# Effects of Raspberry Ketone on the Mating Success of Male Melon Flies (Diptera: Tephritidae)

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**Abstract.** Laboratory tests were conducted to determine whether feeding on raspberry ketone affected the mating success of male melon flies, *Bactrocera cucurbitae* (Coquillett). The effect of this chemical was time-dependent. Males exposed to raspberry ketone obtained a disproportionately small number of matings when tested on the day of exposure but a disproportionately large number of matings when tested 1 d after exposure. The chemical had no detectable effect on mating frequency in tests conducted 3 d or 7 d after feeding by the treated males. Males exposed to raspberry ketone were less likely to be captured in traps than unexposed males. These results are compared with similar studies on other tephritids.

## Introduction

The males of many tephritid species are strongly attracted to particular chemical compounds, termed male lures or parapheromones, which either occur naturally in plants or are (presumed) synthetic analogues of plant-borne substances (Metcalf and Metcalf 1992). Being highly attractive, male lures are commonly used in detection and eradication efforts of tephritid pests. Despite wide usage in control programs, however, the biological significance of male attraction to these compounds remains obscure.

Recent evidence suggests that parapheromones may have an important role in the reproductive biology of male tephritids. In laboratory trials, males given access to the appropriate lure may enjoy increased mating success over males never exposed to the compound. For example, in the oriental fruit fly, *Bactrocera dorsalis* (Hendel), males allowed to feed on methyl eugenol for only 30 s obtained significantly more matings than unexposed males for as long as 35 d after feeding on the lure (Shelly and Dewire 1994). Males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), exposed to trimedlure also had a mating advantage but only in trials conducted immediately after the exposure period (Shelly et al. 1996). Similarly, in the melon fly, *B. cucurbitae* (Coquillett), cue lure conferred a mating advantage of only 1–2 days (Shelly and Villalobos 1995).

The present study further investigates the relationship between parapheromones and the sexual behavior of male tephritids by examining the effect of raspberry ketone (also known as Willison's lure; Nishida et al. 1993) on male mating success in *B. cucurbitae*. Raspberry ketone, the hydrolysis product of cue lure (4-[p-hydroxyphenyl]-2-butanone; Millar 1995), occurs in a variety of plant species and is highly attractive to males of various *Bactrocera* species (Drew 1974; Drew and Hooper 1981). Recently, Nishida et al. (1993) identified this compound from flowers of an orchid species and found that male melon flies that fed on these flowers sequestered raspberry ketone in their rectal gland, suggesting that raspberry ketone may be used to synthesize male sex pheromone. Given the fact that (1) raspberry ketone is structurally similar to cue lure and (2) males fed cue lure also sequester raspberry ketone in their rectal gland (Nishida et al. 1990), I predicted that the effects of raspberry ketone on male mating success would be similar to those previously reported for cue lure

(Shelly and Villalobos 1995). In addition to the mating tests, a field test was conducted to determine whether prior feeding on raspberry ketone affected capture probability of males in traps baited with cue lure.

## Materials and Methods

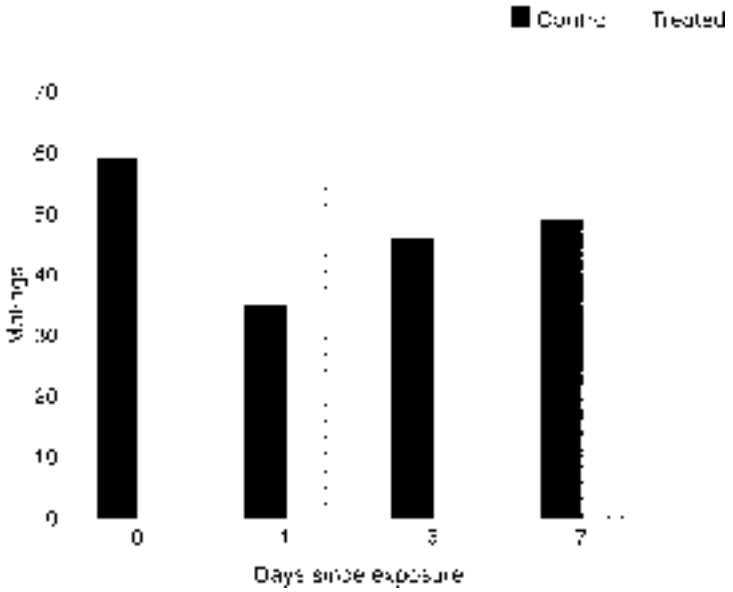
Flies were obtained from a laboratory stock started in April 1994 with 200–300 adults reared from *Coccinia grandis* (L.) collected in Waimanalo, Oahu, Hawaii. The present study was conducted during September–December, 1994, and consequently the flies were 5–7 generations removed from the wild. The colony 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 held in plastic buckets (volume 5 liters) covered with screen mesh and were supplied with ample food and water. For descriptions of mating behavior in this species, see Suzuki and Koyama (1980), Kuba and Koyama (1985), Kuba et al. (1984), Iwahashi and Majima (1986), and Kuba and Sokei (1988).

In the mating trials, 3 (treated) males that had prior access to raspberry ketone and 3 (control) males that had no exposure to the chemical were placed with 3 females in screen cages at least 4 hours before sunset. Six to 9 cages were used on a given day over 27 days, and a total of 196 cages was observed over the entire study. The cages were 30 cm cubes with 1 side open and fitted with a cloth sleeve. Before placement in the cages, males 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. The identity of mating males was checked 3–4 hours after sunset.

Treated males were given 2 hours (0900–1100 h) of unrestricted access to 10 mg of raspberry ketone. The chemical (plus 3 drops of distilled water) was placed on a filter paper disk, which in turn was placed in a petri dish, and then placed in a plastic bucket containing 50 males. Males typically gathered about disk and licked its surface and thus apparently ingested the chemical. Treated males were used 0, 1, 3, or 7 days after exposure. In all trials, control and treated males were of similar age (21–28 days old), and females were 21–25 days old. For statistical analysis, data were pooled within and between days, and a binomial test (with a normal approximation following Zar 1974) was used to test the null hypothesis of random mating.

The field trapping experiment was conducted in a small guava patch (1 ha) at the University of Hawaii Agricultural Experiment Station, Waimanalo, Oahu. Fourteen Steiner traps (Steiner 1957) were placed singly in trees in a circle (20 m radius) around a central release point. Each trap contained a 5 cm long cotton wick to which 1.5 ml of cue lure (5% naled) had been applied. Treated males were exposed to raspberry ketone following the above procedure 4 days prior to release, and control males were not given access to the chemical (all males were between 21–23 days old). Prior to release, males were marked with enamel paint as described above. Flies were released between 0930–1030 h from buckets placed on the ground. Traps were emptied 1 week after release, and captured flies were examined individually for markings. Ten replicates were conducted with 200 males released per group (control or treated) per replicate. A Mann-Whitney test, a non-parametric analogue to the t-test, was used to test for a difference in trap catches between the two groups.

**Figure 1.** Numbers of matings obtained by control vs. treated males in trials conducted 0, 1, 3, or 7 days after treated males were exposed to raspberry ketone. Z values: 0 day Z = 2.0, P < 0.05; 1 day Z = 2.3, P < 0.05; 3 days Z = 0.1, P > 0.05; 7 days Z = 0.6, P > 0.05.




## Results

The effect of raspberry ketone on mating success varied with the time elapsed between exposure to the chemical and the mating trials (Fig. 1). Treated males obtained a disproportionately small number of matings when tested on the day of exposure but achieved a disproportionately large number of matings when tested 1 day after exposure. No difference in mating success was observed between control and treated males in tests conducted 3 or 7 days after treated males were exposed to the chemical.

Males that fed on raspberry ketone were captured in much lower numbers than control males. On average, 45 (SD=21) control males were captured per replicate compared to only 11 (SD=9) treated males (U=97, P < 0.001, Mann-Whitney test).

## Discussion

The present study is the first to show, albeit under laboratory conditions, that consumption of a naturally occurring parapheromone affects mating success in male melon flies. It also confirms that cue lure and raspberry ketone have similar, short-lasting effects on mating success (Shelly and Villalobos 1995). For both chemicals, males enjoyed a mating advantage in trials conducted 1 day after exposure but not in trials conducted 3 or 7 days after exposure. However, the more immediate effects differed between the two lures: when tested

on the day of exposure, males exposed to cue lure mated with the same frequency as control males, whereas those exposed to raspberry ketone actually mated significantly less often than control males. Interestingly, *B. dorsalis* males fed methyl eugenol also achieved very few matings on the day of exposure (Shelly and Dewire 1994). Though the appropriate data were not collected, males of *B. cucurbitae*, like those of *B. dorsalis* (Shelly and Dewire 1994), were perhaps "sluggish" immediately after feeding on raspberry ketone and had reduced levels of signaling.

Cue lure and raspberry ketone also had similar effects on subsequent capture probabilities (Shelly and Villalobos 1995). In both cases, males fed the lure were less likely to be trapped in cue lure-baited traps than were control males. Given the fact that both lures confer only a 1 day mating advantage, it remains unclear why male melon flies would display a reduced tendency to re-visit these chemicals several days after the initial feeding. Given the present results, it is now important to investigate whether feeding on a natural source of raspberry ketone also confers a mating advantage to *B. cucurbitae* males. Studies on *B. dorsalis* (Shelly unpubl. data) have confirmed that males allowed to feed on methyl eugenol-containing flowers of the golden-shower blossom, *Cassia fistula* L., do indeed gain a long-lasting mating advantage over control males. The finding that natural sources of parapheromones affect male mating behavior is significant, because it indicates that (1) the underlying basis of mate attraction is sexual and (2) increased understanding of the biological function of male lures may ultimately be useful in enhancing the effectiveness of control programs, particularly those involving release of sterile males.

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