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Male endogenous pheromonal component of *Bactrocera carambolae* (Diptera: Tephritidae) deterred gecko predation

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Summary. Geckos consumed immature *Bactrocera carambolae* males readily, but their consumption was reduced significantly when offered sexually mature males. They showed a similar aversion for sexually mature males previously fed with methyl eugenol. When male flies at different ages were offered to starved geckos, geckos' consumption showed an age-related anti-feeding effect, which was concomitant with the production trend of 6-oxo-1-nonanol, a major endogenous component synthesized in the male rectal gland. The production of 6-oxo-1-nonanol was also age-related and corresponded with sexual maturity as reported previously. Topical application of the authentic chemical of 6-oxo-1-nonanol onto the thorax of female fruit flies, at the amount normally detected in the rectal gland of a sexually mature male, had the same deterrent effect against geckos.

Key words. *Bactrocera carambolae* – sexual maturation – 6-oxo-1-nonanol – anti-feeding – gecko predation

Introduction

Bactrocera carambolae Drew and Hancock (Diptera: Tephritidae) is a closely related sibling species of the Oriental fruit fly, *B. dorsalis* Hendel complex. This polyphagus species is posing an increasing threat to many fruit growers in Malaysia, Thailand and Indonesia.

Large quantity of an endogenous compound, 6-oxo-1-nonanol (OXO), along with other minor components, was detected in the rectal gland of a sexually mature *B. carambolae* male (Perkins *et al*. 1990). The production and accumulation of these compounds in the male putative pheromone gland were subsequently found to correspond with sexual maturation in this species (Wee & Tan 2005). During courtship period, these compounds were released into the air unchanged and attracted conspecific females for mating (Wee 2000; Wee & Tan 2005). In a wind tunnel bioassay, OXO was shown to elicit sexual response in females that was similar to that of *B. carambolae* males' calling during courtship period. This suggested a pheromonal role for OXO (Wee 2000; Wee & Tan 2005).

Like the Oriental fruit fly, males of *B. carambolae* showed strong affinity to and voraciously fed on methyl eugenol (ME), a natural phenylpropanoid that was found as a component of essential oils from more than 200 plant families (Tan & Nishida 2000; Wee *et al*. 2002). In *B. papayae* [recently shown to be not a distinct species from *B. dorsalis* (Naeole & Haymer 2003; Tan 2003)] and *B. dorsalis* males, consumed ME was metabolized mainly into (*E*)-coniferyl alcohol (CF) and 2-allyl-4,5-dimethoxyphenol (DMP), which were stored in the rectal gland prior to release as sex pheromone to enhance mating competitiveness during courtship (Nishida *et al*. 1988; Tan & Nishida 1996, 1998; Hee & Tan 1998). However, males of *B. carambolae* produced only CF after ME consumption along with endogenously produced OXO and other minor components (Tan & Nishida 1996; Wee 2000).

Grains treated with individual compounds of CF and DMP were shown to deter avian consumption (Nishida & Fukami 1990; Jakubas *et al*. 1992). ME-fed *B. papayae* males, which contained both DMP and CF in the rectal gland, were also demonstrated to avert predation by hungry gecko that have been conditioned to feed on untreated fruit flies (Tan & Nishida 1998; Wee & Tan 2001). When assayed individually, CF was shown to be a weaker deterrent than DMP in deterring vertebrate predators (Nishida & Fukami 1990; Jakubas *et al*. 1992). Therefore, a question arises as to whether ME-fed *B. carambolae* male would be able to deter vertebrate predation? In an attempt to answer the above question, this paper also reports that OXO, apart from its pheromonal role, plays an allomonal role as well in the ecology of *B. carambolae*.

Materials and methods

Insect

Bactrocera carambolae was bred from infested star-fruit, *Averrhoa carambola* L., originally collected from the field since late 1993. Larvae were cultured on a semi-artificial diet containing star-fruit, yeast, protein, wheat bran, sugar, sweet potatoes and water (Wee & Tan 2000). Flies were segregated by sex within 5 days after emergence (DAE). For treatments, virgin male and female flies

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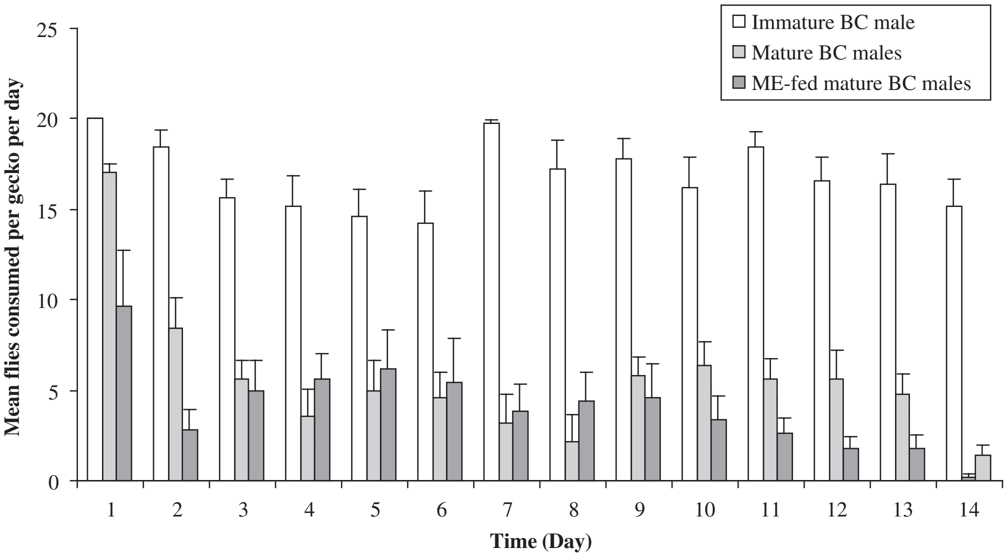

Fig. 1 Mean gecko (*Gekko monarchus*) consumption of immature, mature and methyl eugenol (ME)-fed *B. caramblae* males over a two-week period. (Bars = SE)

were maintained separately at 30 ± 2 °C and relative humidity 73 ± 5 % with a 12L: 12D photoperiod. Adult food mixture, comprised of protein, yeast, sugar and sodium benzoate, as well as water, were provided (unpublished).

Chemicals

Methyl eugenol (1,2-dimethoxy-4-[2-propenyl]benzene; > 99.8 % purity) was obtained from Agrisense-BCS Ltd. (UK). Authentic compound OXO (> 98 % purity) were provided by R. Nishida (Kyoto University, Japan). OXO was diluted to 5 and 10 µg/µl using redistilled ethanol.

Vertebrate Predator

Malayan Spiny gecko, *Gekko monarchus* Dumeril and Bibron (Squamata: Gekkonidae) was captured from University Science Malaysia campus, Penang. Naïve geckos were conditioned to feed on live immature *B. carambolae* fruit flies for more than a month. Geckos that consumed a minimum of 10 flies per day were selected for feeding tests (Wee & Tan 2001). Prior to all feeding experiments, geckos were starved for 3 days to ensure a similar degree of hunger (a fully satiated gecko could cease feeding for 2 days). Each gecko was weighed before and after each experiment, and no individual gecko was reused another feeding experiment.

Effect of methyl eugenol consumption on gecko predation

To examine the effect of ME consumption on gecko predation, geckos, caged individually, were randomly divided into three groups (N = 5 each) and was offered immature males (< 10 day-old), mature males (26–35 day-old) (Wee & Tan 2000) and 1-day post ME-feeding (1-DPT) of mature males as treatments. ME-fed males were prepared a day prior to experimentation by allowing 10 mature males to feed on 20 µg pure ME, dispensed on a piece of filter paper (3 × 3 cm^2; Whatman® No. 1) for 15 min.

Starved geckos from each group were each provided with 25 flies according to their assigned treatment. Each day, all dead and live flies were removed from every cage and were replaced with new flies as assigned. Gecko consumption of flies was recorded daily for a 2-week period.

Age-related anti-predation

For the age effect of *B. carambolae* male on gecko predation, each of the selected geckos (N = 10) was given 25 male flies of a known age (10, 20, 25, 30 and 40 DAE), starting with the youngest age. The number of male flies consumed in a day was recorded; and the remaining flies were then removed and replaced with 25 male (< 8 DAE) and/or female (5–20 DAE) fruit flies each day for 2 consecutive days in between tests. Feeding test was repeated by offering older male flies following 2 days of fly deprivation. Consumption of each gecko was recorded.

Topical application of OXO against gecko predation

In a separate feeding test, each gecko (N = 4) was given 20 mature female fruit flies, each with 1 µl ethanol applied topically after light anesthesia. Daily, for 3 consecutive days, each gecko was offered 20 ethanol-treated females (as controls), after removing all remaining flies. Total flies consumed by each gecko were recorded daily. For treatment, the feeding procedure was repeated in separate experiments by offering 20 treated female fruit flies, each topically treated with 1 µl of 1.0 µg or 5.0 µg OXO, to each of the geckos. Consumption of each gecko was recorded daily for 3 days.

Statistical analyses

Effects of ME consumption by and different ages of *B. carambolae* males on gecko predation were individually subjected to one-way analysis of variance (ANOVA). Factorial ANOVA was used to determine whether topical application of OXO, feeding days and interactions between OXO and feeding days have effects on gecko predation. Means were separated using Tukey's test at $P = 0.05$. Comparison between gecko body weight before and after experimentation was done using Student's *t*-test ($P = 0.05$).

Results

There were significant differences in gecko consumption of immature, mature and ME-fed *B. carambolae* males ($F = 51.37$, df = 2,12; $P < 0.001$) (Fig. 1). Immature males did not deter gecko predation (16.8 ± 0.5 fly per day) throughout the 2-week observation period. Hence, there was a significant increase in the weight of geckos ($t = +4.45$, $P < 0.01$).

When sexually mature males were offered on the first day, the average flies consumed per gecko was 17 males. However, on the second day, there was a significant decrease in the consumption of geckos when compared with the first

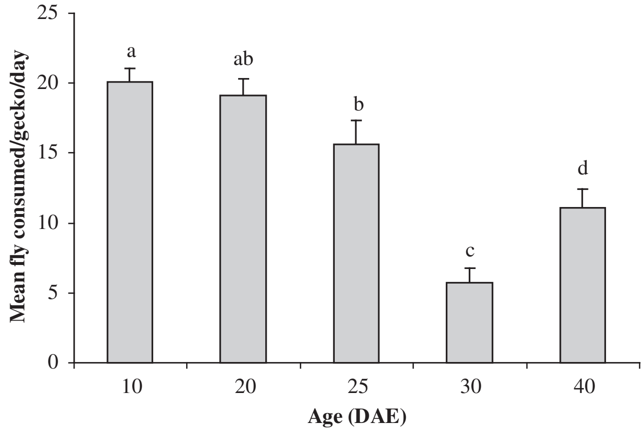

Fig. 2 Mean consumption of *B. carambolae* males at different age, days after adult emergence (DAE), by gecko (*Gekko monarchus*) (N = 10; bars = SE). Bar followed by different letters are significantly different at P = 0.05 (Tukey's test).

Table 1 Mean (SE) consumption of female fruit fly, *B. carambolae*, topically treated with male endogenous compound, 6-oxo-1-nonanol (OXO) by gecko, *Gekko monarchus*

	Mean[*] (SE) treated female fruit flies consumed by gecko		
Treatment	Day-1	Day-2	Day-3
Control (Ethanol)	19.3 (0.8) a	17.3 (1.3) a	15.5 (1.0) a
1.0 µg/µl per female	15.0 (2.1) a	8.5 (2.0) b	6.0 (2.0) b
5.0 µg/µl per female	9.8 (2.8) b	6.0 (1.8) b	5.5 (1.2) b

[*] Mean followed by different letters are significantly different at $P = 0.05$, Tukey's Test (N = 4).

day ($P < 0.05$; Fig. 1). Over the 2-week feeding trial, each gecko consumed an average of 5.6 ± 0.7 flies per day, which was significantly less than the consumption of immature flies ($P < 0.01$; Tukey's test). However, there was no significant difference in the geckos' weight after 2 weeks ($t = -1.09$, paired-*t* test, $P > 0.05$).

Geckos' consumption was reduced significantly during the first encounter with ME-fed *B. carambolae* males ($P < 0.01$; Tukey's test) (Fig. 1). Over the two weeks, geckos' average consumption of ME-fed males (4.2 ± 1.3 fly per gecko per day) was not significantly different from that of the mature males (5.6 ± 0.7 fly per gecko per day) ($P > 0.05$; Tukey's test). There was a significant weight loss for geckos offered ME-fed males, after the feeding experiment ($t = -6.23$, $P < 0.005$).

Age of the fruit flies affected gecko predation ($F = 21.64$, df = 4,45; $P < 0.01$). Male *B. carambolae* aged at 10 and 20 DAE did not deter gecko consumption. However, when older male flies, aged at 25, 30 and 40 DAE, were offered, geckos consumed significantly less male flies ($P < 0.05$; Tukey's test) (Fig. 2). The lowest consumption was observed when flies were at 30 DAE. There was a significant weight increase in the geckos after the feeding test ($t = +5.34$, $P < 0.005$).

Both topical applications of OXO ($F = 27.20$, df = 2,27; $P < 0.01$) and treatment days ($F = 8.26$, df = 2,27; $P < 0.01$) had significant effect on gecko predation. However, there was no interaction between OXO application and treatment days ($F = 0.78$, $F = 4,27$; $P > 0.05$).

Female flies treated with 1.0 µg OXO/fly did not deter gecko predation on the first day of feeding trial but caused a significant reduction of gecko consumption on treated females a day after it was introduced to the treated geckos ($P = 0.05$; Tukey's test) (Table 1). When females treated with a higher dosage, i.e., 5.0 µg OXO/fly, geckos instantly consumed significantly fewer female flies when compared with the controls on the first day of encounter (Table 1). For each treatment, geckos showed significant increase in body weight after feeding trials ($t_{ethanol} = 2.53$, $t_{lowOXO} = 2.85$, $t_{highOXO} = 2.43$; $P < 0.05$).

Discussion

Hungry geckos readily consumed both immature male and female *B. carambolae* fruit flies. However, gecko consumption decreased significantly when mature male flies (≥ 25 DAE) were offered. Hence, with attainment of sexual maturity, *B. carambolae* males developed an inherent anti-predation mechanism against gecko predation. In the case of ME-fed males, although geckos' consumption was also significantly reduced, the reduction was not different from that of sexually mature males. This showed that the presence of an additional component-CF in the rectal gland of ME-fed *B. carambolae* has contributed little/negligible additional deterrent effect against gecko predation when compared with ME-deprived sexually mature males.

The amount of defensive compound that insects produce may determine whether or not they survive a predator's attack; and variation in the amount of defensive chemicals may determine which individuals are killed and eaten and which are rejected (Bower 1992). The rectal gland of a newly emerged *B. carambolae* male lacks volatile compounds. OXO was not present in the male rectal gland until 14 DAE, in trace amount (Wee & Tan 2005). From thereon, the average OXO content increased with age, from 0.4 µg/ male at 17 DAE to 2.5 µg/male at 21 DAE, and peaked at 31 DAE with 26.1 µg/male (Wee & Tan 2005). Subsequently, OXO content decreased as age advanced, to *ca.* 4 µg/male for males aged between 40–50 DAE (Wee & Tan 2005). Therefore, the average OXO content in every mature *B. carambolae* male after 20 DAE is sufficient to deter predation.

Vertebrate predators such as birds and lizards are visual hunters. Thus, prominent characteristic such as warning colouration, which serves to advertise unpalatability, is an effective accompaniment to a distasteful prey. Warning colouration or 'conspicuously-coloured' (e.g., black, red, orange, yellow and white – a characteristic of the unpalatable insects) may enhance the survival of an individual insect (Bower 1992). Warning colour may also reduce the chance that an experienced predator will make an inappropriate attack (Guilford 1986).

Since immature males and females bear identical colour pattern to the mature males, there is likely to be a spectrum of palatability or phenomenon called automimicry – where the more palatable females and immature males in a population may gain benefit in terms of protection in association with the more distasteful individuals. And because the model and the mimic are from the same species, the mimicry is exact. Tan and Nishida (1998) demonstrated that *B. papayae* females were chemically protected through automimicry from starved geckos in the presence of ME-fed males.

From this study, the maximum anti-predation effect was observed for flies around 30 DAE, which coincided with the peak production of OXO in the male rectal gland as reported by Wee and Tan (2005). These quantitative changes in OXO content also corresponded with peak mating performance (15–35 DAE) observed for *B. carambolae* (Wee & Tan 2000). Hence, we suggest that OXO, apart from functioning as a sex attractant, doubles as a highly effective allomone in the chemical ecology of *B. carambolae*.

The melon fly, *B. cucurbitae*, a species of the raspberry ketone(RK)-/cue-lure-sensitive group of *Bactrocera* and a major pest of cucurbit as well as solanaceous fruits, was shown to deter gecko predation with its endogenous production of 1,3-nonanediol (Tan 2000a). This compound was also shown to play a role as sex and aggregation pheromones in the courtship of the melon fly (Kuba & Sokei 1988; Kuba 1991). As OXO and 1,3-nonanediol are structurally related, it is not surprising that OXO possesses strong pheromonal and allomonal activities.

Defensive secretion in animals plays a significant role in ecology and evolution of a prey-predator relationship (Pasteels *et al*. 1983). In insects, defensive chemicals may be synthesized endogenously (*de novo* synthesis) or acquired from plants during larval or adult stage (sequestration). It was suggested that *Bactrocera* species that are attracted to natural RK attractant are less advanced than those attracted to ME (Metcalf 1990; Tan 2000b). The former group may spend more metabolic energy, as enzymes are needed to catalyse the reactions to synthesize the compounds (Pasteels *et al*. 1983). In addition, a specialized gland may be needed for producing these compounds which would entail a 'cost' in terms of using resources that might otherwise be used for growth or reproduction (Pasteels *et al*. 1983). The latter group, such as male *B. dorsalis*, acquires ME from non-host plants and directly converts ME via oxidation into its analogues, which function as pheromone as well as allomone (Tan 2000b). This avoids the requirement of a series of metabolic pathways necessary for *de novo* synthesis of the endogenously produced pheromonal and/or allomonal components – thus, uses much less metabolic energy. In the case of male *B. carambolae*, which produces pheromonal and allomonal components via both *de novo* synthesis as well as by converting ME to CF, it appears to be in between the two groups. Through the production of endogenous components that coincided with attaining sexual maturity, *B. carambolae* males possess a different defensive repertoire compared with its sibling species, *B. dorsalis*. *B. dorsalis* male was shown to deter gecko predation only after pharmocophagy of ME (Tan & Nishida 1998; Wee & Tan 2001). In this context, *B. carambolae* male, besides converting ME to CF also synthesize its own pheromone and allomone endogenously, may be considered evolutionarily more advanced than RK-attracted group but less advanced than *B. dorsalis*. This certainly warrants further investigation.

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