cotton wick or a wafer containing methyl eugenol (with a Plato insecticidal DDVP square). The liquid treatment was prepared fresh at the start of each trapping period (24 h), whereas the wafers (and DDVP squares) were tested after aging for 0 (fresh), 6, 8, 10, or 12 wk. Symbols represent means (± 1 SE); *N* = 15 traps for each treatment for each sampling period.

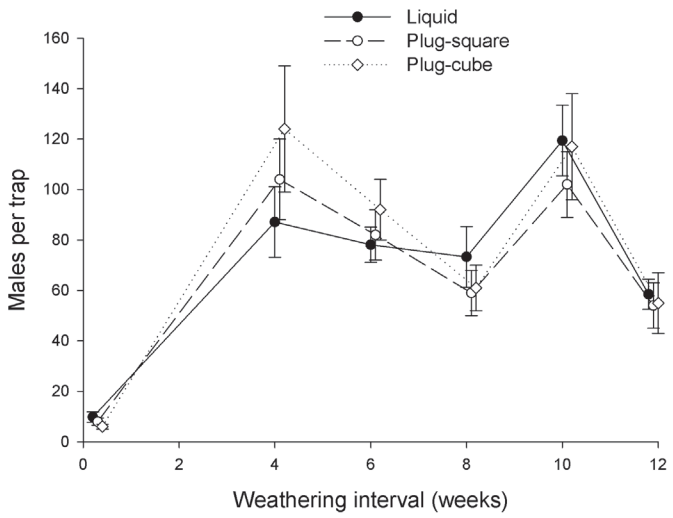

Fig. 2. Captures of *Bactrocera dorsalis* males in Jackson traps baited with liquid methyl eugenol on a cotton wick or a plug containing methyl eugenol with either a Plato insecticidal DDVP square or a Biotrap insecticidal DDVP cube. The liquid treatment was prepared fresh at the start of each trapping period (24 h), whereas the plugs (and DDVP squares or cubes) were tested after aging for 0 (fresh), 4, 6, 8, 10, or 12 wk. Symbols represent means (± 1 SE); *N* = 15 traps for each treatment for each sampling period.

Consistent with the above data, release rates of methyl eugenol were higher for wafers than for plugs (Table 1). Over the first 6 wk of weathering when loss of methyl eugenol was greatest, the cumulative release rate for wafers was approximately twice that for plugs (3.83 mg per h vs. 1.87 mg per h, respectively). After 10 wk, the cumulative release rate for wafers was about 50% greater than for plugs (3.28 mg per h vs. 2.21 mg per h, respectively). Sequential release rates declined over time for wafers but were more consistent over time for plugs, reflecting exponential vs. linear decline of lure content for wafers and plugs, respectively.

Discussion

As noted above, the use of liquid methyl eugenol for detection of *Bactrocera* spp. imposes considerable handling costs and health risks to workers in trapping programs. These problems have long been recognized (Hiramoto et al. 2006), and the USDA-APHIS along with partnering state agencies are now weighing the use of solid methyl eugenol dispensers in domestic detection programs. This would represent a major shift in operations: the fruit fly detection systems in Florida, Texas, and California alone collectively deploy approximately 44,000 methyl eugenol-baited traps (Vargas et al. 2013; A. Fox and G. Gracias, personal communication). In addition, methyl eugenol-baited traps are used in 7 southern states as well as Puerto Rico, the US Virgin Islands, and Hawaii in continuous monitoring programs (IPRFFSP 2006).

The process of switching from liquid methyl eugenol to solid dispensers requires that (1) the solid dispensers (and the associated killing agent) meet the 50% rule adopted by the USDA-APHIS (as described in the Introduction), and (2) the trap/lure dispenser/toxicant

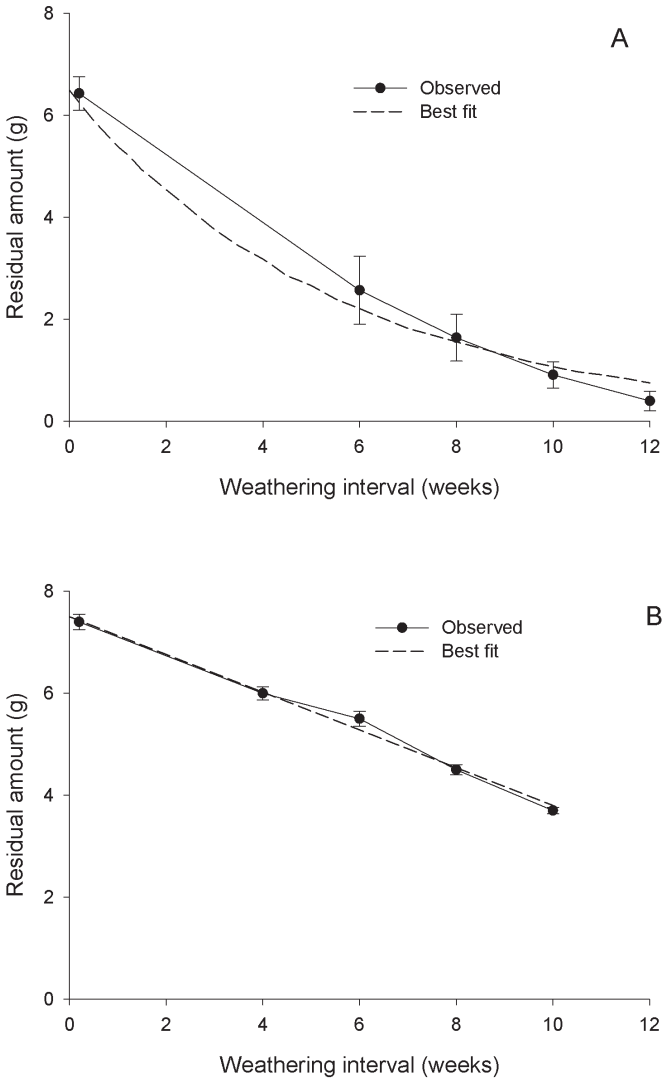

Fig. 3. Residual amount of methyl eugenol in wafers (A) and plugs (B) as a function of weathering interval. Note the initial amounts (wk 0) differed between wafers and plugs (6.43 vs. 7.42 g at wk 0, respectively). Points represent mean values (± SE); *N* = 3 and *N* = 5 per weathering interval for wafers and plugs, respectively. Best fit curves – wafers: $Y = 6.49 * e^{-0.18X}$, $R^2 = 0.91$; plugs: $Y = 7.49 - 0.37X$, $R^2 = 0.98$.

combination tested represents the exact replacement product under consideration. As noted above, a number of studies have found polymeric dispensers to be effective for trapping *B*. *dorsalis* males. From the operational perspective of USDA-APHIS, however, this earlier research has not afforded a clear-cut assessment of the 50% rule. For example, studies often did not include liquid-baited traps as a control for comparison with solid-baited traps (Suckling et al. 2008; Jang 2011) or, if included, liquid-baited wicks were weathered along with the solid dispensers (Vargas et al. 2009; Jang et al. 2013; Shelly et al. 2013), thus precluding comparisons between fresh liquid and weathered solid dispensers. In addition, earlier work typically used trap/lure dispenser/toxicant combinations that differed greatly from the specific combination being appraised as the program-wide replacement for liquid methyl eugenol. Briefly, previous research often used wafers and not plugs (e.g., Vargas et al. 2009; Shelly 2010; Leblanc et al. 2011; Wee & Shelly 2013; Stringer et al. 2019). As noted above, however, wafers are impractical for large-scale trapping programs, because placing them in Jackson traps is a cumbersome and time-consuming process. Also, owing to a difference in surface area, emission rates likely differ between wafers and plugs; consequently data derived from wafer-bearing traps may not allow reliable prediction of the field performance of plug-bearing traps. Moreover, the solid dispensers investigated contained variable quantities of methyl eugenol (e.g., from 2–10 g) (Hiramoto et al. 2006; Jang 2011). Additionally, bucket traps often were used in prior research and not Jackson traps (e.g., Vargas et al. 2010b), which are the standard traps used in large-scale trapping programs in the US (IPRFFSP 2006). Finally, the killing agent used in many earlier studies (i.e., Hercon Vaportape II strip, Hercon Environmental, Emigsville, Pennsylvania, USA) (Hiramoto et al. 2006) contains far greater amounts of DDVP than the devices used in the present study.

Table 1. Release rates of methyl eugenol for wafers and plugs. Release rates were computed based on average residual amounts of methyl eugenol from successive weathering intervals (sequential rates) or from residual amounts observed in wk 0 and week × (cumulative rates in parentheses).

	Release rate (mg per h)	
Weathering interval (wk)	Wafers	Plugs
0	–	–
4	NA	2.16 (2.16)
6	3.83 (3.83)	1.29 (1.87)
8	2.77 (3.56)	3.14 (2.19)
10	2.16 (3.28)	2.30 (2.21)
12	1.53 (2.99)	NA

The present study was conducted within the context of programmatic guidelines of USDA-APHIS and thus had a very specific objective, namely to compare the effectiveness of 6 g methyl eugenol plugs to standard liquid bait via the 50% rule. Field captures of wild *B*. *dorsalis* males showed that, based on the 50% rule, the plugs tested could serve as adequate replacement to liquid-bearing wicks. In fact, Jackson traps containing methyl eugenol-bearing plugs (or wafers) not only met the 50% criterion for captures over all sampling intervals up to 12 wk but captured *B*. *dorsalis* males in numbers that were not statistically significantly different from those captured in Jackson traps baited with fresh liquid lure. This important finding confirms the results of Shelly et al. (2020), and collectively these studies indicate that the 6 g methyl eugenol plugs are an acceptable replacement for the liquid lure.

Because the plugs were effective over the entire weathering period, we were unable to determine the residual level of methyl eugenol at which attractancy declines below the acceptable level. The loss of methyl eugenol from plugs was relatively low, and 50% (3.7/7.42 g) of the initial amount was still present after 10 wk of weathering. This finding agrees with Suckling et al. (2008), who reported only a 25% loss of methyl eugenol from plugs over a 17-wk period. The difference in relative lure loss likely reflects temperature differences: Suckling et al. (2008) weathered plugs in New Zealand at 15 to 20 °C, whereas plugs in Hawaii were weathered at 23 to 30 °C. Although working with wafers, Vargas et al. (2015b) found that solid dispensers weathered for 12 wk and having only 0.5 g residual mass of methyl eugenol were as attractive to *B*. *dorsalis* males as fresh lure. This finding is consistent with our data on wafers, which were attractive when residual methyl eugenol content was only 0.4 g. Collectively, these findings clearly indicate that plug attractancy may extend well beyond 12 wk, and future studies are warranted to examine this possibility.

In conclusion, the present study was undertaken to generate data that would allow concurrent evaluation of field performance and chemical composition of methyl eugenol plugs in a manner consistent with the assessment protocol employed by the USDA-APHIS. The present results show that, if plugs were adopted, the replacement interval

could be increased from 6 to 12 wk. Because of the plugs' long-lasting attractancy, the present study was unable to determine the maximum acceptable replacement interval or the minimum effective residual mass of methyl eugenol plugs. Future work may reveal that effective trapping could be achieved with replacement intervals exceeding 12 wk.

Acknowledgments

We thank the Dole Corporation for allowing fieldwork on their property, and Abbie Fox and Lupita Gracia for supplying trapping information for Florida and Texas, respectively.

References Cited

Cantrell BK, Chadwick B, Cahill A. 2002. Fruit Fly Fighters: Eradication of Papaya Fruit Fly. CSIRO, Collingwood, Australia.

De Villiers M, Hattingh V, Kriticos DJ, Brunel S, Vayssières JF, Sinzogan A, Billah MK, Mohamed SA, Mwatawala M, Abdelgader H, Salah FEE, De Meyer M. 2016. The potential distribution of *Bactrocera dorsalis*: considering phylogeny and irrigation patterns. Bulletin of Entomological Research 106: 19–33.

FAO/IAEA – Food and Agriculture Organization/International Atomic Energy Agency. 2018. Trapping Guidelines for Area-Wide Fruit Fly Programmes, 2nd Edition. Enkerlin WR, Reyes-Flores J [Eds.]. Food and Agriculture Organization, Rome, Italy.

Gilbert AJ, Hoffman KM, Cannon CJ, Cook CH, Chan JK. 2013. Insect Trapping Guide, 13th edition. California Department of Food and Agriculture, Sacramento, California, USA.

Hiramoto MK, Arita-Tsutsumi L, Jang E. 2006. Test of effectiveness of newly formulated plastic matrix with methyl eugenol for monitoring *Bactrocera dorsalis* (Hendel) populations. Proceedings of the Hawaiian Entomological Society 38: 103–110.

IPRFFSP – International Panel for Review of Fruit Fly Surveillance Programs. 2006. Review of the Fruit Fly Surveillance Programs in the United States. USDA/APHIS/PPQ/Fruit Fly Program, Riverdale, Maryland, USA.

Jang EB. 2011. Effectiveness of plastic matrix lures and traps against *Bactrocera dorsalis* and *Bactrocera cucurbitae* in Hawaii. Journal of Applied Entomology 135: 456–466.

Jang EB, Ramsey A, Carvalho LA. 2013. Performance of methyl eugenol + matrix + toxicant combinations under field conditions in Hawaii and California for trapping *Bactrocera dorsalis* (Diptera: Tephritidae). Journal of Economic Entomology 106: 727–734.

Leblanc L, Vargas RI, Putoa R. 2013. From eradication to containment: invasion of French Polynesia by *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and releases of two natural enemies: a 17-year case study. Proceedings of the Hawaiian Entomological Society 45: 31–43.

Leblanc L, Vargas RI, Mackey B, Putoa R, Piñero JC. 2011. Evaluation of cue-lure and methyl eugenol solid lure insecticide dispensers for fruit fly (Diptera: Tephritidae) monitoring and control in Tahiti. Florida Entomologist 94: 510–516.

Leonhardt BA, Cunningham RT, Rice RE, Harte EM, Hendrichs J. 1989. Design, effectiveness, and performance criteria of dispenser formulations of trimedlure, and attractant of the Mediterranean fruit fly (Diptera: Tephritidae). Journal of Economic Entomology 82: 860–867.

National Toxicology Program. 2000. Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-2) in F344/N rats and B6C3F_1 mice (gavage studies). National Institute of Environmental Health Sciences. Research Triangle Park, North Carolina, USA. Technical Report 491: 1–412.

Ohno S, Tamura Y, Haraguchi D, Matsuyama T, Kohama T. 2009. Re-invasions by *Bactrocera dorsalis* complex (Diptera: Tephritidae) occurred after its eradication in Okinawa, Japan, and local differences found in the frequency and temporal patterns of invasions. Applied Entomology and Zoology 44: 643–654.

Schutze MK, Krosch MN, Armstrong KF, Chapman TA, Englezou A, Chomič A, Cameron SL, Hailstones D, Clarke AR. 2012. Population structure of *Bactrocera dorsalis* s.s., *B. papayae*, and *B. philippinensis* (Diptera: Tephritidae) in southeast Asia: evidence for a single species hypothesis using mitochondrial DNA and wing-shape data. BMC Evolutionary Biology 12: 130. doi: 10.1186/1471-2148-12-130

Shelly T, Kurashima R, Fezza T. 2020. Field capture of male oriental fruit flies (Diptera: Tephritidae) in traps baited with solid dispensers containing varying amounts of ethyl eugenol. Florida Entomologist 103: 516–518.

Shelly T, Renshaw J, Dunivin R, Morris T, Giles T, Andress E, Diaz A, War M, Nishimoto J, Kurashima R. 2013. Release-recapture of *Bactrocera* fruit flies (Diptera: Tephritidae): comparing the efficacy of liquid and solid formulations of male lures in Florida, California, and Hawaii. Florida Entomologist 96: 305–317.

Shelly TE. 2010. Capture of *Bactrocera* males (Diptera: Tephritidae) in parapheromone-baited traps: a comparison of liquid versus solid formulations. Proceedings of the Hawaiian Entomological Society 42: 1–8.

Steck GJ, Fox AJ, Carrillo D, Dean D, Roda A, Epsky ND, Smith TR. 2019. Oriental fruit fly eradication in Florida 2015-2016 program implementation, unique aspects, and lessons learned. American Entomologist 65: 108–121.

Stringer LD, Soopaya R, Butler RC, Vargas RI, Souder SK, Jessup AJ, Woods B, Cook PJ, Suckling DM. 2019. Effect of lure combination on fruit fly surveillance sensitivity. Scientific Reports 9: 2653. doi: 10.1038/s41598-018-37487-6

Suckling DM, Jang EB, Holder P, Carvalho L, Stephens AEA. 2008. Evaluation of lure dispensers for fruit fly surveillance in New Zealand. Pest Management Science 64: 848–856.

Tan KH, Nishida R, Jang EB, Shelly TE. 2014. Pheromones, male lures, and trapping of tephritid fruit flies, pp. 15–74 *In* Shelly T, Epsky N, Jang EB, Reyes-Flores J, Vargas R [Eds.], Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies. Springer, Dordrecht, Netherlands.

USDA – United States Department of Agriculture. 2015. National Exotic Fruit Fly Detection Trapping Guidelines. United States Department of Agriculture, Washington, DC, USA.

Vargas RI, Piñero JC, Leblanc L. 2015a. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific region. Insects 6: 297–318.

Vargas RI, Shelly TE, Leblanc L, Piñero JC. 2010a. Recent advances in methyl eugenol and cue-lure technologies for fruit fly detection, monitoring, and control in Hawaii, pp. 575–596 *In* Litwack G [Ed.], Vitamins and Hormones, section: Pheromones, vol. 83. Academic Press, Burlington, Vermont, USA.

Vargas RI, Burns RE, Mau RFL, Stark JD, Cook P, Piñero JC. 2009. Captures in methyl eugenol and cue-lure detection traps with and without insecticides and with a Farma Tech solid lure and insecticide dispenser. Journal of Economic Entomology 102: 552–557.

Vargas RI, Haviland D, Faber B, Kabashima J, Grafton-Cardwell B, Morse JG. 2013. Improving trapping systems for early detection and eradication of fruit flies in California. Citrogragh Summer 2013: 28–34.

Vargas RI, Mau RFL, Stark JD, Piñero JC, Leblanc L, Souder SK. 2010b. Evaluation of methyl eugenol and cue-lure traps with solid lure and insecticide dispensers for fruit fly monitoring and male annihilation in the Hawaii areawide pest management program. Journal of Economic Entomology 103: 409–415.

Vargas RI, Souder SK, Nkomo E, Cook PJ, Mackey B, Stark JD. 2015b. Weathering and chemical degradation of methyl eugenol and raspberry ketone solid dispensers for detection, monitoring, and male annihilation of *Bactrocera dorsalis* and *Bactrocera cucurbitae* (Diptera: Tephritidae) in Hawaii. Journal of Economic Entomology 108: 1612–1623.

Wee SL, Shelly TE. 2013. Capture of *Bactrocera* fruit flies (Diptera: Tephritidae) in traps baited with liquid versus solid formulations of male lures in Malaysia. Journal of Asia-Pacific Entomology 16: 37–42.

White IM, Elson-Harris MM. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. CABI, Wallingford, United Kingdom.