

Use of Ceralure and Trimedlure in Mediterranean Fruit Fly (Diptera: Tephritidae) Mass-Trapping Tests¹

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ABSTRACT Ceralure, a new potent and persistent attractant for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), was evaluated in a new technique which serves as an alternative to aerial application of male annihilation bait-sprays for eradication. Residual ceralure and trimedlure contents, release rates, and medfly captures using panels coated with ceralure and trimedlure in stickem were evaluated for effective attraction of male medflies. The lure/stickem coated panels may be more effective than the Jackson trap baited with the "standard" trimedlure plug dispenser.

KEY WORDS Mediterranean fruit fly, *Ceratitis capitata*, ceralure, trimedlure, controlled-release.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most destructive agricultural pests in the world (Jackson and Lee 1985). In the United States, established populations exist only in Hawaii; however, spot infestations occasionally occur in California and Florida. Monitoring and detection of this pest using synthetic attractants is a key step in signaling the need for eradication. For more than 30 years, trimedlure (TML) [tert-butyl 4(and 5)-chloro-*trans*-2-methylcyclohexane-1-carboxylate], discovered during an intensive U. S. Department of Agriculture screening and testing program (Beroza et al. 1961), has been widely used as a synthetic male *C. capitata* (medfly) attractant. During the past 20 years, several technologies have been used to try to improve the detection capability for this pest (Nakagawa et al. 1971a,b, Nakagawa et al. 1975, Nakagawa et al. 1978, Zervas 1986, Campos and Ramos 1987, Prokopy and Economopoulos 1976). The standard dispenser of 2 ml of TML had been a cotton dental roll which was effective for only 2-4 weeks (King and Landolt 1984, Leonhardt et al. 1984, Rice et al. 1984). Use of this dispenser was discontinued and replaced by a polymer plug dispenser containing 70% TML by weight. The plug dispenser was shown to be effective for approximately 8 weeks (Leonhardt et al. 1987). In the U. S., over 50,000 TML-baited detection traps are

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deployed annually by USDA and by cooperators such as the California Department of Food and Agriculture (CDFA). These detection programs employ both Jackson traps baited with TML, which attract only male flies, and McPhail traps (Steyskal 1977) baited with a protein hydrolysate which attract both sexes. Although TML has been used in different dispensers and traps, its volatility and relatively short residual life prompted the development of ceralure (CER) [ethyl 4(and 5)-iodo-*trans*-2-methylcyclohexane-1-carboxylate]. CER, like TML, is a mixture composed of eight isomers (McGovern and Cunningham 1988) with the *trans* B₁ isomer being the most attractive isomer (McGovern and Cunningham 1988). In contrast, the *trans* C isomer is the most attractive isomer in TML (McGovern et al. 1987, Doolittle et al. 1991). Commercial samples of CER consist of a $\geq 81\%$ mixture of the four *trans* isomers with not less than 22% B₁ content. Synthetic CER is stored over a copper coil to prevent discoloration, and no decomposition products were indicated in the GC analysis (DeMilo et al. unpubl. data). CER is as attractive as TML and more persistent in cotton wick field bioassay studies (McGovern and Cunningham 1988).

This research, in cooperation with the U. S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) and Agricultural Research Service (ARS), evaluated CER for use in detection programs and possibly as a mass-trapping, eradication tool for medflies. Plastic panels coated with a mixture of CER in a sticky coating were evaluated in field tests conducted in Guatemala in a natural infestation of medflies and in Hawaii with sterile released medflies.

Materials and Methods

Field Bioassay-Dose/Response Tests. A 6-week study (Test 1-H) to determine the relationship of CER and TML doses to male and medfly captures was conducted in Hilo, HI from December 1991 to January 4 1992. Each lure was stirred into "Hold Fast Stickem®" (Seabright Laboratory, Emeryville, CA) at 5 concentrations, 24, 12, 4, 1 and 0.2%. Yellow laminated foam boards (15 × 22.5 × 0.1 cm) were coated with 20 g amounts of these mixtures to yield panel traps containing 4.8, 2.4, 0.8, 0.2, and 0.04 g of lure. Jackson traps baited with 2 g TML polymer plugs served as the reference treatment. The treatments were hung from trees 1.5 m above the ground and 20 m apart in a macadamia nut orchard using a balanced lattice field plot design with 5 replicates (Cochran and Cox 1957). Because the population of wild medflies was very low or non-existent, approximately 36,000 laboratory-reared, male and female sterile flies (3 to 5 days old) were released for each bioassay period, and the trap catches were recorded by the procedure described by Rice et al. (1984). The untransformed data were subjected to ANOVA and Fisher's (Fisher 1966) PLSD test. Because of the variations in field environment during the bioassay, the fly capture data for each evaluation period were also normalized to the Jackson trap control by dividing the mean fly capture for CER and TML by the mean fly capture of the Jackson trap control and multiplying by 100.

Small portions of the CER and TML in stickem mixture (0.01 to 0.2 g) were scraped from the panels weekly for gas chromatographic (GC) analysis of residual lure content. The residual lure contents on the panels were used to obtain an

exponential regression formula and correlation coefficient. This regression formula was then used to obtain the respective estimated daily release values.

In Test 1-G, a 7-week study to determine the dose/response of CER and TML at various concentrations was conducted from 29 August 1991 to 10 October 1991, near Antigua, Guatemala. The test site was a grove of mature citrus plants with early fruit in which there was a constant, low population of medflies. The average daily temperature was 18.5°C. Each lure was stirred into "Hold Fast Stickem®" at 3 concentrations (9.10, 4.76, and 0.99%) providing trap coatings on yellow polyethylene panels (15 × 22.5 × 0.1 cm) containing 2, 1, and 0.2 g of attractant in stickem. Ten replicates of eight treatments were compared with a standard Jackson trap equipped with a polymeric plug containing 2 g of TML using a randomized complete block field plot design. The untransformed fly capture data were analyzed by ANOVA and Fisher's PLSD test. The TML plug was replaced with a fresh plug biweekly to provide a constant standard. Small portions of the CER and TML in stickem mixture (0.01 to 0.2 g) were scraped from the panels initially and on days 4 and 7 and weekly thereafter and analyzed by GC for residual attractant and calculation of loss-rates.

Persistence of Ceralure under Field Aging. Yellow polyethylene panel traps (15 × 22.5 × 0.1 cm) were evaluated in a 12-week weathering experiment near Colombo, Guatemala from June to August 1991 (Test 2-G). The test site was a uniformly broken, hilly site with coffee trees at various mature sizes (1.8-3.0 m) with no fruit. The average daily temperature was 22.4°C. The medfly population was high at the beginning of the test and then decreased, ending with low populations. The panels (24 replicates) were coated on both sides with 14-15 g of a mixture of CER (14.5% by weight, 10% by volume) in stickem; each panel contained 2.0 g of CER. The panels were suspended 1.5 m above the ground in coffee trees and 20 m apart in a randomized complete block pattern. Small portions of the sticky mixture (0.01 to 0.2 g) were scraped from the panels weekly for GC analysis of residual CER content.

Controlled Release Dispensers for Ceralure. A new controlled-release CER and plastic formulation was prepared by mixing CER (15% by weight) with commercial polymer (PDI Inc., Blain, MN). The CER and plastic mixture was cast in rectangular molds made from glass slides stacked and glued together to form cavities. Four sizes of slabs [(2.5 × 2.5 × 0.6 cm), (2.5 × 1.87 × 0.36 cm), (7.5 × 3.75 × 0.12 cm), and (10 × 5 × 0.12 cm)] were made, and each slab (3 replicates) was placed on one side of plastic yellow panels, which were previously coated with 20 g of pure stickem. Because one large side of each slab was not exposed, the four slab sizes provided exposed surface areas of 12.25, 7.82, 30.83, 53.6 cm², respectively. The CER contents were 0.18, 0.08, 0.17, and 0.25 g, respectively. The panels were deployed in a citrus grove near Antigua, Guatemala, from August 1991 to September 1991 (Test 3-G). The test site was uniform with mature citrus plants bearing early fruit. Medfly captures were not recorded because populations were too low in the test area. One replicate of each slab was returned to the laboratory biweekly for GC analysis of residual lure content.

Chemical Analysis. In Test 1-G (3 replicates of each concentration), Test 1H (1 replicate of each concentration), and Test 2-G (24 replicates), small portions (0.01 to 0.2 g) of the lure/stickem mixtures were scraped from random

panels weekly for analysis of residual CER or TML by GC. In Test 3-G, one replicate of the rectangular slabs was analyzed for residual lure content. The samples of CER in stickem were mixed with a measured volume of hexane (10 or 20 ml) and allowed to stand overnight in a sealed vial before analysis. The samples of CER in plastic were mixed with hexane (50 ml), and the sealed bottle was allowed to stand in the freezer for 24 h to precipitate dissolved plastic from the mixture; the supernate was used for analysis. A Shimadzu GC9A (Columbia, MD) gas chromatograph, equipped with a capillary injector system and a flame ionization detector, was used for GC analysis. A Supelco (Bellefonte, PA) SPB-1 fused-silica wide-bore column (30 m \times 0.75 mm ID) was used for the residual analysis. Solutions of known concentrations of CER and TML were used as external standards. Peak areas were used for content determinations. A Supelco SPB-608 fused-silica capillary column (60 m \times 0.25 mm ID) was used for determination of isomer ratios in residual CER. Temperatures employed for GC were: injection port 225°C; detector 225°C; SPB-1 column, 100°C for 2 min, then programmed at 10°C/min to 250°C, maintained at 250°C for 6 min; total run time was 23 min. The SPB-608 column was run isothermally at 180°C. The chromatograms showed no evidence of extraneous peaks resulting from decomposition of CER during the field exposure.

Results

The Hawaii dose/response test (Test 1-H) comparing the relative attractiveness of CER and TML in stickem to *C. capitata* was conducted using laboratory-reared, sterile flies. By the end of the first 7 days, the residual content of each lure was 65% of its original amount for concentrations of 24, 12 and 4% lure in stickem and 75% for concentrations of 1 and 0.2% lure in stickem (Fig. 1). By the end of the first 14 days, the residual lure content for all concentrations was slightly less than 50% of its original amount. The residual lure content continued to decrease accordingly for days 21 through 35. The daily release rates of CER and TML were essentially the same at the respective concentrations, although the daily release rate of TML was 5 to 10% higher than that of CER during the first 14 days of exposure at concentrations greater than 4% lure in stickem.

For each lure concentration at each aging period in this dose-response test there was no significant difference in fly capture for TML versus CER (Table 1). For the 0-3 day and 3-7 day aging periods, 24 and 12% TML lure concentrations resulted in significantly higher fly captures than did the 0.2% TML dose. The 4% and 1% doses yielded intermediate fly captures. In the case of the less volatile CER, the same trend was observed. Because the fly capture rates were substantially different at each test interval, all of the fly captures in Table 1 were normalized to that of the appropriate polymeric plug containing 2 g of TML to show relative capture (Fig. 2). The fly captures showed a clear dose dependency only during the first 1-2 weeks of the test. The higher doses (24, 12, and 4%) of TML and CER declined substantially with time over the first 2-3 weeks of the test. The fly capture data for all of the doses of TML and CER were, in general, similar following 3-7 weeks of aging.

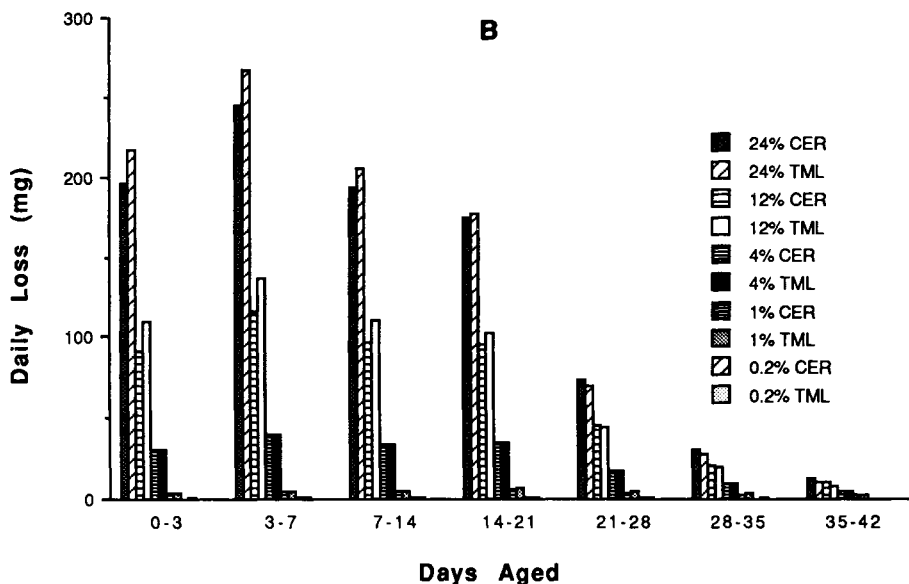
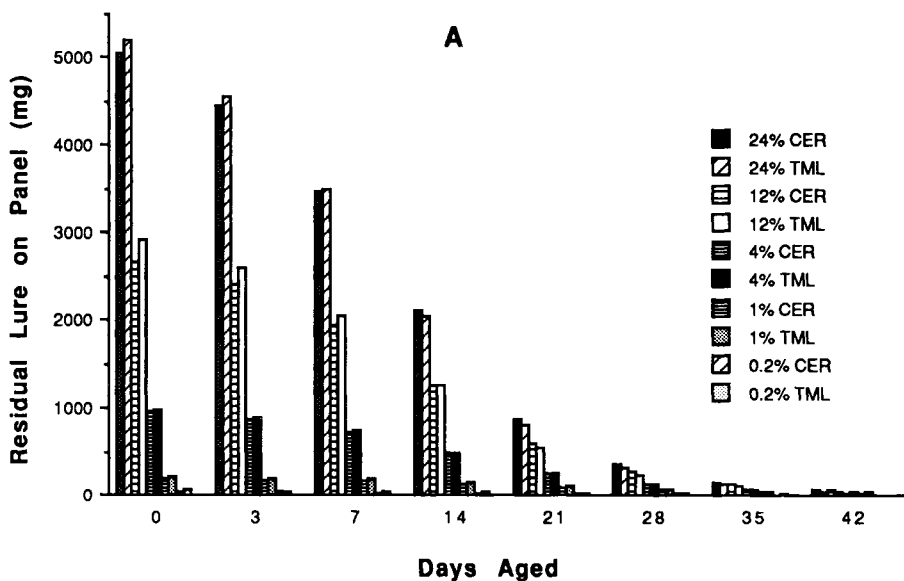


Fig. 1. A. Extrapolated residual TML and CER contents of stickem panels evaluated over a 42-day period (Test 1-H). B. Extrapolated daily release rates of TML and CER from stickem panels evaluated over a 42-day period (Test 1-H).

Table 1. Mean medfly capture for TML and CER Stickem panels evaluated in test 1-H*.

Lure Conc. % in stickem	Lure	Dose	Days Aged†						
			0 - 3	3 - 7	7 - 14	14 - 21	21 - 28	28 - 35	35 - 42
Jackson Trap‡	TML	2.0	86.2 ± 17.8 d	63.4 ± 15.1 cd	78.4 ± 8.4 d	184.2 ± 44.3 cd	128.6 ± 14.5 c	222.4 ± 4.7 ab	239.4 ± 38.4 b
	CER	4.8	446.8 ± 69.9 a	302.0 ± 104.0 ab	175.4 ± 21.1 abcd	226.2 ± 65.6 bcd	71.0 ± 21.9 cd	116.4 ± 31.1 bcd	34.2 ± 22.8 cd
12	TML	2.4	301.4 ± 82.3 abcd	350.2 ± 68.7 a	291.8 ± 43.3 b	374.2 ± 111.0 b	68.4 ± 11.6 cd	103.8 ± 19.6 bcd	65.8 ± 23.5 cd
	CER		419.6 ± 136.7 ab	343.4 ± 83.0 a	267.2 ± 78.1 a	284.0 ± 59.9 bc	105.0 ± 22.5 cd	123.8 ± 37.2 bcd	129.4 ± 67.8 cd
4	TML	0.8	211.4 ± 54.3 bcd	275.6 ± 70.4 ab	282.4 ± 54.9 b	331.4 ± 65.5 bc	85.6 ± 31.9 cd	88.4 ± 21.7 cd	51.2 ± 21.8 d
	CER		351.6 ± 105.4 abc	235.4 ± 71.5 abcd	124.8 ± 10.0 d	240.6 ± 58.4 bcd	103.4 ± 55.3 cd	223.8 ± 65.5 ab	88.0 ± 10.5 cd
1	TML	0.2	330.4 ± 125.5 abc	251.4 ± 94.6 abc	147.8 ± 29.4 cd	273.2 ± 107.8 bc	97.8 ± 31.9 cd	271.6 ± 86.0 a	51.6 ± 17.0 d
	CER		191.4 ± 57.7 cd	114.2 ± 24.2 bcd	171.0 ± 54.3 bcd	245.0 ± 25.1 bcd	68.8 ± 24.3 cd	180.2 ± 45.1 abc	84.4 ± 18.5 cd
0.2	TML	0.04	178.8 ± 46.3 cd	166.8 ± 85.7 abcd	171.8 ± 64.0 bcd	180.0 ± 58.9 cd	57.8 ± 11.7 cd	140.2 ± 39.1 bcd	129.6 ± 52.9 cd
	CER		101.6 ± 12.8 d	79.0 ± 19.9 cd	81.2 ± 32.1 d	152.0 ± 41.1 cd	48.4 ± 13.9 d	156.6 ± 44.9 abcd	163.4 ± 65.1 bc
			84.8 ± 18.1 d	50.6 ± 5.9 d	71.0 ± 10.1 d	83.8 ± 22.3 d	35.4 ± 22.3 d	56.4 ± 6.4 d	79.2 ± 16.5 cd
			F = 2.909	F = 2.793	F = 3.356	F = 1.567	F = 1.105	F = 2.331	F = 2.542
			EMS = 29711.355	EMS = 22748.373	EMS = 9767.373	EMS = 21727.964	EMS = 3481.855	EMS = 9207.482	EMS = 31382.400
			P = 0.0070	P = 0.0091	P = 0.0026	P = 0.1488	P = 0.3798	P = 0.0264	P = 0.0163

* All data were analyzed by Fisher PLSD test; means ± standard error. Five treatments of the original balanced lattice design are not reported here.

† For each aging period, means followed by the same letter are not significantly different ($P > 0.05$; Fisher PLSD test).

‡ The Jackson trap was baited with standard polymeric plug dispenser containing 2.0 g of TML.

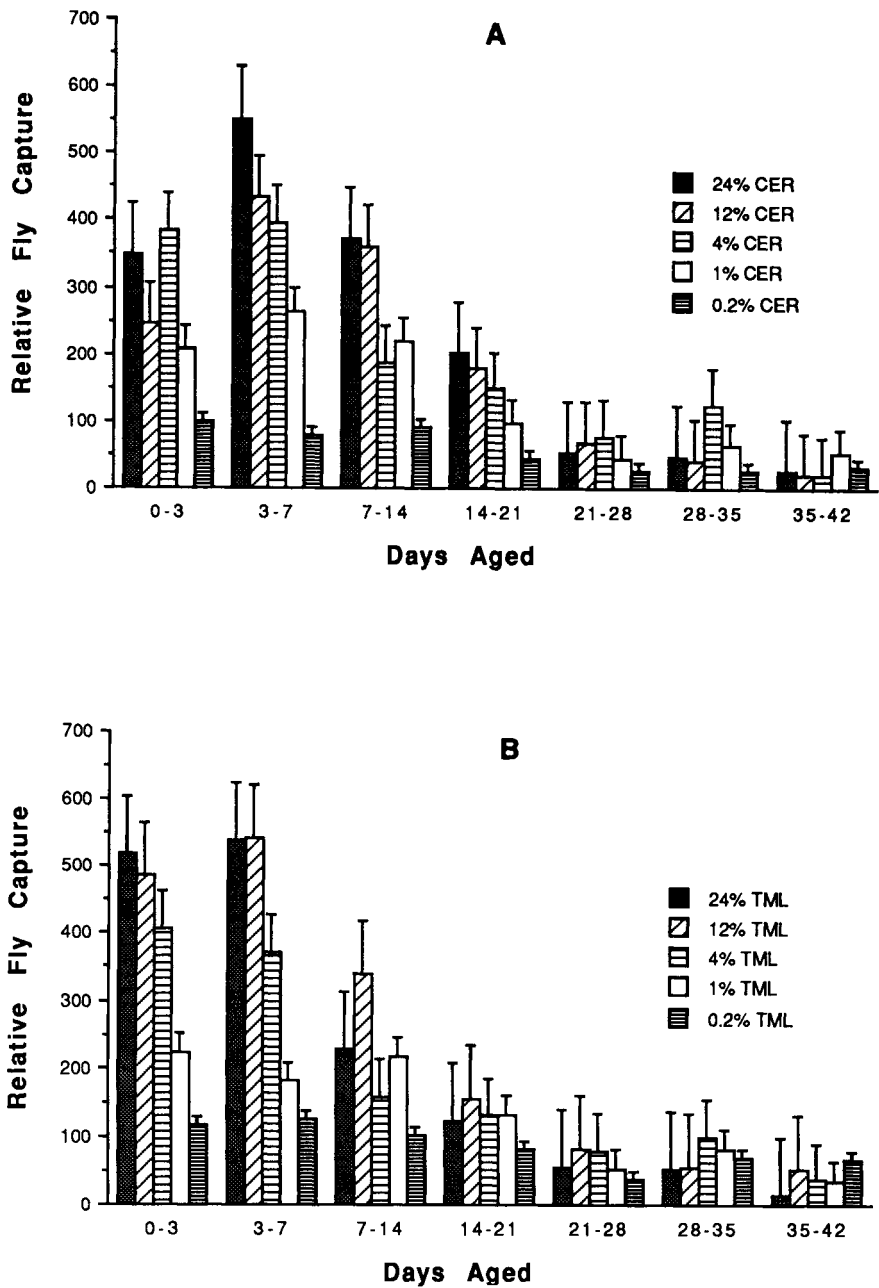


Fig. 2. Relative medfly captures of CER (A) and TML (B) (24, 12, 4, 1, and 0.2% in stickem coated panels) over a 42-day period (Test 1-H). Standard errors of the normalized data indicated by vertical bars; n = 10.

The linear regression of the relative fly capture vs. daily release rate for all concentrations of each lure (Fig. 3) shows a cluster of relative fly captures from 0-200 at release rates below 100 mg/day. Only when the rate exceeded 50 mg/day did the fly capture tend to increase. Only the 24 and 12% concentrations (4.8 and 2.4 g of lure, respectively) produced a release rate of 100 mg/day or higher for 14 days. Fly capture was not significantly improved with a two-fold increase in lure concentration (24 vs. 12%) showing a typical asymptotic dose-response curve for semiochemicals (Cunningham 1981).

In the Guatemala test (Test 1-G) conducted in a natural population of med-flies, the residual CER and TML contents from the 49 day dose/response test (Figure 4) paralleled those in the Hawaii test. The fly population was so low that distinction among treatments was not readily apparent. The results show that a larger number of flies was attracted to the CER and TML sticky panels during the first two weeks, and that, during this period, sticky panels were significantly better than standard Jackson traps (Table 2).

The persistence of CER in stickem was evaluated in a 12-week weathering experiment in Guatemala (Test 2-G). The data from over 450 samples (Fig. 5) indicated that approximately 50% of the CER was released during the first week of exposure. By the end of 3 weeks of exposure, about 80% of the applied lure had been released; only 1-2% of the applied CER remained at week 12.

Gas chromatographic analysis of the isomer composition of CER samples from the weathering test (Test 2-G) in Guatemala showed that the most attractive *trans* B₁ isomer increased from 22 to 29% of the total CER content after 2 weeks of exposure and remained at this level through week 12. These data suggest that the B₁ isomer is less volatile than the other isomers when incorporated in stickem, and this may account for CER's persistence in field tests. Similarly, the most attractive isomer of TML, *trans* C, has been shown to be more persistent than the other TML isomers (McGovern et al. 1987).

The release characteristics of a new controlled-release formulation of CER in a commercial polymer (Test 3-G) were compared with those of panels coated with CER in stickem (Fig. 6). The relative CER contents showed that the release pattern over time for the two thinnest ($7.5 \times 3.75 \times 0.12$ cm and $10 \times 5 \times 0.12$ cm) and highest surface area (30.83 and 53.6 cm²) slabs was similar to that shown by the CER in stickem for weeks 2, 4, and 6. By week 6, all three of these treatments contained only 12 to 19% of their original lure content. However, the two thicker ($2.5 \times 5 \times 0.6$ cm and $1.87 \times 2.5 \times 0.36$ cm) slabs which also had lower surface areas (12.5 and 7.82 cm²) contained 50-70% of their original CER dose at week 6. This suggests that the release rate of the lure is related to the thickness and surface area of the plastic dispenser. The controlled-release formulations using the plastic polymer slabs may offer prolonged effectiveness during exposure for CER, and the slabs can easily be attached to existing panels.

Discussion

The results of this research indicate that there was no significant difference in the persistence of CER and TML when incorporated in stickem and applied to plastic panels. Both CER- and TML-containing panels were highly effective for 2 to 4 weeks and continued to attract flies for 6 to 7 weeks. These coated panels

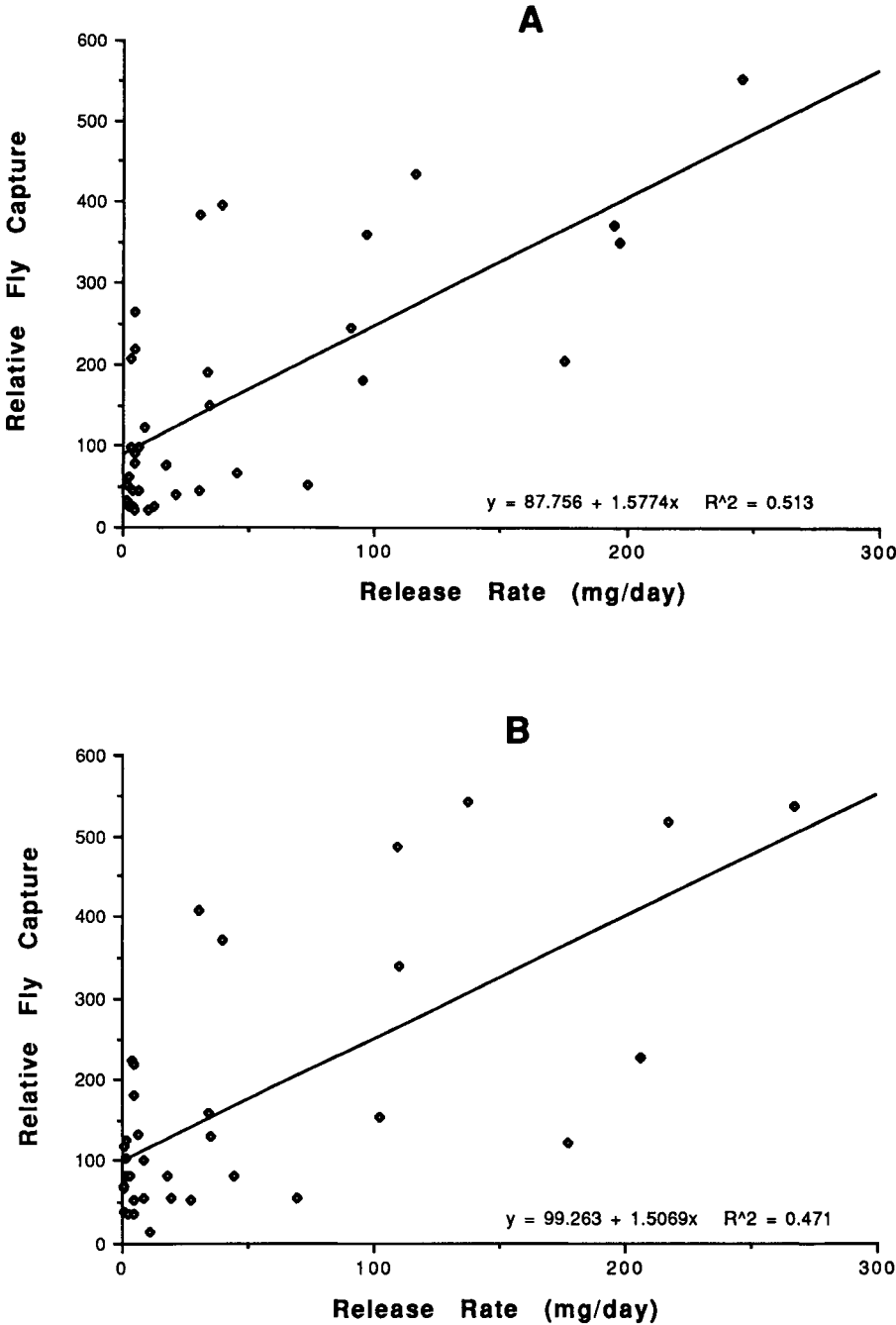


Fig. 3. Linear regression of the relative medfly captures vs. daily release rates for all concentrations of CER (A) and TML (B) (24, 12, 4, 1, and 0.2% in stickem coated panels) over a 42-day period (Test 1-H).

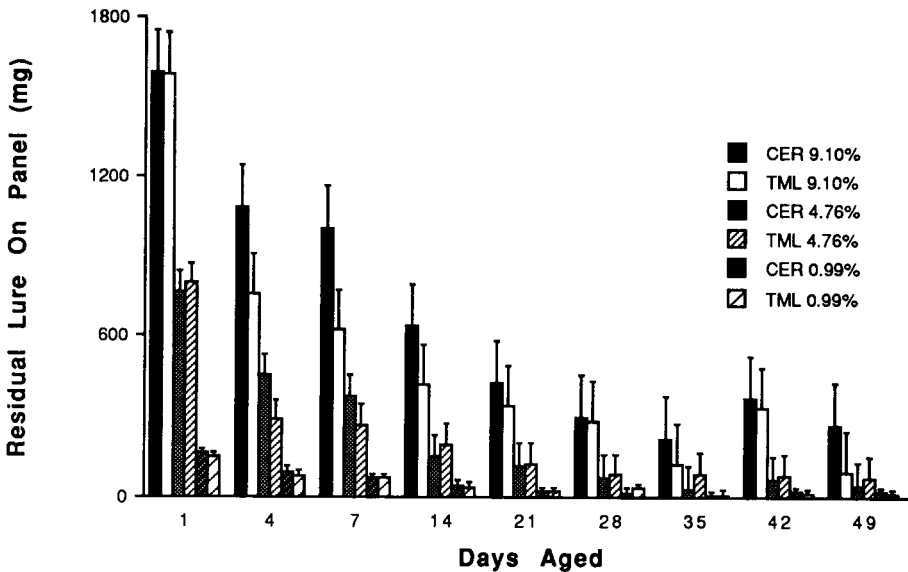


Fig. 4. CER and TML (9.10, 4.76, and 0.99%) in stickem-coated panels; residual contents over a 49-day aging period (Test 1-G). Standard errors of the data indicated by vertical bars; $n = 3$.

were more effective in attracting and capturing medflies than were Jackson traps baited with the standard 2 g TML polymer plug. This is unlike the case when these two lures are dispensed from cotton wicks where CER has a substantially longer residual life. The reported longevity for CER as compared to TML was not observed when CER was incorporated in a stickum mixture. These results suggest the need for a controlled-release dispensing system that regulates the release of lure in amounts which are required to maintain high fly capture over an extended period of time. The results also indicate that the most attractive CER isomer, *trans* B₁, appears to be less volatile than the other isomers, which may contribute to the persistence of CER in the field. Therefore, the use of CER or TML in stickem-coated panels may serve as an integral part in medfly integrated pest management programs for survey and detection and/or eradication via mass trapping without the use of insecticides.

Table 2. Mean medfly capture for TML and CER Stickem panels evaluated in test 1-G*.

Lure Conc. % in stickem	Lure	Dose	Days Aged†						
			0 - 7	7 - 14	14 - 21	21 - 28	28 - 35	35 - 42	42 - 49
Jackson Trap‡ 9.10	TML	2.0	0.1 ± 0.1c	0.1 ± 0.1 c	0.1 ± 0.1 c	0.4 ± 0.3	0.0 ± 0.0 c	0.0 ± 0.0 c	0.1 ± 0.1 c
	TML	2.0	7.0 ± 3.5 b	2.1 ± 0.9 abc	0.5 ± 0.2 bc	0.3 ± 0.2	0.1 ± 0.1 bc	0.4 ± 0.2 c	0.5 ± 0.3 c
	CER	2.0	7.1 ± 2.1 b	4.4 ± 1.9 a	1.30 ± 0.4 a	0.8 ± 0.3	0.1 ± 0.1 abc	0.2 ± 0.1 c	0.2 ± 0.1 c
4.76	TML	1.0	3.6 ± 1.5 bc	1.5 ± 0.5 bc	0.6 ± 0.2 bc	0.3 ± 0.2	0.0 ± 0.0 c	0.0 ± 0.0 c	0.1 ± 0.1 c
	CER	1.0	5.0 ± 1.6 bc	3.5 ± 1.6 bc	0.9 ± 0.3 ab	0.6 ± 0.3	0.8 ± 0.3 a	0.2 ± 0.1 c	0.0 ± 0.0 c
0.99	TML	0.2	1.7 ± 0.4 c	0.4 ± 0.2 c	0.0 ± 0.0 c	0. ± 0.2	0.50 ± 0.2 abc	0.10 ± 0.1 c	0.0 ± 0.0 c
	CER	0.2	2.9 ± 1.9 bc	1.1 ± 0.5 bc	0.4 ± 0.2 bc	0.30 ± 0.2	0.1 ± 0.1 bc	0.1 ± 0.1 c	0.0 ± 0.0 c
			<i>F</i> = 1.865	<i>F</i> = 2.243	<i>F</i> = 2.869	<i>F</i> = 0.679	<i>F</i> = 2.499	<i>F</i> = 3.341	<i>F</i> = 5.847
			EMS = 2395.60	EMS = 696.90	EMS = 43.70	EMS = 40.700	EMS = 0.371	EMS = 0.325	EMS = 0.393
			<i>P</i> = 0.0880	<i>P</i> = 0.0403	<i>P</i> = 0.0107	<i>P</i> = 0.6891	<i>P</i> = 0.0234	<i>P</i> = 0.0039	<i>P</i> = 0.0001

* All data were analyzed by Fisher PLSD test; means ± standard error. Five treatments of the original balanced lattice design are not reported here.

† For each aging period, means followed by the same letter are not significantly different (*P* > 0.05; Fisher PLSD test).

‡ The Jackson trap was baited with standard polymeric plug dispenser containing 2.0 g of TML and was replaced with a fresh dispenser biweekly.

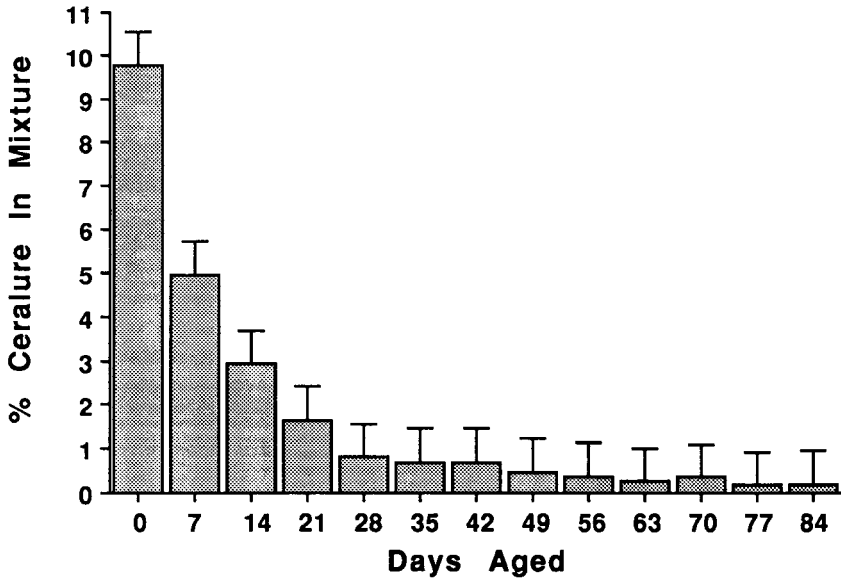


Fig. 5. Averaged residual CER contents in stickem over an 84-day weathering period (Test 2-G).

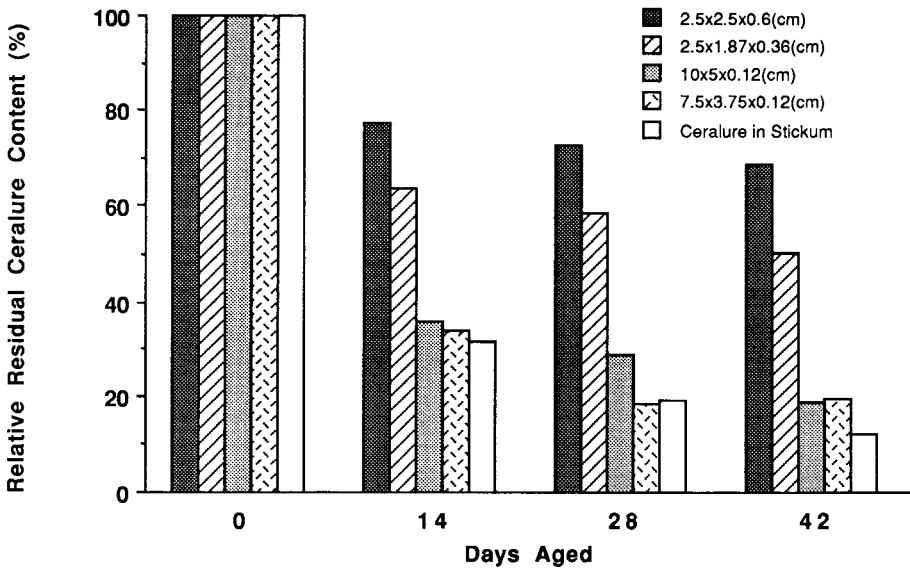


Fig. 6. Relative residual CER contents (% of original dose of lure remaining in plastic formulation) over a 42-day aging period.

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