tert-Pentyl 4 (and 5)-chloro-*trans*-2-methylcyclohexanecarboxylate, a Highly Effective and Persistent Male Specific Attractant for the Mediterranean Fruit Fly (Diptera: Tephritidae)[1,2]

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Abstract The attraction of male *Ceratitis capitata* (Wiedemann) to *tert*-pentyl 4(and 5)-chloro-*trans*-2-methylcyclohexanecarboxylate (or pentamedlure) was compared to its attraction to trimedlure to establish the suitability of pentamedlure as a possible alternate for trimedlure in applications that use a cotton dental-roll wick dispenser. Results from field tests with released sterile *C. capitata* showed that pentamedlure, applied to cotton dental roll wicks, was not only comparable to trimedlure in attractiveness but was more persistent. Batch to batch variations in isomer distribution of pentamedlure appeared to have little effect on attraction. Dose-response data showed that pentamedlure, applied at 0.02 and 0.16 ml/wick, initially caught more flies than trimedlure at comparable doses. Moreover, initial catches for pentamedlure for most dosages tested exceeded, but not significantly, initial catches for trimedlure despite a much higher load (2 ml/wick). Trimedlure initial catches within a natural population of wild *C. capitata* were significantly higher than pentamedlure catches at a comparable dosage (0.2 ml lure/wick). However, pentamedlure-treated wicks were 2 to 3 times more persistent than wicks treated with trimedlure.

Key Words Insecta, *Ceratitis capitata,* lure, pentamedlure, trimedlure analog, attractant.

Trimedlure, *tert*-butyl 4(and 5)-chloro-*trans*-2-methylcyclohexanecarboxylate, has been used as the standard male attractant for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), since the early 1960's when it replaced medlure, 1-methylpropyl 4(and 5)-chloro-*trans*-2-methylcyclohexanecarboxylate (Beroza et al. 1961). Although trimedlure is a highly effective attractant for *C. capitata,* users have identified its tendency to form crystals during cool weather and its high volatility from cotton wicks during warm weather as negative characteristics. Recent technological developments in the use of controlled-release dispensers (Leonhardt et al. 1987) have overcome both of those disadvantages. However, increased costs associated with a controlled-release system might hinder its acceptance and use in certain areas and,

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consequently, other acceptable alternatives are being sought. Data from earlier investigations on the use of additives in trimedlure as crystallization inhibitors (McGovern et al. 1987) and on efficacy evaluation of analogs of trimedlure isomer C (McGovern and Cunningham 1988a), helped identify certain esters as potential replacements for trimedlure when applied to cotton wicks. Those esters not only showed levels of initial attractiveness comparable to that of trimedlure, but they were longer lasting and did not form crystals when cooled. *tert*-Pentyl 4 (and 5)-chloro-*trans*-2-methylcyclohexanecarboxylate (pentamedlure) was selected from that group as the most promising alternative for trimedlure. Results from a more recent study (DeMilo et al. 1994) involving structure-activity correlations of 68 analogs of trimedlure confirmed pentamedlure's status as a promising attractant for *C. capitata.*

In this study, pentamedlure, applied to cotton wick dispensers, was field bioassayed to further define its efficacy and persistence against released laboratory-reared and natural populations of *C. capitata.* Results of those tests are reported herein.

Materials and Methods

Chemicals. Trimedlure was obtained commercially (UOP Chem. Div., East Rutherford, NJ). Pentamedlure was synthesized from 4 (and 5)-chloro-*trans*-2-methylcyclohexanecarboxylic acid using a non-epimerizing method to convert the acid to the corresponding ester through its acid chloride (Green and Beroza 1959). Purification by standard work-up procedures followed by distillation gave a liquid with a boiling point 65°C (0.1 mm Hg) and refractive index (n_D) 1.4598. The purity of the distilled pentamedlure was >95% as determined by capillary gas chromatographic analysis (Leonhardt et al. 1982). Seven batches of pentamedlure were synthesized for bioassay. The percentage of each of the principal *trans* isomers (A, B1, B2, C) was determined by previously described methods (Leonhardt et al. 1982). Typically, isomer distribution was similar to that reported by Leonhardt et al. (1982) for trimedlure: A, 25.4-28.9%; B1, 7.6-12.1%; B2, 18.2-22.0%; C, 27.8-40.5%). Observed differences in isomer distribution (Table 1) between batches were due to variations in synthesis and purification methods.

Field evaluation. Field tests with laboratory-reared sterile flies were conducted in

Table 1. Isomer content in different batches of pentamedlure

	% of the designated isomer*			
Batch	A	B1	B2	C
1	27.6	4.5	13.8	54.1
2	23.9	2.6	7.1	66.4
3	26.8	4.8	10.9	57.6
4	31.3	6.3	10.2	52.2
5	27.7	3.6	7.6	61.1
6	23.9	4.0	10.3	61.8
7	33.1	5.0	10.8	51.1

* A, B1, B2 and C are arbitrary descriptors to designate the relative order of elution of isomers from the gas chromatograph (McGovern et al. 1966).

a macadamia nut, *Macadamia integrifolia* Maiden and Betche, orchard at Keaau, HI. Test chemicals were pipetted neat onto cotton dental-roll wicks (9.53 mm diam × 12.7 mm length; Johnson and Johnson No. 2) secured in Jackson traps (Harris et al. 1971), which are the standard survey traps used in the large-scale detection trapping arrays in the continental U.S. Traps were hung on consecutive trees about 8 m apart and remained in the same position throughout a test. Laboratory-reared sterile adults (DeMilo et al. 1994) were released throughout the test plot at the beginning of each examination period over the course of the test. The removable sticky inserts in each trap were removed at each examination and replaced with unused sticky inserts. Used inserts were returned to the laboratory where the trapped flies were counted.

Three tests were conducted in which the relative attraction and persistence of trimedlure and pentamedlure were compared. Seven different batches of pentamedlure were evaluated. A randomized complete block design with 14 blocks (one replicate per block) was used in test 1, and 12 blocks were used in test 2. Dosages of 0.2 ml and 0.01 ml were used in tests 1 and 2, respectively. In all tests, traps with freshly-baited trimedlure wicks were added at the beginning of each test interval (daily, days 1-4). TML-treated wicks labelled "TML aged" (Table 2) were exposed in the field identically to PML-treated wicks for the seven batches of PML. A dose-response test was included as a third test with released flies; dosages of 0.02, 0.04, 0.08, 0.16, and 0.32 ml were applied to cotton wick dispensers. Traps with a standard 2 ml dose of trimedlure were included for comparison. A randomized complete block design with three blocks (one replicate per block) was used. The remainder of the bioassay procedure was the same as described earlier in this section.

Field tests with wild flies were conducted in coffee fields, *Coffea arabica* L, in the Kona district of the island of Hawaii. A paired *t*-test field plot design was used with 30 replicates. A 0.2 ml dosage was applied to a 9.53 mm diam × 25.4 mm length cotton dental-roll. Trap maintenance was the same as that used in the tests with released flies. Aged trimedlure wicks were replaced with freshly-baited trimedlure wicks at the beginning of the fifth test period (28 d). All catch data from the tests were transformed to the square root prior to analysis of variance. Means reported in the tables are back transformations of the means of the transformed data. Duncan's (1951) multiple range test of mean separation was used where appropriate.

Results and Discussion

The initial and persistent attractiveness of seven different batches of pentamedlure that varied in isomer distribution (Table 1) was determined in two tests using field-aged and fresh trimedlure for comparison. Results are presented in Table 2. The 7 d test period was divided into six test intervals. The dosage used in test 1 was large enough to allow pentamedlure to still catch high numbers of flies at d 7 (termination of test); a lower dosage in test 2 resulted in pentamedlure's almost complete depletion by d 7. Variations in isomer distribution in the seven test batches (Table 1) appeared to have little effect on pentamedlure's attractiveness or persistence.

Of the seven batches of pentamedlure, only batch 2 (test 1) appeared to have a larger initial catch (d 0) than trimedlure, but this difference was not significant. However, the initial catch and the catch at d 1 with pentamedlure was not significantly different from that of trimedlure. Data from these tests showed that pentamedlure was clearly more persistent than trimedlure, i.e., it remained competitive with fresh trimedlure and it lasted 2 to 3 times longer than aged trimedlure.

Table 2. Relative attraction of released *C. capitata* to trimedlure (TML) and pentamedlure (PML), Keaau, Hawaii

Lure	Batch no.	Weighted mean catch* per trap after indicated days exposure in the field					
		0	1	2	3	4	7
				test 1**			
TML fresh		108a	147a	197a	183a	131a	145a
TML aged		115a	139a	118b	41c	1c	0c
PML	1	111a	108a	131b	120b	86b	43b
PML	2	138a	111a	148ab	122b	116a	50b
				test 2†			
TML fresh		160a	184a	169ab	171a	175a	248a
TML aged		181a	137a	21c	0c	0e	0b
PML	3	120a	116a	122b	93b	58d	0b
PML	4	142a	144a	157ab	143a	70cd	0b
PML	5	136a	142a	137ab	117ab	64d	0b
PML	6	158a	153a	159ab	157a	116bc	0b
PML	7	140a	153a	164ab	132ab	92bcd	2b

test 1	test 2
day 0: F=0.94; df = 3, 39; not significant.	day 0: F=1.42; df = 7, 77; not significant.
day 1: F=2.13; df = 3, 39; not significant.	day 1: F=1.68; df = 7, 77; not significant.
day 2: F=3.19; df = 3, 39; $P < 0.05$.	day 2: F=11.97; df = 7, 77; $P < 0.01$.
day 3: F=17.43; df = 3, 39; $P < 0.01$.	day 3: F=36.42; df = 7, 77; $P < 0.01$.
day 4: F=97.09; df = 3, 39; $P < 0.01$.	day 4: F=22.78; df = 7, 77; $P < 0.01$.
day 7: F=56.65; df = 3, 39; $P < 0.01$.	day 7: F=110.37; df = 7, 70; $P < 0.01$.

* Catches followed by the same letter within a column within a test are not significantly different ($P > 0.05$; Duncan's [1951] multiple range test).

** 0.02 ml dosage; 14 replicates.

† 0.01 ml dosage; 12 replicates.

The relative attraction of trimedlure and pentamedlure was evaluated over a series of dosages (0.02 to 0.32 ml), and results are presented in Table 3. Two of the five dosages (0.02, 0.16 ml) tested for pentamedlure elicited a higher initial catch than that observed for trimedlure. Moreover, catches for most dosages at d 0 exceeded, but not significantly, those for traps containing 2 ml trimedlure. Since the catch for one of the lower dosages (0.32 ml) of trimedlure exceeded that of the 2 ml trimedlure trap, it is possible that the 2 ml dose caused a repellent effect; an effect often ascribed to the low initial catches associated with trimedlure dosages exceeding 2 ml (Nakagawa et al. 1971). While lower initial catches with high trimedlure dosages are sometimes observed in certain trap geometries (Nakagawa et al. 1971), it is apparent that high initial catches of *C. capitata* can be achieved within the geometry of the standard Jackson trap with a much lower concentration of trimedlure than the standard 2 ml dosage. Application of controlled-release technology has taken advantage of this fact (Leonhardt et al. 1987). The fact that the attractiveness of two treatments (0.02 and 0.16 ml) of pentamedlure were statistically superior to those of trimedlure at d 0,

Table 3. Relative attraction of released *C. capitata* to trimedlure (TML) and pentamedlure (PML) at various dosages, Keaau, Hawaii

Lure	Dosage (ml)	Weighted mean catch* per trap after indicated days exposure in the field				
		0	1	2	3	4
TML	0.02	53.6c	0.0d	0.0b	0.0c	0.3c
PML	0.02	131.6ab	19.7c	0.3b	2.6c	0.1c
TML	0.04	87.5b	0.2cd	0.2b	0.0c	0.0c
PML	0.04	99.9abc	88.9b	5.0b	0.1c	1.6c
TML	0.08	101.5abc	8.0cd	0.0b	0.1c	0.0c
PML	0.08	132.2ab	137.4ab	16.8b	0.4c	0.8c
TML	0.16	80.1bc	12.0cd	0.0b	0.4c	0.4c
PML	0.16	176.4a	158.2ab	150.1a	19.2c	1.3c
TML	0.32	135.5ab	195.9a	13.7b	0.9c	1.5c
PML	0.32	140.7ab	246.9a	270.7a	137.8b	60.1b
TML	2.00	110.2abc	189.0a	164.1a	271.6a	181.8a

day 0: F=2.65; df = 10, 20; $P < 0.05$.
day 1: F=19.92; df = 10, 20; $P < 0.01$.
day 2: F=17.99; df = 10, 20; $P < 0.01$.
day 3: F=17.62; df = 10, 20; $P < 0.01$.
day 4: F=29.48; df = 10, 20; $P < 0.01$.

* Catches followed by the same letter within a column are not significantly different ($P > 0.05$; Duncan's [1951] multiple range test). Means were determined from 3 replicates.

suggests that the attractiveness of pentamedlure might benefit more from controlled-release technology than would trimedlure.

The relative attractiveness and persistence of trimedlure and pentamedlure were evaluated against natural populations of wild *C. capitata,* and a summary of the data is presented in Table 4. The natural populations of *C. capitata* were low and variable in the Kona area of the island of Hawaii at the time of the test. Early in the test, catches for pentamedlure were significantly lower than those of trimedlure. Despite pentamedlure's lower initial catch, it continued to catch large numbers of flies 2 to 3 times longer than did trimedlure before it gradually lost its activity over a 4-wk period. In contrast, trimedlure lost its activity rather abruptly after 1 wk.

The molecular structure for pentamedlure is very similar to that of trimedlure, the difference being an extra methyl group attached to the ester moiety of pentamedlure. Although this difference may appear inconsequential, the increase that this additional group lends to the molecular weight of pentamedlure most likely is responsible for its lowered volatility and concomitant persistence. High and persistent attraction of ceralure, an iodo analog of trimedlure, to *C. capitata* has also been reported (McGovern and Cunningham 1988b, DeMilo et al. 1994). Ceralure's structure is also quite similar to that of trimedlure, varying only in the halogen and ester moieties. Ceralure's higher molecular weight (27% > than trimedlure) was also claimed as a reason (Warthen et al. 1994) for its low volatility and increased persistence.

In the test against a wild population of *C. capitata,* trimedlure appeared to attract

Table 4. Relative attraction of wild *C. capitata* to trimedlure (TML) and pentamedlure (PML), Kona, Hawaii

	Weighted mean catch* per trap after indicated days exposure in the field					
Lure**	0-1	1-7	7-14	14-21	21-28	28-36
TML	21.3a	48.8a	6.8b	0.0b	43.9a†	7.9a
PML	15.1b	34.8b	36.8a	24.3a	7.6b	0.0b

day 0-1: F=6.52; df = 1, 29; $P < 0.05$.
day 1-7: F=8.38; df = 1, 29; $P < 0.01$.
day 7-14: F=78.66; df = 1, 29; $P < 0.01$.
day 14-21: F=136.50; df = 1, 29; $P < 0.01$.
day 21-28: F=82.47; df = 1, 29; $P < 0.01$.
day 28-36: F=38.49; df = 1, 29; $P < 0.01$.

* Mean catches followed by the same letter within a column are not significantly different. Means were determined from 30 replicates.
** 0.2 ml dosage.
† Aged TML wicks were replaced with freshly baited TML wicks on day 21.

larger numbers of flies early in the test than did pentamedlure, and the difference was significant. While this may be considered a slight disadvantage for pentamedlure, pentamedlure's distinct advantage over trimedlure was its increased persistence while maintaining high levels of attraction. Further testing will be required to determine pentamedlure's future as an alternate for trimedlure. Nonetheless, results from these studies show it has promise. Pentamedlure's high attractiveness and persistence are positive attributes that in selected applications could lead to the use of less lure (an environmental plus) and less frequent rebaiting of traps (distinct advantage in large-scale trap deployment program).

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