# Response of Nontarget Insects to Methyl Eugenol, Cue-Lure, Trimedlure, and Protein Bait Bucket Traps on Kauai Island, Hawaii, USA

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Abstract. Captures of nontarget insects inside fruit fly (Diptera: Tephritidae) traps placed in native habitats at three localities on Kauai Island, Hawaiian Islands were evaluated. Significantly greater nontarget capture rates were found for traps separately baited with four different attractants, methyl eugenol (five species and two morphospecies), cue-lure (two species), trimedlure (three species and one morphospecies), and protein bait traps (one species and one morphospecies) compared to unbaited control traps. These findings suggest that nontarget insects are attracted to those types of fruit fly traps. However, because of the presence of large numbers of scavenger flies captured in our traps, it appears that attraction may have partially been due to lure and protein bait, and, in part, to decaying insects accumulating inside the traps. Nonetheless, fruit fly traps placed in environmentally sensitive habitats should be used sparingly, so as not to place a strain on populations of rare endemic species.

**Key words:** methyl eugenol, cue-lure, trimedlure, protein bait, nontarget insects, Tephritidae, male lures, bucket traps

## Introduction

Fruit fly (Diptera: Tepritidae) lures and baits have long been employed for monitoring and eradicating insect pests. Methyl eugenol (4-allyl-1,2-dimethoxybenzene-carboxylate) is a kairomone (Metcalf and Metcalf 1992) that is attractive to many species of the Subfamily Dacinae (Tephritidae) in the South Pacific Region (Hardy 1979). It is known to occur in more than 200 plant species (Tan and Nishida 1996). Methyl eugenol was used for male annihilation or killing of sexually immature males before they were able to mate with females (Steiner 1952) to eradicate established populations of oriental fruit fly (*Bactrocera dorsalis* (Hendel)) in the Mariana Islands (Steiner et al. 1970) and Okinawa (Koyama et al. 1984). Cue-lure (Beroza et al. 1960), a derivative of anisylacetone (4-[p-methoxyphenyl]-2-butanone) (Barthel et al. 1957, Beroza et al. 1960), is an unstable synthetic compound

that is sensitive to hydrolysis (Metcalf and Metcalf 1992). Cue-lure is not known to occur naturally, but its hydrolyzed form, raspberry ketone (4-[p-hydroxyphenyl]-2-butanone) (Beroza et al. 1960, Metcalf and Metcalf 1992), is known to occur in at least one plant species (Nishida et al. 1993). In an areawide male annihilation test (5.2 km<sup>2</sup> plot) using cuelure in Hawaii, numbers of melon fly (*B. cucurbitae* (Coquillett)) males were reduced by over 99% for 60 days (Cunningham and Steiner 1972). Trimedlure (tert-butyl 4 [and 5]chloro-trans-2-methylcyclohexane-1-carboxylate) is the most attractive synthetic male lure known for Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) (Beroza et al. 1961). Trimedlure is not effective as a standalone tool for male annihilation of *C. capitata* (Barinaga 1991), but it is important for survey, detection, and monitoring purposes (Mitchell and Saul 1990). Protein bait (PIB-7 or Staley's Protein Insecticide Bait No. 7 = Nu-Lure; Miller Chemical and Fertilizer Co., Hanover, PA) (Steiner et al. 1961, Prokopy 1992) has been used as a standard bait in Mexico and California for fruit fly detection (Lopez and Becerril 1967) and control (Jackson and Lee 1985).

Concern for the unintended consequences of insect pest eradication on endemic and beneficial insects grew among scientists and the public during the 1980s (USDA-APHIS 1985). Subsequently, several authors (Asquith and Messing 1992, Asquith and Kido 1994, Kido and Asquith 1995, Kido et al. 1996, Asquith and Burny 1998, Howarth and Howarth 2000) reported that Hawaiian species of nontarget arthropods were attracted to methyl eugenol, trimedlure, and protein bait (PIB-7). Mitchell and Saul (1990) believed that the high economic and environmental costs of fruit fly eradication would cause scientists to abandon eradication and rely on areawide integrated pest management techniques for control of fruit flies. With the implementation of an areawide pest management (AWPM) program (Faust and Chandler 1998) in the Hawaiian Islands in 1999, an environmental component was set up to examine the nontarget effects of fruit fly monitoring traps.

Populations of endemic insect species are rare and aggregated in disturbed, nonnative habitats at lower and middle elevations throughout the Hawaiian Islands and associated with few native plants, isolated remnant native forests, or, less commonly, introduced plants to which a few species of endemic insects have adapted (Zimmerman 1948, Gagne 1988, Howarth and Mull 1992, Asquith and Messing 1992). Most remaining native insect species exist in native rainforests between 609 and 1,524 m elevation (Hardy 1965, Thornton 1984, Asquith and Messing 1992). Thus, our objective was to conduct experiments to determine the possible attraction of methyl eugenol, cue-lure, trimedlure, and protein bait bucket traps to nontarget insects in native habitats where endemic species are abundant.

#### Materials and Methods

Bucket traps (Hafraoui et al. 1980) were fabricated with opaque white plastic cups (Highland Plastics, Pasadena, CA, LT-408-30-N; 11.5 cm diam, 12.3 cm high) and lids (PF-408-32-N; 11.7 cm diameter). Galvanized wire was used for handles and wick holders. Dental cotton wicks (3 cm long, 0.9 cm diam) were used as attractant dispensers. Four evenly spaced 2.2 cm diameter entrance holes were drilled into the upper sides of each cup.

Research was conducted on Kauai Island, which was designated as the first target island for a proposed fruit fly eradication program (Vargas and Nishida 1988). Three localities with predominantly native vegetation were selected (Fig.1) on the basis of variation in vegetation types and terrain, and relative ease of access by 4-wheel drive vehicles and hiking trails. On the northeast side, four trap sites (PL1, PL2, PL3, and PL4) were established in open native scrub communities along Power Line Road. On the south side, one trap site (AD) was established above Alexander Reservoir near Mt. Kahili in open scrub. On the northwest side, six trap sites were selected: AW on Awaawapuhi Trail in a wet ohia

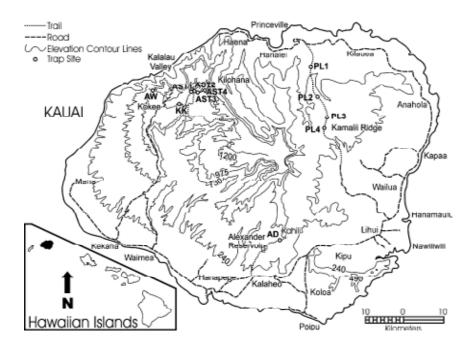


Figure 1. Location of trap sites on Kauai Island.

(*Metrosideros polymorpha* Gaud.) forest above an altitudinal band of koa (*Acacia koa* A. Gray); KK next to Kauaikinana Stream in a disturbed native forest; and AST1, AST2, AST3, and AST4 on Alakai Swamp Trail, on a low narrow ridge in a closed ohia forest.

The responses of nontarget insects to attractant bucket traps were tested from November 1987 to April 1988. Each trap wick was loaded with 2 ml of 5% naled (Dibrom 8 Emulsive, Chevron, San Ramon, CA) plus one of the following: (1) methyl eugenol, (2) cue-lure, (3) trimedlure, (4) protein bait (1 part concentrated PIB-7 to 13.7 parts water), or (5) water (control) with a 1 ml plastic dropping pipette. Traps were hung individually on native shrubs about 10 m or more apart. Trap wicks were replaced with freshly treated wicks during each service date. Long distances separating each locality (Figure 1), remoteness of most trap sites, and difficulty in scheduling field trips made it impossible to service all of the traps at 14-day intervals (Fig. 2). Although many B. dorsalis individuals were collected consistently in every methyl eugenol trap, these flies were not scored. Only one B. cucurbitae was collected in a cue-lure trap at trap site PL4. C. capitata was never collected in trimedlure traps. In this experiment, no effort was made to control the influence of decaying insects collected or to sterilize the traps. Nontarget insects were removed from traps with a sable brush saturated with 75% methanol and transferred to 1-dram vials containing 75% methanol. Collected insects were identified to the lowest taxonomic category possible, but the relative lack of taxonomic information (Beardsley 1990) and the poor condition of some decayed insects made identification to the species level impossible. Unidentified species were grouped according to their morphological similarities and are designated as morphospecies herein. All nontarget insects were deposited in the Insect Museum at the

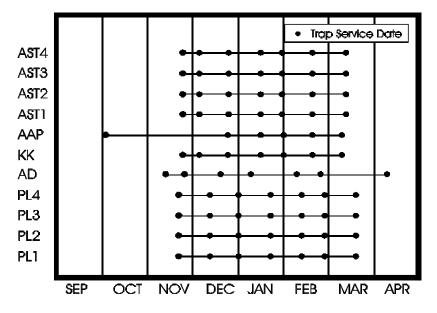


Figure 2. Fruit fly trap collection data points at 11 locations on Kauai Island.

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Data were analyzed using a generalized, linear, mixed models approach with a GLIMMIX.SAS macro (Littell et al. 1996), and a log link function to model count data as a Poisson distribution. Because of the large numbers of zeros, a constant of 0.5 was added to all counts prior to analysis. Whenever possible, count data for attractant types with all zero counts were dropped from the analysis and reported herein as zeros without confidence intervals. When only one attractant type containing nonzero counts was available for a species or morphospecies, it was analyzed with one other attractant type with all zero counts. Estimates and associated asymmetrical confidence intervals are reported herein as either positive or negative values. Depending on which criterion gave minimum fit statistics: Akaike's Information Criterion (AIC), a finite sample corrected version of AIC (AICC), or Schwartz's Bayesian Criterion (BIC), the covariance structure among periods was modeled as independent, first-order autoregressive, or Banded Toeplitz. This repeated measures model was fitted with both random (site and attractant type x site) and fixed (attractant type, day, and attractant type x day) effects. Individual t-tests (Steel and Torrie 1980) were used to find significant differences (P < 0.05) among attractant type x day means. Overall tests of significance for attractant type x day fixed effects, least square means, and 95% confidence intervals transformed back to original counts are reported herein.

#### **Results and Discussion**

Eighty species and 75 morphospecies were collected during this study. In our study, 2,377, 376, 756, 189, and 353 nontarget insect specimens were collected in methyl eugenol, cuelure, trimedlure, protein bait, and control traps, respectively. There were 43.5% endemic,

37.2% introduced, 1.8% biological control, and 17.5% unidentified species plus morphospecies among the nontarget insects collected in all traps. Significant capture means and 95% confidence intervals by attractant type and period (P<0.05), periods with significant means, and residency status are reported for 10 species and two morphospecies of nontarget insects in Table 1.

Four types of effects are observed in the analyzed data. (1) For Drosophila basimacula Hardy, Scatomyza (Tantalia) varipicta Hardy (Drosophilidae), Pseudosmittia maculiventris (Edwards) (Chironomidae), Atherigona orientalis Schiner (Muscidae), and Psychoda *williamsi* Quate (Psychodidae), main effects of attractant type (F = 0.72, df = 2, 24.1; P = (0.4977), (F = 0.99, df = 9, 33.6; P = 0.4075), (F = 0.60, df = 2, 33.5; P = 0.5560), (F = 0.85, 0.4977) df = 1, 21.5; P = 0.3664, and (F = 0.37, df = 3, 53.6; P = 0.7767), respectively, were not significant. Main effects of period (F = 1.84, df = 5, 108; P = 0.1113), (F = 0.97, df = 5, 140; P = 0.4390, (F = 1.46, df = 5, 135; P = 0.2060), (F = 1.35, df = 5, 61.3; P = 0.2558), and (F = 2.02, df = 5, 185; P = 0.0772), respectively, were not significant. Main effects of attractant type x period interaction (F = 5.07, df = 10, 114; P = <0.0001), (F = 4.40, df = 15, 154; P = <0.0001), (F = 2.53, df = 10, 135; P = <0.0081), (F = 2.94, df = 5, 61.3; P = 0.0193), and (F = 3.41, df = 15, 185; P = <0.0001), respectively, were significant. For D. basimacula (periods 1, 2, and 3) and A. orientalis (periods 3 and 5), methyl eugenol traps collected greater numbers than were in control traps. More of S. (T.) varipicta were collected in methyl eugenol (periods 1, 3, and 5), cue-lure (period 4), trimedlure (period 4), and protein bait (period 6) traps than in control traps. In contrast, P. maculiventris and P. williamsi were collected in greater numbers in control traps than in methyl eugenol, cue-lure, trimedlure, and protein bait traps during period 4. (2) For Drosophila suzukii (Matsumura), main effect of period (F = 1.31, df = 5, 189; P = 0.2628) was not significant. Effects of attractant type (F = 11.98, df = 4, 60.6; P = < 0.0001) and attractant type x period interaction (F = 2.39, df = 20, 208; P = 0.0012) were significant. Methyl eugenol (periods 2 and 3) and cue-lure traps (period 3) collected greater numbers than in control traps. (3) For Drosophila perissopoda Hardy, Drosophila sp. 1, Scaptomyza (Trogloscaptomyza) sp. 1, Fannia pusio (Wiedemann) (Fanniidae), and Euxesta annonae (Fabricius) (Otitidae), main effects of attractant type (F = 1.25, df = 2, 19.9; P = 0.3086), (F = 0.29, df = 3, 43.1; P = 0.8320), (F = 0.07, df = 1, 96.9; P = 0.7857), (F = 0.16, df = 1, 27.2; P = 0.6948), and (F = 0.04, df = 2, 16.9; P = 0.9603), respectively, were not significant. But effects of period (F = 3.52, df = 5, 96.5; P = 0.0058), (F = 5.68, df = 5, 185; P = <0.0001), (F = 4.37, df = 5, 96.9; P = 0.0012), (F = 6.85, df = 5, 96.9; P = 0.0012)91.6;  $P = \langle 0.0001 \rangle$ , and  $(F = 8.15, df = 5, 77.6; P = \langle 0.0001 \rangle$ , and attractant type x period interaction (F = 2.16, df = 10, 104; P = 0.0258), (F = 7.44, df = 15, 185; P = <0.0001), (F = 7.83, df = 5, 96.9;  $P = \langle 0.0001 \rangle$ , (F = 7.48, df = 5, 91.6;  $P = \langle 0.0001 \rangle$ , and (F = 3.00, df = 10, 86.6; P = 0.0027), respectively, were significant. For D. perissopoda Hardy (periods 1, 2, and 3) and D. sp. 1 (periods 2 and 4), greater numbers were collected in methyl eugenol traps. Additionally, both species were collected, respectively, in greater numbers during periods 1 and 2 in trimedlure traps than in control traps. Scaptomyza (T.) sp. 1 was collected in greater numbers in methyl eugenol (period 3) and protein bait (period 6) traps than in control traps. Conversely, F. pusio and E. annonae were collected in greater numbers in control traps than in methyl eugenol traps during period 1. (4) For Forcipomyia hardyi Wirth and Howarth, main effects of attractant type (F = 5.89, df = 4, 63.6; P = 0.0004), period (F = 2.55, df = 5, 233; P = 0.0287), and attractant type x period interaction (F = 3.87,  $df = 20, 233; P = \langle 0.0001 \rangle$  were significantly different. Trimedlure traps collected greater numbers than in control traps during periods 2, 5, and 6.

Methyl eugenol traps collected three endemic species, two introduced species, and two endemic morphospecies of nontarget insects in significantly greater numbers than did control traps (Table 1). Our results suggest that methyl eugenol traps are attractive to nontarget

Table 1. Response of nontarget insects to methyl eugenol, cue-lure, trimedlure and protein bait traps, compared with control traps placed in native habitats at three localities on Kaui Island.	s to m (aui Is)	ethyl eugenol, cue-lu land.	ıre, trimedlure and p	orotein bait traps, c	ompared with cont	rol traps placed in
Family Taxon / Status	Period	Methyl eugenol	Cue-lure	Trimedlure	Protein bait	Water (control)
Ceratopogonidae <i>Forcipomyia hardyi</i> Wirth and Howarth / E	0 v 5	N.S. N. S. N. S.	N. S. N. S. N. S.	2.71 (0.90–6.83)a 6.11 (2.52–13.98)a 3.16 (1.11–7.80)a	N. S. N. S. N. S.	-0.25 (-0.45-0.88)b -0.10 (-0.40-1.18)b -0.22 (-0.44-0.97)b
Chironomidae Pseudosmittia maculiventris (Edwards) /1	4	0.00 (-0.11–0.14)b	-0.01 (-0.11-0.13)b	0.00b	0.00b	0.30 (0.15–0.48)a
Drosphilidae Drosophila perissopoda Hardy / E	- 0 4	3.17 (1.01–8.42)a 5.99 (2.37–14.20)a 5.69 (2.22–13.58)a	N. S. S. S.	1.34 (0.07–5.40)a N. S. N. S	N. S. N. S. S. S.	00.00 0000 0000
Drosophila sp. 1 / E	004	0.33 (0.02–0.80)b 0.33 (0.02–0.80)b	N.S.	(0.93–2.58)a	i N N	0.00c 0.00b
Drosophila suzukii (Matsumura) / I	1010	9.70 (4.99–18.45)a 17.56 (9.54–32.01)a	N. S. 6.20 (2.89–12.75)a		i S Z	-0.04 (-0.42–2.29)b 0.06 (-0.39–2.41)b
Scaptomyza (Tantalia) varipicta Hardy / E	n − co 4 w	0.36 (0.09–0.76)a 0.44 (0.17–0.84)a N. S.	N. S. N. S. 0.26 (0.02–0.61)a	0.03-0.62)a	N N N N N N N N N N N N N N N N N N N	00.0 000.0 000.0 000.0
Scaptomyza (Trogloscaptomyza) sp. 1 / E	0 3 0 1	0.20 (0.02–0.00)a N. S. 1.72 (0.80–3.29)a N. S.	N. N		N. S. 1.01 (0.60–1.57)a N. S. 1.10 (0.41–2.32)a	0.000 0.000 0.000 0.000
Muscidae Atherigona orientalis Schine / I	er vi	0.38 (0.16–0.67)a 0.01 (-0.16–0.20)a	N. S. N. S.	N. S. N. S.	N. S. N. S.	0.00 (-0.15–0.21)b 0.20 (0.01–0.45)b

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Fanniidae <i>Fannia pusio</i> (Wiedemann) / I 5 0.00 (-0.26–0.55)b	0.00b	0.00b	0.00b	2.04 (0.99–3.82)a
Otitidae <i>Euxesta annonae</i> (Fabricius) / I 5 N. S.	0.00b	0.00 (-0.21–0.37)b	0.00b	1.07 (0.53–1.90)a
Psychodidae Psychoda williamsi Quate / E 4 0.00 (-0.15–0.21)b	0.10 (-0.07–0.33)b	0.01 (-0.15–0.20)b 0.00b	0.00b	0.72 (0.45–1.07)a

Student's t-test (Agresti 2002)). Only trapping periods with significant means and their 95% confidence intervals are reported. N.S. = means not statistically significant. Status: E = endemic, I = introduced.  $|\Sigma|$ 

insects. Although other experimenters found attraction to methyl eugenol traps, Kido et al. (1996) surmised that a plant-associated species *D. perissopoda* is attracted to methyl eugenol. Asquith and Kido (1994) who used ethylene glycol to remove the influence of decaying insects in traps suggested that *D. perissopoda*, *D. basimacula*, and *S. (T.) varipicta* were attracted to methyl eugenol. Conversely, because drosophilid adults are attracted to decaying plant and animal matter, and their larvae feed on yeast and other microorganisms in decaying matter (Hardy 1965, Goff et al. 1986), we also suspect that *D. perissopoda*, *D. basimacula*, *D. sp. 1, D. suzukii*, *S. (T.) varipicta*, and *S. (T)* sp. 1 were attracted to captured decaying *B. dorsalis* in our methyl eugenol traps. *A. orientalis* is suspected of being common around decaying plant and animal matter, and the larvae were reported feeding on fly larvae including *B. cucurbitae* (Hardy 1981). Therefore, we suspect that *A. orientalis* was not only attracted to methyl eugenol traps, but also to captured decaying *B. dorsalis*.

Cue-lure traps collected significantly greater numbers of one endemic and one introduced species than did control traps, which suggests that these nontarget insects are attracted to cue-lure traps. In contrast, Kido and Asquith (1995) reported no apparent attraction of aquatic species to cue-lure traps placed in swift water habitats. Differences in results may be due to the sampling of insects associated with aquatic habitats by Kido and Asquith (1995), we sampled terrestrial habitats distant from aquatic environments. But, because *D. suzukii* and *S.* (*T.*) *varipicta* are known to breed in decaying plant and animal matter (Hardy 1965), it appears that not only were these species attracted to cue-lure traps, but also to randomly captured decaying insects (Kido et al. 1995), which were the first to enter our traps accidentally.

Trimedlure traps collected two endemic species, one introduced species, and one endemic morphospecies in significantly greater numbers than did control traps. Our results suggest that nontarget insects are attracted to trimedlure traps. As mentioned previously, *D. perissopoda*, *D.* sp. 1, and *D. suzukii* breed in decaying plant and animal matter. Additionally, *E. annonae* was reported as breeding as a scavenger in various types of organic matter, such as banana and manure (Hardy 1980). Also, a saprophagous insect *F. hardyi* was reported as common around decaying plant matter in mountains and wet lowlands (Williams 1944). Because these nontarget insects are known to breed in decaying plant and animal matter, we suspect that these insects were not only attracted to trimedlure traps, but also to randomly captured decaying insects (Kido et al. 1996).

Protein bait traps collected significantly greater numbers of one endemic species and one endemic morphospecies of nontarget insects than did control traps, suggesting that nontarget insects are attracted to protein bait traps. Although we concur with Asquith and Messing (1992) that *S*. (*T*.) *varipicta* is attracted to protein bait traps, the authors suggested that protein bait was the attractive source. Based on the previous discussion, we suspect that *S*. (*T*.) *varipicta*, as well as, *S*. (*T*.) sp. 1 were attracted not only to protein bait traps, but also to randomly captured decaying insects (Kido et al. 1996).

Unbaited control traps collected significantly greater numbers of one endemic species and three introduced species of nontarget insects than did lure and bait traps. Our results suggest that control traps are attractive to nontarget insects. Among the four species, *P. williamsi* and *P. maculiventris* breed in decaying vegetation, and *F. pusio* and *E. annonae* breed in decaying plant and animal matter. Logically, saprophagous insects would be expected to respond to decaying insects collected in lure and bait traps. *Euxesta annonae* was reported to be positively correlated in abundance with either or both *B. dorsalis* and introduced drosophilids (*D. suzukii* and *Drosophila immigrans* Sturtevant) by Kido et al. (1996) who surmised that this carrion associated species was attracted to dead insects in traps. Although the capture means of nontarget insects in control traps were low (Table 1), we suspect that randomly captured insects (Kido et al. 1996) accumulated in sufficient num-

bers in these traps to attract other nontarget insects. Field data show that when *P. williamsi*, *P. maculiventris*, *F. pusio*, and *E. annonae* were present in a control trap, five or more different nontarget individuals were also present.

Traps themselves, quantity of naled, and trap physical characteristics (i. e. color) were constant for all attractant and control traps. Apparently, a positive response to attractant or attractant plus naled and decaying target and nontarget insects in treatment traps, and a positive response to water and decaying nontarget insects in control traps had occurred. A response to water seems unlikely because our experiments were conducted in very wet and humid habitats. More research is needed to resolve this issue.

In conclusion, many other researchers have suggested that nontarget insects were attracted to methyl eugenol and large numbers of lure responding target and nontarget insects, which accumulated in traps (Hardy 1980, Kido et al. 1996), rather than to randomly captured nontarget insects in traps without target species (Kido et al. 1996). Our data suggest that nontarget flies were attracted to traps containing lure- or bait-plus naled. However, based on the presence of large numbers of scavenger flies in our traps, it appears that attraction was not only due specifically to the lure or protein bait traps, but also to decaying insects accumulating inside our traps, which probably confounded our results. Presently, studies are underway to define the exact role of decaying insects in fruit fly bucket traps.

Low trap catches of individuals of nontarget species and taxa reported by earlier experimenters (Asquith and Messing 1992, Asquith and Kido 1994, Kido et al. 1996) and herein, indicate that endemic species would not likely be negatively impacted by fruit fly traps placed in native forests, but traps could place a strain on rare endemic species (Asquith and Kido 1994). Thus, solutions are needed to reduce the risk of collecting rare insects of value (i. e., endemic species). Uchida et al. (2003) stressed the importance of reducing the number of nontarget insects in traps which are intended for use over long periods of time. Howarth and Howarth (2000) demonstrated that trap color can influence the number of individuals collected in methyl eugenol traps. To diminish the influence of decaying insects, Uchida et al. (2003) suggested using male annihilation traps with open bottoms to allow killed insects to drop out of the traps and onto the ground. When using traps with bottoms, Uchida et al. (2003) recommended adding mineral oil to fruit fly trap bottoms to suppress the escape of decaying insect volatiles from collected nontarget insects. Hence, we recognize a need for more research focused on devising a trap with altered physical characteristics (i.e., trap shape) which will collect fewer nontarget individuals than the standard trap currently in use in the AWPM program.

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