Identification of a sex pheromone component for *Pennisetia marginata* (Lepidoptera: Sesiidae)

Gary J.R. Judd,[1] Regine Gries, Carolyn Teasdale, Gerhard Gries

Abstract—Raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is a native North American species and pest of many cane fruits. Using coupled gas chromatographic–electroantennographic detection analyses (GC-EAD) we identified candidate sex pheromone components in pheromone gland extracts and effluvia from calling females. Analysis of gland extracts on a Zebron-5 column revealed four components (A, B, C, and D) that elicited strong responses from male antennae. The two most EAD-active components A and B were hypothesised to be (3*E*,13*Z*)-octadecadienal [(3*E*,13*Z*)-18:Ald] and (3*E*,13*Z*)-octadecadienol [(3*E*,13*Z*)-18:OH], respectively. Their retention times on other GC columns matched those of authentic standards, thus supporting structural assignments. Synthetic (3*E*,13*Z*)-18:Ald, and its GC rearrangement product (2*E*,13*Z*)-octadecadienal [(2*E*,13*Z*)-18:Ald; component C], also induced antennal responses in GC-EAD analyses of female effluvia. Compounds D in pheromone gland extract, and E and F in female effluvia, elicited EAD responses but could not be identified. In field trapping experiments, (3*E*,13*Z*)-18:Ald was the only component that attracted male *P. marginata* when tested alone. Attractiveness of (3*E*,13*Z*)-18:Ald was reduced when combined in binary or ternary blends with any of the other identified components, suggesting one or all may act as pheromone antagonists. In Aldergrove, British Columbia, Canada, peak diel attraction of male *P. marginata* to (3*E*,13*Z*)-18:Ald occurred between 16:00 and 18:00 hours Pacific Daylight Time, at temperatures of 21–23°C, on 30 August 2010, 3 September 2010, and 13 September 2010. We conclude that (3*E*,13*Z*)-18:Ald is the major pheromone component of *P. marginata*.

Résumé—Le rhizophage du framboisier, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), une espèce indigène d'Amérique du Nord, est un ravageur de plusieurs arbustes fruitiers à drageons. Des analyses couplées de chromatographie en phase gazeuse et de détection électroantennographique (GC-EAD) nous ont permis d'identifier des composantes potentielles de la phéromone sexuelle dans des extraits de glandes à phéromone et des effluves émises par des femelles en appel. L'analyse des extraits de glandes sur colonne de Zebron-5 a reconnu quatre composantes (A, B, C et D) qui provoquent de fortes réactions dans les antennes mâles. Notre hypothèse veut que les deux composantes les plus actives à l'EAD, A et B, soient respectivement le (3*E*,13*Z*)-octadécadiénal [(3*E*,13*Z*)-18:Ald] et le (3*E*,13*Z*)-octadécadiénol [(3*E*,13*Z*)-18:OH]. Leurs temps de rétention sur d'autres colonnes GC correspondent à ceux de standards authentiques, ce qui appuie leur assignation structurale. Le (3*E*,13*Z*)-18:Ald synthétique et son produit de réarrangement en GC, le (2*E*,13*Z*)-octadécadiénal [(2*E*,13*Z*)-18:Ald, composante C], produisent aussi des réactions antennaires dans les analyses CG-EAD des effluves de la femelle. Les composantes D dans l'extrait de glande à phéromone et E et F dans les effluves de la femelle provoquent des réactions EAD, mais ne peuvent être identifiées. Dans des expériences de piégeage sur le terrain, le (3*E*,13*Z*)-18:Ald est la seule composante qui attire des mâles de *P. marginata* lorsqu'elle est utilisée seule. L'attraction de (3*E*,13*Z*)-18:Ald est réduite lorsqu'il est combiné en mélanges binaires ou ternaires avec n'importe lesquelles des autres composantes identifiées, ce qui laisse croire que l'une ou l'ensemble de ces composantes peuvent agir comme antagonistes de la phéromone. À Aldergrove, en Colombie-Britannique, Canada, le pic journalier d'attraction des mâles de *P. marginata* par le (3*E*,13*Z*)-18:Ald s'est produit entre 16:00 et 18:00 h, heure avancée du Pacifique, à des températures de 21–23 °C, les 30 août 2010, 3 septembre 2010 et 13 septembre 2010. Nous concluons que le (3*E*,13*Z*)-18:Ald est la composante principale de la phéromone de *P. marginata*.

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Introduction

The raspberry crown borer (RCB), *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is native to North America and the only species of this genus in areas north of Mexico (Eichlin and Duckworth 1988). Throughout its range, larvae of this clearwing moth commonly feed within the crowns and stems of cane fruits in the genus *Rubus* Linnaeus (Rosaceae). Although the incidence of RCB is often sporadic, in some areas and years it can be a highly destructive pest of cultivated raspberry and blackberry (Lovett 1921; Raine 1962; McKern *et al*. 2007). Eggs are laid individually on host-plant leaves from mid-August into October. Larvae begin hatching in early September and initiate feeding within plant crowns located at the base of canes. Larvae eventually tunnel upwards into the pith of the canes where they pupate and emerge as adults almost 2 years later in British Columbia (BC) (Raine 1962). Withering, wilting, and dying cane foliage, sometimes with half-grown fruit still attached, are often the first signs of a RCB infestation. Larval feeding within plant crowns also facilitates entry of pathogens that can produce symptoms similar to cane blight (Lawrence 1904). In regions where RCB has a 2-year life cycle (Raine 1962), feeding by second-year larvae in plant crowns often causes canes to break off when growers tie them up in spring. RCB populations build up slowly in commercial cane berry fields and often go undetected until cane vigour is severely reduced (Raine 1962).

Detection of RCB in commercial plantings is challenging because the insect spends most of its life as a larva tunnelling inside the lower parts of canes (Lawrence 1904; Raine 1962). Adult crown borers are thought to emerge and fly between July and September, but this timing is difficult to confirm from field observations and likely varies across regions and seasons (Eichlin and Duckworth 1988). Without precise knowledge of adult flight phenology and egg laying, growers throughout North America often protect their crops with preventative applications of insecticides. These sprays are referred to as drenches when applied using large volumes of water. In BC, Canada, for example, growers within the Fraser Valley routinely drench raspberry and blackberry crops with diazinon between October and March (British Columbia Ministry of Agriculture and Lands 2009). Growers apply these organophosphate sprays without knowing whether RCB is active in their fields or not. In this region, routine applications of organophosphates put the Fraser Valley aquifer at unnecessary risk of contamination. Diazinon and other organophosphates are being replaced by newer, more expensive, life-stage specific insecticides, but these require accurate information about insect phenology to be most effective.

Insect sex pheromones are useful for developing monitoring systems that provide growers with an early warning of an insect's presence and phenology (McNeil 1991). These systems allow for more accurate targeting of insecticide sprays when and where needed. Berry growers in BC are familiar with pheromone-based monitoring systems used to determine seasonal flight phenology and relative population levels of the obliquebanded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae), and currant borer, *Synanthedon tipuliformis* Clerck (Lepidoptera: Sesiidae) (British Columbia Ministry of Agriculture and Lands 2009). Efforts to develop a similar monitoring system for *P. marginata* have failed because none of the readily available commercial sex attractants identified in other sesiid moths have proved attractive (Tracy Hueppelsheuser, British Columbia Ministry of Agriculture and Lands, Abbotsford, BC, Canada, personal communication). Although related species of sesiid moths often share pheromone components (El-Sayed 2009), molecular phylogeny studies suggest *Pennisetia* is a unique and divergent sesiid genus in North America (McKern *et al*. 2008). Several compounds are postulated to be sex pheromones of *Pennisetia* species but no sex pheromones have been isolated from virgin females in this genus (El-Sayed 2009). We undertook this study to identify the sex pheromone of *P. marginata* in an effort to aid development of pheromone-based management tools for this pest.

Methods and materials

Insects

Adult RCBs were reared from 1.5-year-old larvae, which were collected on 20 April 2008 from a commercial raspberry field in Aldergrove, BC (49°2′22.53″N, 122°27′32.92″W). Host raspberry

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cane stubs showing signs of RCB infestation were dissected and second-year larvae (ca. 2 cm long) were removed and transferred to 2-cm long incisions made into the sides and half way along the lengths of freshly cut, second-year wild blackberry canes (30 × 1.5 cm). Once larvae were transferred, each incision was sealed with wax tape, and severed ends of canes were submerged in water in glass test tubes. Transferred larvae continued to feed, almost always tunnelling upward, and adults emerged from pupae protruding from the side of canes (Raine 1962). Thirty such canes were kept in an environment chamber set at 22°C under a 16L:8D photoregime. On 15 July 2008, canes were transferred to 11.4-L plastic buckets into which adults emerged. Moths emerging from 15 July to 15 August 2008 were collected within 12 hours of emergence to prevent mating and kept singly in capped 29.5-mL plastic cups (Solo Cup Company, Lake Forest, Illinois, United States of America) until analysis.

Extraction and analyses of pheromone glands

The abdominal tips of 1-day to 2-day-old females with intact pheromone glands were removed with fine forceps and solvent-extracted for 15 minutes using ~20 µL of HPLC-grade hexane (EMD Chemicals, Gibbstown, New Jersey, United States of America) per gland. Aliquots of one female equivalent of pheromone gland extract were analysed by coupled gas chromatographic–electroantennographic detection (GC-EAD) (Arn *et al*. 1975; Gries *et al*. 2002; Judd *et al*. 2011) employing a Hewlett Packard 5890A gas chromatograph (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada) equipped with a flame ionization detector (FID) and one of three GC columns (Table 1). Helium was used as a carrier gas (1 mL/minute flow rate) for the GC with temperature programmes as reported in Table 1. EAD-active components were identified by comparing their retention indices with those of authentic standards on three GC columns (Van Den Dool and Kratz 1963; Table 1).

Collection and analysis of pheromone effluvia

For several consecutive days, 1-day to 2-day-old virgin females were held in a vertically positioned, cylindrical, Pyrex glass chamber (10 cm ID × 6 cm length) provisioned with a water-soaked cotton wick. This chamber contained three to eight females at any particular time and dead females were removed daily. A water aspirator drew humidified, charcoal-filtered air at a rate of 1 L/minute through the chamber and an attached glass column (14 × 1.3 cm OD) filled with 150 mg of Porapak Q (50–80 mesh, Waters Associates Inc., Milford, Massachusetts, United States of America). In control aerations the chamber contained no females. A total of 576 female hour equivalents (FHEs) or 24-day equivalents of pheromone emission (1 FHE = amount of pheromone emitted by one female during 1 hour) were captured on Porapak Q. Trapped volatiles were eluted from Porapak Q with 2 mL of redistilled pentane. The eluted material was concentrated under a stream of nitrogen such that 2-µL aliquots contained 10 FHE of pheromone emission, which was then analysed by GC-EAD on a DB-5 column (Table 1).

Pheromone identification

Components in pheromone gland extracts that elicited antennal responses were identified by comparing their respective retention indices on three GC columns (Zebron-5, DB-23, and DB-210) with retention indices of synthetic diene alcohols or acetates (Table 1) that were previously identified as sesiid pheromones. Corresponding aldehydes were synthesised (see below). Components in extracts or effluvia that elicited antennal responses, and their corresponding synthetic standards, were also subjected to GC-mass spectrometry (MS) employing a Saturn 2000 Ion Trap GC-MS (Varian Instrument, Laurent, Quebec, Canada; now part of Agilent Technologies Inc.) fitted with a DB-5 column.

Sources of candidate pheromone components

All candidate pheromone components except (3*E*,13*Z*)-octadecadienal [(3*E*,13*Z*)-18:Ald)] and (2*E*,13*Z*)-octadecadienal [(2*E*,13*Z*)-18:Ald] were purchased from Pherobank (Wageningen, The Netherlands). (3*E*,13*Z*)-18:Ald was synthesised from (3*E*,13*Z*)-octadecadienol [(3*E*,13*Z*)-18:OH] (100 mg, 0.375 mmol) with pyridinium chlorochromate (121 mg, 0.566 mmol) in the presence of anhydrous K_2CO_3 (78 mg, 0.565 mmol) in 3 mL of dichloromethane (Islam *et al.* 2007). After

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Table 1. Retention indices for candidate pheromone components **A**, **B** and, **C** eliciting antennal responses in Figures 1 and 2, and of geometrical isomers of synthetic 2,13- and 3,13-octadecadienol, -octadecadienyl acetate, and -octadecadienal on three gas chromatography (GC) columns. The retention indices of **A**, **B**, and **C** matched those of (*E*,*Z*)-3,13-18:Ald, (*E*,*Z*)-3,13-18:OH, and (*E*,*Z*)-2,13-18:Ald on all three GC columns, respectively.

Compound	Zebron-5	DB-23	DB-210
Component **A**	**2000**	**2506**	**2345**
Component **B**	**2056**	**2627**	**2271**
Component **C**	**2082**	**2714**	**2558**
(3*E*,13*E*)-18:Ald	2000	2489	2327
(3*E*,13*Z*)-18:Ald	**2000**	**2506**	**2345**
(2*E*,13*E*)-18:Ald	2081	2693	2539
(2*E*,13*Z*)-18:Ald	**2082**	**2714**	**2558**
(3*E*,13*E*)-18:OH	2056	2608	2258
(3*E*,13*Z*)-18:OH	**2056**	**2627**	**2271**
(3*Z*,13*E*)-18:OH	2065	2641	2266
(3*Z*,13Z)-18:OH	2065	2662	2279
(2*E*,13*E*)-18:OH	2074	2655	2287
(2*E*,13*Z*)-18:OH	2076	2677	2300
(2*Z*,13*E*)-18:OH	2079	2673	2288
(2*Z*,13*Z*)-18:OH	2079	2696	2303
(3*E*,13*E*)-18:Ac	2180	2591	2461
(3*E*,13*Z*)-18:Ac	2180	2610	2475
(3*Z*,13*E*)-18:Ac	2180	2602	2455
(3*Z*,13*Z*)-18:Ac	2180	2620	2470
(2*E*,13*E*)-18:Ac	2195	2621	2467
(2*E*,13*Z*)-18:Ac	2195	2641	2482
(2*Z*,13*E*)-18:Ac	2185	2604	2450
(2*Z*,13*Z*)-18:Ac	2185	2622	2466

Note: (1) *cis*-double bonds at C2 and C3 of 2,13- or 3,13-octadecadienals, respectively, rearrange during chromatography, and thus these isomers are not reported here; (2) the stationary phase of a Zebron-5 and DB-5 column is identical; (3) the temperature of injection port and flame ionisation detector was 250°C; (4) the temperature programmes on GC columns were as follows:

Zebron 5 (pheromone extract)	(30 m × 0.25 mm ID)	100°C (1 minute) – 20°C to 300°C/minute
DB-5 (effluvium)	(30 m × 0.32 mm ID)	100°C (1 minute) – 20°C to 280°C/minute
DB-23 (pheromone extract)	(30 m × 0.32 mm ID)	100°C (1 minute) – 10°C to 190°C/minute
DB-210 (pheromone extract)	(30 m × 0.32 mm ID)	100°C (1 minute) – 10°C to 220°C/minute

stirring the reaction mixture for 30 minutes at room temperature, 10 mL of hexane was added, and stirring continued for an additional 5 minutes. Silica filtration of the mixture and purification by flash column chromatography with a hexane/ether mixture (97/3) as an eluant afforded the desired aldehyde at 70% yield. (3*E*,13*E*)-18:Ald, (2*E*,13*Z*)-18:Ald, and (2*E*,13*E*)-18:Ald were synthesised following an analogous procedure.

Field experiments

Three field trapping experiments were conducted in a 3.6-ha commercial raspberry planting (cultivar Malahat) with 2-m high canes and a 0.9 × 2.5 m plant × row spacing, in Aldergrove, BC (South Alder Farms Ltd.). All experiments employed a randomised complete block design with 8–10 replicates each. White, sticky, wing traps (Wingtrap-II, Contech Enterprises, Delta, BC, Canada) were hung from the upper trellis wire at a height of 1.3 m within the plant canopy. Traps were deployed at 10-m intervals in linear arrays (blocks) within planted rows and blocks of traps were spaced 20 m apart. Each trap was baited with a grey halobutyl rubber septum (West Pharmaceutical Services, Lionville, Pennsylvania,

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United States of America) impregnated with candidate pheromone components dissolved in 100 µL of HPLC-grade hexane. In experiment 1 (1–7 September 2008), we tested the attractiveness of the two synthetic candidate pheromone components that elicited the strongest EAD responses and their structural analogue all at 100 µg loads and compared with a solvent control, as follows: (1) (3*E*,13*Z*)-18:Ald; (2) (3*E*,13*Z*)-18:OH; (3) (3*E*,13*Z*)-octadecadienyl acetate [(3*E*,13*Z*)-18:Ac]; and (4) an unbaited solvent control. In experiment 2 (8–15 September 2008), we examined the importance of the double bonds at C3 and C13 in the pheromone component (3*E*,13*Z*)-18:Ald by testing four treatments (each component at 100 µg): (1) (3*E*,13*Z*)-18:Ald; (2) (2*E*,13*Z*)-18:Ald; (3) (3*E*,13*Z*)-18:Ald *plus* (2*E*,13*Z*)-18:Ald; and (4) an unbaited solvent control. In experiment 3 (18–25 August 2009), we tested whether other EAD-active pheromone gland components affect the attractiveness of the pheromone component (3*E*,13*Z*)-18:Ald. Specifically, we tested five treatments: (1) 100 µg (3*E*,13*Z*)-18:Ald; (2) 100 µg (3*E*,13*Z*)-18:Ald *plus* 5 µg (3*E*,13*Z*)-18:OH; (3) 100 µg (3*E*,13*Z*)-18:Ald *plus* 5 µg (3*E*,13*Z*)-18:Ac; (4) 100 µg (3*E*,13*Z*)-18:Ald *plus* 5 µg (3*E*,13*Z*)-18:OH *plus* 5 µg (3*E*,13*Z*)-18:Ac; and (5) an unbaited solvent control.

Diel periodicity of pheromone response

In 2010 we conducted an experiment to determine the diurnal periodicity of male pheromone responses in relation to temperature. On each of three test days, 10 sticky wing traps were baited with 100 µg of (3*E*,13*Z*)-18:Ald loaded on grey septa and deployed as before at 10-m intervals within a single row of raspberries transecting an infested field. Moth catches and temperature were recorded hourly from 07:00 to 20:00 hours Pacific Daylight Time (PDT) on 30 August, 3 September, and 13 September, in Aldergrove, BC. Temperatures were measured on site with a thermometer (Fisherbrand Red-Spirit No-Roll Laboratory Thermometer with range: −20°C to +110°C) hung at mid-canopy height within the same row of plants as the traps. The thermometer was shielded from direct sunlight at all times. Moth catches were counted and removed from all traps each hour. On each test day all traps were deployed with new lures and sticky bottoms in a different raspberry field on the same farm.

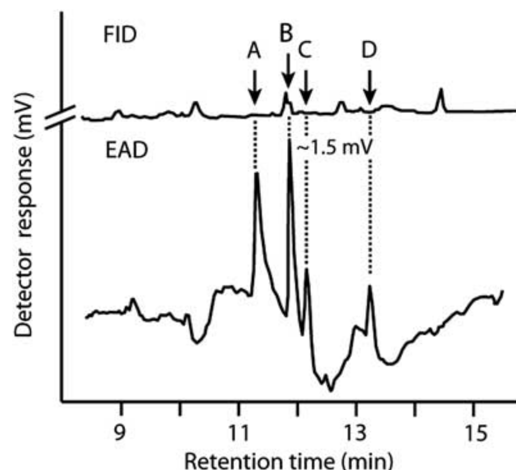
Statistical analyses

In experiments 1–3, trap-catch data were subjected to a two-way randomised block analysis of variance (ANOVA) and mean catches for each treatment were separated using the Student–Newman–Keuls' multiple comparison procedure with the experiment-wise error rate set at $\alpha = 0.05$ (Zar 1984). All analyses were performed using Sigmastat® 3.0.1 (SYSTAT Software Inc., San Jose, California, United States of America).

Results and discussion

Four components (A, B, C, and D in Fig. 1) elicited responses from male antennae in GC-EAD analyses of pheromone gland extracts from females. Component B consistently elicited the strongest response and was hypothesised to be (3*E*,13*Z*)-18:OH based on its relative retention index (corresponding with an antennal response) on Zebron-5, DB-23, and DB-210 columns, which matched the index of authentic (3*E*,13*Z*)-18:OH on

Fig. 1. Representative recording of responses of a gas chromatograph flame ionisation detector (FID) and an electroantennographic detector (EAD: male *Pennisetia marginata* antenna) to one female equivalent of pheromone gland extract from female *Pennisetia marginata*. Components that elicited antennal responses were identified as (3*E*,13*Z*)-octadecadienal (A), (3*E*,13*Z*)-octadecadienol (B), and (2*E*,13*Z*)-octadecadienal (C) on a Zebron-5 column (see the section "Materials and methods"). Component D was tentatively identified as (3*E*,13*Z*)-octadecadienyl acetate. Chromatography as reported in Table 1.



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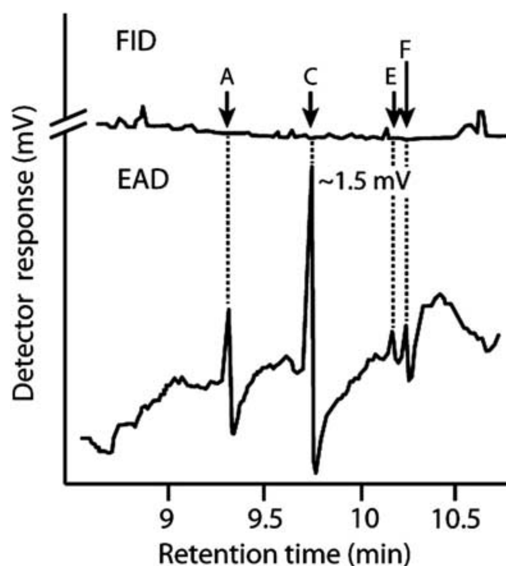
these different columns (Table 1), supporting the structural assignment. Component A had retention indices, and inter-column differentials of retention indices, that supported an aldehyde rather than an alcohol or acetate functionality. Accordingly, component A was hypothesised to be (3*E*,13*Z*)-18:Ald. This aldehyde is heat labile (Islam *et al.* 2007), and readily rearranges to (2*E*,13*Z*)-18:Ald, which elutes later due to the conjugated 2*E* double bond. Component C was therefore hypothesised to be (2*E*,13*Z*)-18:Ald. The assignments of A and C were supported by identical GC retention times of synthetic (3*E*,13*Z*)-18:Ald and (2*E*,13*Z*)-18:Ald, respectively. When the former was injected in the GC, it yielded (2*E*,13*Z*)-18:Ald as an abundant rearrangement product, as supported by GC-MS of synthetic standards. Synthetic (3*E*,13*Z*)-18:Ald, (3*E*,13*Z*)-18:OH, and (2*E*,13*Z*)-18:Ald had retention indices on each of three GC columns that were identical to those appearing as EAD-active components A, B, and C on the same columns, respectively; all three synthetic components elicited strong responses from male antennae. These results strongly supported structural assignments even though all components remained below detection threshold of the mass spectrometer. Compound D was tentatively identified as (3*E*,13*Z*)-18:Ac.

GC-EAD analyses of effluvia from calling females revealed four components (Fig. 2), including (3*E*,13*Z*)-18:Ald (A) and (2*E*,13*Z*)-18:Ald (C). (3*E*,13*Z*)-18:OH, component B in Figure 1, was not detectable in the effluvia (Fig. 2). Alcohols commonly serve as the biosynthetic precursor to aldehyde pheromones in several Lepidoptera and these are often stored in the pheromone gland but not emitted by calling females (Weatherston and Maclean 1974; Teal *et al.* 1984). Components E and F in female effluvia remain unidentified (Fig. 2).

In field experiment 1, (3*E*,13*Z*)-18:Ald was the only candidate pheromone component that attracted male RCBs (Table 2). Although previous studies have reported a few catches of RCB with (3*E*,13*Z*)-18:OH (Solomon *et al.* 1982; Brown and Snow 1985), we did not capture a single moth in traps baited with this alcohol or the acetate analogue (Table 2). Failure of any lure except (3*E*,13*Z*)-18:Ald to attract male moths negated the need for statistical analysis in experiment 1, and clearly indicated that the RCB sex pheromone has an aldehyde functionality, which is rare among sesiid moths (Francke *et al.* 2004). Results of experiment 2 revealed the importance of the double bonds at C3 and C13 in (3*E*,13*Z*)-18:Ald because (2*E*,13*Z*)-18:Ald proved to be unattractive (Table 2). Furthermore, (2*E*,13*Z*)-18:Ald appears to act as a pheromone antagonist because a 1:1 blend of (3*E*,13*Z*)-18:Ald and (2*E*,13*Z*)-18:Ald was less attractive than (3*E*,13*Z*)-18:Ald alone (Table 2).

Fig. 2. Representative recording of responses of a gas chromatograph flame ionisation detector (FID) and an electroantennographic detector (EAD: male *Pennisetia marginata* antenna) to 40 female hour equivalents (FHEs) of pheromone emission (1 FHE=amount of pheromone emitted by one female during 1 hour). Components A and C were identified as (3*E*,13*Z*)-octadecadienal and (2*E*,13*Z*)-octadecadienal, respectively. Components E and F remain unknown. Note that the temperature programme (see Table 1) differed from that of Figure 1.



In experiment 3, we tested the hypothesis that the unattractive, but EAD-active, (3*E*,13*Z*)-18:OH and (3*E*,3*Z*)-18:Ac might be synergists of (3*E*,13*Z*)-18:Ald when combined in two- or three-component blends. In both cases, the addition of as little as 5% of either of these compounds in binary blends with (3*E*,13*Z*)-18:Ald reduced blend attractiveness (Table 2). A ternary blend of all three compounds was also less attractive than (3*E*,13*Z*)-18:Ald alone (Table 2). Results of pheromone analyses and field trapping experiments

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Table 2. Results of field trapping experiments with candidate sex pheromone components and blends (μg per lure) detected in extracts of pheromone glands and effluvia of female *Pennisetia marginata*.

Experiment no.	(3*E*,13*Z*)-18:Ald	(3*E*,13*Z*)-18:OH	(3*E*,13*Z*)-18:Ac	(2*E*,13*Z*)-18:Ald	Trap captures (mean ± SE)*
1	100	–	–	–	16.4 ± 3.1
	–	100	–	–	0
	–	–	100	–	0
	–	–	–	–	0
2	100	–	–	–	13.0 ± 2.7a
	–	–	–	100	0
	100	–	–	100	5.1 ± 3.1b
	–	–	–	–	0
3	100	–	–	–	9.8 ± 3.5a
	100	5	–	–	4.4 ± 1.4b
	100	–	5	–	1.8 ± 0.9b
	100	5	5	–	0.2 ± 0.2b
	–	–	–	–	0

***Note:** Treatments with zero moth catches and no variance were excluded from statistical analyses. Within a column and experiment, means for a given treatment followed by different letters are significantly different (Student–Neuman–Keuls' test, $\alpha = 0.05$) following significant ANOVA ($P \leq 0.05$).

indicate that (3*E*,13*Z*)-18:Ald is the major and perhaps only pheromone component in *P. marginata*. Single-component pheromones are unusual in Lepidoptera, and even *Sesia apiformis* (Clerck) (Lepidoptera: Sesiidae), the only sesiid moth known to use an aldehyde pheromone component (El-Sayed 2009), only responds to (2*E*,13*Z*)-18:Ald when combined with (3*E*,13*Z*)-18:OH (Francke *et al*. 2004). To the best of our knowledge, *P. marginata* is the only sesiid species currently known to use (3*E*,13*Z*)-18:Ald as a pheromone component, and the only sesiid species known to respond to an aldehyde alone. Additional components may be unnecessary in this communication system because (3*E*,13*Z*)-18:Ald appears to be an uncommon pheromone signal (El-Sayed 2009).

Among those *Pennisetia* spp. studied to date, most have been attracted to (3*E*,13*Z*)-18:Ac and (3*E*,13*Z*)-18:OH alone, or in combinations (Sharp and Eichlin 1979; Solomon *et al*. 1982; Brown and Snow 1985; Priesner *et al*. 1986; Szöcs *et al*. 1989). For example, trapping studies showed that the European raspberry clearwing moth, *Pennisetia hylaeiformis* (Laspeyres), is maximally attracted to a 1:1 binary blend of (3*E*,13*Z*)-18:Ac and (3*E*,13*Z*)-18:OH (Priesner *et al*. 1986). These results were supported by electrophysiological studies that found two specialist receptor cells each tuned to one of these components (Priesner *et al*. 1986). Although Szöcs *et al*. (1989) reported catches of three specimens of *P. hylaeiformis* with a binary blend of (3*E*,13*Z*)-18:Ac and (3*E*,13*Z*)-18:Ald, this result should not be considered evidence of a role for the aldehyde in this pheromone system because *P. hylaeiformis* is attracted by (3*E*,13*Z*)-18:Ac on its own (Priesner *et al*. 1986). The degree to which the pheromones used by *P. marginata* and *P. hylaeiformis* appear to differ is intriguing given their close relatedness (Eichlin 1986). Eichlin and Duckworth (1988) stated that the two species share a common host range and bear a superficial resemblance, with minor differences in male genitalia being the only taxonomic difference. Geographic separation of these species provides differences in regional competition that may have caused a divergence of their pheromone blends. The evolution of multi-component pheromones is often driven by sympatric species competing for particular communication channels. Whereas *P. hylaeiformis* must compete with several sympatric *Pennisetia* species in Europe (Spatenka *et al*. 1999), *P. marginata* is the only representative of this genus in areas north of Mexico (Eichlin and

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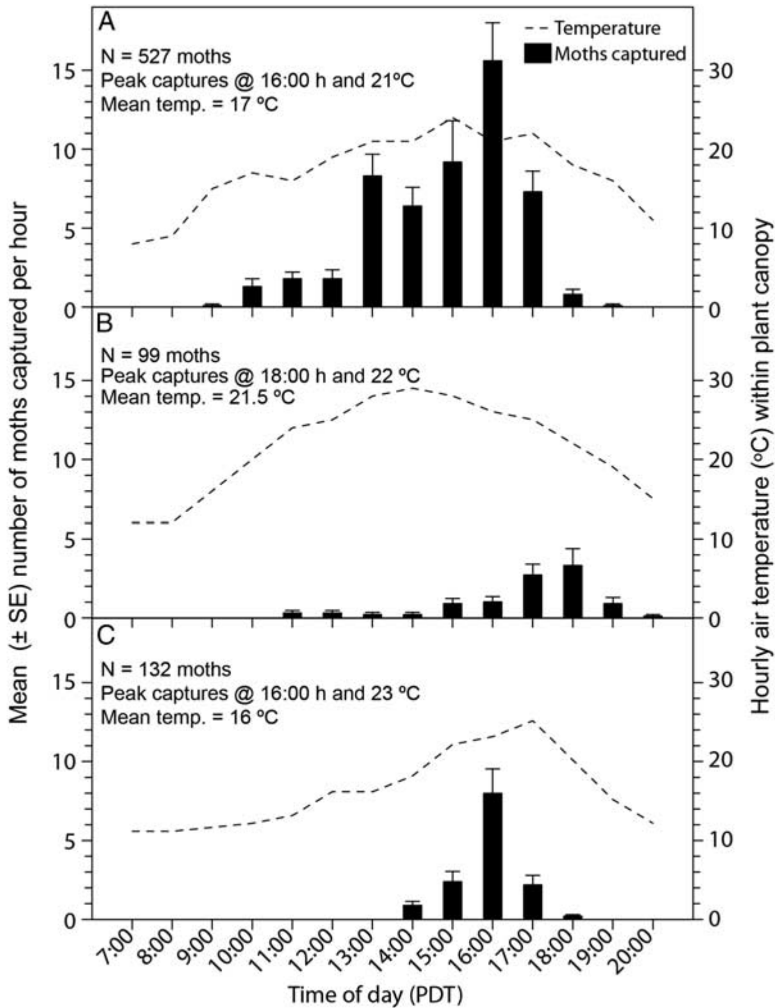
Fig. 3. Mean (± SE) hourly captures of male *Pennisetia marginata* in 10 sticky wing traps each baited with a grey rubber septum loaded with 100 µg of (3*E*,13*Z*)-octadecadienal on 30 August (A), 3 September (B), and 13 September (C) in relation to air temperature within the canopy of three different raspberry fields on the same farm at Aldergrove, BC, in 2010. PDT is Pacific Daylight Time.



Duckworth 1988). We note, however, that the sex-attractant blend used by *P. hylaeiformis* is the same blend that maximally attracts the strawberry crown moth, *Synanthedon bibionipennis* (Boisduval) (Lepidoptera: Sesiidae) (Nielsen *et al.* 1978). *Synanthedon bibionipennis* is a North American species with which *P. hylaeiformis* has likely had no contact, but the distribution and seasonal phenology of the former species partially overlaps that of *P. marginata* (Eichlin and Duckworth 1988). In the Pacific Northwest, *S. bibionipennis* sometimes infests *Rubus* species (Mote *et al.* 1929; Bruck *et al.* 2008). It seems entirely possible that *P. marginata* makes no use of (3*E*,13*Z*)-18:Ac or (3*E*,13*Z*)-18:OH in its pheromone communication system (Figs. 1, 2) because of competition for this pheromone channel. However, a common ancestry with *P. hylaeiformis* may have provided *P. marginata* with an olfactory system capable of detecting these components, thus providing a means for behavioural discrimination between conspecifics and sympatric competitors like *S. bibionipennis*. Given that there are two North American records of *P. marginata* responding to (3*E*,13*Z*)-18:OH (Solomon *et al.* 1982; Brown and Snow 1985),

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it would be interesting to examine the pheromone responses of different populations of *P. marginata* in relation to its overlapping distribution with *S. bibionipennis* and their respective host plants.

Diurnal flight activity of male *P. marginata*, as reflected by pheromone trap catches, occurred throughout the day (09:00–20:00 hours PDT), but had an obvious peak at 16:00 hours on days 1 and 3 (Fig. 3A, 3C). This activity pattern is somewhat different from that of *P. hylaeiformis*, which exhibited a peak of activity before noon and another in late afternoon (Priesner *et al*. 1986). Part of the within-day increase in catches of RCB with increasing temperatures could be related to an expected increase in the release of pheromone from rubber septa lures as air temperatures increased. However, on the second, and hottest of our three test days, peak captures of *P. marginata* were shifted 2 hours later into early evening (Fig. 3B) when air temperature was 22°C and below the daily maximum, but similar to temperatures at peak captures on the other days. This suggests that peak pheromone response, and by inference mating, likely occurs in mid to late afternoon and is modified daily by an interaction between light intensity and temperature, as it is in other moths (Castrovillo and Cardé 1979). We did not monitor captures of *P. marginata* between 20:00 and 07:00 hours PDT, but the patterns observed suggest that like *P. hylaeiformis* (Priesner *et al*. 1986), *P. marginata* is strictly a day-flying moth.

We conclude that any development of monitoring or mass-trapping techniques for management of *P. marginata* in BC, and probably other parts of North America, could use (3*E*,13*Z*)-18:Ald. In our experience, however, this will be challenging because (3*E*,13*Z*)-18:Ald is difficult to synthesise in large quantity and it appears to lose its activity quite quickly when loaded on rubber septa lures and deployed in the field. We did not attempt to use anti-oxidants or ultraviolet (UV) protectants to stabilise (3*E*,13*Z*)-18:Ald on rubber septa because the nature of its instability is not exclusively related to oxidation or UV degradation. Alternatively, it may be worth examining the attraction of formate analogues of this aldehyde pheromone (Todd *et al*. 1992) and/or the use of pheromone antagonists (Table 2) to manage this pest (Leskey *et al*. 2009).

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