Title: Indirect effects of phytochemicals on offspring performance of Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae)

Short heading: Effect of male attractants on fruit fly offspring

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Abstract

Phytochemical lures such as methyl eugenol (ME) and cue-lure are used in the management of *Bactrocera* fruit flies for monitoring and control. These lures are not just attractants, but also trigger physiological changes in males that lead to enhanced mating success. Additionally, in the cue-lure responsive *Bactrocera tryoni*, females mated with lure-fed males exhibit changes in fecundity, remating receptivity and longevity. While the lures show current generation effects, no research has been done on possible multi-generational effects, although such effects have been hypothesized within a 'sexy son' sexual selection model. In this study we test for indirect, cross-generational effects of lure exposure in F1offspring of *B. tryoni* females mated with cue-lure fed, zingerone fed and lure unfed (= control) males. The F1 attributes we recorded were immature development time, immature survival, adult survival and adult male lure foraging. No significant differences were found between treatments for any of the three life history measurements, except that the offspring sired by zingerone fed males had a longer egg development time than cue-lure and control offspring. However, indirect exposure to lures significantly enhanced the lure foraging ability of F1 adult males. More offspring of cue-lure fed males arrived at a lure source in both large flight cages and small laboratory cages over a two-hour period than did control males. The offspring of zingerone fed males were generally intermediate between cue-lure and control offspring. This study provides the first evidence of a next generation effect of fruit fly male lures. While the results of this study support a 'sexy son' sexual selection mechanism for the evolution of lure response in *Bactrocera* fruit flies, our discussion urges caution in interpreting our results in this way.

Key words: cue-lure, zingerone, male lures, sexual selection, offspring effects

Introduction

The males of many fruit fly species from the Tribe Dacini (Diptera: Tephritidae: Dacinae) are strongly attracted to plant derived chemicals or their close chemical analogues (Bateman 1972). These chemicals, known in the fruit fly literature as parapheromones or male lures (referred to in this paper as male lures) elicit strong, positive anemotaxis and chemotactic feeding responses in male flies (Metcalf et al. 1975). The behavioural attraction of flies to male lures has been manipulated and used in monitoring and as part of a lure-and-kill approaches in pest management (Christenson 1963). The two most commonly used male lures are methyl-eugenol (ME) (4-[allyl-1,2-dimethoxybenzene]) and cue-lure (4-[4-acetoxyphenyl]-2-butanone), but other lures occur (Drew and Hooper 1981). Methyl-eugenol is found commonly in nature (Tan and Nishida 2012), while cue-lure is a synthetic chemical with natural analogues (Porter and Christenson 1960).

In addition to the importance of lures in fruit fly management, their functional role(s) and the evolutionary reasons for lure feeding remain only partially understood (Shelly 2010). Males fed on the chemicals accumulate them (or their conversion products) in their rectal glands via the heamolymph, from where they are subsequently released as part of the male sex pheromone (Hee and Tan 2005, Hee and Tan 2006). Presumed to be due to the modified pheromone, lure fed males of many species have a well documented mating advantage over lure unfed males (Shelly and Villalobos 1995, Shelly and Nishida 2004, Wee et al. 2007, Shelly et al. 2010). However, the effects of lure feeding have been studied in detail for only a very small number of the hundreds of fruit fly species which respond to lures, and at least some results have been shown to vary across species. For example male mating advantage following lure feeding has been shown in *Bactrocera dorsalis* (Hendel), *Bactrocera carambolae* (Drew and Hancock) and *Bactrocera tryoni* (Froggatt) (Shelly et al. 2010, Kumaran et al. 2013), but not in *B. cacuminata* (Hering) (Raghu and Clarke 2003); while a

positive male mating effect is long lasting (up to 30days) in the ME responsive *B. dorsalis* (Shelly and Dewire 1994), it is short (2 - 3 days) in the cue-lure responsive *B. cucurbitae* (Coquillett) and in the ME responsive *B. carambolae* (Shelly and Villalobos 1995, Wee et al. 2007). The physiology of how the different lures are handled by the flies is also different, with ME being broken down soon after digestion and before accumulation in the rectal gland, while cue-lure accumulates in the rectal gland unaltered (Shelly 2010).

Nearly all research on fruit fly lures has focused on the direct effects of lure feeding on males, and almost none on the indirect effects on females mated with lure-fed males. For the ME responding *B. dorsalis* there is no evidence of effect on females, with females mated with lure-fed males showing no changes in longevity, total egg production, temporal pattern of egg production or percentage egg hatch compared to females mated with control males (Shelly 2000). In direct contrast, in the only other study of its sort, Kumaran et al. (2013) found all of these measures were significantly different for females mated with lure-fed males over control females for the cue-lure responsive *B. tryoni*. These results, along with the known differences in the internal processing of ME and cue-lure and the different duration of male mating impacts (references above), suggests that more research on cue-lure is warranted, particularly as this lure is generally under-represented in the broader fruit fly lure research area (Shelly 2010).

Given that our previous work (Kumaran et al. 2013) found that mating with lure-fed males altered the physiology of *B. tryoni* females, we wished to investigate if any changes also occurred in the progeny of those females (i.e. the F1 generation). More specifically, by comparing offspring sired by lure-fed and unfed males, the aims of this study are to: i) assess any indirect physiological effects of lures on the F1 life history traits of immature development time, immature survival and adult longevity; and ii) to compare lure foraging

ability of F1 adult males in order to test the cross-generational effect of male lures in the context of 'sexy son' explanation for the evolution of lure response. As for our previous study the cue-lure responsive Queensland fruit fly, *B. tryoni*, served as our model species. As lures we used both the synthetic cue-lure and the naturally occurring phenolic alkanone, zingerone. Zingerone is found in orchid blossoms and other plant sources and attracts both ME and cue-lure responsive flies (Tan and Nishida 2007); physiologically it induces exactly the same changes in male and female *B. tryoni* as does cue-lure (Kumaran et al. 2013). Zingerone is less attractive to *B. tryoni* than cue-lure (Fay 2012); however, this chemical is included in the current study as it is a naturally occurring compound that might help to answer evolutionary reasons for lure response. Also, as a chemical that attracts both cue-lure and ME responsive flies, we had *a priori* reason to suspect that the fly's response to zingerone may not be the same as to cue-lure and so its conclusion increased the breadth of conclusions we could draw from any findings.

Materials and Methods

Insect source

Flies were obtained from a colony maintained by the [Queensland Government] Department of Agriculture, Fisheries and Forestry, Brisbane. Flies were maintained in cages (90 cm x 60 cm x 60 cm high) at 27°C and 70% relative humidity in a room illuminated with fluorescent lights between 0700 and 1600 hours and natural light for the rest of the day. Adults were sexed within three days of emergence and provided with protein hydrolysate, sugar and water *ad libitum*. When 14-days old, 15 randomly chosen males were placed in each of 24 small cages (30 cm x 20 cm x 20 cm high) and provided with 1.5 ml of cue-lure (eight cages), or zingerone (10ug/ul of 95% ethanol) (eight cages) in a cotton wick on inverted petri dish for 2h: the remaining eight cages were lure-unfed controls. The lure concentration was chosen

based on previous studies on *B. cucurbitae* and *B. tryoni* (Shelly and Villalobos 1995; Tan and Nishida 1995). To obtain offspring, 15 females from the same batch of pupae as were the males were released into each of the cages and allowed to mate. The following day females were provided with egging cups for egg laying and eggs were collected over three hours; this was repeated for the following two days (i.e. three days of egging in total). The short duration of sampling was based on previous research which shows the direct effect of cue-lure on fruit flies only lasts for two to three days in *B. cucurbitae* (Shelly & Villalobos, 1995) and *B. tryoni* (Kumaran et al. 2013). For each treatment (i.e. the two lures and control), the eggs were collected for three days and from across the eight cages. Eggs and emerging adults were randomly used for all the life history and foraging experiments (NB the age of all eggs used were known and accounted for in development time calculations). The purpose of splitting treatment flies across cages was so we could more easily observe individual pairs and so ensure mating had occurred.

Experiments

Developmental time and survival of F1 immature stages

To identify differences in the F1 immature developmental time and survivorship for offspring sired by lure-fed males and unfed males, eggs from the three treatment groups were placed individually into the lids of 10ml screw-cap vials filled with carrot media (Heather and Corcoran 1985) as larval food. These were then placed individually into petri-dishes, partially filled with vermiculite to serve as a subsequent pupation site. Individual egg hatching was directly monitored every three hours until all hatching was completed, and then subsequent larval (the three instars combined) and pupal duration monitored every 12 hours. On adult emergence the sex of the individual was recorded for sex ratio calculation. The percentage survival of egg, larval and pupal stages was calculated based on difference between the number of individuals entering a particular developmental stage and number successfully

140 completing. Sixty eggs were initially set up for each treatment, with the total sample size
141 reducing over the life of the trial due to mortality. Petri-dishes were held at room
142 temperature during egg development and at a constant 27°C and 70% relative humidity for
143 the rest of the observations.

144 *F1 adult survival*

145 On the day of emergence, F1 adults from cue-lure, zingerone and lure-unfed treatments were
146 placed into small Perspex cages (30 cm x 20 cm x 20 cm high) and provided with water,
147 sugar and protein hydrolysate (but no lure). Thirty flies (equal sex ratio) were placed into
148 each cage, and there were eight replicate cages per treatment. The mortality of flies within
149 the cages was monitored daily for eight weeks.

150 *F1 adult foraging to a lure*

151 This study tested for differences in the ability of offspring sired by lure-fed or unfed males in
152 foraging to a lure, as measured by the number of F1 adult males landing on a lure source
153 within a two hour observation period. The experiment was carried out in large field cages
154 (7m x 7 m x 3.8m high). For each replicate, 30 F1 males (14 - 17 day old) from each of the
155 cue-lure, zingerone and control treatments were simultaneously released into a cage. Flies
156 were marked with non-toxic paint on their thoraces for identification; preliminary
157 observations showed no effect of marking. Further, the colours were rotated amongst the
158 treatment groups to avoid any hidden effects. The lure sources (either 1.5ml of cue-lure or
159 zingerone on cotton wicks) were exposed at a height of 110 cm on plastic plants at each of
160 three locations randomly chosen with uniform distance to each other and to cage walls; only
161 one lure type was tested in a given trial. The number of flies locating the lure (three sources
162 combined) was recorded for two hours between 0800 and 1000h, which is the major time of
163 lure response for *B. tryoni* (Brieze-Stegeman et al. 1978). Eight replicate cages were run for

each of the cue-lure and zingerone trials, and on a given day four cages (two replicates each for cue-lure and zingerone) were run.

Because of an unexpected result, the experiment was repeated in the laboratory using small cages (30cm x 20 cm x 20 cm high), with 30 males per cage. Flies were not marked this time and the three F1 treatment groups were maintained in separate cages for observation, which were otherwise identical to the field cage trial except that only one lure source was provided. Four replicate cages for each treatment/lure combination (e.g. F1 adults of cue-lure mated females exposed to zingerone source) were run.

Statistical analysis

Differences in F1 egg, larval and pupal duration, as well as response of F1 adults to a lure source, were compared using one-way ANOVA with *post hoc* comparisons of means done using Tukey's HSD test. The probability of survival of immature stages was subjected to logistic analysis with the significance tested using the likelihood ratio test. F1 adult survival was analysed using survival analysis with significance tested using Cox proportional hazard test, and differences in cumulative survival analysed using one-way ANOVA. The sex ratio of emergent F1 adults for each lure treatment was tested for deviation from the 1:1 sex ratio normal for *B. tryoni* (Khan 2013). A Chi-square test of proportions was carried out for females, assuming the expected value was 50% of the sampled population size. All data before analyses were checked for assumptions of homogeneity of variance and any violation was corrected by appropriate data transformation. The probability level was set as $\alpha = 0.05$.

Results

Developmental time of F1 immature stages

Egg developmental time significantly differed among treatments ($F_{2, 154} = 4.39$; $P = 0.041$). Post-hoc analysis showed eggs from females mated with zingerone fed males had significantly longer development duration than control eggs, with the development duration of eggs from females mated with cue-lure fed males intermediate between the two, and not significantly different from either. Larval ($F_{2, 117} = 0.944$; $P = 0.392$), pupal ($F_{2, 110} = 1.654$; $P = 0.196$) and total immature ($F_{2, 110} = 3.152$; $P = 0.057$) development time did not significantly differ among treatments (Table 1).

Survival of F1 immature stages and sex ratio

There were no significant differences in likelihood of survival for eggs (b= 1.83 ± 0.22; $\chi^2 = 1.21$; $P = 0.547$), larvae (b= 1.19 ± 0.19; $\chi^2 = 3.21$; $P = 0.200$) and pupae (b= 2.76 ± 0.39; $\chi^2 = 1.62$; $P = 0.445$) among offspring sired by cue-lure fed, zingerone fed and unfed males (Table 1). The observed sex ratio of emergent adults was 1.5:1 (female: male), 1.3:1 and 1.2:1 for cue-lure, zingerone and control offspring respectively; none of which differed significantly from a 1:1 expected sex ratio (cue-lure $\chi^2 = 1.225$; zingerone $\chi^2 = 0.658$; control $\chi^2 = 0.121$; for df = 1, $P = 0.05$, critical $\chi^2 = 3.84$).

F1 adult survival

Adult longevity did not differ among treatments for either sex (male: $F_{2, 23} = 0.381$; $P = 0.688$; female: $F_{2, 23} = 0.452$; $P = 0.643$). During the eight week observation period, the cumulative male mortality observed in cue-lure, zingerone and control offspring was 17.9 ± 2.6, 15.8 ± 1.8 and 18.3 ± 3.1%, respectively; the same data for females was, respectively, 28.8 ± 2.1, 26.3 ± 1.6 and 25.4 ± 2.8%. Cox proportional hazard analysis showed no significant difference among treatments with time lapsed (male: $\chi_2^2 = 0.376$; $P = 0.540$; female: $\chi_2^2 = 0.128$; $P = 0.720$), with the mortality trend over time similar among treatment for both males and females (Fig. 1).

F1 adult foraging to a lure

In the large field cages, there were significant treatment effects with respect to F1 adults foraging to cue-lure ($F_{2, 23} = 3.81$; $P = 0.039$). A significantly higher proportion of offspring from females mated with cue-lure exposed males located the cue-lure source than either the zingerone or control offspring, which were not significantly different to each other (Fig. 2A). A similar pattern was seen in the trials testing fly response to zingerone, where again significant treatment effects were observed ($F_{2, 23} = 4.29$; $P = 0.027$). In this trial F1 male adults of the cue-lure treatment again showed a significantly stronger response than control males, but additionally in this trial the response of F1 males sired by zingerone fed flies were intermediate between the cue-lure and control males, and not significantly different from either (Fig. 2B).

When repeating the experiments in small cages, similar results were obtained to the large cages. There were significant treatment effects in both the cue-lure ($F_{2, 11} = 3.93$; $P = 0.039$) and zingerone ($F_{2, 11} = 6.78$; $P = 0.016$) lure trials and the general patterns of fly response were identical between trials. Significantly higher proportions of cue-lure offspring foraged to cue-lure or zingerone than the respective control offspring; while the zingerone offspring were intermediate in lure response to the cue-lure and control offspring and were not statistically different to either (Fig. 3).

Discussion

Summary of results

In *B. tryoni* we studied possible indirect effects of two male lures (cue-lure and zingerone) on the lure foraging ability and selected life history traits of F1 offspring sired by lure-fed and unfed males. In both large field cages and small laboratory cages more offspring sired by cue-lure fed males arrived at cue-lure and zingerone sources than offspring of lure

unfed males (= control flies), with the offspring of zingerone fed males generally intermediate between the two. Lure-feeding by the sire had few if any effects on the measured F1 life-history traits, with a minor increase in F1 egg development time for the zingerone fed treatment being the only significant difference between treatment and control flies. While restricted to changes in lure foraging, this study still provides the first evidence for multi-generation effects of a male lure on a tephritid fruit fly and, when combined with our earlier work (Kumaran et al. 2013), suggests that lures have positive effects for *B. tryoni* on both the parental generation and, in the context of the 'sexy son hypothesis', on the F1 generation.

Evolutionary reasons for lure feeding

Lure feeding in tephritid fruit flies is presumed to be a trait associated with sexual selection as males of most species have enhanced mating success when fed on either methyl-eugenol or cue-lure (depending on the fruit fly species) (Shelly 2010). However, no direct female benefits were detected when *B. dorsalis* females mated with ME-fed males and, assuming that increased mating success was due to modified female choice, this led Shelly (2000) to hypothesise two reasons as to why females may select lure-fed males. Firstly, he suggested that females may have inherent sensory bias to the lures and so were simply responding to males which produced those chemicals. Secondly, he suggested that females may selectively mate with lure-fed males because such matings may confer indirect benefits to the females via sexy sons (sensu Weatherhead and Robertson 1979), i.e. the sons inherit their father's lure foraging ability and so ultimately have a greater chance of passing on the female's genes. In *B. tryoni* we have previously found enhanced male mating success following male lure feeding, but we have also found direct female benefits. As definitions of 'sexy-son' sexual selection require that there be no direct parental female benefits (Weatherhead and Robertson 1979), this led us to propose that while lure feeding is indeed a trait involved in sexual

selection for *B. tryoni*, the evolution of the lure response in this species is unlikely to be driven by sexy-son selection (Kumaran et al., 2013).

In contrast to our earlier work, the data presented in this paper clearly supports a 'sexy son' effect of lures; matings between cue-lure fed males and mothers produced sons which had (at least in cages) an enhanced ability to locate lures. This means, for *B. tryoni* and cue-lure, that the 'sexy son' concept demands further attention. However, it is important to note that the aim of the current study was not to explicitly test sexy-son theory in dacine fruit flies; rather it was to test for possible physiological effects of lure exposure on the parental population to the F1 generation. To test the sexy-son hypothesis robustly requires a complex multi-generation study, assessing trait heritability and changes in mating and foraging success within known lineages. For *B. tryoni*, regardless of the outcome of such a trial, the sexy-son theory as the evolutionary driver for lure response in dacine tephritids cannot be the exclusive answer because of known direct female benefits, such as increased life-time fecundity of females mated with lure-fed males (Kumaran et al. 2013). However, in a species such as *B. dorsalis* where no current generation female affects are known (Shelly 2000), then sexy-son selection may still be an exclusive evolutionary mechanism for the development of lure response if a multi-generational lure effect is demonstrated.

Phytochemical lures appear to be an integral part of fruit fly ecology and, following the outcomes of this paper, there are now documented direct or indirect effects of lures on males feeding on those lures, females mating with lure-fed males and offspring sired by lure-fed males. Lures are indeed likely a sexual selection trait that provides various fitness and genetic benefits, but differences in how the lures act at an individual species level suggests that trying to slot the evolution of lure response into a particular theoretical sexual selection 'pigeon-hole' is unlikely to be helpful. Rather, as detailed studies are carried out on more species of

fruit fly, it appears that most progress will be made through independent assessments of how the lures directly and indirectly affect the physiology and behavior of individual species. We suggest that to fully understand the evolution of lure response and their functional significance to fruit flies, future studies should concentrate on how lures directly impact on male physiology and behavior leading to mating benefits, and the underlying mechanisms (e.g. accessory gland proteins, Radhakrishnan and Taylor 2007, Avila et al. 2011) that trigger indirect responses in females and their offspring.

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References

Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF, 2011. Insect seminal fluid proteins: Identification and function. Annu. Rev. Entomol. **56**, 21-40.

Bateman MA, 1972. The ecology of fruit flies. Annu. Rev. Entomol. **17**, 493-518.

Brieze-Stegeman R, Rice MJ, Hooper GHS, 1978. Daily periodicity in attraction of male tephritid fruit flies to synthetic chemical lures. Aust. J. Entomol. **17**, 341-346.

Christenson LD, 1963. The male annihilation technique in the control of fruit flies. In: New approaches to pest control and eradication. Ed. by SA Hall, American Chemical Society, Washington, 31-35.

Drew RAI, Hooper GHS, 1981. The responses of fruit fly species (Diptera: Tephritidae) in Australia to various attractants. Aust. J. Entomol. **20**, 201-205.

308 Fay HAC, 2012. A highly effective and selective male lure for *Bactrocera jarvisi* (Tryon)
309 (Diptera: Tephritidae). Aust. J. Entomol. **51**, 189-197.
310 Heather NW, Corcoran RJ, 1985. *Dacus tryoni*, pp. 41-48. In P. Singh & R.F. Moore [eds.],
311 Hand book of insect rearing. Elsvier Science Publishers B.V., Amsterdam.
312 Hee AKW, Tan KH, 2005. Bioactive fractions containing methyl eugenol-derived sex
313 pheromonal components in haemolymph of the male fruit fly *Bactrocera dorsalis*
314 (Diptera: Tephritidae). Bull. Entomol. Res. **95**, 615-620.
315 Hee AKW, Tan KH, 2006. Transport of methyl eugenol-derived sex pheromonal components
316 in the male fruit fly, *Bactrocera dorsalis*. Comp. Biochem. Physiol., Part C: Toxicol.
317 Pharmacol. **143**, 422-428.
318 Khan M, 2013. Potential of liquid larval diets for mass rearing of Queensland fruit fly,
319 *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). Aust. J. Entomol., In Press. DOI:
320 10.1111/aen.12024.
321 Kumaran N, Balagawi S, Schutze MK, Clarke AR, 2013. Evolution of lure response in
322 tephritid fruit flies: Phytochemicals as drivers of sexual selection. Anim. Behav. **85**, 781-
323 789.
324 Metcalf RL, Mitchell WC, Fukuto TR, Metcalf ER, 1975. Attraction of the oriental fruit fly,
325 *Dacus dorsalis*, to methyl eugenol and related olfactory stimulants. Proc. Natl. Acad. Sci.
326 **72**, 2501-2505.
327 Porter B, Christenson L, 1960. A review of the fruit fly research program of the US
328 Department of Agriculture Entomology research division. Fla. Entomol. **43**, 163-169.
329 Radhakrishnan P, Taylor PW, 2007. Seminal fluids mediate sexual inhibition and short
330 copula duration in mated female Queensland fruit flies. J. Insect Physiol. **53**, 741-745.
331 Raghu S, Clarke AR, 2003. Sexual selection in a tropical fruit fly: Role of a plant derived
332 chemical in mate choice. Entomol. Exp. Appl. **108**, 53-58.

333 Shelly TE, 2010. Effects of methyl eugenol and raspberry ketone/cue lure on the sexual
334 behavior of *Bactrocera* species (Diptera: Tephritidae). Appl. Entomol. Zool. **45**, 349-361.
335 Shelly TE, 2000. Fecundity of female oriental fruit flies (Diptera: Tephritidae): Effects of
336 methyl eugenol-fed and multiple mates. Ann. Entomol. Soc. Am. **93**, 559-564.
337 Shelly TE, Dewire AM, 1994. Chemically mediated mating success in male oriental fruit flies
338 (Diptera: Tephritidae). Ann. Entomol. Soc. Am. **87**, 375-382.
339 Shelly TE, Edu J, McInnis D, 2010. Pre-release consumption of methyl eugenol increases the
340 mating competitiveness of sterile males of the oriental fruit fly, *Bactrocera dorsalis*, in
341 large field enclosures. J. Insect Sci. **10**, 1-16.
342 Shelly TE, Nishida R, 2004. Larval and adult feeding on methyl eugenol and the mating
343 success of male oriental fruit flies, *Bactrocera dorsalis*. Entomol. Exp. Appl. **112**, 155-
344 158.
345 Shelly TE, Villalobos EM, 1995. Cue lure and the mating behavior of male melon flies
346 (Diptera: Tephritdae). Fla. Entomol. **78**, 473-482.
347 Tan KH, Nishida R, 1995. Incorporation of raspberry ketone in the rectal glands of males of
348 the Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae). Appl.
349 Entomol. Zool. **30**, 494-497.
350 Tan KH, Nishida R, 2012. Methyl eugenol: Its occurrence, distribution, and role in nature,
351 especially in relation to insect behavior and pollination. J. Insect Sci. **12**, 1-60.
352 Tan KH, Nishida R, 2007. Zingerone in the floral synomone of *Bulbophyllum baileyi*
353 (orchidaceae) attracts *Bactrocera* fruit flies during pollination. Biochem. Syst. Ecol. **35**,
354 334-341.
355 Weatherhead PJ, Robertson RJ, 1979. Offspring quality and the polygyny threshold:" The
356 sexy son hypothesis". Am. Nat., 201-208.

Wee SL, Tan KH, Nishida R, 2007. Pharmacophagy of methyl eugenol by males enhances sexual selection of *Bactrocera carambolae*. J. Chem. Ecol. **33**, 1272-1282.

Figure Legends

Figure 1. Proportionate survival over eight weeks from initial emergence of adult *Bactrocera tryoni* A) males and B) females. The flies are the F1 offspring of females mated with cue-lure fed, zingerone fed or lure unfed males.

Figure 2. Mean percentage (+SE) of offspring sired by cue-lure fed, zingerone fed and unfed males foraging to (A) cue-lure and (B) zingerone in large field cages.

Figure 3. Mean percentage (+SE) of offspring sired by cue-lure fed, zingerone fed and unfed males foraging to (A) cue-lure (B) zingerone in small cages in the laboratory.