



**SYNERGISTIC AND INHIBITORY INTERACTIONS  
BETWEEN METHYL EUGENOL AND CUE LURE  
INFLUENCE TRAP CATCH OF MALE FRUIT FLIES,  
BACTROCERA DORSALIS (HENDEL) AND B.  
CUCURBITAE (DIPTERA: TEPHRITIDAE)**

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SYNERGISTIC AND INHIBITORY INTERACTIONS BETWEEN METHYL EUGENOL AND CUE LURE INFLUENCE TRAP CATCH OF MALE FRUIT FLIES, *BACTROCERA DORSALIS* (HENDEL) AND *B. CUCURBITAE* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) and the melon fly, *B. cucurbitae* (Coquillett), are attracted to methyl eugenol (ME) and cue lure (CL), respectively. These lures, when mixed with a toxicant, are widely used to detect and suppress populations of these agricultural pests. The objective of this study was to assess the effectiveness of (1) traps baited with both ME and CL (mixed or presented separately on adjacent wicks), and (2) traps baited with a single lure but placed in the same tree as a trap containing the alternate lure (1 or 3 m apart). Jackson traps were placed in a mixed orchard on Oahu, Hawaii, and the numbers of released (marked) and wild males were recorded. Traps baited with ME and CL (mixed or separate) captured significantly fewer *B. dorsalis* males than traps baited with ME alone. CL placed 1 m from ME-baited traps in the same tree also reduced the number of *B. dorsalis* males captured. Conversely, ME appeared to increase capture of *B. cucurbitae* males, and traps baited with the 2 lures (mixed but not separate) captured significantly more released males than traps baited with CL alone. Also, ME placed 1 m (but not 3 m) from CL-baited traps increased the trap catch of released *B. cucurbitae* males. Results are discussed from the perspectives of management and evolution of *Bactrocera* species.

Key Words: *Bactrocera* spp., oriental fruit fly, melon fly, methyl eugenol, cue-lure, Diptera.

RESUMEN

Los machos de la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel) y de la mosca del melón, *B. cucurbitae* (Coquillett), son atraídos al eugenol metil (EM) y al atrayente “cue lure” (CL), respectivamente. Estos atrayentes, cuando están mezclados con una toxina, son usados en muchos lugares para detectar y suprimir las poblaciones de estas plagas agrícolas. El objetivo de este estudio fue para evaluar la eficacia de 1) trampas de cebo con ambos EM y CL (mezcladas o presentadas separadamente en mechas adyacentes) y 2) trampas de cebos con un solo atrayente pero puestos en el mismo árbol de una trampa que tiene un atrayente alternativo (separadas por 1 o 3 metros). Las trampas “Jackson” fueron puestas en un huerto mezclado en Oahu, Hawaii, y el número de los machos liberados (marcados) y los machos naturales fueron registrados. Las trampas con EM y CL (mezclados o separados) capturaron significativamente menos machos de *B. dorsalis* que las trampas con solo EM. También, el CL puesto 1 m de las trampas con EM en el mismo árbol redujó el número de los machos de *B. dorsalis* capturados. Al contrario, el EM aparece que se aumenta el número capturado de los machos de *B. cucurbitae*, y las trampas con los dos atrayentes (mezclados pero no separados) capturaron significativamente más machos liberados que las trampas con solo CL. También, el EM puesto 1 m (pero no 3 m) de las trampas cebadas con el CL aumentó el número capturado de machos de *B. cucurbitae* liberados. Se discuten los resultados desde el punto de vista de manejo y evolución de las especies de *Bactrocera*.

The genus *Bactrocera* contains approximately 440 species distributed primarily in Southeast Asia, the South Pacific, and Australia (White & Elson-Harris 1992). Males of many *Bactrocera* species are attracted to either methyl eugenol (4-allyl-1,2-dimethoxybenzene) or cue lure [4-(4-ace-toxyphenyl)-2-butanone] (Drew & Hooper 1981). Methyl eugenol (ME hereafter) is a widely distributed plant natural product and occurs in over 200 plant species representing 32 families (Tan & Nishida 1996). Cue lure (CL hereafter) has not been isolated as a natural product but is rapidly hydrolyzed to form raspberry ketone (RK hereaf-

ter), which is found in a variety of plants (Metcalf 1990). In Hawaii, the site of the present study, two major fruit fly pests are the Oriental fruit fly, *B. dorsalis* (Hendel), and the melon fly, *B. cucurbitae* (Coquillett), males of which respond to ME and CL, respectively.

Control programs against *Bactrocera* species use male lures to both detect and suppress pest populations. For example, ME- and CL-baited traps are monitored year-round in southern California to detect the presence of incipient infestations of *B. dorsalis* and *B. cucurbitae*, respectively (Gilbert & Bingham 2002). In a well-known case

of male annihilation, Steiner et al. (1965) eradicated *B. dorsalis* from Rota Island by distributing thousands of fiber board blocks soaked with ME and a toxicant. Although a less powerful attractant than ME, and hence less effective at male annihilation, CL (plus a toxicant) has similarly been used to suppress populations of *B. cucurbitae* prior to the start of sterile release programs (e.g., Miyako Island, Kuba et al. 1996).

Several studies have investigated the effectiveness of traps baited with a mixture of ME and CL for possible use in detection and suppression programs in areas containing both ME- and CL-responding *Bactrocera* species. Combining lures in such a manner could potentially reduce the number of traps needed in a given area and the associated manpower required to service them. In addition, combining lures may reduce the amount of pesticide used, thus lessening economic and environmental costs. Previous studies suggest an asymmetry in the effectiveness of ME + CL mixtures for ME- versus CL-responding species. On one hand, data uniformly show that CL, either mixed with or placed immediately adjacent to ME, reduces trap capture of ME-responding species (Hooper 1978; Vargas et al. 2000). In contrast, data regarding lure mixtures and the response of CL-responding species have been inconsistent. Data on *B. cucurbitae* from Taiwan (cited by Hooper 1978) showed that adding ME to CL nearly doubled the number of males captured compared with traps baited with CL alone. In contrast, Hooper (1978) found that the mixture of ME + CL reduced trap capture of CL-responding species in Queensland, Australia. However, Hooper (1978) did report an increase in male numbers for traps in which ME and CL were applied to separate wicks in the same trap. More recently, Vargas et al. (2000) found that lure mixtures containing at least 25% CL by volume attracted similar numbers of *B. cucurbitae* males as traps baited solely with CL.

The objective of the present study was to provide additional data on the responsiveness of *B. dorsalis* and *B. cucurbitae* males to traps baited only with ME or CL, respectively, relative to traps containing the 2 lures mixed together or on separate wicks. In addition, we monitored the effect of ME on CL, and vice versa, on trap captures when 2 traps containing the respective lures were placed in the canopy of the same tree.

## MATERIALS AND METHODS

### Study Site

Fieldwork was conducted during January - November, 2003, at the University of Hawaii Agricultural Experiment Station, Waimanalo, Oahu, in a mixed fruit orchard that contained mango (*Mangifera indica* L.), guava (*Psidium guajava* L.), orange (*Citrus sinensis* (L.) Osbeck), lime

(*C. aurantiifolia* (Christm.) Swingle), and breadfruit (*Artocarpus altilis* (Parkins.) Fosb.) along with other non-host trees. Daily maximum and minimum temperatures ranged between 25-33°C and 19-25°C, respectively, during this period.

### Study Insects

Males of *B. dorsalis* and *B. cucurbitae* were from laboratory colonies maintained by the USDA-ARS Tropical Fruit, Vegetable, and Ornamental Crop Laboratory, Honolulu, since the mid-1980s and mid-1960s, respectively (D. McInnis, personal communication). We obtained *B. cucurbitae* as pupae and *B. dorsalis* as eggs, which were placed on standard larval medium (Tanaka et al. 1969) in plastic containers over vermiculite for pupation. In both species, males were collected within 48 h of eclosion (males from laboratory colonies attain sexual maturity at 6-7 days of age, Vargas et al. 1984), held in screen-covered plastic buckets (volume 5 liters; 100-150 males per bucket), and provided water and food (a sugar-yeast hydrolysate mixture, 3:1 v/v). Flies were maintained at 23-27°C and 60-90% RH under a natural photoperiod (approximately 12:12 L:D). One day before release, we marked males by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no obvious adverse effects, and males resumed normal activities within minutes of handling. When used in the field experiments, males of both species were 10-19 days old.

### Effect of CL on Trap Catch of *B. dorsalis*

We conducted 4 experiments to assess the effect of CL on the capture of *B. dorsalis* males in ME-baited traps. Lures (2 ml of either mixed or pure lures, see below) were applied to cotton wicks (1.2 cm diameter, 4.0 cm length) with a pipette. Wicks were placed individually in perforated, plastic baskets, which were suspended inside Jackson traps above a sticky insert resting on the trap floor. In each experiment, we placed traps in 8 trees arranged in a circle (radius 40 m) about a central tree, which served as the release point. In any given replicate, 4 of the trees contained a single ME-baited Jackson trap. The remaining 4 trees contained the following: in Experiment 1, a single trap baited with a mixture of CL and ME (2 ml of each lure were mixed, and the mixture was apportioned equally between 2 wicks held in 2 separate baskets); in Experiment 2, a single trap baited with CL and ME applied separately to 2 wicks (and housed in separate baskets); in Experiment 3, 2 traps baited with CL and ME, respectively, placed 1 m apart; or, in Experiment 4, 2 traps baited with CL and ME, respectively, placed 3 m apart. Traps were placed 2 m above ground in shaded locations within the

tree canopy. For a given replicate, adjacent test trees contained different treatments (i.e., ME only, or some combination of ME and CL).

For a given replicate, we set out the traps between 0900-1000 h and then released 300 males at the central release point. Flies were released by placing 2 buckets (150 males per bucket) on the ground beneath the release tree and gently removing the screen cover from the bucket. The buckets were not tapped or shaken, and the flies exited the bucket on their own volition. Traps were collected 48 h after release, and the numbers of released (marked) and wild (unmarked) *B. dorsalis* males were recorded. In general, successive releases were separated by an interval of 2-4 days to allow previously released flies time to disperse from the study area. The same test trees were used in all experiments, but the treatment assigned to a particular tree was alternated between successive replicates in a given experiment. Ten replicates were conducted for each experiment.

#### Effect of ME on Trap Catch of *B. cucurbitae*

We repeated Experiments 1-4 to assess the effect of ME on capture of *B. cucurbitae* males in CL-baited traps. These experiments followed the procedures described above, except that 300 *B. cucurbitae* males were released, and CL was used to bait traps on trees that had only a single male attractant present. Experiments involving release of *B. cucurbitae* were replicated 8 times except Experiment 4 for which 6 replicates were performed.

#### Statistical Analysis

For both species, pairwise comparisons were made by the 2-tailed *t*-test. Raw data were used for both species as the assumptions of normality and homoscedasticity were met, except for Experiment 1 with *B. cucurbitae* for which a  $\log_{10}$  transformation of the raw data was performed to meet these criteria. Calculations were performed with SigmaStat Statistical Software (Version 2.0).

## RESULTS

Data regarding trap captures of *B. dorsalis* males are presented in Table 1. Traps containing both ME and CL captured significantly fewer *B. dorsalis* males than traps baited with ME only. This result was evident for released and wild males both when the lures were mixed together (Experiment 1) and when they were presented separately (i.e., on different wicks) in the same trap (Experiment 2). In addition, CL-baited traps placed 1 m from ME-baited traps on the same tree reduced trap catch of released *B. dorsalis* males relative to ME-baited traps occurring singly on trees (Experiment 3). A similar result was ob-

tained for wild males, but in this case the effect was only marginally significant. No reduction in trap catch of *B. dorsalis* males (released or wild) was apparent when CL- and ME-baited traps were separated by 3 m on the same tree (Experiment 4). Wild males of *B. cucurbitae* were not found in any trap baited solely with ME in any of the experiments.

Data from the experiments involving *B. cucurbitae* are presented in Table 2. Traps containing mixed lures (Experiment 1) captured significantly more released *B. cucurbitae* males than traps baited with CL only, while a similar, but marginally significant, result was found for wild males. When CL and ME were presented separately (i.e., on different wicks) in the same trap (Experiment 2), a marginally significant increase in released males was recorded, but no effect was noted for wild males. The mean number of released *B. cucurbitae* males captured in CL-baited traps placed 1 m from ME-baited traps was significantly greater than that recorded for CL-baited traps placed singly on trees, but no difference was evident for wild males (Experiment 3). ME-baited traps placed 3 m from CL-baited traps had no effect on trap catch of wild or released males (Experiment 4). Wild males of *B. dorsalis* were not found in any trap baited solely with CL in any of the experiments.

## DISCUSSION

### Management

The present results for *B. dorsalis* are consistent with those of previous studies (Hooper 1978; Vargas et al. 2000), which showed that when presented in the same trap as ME (mixed or separate), CL significantly reduced capture of *B. dorsalis* males. Our study further showed that trap catch of *B. dorsalis* males was reduced (though the effect was only marginally significant for wild males) even when CL was placed 1 m (but not 3 m) from ME-baited traps. With respect to *B. cucurbitae*, the present findings agree with the Taiwan study (cited by Hooper 1978) in which the 2 lures mixed together increased capture of *B. cucurbitae* males (though the effect was only marginally significant for wild males). Also, when ME was placed 1 m from CL-baited traps, there was an increase in the number of released (but not wild) males captured. There were, however, no significant effects detected when ME was presented separately in the same trap or 3 m away from CL-containing wicks. Thus, in general, the inhibitory effect of CL on capture of *B. dorsalis* males appears greater than the synergistic effect of ME on capture of *B. cucurbitae* males. The absence of *B. dorsalis* males in traps baited with CL only and *B. cucurbitae* males in traps baited with ME confirms earlier laboratory results (Metcalf et al. 1983).



TABLE 1. NUMBER OF RELEASED AND WILD *B. DORSALIS* MALES CAPTURED IN JACKSON TRAPS BAITED WITH METHYL EUGENOL ONLY AND PLACED SINGLY ON TREES (DESIGNATED ME) VERSUS JACKSON TRAPS BAITED WITH A METHYL EUGENOL-CUE LURE MIXTURE (ME+CL, EXPERIMENT 1), METHYL EUGENOL AND CUE LURE PRESENTED SEPARATELY (ME+CL, EXPERIMENT 2), OR METHYL EUGENOL ONLY BUT PLACED ON A TREE WITH CUE LURE-BAITED JACKSON TRAP EITHER 1 M (ME+CL, EXPERIMENT 3) OR 3 M (ME+CL, EXPERIMENT 4) APART. VALUES REPRESENT MEANS  $\pm$ 1 SD; 10 REPLICATES WERE CONDUCTED FOR EACH EXPERIMENT. SIGNIFICANCE LEVELS ( $df = 18$  IN ALL TESTS): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , <sup>ns</sup> $p < 0.10$ , <sup>ns</sup> $p > 0.10$ .

Experiment	Cue lure placement relative to methyl eugenol	Trap type		
		ME	ME+CL	<i>t</i>
A. Released males				
1	Same trap, same wick	36.8 (14.2)	8.6 (3.4)	6.1***
2	Same trap, separate wick	36.6 (16.7)	19.3 (12.1)	2.2*
3	Separate trap, 1 m away	39.8 (13.9)	22.2 (13.0)	2.9**
4	Separate trap, 3 m away	31.5 (17.4)	33.7 (21.9)	0.2 <sup>ns</sup>
B. Wild males				
1	Same trap, same wick	182.0 (76.7)	81.1 (56.6)	3.3**
2	Same trap, separate wick	239.2 (65.8)	143.8 (47.4)	3.7**
3	Separate trap, 1 m away	128.2 (54.3)	82.9 (49.9)	1.8 <sup>ns</sup>
4	Separate trap, 3 m away	199.5 (69.6)	159.1 (79.7)	1.0 <sup>ns</sup>

These data imply that where *B. dorsalis* (or another ME-responding species) is dominant, the most effective trapping procedure would involve placement of separate ME and CL traps in different trees. However, where *B. cucurbitae* (or another CL-responding species) is most abundant, mixing ME with CL in the same trap may be most effective. In addition, the price of ME is only about 20% that of CL (J. Knapp, pers. comm.), and therefore use of a ME-CL mixture could reduce costs considerably.

#### Lure Interactions and Evolution

Plants containing ME or RK are attractive to *Bactrocera* males, apparently because they signal the presence of pheromone precursors. Males of *B. dorsalis* that feed on ME (Nishida et al. 1988) or flowers containing ME (Nishida et al. 1997) contain ME metabolites in the rectal gland, the site of pheromone synthesis and storage. Likewise, males of *B. cucurbitae* that feed on CL (Nishida et al. 1990) or plants containing RK (Nishida et al. 1993) sequester RK in their rectal gland. To the extent that the incorporation of ME or RK in the sex pheromone promotes a species-specific response, we might expect males of *B. dorsalis* and *B. cucurbitae* to possess specific receptors only for ME and RK/CL, respectively, and (as reported by Drew & Hooper 1981) to respond to either ME or RK/CL but not both.

The present study shows clearly, however, that males of *B. dorsalis* and *B. cucurbitae* respond to the "alternate" lure when ME and CL are mixed or presented in close proximity. Although pheromones, and not plant volatiles, are involved, similar types of interspecific interactions are well-documented for moths (Phelan 1992) and bark

beetles (Byers 1995). It seems unlikely that the same single receptor cells are responding to both compounds, resulting in competitive blocking on the surface of the receptor cell membrane. Rather, as described for other instances of olfactory interaction (Mustaparta 1984), the compounds probably activate different receptor cells and the concurrent transduction from these different cells interrupts, in the case of *B. dorsalis*, or enhances, in the case of *B. cucurbitae*, the response at a more central neurophysiological level.

Why the between-lure interaction is inhibitory for *B. dorsalis* but synergistic for *B. cucurbitae* is unknown but may reflect evolutionary relationships. Based on biochemical changes accompanying the evolution of higher plants, it appears that both ME and RK are derived from the same, widely distributed compound (*p*-hydroxycinnamic acid), and speciation within the Dacinae subsequently led to ME- and RK/CL-responding species (Metcalf 1979). Because contemporary RK/CL-responding species also respond to this ancestral compound, whereas ME-responding species do not, it further appears that the RK/CL-responding species are more closely related to the ancestral dacines and that ME-responding species are derived taxa (Metcalf et al. 1983).

If ME-sensitive species arose from an RK-responding lineage, then there may have been strong selection for RK inhibition in the incipient species to avoid hybridization (and unsuccessful reproductive attempts) with females from basal, RK-sensitive species. Once again, data from pheromonal interactions are illustrative. Specific components of the pheromone of *Heliothis virescens* inhibit response by *H. zea* and vice versa (Stadelbacher et al. 1983). Cross-inhibition is highly adaptive in this case, as heterospecific matings

TABLE 2. NUMBER OF RELEASED AND WILD *B. cucurbitae* MALES CAPTURED IN JACKSON TRAPS BAITED WITH CUE LURE ONLY AND PLACED SINGLY ON TREES (DESIGNATED CL) VERSUS JACKSON TRAPS BAITED WITH A CUE LURE-METHYL EUGENOL MIXTURE (CL + ME, EXPERIMENT 1), CUE LURE AND METHYL EUGENOL PRESENTED SEPARATELY (CL + ME, EXPERIMENT 2), OR CUE LURE ONLY BUT PLACED ON A TREE WITH A METHYL EUGENOL-BAITED JACKSON TRAP EITHER 1 M (CL + ME, EXPERIMENT 3) OR 3 M (CL + ME, EXPERIMENT 4) APART. VALUES REPRESENT MEANS  $\pm$ 1 SD ( $\log_{10}$  TRANSFORMED DATA WERE USED IN T TEST IN EXPERIMENT 1). EIGHT REPLICATES WERE CONDUCTED FOR EXPERIMENTS 1-3, AND 6 REPLICATES WERE CONDUCTED FOR EXPERIMENT 4. SIGNIFICANCE LEVELS ( $df = 14$  IN EXPERIMENTS 1-3 AND 10 IN EXPERIMENT 4): \* $p < 0.05$ , \*\* $p < 0.01$ ; <sup>ms</sup> $p < 0.10$ ; <sup>ns</sup> $p > 0.10$ .

Experiment	Cue lure placement relative to methyl eugenol	Trap type		
		ME	ME+CL	<i>t</i>
<b>A. Released males</b>				
1	Same trap, same wick	16.8 (5.2)	33.3 (11.2)	3.1*
2	Same trap, separate wick	14.9 (8.1)	22.9 (9.2)	1.8 <sup>MS</sup>
3	Separate trap, 1 m away	10.2 (3.2)	20.3 (8.2)	3.2**
4	Separate trap, 3 m away	15.0 (5.9)	17.5 (6.1)	0.7 <sup>NS</sup>
<b>B. Wild animals</b>				
1	Same trap, same wick	29.6 (17.2)	42.7 (21.0)	1.9 <sup>MS</sup>
2	Same trap, separate wick	40.5 (13.8)	32.7 (13.2)	1.4 <sup>NS</sup>
3	Separate trap 1 m away	55.8 (23.4)	54.0 (21.1)	0.3 <sup>NS</sup>
4	Separate trap, 3 m away	57.3 (48.4)	46.2 (24.9)	0.5 <sup>NS</sup>

are unsuccessful due to incompatible genitalia (Stadelbacher et al. 1983). The synergism noted between ME and CL apparently reflects the absence of any evolutionary cost to *B. cucurbitae* of responding to this chemical blend, which, in turn, may reflect the infrequency with which ME and RK co-occur in the same plant species. Without natural exposure to an ME-RK blend, males of *B. cucurbitae* have not evolved avoidance of this stimulus. Comparing the relative strength of inhibitory and synergistic effects supports the reliance on evolutionary costs to explain the observed interactions, namely that the inhibitory effect of CL on ME is stronger (reflecting the high cost of inviable matings) than is the synergistic effect of ME on CL (reflecting the absence of benefits).

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