Comparison of Ceralure and Trimedlure Attractants for the Male Mediterranean Fruit Fly (Diptera: Tephritidae)[1, 2]

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ABSTRACT The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a major pest of fruits and vegetables in the world but, through an extensive trapping program, the continental United States has remained free of established populations. These traps are baited with 2 g of trimedlure which is highly attractive to the male flies. This study contrasts the effectiveness of trimedlure and a new attractant, ceralure, which is an iodo-analog of trimedlure. Field tests in Hawaii using released sterile flies showed that the most attractive B1 isomer of ceralure caught 2 to 3 times as many male flies per mg as did the most attractive C isomer of trimedlure.

KEY WORDS Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), trimedlure, ceralure, attractant.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a worldwide agricultural pest, but an extensive surveillance program has maintained the continental United States free of established populations. A network of about 40,000 traps is used to detect accidental introductions of this pest. Each trap is baited with the synthetic attractant, trimedlure, which is a mixture of the *tert*-butyl esters of 4-(and 5-)chloro-(*E* and *Z*)-2-methylcyclohexane-1-carboxylic acids (Beroza et al. 1961). Only male Mediterranean fruit flies are attracted. For effective and convenient use, the trimedlure is formulated in controlled-release dispensers which prolong evaporation and thus the effectiveness of the attractant (Leonhardt et al. 1987, 1989). Each polymeric plug-type dispenser contains 2 g (70% by weight) of trimedlure.

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[2] This paper reports the results of research only. Mention of commercial products in this paper does not constitute a recommendation by USDA.

In a continuing search for more effective attractants, ceralure, a mixture of the ethyl esters of 4-(and 5-)iodo-(*E* and *Z*)-2-methylcyclohexane-1-carboxylic acids, was found to be a highly effective lure for male Mediterranean fruit flies (McGovern and Cunningham 1988, DeMilo et al. 1994a). This study reports the relative attractancy of trimedlure and ceralure as dispensed from cotton wicks and from polymeric plug-type dispensers.

Materials and Methods

Attractants. The trimedlure (lot 3702, UOP Chemical Div., East Rutherford, NJ) was 98% pure (density: 1.02 g/ml) and contained 42.1% of the most attractive trimedlure-C isomer (McGovern et al. 1990). The ceralure (lot 006118, AgriSense/Biosys, Palo Alto, CA) was 88.0% pure (density: 1.43 g/ml) and contained 23.4% of the most attractive ceralure-B_1 isomer (DeMilo et al. 1994b, Warthen et al. 1994). In Test 1 (Table 1), the reference treatment was a solid plug-style polymeric dispenser (1.7 cm l × 1.3 mm diam; 2 g trimedlure; AgriSense/Biosys) similar to those routinely used in fly detection programs (Leonhardt et al. 1989). Each reference dispenser was replaced with a fresh plug every 4 wk to maintain a constant level of attraction in the reference traps. Trimedlure and ceralure were compared on the basis of: equal weight (2.0 g), equal volume (2.0 ml), and equal moles (8.6 mmoles) by applying the liquid attractant to cotton dental rolls (3.7 cm l × 9.5 mm diam). The amounts of trimedlure and ceralure deployed were not corrected for chemical purity. In Test 2 (Table 2), the reference treatment was again the polymer plug dispenser with 2 g trimedlure, replaced monthly. Equal weight (2.0 g) and equal volumes (2.0 ml) of liquid trimedlure and ceralure were compared. In addition, the two attractants (trimedlure and ceralure) were compared at equal weight (2.0 g) in solid, plug-style, polymeric dispensers (1.7 cm l × 1.3 mm diam; AgriSense/Biosys).

Bioassay. In each test, plug and cotton wick dispensers were placed in tent-shaped Jackson traps with unobstructed ends and removable sticky insert floors (Harris et al. 1971). The traps were deployed in a randomized complete block design in a mature *Macadamia integrifolia* Maiden and Betche (macadamia nut) orchard near Hilo, HI. There were no wild flies present in the test area. In Test 1, 16 replicates of 5 treatments were deployed 25 February. About 50,000 male and female sterile unmarked laboratory-reared flies were released throughout the plot immediately after deployment and immediately after each weekly examination thereafter. The flies used for these tests had emerged from pupae held in large screened tote boxes with sugar, hydrolyzed protein, and water (in an agar block) laid on the top screen. Flies were released uniformly by walking through the plot carrying the fly boxes with lids partially ajar to allow flies to escape. The traps were examined the day after deployment and at weekly intervals thereafter over the next 6 months. The sticky inserts were removed at each examination and the captured flies were counted. Fresh sticky inserts were put into the traps at the time of removal of the old ones. Recovery of sterile flies varies due to changing weather conditions with captures particularly depressed during periods of cool temperatures and heavy rain. The captures within each replicate were summed over 4-wk intervals for

Table 1. Mean numbers (S.E.; n = 16) of male Mediterranean fruit flies captured in Test 1 in Hilo, HI and amounts (S.E.; n = 10) of residual attractant remaining at the end of the test.*

Treatment	Initial Amount of Attractant			Mean Numbers Male Flies (S.E.) Captured Monthly						Residue**
	g	ml	mmoles	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	mg Attractant (S.E.)
Fresh TML plug†	2.00	2.0	8.6	513.4 (63.0)	517.2 (43.9) a	445.6 (42.0) a	259.3 (24.5) a	507.1 (35.4) a	928.1 (26.3) a	864.‡ (43.5)
TML on cotton wick	2.00	2.0	8.6	525.6 (52.8)	335.7 (14.2) b	261.8 (27.4) b	135.1 (22.3) c	163.7 (33.8) c	47.3 (17.5) d	< 0.4
CER on cotton wick	2.00	1.4	6.7	569.9 (60.8)	514.2 (37.1) a	393.0 (46.9) a	190.6 (29.3) bc	364.6 (59.8) b	341.9 (66.7) c	1.6 (0.2)
CER on cotton wick	2.86	2.0	9.6	677.3 (54.7)	492.5 (31.3) a	421.0 (34.1) a	255.3 (17.8) a	607.4 (49.0) a	725.6 (65.3) b	16.8 (2.8)
CER on cotton wick	2.55	1.8	8.6	572.1 (64.5)	504.3 (23.4) a	423.8 (39.6) a	240.1 (22.5) ab	579.4 (59.5) a	720.7 (59.8) b	12.1 (1.2)
F(df)				1.626 (4, 60)	7.029 (4, 60)	5.271 (4, 60)	5.898 (4, 60)	17.651 (4, 60)	54.403 (4, 60)	
k				Not Significant	79.35	90.19	60.48	112.01	120.40	

* All data untransformed were analyzed by ANOVA; means followed by the same letter in a column are not significantly different ($P > 0.05$) by the Waller-Duncan (1969) LSD test. Other treatments were included in the test but the data are not included in this report.

** Residual attractant (mg/dispenser) remaining on dispenser after exposed for 6 months in the field; mean of 5 replicates.

† Reference treatment. Replaced monthly; all other treatments allowed to remain in field for duration of test.

‡ Treatment replaced monthly; residual attractant measured after only one month of exposure in the field.

Table 2. Mean numbers (S.E.; n = 6) of male Mediterranean fruit flies captured in Test 2 in Hilo, HI and amounts (S.E.; n = 7) of residual attractant remaining at the end of the test.*

Treatment	Initial Amount of Attractant			Mean Numbers Male Flies (S.E.) Captured Monthly				Residue**
	g	ml	mmoles	Month 1	Month 2	Month 3	Month 4	mg Attractant (S.E.)
Fresh TML plug†	2.00	2.0	8.6	731.3 (103.5) ab	277.5 (29.3) a	179.5 (29.4) a	531.0 (49.9) a	274. (25.0)
TML plug	2.00	2.0	8.6	569.6 (71.4) b	155.5 (18.8) b	44.0 (13.8) b	52.9 (15.1) d	848. (27.2)
CER plug	2.00	1.4	6.7	504.1 (51.8) c	154.8 (11.6) b	120.0 (34.3) ab	175.0 (42.0) cd	9.9 (3.8)
TML on cotton wick	2.00	2.0	8.6	778.6 (110.8) a	164.8 (21.3) b	79.9 (22.0) b	146.9 (38.4) cd	11.1 (2.7)
CER on cotton wick	2.00	1.4	6.7	621.5 (78.6) abc	240.8 (29.0) a	116.3 (34.2) ab	246.3 (80.5) c	448. (37.3)
CER on cotton wick	2.86	2.0	9.6	645.0 (56.8) abc	217.6 (32.8) ab	194.6 (23.7) a	378.6 (43.0) b	
F(df)				3.230 (5, 35)	4.518 (5, 35)	4.308 (5, 35)	13.319 (5, 35)	
k				178.35	71.50	82.45	127.06	

* All data untransformed were analyzed by ANOVA; means followed by the same letter in a column are not significantly different ($P > 0.05$) by the Waller-Duncan (1969) LSD test. Other treatments were included in the test but the data are not included in this report.

** Residual attractant (mg/dispenser) remaining on dispenser after exposed for 4 months in the field; mean of 7 replicates.

† Reference treatment. Replaced monthly; all other treatments allowed to remain in field for duration of test.

analysis. The totals were subjected to analysis of variance (ANOVA) and to the Waller and Duncan (1969) k-ratio test for separation of means ($P < 0.5$). In Test 2, eight replicates of 6 treatments were evaluated as above from 25 March-30 July in the same location. The use of sterile flies increases the sensitivity of the test by decreasing the high variance below that which occurs when testing is conducted with the highly contagious distributions of wild fly populations. The relative response among differing traps or lures has been observed to be the same whether wild flies or sterile laboratory flies were used for the test (Unpublished data, R.T.C.)

Chemical Analysis. The amounts of each attractant that evaporated from the dispensers were determined to assess the relative attractancy. At the completion of the first test, 5 replicate dispensers were each extracted with measured volumes (20-800 ml) of 1:1 hexane-acetone solvent. Recovery of the attractants was 98-100% following 2 days of such extraction at room temperature. The residual quantities of trimedlure and ceralure were measured by gas chromatography on an SPB-1 (Supelco, Bellefonte, PA) wide-bore, fused-silica, capillary column (30 m × 0.75 mm, i.d.) on a Shimadzu (Columbia, MD) Model GC-9A chromatograph. The column was operated at 100°C for 2 min and then programmed at 10°C/min to 250°C where it was held for 6 min. The two peaks corresponding to all of the isomers (retention times: 6.8 and 7.0 min for ceralure and 5.6 and 5.8 min for trimedlure) were summed for calculation of total amounts of residual attractant in each replicate. Solutions of re-distilled ceralure and of pure, cyrstalline, trimedlure-C isomer were used as external standards to calculate the residual quantities of the two attractants. Standard errors (n = 5) were calculated. At the end of Test 2, the residual trimedlure or ceralure contents were determined by gas chromatography as described above on each of seven replicates following overnight, room-temperature extraction with 100-800 ml of 1:1 hexane-acetone solvent. For each extraction, the volume of solvent was measured but the amount was varied depending on the level of residual attractant expected. Standard errors (n = 7) were calculated. No other major peaks related to the attractants were detected.

Results and Discussion

In Test 1 (Table 1), there were no significant differences in male fly captures in the first month. However, aged ceralure was significantly more attractive than aged trimedlure at months 2 to 6 when an equal volume (2.0 ml) or an equal molar (8.6 mmoles) quantity was initially deployed on cotton wicks. When compared at equal initial weight (2.0 g), aged ceralure remained significantly more attractive than aged trimedlure at months 2, 3, 5, and 6. Very little of either attractant was left on the wick dispenser after 6 months of exposure (Table 1). More (1.6-16.8 mg) ceralure remained on the wicks at the end of the test than on the trimedlure-treated wicks (<0.4 mg).

An initial dose of 2.0 g of ceralure on a cotton wick and allowed to age was as attractive for the first 3 months as was the reference 2.0 g trimedlure plug dispenser which was replaced monthly to assure high attractancy. Aged ceralure on cotton wick dispensers, which had initially been treated with amounts equal in moles (8.6 mmoles) and volume (2.0 ml) to the trimedlure in the plug

dispenser, was as, or more, attractive than the reference fresh plug for the first 5 months of the test; only at 6 months when the ceralure was essentially depleted from the cotton wicks were captures significantly lower than those with the reference trimedlure plug. Beyond the first month, aged trimedlure on a cotton wick was significantly less attractive than the reference, fresh trimedlure plug dispenser.

The Test 2 comparison of aged ceralure and trimedlure as dispensed from cotton wicks gave results (Table 2) that were similar to those in Test 1. Comparison of initial doses of 2.0 g of ceralure and trimedlure in plug dispensers showed significantly higher captures with trimedlure in the first month and no significant differences in month 2. At months 3 and 4 of aging, the ceralure plug with its longer persistence (848 mg ceralure vs 274 mg trimedlure residual contents) gave higher male captures but the differences were not significant. In general, the aged plug dispensers (both ceralure and trimedlure) caught fewer male flies than did the reference fresh trimedlure plug dispenser.

The results of both tests show that when equal weights of ceralure and trimedlure were compared over a duration of 4 to 6 months in the field, male fly captures with aged ceralure were generally as high or higher than those with aged trimedlure. Because the ceralure evaporates at a slower rate than does trimedlure from cotton wicks, the differences in fly captures suggest that ceralure is inherently more attractive than trimedlure as well as more persistent. The quantity of attractive isomer must also be considered; ceralure contains 23.4% of its attractive isomer, ceralure-B_1 (Warthen et al. 1994), and thus an initial dose of 2.0 g of ceralure contains only 0.468 g of ceralure-B_1. Trimedlure contains 40% of its most attractive isomer, trimedlure-C (Leonhardt et al. 1982), and therefore the 2.0 g dose contains only 0.8 g of trimedlure-C. Of the other major isomers in ceralure and trimedlure, only trimedlure-A has weak to moderate attractiveness and its activity declines more rapidly than does that of trimedlure-C (McGovern et al. 1987, McGovern et al. 1990). Because the other major isomers of both ceralure and trimedlure have low, if any, attractancy, the total fly captures obtained can be ascribed to isomers ceralure-B_1 or trimedlure-C. On this basis, the total mean captures over the six months of Test 1 and over 4 months in Test 2 were calculated per milligram released of the attractive isomer (Table 3). The results show that ceralure-B_1 captures about 2 to 3 times more flies per mg than does trimedlure-C. If the quantity of trimedlure-A isomer (about 27% of trimedlure) was also considered, the total mean capture per mg of attractive isomers (A plus C) would be even lower for trimedlure. Thus, the use of the more attractive ceralure rather than trimedlure in traps might enhance the detectability of incipient Mediterranean fruit fly populations, particularly if the concentration of the active ceralure-B_1 isomer could be increased.

Table 3. Total mean captures (S.E.) of male Mediterranean fruit flies over the duration of Tests 1 and 2 per milligram of attractive TML-C and CER-B_1 isomers released.

Treatment	Total Mean Capture per mg TML-C* (S.E.)		Total Mean Capture per mg CER-B_1**	
	Test 1 (n = 5)	Test 2 (n = 7)	Test 1 (n = 5)	Test 2 (n = 7)
2.0 g TML on cotton wick	1.84 (0.000)	1.47 (0.056)		
2.0 g CER on cotton wick			5.08 (0.001)	2.63 (0.032)
2.86 g CER on cotton wick			4.78 (0.006)	2.54 (0.041)
2.55 g CER on cotton wick			5.12 (0.002)	
2.0 g TML in polymer plug		1.19 (0.465)		
2.0 g CER in polymer plug				3.55 (0.188)

* mg TML-C released = (mg TML initial dose - residual mg TML) × 0.40.
** mg CER-B_1 released = (mg CER initial dose - residual mg CER) × 0.234.

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