The Role of Sound and Pheromone in the Sexual Communication of the Raspberry Crown Borer (Lepidoptera: Sesiidae): Implications for Monitoring and Management

by

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Abstract

The raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is a pest of caneberries in the genus *Rubus*. Understanding its sexual communication may lead to the development of sound- and/or semiochemical-based monitoring and management tools. Newly-eclosed females wing fan. By testing playback of recorded sounds in pheromone-baited traps, I revealed that wing fanning is not involved in long-range sexual communication. Trap and pheromone lure attributes affected capture of males. Most notably, freshly prepared pheromone lures were significantly more attractive than lures aged at room temperature for 2-10 days. Mass trapping with high-dose (100µg) pheromone lures reduced capture of males in traps baited with low-dose (10µg) pheromone lures by 93% in 2010 and by 75% in 2011. Fifty percent male flight in raspberry occurred between 838 and 892 degree days >10°C from January 1. A trap-catch threshold of 19 moths captured between 652 and 842 degree days consistently indicated egg-infested canes.

Keywords: clearwing moth; trap and lure attributes; pheromone antagonist; monitoring; mass trapping

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1. Introduction

1.1. Distribution

The raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is native to Canada and the USA. It is the only species in the genus *Pennisetia* in North America and the only genus in the tribe Pennisetiini (Duckworth and Eichlin 1977). Its distribution extends from British Columbia (BC) to Nova Scotia in Canada, and south through Washington, Oregon, California, New Mexico, Ohio, Illinois, Missouri, Kansas, Nebraska, Mississippi and Florida (Essig 1946, Putnam 1955, Raine 1962, Solomon et al. 1982, Brown and Snow 1985). *Pennisetia marginata* was previously named *Bembecia marginata* (Edwards 1881) and has been referred to as the raspberry root borer and blackberry clearwing borer (Raine and Andison 1960, Brown and Snow 1985). Phylogenetically, *P. marginata* is more divergent than other sesiid genera in North America and may be a more ancient species (McKern et al. 2008).

1.2. Biology

The raspberry crown borer has a narrow host range of *Rubus* species, including cultivated red raspberry, *R. ideaus* (L.), cultivated blackberry, *R. fruticosus* (L.), black raspberry, *R. occidentalis* (L.), salmonberry, *R. spectabilis* (Pursh), thimbleberry, *R. parvioflorus* (Nutt), cutleaf blackberry, *R. lacineatus* (Willd), and himalayan blackberry¸ *R. discolor* (Weihe & Nees). It has a two-year life cycle in northern latitudes such as British Columbia, Washington, Oregon and Michigan, but a one-year life cycle in Arkansas (Lawrence 1904, Breakey 1963, Raine 1962, McKern et al. 2007). Eggs are laid singly on new leaves near the top of canes in August and September, usually no more than two or three eggs per plant. Eggs hatch following precipitation events from early September to late November. Neonate larvae do not feed on the leaf but instead crawl along the leaf margin, down the petiole and down the cane to the base of plants where they construct hibernacula just below the soil surface. The larvae feed within hibernacula during the first winter, and then tunnel directly from hibernacula into the cambia of the plants the following spring (Raine 1962). In the second winter, the larvae tunnel 2.5-13 cm upward inside the base of the canes (Lawrence 1904) before pupating in July. Moths emerge from the base of canes over four weeks from mid-August to mid-September and fly during the day. Both males and females exhibit Batesian mimicry and are often mistaken for wasps due to their yellow and black colouration and hovering flight patterns. This species is protandrous, with males emerging a few days before females. The sex ratio within populations of *P. marginata* is 1:1. Before mating, newly-eclosed females wing fan, which produces a distinct humming sound. Female *P. marginata* mate on the same day that they emerge and usually mate only once. Mating occurs in the plant canopy with males and females side by side on the upper surface of a leaf. Females usually oviposit the day after they have mated (Raine 1962).

1.3. Assessment of Pest Risk

Larval feeding interferes with nutrient uptake and reduces cane vigour. Infestations of *P. marginata* can lead to a 30% loss in plants (Lovett 1921). Wounding to the crown can facilitate the entry of pathogens, which contributes to further plant decline (Lawrence 1904, Schaefers 1974). Populations of *P. marginata* tend to build up slowly (Raine 1962). The action threshold for treating fields with insecticides is 5% of canes damaged (British Columbia Ministry of Agriculture and Lands 2009). However, infestations in commercial raspberry fields are rarely detected until populations exceed this threshold as the larvae are cryptic, making it difficult to monitor populations. When infestations are large, larvae are often detected while pruning in the spring. Damaged canes break away from the crown when tied to the trellis wires (Raine and Andison 1960). In recent years, *P. marginata* has caused severe cane damage on some raspberry farms in Langley and Abbotsford, BC (C.T. personal observation).

1.4. Previous Research

As a sporadic and difficult to monitor pest, research on *P. marginata* has been limited to management of populations with biological and insecticidal controls. The only documented cultural control is to remove and burn infested canes (Breakey 1963). Although *P. marginata* is a native species with co-evolved predators and parasitoids, few natural enemies have been observed. A parasitic wasp in the family Eulophidae was found in field-collected eggs (J.A.M. unpublished data in McKern et al. 2007), and pupae have been parasitized by the ichneumonid wasps *Pterocormus chasmadops* (Heinrich) (Pavuk and Williams 1988) and *Barichneumon* sp. (Raine 1962). Drenches with the entomopathogenic nematodes *Steinernema feltiae, S. carpocapsae*, and *Heterorhabditis bacteriophora* have shown promise in reducing numbers of larvae (Capinera et al. 1986, McKern et al. 2007). Beginning in the 1950s, broad spectrum insecticides such as dichlorodiphenyltrichloroethane (DDT), parathion and diazinon were tested and applied as basal sprays or drenches to target first-year *P. marginata* larvae (Wallace 1956, Raine and Andison 1960, Schaefers 1974). Until 2005, the organophosphates diazinon and azinphos-methyl were the industry standards for control of *P. marginata* in Canada and the USA, respectively (McKern et al. 2007, British Columbia Ministry of Agriculture and Lands 2009). These products are currently being phased out and more selective insecticides such as Altacor (35% chlorantraniliprole) (DuPont) are being registered for use against *P. marginata* in Canada (British Columbia Ministry of Agriculture and Lands 2009).

1.5. Research Challenge

Without tools to easily monitor *P. marginata*, commercial growers often apply insecticides on an annual preventative basis to protect their fields (C.T. personal observation). In the Fraser Valley, British Columbia, applications of organophosphates over the unconfined Abbotsford aquifer put the quality of groundwater at risk. As newer, more expensive and more selective insecticides replace diazinon in Canada, accurate timing of application will be critical to ensure efficacy against the most susceptible life stages. A pheromone-based monitoring and management tool for the raspberry crown borer would be economically and environmentally beneficial.

1.6. Sexual Communication

Moths in the family Sesiidae are known to use female-produced sex pheromones in their long-distance sexual communication (Tumlinson et al. 1974, Yonce et al. 1974, Nielsen et al. 1975, Karandinos et al. 1977, Priesner et al. 1986, Zhang et al. 2005, Frank et al. 2011). Many sesiid pheromones are 2,13- and 3,13-octadecadien-1-yl acetates and the corresponding alcohols (Solomon et al. 1982, El-Sayed 2011). Although sporadic catches of male *P. marginata* have been reported in traps baited with (*E*,*Z*)-3,13-octadecadien-1-ol (Solomon et al. 1982, Brown and Snow 1985), pheromone-based trapping of this species has not been reliable. The sex pheromone of *P. marginata* was recently identified as (*E*,*Z*)-3,13-octadecadienal (Judd et al., in press). With the identification of this pheromone component, the sexual communication of *P. marginata* can be examined. Before mating, newly-eclosed female *P. marginata* wing fan producing a distinct humming sound (Raine 1962). Sound signals in combination with pheromones play a role in the sexual communication systems of many Lepidoptera, including the Arctiidae (Conner 1987; Krasnoff and Yager 1988), Crambidae (Nakano et al. 2008; Orci and Szöcs 2009), Geometridae (Nakano et al. 2009), Noctuidae (Heller and Achmann 1993; Alcock and Bailey 1995; Rowland et al. 2011) and Pyralidae (Bennett et al. 1991; Heller and Krahe 1994). The wing fanning sound produced by female raspberry crown borers could enable male moths to better locate a calling female within the plant canopy.

1.7. Outline of Research Chapters

My thesis is organized into five chapters. Following this introductory chapter there are three research chapters and a concluding chapter that summarizes my research results and directions for future research. The dissertation is organized as an article-style thesis. Research chapters closely resemble manuscripts that have or will be submitted for publishing. Each research chapter is written in the preferred style of the journal that it has or will be submitted to and includes an abstract, introduction, methodology, results, discussion and a list of literature cited. I have presented tables and figures at the end of each chapter.

The first research chapter of my thesis explores whether sounds produced by wing-fanning females function as signals in the sexual communication system of *P. marginata.* Wing-fanning events of both female and male moths were recorded in the laboratory and played back in the field. The objectives of this chapter were:

1. To record and characterize the wing fanning sounds of female and male *P. marginata*;
2. To determine whether the wing fanning sound produced by females enhances attraction of males to pheromone sources; and
3. To conduct field observations of emerging wild females for evidence that sounds produced by wing fanning have a signal function.

With no apparent role of sound in the sexual communication of this species, the focus of my research shifted to evaluate the sex pheromone as a management tool. In my second research chapter I carried out experiments to evaluate the effect of trap and lure attributes on captures of male *P. marginata*. Capture of other Sesiid species in pheromone-baited traps is affected by trap colour, trap type, trap placement in the plant canopy, pheromone concentration and pheromone longevity (Yonce et al. 1976, Rocchini et al. 2003, Suckling et al. 2005, Brown et al. 2006, Karalius and Buda 2007, Weihman and Liburd 2007, Roubos and Liburd 2008, Cottrell et al. 2010). Two potential pheromone-based management strategies for Lepidoptera with unstable pheromone compounds are mass trapping and mating disruption with antagonist pheromones. Pheromone-based mass trapping involves placement of numerous pheromone-baited traps throughout an area to capture a large proportion of male moths before they mate with females. This strategy can reduce economic damage to the crop if the density of traps and lure dose is sufficient to remove a significant number of insects (El-Sayed et al. 2006). Pheromone antagonists, such as the pheromones of closely related species, can be used to disorient or repel mate-seeking males from a field (Priesner and Witzgall 1984, Rumbo et al. 1993, Witzgall et al. 1993, Miller et al. 2006). The objectives of my second research chapter were:

1. To test the effects of trap type, colour and placement as well as lure dose and lure age on captures of male *P. marginata*;

2. To assess the potential of pheromone antagonists to interfere with pheromonal communication of *P. marginata*; and
3. To examine the potential of mass trapping to interfere with mate-seeking behaviour of male *P. marginata.*

My third research chapter uses the sex pheromone to gather information on seasonal flight phenology and to facilitate the development of a pheromone-based monitoring system. Pheromone trapping enables the detection of pest populations even at low levels. Early detection of *P. marginata* at sub outbreak levels may help to extend the life of raspberry and blackberry plantings, as insecticide applications could be targeted to infested field areas. Pheromone-based monitoring has been successfully used to survey species distribution and to predict the timing of egg hatch and insecticide application for other Lepidopteran pests (Sanders 1988, Hoffman et al. 1992, Reddy and Guerrero 2001, Bazok et al. 2009). While pheromone trap captures of some species have been positively correlated with population density of the subsequent larval generation, this correlation is often poor when populations are low (Sanders 1983, 1988). Outbreaks of *P. marginata* have occurred sporadically in isolated fields in the Fraser Valley, despite the high density of raspberry plantings. For Fraser Valley raspberry growers, a pheromone-based monitoring tool that provides information on the presence or absence of *P. marginata* eggs would be helpful in making spray decisions.

Degree-day models that use field temperatures to predict adult eclosion and flight have been developed for other Lepidoptera (Riedl et al. 1976, Potter and Timmons 1983, McBrien and Judd 1998, Evenden and Judd 1999, Doerr et al. 2005). Relating captures of male RCB in pheromone-baited traps with temperature sums, may enable the development of predictive models for RCB eclosion, peak flight, oviposition and egg hatch. Temperature-based knowledge of the seasonal moth flight is a valuable first step in developing these predictive capabilities that may enable pest managers to time pheromone-based monitoring activities. The objectives of my third research chapter were:

1. To survey the distribution of *P. marginata* in commercial raspberry fields and unmanaged blackberry patches in the Fraser Valley;

2. To determine the peak seasonal flight of *P. marginata* in the Fraser Valley, and to relate it to accumulated degree days; and

3. To determine a baseline pheromone trap catch threshold that corresponds with egg detection in the fields.

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2. Does wing fanning play a role in the long-range sexual communication of the raspberry crown borer?

2.1. Abstract

It has been reported that newly-eclosed female raspberry crown borers, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), wing fan prior to mating. We tested the hypothesis that wing fanning plays a role in long-range mate attraction. Wing fanning events of both female and male moths were recorded within 24 h of their emergence in the laboratory, using sonic and ultrasonic microphones. The mean fundamental frequency of the females' wing fanning sound (92.9 ± 1.5 Hz) was significantly ($P < 0.05$) lower than that of the males' (112.0 ± 1.9 Hz). There was no evidence of an ultrasonic frequency component in either sex. In field experiments, traps baited with synthetic pheromone and equipped with speakers playing back either recorded female wing fanning sound or silence attracted similar numbers of male moths, indicating that wing fanning sound is not part of the long-range sexual communication system in *P. marginata,* however it may serve another as yet unknown function.

2.2. Introduction

Sound signals play a role in the sexual communication system of many Lepidoptera, including species in the Arctiidae (Conner 1987; Krasnoff and Yager 1988), Crambidae (Nakano *et al.* 2008; Orci and Szöcs 2009), Geometridae (Nakano *et al.* 2009), Noctuidae (Heller and Achmann 1993; Alcock and Bailey 1995; Rowland *et al.* 2011) and Pyralidae (Bennett *et al.* 1991; Heller and Krahe 1994). Typically, the males signal and females respond (Conner 1999), but females may also produce sound signals. For example, females of the banded woolly bear, *Pyrrharctia isabella* (Smith) (Lepidoptera: Arctiidae), and the Eurasian nun moth, *Lymantria monarcha* (L.) (Lepidoptera: Noctuidae), produce sound that attracts males (Krasnoff and Yager 1988; Rowland *et al.* 2011). In other species, males and females exchange acoustic signals. Sexually dimorphic sound signals are used in the courtship of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), and the polka-dot wasp moth, *Syntomeida epilais* (Walker) (Lepidoptera: Arctiidae) (Spangler 1985; Sanderford and Conner 1990, 1995).

Ultrasonic (>20 kHz) frequency components prevail in sound signals of nocturnal moths, likely because their ears evolved primarily to detect ultrasonic foraging cues of bats (Roeder 1965, 1966). The ability to detect and evade bats is considered the most significant evolutionary factor in the pre-adaptation of moths to evolve intraspecific acoustic communication systems (Conner 1999). Sonic, low-frequency sound signals (< 20 kHz) are less common but have been reported in the webbing clothes moth, *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae) (Takács *et al.* 2003), greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Spangler 1985, 1987), and European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Orci and Szöcs 2009). Low-frequency sounds are often produced by wing fanning or wing fluttering (Spangler 1985, 1987; Takács *et al.* 2003; Orci and Szöcs 2009).

Acoustic communication is not restricted to nocturnal moths. In the diurnal gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Noctuidae), males flying toward a pheromone-emitting female produce wing-fanning sound which induces the female to move which makes her visually conspicuous (E. Rowland, Simon Fraser University, personal communication). In the Australian whistling moth, *Hecatesia exultans* Walker (Lepidoptera: Noctuidae), males mark their territory and attract females with ultrasonic sounds that they produce in the late afternoon (Alcock and Bailey 1995).

The intensity of sound signals in moths is low (<100 dB) (Fullard and Fenton 1977; Spangler 1985; Krasnoff and Yager 1988; Bennett *et al.* 1991; Nakano *et al.* 2009; Rowland *et al.* 2011) and thus may help avoid eavesdropping by predators. Low-intensity sounds are perceptible only over a few metres (Spangler 1984; Krasnoff and Yager 1988). For example, female *P. isabella* produce sounds in response to males approaching within 15 cm (Krasnoff and Yager 1988), and many noctuid, arctiid, geometrid and crambid moths use low-intensity ultrasounds just before copulation (Nakano *et al.* 2009). In this paper, we define long-range communication to occur over distances longer than 30cm.

Newly-eclosed females of the diurnal raspberry crown borer (RCB), *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), wing fan with a distinct humming sound before mating (Raine 1962). Female RCBs emit sex pheromone that attracts males (Judd et al. in press) but wing fanning sound could be part of their sexual communication system. In commercial raspberry and blackberry plantings, RCBs can be a serious pest often requiring chemical control (British Columbia Ministry of Agriculture and Lands 2009). Before developing potential pheromone-based control methods we considered it important to explore whether wing-fanning by female RCB has a signal function during sexual communication. Our objectives were to (1) conduct field observations of emerging feral females for evidence that wing fanning sound has a signal function; (2) record and characterize the wing fanning sound of female and male RCBs*;* and (3) determine whether wing fanning sound of females enhances the attraction of males to pheromone sources.

2.3. Materials and methods

2.3.1. Experimental insects

Adult insects were reared from 19-20 month-old larvae which were collected on 20 May, 3 June and 30 June 2010 from a commercial raspberry field in Aldergrove, British Columbia (49° 2'23"N, 122°27'33"W). Host raspberry cane stubs were cut open, and larvae were transferred to pieces of non-infested blackberry canes (30 × 1.5 cm) (Raine 1962), within which they developed and pupated. The severed sections of these canes were submerged in water in plastic tubs (57 × 40 × 30 cm). Sixty such canes were kept in the insectary under a 16:8 (light: dark) regime at 21°C, and an additional 50 canes were kept in a sheltered outdoor location at Simon Fraser University (SFU). On 26 July 2010, canes were transferred to 4-l buckets which were placed into mesh rearing cages (42 × 30 × 30 cm) in a sheltered outdoor location at SFU. Moths emerging from 20 July to 1 September 2010 were collected within 12 h to prevent mating and kept individually in capped plastic cups (30 mL). Their wing fanning sound was recorded within 24 h of emergence.

2.3.2. Recording and analyses of potential sound signals

Wing-fanning sounds of 12 females and eight males were recorded in a Perspex® box (Fig. 2.1) in a soundproof room under a light source of one Sylvania Daylight Deluxe F40/DX 40-watt, 121.9-cm long tube light and one Philips Plant and Aquarium F40T12 40-watt, 121.9-cm long tube light. Recordings employed an AKG CK 61-ULS condenser sonic microphone (sensitivity: 20.0 mV/Pa; frequency response: 20 Hz to 20 kHz +/- 1 dB, AKG Acoustics, Nashville, TN, USA) and a Knowles BT1759 high-performance ultrasonic microphone (sensitivity: 40 mV/Pa; frequency response: 100 Hz to 10 kHz; Knowles Acoustics, Itasca, Illinois, USA). Microphones were inserted through 2.5-cm diameter holes in a Perspex box and positioned 2-5 cm from, and on a level plane with, wing-fanning moths (Fig. 2.1). Initial recordings at a 500-kHz sampling frequency employed sonic and ultrasonic microphones concurrently to check for the presence of both sonic and ultrasonic frequency components associated with wing fanning. With no evidence for ultrasonic frequency components, subsequent recordings at a 100-kHz sampling frequency deployed only the sonic microphone. Sounds were recorded throughout each wing-fanning event which ranged from 12 to 120 s, after which background noise was recorded. The signal to noise ratio was improved by pre-amplifying sounds [SC-2040 amplifier National Instruments (NI), Austin, TX, USA] prior to digitizing with a with an NI PCI-MIO-16XE-10 data acquisition (DAQ) card. Recordings were saved to an Intel Pentium 2.54 GHz computer. The waveform, frequency, and time-frequency sound intensity (sonogram) of each recording were analyzed with Joint Time-

Frequency Analysis (JTFA) 7.11 software in LabVIEW to determine the dominant frequency (Hz) of sounds associated with male and female wing fanning. Sound files were filtered with LabVIEW to remove background noise above 120 Hz. The sound intensity was measured with a 1551-C sound level decibel meter (General Radio Company, Massachusetts, USA) at a distance of 5 cm from wing-fanning females (N=8) and males (N=8).

2.3.3. Field experiment

Attraction of males to play back of recorded female wing-fanning sounds was tested in a commercial raspberry field in Aldergrove, British Columbia (49° 2'23"N, 122°27'33"W) between 18 and 25 August, 2010. The experiment deployed paired traps (N = 15) which were suspended 2 m apart at a height of 1.3 m within the same row from the upper trellis wire in the plant canopy. Traps were made of 2-l milk cartons coated on the inside with an adhesive (Tanglefoot, Contech, Canada). Each trap was baited with a rubber septum (The West Company, Pennsylvania, USA) impregnated with synthetic pheromone [(*E*,*Z*)-3,13-octadecadienal (100 µg); Gries-laboratory] and equipped with a Panasonic WM-R57A speaker (flat frequency response: 0.15-100 kHz) attached to a battery-operated Sony CD Walkman D-EJI20 which was set to continuous play mode. By random assignment, one Walkman played back a looped female wing-fanning recording, while the other played a blank CD. The playback recording consisted of female wing-fanning sound (9 s) followed by silence (10 s). The sound intensity 5 cm from the speaker was equivalent to the sound intensity 5 cm from a wing-fanning female.

2.3.4. Field behavioural observations

Three feral females were observed for 120 min after emergence in a commercial raspberry field on 12 and 17 August, 2010. Observations were carried out from 14:30 to 19:30 h [Pacific Daylight Time (PDT)] under partly cloudy conditions. Mate-calling of one female was recorded with a Canon FS100A video camera equipped with digital zoom. In addition, two laboratory-reared females, 24 to 48 h old, were taken to the field, placed on raspberry foliage, enclosed on three sides by a mesh bag (20 × 20 cm), and observed and video recorded from 12:00 to 15:00 h PDT while wing fanning to document wild males' response to the female's behaviour.

2.4. Statistical analyses

Statistical analyses were carried out with JMP® software (Version 7, SAS Institute). The mean dominant frequency of male and female wing-fanning recordings was analyzed with a two-sample *t*-test. Captures of moths in the field experiment were analyzed with a Student's two-sample *t*-test. For both statistical tests, the alpha value was set at 0.05.

2.5. Results

2.5.1. Recording and analyses of potential sound signals

Waveform, frequency, and time-frequency sound intensity (sonogram) of male and female sound are shown in Figure 2.2. The mean (± SE) fundamental frequency of the females' wing-fanning sound (92.9 ± 1.5 Hz) was lower than that of the males' (112.0 ± 1.9 Hz) (Fig. 2.3: $t_{1,18} = 7.86$, $P < 0.0001$). Female and male sounds had a harmonic frequency of 186 Hz and 224 Hz, respectively (Fig. 2.2). The males' sound 5 cm from the microphone [Sound Pressure Level (SPL): ~58 dB (C weighted)] was quieter than the females' [(SPL: ~60 dB (C weighted)]. The lower frequency and higher sound pressure of female wing fanning was likely due to the larger size of females.

2.5.2. Field experiment

Pheromone traps with speakers playing back female wing-fanning sound recordings attracted a similar number of male moths as did traps with speakers playing silence (Fig. 2.4: $t_{1,28}$: 0.51, $P = 0.62$).

2.5.3. Field behavioural observations

Between 14:30 and 16:30 h PDT, feral females eclosed from pupae extruding from the base of raspberry canes and they immediately climbed up the canes to a height of 50-75 cm. While expanding their wings for 10-15 min, females stayed in a vertical position on the cane. The females then lowered their wings and remained motionless for an additional 30-60 min before continuously wing fanning while in a vertical position on the cane for 30-120 s. Following wing fanning, females flew a few metres within the raspberry canopy and landed on the upper surface of a leaf. Two of three females stayed on this leaf for an additional 15-25 min before they wing fanned and took another short flight within the canopy. At the end of several wing-fanning episodes, and immediately before females took flight, they released a droplet of white substance (meconium) from their ovipositor. Laboratory-reared wing-fanning females exhibited similar behaviour.

Ovipositor extension (mate-calling) was recorded for one feral female that had been observed from eclosion through episodes of wing fanning. This female called at 18:00 h PDT from a height of 110 cm above the ground and while positioned horizontally on the upper surface of a raspberry leaf but shaded within the raspberry canopy. Without wing fanning or other significant movement, this female extended her ovipositor with its pheromone gland and kept it protruded for ~ 5 s. Within 10 s of ovipositor extension, three males approached, wing fanned, and walked on nearby leaves in the plant canopy before locating the female. The first male to locate the female copulated successfully; the other males attempted to copulate but did not succeed. The mating pair remained in *copula* on the upper surface of the leaf for > 1 h.

Laboratory-reared females that wing fanned within a mesh bag in the field did not attract any males. They wing fanned intermittently, while remaining stationary for hours between wing-fanning events. Feral females that were not newly eclosed exhibited the same behaviour in the field. Laboratory-reared females did not extend their ovipositors while under observation.

2.6. Discussion

Our results show that female RCBs wing fan before mating but the low-frequency sound associated with it has no effect on long distance attraction of conspecific males. Wing fanning precedes pheromone release and does not play a role in the long-range sexual communication of RCB.

Wing fanning is likely used in preparation for flight, possibly to raise the pre-flight thoracic temperature. Wing flapping or wing vibration by the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), rapidly increases the thoracic temperature by 2 to 7.6°C per min (up to 38.8°C), depending on ambient temperature (Heinrich and Bartholemew 1971). Similarly, the duration of pre-flight wing fanning by female Asian gypsy moth, *L. dispar*, is dependent on ambient temperature, and can raise the pre-flight thoracic temperature by up to 10°C (Charlton *et al.* 1999).

Following wing fanning, RCBs often released meconium which is metabolic waste from the pupal stage. Many geometrid, notodontid and pierid moths are known to release meconium within hours of eclosion (Shapiro 1982). Release of meconium during wing fanning has been described in *M. sexta* (Reinecke *et al.* 1980) and *L. dispar* (Charlton *et al.* 1999).

The Batesian mimicry of RCBs, including their resemblance to vespid wasps and comparable hovering flight, may help reduce predation. We considered that wing fanning, which can readily be triggered by touch before mating, may reinforce wasp mimicry. However, the fundamental frequency of flight sounds of the common wasp, *Vespula vulgaris* (L.), the aerial yellowjacket, *Dolichovespula arenaria* (F.), and the German yellowjacket, *V. germanica* (F.) (all Hymenoptera: Vespidae), ranges between 152 Hz and 171 Hz (Rashed *et al.* 2009), and significantly exceeds the frequency we recorded from RCB females (92.9 ± 1.5 Hz) and males (112.0 ± 1.9 Hz). Moreover, vespid wasps under attack tend to sting or fly away rather than buzz on the spot (Rashed *et al.* 2009). We conclude that wing fanning by RCBs is not “intended” to mimic the sounds of the vespid wasps that they morphologically resemble.

Sex pheromone appears to be the primary signal in the sexual communication of RCB. With their bipectinate antennae (Engelhardt 1946), male RCB readily oriented toward pheromone-releasing females in field observations. With sound having no detectable role in the RCB communication system, the development of a non-pesticidal control strategy for RCB in commercial raspberry and blackberry fields should probably now focus on synthetic pheromone for mass trapping or disorientation of male moths.

2.7. Acknowledgements

We thank South Alder Farms for providing access to commercial raspberry fields; Melanie Hart and Adela Danci for technical advice on sound recordings, and Eloise Rowland for assistance in filtering sound files. This research was funded by an Industrial Postgraduate Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) to C.T., with funding from the BC Raspberry Industry Development Council, the Oregon Raspberry and Blackberry Commission, and the Lower Mainland Horticulture Improvement Association, and by an NSERC–Industrial Research Chair to G.G, with Contech Enterprises and Global Forest Science as industrial sponsors.

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2.9. Figures

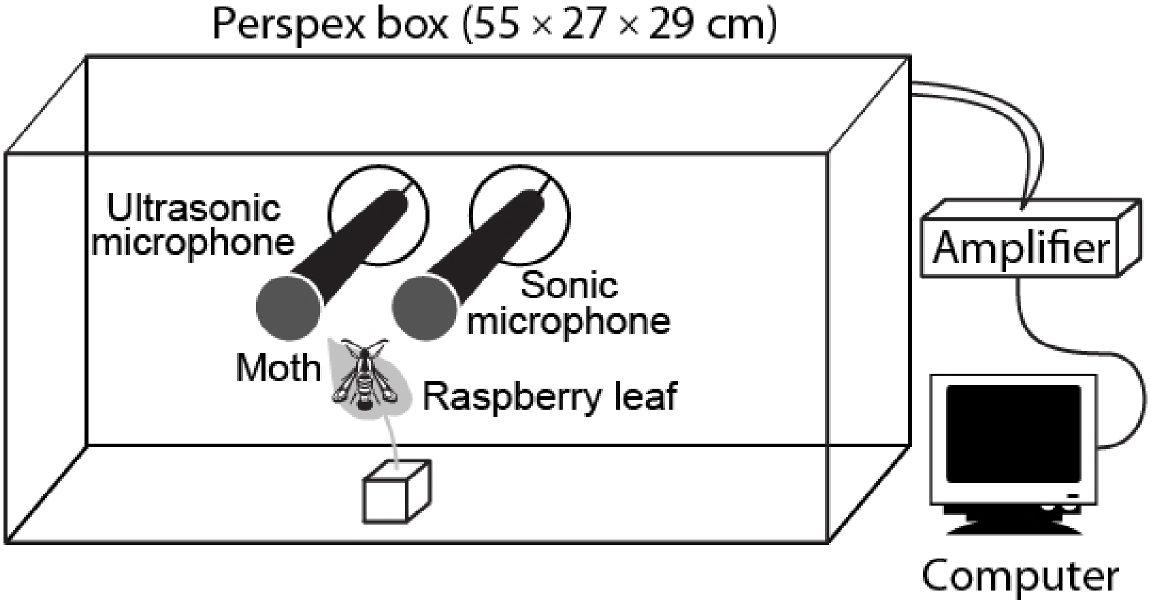

Figure 2.1. Experimental apparatus used to record sound from wing fanning male and female Pennisetia marginata.

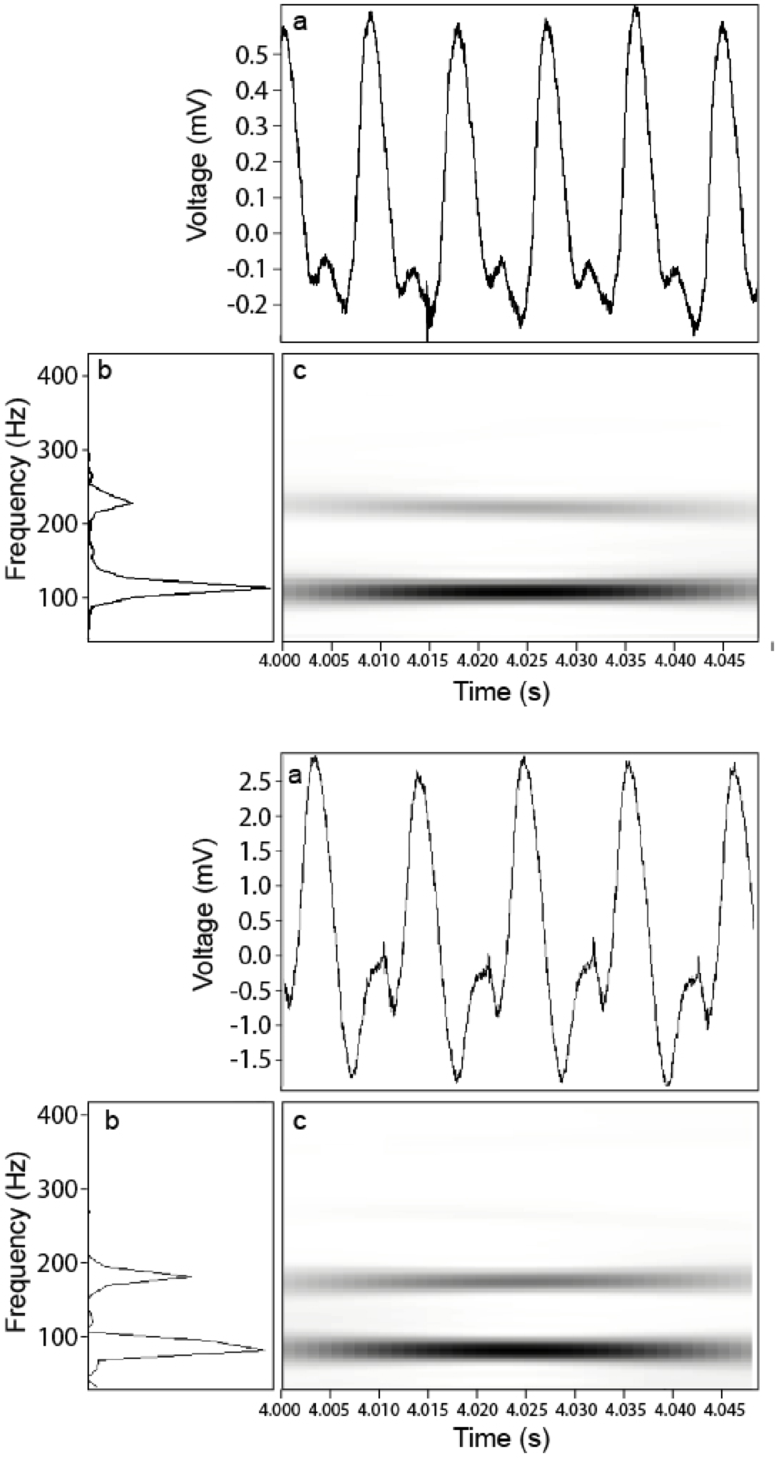

Figure 2.2. Analysis of waveform (a), frequency (b), and time-frequency sound intensity (sonogram) (c) of wing fanning sound recorded from male (top) and female (bottom) Pennisetia marginata.

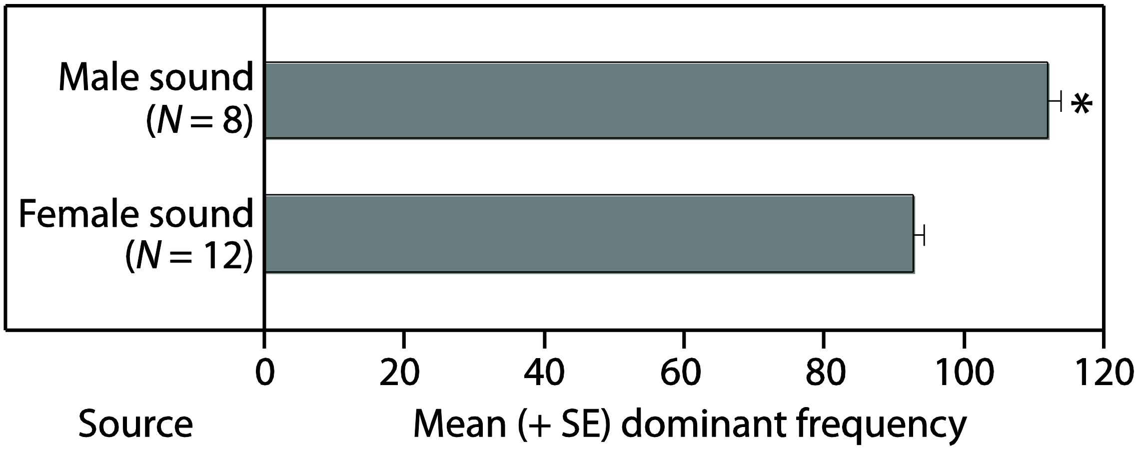

Figure 2.3. Mean (+ SE) dominant frequency of male and female Pennisetia marginata wing fanning sound. The asterisk (*) indicates a significantly higher frequency **(two sample *t-test*, df = 18, *P<0.05*).**

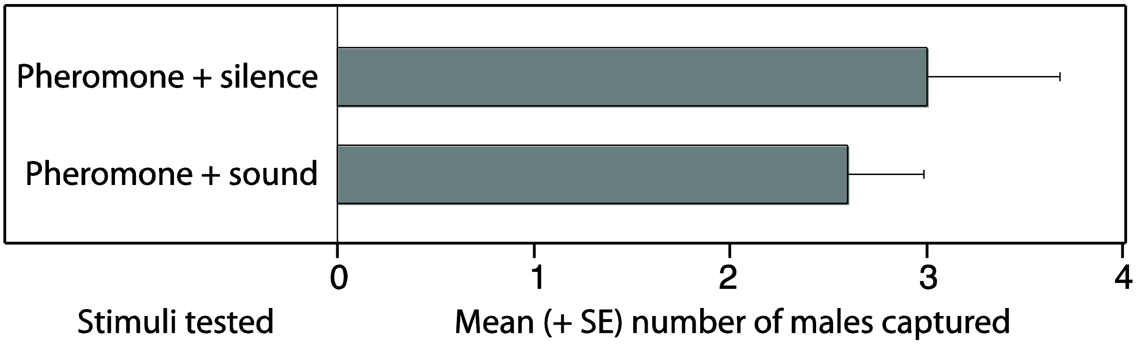

Figure 2.4. Mean (+ SE) number of male Pennisetia marginata attracted to paired traps (N=15) baited with synthetic pheromone and equipped with a speaker playing back recordings of female wing fanning sound or silence. There was no statistically significant difference between test stimuli (*two sample *t-test,* df = 28*, P>0.05).

3. Evaluation of synthetic sex pheromone for monitoring and management of raspberry crown borer[1]

3.1. Abstract

(*E*,*Z*)-3,13-Octadecadienal (*E*3,*Z*13-18:Ald) was recently identified as a sex pheromone component of the raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae). This pheromone may have utility for monitoring the seasonal abundance and flight phenology of adult *P. marginata*, and for developing control tactics, such as mass trapping or disorienting mate-seeking males. Experiments conducted in raspberry and blackberry crops tested the effect of trap and lure attributes on captures of male *P. marginata*. White wing traps and white delta traps were more effective than green delta or green bucket traps. Trap height in the crop canopy had no effect on captures of males. Increasing lure loads (10, 100 or 1000 µg) of *E*3,*Z*13-18:Ald significantly increased trap captures. Freshly prepared lures were significantly more attractive than lures aged for 2-10 days at room temperature. When sex pheromone components of three other sesiid moth species were added to *E*3,*Z*13-18:Ald, attraction of male *P. marginata* was reduced, and thus these components were identified as pheromone antagonists. Pheromone-based mass trapping with 25 wing traps per hectare reduced captures of males in traps baited with a low-dose (10 µg) pheromone lure by 93% in 2010, and by >75% in 2011.

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[1] This chapter will be submitted to the Journal of Integrated Pest Management with authors as follows: Teasdale, C., Judd, G.J.R., Gries, R., and Gries, G.

3.2. Introduction

The raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is a sporadic but severe pest of caneberries in the genus *Rubus.* It is the only species in the genus *Pennisetia* in Canada and the USA, and this is the only genus in the tribe Pennisetiini (Duckworth and Eichlin 1977). Its distribution extends from British Columbia to Nova Scotia in Canada, and south through Washington, Oregon, California, New Mexico, Ohio, Illinois, Missouri, Kansas, Nebraska, Mississippi and Florida (Essig 1946, Putnam 1955, Raine 1962, Solomon et al. 1982, Brown and Snow 1985). *Pennisetia marginata* has a two-year life cycle in northern latitudes such as British Columbia, Washington, Oregon and Michigan, but a one-year life cycle in Arkansas (Lawrence 1904, Breakey 1963, Raine 1962, McKern et al. 2007). Eggs are laid singly on new leaves near the top of canes. Neonate larvae immediately crawl to the base of plants where they construct hibernacula just below the soil surface. The larva feeds within a hibernaculum during the first winter, and then tunnels directly from the hibernaculum into the cambium of the plant the following spring (Raine 1962). In the second winter, the larva tunnels 2.5-13 cm upward inside the base of the canes (Lawrence 1904) before pupating in July. Larval feeding reduces cane vigour and yield (Schaefers 1974). Infestations are difficult to detect due to the protected and hidden location of the feeding larvae. Moths emerge from the base of the canes over four weeks from mid-August to mid-September, and fly during the day (Raine 1962). The sex pheromone of female raspberry crown borers was recently identified (Judd et al. in press), and synthetic pheromone may offer new opportunities to describe the seasonal life cycle in more detail and to monitor seasonal abundance or potentially manage adult populations of *P. marginata*.

Pheromone-based trapping enables the detection of insect populations even at low densities. Finding a suitable trap and bait is an important step towards the development of pheromone-based trapping. Trap attributes including type, colour and placement in the plant canopy, as well as pheromone load and longevity, affect captures of other sesiid moths in pheromone-baited traps (Yonce et al. 1976, Rocchini et al. 2003, Suckling et al. 2005, Brown et al. 2006, Karalius and Buda 2007, Weihman and Liburd 2007, Roubos and Liburd 2008, Cottrell et al. 2010).

Trap colour has been shown to influence captures of male sesiid moths in pheromone-baited traps. For example, males of the currant borer, *Synanthedon tipuliformis* (Clerck), show spectral sensitivity in the green range (λ_{max}= 530 nm) and demonstrate a preference for the spectral reflectance of a trap rather than contrast against background (Karalius and Buda 2007). Male apple clearwing moths, *S. myopaeformis* (Borkhausen)*,* orient towards the red stripe on the female's abdomen, and towards a red stripe on black traps (Stüber and Dickler 1988). Males of the grape root borer, *Vitacea polistiformis* (Harris), prefer green or yellow bucket traps over white or blue traps (Roubos and Liburd 2008), and more male grape root borers and currant borers were captured in multi-coloured (green-yellow-white) bucket traps than in all-yellow or all-green bucket traps (Suckling et al. 2005, Roubos and Liburd 2008).

Trap design can affect entry and capture of moths. The most commonly used traps for moths are delta traps, wing traps, and bucket traps. For sesiids, the optimal trap type varies by species. For example, delta traps are more effective than bucket traps for capturing males of the western poplar clearwing moth, *Paranthrene robiniae* (Hy. Edwards) (Brown et al. 2006), males of the grape root borer are captured more readily in bucket traps than in wing traps (Weihman and Liburd 2007), and males of the Douglas-fir pitch moth, *S. novaroensis* (Hy. Edwards), are captured equally well in wing- and bucket traps (Rocchini et al. 2003). The moth-retaining capacity of a trap is important for monitoring or mass trapping when sesiid populations are high. Sticky inserts in wing traps and delta traps can become saturated (Trematerra 1993), whereas bucket traps have a large holding capacity. By-catch of beneficial and non-target insects (i.e., Apoidae) must also be a considered when evaluating the optimal trap type (Meagher 2001).

The height at which a pheromone lure is positioned in the plant canopy may affect male moth attraction and capture. In commercial raspberry fields, canes grow to a height of 2.5 m and are trellised with horizontal wires at a height of circa 0.3 and 1.3 m (C.T. personal observation). Trellis wires provide an ideal structure from which to hang traps. A feral female *P. marginata* was observed calling at a height of 1.1 m (C.T. personal observation) suggesting that this height might be suitable for placement of pheromone-baited traps. Other sesiids have responded best to pheromone sources placed at mid-heights in the plant canopy. For example, when caged virgin females of the lesser peachtree borer, *S. pictipes* (Grote and Robinson), were placed at a height of 2 m, they attracted more males than did females placed at heights of 0, 1 or 3 m (Yonce et al. 1976). Similarly, pheromone-baited traps at a height of 1.8 m captured more males than did traps at heights of 0, 3.6 or 5.5 m (Cottrell et al. 2010). Moreover, a greater number of male dogwood borers, *S. scitula* (Harris), were captured in traps placed at a height of 1.8-2.4 m than did traps 0.6 m above ground (Bergh et al. 2006).

Increasing the pheromone load enhances attractiveness of the lure in many sesiid species. For example, increasing lure loads of synthetic dogwood borer pheromone (0.01, 0.1, 0.5, 1.0 or 2.0 mg), lesser peachtree borer pheromone (0.01-10 mg) or currant borer pheromone (0.2-10 mg), increased trap captures of male moths, typically in a near-linear relationship (Yonce et al. 1976; Bergh et al. 2004; Suckling et al. 2005). This is important to know because the cost of pheromone is often the limiting factor when selecting a pheromone load for trapping activities.

The chemical structure of a sex pheromone and its stability at field temperatures affects the longevity of lure attractiveness. The pheromone (*E*,*Z*)-3,13-octadecadienal (*E*3,*Z*13-18:Ald) of *P. marginata,* readily rearranges to (*E,Z*)-2,13-octadecadienal (*E2*,*Z*13-18:Ald) when heated, and may be unstable at field temperatures (Francke et al. 2004, Islam et al. 2007). Most known sesiid pheromones are acetates which are less susceptible to oxidization and are more stable than aldehydes. For example, the synthetic currant borer sex pheromone [(*E*,*Z*)-2,13-octadecadien-1-yl acetate (97%), (*E*,*Z*)-3,13-octadecadien-1-yl acetate (3%)] has a half-life of 110 days (Suckling et al. 2005). This renders lures attractive during the entire seasonal flight period of the moths. Similarly, synthetic dogwood borer pheromone lures [(*Z*,*Z*)-3,13-octadecadien-1-yl acetate (88%), (*E*,*Z*)-2,13-octadecadien-1-yl acetate (6%), (*Z,E*)-3,13-octadecadien-1-yl acetate (6%)] remain attractive for the duration of the entire seasonal moth flight (Zhang et al. 2005). Attractiveness of some acetate pheromone lures peak weeks after lure deployment in the field. For example, synthetic lesser peachtree borer pheromone lures [(*E*,*Z*)-3,13-octadecadien-1-yl acetate] are more attractive to male moths 30 days after field deployment than when aged for 0 or 90 days (Yonce et al. 1976).

Once a suitable trap and bait for *P. marginata* have been identified, pheromone-based management strategies can be evaluated. Two such strategies are pheromone-based mass trapping, and mating disruption with antagonist pheromones. Pheromone-based mass trapping involves placement of numerous pheromone-baited traps throughout an area to capture a large proportion of male moths before they mate with females. This strategy can reduce economic damage to the crop if the number of traps and the dose of lures are sufficient to remove a significant number of males from a population (El-Sayed et al. 2006). According to Knipling and McGuire (1966), mass trapping needs to remove 80-95% of the wild male population to be effective. Combining the sex pheromone with a pheromone antagonist, such as the pheromone of a closely related species, can also disorient mate-seeking males or repel them from a field (Priesner and Witzgall 1984, Rumbo et al. 1993, Witzgall et al. 1993). Pheromone antagonists reduce upwind orientation of male moths to attractive pheromone sources (Miller et al. 2006). For example, pheromonal attraction of male lesser peachtree borers is strongly reduced when the peachtree borer pheromone is added to the same lure and *vice versa* (Tumlinson et al. 1974), and pheromonal attraction of male dogwood borers is reduced in the presence of the currant borer and lesser peachtree borer pheromones (Greenfield and Karandinos 1979, Zhang et al. 2005, Leskey et al. 2009). The seasonal flight period of *P. marginata* overlaps with that of the currant borer, dogwood borer and the apple clearwing moth (James et al. 2001, Bergh et al. 2009, Judd, pers. comm.). If the acetate pheromones of these other sesiids were detected by male *P. marginata*, they could be effective antagonists in the *P. marginata* sexual communication system. The rearrangement product of *P. marginata* pheromone, (*E*,*Z*)-2,13-octadecadienal, is also repellent to male *P. marginata* (Judd et al. in press).

The overall objective of this research was to gather information that may facilitate the development of pheromone-based monitoring or management of *P. marginata* populations. Our specific objectives were to (1) test the effects of trap type, colour and placement as well as lure load and lure age on captures of male *P. marginata,* (2) assess the potential of pheromone antagonists to interfere with pheromonal communication of the raspberry crown borer, and (3) examine the potential of mass trapping to interfere with mate-seeking behaviour of male *P. marginata*.

3.3. Materials and Methods

3.3.1. General methods

All experiments were conducted in commercial raspberry and blackberry fields in the Fraser Valley, British Columbia, Canada (Table 3.1). Unless otherwise stated, traps (Contech Enterprises, Delta, British Columbia, Canada) were hung from the upper trellis wire (circa 1.3 m above ground) of the plant canopy and baited with grey halobutyl rubber septa (West Pharmaceutical Services, Lionville, PA, USA) impregnated with 100 µg of synthetic pheromone [(*E*,*Z*)-3,13-octadecadienal; Gries-laboratory] dissolved in HPLC-grade hexane.

Adult raspberry crown borers were reared in the laboratory at Simon Fraser University from 19-21-month-old larvae collected from commercial raspberry fields in Ladner, Langley and Abbotsford in June and July 2011. Host raspberry cane stubs were cut open and larvae were transferred to pieces of fresh 30-cm segments of blackberry canes (Raine 1962), within which they developed and pupated. Females were separated from males within 12 hours of emergence to prevent mating.

3.3.2. Experiments 1, 2: Effect of colour on catches in bucket traps

In experiment 1 (Table 3.1), we tested the effect of colour (all-white, -yellow, or -green and multi-coloured: green lid, yellow funnel, white bucket) on captures of male *P. marginata* in bucket traps. A pheromone lure was placed in a translucent white pheromone basket, inserted through the lid of each trap. A 2 × 2-cm piece of Vaportape™ II (Hercon Environmental, Emigsville, PA, USA) was placed in the bottom of each trap to kill captured moths. Traps were arranged in a 4 × 4 Latin square, spaced 15 m apart, and ≥ 15 m from the field's edge. This experiment was conducted on 2 farms. Traps were deployed on August 6, 2010 and pheromone lures were replaced on August 14, 2010. Total trap captures were recorded on August 20, 2010. In experiment 2 (Table 3.1), trap spacing was reduced to 3 m to increase the likelihood of male *P. marginata* discerning between trap colour rather than responding to the nearest pheromone source in the field. One trap of each colour (see experiment 1) was placed in the corner of a 4 × 4 Latin square with 3-m spacing between traps. The position of each trap was rotated every 3 days until each trap had been in each position of the square.

Pheromone lures were replaced every 3 days. The experiment was replicated at four locations within the same field, with replicates spaced 50 m apart. Traps were set up on August 27, 2011, and captures of male *P. marginata* moths were recorded on August 30, and on September 2, 5 and 8, 2011.

3.3.3. Experiments 3, 4: Effect of trap type

In experiment 3 (Table 3.1), we tested the effect of commercial trap type (white wing traps, green delta traps, white delta traps, green bucket traps) on captures of male *P. marginata*. The colour of the first three trap types was a fixed attribute of the commercial device. Green bucket traps were deployed based on results of experiment 1. Traps were arranged in two 4 × 4 Latin squares, spaced 15 m apart and ≥ 15 m from field edges. Traps were set up on August 14, 2010, and captures were recorded on August 17, 2010. Lures were not changed. Captures in white wing traps, green delta traps and white delta traps were adjusted for differences in adhesive surface area. Captures in green bucket traps were not adjusted.

In experiment 4 (Table 3.1), we tested the same four trap types as in experiment 3 but with 3-m trap spacings. One trap of each type was placed in the corner of a Latin square with replicates spaced 50 m apart. Traps were set up on September 20, 2011, and captures of male *P. marginata* were recorded two days later. Lures were not changed. Captures in white wing traps, green delta traps and white delta traps were adjusted for differences in adhesive trapping area. Captures in green bucket traps were not adjusted.

3.3.4. Experiment 5: Effect of trap height

In experiment 5 (Table 3.1), we tested the effect of trap height on captures of male *P. marginata*. In each replicate, two green cardboard delta traps were hung from trellis wires at a height of 0.3 m or 1.3 m in two adjacent rows, which were 3 m apart. Six replicates were spaced at 20 m intervals within the same field. Traps were kept in the field for 2-3 days from August 12-14, 20-23 and 23-25, 2010. Pheromone lures were replaced for each 2-3 day trapping interval. Mean trap captures per day at the two heights were compared.

3.3.5. Experiment 6: Effect of pheromone load

In experiment 6 (Table 3.1), we tested the effect of different pheromone lure loads on captures of male *P. marginata*. Each of thirty traps was baited with a grey halobutyl rubber septum impregnated with 10, 100 or 1000 µg of synthetic pheromone. Traps were arranged in ten randomized complete blocks with 15 m between blocks. Each block contained three adjacent rows of raspberry with a 3-m inter-row spacing, and one wing trap per row. Traps were deployed on August 16, 2010 and remained in the field for only 24 hours.

3.3.6. Experiment 7: Longevity of pheromone lures

In experiment 7 (Table 3.1), we tested whether aging pheromone lures decreases their attractiveness to male *P. marginata*. Sixty grey halobutyl rubber septa were impregnated with 100 µg of synthetic pheromone on August 27, 2010, and stored in the freezer at -15°C. Every two days, 10 septa were removed from the freezer and held at 20-22°C in the laboratory. Wing traps were baited with lures aged for 0, 2, 4, 6, 8, or 10 days and deployed on September 6, 2010, in traps set out in randomized complete blocks, with 15 m between blocks and traps. Captures of male *P. marginata* were recorded four days later.

3.3.7. Experiment 8: Effect of pheromone antagonists on trap captures

In experiment 8 (Table 3.1), we tested the effect of four potential pheromone antagonists on reducing the attractiveness of the *P. marginata* pheromone to males. Grey rubber septa were baited with 100 µg of *P. marginata* pheromone (*E*,*Z*)-3,13-octadecadienal plus 100 µg of one of five treatments: hexane (control), (*E*,*Z*)-2,13-octadecadien-1-yl acetate, (*E*,*Z*)-3,13-octadecadien-1-yl acetate, (*Z*,*Z*)-3,13-octadecadien-1-yl acetate, or (*E*,*Z*)-2,13-octadecadienal. All compounds were diluted in 50 µl of hexane. All compounds were selected from pheromones of sesiid species present in North America with flight periods overlapping with that of the raspberry crown borer. Wing traps were arranged in a 5 × 5 Latin square with 15 m between traps. Traps were deployed on September 3, 2011, and captures of male *P. marginata* were recorded on September 6, 2011.

3.3.8. Experiments 9-11: Mass trapping male P. marginata

Experiment 9 (Table 3.1) was designed to explore the potential of mass trapping as a means of controlling populations of *P. marginata*. Paired treatment and control plots, (1 hectare, 95 × 105 m), separated by a 30-m buffer (Fig. 3.1, top) were set up in two raspberry fields. Fields were selected that had a history of severe *P. marginata* infestation. Each treatment (mass trapping) plot received 25 wing traps arranged in a 5 × 5 grid with 20 m between traps. Each of these 25 wing traps was baited with a 100 µg pheromone lure. An additional six wing traps, each baited with a low-dose (10-µg) pheromone lure, were hung in the centre of both treatment and control plots and spaced 20 m apart. Low-dose lures were meant to simulate the pheromone release rates of calling females. These low-dose lure traps were set out to evaluate the ability of mass trapping to reduce male response to calling females within the plots. All traps were deployed on August 31, 2010 and captures of males were recorded on September 3, 2010. Mean captures of *P. marginata* in low-dose lure traps in treatment and control plots were compared, and the percent reduction of catches within treatment blocks was calculated as 1-(average plot catch in low-dose traps in treated fields/average plot catch in low-dose traps in control fields) × 100 (Teixeira et al. 2010).

In experiment 10 (Table 3.1), the potential for mass trapping *P. marginata* was tested in five commercial raspberry fields in Langley and Abbotsford. These fields had >10 males per trap during the 2010 peak flight, or had larvae and damage in the spring of 2011. Paired treatment and control plots (0.36 ha, 50 × 72 m) separated by a 25-m buffer were set up in each of five fields. Each mass-trapping-treatment plot received nine wing traps arranged in a 3 × 3 grid with 20 m between traps (Fig. 3.1, bottom). Each of these nine traps was baited with a 100-µg pheromone lure. An additional four wing traps, each baited with a low-dose (10-µg) pheromone lure, were hung in the centre of both treatment and control plots and spaced 20 m apart. Low-dose lures were meant to simulate the pheromone release rates of calling females and were set out to evaluate the ability of mass trapping to reduce male response to calling females within the plots. All traps were deployed on August 10, 2011 and captured male *P. marginata* were counted and removed from traps every seven days until October 12, 2011. Adhesive trap inserts were replaced as needed. Pheromone lures were replaced every seven days by placing them directly on the sticky inserts for ease of handling. Season-long mean male *P. marginata* captures in low-dose (10 µg) lure traps in treatment and control plots were compared, and the percent reduction in trap catches in treated plots was calculated as described above for each of the nine weeks during which experiment 10 was run.

In experiment 11, one pair of virgin females which had emerged within 48 hours of one another, was used to replace a pair of low-dose pheromone trap lures in a mass trapping treatment and control plot in one of the fields used in experiment 10. Each female was caged in a green pheromone basket from a bucket trap, wrapped in wire mesh. This experiment was not replicated because no additional females emerged within 48 hours of each other in the laboratory.

3.4. Statistics

Data were analyzed statistically, using JMP-IN Version 8 software (SAS Institute). Data in experiments 2, 3, 7, 9 and 10 were square root + 0.5 transformed to meet the equal variance assumption of parametric tests (Zar 2010). Experiments 1, 3 and 8 were analyzed with a Latin Square analysis of variance (ANOVA). Experiments 2, 4, 5, 6 and 7 were analyzed with a two-way ANOVA blocked by field location. In mass trapping experiments 9 and 10, mean trap captures in low-dose lure traps were compared with a paired *t*-test (Exp. 9) and a two-way ANOVA blocked by field site (Exp. 10). When required, all post-hoc means were compared using the Tukey-Kramer HSD test. The level of significance was set at 5% for all statistical tests.

3.5. Results

3.5.1. Experiments 1, 2: Effect of colour on catches in bucket traps

When bucket traps were spaced 15 m apart (experiment 1, 2010), their colour had no effect on captures of male *P. marginata* (Fig. 3.2: *Farm 1:* $F_{(3,3,3)} = 0.97$; $P = 0.47$; *Farm 2:* $F_{(3,3,3)} = 0.97$; $P = 0.46$). When bucket traps were spaced 3 m apart (experiment 2, 2011) catches of raspberry crown borers varied among differently-coloured traps (Fig.3.2: $F_{(3,9)} = 7.09$; $P = 0.01$). Green bucket traps captured more male *P. marginata* than identical white ($P = 0.03$) or yellow ($P = 0.02$) traps but not more than multi-coloured bucket traps.

3.5.2. Experiments 3, 4: Effect of trap type

In experiment 3 (2010; traps 15 m apart), white-coloured traps (wing traps or delta traps) caught significantly more *P. marginata* than green-coloured traps (delta traps or bucket traps). Among green traps, delta traps caught significantly more moths than bucket traps in one of the two fields (Fig 3.3: Field 1: *Trap Type:* $F_{(3,3,3)} = 14.23$, $P < 0.01$, Field 2: *Trap Type:* $F_{(3,3,3)} = 102.27$, $P < 0.0001$; *Location:* $F_{(3,3,3)} = 11.64$, $P = 0.01$). More male *P. marginata* were captured on the south side of the field than on the north side, highlighting an infestation gradient through the field or an effect of wind direction on trap captures. In experiment 4 (2011; traps 3 m apart), trap captures did not differ between trap types (Fig 3.3: $F_{(3,9)} = 2.35$, $P = 0.14$).

3.5.3. Experiment 5: Effect of trap height

Placement of traps at different heights within the raspberry crop canopy (0.3 m or 1.3 m above ground) did not affect capture of male *P. marginata* ($F_{(1, 32)} = 1.35$, $P = 0.25$).

3.5.4. Experiment 6: Effect of pheromone lure load

Pheromone load on septa had a significant effect on captures of *P. marginata* (Fig. 3.4: $F_{(2,18)} = 85.62$, $P < 0.001$). Wing traps baited with increasing amounts of pheromone (10, 100 or 1000 µg) captured increasingly more male *P. marginata*.

3.5.5. Experiment 7: Longevity of pheromone lures

Fresh (0-day-old) pheromone lures attracted more *P. marginata* than lures which had been aged at room temperature for 2-10 days (Fig.3.5: $F_{(5,45)} = 78.73$, $P < 0.001$). No moths were captured in traps baited with 8- or 10-day-old lures.

3.5.6. Experiment 8: Effect of pheromone antagonists on trap captures

Each of the four potential pheromone antagonists reduced attractiveness of the *P. marginata* pheromone in field 1 (Fig. 3.6: $F_{(4,4,4)}$ = 12.52, $P < 0.001$). Trap captures were too low in the second field to see a treatment effect ($F_{(4,4,4)}$ = 2.56, P = 0.09).

3.5.7. Experiments 9-11: Mass trapping male P. marginata

In experiment 9 (2010), low-dose (10 µg) pheromone traps captured a mean (± SE) of (0.2 ± 0.2) and (5.0 ± 2.6) of male raspberry crown borers in treatment (mass trapping) and control plots, respectively. However, with only two replicates, mean trap captures were too variable to detect a significant difference between treatment and control plots (*t* = 2.12, df = 1, P = 0.34). In experiment 10 (2011), the seasonal total capture of males in low-dose pheromone traps was significantly lower in mass trapping plots than in paired control plots ($F_{(1,4)}$= 9.27, P = 0.04, Tukey HSD P = 0.04). Seasonal mean (± SE) trap captures in low-dose (10 µg) pheromone traps plots were (2.3 ± 0.9) and (11.3 ± 4.6) in treatment (mass trapping) and control plots, respectively. Mean weekly captures of *P. marginata* in low-dose traps varied over the flight period, with the greatest difference between treatment and control plot trap captures occurring on August 31 and September 7, 2011 (Fig 3.7). Mass trapping reduced *P. marginata* captures in low-dose pheromone (false-female) traps by a mean (± SE) of 93.0 ± 7.0% in 2010 and 75.0% ± 3.4% in 2011. In 2011, the season-long percent reduction in low-dose trap captures ranged from 67-87% in the 5 fields where mass trapping was evaluated (Table 3.2).

In experiment 11, when a pair of low-dose (10 µg) pheromone lures was replaced with a pair of virgin female *P. marginata*, 34 males were attracted in the control plot and eight males in the treatment plot.

3.6. Discussion

Our data allow several conclusions regarding (*i*) the effect of trap and lure attributes on captures of male *P. marginata*, (*ii*) the potential for developing pheromone-based management tactics, and (*iii*) the most promising directions for future research.

There was no upper pheromone lure load tested which interfered with the attraction of male *P. marginata*. This is consistent with findings in other sesiids (Yonce et al. 1976, Bergh et al. 2004, Suckling et al. 2005). Wing traps baited with increasing amounts of synthetic sex pheromone captured increasingly more male *P. marginata* (Fig. 3.4). However, traps baited with a 1000-μg lure captured only twice as many males than traps baited with a 100-μg lure, which does not justify the greater cost of producing high-dose lures.

The synthetic pheromone of *P. marginata*, *E*3,*Z*13-18:Ald, appears unstable and remains attractive to males for ≤ 6 days (Fig. 3.5). Under field conditions, *E*3,*Z*13-18:Ald likely partially or fully rearranges to (*E*,*Z*)-2,13-octadecadienal (*E2*,*Z*13-18:Ald) which, when added to septa impregnated with pheromonal *E*3,*Z*13-18:Ald, inhibited attraction of males (Fig. 3.6). The repellency of this rearrangement product could explain why *P. marginata* pheromone lures quickly become unattractive to males in the field (Judd et al. in press). This finding has implications for pheromone-based monitoring or management of *P. marginata*. Pheromone lures will need to be replaced at least weekly rather than every 6-12 weeks, as implemented for other sesiid pheromone lures (Yonce et al. 1976, Suckling et al. 2005, Zhang et al. 2005).

The two congeneric pheromones (*E*,*Z*)-3,13-octadecadienyl acetate and (*Z*,*Z*)-3,13-octadecadienyl acetate were also repellent to male *P. marginata* (Fig. 3.6). As both of these acetate pheromones are stable, they may have potential for operational use to disorient mate-seeking male *P. marginata*, in the presence or absence of synthetic *P. marginata* pheromone. Such an approach has been tested with populations of the pea moth, *Cydia nigricana* (F.), dogwood borer, *Synanthedon scitula* (Harris), oblique-banded leafroller, *Choristoneura rosaceana* (Harris), and three-lined leafroller, *Pandemis limitata* (Robinson), and has met with some success (Bengtsson et al. 1994, Evenden et al. 1999, Leskey et al. 2009).

Mass trapping male *P. marginata* (Fig. 3.7) as a management tool has challenges. Mass trapping with 25 traps per hectare, each baited with a 100-µg pheromone lure, reduced mean captures of males in low-dose (false-female) pheromone traps by 75 and 93% in 2011 and 2010 respectively. A similar reduction (80-83.5%) of captures in lower dose pheromone traps was found when males of the American plum borer, *Euzophera semifuneralis* (Walker), and peachtree borer, *Synanthedon exitiosa* (Say) were subjected to mass trapping with 20 high-dose pheromone traps per hectare (Teixeira et al. 2010).

Mass trapping as a management strategy has been most successful for Lepidoptera when pest population densities are low and populations are isolated (El-Sayed et al. 2006). At high densities, there is more competition between feral females and synthetic pheromone lures for male moths. In our five-field mass trapping experiments, population densities of *P. marginata* ranged from low to high based on the 30 and 223 males, respectively, which were captured in nine 100 µg-baited traps over 12 weeks. Interestingly, we found mass trapping was most successful (87% reduction) in the field with the highest population density (223 males). In a moderately infested field, traps baited with a virgin female *P. marginata* were still able to attract and capture males, although captures were lower in the mass-trapped plot than in the untreated control plot. The females' ability to remain attractive even in the mass-trapped plot was likely due to deterioration of synthetic pheromone over time. Had additional pairs of virgin females been available, it would have been interesting to investigate their attractiveness in mass-trapped fields with lower and higher *P. marginata* densities. Irrespectively, replacing synthetic pheromone lures once or even twice per week renders mass trapping *P. marginata* a labour-intensive management tactic that may appeal only to small-scale or organic growers.

An alternative to using synthetic pheromone to monitor or manage *P. marginata* may be to use its corresponding formate [(*E*,*Z*)-3,13-octadecadien-1-yl formate]. Formates in general are more stable than their corresponding aldehydes, and in some species elicit the same behavioural response as the major pheromone component. For example, males of the tobacco budworm moth *Heliothis virescens* (Fabricius) and corn earworm *Helicoverpa zea* (Boddie), respond equally well to (Z)-9-tetradecen-1-yl formate as they do to the aldehyde pheromone component (Z)-11-hexadecenal (Mitchell et al.

1975, Berg et al. 1995). Furthermore, males of the carob moth, *Ectomyelois ceratoniae* (Zeller), were attracted to (*Z*,*E*)-7,9,11-dodecatrien-1-yl formate as the analogue of the major aldehyde pheromone component (*Z*,*E*)-9,11,13-tetradecatrienal (Todd et al. 1992, Vetter et al. 2006). If male *P. marginata* respond equally well to the pheromone *E*3,*Z*13-18:Ald and its formate analogue, the analogue could be developed for monitoring and control of *P. marginata*.

In conclusion, if frequently replaced and deployed in appropriate traps, synthetic sex pheromone lures will be useful for tracking the seasonal flight of *P. marginata*. Captures in pheromone-baited traps may help predict population densities and aid management decisions (see Chapter 4). However, the instability of the sex pheromone does not lend itself to deployment for control of *P. marginata* populations. Instead, semiochemical-based tactics with pheromone antagonists or the formate analogue may have considerably more potential and should be investigated in further studies.

3.7. Acknowledgements

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3.9. Tables

Table 3.1. Time period and geographical location of field experiments 1-11

Exp.	Time	N[1]	Crop	Cultivar (s)[2]	City	Location(s)[3]
1	6–20 Aug. 2010	8 (2)	Raspberry	Cascade D	Abbotsford	49°02’24”N, 122°27”23”W 49°00’28”N, 122°22”58”W
2	27Aug.–8 Sept. 2011	4 (1)	Raspberry	Cascade D	Ladner	49°05’26”N, 123°08’45”W
3	14–17 Aug. 2010	8 (1)	Raspberry	Cascade D	Abbotsford	49°02’24”N, 122°27”22”W
4	20–22 Sept. 2011	4 (1)	Raspberry	Coho	Langley	49°07’43”N, 122°32’59”W
5	12–25 Aug. 2010	24 (2)	Raspberry	Cascade D	Abbotsford	49°02’24”N, 122°27”22”W
				Cascade D	Abbotsford	49°00’27”N, 122°22’56”W
6	16–17 Aug. 2010	10 (1)	Raspberry	Cascade D	Abbotsford	49°02’24”N, 122°27”22”W
7	06–10 Sept. 2010	10 (1)	Raspberry	Cascade D	Abbotsford	49°02’29”N, 122°26’56”W
8	3–6 Sept. 2011	10 (2)	Blackberry	Chester	Langley	49°07’36”N 122°32’39”W 49°07’36”N, 122°32’34”W
9	31 Aug –3 Sept. 2010	2 (2)	Raspberry	Cascade D	Abbotsford	49°02’22”N, 122°27’24”W 49°02’22”N, 122°27’18”W
10-11	10 Aug.–12 Oct. 2011	5 (5)	Raspberry	Tulameen	Langley	49°07’28”N, 122°32’51”W
			Raspberry	Malahat	Langley	49°06’53”N, 122°32’22”W
			Raspberry	Cascade D	Abbotsford	49°02’28”N, 122°27’01”W
			Raspberry	Meeker	Abbotsford	49°00’34”N, 122°22’38”W
			Raspberry	Malahat	Abbotsford	49°00’27”N, 122°22’56”W

[1] number of replicate traps; number of fields in parentheses;

[2] D = Delight

[3] Elevation ranged between 49-114 m

Table 3.2. Seasonal percent reduction in captures of male Pennisetia marginata in traps baited with a low-dose (10 µg) pheromone lure in mass trapping plots treated with 25 high-dose pheromone traps per ha or in untreated control plots in five fields from August 17 to October 12, 2011

Field	**Seasonal % reduction in trap captures**
1	77.5%
2	66.7%
3	86.9%
4	71.4%
5	72.6%

3.10. Figures

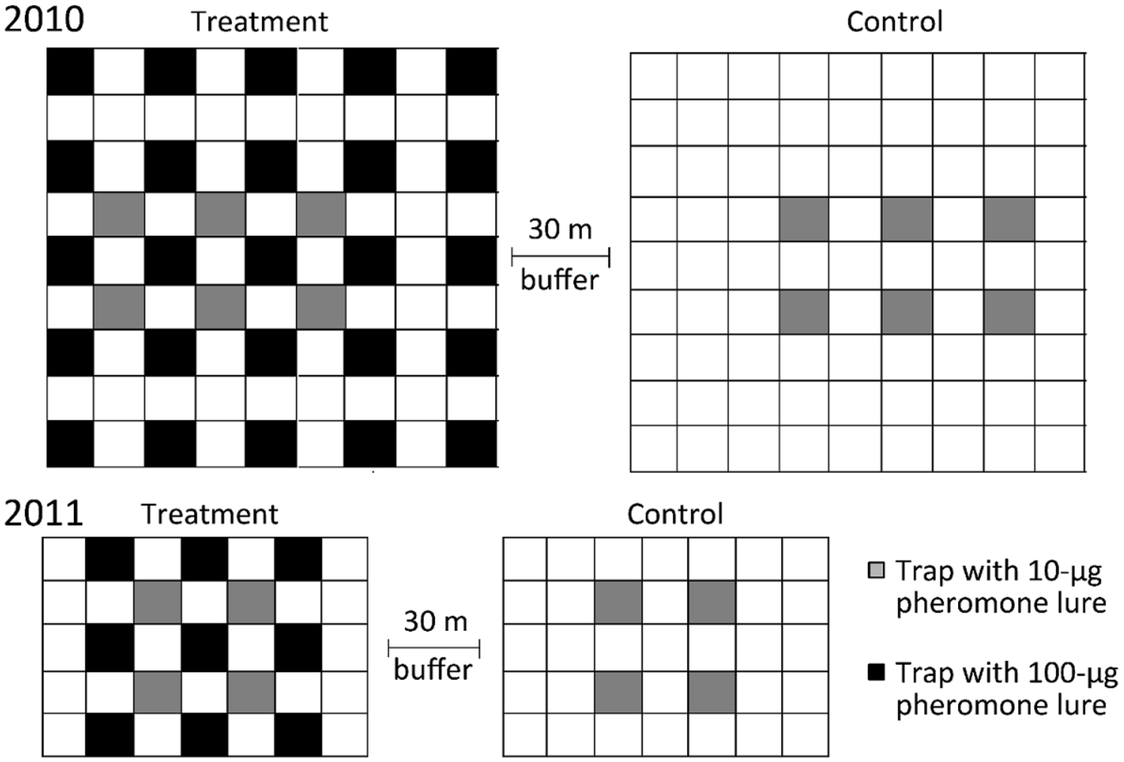

Figure 3.1. Layout of field plots in experiments 9 and 10 (Table 3.1). The lower pheromone dose was meant to simulate pheromone release rates of a calling female Pennisetia marginata.

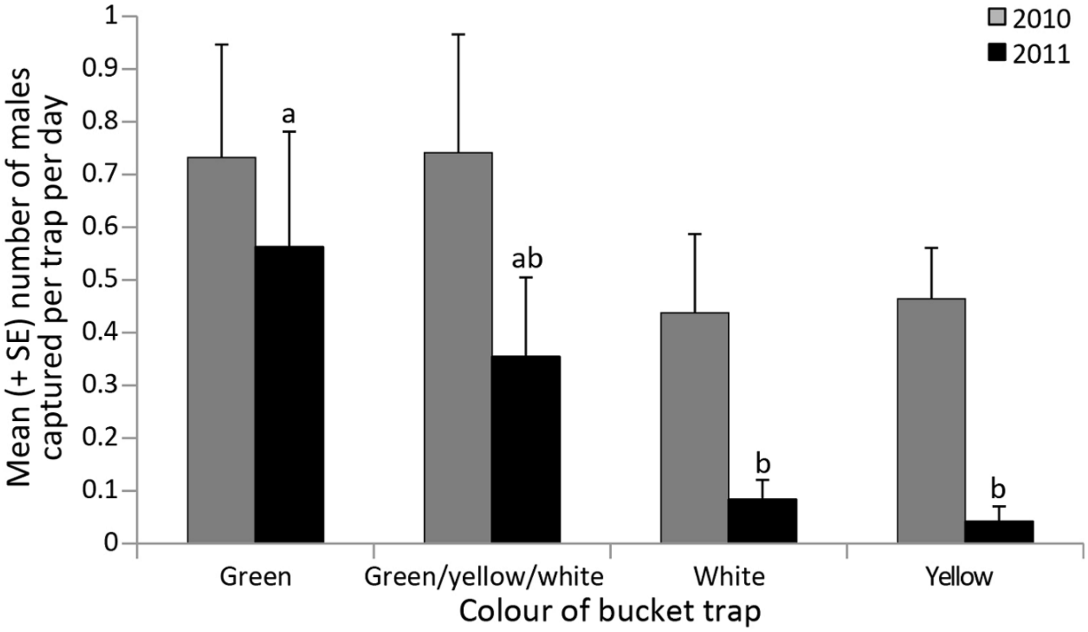

Figure 3.2. Effect of colour on captures of male Pennisetia marginata in bucket traps in experiments 1 and 2 (Table 3.1) (ANOVA, 2010: N = 8, P = 0.46; 2011: N = 4, P = 0.01). Bars with the same letter superscript are not significantly different from one another (Tukey HSD P<0.05)

Figure 3.3. Effect of trap type on captures of male Pennisetia marginata in experiments 3 and 4 (Table 3.1) (ANOVA, 2010: N= 8, P<0.01; 2011: N=4, P=0.14). Bars with the same letter superscript are not significantly different from one another (Tukey HSD P<0.05)

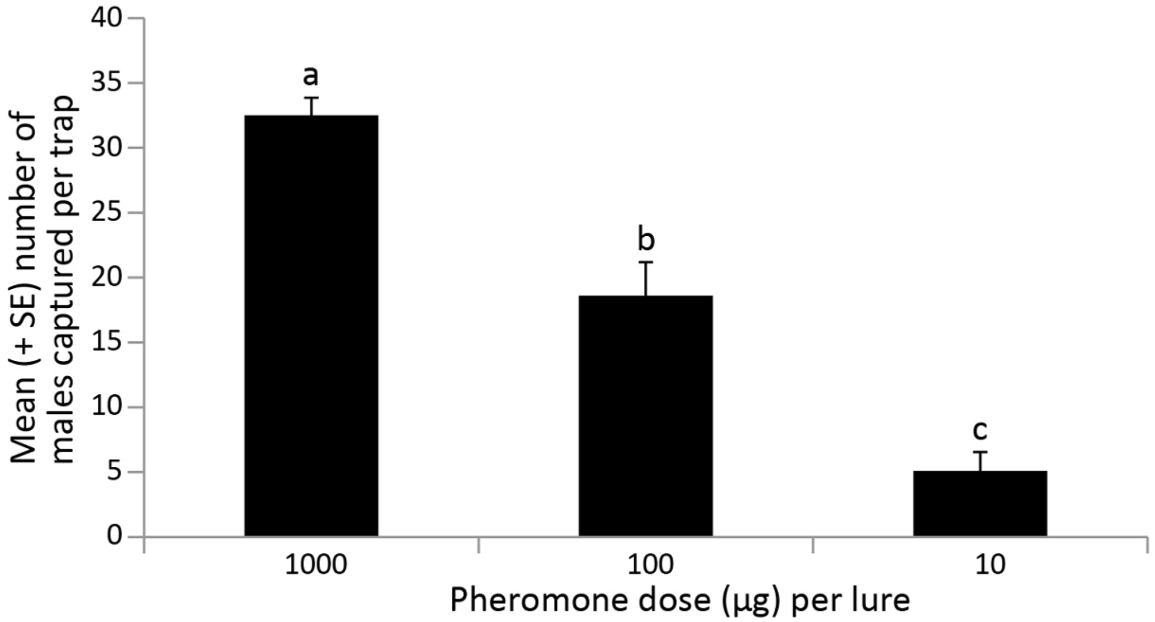

Figure 3.4. Effect of pheromone lure load on captures of male Pennisetia marginata in experiment 6 (Table 3.1) (ANOVA, N=10, P < 0.001). Bars with the same letter superscript are not significantly different from one another (Tukey HSD P<0.05)

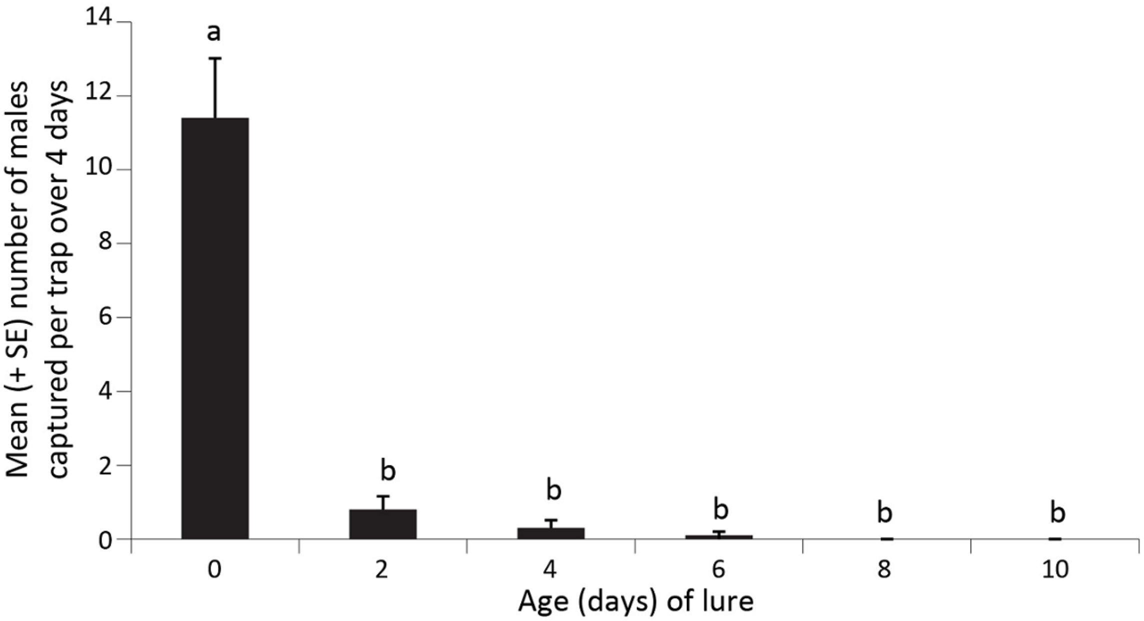

Figure 3.5. Effect of lure age on captures of male Pennisetia marginata in experiment 7 (Table 3.1) (ANOVA, N=10, P < 0.001). Bars with the same letter superscript are not significantly different from one another (Tukey HSD P<0.05)

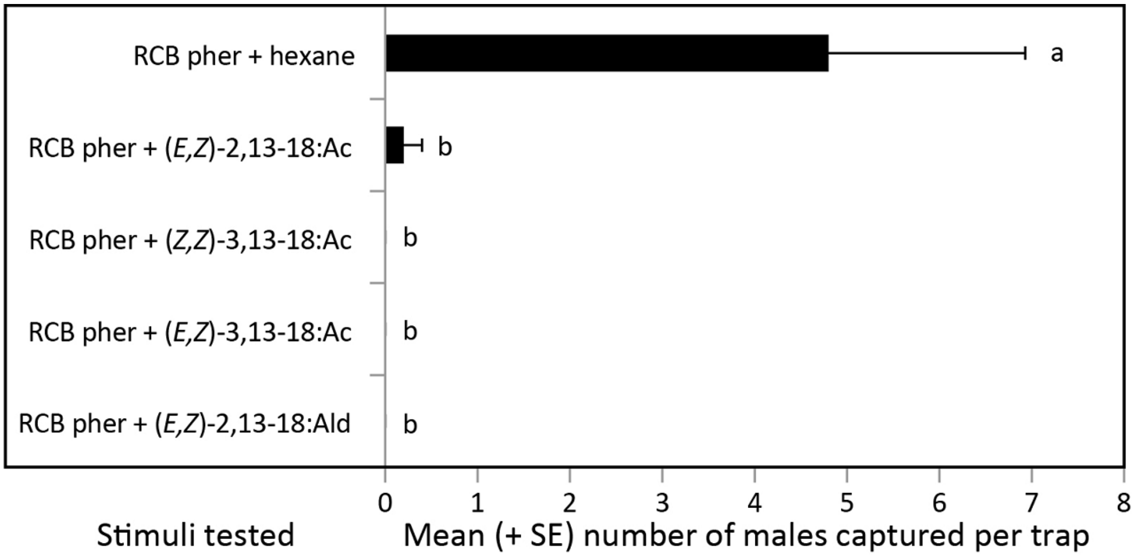

Figure 3.6. Effect of potential pheromone antagonists in experiment 8 (Table 3.1) on reducing captures of male Pennisetia marginata in traps baited with the P. marginata sex pheromone (ANOVA, N = 10, P < 0.001). Bars with the same letter superscript are not significantly different from one another (Tukey HSD P<0.05)

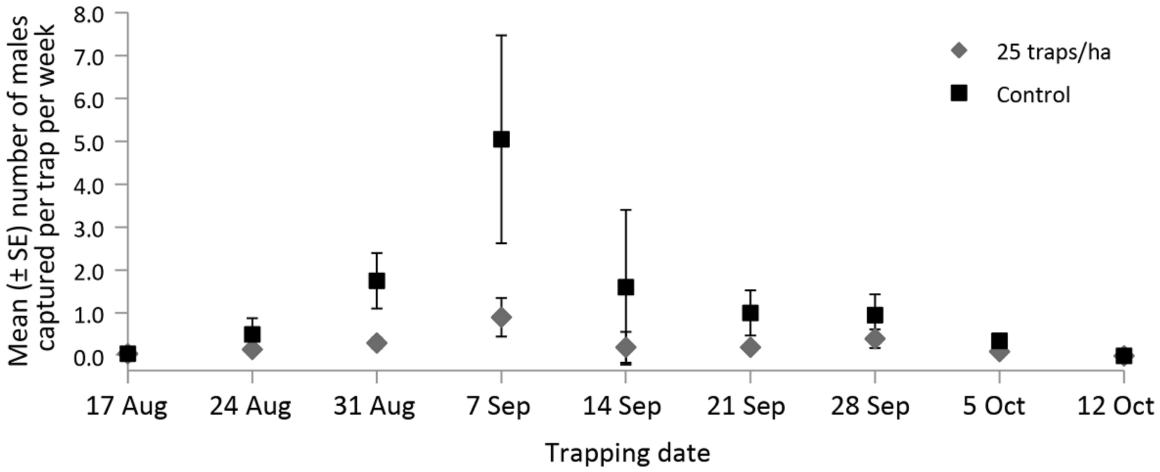

Figure 3.7. Effect of mass trapping on weekly captures of male Pennisetia marginata in low-dose (10µg) pheromone traps between August 10 and October 12, 2011 in experiment 10 (Fig. 3.1; Table 3.1) (ANOVA on season-long treatment and control trap catches, N=5, P=0.04).

4. Development of a trap-catch threshold to detect egg-infested raspberry canes[2]

4.1. Abstract

The raspberry crown borer (RCB), *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is a challenging pest to monitor as it spends most of its two-year life cycle in the larval stage inside the base of host-plant canes. Without a monitoring tool, RCB populations have been managed with preventative insecticide drenches. Using a synthetic version of the female-produced sex pheromone, we surveyed the distribution of RCBs in cultivated raspberry fields and wild Himalayan blackberry patches in the Fraser Valley, British Columbia (BC). Similar numbers of male moths were captured in traps placed in commercial raspberry fields and wild blackberry patches, and in traps in the centre and at the edge of raspberry fields. In 2010 and 2011, two traps were deployed in 20 to 25 raspberry fields in the Fraser Valley, BC, and Whatcom and Skagit counties, Washington, to compare captures of RCB males with our detection of eggs on 100-200 primocane leaves around the traps. In Fraser Valley raspberry fields, seasonal peak flight occurred 779-892 degree days above 10°C after 1 January, between 25 August and 3 September 2010, and 1 and 8 September 2011. A mean trap-catch threshold of 10-11 moths in a one-week period between 779-843 degree-days, when ~50% of trap captures had taken place, was indicative of egg presence on 100-200 leaves in a field in both years. At or above this trap-catch threshold, but not below it, eggs were consistently found. In 2011, when ≥19 moths were captured over a three-week period between 652 to 842 degree-days, <1% of canes were infested with eggs. Seventy-five percent of

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[2] This chapter will be submitted to the Journal of the Entomological Society of British Columbia with authors as follows: Teasdale, C., Judd, G.J.R., and Gries, G.

raspberry fields monitored in 2011 had trap-catch levels below this cumulative threshold. Implementation of a trap-catch threshold may translate into considerably fewer growers applying insecticide drenches.

4.2. Introduction

The raspberry crown borer (RCB), *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae), is a sporadic but severe pest of commercial raspberries. In recent years, RCB has caused severe cane damage on some raspberry farms in Langley and Abbotsford, British Columbia (BC) (C.T. personal observation). This pest is challenging to monitor because it has a two-year life cycle and larvae feed within the crown and base of canes (Lawrence 1904, Raine 1962). Adult RCBs, which mimic wasps in their appearance and flight patterns, are thought to fly between July and September (Raine 1962) although their seasonal peak flight is yet to be determined. A female-produced sex pheromone component from RCB was recently identified as (*E*,*Z*)-3,13-octadecadienal (Judd et al. in press). This pheromone is not yet commercially available but may have the potential to be used as a tool to monitor RCB populations and flight phenology in the Fraser Valley and other raspberry growing regions of North America. Pheromone-based trapping of lepidopteran pests is used operationally to survey the distribution of populations, predict the timing of egg hatch, and time insecticide application (Sanders 1988, Hoffman et al. 1992, Reddy and Guerrero 2001, Bazok et al. 2009).

The current action threshold for RCB control is 5% of plants with hollow canes, as detected when pruning in the winter (British Columbia Ministry of Agriculture and Lands 2009). With no tools for effectively monitoring RCB populations before this damage occurs, many raspberry growers apply preventative insecticide drenches to protect their crops (C.T. personal observation). Growers might benefit from a pheromone-based monitoring system that indicates whether RCBs are present and when they are active before fall or spring drenches are applied. As more selective and life-stage specific insecticides are used as replacements for the commonly recommended organophosphate diazinon (British Columbia Ministry of Agriculture and Lands 2009), accurate timing of insecticide application will be critical to ensure efficacy against the most susceptible life stages of RCB. Early detection of RCB populations at sub-economic levels may help to extend the life of raspberry plantings, as many infested fields are removed within three years of larval detection (C.T. personal observation).

Positive correlations between captures of adult moths in pheromone-baited traps and population densities of subsequent generations of larvae have been reported for the spruce budworm, *Choristoneura fumiferana* (Clemens), the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) and the western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst) (Sanders 1988, Evenden et al. 1995, Asaro and Berisford 2001), but these correlations can be weak when populations are small (Sanders 1983, 1988). The incidence of severe damage by RCB to commercial raspberry plantings in the Fraser Valley is sporadic and occurs in isolated fields despite the large acreage planted with raspberries. RCBs may emigrate from adjacent raspberry fields or from alternate host plants such as Himalayan blackberries, *Rubus discolor* (Weihe & Nees) and salmonberries, *R. spectabilis* (Pursh), that commonly grow within the hedgerows surrounding commercial raspberry fields (C.T. pers observ). Male moths of many species disperse farther than females (Cardé and Elkington 1984), and sesiid moths in particular are strong fliers that can fly hundreds of metres to upwind pheromone sources (Karandinos 1974, Priesner et al. 1986). If captures of male RCBs in pheromone traps were shown to be indicative of the presence of RCB eggs in commercial crops, then Fraser Valley raspberry growers could decide whether or not to apply insecticides before RCB damage ensued.

Degree days are often more useful than calendar dates in predicting the timing of insect development. Degree-day models that use field temperatures to predict adult eclosion and flight have been developed for other Lepidoptera including the lilac borer, *Podosesia syringae* (Harris) (Potter and Timmons 1983), codling moth, *Cydia pomonella* (L.) (Riedl et al. 1976), eye-spotted bud moth, *Spilonota ocellana* (Denis & Schiffermüller) (McBrien and Judd 1998), obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Evenden and Judd 1999), and speckled cutworm moth, *Lacanobia subjuncta* (Grote and Robinson) (Doerr et al. 2005). Relating capture of RCBs in pheromone-baited traps to degree days would enable the development of a predictive model for RCB adult eclosion and peak flight. Knowledge of the seasonal moth flight will enable pest managers to time pheromone-based monitoring activities.

Our objectives were to (1) survey the distribution of RCBs in commercial raspberry fields and unmanaged blackberry patches in the Fraser Valley, (2) determine the peak seasonal flight of RCBs in the Fraser Valley, and (3) determine a baseline pheromone trap-catch threshold that might correspond with the presence of eggs in monitored fields.

4.3. Methods

All experiments were conducted in commercial raspberry and blackberry fields in the Fraser Valley, British Columbia, Canada. Pheromone traps (Contech Enterprises, Delta, British Columbia, Canada) were hung from the upper trellis wire (circa 1.3 m above ground) of the plant canopy. Each trap was baited with a grey halobutyl rubber septum (West Pharmaceutical Services, Lionville, PA, USA) impregnated with 100 µg of synthetic RCB sex pheromone [(*E*,*Z*)-3,13-octadecadienal; Gries-laboratory] dissolved in HPLC-grade hexane. Pheromone dispensed from lures deployed 12 September 2009 was purified with a Nova-Pak C-18 reverse phase HPLC column (3.9 × 300 mm; Waters, Massachusetts, USA). The solvent was 100% acetonitrile.

4.3.1. Captures of RCBs in raspberry fields and blackberry patches

Green delta traps were placed in 12 commercial raspberry fields and in six patches of unmanaged Himalayan blackberry in the Fraser Valley on 29 July 2009 (Table 4.1). These traps are sufficiently narrow to fit through a machine harvester and are easy to hang in a dense raspberry canopy. Raspberry fields ranged in size from 3 to 25 acres. Two traps were placed in each field, one in a corner 3 m from both field edges, and the other 30 m from the field edge and 100 m from the row end. Blackberry patches selected for this study were at least 100 m^2 in size and ≥ 200 m from any commercial raspberry field. One trap was placed in the centre of each blackberry patch. Pheromone lures were replaced and captured moths recorded on 18 August and 12 September 2009. The 12-September lures were HPLC-purified (see above) as previous low trap captures were thought to be the result of pheromone impurity.

4.3.2. *Seasonal phenology of RCB flight*

In 2010, two white delta traps were placed 30 m apart in each of two raspberry and two blackberry fields in the Fraser Valley (Table 4.2). Captured RCB males were counted and removed every 4-5 days from 29 July to 1 October 2010. Adhesive trap inserts were replaced as needed. Pheromone lures were replaced on 16 and 25 August and on 3, 13 and 22 September 2010. Mean daily trap captures were calculated for the 8-10 days following each lure replacement. In 2011, one wing trap was placed 30 m from the edge of each of five raspberry and five blackberry fields (Table 4.2). Pheromone lures were replaced every 7 days and captured RCB males were counted and removed twice each week from 28 July to 17 October 2011. Adhesive trap inserts were replaced as needed. Trap captures in white delta traps (2010) and wing traps (2011) were adjusted for differences in adhesive surface area. First sustained male RCB capture was defined as the beginning of the continual period of moth trap catches, when at least one moth was caught on consecutive trapping dates. Mean weekly trap captures were converted to cumulative percentages of total seasonal moth captures. Daily maximum and minimum air temperatures were recorded at the Abbotsford airport, within 16 km of all field sites. Accumulated degree days from 1 January 2010 and 2011 were calculated using a modified double sine wave method developed by Allen (1976). As lower and upper temperature thresholds for RCB development have not been identified, a lower base temperature of 10°C was used, which is a common base temperature for insect degree-day models in warm-season crops (Pruess 1983). No upper temperature threshold was used in the degree-day calculations.

4.3.3. *Developing a trap-catch threshold indicative of egg-infested canes*

On 29 July 2010, green delta traps were placed in 20 raspberry fields in the Fraser Valley (Table 4.3). Fields ranged from 3-10 acres in size. Two traps were placed per field, one trap in a corner 3 m from field edges, and the other 30 m from the field edge and 100 m from the row end. Field trapping sites were >400 m apart, although often located directly adjacent to non-test raspberry fields. Pheromone lures were replaced on 19 August and trap captures were recorded on 19 August and 15 September. Egg counts were carried out in each field from 22 September to 1 October by examining a total of 100 leaves on 20 primocanes around each trap. Sampling during this time frame ensured that most oviposition but minimum egg hatch, which occurred in October in both 2010 and 2011 (CT personal observation), had taken place. Egg counts instead of larval counts were carried out because larval hibernacula were difficult to detect at or below the soil line. For egg counts around centre-field traps, the underside and margin of the upper five leaves on each of five primocanes at four cardinal locations (NE, NW, SE and SW) within a 12-m circular radius of each trap were closely examined for the presence of eggs. Egg counts around edge-of-field traps were carried out on the same number of primocanes but at four locations 10 and 20 m down-row or cross-row from the corner trap (N and E, N and W, S and E or S and W). One field was ploughed under before trap captures could be recorded and two additional fields were removed before egg counts could be carried out.

In 2011, pheromone traps were placed in 20 raspberry fields in the Fraser Valley and five raspberry fields in Whatcom and Skagit counties, Washington, on 28 and 29 July, respectively (Table 4.4). Because eggs were not consistently detected around edge-of-field traps that caught moths in 2010, pheromone traps were placed 50 m apart in a single row ≥30 m from the field edge and 50 m from each row end in each field in 2011. Traps were set up (28-29 July) and pheromone lures were changed on the same dates as in 2010 (18-19 August). Moreover, as cool spring and summer weather delayed the seasonal flight of RCB in 2011, lures were also replaced later in the season (on 25-26 August and 1-2 September) to ensure trap captures during the expected peak of RCB flight. Captures were recorded on 18-19 and 25-26 August, and on 1-2 and 8-9 September. Traps were serviced weekly when all moths were removed and lures were replaced. Sticky inserts were replaced as needed (Contech Inc., Delta, BC, Canada). One field was ploughed under before trap captures could be recorded.

Egg counts were carried out from 29 September to 6 October, 2011. The number of sampling locations increased from four to eight cardinal locations (N, NE, E, SE, S, SW, W, NW) in 2011 to improve potential detection of eggs. The underside of five upper leaves on each of five primocanes was examined at each location for a total of 200 leaves on 40 primocanes. The mean weekly trap catch and cumulative trap catch over three weeks were compared to the percentage of egg-infested canes to determine a trap-catch threshold indicative of egg-presence in a field.

Trap catches were retrospectively compared to cumulative degree days from 1 January each year. Daily maximum and minimum air temperatures were recorded at the Abbotsford airport, located <80 km of all trapping sites. Cumulative degree days were calculated as described in our phenology experiment above.

4.4. Statistics

Data were analyzed using JMP-IN Version 8 software (SAS Institute). Mean cumulative trap captures in raspberry and blackberry patches were compared with a Student's two-sample *t*-test, and mean cumulative trap captures in traps at field edges and centres were compared with a paired *t*-test; in both tests the level of significance was set to 5%. Regression or correlation analyses on trap catch and egg count data were not carried out as our objective was to identify an egg-presence- or absence trap-catch threshold and not to develop a predictive model.

4.5. Results

4.5.1. Captures of RCBs in raspberry fields and blackberry patches

Mean (± SE) trap captures of RCB in raspberry fields (3.71 ± 1.25) and unmanaged Himalayan blackberry patches (1.17 ± 0.79) were similar (*t*=1.35; df=16; *P*=0.19). Cumulative RCB captures from 29 July to 8 October 2009 ranged from 0 to 23 in raspberry fields and from 0 to 5 in blackberry patches. The only catch from 29 July to 18 August 2009 was in an unmanaged blackberry patch. Within raspberry fields, mean RCB captures in centre-traps (2.50 ± 0.93) and edge-of-field traps (4.92 ± 1.95) did not differ statistically (*t*=1.39; df=11; *P*=0.19).

4.5.2. Seasonal phenology of RCB flight

Male RCBs were captured from 2 August to 27 September 2010 and from 1 August to 3 October 2011. The number of degree days >10°C from 1 January to first sustained male RCB capture was 619 and 582 in 2010 and 2011, respectively. In raspberry fields, 50% of cumulative seasonal trap catch occurred between 838-892 degree days in 2010 (25 August to 3 September) and between 779-843 degree days in 2011 (1-8 September) (Fig. 4.1). In raspberry fields, there was one distinct flight peak and a sharp decline in trap captures afterwards. In blackberry fields, 50% trap catch occurred between 892-944 degree days in 2010 (3-13 September) and between 779-843 degree days in 2011 (1-8 September). Peak flight was less well defined in blackberry fields than in raspberry fields.

4.5.3. Developing a trap-catch threshold indicative of egg-infested canes

In 2010, trap captures and egg counts were more consistent for centre-field traps than for edge-of-field traps. RCBs were captured in centre-field traps in 17 out of 19 fields. Eggs were detected around centre-field traps in 9 out of these 19 fields, with the percentage of egg-infested canes ranging between 0 to 55%. Eggs were detected in all fields where ≥10 moths were captured in the centre-field trap between 789-958 degree-days (19 August to 15 September 2010) (Fig. 4.2). When centre-field traps caught ≤ 10 moths during this period, eggs were detected in three fields, and 0-20% of canes were egg-infested. In one field, no moths were captured in the centre-field trap but 15% of the canes were infested with eggs.

In 2011, overall trap captures and egg counts were lower per field than in 2010, probably because growers removed many infested fields. RCBs were captured in 23 out of 24 fields, and eggs were found in 10 of these fields. Weekly trap catches that were indicative of egg presence varied depending on the time of the season and cumulative degree-days. These thresholds were 3, 9.5 and 11 RCBs captured per trap for the period of 652-724 degree days (18-25 August), 724-779 degree days (25 August to 1 September) and 779-842 degree days (1-8 September), respectively. Between 779-842 degree days, when ≥ 11 moths were caught per trap in Fraser Valley and Whatcom county fields, eggs were detected in all fields (Fig. 4.3). Below this threshold, eggs were detected in six fields, and 1-3% of canes were egg-infested. Moth flight seemed to peak one week earlier in Rockport, Washington, although degree days were not calculated for this location. When the mean cumulative number of moths captured per trap per field between 652-842 degree days (18 August to 8 September 2011) was plotted against the percentage of egg-infested canes, a trap-catch threshold of 19 moths per trap was indicative of egg presence in a field. In fields with captures of ≤19 moths per trap, ≤ 1% of canes were egg-infested (Fig. 4.4).

4.6. Discussion

To reliably predict the presence or absence of RCB eggs through pheromone-based trapping of adult moths, traps should be placed in the centre of fields. Traps located on field edges likely attract relatively more moths from neighbouring fields or wild blackberry and salmonberry patches. If RCB males fly as well as male raspberry clearwings, *Pennisetia hylaeiformis* (Laspeyres), which reportedly move 500-1000 m upwind in response to a pheromone source (Priesner et al. 1986), then RCB males could easily have moved between patches of cultivated or wild host plants in this study. This may explain why trap captures in raspberry fields and blackberry patches did not differ. Alternatively, potential differences in population densities due to host-plant preference may have been masked by different acreages of raspberry fields (≥ 3 acres) and blackberry patches (≤ 0.1 acre), or by RCB control measures implemented only in commercial crops.

Female RCBs may discriminate between different cultivars of raspberries, and males may respond differently to pheromone in the presence of different host-plant volatiles. Overall, we observed lower trap captures and egg counts in 'Meeker' than in other cultivars. In apple and currant crops, infestation levels of the apple clearwing, *Synanthedon myopaeformis* (Borkhausen), the dogwood borer, *Synanthedon scitula* (Harris), and the currant borer, *Synanthedon tipuliformis (*Clerck), vary by cultivar and rootstock (Stüber and Dickler 1988, Hummer and Sabitov 2003, Ateyyat 2006, Frank et al. 2011). Further investigation into RCB preference for raspberry cultivars is warranted.

Flight activity of male RCBs extended from late July to early October in both years, with peak flight occurring between 25 August and 8 September. In raspberry fields, 50% cumulative seasonal trap catch, thought to reflect 50% seasonal moth flight, occurred around 840 degree days in both years (Fig. 4.1). In blackberry fields, the range in accumulated degree days at which 50% trap capture occurred in 2010 and 2011 did not overlap. Additional years of flight phenology data are required to reliably predict adult eclosion and peak flight in raspberries and blackberries based on degree-day accumulations.

Using accumulated degree days rather than calendar dates to time pheromone-based monitoring activities makes trap-catch thresholds relevant even in unseasonably early or late cropping seasons. The RCB pheromone has a field life of about 6 days following deployment in the field (Chapter 3). Thus, we can assume that moths caught in monitoring traps from 18 August to 15 September 2010 were attracted only between 18 and 25 August 2010, approximately 789-843 degree days after 1 January. In 2011, a similar number of degree-days accumulated between 1 and 8 September. In both years, a mean trap-catch threshold of 10-11 moths at 779-843 degree days, ~ 50% seasonal moth flight, was indicative of egg presence in a field. At or above this trap-catch threshold, but not below it, eggs were consistently found.

The cumulative number of moths captured over a greater degree-day accumulation (i.e., multiple weeks of moth flight) may provide a better estimate of the population size and a more accurate trap-catch threshold. In 2011, when ≥19 moths were captured from 652 to 842 degree days, < 1% of canes were infested with eggs. Using this cumulative trap-catch threshold, RCB populations were below threshold in 18 out of 24 fields monitored in 2011. Operational implementation of our cumulative trap-catch threshold would translate into considerably fewer growers applying insecticide drenches. Our cumulative trap-catch threshold would still provide growers time to make a spray decision before egg hatch in October.

The trap-catch thresholds that we have identified are specific to a trap type, lure dose and lure replacement schedule with relation to 50 % seasonal moth flight or cumulative degree days. In addition, the threshold may vary with the density of raspberry plants in the field, insecticide applications for other pests during the RCB flight period and cultural practices such as pruning the canes before egg hatch.

Our trap-catch threshold could be used as an early warning system to identify fields that require more thorough scouting for egg presence and larval hatch in October. We did not attempt to correlate trap captures with the population density of the subsequent generation of larvae because more intensive egg sampling would be necessary to establish a strong correlation. Strong correlations between trap captures of adult moths and population densities of subsequent generations, as indicated by counts of eggs, larvae or pupae, have been reported for other Lepidoptera, such as the spruce budworm, *C. fumiferana* (Clemens), the Nantucket pine tip moth, *R. frustrana* (Comstock) and the western hemlock looper, *L. fiscellaria lugubrosa* (Hulst) (Sanders 1988, Evenden et al. 1995, Asaro and Berisford 2001). A potential correlation between RCB trap catches and larval numbers could be investigated in the future when high population densities of RCB are identified through pheromone-based trapping.

Future research should focus on developing a degree-day model that predicts the timing of egg hatch. RCB eggs are laid on the upper leaves of the primocanes but neonate larvae crawl to the base of canes (Raine 1962). The new insecticide Altacor (35% chlorantraniliprole) (DuPont) was recently registered to control RCB populations. This insecticide is registered for application against first-instar larvae feeding in the cambium at the base of canes before they have tunneled into the crown (British Columbia Ministry of Agriculture and Lands 2009, Health Canada 2011). Degree-day models that use the capture of male moths in pheromone traps to predict egg hatch have been developed for many Lepidoptera including the codling moth, *Cydia pomonella* (L.) (Riedl et al. 1976, Beers and Brunner 1992, Knight 2007), eye-spotted budmoth, *Spilonota ocellana* (Denis & Schiffermüller) (McBrien and Judd 1998), obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Evenden and Judd 1999), oriental fruit moth, *Grapholita molesta* (Busek) (Borchert et al. 2004) and pecan nut casebearer, *Acrobasis nuxvorella* Nuenzig (Knutson and Meugge 2010). For RCB, a degree-day model that predicts egg hatch may need to consider rainfall events, as Raine (1962) observed egg hatch occurring after precipitation events.

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4.9. Tables

Table 4.1. Location of pheromone traps in raspberry fields and unmanaged blackberry patches in the Fraser Valley, BC

Site #	Crop	Cultivar	Region	Location	Elevation (m)
1	Raspberry	Meeker	Abbotsford	49°00'41"N 122°17'05"W	50
2	Raspberry	Meeker	Abbotsford	49°01'57"N 122°21'26"W	63
3	Raspberry	Cascade Delight	Abbotsford	49°00'29"N 122°22'48"W	50
4	Raspberry	Meeker	Abbotsford	49°00'16"N 122°24'18"W	47
5	Raspberry	Chemainus	Abbotsford	49°00'24"N 122°26'13"W	47
6	Raspberry	Cascade Delight	Abbotsford	49°02'29"N 122°26'58"W	113
7	Raspberry	Meeker	Langley	49°04'51"N 122°34'05"W	92
8	Raspberry	Malahat	Langley	49°06'53"N 122°32'21"W	93
9	Raspberry	Cascade Delight	Langley	49°07'34"N 122°33'08"W	64
10	Raspberry	Tulameen	Langley	49°08'09"N 122°33'33"W	61
11	Raspberry	Cascade Delight	Ladner	49°03'35"N 123°08'28"W	3
12	Raspberry	Cascade Delight	Ladner	49°04'34"N 123°09'23"W	1
13	Blackberry	Himalayan	Abbotsford	49°00'59"N 122°16'57"W	63
14	Blackberry	Himalayan	Abbotsford	49°01'39"N 122°17'36"W	58
15	Blackberry	Himalayan	Abbotsford	49°01'51"N 122°20'13"W	67
16	Blackberry	Himalayan	Abbotsford	49°02'49"N 122°28'13"W	105
17	Blackberry	Himalayan	Langley	49°07'48"N 122°33'38"W	62
18	Blackberry	Himalayan	Ladner	49°05'01"N 123°09'10"W	3

Table 4.2. Location of seasonal flight phenology traps in 2010 and 2011

Time	Crop	Cultivar (s)	City	Location(s)[1]
29 July – 1 Oct. 2010	Raspberry	Cascade Delight	Abbotsford	49°02’22”N, 122°27’27”W
	Raspberry	Malahat	Langley	49°00’26”N, 122°22’59”W
	Blackberry	Lochness	Abbotsford	49°07’33”N, 122°33’05”W
	Blackberry	Lochness	Langley	49°00’36”N, 122°23’13”W
28 July – 17 Oct. 2011	Raspberry	Tulameen	Langley	49°07’32”N, 122°32’49”W
	Raspberry	Malahat	Langley	49°06’50”N, 122°32’24”W
	Raspberry	Cascade Delight	Abbotsford	49°02’31”N, 122°26’57”W
	Raspberry	Meeker	Abbotsford	49°00’34”N, 122°22’50”W
	Raspberry	Malahat	Abbotsford	49°00’24”N, 122°23’02”W
	Blackberry	Chester	Langley	49°07’35”N, 122°32’39”W
	Blackberry	Lochness	Langley	49°06’46”N, 122°32’15”W
	Blackberry	Chester	Langley	49°02’23”N, 122°27’47”W
	Blackberry	Obsidian	Abbotsford	49°02’22”N, 122°27’04”W
	Blackberry	Lochness	Abbotsford	49°00’36”N, 122°23’01”W

[1] Elevation ranged between 49-114 m

Table 4.3. Location of monitoring traps in the Fraser Valley, BC in 2010

Site #	Crop	Cultivar	Region	Location	Elevation (m)
1	Raspberry	Meeker	Abbotsford	49°01'44”N 122°13'01”W	11
2	Raspberry	Meeker	Abbotsford	49°00'57”N 122°17'16”W	58
3	Raspberry	Meeker	Abbotsford	49°01'06”N 122°18'28”W	59
4	Raspberry	Meeker	Abbotsford	49°00'35”N 122°18'20”W	55
5	Raspberry	Meeker	Abbotsford	49°01'59”N 122°21'05”W	72
6	Raspberry	Meeker	Abbotsford	49°01'42”N 122°23'29”W	58
7	Raspberry	Meeker	Abbotsford	49°00'14”N 122°17'19”W	25
8	Raspberry	Chemainus	Abbotsford	49°02'31”N 122°27'18”W	106
9	Raspberry	Malahat	Abbotsford	49°00'32”N 122°22'47”W	49
10	Raspberry	Meeker	Abbotsford	49°00'26”N 122°23'16”W	48
11	Raspberry	Meeker	Abbotsford	49°01'41”N 122°28'53”W	91
12	Raspberry	Cascade Delight	Abbotsford	49°02'28”N 122°26'56”W	114
13	Raspberry	Meeker	Langley	49°04'50”N 122°34'05”W	91
14	Raspberry	Malahat	Langley	49°07'32”N 122°32'56”W	63
15	Raspberry	Malahat	Langley	49°06'53”N 122°32'23”W	92
16	Raspberry	Chemainus	Langley	49°07'49”N 122°33'09”W	65
17	Raspberry	Tulameen	Langley	49°08'14”N 122°33'50”W	66
18	Raspberry	Cascade Delight	Ladner	49°05'24”N 123°08'46”W	2
19	Raspberry	Cascade Delight	Ladner	49°04'33”N 123°09'24”W	1
20	Raspberry	Cascade Delight	Ladner	49°03'34”N 123°08'27”W	3

Table 4.4. Location of monitoring traps in the Fraser Valley, BC and Whatcom and Skagit counties, WA, in 2011

Site #	Crop	Cultivar	Region	Location	Elevation (m)
1	Raspberry	Meeker	Abbotsford, BC	49°00’35”N 122°17’37” W	55
2	Raspberry	Meeker	Abbotsford, BC	49°01’06”N 122°18’27” W	63
3	Raspberry	Meeker	Abbotsford, BC	49°01’01”N 122°19’21” W	66
4	Raspberry	Meeker	Abbotsford, BC	49°00’14”N 122°19’55” W	59
5	Raspberry	Meeker	Abbotsford, BC	49°01’53”N 122°21’28” W	64
6	Raspberry	Cascade Delight	Abbotsford, BC	49°00’32”N 122°22’47” W	50
7	Raspberry	Chemainus	Abbotsford, BC	49°00’27”N 122°23’11” W	49
8	Raspberry	Meeker	Abbotsford, BC	49°00’38”N 122°24’38” W	56
9	Raspberry	Meeker	Abbotsford, BC	49°00’12”N 122°26’44” W	52
10	Raspberry	Cascade Delight	Abbotsford, BC	49°02’31”N 122°27’20” W	106
11	Raspberry	Meeker	Langley, BC	49°01’33”N 122°28’42” W	94
12	Raspberry	Chemainus	Langley, BC	49°02’23”N 122°27’36” W	107
13	Raspberry	Meeker	Langley, BC	49°04’48”N 122°33’55” W	90
14	Raspberry	Malahat	Langley, BC	49°06’48”N 122°32’26” W	92
15	Raspberry	Coho	Langley, BC	49°07’44”N 122°32’58” W	63
16	Raspberry	Cascade Delight	Langley, BC	49°07’45”N 122°33’09” W	63
17	Raspberry	Chemainus	Langley, BC	49°08’15”N 122°33’51” W	60
18	Raspberry	Malahat	Langley, BC	49°08’08”N 122°33’39” W	64
19	Raspberry	Cascade Delight	Ladner, BC	49°05’33”N 123°08’39” W	3
20	Raspberry	Cascade Delight	Ladner, BC	49°04’33”N 123°09’24” W	1
21	raspberry	Tulameen	Everson, WA	48°56’41”N 122°17’47” W	45
22	raspberry	Cascade Bounty	Nooksack, WA	48°53’21”N 122°19’08” W	34
23	raspberry	Meeker	Lynden, WA	48°56’24”N 122°32’29” W	26
24	raspberry	Meeker	Burlington, WA	48°30’06”N 122°23’04” W	7
25	raspberry	Cascade Dawn	Rockport, WA	48°28’39”N 121°34’41” W	72

4.10. Figures

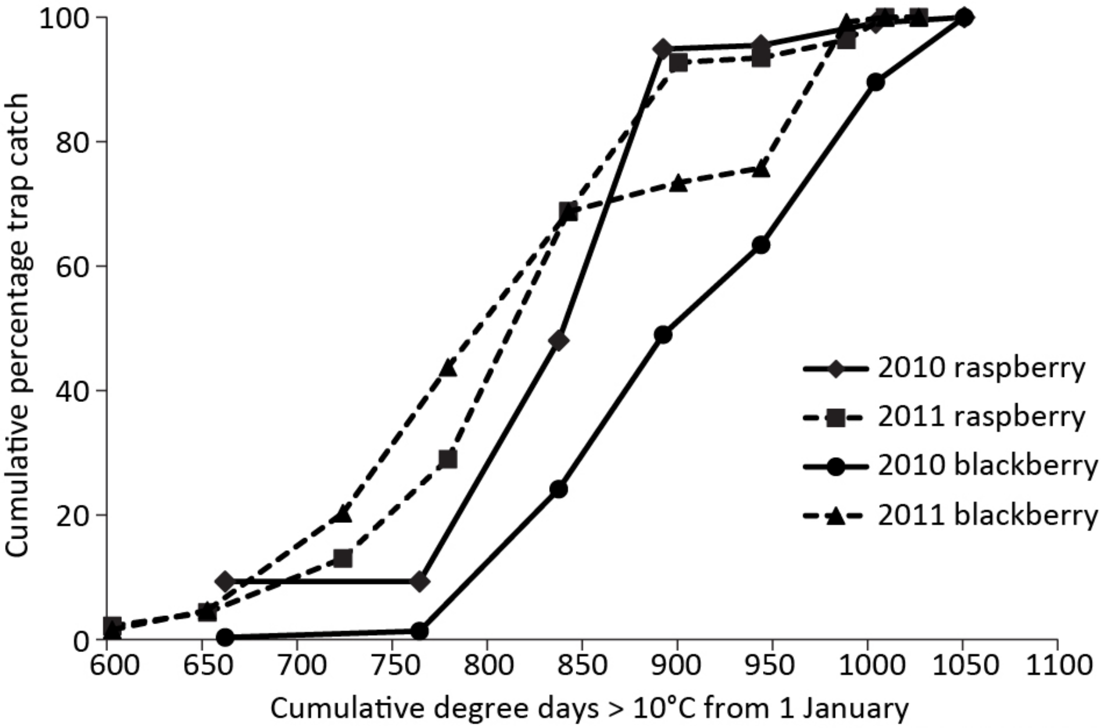

Figure 4.1. Seasonal flight phenology of Pennisetia marginata expressed as cumulative trap catches in raspberry and blackberry fields (Table 4.2) (N=2 in 2010; N=5 in 2011).

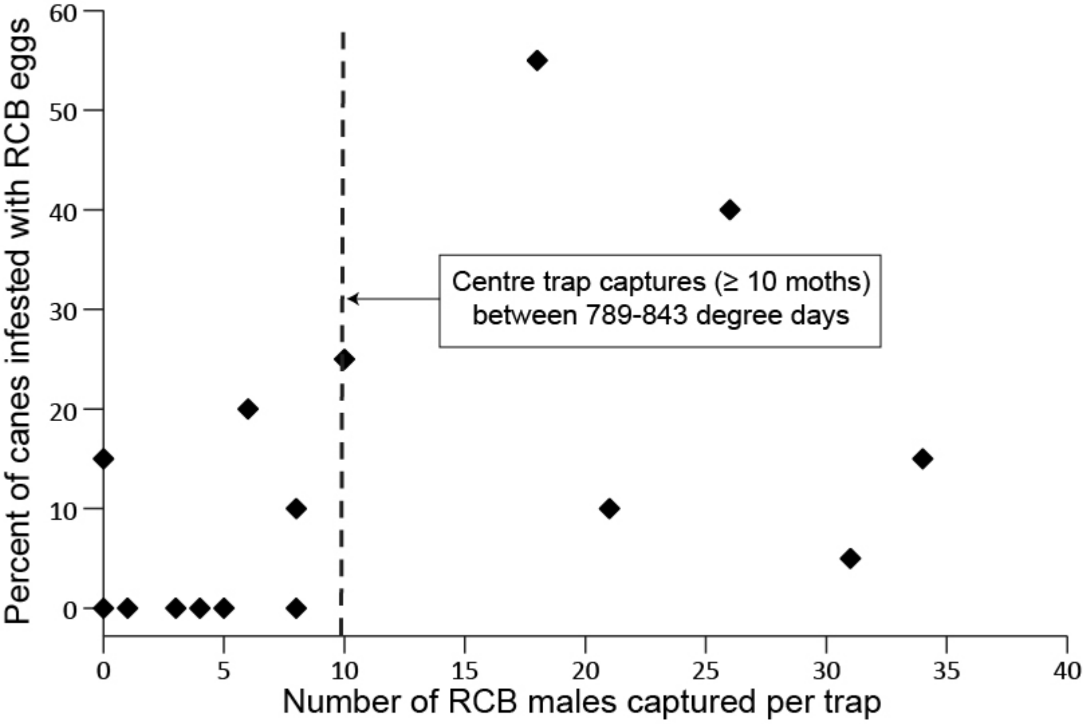

Figure 4.2. Number of male Pennisetia marginata captured between 789-843 degree days (19 August to 25 August 2010) in centre-field traps and counts of P. marginata eggs from 22 September to 1 October 2010. The dotted line indicates the trap-catch threshold above which eggs were consistently detected in the field (N=17).

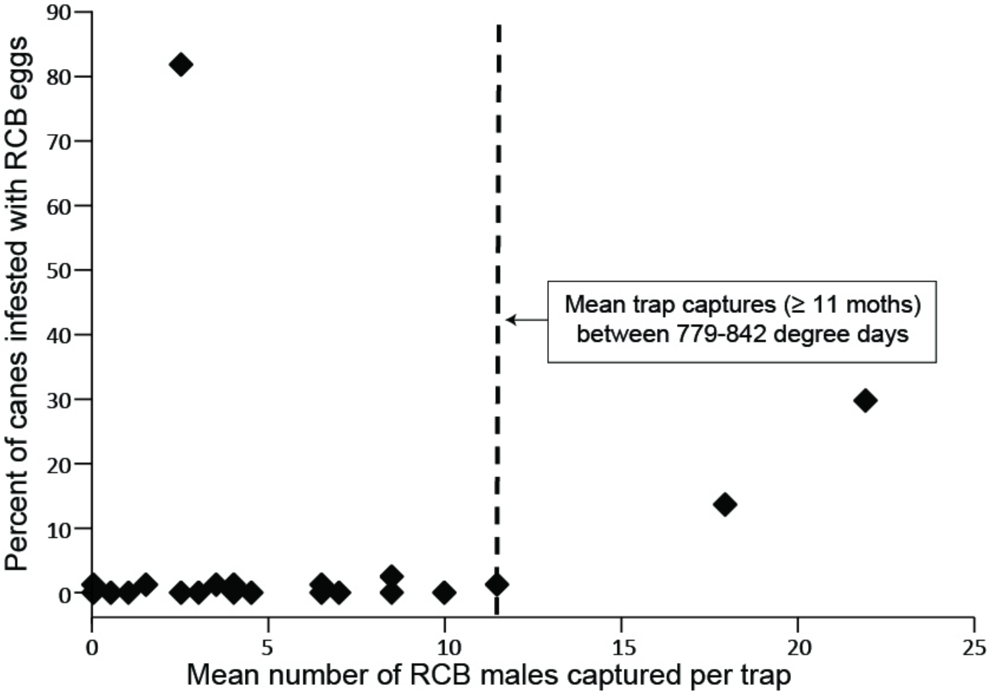

Figure 4.3. Mean number of male Pennisetia marginata captured between 779-842 degree days (1-8 September 2011) in centre-field traps and counts of P. marginata eggs from 29 September to 6 October 2010. The dotted line indicates the trap-catch threshold above which eggs were consistently detected in the field (N=24).

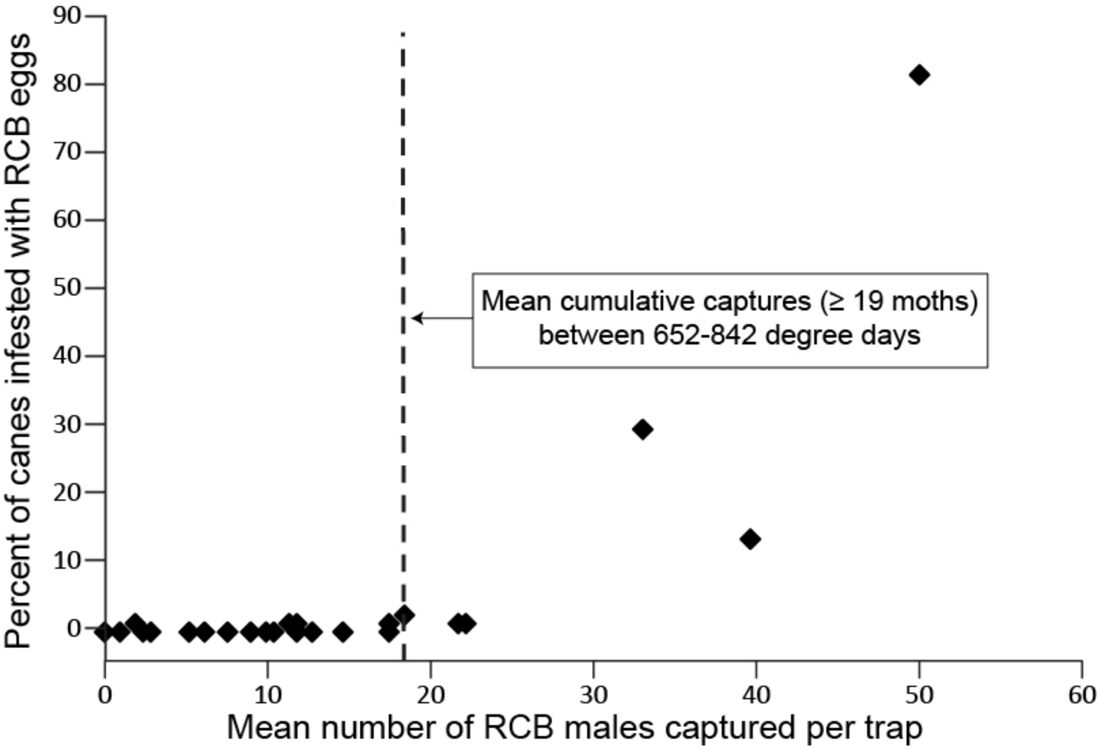

Figure 4.4. Mean cumulative number of male Pennisetia marginata captured between 652-842 degree days (18 August to 8 September 2011) in centre-field traps and counts of P. marginata eggs from 29 September to 6 October 2010. The dotted line indicates the trap-catch threshold above which eggs were consistently detected in the field (N=24).

5. Conclusions

This research has expanded the knowledge of the sexual communication of *P. marginata* and evaluated the potential of pheromone-based monitoring and management tools. Based on the experiments conducted, the following conclusions can be drawn:

- Female *P. marginata* wing fan prior to mating but the low-frequency sound associated with it has no effect on attraction of conspecific males.
- Wing traps hung within the plant canopy at either 0.3 or 1.3 m above ground are best suited for capturing male *P. marginata.*
- More male *P. marginata* were captured in traps baited with 1000-µg pheromone lures than in traps baited with 100- or 10- or µg lures. However, this 10–fold increase in pheromone dose results in less than a 2-fold increase in moth captures. For economic reasons, 100-µg pheromone lures are recommended for pheromone-based monitoring of *P. marginata.*
- The pheromone of *P. marginata* degrades quickly over time in the field. More males were captured in traps baited with fresh lures than in traps baited with lures that had been held at room temperature for 2, 4, 6, 8 or 10 days.
- Based on two seasons of trapping, the number of degree days >10°C from 1 January to first sustained captures of male *P. marginata* in raspberry and blackberry fields ranges between 582 and 619.
- Based on two seasons of trapping, the number of degree days >10°C from 1 January to 50% trap captures occurs around 840 degree days in raspberry fields. In blackberry fields, the cumulative number of degree days to 50% trap captures is more variable.
- For monitoring purposes, a mean trap-catch threshold of 10-11 moths captured per green delta trap in a one-week period between 779-843 degree-days, or a cumulative trap-catch threshold of 19 moths captured in a three-

week period between 652 to 842 degree days, is probably indicative of egg presence in raspberry fields.

- Mass trapping can reduce the males' response to low-dose (false-female) pheromone-baited traps, although the short field life of the pheromone makes mass trapping a labour-intensive management tactic.
- The pheromones [(*E*,*Z*)-3,13-octadecadienyl acetate, (*Z*,*Z*)-3,13-octadecadienyl acetate, and (*E*,*Z*)-2,13-octadecadienal] are repellent to *P. marginata* when released in combination with (*E*,*Z*)-3,13-octadecadienal, and may have use in mating disruption.

5.1. Implications for pest management

Synthetic sex pheromone lures for *P. marginata*, if frequently replaced and deployed in appropriate traps, will be useful for tracking seasonal flight phenology. Captures in pheromone-baited traps may help predict population densities and aid in management decisions. However, the instability of the sex pheromone does not lend itself to deployment for control of *P. marginata* populations through mass trapping.

5.2. Recommendations for Further Study

- Because the pheromone degrades quickly over time in the field, pheromone-based management strategies that employ more stable compounds need to be evaluated. The pheromone antagonists [(*E*,*Z*)-3,13-octadecadienyl acetate, (*Z*,*Z*)-3,13-octadecadienyl acetate, and (*E*,*Z*)-2,13-octadecadienal] should be field-tested separately from (*E*,*Z*)-3,13-octadecadienal to examine effect on male *P. marginata* behaviour. Electrophysiological and behavioural responses of male *P. marginata* to the corresponding formate [(*E*,*Z*)-3,13-octadecadien-1-yl formate] should also be evaluated.
- Trap catches and egg counts have consistently been low in the cultivar Meeker, and higher in other cultivars such as Cascade Delight. It would be worthwhile to investigate the cultivar preference of *P. marginata.*

- Pheromone trap catches were very low in fields under poly-tunnels. When the plastic was removed in mid-September, moth trap catches surged, likely due to the increase in air flow. This suggests that pheromone trapping for monitoring purposes may not be effective in raspberries grown under tunnels. However, if the plastic remains until the end of the flight period, poly-tunnels may also help protect a raspberry field from infestation.
- In fields with large numbers of *P. marginata* eggs, up to 30% of the eggs were black instead of red. These black eggs may be parasitized and the parasitoid should be identified.
- Egg hatch appears to occur in the last few weeks of October. Growers would benefit from a degree-day model based on moth flight that predicts egg hatch.