CONTACT PHEROMONE COMPONENTS AND DIEL PERIODICITY OF SEXUAL COMMUNICATION IN PEACH TWIG BORERS, *ANARSIA LINEATELLA* (LEPIDOPTERA: GELECHIIDAE)

by

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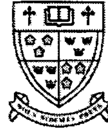

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ABSTRACT

Sexual communication of the peach twig borer moth, *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae), was re-investigated. In a field-trapping experiment, females were more attractive to foraging males than synthetic sex pheromone [(*E*)-5-decen-1-yl acetate and (*E*)-5-decen-1-ol], suggesting that females use additional communication signals. Three new pheromone components [octadecanyl acetate, (*R*)-11-methyltricosane, (*S*)-11-methyltricosane] were identified in extracts of female bodies. In laboratory experiments, these body components together with sex pheromone components induced contact by males. Body pheromone components did not enhance attractiveness of sex pheromone components in field experiments, suggesting that they are effective only at close range. Females retained a constant titre of sex pheromone in pheromone glands, but emitted pheromone and attracted males only during periods of sexual communication in the early morning hours (3:00 to 6:00 Pacific Standard Time).

We all need sustenance, he told me. The wolf, the puma, the eagle as much as the rabbit, the deer, the salmon. Even the trees and grass require nourishment that's dependent on the lives of others. Nature was never benevolent or fair. But by the same token, we have to live together in this world and cruelty is neither gracious nor defensible. So when you take from the bounty that others provide for you, bless their gift, treat it with respect, give it dignity. And always ask before you take, give thanks for what you receive.

"The Onion Girl"
Canadian author Charles de Lint

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CHAPTER 1. INTRODUCTION

1.1 Current knowledge about sexual communication in *A. lineatella*

"Chemical communication is arguably the primary mode of information transfer in the Insecta, and is well documented. Semiochemicals (message bearing chemicals) are employed for both intra- and interspecific communication" (Jutsum and Gordon, 1989). Pheromone-based sexual communication in moths is one of the best-studied communication systems in the Insecta (Phelan, 1997). Moths utilize sex pheromones as communication cues to attract or locate mates (Wyatt, 2003).

The peach twig borer, *Anarsia lineatella* Zeller, is a pest insect of almond and stone fruit worldwide. Evidence for the presence of a sex attractant pheromone in *A. lineatella* was first described by Anthon *et al.* (1971). It was identified as a 2-component blend, consisting of (*E*)-5-decen-1-ol (*E*5-10:OH) and (*E*)-5-decen-1-yl acetate (*E*5-10:OAc) (Roelofs *et al.,* 1975). Synthetic pheromone has been extensively used to monitor *A. lineatella* populations in almond and stone fruit orchards (Millar and Rice, 1996; Kehat *et al.*, 1994; Rice and Jones, 1988; Youngman and Barnes, 1985; Hathaway, 1981). It has also been assessed for pheromone-based mating disruption (Mayer and Lunden, 1996; Nicolli *et al.*, 1990) and mass trapping (Hathaway *et al.*, 1985), but the efficacy of control remained economically unacceptable. In contrast, pheromone-based mating disruption has been successfully deployed for the control of two other economically important gelechiid moths: the pink bollworm, *Pectinophora gossypiella*

Saunders (Baker *et al.*, 1990), and tomato pinworm, *Keiferia lycopersicella* Walsingham (Jenkins *et al.*, 1990).

Millar and Rice (1992) re-analyzed the sex pheromone of *A. lineatella*, hypothesizing that secondary pheromone components might play a role in courtship or copulation. Although such pheromones were not identified in *A. lineatella* they occur in other moths, such as the white-marked tussock moth, *Orgyia leucostigma* Smith (Grant *et al.*, 1987).

The periodicity of pheromone production and emission by female *A. lineatella* has hardly been studied. In almond orchards of the San Joaquin Valley (California), response periods by male *A. lineatella* commence during late evening and peak during early morning (Rice and Jones, 1975). However, the periodicity of pheromone production or emission by females, and potential correlations with the males' response periodicity, have not yet been studied. There is also no knowledge whether calling females communicate with mate-seeking males. For example, if males were to produce signals when approaching calling females, females might lower pheromone emission, and even produce (bioacoustic) reply signals, assisting males to pinpoint the microlocation of females. Such exchange of communication signals has been reported between males and females of the lesser wax moth, *Achroia grisella* Fabricius (Snedden *et al.*, 1994; Spangler *et al.*, 1984), and the greater wax moth, *Galleria mellonella* Linnaeus (Skals and Surlykke, 2000). If such a system existed in *A. lineatella*, traps baited with virgin females would be expected to be significantly more attractive to foraging males than traps baited with synthetic pheromone.

1.2 Life history of *A. lineatella*

Female moths can lay up to 115 eggs which hatch in 5-7 days. Larval development takes 10-20 days, and pupation takes 6-7 days, for an approximate complete generation time of 28 days. The pre-oviposition period can last for 1-4 days. Newly deposited eggs are white or cream coloured, acquiring an orange tint after 24 hours. They are bluntly oval, and approximately 0.4 mm by 0.2 mm, with reticulations. Eggs are deposited singly, or in groups of 2-5 on bark, leaves, or fruit of the host plant.

First instar larvae are 0.5 mm long and grow to 11 mm. There are 4 or 5 instars, depending on the rate of growth (Bailey, 1948). Neonate larvae are yellow, turning reddish-brown with a black head and black cervical and anal plates. Overwintering first and second instar larvae hibernate in cavities (hibernacula) in the bark of stems, in crotches of two- to four- year-old branches, and (rarely) in buds (Bailey, 1948; Summers, 1955) for up to 7-9 months (Bailey, 1948; Ponomarenko, 1990). Larvae resume development in the spring. They tend to enter buds and terminal shoots, boring a path downward towards the center, often until they reach the previous year's wood (Ponomarenko, 1990). A larva can damage up to five young peach shoots before completing development.

Adult moths are about 8 mm in length with a wingspan of 14 mm. The head, dorsum and forewing are light grey, with 2 distinct dark spots on the forewing, one at the middle of the costal margin and the second just behind. At rest, the anterior portion of the body is slightly raised and the large, sexually dimorphic labial palps are held upright in front of the head. When disturbed, the moths often move erratically over bark and leaves, or make short flights and alight nearby.

Depending on the geographic region and host species, *A. lineatella* can have 1-4 generations per year. Three and four generations have been recorded in California (Price and Summers, 1961), and Washington state (Brunner and Rice, 1984), three generations in the Okanagan Valley of British Columbia (Sarai, 1966), and two generations and a partial third in the Similkameen Valley of British Columbia.

In British Columbia, adults of the first generation eclose from mid-May to mid-June when developing (green and hard) peaches and apricots are only 2.5 cm in diameter. At this time, gravid females lay eggs in groups of 2-5 at the base of leaves near the petiole, at the tip of twigs, and on fruit (Bailey, 1948). Larvae of the second generation (or first summer generation) feed on apical branches at the base of leaves and enter green fruit. At the end of July, pupation usually occurs next to the stem immediately outside the fruit that was attacked previously, often concealed by floral residues.

The second generation of adults ecloses from mid-July to mid-August, and has the greatest propensity to cause damage in peach orchards. It is at this time that females will lay the greater proportion of eggs on ripening fruit (Bailey, 1948; Ivanova, 1995). Late-ripening peach varieties have a higher incidence of 'stung' peaches (Bailey, 1948; Weakley *et al.*, 1990). Development of third-generation larvae depends on the location of the eggs. Larvae hatching from eggs on mature fruit re-enter the fruit and develop into the third generation of adults (Ponomarenko, 1990). However, larvae hatching from eggs on the bark of trunks and branches make cavities for hibernation and remain in the bark.

1.2.1 Distribution

Anarsia lineatella is of European or Western Asian origin (Marlatt, 1898) and was first described by Zeller in Germany in 1839. Since then, it has been reported in all major growing areas of its host trees in North America, Europe, and Asia (Marlatt, 1898; Jones, 1935, Bailey, 1948; Ahmad, 1988; Ponomarenko, 1990). It was first found in New York state (US) in 1872, and was first reported in Canada in 1902 (Belton, 1988). It has been a pest in the coastal and interior areas of BC since 1908 (Madsen and Arrand, 1971). All major host plants belong to the genus *Prunus* in the family Rosaceae, which comprises approximately 400 species of trees and shrubs. There are seven subgenera in *Prunus* (Strasburger *et al.*, 1991), four of which contain the main host plants of *A. lineatella*; *Amygdalus* (almonds), *Persica* (peaches), *Armenica* (apricots), and *Prunus* (plums and prunes). The principal host plants of *A. lineatella* are almond and peach, but it has been reported on apricot, nectarine, plum and prune (Summers, 1955), and also on sweet and sour cherry, apple and persimmon (Ponomarenko, 1990). These fruit are valued worldwide as fresh products for immediate consumption, or for the production market, including drying, distilling, canning, and producing jams, syrups and juices.

1.2.2 Pest status

Anarsia lineatella is considered one of the most important pests of almonds and stonefruit in North America, Europe and Asia (Bailey, 1948; Summers *et al.*, 1959; Millar and Rice, 1992). In peach orchards, most economic damage is caused when larvae burrow into fruit. They typically mine cavities just beneath the skin, discolouring the fruit and causing the exudation of gum and frass. Although the extent of damage can be minor, the aesthetic value of the fruit is reduced, thereby increasing picking and culling costs.

Fruit damage also increases susceptibility to other pests and premature putrefaction. In almond orchards, economic damage occurs primarily in soft-shelled varieties because larvae can advance into the kernel, consuming the meat, and also providing access for weaker borers like the navel orangeworm, *Ameylois transitella* Walker (Curtis, 1983; Legner and Gordh, 1992). In both peach and almond orchards, severe shoot damage can stunt and kill small trees, leading to recommendations for prophylactic sprays, even in young non-bearing orchards (Summers, 1955).

The greatest damage caused by *A. lineatella* occurs in peach orchards. Before the introduction of the oriental fruit moth, *Grapholita molesta* Busck, *A. lineatella* was the most serious pest of peaches in California (Weakley *et al.*, 1990). In 1931, infestation levels reached an average of 10% in California peaches, and more recently, "in severe cases, a grower can lose between 60 percent and the entire crop" (Abbott, 1996). Since the early 1970s, the recommended control of *A. lineatella* has consisted of dormant sprays of organophosphate insecticide and oil on overwintering larvae (Rice *et al.*, 1972). Further insecticide sprays, pre- or post-bloom, target larvae of spring and summer generations, respectively. However, this pesticide use has created health and environmental concerns, led to development of insecticide resistance (Summers *et al.*, 1959), and also reduced natural enemies of other pests such as the green peach aphid (Tamaki, 1973). Moreover, because other orchard pests, such as *G. molesta*, are controlled without the use of insecticides (Rice and Kirsch, 1990), there is increasing pressure to employ alternative tactics for control of *A. lineatella*.

Bacillus thuringiensis has been applied when overwintering larvae emerge and initiate surface feeding (Barnett *et al.*, 1993). Dormant season application of nematodes

reduced levels of *A. lineatella* but not to commercially acceptable levels (Agudelo-Silva *et al.*, 1995). Semiochemical-based control has to date met with little success (Hathaway *et al.*, 1985; Nicolli *et al.*, 1990). Removal of males using pheromone-baited traps did not reduce damage to commercially acceptable levels (Hathaway *et al.*, 1985). Sex pheromone-based mating disruption has produced inconclusive results (Millar and Rice, 1992; Rotundo and Viggiani, 1996). The aim of mating disruption is to stop fertilization of eggs by preventing adult males and females from finding each other (Wyatt, 2003). If improved, this method could be an important part of an integrated management system for *A. lineatella*.

1.2.3 Rearing

The first rearing method for *A. lineatella* (Anthon *et al.*, 1971) was complicated and labour intensive, prompting McElfresh and Millar (1993) to develop a much improved protocol which formed the basis for the method by Sidney (in preparation) followed in this study.

1.3 Objectives

My objectives were to:

1) Test the hypothesis that the known two-component (*E*5-10:OAc and *E*5-10:OH) synthetic sex pheromone is as effective as virgin female *A. lineatella* in attracting conspecific males;
2) Test the hypothesis that pheromone components derived from body scales of females are part of the sexual communication system in *A. lineatella*;
3) Investigate the diel periodicity of sexual communication in *A. lineatella*; and,
4) Test the hypothesis that the presence of male *A. lineatella* affects pheromone emission by female *A. lineatella*.

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CHAPTER 2. CONTACT PHEROMONE COMPONENTS OF FEMALE *A. LINEATELLA*

2.1 Introduction

Pioneering work by Roelofs *et al.* (1975) identified a two-component sex pheromone consisting of (*E*)-5-decen-1-yl acetate (*E*5-10:OAc) (87%) and (*E*)-5-decen-1-ol (*E*5-10:OH) (13%) in pheromone gland extracts of female *A. lineatella* that attracted conspecific males. Synthetic pheromone released from various dispensers and traps were tested (Rice and Jones, 1975; Hathaway, 1981, Kehat *et al.*, 1994) as tools for monitoring populations of *A. lineatella* in commercial fruit orchards. Deployment of synthetic pheromone for control of *A. lineatella* by pheromone-based mating disruption yielded unsatisfactory results (R. E. Rice, personal observation; cited in Millar and Rice, 1992). Although comparative attractiveness of virgin female *A. lineatella* and synthetic sex pheromone had not been tested, it was concluded that there might be additional as yet unknown pheromone components. Re-analysis of the pheromone of *A. lineatella* led to the identification of several candidate pheromone components [decyl acetate, (*E*)- and (*Z*)-4-decenyl acetate, (*E,E*)-3,5- and (*Z,E*)-3,5-decadienyl acetates] (Millar and Rice, 1992) but none enhanced long-range attractiveness of the previously identified two-component blend (Roelofs *et al.*, 1975). These results suggested that if additional pheromonal communication signals existed, they probably were present in, or released from, body parts other than abdominal pheromone glands.

Contact- or copulation-inducing pheromones are typically present on the body surface of (signalling) insects. Although they appear effective only at short range, they often complement attractiveness of long-range sex or aggregation pheromones in the mating sequence. Close-range pheromones have been noted, or the pheromones have been identified, in several orders of the Insecta, including Diptera (Stoffolano *et al.*, 1997), Hymenoptera (Kimani and Overholt, 1995), Coleoptera (Ginzel *et al.*, 2003), Isoptera (Clement, 1982), and Lepidoptera (Grant *et al.*, 1987). In white-marked tussock moths, *Orgyia leucostigma*, female body scales contain straight-chain hydrocarbons that induce copulatory attempts by males (Grant *et al.*, 1987). Conceivably, similar pheromone components may exist in *A. lineatella,* and play a role in short-range communication among males and females.

My objectives were to test the hypotheses (1) that the known two-component (*E*5-10:OAc and *E*5-10:OH) synthetic sex pheromone is as effective as virgin female *A. lineatella* in attracting conspecific males; and (2) that pheromone components derived from body scales of females are part of the sexual communication system in *A. lineatella.*

2.2 Materials and methods

2.2.1 Field testing relative attractiveness of females and synthetic pheromone

The relative attractiveness of female *A. lineatella* and synthetic pheromone was tested in experiment 2.1 in a 2-ha peach orchard 5 km east of Keremeos, BC. The fruit on five varieties of trees (12-15 years old, spaced 3 × 4.5 m apart) ripened consecutively from mid-July to mid-September). Delta-type traps made from 2-L milk cartons (Gray *et al.*, 1991) were coated with Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI,

USA) and suspended from trees 1 m above ground. One of four treatments was assigned to each trap: (1) two virgin female *A. lineatella* (2-4 days old) in a plastic cage (3.25 × 5.25 cm) with mesh top and bottom; (2) an empty cage as control; (3) a gray rubber septum (The West Company, Lionville, PA, USA) impregnated with synthetic *E*5-10:OAc (1000 μg) and *E*5-10:OH (100 μg) dissolved in 40 μl of HPLC-grade hexane; and (4) a control septum impregnated with 40 μl of HPLC grade hexane. Two traps were suspended from each of 20 trees, with treatment and corresponding control stimuli randomly assigned to east or west sectors of each tree. These paired replicates were separated by one tree without traps. Captures of male moths were analyzed statistically using Friedman's test, a nonparametric randomized block analysis of variance based on ranks, followed by a Student-Newman-Keuls multiple comparison of means (Zar, 1996) using SAS® Version 8 (SAS Institute, Cary, NC, USA).

2.2.2 Extraction of pheromone glands and moth bodies

Separate groups of 2-5 day old, 10 male or 10 female moths each were submerged in pentane. After 5 min, the supernatant was withdrawn, and pipetted into a new vial. This procedure was repeated twice with the same group of moths and extracts were then combined and concentrated such that 12.5 μl equalled one body-extract equivalent.

2.2.3 Analyses of extracts

Aliquots of body extracts were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn *et al.*, 1975; Gries *et al.*, 2002), employing a Hewlett-Packard (HP) 5890 gas chromatograph fitted with a GC column (30 m × 0.25 or 0.32 mm ID) coated with DB-5, DB-23, DB-210 (J&W Scientific, Folsom,

CA, USA) or SP-1000 (Supelco, Bellefonte, PA, USA). For GC-EAD recordings, an antenna was gently pulled from an insect's head, the distal segment removed, and then suspended between glass capillary electrodes filled with Ringer's solution [NaCl (6.5g/L), KCl (1.4 g/L), $CaCl_2$ (0.12 g/L), Na_2CO_3 (0.1 g/L), Na_2HPO_4 (0.01 g/L)] in distilled water. Coupled GC-mass spectrometric (MS) analyses of pheromone extract [300 females equivalent (FE)] and of synthetic standards employed a Varian Saturn 2000 Ion Trap GC-MS fitted with the above-referenced DB-5 column.

2.2.4 Syntheses of candidate pheromone components*

2.2.4.1 Syntheses of (Z)-11-eicosenyl acetate and racemic methylated hydrocarbons

(*Z*)-11-Eicosenyl acetate (Sugarawa *et al.*, 1978) was obtained by reduction of (*Z*)-11-eicosanoic acid (Aldrich) with lithium aluminum hydride in THF to (*Z*)-11-eicosen-1-ol, and acetylation of this alcohol (Pederson *et al.*, 2002) with acetic anhydride in the presence of pyridine.

Previously reported methylated hydrocarbons 11-methyltricosane (11me-23Hy), 2-methyltetracosane (2me-24Hy), 11-methylpentacosane (11me-25Hy), and 13-methylheptacosane (13me-27Hy) (Jackson, 1970; Tarvita and Jackson, 1970; Howard *et al.*, 1978; Tsuda *et al.*, 1981; Lange, 1993; Szafranek *et al.*, 1994; Finidori-Logli *et al.*, 1996; Wagner *et al.*, 1998; Haverty *et al.*, 2000) were synthesized from corresponding carbonyl precursors and ylids by Wittig reactions, and by subsequent hydrogenation of the resulting olefins in the presence of platinum oxide.

* All syntheses were conducted by Dr. Grigori Khaskin, Gries - Laboratory, Simon Fraser University

2.2.4.2 Syntheses of (*R*)- and (*S*)-11-methyltricosanes (8 and 12; Figure 2.1)

tert-Butyldimethylsilylchloride (3.50 g; 1.1 equiv.) and 1.60 g (1.1 equiv.) of imidazole were added to 2.50 g of methyl (*R*)-3-hydroxy-2-methyl propionate **1** (21.2 mmol; Aldrich) dissolved in 10 ml DMF. After stirring overnight at room temperature, methyl (*R*)-3-*tert*-butyldimethylsilyloxy-2-methyl-propionate **2** was obtained in quantitative yield. Borane reduction of silyl ether **2** with 45 ml of a 1.0 M solution of BH_3 in a THF matrix under argon yielded known (*S*)-2-methyl-3-*tert*-butyldimethylsilyloxy-1-propanol (**3**) (King *et al.*, 1995) after 48 hr. Ether **3** (quantitative yield) was isolated by quenching the reaction mixture with concentrated aq. $NaHCO_3$. The product was extracted with a 1:1 mixture of ether/hexane (3 × 50 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*.

All monosilyl ether **3** (> 99% pure, GC) was converted to (*R*)-mesylate **4** (King *et al.*, 1995) at 0°C in dichloromethane with 1.1 equiv. of methanesulfonyl chloride and 1.5 excess of triethylamine. After 30 min of vigorous stirring at 0°C, the mixture was allowed to warm to room temperature (rt) and quenched with water. The organic layer was extracted with hexane, washed with 0.5 M HCl, conc. aq. $NaHCO_3$, and brine, and dried ($MgSO_4$). After removal of excess solvents at 15 mm Hg, 10 ml of dry THF was added to the sulfonate. The mixture was transferred slowly *via cannula* under argon pressure to a stirred suspension of Grignard reagent [freshly prepared from 10.5 ml (55 mmol) of *n*-nonyl bromide and 2.7 g (111 mmol) of Mg] and CuI (0.84 g, 4.4 mmol) in 100 ml of THF at −23°C]. After 1 hr, the reaction mixture was warmed to rt and quenched with a concentrated aqueous NH_4Cl solution. The organic layer was extracted with hexane (2 × 75 ml), washed with water and brine, and dried (Na_2SO_4). The product was concentrated

in vacuo and filtered through 10 g of silica to yield crude (*S*)-2-methyl-1-(*tert*-butyldimethylsilyloxy)-dodecane (**5**). Without any further purification, the silyl protective group was removed by stirring **5** with an excess of tetrabutylammonium fluoride in THF/H_2O overnight. Alcohol **6** was extracted from the reaction mixture with 100 ml of ether/hexane (1:1), and washed with water and brine. The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. Flash column chromatography [50 g of silica, hexane/ether as eluent with gradual increase (5 to 15%) of the ether content] afforded 2.30 g (11.5 mmol, 54% yield based on propionate **1**) of 96% pure (*S*)-2-methyl-1-dodecanol (**6**) $[\alpha]^{23}_D = -8.4$ °C (c 1.0; $CHCl_3$). Anal. calcd. for $C_{13}H_{28}O$ (%): C, 77.93; H, 14.09, found: C, 77.80, H, 14.01. ^1H NMR ($CDCl_3$) δ (ppm): 0.87 (t, 3H, J = 7.0 Hz), 0.90 (d, 3H, J = 6.7 Hz), 1.22-1.40 (m, 17H), 1.59 (m, 2H), 3.39 (dd, 1H, J = 6.2, 10.5 Hz), 3.49 (dd, 1H, J = 6.2, 10.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 14.08, 16.54, 22.66, 26.96, 29.32, 29.62, 29.63, 29.65, 29.93, 31.90, 33.13, 35.73, 68.35.

Mesylation of alcohol **6** (2.00 g, 10.0 mmol) (conditions, reagent ratio, and work-up as described for conversion of alcohol **3** to mesylate **4**), and immediate Grignard coupling of methanesulfonate **7** with 10-undecen-1-yl-magnesium bromide in the presence of CuI [7.60 ml (35.0 mmol) of 11-bromo-undec-1-ene (Aldrich), 1.70 g (70 mmol) of Mg, and 0.57 g (3.0 mmol) of CuI; reaction conditions and work-up as described for the synthesis of ether **5**] yielded (*S*)-13-methyl-1-tridecene **9** (28%) with the following impurities in the mixture: 1,9-undecadiene (7%), 1-undecene (52%), 10-undecen-1-ol (2%), 1,21-docosadiene (7%), alcohol **6** (2%), and mesylate **7** (1%). Polar impurities were removed by filtering the mixture through 10 g of silica with hexane. Filtrates containing hydrocarbons were concentrated *in vacuo* and added to a cold

solution of 11.2 g (77% pure, 50 mmol) of *m*-chloroperbenzoic acid (Aldrich) in 20 ml of CH_2Cl_2. The mixture was stirred for 3 hr at 0 °C, allowed to warm to rt, and then quenched with 100 ml of 1N NaOH. The organic layer was extracted with ether (2 × 50 ml), washed twice with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*, yielding a mixture of mono- and di-epoxides. Flash column purification (50 g of silica, 2% ether in hexane as eluent) of this mixture gave 3.10 g of epoxide **10** (61% pure by GC) with 1,2-epoxyundecane as the main impurity (30%). No di-epoxides were present as impurities. De-epoxidation of the complex mixture containing **10** was carried out with freshly prepared triphenylphosphonium selenide [obtained by stirring 8.26 g (31.5 mmol) of TPP and 2.49 g (31.5 mmol) of Se for 30 min] in 50 ml of CH_2Cl_2 with 1 ml of trifluoroacetic acid (Clive, 1978). After 1 hr of stirring at rt, solvents were removed *in vacuo*. The mixture was filtered through 20 g of silica with 150 ml of hexane. Olefin **9** (65% pure by GC) was then hydrogenated in hexane with 10% Pd/C (3 hr). The catalyst was eliminated by filtering through 5 g of silica, and solvent was removed *in vacuo* at 15 mm Hg. Undecane and other low-boiling impurities were removed at 2-3 mm Hg (70 °C, 2 hr), yielding >98% pure (*S*)-11-methyltricosane (**8**) (1.68 g, 4.96 mmol, 50% yield based on alcohol **6**, overall yield 26.5%). Anal. Calcd. for $C_{24}H_{50}$ (%): C, 85.12; H, 14.88, found: C, 85.06; H, 15.08. ^{1}H NMR (in $CDCl_3$): δ (ppm) 0.83 (d, 3H, J = 6.6 Hz), 0.88 (t, 6H, J = 6.9 Hz), 1.18-1.37 (m, 41 H); ^{13}C NMR (in $CDCl_3$): δ (ppm) 14.09; 19.70; 22.67; 27.06; 29.34; 29.63-29.68 (several unresolved peaks), 30.00, 31.90, 32.72, 37.07.

Coupling of the mesylate **7** with 1-undecylmagnesium bromide leads directly to hydrocarbon **8** which was impossible to separate from by-product docosane. In the

reaction mixture, **8** comprised 20%; after the removal of low-boiling and polar impurities it was ~60% pure.

(*R*)-11-Methyltricosane (**12**) was synthesized through the same route, starting with methyl (*S*)-3-hydroxy-2-methylpropionate (**11**) (overall yield 20%). GC retention times and NMR data matched those of (*S*)-11-methyltricosane (**8**). Optical rotation for intermediate (*R*)–2-methyl-1-dodecanol: $[\alpha]^{23}_{D} = +6.1$ °C (c 7.7; $CHCl_3$).

2.2.5 Laboratory experiments of candidate contact pheromone components

Candidate body pheromone (BP) components were tested in laboratory bioassays, employing a mesh (200 μm) cage (90 × 90 × 100 cm) (BioEquip Products Inc, Rancho Dominguez, CA, USA), with one of two test stimuli randomly assigned to opposite corners of the cage. A test stimulus consisted of a white Teflon® decoy (0.25 × 0.75 cm) pinned to the center of an inverted Petri dish (10 × 2 cm), and impregnated with gland pheromone (GP) components or GP plus candidate BP components. For each replicate, ten 2-4 day old males were introduced into each cage and acclimatized for 12 hr to environmental conditions (23 °C; >70% rh; 16L:8D) before bioassays. Bioassays were initiated by introducing test stimuli, starting a custom-designed computer program (Raymond G. Holland, Electronic Supervisor, Science Technical Centre, SFU, unpublished) that simulated a dawn environment by increased intensity of the light source (60 watt Phillips incandescent light bulb) from 0 to 600 lux within 15 min, and by manually turning on a desk swing fan (Windmere, Miramar, FL, USA) behind the bioassay cage which delivered intermittent pulses of moving air (0.3 m/sec). For each bioassay, numbers of contacts with test stimuli were recorded for 15 min. Repeated contacts by the same male were recorded, if that male was further than 1 body length

from the stimulus between consecutive contacts. Each of 15 replicates per experiment employed a new set of 10 males and test stimuli.

In experiments 2.2-2.7, two synthetic GP components [*E*5-10: OAc (100 ng) and *E*5-10:OH (10 ng)] were tested alone or in combination with: a) body extract of females at 10 FE (Exp. 2.2); b) a complete synthetic blend of candidate BP components, consisting of two acetates [octadecanyl acetate (18:OAc), (*Z*)-11-eicosenyl acetate (*Z*11-20:OAc)], four methylated hydrocarbons [11-methyltricosane (11me-23Hy); 2-methyltetracosane (2me-24Hy); 11-methylpentacosane (11me-25Hy), 13-methylheptacosane (13me-27Hy)], and eight straight-chain hydrocarbons [docosane (22Hy), tricosane (23Hy), tetracosane (24 Hy), pentacosane (25Hy), octacosane (28Hy), nonacosane (29Hy) and triacontane (30Hy)] (Exp. 2.3); c-f) BP *minus* the two acetates 18:OAc and *Z*11-20:OAc (Exp. 2.4), BP *minus* all hydrocarbons (Exp. 2.5), BP *minus* methylated hydrocarbons (Exp. 2.6), or BP *minus* aliphatic hydrocarbons (Exp. 2.7).

Taking into account that BP blends lacking acetates (Exp. 2.4) or methylated hydrocarbons (Exp. 2.6) were not effective in increasing the number of body contacts, follow-up experiments 2.8-2.13 explored which acetate (Exps. 2.8-2.9), or methylated hydrocarbon(s) (Exps. 2.10-2.13) contributed to behavioural activity of the BP blend. Considering that 18:OAc appeared more effective than *Z*11-20:OAc (Exp. 2.8-2.9), and that 11me-23Hy was the single most effective methylated hydrocarbon (Exp. 2.12), experiments 2.14-2.16 investigated which enantiomer of 11me-23Hy was behaviourally active, by testing GP alone or in combination with 18:OAc plus (*S*)-11-methyltricosane [(*S*)-11me-23Hy] (Exp. 2.14), (*R*)-11-methyltricosane [(*R*)-11me-23Hy) (Exp. 2.15), or both (1:1) (Exp. 2.16). With the presence of both the *R*- and *S*-enantiomers of 11-

methyltricosane needed for males to respond (Exp. 2.16), experiment 2.17 tested GP plus female body extract *versus* GP plus synthetic 18:OAc and (*R*)- and (*S*)-11me-23Hy at equivalent ratios and quantities. Paired mean contacts of paired stimuli by male moths were analyzed statistically using paired *t*-tests (Zar, 1996). All statistical analyses were performed with JMP® Version 4 (SAS Institute, Cary, NC, USA).

2.2.6 Field testing of candidate pheromone components

Field experiments were conducted in apricot, peach, almond, plum, and nectarine orchards in British Columbia, Washington, and California, using Delta-type traps as described above. For each experiment, two traps were suspended 1-3 m above ground from each of 20 trees, with treatment and control stimuli randomly assigned to traps. Trees carrying paired traps were separated by 4-5 m. Traps were baited with gray rubber septa impregnated with the known pheromone blend [*E*5-10:OAc (1000 μg) and *E*5-10:OH (100 μg)] alone or in combination with additional candidate pheromone components. Captures of male moths were recorded 24 or 48 hours after experiment initiation, and data were analyzed by paired t-tests or ANOVA, using JMP® Version 4 (SAS Institute, Cary, NC, USA). Results are tabulated in the appendix.

2.3 Results

2.3.1 Relative attractiveness of female moths and synthetic pheromone

In the field, traps baited with virgin female *A. lineatella* captured significantly more males than those baited with synthetic pheromone, which in turn captured more males than unbaited traps (Figure 2.2).

2.3.2 GC-EAD and GC-MS analyses of female body extracts

GC-EAD analyses of body extracts from female *A. lineatella* revealed numerous compounds that elicited responses from male antennae and several that did not (Figure 2.3). In GC-MS analyses, two of these EAD-active compounds with fragmentation ion *m/z* 61 (indicative of an acetate functionality) and with molecular ions *m/z* 312 and *m/z* 338 were identified as octadecanyl acetate (18:OAc) and eicosenyl acetate, respectively. Dimethyl-disulfide treatment (Dunkelblum *et al.*, 1985) of the latter without prior isolation yielded an adduct with GC-MS fragmentation ions *m/z* 173 and *m/z* 259, indicative of a double bond at C11. This compound was thus postulated and, through comparative GC-MS of an authentic standard, confirmed to be (*Z*)-11-eicosenyl acetate (*Z*11-20:OAc).

Mass spectra of other EAD-active compounds in female body extracts suggested they were saturated hydrocarbons. Four of these compounds had retention indices (Van den Dool and Kratz, 1963) indicative of methyl branches. Their mass spectra revealed fragmentation ions diagnostic of methyl branch positions (Pomonis *et al.*, 1980; Francke *et al.*, 1987, 1988; Gries *et al.*, 1991, 1993, 1994), and suggested that these compounds were 11me-23Hy, 2me-24Hy, 11me-25Hy and 13me-27Hy, respectively. Comparative GC-MS of insect-produced and authentic standards confirmed these structural assignments.

2.3.3 Laboratory experiments with candidate contact pheromone components

Teflon® decoys impregnated with body extracts from female *A. lineatella* at 10 FE plus GP components provoked more decoy contacts by male *A. lineatella* than GP components alone (Figure 2.4; Exp. 2.2). Similarly, a synthetic blend of all candidate BP

components at ratios and concentrations equivalent to10 FE plus GP provoked more contacts by males than GP alone (Figure 2.4; Exp. 2.3). Synthetic BP blends lacking aliphatic hydrocarbons were still bioactive (Figure 2.4; Exp. 2.7), but BP blends lacking acetates (Figure 2.4; Exp. 2.4), all hydrocarbons (Figure 2.4; Exp. 2.5), or all methylated hydrocarbons (Figure 2.4; Exp. 2.6) were not. Neither *Z*11-20:OAc nor 18:OAc alone significantly enhanced the attractiveness of GP (Figure 2.5; Exps. 2.8, 2.9), but 18:OAc did, combined with four methylated hydrocarbons (Figure 2.5; Exp. 2.10).

Deleting a group of two or single methylated hydrocarbons from the BP blend determined that only 11me-23Hy, in addition to 18:OAc, is needed to retain the blend's behavioural activity (Figure 2.5; Exps. 2.11-2.13).

The *R*- and *S*-enantiomer of 11me-23Hy in combination, but not singly, are BP pheromone components (Figure 2.6; Exp. 2.16). A BP blend with the *S*-enantiomer alone was benign (Figure 2.6; Exp. 2.14), and with the *R*-enantiomer alone inhibitory (Figure 2.6; Exp. 2.15). The three-component BP blend consisting of 18:OAc, (*R*)-11me-23Hy, and (*S*)-11me-23Hy was as effective as a body extract in provoking contacts by male *A. lineatella* (Figure 2.6; Exp. 2.17).

2.4 Discussion

Significantly more captures of male *A. lineatella* in traps baited with conspecific virgin females than in those baited with a two-component synthetic sex pheromone (Figure 2.2) strongly suggests that females use additional communication signals to attract mate-seeking males.

Close-range signals include pheromone components from the females' body surface that provoke contact by males. These contact pheromone components are perceived by the males' antennae (Figure 2.3), and enhance the behavioural activity of long-range sex pheromone components (Roelofs *et al.*, 1975) released from abdominal pheromone glands.

Although numerous compounds are extractable from the females' body surface and elicit responses from male antennae (Figure 2.3), the females' body pheromone seems to comprise only three components: 18:OAc, (*R*)-11me-23Hy and (*S*)-11me-23Hy (Figures 2.5, 2.6). Positive responses by males only to BP blends containing both the *R*- and *S*-enantiomer of 11me-23Hy (Figure 2.6; Exps. 2.14-2.16), and even inhibition of response to blends containing only the *R*-enantiomer (Figure 2.6; Exp. 2.15), clearly indicate that (*R*)- and (*S*)-11me-23Hy are BP components of female *A. lineatella.*

Body pheromone components may serve as ultimate cues to confirm the proper species, and sex, of a prospective mate. The two acetates, 18:OAc and *Z*11-20:OAc, are indeed present only in body extracts of female but not male *A. lineatella* (data not shown), suggesting that they may help males recognize females. In contrast, 11me-23Hy is present in body extracts of both males and females (data not shown), suggesting that it is not suitable for mate recognition. However, considering that both enantiomers of 11me-23Hy were required to induce positive responses by males, the presence of only one (*R*) or both enantiomers may help reveal the signaller's sex. In field experiments, those contact pheromone components had no effect on long-range attraction of male *A. lineatella*, clearly indicating that they play a role only at close-range before or during courtship.

This type of close- and long-range communication system with components from sex pheromone glands and body scales has also been reported in other species of moths. Live female gypsy moths, *Lymantria dispar*, or physical models of female *L. dispar* baited with sex pheromone and covered with female abdominal scales, elicited copulatory responses by males, whereas exposure of males to sex pheromone alone did not (Charlton and Cardé, 1990). Similarly, males of the smaller tea tortrix moth, *Adoxophyes orana,* will attempt copulation only in the presence of female-produced sex pheromone and scales (Shimizu and Tamaki, 1980).

In conclusion, this study has revealed contact pheromone components derived from scales of female *A. lineatella* which, together with gland-derived sex pheromone components, induce contacts by males. Contact pheromone components do not enhance the efficacy of sex pheromone in attracting males in the field (Appendix). Thus, the superior attractiveness of virgin female *A. lineatella* as a trap-bait, compared to synthetic sex pheromone (Figure 2.2), must be due to other signals from females.

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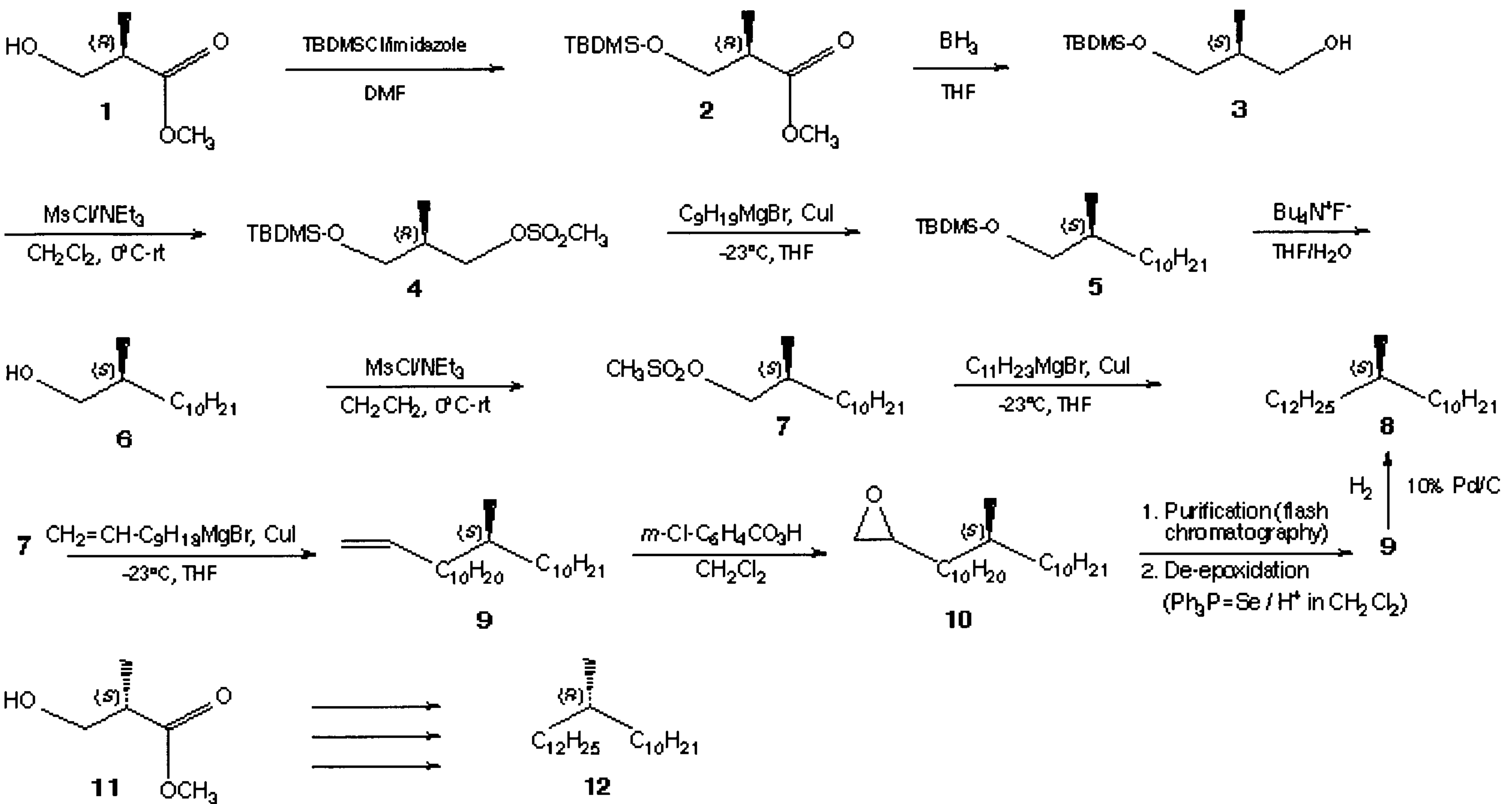

Figure 2.1. Scheme for the syntheses of (*R*)- and (S)-11-methyltricosane.

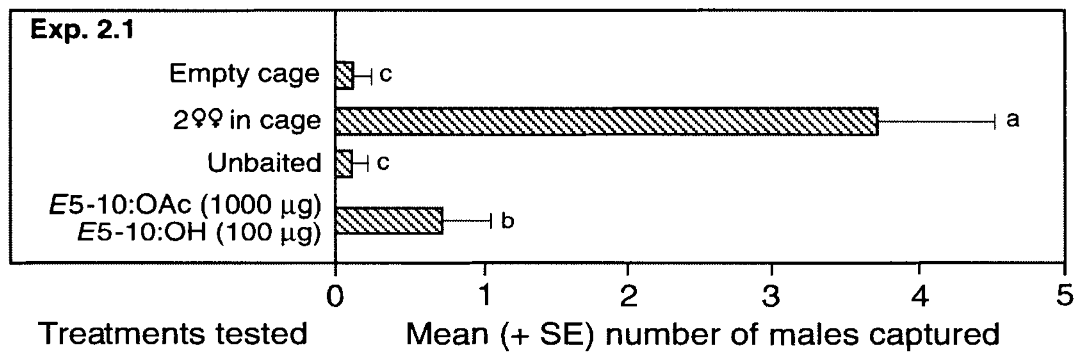

Figure 2.2. Comparison of captures of male *Anarsia lineatella* in experiment 2.1 (10 replicates; 21 June 2002) in sticky Delta traps baited with a 2-component synthetic pheromone blend or two caged virgin female *A. lineatella* in a peach orchard near Keremeos, BC. Bars with different letter superscripts are significantly different; nonparametric analysis of variance by ranks (Friedman's test; F-ratio = 16.76; df = 3; $P<0.0001$) followed by comparison of means (Student-Newman-Keuls' test) (Zar, 1996) using SAS® Version 8 (SAS Institute, Cary, NC, USA).

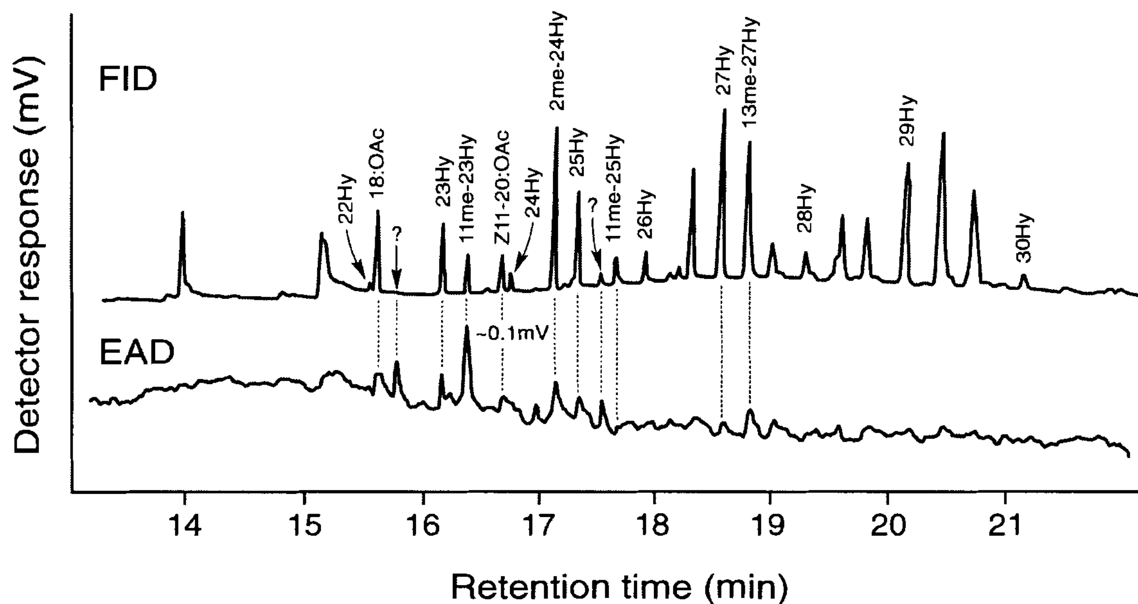

Figure 2.3. Representative recording (n = 5) of flame ionization detector (FID) and electroantennographic detector (EAD: male *Anarsia lineatella* antenna) responses to 10 equivalents of body extract of female *A. lineatella*. Chromatography: splitless injection; injector and FID detector: 240 °C, DB-5 column (30 m × 0.32 mm ID); temperature program: 50 °C (2 min), then 15 °C per min to 280°C (10 min). Compound abbreviation as follows: 22Hy = docosane; 18:OAc = octadecanyl acetate; 23Hy = tricosane; 11me-23Hy = 11-methyltricosane; *Z*11-20:OAc = (*Z*)-11-eicosenyl acetate; 24Hy = tetracosane; 2me-24Hy = 2-methyltetracosane; 25Hy = pentacosane; 11me-25Hy = 11-methylpentacosane; 26Hy = hexacosane; 27Hy = heptacosane; 13me-27Hy =13-methylheptacosane; 28Hy = octacosane; 29Hy = nonacosane; 30Hy = triacontane.

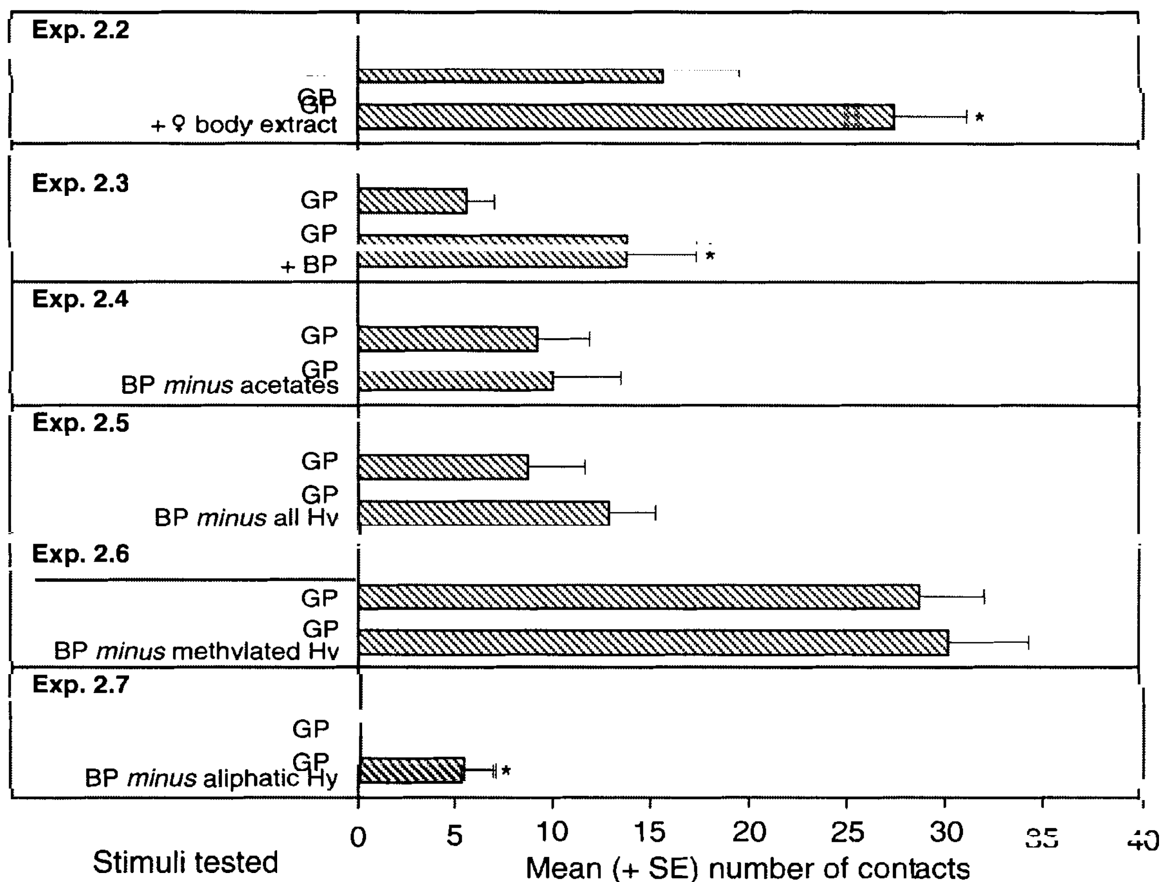

Figure 2.4. Mean (+ SE) number of contacts made by male *Anarsia lineatella* in experiments 2.2-2.7 (15 replicates each) with a Teflon® decoy impregnated with various test stimuli. In each experiment, an asterisk (*) indicates a significant preference for a particular stimulus; paired t-test, $P < 0.05$. Abbreviations as follows: GP = synthetic gland pheromone components [(*E*)-5-decen-1-yl acetate (100 ng) and (*E*)-5-decen-1-ol (10 ng)]; female body extract = body extract of female *A. lineatella* tested at 10 female equivalents; BP = synthetic body pheromone components consisting of two acetates [18:OAc, *Z*11-20:OAc], four methylated hydrocarbons (Hy) [11me-23Hy, 2me-24Hy, 11me-25Hy, 13me-27Hy] and eight aliphatic hydrocarbons [22Hy, 23Hy, 24Hy, 25Hy, 26Hy, 28Hy, 29Hy, 30Hy]. For full names of chemicals see caption of Figure 2.3.

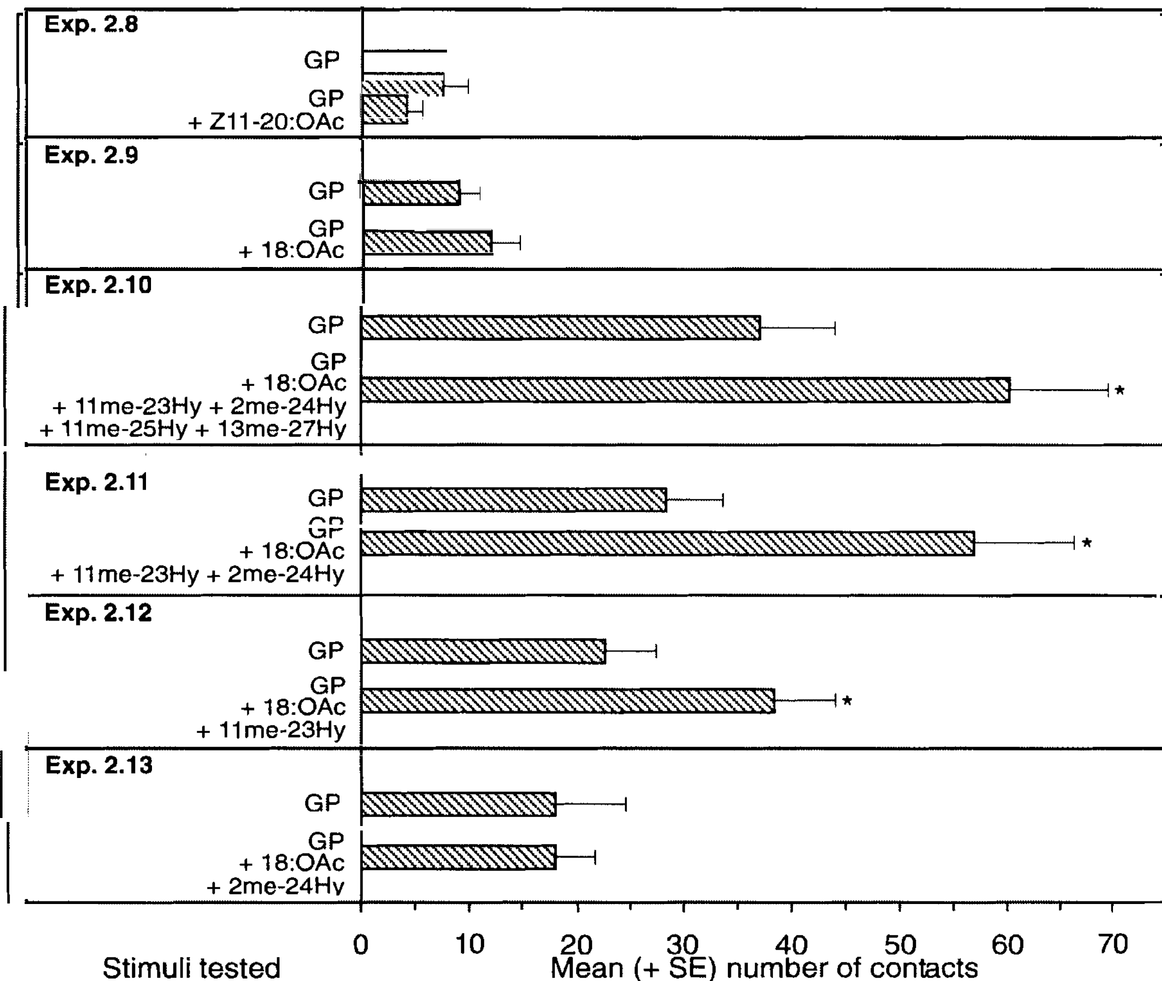

Figure 2.5. Mean (+ SE) number of contacts made by male *Anarsia lineatella* in experiments (Exp.) 2.8-2.13 (15 replicates each) with a Teflon® decoy impregnated with various test stimuli. In each experiment, an asterisk (*) indicates a significant preference for a particular treatment; paired *t*-test, $P < 0.05$. Abbreviations as in captions of Figures 2.3 and 2.4.

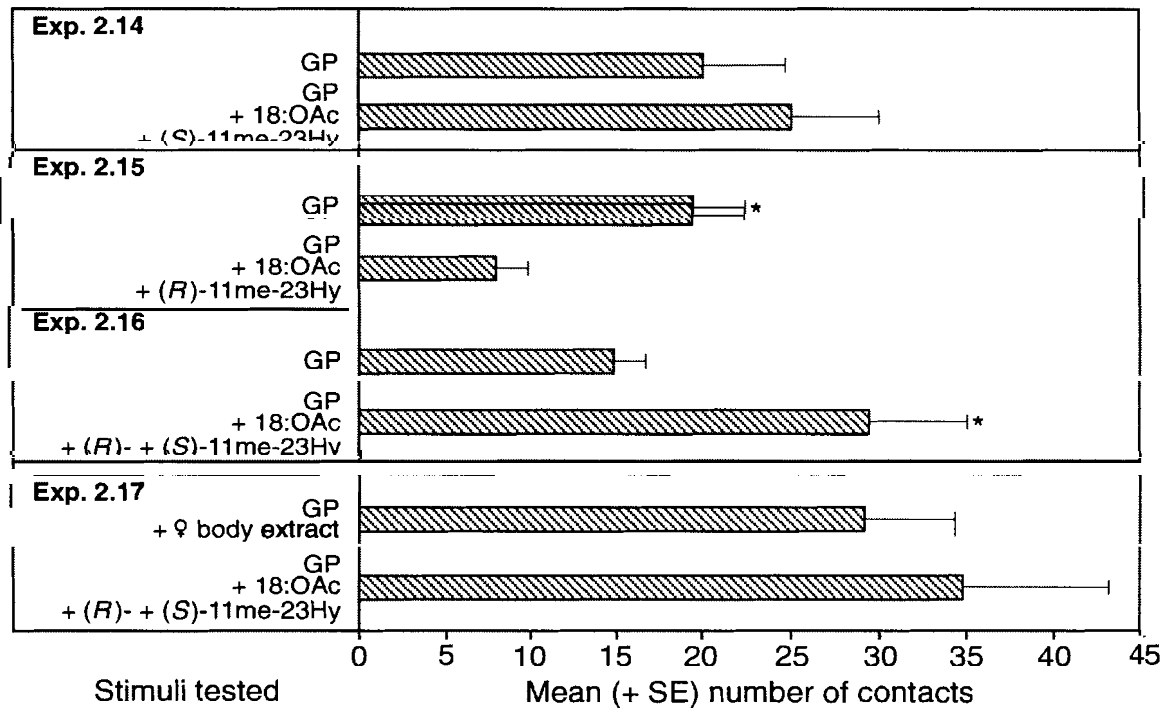

Figure 2.6. Mean (+ SE) number of contacts made by male *Anarsia lineatella* in experiments 2.14-2.17 (15 replicates each) with a Teflon® decoy impregnated with various test stimuli. In each experiment, an asterisk (*) indicates a significant ($P < 0.05$) preference for a particular treatment using a paired *t*-test. (*R*)- and (S)-11me-23 = (*R*)- and (*S*)-11-methyltricosane, respectively; other abbreviations as in captions of Figures 2.3 and 2.4.

CHAPTER 3. PHEROMONE PRODUCTION AND EMISSION IN RELATION TO DIEL PERIODICITY AND PRESENCE OF MALES

3.1 Introduction

Many insects use sexual communication signals to attract or locate mates. The communication signals themselves, or diel periodicity of signalling, may impart specificity to the communication system of the respective species (Jutsum and Gordon, 1989). Artichoke plume moths, *Platyptila carduidactyla* Riley, and *P. williamsii* Grinnell (Lepidoptera: Pterophoridae), for example, use the same pheromonal communication signal but signal at different times (Haynes and Birch, 1986) and thus remain reproductively isolated. The periodicity of signalling differs among heterospecifics, but may vary even within the same species possibly in response to the presence of congeners in the community. For example, nun moths, *Lymantria monacha* L. (Lepidoptera: Lymantriidae), in forest communities in Bohemia signal between 21:00 to 24:00 hours, whereas *L. monacha* in Honshu signal between 24:00 to 6:00 hours, likely due to the presence of *L. fumida* Butler which signal during the first half of the night (Gries *et al.*, 2001). Similarly, four species of limacodid moths, *Darna bradleyi* Hell., *D. trima* (Moore), *Setothosea asigna* (Van Eecke), and *Setora nitens* Walker (Lepidoptera: Limacodidae), in oil palm plantations of Borneo (southeast Asia), maintain reproductive isolation through specificity in their communication signal, diel periodicity of, and/or microlocation for communication (Sasaerila *et al.*, 2000).

The periods during which female moths emit and males respond to pheromone are typically synchronized (Sasaerila *et al.,* 2000, and references cited therein; Haynes and Birch, 1984). The pheromone is either present continuously in pheromone glands at fairly constant levels and is emitted only during signalling (del Mazo-Cancino *et al.,* 2004; Rojas, 2004), or the pheromone titre rises just before and during pheromone emission (Valles *et al.,* 1992).

Quantities of pheromones produced or released by (signalling) insects vary intra- and inter-specifically (Guibiao *et al.,* 1999; Griepink *et al.,* 2000), and range between femtograms and micrograms per insect, or calling period and age (Delisle and Royer, 1994). Large quantities of pheromone emitted by females may increase the active space over which foraging males are recruited (Kehat *et al.,* 1999), but at short range a high pheromone concentration may hinder rather than help males to pinpoint the micro-location of calling females (Schmitz *et al.,* 1995; Suckling, 2000). If so, females may cease or reduce pheromone emission in response to signals from mate-seeking males.

Pheromone components emitted by female peach twig borers, *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae), have been identified (Roloefs *et al.,* 1975; Millar and Rice, 1992), but little else is known about the communication biology of this species. In an effort to better understand sexual communication in *A. lineatella* and perhaps improve control techniques, I tested the following hypotheses: (1) that there is diel periodicity in pheromone production and emission by females; (2) that periods of pheromone emission by females and attraction response by males are synchronized; and (3) that females alter pheromone emission in the presence of males.

3.2 Materials and methods

3.2.1 Diel periodicity of sexual communication

Diel periodicity of the males' response to synthetic sex pheromone, or live conspecific females, was investigated in four field experiments. Experiments 3.1 and 3.2 were conducted on 20 and 21 June 2002 in an organic peach orchard 5 km east of Keremeos (N 49° 21', W 119° 83'), British Columbia (BC). Ten Delta-type traps made from 2-L milk cartons (Gray *et al.,* 1984) were coated with Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI, USA) and suspended from trees at a height of 1.5 m and spaced 3.6 m apart. Each trap was baited with a cage (3.25 × 5.25 cm) containing two 3- to 4-day-old females reared in the laboratory at a photoperiod of 16L:8D (20 ± 2°C, 70% ± 5% relative humidity) (Sidney, in preparation). Experiments 3.3 and 3.4 were conducted on 5 and 6 July 2001 in an almond orchard near Livingston (N 37° 38', W 120° 73'), California (CA). Delta-type traps were deployed as in experiments 3.1 and 3.2, and baited with gray rubber septa (The West Company, Lionville, PA, USA) impregnated with synthetic *E*5-10:OAc (1000 μg) and *E*5-10:OH (100 μg) in HPLC-grade hexane (0.2 mL). In all experiments, captures of males were recorded every hour for 12 hours.

3.2.2 Diel periodicity of pheromone production

In experiment 3.5, 220 females (1- to 3-day-old) were kept individually in filter paper-lined Petri dishes (10 × 2 cm) (Fisher Scientific International Inc., Hampton, NH) and maintained at a temperature of 23°C and at a photoperiod of 16 L:8D. Every hour for each of 24 hours, five females were randomly selected, and their abdominal tip with pheromone gland extruded was removed with micro-scissors and placed for extraction into separate ampoules, containing 10 μl of pentane and 1 ng of (*E*)-8-undecenyl acetate

as an internal standard. Aliquots (2-μL) of each of the 120 samples were then analyzed by gas chromatography, employing a Hewlett-Packard (HP) 5890 gas chromatograph fitted with a DB-5 GC column (30 m × 0.25 mm ID; J&W Scientific, Folsom, CA, USA) to determine the amount of pheromonal *E*5-10:OAc.

3.2.3 Diel periodicity of pheromone emission

In experiment 3.6, 30 females (1- to 3-day-old) were placed in each of two Pyrex glass aeration chambers (15.5 × 28.0 cm ID), and maintained at 23°C and a photoperiod of 16 L:8D. A water aspirator drew humidified, charcoal-filtered air at a rate of 1.2 L/min for 24 hours through each chamber and a corresponding glass column (30 mm × 13 mm OD) downwind filled with 50-80 mesh Porapak Q. The Porapak Q volatile traps were replaced every hour, and eluted with 2 ml of redistilled pentane. After (*E*)-8-undecenyl acetate (1 μg) was added as an internal standard, extracts were concentrated and aliquots analyzed by gas chromatography-mass spectrometry, employing a Varian Saturn 2000 Ion Trap GC-MS fitted with a DB-5 GC column to determine the amount of pheromonal *E*5-10:OAc.

3.2.4 Volatile emission by females in the presence or absence of males

In experiment 3.7, 30 females (1- to 3-day-old) were kept for 24 hours (16L:8 D) in each of two paired Pyrex glass aeration chambers (22 × 28 cm), each containing a smaller Pyrex glass chamber (15.0 × 17.5 cm) perforated with 60 1-mm holes. The perforated chamber housed 10 males (3- to 5-day-old), or was left empty. Volatiles emitted by females in the absence or presence of males were captured in, and eluted from Porapak traps, and aliquots of Porapak Q extracts were analyzed as described above.

3.3 Results

In field experiments 3.1 and 3.2 near Keremeos (BC), male *A. lineatella* responded to female-baited traps between 4:00 and 7:00 hours Pacific Standard Time (PST) (Figure 3.1). Similarly, in field experiments 3.3 and 3.4 near in Livingston (CA), males responded to pheromone-baited traps between 3:00 to 6:00 hours PST (Figure 3.1). In the laboratory, female *A. lineatella* emitted pheromone only between 4:00 and 6:00 hours (Figure 3.2, Exp. 3.6), whereas pheromone was present in pheromone glands at similar quantities throughout the 24-hour recording period (Figure 3.2; Exp. 3.5). In the presence of conspecific males (Exp. 3.7), females emitted less pheromone than females in the absence of males (Figure 3.3).

3.4 Discussion

My data demonstrate diel periodicity of sexual communication in *A. lineatella* (Figure 3.1) and likely synchrony between periods of pheromone emission by females in the laboratory (Figure 3.2) and attraction by males in the field (Figure 3.1). Similar findings were previously obtained with other species of moths, including the Oriental tobacco budworm, *Helicoverpa assulta* Guenée (Lepidoptera: Noctuidae) (Kamimura and Tatsuki, 1993), *P. carduidactyla* (Haynes and Birch, 1984), and the carpenterworm, *Holcocerus insularis* L. (Lepidoptera: Cossidae) (Jin-Tong and Xian-Zuo, 2001).

Although female *A. lineatella* maintained a constant presence of pheromone in their glands throughout the 24-hour study period (Figure 3.2), they emitted pheromone only during early dawn (Figure 3.2). This suggests that pheromone production and emission are not tightly linked, and that pheromone emission, but not production, is instigated by external cues, such as photoperiod. These results are consistent with those

obtained for the saltmarsh caterpillar, *Estigmene acrea* Drury (Lepidoptera: Arctiidae) (del Mazo-Cancino *et al.,* 2004; Rojas, 2004), the black army cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) (Gemeno and Haynes, 2001), and the vine bud moth, *Theresimima ampellophaga* Bayle-Barelle (Lepidoptera: Zygaenidae) (Toshova and Subchev, 2003), but contrast with those obtained for *H. insularis* (Zhang and Meng, 2001). Female *H. insularis* produce and emit pheromone only during a 3-hour early-dawn calling period. Our results also differ from those obtained for female jujube leafrollers, *Ancylis sativa* Liu (Lepidoptera: Tortricidae), which exhibit diel periodicity for both pheromone production and emission (Guibiao *et al.,* 1999).

Reduced pheromone emission by females in the presence of males (Figure 3.3) suggests that males communicate their presence to females. To the best of my knowledge, such a phenomenon has not yet been reported in the Insecta. The males' communication signals have not yet been studied, but one might speculate that they are not likely pheromones. Flying upwind towards calling females, any potential male pheromones, or body odours, would not readily be perceived by females. In contrast, visual displays or bioacoustic signals might allow males to announce their arrival and provoke females to lower their pheromone emission, or even emit "reply" signals. Bioacoustic signals might be particularly useful because their perception by females would not be as dependent on wind direction as pheromones, and they might more readily reveal the micro-location of the signaller.

3.5 References

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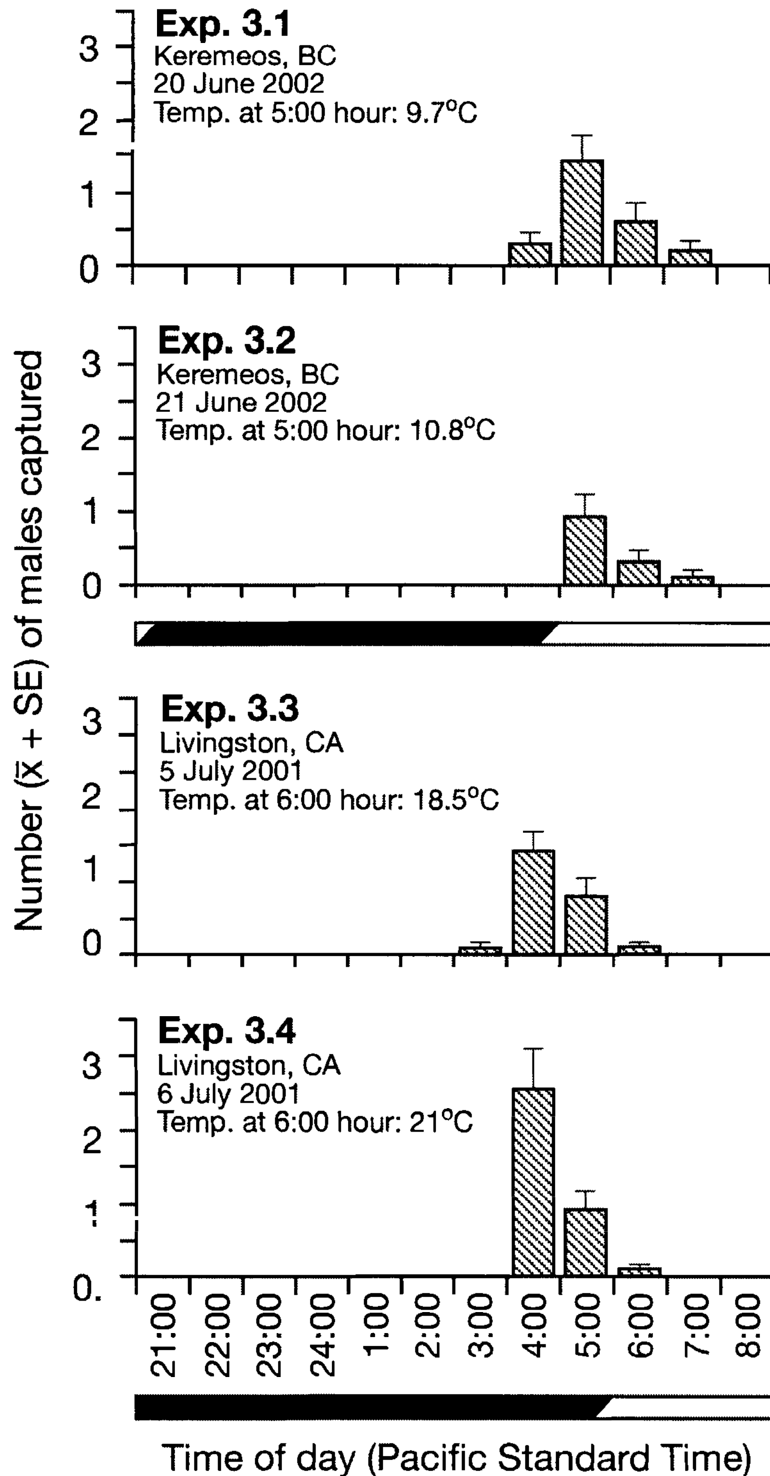

Figure 3.1. Response periods by male Anarsia lineatella to traps baited with two caged virgin female A. lineatella (experiments 3.1-3.2; 10 replicates each), or baited with a 2-component blend of synthetic pheromone [(E)-5-decen-1-yl acetate (1,000 μg) and (E)-decen-1-ol (100 μg)] (experiments 3.3-3.4; 10 replicates each). Black horizontal bars indicate scotophase.

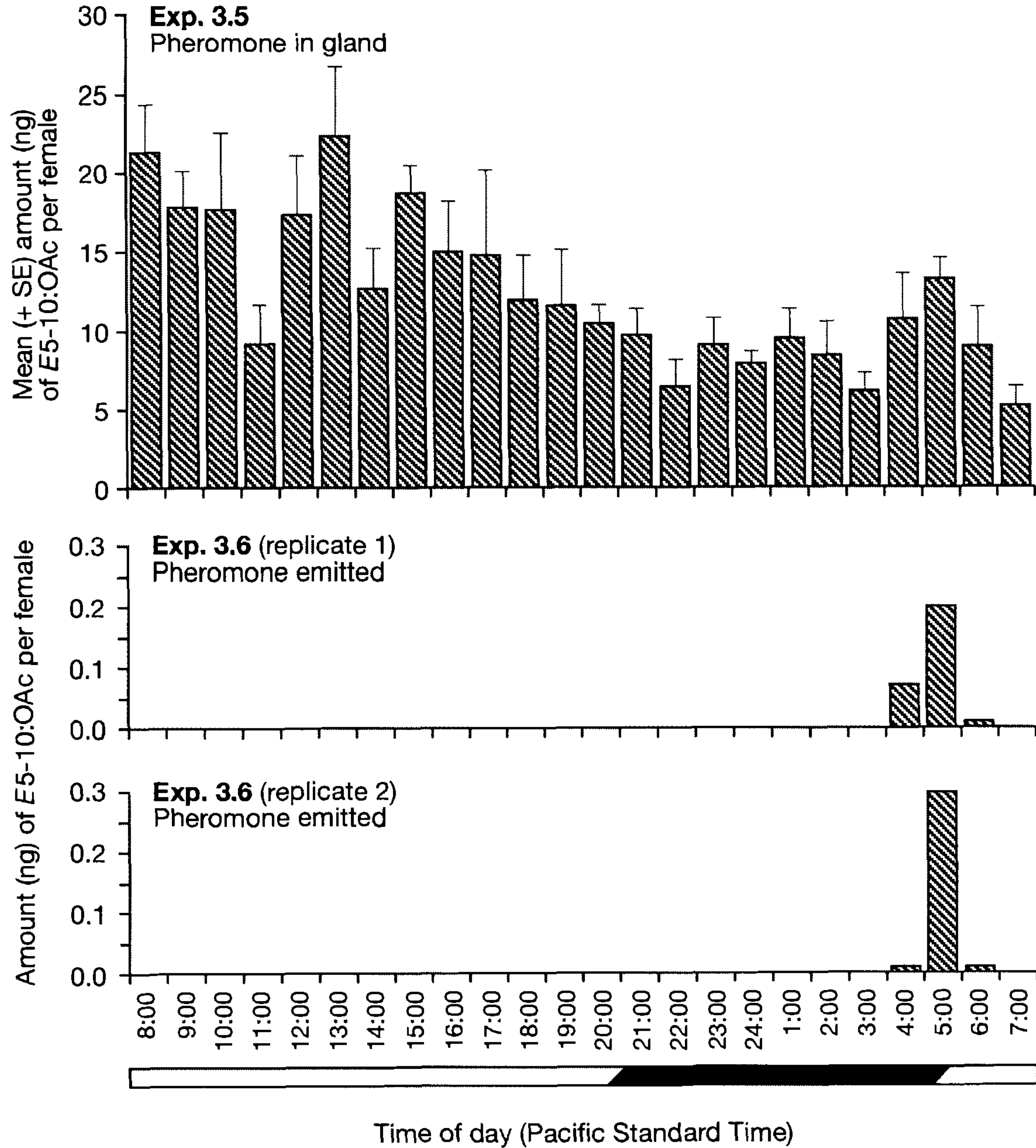

Figure 3.2. Periodicity of pheromone content in pheromone gland extracts of 220 separately kept virgin female *Anarsia lineatella* in experiment 3.5 and periodicity of pheromone emissions by 30 virgin females in each of replicates 1 and 2 of experiment 3.6. Each bar in experiment 3.5 represents the mean (+ SE) pheromone content in five pheromone gland extracts. Black horizontal bar indicates scotophase.

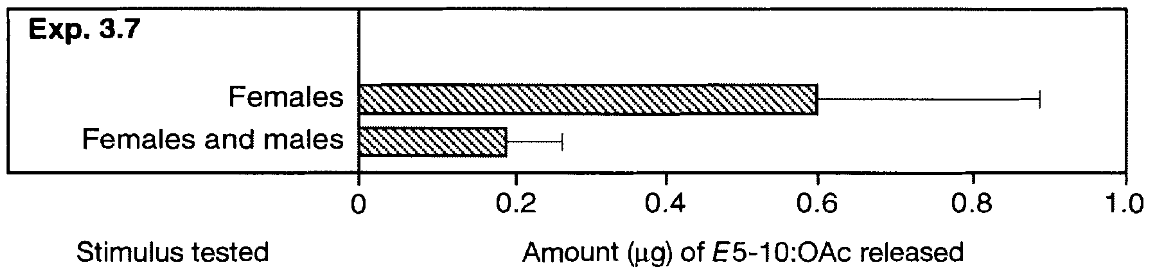

Figure 3.3. Mean amount of pheromonal (*E*)-5-decen-1-yl acetate (*E*5-10:OAc) released by 30 female *Anarsia lineatella* in each of two replicates in the absence or presence of 10 conspecific males that were physically separated from females through a perforated glass container.

CHAPTER 4. CONCLUDING SUMMARY

My study has provided new insights into sexual communication of *A. lineatella*. The data support the following conclusions:

Females have three body (scale)-derived pheromone components [octadecanyl acetate, (*R*)-11-methyltriocosane, (*S*)-11-methyltricosane] that induce contact by males.

In field experiments, these three body pheromone components do not enhance the attractiveness of sex pheromone components [(*E*)-5-decen-1-yl acetate and (*E*)-5-decen-1-ol] previously identified in pheromone glands.

The preceding results, coupled with stronger attraction of males to virgin females than to synthetic sex pheromone as trap baits, suggest that females use additional pheromonal and bioacoustic signals for sexual communication.

In the presence of males, females reduce pheromone emission, suggesting that searching males communicate their arrival to (calling) females.

The titre of pheromone in sex pheromone glands of females remains fairly constant, but females emit pheromone only during periods of sexual communication.

Females emit and males respond to pheromone (and possibly other communication signals) in the early morning hours (3:00 to 6:00 Pacific Standard Time).

APPENDIX. FIELD EXPERIMENTS

Experiments in British Columbia (BC)

Exp. # BC1	Keremeos & Cawston (Apricot Orchard; 3 June, 2001)				
	Lure Composition				
	*E*5-10:Ac	*E*5-10:OH	18:OAc	*Z*11-20:Ac	11-me-23Hy
Treatment 1	1000µg	100 µg	-	-	-
Treatment 2	1000 µg	100 µg	700 µg	350 µg	20 µg
Numbers of male moths captured					
Replicate		Treatment 1		Treatment 2	
1		1		1	
2		1		1	
3		1		2	
4		0		1	
5		0		1	
6		1		1	
7		1		0	
8		0		2	
9		0		0	
10		0		0	
Mean ± SE		0.5 ± 0.17		0.9 ± 0.23	
		t-ratio = 1.5		p-value = 0.17	

Exp. # BC2	Keromeos & Cawston (Apricot Orchard; 3 June, 2001)		
	Lure Composition		
	*E*5-10:Ac	*E*5-10:OH	18:OAc
Treatment 1	1000µg	100 µg	-
Treatment 2	1000 µg	100 µg	1000 µg
Numbers of male moths captured			
Replicate	Treatment 1	Treatment 2	
1	0	1	
2	0	0	
3	0	1	
4	0	0	
5	0	0	
6	0	0	
7	1	0	
8	0	0	
9	1	0	
10	0	0	
Mean ± SE	0.2 ± 0.13	0.2 ± 0.13	
	t-ratio = 0	p-value = 1.00	

Exp. # BC3	Keromeos & Cawston (Apricot Orchard: 3 June, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	*Z*11-20:Ac
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	1000 μg
Numbers of male moths captured			
Replicate	Treatment 1	Treatment 2	
1	2	0	
2	3	1	
3	0	1	
4	0	0	
5	0	1	
6	1	2	
7	1	0	
8	1	0	
9	0	0	
10	0	0	
Mean ± SE	0.8 ± 0.33	0.5 ± 0.22	
	t-ratio = -0.82	p-value = 0.43	

Exp. # BC4	Keromeos & Cawston (Peach Orchard; 3 June, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	11me-23Hy
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	1000 μg
Numbers of male moths captured			
Replicate	Treatment 1	Treatment 2	
1	1	1	
2	0	0	
3	1	1	
4	0	0	
5	1	0	
6	1	0	
7	0	0	
8	0	0	
9	0	0	
10	0	0	
Mean ± SE	0.4 ± 0.16	0.2 ± 0.13	
	t-ratio = -1.5	p-value = 0.17	

Experiments in Washington (W)

Exp. # W1	Yakima (Plum Orchard; 12 June, 2001)				
	Lure Composition				
	*E*5-10:Ac	*E*5-10:OH	18:OAc	*Z*11-20:Ac	11me-23Hy
Treatment 1	1000μg	100 μg	-	-	-
Treatment 2	1000 μg	100 μg	700 μg	350 μg	20 μg
Numbers of male moths captured					

Replicate	Treatment 1	Treatment 2
1	4	3
2	10	1
3	6	4
4	7	5
5	3	0
6	2	10
7	6	6
8	8	4
9	4	4
10	6	12
Mean ± SE	5.6 ± 0.76	4.9 ± 1.17
	t-ratio = -0.46	p-value = 0.66

Exp. # W2	Wapato (Peach Orchard; 12 June, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	11me-23Hy
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	1000 μg
Numbers of male moths captured			
Replicate	Treatment 1	Treatment 2	
1	2	3	
2	10	0	
3	3	1	
4	3	1	
5	2	0	
6	0	7	
7	2	0	
8	4	3	
9	1	2	
10	2	5	
Mean ± SE	2.9 ± 0.86	2.2 ± 0.74	
	t-ratio = -0.51	p-value = 0.62	

Experiments in California (C)

Exp. # C1	Livingston, Keyes Road (Peach Orchard; 15 May, 2001)				
	Lure Composition				
	*E*5-10:OAc	*E*5-10:OH	18:OAc	*Z*11-20:OAc	11me-23Hy
Treatment 1	1000μg	100 μg	-	-	-
Treatment 2	1000 μg	100 μg	700 μg	350 μg	20 μg

Numbers of male moths captured		
Replicate	Treatment 1	Treatment 2
1	4	5
2	1	0
3	0	0
4	0	0
5	0	0
6	1	0
7	0	2
8	1	0
9	0	0
10	3	0
Mean ± SE	1.0 ± 0.45	0.7 ± 0.52
	t-ratio = -0.71	p-value = 0.50

Exp. # C2	Fresno (Unmanaged Apricot Orchard; 15 May, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	18:OAc
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	10 μg
Treatment 3	1000 μg	100 μg	100 μg
Treatment 4	1000 μg	100 μg	1000 μg

Numbers of male moths captured				
Replicate	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	4	1	1	4
2	4	0	4	2
3	1	2	6	0
4	0	4	6	1
5	4	1	1	0
6	5	3	6	4
7	2	5	2	3
8	0	2	1	4
9	3	1	0	1
10	4	2	0	5
Mean ± SE	2.7 ± 0.58	2.1 ± 0.48	2.7 ± 0.80	2.4 ± 0.58
	ANOVA F-value = 0.21		p-value = 0.89	

Exp. # C3	Livingston, Hammatt Exit (Almond Orchard; 15 May, 2001)			
		Lure Composition		
		*E*5-10:OAc	*E*5-10:OH	*Z*11-20:OAc
Treatment 1		1000μg	100 μg	-
Treatment 2		1000 μg	100 μg	10 μg
Treatment 3		1000 μg	100 μg	100 μg
Treatment 4		1000 μg	100 μg	1000 μg
Numbers of male moths captured				
Replicate	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	10	6	10	24
2	11	10	9	7
3	14	11	13	10
4	7	6	10	8
5	0	16	5	2
6	0	22	3	20
7	9	7	5	9
8	11	14	6	14
9	11	2	14	2
10	3	14	14	9
Mean ± SE	7.6 ± 1.56	10.8 ± 1.86	8.9 ± 1.27	10.5 ± 2.24
	ANOVA F-value = 0.71		p-value = 0.55	

Exp. # C4	Livingston, Bradbury Exit (Almond Orchard; 15 May, 2001)			
		Lure Composition		
		*E*5-10:OAc	*E*5-10:OH	11me-23Hy
Treatment 1		1000μg	100 μg	-
Treatment 2		1000 μg	100 μg	10 μg
Treatment 3		1000 μg	100 μg	100 μg
Treatment 4		1000 μg	100 μg	1000 μg
Numbers of male moths captured				
Replicate	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	15	16	8	16
2	9	18	16	4
3	10	12	7	9
4	14	9	20	3
5	28	7	18	9
6	10	5	6	1
7	8	6	8	10
8	6	1	0	18
9	12	4	8	3
10	18	2	10	5
Mean ± SE	13.0 ± 2.01	8.0 ± 1.81	10.1 ± 1.93	7.8 ± 1.81
	ANOVA F-value = 1.6		p-value = 0.20	

Exp. # C5	Livingston, Bradbury Exit (Almond Orchard; 25 June, 2001)				
	Lure Composition				
	*E*5-10:OAc	*E*5-10:OH	18:OAc	*Z*11-20:Ac	11me-23Hy
Treatment 1	1000μg	100 μg	-	-	-
Treatment 2	1000 μg	100 μg	700 μg	350 μg	20 μg

Numbers of male moths captured		
Replicate	Treatment 1	Treatment 2
1	12	17
2	9	11
3	9	10
4	13	3
5	2	1
6	10	10
7	7	8
8	6	12
9	22	11
10	24	8
Mean ± SE	11.4 ± 2.17	9.1 ± 1.43
	t-ratio = -0.98	p-value = 0.35

Exp. # C6	Livingston, Hammatt Exit (Almond Orchard; 25 June, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	18:OAc
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	1000 μg

Numbers of male moths captured		
Replicate	Treatment 1	Treatment 2
1	10	66
2	34	18
3	19	29
4	18	23
5	44	15
6	35	24
7	58	25
8	28	27
9	42	37
10	65	68
Mean ± SE	35.3 ± 5.56	33.2 ± 5.94
	t-ratio = -0.27	p-value = 0.80

Exp. # C7	Livingston Hammatt Exit (Peach Orchard; 25 June, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	*Z*11-20:OAc
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	1000 μg

Numbers of male moths captured		
Replicate	Treatment 1	Treatment 2
1	6	1
2	5	3
3	8	2
4	2	5
5	10	0
6	3	2
7	4	1
8	4	2
9	2	0
10	6	3
Mean ± SE	5.0 ± 0.82	1.9 ± 0.48
	t-ratio = -2.87	p-value = 0.02

Exp. # C8	Fresno (Apricot Orchard; 25 June, 2001)		
	Lure Composition		
	*E*5-10:OAc	*E*5-10:OH	11me-23Hy
Treatment 1	1000μg	100 μg	-
Treatment 2	1000 μg	100 μg	1000 μg

Numbers of male moths captured		
Replicate	Treatment 1	Treatment 2
1	0	4
2	5	1
3	4	3
4	1	3
5	0	1
6	1	3
7	1	0
8	0	2
9	4	1
10	2	2
Mean ± SE	1.8 ± 0.59	2.0 ± 0.39
	t-ratio = 0.25	p-value = 0.80