the cocoons. This suggests that the parasites were able to develop on immature *M. flavescens*, but adults were not able to puncture the wall of the cocoon to emerge.

DISCUSSION

CLAUSEN (1940) mentioned that oviposition of *Eurytoma* and adult emergence at maturity are both strictly dependent on the presence of the *Chrysis* oviposition hole. However, our experiments indicate that adults of *E. monemae* are not able to puncture the cocoon of *M. flavescens* to oviposit because of the thickness of the wall. Even if oviposition is successful using holes made by *Chrysis* adults, developed adults are not able to emerge from the cocoon because of the thickness of the wall. Also, the presence of a *Chrysis* oviposition hole is apparently ineffective for oviposition or adult emergence. Adults of *E. monemae*

are able to oviposit through the thin-walled cocoon of *P. sinica* and adults are able to puncture the cocoon for emergence. Our findings suggest that *E. monemae* may be effective for natural control of *P. sinica*, but an ineffective parasite for control of *M. flavescens*.

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Incorporation of Raspberry Ketone in the Rectal Glands of Males of the Queensland Fruit Fly, *Bactrocera tryoni* FROGGATT (Diptera: Tephritidae)¹

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Most males of dacine fruit fly species respond either to methyl eugenol or cue-lure/raspberry ketone (DREW, 1974; FLETCHER, 1987). Methyl eugenol was shown to be a precursor of the male sex pheromone

which also acts as an allomone against vertebrate predators in the Oriental fruit fly complex, *Bactrocera dorsalis* HENDEL and *B. papayae* DREW & HANCOCK (NISHIDA et al., 1988; TAN and NISHIDA, 1994). On the other hand, males of the melon fly, *B. cucurbitae* COQUILLET, feed on and sequester raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) for storage in their rectal glands (NISHIDA et al., 1993). The ketone was suggested to play a pheromonal role in the courtship sequence, although the actual function has not been clarified (NISHIDA et al., 1990, 1993). These findings suggest the biological importance of male rectal substances, particularly in association with male attractants, in the life history of dacine fruit flies, since those attractant chemicals are frequently found in nature as essential oil components from various plants.

Males of the Queensland fruit fly, *B. tryoni* FROGGATT, are strongly attracted to both cue-lure and raspberry ketone. A series of several endogenous aliphatic amides have been identified from the rectal glands of *B. tryoni* (BELLAS and FLETCHER, 1979). This paper demonstrates the selective accumulation of raspberry ketone in the rectal glands and discusses its

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possible role in the behavior of the Queensland fruit fly.

MATERIALS AND METHODS

Spectral analysis and authentic samples. Gas chromatography-mass spectroscopy (GC-MS) was done with a Hitachi M-80 mass spectrometer (70 eV) connected to a GC-column (25 m $\times$ 0.20 mm fused silica column coated with cross-linked-bonded methyl silicone HP-1, 0.33 μ m thick) programmed from 80°C (approximately 2 min holding) to 240°C at a rate of 10°C/min. Authentic samples of *N*-3-methylbutyl acetamide (**1**) and *N*-3-methylbutyl propanamide (**2**) were obtained by acylation of *N*-3-methylbutylamine by the method of BAKER et al. (1982). 4-(4-Hydroxyphenyl)-2-butanone (**4**) was obtained from Tokyo-Kasei Chemical Industries Co., Ltd. 4-(4-Hydroxyphenyl)-2-butanol (**5**) was obtained by reduction of 4-(4-acetoxyphenyl)-2-butanone (Wako Pure Chemical Industries Ltd.) with lithium aluminium hydride in ether [MS: m/z (%): 45 (35), 77 (51), 107 (96), 133 (100), 148 (45), 166 (55); ¹H-NMR (90 MHz): δ 7.04 (2H doublet, J = 8.5 Hz), 6.73 (2H, doublet, J = 8.5 Hz), 3.83 (1H, sextet, J = 6 Hz), 2.62 (2H, multiplet), 1.89 (2H, multiplet), 1.23 (3H, doublet, J = 6 Hz); ¹³C-NMR (22.5 MHz): δ 153.96, 135.92, 129.42, 115.39, 67.83, 41.01, 31.20, 23.51].

Feeding assay. The feeding experiment of *B. tryoni* was conducted at the Faculty of Agriculture, University of Sydney, Australia, using a strain raised at the university. Males of *B. tryoni* (24 d old) were allowed to feed on raspberry ketone (**4**) for 15 min and then kept in a cage containing adult food (protein hydrolysate and sugar) and water. As controls, males from the same cohort were similarly treated in a separate cage but without ketone, **4** under summer ambient room temperature. Rectal glands were individually extracted one day after treatment and subjected to GC-MS analysis. To examine the quantities of ketone **4**, cue-lure (2 μ l) on a filter paper was given to 14 d-old males for 15 min. Those that fed were isolated in separate containers, with adult food and water, under ambient temperature and humidity. The rectal gland was extracted individually from treated males after 6 h, 1 d and 3 d. As controls, males of the same age were similarly treated but not exposed to cue-lure, and the excised rectal glands were extracted after 6 h and 3 d. Rectal gland extracts were individually subjected to quantitative GC analyses, using an HP 5790A gas chromatograph with an HP-1 capillary column (16 m, programmed from 90 to 220°C

at a rate of 10°C/min) by comparing the FID-intensities with those of standard samples of known concentrations.

Behavioral observations. The behavioral response of males and females (15–26 d-old) was noted over a period of 30 min under exposure to 8 μ g of raspberry ketone (**4**) on a glass slide in the morning, at noon and at dusk.

RESULTS

Identification of rectal volatiles. Figure 1 shows typical capillary gas chromatograms (total ion mass chromatogram) of the rectal gland extract of *B. tryoni* males unfed and fed with raspberry ketone. Three peaks (1, 2, and 3) commonly seen both in fed and unfed males as endogenous volatile components were identified as *N*-3-methylbutyl acetamide (**1**), *N*-3-methylbutyl propanamide (**2**) and *N*-3-methylbutyl 2-methylpropanamide (**3**) from the diagnostic mass spectra. The chromatogram from males fed on ketone **4** indicated selective accumulation of the compound in the rectal glands [MS m/z (%): 43 (40), 77 (24), 107 (100), 121 (15), 164 (56)]. A small peak 5, accompanying ketone **4** at a retention time of 12.7 min [MS: m/z (%): 45 (29), 77 (48), 107 (99), 133 (100), 148 (44), 166 (48)], was identified as 4-(4-hydroxyphenyl)-2-butanol (**5**) by comparison with the authentic specimen.

Change in contents of rectal volatiles after feeding on cue-lure. The contents of the rectal gland volatiles at 6 h, 1 d and 3 d after feeding with cue-lure were quantified by GC analysis. Table 1 shows the contents of the two major amides (**1** and **2**) and ketone **4** at various times after treatment. Both amide components showed significant increases with sexual maturation. The rectal gland of males fed on cue-lure was loaded with ketone **4**, but no cue-lure was detected. At 6 h post-feeding, the quantity of ketone **4** in the rectal organs was relatively low. Although alcohol **5** could not be quantified precisely, its relative abundance was estimated to be in the range of 3 to 10% of the total phenylbutanoid components (**4** + **5**).

Behavior towards raspberry ketone. Sexually mature females showed no response to authentic ketone **4**. However, under laboratory conditions mature males were attracted to and fed on the ketone, even during dusk in summer.

DISCUSSION

The three amides detected in this study are identical to the three major components of the six amides

Fig. 1. Gas chromatograms of volatiles in the rectal gland complex of *Bactrocera tryoni* males (24 day-old), unfed (upper) and fed (lower) with raspberry ketone (**4**). **1**: *N*-3-Methylbutyl acetamide, **2**: *N*-3-methylbutyl propanamide, **3**: *N*-3-methylbutyl 2-methylpropanamide, **4**: 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone), **5**: 4-(4-hydroxyphenyl)-2-butanol. These chromatograms were done on a capillary HP-1 column (25 m $\times$ 0.20 mm) with a total ion monitor (m/z 33–250).

Table 1. Mean contents ($\pm$ S.D., μ g/male) of *N*-3-methylbutyl acetamide (**1**), *N*-3-methylbutyl propanamide (**2**), 4-(4-hydroxyphenyl)-2-butanone (**4**) in the rectal gland complex of *Bactrocera tryoni* males post-feeding with cue-lure and unfed control

Days after treatment		Compound 1	Compound 2	Compound 4	<i>N</i>
Fed with cue-lure	0.25	0.6 ± 0.8	3.0 ± 2.8	13.5 ± 7.6	3
	1	2.7 ± 1.9	6.8 ± 3.7	30.8 ± 22.2	3
	3	6.7 ± 4.8	16.8 ± 3.5	33.3 ± 26.6	4
Unfed control	0.25	3.0 ± 1.0	6.3 ± 3.2	0	3
	3	9.5 ± 0.7	26.7 ± 4.4	0	3

detected in *B. tryoni* and *B. neohumeralis* PERKINS (BELLAS and FLETCHER, 1979). The mixture of amides found in *B. tryoni* male rectal glands was suggested to act as a short-range pheromone which excited females in the vicinity of males (BELLAS and FLETCHER, 1979). Amides **1** and **2** increased dramatically from 14 to 17 d-old male *B. tryoni*, which corresponded with increasing sexual maturity.

Males of *B. tryoni*, like the melon fly males, are attracted to and actively ingest cue-lure and raspberry ketone (DREW, 1974; FLETCHER, 1987). Ketone

4 was selectively accumulated in the rectal gland when males were fed with either cue-lure or ketone **4**, while no cue-lure was detected in the rectal gland of males fed with the lure. Cue-lure seems to be readily hydrolyzed to **4** in the body tissues. The relatively low quantity of the ketone at 6 h post-feeding suggests a slow incorporation into the rectal gland. Incorporation of the ketone reached its maximum one day post-feeding. It was also noted that 4-(4-hydroxyphenyl)-2-butanol (**5**), although in minute quantity, was accompanied by ketone in the male

rectal gland after feeding on raspberry ketone or cue-lure.

The ketone sequestered by *B. tryoni* males was suggested to play a role in male-male interactions, possibly as the pheromone primarily responsible for lek formation, because only males were responsive to the compound even during the mating period around sunset. Similar evidence was also observed in the melon fly whose females are not responsive to the ketone (NISHIDA et al., 1993). We also suggest that the release of this substance is secondary to distract "unfed" males from mating with responsive females, as observed in *B. cucurbitae* (NISHIDA et al., 1993). In *B. dorsalis* and *B. papayae*, males fed on methyl eugenol perform significantly better in male-male mating competition for females (SHELLY and DEWIRE, 1994; TAN and NISHIDA, 1994). The role of the ketone together with other rectal volatiles in the courtship sequence of *B. tryoni*, including lek formation, male-male competition and acceptance by females at the final step, certainly warrants further studies.

Although cue-lure is not known as a naturally occurring substance (BEROZA et al., 1960), raspberry ketone has been detected in several plants and berries (HONKANEN et al., 1980; HIRVI et al., 1981; GALLOIS, 1982; HIRVI and HONKANEN, 1984; LIN and CHOW, 1984; MARCO et al., 1988), and orchid flower fragrance of *Dendrobium superbum* RCHB. (a cultivar in Japan, NISHIDA et al., 1993) and three varieties of *D. anosmum* L. (raspberry jam orchid, synonymous with *D. superbum*) found in Malaysia (TAN, 1993). The role of male fruit fly feeding on the ketone in the plant-fruit fly relationship is still uncertain (NISHIDA et al., 1993). However, wild orchid flowers of several *Bulbophyllum* species attract males of fruit fly species that respond to methyl eugenol. Individual flowers of *B. cheiri* L. (fruit fly orchid) produce a synomone containing methyl eugenol to attract male fruit flies for pollination (Y.G. TOONG and K.H. TAN, unpubl.). The male uses methyl eugenol for its own benefit (TAN, 1993). In this instance, the plant's methyl eugenol is not a kairomone as claimed by METCALF (1990). Ecological association of *B. tryoni* with wild plants has not been observed in their natural habitat.

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