

CONSUMPTION OF METHYL EUGENOL BY MALE
BACTROCERA DORSALIS (DIPTERA: TEPHRITIDAE):
LOW INCIDENCE OF REPEAT FEEDING

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ABSTRACT

The tendency of male *Bactrocera dorsalis* (Hendel) to re-visit a methyl eugenol source following initial exposure was examined. The first field test investigated the effect of duration of exposure on subsequent capture probability. "Treated" males were allowed to feed on methyl eugenol for 30 s or had access to methyl eugenol for 1 h, 4 h, or 24 h immediately prior to release. Capture probabilities (1%-4%) did not differ significantly among the different treatments but were significantly below that (22%) recorded for "control" (unexposed) males. In a second field test, treated males were released 7 d, 21 d, or 35 d after an initial exposure (2 h) to methyl eugenol. Capture probabilities (11%-18%) did not differ significantly among the different treatments but were significantly below that (34%) recorded for control males. Laboratory tests yielded similar results as both the incidence and duration of re-feeding on methyl eugenol were uniformly low for males held 7 d, 21 d, or 35 d after their initial exposure. By exposing sterile males to the lure prior to release, it may be possible to combine programs of male annihilation and sterile insect release. The present findings also suggest that the effectiveness of male annihilation efforts may be reduced in areas where wild males have consumed sufficient amounts of methyl eugenol from natural sources.

Key Words: Oriental fruit fly, parapheromone.

RESUMEN

Se estudió la tendencia de las visitas continuas del macho de *Bactrocera dorsalis* a una fuente de methyl eugenol. En el primer ensayo se investigó el efecto de el tiempo de exposición en la posibilidad de captura. Los machos tratados fueron alimentados con methyl eugenol por 30 segundos y tuvieron acceso a el methyl eugenol por 1, 4 o 24 horas inmediatamente antes de la liberación de los machos. No hubo diferencias significativas para la captura (1-4%) entre los diferentes tratamientos, pero hubo diferencias entre los machos que habían sido expuestos al eugenol comparadas con los machos sin tratamiento (22%). En el segundo experimento, los machos tratados por dos horas con methyl eugenol fueron liberados a los 7, 21 o 35 días después de tratamiento. No hubo diferencias de captura entre tratamientos, pero la captura fué menor (34%) que en el testigo. Los ensayos de laboratorio dieron resultados similares en cuanto a la incidencia y la duración de la re-alimentación con methyl eugenol y la captura fué baja para los machos expuestos por 7, 21 o 35 días.

Al exponer los machos estériles a el atrayente antes de su liberación, puede ser posible el combinar programas de eliminación de machos y de liberación de machos estériles. Los resultados sugieren que la eficiencia de la eliminación de machos puede aumentar en aquellas áreas en las cuales los machos salvajes han consumido cantidades suficientes de methyl eugenol proveniente de fuentes naturales.

The males of several economically important tephritid species are strongly attracted to particular chemical compounds, termed "male lures" or "parapheromones", that either occur naturally in plants or are (presumed) synthetic analogues

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of plant-borne substances (Chambers 1977; Sivinski & Calkins 1986; Fletcher 1987). Several well-known examples include the attraction of male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), to trimedlure, male melon flies, *Bactrocera cucurbitae* (Coquillett), to cue-lure, and male Oriental fruit flies, *B. dorsalis* (Hendel), to methyl eugenol. Owing to their powerful attractancy, parapheromones play an important role in current control programs of tephritid pests, both in detecting incipient population outbreaks and eradicating already established populations via male annihilation (Chambers 1977).

Despite the wide use of male lures in control efforts, relatively little attention has been given to explaining the underlying biological basis of this sex-specific, chemical attraction. In a recent study on the Oriental fruit fly, Shelly & Dewire (1993) found that "treated" males that fed on methyl eugenol achieved significantly more matings than "control" males deprived of methyl eugenol. Interestingly, treated males had a mating advantage even when they fed on methyl eugenol for only 30 s and were tested 35 d post-feeding.

The present study investigates the tendency of *B. dorsalis* males to re-visit a methyl eugenol source following an initial exposure. Specifically, two field experiments and one laboratory experiment were conducted to examine whether the duration of the initial exposure and the time elapsed since the initial exposure affected the incidence and duration of re-feeding. Based on the results of mating trials (Shelly & Dewire 1993), I predicted that neither the duration of the initial exposure (at least for exposure periods exceeding 30 s) nor the time elapsed since the initial feeding (at least for intervals up to 35 d) would significantly affect the tendency for re-feeding.

MATERIALS AND METHODS

Field Experiments

All flies used in field tests were from a colony maintained by the USDA/ARS Tropical Fruit and Vegetable Laboratory, Honolulu, for approximately 70 generations (M. Fujimoto, pers. comm.) using standard rearing procedures (Tanaka et al. 1969). Non-irradiated pupae were obtained 2 d prior to eclosion, and adults were sexed within 5 d of eclosion [(sexual maturity in this stock is attained at about 10 d of age, (M. Fujimoto, pers. comm.)]. Males were kept in 5-liter plastic buckets (50 per bucket) covered with screen mesh and given food and water ad libitum.

Experiments were conducted at 2 locations on the island of Oahu, Hawaii. During September-October, 1991, I used a 0.6-ha citrus grove in the University of Hawaii Agricultural Experiment Station, Waimanalo, that contained approximately 60 orange trees (*Citrus sinensis* (L.)). The grove was bordered on two sides by an open field containing small patches of guava (*Psidium guajava* L.) and coffee (*Coffea arabica* L.) and on the other two sides by highly disturbed, second-growth forest. During May-July, 1992, field-work was conducted at the Kanewai Garden near the campus of the University of Hawaii, Honolulu. This small area (0.4 ha) contained six large mango trees (*Mangifera indica* L.) and was bordered by an open lot on one side and lawns containing non-host vegetation on the remaining sides.

Two field experiments were performed. At Waimanalo, I examined whether the duration of exposure to methyl eugenol affected capture probability. As described below, treated males fed on methyl eugenol for only 30 s or had access to methyl eugenol for 1 h, 4 h, or 24 h immediately prior to release. At the Kanewai Garden, I examined the effect of time lapse following initial feeding on capture probability. Treated males had access to methyl eugenol for 2 h and were released 7 d, 21 d, or 35 d later. An additional set of treated males was permitted to feed on methyl eugenol for only 30 s and was released 35 d later. Control males that had no exposure to methyl eugenol were also released in both experiments.

To obtain treated males, 1.5 ml of methyl eugenol was applied to 5-cm long cotton wicks, and the wicks, held upright in small plastic containers, were placed singly in the appropriate buckets during midday. Buckets were placed on a shaded outdoor porch where air temperatures varied between 29-31 °C (or 23-31 °C during 24 h exposure periods). The feeding activity of individual males was not monitored during exposure periods of 1 h or more. To obtain males with 30 s feeding times, groups of 5-10 males were observed in screen cages (30 cm cubes with a cloth sleeve on one side) containing a single wick. Individuals were removed after 30 s of feeding by gently "coaxing" them into a vial. In all cases, treated males were exposed to methyl eugenol at 14 d of age and correspondingly were released at the age of 14 d at the Waimanalo site and 21 d, 35 d, or 49 d at Kanewai Garden. At Waimanalo, control males were 14 d old at release, while at Kanewai Garden separate control groups of males aged 21 d, 35 d, and 49 d, respectively, were used for the two treatment categories. Prior to release, control males and the males in the different treatment groups were cooled and marked on the thorax with different color combinations of enamel paint (a given combination was used only once at either field site). The cooling and painting procedures had no apparent adverse effects on male behavior, and individuals resumed "normal" activities within minutes of handling.

The following protocol was used for the tests conducted at Waimanalo. On the day prior to a release, Steiner traps were placed singly in 16 different trees located throughout the grove. The same trees were used in all tests. Traps were suspended in the canopy by a 30-cm long wire fastened to a branch. Each trap contained a 5-cm long cotton wick to which 1.5 ml of methyl eugenol (3% naled) had been applied. For all tests, the males were released beneath a centrally located orange tree between 1500-1700 hours. The actual release was accomplished by removing the screen top and gently tapping the bucket to induce flight. Males that were unable to fly were not counted in the release sample. Traps were checked 5 d after release, and in the laboratory captured flies were examined individually for markings. Six replicates were conducted with 75-112 males released per group (control or treatment) per replicate.

A similar release protocol was employed at the Kanewai Garden site. However, owing to the small size of the garden, only eight Steiner traps were used at this site. The traps were placed in a circle (70-m radius) around a central release point (a mango tree). Eight replicates were conducted for tests involving treated males exposed to methyl eugenol for 2 h and released 7 d or 21 d later, with 122-143 males released per group (control or treatment) per replicate. Four replicates were conducted for tests involving treated males released 35 d after either exposure to methyl eugenol for 2 h (82-113 males per group per replicate) or feeding on methyl eugenol for only 30 s (79-120 males released per group per replicate).

Laboratory Observations

The effects of feeding duration and time since first feeding on the incidence and duration of repeat feeding were also investigated in the laboratory. Males used in these tests were from a laboratory stock started in November, 1991, with 200-300 adults reared from mangos collected in Waimanalo. Data were collected in July-September, 1992; consequently, the individuals observed were approximately eight generations removed from the wild. Larvae were reared on papaya, and adults were separated by sex within 7 d of eclosion, well before reaching sexual maturity (at approximately 15-20 d of age, Foote & Carey 1987).

Treated males fed on methyl eugenol for only 30 s (following the protocol described above) or had access to methyl eugenol for a 30-min period during which their feeding activity was monitored. To obtain this latter group, five uniquely marked individuals were placed in screen cages (30-cm cubes), allowed a 1-2 h "acclimation period", and then given free access to a 5-cm long cotton wick to which 1.5 ml of methyl eugenol had been applied. The amount of time that individual males fed on

the wick was then recorded to the nearest second. All observations were made between 1100-1330 hours on a shaded outdoor porch at temperatures between 29-31 °C. Following the initial exposure, treated males were kept in 5-liter plastic buckets and given ample food and water.

Treated males - both those restricted to 30 s feeding and those given 30 min access - were held 7 d, 21 d, or 35 d before a second exposure (30 min) during which feeding times of individual males were recorded. All treated males were initially exposed to methyl eugenol at 25 d of age. To investigate the possibility that male age was partly responsible for any feeding differences observed between the first and second exposures, I recorded the feeding times of uniquely marked, control males given their first exposure (30 min) to methyl eugenol at ages 32 d, 46 d, and 60 d, respectively (ages correspond to those of treated males held 7 d, 21 d, or 35 d, respectively).

RESULTS

Field Experiments

In the Waimanalo experiment, no significant differences in capture probability were found among males in the different treatment groups ($H=6.1$; $P > 0.05$; Kruskal-Wallis test; Fig. 1). Among the different exposure groups, only 1%-4% of the males were captured, on average, in a given replicate. In contrast, 22% of control

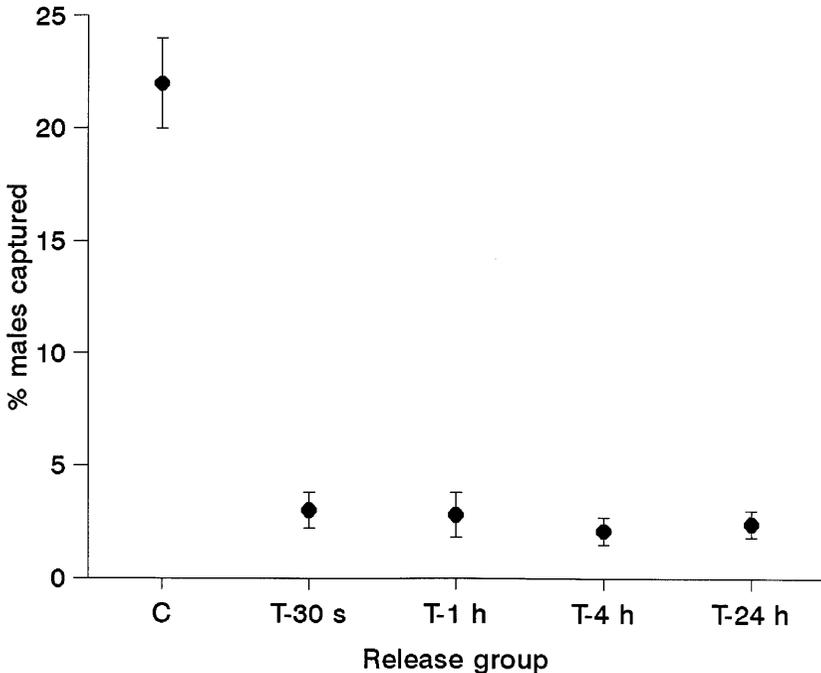


Fig. 1. Capture probabilities of *B. dorsalis* males exposed to methyl eugenol for varying lengths of time. Points represent average proportion of males captured per replicate; vertical lines indicate \pm standard error. Release groups: C=control, T=treated. T-30 s males were restricted to 30 s of feeding on methyl eugenol; the remaining groups of treated males had access to methyl eugenol for 1 h, 4 h, or 24 h, respectively. See text for sample sizes.

males were captured, on average, in a given replicate. The capture probability of control males differed significantly from males exposed for 1 h ($q=5.6$), 4 h ($q=6.0$), or 24 h ($q=5.2$) as well as from males whose feeding was restricted to 30 s ($q=7.9$; $P < 0.005$ in all cases; multiple comparisons test, Zar 1974: 156).

At the Kanewai Garden, no significant differences in capture probability were detected among males exposed to methyl eugenol for 2 h but released after differing time intervals ($H=5.1$; $P > 0.05$; Kruskal-Wallis test; Fig. 2). Over the different intervals, only 11%-18% of the treated males were captured, on average, in a given replicate. Similarly, capture probabilities did not differ among control males held for varying periods before release ($H=0.5$; $P > 0.05$; Kruskal-Wallis test; Fig. 2). On average, approximately 33% of control males were trapped over all pre-release intervals. Based on data pooled over all pre-release intervals, the capture probability for control males was significantly higher than that observed for males given 2 h access to methyl eugenol before release ($U=387.5$; $P < 0.001$; Mann-Whitney test). Treated males that fed for only 30 s prior to their release 35 d later also had low capture probability (Fig. 2). An average of 11% of these males was captured per replicate, the same proportion observed for males released 35 d after 2 h exposure to methyl eugenol ($U=9$; $P > 0.05$; Mann-Whitney test).

Laboratory Observations

Among treated males given an initial 30-min exposure period, feeding durations were significantly shorter during the second exposure for males tested 7 d ($T=276$; $n=54$), 21 d ($T=87$; $n=53$), or 35 d ($T=3$; $n=27$) after the initial feeding ($P < 0.001$ in all

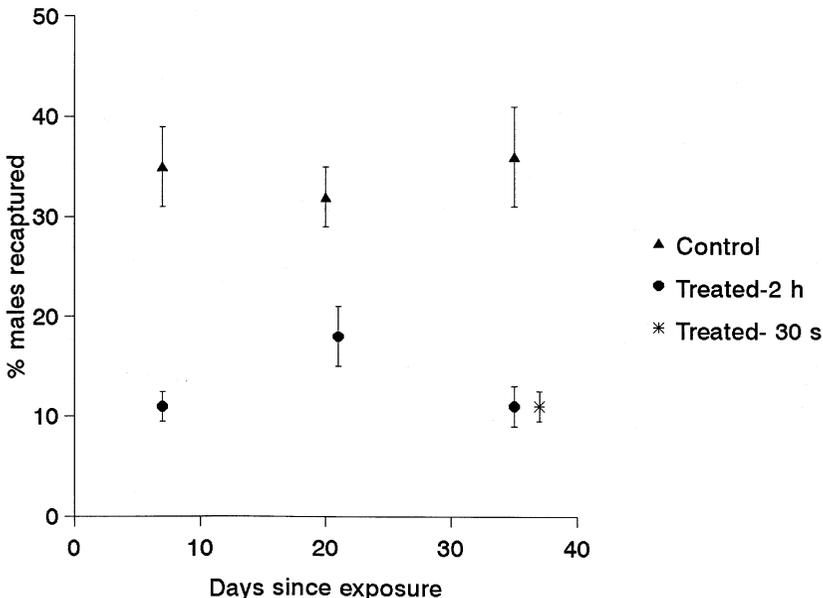


Fig. 2. Capture probabilities of *B. dorsalis* males held varying lengths of time after exposure to methyl eugenol. Points represent average proportion of males captured per replicate; vertical lines indicate \pm standard error. One set of treated males (held 35 d) was restricted to 30 s of feeding on methyl eugenol; all other treated males had access to methyl eugenol for 2 h. See text for sample sizes.

cases; Wilcoxon on paired-sample test; Fig. 3). Moreover, for these males, feeding durations during the second exposure were independent of time elapsed since the initial feeding ($H=1.1$; $P > 0.05$; Kruskal-Wallis test). Among the different trials, 85%-91% of the males consumed methyl eugenol during the initial exposure compared to only 32%-38% during the second exposure. Decreased feeding during the second exposure was apparently not age-related: average feeding durations were similar among control males aged 32 d ($n=35$), 46 d ($n=40$), and 60 d ($n=40$; $H=3.9$; $P > 0.05$; Kruskal-Wallis test; Fig. 3). Data pooled over the different inter-exposure intervals (or, equivalently, male ages) revealed that, during their second exposure period, treated males fed for shorter periods of time, on average, than control males ($Z=11.1$; $P < 0.05$; $n_1=134$, $n_2=115$; Mann-Whitney U-test).

Among treated males given an initial 30-min exposure, there was no correlation in the feeding times of individual males between the first and second exposure periods ($r_s=0.05$; $P > 0.05$; $n=134$; Spearman rank). Even if only the incidence of feeding is considered (i.e., regardless of duration), feeding activity during the first exposure period was still not a reliable predictor of subsequent feeding activity: males that fed during the first exposure period were as likely to feed during the second period (48 of 118=41%) as were males that did not feed at all during the initial exposure (8 of 16=50%; $G=0.4$; $P > 0.05$; G test with Yates correction). Among treated males given two 30-min exposure periods, 6% (8/134) did not feed on methyl eugenol during either period.

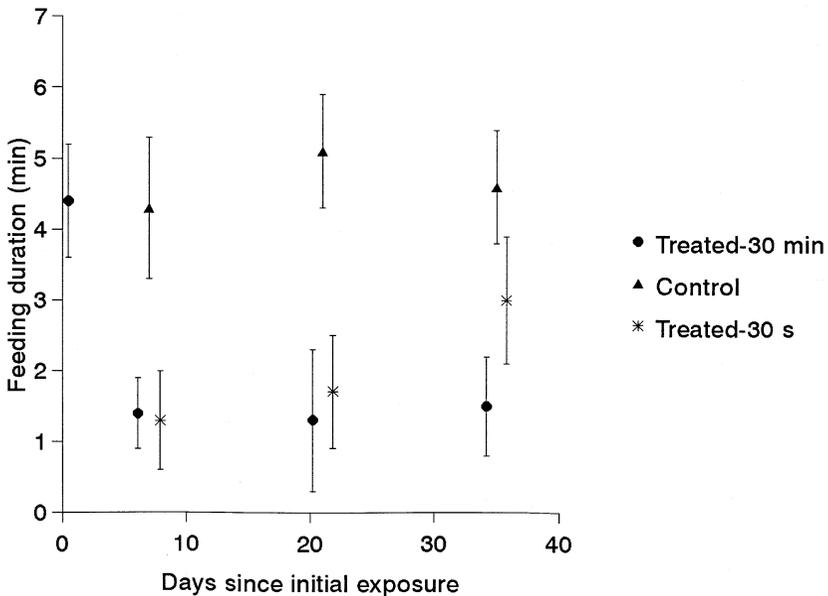


Fig. 3. Feeding times of *B. dorsalis* males during their second exposure to methyl eugenol 7 d, 21 d, or 35 d after the initial exposure. One set of treated males was given an initial 30 min exposure period, while another set was restricted to an initial feeding of 30 s; for both sets of treated males, the second exposure period was 30 min. Data for control males represent feeding durations during initial 30-min exposure periods at ages corresponding to males in different treatment groups. Points represent average values; vertical lines indicate \pm standard error. The value plotted for the initial exposure was calculated over all treated males given an initial 30-min exposure period. See text for sample sizes.

Treated males limited to an initial feeding of 30 s also displayed low feeding activity during the second exposure period (Fig. 3). In fact, when re-exposed to methyl eugenol 7 d (n=35 males) or 21 d (n=35 males) after the first feeding, these individuals had feeding durations that were similar to (and not greater than, as might be expected) males given an initial access of 30 min (7 d - $Z=0.6$; $n_1=35$, $n_2=54$; 21 d - $Z=0.5$; $n_1=35$, $n_2=53$; $P > 0.05$ in both cases; Mann-Whitney U-test). However, at 35 d after the initial exposure, males (n=40) limited initially to a 30 s feeding fed longer, on average, than males first given a 30 min exposure period ($Z=2.7$; $P < 0.01$; $n_1=40$, $n_2=27$; Mann-Whitney U-test). Though feeding durations of these males increased after 35 d, they were still significantly lower than those of control males of the same age ($Z=2.1$; $P < 0.05$; $n_1=n_2=40$; Mann-Whitney U-test).

DISCUSSION

Results of the present study indicate that after an initial exposure, *B. dorsalis* males have a greatly reduced tendency to re-visit a methyl eugenol source. In the field experiments, males that were permitted only 30 s feeding on methyl eugenol were rarely captured in methyl eugenol-baited traps even when released 35 d after feeding. Similarly, in the laboratory most males given an initial exposure of 30 min "ignored" a methyl eugenol source placed directly in their cage 35 d later.

Though data are scant, it appears that a dramatic reduction in male responsiveness to lures following exposure characterizes other tephritid species as well. Using a large outdoor cage, Chambers et al. (1972) reported that, after initial exposure to cue-lure, only 14% of male *B. cucurbitae*, on average, responded to cue-lure-baited traps compared to 50% of control (unexposed) males. Similarly, Brieze-Stegeman et al. (1978) placed dye in a methyl eugenol-baited trap (lacking poison) and found that only 13% (daily average) of the *B. cacuminatus* (Hering) males seen at the trap over the next several days were marked.

The major difficulty in interpreting laboratory studies on male attraction to lures is the scarcity of field data regarding both the availability of parafformones in natural sources and the feeding behavior of males at these sources. To my knowledge, no data exist regarding either the incidence and duration of feeding bouts or the rate and amount of parafformone consumption during these bouts. It is likely that the 1-2 ml doses of parafformones used by experimenters (Chambers et al. 1972; Brieze-Stegeman et al. 1978; present study) exceed levels available in natural sources (e.g., Kawano et al. 1968). Despite this possible discrepancy, it is certainly conceivable that in the wild, males initially make frequent or prolonged feeding bouts and in so doing eventually consume parafformone in amounts similar to males observed in laboratory studies. In other words, though the feeding time required to inhibit subsequent feeding is reduced in laboratory studies, the basic pattern of decreased responsiveness to parafformones may nonetheless be characteristic of wild males.

The present study has three major implications for control or eradication projects of tephritid pests. First, by exposing sterile males to the lure prior to their release, workers may be able to combine programs of male annihilation and sterile insect release. As noted by Chambers et al. (1972), pre-exposure of sterile males may increase the efficiency of achieving effective overflooding ratios, since wild males would respond to lure-baited traps, whereas sterile males would not. Pre-exposure to the parafformone may also increase the mating competitiveness of sterile males (Shelly & Dewire 1993), further enhancing the effectiveness of the sterile insect release method. Second, the present findings suggest the possibility that wild males that have consumed sufficient amounts of parafformone from natural sources may show reduced attraction to lure-baited traps, thus potentially reducing the effectiveness of male annihilation programs. Finally, and somewhat unexpectedly, 6% of the

males observed in the laboratory tests were not attracted to methyl eugenol in two separate exposure periods. The possibility that some males in a population may respond only slightly or not at all to parapheromones implies that in certain situations male annihilation may fail to achieve total eradication. Studies in our laboratory are currently investigating the genetic basis of male responsiveness to parapheromones using the *B. dorsalis*-methyl eugenol association.

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REFERENCES CITED

- BRIEZE-STEGEMAN, R., M. J. RICE, AND G. H. S. HOOPER. 1978. Daily periodicity in attraction of male tephritid fruit flies to synthetic chemical lures. *J. Australian Entomol. Soc.* 17: 341-346.
- CHAMBERS, D. L., K. OHINATA, M. FUJIMOTO, AND S. KASHIWAI. 1972. Treating tephritids with attractants to enhance their effectiveness in sterile-release programs. *J. Econ. Entomol.* 65: 279-282.
- CHAMBERS, D. L. 1977. Attractants for fruit fly survey and control, pp. 327-344 in H. H. Shorey and J. J. McKelvey [eds.], *Chemical control of insect behavior*. Wiley, New York.
- FLETCHER, B. S. 1987. The biology of dacine fruit flies. *Ann. Rev. Entomol.* 32: 115-144.
- FOOTE, D., AND J. R. CAREY. 1987. Comparative demography of a laboratory and a wild strain of the Oriental fruit fly, *Dacus dorsalis*. *Entomol. Exp. Appl.* 44: 263-268.
- KAWANO, Y., W. C. MITCHELL, AND H. MATSUMOTO. 1968. Identification of the male Oriental fruit fly attractant in the golden shower blossom. *J. Econ. Entomol.* 61: 986-988.
- SHELLY, T. E., AND A. DEWIRE. 1993. Chemically mediated mating success in male Oriental fruit flies, *Bactrocera dorsalis* (Diptera: Tephritidae). *Ann. Entomol. Soc. America*. In press.
- SIVINSKI, J. M., AND C. CALKINS. 1986. Pheromones and parapheromones in the control of tephritids. *Florida Entomol.* 69: 157-168.
- TANAKA, N., L. F. STEINER, K. OHINATA, AND R. OKAMOTO. 1969. Low-cost larval rearing medium for mass production of Oriental and Mediterranean fruit flies. *J. Econ. Entomol.* 62: 967-968.
- Zar, J. H. 1974. *Biostatistical analysis*. Prentice-Hall, Inc. Englewood Cliffs, NJ.