Testing the Temporal Limits of Lures and Toxicants for Trapping Fruit Flies (Diptera: Tephritidae): Additional Weathering Studies of Solid *Bactrocera* and *Zeugodacus* Male Lures and Associated Insecticidal Strips

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Abstract. Detection of pestiferous fruit flies (Diptera: Tephritidae) relies largely on traps baited with male-specific attractants. Surveillance programs in Florida and California use liquid methyl eugenol (ME, attractive to males of *Bactrocera dorsalis* (Hendel)) and liquid cue-lure (CL, attractive to males of *Zeugodacus cucurbitae* (Coquillett)) mixed with the toxicant naled to bait detection traps. However, this practice requires considerable time and may subject personnel to health risks. Recent work indicates that solid male lures deployed with a separate insecticidal (DDVP) strip are as effective as the standard liquid formulations. Specifically, solid ME and CL dispensers and DDVP strips were weathered for 6 or 12 weeks under summer conditions in AZ and FL and subsequently field tested in Hawaii. Results showed that (i) solid ME dispensers weathered for 6 weeks, but not 12 weeks, were as attractive as fresh liquid ME, and (ii) solid CL dispensers and the insecticidal strips were as effective as fresh liquid formulation for at least 12 weeks. The present study expands upon these earlier findings and addresses two specific questions: Could solid ME dispensers be deployed for 8 or 10 weeks without loss of effectiveness? Could solid CL dispensers and insecticidal strips be deployed for intervals even longer than 12 weeks? Adopting the same protocol noted above, the present study indicates that effective field longevities are 10 weeks for solid ME dispensers, at least 20 weeks for solid CL dispensers, and 12 weeks for the DDVP strips. Comparisons are drawn with related studies, and implications for tephritid surveillance programs are discussed.

The subtribe Dacina of fruit flies (Diptera: Tephritidae) contains numerous pest species that attack a wide range of vegetables and fruits (Virgilio et al. 2015). Two of these species, the oriental fruit fly *Bactrocera dorsalis* (Hendel) and the melon fly, *Zeugodacus cucurbitae* (Coquillett) (the new generic classification proposed by Virgilio et al. [2015] and De Meyer et al. [2015] is here adopted), pose severe invasive threats to US agriculture, and several states operate continuous trapping programs to detect incipient infestations. Detection of these species relies on male-specific attractants (termed male lures), namely methyl eugenol (ME) for *B. dorsalis* and cue-lure (CL) for *Z. cucurbitae*, which are applied as liquids (containing the insecticide naled as well) to cotton wicks, which are then placed in traps and replaced at 6-week intervals (IPRFFSP 2006).

In addition to being time-consuming, the application of liquid lures entails health risks arising from inadvertent exposure to both the lures and the toxicant

(National Toxicology Program 2000). Recent studies (Vargas et al. 2012, Jang et al. 2013, Shelly 2013) have shown that solid polymeric ME- or CL-containing wafers or plugs presented with separate insecticidal (DDVP) strips are at least as effective as the standard lure plus toxicant formulations. These results are important, because (i) they suggest a safer and more time effective alternative to use of liquid formulations and (ii) the use of separately presented solid lures and toxicant in traps would avoid a lengthy approval and registration process (J. Crowe, pers. comm.) and be immediately permissible.

To further explore the feasibility of replacing the standard liquid formulations for detection of pest tephritid species, Shelly et al. (2015) assessed the effectiveness of solid male lures and DDVP strips after weathering in two southern locations in the continental US. Solid dispensers containing ME or CL and DDVP strips were aged for 6 or 12 weeks under summer conditions in Florida and Arizona, respectively, then shipped to Oahu, Hawaii, and compared with fresh liquid formulations for trap catch of wild males of *B. dorsalis* and *Z. cucurbitae*. Results for the Florida and Arizona materials were similar and revealed (i) solid ME dispensers (wafers) weathered for 6 weeks were as effective as the fresh standard liquid formulation, but those weathered for 12 weeks were less effective than the fresh standard; (ii) solid CL dispensers (plugs) weathered for 6 or 12 weeks were as effective as the fresh standard liquid formulation; and (iii) the DDVP strips (2.54 cm squares) weathered for 6 or 12 weeks were as effective as fresh naled or fresh insecticidal strips.

The present study was undertaken to expand upon these previous data and address two additional questions. First, although ME wafers lost significant attractancy after 12 weeks, could they be deployed for 8 or 10 weeks without loss of effectiveness? Second, as CL plugs and the DDVP strips were effective after 12 weeks of ageing, could they be deployed for even longer intervals without loss of effectiveness? These questions are of considerable importance to large-scale detection programs that operate large numbers of traps (e.g., approximately 25,000 traps in southern California; Vargas et al. 2013) each of which requires periodic visits and servicing. Lengthening the trap servicing interval without compromising the integrity of monitoring programs could yield large cost savings, an important factor in light of limited funding for such programs.

Materials and Methods

The methods used were essentially identical to those described in Shelly et al. (2015), consequently we provide only a brief overview and refer the reader to that earlier study for details.

Mainland weathering. *Study sites.* Weathering of lures and toxicants was conducted in Nogales, AZ, and Sarasota, FL. Climatological data were obtained from the National Climatic Data Center (NCDC) with measurements taken at the Nogales International Airport and the Sarasota-Bradenton International Airport.

Traps, lures, and toxicants. Materials were weathered in Jackson traps (Better World Mfg., Fresno, CA) in both Arizona and Florida. The solid ME dispensers were rectangular wafers each containing 6 g of ME (Farma Tech International, North Bend, WA), while the solid CL dispensers were plugs each containing 3 g of CL (Scentry Biologicals Inc., Billings, MT). Within a Jackson trap, the ME wafer was suspended directly from the metal hangar supporting the interior apex of the trap, while the CL plug was placed in a perforated basket fastened to the metal hangar. DDVP strips (2.54 cm squares, 0.09 g a.i.; Plato Industries Inc., Houston, TX) were weathered only in traps with CL plugs and were placed in the same basket as the plug.

Weathering procedures. In both Arizona and Florida, two sets of 15 ME wafers each were placed in the field, one set being aged for 8 weeks and the other for 10 weeks. Similarly, in both locations three sets of 12 CL plugs each (and their associated DDVP strips) were placed in the field for ageing of 14, 16, or 20 weeks, respectively. As noted above, no DDVP strips were included with the weathered ME wafers, because earlier results (Shelly et al. 2015) showed that DDVP strips were effective for at least 12 weeks, i.e., an interval longer than the ME weathering periods (i.e., 8 or 10 weeks) examined here. Jackson traps used in the mainland weathering were placed in trees (primarily, but not exclusively, host plants) at a height of 1.5–2.0 m above ground.

The time course for mainland weathering was as follows. In Arizona, weathering of the first set of ME wafers (10-week ageing) commenced on July 1, 2015, with the second set (8-week ageing) started two weeks later. All ME wafers were collected and shipped to Hawaii on September 9, 2015. Weathering of CL plugs (and DDVP strips) started on July 17, 2015 (20-week ageing), with the second (16-week ageing) and third (14-week ageing) sets started four and six weeks later. All CL plugs and DDVP strips were collected and shipped to Hawaii on December 3, 2015. In Florida, weathering of the first set of ME wafers began on July 15, 2015, with the second set (8-week ageing) started two weeks later. All ME wafers were collected and shipped to Hawaii on September 23, 2015. Weathering of CL plugs (and DDVP strips) also started on July 15, 2015 (20-week ageing), with the second (16-week ageing) and third (14-week ageing) sets started four and six weeks later. All CL plugs and DDVP strips were collected and shipped to Hawaii on December 2, 2015.

Field testing in Hawaii. Trapping was conducted at two locations on Oahu, a coffee (*Coffea arabica* L.) field near Haleiwa with high numbers of *B. dorsalis* and Aloun Farm near Kapolei with a large population of *Z. cucurbitae*. Regarding ME wafers, weathering was staggered between Arizona and Florida (as described above), consequently the associated field tests were run sequentially, with ME wafers from Florida tested September 14–15, 2015, and those from Florida tested September 28–29, 2015. Regarding CL plugs and the associated DDVP strips, weathering was coincident in Arizona and Florida. Consequently, CL plugs from both locations were tested simultaneously on December 7–8, 2015, and the DDVP strips were tested simultaneously on December 9–10, 2015. In all tests, Jackson traps were arranged in a grid with different treatments (see below) assigned positions randomly. Neighboring traps were separated by approximately 50 m in tests involving ME lures and 30 m in tests involving CL lures. At Haleiwa, traps were placed on coffee plants approximately 1.0 m above ground, and those at Kapolei were placed 1.5–2.0 m above ground in a citrus grove surrounded by commercial plantings of zucchini (*Cucurbita pepo* L.) and melons (*Cucumis* spp.).

In testing the ME wafers, the following treatments were used: (i) 6 ml fresh liquid ME (containing 1% naled, i.e., the standard formulation) on a cotton wick, (ii) fresh ME wafer with fresh DDVP strip, (iii) 8-week-aged ME wafer with fresh DDVP strip, and (iv) 10-week-aged ME wafer with fresh DDVP strip. Thus, a total of 60 traps (4 treatments x 15 traps/treatment) were deployed for the tests involving ME wafers weathered in Arizona and Florida, respectively. In testing the CL plugs, the following treatments were used: (i) 6 ml fresh liquid CL (containing 5% naled, i.e., the standard formulation) on a cotton wick, (ii) fresh CL plug with fresh DDVP strip, (iii) 14-week-aged CL plug with fresh DDVP strip, (iv) 16-week-aged CL plug with fresh DDVP strip, and (v) 20-week-aged CL plug with fresh DDVP strip. Thus, a total of 120 traps (5 treatments x 12 traps/treatment x 2 locations) were deployed for the single test that included CL plugs weathered in both Arizona and Florida. Thus, in testing both ME wafers and CL plugs, the toxicant was fresh in all traps (the DDVP strips used in Hawaii were the same type and size used on the mainland), while the lures were either fresh (no weathering) or had been weathered 8 or 10 weeks for ME or 14, 16, or 20 weeks for CL. Given the large fly populations at both study sites, traps were collected 1 d after deployment, and numbers of captured males were tallied for each trap.

In testing the DDVP strips associated with the weathered CL plugs, the following treatments were used: (i) 6 ml fresh liquid CL (containing 5% naled) on a cotton wick, (ii) fresh CL plug with fresh DDVP strip, (iii) 6 ml fresh liquid CL with 14-week-aged DDVP strip, (iv) 6 ml fresh liquid CL with 16-week-aged DDVP strip, and (v) 6 ml fresh liquid CL with 20-week-aged DDVP strip. Thus, in all treatments the CL lure was fresh, while the DDVP strips were either fresh (no weathering) or had been weathered 14, 16, or 20 weeks. As above, traps were collected 1 d after deployment, and numbers of captured males were tallied for each trap.

Data analysis. One-way ANOVA (with $\log_{10}$ transformed data) was used to examine variation in trap capture among lure treatments, as transformed data met the parametric assumptions for the Arizona and Florida comparisons, respectively. In contrast, for the weathered DDVP strips, neither raw nor $\log_{10}$ transformed data met the assumptions of normality or equal variances. Consequently, the non-parametric Kruskal-Wallis test was employed followed by the Student-Newman-Keuls multiple comparisons test ($\alpha = 0.05$).

Results

Mainland weathering. Average daily temperatures exceeded 25°C in both Arizona and Florida during the summer months (10 weeks) when ME wafers were weathered (Table 1). Not surprisingly, humidity and rainfall were considerably higher in Florida than Arizona during this period. The much longer weathering interval (20 weeks) used for CL plugs and DDVP strips extended from summer to early winter, and correspondingly average daily temperatures were slightly below summer values in both locations. Again, the Florida site had higher humidity and more rain than the Arizona site.

ME wafers. In the field trial involving ME wafers weathered in Arizona, there was no significant variation detected in the numbers of *B. dorsalis* males captured in the different treatments ($F_{3, 56} = 2.18$, $P = 0.10$; Table 2). Likewise, captures of *B. dorsalis* males did not vary significantly among treatments in the field trial involving ME wafers weathered in Florida ($F_{3,56} = 0.92$, $P = 0.44$; Table 2).

CL plugs. There was no significant variation in captures of *Z. cucurbitae* males among the 2 fresh (liquid, plug) and 6 weathered treatments (14-, 16-, or 20-week weathered plugs from Arizona and Florida, respectively) ($F_{7, 88} = 0.30$, $P = 0.95$; Table 3).

DDVP strips. Significant variation in trap catch was evident among traps containing fresh or weathered toxicants ($H = 22.6$, $df = 7$, $P = 0.002$; Fig. 1). The multiple comparisons test revealed that captures of *Z. cucurbitae* males did not differ significantly between traps containing fresh naled or a fresh DDVP strip but that both types of fresh toxicants resulted in significantly higher captures than weathered DDVP strips from Arizona or Florida.

Table 1. Climatological data for Nogales, AZ, and Sarasota, FL, over the weathering intervals for the ME wafers (July-September, 2015) and the CL plugs and DDVP strips (July-November, 2015). Temperature and relative humidity values are daily averages based on hourly measurements; rainfall represents total amount of precipitation over the weathering intervals.

	Arizona		**Florida**	
	July–Sept	July–Nov	July–Sept	July–Dec
Parameter				
Temperature (°C)				
Minimum	18.3	14.0	23.9	22.2
Maximum	33.3	29.8	32.2	30.9
Average	25.4	21.7	28.3	26.8
Relative humidity (%)	57.5	54.8	80.2	78.8
Rainfall (cm)	29.3	33.6	50.0	62.6

Table 2. Captures of *Bactrocera dorsalis* males in Jackson traps baited with different ME treatments deployed in a coffee field, Oahu, Hawaii. The fresh treatments were prepared in Hawaii at the start of each field test for comparison with the aged lures. The lures weathered in Arizona were tested on September 14–15, 2015, and those from Florida were tested on September 28–29, 2015. Values represent means (± 1 SE); 15 traps were deployed per treatment.

Treatment	**Arizona**	**Florida**
Fresh ME liquid	84.5 (10.3)	60.5 (7.9)
Fresh ME wafer	130.7 (14.4)	74.2 (10.1)
8-week-aged ME wafer	111.8 (19.2)	60.3 (12.5)
10-week-aged ME wafer	90.1 (16.8)	59.3 (9.7)

Table 3. Captures of *Zeugodacus cucurbitae* males in Jackson traps baited with different CL treatments deployed at Aloun Farm, Oahu, Hawaii. The fresh treatments were prepared in Hawaii at the start of the test for comparison with the aged lures. The lures weathered in Arizona and Florida were tested during the same 1 day period (December 7–8, 2015). Values represent means (± 1 SE); 12 traps were deployed per treatment.

Treatment	**Fresh**	**Arizona**	**Florida**
Fresh CL liquid	75.0 (9.0)	-	-
Fresh CL plug	82.7 (11.1)	-	-
14-week-aged CL plug	-	70.9 (8.5)	85.3 (11.9)
16-week-aged CL plug	-	73.1 (9.7)	72.3 (6.6)
20-week-aged CL plug	-	72.4 (7.8)	84.1 (8.3)

No significant differences were detected among any of the weathered treatments.

Discussion

Coupled with the results of Shelly et al. (2015), the current study allows greater resolution regarding the effective field longevity of *Bactrocera* and *Zeugodacus* solid male lures and the associated insecticidal strips. The earlier study reported that ME wafers weathered for 6 weeks, but not 12 weeks, were as attractive as the standard liquid ME formulation. Here, we examined ME wafers aged 8 or 10 weeks and found them to be as effective as ME liquid. Similarly, the earlier study reported that CL plugs weathered for 6 or 12 weeks were as attractive as the standard CL liquid formulation. Here, we tested CL plugs aged for 14, 16, or 20 and found that all were as effective as CL liquid. Finally, the earlier study indicated that the DDVP strips weathered up to 12 weeks were as effective as the naled + male lure mixture used in the standard formulation. Here, we investigated the effectiveness of DDVP strips aged 14, 16, or 20 weeks and found that, for both Arizona and Florida materials, none of the weathered strips were as effective as fresh toxicants.

Although the use of different traps, lure dispensers, and toxicants makes comparisons problematic, the present findings on the longevity of *Bactrocera* and *Zeugodacus* solid male lures are in general agreement with previous studies. For example, Hiramoto et al. (2006) found that bucket traps containing plugs with 2 g of ME (plus a Hercon® Vaportape™ II strip, Hercon Environmental, Emigsville, PA) captured similar numbers of *B. dorsalis* males as bucket traps containing 4 ml liquid ME on a wick (plus a Vaportape™ II strip) over a 2-month period. Similarly, using Jackson traps, Jang et al. (2013) observed that traps containing a cone with 5 g of ME (plus a Vaportape™ II strip) captured similar numbers of *B. dorsalis* males as traps containing 6 ml of ME on a wick (with 5% naled) over an 8-week interval. Fewer data are available on the performance of separate CL dispensers + toxicant, but Jang (2011) suggested extreme longevity of solid CL dispensers. In that study, traps containing plugs with 2 g CL had a relatively constant catch rate of *Z. cucurbitae* males over a period of 11 months, which Jang (2011) interpreted as evidence of stable attractiveness of the CL plug.

Assessing the effectiveness of the insecticidal strips is complicated by the fact that previous studies used different insecticidal devices with different DDVP loading. Vargas et al. (2003), for example, used a 2 g DDVP cube (a.i. 18.6% or 0.37 g DDVP) and reported that it was as effective as naled (mixed with liquid male lure) for 10 weeks against *B. dorsalis* and 5 (one trial) or 20 (a second trial) weeks against *Z. cucurbitae*. In most cases, when lure and toxicant have been presented separately in traps, the insecticide employed is a Hercon® Vaportape™ II strip, which is larger than the DDVP strips used in the present study (2.5 x 10 cm vs. 2.54 x 2.54 cm, respectively) and contains approximately 6 times as much DDVP (0.59 g/strip vs. 0.09 g/strip, respectively; Shelly 2013). Based on the release rate of DDVP, Durkin and Follansbee (2004, cited in Jang [2011]) claimed that Vaportape™ II strips are effective for 25 d, but based on field captures of tephritid fruit flies, Jang (2011) suggested these strips are effective for at least 100 d. In fact, Jang (2011) and Jang et al. (2013) proposed that the release of DDVP from fresh Vaportape™ II strips results in such a high concentration of toxicant in and around the trap that flies are either repelled or killed before entering the trap. The present findings, along with Shelly et al. (2015), show that smaller DDVP strips, which contain less toxicant,

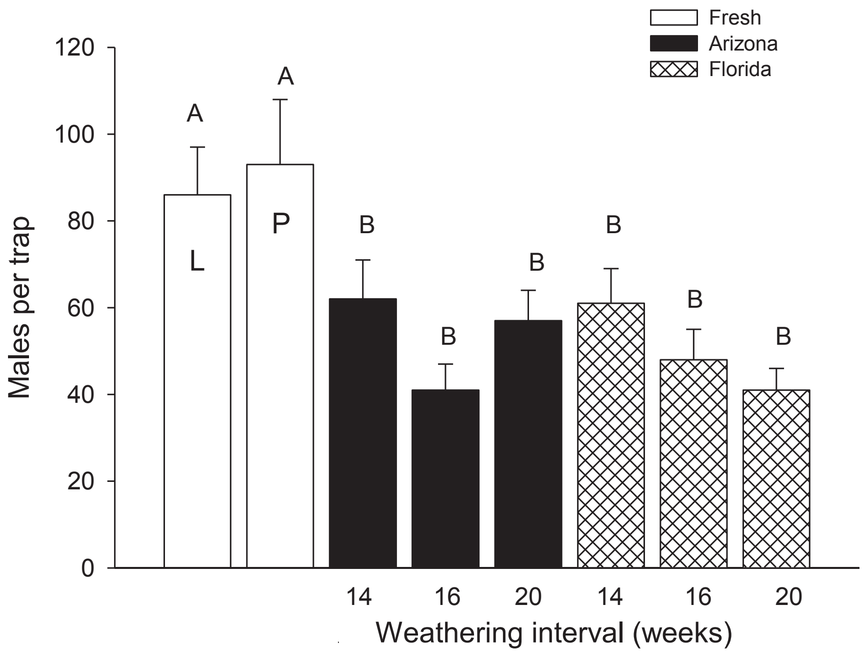

Figure 1. Captures of *Zeugodacus cucurbitae* males in Jackson traps containing toxicants of variable age deployed at Aloun Farm, Oahu, Hawaii. The lures were fresh in all traps and were prepared in Hawaii at the start of the test. Two fresh toxicants were included: naled in liquid CL (bar labelled L) and a DDVP strip with a CL plug (bar labelled P). The DDVP strips weathered in Arizona and Florida were tested during the same 1-day period (December 9–10, 2015). Values represent means (± 1 SE); 12 traps were deployed per treatment. Bars marked by different letters were significantly different (Student-Newman-Keuls multiple comparisons test).

can be used effectively for up to 14 weeks without apparent repulsion or extra-trap mortality as suggested for the Vaportape™ II strips.

In conclusion, the present findings, along with other aforementioned studies, indicate that solid dispensers of *Bactrocera* and *Zeugodacus* male lures with an associated insecticidal strip effectively attracts and kills flies for intervals markedly longer than 6 weeks, which is the accepted servicing schedule for fruit fly detection traps (IFFRP 2006). Thus, with respect to *Bactrocera* and *Zeugodacus* monitoring at least, there exists the potential for lengthening the field "life" of detection traps. Of course, large-scale detection programs in the USA also monitor invasive tephritid species in the genera *Anastrepha*, *Ceratitis*, and *Dacus* using food-baited traps and in the case of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), with the male-specific attractant trimedlure as well (Jang and Light 1996). Whether trapping programs can adjust their servicing schedule of these other traps to take advantage of the long-lived solid male lures (and the associated reduction in supply and labor costs) is an operational issue that may or may not be feasible. The message here is that—from the perspective of *Bactrocera* and *Zeugodacus* monitoring—longer servicing intervals would not compromise detection sensitivity.

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