CUE LURE AND THE MATING BEHAVIOR OF MALE MELON FLIES (DIPTERA: TEKRITIDAE)

TODD E. SHELLY1 AND ETHEL M. VILLALOBOS2
1Hawaiian Evolutionary Biology Program, University of Hawaii, Honolulu, HI 96822
2Biology Department, Chaminade University, Honolulu, HI 96816

ABSTRACT

Laboratory tests were conducted to assess the effect of the parapheromone cue lure on the mating behavior of male Bactrocera cucurbitae (Coquillett). Exposure to cue lure resulted in a short-term mating advantage. For wild flies, treated males that fed on cue lure on the day of testing, or 1 day prior to testing, mated more frequently than control males that had no prior exposure to cue lure. However, control and treated males had similar mating success in tests performed 3 or 7 days after the treated males were exposed to the lure. Exposure to cue lure also increased the mating success of mass-reared, irradiated males relative to unexposed wild males, though this advantage was evident for only 1 day following exposure. Cue lure appeared to enhance mating performance by increasing male wing-fanning activity but not the attractiveness of the signal per se. A field study revealed that irradiated males exposed to cue lure 1 week prior to release were less likely to be captured (in Steiner traps baited with cue lure and naled) than unexposed males. These findings suggest that exposure of sterile males to cue lure might improve the effectiveness of sterile insect release as well as enable simultaneous control programs of sterile insect release and male annihilation.

Key Words: Bactrocera cucurbitae, parapheromone, sterile insect release, Hawaii.

RESUMEN

Se estudió en el laboratorio el efecto de la paraferomona “cue lure” en el comportamiento sexual de los machos de Bactrocera cucurbitae (Coquillett). La exposición a la “cue lure” tuvo como resultado ventajas en los apareamientos a corto plazo. Los machos salvajes que fueron alimentados con “cue lure” el mismo día, o el día anterior al experimento se aparearon más frecuentemente que los machos del grupo control que no habían sido expuestos a la paraferomona. Sin embargo, los machos de ambos grupos tuvieron éxitos de copulación similares en experimentos conducidos 3 o 7 días después de haber sido expuestos a la “cue lure”. La exposición a la “cue lure” de los
machos criados en el laboratorio e irradiados también aumentó el éxito de copulación en comparación con los machos salvajes que no habían recibido la parapheromona, pero esta ventaja sexual fue efectiva sólo un día después del tratamiento. Al parar la “cue lure” aumenta la efectividad del comportamiento sexual porque estimula la actividad vibratoria de las alas, pero no afecta la calidad atractiva de la señal química en sí. Un estudio de campo indicó que los machos irradiados expuestos a la “cue lure” la semana anterior a ser liberados fueron capturados con menor frecuencia (en trampas de Steiner cebadas con “cue lure” y naled) que los machos que no fueron tratados. Los resultados sugieren que el exponer machos estériles a la “cue lure” podría aumentar la efectividad de las liberaciones de insectos estériles, así como permitir el uso simultáneo de programas de control basados en la liberación de insectos estériles y aniquilación de machos.

The males of many tephritid species are strongly attracted to specific chemical compounds, termed male lures or parapheromones, which either occur naturally in plants or are (presumed) synthetic analogues 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 oriental fruit flies, Bactrocera dorsalis (Hendel), to methyl eugenol, and male melon flies B. cucurbitae (Coquillett) to cue lure. Because they are powerful attractants, parapheromones are used in current control programs of tephritid pests for detection and monitoring of populations and for eradication via male annihilation (Chambers 1977; Economopoulos & Haniotakis 1994).

Recent studies (Shelly & Dewire 1994; Shelly 1994) on B. dorsalis suggest another potential use of male lures in control efforts. Data collected in the laboratory showed that (treated) males exposed to methyl eugenol mated more frequently than (control) males that had no prior exposure to the lure. Moreover, the effect of methyl eugenol was long-lasting, and males that fed for only 30 s on methyl eugenol had a mating advantage as long as 35 days later. Enhanced mating success appeared to result from 2 factors: treated males signaled (wing-fanned) more frequently and for a given level of signaling attracted more females per min than control males. In addition, field tests showed that treated males were less likely to be captured (in traps baited with methyl eugenol and naled) than control males for as long as 35 days after exposure. These studies suggest that the effectiveness of control efforts might be enhanced by exposing sterile males to parapheromone prior to release, making it possible to combine programs of male annihilation and sterile insect release.

The present study further examines the relationship between parapheromones and sexual behavior by investigating the influence of cue lure on the mating behavior of male B. cucurbitae under laboratory conditions. Using laboratory-reared wild flies, we initially tested whether exposure to cue lure enhances mating success and, if so, for how long after exposure. We then examined whether exposure to cue lure increased the mating competitiveness of irradiated (sterile) males relative to wild males and, if so, for how long after exposure. As will be described, the mating trials did, in fact, show that cue lure enhanced mating performance, and data were then gathered to assess the effect of cue lure on male signaling effort and signal attractiveness. Finally, to investigate the possibility of conducting sterile insect release and male annihilation simultaneously, we tested whether exposure to cue lure reduced the probability of capturing (in traps baited with cue lure and naled) irradiated males following their release in the environment.
Mating Behavior of B. cucurbitae

Several workers have described the mating behavior of B. cucurbitae (Suzuki & Koyama 1980, 1981; Kuba & Koyama 1982, 1985; Kuba et al. 1984; Iwahashi & Majima 1986; Kuba & Sokei 1988), and the following summary derives from these earlier reports. Mating occurs at dusk in male aggregations termed leks. Males perch singly on leaf under surfaces of both host and non-host plants and defend their site against intruding males. While perching, males fan their wings rapidly, producing a high-pitched buzzing sound. Wing-fanning also enhances dispersal of a pheromone, produced in the rectal gland, attractive to females (Schultz & Bousch 1971; Kobayashi et al. 1978; Baker et al. 1982). (Because acoustic, visual, and olfactory cues may be involved, the terms “signaling” and “calling”, which are used synonymously with wing-fanning, refer to the composite stimulus produced by all communicative modes.) After several seconds of wing-fanning, the male pounces on the female, and copulation ensues.

At present, the role of cue lure in male mating behavior is obscure, particularly because cue lure is a synthetic compound (unlike methyl eugenol; Metcalf et al. 1975) that has not yet been recorded from any natural source (Beroza et al. 1960). However, studies by Nishida and co-workers (Nishida et al. 1990, 1993) suggest that cue lure resembles a natural plant-borne substance, whose metabolites are sequestered in the rectal gland for pheromone synthesis. Initial work showed that males fed cue lure accumulated in the rectal gland a particular ketone [4-(4-hydroxyphenyl)-2-butanone; also known as Willison’s lure and raspberry ketone] known to occur in various plants and to be attractive to male B. cucurbitae. More recently, male melon flies were observed to feed voraciously on flowers of an orchid that contained raspberry ketone and to subsequently sequester it in the rectal gland. Though the exact role of this compound in female attraction remains unknown, its accumulation at the site of pheromone production clearly suggests a role in mate attraction and courtship.

Mating Trials

Wild flies were derived from a laboratory stock started in April 1994 with 200-300 adults reared from Coccinia grandis (L.) collected in Waimanalo, Oahu, Hawaii. The present study was conducted during July-September, 1994, and consequently the flies were 3-5 generations removed from the wild. (Though this stock may have undergone genetic change as a result of colonization, the flies will be referred to as “wild” as terminological shorthand). The colony was housed in a large screen cage (1.2 m by 0.6 m by 0.6 m) with superabundant food (a mixture of honey and protein hydrolysate) and water. Italian squash (Cucurbita pepo L.) was provided periodically for oviposition. Room temperature was maintained at 20-22°C and relative humidity at 65-75%. Infested squash were placed in plastic buckets containing vermiculite, and larval development and pupation proceeded in situ. Adults were separated by sex within 5 days of eclosion, well before reaching sexual maturity [at 18-20 days of age (Wong et al. 1986)]. Adults were maintained in plastic buckets (5 liters volume; 40-60 individuals per bucket) covered with screen mesh and were supplied with ample food and water.

Mass-reared, irradiated flies were obtained from a colony started by the USDA/ARS Tropical Fruit and Vegetable Laboratory, Honolulu, in 1958 (H. Chang, pers. comm.) using standard rearing procedures (Tanaka et al. 1969; males from this stock will hereafter be referred to as “sterile males”). Pupae that had been exposed to 10 krad of gamma radiation from a 60Co source were obtained 1 day before eclosion. Sexes
were separated within 3 days of eclosion [sexual maturity in this stock is attained at about 8-10 days of age (Vargas et al. 1984; Wong et al. 1986)].

In the mating tests, 3 males from each of the 2 groups being tested were placed with 3 wild females in screen cages at least 4 h before sunset. Six to 9 cages were used on a given day. The cages were 30-cm cubes with one side open and fitted with a cloth sleeve. Before placement in the cages, males from the 2 groups were cooled for several minutes and then marked on the thorax with different colors of enamel paint. Females were not marked. The cooling and painting procedures had no apparent adverse effects, and males resumed normal activities within minutes of handling.

Three experiments were conducted. In the first, mating success was compared between wild males that had previous exposure to cue lure (treated males) and wild males denied access to cue lure (control males). Treated males (21-25 days old) were given unrestricted access during a 2-h period to a cotton wick (5 cm long) to which 1.5 ml of pure cue lure had been applied (cue lure obtained from the USDA/ARS Fruit and Vegetable Laboratory, Honolulu). The wicks, held upright in small plastic containers, were placed singly in the plastic buckets during midday. Treated males were used 0, 1, 3 or 7 days after exposure to cue lure. In an additional test, treated males were exposed to the wick for 2 h, but the wick was covered with screen mesh so that direct contact with it was prohibited. In these tests, control and treated males were approximately the same age. Females used in this and all subsequent experiments were 21-28 days old. In the second experiment, the mating frequency of wild vs. sterile males was compared without previously exposing either group to cue lure. In the final experiment, cue lure was provided to either wild or sterile males during a 2-h midday period 1 day or 3 days prior to testing. In the latter two experiments, males of both groups were used during their first 2 weeks of sexual maturity (21-30 days and 12-21 days of age for wild and sterile males, respectively). In the final experiment, wild and sterile males were exposed to cue lure at 21-23 days and 12-14 days of age, respectively.

Signal Production and Attractiveness

To investigate the possibility that exposure to cue lure increased signal production and attractiveness, we placed treated and control males in “minicages” within a larger flight cage and simultaneously monitored signalling by, and female attraction to, males (following the basic protocol of Poramarcon & Boake [1991]). Because wild males called only infrequently in the mini-cages, males (and females) used in this experiment were from the USDA/ARS colony mentioned above. In this case, however, we used non-irradiated, “normal” flies. Sexes were separated within 3 d of eclosion.

Because single males were reluctant to call, groups of three males were placed in the minicages. Four groups—two control and two treated—were tested on a given day. All treated males fed for 1-1.5 min on a wick containing 1.5 ml of cue lure one day prior to use, and in all tests control and treated males were between 12-15 days old. The minicages were transparent, plastic cylinders (12 cm by 7 cm) whose ends were covered with nylon screening.

The tests were conducted in a large screen cage (1.2 m by 0.6 m by 0.6 m) that contained three potted plants (Ficus sp.). The minicages were hung at four selected branches on these plants, and the individual cages were placed randomly at these locations for each observation day. On a given day, 45 mature virgin females (12-15 days of age) were placed in the cage 4-6 h before sunset. At the same time, the minicages (containing males) were placed at their assigned locations on the plants. Room lights were then extinguished, and the flight cage, which was near a west-facing window, received only natural light.
Starting 45 min before sunset, we checked the individual minicages at 1-min intervals and recorded the numbers of wing-fanning males and females resting on the minicages. Attractiveness of male signaling was thus based on the number of female sightings for a given minicage; because females were not marked, no data are available on the number of different females that alighted on the minicages. At the end of an observation period, individuals of both sexes were discarded, and a new set of males and females was used each day of testing. Observations were made on 7 days, and thus data were gathered for 14 minicages of control and treated males, respectively.

Capture of Sterile Males Exposed to Cue Lure

A trapping experiment was conducted on the campus of the University of Hawaii at Manoa, Honolulu, Hawaii. The study area was a large, grassy lawn containing numerous ornamental trees and shrubs. Ten Steiner traps were placed singly in trees in a circle (50-m radius) around a central release point. The same plants were used in all replicates. Traps were suspended in the canopy (2 m above ground) by a 30-cm wire fastened to a branch. Each trap contained a 5-cm long cotton wick to which 2.0 ml of cue lure (5% naled) had been applied.

Treated sterile flies (12-15 days old) were given access to a wick containing 1.5 ml of cue lure for a 2-h period 1 day prior to release. Similarly-aged control males (18-23 day old) that had no exposure to cue lure were also released. Prior to release, control and treated males were marked on the thorax with different color combinations of enamel paint (a given combination was used only once). Flies were released between 1000-1100 hours by removing the screen top and gently tapping the bucket to induce flight. Traps were checked 48 h after release, and captured flies were examined individually for markings in the laboratory. Seven replicates were conducted with 100 males released per group (control or treatment) per replicate. During the study period, days were generally sunny or only partly cloudy, and ambient daylight temperatures ranged between 25-33°C.

RESULTS

Mating trials

Cue lure conferred a short-term mating advantage (Fig. 1). In the first experiment, treated males tested on the same day they were exposed to cue lure achieved 62% (55/88) of all matings recorded (t=2.3; P < 0.05; binomial test). Similarly, treated males tested on the day following exposure obtained 67% (58/86) of the matings (t=3.4; P < 0.001; binomial test). However, no difference in mating success was observed between control and treated males when treated males were tested 3 days (t=0.8) or 7 days after exposure to cue lure (P > 0.05 in both cases; binomial test). Also, treated males that were permitted only to approach a cue lure source without contacting it did not have a mating advantage over control males. Treated males obtained only 51% (38/74) of all matings (t=0.2; P > 0.05; binomial test).

Cue lure also influenced the relative mating competitiveness of wild and sterile males. In the second experiment (where cue lure was not given to either wild or sterile males), sterile males had a mating advantage over wild males and obtained 86% (75/87) of all matings (t=9.6; P < 0.001; binomial test). In the final experiment, sterile flies exposed to cue lure 1 day prior to testing obtained 98% (93/95) of all matings, a proportion significantly greater than that observed in the preceding experiment (G=8.0; P < 0.01; G test with Yates correction). Wild flies exposed to cue lure 1 day before test-
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Figure 1. Numbers of matings obtained by control vs. treated wild males in trials conducted 0, 1, 3, or 7 days after treated males were exposed to cue lure.

Signal Production and Attractiveness

Males exposed to cue lure called more frequently than control males (Fig. 2). An average of 70 instances of wing-fanning (maximum value possible = 135 = 3 males per minicage over 45 checks) was recorded for minicages with treated males compared to only 54 instances for minicages with containing control males (U = 149; P < 0.001; Mann-Whitney test). In addition, more females were sighted on minicages with treated males than control males (Fig. 2). On average, 56 female sightings were recorded for minicages with treated males but only 34 were observed for minicages with control males (U = 161; P < 0.001; Mann-Whitney test). This difference in female arriv-
als appeared to reflect the differential calling activity between control and treated males and not a difference in signal attractiveness per se between the two groups, since the rate at which female sightings increased with male signaling was similar for control and treated males (t=0.7; P > 0.05; Fig. 2).

**DISCUSSION**

The present study shows that, under laboratory conditions, exposure to cue lure confers a mating advantage to male *B. cucurbitae*. However, whereas a positive effect of methyl eugenol was evident one month after exposure (Shelly & Dewire 1994), cue lure provided a mating advantage for only one day following feeding. In the melon fly,
ingestion of cue lure appeared to increase mating success via an increased level of sexual signaling. Though metabolites of cue lure (or its natural analogue) may be used for pheromone synthesis (Nishida et al. 1990, 1993), we found no evidence that the signals produced by males exposed to cue lure were more attractive than those generated by control males: for a given level of wing-fanning, similar numbers of females were sighted on minicages with control and treated males. This situation differs from that reported for B. dorsalis, where (independent of signaling effort) females showed a striking preference for males that fed on methyl eugenol over control males (Shelly & Dewire 1994).

A separate study (Whittier & Shelly 1995) indicates that trimedlure has an effect on male medflies similar to that described here for cue lure and male melon flies. Though they do not feed on the chemical, C. capitata males exposed to trimedlure displayed higher calling levels and mated more often than control males. However, based on the number of female arrivals to calling males, treated males did not produce more attractive signals than control males. Also, as with the melon fly, the positive effect of the lure was short-lived and was, in fact, evident only on the day that males were exposed to the lure.

Cue lure appears to be far less attractive to B. cucurbitae males than is methyl eugenol to B. dorsalis males. When exposed to the lure for a 30-min period in screen cages, nearly 90% of wild B. dorsalis males fed on methyl eugenol at their first opportunity (Shelly 1994), but only 33% (22/64) of wild B. cucurbitae males fed on cue lure during initial exposure (T.E.S., unpublished data). Similarly, trapping experiments (using identical procedures at the same site) with untreated, irradiated males in the two species showed a pronounced difference in capture probability: an average of 32% of B. dorsalis males were captured per replicate (Shelly, unpublished data) compared to only 19% of B. cucurbitae males. The comparatively low attractiveness of cue lure is consistent with its short-lived effect on mating performance. Alternatively, and independent of its effects on male behavior, cue lure may be an "imperfect" mimic of a natural analogue and hence a less potent attractant than methyl eugenol, a natural plant product.

As in B. dorsalis, B. cucurbitae males that fed on cue lure were unlikely to re-visit a cue lure source for additional feeding. In the field experiment, an average of only 6 previously exposed males were captured per replicate compared to 19 control males per replicate. Similarly, among the 22 males that fed on cue lure during an initial 30-min trial (see above), not a single individual fed on cue lure during a second trial conducted 7 days later. These results are consistent with Chambers et al. (1972), who reported reduced trap captures of B. cucurbitae males for 3 weeks following exposure to cue lure. Given that cue lure confers only a short-term mating advantage, it remains unclear why male melon flies show a reduced tendency to re-visit a cue lure source.

Regarding potential application to control efforts, the present study suggests that pre-release exposure of sterile B. cucurbitae males to cue lure may both enhance their mating competitiveness in the wild and decrease their attraction to lure-baited traps. Thus, pre-release exposure to lure may represent a relatively simple and inexpensive means to combine programs of sterile insect release and male annihilation (see also Chambers et al. 1972). However, compared to the oriental fruit fly, the effectiveness of pre-release exposure may be limited for the melon fly given the short-lived mating advantage conferred by the lure. For both species, assessing the value of this technique will ultimately require field tests in which wild populations are compared between areas receiving, for example, (1) sterile insect release with or without pre-release exposure to lure or (2) male annihilation alone or in combination with sterile insect release with pre-release exposure to lure.
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REFERENCES CITED


