Rectal Gland Exudates and Emissions of *Bactrocera bryoniae*: Chemical Identification, Electrophysiological and Pheromonal Functions

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Abstract

Bactrocera bryoniae is a polyphagous and economically significant fruit fly found in Indonesia, Papua New Guinea and Australia. To understand chemical-mediated sexual communication, and the potential for novel pheromone-based attractants for monitoring and mass-trapping of *B. bryoniae*, rectal gland exudates and emissions from sexually mature males and females were investigated. Gas chromatography-mass spectrometry showed that male rectal glands contained six compounds, of which 1,7-dioxaspiro[5,5]undecane elicited electroantennographic (EAD) and electropalpographic (EPD) responses in both sexes, ethyl 3-acetoxybutanoate elicited EPD responses in both sexes, *N*-(3-methylbutyl)acetamide elicited EAD response from males and 4-hydroxy-1,7-dioxaspiro[5.5]undecane elicited EAD responses in males and females and EPD responses in females. Female rectal glands contained 23 compounds with the esters ethyl laurate and ethyl myristate as major components. Amongst the female rectal gland constituents, ethyl laurate, ethyl myristate and ethyl palmitate elicited EAD responses in males and females, *N*-(3-methylbutyl)acetamide elicited EAD responses in males only, (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane elicited EAD responses in males and EPD responses in females, and 2,7-dimethyl-1,6-dioxaspiro[4.5]decane, (*E*,*E*)-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane, (*E*,*E*)-2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane, (*Z*,*Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, (*E*,*E*)-2-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane and ethyl caprate elicited EPD responses in females only. Y-tube bioassays indicated that male rectal gland extracts and headspace volatiles attracted females and males, while female rectal gland extracts and headspace volatiles only attracted males. The results suggest that ethyl 3-acetoxybutanoate, 1,7-dioxaspiro[5,5]undecane and 4-hydroxy-1,7-dioxaspiro[5.5]undecane may be components of male-produced sex pheromone in *B. bryoniae* while (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, *N*-(3-methylbutyl)acetamide, ethyl laurate, ethyl myristate and ethyl palmitate may be components of female-produced sex pheromone. Ethyl 3-acetoxybutanoate, *N*-(3-methylbutyl)acetamide, 1,7-dioxaspiro[5,5]undecane and 4-hydroxy-1,7-dioxaspiro[5.5]undecane may be components of male aggregation pheromone. These findings contribute to the understanding of pheromone communication in *B. bryoniae* and provide a foundation for developing pheromone-based monitoring and control methods.

Keywords: Fruit Fly, Pheromone, Attractants, Rectal Gland, Olfaction, GC–MS, EAG, EPG

Introduction

Many *Bactrocera* fruit flies (Tephritidae) are economically important pests of fruits and vegetables, and some pose serious quarantine risks with potential to cause major disruptions in the international trade of fresh fruits and vegetables (Vijaysegaran 1997; Hickey et al. 2010; Clarke et al. 2011; Benelli et al. 2014; Dominiak and Mapson 2017). Diverse methods have been used in fruit fly control programs, including cover sprays, sterile insect technique (SIT), bait sprays, male annihilation technique (MAT), and biological control (Clarke et al. 2011; Ero et al. 2011; Zamek et al. 2012; Lauzon and Potter 2012; Dominiak and Ekman 2013). Fruit fly management relies on effective monitoring tools, as well as attractants for lure-and-kill methods. Sex and aggregation pheromones are central to the mating systems of many *Bactrocera* species and have been considered as potential attractants for management of some species. For example, 1,7-dioxaspiro[5,5]undecane, a female-produced sex pheromone, has been used for monitoring and mass trapping of *Bactrocera oleae* (Rossi) (Haniotakis et al. 1977).

The rectal gland is well known as a sex pheromone-secreting organ in fruit flies (Fletcher 1968, 1969; Piccardi 1980; Perkins et al. 1990a; Wee and Tan 2005; Tokushima et al. 2010). The volatile compounds emitted during calling and courtship, especially by males, are known as short and long range attractants for the opposite sex in some species (Nation 1972; Perkins 1990; Sivinski et al. 2000; Cruz-López et al. 2015). Male-produced volatiles are also known to function as mating aggregation pheromones in some species (Sivinski and Calkins 1986; Nishida et al. 1988b; Hendrichs et al. 2002). Among the more than 450 species of *Bactrocera*, the sex and aggregation pheromones of only a few pest species have been investigated (Doorenweerd et al. 2018). Some of the first investigations described production and release of sex pheromones by male *Bactrocera tryoni* (Froggatt) (Fletcher 1968, 1969; Bellas and Fletcher 1979). Males are thought to be the main pheromone producers in most fruit flies (Heath et al. 2000; El-Sayed 2007), and studies of volatile compounds in *Bactrocera* have mainly focused on males (Bellas and Fletcher 1979; Kitching et al. 1986, 1989; Perkins et al. 1990b; Krohn et al. 1991; Hayes et al. 2001; Tokushima et al. 2010). However, there are at least three examples of fruit fly species in which females are also known to produce sex pheromones. In the olive fruit fly, *Bactrocera oleae,* sex pheromones are mainly produced by females (Haniotakis 1974; Mazomenos and Haniotakis 1981, 1985) while males produce a compound, (*Z*)-9-tricosene, that only acts as a close range attractant

for females (Carpita et al. 2012; Canale et al. 2013). Similarly, in the melon fly, *Zeugodacus cucurbitae* (Coquillett) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel), both male and female volatile emissions have been reported to attract the opposite sex (Baker et al. 1982a; Baker and Bacon 1985; Nishida et al. 1988a, b).

Bactrocera bryoniae (Tryon) (Diptera: Tephritidae) is an economically important pest species in Indonesia (Papua, formerly part of Irian Jaya), Papua New Guinea (every province except Bougainville and Manus) and Australia (South East Queensland, Central Queensland, Northern Queensland, Northern Western Australia, Northern Territory, east coast south to Sydney, New South Wales, and the Torres Strait Islands) (Drew and Romig 2013; Leblanc et al. 2013; Schutze et al. 2018). *Bactrocera bryoniae* is polyphagous, having been reported to infest nine fruits and vegetables from five families including Cucurbitaceae, Loganiaceae, Musaceae, Passifloraceae and Solanaceae. Chilli pepper is the main commercial host of *B. bryoniae* (Leblanc et al. 2013). Chemical communication of *B. bryoniae* has not been investigated previously. The present study identifies and characterizes rectal gland secretions and volatiles released by both males and females, and evaluates the attractiveness of rectal gland volatiles to the opposite and same sex. This information about the chemistry of *B. bryoniae* not only provides insight into the functional role of rectal gland volatiles but also the potential application of *B. bryoniae* volatiles as attractants for monitoring and control.

Methods and Material

Bactrocera bryoniae rearing

A laboratory-reared population of *B. bryoniae* (G68) was obtained from the Queensland Department of Agriculture and Fisheries (Cairns, Queensland). Approximately 500 pupae were placed in a 47.5 × 47.5 × 47.5 cm fine mesh cage (Megaview Bugdorm 4S4545, Taiwan) for emergence and kept in a controlled-environment room at 25 ± 0.5 °C, 65 ± 5% relative humidity (RH) and 11.5:0.5:11.5:0.5 hour light/dusk/dark/dawn photoperiod at Macquarie University. Adult flies were provided sugar and yeast hydrolysate (MP Biomedicals LLC) as food in separate dishes, and were provided tap water through a soaked sponge. Flies were separated by sex within 3 days of emergence and transferred to 12.5 L clear plastic cages that had two 10 cm diameter mesh-covered openings for ventilation (180

flies per cage). No mating was observed before separating the flies. All cages were maintained with the same diet and environmental conditions described above. All experiments used 13-18 days old virgin flies.

Rectal gland extraction

Gland extracts were obtained from sexually mature males and females of *B. bryoniae*. Handling of the flies and the gland extractions followed the procedure of Kitching et al. (1989). Flies were chilled on dry ice to kill them. The abdomen was gently squeezed with tweezers such that the glands protruded slightly. The glands were then gently pulled out with tweezers, and the secretory sac separated. Glands were carefully placed in a 1.1 mL tear-drop vial in dry ice. Once 10 glands were collected, the vials were removed from the dry ice and the contents were extracted into 100 µL of *n*-hexane (HPLC grade, Sigma-Aldrich) by saturating the glands with solvent and leaving them to stand at room temperature for 10 minutes. Ten replicates per sex were collected using 10 glands per replicate. Samples were stored at –20 °C until analysed.

Collection of airborne volatiles

Headspace collections were conducted during the dusk period in the controlled-environment room. This time of the day was selected based on our observations of male calling behaviour and mating. Ten sexually mature males or females were separately placed into a glass chamber (150 mm long × 40 mm ID) 30 minutes before dusk and charcoal-filtered air at a flow rate of 0.5 L per minute was drawn over the flies for a period of one hour, starting from beginning of dusk. Released volatiles were adsorbed onto 50 mg of Tenax adsorbent (Scientific Instrument Services, Inc, Tenax-GR Mesh 60/80) packed into glass cartridges (6 mm ID × 50 mm) and fitted with glass wool plugs. Volatiles were subsequently extracted from the absorbent with 1 mL of *n*-hexane. Samples were stored at –20 °C until analysis. Nine replicates per sex were collected. To identify any possible contaminants, an air control sample comprising an empty glass chamber, was run and analyzed along with every volatile collection. Tenax traps were conditioned at 200 °C for three hours under a nitrogen stream (75 mL/min) prior to each headspace collection. The glass chambers were washed with 5% Extran aqueous solution, rinsed with hot tap water, and heated at 200 °C for 18 hours.

Activated charcoal filters were thermally conditioned by heating them at 200 °C for 18 hours prior to each headspace collection (El-Sayed et al. 2008).

Analysis of rectal gland extracts and headspace collections

Mass spectra were recorded by gas chromatography-mass spectrometry (GC-MS) on a Shimadzu GCMS-TQ8040 instrument equipped with a split/splitless injector and SH Rtx-5MS (30 m × 0.25 mm ID × 0.25 μm film thickness) fused silica capillary column as the stationary phase. The carrier gas was helium (99.999%) (BOC, North Ryde, NSW, Australia) at a constant flow rate of 1 mL/min. The injection port temperature was 270 °C. The initial column temperature was 40 °C, held for 1 minute, followed by an increase to 250 °C at a rate of 10 °C/min. The final temperature was held for 3 minutes. The ionisation method was electron impact at a voltage of 70 eV, and the spectra were obtained with scanning from 45 to 500 *m/z*. The ion source and transfer line temperatures were 200 °C and 290 °C, respectively. The relative percentage of each compound in the rectal gland blend or headspace was obtained by dividing its individual peak area by the total peak area and multiplying the result by 100. All compounds were identified through comparison with retention times and mass spectra of authentic samples, where available, or NIST library (NIST17-1, NIST17-2 and NIST17s) and mass spectra published in the literature, where authentic samples were not available.

Electrophysiology

Electrophysiological recordings were performed using antennae and maxillary palps of sexually mature virgin females and males using the rectal gland extracts of both sexes as stimuli. Male rectal gland extracts and female rectal gland extracts were separately subjected to both female and male *B. bryoniae* to detect active compounds.

The responses were evaluated by gas chromatography-electroantennogram detection (GC-EAD) or gas chromatography-electropalpogram detection (GC-EPD). The system comprised of an Agilent 7890B gas chromatograph equipped with an SH-Rtx-35 (30 m × 0.25 mm ID × 0.25 μm film thickness) fused silica capillary and FID detector. The carrier gas was hydrogen (99.999% pure) with a constant flow of 2.5 mL/min. The injection port temperature was 270 °C. The initial temperature of the column was 50 °C, held for 1 minute,

ramped to 250 °C at a rate of 10 °C/min, and held for 3 minutes. The detector temperature was 290 °C. The effluent of the column was mixed with 30 mL/min make-up nitrogen gas and split at 1:1.5 (*v/v*) ratio, with one part going to the internal FID and the other through a heated transfer line (TC-02, Syntech, Hilversum, The Netherlands), kept at a constant temperature of 200 °C.

The head of a male or female fly was mounted between two silver wires with capillary electrodes filled with phosphate-buffer saline and electrically conductive gel (Spectra 360). In both the GC-EAD and GC-EPD experiments, the electrode with phosphate-buffered saline was placed at the tip of an antenna or a maxillary palp as the recording electrode and the other electrode, filled with electrically conductive gel, at the back of the head as the reference electrode. The mounted heads were subjected to a charcoal-filtered and humidified air-flow (400 mL/min) controlled by a flow controller (Syntech Stimulus Controller CS-55, Syntech, Hilversum, The Netherlands). All signals were captured and processed with a data acquisition controller (IDAC-4, Syntech, Hilversum, The Netherlands) and analysed using GC-EAD 2014 software version 1.2.5. Before injection of a sample, the antenna or maxillary palp were stimulated with 1-hexanol to check sensitivity, then 1 µL of the rectal gland extract from the opposite sex as well as the same sex was injected. Nine GC-EAD and nine GC-EPD recordings per sex were obtained. Responses were considered genuine if present in at least six of the nine replicates collected. The identity of each compound eliciting an electrophysiological response was confirmed by comparing retention time with that of the GC-MS chromatograms.

Behavioural assays

The response of sexually mature virgin (13-18 days old) *B. bryoniae* males and females toward volatiles released from the rectal glands of the same and opposite sex was evaluated using Y-tube olfactometers. The Y-tube olfactometer comprised of a clear acrylic Y shaped tube with one central arm (6.5 cm × 4.5 cm × 5 cm) in which the release chamber (5 cm × 5 cm × 5 cm) was located, and two upwind lateral arms (12.5 cm × 4.5 cm × 5 cm), each of them connected to a rectangular chamber (7.5 cm × 5 cm × 5 cm) (see Online Resource). The Y-tube olfactometer was positioned horizontally on a white table and a humidified and charcoal-filtered air stream was passed through the Y-tube at a flow rate of 140 ± 5 mL/min. For the response of flies toward the rectal gland volatiles, the stimulus cartridge was prepared

by crushing 15 rectal glands of male or female *B. bryoniae* on a 1 cm^2 section of filter paper (Advantec, Japan) inserted in a glass Pasteur pipette (145 mm long). The control cartridge was prepared using 1 cm^2 filter paper inserted in the same type of glass Pasteur pipette. One cartridge of each type was fitted to one of the Y-tube upwind arms using Tygon tubing (Tygon® formula E-3603, Sigma-Aldrich). For the response of flies towards the natural blend of volatile compounds released from live flies, four *B. bryoniae* males or females were separately placed into a glass chamber (150 mm long × 40 mm ID) 30 minutes before experiments started at dusk in a controlled-environment room, under the same conditions the flies had been maintained in. The control unit was prepared using an empty glass chamber. One chamber of each type was fitted to one of the Y-tube upwind arms using 12 cm of Tygon tubing. For both experiments, an individual fly was placed in the release chamber to acclimatize 30 minutes before dusk. Every trial lasted 30 minutes. Once the two cartridges (stimulus and control) or chambers (flies and control) were connected to the upwind arms, the system was equilibrated for two minutes and then the barriers of the two upwind arms and the release chamber were removed. Behaviours of flies were observed every 5 minutes and the arm in which the fly was located was recorded. Overall, six observations were made until dark and the arm in which a fly spent most time was recorded as a final choice. Flies that did not make any choice, *i.e.*, remained in the release chamber and did not reach one of the two upwind arms, were not counted. For the rectal gland attraction experiments, at least 56 replicates/treatment and for headspace attraction 30-35 replicates/treatment were carried out (non-responsive flies were not counted). To compare the number of flies choosing the stimulus over the control, a binomial test was used ($\alpha = 0.05$).

The position (left or right) of the stimulus and the control was alternated every trial to counter potential positional effects. Each fly was tested only once and fresh rectal glands were used each day. Before each replicate, the Y-tube olfactometer and tubes were washed with 5% Extran aqueous solution, rinsed with hot tap water and air-dried. The glass chambers were washed with 5% Extran aqueous solution, rinsed with hot tap water, and heated at 200 °C for 3 hours.

233 Chemicals
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235 The following chemicals were purchased from Sigma-Aldrich (St Louis, MO, US), Alfa-
236 Aesar (Ward Hill, MA, US) and Chem-Supply (Bedford St, Gillman, SA), with the purities
237 noted in parentheses, and were used without further purification: ethyl 3-acetoxybutanoate
238 (98%), ethyl caprate (98%), methyl laurate (98%), ethyl laurate (98%), ethyl tridecanoate
239 (99%), methyl myristate (99%), ethyl myristate (98%), methyl palmitate (99%), ethyl
240 palmitate (99%), ethyl oleate (98%), lauric acid (98%), palmitoleic acid (98.5%), palmitic
241 acid (98%), oleic acid (99%) and 1,7-dioxaspiro[5,5]undecane (97%). Propyl laurate, ethyl
242 palmitoleate, *N*-(2-methylbutyl)acetamide, *N*-(3-methylbutyl)acetamide, (*E*,*E*)-2,8-dimethyl-
243 1,7-dioxaspiro[5.5]undecane and (*E*,*E*)-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane were
244 synthesised (see Online Resource for synthesis details).
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246
247 **Results**
248
249 Rectal gland and released volatile components of *B. bryoniae*
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251 GC-MS analyses identified a total of 26 compounds that were produced and released by male
252 and female *B. bryoniae* (Table 1). All compounds were tentatively identified based on their
253 mass spectral fragmentation patterns. Identities of compounds **1**, **3**-**7**, **12**-**14** and **15**-**26** were
254 confirmed by comparison of GC retention times and mass spectral fragmentation patterns
255 with authentic samples. These were identified as ethyl 3-acetoxybutanoate (**1**), 1,7-
256 dioxaspiro[5,5]undecane (**3**), (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**4**), (*E*,*E*)-2-
257 ethyl-7-methyl-1,6-dioxaspiro[4.5]decane (**5**), *N*-(2-methylbutyl)acetamide (**6**), *N*-(3-
258 methylbutyl)acetamide (**7**), ethyl caprate (**12**), methyl laurate (**13**), lauric acid (**14**), ethyl
259 laurate (**15**), ethyl tridecanoate (**16**), propyl laurate (**17**), methyl myristate (**18**), ethyl
260 myristate (**19**), ethyl myristate (**20**), palmitoleic acid (**21**), palmitic acid (**22**), ethyl
261 palmitoleate (**23**), ethyl palmitate (**24**), oleic acid (**25**) and ethyl oleate (**26**). Five compounds,
262 2,7-dimethyl-1,6-dioxaspiro[4.5]decane (**2**), (*E*,*E*)-2-ethyl-8-methyl-1,7-
263 dioxaspiro[5.5]undecane (**8**), (*Z*,*Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**9**), (*E*,*E*)-2-
264 propyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**10**) and 4-hydroxy-1,7-
265 dioxaspiro[5.5]undecane (**11**), which were not commercially available or synthesized, were
266 tentatively identified based on the literature mass spectral fragmentation patterns (Baker et al.

1982b; Perkins 1990; Fletcher et al. 1992; Booth et al. 2006, 2007; Schwartz et al. 2008; Mitchell et al. 2017). For both males and females, all compounds that were detected in the headspace were also detected in the rectal gland extracts.

Six compounds were identified in male rectal glands, including ester **1**, spiroacetals **3** and **4** and **11**, and the amides **6** and **7**. Of these, all compounds except the spiroacetal (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**4**) were present in the male headspace samples. The most abundant compound in male gland extracts and headspace samples, being found in a similar proportion (~85%), was spiroacetal **4**. Females produced a more complex blend than males. Of the 23 compounds that were found in females, eight compounds were detected only in rectal gland extracts (Table 1). This included 2,7-dimethyl-1,6-dioxaspiro[4.5]decane, *N*-(2-methylbutyl)acetamide, lauric acid, palmitoleic acid, palmitic acid, oleic acid, methyl palmitate and ethyl oleate. The main compounds produced and released by females were ethyl laurate (~40% and 67%, respectively) and ethyl myristate (~32% and 21%, respectively). More compounds were detected in female rectal glands than in female headspace collections. Compounds found in both rectal gland extracts and headspace include spiroacetals **4**, **5**, **8**, **9** and **10**, amide **7** and esters **12**, **13**, **15**, **16**, **17**, **18**, **19**, **23** and **24**.

Electrophysiological responses of antennae

Males and females shared the EAD response to five compounds; 1,7-dioxaspiro[5,5]undecane (**3**) and 4-hydroxy-1,7-dioxaspiro[5.5]undecane (**11**) from male rectal glands (Fig. 1) and ethyl laurate (**15**), ethyl myristate (**19**) and ethyl palmitate (**24**) from female rectal glands (Fig. 2). Other compounds only elicited responses in the antennae of one sex; *N*-(3-methylbutyl)acetamide (**7**) from male and female rectal glands elicited EAD responses in males and (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**4**) from female rectal glands elicited EAD responses in males. There were no compounds that only females responded to in the rectal glands of either males or females.

Electrophysiological responses of maxillary palps

Males and females shared EPD responses to two compounds from the rectal glands of males (Fig. 1); ethyl 3-acetoxybutanoate (**1**) and 1,7-dioxaspiro[5,5]undecane (**3**). These are the only compounds that elicited EPD responses in males from male rectal glands. 4-Hydroxy-

1,7-dioxaspiro[5.5]undecane (**11**) from male rectal gland extracts elicited EPD response only in females.

There were no compounds in female rectal glands that elicited EPD response in males. In contrast, seven compounds in female rectal glands elicited EPD responses in females; 2,7-dimethyl-1,6-dioxaspiro[4.5]decane (**2**), (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**4**), (*E*,*E*)-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane (**5**), (*E*,*E*)-2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**8**), (*Z*,*Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**9**), (*E*,*E*)-2-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**10**) and ethyl caprate (**12**). The ten EPD active compounds in females belong to two functional groups; eight spiroacetals and two esters.

Behavioural assays

Sexually mature males were attracted to emissions from female rectal glands ($P = 0.001$), while females did not show any preference for female rectal gland emissions over the control ($P > 0.05$). The percentage of non-responders was 5.3% and 9.2% when males and females, respectively, were presented with emissions from female rectal glands. Both males and females were attracted to male rectal gland emissions ($P = 0.03$ and $P < 0.001$, respectively). The percentage of non-responders was 9.3% and 6.6% when males and females, respectively, were presented with male rectal gland emissions (Fig. 3A).

The behavioural responses of sexually mature male and female *B. bryoniae* to headspace volatiles from live conspecific males and females were very similar to responses to rectal gland emissions (see above). Females were attracted to the volatiles released by males ($P < 0.001$), but were not attracted to the volatiles released by females ($P > 0.05$). Males were attracted to the volatiles released by males and females ($P = 0.005$ and $P = 0.04$, respectively) (Fig. 3B).

Discussion

We identified the composition of rectal gland secretions in male and female *B. bryoniae* and evaluated electrophysiological and behavioural responses of both sexes to the volatiles released by males and females of this species for the first time. Chemical analyses revealed

that female *B. bryoniae* produced and released a more complex blend than males. The volatiles from female *B. bryoniae* consisted of two aliphatic amides, six spiroacetals, eleven saturated/unsaturated esters and four fatty acids of which all except *N*-(2-methylbutyl)acetamide, *N*-(3-methylbutyl)acetamide and (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane are female specific.

The aliphatic amides found in this study, *N*-*(*2-methylbutyl)acetamide and *N*-(3-methylbutyl)acetamide, have been reported as part of rectal gland compositions of other species, including *B. tryoni* (Bellas and Fletcher 1979; El-Sayed et al. 2019), *B. dorsalis*, *Z. cucurbitae* (Baker and Bacon 1985), *Zeugodacus cucumis* (French) (Fletcher and Kitching 1995) and *Bactrocera zonata* (Saunders) (Levi-zada et al. 2020). Different isomers of the spiroacetals were also identified in rectal glands of other *Bactrocera* and species from the closely related genus *Zeugodacus*. For instance, 2,7-dimethyl-1,6-dioxaspiro[4.5]decane has been previously reported in the *n*-pentane extract of female *B. tryoni* (Booth et al. 2006). Isomers of 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane have been reported in the previous investigation of rectal glands of *B. dorsalis* (*E*,*E* isomer), *Bactrocera nigrotibialis* (Perkins) (*E*,*E* isomer), *Bactrocera albistrigata* (Meijere) (*E*,*E* isomer), *Bactrocera jarvisi* (Tryon) (*E*,*E* isomer), *Bactrocera kirki* (Froggatt) (*E*,*E* isomer), *Bactrocera kraussi* (Hardy) (*E*,*E* isomer), *Z. cucumis* (*E*,*E*, *E*,*Z*, *Z*,*Z* isomers), *B. tryoni* (*E*,*E* isomer) and *B. musae* (Tryon) (*E*,*E* isomer) (Baker and Bacon 1985; Kitching et al. 1989; Fletcher et al. 1992; Fletcher and Kitching 1995; Booth et al. 2006; El-Sayed et al. 2019; Noushini et al. 2019). 2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane has been reported in the rectal gland of male *Bactrocera kraussi* (Hardy) (*E*,*E* or *E*,*Z* isomer, unidentified) and female *B. tryoni* (*E*,*Z* isomer) (Fletcher et al. 1992; Booth et al. 2006). (*E*,*E*)-2-Ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane has been previously reported as part of male emanations of *B. nigrotibialis*, *Bactrocera halfordiae* (Tryon), *B. dorsalis*, *B. kirki*, *Bactrocera latifrons* (Hendel) and *Bactrocera occipitalis* (Bezzi) as well as female of *B. tryoni* and *B. musae* (Perkins et al. 1990b; Symonds et al. 2009; Benelli et al. 2014; El-Sayed et al. 2019; Noushini et al. 2019). 2-Propyl-8-methyl-1,7-dioxaspiro[5.5]undecane has been identified in *n*-pentane extracts of the abdomen of female *B. tryoni* (Booth et al. 2006). With respect to fatty acid esters, ethyl caprate (**12**), methyl laurate (**13**), ethyl laurate (**15**), methyl myristate (**18**), ethyl myristate (**19**), methyl palmitate (**20**), ethyl palmitoleate (**23**), ethyl palmitate (**24**) and ethyl oleate (**26**) have also been found in *B. oleae, B. tryoni* and *B. correcta* female rectal gland extracts (Canale et al. 2015; El-

Sayed et al. 2019; Zhang et al. 2019). Of these, ethyl caprate and methyl palmitate have been reported as attractants for both *B. oleae* males and females (Canale et al. 2015).

Mature male *B. bryoniae* produced a simple blend of six volatiles (Table 1) with 1,7-dioxaspiro[5,5]undecane (**3**) as the major component and being detected by both antenna and maxillary palps of both males and females. The headspace samples contained five of the same components, **1**, **3**, **6**, **7** and **11**, with the same dominant compound, 1,7-dioxaspiro[5,5]undecane, which has been identified as the major component of the female sex pheromone of *B. oleae* (Mazomenos and Haniotakis 1981). Although young male *B. oleae* also produce this spiroacetal in their rectal gland as a male aggregation pheromone, 1,7-dioxaspiro[5,5]undecane does not attract female *B. oleae* (Haniotakis et al. 1986). This spiroacetal has also been reported as the major volatile component of *Bactrocera cacuminata* (Hering) males (Kitching et al. 1986). The male specific compound, ethyl 3-acetoxybutanoate, elicited electrophysiological responses in palps of both males and females. Ethyl 3-acetoxybutanoate has not been identified in rectal glands or emissions of other tephritids but is found in pineapple (Zheng et al. 2012). 4-Hydroxy-1,7-dioxaspiro[5.5]undecane (**11**) was unique to males and elicited EAD responses in males and females as well as EPD responses in females. 4-Hydroxy-1,7-dioxaspiro[5.5]undecane has also been isolated from rectal glands of female *B. oleae* (Baker et al. 1982b).

Although males of many tephritid fruit flies have been reported as sex pheromone producers (Nation 1972, 1990; Baker et al. 1985; Fletcher and Kitching 1995), in some species such as *Z. cucurbitae* and *B. dorsalis,* both male and female volatile emissions have been reported to attract the opposite sex (Baker et al. 1982a; Baker and Bacon 1985; Nishida et al. 1988a, b). Our Y-maze olfactometer results demonstrated that males and females of *B. bryoniae* are attracted to the rectal gland emissions of opposite sex conspecifics. This suggests that rectal gland secretions of both sexes may function as mate-attracting sex pheromones in this species. We showed that males are attracted to male rectal gland odour whereas females did not exhibit a significant preference for female rectal gland odour. This suggests rectal gland secretions may play a role in aggregation of males only. In some fruit fly species, male rectal gland secretions function both as sex pheromones and as aggregation pheromones. For example, in *B. dorsalis* volatiles produced by males are known to act as aggregation pheromones for males as well as attractant for females (Nishida et al. 1988b).

Similarly in *Anastrepha suspensa* (Loew) male volatiles attract both males and females (Perdomo et al. 1976).

Our GC-EAD and GC-EPD results demonstrate differences in the detection of compounds between antennae and maxillary palps, suggesting different olfactory function of antenna from that of palps. Independent olfactory roles of maxillary palps and antenna have been reported in other tephritid fruit flies. For example, in male *B. tryoni* and *Bactrocera depressa* (Shiraki) the palps exhibit stronger electrophysiological responses to cuelure than the antennae (Verschut et al. 2018; Oh et al. 2019). In combination with our findings from Y-tube olfaction studies, the electrophysiological results suggest that ethyl 3-acetoxybutanoate, 1,7-dioxaspiro[5,5]undecane and 4-hydroxy-1,7-dioxaspiro[5.5]undecane are likely components of male-produced sex pheromone in *B. bryoniae* and ethyl 3-acetoxybutanoate, *N*-(3-methylbutyl)acetamide, 1,7-dioxaspiro[5,5]undecane and 4-hydroxy-1,7-dioxaspiro[5.5]undecane are likely components of male aggregation pheromone. Our findings also suggest (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane, *N*-(3-methylbutyl)acetamide, ethyl laurate, ethyl myristate and ethyl palmitate as components of female-produced sex pheromone in *B. bryoniae*. Further behavioural studies are needed to clarify the function of each these compounds.

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Declarations

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Availability of data and material: The authors confirm that data supporting the findings of this study are available within the manuscript and Online Resource.

Authors' contributions: S.N., P.T., J.J., I.J., designed the experiment. S.N. and S.J.P. performed the experiments. S.N. analysed the data and wrote the original draft. P.T., J.J., I.J.

and S.J.P reviewed and edited the manuscript. All authors read and approved the final manuscript.

References

Baker R, Bacon AJ (1985) The identification of spiroacetals in the volatile secretions of two species of fruit fly (*Dacus dorsalis, Dacus curcurbitae*). Experientia 41:1484–1485. doi: 10.1007/BF01950049

Baker R, Herbert RH, Grant GG (1985) Isolation and identification of the sex pheromone of the Mediterranean fruit fly, *Ceratitis capitata* (Wied). J Chem Soc Chem Commun 824–825

Baker R, Herbert RH, Lomer RA (1982a) Chemical components of the rectal gland secretions of male *Dacus cucurbitae*, the melon fly. Experientia 38:232–233. doi: 10.1007/BF01945082

Baker R, Herbert RH, Parton AH (1982b) Isolation and synthesis of 3- and 4-hydroxy-1,7-dioxaspiro[5.5]undecanes from the olive fly (*Dacus oleae*). J Chem Soc Chem Commun 601. doi: 10.1039/c39820000601

Bellas TE, Fletcher BS (1979) Identification of the major components in the secretion from the rectal pheromone glands of the Queensland fruit flies *Dacus tryoni* and *Dacus neohumeralis* (Diptera: Tephritidae). J Chem Ecol 5:795–803. doi: 10.1007/BF00986564

Benelli G, Daane KM, Canale A, et al (2014) Sexual communication and related behaviours in Tephritidae: current knowledge and potential applications for integrated pest management. J Pest Sci 87:385–405. doi: 10.1007/s10340-014-0577-3

Booth YK, Hayes PY, Moore CJ, et al (2007) Synthesis and absolute configuration of a constitutionally-new [5.6] spiroacetal from *B. tryoni* (Queensland fruit fly). Org Biomol Chem 5:1111–1117. doi: 10.1039/B701833A

Booth YK, Schwartz BD, Fletcher MT, et al (2006) A diverse suite of spiroacetals, including a novel branched representative, is released by female Bactrocera tryoni (Queensland fruit fly). Chem Commun 3975–3977. doi: 10.1039/B611953K

Canale A, Benelli G, Germinara GS, et al (2015) Behavioural and electrophysiological responses to overlooked female pheromone components in the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). Chemoecology 25:147–157. doi: 10.1007/s00049-014-0183-0

469 Canale A, Germinara SG, Carpita A, et al (2013) Behavioural and electrophysiological
470 responses of the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), to male-
471 and female-borne sex attractants. Chemoecology 23:155–164. doi: 10.1007/s00049-013-
472 0131-4
473 Carpita A, Canale A, Raffaelli A, et al (2012) (*Z*)-9-tricosene identified in rectal gland
474 extracts of *Bactrocera oleae* males: first evidence of a male-produced female attractant
475 in olive fruit fly. Naturwissenschaften 99:77–81. doi: 10.1007/s00114-011-0868-y
476 Clarke AR, Powell KS, Weldon CW, Taylor PW (2011) The ecology of *Bactrocera tryoni*
477 (Diptera: Tephritidae): what do we know to assist pest management? Ann Appl Biol
478 158:26–54. doi: 10.1111/j.1744-7348.2010.00448.x
479 Cruz-López L, Malo EA, Rojas JC (2015) Sex pheromone of *Anastrepha striata*. J Chem
480 Ecol 41:458–464. doi: 10.1007/s10886-015-0581-y
481 Dominiak BC, Ekman JH (2013) The rise and demise of control options for fruit fly in
482 Australia. Crop Prot 51:57–67. doi: 10.1016/j.cropro.2013.04.006
483 Dominiak BC, Mapson R (2017) Revised distribution of *Bactrocera tryoni* in eastern
484 Australia and effect on possible incursions of Mediterranean fruit fly: development of
485 Australia's eastern trading block. J Econ Entomol 110:2459–2465. doi:
486 10.1093/jee/tox237
487 Doorenweerd C, Leblanc L, Norrbom AL, et al (2018) A global checklist of the 932 fruit fly
488 species in the tribe Dacini (Diptera, Tephritidae). Zookeys 730:19–56. doi:
489 10.3897/zookeys.730.21786
490 Drew RAI, Romig MC (2013) Tropical Fruit Flies of South-East Asia. CAB International,
491 Wallingford
492 El-Sayed AM (2007) The Pherobase: Database of pheromones and semiochemicals.
493 http://www.pherobase.com/. Accessed 17 Mar 2019
494 El-Sayed AM, Byers JA, Manning LM, et al (2008) Floral scent of Canada thistle and its
495 potential as a generic insect attractant. J Econ Entomol 101:720–727. doi:
496 10.1093/jee/101.3.720
497 El-Sayed AM, Venkatesham U, Unelius CR, et al (2019) Chemical composition of the rectal
498 gland and volatiles released by female Queensland fruit fly, *Bactrocera tryoni* (Diptera:
499 Tephritidae). Environ Entomol. doi: 10.1093/ee/nvz061
500 Ero MM, Hamacek E, Clarke AR (2011) Foraging behaviours of *Diachasmimorpha kraussii*
501 (Fullaway) (Hymenoptera: Braconidae) and its host *Bactrocera tryoni* (Froggatt)
502 (Diptera: Tephritidae) in a nectarine (*Prunus persica* (L.) Batsch var. *nectarina* (Aiton)

503 Maxim) orch. Aust J Entomol no-no. doi: 10.1111/j.1440-6055.2011.00821.x
504 Fletcher BS (1968) Storage and release of sex pheromone by the Queensland fruit fly, *Dacus*
505 *tryoni* (Diptera:Trypetidae). Nature 219:631–632. doi: 10.1038/219631a0
506 Fletcher BS (1969) The structure and function of the sex pheromone glands of the male
507 Queensland fruit fly, *Dacus tryoni*. J Insect Physiol 15:1309–1322. doi: 10.1016/0022-
508 1910(69)90193-0
509 Fletcher MT, Kitching W (1995) Chemistry of fruit flies. Chem Rev 95:789–828. doi:
510 10.1021/cr00036a001
511 Fletcher MT, Wells JA, Jacobs MF, et al (1992) Chemistry of fruit-flies. Spiroacetal-rich
512 secretions in several *Bactrocera* species from the South-West Pacific region. J Chem
513 Soc Perkin Trans 1 2827–2831. doi: 10.1039/P19920002827
514 Haniotakis G, Francke W, Mori K, et al (1986) Sex-specific activity of (*R*)-(−)- and (*S*)- (+)-
515 1,7-dioxaspiro[5.5]undecane, the major pheromone of *Dacus oleae*. J Chem Ecol
516 12:1559–1568. doi: 10.1007/BF01012372
517 Haniotakis GE (1974) Sexual attraction in the olive fruit fly, *Dacus oleae* (Gmelin). Environ
518 Entomol 3:82–86. doi: 10.1093/ee/3.1.82
519 Haniotakis GE, Mazomenos BE, Tumlinson JH (1977) A sex attractant of the olive fruit fly,
520 *Dacus oleae* and its biological activity under laboratory and field conditions. Entomol
521 Exp Appl 21:81–87. doi: 10.1111/j.1570-7458.1977.tb02660.x
522 Hayes P, Fletcher MT, Moore CJ, Kitching W (2001) Synthesis and absolute stereochemistry
523 of a constitutionally new spiroacetal from an insect. J Org Chem 66:2530–2533. doi:
524 10.1021/jo015502p
525 Heath RR, Landolt PJ, Robacker DC, et al (2000) Sexual pheromones of tephritid flies: clues
526 to unravel phylogeny and behavior. In: Aluja M, Norrbom AL (eds) Fruit Flies
527 (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, pp 793–809
528 Hendrichs J, Robinson AS, Cayol JP, Enkerlin W (2002) Medfly areawide sterile insect
529 technique programmes for prevention, suppression or eradication: the importance of
530 mating behavior studies. Fla Entomol 85:1–14. doi: 10.1653/0015-
531 4040(2002)085[0001:MASITP]2.0.CO;2
532 Hickey P, Woods B, Windle B, et al (2010) National fruit fly strategy implementation action
533 plan. Plant Health Australia, Canberra, Australia
534 Kitching W, Lewis JA, Fletcher MT, et al (1986) Spiroacetals in rectal gland secretions of
535 Australasian fruit fly species. J Chem Soc Chem Commun 11:853. doi:
536 10.1039/c39860000853

537 Kitching W, Lewis JA, Perkins M V, et al (1989) Chemistry of fruit flies. Composition of the
538 rectal gland secretion of (male) *Dacus cucumis* (cucumber fly) and *Dacus halfordiae*.
539 Characterization of (*Z,Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane. J Org Chem
540 54:3893–3902. doi: 10.1021/jo00277a028
541 Krohn S, Fletcher MT, Kitching W, et al (1991) Chemistry of fruit flies: Nature of glandular
542 secretion and volatile emission of *Bactrocera (bactrocera) cacuminatus* (Héring). J
543 Chem Ecol 17:485–495. doi: 10.1007/BF00994347
544 Lauzon CR, Potter SE (2012) Description of the irradiated and nonirradiated midgut of
545 *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and *Anastrepha ludens* Loew
546 (Diptera: Tephritidae) used for sterile insect technique. J Pest Sci 85:217–226. doi:
547 10.1007/s10340-011-0410-1
548 Leblanc L, Tora E, Drew RAI, Allwood AJ (2013) Host plant records for fruit flies (Diptera:
549 Tephritidae: Dacini) in the Pacific Islands. Proc Hawaiian Entomol Soc 44:11–53. doi:
550 10.1002/ana.21927.Dicer
551 Levi-zada A, Levy A, Rempoulakis P, et al (2020) Diel rhythm of volatile emissions of males
552 and females of the peach fruit fly *Bactrocera zonata*. J Insect Physiol 120:103970. doi:
553 10.1016/j.jinsphys.2019.103970
554 Mazomenos BE, Haniotakis GE (1985) Male olive fruit fly attraction to synthetic sex
555 pheromone components in laboratory and field tests. J Chem Ecol 11:397–405. doi:
556 10.1007/BF01411425
557 Mazomenos BE, Haniotakis GE (1981) A multicomponent female sex pheromone of *Dacus*
558 *oleae* Gmelin: Isolation and bioassay. J Chem Ecol 7:437–444. doi:
559 10.1007/BF00995766
560 Mitchell RF, Curkovic T, Mongold-Diers JA, et al (2017) Evidence that cerambycid beetles
561 mimic vespid wasps in odor as well as appearance. J Chem Ecol 43:75–83. doi:
562 10.1007/s10886-016-0800-1
563 Nation JL (1990) Biology of pheromone release by male Caribbean fruit flies, *Anastrepha*
564 *suspensa* (Diptera: Tephritidae). J Chem Ecol 16:553–572. doi: 10.1007/BF01021786
565 Nation JL (1972) Courtship behavior and evidence for a sex attractant in the male Caribbean
566 fruit fly, *Anastrepha suspensa*. Ann Entomol Soc Am 65:1364–1367. doi:
567 10.1093/aesa/65.6.1364
568 Nishida R, Tan KH, Fukami H (1988a) *Cis*-3,4-dimethoxycinnamyl alcohol from the rectal
569 glands of male oriental fruit fly, *Dacus dorsalis*. Chem Express 3:207–210
570 Nishida R, Tan KH, Serit M, et al (1988b) Accumulation of phenylpropanoids in the rectal

571 glands of males of the Oriental fruit fly, *Dacus dorsalis*. Experientia 44:534–536. doi:
572 10.1007/BF01958941
573 Noushini S, Perez J, Park SJ, et al (2019) Rectal gland chemistry, volatile emissions, and
574 antennal responses of male and female banana fruit fly, Bactrocera musae. Insects 11:32.
575 doi: 10.3390/INSECTS11010032
576 Oh H, Jeong SA, Kim J, Park KC (2019) Morphological and functional heterogeneity in
577 olfactory perception between antennae and maxillary palps in the pumpkin fruit fly,
578 *Bactrocera depressa*. Arch Insect Biochem Physiol 101:. doi: 10.1002/arch.21560
579 Perdomo AJ, Nation JL, Baranowski RM (1976) Attraction of female and male Caribbean
580 fruit flies to food-B\baited and male-baited traps under field conditions. Environ
581 Entomol 5:1208–1210. doi: 10.1093/ee/5.6.1208
582 Perkins MV (1990) Characterisation and synthesis of Bactrocera fruit fly pheromones. PhD
583 Thesis, Department of Chemistry.The University of Queensland, Australia
584 Perkins MV, Fletcher MT, Kitching W, et al (1990a) Chemical studies of rectal gland
585 secretions of some species of *Bactrocera dorsalis* complex of fruit flies (diptera:
586 Tephritidae). J Chem Ecol 16:2475–2487. doi: 10.1007/BF01017470
587 Perkins MV, Kitching W, Drew RAI, et al (1990b) Chemistry of fruit flies: composition of
588 the male rectal gland secretions of some species of South-East Asian Dacinae. Re-
589 examination of *Dacus cucurbitae* (melon fly). J Chem Soc Perkin Trans 1 1111–1117.
590 doi: 10.1039/P19900001111
591 Piccardi P (1980) Insect sex-communication and prospects for pheromones in pest
592 management. Bolletino di Zool 47:397–408. doi: 10.1080/11250008009438696
593 Schutze M, McMahon J, Krosch M, et al (2018) The Australian Handbook for the
594 Identification of Fruit Flies. Version 3.1. Plant Health Australia, Canberra, ACT
595 Schwartz BD, Moore CJ, Rahm F, et al (2008) Spiroacetal biosynthesis in insects from
596 Diptera to Hymenoptera: The giant ichneumon wasp *Megarhyssa nortoni nortoni*
597 Cresson. J Am Chem Soc 130:14853–14860. doi: 10.1021/ja8036433
598 Sivinski J, Aluja M, Dodson GN, et al (2000) Topics in the evolution of sexual behavior in
599 the Tephritidae. In: Aluja M, Norrbom AL (eds) Fruit Flies (Tephritidae): Phylogeny
600 and Evolution of Behavior. CRC Press, pp 751–792
601 Sivinski JM, Calkins C (1986) Use of pheromones in tropical crops: pheromones and
602 parapheromones in the control of tephritids. Florida Entomol 69:157–168. doi:
603 10.2307/3494757
604 Symonds MRE, Moussalli A, Elgar MA (2009) The evolution of sex pheromones in an

ecologically diverse genus of flies. Biol J Linn Soc 97:594–603

Tokushima I, Orankanok W, Tan KH, et al (2010) Accumulation of phenylpropanoid and sesquiterpenoid volatiles in male rectal pheromonal glands of the guava fruit fly, *Bactrocera correcta*. J Chem Ecol 36:1327–1334. doi: 10.1007/s10886-010-9874-3

Verschut TA, Farnier K, Cunningham JP, Carlsson MA (2018) Behavioral and physiological evidence for palp detection of the male-specific attractant cuelure in the Queensland fruit fly (*Bactrocera tryoni*). Front Physiol 9:. doi: 10.3389/fphys.2018.00990

Vijaysegaran S (1997) Fruit fly research and development in tropical Asia. In: Allwood, A., Drew, R E (ed) Management of Fruit Flies in the Pacific. Australian Centre for International Agricultural Research Proceedings, Canberra, pp 21–29

Wee SL, Tan KH (2005) Female sexual response to male rectal volatile constituents in the fruit fly, *Bactrocera carambolae* (Diptera: Tephritidae). Appl Entomol Zool 40:365–372. doi: 10.1303/aez.2005.365

Zamek AL, Spinner JE, Micallef JL, et al (2012) Parasitoids of Queensland fruit fly *Bactrocera tryoni* in Australia and prospects for improved biological control. Insects 3:1056–1083

Zhang X, Wei C, Miao J, et al (2019) Chemical compounds from female and male rectal pheromone glands of the guava fruit fly, *Bactrocera correcta*. Insects 10:. doi: 10.3390/insects10030078

Zheng L-Y, Sun G-M, Liu Y-G, et al (2012) Aroma volatile compounds from two fresh pineapple varieties in China. Int J Mol Sci 13:7383–7392. doi: 10.3390/ijms13067383

Table 1. Rectal gland (RG) and headspace (HS) volatile compounds of *B. bryoniae* adults. No = number, FRG = female rectal gland, FHS = female headspace, MRG = male rectal gland, MHS = male headspace, RI = retention index, MW = molecular weight, ND = not detected

No	Name	FRG (%)	FHS (%)	MRG (%)	MHS (%)	Characteristic EI ions *m/z* (%)	RI
1	Ethyl 3-acetoxybutanoate	ND	ND	2.88	0.49	174 (M^+, 0.02), 131 (M – CH_3C=O, 32.84), 129 (M – OC_2H_5,16.93), 117 (10.7), 114 (27.4), 85 ($CH_3(CO)$ CH_2C=O, 25.26), 69 (100)	1109
2	2,7-Dimethyl-1,6-dioxaspiro[4.5]decane	0.01	ND	ND	ND	170 (M^+, 1.9), 155 (M – CH_3, 1.7), 126 (7.8), 115 (13.1), 111 (4.9), 101 ($CH_3(C_4H_5O)$=OH^+, 100), 98 ($CH_3(C_4H_5O)$=CH_2, 77.32), 83 (31.2), 69 (12.7), 55 (49.1)	1087
3	1,7-Dioxaspiro[5,5]undecane	ND	ND	87.335	84.81	156 (M+, 8.2%), 128 (7.5), 111 (12.7), 102 (6), 101 ((C_5H_7O)=OH^+, 100), 100 (64.7), 99 (7.8), 98 ((C_5H_7O)=CH_2, 85.1), 97 (3.6), 83 (33.4), 70 (5.9), 56 (16), 55 (36.52)	1135
4	(*E*,*E*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	4.85	5.78	0.374	ND	184 (M^+, 6.6), 169 (M – CH_3, 1.7), 140 (13.7), 125 (8.6), 115 ($CH_3(C_5H_7O)$=OH^+, 92), 112 ($CH_3(C_5H_7O)$=CH_2, 100), 97 (73.9), 69 (37), 55 (36.8)	1147
5	(*E*,*E*)-2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane	0.03	0.12	ND	ND	184 (M^+, 3.3), 155 (M – C_2H_5, 30.2), 140 (11.8), 115 (M – $C_5H_9^-$, 100), 112 (M – C_4H_8O, 60.5), 97 (90.4), 85 (56.2), 69 (47.2), 55 (56.8)	1160
6	*N*-(2-Methylbutyl)acetamide	0.01	ND	0.11	0.057	129 (M^+, 9.8), 100 (M – C_2H_5, 61.2), 73 (β-cleavage product, 76.8), 72 (β-cleavage product,100), 60 ($CH_3C(OH)NH^+$, 56.4)	1132
7	*N*-(3-Methylbutyl)acetamide	0.43	0.30	8.26	13.56	129 (M^+, 6.1), 114 (M – CH_3, 16.9), 86 (M – C_3H_7, 29.1), 73 (β-cleavage product, 100), 72 (β-cleavage product, 69.7), 60 ($CH_3C(OH)NH^+$, 25.3)	1136
8	(*E*,*E*)-2-Ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane	0.39	2.14	ND	ND	198 (M^+, 6.6), 169 (M - C_2H_5, 14.8), 140 (13.7), 129 ($CH_3CH_2(C_5H_7O)$=OH^+, 45.8), 126 ($CH_3CH_2(C_5H_7O)$=CH_2, 37.3), 115 $CH_3(C_5H_7O)$=OH^+, 92.8), 112 ($CH_3(C_5H_7O)$=CH_2, 100), 97 (84.9), 83 (70), 69 (60.9), 55 (71.2)	1236
9	(*Z*,*Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	0.08	0.197	ND	ND	184 (M^+, 3.5), 169 (M – CH_3, 1.1), 140 (4.7), 125 (6), 115 ($CH_3(C_5H_7O)$=OH^+, 100), 114 (42.4) 112 ($CH_3(C_5H_7O)$=CH_2, 45.4), 97 (87.6), 69 (56.3), 55 (40)	1221
10	(*E*,*E*)-2-Propyl-8-methyl-1,7-dioxaspiro[5.5]undecane	0.09	0.52	ND	ND	212 (M^+, 4.9), 169 (M – C_3H_7, 16.7), 143 ($CH_3CH_2CH_2(C_5H_6O)$=OH^+, 28.2), 140 ($CH_3CH_2CH_2(C_5H_6O)$=$CH_2CH_2CH_3$, 29.4), 125 (47.1), 115 ($CH_3(C_5H_7O)$=OH^+, 100),112 ($CH_3(C_5H_7O)$=CH_2, 88), 97 (96.9), 83 (38.6), 82 (31.5), 69 (40.2), 55 (63.2)	1322
11	4-Hydroxy-1,7-dioxaspiro[5.5]undecane	ND	ND	0.81	0.47	172 (M^+, 0.4), 155 (M – OH, 11.1), 127 (31.6), 117 (OH(C_5H_7O)=OH^+, 96.2), 114 (OH(C_5H_7O)=CH_2, 39.4), 101 ((C_5H_7O)=OH^+, 100), 98 ((C_5H_7O)=CH_2, 57.1), 83 (22.6), 55 (35.8)	1357
12	Ethyl caprate	0.21	0.59	ND	ND	200 (M^+, 1.2), 171 (M – C_2H_5, 2.4), 157 (11.9), 155 (M – OC_2H_5, 9.9), 115 (7.7), 101 (36.5), 88 (McLafferty rearrangement product, 100), 73 ($COOC_2H_5$, 22.6), 70 (27.1), 55 (24.5)	1395

13	Methyl laurate	0.21	0.42	ND	ND	214 (M^+, 2.1), 183 (M – OCH_3, 4.3), 171 (8.4), 143 (11.8), 87 (59.3), 74 (McLafferty rearrangement product, 100), 69 (11.6), 59 ($COOCH_3$, 9.6), 55 (24.8)	1524
14	Lauric acid	0.29	ND	ND	ND	200 (M^+, 5.6), 171 (9.2), 157 (25), 129 (35.1), 115 (18.8), 101 (15.7), 97 (17.1), 85 (35.2), 73 (100), 69 (28.7), 57 (54.7), 55 (63.3)	1559
15	Ethyl laurate	39.9	66.59	ND	ND	228 (M^+, 3.3), 199 (M – C_2H_5, 3.7), 183 (M – OC_2H_5, 9.5), 157 (14.8), 101 (48.4), 88 (McLafferty rearrangement product, 100), 73 ($COOC_2H_5$, 20), 70 (23.4), 61 (11.3), 55 (22)	1594
16	Ethyl tridecanoate	0.03	0.08	ND	ND	242 (M^+, 2.8), 213 (M – C_2H_5, 5.1), 199 (6.8), 197 (M – OC_2H_5, 2.1), 157 (13.7), 101 (58.6), 88 (McLafferty rearrangement product, 100), 83 (27), 73 ($COOC_2H_5$, 13.9), 57 (18.8), 55 (30.8)	1665
17	Propyl laurate	0.09	0.10	ND	ND	242 (M^+, 2.4), 201 (23.4), 199 (M – C_3H_7, 1.6), 183 (M – OC_3H_7, 22.6), 157 (8.3), 129 (9.8), 115 (17.9), 102 (McLafferty rearrangement product, 23.9), 87 ($COOC_3H_7$, 10.4), 61 (100), 60 (38.5), 59 (6.1), 55 (30.4)	1690
18	Methyl myristate	0.28	0.25	ND	ND	242 (M^+, 2.8), 211 (M – OCH_3, 2.4), 199 (8.1), 143 (16.8), 125 (7.3), 129 (5.8), 101 (7.9), 87 (65.9), 74 (McLafferty rearrangement product, 100), 69 (13.5), 59 ($COOCH_3$, 8.7), 55 (26.5)	1725
19	Ethyl myristate	31.99	20.78	ND	ND	256 (M^+, 5.7), 213 (10.9), 211 (M – OC_2H_5, 6.6), 157 (18.8), 101 (52.5), 88 (McLafferty rearrangement product, 100), 73 ($COOC_2H_5$, 18.2), 70 (21.7), 69 (12.3), 55 (22.1)	1794
20	Methyl palmitate	0.15	ND	ND	ND	270 (M^+, 5.1), 227 (6.9), 143 (15.7), 87 (68.4), 74 (McLafferty rearrangement product, 100), 69 (15.9), 59 ($COOCH_3$, 8.8), 55 (29.4)	1926
21	Palmitoleic acid	0.24	ND	ND	ND	254 (M^+, 1.7), 236 (5.8), 152 (7.2), 111 (22.2), 98 (30.1), 97 (45.8), 96 (32.7), 83 (54.7), 73 (17.6), 69 (75.5), 60 (McLafferty rearrangement product, 12.8), 57 (33.7), 55 (100)	1943
22	Palmitic acid	0.39	ND	ND	ND	256 (M^+, 14.4), 227 (5.6), 213 (M – COOH, 15.9), 185 (15.1), 157 (20.6), 129 (41.3), 115 (22.8), 97 (25.5), 87 (37.4), 85 (35), 83 (38.3), 73 (100), 69 (45.6), 60 (McLafferty rearrangement product, 89.3), 57 (80.9), 55 (83.1)	1960
23	Ethyl palmitoleate	2.46	0.19	ND	ND	282 (M^+, 2.8), 237 (M – OC_2H_5, 10.1), 236 (M – C_2H_5OH, 10.2), 194 (11.9), 152 (17.6), 88 (McLafferty rearrangement product, 52.1), 73 ($COOC_2H_5$, 15.3), 69 (69.8), 55 (100)	1975
24	Ethyl palmitate	15.9	0.96	ND	ND	284 (M^+, 7.8), 255 (M – C_2H_5, 2.9), 241 (9.1), 239 (M – OC_2H_5, 5.3), 157 (17.3), 101 (55.2), 88 (McLafferty rearrangement product, 100), 73 ($COOC_2H_5$, 17.3), 55 (23.5)	1994
25	Oleic acid	0.25	ND	ND	ND	282 (M^+, 2.3), 264 (10), 221 (2.9), 180 (4), 165 (5.6), 137 (7.6), 1151 (29.8), 97 (61.5), 87 (69.9), 69 (77.6), 60 (McLafferty rearrangement product, 10.6), 57 (40.3), 55 (100)	2141
26	Ethyl oleate	1.5	ND	ND	ND	310 (M^+, 3.4), 265 (M – OC_2H_5, 12.4), 264 (M – C_2H_5OH, 14.8), 222 (9.6), 180 (10), 123 (15.9), 110 (23.2), 97 (56.5), 88 (McLafferty rearrangement product, 57), 83 (61.9), 73 ($COOC_2H_5$, 15.9), 69 (68.1), 55 (100)	2171

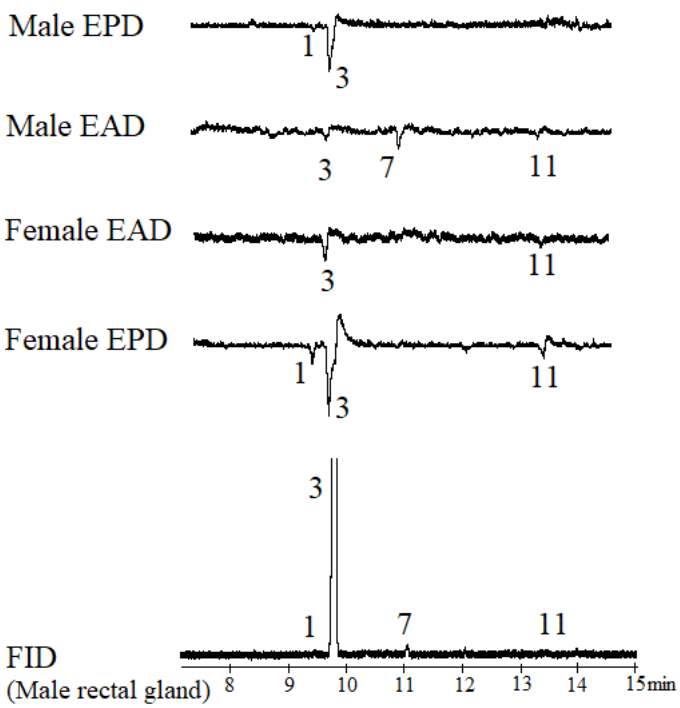

Fig. 1 Flame ionization detector (FID) response and electrophysiological responses of antennae (EAD) and palps (EPD) of *Bactrocera bryoniae* males and females to the rectal gland extracts from conspecific males. Numbered peaks indicate EAD- and/or EPD-active compounds: ethyl 3-acetoxybutanoate (**1**), 1,7-dioxaspiro[5,5]undecane (**3**), *N*-(3-methylbutyl)acetamide (**7**), 4-hydroxy-1,7-dioxaspiro[5.5]undecane (**11**)

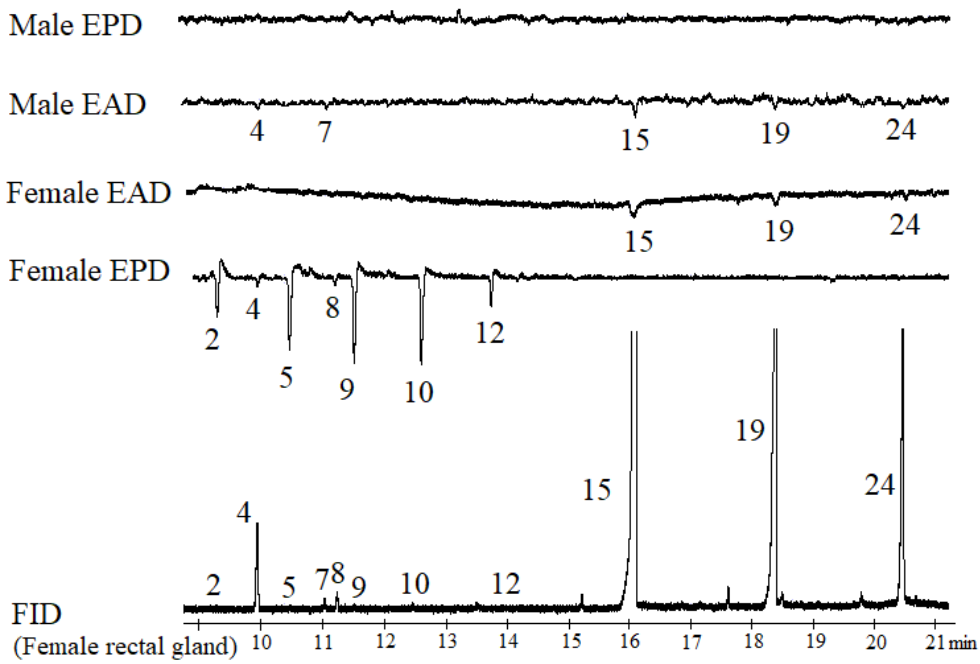

Fig. 2 Flame ionization detector (FID) response and electrophysiological responses of antennae (EAD) and palps (EPD) of *Bactrocera bryoniae* males and females to the rectal gland extracts from conspecific females. Numbered peaks indicate EAD- and/or EPD-active compounds: 2,7-dimethyl-1,6-dioxaspiro[4.5]decane (**2**), (*E*,*E*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**4**), (*E*,*E*)-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]decane (**5**), *N*-(3-methylbutyl)acetamide (**7**), (*E*,*E*)-2-ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**8**), (*Z*,*Z*)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**9**), and (*E*,*E*)-2-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**10**), ethyl caprate (**12**), ethyl laurate (**15**), ethyl myristate (**19**) and ethyl palmitate (**24**)

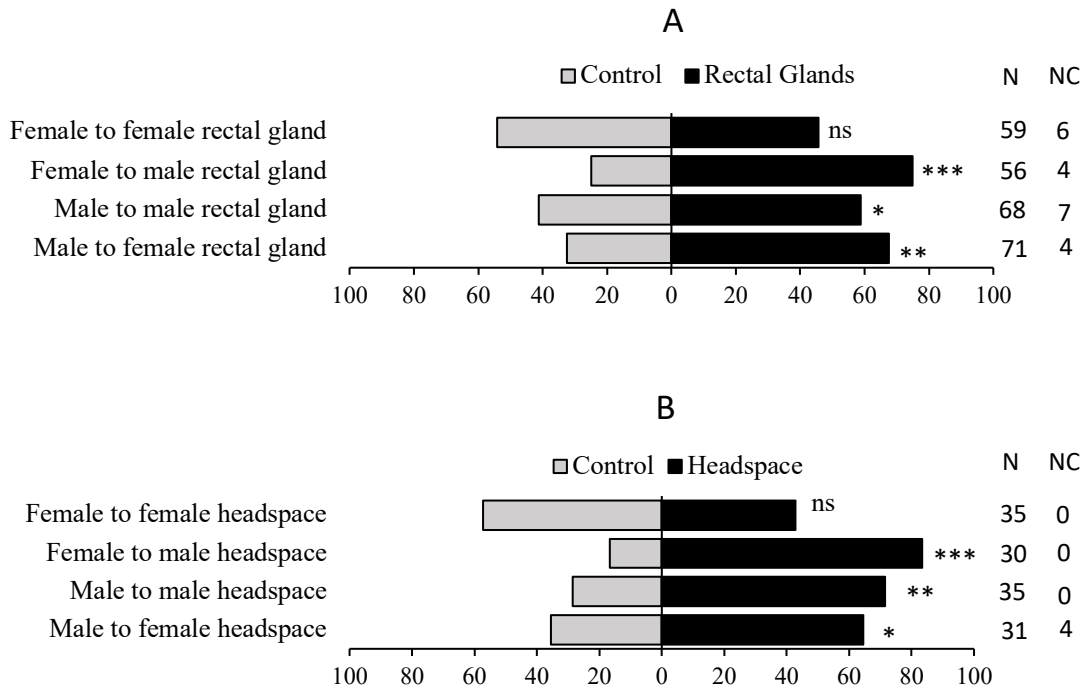

Fig. 2 Behavioural response of virgin adults of *Bactrocera bryoniae* males and females to (A) the rectal gland volatiles and (B) the headspace volatiles of males and females in a Y-tube olfactometer. N total number of responders, NC number of non-responders (excluded from statistical analysis), ns $P > 0.05$, * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$