METHYL EUGENOL AND MATING COMPETITIVENESS OF IRRADIATED MALE *BACTROCERA PHILIPPINENSIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Males of many dacine tephritids are strongly attracted to methyl eugenol, a natural compound occurring in a variety of plant species. Here, we investigated the effect of methyl eugenol on male reproductive behavior in *Bactrocera philippinensis* (Drew & Hancock). In mating trials conducted on caged host plants, irradiated males that fed on methyl eugenol 3 or 5 days before testing had a mating advantage over wild males that were not given access to the lure. Additional tests showed that feeding on methyl eugenol increased male signaling activity (wing-fanning) and hence male attractiveness to females. The tendency of males to feed on methyl eugenol following an initial exposure was also examined. Following an initial feeding on the lure, irradiated (but not wild) males were less likely to feed in tests conducted 5 days later. The possibility of releasing methyl eugenol-exposed, irradiated males as a control strategy is discussed.

Key Words: Sterile insect technique, male replacement, fruit flies, male attractant

RESUMEN

Los machos de muchos tefrítidos daquinos son fuertemenmte atraidos por el methyl eugenol, un compuesto natural que aparece en varias especies de plantas. Investigamos aquí el efecto del methyl eugenol en el comportamiento reproductivo de los machos de *Bactrocera philippinensis* (Drew & Hancock). En ensayos de apareo conducidos en plantas hospedantes en jaulas, los machos irradiados que se alimentaron de eugenol de 3 a 5 días antes del ensayo tuvieron ventajas de apareo sobre los machos salvajes que no tuvieron acceso al cebo. Pruebas adicionales mostraron que la alimentación con methyl eugenol aumentó la actividad de emisión de señales (aleteo) y la atracción de los machos hacia las hembras. La tendencia de los machos a alimentarse de methyl eugenol luego de una exposición inicial también fue examinada. Luego de una alimientación inicial con el cebo, los machos irradiados (pero no los salvajes) tendieron menos a volver a alimentarse en las pruebas llevadas a cabo 5 dias más tarde. Es discutida la posibilidad de liberar machos irradiados expuestos al methyl eugenol como estrategia de control.

Males of many dacine tephritids are strongly attracted to methyl eugenol, a compound found naturally in a variety of plant species (Metcalf & Metcalf 1992). Though

the basis of this attraction is still unknown, two lines of evidence suggest that methyl eugenol plays a major role in male mating behavior by serving as a pheromonal precursor. Working with *Bactrocera opiliae* (Drew & Hardy) and *B. dorsalis* (Hendel), respectively, Fitt (1981) and Nishida et al. (1988) reported that males fed on methyl eugenol produced volatiles which contained metabolites of this compound, whereas unfed males lacked these metabolites. Additionally, Shelly & Dewire (1994) showed that wild males of *B. dorsalis* which ingested methyl eugenol exhibited increased signaling effort, signal attractiveness, and mating success compared with males not given access to the lure. Additional tests similarly revealed that irradiated males exposed to methyl eugenol gained a mating advantage over unexposed wild males for up to three weeks after feeding on the lure (Shelly 1995). Moreover, irradiated males exposed to methyl eugenol were less likely to be captured in lure-baited traps than were unexposed irradiated males (Shelly 1995).

These results suggest that the methyl eugenol-male association could potentially be incorporated in the sterile insect technique (SIT). Specifically, the abundance of wild males could be reduced initially via male annihilation, and then lure-fed irradiated males could be released concurrent with continued male annihilation. If successful, this approach would effectively replace wild males with irradiated males and thereby generate a high proportion of irradiated male by wild female matings.

The purpose of this study was to examine relationships between methyl eugenol consumption and male mating behavior in *Bactrocera philippinensis* (Drew & Hancock). Although field data are lacking, laboratory observations suggest that the reproductive behavior of this species is similar to that described for *B. dorsalis* (Kobayashi et al. 1978, Arakaki et al. 1984, Shelly & Kaneshiro 1991): sexual activity is restricted to a brief interval immediately preceding sunset, and males exhibit rapid wing-fanning (to facilitate pheromone dispersal) to attract females for mating. Three sets of experiments were conducted. First, we examined whether consumption of methyl eugenol affected the mating performance of irradiated males relative to wild males. Second, the effects of methyl eugenol consumption on male wing-fanning activity and female attraction were monitored. Finally, we measured the tendency of males to feed on methyl eugenol following initial exposure.

MATERIALS AND METHODS

Study Animals

Wild flies were reared from mango (*Mangifera indica* L.) fruits collected at various localities on Guimaras Island, Philippines. Adults were separated by sex within 7 days of eclosion. Irradiated (150 Gy 2 days before adult emergence) and nonirradiated mass-reared flies were obtained from the Philippines Nuclear Research Institute, where the sexes were separated within 2-4 days of eclosion. Flies were held, and all experiments were conducted, at the National Mango Research and Development Center (NMRDC), Guimaras Island, at 24-29°C under a natural 12:12 (L:D) photoperiod. Flies were provided water and a honey-protein hydrolysate mixture ad libitum.

Mating Tests

Most of the tests were conducted in two outdoor field cages (4 m by 2 m by 2 m) containing potted guava (*Psidium guajava* L.) and mango plants. Thirty irradiated males, 30 wild males, and 30 wild females were released into each cage between 1500-1530 hours, and mating pairs were collected from the onset of mating (1630-1730

hours) until dark (about 1830 hours). Irradiated males were 10-20 days old, and wild flies were 21-35 days old. To distinguish male type, irradiated and wild males were marked a minimum of 4 h prior to release by placing enamel paint on the thorax of flies chilled for 60-90 s in a freezer. This procedure had no obvious adverse effects, and males resumed normal activities within minutes of being marked.

Mating tests were occasionally conducted in a non-air-conditioned laboratory using large (75 cm by 75 cm by 60 cm) and small (25 cm by 25 cm by 45 cm) screen cages. We placed six irradiated males, six wild males, and six wild females in the large cages, and three irradiated males, three wild males, and three wild females in the small cages. The cages were placed by open, west-facing windows, thus ensuring natural conditions of temperature and light. Room lights were turned off at 1530 hours. No plants were placed in the screen cages.

We ran 5 different mating experiments. In the first, the mating success of wild and irradiated males was compared in the absence of methyl eugenol. In the second, wild males with no prior exposure to methyl eugenol (control) were tested against irradiated males exposed to methyl eugenol 1 day before the trial (treated). During exposure, 30-40 irradiated males were placed in large screen cages between 0930-1100 hours and given 2 h access to a cotton wick containing 1.5 ml of methyl eugenol. Feeding times of individual males were not recorded during the exposure period. Because treated males in the second experiment had unexpectedly low mating success, we exposed irradiated males in the third experiment to the lure 1 day prior to testing but removed males from the cotton wick after only 30 s of feeding. In the fourth and fifth experiments, irradiated males were exposed to methyl eugenol for 2 h 3 and 5 days before testing, respectively. Individual flies were used in only 1 trial.

Male Wing-Fanning and Female Attraction

Tests were conducted in large screen cages placed outside the laboratory building. Each cage held 5-7 mango seedlings. Groups of four treated or four control males were placed into transparent plastic cups (10 cm high by 6 cm diam). Treated males were exposed to methyl eugenol for 2 h on the day preceding a trial following the above procedure. Many small holes were made in the bottom of the cups, and the top was covered with nylon screening.

In a given experimental cage, two cups, one containing treated males and one containing control males, were suspended from the plants at 1600 hours. The cups were placed at opposite sides of the cage (a minimum of 50 cm apart), and the positions of treated and control males were alternated between days to compensate for any location effect. Fifteen females were then introduced into the cage. At the initiation of wing-fanning, we recorded (1) the number of males wing-fanning in each cup and (2) the number of females resting on each cup at 1 min intervals over a 30 min period. All flies used in this experiment were nonirradiated, mass-reared individuals between 16-22 days old used only once.

Repeat Feeding on Methyl Eugenol

To monitor the incidence of repeat feeding on methyl eugenol, groups of 6 individually marked males were placed in small screen cages along with a cotton wick containing 1.5 ml of methyl eugenol. The wild males used were 22-35 days old, and the irradiated males were 15-22 days old. Males were placed in the cages between 1000-1100 hours. The wick was introduced 20-30 min later, and the amount of time individual males spent feeding on the wick was recorded over a 30 min period. The wick was

then removed, and the flies were held in the cage (with food and water) for re-testing 5 days later using the same protocol.

Statistical Analyses

In the mating experiments, relatively low numbers of matings were observed per cage per day; consequently, data were pooled within and between days. Deviation from random mating was tested using the binomial test on the entire data set (using normal approximation with test statistic Z and df=1). Over the 5 different mating experiments, tests in field cage tests were conducted on 4-10 different days. In comparisons of male wing-fanning and female attraction, we used the non-parametric Mann-Whitney test (test statistic U) to avoid assumptions of normality and homoscedasticity (Zar 1974). Simple linear regression was calculated to describe the relationship between female arrivals and male wing-fanning activity. The Student's t-test was used to test for a difference between the slopes obtained for control vs. treated males. The incidence of repeat feeding on methyl eugenol was examined using the G test (df=1) with Yates correction. All statistical procedures followed Zar (1974).

RESULTS

Mating Tests

Results of the mating experiments are presented in Fig. 1. In the absence of methyl eugenol, wild and irradiated males had similar mating success ($Z=0.5$; $P>0.05$). Contrary to expectations (see below), irradiated males exposed to methyl eugenol 1 day prior to testing obtained a disproportionately small number of matings both when given 2 h access ($Z=2.2$; $P<0.05$) or when limited to 30 s feeding ($Z=2.1$; $P<0.05$). However, increasing the length of the post-exposure interval greatly increased the mating frequency of irradiated males, and they obtained 79% and 70% of all matings in tests conducted 3 days ($Z=4.6$; $P<0.001$) and 5 days ($Z=4.0$; $P<0.001$) after exposure, respectively.

Male Wing-Fanning and Female Attraction

Methyl eugenol had a pronounced effect on male wing-fanning activity (Fig. 2). A mean of 42 instances of wing-fanning (maximum value possible=120 for four males per cup over 30 checks) was recorded for cups with treated males compared to only 23 instances for cups containing control males (n=7 replicates; $U=42$; $P<0.05$). Also, more females were sighted on cups with treated males than control males (Fig. 2). A mean of 36 female sightings was recorded for cups with treated males, while only eight was observed for cups with control males ($U=49$; $P<0.001$). This difference in female arrivals apparently resulted from the increased wing-fanning of treated males and not to a difference in signal attractiveness per se between control and treated males, because the slopes relating female arrivals to male signaling were not significantly different between control ($Y=0.2+0.32X$; $r^2=0.40$) and treated ($Y=1.3+0.9X$; $r^2=0.58$) males ($t=1.5$; $P>0.05$).

Repeat Feeding on Methyl Eugenol

Large, and statistically similar, proportions of both wild (25/62=40%) and irradiated (43/94=46%) males failed to feed on methyl eugenol during either the first or sec-

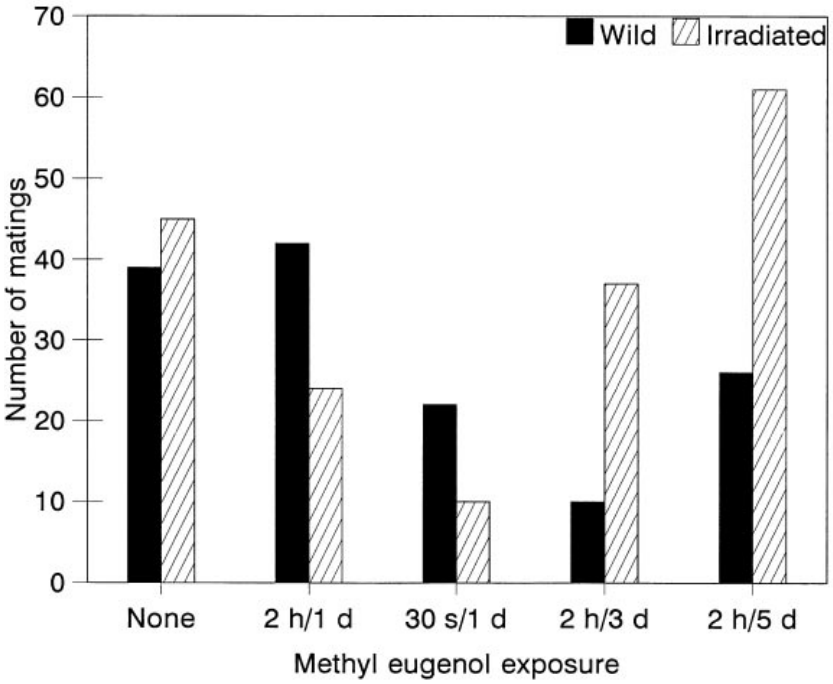

Fig. 1. Number of matings obtained by wild and irradiated males. Designations along the abscissa refer to different exposure regimes for irradiated males; wild males were not exposed to methyl eugenol in any test. First value represents duration of exposure period, and second value represents interval length between exposure period and testing. See text for additional details.

ond exposure periods (G=0.2; P>0.05). Moreover, among wild males there was no reduction in the incidence of feeding following an initial feeding: 35% (22/62) of wild males fed during the first exposure, and of these 32% (7/22) fed again during the second exposure (G=0.2; P>0.05). In contrast, among irradiated males initial feeding resulted in a reduced tendency to feed again. Over 40% (41/94) of irradiated males fed during the first exposure period, but of these only 24% (10/41) also fed during the second exposure period (G=6.0; P<0.05).

For both types of males, the likelihood of repeat feeding was inversely related to feeding duration during the initial exposure. Feeding durations in the first period were significantly longer for individuals that fed during the first period only than for individuals that fed during both periods for both wild (5.8 vs. 3.1 min, respectively; U=91.5; n_1=15, n_2=7) and irradiated (5.7 vs. 2.9 min, respectively; U=241; n_1=31, n_2=10) males (P<0.01 in both cases; Mann-Whitney test). These data suggest that the difference in the incidence of repeat feeding noted above between wild and irradiated males resulted from the fact that, among individuals that fed only briefly at first, wild males were more likely to feed again than irradiated males. Though samples were small, this trend is indicated: among males that fed very little initially (<3 min), 80% (4/5) of wild males were repeat feeders compared to only 30% (3/10) of irradiated males.

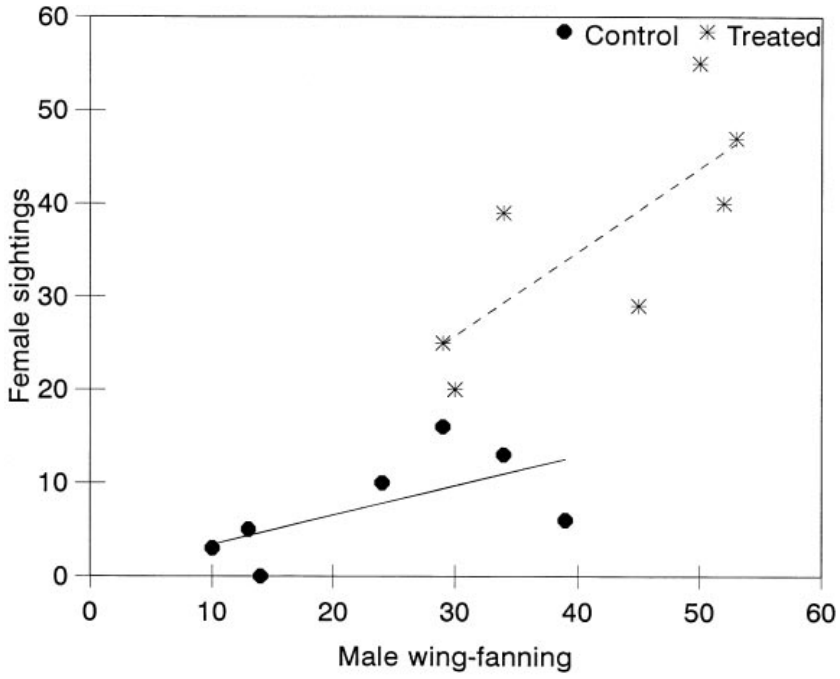

Fig. 2. Relationship between female sightings and wing-fanning for control (●) and treated (✳) males. Each point represents a cup that contained four males. The abscissa represents the total number of wing-fanning instances recorded for all four males per cup over the 1-min observations. The ordinate represents the total number of female sightings on a cup over the 1-min observations.

DISCUSSION

The present study reveals that, as in *B. dorsalis* (Shelly 1995), methyl eugenol greatly increased the mating success of irradiated *B. philippinensis* males relative to control wild males. Nonetheless, several differences were evident between these species. First, the positive effect of methyl eugenol on mating performance was evident sooner in *B. dorsalis* than *B. philippinensis*. Male *B. dorsalis* given 2 h access to the lure had low mating success when tested on the same day but enhanced mating success when tested 1 day later, and males whose feeding was restricted to 30 s had a mating advantage when tested on the same day (Shelly & Dewire 1994). In *B. philippinensis*, however, males in both treatment types had low mating frequency even 1 day after exposure. Initially, at least, methyl eugenol appears to reduce male sexual activity (e.g., wing-fanning, Shelly & Dewire 1994), and it appears that these negative effects are longer lasting in *B. philippinensis* than *B. dorsalis*.

In addition, methyl eugenol influenced both signaling effort and signal attractiveness in *B. dorsalis* (Shelly & Dewire 1994), but apparently enhanced only signaling effort in *B. philippinensis*. However, the relative difference in female sightings vs. male wing-fanning (Fig. 2) is quite large between control vs. treated males (0.32 vs. 0.9, respectively), and a larger sample may have shown a statistically significant difference in attractiveness.

Third, the level of feeding activity on methyl eugenol differed between the species. In tests conducted in the same manner, over 90% (126/134) of *B. dorsalis* males (mass-reared, non irradiated; Shelly 1994) fed on the lure during at least 1 of 2 exposure periods compared to only 57% (88/156) of *B. philippinensis* males (wild and mass-reared, irradiated males combined; $G=56.8$; $P<0.001$; G test with Yates correction). However, irradiated males of both *B. dorsalis* (Shelly 1995) and *B. philippinensis* showed a reduced tendency to re-visit a methyl eugenol source following an initial feeding.

Despite the lower incidence of methyl eugenol feeding, the enhanced mating success and low tendency for repeat feeding of lure-fed irradiated males suggest that *B. philippinensis* is a potential candidate for the method of "male replacement" mentioned above. This strategy has several potential merits. Exposure to methyl eugenol is a logistically simple and relatively inexpensive means to increase the mating competitiveness of irradiated males. Also, lure-induced enhancement of mating performance may compensate for genetic changes accompanying colonization that lessen the attractiveness of mass-reared males to wild females (Calkins 1984). Perhaps the greatest obstacle to implementation is the fact that *Bactrocera* males do not respond to methyl eugenol until sexually mature (Metcalf 1990). Consequently, additional space and rearing supplies would be needed to hold eclosed adults for 7-10 days prior to exposure and release.

The present study furnishes evidence for another tephritid species in which male attractants, or structurally similar compounds, function in mate attraction and mating success. Previous studies with cue lure and *B. cucurbitae* (Coquillett) (Shelly & Villalobos 1995) and trimedlure and *Ceratitis capitata* (Wiedemann) (Shelly et al. 1996) also reveal a positive relationship between exposure to male lure and male mating performance. Interspecific variation is apparent in (1) the time elapsed between exposure and heightened mating success, (2) the "potency" of the lure in terms of the duration of mating advantage, and (3) the effect of the lure on signaling effort and signal quality. Clearly, data from more species are required before any pattern emerges regarding general relationships between parapheromones and male mating behavior. Nonetheless, the few existing studies will, we hope, stimulate additional research in this area from both basic and applied perspectives.

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