Zingerone feeding affects mate choice but not fecundity or fertility in the melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae)

Jess R. Inskeep[1,]*, Todd E. Shelly[2], Roger I. Vargas[3], and Helen Spafford[4]

Abstract

Consuming natural male lure compounds enables the males of some fruit flies (Diptera: Tephritidae) to mate more successfully within their female-choice mating systems. However, it remains unclear what benefits females derive from mating with lure-fed males. With *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), feeding on the lures cue-lure and zingerone is associated with increased fecundity of mated females, but this direct fitness benefit was not apparent with *B. dorsalis* (Hendel) (with the lure methyl eugenol) or *Zeugodacus cucurbitae* (Coquillett) (with the lure cue-lure). Expanding on previous observations, we fed *Z. cucurbitae* males zingerone, but we observed no evidence of direct fitness benefits to males feeding on zingerone (i.e., mating success and virgin longevity), or to females mated to zingerone-fed males (i.e., longevity, fecundity, and egg viability). We therefore find no reason to reject the runaway selection hypothesis that previously has been proposed to explain lure attraction in *B. dorsalis* and *Z. cucurbitae*.

Key Words: sexual selection; fitness; male lure; *Bactrocera cucurbitae*

Resumen

El consumo de compuestos naturales para señuelos de machos permite que los machos de algunas moscas de la fruta (Diptera: Tephritidae) puedan aparearse y escoger con más éxito dentro de su sistema de apareamiento con las hembras. Sin embargo, todavia no esta claro qué beneficios obtienen las hembras de aparearse con machos alimentados con señuelos. Con *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), la alimentación con los señuelos, el "cue-lure" y la zingerona se asocia con un aumento de la fecundidad de las hembras apareadas, pero este beneficio directo a su desempeño no fue evidente con *B. dorsalis* (Hendel) (con el señuelo de eugenol metilo) ni en *Zeugodacus cucurbitae* (Coquillett) (con el señuelo "cue-lure"). Ampliando sobre observaciones hechas anteriormente, alimentamos a los machos de *Z. cucurbitae* con zingerona, pero no observamos evidencia de beneficios directos sobre el desempeño de los machos que se alimentaron de zingerona (en cuanto de su éxito de apareamiento y longevidad de las hembras vírgenes), ni tampoco en las hembras apareadas con machos alimentados con zingerona (en cuanto de su longevidad, fecundidad y viabilidad de los huevos). Por lo tanto, no encontramos ninguna razón para rechazar la hipótesis que la selección de su pareja esta fuera de control que se propuso anteriormente para explicar la atracción de señuelos en *B. dorsalis* y *Z. cucurbitae*.

Palabras Clave: selección sexual; desempeño; señuelo masculino; *Bactrocera cucurbitae*

Female choice is a common element of sexual selection among animals, whereby males compete to attract females to mate (Darwin 1871). In response, females perform a "cost-benefit analysis" of either mating with a courting male or moving on in search of a more favorable male, all the while risking death or scarcity of acceptable males in the process. Female choice characterizes the mating systems of many dacine fruit flies (Diptera: Tephritidae), and males display complex courtship behaviors consisting of acoustic, visual, and chemical displays that presumably serve as indicators of their genetic quality and their condition (Sivinski et al. 2000). An interesting aspect of the sexual selection process that is prevalent in many dacine flies is the attraction to, and consumption of, naturally occurring phenylpropanoids and phenylbutanoids by males (Shelly 2010). These compounds (hereafter referred to as "male lures" or simply "lures")

are consumed and then released along with endogenously produced pheromones during courtship (Tan et al. 2014). Consuming male lures (or in some cases simple exposure to the volatiles) improves the mating success of males in a variety of species (Shelly & Dewire 1994; Shelly & Villalobos 1995; Wee et al. 2007; Orankanok et al. 2011; Kumaran et al. 2013; Haq et al. 2014, 2016, 2018), and improves the attractiveness of the male pheromone blend to conspecific females (Hee & Tan 1998; Khoo & Tan 2000; Wee et al. 2007). Males presumably are attracted to the lures because it enhances their chance of mating success, but less is known about the benefits to the female derived from mating with lure-fed males.

Past research on the biological effects of lure consumption in fruit flies has focused on methyl eugenol, raspberry ketone (and its synthetic analog cue-lure), and zingerone primarily because these lures

[1]Department of Biological Sciences, Macquarie University, North Ryde, Sydney, NSW 2109, Australia; E-mail: inskeep.jessr@gmail.com (J. R. I.)
[2]USDA-APHIS, P.O. Box 1040, Waimanalo, Hawaii 96795, USA; E-mail: todd.e.shelly@aphis.usda.gov (T. E. S.)
[3]Daniel K. Inouye, U.S. Pacific Basin Agricultural Research Center, United States Department of Agriculture, Agricultural Research Service, 64 Nowelo Street, Hilo, Hawaii 96720, USA
[4]Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA; E-mail: hspaffor@hawaii.edu (H. S.)
*Corresponding author; E-mail: inskeep.jessr@gmail.com

are readily available to fruit flies in nature and have tremendous value to the management of fruit fly pests as attractants (Tan et al. 2014). Much recent attention has been given to zingerone due to its ability to attract a wide variety of dacine fruit fly species (Tan & Nishida 2000, 2007; Fay 2012; Dominiak et al. 2015; Royer 2015; Manrakhan et al. 2017; Royer et al. 2018). Zingerone shares a similar molecular structure to capsaicin (in chili pepper), piperine (in black pepper), and vanillin (in vanilla) (Liu & Simon 1996), and though not widely distributed in nature, it is produced by the flowers of at least 6 species of orchids in the genus *Bulbophyllum* (Orchidaceae). These orchids are endemic to Malaysia, Indonesia, Papua New Guinea, and Australia, and attract male fruit flies for pollination (Tan 2009). Recently, other studies on dacine flies that have investigated potential benefits to females mating with lure-fed males have largely focused on *Bactrocera dorsalis* (Hendel) (using the lure methyl eugenol), *Bactrocera tryoni* (Froggatt) (using the lures raspberry ketone and zingerone), and *Zeugodacus cucurbitae* (Coquillett) (all Diptera: Tephritidae) (using the lure cue-lure).

In the case of *B. dorsalis*, Shelly (2000a) found no difference in the number of eggs laid by females mated with methyl eugenol-fed males compared to females mated with control males. Shelly (2000a, b) proposed 2 scenarios that could explain the enhanced mating success of lure-fed males in the absence of direct fitness benefits. First, a "sensory bias" may trigger a pre-existing sensory response in females that evolved in a context unrelated to mating (e.g., host searching). Second, mating females may prefer lure-fed males due to Fisherian runaway selection (Fisher 1930). In this scenario females receive no direct fitness benefits from mating with lure-fed males, but instead increase the likelihood that their sons will have a greater ability to seek out and consume lures in nature, and therefore have enhanced mating success. Presumably foraging for lures increases the chance of mortality for males, but the benefit garnered from enhanced mating success outweighs the possibility of death. Assuming the fitness cost does not outweigh the mating benefit, Fisherian selected traits can continue exponentially and without limit, leading to an exaggerated trait by the males (Fisher 1930; Dawkins 1986). In *B. dorsalis*, females preferentially mating with lure-fed males could reinforce a runaway selection scenario by producing (1) sons that are more likely to respond to and feed on lures due to paternal habits, and (2) daughters that are more likely to mate with lure-fed males due to maternal habits.

In a study of *B. tryoni*, Kumaran et al. (2013) observed that females laid more eggs when mating with cue-lure- and zingerone-fed males. This suggests a simple explanation for mate choice in this species: females select lure-fed males because this results in higher fecundity, and presumably more surviving adult offspring. Furthermore, Kumaran et al. (2014) found zingerone feeding by males induced weight loss, and resulted in the upregulation of genes believed to influence aggression, pheromone synthesis, mating, and accessory gland protein synthesis in various *Drosophila* (Drosophilidae) species. Kumaran et al. (2013) observed reduced lifespans for males that fed on lures and for females that mated to lure-fed males. Also lure-fed males were more likely to mate with multiple females. Akter et al. (2017a, b) found that feeding raspberry ketone to immature male and female *B. tryoni* increased the rate of sexual development, potentially a result of increased metabolism. Increased metabolism may allow males to display courtship behaviors more energetically, which could increase the male's chance of securing a mate if the female-choice mating system involves elements of scramble competition or endurance rivalry (Andersson 1994).

More recently, Shelly and Nishimoto (2017) fed cue-lure to *Z. cucurbitae* males, and compared similar parameters of fitness as the previously described studies on *B. dorsalis* and *B. tryoni*. In this study, fecundity and survival were not affected, and egg hatch rate was negatively affected by male cue-lure feeding. These findings suggest that cue-lure feeding by males does not confer direct fitness benefits, as were observed in *B. tryoni*, and the cue-lure mating bias in *Z. cucurbitae* may be due to runaway selection, as was suggested to explain the methyl eugenol mating bias with *B. dorsalis*.

Here we expand upon existing work by examining lure feeding and some fitness benefits in the melon fly, *Z. cucurbitae*. The male lure zingerone was chosen due to previously compelling observations by Kumaran et al. (2013, 2014) when using this lure to study fitness benefits conferred to *B. tryoni*. This new study tested fitness parameters associated with zingerone feeding by male *Z. cucurbitae*, including female mate choice, direct female and male benefits (= mated female longevity, female fecundity, and egg viability), and a direct male-only benefit (= virgin male longevity).

Materials and Methods

MATING SUCCESS

Insects

Adult *Zeugodacus cucurbitae* were obtained from a laboratory colony (3–9 generations removed from the wild) maintained at the University of Hawaii at Manoa. This colony began from an initial stock of 300 to 350 adults reared from naturally infested cherry tomatoes (*Solanum lycopersicum* L.) (Solanaceae) and Japanese cucumber (*Cucumis sativus* L.) (Cucurbitaceae) collected at a farm near Kahuku, Oahu, Hawaii. Mature adults were given access to punctured zucchini (*Cucurbita pepo* L.) (Cucurbitaceae) for 20 min for oviposition. The infested fruits were placed on newspaper and suspended over a layer of fine corncob granules (Grit-o'cobs®, The Andersons, Maumee, Ohio, USA). Larvae fed on the fruits and pupated in the corncob granules below, which were sifted twice per wk to collect pupae. The colony was maintained in a mesh cage (0.6 × 0.6 × 1.0 m) in a large room illuminated with artificial (fluorescent) and natural (window) light throughout the daytime. At 5:00 PM, the artificial lights were extinguished, and only natural lighting was available until sunset. The room was maintained at (mean ± SD) 23.4 ± 0.3 °C and 69.0 ± 3.0% RH.

Experimental flies derived from this colony were sexed and separated within 8 d of eclosion. Flies were given unrestricted access to a 3:1 (vol:vol) mixture of white granulated sugar and hydrolyzed yeast protein as a food source, and cotton dental wicks placed upright in small cups filled with water. Food and water were replaced once per wk.

Mating Trials

Males were captured in vials and placed on ice for several min; after they were immobilized, a small dot of enamel paint was carefully placed on the thorax. Male flies were marked with different colors to distinguish between control and treated males, and marked males resumed normal activity within 2 min of exposure to ambient temperature. Females were not cooled or marked. Previous studies have not observed any noticeable effects of cooling and marking males (Shelly & Villalobos 1995; Shelly et al. 2005; Wee et al. 2007; Kumaran et al. 2013), and preliminary mating trials of marked males in this study likewise revealed no effect.

At least 2 d following marking, males (18–20 d after eclosion) of 1 color group were exposed to a filter paper (7 cm diam) soaked in 5 mL of a zingerone solution (5 g of crystalline lure mixed with 50 mL of

distilled water) for 2 h (hereafter referred to as "treated males"). This quantity of lure solution saturated the filter paper. Within 15 s of exposure to the zingerone-treated filter paper, a high feeding response from the males was observed. Males were exposed to the lure from 8:00 to 10:00 AM, in groups of 50 to 100 individuals, in wooden cages (27 cm cubes) with mesh sides. The males of the other color group were not exposed to zingerone, but instead received only water (hereafter referred to as "control males").

Mating trials were performed using the aforementioned treated and control males. Treated males (n = 2), control males (n = 2), and a single female were placed into clear plastic cages (30 cm cubes), using a male-biased sex ratio to mimic a lek. An artificial plant with green leaves in the cages also helped mimic the natural mating environment. Mating occurs at dusk in *Z. cucurbitae*; therefore, cages were placed in front of a west-facing window and left undisturbed for 8 h prior to sunset. Artificial lights were extinguished at 4:00 PM, and the cages received direct sunlight at dusk via the window. Cages were checked for mating pairs at 10:00 PM, after complete darkness, and the mated pairs were captured in vials. The mated males were identified to treatment group by observing the color of enamel paint on their thorax.

Observations of mating between females with treated males and control males were carried out 0 (same d), 1, 2, and 3 d after treated males were exposed to the zingerone solution. Data from 70 mated females were collected from each of the 4 lure exposure periods.

FECUNDITY, EGG HATCH, AND MORTALITY

Insects

Zeugodacus cucurbitae pupae were obtained from a colony maintained at the USDA-ARS facility in Hilo, Hawaii. These flies were > 300 generations removed from the wild, and reared on an artificial diet using standard mass-rearing procedure (Vargas 1989). Adults were sexed and separated within 3 d of eclosion, well before sexual maturity at 12 to 14 d for this strain. Preliminary tests revealed that males of this rearing colony were most responsive to zingerone at 14 d after eclosion. Thus, at this age males were provided access to zingerone following the previously described protocol. Feeding was observed, and males that fed on the zingerone for 3 min were gently collected by placing a cup over the feeding males and moving them to a separate cage. Control males were not exposed to zingerone liquid or volatiles.

Mating was performed with groups of 150 males (14 d after eclosion) and 150 females (14 d after eclosion) together in large cages. Females were placed in cages containing either zingerone-fed males or control males. These cages were placed in front of a window receiving natural light. Artificial lights were extinguished 4 h before sunset, and mating began immediately prior to sunset. Sperm transfer in *Z. cucurbitae* is completed after 4 h from initiation of copulation (Yamagishi & Tsubaki 1990), so mating pairs were collected 4 to 5 h after the mating period to allow ample time for sperm and seminal fluid transfer. Females mated to zingerone-fed males were referred to as "treated females," and females mated to non-lure fed males were referred to as "control females."

Female Fecundity and Mortality

Fifteen mated females were placed into clear plastic cages (30 cm cubes) with mesh ventilation holes. There were 6 cages for both treatment groups, for a total of 12 cages arranged randomly on 4 shelves placed directly in front of a window receiving natural light throughout the daytime and dusk. All females were given unrestricted access to food (protein hydrolysate and sugar in a 3:1 [vol:vol] mixture) and water (cotton wicks placed in cups of water). Food and water sources were replaced once per wk.

Three times per wk females in each cage were provided access to egg collection cups (266 mL [9 oz.] paper cups coated in a 1:4 [vol:vol] mixture of green and yellow gloss enamel paints) that were punctured with holes and lined with a paper towel soaked with 1.5 mL of pure cucumber juice. Females were allowed to oviposit into the cups for 4 h, and eggs were counted within 6 h of collection. The number of eggs laid by females in each cage was recorded for 6 wk. Dead females were removed and recorded on egg collection d.

Egg Hatch

Egg hatch was assessed for each of the oviposition d over the 6 wk period. On oviposition d, 50 eggs were taken from each egg collection cup and placed onto moist black filter papers. All eggs were collected if fewer than 50 were deposited. The filter paper was placed in an enclosed Petri dish and sealed with parafilm to avoid egg desiccation. Egg hatch rates were observed under a microscope 48 to 52 h after the eggs were laid.

Male Mortality

Virgin males were sexed 3 d after eclosion and moved to a large cage provisioned with food and water. At 13 d after eclosion, the males were randomly separated into 1 of 2 large cages and again provided food and water. The next d the males in 1 cage were provided access to zingerone following the protocol previously described. Males that fed on the zingerone for 3 min were collected and moved to a separate cage. Zingerone-exposed males were referred to as "treated males" and males from the second cage that were never exposed to zingerone were referred to as "control males." Males were then placed in clear plastic experimental cages (30 cm cubes) with ventilation holes covered in mesh. Each experimental cage received 30 males of the same treatment (zingerone-fed or control), and 6 cages were allotted to each treatment. Cages were supplied with food and water, which were replaced once per wk. Each cage was checked daily (Mon to Fri) for dead males, which were recorded and removed from the cage. All flies that died over the weekend (Sat and Sun) were counted on Mon and removed. Male deaths were recorded for 10 continuous wk.

STATISTICS

A binomial test assessed the null hypothesis of random mating (i.e., equal mating frequencies) of treated and control males. To compare female fecundity, the number of eggs laid per living female per collection date (3 per wk) were averaged on a weekly basis and analyzed using a 2-way ANOVA with wk and lure treatment as principal effects. Egg hatch rates also were computed on a weekly basis and analyzed with a 2-way ANOVA with wk and lure treatment as principal effects. Fecundity and egg-hatch rate were log-normal transformed to obtain normal distributions. Female and male mortality over time was assessed using a chi-squared log-rank test, and the number of flies living at the conclusion of the observations were compared using a 1-way ANOVA testing lure treatment. All statistics were computed using SAS JMP (SAS Institute 2012) and R Statistical Language (R Core Team 2017).

Results

MATING SUCCESS

Allowing adult males to feed on zingerone improved their mating success but only on the first day after exposure (Fig. 1). On this day,
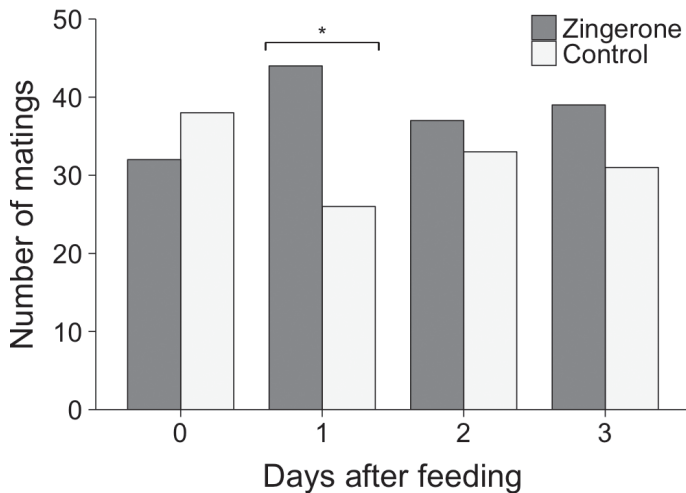
Fig. 1. Number of copulations by zingerone-fed and unfed (control) males in cages containing 1 female, 2 zingerone-fed males, and 2 control males. Copulations were observed 0 (same d), 1, 2, and 3 d after zingerone-fed males consumed zingerone. An asterisk (*) denotes statistical significance at $P < 0.05$.

62.9% (44/70) of the treated males mated compared to 37.1% (26/70) of the control males ($P < 0.05$). On all other test days (i.e., 0, 2, and 3 d after zingerone exposure), there were no detectable differences in the mating success of treated and control males.

FECUNDITY, EGG HATCH, AND MORTALITY

Female Fecundity

The number of eggs laid by females varied independently of lure treatment ($F = 2.49$; df = 1,7; $P = 0.12$) (Fig. 2). The number of eggs laid was highest in the first wk after mating and declined weekly ($F = 37.76$; df = 5,71; $P < 0.0001$). After 6 wk, the cumulative number of eggs laid by females mated to treated or control males was (mean ± SE) 430.53 ± 15.29 and 404.23 ± 25.13, respectively. The average number of eggs laid per wk by females mated to treated and control males was (mean ± SE) 71.75 ± 4.47 and 67.37 ± 4.79, respectively.

Egg Hatch

The rate of egg hatch did not differ with lure treatment ($F = 0.11$; df = 1,71; $P = 0.74$), and the hatch rate declined weekly ($F = 33.73$; df = 5,71; $P < 0.0001$) (Fig. 3). Over the first wk, the hatch rate of eggs laid by females mated to treated males was (mean ± SE) 91.22% ± 4.03%, and the hatch rate of eggs laid by control males was (mean ± SE) 93.22% ± 1.66%. In the sixth wk, the hatch rate of eggs laid by females mated to treated males was (mean ± SE) 22.89% ± 6.90%, and the hatch rate of eggs laid by control males was (mean ± SE) 25.43% ± 5.16%. Egg hatch rates declined by an average of 13.67% per wk for females mated to treated males and 13.56% for females mated to control males.

Female Mortality

Survival differed significantly between females mated to either treated or control males ($\chi^2 = 4.08$; $P < 0.05$) (Fig. 4). This difference reflected the high mortality of females mated to treated males between wk 1 and 2. During this period 10.0% of females mated to treated males died compared to 1.1% of females mated to control males. After 6 wk, there was a difference ($F = 5.46$; df = 1,71; $P < 0.05$) in the number of surviving females mated to treated males (mean ± SE, 66.67% ± 2.43%) and females mated to control males (mean ± SE, 73.33% ± 4.21%).

Male Mortality

There was no difference in the survival of treated and control males ($\chi^2 = 0.0032$; $P > 0.05$) (Fig. 5). Between wk 1 and 2, there was a greater rate of mortality in treated males (i.e., 2.25% of the population died) relative to control male (no deaths), but this decline was not statistically significant ($P > 0.05$, Tukey HSD test). After 10 wk, the survival of treated males (mean ± SE, 89.22% ± 2.62%) and control males (mean ± SE, 90.12% ± 3.11%) was not different ($F = 0.078$; df = 1,116; $P > 0.05$).

Discussion

Various lures enhance the mating success of male fruit flies that feed on them (Shelly 2010; Kumaran et al. 2013). This study is the first to demonstrate a mating effect of zingerone feeding by *Z. cucurbitae*, although zingerone has been assumed to confer a mating advantage
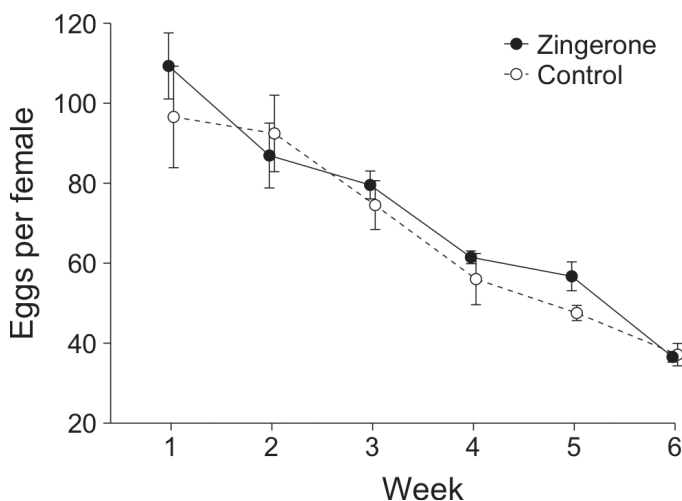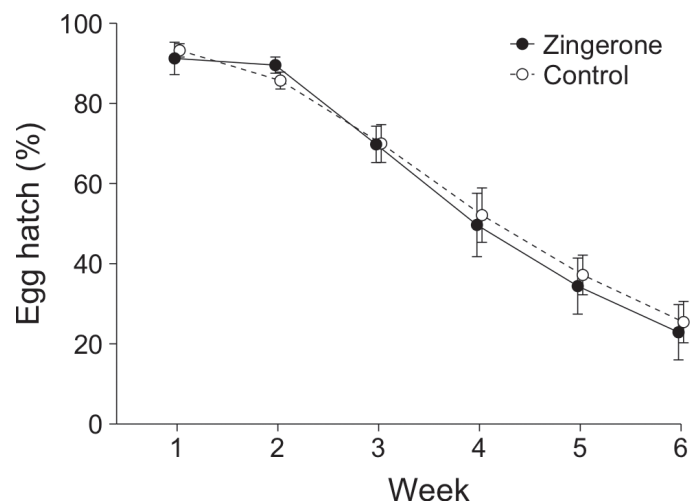

Fig. 2. Number of eggs laid by females mated with zingerone-fed and unfed (control) males over 6 wk. Means represent eggs laid by living females to account for mortality over the 6 wk trial.

Fig. 3. Percent hatch rate (mean ± SE) of eggs laid by females mated to zingerone-fed and unfed (control) males. Fifty eggs were observed 3 times each wk and the hatch rate was averaged weekly. Means represent the weekly hatch rate of eggs collected from 6 cages containing 15 mated females each.
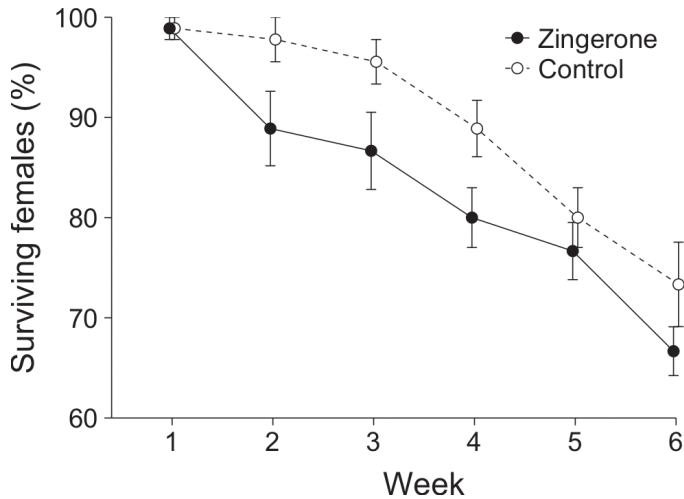
Fig. 4. Survival (mean ± SE) of females mated with zingerone-fed and unfed (control) males over 6 wk. Means represent the weekly percentage of females living in 6 cages containing 15 mated females each. Females were allowed to oviposit into egging cups 3 times per wk and the numbers of eggs laid were recorded.

in this species due to its enhanced mating effects in other fruit fly species (Khoo & Tan 2000; Tan & Nishida 2000; Kumaran et al. 2013) and its similarity in molecular structure to other male lures (Tan & Nishida 2000).

We found that zingerone feeding enhanced the mating success of males only on the first d after lure feeding. This 1-d mating advantage has been similarly demonstrated when *Z. cucurbitae* males feed on raspberry ketone (Shelly 2000c) and cue-lure (Shelly & Villalobos 1995). However, Shelly (2017) did not observe a mating effect of zingerone feeding on *Z. cucurbitae* in field cage trials. Differing effects could be due to the amount of lure presented to the flies (1 mL liquefied zingerone at the highest dose in the aforementioned study compared to 5 g of liquefied zingerone in our study), or differences in the mating arena (outdoor field cages [2.5 m high × 3 m diam] with 240 flies per cage in the aforementioned study, compared to small indoor cages [30 cm³] with 5 flies per cage in our study). On the same day of zingerone feeding, observations during this study suggested that males mated less often than unfed males (although the difference was non-significant),
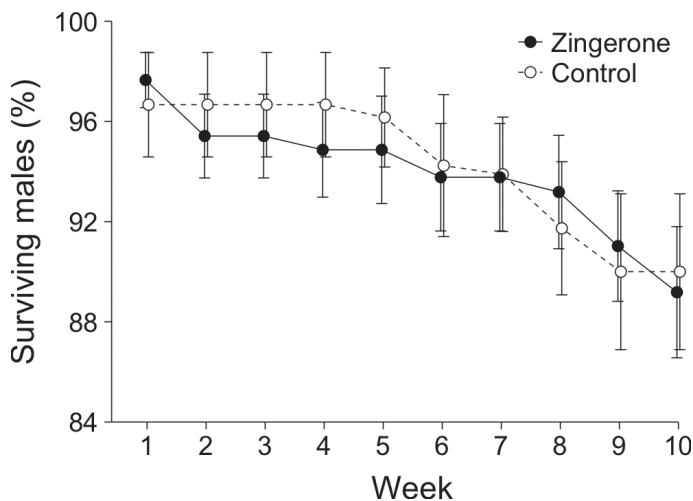

Fig. 5. Survival (mean ± SE) of zingerone-fed and unfed (control) males over 10 wk. Means represent the weekly percentage of males living in 6 cages containing 30 males each.

a result also observed for raspberry ketone-fed *Z. cucurbitae* (Shelly 2000c) and methyl eugenol-fed *B. dorsalis* (Shelly & Dewire 1994). Currently there is no explanation for this effect; however, *Z. cucurbitae* and *B. dorsalis* are described as appearing "sluggish" or lethargic shortly after consuming a male lure (see aforementioned studies), and this may explain fewer matings on the same d of lure feeding. This explanation would run contrary to recent indications that lure-feeding increases the metabolism of males (Kumaran et al. 2014; Akter et al. 2017b). The current study did not include behavioral observations of males after feeding on zingerone; thus, it is unknown whether consumption of zingerone similarly induces male lethargy.

It has been suggested that feeding male lures to sterile flies may improve their mating success with wild fertile females in the field. The benefit to application of the sterile insect technique (SIT) to insect management may be substantial for some methyl eugenol-responding species, such as *B. dorsalis* that mate more often for > 35 d after feeding on methyl eugenol (Shelly & Dewire 1994). However, our results suggest that feeding zingerone to sterile male *Z. cucurbitae* would likely have a minimal effect as mating success is enhanced for only 1 d. Nevertheless, feeding lures to male flies has the additional benefit of reducing the attraction of lure-fed males to lure traps in the field, enabling both the sterile insect technique and the male annihilation technique to be used in conjunction when males are released during sterile insect technique-based programs, and lures are added to their pre-release diet. Zingerone feeding may reduce male attraction to cue-lure traps when added to diet consumed by the immature or mature adults (for examples with cue-lure and *B. tryoni*, see Akter et al. 2017a; Khan et al. 2017), or to diet consumed by the larvae (for example with methyl eugenol and *B. dorsalis*, see Manoukis et al. 2018).

Female Fecundity

This study provided no evidence that female fecundity is affected when *Z. cucurbitae* females mate with zingerone-fed males. The ineffectiveness of zingerone-fed males to modify female fecundity in our study also was observed with *Z. cucurbitae* when males fed on cue-lure (Shelly & Nishimoto 2017), and with *B. dorsalis* when males fed on methyl eugenol (Shelly 2000a). However, observations with *Z. cucurbitae* and *B. dorsalis* differ from those for *B. tryoni*, whereby females mated to cue-lure-fed or zingerone-fed males laid more eggs during their lifetime (Kumaran et al. 2013).

The effects of lure feeding may differ between species, whereby some experience enhanced fecundity (e.g., *B. tryoni*), but others do not (e.g., *Z. cucurbitae* and *B. dorsalis*). Also, it is possible that lure-feeding has a similar effect on the different species, and the observed differences can be, at least partially, attributed to differing experimental designs. For example, in the study with *B. tryoni* (Kumaran et al. 2013), male lures remained in the experimental cages for the entire 8 wk experiment, and males may have consumed more lure given their unrestricted access to it. In contrast, in our study, the Shelly and Nishimoto (2017) study on *Z. cucurbitae*, and the Shelly (2000a) study on *B. dorsalis*, males were allowed to feed on zingerone (5 g diluted in 50 mL water) for 3 min, cue-lure (0.5 mL, undiluted) for 2 h, and methyl eugenol (1.5 mL, undiluted) for 30 s, respectively, during restricted feeding periods when the adult males were sexually mature. In our study, similar to Shelly (2000a), we collected and counted only males that fed on the lure as "treated" males, whereas Kumaran et al. (2013) and Shelly and Nishimoto (2017) exposed the males to the lure without documenting how many mated males fed on the lures. Whereas lure feeding did not affect fecundity in *Z. cucurbitae* and *B. dorsalis*, it is possible that the males did not consume enough lure to influence female fecundity (although mating success was affected) since feeding

was restricted to a single period in these studies. This is a notable departure in methodology from the study with *B. tryoni*, where fecundity was affected when males were given unrestricted access to the lures for 8 wk. Furthermore, male lures have a similar molecular structure to compounds that stimulate oviposition in female fruit flies (Metcalf et al. 1983), and it is possible that in the study with *B. tryoni* the volatiles emitted from the lure source stimulated females to lay eggs over the course of the study.

In addition, in our study, as well as those of Shelly and Nishimoto (2017) and Shelly (2000a), female fecundity was measured following a single mating with either a lure-fed or control male. However, in the Kumaran et al. (2013) study, *B. tryoni* females and males were placed in the same cages for the duration of the 8 wk study. Male and female *B. tryoni* may have mated multiple times during this time, as female *B. tryoni* are known to mate more than once (Harmer et al. 2006; Radhakrishnan & Taylor 2008; Radhakrishnan et al. 2009). Kumaran et al. (2013) also noted that female *B. tryoni* were more likely to remate when presented with a lure-fed male. Frequent mating may shorten female lifespan and increase fecundity in dacine fruit flies (Whittier & Shelly 1993; Shelly 2000a; Chinajariyawong et al. 2010) and broadly in insects (Arnqvist & Nilsson 2000). If multiple mating occurred more often in cages where females cohabited with lure-fed males, then an increase in fecundity could have resulted, and subsequently a shorter lifespan in females would have been a likely effect of increased fecundity early in life.

Egg Hatch

We found no difference in egg hatch for females mated to zingerone-fed males versus control males. Similar observations were made for *B. dorsalis* (Shelly 2000a) when females were mated with methyl eugenol-fed males, and *B. tryoni* (Kumaran et al. 2013) when females were mated with cue-lure or zingerone-fed males. However, in their study of *Z. cucurbitae*, Shelly and Nishimoto (2017) found that females mated to cue-lure-fed males had reduced egg hatch compared with females mated to control males. Different results in the 2 studies suggest that cue-lure and zingerone feeding by males may have different effects on mated females. We found that egg hatch rate declined gradually over time; this effect was likely due to depletion of sperm reserved by the singly mated females, as was the case for *B. tryoni* (Perez-Staples et al. 2007) and *Ceratitis capitata* (Wiedemann) (Blay & Yuval 1999).

Female and Male Mortality

The number of surviving females mated to zingerone-fed males declined sharply between wk 1 and 2 after mating. During this period there also was a decline in the number of surviving treated males, but this trend was not statistically significant. It is possible that zingerone has some toxic properties, and some of the lure may have remained on the male after lure feeding and could have been transferred to the female while mating. If true, small amounts of the lure could have harmed the flies during the experiment, leading to higher mortality. Lure-feeding has been suggested to increase the activity of males (Kumaran et al. 2013), which could lead to increased aggression when housed together in a cage, and potentially increase stress and mortality. However, increased activity of females mated with lure-fed males has not been tested. Another explanation is that lure feeding enhances the male seminal fluids, either causing the male to produce more proteins or a higher quality of proteins, which could affect the fitness of the mated female. For example, in *Drosophila melanogaster* (Macquart) (Diptera: Drosophilidae) at least 85 different proteins are

passed from the male to the female during copulation, and these have a wide range of effects on female behavior (Chen 1996). Many of these proteins reduce the lifespan of the female when passed from the male (Chapman 2001). In male *B. tryoni*, Kumaran et al. (2014) demonstrated that zingerone feeding altered the expression of genes with homologues linked to various seminal proteins in *Drosophila* species, and some of these proteins are believed to impact female longevity when transferred from the male during copulation. It is currently unknown if zingerone feeding alters male seminal proteins in *Z. cucurbitae*, or if female longevity would be impacted, but investigations into such an effect may be insightful. The current results suggest that females do not benefit from mating with zingerone-fed males, because there is no positive benefit associated with fecundity and egg hatch, but there is a negative effect of increased mortality. Given these results, at present it is not clear why *Z. cucurbitae* females select zingerone-fed males as mates. Future work might consider whether this mate reference confers indirect fitness benefits to females, or alternatively, whether it represents an example of sexual conflict.

Acknowledgments

References Cited

Akter H, Adnan S, Morelli R, Rempoulakis P, Taylor PW. 2017a. Suppression of cuelure attraction in male Queensland fruit fly provided raspberry ketone supplements as immature adults. PLoS One 12: e0184086. doi.org/10.1371/journal.pone.0184086

Akter H, Mendez V, Morelli R, Perez J, Taylor PW. 2017b. Raspberry ketone supplement promotes early sexual maturation in male Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). Pest Management Science 73: 1764–1770.

Andersson MB. 1994. Sexual Selection. Princeton University Press, Princeton, New Jersey, USA.

Arnqvist G, Nilsson T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. Animal Behaviour 60: 145–164.

Blay S, Yuval B. 1999. Oviposition and fertility in the Mediterranean fruit fly (Diptera: Tephritidae): effects of male and female body size and the availability of sperm. Annals of the Entomological Society of America 92: 278–284.

Chapman T. 2001. Seminal fluid-mediated fitness traits in *Drosophila*. Heredity 87: 511–521.

Chen PS. 1996. The accessory gland proteins in male *Drosophila*: structural, reproductive, and evolutionary aspects. Experientia 52: 503–510.

Chinajariyawong A, Drew RAI, Meats A, Balagawi S, Vijaysegaran S. 2010. Multiple mating by females of two *Bactrocera* species (Diptera: Tephritidae: Dacinae). Bulletin of Entomological Research 100: 325–330.

Darwin C. 1871. The descent of man and selection in relation to sex. J. Murray, London, United Kingdom.

Dawkins R. 1986. The Blind Watchmaker. W.W. Norton & Company, New York, USA.

Dominiak BC, Fay HAC, Fanson BG. 2015. Field evaluation of the efficacy of zingerone and other chemical lures on seven species of fruit fly in Sydney, New South Wales, Australia. Plant Protection Quarterly 30: 67–71.

Fay HA. 2012. A highly effective and selective male lure for *Bactrocera jarvisi* (Tryon) (Diptera: Tephritidae). Australian Journal of Entomology 51: 189–197.

Fisher RA. 1930. Genetical Theory of Natural Selection. Oxford University Press, Oxford, United Kingdom

Harmer AMT, Radhakrishnan P, Taylor PW. 2006. Remating inhibition in female Queensland fruit flies: effects and correlates of sperm storage. Journal of Insect Physiology 52: 179–186.

Haq IU, Caceres C, Meza JS, Hendrichs J, Vreysen MJB. 2018. Different methods of methyl eugenol application enhance the mating success of male oriental fruit fly (Diptera: Tephritidae). Scientific Reports 8: 1–8.

Haq IU, Vreysen MJB, Caceres C, Shelly TE, Hendrichs J. 2014. Methyl eugenol aromatherapy enhances the mating competitiveness of male *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae). Journal of Insect Physiology 1: 1–6.

Haq IU, Vreysen MJB, Schutze M, Hendrichs MJ, Shelly T. 2016. Effects of methyl eugenol on mating compatibility of Asian populations of *Bactrocera dorsalis* (Diptera: Tephritidae) with African population and with *B. carambolae*. Journal of Economic Entomology 109: 148–153.

Hee AKW, Tan KH. 1998. Attraction of female and male *Bactrocera papayae* to conspecific males fed with methyl eugenol and attraction of females to male sex pheromone components. Journal of Chemical Ecology 24: 753–764.

Khan MAM, Manoukis NC, Osborne T, Barchia IM, Gurr GM, Reynolds OL. 2017. Semiochemical mediated enhancement of males to complement sterile insect technique in management of the tephritid pest *Bactrocera tryoni* (Froggatt). Scientific Reports 7: 13366. DOI:10.1038/s41598-017-13843-w

Khoo CCH, Tan KH. 2000. Attraction of both sexes of melon fly, *Bactrocera cucurbitae* to conspecific males: a comparison after pharmacophagy of cue-lure and a new attractant – zingerone. Entomologia Experimentalis et Applicata 97: 317–320.

Kumaran N, Balagawi S, Schutze MK, Clarke AR. 2013. Evolution of lure response in tephritid fruit flies: phytochemicals as drivers of sexual selection. Animal Behaviour 85: 781–789.

Kumaran N, Prentis PJ, Mangalam KP, Schutze MK, Clarke AR. 2014. Sexual selection in true fruit flies (Diptera: Tephritidae): transcriptome and experimental evidence for phytochemicals increasing male competition ability. Molecular Ecology 23: 4645–4657.

Liu L, Simon SA. 1996. Similarities and differences in the currents activated by capsaicin, piperine, and zingerone in rat trigeminal ganglion cells. Journal of Neurophysiology 76: 1858–1869.

Manoukis NC, Cha DH, Collignon RM, Shelly TE. 2018. *Terminalia* larval host fruit reduces the response of *Bactrocera dorsalis* (Diptera : Tephritidae) adults to the male lure methyl eugenol. Journal of Economic Entomology 111: 1644–1649.

Manrakhan A, Daneel JH, Beck R, Virgilio M, Meganck MK, De Meyer M. 2017. Efficacy of trapping systems for monitoring of Afrotropical fruit flies. Journal of Applied Entomology 141: 825–840.

Metcalf RL, Mitchell WC, Metcalf ER. 1983. Olfactory receptors in the melon *Dacus cucurbitae* and the oriental fruit fly *Dacus dorsalis*. Proceedings of the National Academy of Sciences 80: 3143–3147.

Orankanok W, Chinvinijkul S, Sawatwangkhoung A, Pinkaew S, Orangkanok S. 2011. Methyl eugenol and pre-release diet improve mating performance of young *Bactrocera dorsalis* and *Bactrocera correcta* males. Journal of Applied Entomology 137: 200–209.

Perez-Staples D, Harmer AM, Taylor PW. 2007. Sperm storage and utilization in female Queensland fruit flies (*Bactrocera tryoni*). Physiological Entomology 32: 127–135.

R Core Team. 2017. R: a language and environment for statistical computing v.1.1.419. R Foundation for Statistical Computing, Vienna, Austria.

Radhakrishnan P, Perez-Staples D, Weldon CW, Taylor PW. 2009. Multiple mating and sperm depletion in male Queensland fruit flies: effects on female remating behaviour. Animal Behaviour 78: 839–846.

Radhakrishnan P, Taylor PW. 2008. Ability of male Queensland fruit flies to inhibit receptivity in multiple mates, and the associated recovery of accessory glands. Journal of Insect Physiology 54: 421–428.

Royer JE. 2015. Responses of fruit flies (Tephritidae: Dacinae) to novel male attractants in north Queensland, Australia, and improved lures for some pest species. Austral Entomology 54: 411–426.

Royer JE, Agovaua S, Bokosou J, Kurika K, Marasuai A, Mayar DG, Niangu B. 2018. Responses of fruit flies (Diptera: Tephritidae) to new attractants in Papua New Guinea. Austral Entomology 57: 40–49.

SAS Institute. 2012. SAS/STAT v.10 users guide: statistics. SAS Institute, Cary, North Carolina, USA.

Shelly T. 2000a. Fecundity of female oriental fruit flies (Diptera: Tephritidae): effects of methyl eugenol-fed and multiple mates. Annals of the Entomological Society of America 93: 559–564.

Shelly T. 2000b. Flower-feeding affects mating performance in male oriental fruit flies (Diptera: Tephritidae): low incidence of repeat feeding. Florida Entomologist 77: 201–208.

Shelly T. 2000c. Effects of raspberry ketone on the mating success of male melon flies (Diptera: Tephritidae). Proceedings of the Hawaiian Entomological Society 34: 143–147.

Shelly T. 2010. Effects of methyl eugenol and raspberry ketone/cue lure on the sexual behavior of *Bactrocera* species (Diptera: Tephritidae). Journal of Applied Entomology and Zoology 45: 349–361.

Shelly TE. 2017. Zingerone and the mating success and field attraction of male melon flies (Diptera: Tephritidae). Journal of Asia-Pacific Entomology 20: 175–178.

Shelly TE, Dewire AM. 1994. Chemically mediated mating success in male oriental fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America 87: 375–382.

Shelly TE, Nishimoto J. 2017. Does female mate choice confer direct fitness benefits? Results from a Tephritid fruit fly. Annals of the Entomological Society of America 110: 204–211.

Shelly TE, Villalobos EM. 1995. Cue lure and the mating behavior of male melon flies (Diptera: Tephritidae). Florida Entomologist 78: 473–482.

Shelly TE, Edu J, Pahio E. 2005. Influence of diet and methyl eugenol on the mating success of males of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Florida Entomologist 88: 307–313.

Sivinski J, Aluja M, Dodson G, Freidberg A, Headrick D, Kaneshiro K, Landolt P. 2000. Topics in the evolution of sexual behavior in the Tephritidae, pp. 751–792 *In* Aluja M, Norrbom AL [eds.], Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, Florida, USA.

Tan KH. 2009. Fruit fly pests as pollinators of wild orchids, pp. 195–206 *In* Sugayama RL, Zucchi RA, Ovruski SM, Sivinski J [eds.], Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance, 10–15 Sep 2009, Salvador, Brazil.

Tan KH, Nishida R. 2000. Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens*, and *Bactrocera* fruit flies via a floral synomone. Journal of Chemical Ecology 26: 533–546.

Tan KH, Nishida R. 2007. Zingerone in the floral synomone of *Bulbophyllum baileyi* (Orchidaceae) attracts *Bactrocera* fruit flies during pollination. Biochemical Systematics and Ecology 31: 334–341.

Tan KH, Nishida R, Jang EB, Shelly TE. 2014. Pheromones, male lures, and trapping of tephritid fruit flies, pp. 15–74 *In* Shelly TE, Epsky N, Jang EB, Reyes-Flores J, Vargas R [eds.], Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies. Springer, New York, New York, USA.

Vargas RI. 1989. Mass production of tephritid fruit flies, pp. 141–151 *In* Robinson AS, Hooper G [eds.], World Crop Pests, Volume 3B, Fruit Flies, their Biology, Natural Enemies and Control. Elsevier Science Publishers, Amsterdam, The Netherlands.

Wee SL, Tan KH, Nishida R. 2007. Pharmacophagy of methyl eugenol by males enhances sexual selection of *Bactrocera carambolae*. Journal of Chemical Ecology 33: 1272–1282.

Whittier TS, Shelly TE. 1993. Productivity of singly vs. multiply mated female Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae). Journal of the Kansas Entomological Society 66: 200–209.

Yamagishi M, Tsubaki Y. 1990. Copulation duration and sperm transfer in the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Japanese Society of Applied Entomology and Zoology 25: 517–519.