Design, Effectiveness, and Performance Criteria of Dispenser Formulations of Trimedure, an Attractant of the Mediterranean Fruit Fly (Diptera: Tephritidae)

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USDA-ARS


ABSTRACT Controlled-release dispensers of trimedure (TML), a synthetic attractant of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), were evaluated in seven aging tests in natural and released populations. Insect captures and laboratory-measured release rates and residual contents showed that the cotton wick dispensers, which were formerly used for 2 g liquid TML (density = 1.0 g/ml), were generally ineffective after 2-4 wk of field exposure; a 4-g dose of TML increased the longevity, but not the initial biological activity of the cotton wick. Two controlled-release formulations, a polymeric plug (70% by weight TML) and a plastic laminate, extended the effective lifetime of 2 g TML to about 8 wk. A polymeric plug with 4 g TML further extended the duration of effectiveness to 12 wk or longer. Laboratory and field data suggested definitive values for relative insect capture, release rate, and residual trimedure content, which have been used as performance criteria in the purchase of trimedure dispensers.

KEY WORDS Insecta, Ceratitis capitata, trimedure, controlled release

The Mediterranean fruit fly, Ceratitis capitata (Wiedemann), is an established agricultural pest in many parts of the world, but a vigilant surveillance program has kept the continental United States free of established populations. About 40,000 traps are used to detect accidental introduction of this pest into the United States. Such introductions, including several in recent years, have been detected and then eradicated in Florida, Texas, and California.

The bait in the detection traps is trimedure (TML), a synthetic attractant, consisting of a mixture of eight isomers of the tert-butyl esters of 4- and 5-chloro-2-methylcyclohexane-carboxylic acids (Beroza et al. 1961). The four trans isomers constitute 90-95% of trimedure, the isomer designated as "C" (McGovern & Beroza 1966) is not only the most abundant (35-44%), but also the most attractive (McGovern et al. 1966, 1966, 1967).

Previous work showed that solid, controlled-release dispensers containing trimedure prolonged the effectiveness of the attractant and eliminated the frequent rebaiting that was necessary every 2-4 wk when the original cotton dental roll was used to dispense 2 g liquid TML (King & Landolt 1984; Leonhardt et al. 1984, 1987; Rice et al. 1984). Site contamination caused by accidental spillage of liquid trimedure when cotton wicks were rebaited also was eliminated by the use of solid dispensers. Because doses that are higher than 2 g TML are repellant to the Mediterranean fruit fly (Nakagawa et al. 1971), the 2-g dose, which is routinely used in detection programs, was selected for incorporation in most of the controlled-release formulations. Trimedure is released more slowly by these polymeric dispensers and, as a result, they generally attracted somewhat fewer insects than did the freshly treated cotton wick used as the reference. This disadvantage was overshadowed by an effective lifetime of about 5 wk for the solid polymeric plug dispenser containing 70% by weight TML. In contrast, the cotton wick dispenser was attractive for just 2-4 wk (Leonhardt et al. 1987).

Here we describe the evaluation of polymeric plug modifications to increase insect capture. A 4-g rather than 2-g dose in a polymeric plug and dispensers from two other commercial sources were included in the tests. Field tests were done in Hawaii with released insects, and in Egypt and Lebanon in natural populations. We describe a laboratory method for measurement of release rates under standard conditions of airflow and temperature. This method was used to measure the release rate of trimedure as a function of aging time in the field, as well as the effect of temperature on...
release rates from the cotton wick and solid dispensers. We used comparative laboratory and field data to rank dispensers and to develop preliminary performance criteria to be used for bid solicitation and procurement of dispensers for the USDA's detection program for the Mediterranean fruit fly.

**Materials and Methods**

Table 1 describes the six field tests that were done. The weathering site for the dispensers of Tests I and II was Parlier, Calif.; at the Kearney Agricultural Research and Extension Center, after each aging interval, 10 replicates of each formulation were sent to Hilo, Hawaii, for bioassay and 4 were sent to Beltsville, Md., for chemical analyses. A single exception was the membrane/disk dispenser in Test II; it was weathered in Hilo, under conditions similar to those for Test III. In Test III, the dispensers were aged and tested in mature *Macadamia integrifolia* Maiden & Betchey (macadamia nut) orchards near Hilo. The weathering and bioassay site for Test IV was a semi-isolated area of mixed, managed orchards (mainly guava, *Psidium guajava* L.) located about 800 km south of Alexandria, Egypt; no insecticides were applied in this area and the Mediterranean fruit fly population was moderate and well established. At each aging interval (Table 1) of Tests III and IV, two replicates of each formulation were chemically analyzed. In Test V, the weathering and bioassay site was a neglected orchard near the Bmeleh Coast of Lebanon; Test VI was done in a commercial orchard of peaches (*Prunus persica*) and citrus fruits (*Citrus spp.*) in Mazaat Khaish, Lebanon. Dispensers were not analyzed for content or release rate after the field tests in Lebanon.

**Bioassay.** The dispensers were mounted in Jackson traps with unobstructed ends (Harris et al. 1971). The traps were arranged in a randomized complete block design. In Tests I-III (at Hilo), the insect releases and captures were made by the procedure described by Leonhardt et al. 1987. Captures of native flies were recorded similarly in Tests IV-VI in Egypt and Lebanon. Weight averages of insects captured were back-calculated from square-root-transformed data, $\hat{\xi} = (\Sigma \sqrt{\hat{x}} / n)^{1/2}$. Data were subjected to analysis of variance and Duncan's (1955) multiple range test ($P > 0.05$).

**Dispensers.** Table 2 describes the dispensers that were evaluated. Three different sizes of polymeric plugs/rods (Dow Corning Corporation, formerly Agron, Montgomery, N.Y.) contained 70% by weight TML (density of TML = 1.0 g/ml) incorporated within a thermoset matrix (Polytrap); one (PP-2) was the standard plug design with 2 g TML that was previously shown to be effective (Leonhardt et al. 1987), and another was a double length of the same plug with 4 g TML (PP-4). The third size (PR-2) was a rodlike, longer version of the plug but with one-half its diameter; the greater surface area intended to release trimebule at a higher rate than the plug. Another type of formulation was a plastic laminate in which the polymeric inner.

<table>
<thead>
<tr>
<th>Code</th>
<th>Treatment</th>
<th>Nominal TML*</th>
<th>Size, cm$^3$</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
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<tbody>
<tr>
<td>CF-2</td>
<td>Cotton dental roll</td>
<td>2</td>
<td>1.3 x 5.01</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
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<td>Cotton dental roll</td>
<td>2</td>
<td>1.3 x 5.01</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CF-4</td>
<td>Cotton dental roll</td>
<td>4</td>
<td>1.3 x 5.01</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CA-4</td>
<td>Cotton dental roll</td>
<td>4</td>
<td>1.3 x 1.7</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>PP-2</td>
<td>Polymeric plug</td>
<td>4</td>
<td>1.3 x 3.4</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>-</td>
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<tr>
<td>PF-2</td>
<td>Polymeric plug</td>
<td>4</td>
<td>0.65 x 0.85</td>
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<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PR-2</td>
<td>Polymeric rod</td>
<td>2</td>
<td>4.5 x 8.2</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PL</td>
<td>Plastic laminate</td>
<td>2</td>
<td>1.3 x 3.4</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MD</td>
<td>Membrane disk</td>
<td>2</td>
<td>1.3 x 3.4</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Initial dose

$^{a}$d. diameter, l. length, and w. width

$^{b}$ Replaced with freshly treated cotton dental roll after each bioassay reading.
layer contained the trimekure and the outer layers were 200 μm polyvinyl chloride (PVC) film (Hercon Laboratories Corporation, South Plainfield, N.J.); this formulation was also tested previously (Leonhardt et al. 1987). Two tests included a disk-shaped dispenser (M-D) in which a black, semi-permeable membrane covering a microporous reservoir with the trimekure was sealed to a stiff, foil-faced backing (Bend Research, Bend, Ore.).

Chemical Analyses. The residual quantity of trimekure in each dispenser after the aging intervals was measured by gas chromatography on solvent extracts (50-800 ml of 1:1 hexane/acetone) of 10 dispensers. The external trimekure standard was a solution of pure, crystalline “C” isomer. A 30 by 155 mm innerdiameter wide-bore capillary column (Supelco, Bellefonte, Pa.) coated with SPB-1 non-polar) was used for analyses; it was set at 200°C for 12 min and then programmed to increase 50°C per min to 280°C where it was held for 10 min. All of the trimekure isomers were included in two peaks (7.4 and 8.0 min retention times) whose areas were summed for determination of trimekure contents.

Release rates were determined by weight loss in a recirculating oven (Thermo Model 2, Precision Scientific, Chicago) set at 28 ± 1°C, with 100 ml/min air stream from each of six ports within the oven purged to maintain the outside through holes drilled through the oven wall. Weight loss was recorded at 2-h intervals after an initial 1-h equilibration period; the average loss over a 6-h period was considered to be the emission rate.

Temperature Effect. Weight losses at oven temperatures from 23 to 40°C were used to measure the effect of temperature on the release rate from the dispensers.

Changes to the physical surface of the dispensers were observed in a 5-mo laboratory storage test at temperatures of 40°C (oven), 25°C (room), 3°C (refrigerator), and −26°C (freezer). The plugs were sealed in individual foil packages; the laminates and membrane disks were each packaged in foil pouches with 10 and 5 dispensers per pouch, respectively.

Results and Discussion

Test 1. The insect captures with the four controlled-release formulations were calculated relative to those with the reference, freshly treated cotton wick (C-F-2), and were generally below equivalency or 1.0 (Fig. 1). This reduced insect capture is consistent with the fact that the polymeric formulations prolong the effectiveness of the trimekure by reducing the release rate. The higher trimekure release rate by C-F-2 (Fig. 1) resulted in higher insect captures because of the dose-response relationship. However, we decided that fruit fly captures that are at least one-half (≥0.5) of those with C-F-2 were an acceptable compromise considering the sustained performance and other cited advantages achieved with the solid formulations. The polymeric rod (PR-2) and the aged cotton wick (C-A-2) dropped below 0.5 relative catch by week 6 and the polymeric plug PP-2 by week 10. The relative captures with the plastic laminate (PL) and the polymeric plug with 4 g TML (PP-4) were 0.5-1.0 of the number caught by the freshly treated reference C-F-2 throughout the 12 wk.

The residual trimekure contents (Fig. 1) showed the same relative performance as did the bioassays. After 6 wk of aging, the cotton wick (C-A-2) was essentially depleted of trimekure. At week 6, PR-2 contained only about 300 mg residual TML and the double-sized, polymeric plug (PP-4), the laminate (PL) and the standard plug (PP-2) had >500 mg; the formulations ranked in the same order by residual contents as by insect captures. After 12 wk, only two treatments (PP-4 and PL) had residual trimekure contents >400 mg; these caught at least one-half the number of insects as did the reference.

The release rates, as measured by weight loss in the laboratory oven, were also consistent with the
Table 4. Insect captures and residual contents for dispensers evaluated in Test III

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg no. insects captured during aging period, wk*</th>
<th>Residual content mg at wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-3</td>
<td>4-6</td>
</tr>
<tr>
<td>C-F-2</td>
<td>533.5a</td>
<td>142.9a</td>
</tr>
<tr>
<td>C-A-2</td>
<td>550.5a</td>
<td>45.5b</td>
</tr>
<tr>
<td>C-A-4</td>
<td>539.8a</td>
<td>135.9a</td>
</tr>
<tr>
<td>PP-2</td>
<td>582.0a</td>
<td>115.5a</td>
</tr>
</tbody>
</table>

*Weighted averages of insects caught as back-calculated from square-root transformed data. \( t = \frac{S}{\sqrt{n}} \). For each aging period, averages followed by the same letter are not significantly different (P > 0.05; Duncan’s [1955] multiple range test).

bioassay data and residual contents (Fig. 1). The rates of tridemtrile release from the laminate PL for the first 4–6 wk are artificially high, because one or more components in the laminate formulation other than tridemtrile also evaporated during the oven measurements. When volatile from PL were trapped for analysis, results showed that the initial release rate of tridemtrile (only) was about 1.2 mg/h rather than the 2–4 mg/h indicated by weight loss (Tables 3 and 4, Fig. 1). In general, when treatments gave a release rate ≥0.7 mg/h, insect catch did not differ significantly compared with that of the reference (C-F-2). However, dispensers with release rates of ≤0.1 mg/h captured less than one-half (<0.5) of those with the reference. Treatments with intermediate rates (about 0.3–0.6 mg/h) usually yielded captures that were ≥0.5 of those with the reference. On the basis of bioassay, residual contents and release rates, the aged formulations ranked as follows: PP-4 > PL > PP-2 > PR-2 > C-A-2.

Test II. Comparison of 4 g TML with 2 g TML on freshly treated wicks (C-F-4 and C-F-2) showed no significant difference in insect catch at any time. The release rates from the two doses were similar (Table 3), because the evaporation was limited by the surface area of the cotton wick. Insect captures among the treatments did not differ significantly after 0, 1, or 2 wk of aging. After 4 wk of aging C-A-2 was significantly less effective than all other in the bioassay (Fig. 2); treatment C-A-4 showed similar poor performance by week 6. At week 8 relative insect captures with the polymeric rox (PR-2) and the plug with 2 g TML (PP-2) were <0.5 compared with those with the freshly treated reference, C-F-2. The release rates were 0.2 am 0.1 mg/h, respectively. The laminate (PL) continued to be effective through week 12, but a relatively low insect catch at week 4 cannot be explained (Fig. 2). Through week 12, the insect captures with the 4-g TML plug (PP-4) were significantly different from those by C-F-4, th emission rate (0.7 mg/h) and residual content (77 mg) of PP-4 remained high at week 12. These results suggest that this treatment would be remains highly attractive for at least 12 weeks. Overall ranking of the aged test was PP-4 > PL > PP-2 > PR-2 > M-4 > C-A-4 > C-A-2.

Test III. No significant differences occur among the four treatments for the first 4 wk of this test (Table 4). Compared with the freshly treat reference, C-F-2, the aged cotton wicks with 2 (C-A-2) and 4 g (C-A-4) TML were depleted.

![Fig. 2. Insect captures relative to those with cotton dental roll, freshly treated with 2 g TML (C-F-2) at aging intervals of 0-2, 4, 6, 8, and 10-12 wk in Test II (15 July 1985–7 October 1985). Solid and dotted lines show equivalency to 1.0 and 0.5, respectively, of the capture with the reference, C-F-2.](image-url)
Table 5. Insect captures, residual contents, and release rates for dispensers evaluated in Test IV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg % insects captured during aging period, wk*</th>
<th>Residual content mg/cm² at wk</th>
<th>Release rate mg/hr at wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-3</td>
<td>4-5</td>
<td>6-10</td>
</tr>
<tr>
<td>C-F-2</td>
<td>14.8</td>
<td>45.2</td>
<td>622.9</td>
</tr>
<tr>
<td>C-A-2</td>
<td>13.6</td>
<td>35.0</td>
<td>251.9</td>
</tr>
<tr>
<td>PP-2</td>
<td>12.0</td>
<td>36.5</td>
<td>372.5</td>
</tr>
<tr>
<td>PL</td>
<td>12.0</td>
<td>35.0</td>
<td>254.0</td>
</tr>
</tbody>
</table>

*Weighted averages of insects captured as back-calculated from square-root-transformed data. $t = (\Sigma \sqrt{r_i}/n)^2$. For each aging period, averages followed by the same letter are not significantly different (P > 0.05, Duncan's 1955 multiple range test).

trime dulure and ineffective after 4 and 6 wk of aging, respectively. The polymeric plug with 2 g TML (PP-2) remained as attractive as the reference, C-F-2, for 9 wk and caught $\geq 0.5$ the number of flies as did the reference C-F-2 throughout 14 wk. Release rates were not measured in this test. The ranking of the aged treatments was PP-2 > C-A-4 > C-A-2.

Test IV. This test, done in a relatively low insect population in Egypt during hot weather, showed that the aged cotton wick (C-A-2) was ineffective after 3 wk, when the trime dulure release rate dropped below 0.5 mg/h (Table 5). The polymeric plug (PP-2), laminate (PL), and membrane/disk (M-D) remained as attractive as the freshly treated reference (C-F-2) for 5 wk. The higher residual contents at week 10 and the resulting higher release rates of PL as compared with PP-2 and M-D resulted in the higher insect captures for weeks 6-10. Treatment M-D was essentially deplete in trime dulure by week 8. Overall ranking of the aged dispensers based on insect capture was PL > PP-2 > M-D > C-A-2.

Tests V and VI. A comparison of dispenser formulations in Lebanon coastal orchards showed no significant differences in insect capture over a 9-wk period in a relatively high, natural population of C. capitata (Test V). The total number of flies captured with treatments PP-2, PL, and C-F-2 were 2,054, 1,864, and 1,599, respectively. In a light, natural infestation in Lebanon (Test VI), the laminate (PL) and the 2- and 4-g plugs (PP-2 and PP-4) caught 176, 167, and 160 flies, respectively, over 17 wk; the freshly treated cotton wick caught somewhat fewer flies (135). These tests indicated that the laminate and polymeric plug formulations were highly effective dispensers for trapping C. capitata in high and low natural populations.

Physical Features of Dispensers. The polymeric plug dispensers were easy to handle, particularly when placed in small weblike plastic baskets supplied by the manufacturer. With minimal precautions, contamination of the trap or the hands of the trapper was easily avoided. A laboratory test showed that the plugs did not deteriorate when stored in individual foil packages for 5 mo at -26°C (freezer) to 40°C (oven). Although some trime dulure (25-90 mg) condensed as crystals inside each foil pack-

age, this condensation could be minimized by reducing the air space in the package. In field use, the plug dispensers shrunk in size as the trime dulure was released. For example, the size of the PP-2 dispensers was reduced (± SEM) to 10.4 ± 0.3 mm in diameter and 13.1 ± 0.7 mm in length when the trime dulure content dropped to about 400 mg. At this 400-mg content, the release of the attractant essentially stopped and the plugs were transparent and glasslike in appearance; initially, they were opaque and chalklike.

The laminate dispensers required greater care in handling to avoid contamination and were difficult to mount in the trap, because they were so large. The laboratory storage test showed that the laminates developed some bubbles in the PVC outer layers after 5 mo at 3°C and -26°C in foil packages of 10 dispensers; this effect was less apparent when the dispensers were stored at 25° and 40°C. Effects of these bubbles on the performance of the laminate are unknown.

![Fig. 3 Laboratory-measured release rates as a function of temperature. Linear correlation coefficients, $r^2$. For PL and PP-2 were 0.97 and 0.95, respectively.](image-url)
The membrane-disk dispensers were also very difficult to place in the traps because of their large size and the presence of a film of liquid trimedlure on the membrane surface after the protecting film was removed. Storage of these dispensers in foil packages of five dispensers per package for 5 mo caused substantial leakage of trimedlure at all temperatures (−26°C to 40°C); liquid trimedlure coated the dispensers and the inside of the packages. Overall, the handling properties of the dispensers were ranked: PP-2 > PL > M-D.

**Effect of Temperature.** Laboratory-measured emission rates at temperatures from 24–40°C (Fig 3) showed linear correlation of the logarithm of rate with temperature for the laminate (PL) and polymeric plug (PP-2) dispensers. The equations for the log, rate of PL and PP-2 were (−0.971 + 0.05257) and (−1.375 + 0.04777), respectively, with T expressed as °C. The corresponding r² values for the two lines were 0.97 and 0.95, respectively. These results indicate that the emission rate increased 3.3 and 3.0 times for PL and PP-2, respectively, by a 10°C increase from 25 to 35°C. Therefore, the active lifetime of the dispensers is significantly affected by field temperatures, and dispensers should be changed more frequently in hot climates than in cool climates. This conclusion is in agreement with the bioassay results reported here and in Leonhardt et al. 1984.

**Conclusion.** The polymeric plug and laminate dispensers offer significant advantages compared with the cotton wicks by extending the useful life of a 2-g dose of TML and by substantially reducing the risk of contamination during the rebaiting process. These two solid dispensers gave similar performance in the series of six field tests with released and natural populations of *C. capitata*. Handling properties give the plug dispensers a significant advantage as compared with the laminate.

The performance of plugs with 4 g TML (PP-4) was best in Tests I and II; plugs generally overcame the initial reduction in insect captures caused by the lower emission rates from the solid dispensers. PP-4 dispensers remain highly attractive for at least 8–12 wk. The repellent effect caused by high doses of trimedlure (Nakagawa et al. 1971) was not apparent with PP-4 dispensers, probably because their emission rates were lower than those from cotton wicks loaded with just 2 g TML (Table 3). Notwithstanding the apparent advantages of PP-4, the USDA Animal and Plant Health Inspection Service (APHIS) detection program will continue to use the 2-g TML dispenser until experience with solid dispensers is gained in large-scale trapping arrays. The data presented in this manuscript have led to additional tests with PP-4 dispensers in anticipation that this dispenser will be adopted for general use in the future. The additional cost of trimedlure for a 4-g dose may be offset by the higher attractancy, the increased longevity, and reduced frequency of rebaiting the traps. This 4-g polymeric plug offers no disadvantage in handling, but a laminate with 4 g TML would be too large for the trap unless the formulation was effectively altered.

The rodlike, polymeric dispensers (PL-2) were relatively large surface area released trimedlure too rapidly during the first few weeks of aging and were more difficult to handle in baiting traps. Performance, handling, and storage characteristics of membrane-disk dispensers were poor.

**Performance Criteria.** On the basis of the data collected, we proposed performance criteria for trimedlure dispensers to be used in the USDA detection program; these criteria will be modified the PP-4 dispenser is adopted for use in the future. For purposes of convenience and year-round operation at temperatures, Hilo, Hawaii, was selected as the site for aging and bioassay of the dispenser. The following criteria were used in a solicitation of bids by USDA–APHIS as August 1986 for procurement of 500,000 polymeric plug dispensers at by the California Department of Food and Agriculture in 1988 for a similar number of plug dispensers.

The dispensers shall
1. initially contain 2.0 g TML;
2. after 1 wk field aging in Hilo, Hawaii, contain at least 1.0 g residual TML and release trimedlure at a rate of ≥0.7 mg/h at 28°C in a laboratory test;
3. after 6 wk field aging in Hilo, Hawaii, contain at least 0.4 g residual TML and release trimedlure at a rate of ≥0.4 mg/h at 28°C in a laboratory test;
4. after 1 and 6 wk field aging in Hilo, Hawaii, capture at least one-half the number of flies caught by a standard, freshly treated cotton wick with 2 g TML in a bioassay test with released flies in Hilo;
5. have handling and storage characteristics that substantially reduce the risk of contamination in baiting traps and allow storage without deterioration for at least 1 yr.

**Acknowledgment.**

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**References Cited**


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