

# Enhancement of Attraction of Alpha-Ionol to Male *Bactrocera latifrons* (Diptera: Tephritidae) by Addition of a Synergist, Cade Oil

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**ABSTRACT** Male lures are known for many tephritid fruit fly species and are often preferred over food bait based traps for detection trapping because of their high specificity and ability to attract flies over a wide area. Alpha-ionol has been identified as a male lure for the tephritid fruit fly *Bactrocera latifrons* (Hendel). The attraction of this compound to male *B. latifrons* individuals, however, is not as strong as is the attraction of other tephritid fruit fly species to their respective male lures. Cade oil, an essential oil produced by destructive distillation of juniper (*Juniperus oxycedrus* L.) twigs, synergizes the attraction of  $\alpha$ -ionol to male *B. latifrons*. Catches of male *B. latifrons* at traps baited with a mixture of  $\alpha$ -ionol and cade oil were more than three times greater than at traps baited with  $\alpha$ -ionol alone. Substitution of  $\alpha$ -ionol + cade oil for  $\alpha$ -ionol alone in detection programs could considerably improve the chance of detecting invading or incipient populations of *B. latifrons*. However, detection programs should not rely solely on this lure but also make use of protein baited traps as well as fruit collections. Further work with fractions of cade oil may help to identify the active ingredient(s), which could help to further improve this male lure for *B. latifrons*.

**KEY WORDS** *Bactrocera latifrons*, parapheromone, cade oil, alpha-ionol

*Bactrocera latifrons* (Hendel) is a tephritid fruit fly native to south and Southeast Asia (White and Elson-Harris 1992). It was introduced to Hawaii in approximately 1983 (Vargas and Nishida 1985). It primarily infests fruits of solanaceous plants, but has also been found to infest fruits of some species of cucurbitaceous plants (White and Elson-Harris 1992, Liquido et al. 1994). Detection, monitoring, and suppression of tephritid fruit fly populations are typically based on food baits, attractive to both sexes, or male parapheromone lures. For detection purposes, male lures are often preferred because of their high specificity and ability to attract flies over a wide area (Flath et al. 1994). Male lures, however, are not available for all tephritid species and the strength of known male lures varies considerably (White and Elson-Harris 1992). The search for a male lure for *B. latifrons* involved bioassays of compounds with a structure similar to other established male tephritid fruit fly lures as well as essential oils and commercial, synthetic aroma formulations (Flath et al. 1994). This screening program led to the discovery of  $\alpha$ -ionol, now commonly referred to as latilure (McGovern et al. 1989). *Bactrocera latifrons* males are not as strongly attracted to this compound, however, as many other tephritid fruit fly species are attracted to their respective male lures, such as the

attraction of *B. dorsalis* to methyl eugenol or the attraction of *B. cucurbitae* to cue-lure. Among the essential oils tested with *B. latifrons*, cade oil, a commercially available essential oil produced by destructive distillation of juniper (*Juniperus oxycedrus* L.) twigs, was found to show some attraction to *B. latifrons*. Subsequent research showed that cade oil synergized the attraction of  $\alpha$ -ionol to male *B. latifrons* and this synergistic attraction has since been patented (Liquido et al. 2000). In this article we present results of field studies using both sterile laboratory-reared flies and wild flies that show enhanced attraction of male *B. latifrons* to  $\alpha$ -ionol through the addition of cade oil as a synergist.

## Materials and Methods

**Chemicals.** The chemical  $\alpha$ -ionol, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-ol was obtained from Bedoukian Research (Danbury, CT). Rectified cade oil was obtained from Penta (West Caldwell, NJ).

**Insects.** Sterile *B. latifrons* pupae used to provide adult flies for field tests were obtained from a laboratory colony at the USDA-ARS Tropical Fruit, Vegetable and Ornamental Crop Research Laboratory (now part of the U.S. Pacific Basin Agricultural Research Center) in Honolulu, HI. Fruit flies used in our tests were kept in an insectary at 24–27°C, 65–70% RH, and a photoperiod of 12:12 (L:D) h. Adults were fed water and a diet of six parts sucrose, two parts protein yeast hydrolysate (Enzymatic, United States Bio-

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**Table 1.** Relative concentrations and wick loadings for field tests (2A and 2B) of  $\alpha$ -ionol and cade oil blends with sterile *B. latifrons*

Treatment no.	$\alpha$ -ionol:cade oil	Volume, ml		
		$\alpha$ -ionol	Cade oil	Dibrom
1	0:1	0.0	0.6	0.1
2	1:0	0.6	0.0	0.1
3	12:1	0.6	0.05	0.1
4	6:1	0.6	0.1	0.1
5	3:1	0.6	0.2	0.1
6	1:1	0.6	0.6	0.1
7	1:3	0.6	1.8	0.1

All treatments listed were included in test 2A, whereas all but treatment number 3 were included in test 2B.

chemical, Cleveland, OH), and one part torula yeast (Lake States Division, Rhinelander Paper, Rhinelander, WI). Ten- to 18-d-old, sexually mature flies were used in the field studies.

**Field Tests with Sterile Laboratory Flies.** *Study 1. Initial Field Trial.* Wires were inserted through the top of yellow-bottom plastic dome traps (Biosys, Palo Alto, CA) and used to suspend cotton wicks (2.5 cm long by 1.0 cm diameter) above a solution of 100 ml of distilled water with two drops of Tween 20 (ICN Biomedicals, Aurora, OH) with or without the addition of 2–5.0 g torula yeast pellets (ERA International, Freeport, NY). The torula yeast pellets were composed of four parts torula yeast and five parts borax decahydrate by weight. Wicks were treated with either 0.5 ml  $\alpha$ -ionol, 0.5 ml cade oil, or with 0.5 ml  $\alpha$ -ionol and 0.5 ml cade oil at opposite ends of the wick. Five traps of each treatment were set out in a random complete block design in a macadamia nut orchard (a nonhost environment). Traps were placed in every tree down a row (4.6-m spacing) with replicate blocks in adjacent rows (9.2-m spacing). Approximately 500 *B. latifrons* adults were released from holding containers below each trap in the grid and allowed to fly or walk out. Traps were retrieved 24 h after the fly release. This test was conducted on 3–4 and 21–22 November 1994, and on 9–10 January 1995, for a total of three repetitions.

*Study 2A. Test of  $\alpha$ -ionol and Cade Oil Ratio.* On 23 September 1996, cotton wicks (3.8 cm long by 1.0 cm diameter) were treated with mixtures prepared with different proportions of  $\alpha$ -ionol and cade oil, with 0.1 ml dibrom (Valent, Walnut Creek, CA) added to each wick as a knockdown toxicant. The relative concentrations of  $\alpha$ -ionol and cade oil, and the quantities added to individual wicks, are presented in Table 1. Treated wicks were attached with metal clips to Jackson traps (Biosys). Eight traps per treatment were set out in a macadamia nut orchard in a randomized complete block design. Traps were set out in every tree down a row (4.6 m apart) in two adjacent rows (9.2 m apart). Approximately 5,000, 10-d-old, sterile male *B. latifrons* were released uniformly throughout the trapping grid. Two days after the initial fly release, sticky inserts from the Jackson traps were retrieved, replaced with new inserts, and flies were again released. Sub-

sequent insert replacements followed by fly release were done after 2 d, then after 5 d, then weekly thereafter to 26 December 1996. The test was terminated with final insert collection on 2 January 1997. To minimize numbers of analysis of variance (ANOVA) performed on nonindependent data, trap catches were combined for collections 1–4 (23 September to 9 October 1996; period 1), 5–8 (9 October to 6 November; period 2), 9–12 (6 November to 4 December; period 3), and 13–16 (4 December to 2 January 1997; period 4). This permitted determination of relative effectiveness of the different blends at quarterly periods throughout the weathering process.

*Study 2B. Test of  $\alpha$ -ionol and Cade Oil Ratio.* On 14 November 1996, a repeat of study 2A was begun to provide additional field results of the relative effectiveness of the different blends. This study differed from study 2A only in the schedule of release dates and the lack of a 12:1 ( $\alpha$ -ionol:cade oil) treatment. Four days after the initial fly release, sticky inserts from the Jackson traps were retrieved, replaced with new inserts, and flies were again released. Subsequent insert replacements followed by fly release were done weekly thereafter to 3 March 1997. The test was terminated with final insert collection on 10 March 1997. As described in study 2A above, trap catches were combined for collections 1–4 (14 November to 9 December 1996; period 1), 5–8 (9 December to 6 January 1997; period 2), 9–12 (6 January to 3 February; period 3), and 13–17 (3 February to 10 March 1997; period 4).

*Study 3. Test of Distance of Attraction.* To test for distance of attraction of  $\alpha$ -ionol + cade oil relative to that of protein bait traps, 17- to 18-d-old sterile *B. latifrons* were released in a macadamia nut orchard down the middle of the alley on either side of a row of trees with six traps placed in line with the tree trunks in every other tree, making the release line  $\approx$ 4.9 m away from the trap line. Concurrently, in other parts of the orchard, fly releases were done one alley away on each side (14.6 m away) and two alleys away on each side (24.4 m away). Traps were either yellow-bottom plastic dome traps baited with 350 ml of a protein bait solution composed of 10% Provesta 621 (an autolyzed yeast extract from Integrated Ingredients, Bartlesville, OK), 3% borax, and 87% water, or were Jackson traps baited with 1.0 ml  $\alpha$ -ionol + 0.5 ml cade oil with a 2.0 g strip of Revenge bug strip (18.6% DDVP [2–2-dichlorovinyl dimethyl phosphate]; Roxide International, New Rochelle, NY) added as a knock-down toxicant. Traps were retrieved 24 h after fly release. The test of each distance for each bait was conducted in separate areas of a large macadamia nut orchard. Two replicates of each distance for each bait were tested on 12–13 April 2000 and an additional replicate was completed on 25–26 April 2000. The number of flies released in each area was estimated by the percentage of 'fliers' determined in a quality control test using pupae from the batch packed for field release on 25–26 April.

*Study 4. Test for Synergism with Related Compounds.*  $\beta$ -ionol, which previously was shown to have good attraction to *B. latifrons* males, but was not as stable as

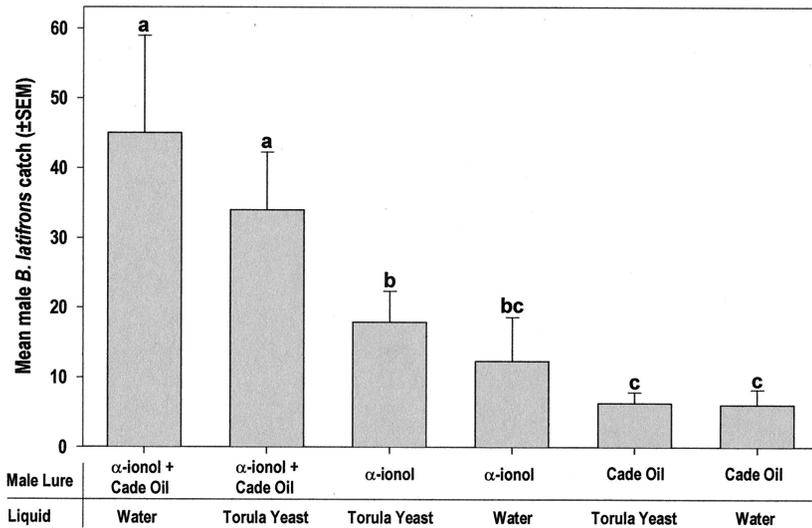


Fig. 1. Male *B. latifrons* catch (mean  $\pm$  SEM) at yellow bottom plastic dome traps baited with  $\alpha$ -ionol + cade oil,  $\alpha$ -ionol alone, or cade oil alone with either water alone or a torula yeast solution. Results presented are the means of three trials. Means with the same letter are not significantly different (at the  $\alpha = 0.05$  level) based on ANOVA of square-root transformed trap catch data.

$\alpha$ -ionol (Flath et al. 1994), was also tested for synergistic enhancement of attraction with cade oil. Jackson traps baited with cotton wicks holding 1.0 ml  $\alpha$ -ionol, 1.0 ml  $\alpha$ -ionol + 0.5 ml cade oil, 1.0 ml  $\beta$ -ionol, 1.0 ml  $\beta$ -ionol + 0.5 ml cade oil, 0.5 ml cade oil, or 1.0 ml water (blank) were set out in a randomized complete block design in a macadamia nut orchard, with 12 replicates of each treatment. Each trap had a 2.0-g strip of Revenge bug strip attached as a knockdown toxicant. Sexually mature sterile *B. latifrons* adults were then uniformly released throughout the trapping grid with traps recovered 24 h after the fly release.

**Field Validation Test with Wild Flies.** Relative attraction of  $\alpha$ -ionol with and without cade oil to wild male *B. latifrons* was tested with paired traps set out at two sites on Maui (Hawaii, U.S.A.) known to have established populations. One site included part of Huluhulunui Gulch north of Kokomo, with traps set within this gulch or in a smaller, adjacent gulch. The other site was along Iao Stream on the west side of Wailuku. These sites had scattered populations of both Sodom apple (*Solanum linnaeanum* Hepper & P. Jaeger) and turkey berry (*Solanum torvum* Sw.) as known hosts of *B. latifrons*. Each trapping station at each site had two Jackson traps. One trap had an attached cotton wick (3.8 cm long by 1.0 cm diameter) holding 1.0 ml of  $\alpha$ -ionol with 0.5 ml of cade oil. The wick in the other trap held only 1.0 ml of  $\alpha$ -ionol. Each trap also held a 2.0-g strip of Revenge pest strip to serve as a knock-down toxicant. Paired traps were separated by  $\approx 10$  m. Each trap was hung in a turkey berry plant with the order of placement of the pair randomized. Traps were set out at the Huluhulunui Gulch site (14 pairs) on 17–18 May 1999. Traps were set at the Iao stream site (10 pairs) on 24 May 1999. At both sites, traps were serviced weekly for three consecutive weeks.

**Statistical Analyses.** For studies 1, 2A, 2B, and 4 with sterile laboratory flies, all trap catch results were square root transformed [ $\sqrt{x + 0.5}$ ] before analysis. For study 1, the difference in catch among treatments was tested using an ANOVA on the transformed values followed by a Waller–Duncan *K*-ratio *t*-test for separation of means. For studies 2A and 2B, the difference in catch among treatments was tested using an ANOVA on the transformed values of trap catches over four time periods, with Waller–Duncan *K*-ratio *t*-tests for separation of means. For studies 1, 2A, and 2B, untransformed trap catch results are presented together with statistical results based on transformed values. For study 3, percentage of fly recovery data were arcsine transformed before submitting to *t*-tests (SAS Institute 1998) for each distance to test for significance of difference in catch between protein-baited traps and  $\alpha$ -ionol + cade oil-baited traps. For the field validation test with wild flies, separate signs tests (Steel et al. 1997) were conducted on the difference in trap catch values of the paired traps for each week of trapping at each site.

## Results

**Field Tests with Sterile Laboratory Flies. Study 1. Initial Field Trial.** Average catch results are summarized in Fig. 1. There was a significant difference among treatments in male catch ( $F = 14.46$ ;  $df = 5, 82$ ;  $P < 0.0001$ ). Catch was significantly greater in the  $\alpha$ -ionol + cade oil treatments than in  $\alpha$ -ionol only treatments, whether the wick was suspended above a torula yeast solution or above water. Catch was also significantly greater in the treatment with the  $\alpha$ -ionol only wick suspended over a torula yeast solution than in the treatment with the cade oil only wick suspended

**Table 2.** Average catch ( $\pm$ SEM) in study 2A of male *B. latifrons* during four periods, at traps baited with  $\alpha$ -ionol only, cade oil only, or various proportions of these two compounds

Source of variation	Treatment	Period				% of period 1
		1	2	3	4	
	No. of trap recoveries	4	4	4	4	
	No. of days in period	16	28	28	29	
	$\alpha$ -ionol:cade oil [1:3]	47.7 $\pm$ 3.9a	39.3 $\pm$ 4.1ab	37.7 $\pm$ 3.4a	33.7 $\pm$ 2.9a	70.6
	$\alpha$ -ionol:cade oil [1:1]	50.5 $\pm$ 6.6a	42.1 $\pm$ 4.8a	28.2 $\pm$ 2.1b	25.7 $\pm$ 2.0b	50.9
	$\alpha$ -ionol:cade oil [3:1]	47.5 $\pm$ 4.0a	30.5 $\pm$ 4.3c	23.2 $\pm$ 2.2c	21.8 $\pm$ 2.3bc	45.9
	$\alpha$ -ionol:cade oil [6:1]	54.0 $\pm$ 5.6a	32.6 $\pm$ 4.0bc	24.1 $\pm$ 2.4bc	19.6 $\pm$ 1.8c	36.3
	$\alpha$ -ionol:cade oil [12:1]	48.5 $\pm$ 4.7a	29.4 $\pm$ 3.8c	21.6 $\pm$ 1.7c	21.3 $\pm$ 2.0bc	43.9
	$\alpha$ -ionol only [1:0]	25.1 $\pm$ 2.2b	17.4 $\pm$ 1.8d	13.5 $\pm$ 1.0d	13.0 $\pm$ 1.4d	51.8
	cade oil only [0:1]	14.8 $\pm$ 1.1c	7.4 $\pm$ 0.7e	6.2 $\pm$ 0.9e	2.9 $\pm$ 0.5e	19.6
Treatment	<i>F</i>	20.18	23.94	31.55	47.76	
	<i>df</i>	6, 196	6, 196	6, 196	6, 196	
	<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	
Days	<i>F</i>	11.89	36.51	1.95	9.05	
	<i>df</i>	3, 196	3, 196	3, 196	3, 196	
	<i>P</i>	<0.0001	<0.0001	0.1227	<0.0001	
Treat $\times$ days	<i>F</i>	0.73	1.26	0.76	0.88	
	<i>df</i>	18, 196	18, 196	18, 196	18, 196	
	<i>P</i>	0.7767	0.2158	0.7492	0.6090	

Numbers in a column followed by the same letter are not significantly different (at the  $\alpha = 0.05$  level) based on ANOVA of square-root transformed trap catch data. Final column presents the trap catch in period 4 as a percentage of that in period 1.

over a torula yeast solution. For all treatments, there was no significant difference whether there was a torula yeast solution or water only in the trap. Male catch in traps baited with  $\alpha$ -ionol + cade oil and water averaged over 3.6 times greater than in traps baited with alpha-ionol alone and water (45.0 versus 12.3 male flies per trap, respectively).

**Study 2A. Test of  $\alpha$ -ionol and Cade Oil Ratio.** There were significant differences in catch among treatments for each of the four time periods. There were also significant differences in catch based on days of exposure for each period except for period three (Table 2). The significance of days of exposure is not surprising because the total catch clearly drops off for each treatment over the course of the study (Table 2). The 'treatment  $\times$  days' interaction term was not significant for any of the periods, suggesting that the selected clumping of trap recovery days was reasonable. For each of the four periods, catch in the cade oil only treatment was significantly less than in the  $\alpha$ -ionol only treatment. Catch in both of these treatments was significantly less than in any of the other treatments, which included both cade oil and  $\alpha$ -ionol.

The results show that synergistic effects of  $\alpha$ -ionol plus cade oil occur even with small additions of cade oil. Over shorter weathering times (period 1), trap catch tends to be greater (though not significantly) with lower cade oil loadings, whereas catches at traps with higher cade oil loadings are significantly greater over longer weathering times (periods 3 and 4). It is also interesting to note that the average trap catch for each treatment in period 4, expressed as a percentage of the average trap catch in period 1 (Table 2), tends to decline in direct correlation with the decline in cade oil concentration ( $r^2 = 0.58$ ).

**Study 2B. Test of  $\alpha$ -ionol and Cade Oil Ratio.** There were significant differences in catch among treatments for each of the four periods (Table 3). There

were also significant differences in catch based on days of exposure for each period (Table 3). The 'treatment  $\times$  days' interaction term was not significant except for period 3. In period 3, one of the four trap recoveries had very low catch for all treatments, although the trends were generally the same, which could account for the significance of the interaction term. Overall, the selected clumping of trap recovery days seems reasonable for this study also. For each of the four periods, catch in the cade oil only treatment was significantly less than in the  $\alpha$ -ionol only treatment. Catch in both of these treatments was significantly less than in any of the other treatments which included both cade oil and  $\alpha$ -ionol. The lowest cade oil loading (1:6) again performed well in period 1 but led to significantly lower catches by period 4. In this case, the highest cade oil loading had significantly less catch in period 1, but was not significantly different than the highest catches in periods 2, 3, and 4, and had, numerically, the highest catch in both periods 3 and 4. The average trap catch for each treatment in period 4, expressed as a percentage of the average trap catch in period 1 (Table 3), showed a stronger correlation with the decline in cade oil concentration ( $r^2 = 0.90$ ) than in study 2A.

**Study 3. Test of Distance of Attraction.** The average percentage recovery of flies from each distance of release for each bait is presented in Fig. 2. There were more flies caught at each distance with the  $\alpha$ -ionol + cade oil baited traps than with the protein-baited traps, with the difference in catch being significant at 4.9 m ( $t = 3.09$ ,  $df = 4$ ,  $P = 0.037$ ), nearly significant at 14.6 m ( $t = 2.57$ ,  $df = 4$ ,  $P = 0.062$ ), and not significant at 24.4 m ( $t = 0.81$ ,  $df = 4$ ,  $P = 0.463$ ). Flies recovered were 99.9% males for the  $\alpha$ -ionol + cade oil traps and 97.7% females for the protein bait traps.

**Study 4. Test for Synergism with Related Compounds.** Catch was significantly different among treatments

**Table 3.** Average catch ( $\pm$ SEM) in study 2B of male *B. latifrons*, over four periods, at traps baited with  $\alpha$ -ionol only, cade oil only, or various proportions of these two compounds

Source of variation	Treatment	Period				% of period 1
		1	2	3	4	
	No. of trap recoveries	4	4	4	5	
	No. of days in period	25	28	28	35	
	$\alpha$ -ionol:cade oil [1:3]	39.3 $\pm$ 3.7b	36.1 $\pm$ 3.6ab	30.5 $\pm$ 4.3a	30.2 $\pm$ 5.0a	76.8
	$\alpha$ -ionol:cade oil [1:1]	48.3 $\pm$ 3.8a	38.6 $\pm$ 3.0a	28.0 $\pm$ 3.9a	27.5 $\pm$ 4.2a	56.9
	$\alpha$ -ionol:cade oil [3:1]	51.7 $\pm$ 3.2a	40.0 $\pm$ 3.2a	30.3 $\pm$ 4.6a	27.6 $\pm$ 2.7a	53.4
	$\alpha$ -ionol:cade oil [6:1]	50.1 $\pm$ 4.4a	32.7 $\pm$ 3.3b	20.9 $\pm$ 3.0b	18.4 $\pm$ 2.6b	36.7
	$\alpha$ -ionol only [1:0]	25.4 $\pm$ 2.2c	17.7 $\pm$ 2.1c	10.3 $\pm$ 1.6c	10.1 $\pm$ 1.4c	39.8
	cade oil only [0:1]	9.9 $\pm$ 1.1d	5.2 $\pm$ 1.0d	3.3 $\pm$ 0.6d	3.6 $\pm$ 0.9d	36.4
Treatment	<i>F</i>	43.99	49.08	29.63	27.73	
	<i>df</i>	5, 168	5, 168	5, 168	5, 210	
	<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	
Days	<i>F</i>	8.93	13.94	71.23	24.82	
	<i>df</i>	3, 168	3, 168	3, 168	4, 210	
	<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	
Treat $\times$ days	<i>F</i>	1.55	0.64	2.04	0.83	
	<i>df</i>	15, 168	15, 168	15, 168	20, 210	
	<i>P</i>	0.0936	0.8428	0.0155	0.6699	

Numbers in a column followed by the same letter are not significantly different (at the  $\alpha = 0.05$  level) based on ANOVA of square-root transformed trap catch data. Final column presents the trap catch in period 4 as a percentage of that in period 1.

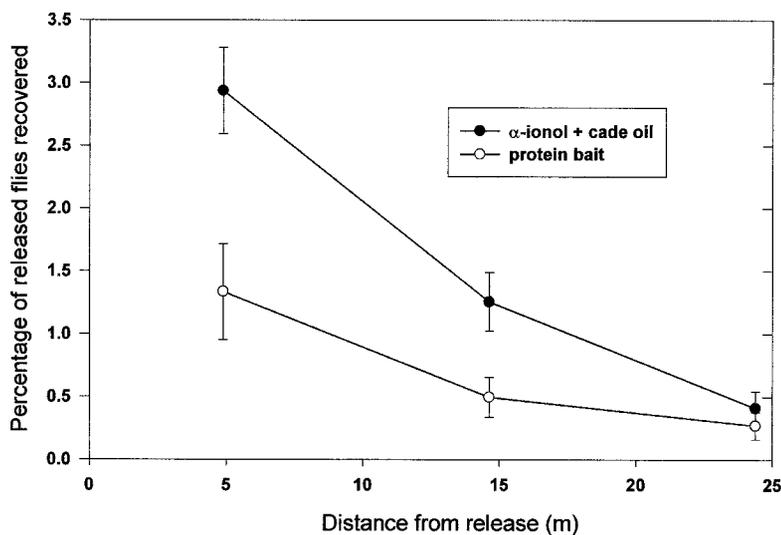
( $F = 46.41$ ;  $df = 5, 66$ ;  $P < 0.0001$ ). Catch at  $\alpha$ -ionol + cade oil and  $\beta$ -ionol + cade oil traps was not significantly different, whereas both  $\alpha$ -ionol + cade oil and  $\beta$ -ionol + cade oil had significantly higher catches than their respective treatments that lacked cade oil.  $\alpha$ -ionol and  $\beta$ -ionol alone treatments had significantly greater catch than the cade oil only treatment and the cade oil only treatment had significantly higher catch than the water only blank traps (Fig. 3).

**Field Validation Test with Wild Flies.** Catch results and results of signs tests for paired traps from both Iao Stream and Huluhulunui Gulch sites are presented in Fig. 4. Average trap catch in  $\alpha$ -ionol + cade oil baited traps was significantly greater than in traps baited with

$\alpha$ -ionol alone at five of the six recovery times. Only trap catch in week 3 at Iao Stream was not significantly different, but, even there, the average  $\alpha$ -ionol + cade oil trap catch was numerically greater than the average for the  $\alpha$ -ionol only trap. Overall, from these tests, the average catch at  $\alpha$ -ionol + cade oil baited traps was  $>3.5$  times greater than catches at traps baited with  $\alpha$ -ionol alone.

## Discussion

The field validation test confirms the comparable results of the trials with sterile laboratory flies. An increase in catch occurred even with small additions



**Fig. 2.** Average ( $\pm$ SEM) percentage *B. latifrons* recovery, males and females combined, at three distances of release from a row of six Jackson traps baited with 1.0 ml  $\alpha$ -ionol + 0.5 ml cade oil versus a row of six yellow-bottom plastic dome traps baited with protein bait.

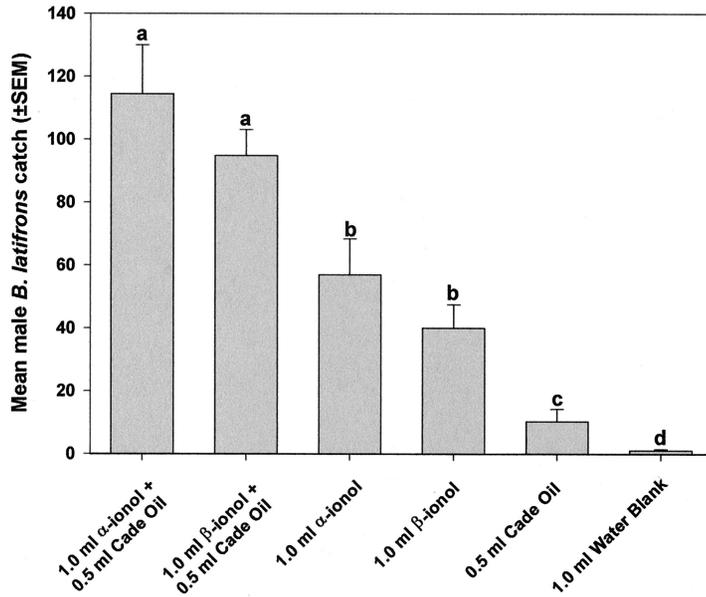


Fig. 3. Average catch ( $\pm$ SEM) of *B. latifrons* at traps baited with 1.0 ml  $\alpha$ -ionol + 0.1 ml cade oil, 1.0 ml  $\beta$ -ionol + 0.1 ml cade oil, 1.0 ml  $\alpha$ -ionol, 1.0 ml  $\beta$ -ionol, 0.1 ml cade oil only, or 1.0 ml water (blank). Catch was not significantly different (at the  $\alpha = 0.05$  level) where adjacent columns have the same letter, based on ANOVA of square-root transformed trap catch data.

of cade oil to  $\alpha$ -ionol. The ratio of  $\alpha$ -ionol to cade oil, however, affects the level of improved catch over time. Over periods of exposure up to  $\approx 1$  mo, intermediate loadings of cade oil (1:1–3:1,  $\alpha$ -ionol:cade oil)

typically perform well. Relative effectiveness of baits with lower cade oil loadings drops off over 2–3 mo of aging. Over these longer periods of aging, baits with higher loadings of cade oil tend to be more effective.

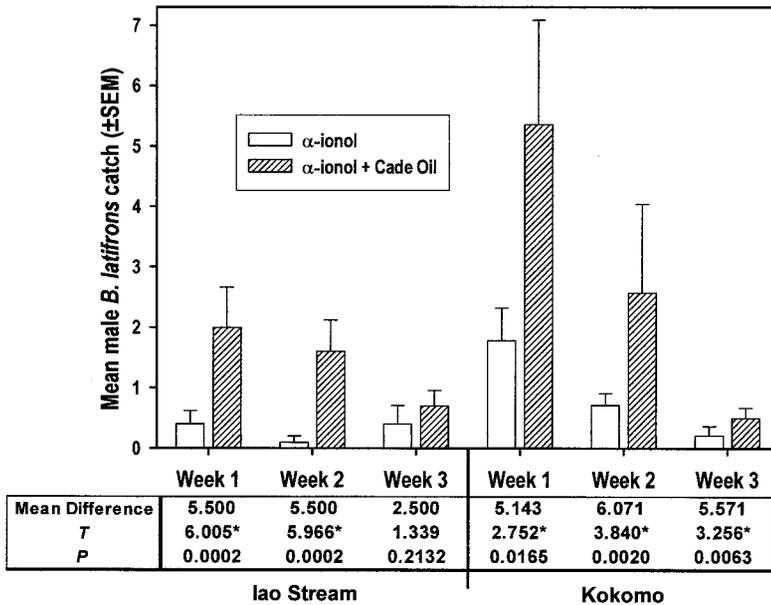


Fig. 4. Relative attraction (mean  $\pm$  SEM) of  $\alpha$ -ionol with and without cade oil to wild male *B. latifrons* based on paired Jackson traps, each including a 2.0 g strip of Revenge pest strip as a knockdown toxicant. Traps were surveyed weekly for three consecutive weeks both near Kokomo and along Iao Stream on the island of Maui. Results of signs tests for each week's paired catches is also presented. Average rank, in the signs test, of the treatment catch difference is presented, together with actual P values listed below the T values. T values followed by an asterisk are significant at the  $\alpha = 0.05$  level.

Given these trends, the choice of ratio of cade oil to  $\alpha$ -ionol to use in a detection program will depend on the frequency of recharging the bait mixture. For a 1-mo recharging interval, intermediate loadings of cade oil would be appropriate.

Synergistic enhancement of attraction has not commonly been found among male lures for tephritid fruit flies of the subfamily Dacinae. Known male attractants in this subfamily include methyl eugenol [4-allyl-1,2-dimethoxybenzene], which is known to attract at least 58 different species; cue-lure [4-(*p*-hydroxyphenyl)-2-butanone acetate], which is known to attract at least 176 different species (Metcalf 1990); trimedlure [1,1-dimethyl 4 (and 5)-chloro-2-methyl-cyclohexane-1-carboxylate] and  $\alpha$ -copaene [a tricyclic sesquiterpene] attractive to *Ceratitis capitata* Wiedemann (Cunningham 1989); and "vert-lure" [methyl-*p*-hydroxybenzoate], attractive to *Dacus vertebratus* Bezzi (Cunningham 1989). Among these male attractants, synergistic enhancement of attraction of  $\alpha$ -copaene by a number of different terpenoid compounds has been found (T. W. Phillips, Department of Entomology, Oklahoma State University, Stillwater, OK, personal communication), but synergistic enhancement of attraction with nonfood-based compounds has not been reported with the other male lures, although there have been a number of attempts to mix a male lure with a food-based attractant (Liquido et al. 1993, Zervas 1987), with mixed results. Alpha-copaene is a naturally occurring botanical compound with a much more complex molecular structure (a 3-ring hydrocarbon molecule) than the other male attractants (Cunningham 1989). Improvements to the other male attractants have typically been sought through the production of synthetic analogs, an example being a number of fluoro-substituted analogs of methyl eugenol (Liquido et al. 1998). Analogs of cue-lure have also been prepared, with some promising results (G.T.M., unpublished data). Trimedlure has greatly improved attraction over its precursor compounds. It is a tertiary butyl analog of 'Medlure,' which is a chloro-substituted analog of 'Siglure' (Cunningham 1989). Many analogs of trimedlure have been synthesized and tested (DeMilo et al. 1994), including the iodo-analog, ceralure (Leonhardt et al. 1996). Results of tests of attractancy of compounds structurally similar to  $\alpha$ -ionol were presented along with those of  $\alpha$ -ionol by Flath et al. (1994). No further work on synthesis and testing of other analogs of  $\alpha$ -ionol has been done since that publication. We have shown here that  $\beta$ -ionol, found by Flath et al. (1994) to provide comparable initial attraction to *B. latifrons* as does  $\alpha$ -ionol, also shows synergistic enhancement of attraction with cade oil. We have not tested for synergism with cade oil of any of the other closely related compounds, which had also been found by Flath et al. (1994) to be attractive to *B. latifrons*. We have conducted preliminary field tests with methyl eugenol (and *B. dorsalis*), cue-lure (and *B. cucurbitae*), and trimedlure (and *C. capitata*) but have not found any synergistic effects of cade oil with these other male lures (G.T.M., unpublished data).

At this point, it is not known how cade oil synergizes the attraction of  $\alpha$ -ionol for male *B. latifrons*. The determination of method of synergism is complicated because cade oil is a multi-compound distillation product and not a pure chemical. If the active ingredient(s) of cade oil can be determined, it may be possible to suggest reasons for this synergism, which could then help to seek further improvements to  $\alpha$ -ionol. Further understanding of this synergism may also help to point to means of further improving other male lures because  $\alpha$ -ionol, methyl eugenol, cue-lure, and trimedlure all have some commonality of structure in having an aromatic or aliphatic six-carbon ring with at least one multi-atom side chain containing a double bond or ester linkage (Flath et al. 1994), although, as noted above, there seems to be no synergistic effect of the other male lures with the full spectrum of compounds in cade oil.

Substitution of  $\alpha$ -ionol + cade oil for  $\alpha$ -ionol alone in detection programs could considerably improve the chance of detecting invading or incipient populations of *B. latifrons*. Although the addition of cade oil significantly improves the attraction of  $\alpha$ -ionol for male *B. latifrons*, it is still a fairly weak male lure relative to the attractancy of methyl eugenol or cue-lure to other tephritid fruit fly species. Consequently, we recommend that detection programs not rely solely on this lure but also make use of protein baited traps, as well as collections of solanaceous fruits (Liquido et al. 1994). We also acknowledge, as did Flath et al. (1994), that further search is needed for a more potent male attractant for *B. latifrons*. We are currently seeking to further improve this lure through efforts to identify the active ingredient(s) in cade oil responsible for the observed synergism with  $\alpha$ -ionol.

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