

# Use of Alpha-Ionol + Cade Oil for Detection and Monitoring of *Bactrocera latifrons* (Diptera: Tephritidae) Populations

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**ABSTRACT:** *Bactrocera latifrons* (Hendel) is a tephritid fruit fly that primarily infests solanaceous fruits. Although primarily of Asian distribution, it has invaded Hawaii and, more recently, the continent of Africa (Tanzania and Kenya). Male *B. latifrons* uniquely respond to alpha-ionol + cade oil, rather than to either methyl eugenol or cue lure, to which males of the majority of other Dacine fruit flies respond. Here we present research results detailing the age of male *B. latifrons* response to alpha-ionol + cade oil, the persistence of wick attractiveness, and the effectiveness of alpha-ionol + cade oil in detecting *B. latifrons* populations. Based on wind tunnel studies with wild flies, male response steadily increased from 5% at age 2 to 45% at age 28, with male response exceeding 50% of the peak response by Day 7 and exceeding 75% and 90% by days 14 and 21, respectively. The attractiveness of wicks treated with 2.0 ml alpha-ionol and 1.0 ml cade oil (on separate wicks) declined over time, with wick response reduced to about 50% of the fresh catch after 6 ½ weeks. Based on concurrent alpha-ionol + cade oil – based trapping and collections of turkeyberry, *Solanum torvum* (Solanaceae), fruits, the presence of *B. latifrons* was detected at the time of fruit collection, 75.5 % of the time.

Key Words: male lure, *Solanum torvum*

## INTRODUCTION

*Bactrocera latifrons* (Hendel) is a tephritid fruit fly of primarily Asian distribution (e.g., Pakistan, India, Sri Lanka, China, Thailand, Laos, Malaysia, and Taiwan) (White and Elson-Harris 1992). It is, also, the most recent alien tephritid fruit fly species of economic importance to become established in Hawaii, being first detected in 1983 (Vargas and Nishida 1985a). Although, at present, little economic damage has been attributed to this species in Hawaii, it has the potential to impact production of solanaceous crops such as peppers (*Capsicum annuum* L. and *C. frutescens* L.), eggplant (*Solanum melongena* L.) and tomatoes (*Lycopersicon esculentum* Mill.) and some cucurbitaceous crops (Liquido et al. 1994). Although males of the majority of Dacine fruit flies respond to either methyl eugenol or cue lure (Metcalf 1990; Cunningham 1989; Drew and Hooper 1981; Drew 1974), *B. latifrons* is one species which shows little to no response to these lures but was

found to respond to alpha-ionol, a compound used in the perfume and flavor industry and is included on the 'Flavor and Extract Manufacturers' (FEMA) list of compounds (FEMA 3624) that are "generally regarded as safe" (GRAS) (Flath et al. 1994, Heath 1981). It was later found that the response to alpha-ionol was synergistically enhanced by the addition of cade oil, a commercially available essential oil produced by destructive distillation of juniper, *Juniperus oxycedrus* L., twigs (McQuate et al. 2004; McQuate and Peck 2001; Liquido et al. 2000). Several important aspects of male lures, however, have not been researched for this male attractant. Here we present research results on (A) The age of male response to alpha-ionol + cade oil; (B) Field persistence over time of treated wicks; and (C) Effectiveness of alpha-ionol + cade oil in detecting *B. latifrons* populations and relationship of trap captures with fruit infestation.

## MATERIALS AND METHODS

**A. Age of Response.** Mature green and ripe (yellow) turkeyberry (*Solanum torvum* Sw. [Solanaceae]) fruits were collected from the vicinity of Haiku, Maui and brought to Hilo, HI,

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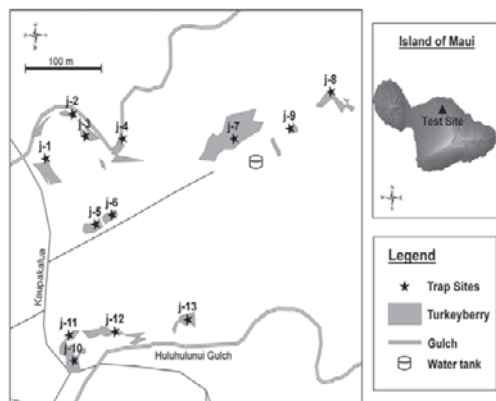
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where they were counted and weighed and held in closed containers with a screened opening to permit aeration and with sand on the bottom to serve as a pupation medium. The sand was sieved weekly for 4 weeks to recover any pupae emerging from the collected fruits. Recovered pupae were transferred to small, screened, plastic cups and held at an average temperature ( $\pm$  SEM) of  $22.7 (\pm 0.1) ^\circ\text{C}$ , an average %RH ( $\pm$  SEM) of  $56.9 \pm 0.4$ , and a photoperiod of 12:12 (L:D) h until adult emergence. Adults emerging on a given day were transferred to holding buckets held under the same temperature, %RH and photoperiod as the pupae. Adults were fed water and a diet of three parts sucrose, one part protein yeast hydrolysate (Enzymatic, United States Biochemical Corporation, Cleveland, OH), and 0.5 part torula yeast (Lake States Division, Rhineland Paper Co., Rhineland, WI). Each of 14 age groups (ages 2 through 28 days, in 2-day intervals) were tested for lure response. For each age group, twenty male flies were released between 1:00 and 1:30 p.m. at the downwind end of a 2.8 m long x 0.9 m x 0.9 m rectangular glass flight tunnel, from a platform situated at mid-height of the tunnel (40.5 cm). Two Jackson traps, each holding sticky inserts, were suspended behind (upwind of) two Hawaiian chili pepper (*Capsicum frutescens* L.) plants (from which flowers had been removed so that there would be no possibility of attraction to flowers or any formed fruits) placed, side by side, at the upwind end of the tunnel. Each trap held a cotton wick baited with either 0.15 ml water (control trap) or 0.1 ml alpha-ionol ("latilure:" 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-ol, obtained from Bedoukian Research, Inc., Danbury, CT) at one end of the wick and 0.05 ml cade oil (obtained from Penta Manufacturing, West Caldwell, NJ) at the other end of the wick (treatment trap). A 12:12 (L:D) h photoperiod was maintained. Inserts were retrieved

after 24 hours. Total response was calculated as the difference between flies recovered in the treatment and control traps. Each fly age tested was replicated a total of 4 times, with relative position of the treatment and control traps reversed for half of the replicates.

**B. Wick Persistence.** Every 2 weeks, 8 Jackson traps, holding wicks treated with 2.0 ml alpha-ionol and 1.0 ml cade oil (separate wicks), were set out in a macadamia nut (*Macadamia integrifolia* Maiden and Betche; a nonhost) orchard, one trap per tree. At initial placement all traps had blank (nonsticky) inserts to maintain the trap in an open condition. Traps from each week's deployment remained in the field until the time of assessment (Week 14). This gave 8 replicates for each aging time. At Week 14, the blank inserts were replaced with sticky inserts and traps were set in a randomized complete block design. Traps were placed in every tree (one trap per tree) down a row with replicate blocks in adjacent rows. Sterile adult *B. latifrons* for use in the assessment of lure persistence were obtained as pupae from a laboratory colony at the USDA-ARS U.S. Pacific Basin Agricultural Research Center in Honolulu, HI. We used sterile flies so as to not add fertile flies which could infest solanaceous crops in the environs of the release site. Emerged adults were kept in an insectary at  $24\text{--}27 ^\circ\text{C}$ , 65–70% RH, and a photoperiod of 12:12 (L:D) h and fed as described above for the Age of Response study. About 20,000 two week old sterile *B. latifrons* adults were then uniformly released throughout the trapping grid in the macadamia nut orchard. Uniformity of the release was provided by partially opening each holding container and tapping on the container over the course of the row of traps so that a steady release rate was achieved. Separate holding containers were similarly used for successive rows. Inserts were retrieved 24 h after the fly release. This trial was repeated for a total of three replications.

**C. Effectiveness of Detection.** Wild adult *B. latifrons* populations were monitored at 13 separate turkeyberry patches in the vicinity of Huluhulunui Gulch on the island of Maui, Hawaii, USA (see Figure 1), through the use of Jackson traps baited with 2.0 ml alpha-ionol and 1.0 ml rectified cade oil. The traps were deployed on separate 3.8 cm long x 1.0 cm diameter cotton wicks each enclosed in separate plastic baskets hung from the middle of the trap hanger. All traps were placed in separate turkeyberry patches. Patch size averaged about 457 m<sup>2</sup>, ranging from about 93 to 2194 m<sup>2</sup>. Four traps were placed in turkeyberry patches in Huluhulunui Gulch, with 4 additional traps set in turkeyberry patches within a smaller, adjacent gulch, and 5 additional traps set in turkeyberry patches along a ridge between the two gulches. Twelve of the 13 traps were deployed on 14 Jan., 2003. The 13<sup>th</sup> trap was deployed on 11 Mar., 2003. Traps were serviced (i.e., captured flies counted and sticky cards replaced) every two weeks from the date of deployment until trapping was terminated on 7 September, 2004, about 1.5 years later. All wicks were concurrently replaced with freshly charged wicks every 8 weeks. At each trap service, both mature green and ripe (yellow) turkeyberry fruits, as available, were collected from each of the 13 turkeyberry patches.



**Figure 1.** Map showing location of traps and turkeyberry patches where tests for detectability of *B. latifrons* populations were conducted.

Fruits were counted and weighed and placed in screened plastic containers containing sand as a pupation medium. The contained sand was screened weekly for four weeks to recover any tephritid fruit fly pupae.

**Statistical Analysis.** Significance of differences, by age, in percentage response to alpha-ionol + cade oil was tested by ANOVA, following arcsine transformation of the percentage data (Sokal and Rohlf 1981), with Tukey HSD used for separation of means (SAS Institute Inc. 2002). The increased male lure response with age was fitted to a four parameter sigmoidal curve of the form:

$$y = y_0 + \frac{a}{1 + e^{-\left(\frac{age - x_0}{b}\right)}}$$

where  $y$  is the % of males responding to alpha-ionol + cade oil, and  $a$ ,  $b$ ,  $x_0$ , and  $y_0$  are all constants. The loss of attractiveness of alpha-ionol + cade oil wicks over time was fitted to a decay curve. The significance of the fit of both generated response curves were tested by ANOVA (SPSS 2002). For the test of effectiveness of detection, the percentage of times where trap catch occurred when (or 2, 4 or 6 weeks after) pupae were recovered from fruit collections was calculated for each of the thirteen study sites, and the percentage detection was averaged across all sites.

## RESULTS

**(A) Age of response.** The percentage of males caught versus fly age, together with the fitted sigmoidal curve, are presented in Figure 2. Because of high variability in response at most ages, only differences between age 2 and ages 18, 24, 26, and 28 are significantly different. However, the trend for increased response over time is apparent, with the net male catch increasing steadily from 5% at age 2 to 45% at age 28, and the sigmoidal curve generated to re-

**Table 1.** Relationship of catch of *B. latifrons* males at traps baited with alpha-ionol + cade oil to recovery of *B. latifrons* pupae from mature green (MG) and ripe (R) turkeyberry fruits collected at 13 sites on Maui, HI (with a maximum of 40 collections per site).

Description	Average	SEM
1. Average total number of attempted fruit collections per site	39.8	0.2
2. Average number of fruit collections per site without fruit	2.3	0.4
3. Average number of fruit collections per site with recovery of fruit but no pupae	5.7	1.4
4. Average number of fruit collections per site with recovery of both fruit and pupae	31.8	1.7
5. Number of collections with fruit, pupae & trap catch at Week 0	24.5	2.2
6. Number of collections with fruit, pupae & trap catch at Week 0 &/or Week 2	27.6	2.2
7. Number of collections with fruit, pupae & trap catch at Week 0 &/or Week 2 &/or Week 4	29.1	2.2
8. Number of collections with fruit, pupae & trap catch at Week 0 &/or Week 2 &/or Week 4 &/or Week 6	29.8	2.1
9. % of collections with recovery of fruit	94.2	1.1
10. % of collections with recovery of both fruit & pupae	84.6	3.9
11. % of collections with recovery of both fruit & pupae & trap catch at Week 0	75.5	3.8
12. % of collections with recovery of fruit & pupae & trap catch at Week 0 &/or Week 2	85.3	3.2
13. % of collections with recovery of fruit & pupae & trap catch at Week 0 &/or Week 2 &/or Week 4	90.0	2.9
14. % of collections with recovery of fruit & pupae & trap catch at Week 0 &/or Week 2 &/or Week 4 &/or Week 6	92.6	2.7
15. Average number of MG fruits per site from collections with pupal recovery	113.8	8.8
16. Average weight (g) of MG fruits per site from collections with pupal recovery	213.4	17.9
17. Average number of <i>B. latifrons</i> per site from MG fruits	14.5	1.4
18. Average number of <i>B. latifrons</i> per site per 100 g MG fruits	6.8	0.5
19. Average number of R fruits per site from collections with pupal recovery	188.7	17.0
20. Average weight (g) of R fruits per site from collections with pupal recovery	356.5	29.0
21. Average number of <i>B. latifrons</i> per site from R fruits	34.5	3.8
22. Average number of <i>B. latifrons</i> per site per 100 g R fruits	9.5	0.6
23. Turkeyberry patch size (m <sup>2</sup> )	457	150
24. Average trap catch on weeks with pupae	12.1	2.7

late the increase in age to the male lure response was significant ( $F = 9.79$ ,  $df = 3, 13$ ;  $P = 0.0025$ ), with an r-square of 0.75. Percentage trap catch in the untreated control trap, on the other hand, averaged only  $5.2 (\pm 0.7)$  % across all ages tested with a tendency to decrease with increasing age. Comparing response data from the fitted curve to the peak response observed at Day 28, male response exceeded 50% of the peak response by Day 7 and exceeded 75% and 90% by days 14 and 21, respectively.

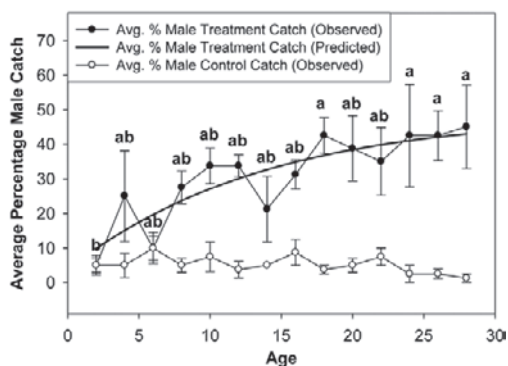
**(B) Wick Persistence.** The decline in trap catch over time, together with a representative decay curve, are presented in Figure 3. The decay curve generated to represent the loss in lure effectiveness over time was significant ( $F = 123.85$ ,  $df = 1, 6$ ;  $P < 0.0001$ ), with an r-square of 0.95. The attractancy of the treated wicks declined to 50% of the fresh catch after 6 ½ weeks.

**(C) Effectiveness of Detection.** Results pertaining to *B. latifrons* population detection are presented in Table 1. Mature green and/or ripe turkeyberry fruits were recovered 94.2 ( $\pm 1.1$ ) % of collection times. Of those collections where turkeyberry fruits were recovered, 84.6 ( $\pm 3.9$ ) % included fruits infested by *B. latifrons*. Of those collections including fruits infested by *B. latifrons*, the presence of *B. latifrons* was detected at the time of fruit collection, with traps baited with alpha-ionol + cade oil, 75.5 ( $\pm 3.8$ ) % of the time. Percentage of times the population was detected increased to 85.3 ( $\pm 3.2$ ) %, 90.0 ( $\pm 2.9$ ) %, and 92.6 ( $\pm 2.7$ ) of the time when trap catch 2 weeks, 4 weeks, and 6 weeks, respectively, after the fruit collection were also considered.

## DISCUSSION

The response of *B. latifrons* to alpha-ionol + cade oil seems to develop at a younger age than does the comparable response of *B. dor-*

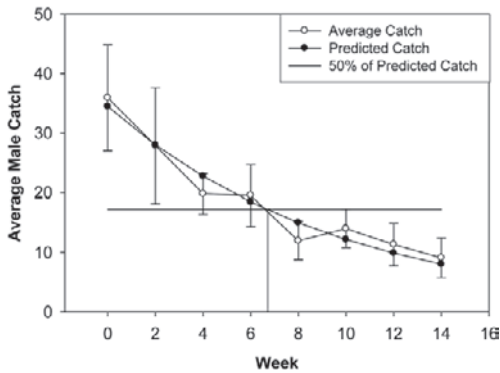
*salis* to methyl eugenol and *B. cucurbitae* to cuelure. Based on data generated in outdoor screened cages, wild oriental fruit fly (Wong et al. 1989) and wild melon fly (Wong et al. 1991) did not reach 50% of their peak response until over 12 days old and over 13 days old, respectively, whereas we found over 50% of the peak response for *B. latifrons* by 7 days old (see Figure 2). The respective responses, however, became more similar for the higher percentages of peak response, with 75% of peak response reached by 17 days for both *B. dorsalis* and *B. cucurbitae* (compared to 14 days for *B. latifrons*; see Figure 2) and over 90% of peak response reached by 21 days by *B. dorsalis* and by 20 days by *B. cucurbitae* (compared to 21 days for *B. latifrons*; see Figure 2).



**Figure 2.** Average percentage male treatment response (- control response) ( $\pm$  SEM) by age, to Jackson traps holding a cotton wick baited with 0.1 ml alpha-ionol and 0.05 ml cade oil, with sigmoidal regression line of the observed treatment response ( $r^2 = 0.75$ ). Points with the same letter are not significantly different by Tukey HSD. Also shown is the average percentage male control response ( $\pm$  SEM) to Jackson traps holding a cotton wick baited with 0.15 ml water.

The 2.0 ml alpha-ionol + 1.0 ml cade oil wick loading used in the studies reported here provides comparable catch with aging to the performance criteria proposed for trimed-lure dispensers to be used in USDA detection programs for Mediterranean fruit fly. Those performance criteria called for capture, after 6 weeks of aging in Hilo, Hawaii, U.S.A., of at

least one-half of the number of flies caught by a standard cotton wick freshly treated with 2.0 g trimedlure (Leonhardt et al. 1989). The average catch of male *B. latifrons* in the present study, after 6 weeks of aging, exceeded 50% of the catch at freshly treated wicks (see Figure 3).



**Figure 3.** Average decline in catch of male *B. latifrons* in Jackson traps baited with 2.0 ml alpha-ionol + 1.0 ml cade oil wicks. Also shown is predicted decay curve ( $r^2 = 0.95$ ) and "T" indicating the point where aged catch is 50% of the fresh catch.

Based on laboratory data (26.6° C, 60% RH) of Vargas and Nishida (1985b), mean duration of egg, larval, and pupal stages for *B. latifrons* are estimated to be 2.3, 8.5 and 10.2 days, respectively, for a total egg-larval-pupal development time of 21 days. With 75% - 90% of adult flies responding to alpha-ionol + cade oil by 14 - 21 days of age (see "Age of Response" results, above), one would expect response of adults developing from eggs or larvae in collected fruits within 6 weeks of fruit collection. The development times recorded in the laboratory also seem consistent with field results of *B. latifrons* in turkeyberry patches as reported by McQuate et al. (2007).

Although the strength of attraction of male *B. latifrons* to alpha-ionol + cade oil is less than that of *B. dorsalis* to methyl eugenol (McQuate and Peck 2001), alpha-ionol + cade oil provides a good detection

tool for *B. latifrons* populations, provided that traps baited with this attractant are deployed in patches of fruit hosts of *B. latifrons*. Although comparisons of attractiveness with all potential protein baits have not been made, alpha-ionol + cade oil has been shown to be more attractive than a protein bait solution based on Provista 621 (an autolyzed yeast extract previously available from Integrated Ingredients, Bartlesville, OK) (McQuate and Peck 2001), a protein bait to which oriental fruit fly has shown good response (McQuate et al. 1999).

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