TREE-DERIVED STIMULI AFFECTING HOST-SELECTION RESPONSE OF LARVA AND ADULT PEACH TWIG BORERS, *Anarsia lineatella* (ZELLER) (LEPIDOPTERA: GELECHIIDAE)

by

Mark Christopher Sidney

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Name: Mark Christopher Sidney

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Examining Committee:

Chair: Dr. E. Elle, Assistant Professor

Dr. G. Gries, Professor
Department of Biological Sciences, S.F.U.

Dr. G. Judd, Research Scientist
Agriculture and Agri-Food Canada, Summerland

Dr. M. Winston, Professor
Department of Biological Sciences, S.F.U.

Dr. S. Lindgren, Professor
Ecosystem Science and Management Program, University of Northern British Columbia
Public Examiner

Date Approved July 26, 2005

SIMON FRASER UNIVERSITY

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ABSTRACT

Volatiles from ripening peach fruit reportedly mediate host-finding by adult peach twig borers, *Anarsia lineatella* (Lepidoptera: Gelechiidae). However, moths were repelled by *in-situ* ripe peach fruits, and by a blend of 22 synthetic volatiles associated with ripe peach fruits. In laboratory experiments, females preferred hairy and creviced surfaces over glabrous surfaces as oviposition sites. Volatiles from almond and peach shoots induced oviposition, as did volatiles from immature, green mature, and hard-ripe peach fruits. Soft-ripe peach fruits, in contrast, did not induce oviposition, and when tested against immature peach fruits received three times fewer eggs. In laboratory olfactometer experiments, larvae of *A. lineatella* were found to orient chemoanemotactically toward Porapak Q extracts of peach shoot or almond shoot and fruit volatiles. β-Bourbonene and (*E*,*E*)-α-farnesene identified in almond shoot and fruit volatile extracts, and tested as a 2-component blend, were as attractive to larvae as was the entire extract.

EXECUTIVE SUMMARY

In my thesis, I present data that refute reports that adult *Anarsia lineatella* (Lepidoptera: Gelechiidae) are attracted by olfactory stimuli from ripe peach fruit. Instead, I show that adult moths were repelled by *in-situ* soft-ripe peach fruits, and by a blend of 22 synthetic volatiles associated with soft-ripe fruits. Modifications of the fruit volatile blend composition, or dosage, and addition of nine synthetic volatiles from peach shoots, all failed to result in an attractive semiochemical blend. The selection of oviposition sites was found to be mediated by both tactile and semiochemical stimuli. Hairy and creviced surfaces were strongly favoured over glabrous surfaces as oviposition sites by female *A. lineatella.* Volatiles from almond and peach shoots induced oviposition, as did volatiles from immature, green mature, and hard-ripe peach fruits. Soft-ripe peach fruits, in contrast, did not induce oviposition, and when tested against immature peach fruits received three times fewer eggs. In field experiments, female *A. lineatella* laid more eggs on mechanically damaged *in-situ* peach fruits than on undamaged ones, as long as fruits were not yet at the soft-ripe maturation stage. Once damaged *in-situ* fruits had matured to the soft-ripe stage, they became repellent to *A. lineatella,* as shown for undamaged *in-situ* soft-ripe peach fruits. In laboratory experiments, larvae of *A. lineatella* were found to orient chemoanemotactically toward Porapak Q extracts of almond shoot and fruit volatiles. β-Bourbonene and (*E*,*E*)-α-farnesene identified in such volatile extracts, and tested as a 2-component blend, were as attractive to larvae as was the entire extract.

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I INTRODUCTION

1.1 SEMIOCHEMICAL-MEDIATED HOST SELECTION IN LEPIDOPTERAN INSECTS

Selection of host plants is mediated by complex and diverse visual, mechanical, gustatory, and olfactory stimuli (Städler 1976). Because olfactory stimuli have been considered most important (Städler 1986, Hansson 1995), plant odours have received much attention. Indeed, there has been a long-standing interest in plant chemicals. In 1888, the German botanist Stahl explained the role of secondary compounds in plants (translated by Fraenkel in 1959): "The great differences in the nature of chemical products ... are brought nearer to our understanding, if we regard these compounds as a means of protection, acquired in the struggle with the animal world." Dethier (1957) and Fraenkel (1959) supported this hypothesis and contended that insects may use the plants defensive compounds as host-finding clues.

Orientation to, and recognition and acceptance of hosts by Lepidoptera are still thought to be guided largely by secondary plant metabolites (Honda 1995). Because of their importance in host-plant selection, these chemicals are classified according to their effects on the behaviour of insects (Bernays and Chapman 1994). Attractants and repellents have been defined as chemicals that cause insects to make oriented movements toward and away, respectively, from the source of the stimuli (Dethier et al. 1960). These stimuli are effective at some distance away from the plant. Feeding or oviposition stimulants and deterrents have been defined as chemicals that elicit or inhibit, respectively, feeding or oviposition (Dethier et al. 1960). These compounds are important in the recognition and acceptance of hosts and require contact with the insect; they have no orientation component.

Oriented flight by moths to host plant semiochemicals has been shown for several moths including the navel orangeworm, *Amyelois transitella* (Phelan et al. 1991), the codling moth, *Cydia pomonella* (Hern and Dorn 1999, 2004, Reed and Landolt 2002), the oriental fruit moth, *Cydia molesta* (Natale et al. 2004), the noctuids *Trichoplusia ni*, *Heliothis subflexa*, and *Mamestra brassicae*, and the diamondback moth, *Plutella xylostella* (Reed and Landolt 2002 and references therein). However, the only field study to date measuring attraction of host-plant derived volatiles to Lepidoptera was the capture of codling moth in traps baited with the pear-derived volatile ethyl-(*E,Z*)-2,4-decadienoate (Light et al. 2001).

In many studies, a mixture of plant semiochemicals was needed to attract insects (Bernays and Chapman 1994). Attraction of the red sunflower seed weevil, *Smicronyx fulvus*, e.g., required five terpenoids, with any combination of up to three volatiles being completely unattractive (Roseland et al. 1992). The abundance of plant semiochemicals is also important to elicit a behavioural response by insects. Low concentrations of allyl-isothiocyanate attracted cabbage webworms, *Hellula undalis*, whereas high concentrations were repellent (Mewis et al. 2002). Few other plant semiochemicals repellent to the Lepidoptera have been reported (Peterson et al. 1994, De Moraes et al. 2001).

After alighting on potential host plants, gravid female insects assess the plant's suitability for oviposition based on physical and chemical stimuli at the surface (Renwick 1989, Renwick and Chew 1994). Contact chemicals that induce oviposition in several species of the Lepidoptera (reviewed by Honda 1995, Binder and Robbins 1997, Hora and Roessingh 1999, Grant et al. 2000) are thought to consist of non-volatile chemicals (Renwick 1989) originating from plant trichomes, leaf waxes or leachings from the plant's interior (Städler 1986).

Surface texture is a physical stimulus affecting the acceptance of host plants and choice of oviposition sites (Kan and Waku 1985, Manjulakumari and Geethabali 1991, Kumar 1992, Foster and Howard 1999). Methods for rearing insects often include specific descriptions about the appropriate texture of oviposition substrate (e.g., Yokoyama et al. 1987, McElfresh and Millar 1993).

Other factors have been found to modulate the attractiveness of host-plants. Mechanical damage may evoke or increase the release of particular volatiles (Boeve et al. 1996, Scutareanu et al. 1997, Landolt et al. 2000). This, in turn, could increase the apparency of plants, or plant parts, and thus affect host selection by foraging insects. Female *C. pomonella*, e.g., readily detect fruit damaged by conspecific larvae (Hern and Dorn 2002, Reed and Landolt 2002). Similarly, cabbage moths, *M. brassicae*, orient toward, and oviposit more often on, host plants damaged by conspecific larvae than on undamaged plants (Rojas 1999a). The same response was noted when the damage was induced mechanically.

Environmental conditions, the insect's mating status and prior experience, and the presence of con- or heterospecifics may further influence the insect's decision to accept or reject a potential host. A female's sensitivity to a plant-derived stimulus has been found to be dependent on her physiological state (Städler 1992, Ramaswamy 1994). Age and mating status affected host selection by *M. brassicae* (Rojas 1999b) and *A. transitella*

(Phelan and Baker 1987), with mated rather than virgin females orienting toward and ovipositing most often on test stimuli.

Lepidopteran larvae too must be able to recognize suitable host plants and parts thereof. Even if a female's choice of host plant was appropriate, larvae must discern between different plant parts, or move to new parts of a plant or new plants as their food supply diminishes. The ability of larvae to discern among plant substrates is based, in part, on olfactory stimuli (Dethier 1969, Bradley and Suckling 1995, Landolt et al. 1998, Carroll and Berenbaum 2002).

Knowledge about plant semiochemicals and host selection by foraging insects could enhance integrated management of pest insects. Semiochemicals could be used to increase the attractiveness of monitoring traps, attract insects to inappropriate host plants or toxic baits, repel them from appropriate ones and disrupt searching behaviour of adult and larval life stages (Finch 1980). Landolt (1994) showed increased attraction of *T. ni* to pheromone when combined with a host-plant kairomone, as hypothesized by Visser (1986). As Bruce and Cork (2001) noted, lures based on plant odours do not have to be as attractive as the female's sex pheromone because removing one female moth is equivalent to killing many larvae.

Female peach twig borers, *Anarsia lineatella* Zeller, reportedly respond to host-derived semiochemicals (Bailey 1948, Weakley et al. 1990). According to these reports, females immigrate into peach orchards with ripening fruit and lay many eggs directly on the fruit rather than on leaves, as earlier in the season. If true, physical and chemical changes during the maturation of peach fruits (Chapman et al. 1991) may generate a semiochemical blend more attractive than that of leaves. Changes in fruit colour are unlikely to provide cues for nocturnal foraging *A. lineatella*. None of the semiochemicals that seem to mediate selection of oviposition sites by female *A. lineatella* has been identified.

In my thesis, I present data that refute reports that adult *A. lineatella* are attracted by olfactory stimuli from ripe peach fruit. Instead, I show that semiochemicals from ripe peach fruit repel and deter oviposition by *A. lineatella*. In addition, I show that semiochemicals from peach shoots and unripe fruits serve as oviposition stimulants. I further show that tactile stimuli from, and mechanical damage to, unripe peach fruit affect oviposition decisions. Finally, I present data demonstrating that *A. lineatella* larvae are attracted chemotactically to Porapak Q extracts of almond and peach shoots.

1.2 DISTRIBUTION AND PEST STATUS OF *Anarsia lineatella*

Anarsia lineatella was chosen for this study due to its status as a serious pest of almond and stone fruits worldwide. Peach twig borer 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 of the major growing areas of its host trees in North America, Europe, and Asia (Marlatt 1898, Jones 1935, Bailey 1948, Ahmad 1988, Ponomarenko 1990).

The principal host plants of *A. lineatella* are almond and peach, but it has been reported in apricot, nectarine, plum and prune (Summers 1955) and even sweet and sour cherry, apple and persimmon (Ponomarenko 1990). All major host plants belong to the genus *Prunus* in the family Rosaceae.

Anarsia lineatella is considered to be one of the most important pests of peaches and almonds in the western United States. In peach orchards, most economic damage is caused when larvae burrow into fruits. They typically mine cavities just beneath the skin, discolouring the fruit and causing the exudation of gum and frass. Even when only minor damage is inflicted, cosmetic alterations reduce the fruits' value and increase picking and culling costs. Fruit damage also increases susceptibility to other pests and putrefaction. In almond orchards, economic damage occurs primarily in soft-shelled varieties in which larvae are able to proceed to the kernel. Larval tunnelling also provides access to weaker borers like the navel orangeworm, *Ameylois transitella* (Curtis 1983), along with *A. lineatella* the most important pests of almonds in California (Legner and Gordh 1992). In both peach and almond orchards, severe shoot damage can stunt and kill small trees, leading to the recommendation of 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. 1990b). In 1931, infestation levels reached an average of 10% in peach fruit in California, and more recently Abbott (1996) stated that "in severe cases, a grower can lose between 60 percent and the entire crop". 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, pesticide use against *A. lineatella* has created health and environmental concerns, caused development of insecticide resistance

(Summers et al. 1959), and has been shown to reduce natural enemies of other pests such as the green peach aphid (Tamaki 1973), thus aggravating other pest problems. Moreover, as 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. Removal of males using pheromone-baited traps did not reduce damage to commercially acceptable levels (Hathaway et al. 1985). Pheromone-based mating disruption has produced inconclusive results (Millar and Rice 1992, Rotundo and Viggiani 1996). Host plant semiochemicals could be used in an integrated management system to improve monitoring of *A. lineatella* infestations and to remove female moths from the population.

1.3 BIOLOGY AND LIFE HISTORY

1.3.1 *Anarsia lineatella*

There are 1-4 generations per year, dependant upon the geographic region and host species. Three and four generations have been recorded in both California (Price and Summers 1961) and Washington (Brunner and Rice 1984). There are also three generations in the Okanagan valley of British Columbia (Sarai 1966) and two generations and a partial third in the Similkameen valley of B.C.

For mid-summer generations, eggs hatch in 5-7 days, larval development takes 10-20 days, and pupation 6-7 days, for an approximate generation time of 28 days. Larvae which overwinter remain in the first or second instar for 7-9 months (Bailey 1948, Ponomarenko 1990). The pre-oviposition period lasts for 1-4 days.

Females lay up to 115 bluntly oval eggs (ca. 0.4 by 0.2 mm) with reticulations. Newly deposited eggs are white or cream coloured, acquiring an orange tint after *ca.* 24 hours. Eggs are deposited singly or in groups of 2-5 on bark, leaves, or fruit depending on host phenology.

First instar larvae are 0.5 mm and grow to 11 mm. There are 4 or 5 larval instars before pupation, depending on the rate of growth of the larvae (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). Larvae resume development in the spring. By the end of apricot blossoming, >50% of overwintering larvae have abandoned their hibernacula (Ivanova 1995). Larvae predominantly enter buds and terminal shoots, boring a path toward the centre, and then downward, often until they reach the previous year's wood (Ponomarenko 1990). In severe outbreaks, due to a shortage of unattacked shoots, mature larvae of the overwintering generation were even observed inside green apricots measuring only 2 cm in diameter. Due to a later onset of bud flush in peach than in apricot trees, an overwintering larva will damage up to five young peach shoots before completing development. Larvae of the overwintering generation pupate in cracks or rolls of bark and in branch crotches.

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

In British Columbia, adults of the first generation eclose from mid-May to mid-June, when developing peach and apricot fruits are only 2.5 cm in diameter and are green and hard. At this time, gravid females lay eggs in groups of two to five at the base of leaves near the petiole, at the tip of twigs, and on fruit (Bailey 1948). Larvae of the second generation (first summer generation) feed on apical branches at the base of leaves and enter green fruit. In B.C., these larvae can be readily found in ripe apricot fruits. At the end of July, pupation usually occurs next to the stem immediately outside the fruit that was attacked, often concealed by floral residues. The second generation of adults, emerging in B.C. from mid-July to mid-August, has the greatest propensity to cause damage in peach fruits. It is at this time that females will lay the greater proportion of their eggs on the ripening fruit (Bailey 1948, Ivanova 1995). Varieties of peaches that ripen later have a higher incidence of 'stung' peach fruits (Bailey 1948, Weakley et al. 1990b). Development of third generation larvae depends on the location of the eggs. Larvae hatching from eggs on mature fruits enter them and develop into the third generation of adults (Ponomarenko 1990). Larvae emerging from eggs on the bark of trunks and branches make cavities for hibernation and remain in them. It may be noted that some larvae enter the bark in May, June, and July and make a cavity similar to the one made by the larvae for hibernation. After some period of summer diapause, they return to active feeding (Price and Summers 1961).

1.3.2 *Prunus* spp. host plants

The genus *Prunus* in the family Rosaceae is comprised of 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). Their fruits are appreciated world-wide either for fresh consumption, or for processing such as drying, distillation, canning, production of jams, syrups and fruit juices. Worldwide production of these fruits has been increasing (Table 1.1).

Prunus, evolutionarily the most advanced taxon of the Rosacean family, has a pistil reduced to one carpel only. In the pistil, there are regularly two ovules but usually only one grows to seed. The rate of two (or even more) seeds per stone are sometimes typical for particular varieties in pomaceous pistils but are rare in *Prunus*. Flowering and consequent fruit production in *Prunus* only occurs on one-year old wood. The stone fruits or 'drupes' develop from a superior ovary and have no floral residues around the pedicel, though occasionally detached floral tissue is present around the stem. The endocarp is characteristically lignified, and the flesh consists of the mesocarp with a thin exocarp comprising the skin.

There is a distinct 3-stage development in stone fruits (Tukey 1936). In stage I, cell division is rapid and accompanied by cell expansion so there is a rapid increase in pericarp volume. Stage II is a period of quiescence in the pericarp and rapid development of the embryo. In stage III the endocarp completes its development and the pericarp resumes a rapid increase in volume predominantly due to further cell expansion. About two thirds of the weight increase in a peach takes place during this period.

A peach fruit is mature when it has completed development and grown to full size. The change in ground colour of a peach fruit from green to yellow is a good indication that the ripening process has begun. After this, the ripening process begins with softening of the fruit and development of flavour. The stages of maturity are broken down into three categories: hard-ripe, firm-ripe, and soft-ripe. Hard-ripe peach fruit do not yield to moderate pressure, firm-ripe peach fruit yield only slightly, whereas soft-ripe peach fruit yield readily to moderate pressure and are in the prime stage for consumption. However, due to the limited shelf life of soft-ripe peach fruit, all peach fruit destined for shipping are picked in the hard or firm-ripe stages, ripening fully to satisfactory eating quality in transit.

1.4 RESEARCH OBJECTIVES

My research objectives were to:

1. Determine whether adult *A. lineatella* are attracted to ripe peach fruits or their volatiles, as previously reported;

2. Identify ovipositional preferences of female *A. lineatella* and identify the stimuli responsible; and
3. Identify host semiochemicals that are attractive to neonate larvae of *A. lineatella*

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Table 1.1 Global stone fruit production (million metric tonnes) in 1989 and 1999.

Subgenus	Global production (million metric tonnes) 1989	 1999
Persica (peach)	9.317	12.044
Prunus (plum)	6.270	7.346
Armenica (apricot)	2.226	2.720
Amygdalus (almond)	1.288	1.632

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II EVIDENCE FOR OLFACTORY RECOGNITION OF HOST SEMIOCHEMICALS

2.1 INTRODUCTION

Plant-derived semiochemicals mediate attraction, or landing, of many phytophagous Lepidoptera (Visser 1986, Bernays and Chapman 1994, Honda 1995). Orientation to plants may depend on the presence of semiochemical attractants and arrestants, or the absence of repellents emanating from the plant (Miller and Strickler 1984, Renwick 1989). In laboratory experiments several species of moths have been shown to be attracted to host extracts or host-derived semiochemicals (Phelan et al. 1991, reviewed by Bernays and Chapman 1994, Takacs 2001, Reed and Landolt 2002, and references therein). In a pioneering field study, a pear-derived semiochemical was shown to attract *C. pomonella* in walnut orchards (Light et al. 2001).

Anecdotal reports (Bailey 1948) have inferred that *A. lineatella* are attracted to ripening peach fruits in peach orchards. According to Bailey (1948), moths migrate to adjacent orchards seeking unpicked fruit once the orchard they originated in has been harvested.

My objectives were: (1) to determine whether *A. lineatella* discern between host *Prunus persica* trees and non-host *Malus domestica* trees; (2) to test whether *A. lineatella* are attracted to ripe peach fruits in orchard settings; and (3) to identify potential semiochemicals associated with ripe peach fruits.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Insects

The first rearing method described 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 rearing method described here.

Pupae of *A. lineatella* were collected in May 1999 from an organic peach orchard (*Prunus persica*) in Cawston, 5 km east of Keremeos, B.C. Approximately 100 adults were used to establish the laboratory colony.

The original artificial diet (McElfresh and Millar 1993) was modified by substituting ground lima beans for large lima beans, reducing levels of the preservatives methyl-*p*-hydroxy benzoate and sorbic acid, and by adding D-(-)-fructose (Table 2.1).

Ground lima beans were added to 1.2 L of distilled water and heated on a hot plate to 50°C. The remaining ingredients were mixed in a beaker and then blended into the warm lima bean slurry. Agar was added to 1 L of distilled water, and autoclaved to form a solution. This hot solution was added as a final ingredient to the lima bean slurry which was then blended thoroughly, and immediately dispensed into 1 oz cups (BioServ, Frenchtown, NJ) until each cup was *ca.* two thirds full. One hundred and thirty cups were filled in this manner from one batch of diet. Each cup was then sealed with a paper lid (BioServ). Diet gelled in the cups (and not before), so that it felt relatively dry to the touch. This is important because neonate larvae are incapable of coping with wet surfaces. In addition, pouring diet down one of the walls causes a thin layer of drier diet to form on the wall, just above the fill line, on which neonate larvae will feed and complete first-instar development. Once the diet has gelled, any mechanical adjustment of its shape will bring water to the surface, rendering it unusable.

Ten neonate larvae were gently transferred into each cup using a paintbrush with just a few fine hairs. After larvae were placed on the inside of the cup wall, rather than directly on the surface of the diet, the cup was immediately capped and incubated for 21 days in an environmentally-controlled room maintained at 25 ± 3°C, with 75 ± 5% relative humidity, with constant darkness.

The cups were not checked these 21 days because the volume of diet in each cup ensured a sufficient level of moisture throughout larval development. Pupation occurred mostly on or near the paper lids. Pupae were removed daily and transferred to Petri dishes (9 cm diam.) in an environmentally-controlled chamber maintained at 20 ± 2°C, with 70 ± 5% relative humidity and a photoperiod of 16:8h (L:D) using six 40W fluorescent lights.

Adults enclosed within 10 days of transfer and were separated by sex (males have terminalia cloaked with scales and females have an extra spur distally on the labial palps). Adults were kept in Petri dishes with moist filter paper under the same environmental conditions as pupae.

For colony propagation, every 7 days 60 – 80 moths were placed into a cylindrical plastic chamber (20 cm x 20 cm diam.), under the same environmental conditions as described above. Adults were sustained with an 8% sugar water solution that was dispensed from a braided cotton roll (Richmond Dental, Charlotte, NC) inside an 18 ml vial and changed every 3-5 days as needed. A piece of black felt (3 x 4 cm) was placed over a mesh-covered hole (15 cm diam.) in the chamber's lid to induce oviposition

(McElfresh and Millar 1993). However, females preferred to oviposit in crevices between mesh and the underside of the lid. Thus, after 10 days egg-bearing lids were removed and placed into sealable clear plastic bags, and hatching neonate larvae were transferred daily to diet cups.

2.2.2 Landing response of *A. lineatella* on host and nonhost trees

Comparative attractiveness of host peach trees and non-host apple trees (*Malus domestica*), was tested in two-choice bioassay experiments using field cages (3.6 x 3.6 x 3.0 m tall) each containing a potted 2.2 m tall apple tree and peach tree 2 m apart from each other. In Exp. 2.1 trees were readily accessible, whereas in Exp. 2.2 each tree was enclosed separately in mesh fabric (Synthetic Industries, Gainesville, GA) such that there was approximately 5 cm of clearance between mesh and tree. This mesh prevented moths from physically contacting trees, and partially obscured visual cues.

For each replicate, 50-100, 2-5-day-old, laboratory-reared male and female *A. lineatella* were released into a cage. All *A. lineatella* on each tree, or mesh, were counted the following morning and removed from the cage. To complete the replicate, the positions of the two trees were reversed, and moths on trees counted again the following morning.

The two-tailed Wilcoxon paired-sample (signed rank) test was used to test the null hypothesis that there was an equal number of *A. lineatella* on (meshed) host and nonhost trees. A non-parametric test was used because it could not be assumed that the data were from a normal distribution (Zar 1996).

2.2.3 Response to *ex-situ* ripe peach fruits in field experiments

To test whether *A. lineatella* are attracted to ripe peach fruits, traps were baited with such fruits and deployed in Exp. 2.3 in an organic apricot orchard in Cawston, 5 km east of Keremeos, B.C. Conducting Exp. 2.3 in an apricot instead of peach orchard, and commencing the experiment before the appearance of mature apricots, helped eliminate competition from *in-situ* ripe fruits. Each trap consisted of a sheet of Plexiglas™ (20 x 20 cm) with a round hole (8 cm) in the centre. Ripe peach fruits were suspended in holes of treatment traps with a 1 cm-wide strip of clear plastic. Control traps received only a plastic strip. All traps were coated with adhesive Tanglefoot™ (The Tanglefoot Company, Grand Rapids, MI) on both sides and hung vertically from two wires, 1 m above ground. Each tree received a treatment and a control trap, randomly assigned to east or west sectors. Peach fruits were changed, and moths counted and removed, every 7 days for three weeks. A two-tailed Wilcoxon two-sample test was used to test the null hypothesis that an equal number of *A. lineatella* were caught on treatment and control traps (Zar 1996).

Exp. 2.4 was conducted in two apricot orchards, each receiving 12 replicates. In each replicate, three traps as described above were suspended from a tree, baited with either a ripe peach, or a plastic red sphere, or left unbaited. Trap catches were counted after 9 days. Data from the two orchards were pooled and Friedman's test was used to test the null hypothesis that trap captures were equal for all three treatments (Zar 1996).

2.2.4 Response to synthetic volatiles from soft-ripe peach fruits and shoots in field experiments

2.2.4.1 Acquisition of soft-ripe peach fruit and shoot volatiles

Ripe peach fruits and shoots (Cawston, B.C.) were aerated separately for 3-7 days in a cylindrical Pyrex™ glass chamber. Charcoal-filtered air was drawn at 2 L/min with a water-aspirator through the chamber and a glass column (14 cm x 0.40 cm ID) containing 3 cm of 50-80 mesh Porapak Q (Waters Associates Inc., Milford, MA). Volatiles were eluted from the Porapak Q with 3 ml of redistilled pentane and refrigerated (4 °C) until use.

2.2.4.2 Identification of antennally-active soft-ripe peach fruit and shoot volatiles

Aliquots (1µL) of Porapak Q-captured volatiles were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al. 1975), using a Hewlett Packard 5890A gas chromatograph equipped with a fused silica column (30 m x 0.32 mm ID) coated with DB-5 (J&W Scientific, Folsom, CA). Full scan electron impact mass spectra of EAD-active compounds were obtained by coupled GC-mass spectrometry (MS), using a Varian Saturn II Ion Trap GC-MS fitted with the DB-5 coated column referred to above. For both GC-EAD and GC-MS, the GC oven was held for 2 min at 50°C, followed by a temperature increase of 10°C/min to 240°C. GC retention times of components eliciting antennal responses were converted into retention indices (Van den Dool and Kratz 1963) relative to saturated, aliphatic hydrocarbons. EAD-active compounds were identified by comparing their GC, GC-MS and GC-EAD characteristics with those of authentic standards. The quantity of each compound in an aliquot was calculated by comparison of peak area with the formula 100,000 (peak area) = 1 ng. For field testing, (*E,E*)-α-farnesene (TCI) and (*E*)-β-ocimene (IFF) were separated from other isomers via preparative GC under the same conditions as above.

2.2.4.3 Field testing of candidate semiochemicals

Candidate synthetic soft-ripe peach fruit semiochemicals (Table 2.2) were bioassayed at ratios equivalent to those found in soft-ripe peach fruits. Complete or partial blends dissolved in hexane were micropipetted onto grey rubber septa (The West Company, Lionville, PA) or Whatman No. 1 filter paper (4.25 cm diam.) which were kept refrigerated (4°C) until use, whereas control septa and filter paper received an equivalent volume of hexane. Rubber septa and filter paper were used for the release of compounds with relatively low and high boiling points, respectively (Table 2.2). Both types of dispensers served as baits in delta traps (Gray et al. 1984) constructed from milk cartons in our laboratory.

Exp. 2.5 tested attractiveness of the complete blend of candidate peach semiochemicals (Table 2.2), with each tree receiving a treatment (baited) and control (unbaited) trap randomized for axial direction. The experimental design for Exp. 2.6 was identical except that both treatment and control traps also received a separate rubber septum impregnated with sex pheromone components (*E*)-5-decen-1-yl acetate and (*E*)-5-decen-1-ol. The addition of pheromone ensured that sufficient numbers of males were captured for statistical analyses. Exps. 2.7-2.10 also included pheromone lures in both treatment and control traps, and paired a treatment and control trap in the same tree.

To determine attractants or repellents in the blend of candidate semiochemicals, compounds were separated into three functional groups: a) methyl or ethyl esters; b) lactones, and c) "rest", consisting of aldehydes, hydrocarbons and non methyl or ethyl esters. Exp. 2.7 tested the three possible binary combinations of these three groups, whereas Exp. 2.8 tested each of the three functional groups alone.

To determine whether the amount of total chemical load per lure affected lure attractiveness, Exp. 2.9 tested methyl and ethyl esters plus lactones at four doses.

To determine whether EAD-active peach shoot volatiles (Table 2.2) could enhance the attractiveness of soft-ripe peach fruit volatiles, Exp. 2.10 tested both groups of candidate semiochemicals in combination.

Stimuli tested in Exps. 2.5-2.10 are summarized in Table 2.3.

The two-tailed Wilcoxon paired-sample test was used to test the null hypothesis that an equal number of *A. lineatella* were captured in paired treatment and control traps in Exps. 2.4-2.10. In addition, data from Exps. 2.4, 2.7, 2.8, and 2.9, were analyzed using the Kruskal-Wallis test ("analysis of variance by ranks") under the null hypothesis that trap catch differences between treatment and control pairs were equal for all pairs in an experiment. The Nemenyi test (nonparametric Tukey-type multiple comparison test) was then applied to determine such differences between treatment and control pairs (Zar 1996).

2.2.5 Response to *in-situ* peach fruits in field cage experiments

Attractiveness of *in-situ* peach fruits was tested in Exp. 2.11 by enclosing them in traps. The experimental peach trees were located in the entomology orchard of the Agriculture and Agri-Food Canada (AAFC) Pacific Agri-Food Research Centre (PARC), Summerland, B.C. A field cage (Chapter 2.2) was set up encompassing a fruit bearing peach tree ca. 3.5 m tall. To accommodate the tree in the 3.0 m tall field cage, a pole was used to raise the centre of the roof to the appropriate height.

In early June, delta traps were constructed around peach fruits in stages I and II of development by cutting a fruit-size hole in traps and inserting the peach fruit through it. Traps were then firmly attached to branches to avoid mechanical injury to peach fruits or their stems. The hole for peach insertion was covered with material from spare traps, being careful not to damage the stem. A control trap without fruit was hung in the same axial orientation as, and 30-50 cm from, the treatment trap. Thirteen such replicates, at least 1 m apart from each other, were set up around one caged tree. All other fruits were removed from the tree. Biweekly, 100-150 laboratory reared male and female *A. lineatella* were released into the cage, and trap captured moths removed and recorded weekly.

After six weeks, in late July, 10 of the 13 fruits had become infested with *A. lineatella* larvae and the field cage had to be moved to encompass a new tree. On the second tree, 15 replicate pairs were prepared as described above except that traps were of rectangular shape to accommodate the larger fruit in development stage III. A third tree with 11 replicate pairs was enclosed in late August, because a wind storm had knocked most of the yellow/red, firm-ripe peach fruits of the second tree to the ground. Exp. 2.11 was concluded two weeks later when the peach fruits of the third tree were soft-ripe and began to fall from the tree/traps.

Weekly counts of trap-captured moths on each tree were summed for the periods: early June, late June, early July, mid July, late July, early August, mid August, and late August and early September. Each period's counts were analyzed using the two-tailed

Wilcoxon paired-sample test under the null hypothesis that equal numbers of *A. lineatella* were caught in treatment and control traps.

2.3 RESULTS

2.3.1 Landing response of *A. lineatella* on host and nonhost trees

In field cage bioassays three times as many male and female *A. lineatella* were found on host peach trees than on non-host apple trees ($P < 0.005$, Figure 2.1, Exp 2.1). When the trees were enclosed in mesh fabric, male and female *A. lineatella* were still found significantly more often on the mesh surrounding peach trees than on the mesh surrounding apple trees ($P < 0.005$, Figure 2.1, Exp. 2.2).

2.3.2 Response to *ex-situ* ripe peach fruits in field experiments

More *A. lineatella* (sex undetermined) were found on sticky Plexiglas™ traps baited with ripe peach fruits than on empty Plexiglas™ traps ($P < 0.01$, Figure 2.2, Exp. 2.3). However, no differences in captures were obtained in a follow-up experiment when traps were provisioned with a ripe peach fruit, a red sphere, or left unbaited (Figure 2.2, Exp. 2.4).

2.3.3 Response to synthetic volatiles from soft-ripe peach fruits and shoots in field experiments

2.3.3.1 Identification of antennally-active soft-ripe peach fruit and shoot volatiles

In coupled GC-EAD analyses, 22 compounds from soft-ripe peach fruits and nine compounds from peach shoots consistently elicited responses from male or female *A. lineatella* antennae (Figure 2.3, Table 2.2). The soft-ripe peach fruit volatiles were classed into three groups: a) methyl and ethyl esters, b) lactones, and c) "rest", consisting of aldehydes, saturated hydrocarbons and non methyl or ethyl esters.

2.3.3.2 Field testing of candidate semiochemicals

Traps baited with synthetic soft-ripe peach fruit volatiles at a total dose of 50 mg caught significantly fewer *A. lineatella* than paired empty control traps ($P < 0.01$, Figure 2.4, Exp. 2.5). The same result was obtained when synthetic sex pheromone had been added to treatment and control traps ($P < 0.005$, Figure 2.4, Exp. 2.6).

In Exp. 2.7 (Figure 2.5) two treatments; minus lactones, and minus esters, caught significantly fewer moths than their paired controls ($P < 0.005$ and $P < 0.05$ respectively). Trap captures in the two treatments, complete volatile blend and minus "rest", did not significantly differ from those of their paired controls. The Kruskal-Wallis test indicated that combined trap captures for each treatment-control pair differed between the four pairs ($P < 0.05$). The Nemenyi multiple comparison test determined that combined trap captures in the treatment-control pair with the complete volatile blend bait differed from those of the other three pairs ($P < 0.05$), which did not differ among each other ($P > 0.05$).

In Exp. 2.8 (Figure 2.6), only the treatment "rest" caught fewer moths than its paired control ($P < 0.005$). The Kruskal-Wallis test indicated that combined trap catches for each treatment-control pair differed between the three pairs ($P < 0.005$). The Nemenyi test determined that combined trap catches in the treatment-control pair with the "rest" volatile bait differed from those of the other two pairs ($P < 0.05$), which did not differ among each other ($P > 0.05$).

In Exp. 2.9 (Figure 2.7) traps baited with the full dose and the 1/10 dose of esters plus lactones caught fewer moths than control traps ($P < 0.05$), whereas traps baited with the 1/100 and 1/1000 dose caught a similar number of moths as did control traps ($P > 0.05$). The Kruskal-Wallis test indicated that combined trap catches for each treatment-control pair differed between the four pairs ($P < 0.05$). The Nemenyi test determined that combined trap captures in the treatment-control pair baited with 1/1000 dose differed from the full dose and the 1/10 dose pairs ($P < 0.05$) but not the 1/100 dose pair. The full dose, 1/10 dose and 1/100 dose pairs did not differ among each other ($P > 0.05$).

A blend of synthetic peach shoot volatiles failed to increase the attractiveness of synthetic soft-ripe peach fruit volatiles (Exp. 2.10), with control traps capturing more moths than volatile baited treatment traps ($P < 0.005$, Figure 2.8).

2.3.4 Response to *in-situ* peach fruits in field cage experiments

In the periods early June to mid July and late July to mid August, similar numbers of *A. lineatella* were caught in traps baited with *in-situ* unripe peach fruits and in unbaited control traps (Figure 2.9, Exp. 2.11). However, in the period late August to early

September, 70% more moths were captured in empty control traps than in traps baited with *in-situ* ripe peach fruits ($P < 0.05$, Figure 2.9, Exp. 2.11).

2.4 DISCUSSION

Selection of host trees by *A. lineatella* is mediated by airborne semiochemicals that are detectable at a distance of at least 5 cm (Exp. 2.2). Contact cues might also play a role as a higher proportion of moths were recorded on peach trees that could be physically contacted (Exp. 2.1). Collectively, all my data refute previous reports by Bailey (1948) and Weakley et al. (1990) that *A. lineatella* are attracted to ripe peach fruits, as follows: (1) There was no consistent attraction of *A. lineatella* to traps baited with ripe peach fruits (Exps. 2.3-2.4); (2) *A. lineatella* were not attracted to *in-situ* unripe peach fruits and were repelled by *in-situ* soft-ripe peach fruits (Exp. 2.11); and (3) *A. lineatella* were repelled by a blend of 22 EAD-active synthetic soft-ripe peach volatiles, whether they were tested alone or in combination with synthetic sex pheromone (Exps. 2.5-2.6). Neither removal of blend components, modification of blend dosage, nor addition of antennally-active synthetic peach shoot volatiles resulted in an attractive blend (Exps. 2.7-2.10).

The conclusion that volatiles from soft-ripe peach fruits are repellent to *A. lineatella* is based on results from an exhaustive series of experiments that took many considerations into account.

Synthetic sex pheromone was included in Exp. 2.6-2.10 to ensure some level of attractiveness from which to measure the effect of fruit volatiles. Several experiments conducted without synthetic sex pheromone had to be discarded due to trap catches too low to warrant statistical analyses of data. Moreover, mate-foraging males will likely respond to a combination of host semiochemicals and female-produced pheromone (Hansson 1995). In some insect species, males must find host plants because females are only receptive when in contact with host plants (e.g., Carriere and McNeil 1988).

To address the possibility that the attractiveness of soft-ripe peach fruit semiochemicals might be dose-dependent, synthetic methyl and ethyl esters and lactones were tested at four doses (Exp. 2.9). Methyl and ethyl esters and lactones were selected because they appeared not to have adverse behavioural activity (Figure 2.6, Exp. 2.8). Exp. 2.9 was justified because the semiochemical allyl-isothiocyanate was found to repel cabbage webworms, *Hellula undalis* at high concentrations, but to induce anemotaxis in females at low concentrations (Mewis et al. 2002). However, none of the four doses tested (34.5 mg; 3.45 mg; 0.345 mg; 0.0345 mg) significantly attracted male or female *A. lineatella*.

Considering that synthetic soft-ripe peach fruit semiochemicals might attract *A. lineatella* only when released together with synthetic peach shoot semiochemicals, both types of semiochemicals were tested in combination. However, synthetic peach shoot semiochemicals did not affect the behavioural activity of synthetic soft-ripe peach fruit semiochemicals (Figure 2.8, Exp. 2.10), nor were they attractive on their own. In retrospect, this result could have been expected because tree-derived shoot volatiles were present in all experiments with ripe peach fruits or equivalents of synthetic semiochemicals.

The repellency of ripe peach fruits was most evident when peach fruits were tested *in-situ* (Figure 2.9, Exp. 2.11). While *in-situ* submature peach fruits had no discernible behavioural effect on foraging *A. lineatella*, firm to soft-ripe mature *in-situ* peach fruits as trap baits deterred insects from entering traps. Olfactory cues from these fruits must have been responsible for the deterrent effect, because visual stimuli of the fruits were largely masked by the traps.

The biological explanation for the repellency of ripe peach fruits to *A. lineatella*, as demonstrated in many of my experiments, lies in the phenology of *A. lineatella* and host peach fruits. Larval development to the pupal stage takes 15 – 27 days. Any eggs laid on a ripe fruit two weeks before it falls from the tree will not likely develop into adult insects. Only first and second instar larvae overwinter. A larva that has reached third instar must mature to adulthood or will perish. Therefore, female *A. lineatella* or even neonate larvae should have evolved the ability to determine whether peach fruits are viable oviposition or feeding sites, respectively. Once peach fruits have reached the firm-ripe to soft-ripe stage of maturity, female *A. lineatella* should lay their eggs on bark, and neonates should excavate hibernacula to overwinter. The final phase of Exp. 2.11 paralleled the final two weeks before ripe peach fruits fall from the tree. Repellency of such fruits could serve as a mechanism to prevent contact of *A. lineatella* with, and subsequent oviposition upon, ripe peach fruits.

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Table 2.1 Amounts and sources of ingredients for *A. lineatella* artificial diet.

Ingredient	Amount	Source[a]
Water, distilled	2200 ml	
Ground lima beans	400 g	BioServ
Wheat germ	100 g	BioServ
Torula yeast	80 g	ICN
Agar	30 g	Anachemia
Formalin (10% formaldehyde solution)	5 ml	Sigma
Cholesterol	1.5 g	ICN
Total	approx 2.5 L	

[a] BioServ, Frenchtown, NJ; ICN, Aurora, OH; Anachemia, Montreal, QC; Sigma, St. Louis, MO.

Table 2.2 Name, amount, chemical purity and source of soft-ripe peach fruit and shoot synthetic test stimuli used in experiments 2.5 – 2.10.

Compound[a]	Amount (mg / lure)	Chemical purity (%)	Source[b]
Soft-Ripe Peach Fruit Volatiles	50.00 (total)		
Esters	30.35		
1 Ethyl octanoate[c]	12.00	99	Acid from Sigma[d]
2 Methyl octanoate[c]	7.68	99	Acid from Sigma[d]
3 Ethyl (Z4)-decenoate[e]	6.72	95	Alcohol from Bedoukian[d]
4 Methyl (Z4)-decenoate[e]	3.60	95	Alcohol from Bedoukian[d]
5 Methyl decanoate[e]	0.12	99	Acid from Sigma[d]
6 Methyl dodecanoate[e]	0.15	99	Sigma
7 Methyl tetradecanoate[e]	0.03	95	BDH
8 Methyl hexadecanoate[e]	0.05	95	Sigma
Lactones	4.15		
9 γ-Decalactone[e]	3.15	98	Aldrich
10 δ-Decalactone[e]	0.31	98	Aldrich
11 γ-Dodecalactone[e]	0.36	98	Bedoukian
12 6-Amyl-α-pyrone[e]	0.33	96	Aldrich
"Rest"	15.50		
13 Pentadecane[e]	3.61	95	Sigma
14 Heptadecane[e]	4.82	95	Sigma
15 Nonadecane[e]	1.44	99	Aldrich
16 Heneicosane[e]	0.24	98	Aldrich

Table 2.2 continued

Compound[a]	Amount (mg / lure)	Chemical purity (%)	Source[b]
17 (*Z*3)-Hexenyl acetate[c]	3.61	98	Alcohol from Aldrich[d]
18 (*Z*3)-Hexenyl octanoate[e]	0.48	98	Alcohol from Aldrich[d]
19 Hexyl octanoate[e]	0.72	95	Acid from Sigma[d]
20 Nonanal[c]	0.48	95	Aldrich
21 Hexadecanal[e]	0.07	95	Alcohol from Aldrich[d]
22 Octadecanal[e]	0.03	95	Alcohol from Aldrich[d]
Peach Shoot Volatiles	10.00 (total)		
(*Z*3)-Hexenyl acetate[c]	1.87	98	Alcohol from Aldrich[d]
(*Z*3)-Hexenyl butanoate[c]	0.38	98	Alcohol from Aldrich[d]
(*Z*3)-Hexenyl tiglate[e]	0.50	95	Bedoukian
Methyl salicylate[c]	0.75	98	Aldrich
Nonanal[c]	2.10	95	Aldrich
Decanal[c]	0.94	95	Aldrich
(*E*,*E*)-α-Farnesene[e]	1.61	99	TCI[f]
(*Z*)-Jasmone[e]	0.25	95	Bedoukian
(*E*)-β-Ocimene[c]	1.60	99	IFF[f]

---

[a] Number refers to GC-EAD numbering (Figure 2.1)

[b] Sigma, St. Louis, MO; Bedoukian, Danbury, CT; BDH = British Drug Houses, a division of VWR International, Mississauga; Aldrich, St. Louis, MO; TCI = Tokyo Chemical Industry, Portland, OR; IFF = International Flavours and Fragrances, New York, NY.

[c] Denotes relatively low boiling point compounds added to rubber septa.

[d] Synthesis from precursors by Grigori Khaskin, S.F.U.

[e] Denotes relatively high boiling point compounds added to filter paper.

[f] Purified to 99% by preparative Gas Chromatography (Regine Gries, S.F.U.).

Table 2.3 Stimuli tested in experiments 2.5 – 2.10 in almond and peach orchards

Experiment No.	Test Stimuli[a]		Reps
	Treatment[b]	Control[c]	
2.5	Testing synthetic peach soft-ripe fruit volatiles		
	PV	Hexane	10
2.6	Testing the addition of pheromone to treatment and control		
	PV + pheromone	pheromone	10
2.7	Testing the removal of one class of chemical		
	PV + pheromone	pheromone	10
	PV (minus E) + pheromone	pheromone	10
	PV (minus L) + pheromone	pheromone	10
	PV (minus R) + pheromone	pheromone	10
2.8	Testing each class alone		
	PV (E only) + pheromone	pheromone	9
	PV (L only) + pheromone	pheromone	9
	PV (R only) + pheromone	pheromone	9
2.9	Testing the reduction of the dose of esters and lactones only		
	PV (E + L only) (34.5 mg) + pheromone	pheromone	10
	1/10 PV (E + L only) (3.45 mg) + pheromone	pheromone	10
	1/100 PV (E + L only) (345 μg) + pheromone	pheromone	10
	1/1000 PV (E + L only) (34.5 μg) + pheromone	pheromone	10
2.10	Testing the addition of peach shoot volatiles		
	PV + PSV + pheromone	pheromone	10

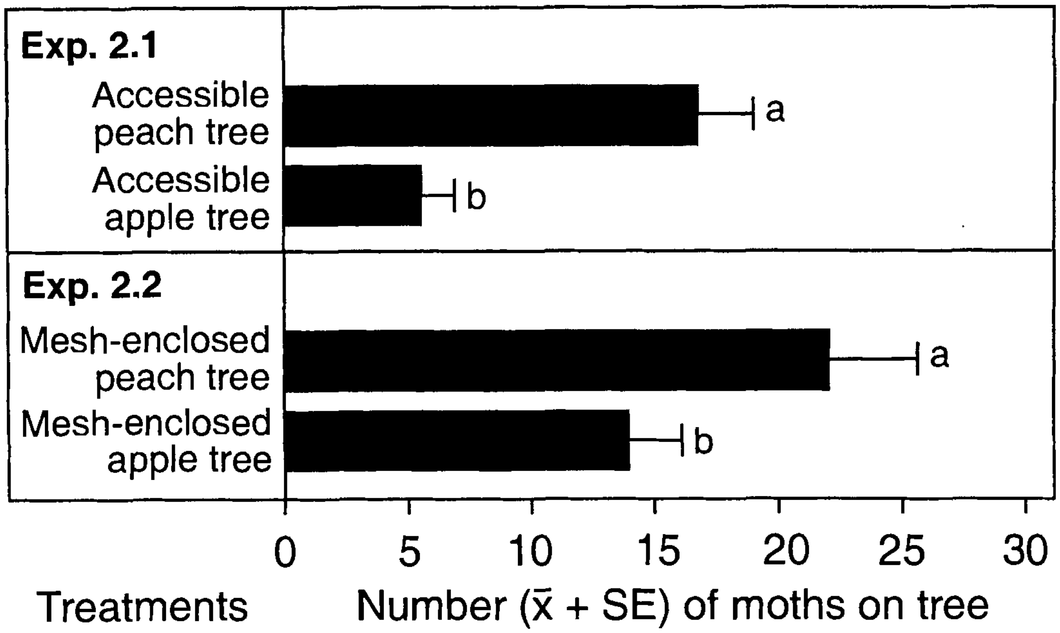
[a] For amounts of volatiles used per experiment see Table 2.2

[b] Abbreviations (see Table 2.2):

PV = soft-ripe Peach fruit Volatiles.
L = lactones.
E = esters.
R = "rest".
PSV = Peach Shoot Volatiles.
Pheromone = 1.00 mg (E5)-decenyl acetate and 100 μg (E5)-decenol.

[c] All controls received a volume of hexane identical to corresponding treatment volumes.

Figure 2.1 Mean number (+ SE) of male and female *Anarsia lineatella* recorded on a paired peach or apple tree (ca. 2.2 m tall) that was accessible to foraging insects (Experiment 2.1, n = 10) or mesh-enclosed (Experiment 2.2, n = 12) in a mesh cage (3.6 x 3.6 x 3.0 m tall). SFU, June 2002. For each replicate, 50-100 *A. lineatella* were released into the field cage and their choice of tree recorded 24 hours later. In each experiment, bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test ($P < 0.005$).



Exp. 2.1
Accessible peach tree
Accessible apple tree
Exp. 2.2
Mesh-enclosed peach tree
Mesh-enclosed apple tree
a
b
a
b
0
5
10
15
20
25
30
Treatments
Number (x̄ + SE) of moths on tree

Figure 2.2 Mean number (+ SE) of male and female *Anarsia lineatella* captured on Plexiglas™ traps left unbaited or baited with ripe peach fruits (Experiment 2.3, n = 10); or ripe peach fruits or red plastic spheres (Experiment 2.4, n = 24). Apricot orchard, Cawston, 5 km east of Keremeos, B.C. June 2000 (Experiment 2.3), June 2002 (Experiment 2.4). In each experiment, bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test (Experiment 2.3, $P < 0.01$), Friedman's test (Experiment 2.4).

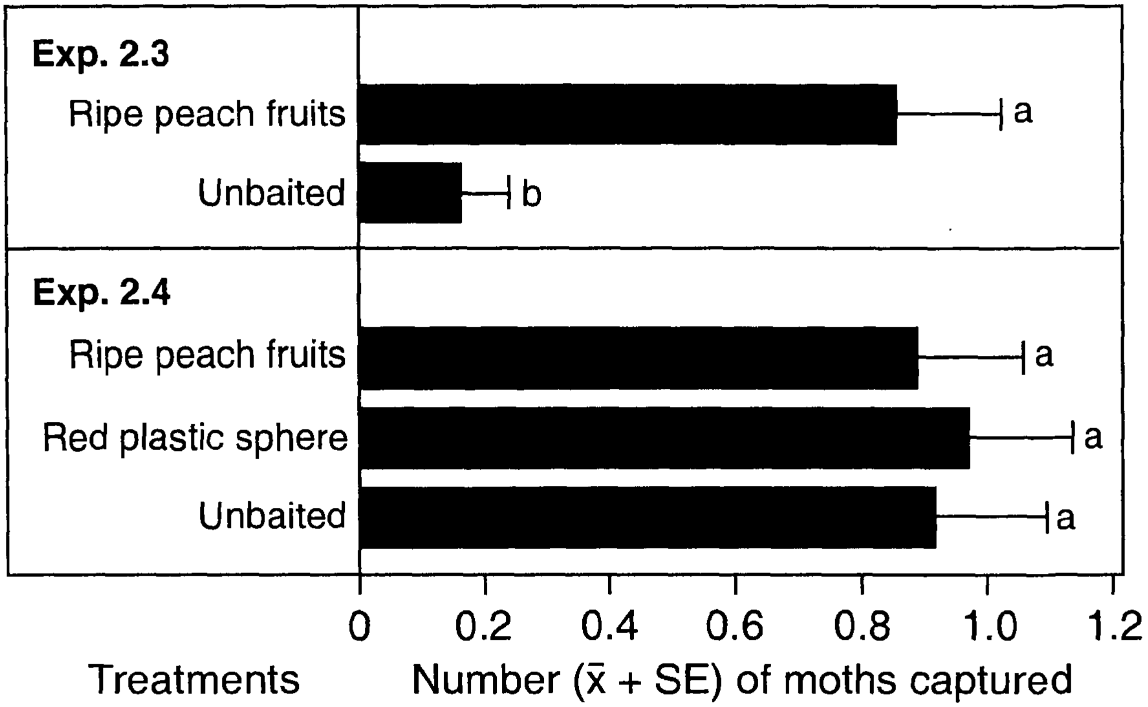

Exp. 2.3
Ripe peach fruits
a
Unbaited
b
Exp. 2.4
Ripe peach fruits
a
Red plastic sphere
a
Unbaited
a
0
0.2
0.4
0.6
0.8
1.0
1.2
Treatments
Number (x̄ + SE) of moths captured

Figure 2.3 Flame ionization detector (FID) and electroantennographic detector (EAD: female and male *Anarsia lineatella* antenna) responses to aliquots of 1.0 peach hour equivalents of airborne volatiles from soft-ripe peach fruits. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with a GC column (30 m x 0.32 mm ID) coated with DB-5; temperature program: 2 min at 50°C, 10°C/min to 240°C. Note: **1** One peach hour equivalents = volatiles released from one peach during one hour at 23 °C; **2** Numbering of compounds as in Table 2.2.

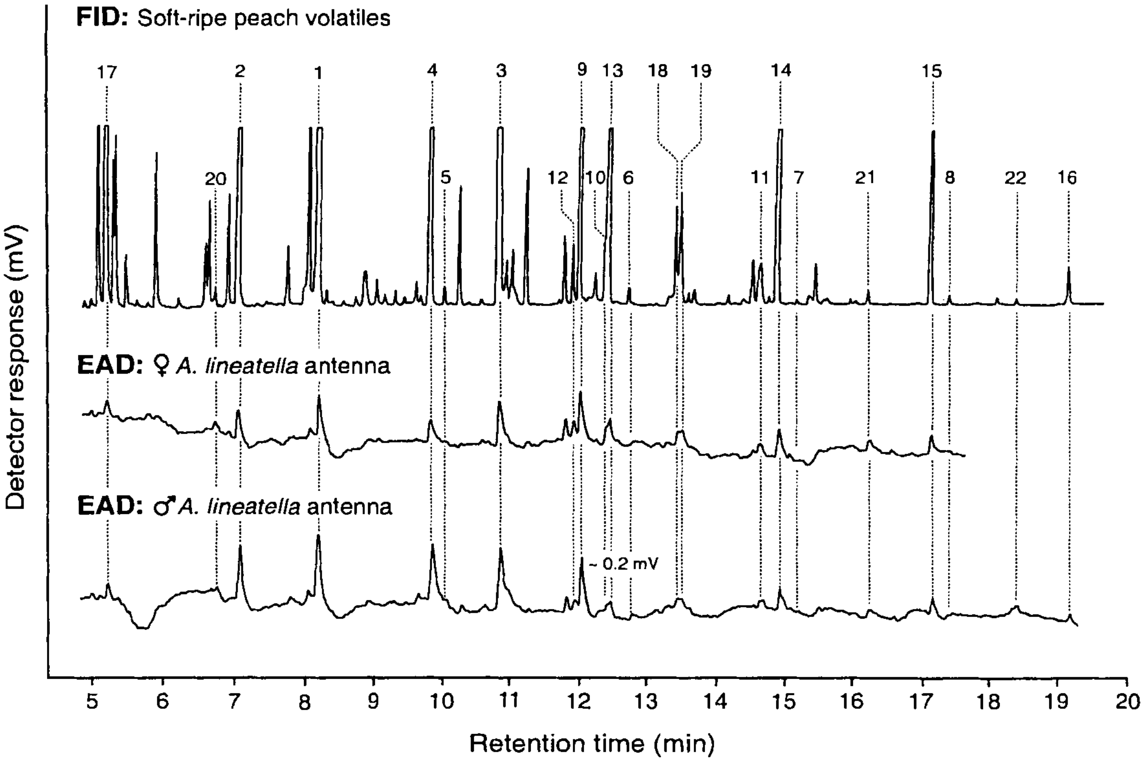

FID: Soft-ripe peach volatiles
17
2
1
4
3
9
13
18
19
14
15
20
5
12
10
6
11
7
21
8
22
16
EAD: ♀ A. lineatella antenna
EAD: ♂ A. lineatella antenna
~ 0.2 mV
Detector response (mV)
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Retention time (min)

Figure 2.4 Mean number (+ SE) of male and female *Anarsia lineatella* captured in sticky Delta traps baited with a blend of 22 synthetic soft-ripe peach fruit volatiles (50 mg) (Table 2.2) singly (Experiment 2.5, n = 10) or in combination with synthetic sex pheromone (Table 2.3) (Experiment 2.6, n = 10), with unbaited or pheromone-baited traps, respectively, serving as control stimuli. Almond orchard, Fresno, CA, June 2001. In each experiment, bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test (Experiment 2.5, $P < 0.01$; Experiment 2.6, $P < 0.005$).

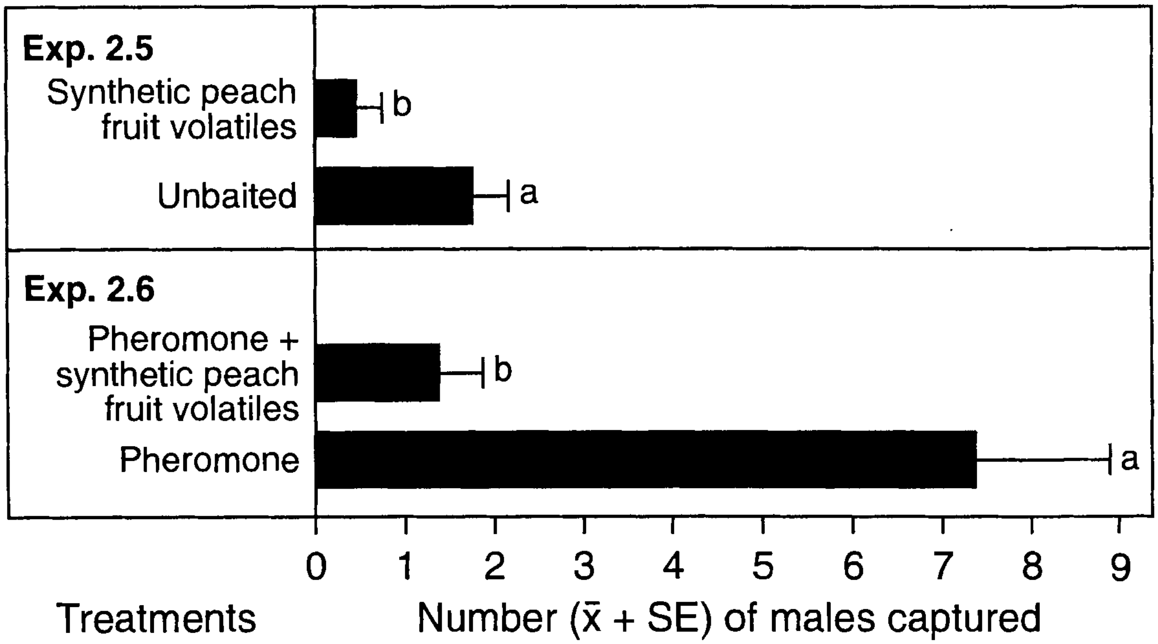

Exp. 2.5
Synthetic peach fruit volatiles
b
Unbaited
a
Exp. 2.6
Pheromone + synthetic peach fruit volatiles
b
Pheromone
a
0
1
2
3
4
5
6
7
8
9
Treatments
Number ($\bar{x}$ + SE) of males captured

Figure 2.5 Mean number (+ SE) of male *Anarsia lineatella* captured in experiment 2.7 (n = 10) in sticky Delta traps baited with synthetic sex pheromone (Table 2.3) plus complete or partial blends of synthetic soft-ripe peach fruit volatiles (Table 2.2). Paired control traps contained synthetic sex pheromone alone. Young (pre-fruit) peach orchard, Moxee, WA, July 2001. Bars with asterisks indicate a significant preference for a particular treatment within a treatment-control pair; two-tailed Wilcoxon paired-sample test, * $P < 0.005$, ** $P < 0.05$. Brackets with different letters indicate significant differences between treatment-control pairs; Kruskal-Wallis test, followed by the Nemenyi multiple comparison test ($\alpha = 0.05$).

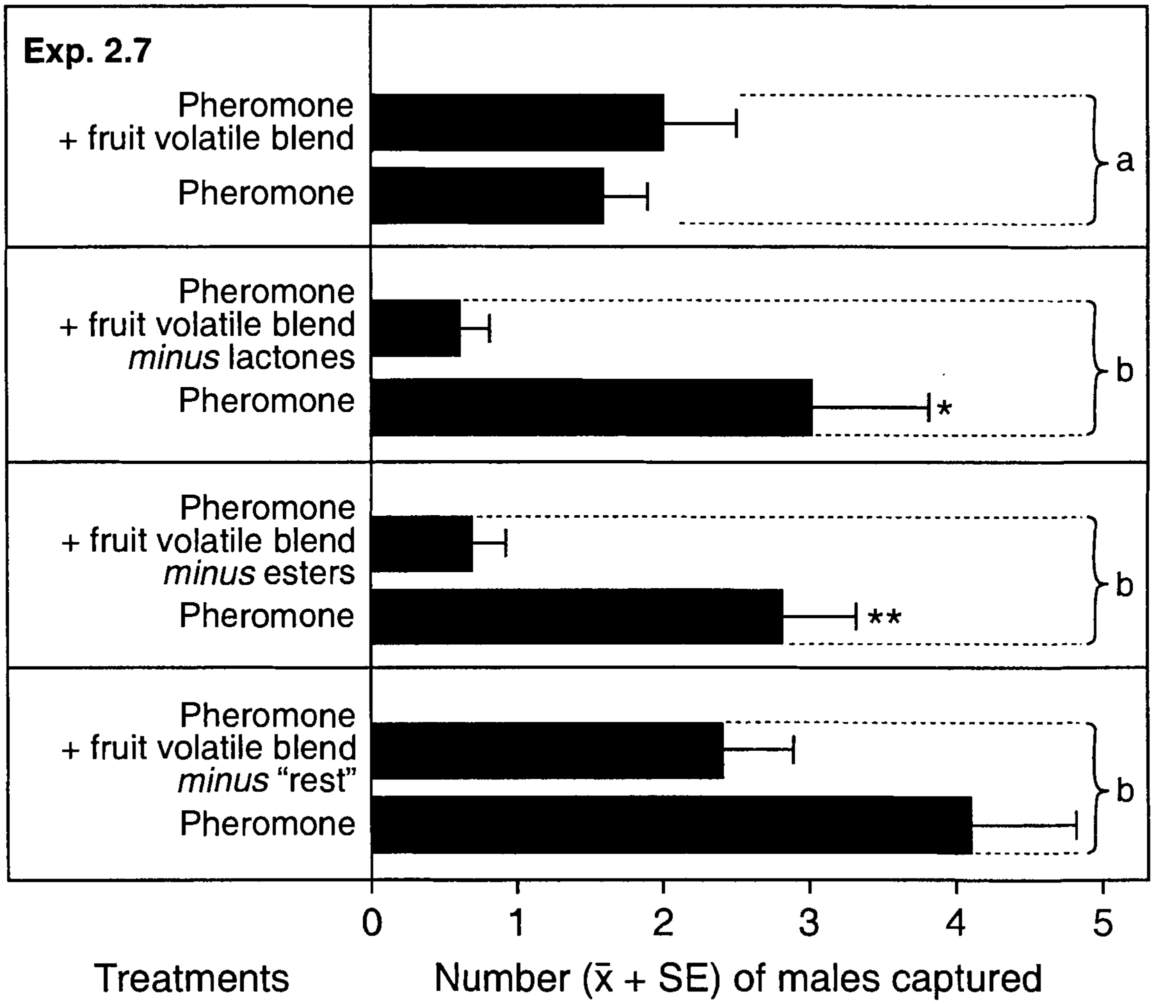

Exp. 2.7
Pheromone
+ fruit volatile blend
Pheromone
a
Pheromone
+ fruit volatile blend
minus lactones
Pheromone
*
b
Pheromone
+ fruit volatile blend
minus esters
Pheromone
**
b
Pheromone
+ fruit volatile blend
minus "rest"
Pheromone
b
0
1
2
3
4
5
Treatments
Number ($\bar{x}$ + SE) of males captured

Figure 2.6 Mean number (+ SE) of male *Anarsia lineatella* captured in experiment 2.8 (n = 9) in sticky Delta traps baited with a rudimentary blend of synthetic soft-ripe peach fruit volatiles (Table 2.2) in combination with synthetic sex pheromone (Table 2.3). Pheromone-baited traps served as control stimuli. Almond orchard, Fresno, CA, July 2001. Bars with asterisks indicate a significant preference for a particular treatment within a treatment-control pair; two-tailed Wilcoxon paired-sample test, * $P <$ 0.005. Brackets with different letters indicate significant differences between treatment-control pairs; Kruskal-Wallis test, followed by the Nemenyi multiple comparison test ($\alpha = 0.05$).

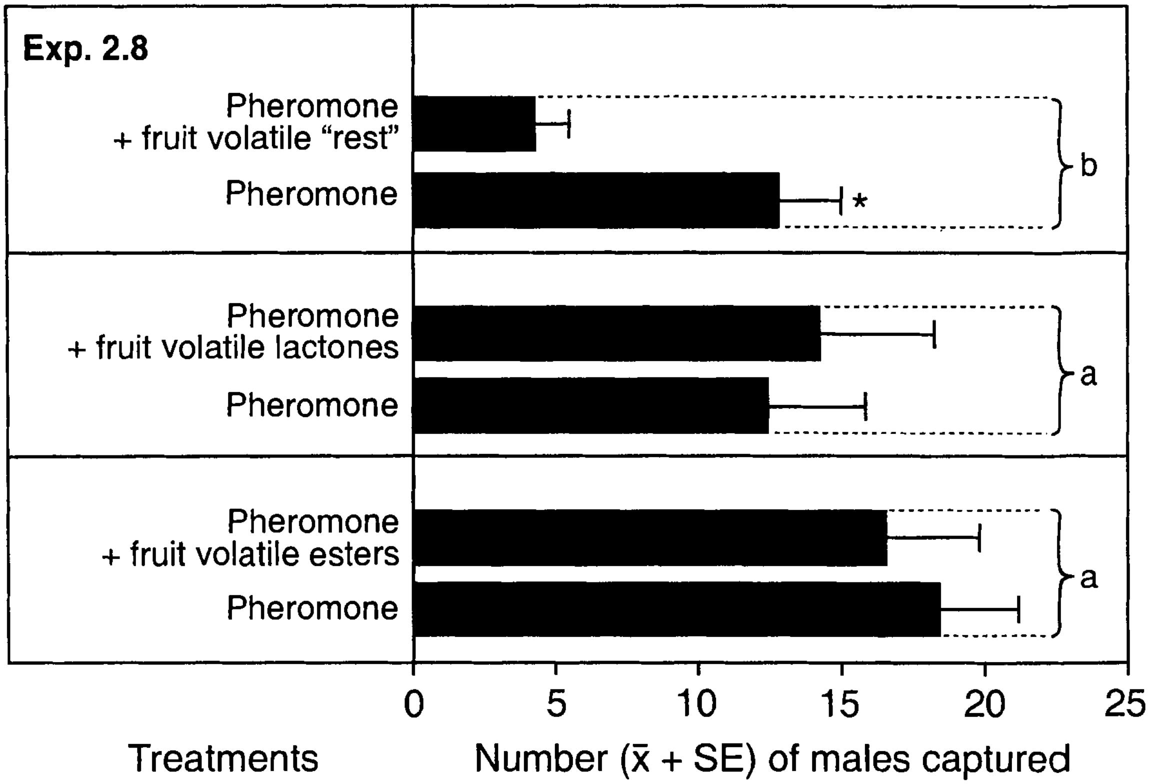

Exp. 2.8
Pheromone + fruit volatile "rest"
Pheromone
*
b
Pheromone + fruit volatile lactones
Pheromone
a
Pheromone + fruit volatile esters
Pheromone
a
0
5
10
15
20
25
Treatments
Number (x̄ + SE) of males captured

Figure 2.7 Mean number (+ SE) of male *Anarsia lineatella* captured in experiment 2.9 ($n = 10$) in sticky Delta traps baited with a rudimentary blend of 12 synthetic soft-ripe peach fruit volatiles (Table 2.2) at lure loadings of 34.5, 3.45, 0.345, or 0.0345 mg. All treatment traps were also baited with synthetic sex pheromone (Table 2.3) and had a paired control trap containing synthetic sex pheromone alone. Almond orchard, Fresno, CA, August 2001. Bars with asterisks indicate a significant preference for a particular treatment within a treatment-control pair; two-tailed Wilcoxon paired-sample test, * $P < 0.05$. Brackets with different letters indicate significant differences between treatment-control pairs; Kruskal-Wallis test, followed by the Nemenyi multiple comparison test ($\alpha = 0.05$).

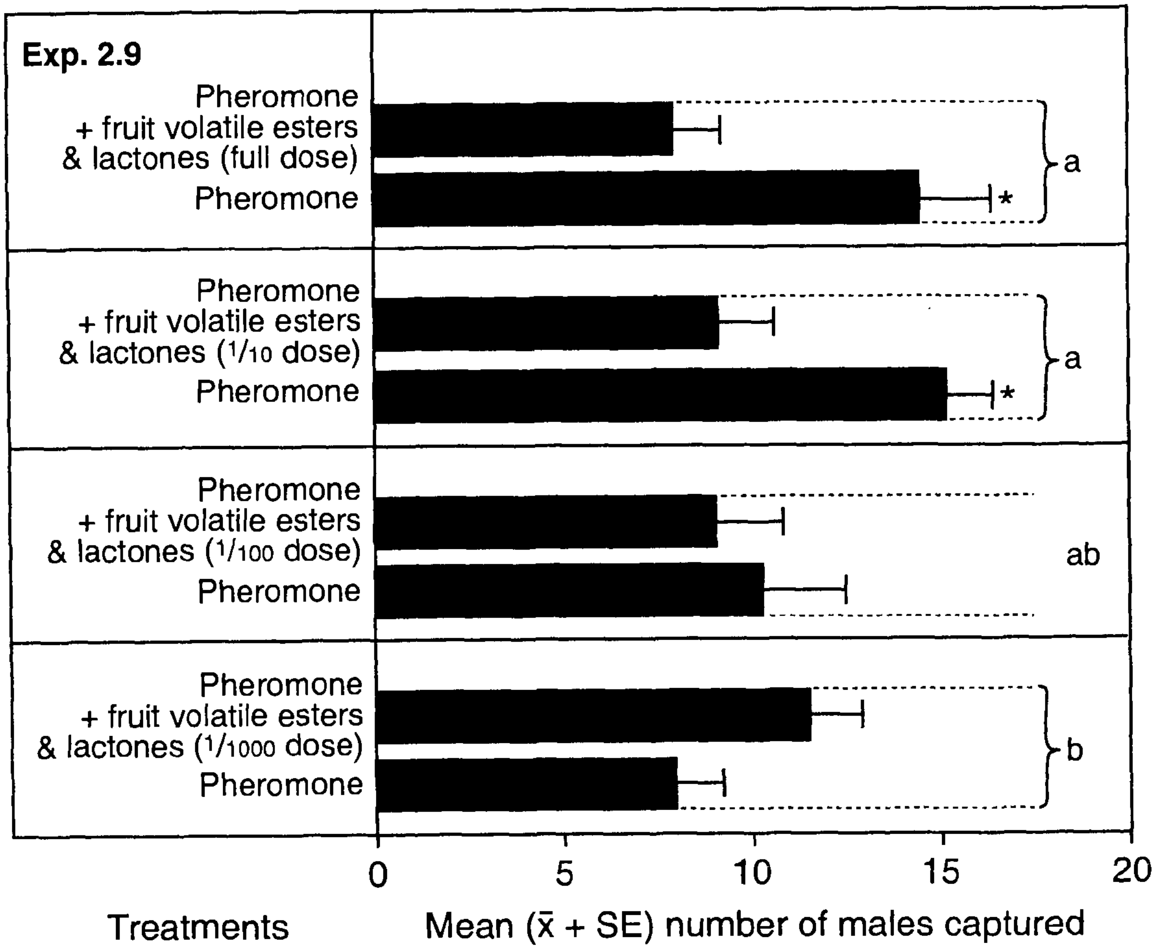

Exp. 2.9
Pheromone
+ fruit volatile esters
& lactones (full dose)
Pheromone
*
a
Pheromone
+ fruit volatile esters
& lactones (1/10 dose)
Pheromone
*
a
Pheromone
+ fruit volatile esters
& lactones (1/100 dose)
Pheromone
ab
Pheromone
+ fruit volatile esters
& lactones (1/1000 dose)
Pheromone
b
0
5
10
15
20
Treatments
Mean (x̄ + SE) number of males captured

Figure 2.8 Mean number (+ SE) of male *Anarsia lineatella* captured in experiment 2.10 (n = 10) in sticky Delta traps baited with blends of 22 synthetic soft-ripe peach fruit volatiles (50 mg) (Table 2.2), 9 synthetic peach shoot volatiles (10 mg) (Table 2.2), and synthetic sex pheromone (Table 2.3), or baited with synthetic sex pheromone alone. Almond orchard, Fresno, CA, July 2001. Bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test ($P < 0.005$).

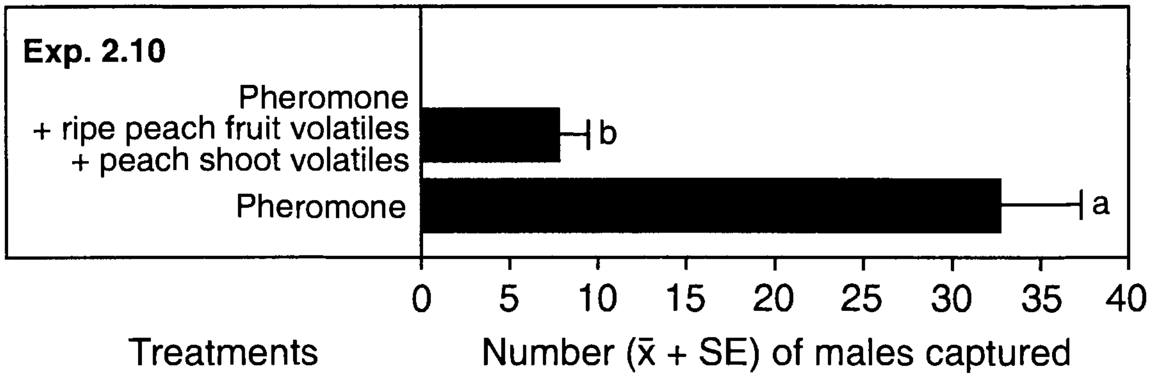
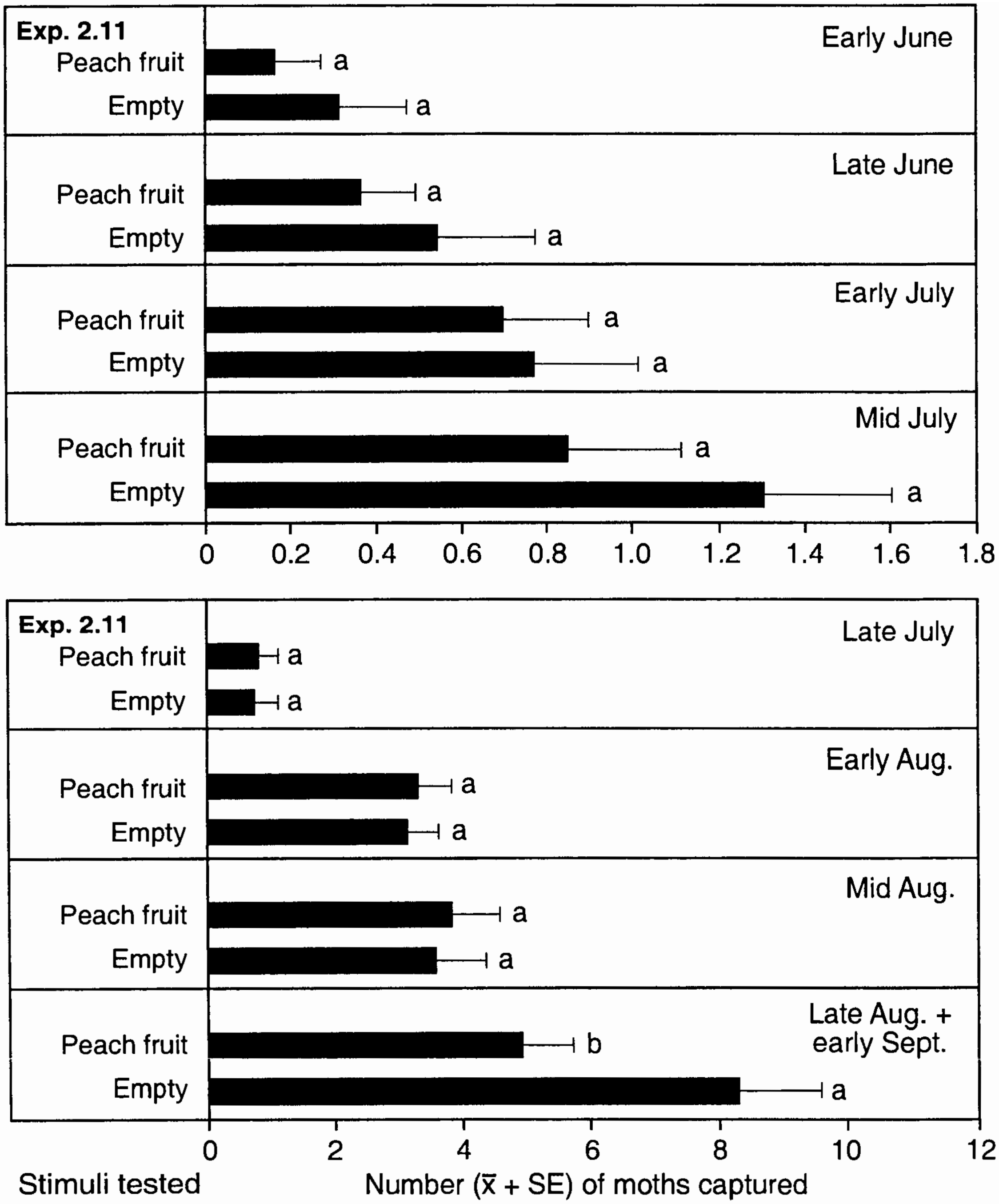

Exp. 2.10
Pheromone
+ ripe peach fruit volatiles
+ peach shoot volatiles
b
Pheromone
a
0
5
10
15
20
25
30
35
40
Treatments
Number ($\bar{x}$ + SE) of males captured

Figure 2.9 Mean number (+ SE) of male and female *Anarsia lineatella* captured in experiment 2.11 in paired traps baited with *in-situ* peach fruit or left unbaited. Traps were suspended from a peach tree (ca. 3.5 m tall) inside a mesh cage (3.6 x 3.6 x 3.0 m tall). AAFC Research Station, Summerland, June - September 2002, early June – mid July (n = 13), late July – mid August (n = 15), late August – early September (n = 11). Biweekly from early June, 100-150 *A. lineatella* were released into the field cage. Paired bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test ($P < 0.05$). Note: Paired bars represent cumulative catches.


Exp. 2.11
Peach fruit
Empty
Early June
Late June
Early July
Mid July
a
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Exp. 2.11
Peach fruit
Empty
Late July
Early Aug.
Mid Aug.
Late Aug. + early Sept.
a
b
0
2
4
6
8
10
12
Stimuli tested
Number ($\bar{x}$ + SE) of moths captured

III STIMULI AFFECTING SELECTION OF OVIPOSITION SITES

3.1 INTRODUCTION

Selecting a suitable oviposition site is critical for many phytophagous insects. Neonate larvae may depend upon their mother's proper choice of oviposition site to complete development to adulthood. Chemical stimuli play major roles in the behavioural sequence leading to oviposition (reviewed by Renwick and Chew 1994, Honda 1995, Binder and Robbins 1997, Hora and Roessingh 1999, Grant et al. 2000). Both positive and negative chemical stimuli affect the insect's decision to accept or reject a particular plant, with physical characteristics adding to the diversity of the stimulus complex (Dethier 1982). Moreover, damaged parts of the host plant have been shown to be visited and oviposited on most frequently by many lepidopteran pests (Reed and Landolt 2002, and references therein).

Female *A. lineatella* deposit and cement single eggs to twigs and leaves of new growth (Jones 1935, Bailey 1948). As peach fruits ripen, most eggs are deposited on the fruit with eggs readily found in the fuzz (Bailey 1948). However, nothing is known about the stimuli that mediate selection of oviposition sites by female *A. lineatella*.

The objectives of this study were: (1) to reinvestigate reported general ovipositional preferences in *A. lineatella* with respect to host and non-host plants; (2) to test the hypothesis that semiochemical and mechanical stimuli serve as ovipositional cues; and (3) to test the hypothesis that mechanically damaged peach fruits are particularly attractive oviposition sites.

3.2 MATERIALS AND METHODS

3.2.1 Evidence for selection of oviposition sites

In Exp. 3.1, fruit-bearing branches (*ca.* 50 cm) of three host plants; apricot, peach, and plum, and one non-host plant, apple, in four separate water-containing Erlenmeyer flasks (250 mL), were placed in small mesh fabric cages (0.9 x 0.9 x 1.0 m high) with 10 male and 10 female *A. lineatella.* Cages were kept in an environmentally controlled room maintained at 20 ± 2°C, and 60 ± 10% relative humidity with a photoperiod of 16:8 h (L:D) using four 40W fluorescent lights and four 40W incandescent lights. Insects were sustained with sugar water dispensed from an Erlenmeyer flask (125 ml) with braided cotton rolls (Chapter II, 2.2.1). After one week, the branches were removed and eggs on stems, leaves and fruits counted. Using two cages, four replicates were conducted over two weeks. Each replicate used new branches, with the corner locations of the four branches randomized. The experiment was run in late July to coincide with the flight of the first summer generation of *A. lineatella.* According to the phenology of the four plants, apricot fruits were firm or soft-ripe, whereas peach, plum and apple fruits were still immature.

In Exp. 3.2, female *A. lineatella* were offered a choice of immature peach fruits (*ca.* 3 cm diam.), green mature peach fruits (*ca.* 6 cm diam.), or soft-ripe peach fruits (*ca.* 6 cm diam.) for oviposition. One peach fruit of each type was placed in a cylindrical plastic chamber (20 cm x 20 cm diam.) (Chapter II, 2.2.1) with 10 males and 10 females, and kept in an environmentally controlled chamber (Chapter II, 2.2.1). The insects were sustained with 8% sugar water dispensed from an 18 ml vial with a braided cotton roll (Chapter II, 2.2.1). After one week, peach fruits were removed and eggs counted. Each replicate employed a Sparkleen™ (Fisher, Pittsburgh, PA) washed cage and new fruits. The immature and green mature peach fruits were of the same cultivar, whereas soft-ripe peach fruits (imported from California) were purchased at a local grocer.

Exp. 3.1 was analyzed by pooling the leaf, stem, and fruit egg counts for each of apricot, peach, plum, or apple branches. The pooled data were then analyzed using Friedman's test under the null hypothesis that an equal number of eggs were deposited on each of the four branches irrespective of species. Exp. 3.2 was analyzed using Friedman's test under the null hypothesis that an equal number of eggs were deposited on each of the three fruits irrespective of their degree of maturity. For both Exp. 3.1 and 3.2, a nonparametric Tukey-type multiple comparison test [similar to the Nemenyi test (Chapter II, 2.2.4.3)] was then applied to determine such differences between treatments (Zar 1996).

3.2.2 Tactile stimuli

Exp. 3.3 tested the hypothesis that *A. lineatella* prefer hairy surfaces to oviposit on. One half of immature peach fruits (*ca.* 3 cm diam.) were razor-shaved, whereas the other half were subjected to mechanical scraping without removing the hair. Four such treated peach fruits were placed in a cage (Chapter II, 2.2.1) with 10 males and 10 females kept in an environmentally controlled chamber, and insects were sustained as described above (Chapter II, 2.2.1). The number of eggs present on shaved and unshaved halves was scored after one week.

Exp. 3.3 was analyzed using the two-tailed Wilcoxon paired-sample test under the null hypothesis that an equal number of eggs were deposited on shaved and unshaved halves of fruits.

3.2.3 Olfactory stimuli

Exps. 3.4-3.11 tested the hypothesis that oviposition by *A. lineatella* is also mediated by olfactory cues. The experiments employed an inverted plastic arena (Rubbermaid, Wooster, OH) (51 x 36 x 15 cm high) with two mesh fabric (Chapter II, 2.2.2) sections (each 20 x 15 cm) in the lid, and two holes (each 1.3 cm diam.) 30 cm apart from each other in the bottom (Figure 3.1). Each hole was covered with a piece (3 x 3 cm) of black felt providing oviposition sites for female *A. lineatella*. A black plastic square (10 x 10 cm) with a central hole (1.5 cm) was placed on each felt square. Three thin spacers (2.0 mm high) were placed on each plastic square around the hole to allow passage of volatiles into the arena, especially in Exps. 3.4-3.5 where Erlenmeyer flasks were placed over the holes. An inverted glass jar, (14 cm x 8.5 cm diam.) covered with black card to eliminate visual stimuli was placed over each plastic square. Both treatment and control jars contained moistened paper towels to even humidity levels. For each replicate, 10 male and 10 female *A. lineatella* were introduced into the arena and sustained as described above. The arena was kept in an environmentally controlled chamber under conditions described for colony propagation in Chapter II, 2.2.1.

Exps. 3.4-3.5 tested whether semiochemicals from almond (Merced, CA) and peach (Cawston, B.C.) shoots respectively, serve as oviposition stimulants for *A. lineatella*. In both experiments, the shoots were placed in a 50 ml Erlenmeyer flask filled with water, whereas a flask filled with water served as a control. Exps. 3.6-3.9 tested semiochemicals from immature peach fruits (*ca.* 3 cm diam.) (Exp. 3.6), green mature peach fruits (*ca.* 6 cm diam.) (Exp 3.7), hard-ripe peach fruits (*ca.* 6 cm diam.) (Exp. 3.8) and soft-ripe peach fruits (*ca.* 6 cm diam.) (Exp. 3.9) as oviposition stimulants, with numbers of fruits adjusted to equalize the weight of test stimuli. Exp. 3.10 tested five immature peach fruits vs. a hard ripe peach fruit, and Exp. 3.11 tested five immature peach fruits vs. a soft ripe peach fruit. Stimuli tested in Exps. 3.4-3.10 are summarized in Table 3.1. The immature, green mature, and hard ripe peach fruits were of the same cultivar (Cawston, B.C.), whereas soft-ripe peach fruits (imported from California) were purchased at a local grocer. In each replicate of all experiments, felt squares were changed daily and scored for eggs, and egg counts were summed over 3-4 days. Between replicates, arenas and jars were washed thoroughly with Sparkleen™, and plastic squares were replaced.

Exps. 3.4-3.10 were analyzed with the two-tailed Wilcoxon paired-sample test under the null hypothesis that an equal number of eggs were laid on felt squares under treatment stimuli and control stimuli. Exp. 3.11 was analyzed with a paired t-test because the number of replicates was too low for analysis with the Wilcoxon paired-sample test to conclude significance at the $\alpha = 0.05$ level.

3.2.4 Ovipositional response to *in-situ* fruit in field cage experiments

Exp. 3.12 tested the hypothesis that female *A. lineatella* lay more eggs on damaged than on undamaged fruit. From early to late July, three peach trees (*ca.* 3.5 m tall) in the entomology orchard of the AAFC Research Station in Summerland were encompassed separately in a field cage (Chapter II, 2.2.5). On each tree, 28-30 pairs of peach fruits were selected, with peach fruits within pairs being 30-50 cm apart from each other and of similar size and maturity. All other peach fruits were removed from trees. One peach per pair was randomly selected to be mechanically damaged on the bottom by five cuts (each 5 cm long x 0.5 cm deep) with a sharp knife. Three days later, 100-150 laboratory-reared male and female *A. lineatella* were released into the cage. Seven days after the release of moths, peach fruits were removed and scored for oviposition. Exp 3.13 assessed whether damaged peach fruits were more attractive and were being visited more often than undamaged peach fruits. Ten pairs of fruit were chosen, with one fruit per pair damaged as in Exp. 3.12, and each fruit of each pair enclosed in a rectangular trap (Chapter II, 2.2.5). An empty control trap was paired with each trap containing a fruit. Exp. 3.13 was run from August 3 to September 7, while peach fruits went from green mature to soft-ripe in the traps. On August 4 and 20, 100-150 laboratory reared male and female *A. lineatella* were released into the cage, moths were counted and removed from the traps weekly.

Results from each of the three trials of Exp. 3.12 were analyzed with the two-tailed Wilcoxon paired-sample test under the null hypothesis that an equal number of eggs were laid on damaged and undamaged peach fruits. Exp. 3.13 was analyzed using Friedman's test under the null hypothesis that an equal number of moths were captured in traps of the three treatments. A nonparametric Tukey-type multiple comparison test (Chapter III, 3.2.1) was then applied to determine such differences between treatments.

3.3 RESULTS

3.3.1 Evidence for selection of oviposition sites

In Exp. 3.1 female *A. lineatella* laid most eggs on hairy or creviced surfaces when presented with apple, almond, peach and plum branches. The favoured oviposition site consisted of hairy peach fruits, followed closely by hairy leaves of plum branches. The glabrous surfaces of apricot, plum, and apple fruits were not selected for oviposition, and the relatively glabrous surfaces of apricot and peach leaves were seldom oviposited upon. Interestingly, the nonhost (hairy) apple leaves were more often selected as oviposition sites than host peach or apricot leaves. The wooden stems of all four plant species were oviposited upon evenly, but at a lower level compared to peach fruits and plum leaves (Figure 3.2, Exp. 3.1). When analyzing statistically the total egg counts on apricot, peach, plum, or apple branches, peach branches received significantly more eggs than apricot branches ($P < 0.05$, Figure 3.2).

In Exp. 3.2, immature green peach fruits invariably received more eggs than green mature peach fruits which, in turn, received more eggs than soft-ripe peach fruits ($P < 0.05$, Figure 3.3).

3.3.2 Tactile stimuli

In Exp. 3.3, female *A. lineatella* oviposited 10 times more eggs on the hairy halves of peach fruits than on the shaved hairless halves ($P < 0.01$, Figure 3.4).

3.3.3 Olfactory stimuli

In Exp. 3.4-3.5, semiochemicals from both almond and peach shoots induced more oviposition by female *A. lineatella* than control stimuli ($P < 0.05$, $P < 0.01$ respectively, Figure 3.5, Exps. 3.4-3.5). Similarly, semiochemicals from immature peach fruits ($P < 0.05$, Exp. 3.6), green mature peach fruits ($P < 0.05$, Exp. 3.7), and hard-ripe peach fruits ($P < 0.05$, Exp. 3.8) induced more oviposition than their respective control stimuli (Figure 3.6). In contrast, semiochemicals from soft-ripe peach fruits did not induce oviposition compared to its control ($P < 0.2$, Figure 3.6, Exp. 3.9). Semiochemicals from immature peach fruits and hard-ripe peach fruits were equally effective in inducing oviposition ($P < 0.1$, Figure 3.6, Exp. 3.10) but semiochemicals from immature peach fruits stimulated three times more oviposition than those from soft-ripe peach fruits ($P < 0.05$, Figure 3.6, Exp 3.11).

3.3.4 Ovipositional response to *in-situ* fruit in field cage experiments

In Exp. 3.12, female *A. lineatella* laid more eggs on mechanically damaged peach fruits than on undamaged peach fruits in early July ($P < 0.01$) and mid July ($P < 0.0005$) but not in late July ($P < 0.1$) (Figure 3.7, Exp. 3.12).

In Exp. 3.13, from August 3 to September 7, similar numbers of moths were captured in traps baited with in-situ damaged or undamaged peach fruits. However, empty control traps caught 50% and 85% more moths than traps containing damaged or undamaged peach fruits, respectively ($P < 0.05$, Figure 3.8, Exp. 3.13).

3.4 DISCUSSION

My data provide evidence that female *A. lineatella* discern between potential oviposition sites, and that both tactile and semiochemical stimuli mediate selection of oviposition sites. Mechanically damaged fruits do not attract *A. lineatella* but induce oviposition more so than undamaged fruits, provided that fruits are not yet in the soft-ripe stage of development.

Host specificity did not appear to be a prime factor in oviposition choices by female *A. lineatella* because both apple leaves and wooden stems were readily oviposited upon. Such ovipositional "mistakes" have been previously reported from other Lepidoptera, including *Papilio glaucus* (Berenbaum 1981) and *Helicoverpa zea* (Breeden et al. 1996). Mistakes by polyphagous *H. zea* have been explained as mechanisms to even further diversify the host range (Breeden et al. 1996). This is not a plausible explanation for oligophagous *A. lineatella*, although *A. lineatella* larvae have been observed to enter and mature to adulthood in immature apples in the laboratory (personal observation).

Reduced discrimination between hosts also may have been influenced by egg loads of ovipositing females. Such a phenomenon has been observed in two species of butterflies and several species of flies where females had not recently oviposited (Minkenberg et al. 1992). Conceivably, female *A. lineatella* in Exp. 3.1 may have had a high egg load due to pre-experiment confinement in Plexiglas™ containers, resulting in less selective oviposition decisions. Alternatively, nonhost *Malus domestica* may still be sufficiently closely related to *Prunus* spp. to contain and release chemicals similar to those found in *Prunus* host plants of *A. lineatella*. However, the lack of reported damage to *Malus domestica* by *A. lineatella* suggests that *A. lineatella* typically avoid this nonhost. It is also possible that avoidance mechanisms or responses may not have been effective in the confined space in which Exp. 3.1 was conducted. Finally, lack of oviposition sites could not have caused oviposition on nonhost apple branches in Exp. 3.1, because eggs in Exp. 3.2 were laid on peach fruits at 10 times the density as in Exp. 3.1.

Hairy and creviced surfaces, as provided by hairy peach fruits, hairy plum leaves and cracks in the bark of 0-2 year old growth (Figure 3.2, Exp. 3.1), are strongly favoured as oviposition sites by *A. lineatella*, while glabrous leaves and fruits are seldom or never oviposited upon. When olfactory cues were held constant, most eggs laid by *A. lineatella* are on hairy sites (Figure 3.4, Exp. 3.3).

Preference for hairy or rough oviposition sites, as provided by apple leaves or branches, has also been reported for many other species in the Lepidoptera (reviewed by Ramaswamy 1988), including another gelechiid, the potato moth *Phthorimaea operculella* (Fenemore 1988). Hairy surfaces provide ovipositing female *H. zea* a secure grip on the substrate's surface (Callahan 1957, Bernays and Chapman 1994). The much smaller female *A. lineatella* do not seem to need that grip as they readily walk upside-down on glabrous surfaces, but their eggs may be better retained on hairy than smooth leaves (Ramaswamy 1988, Renwick and Chew 1994). Physical characteristics of plant trichomes also reduce searching efficiency of parasitoids (e.g. Romeis et al. 1998). Preference for hairy sites by ovipositing female *A. lineatella* could represent a defence mechanism, as up to 75% of their eggs were parasitized by *Paralitomastix pyralidis* (Ashmead) in organic orchards near Cawston, B.C. (personal observation 1999).

Chemical signals from plants in addition to physical properties of plant surfaces convey critical information for female *A. lineatella* seeking oviposition sites, as shown in Exps. 3.4-3.11. The odours of almond (Exp. 3.4) and peach (Exp. 3.5) shoots were found to promote oviposition by *A. lineatella.* Similarly, the odours of immature peach fruits (Exp. 3.6), green mature peach fruits (Exp. 3.7), and hard-ripe peach fruits (Exp. 3.8) all resulted in oviposition nearby. Only soft-ripe peach fruits (Exp. 3.9) did not have this effect, consistent with the repellency of synthetic soft-ripe peach fruit volatiles seen in Chapter II.

The volatile chemical cues tested in Exps. 3.4-3.11 more likely attracted or arrested female *A. lineatella* than induced oviposition. This conclusion is based on previous findings that non-volatile contact stimuli are responsible for inducing oviposition (reviewed in Renwick 1989). The fact that soft-ripe peach fruits moderately deterred oviposition by female *A. lineatella* in Exps. 3.9 and 3.11 substantiates the conclusion that negative stimuli help discern between potential oviposition sites (Renwick 1989, Honda 1995).

Selection of hosts and oviposition sites is also influenced by host injuries. Female *A. lineatella* preferred to oviposit on injured peach fruits in early and mid July (Exp. 3.12), possibly due to qualitative or quantitative changes in fruit volatile blends attracting or arresting females. Similarly, the false codling moth, *Cryptophlebia leucotreta*, is attracted to mechanically damaged citrus fruit (Gerhard Bowsen, South Africa, personal communication), and *A. transitella* is often found in almonds previously damaged by *A. lineatella* (Curtis 1983). Also, injury to peach fruits provides the larvae with points of sheltered entry into the fruit and allows them to bypass the exocarp.

That damaged and undamaged peach fruits repelled *A. lineatella* (Exp. 3.13) in late August is consistent with the results of experiment 2.11 which also showed that soft-ripe peach fruits are repellent to *A. lineatella*. That damaged peach fruits did not attract more moths than undamaged peach fruits suggests that the increased oviposition on damaged peach fruits in experiment 3.12 is not due to increased attraction of *A. lineatella* to the peach fruits. However, experiment 3.13 was conducted with ripe peach fruits rather than immature fruit that stimulated oviposition (Exp. 3.12). The repellency of ripe peach fruits may have masked any increased attractiveness of damaged peach fruits.

In summary, female *A. lineatella* use both tactile and olfactory stimuli to select host plants and oviposition sites. They also prefer injured over intact immature fruits for oviposition. Whether this preference is based on olfactory or contact chemical stimuli, and their molecular structure, remains to be determined.

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Table 3.1 Stimuli tested in experiments 3.4 – 3.10 in olfactory oviposition bioassay.

Experiment No.	Test Stimuli		Reps
	Treatment	Control	
3.4	Almond shoots[a]	Empty	6
3.5	Peach shoots[a]	Empty	8
3.6	5 immature peach fruits[b]	Empty	6
3.7	Mature green peach fruit[c]	Empty	6
3.8	Hard-ripe peach fruit[c]	Empty	7
3.9	Soft-ripe peach fruit[c]	Empty	6
3.10	5 immature peach fruits[b]	Hard-ripe peach fruit[c]	6
3.11	5 immature peach fruits[b]	Soft-ripe peach fruit[c]	4

[a] Almond and peach shoots measured *ca.* 10 cm.
[b] Immature peach fruits measured *ca.* 3 cm in diameter.
[c] Mature green, hard-ripe, and soft-ripe peach fruits all measured *ca.* 6 cm in diameter.

Figure 3.1 Olfactometer used for testing ovipositional choices of female *Anarsia lineatella* in response to various odour stimuli in experiments 3.4-3.11. The olfactometer consisted of an inverted plastic container (51 x 36 x 15 cm high) with two mesh sections (each 20 x 15 cm) in the lid. Note: **1** hole (1.3 cm diam.); **2** black felt square (3 x 3 cm) placed over hole; **3** black plastic square (10 x 10 cm) with a central hole (1.5 cm diam.) placed over black felt; **4** spacers (2.0 mm high) carrying peach fruits or Erlenmeyer (50 mL) flask with a peach or almond shoot, enclosed in an inverted glass jar; **5** black card control. Moistened paper towel lined the inside of the glass jars to control for any humidity difference between treatment and control stimuli.

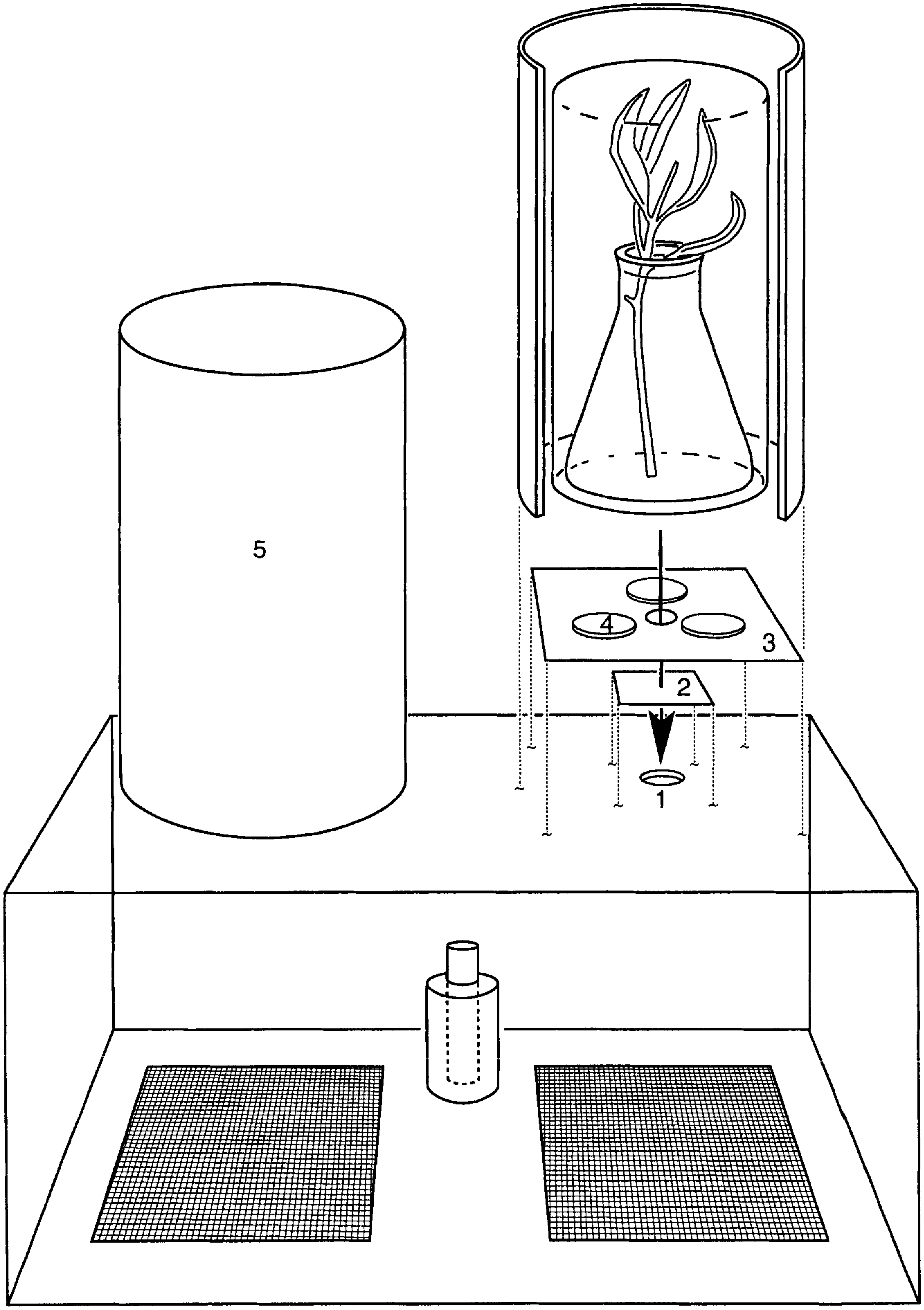

5
4
3
2
1

Figure 3.2 Mean number (+ SE) of eggs laid by female *Anarsia lineatella* in experiment 3.1 on leaves, stem, or fruit of a single branch of three host and one nonhost plant species presented in four separate Erlenmeyer flasks, inside a mesh cage (0.9 x 0.9 x 1.0 m high). Four replicates conducted in July 2002, (coinciding with the flight of the first summer generation of *A. lineatella*), ten male and ten female *A. lineatella* were released into the cage and numbers of eggs laid were recorded seven days later. Triplets of bars with different letters are significantly different; Friedman's test, followed by a nonparametric Tukey-type multiple comparison test ($\alpha = 0.05$).

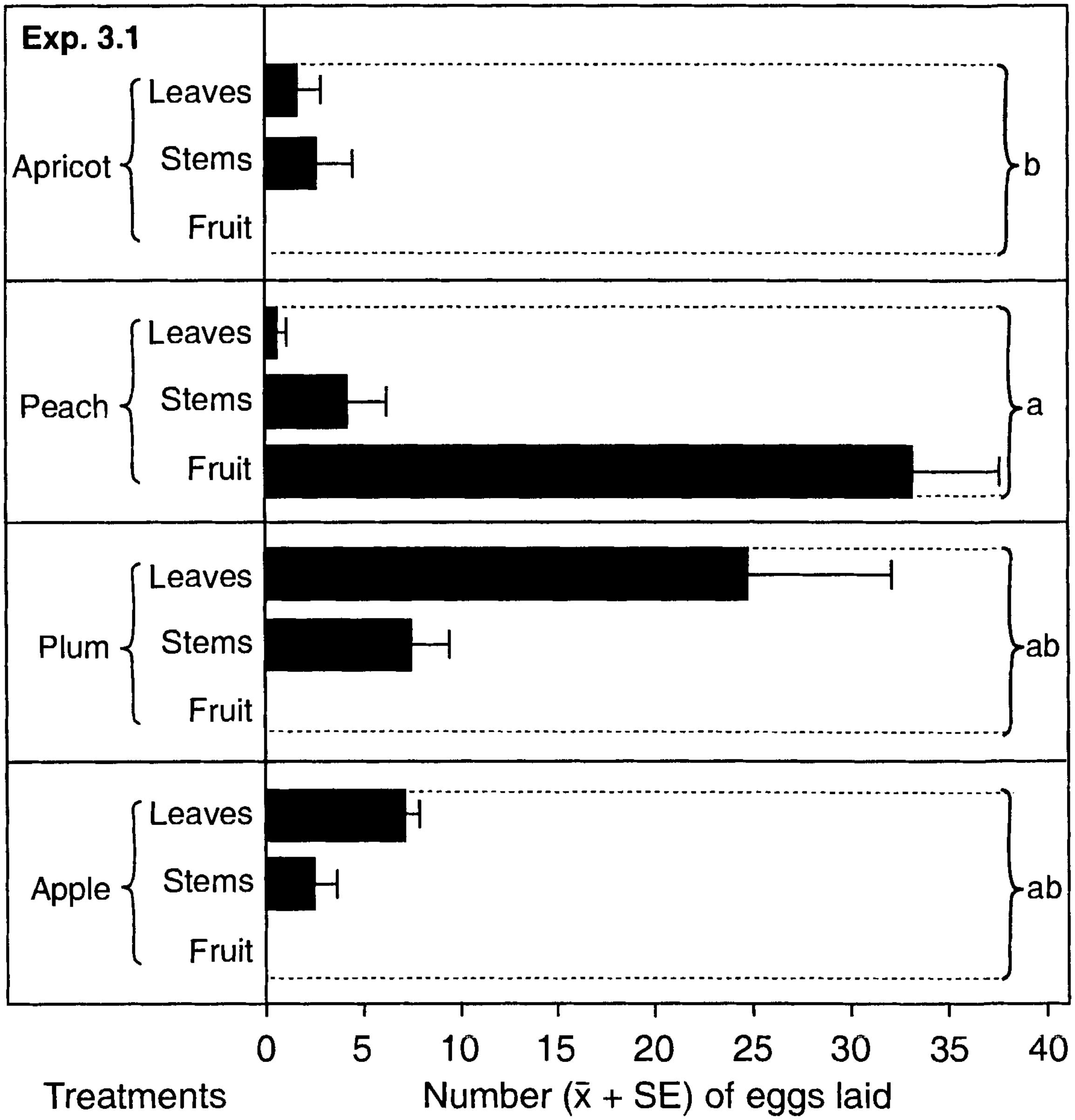

Exp. 3.1
Apricot
Leaves
Stems
Fruit
b
Peach
Leaves
Stems
Fruit
a
Plum
Leaves
Stems
Fruit
ab
Apple
Leaves
Stems
Fruit
ab
0
5
10
15
20
25
30
35
40
Treatments
Number (x̄ + SE) of eggs laid

Figure 3.3 Mean number (+ SE) of eggs laid in experiment 3.2 ($n = 8$) by female *Anarsia lineatella* on one immature, green mature, or soft-ripe peach fruit presented simultaneously in a cylindrical plastic chamber (20 cm x 20 cm diam.), containing ten male and ten female *A. lineatella* for one week. Bars with different letters are significantly different; Friedman's test, followed by a nonparametric Tukey-type multiple comparison test ($\alpha = 0.05$).

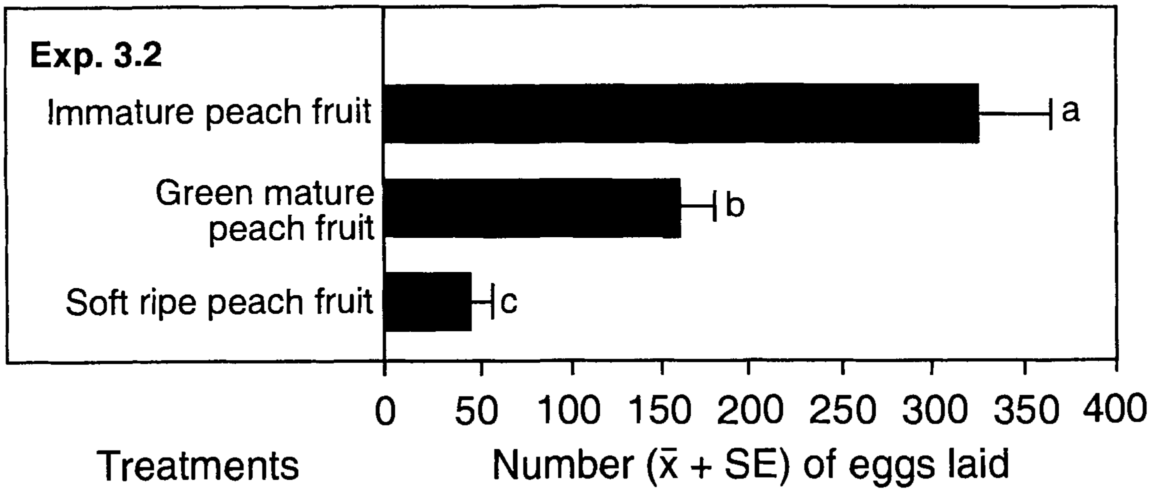

Exp. 3.2
Immature peach fruit
Green mature peach fruit
Soft ripe peach fruit
a
b
c
0
50
100
150
200
250
300
350
400
Treatments
Number (x̄ + SE) of eggs laid

Figure 3.4 Mean number (+ SE) of eggs laid in experiment 3.3 (n = 8) by female *Anarsia lineatella* on shaved and unshaved halves of four immature peach fruits in a cylindrical plastic chamber (20 cm x 20 cm diam.) containing ten male and ten female *A. lineatella* for one week. Bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test ($P < 0.01$).

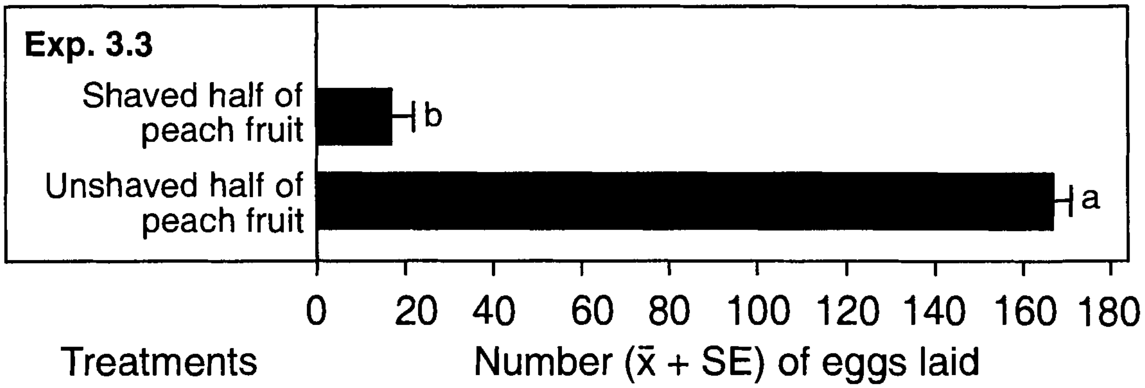

Exp. 3.3
Shaved half of peach fruit
Unshaved half of peach fruit
b
a
0
20
40
60
80
100
120
140
160
180
Treatments
Number (x̄ + SE) of eggs laid

Figure 3.5 Mean number (+ SE) of eggs laid by female *Anarsia lineatella* on felt squares in olfactometers (Fig. 3.1) in the presence (treatment) or absence (control) of volatiles from almond shoots (Experiment 3.4, $n = 6$) or peach shoots (Experiment 3.5, $n = 8$). For each replicate, ten male and ten female *A. lineatella* were released into the olfactometer; felt squares were changed daily with egg counts summed over 3-4 days. In each experiment, bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test (Experiment 3.4, $P < 0.05$; Experiment 3.5, $P < 0.01$).

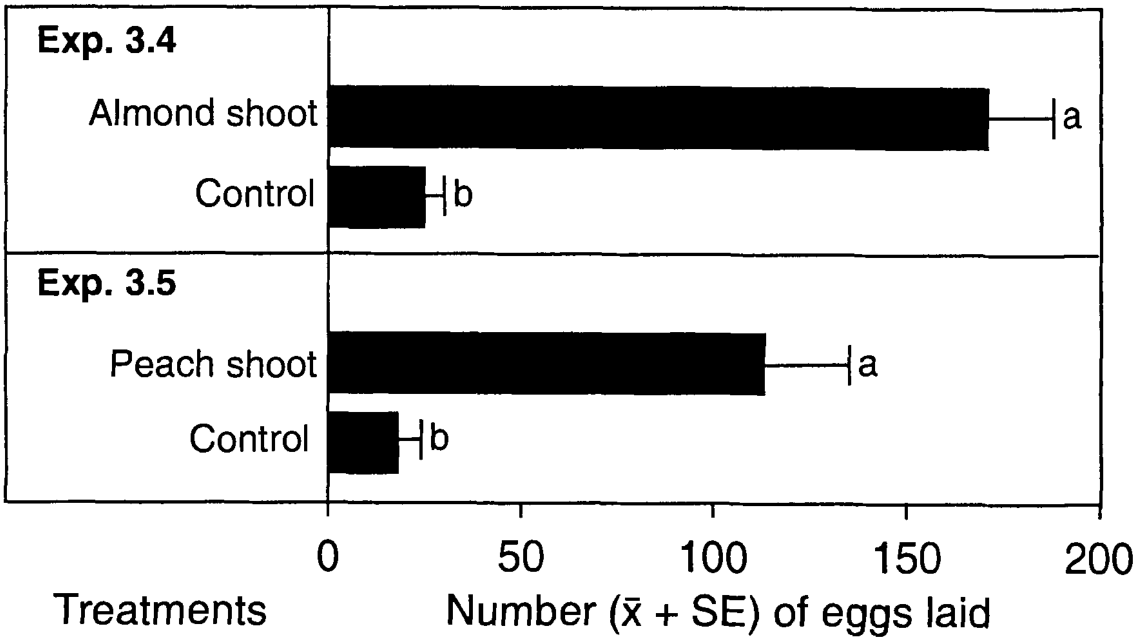

Exp. 3.4
Almond shoot
a
Control
b
Exp. 3.5
Peach shoot
a
Control
b
0
50
100
150
200
Treatments
Number (x̄ + SE) of eggs laid

Figure 3.6 Mean number (+ SE) of eggs laid by female *Anarsia lineatella* on felt squares in olfactometers (Fig. 3.1) in the presence (treatment) or absence (control) of volatiles from immature peach fruits (Experiment 3.6), green mature peach fruits (Experiment 3.7), hard-ripe peach fruits (Experiment 3.8), soft-ripe peach fruits (Experiment 3.9), or in the presence of volatiles from two sources (Experiments 3.10 – 3.11). For each replicate, ten male and ten female *A. lineatella* were released into the olfactometer, felt squares were changed daily with egg counts summed over 3-4 days. In each experiment, bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test (Exps. 3.6 – 8, $P < 0.05$); ANOVA (Experiment 3.11, $P < 0.05$).

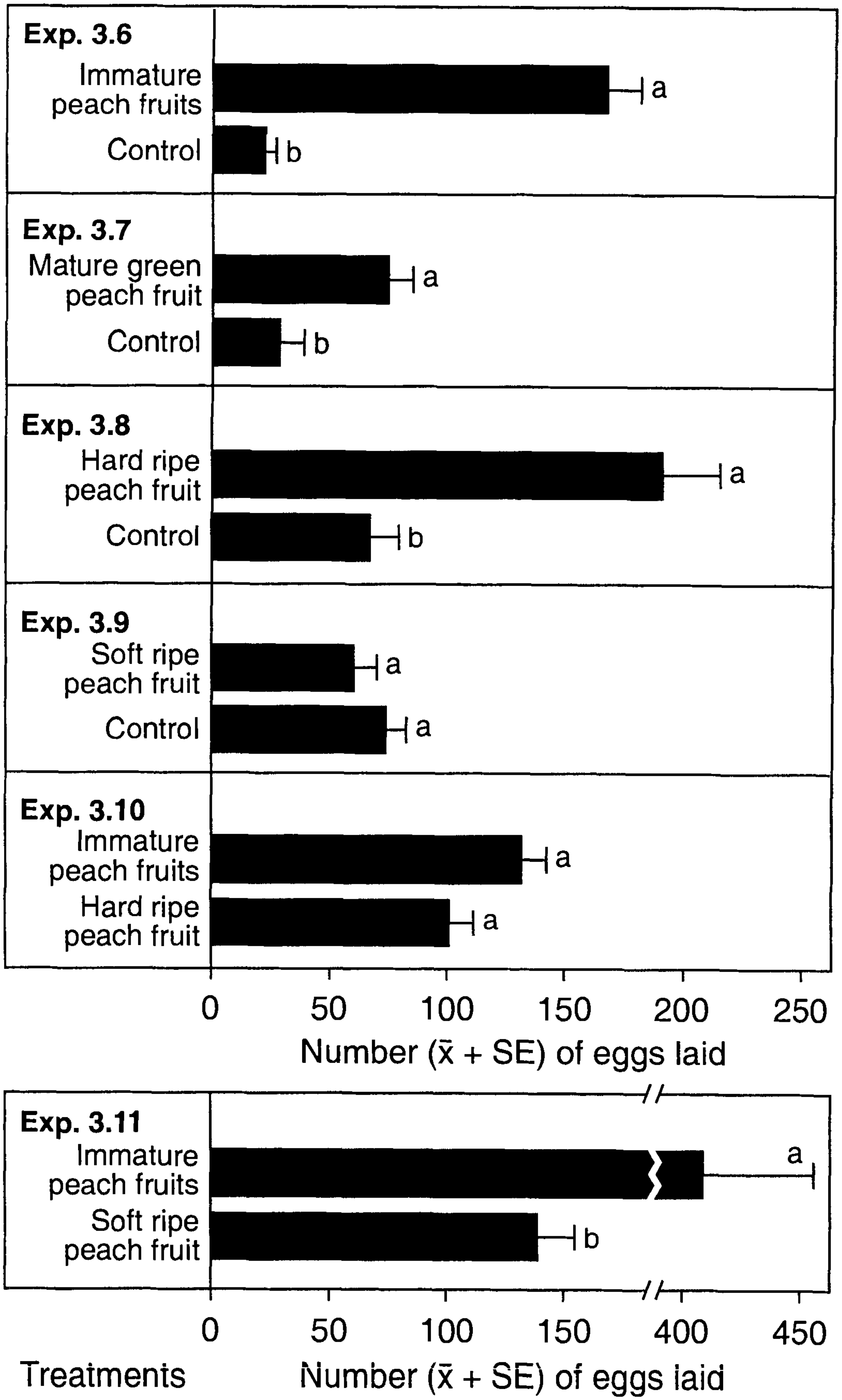

Exp. 3.6
Immature peach fruits
a
Control
b
Exp. 3.7
Mature green peach fruit
a
Control
b
Exp. 3.8
Hard ripe peach fruit
a
Control
b
Exp. 3.9
Soft ripe peach fruit
a
Control
a
Exp. 3.10
Immature peach fruits
a
Hard ripe peach fruit
a
0
50
100
150
200
250
Number ($\bar{x}$ + SE) of eggs laid
Exp. 3.11
Immature peach fruits
a
Soft ripe peach fruit
b
0
50
100
150
400
450
Treatments
Number ($\bar{x}$ + SE) of eggs laid

Figure 3.7 Mean number (+ SE) of eggs laid by female *Anarsia lineatella* in experiment 3.12 on mechanically damaged or undamaged *in-situ* peach fruits of a 3.5 m tall peach tree inside a mesh cage (3.6 x 3.6 x 3.0 m tall). AAFC Research Station, Summerland, July 2002, n = 28–30. For each time period, a different peach tree was used and 100-150 *A. lineatella* were released into the cage; peach fruits were removed and eggs counted after one week. Paired bars with different letters are significantly different; two-tailed Wilcoxon paired-sample test (early July, $P < 0.01$; mid July, $P < 0.0005$).

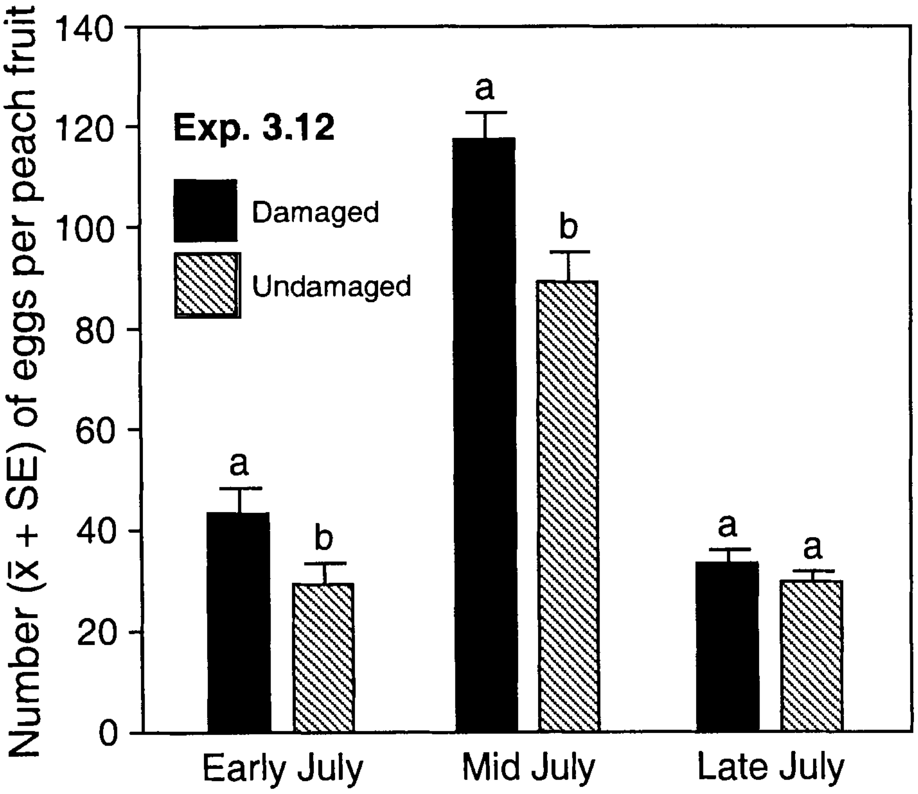
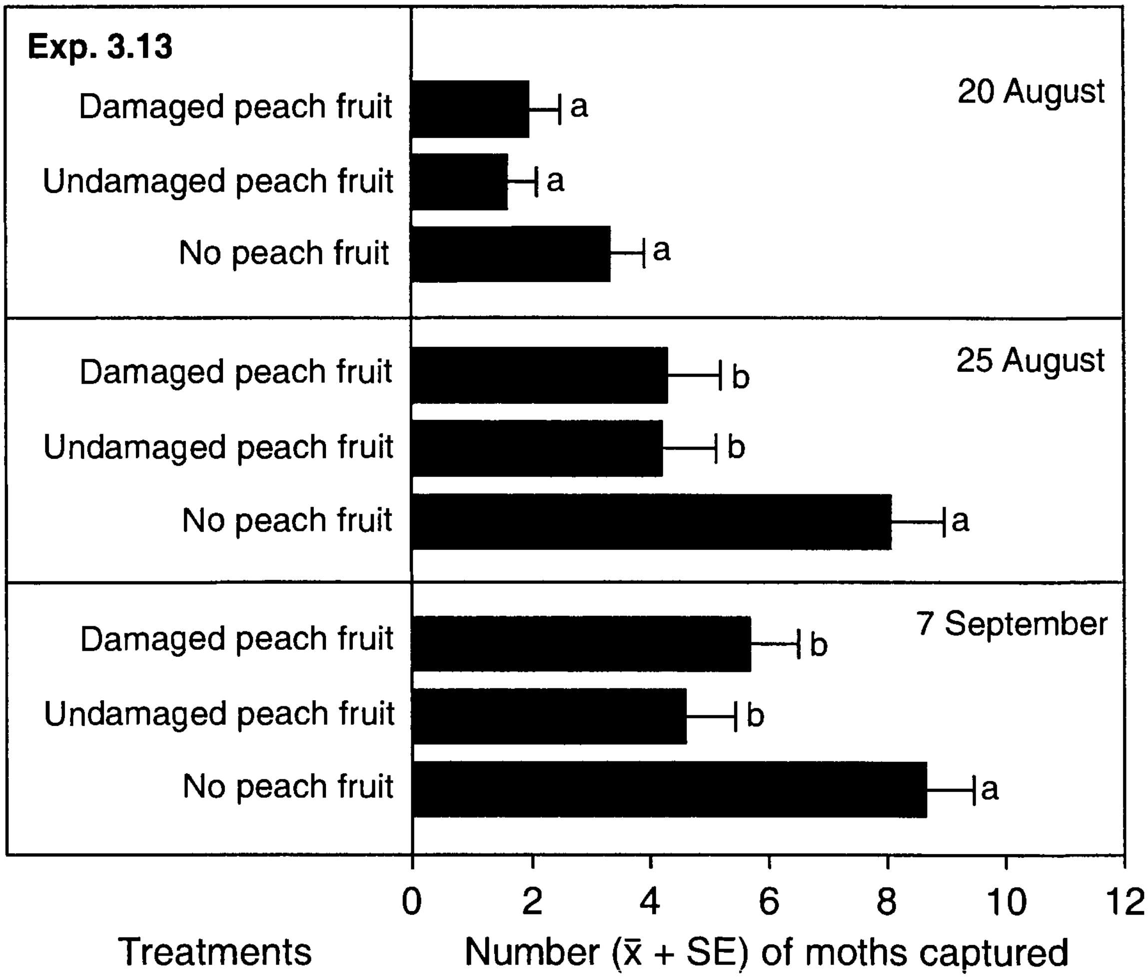

Number (x̄ + SE) of eggs per peach fruit
0
20
40
60
80
100
120
140
Exp. 3.12
Damaged
Undamaged
a
b
a
b
a
a
Early July
Mid July
Late July

Figure 3.8 Mean number (+ SE) of male and female *Anarsia lineatella* captured in experiment 3.13 in traps constructed around mechanically damaged or undamaged *in-situ* peach fruits of a 3.5 m tall peach tree inside a mesh cage (3.6 x 3.6 x 3.0 m tall). AAFC Research Station, Summerland, August – September 2002, n = 10. On August 4 and 20, 100 – 150 *A. lineatella* were released into the cage. For each date, bars with different letters are significantly different; Friedman's test, followed by a nonparametric Tukey-type multiple comparison test ($\alpha = 0.05$). Note: Bars represent cumulative catches for the respective date.


Exp. 3.13
20 August
Damaged peach fruit
a
Undamaged peach fruit
a
No peach fruit
a
25 August
Damaged peach fruit
b
Undamaged peach fruit
b
No peach fruit
a
7 September
Damaged peach fruit
b
Undamaged peach fruit
b
No peach fruit
a
0
2
4
6
8
10
12
Treatments
Number (x̄ + SE) of moths captured

IV ATTRACTION OF NEONATE LARVAE TO PEACH SHOOT AND ALMOND FRUIT AND SHOOT VOLATILES

1. INTRODUCTION

Host-foraging and selection by *A. lineatella* may be achieved by both female moths and larvae. Females select host plants as oviposition sites and lay eggs primarily on fruits or bark of host trees. When eggs are laid on bark, neonate larvae must locate shoots or fruits. Particularly larvae that have overwintered in hibernacula may need to forage considerable distances to locate new growth (Bailey 1948).

Such foraging behaviour, may be mediated, in part, by airborne semiochemicals, as shown for codling moth larvae, *C. pomonella* (Bradley and Suckling 1995, Landolt et al. 1998, Knight and Light 2001), and parsnip webworm larvae, *Depressaria pastinacella* (Carroll and Berenbaum 2002). Lepidopteran larvae can detect semiochemicals from host plants (Dethier 1980, Landolt et al. 1998, 2000, Singh and Mullick 2002) with semiochemical receptors residing on simple antennae or maxillae (Dethier and Kuch 1971, Dethier and Schoonhoven 1969, 1987).

My objectives were: (1) to determine whether neonate *A. lineatella* larvae orient chemoanemotactically toward Porapak Q extracts of host almond and peach shoot volatiles; and (2) if so, to identify the semiochemical(s) responsible for larval attractiveness.

2. MATERIALS AND METHODS

2.1 Identification of antennally-active almond fruit and shoot volatiles

Aliquots of Porapak Q-captured almond fruit and shoot volatiles (Chapter II, 2.2.4.1) were analyzed by GC-EAD and GC-MS (Chapter II, 2.2.4.2). Antennae of adults were used for GC-EAD analyses because larval antennae were deemed too small and thus unsuitable. Five compounds eliciting antennal responses were identified (Table 4.1) and made available for larval bioassays. β-Bourbonene was purified from Geranium, *Pelargonium graveolens*, oil (Saje, Langley, B.C.) by High Performance Liquid Chromatography (HPLC) using a Waters LC 626 HPLC equipped with a Waters 486 UV-visible detector and a reverse phase Nova-pak C_{18} column (0.3 m x 3.9 mm ID). The eluent was further purified with preparative GC under the same GC conditions as described in Chapter II, 2.2.4.2. (*E,E*)-α-Farnesene (TCI) and (*E*)-β-ocimene (IFF) were separated from other isomers also via preparative GC. Nonanal and decanal were purchased from Sigma-Aldrich. The amounts of Porapak Q extract and individual almond volatile components used per replicate are summarized in Table 4.1.

2.2 Y-tube experiments testing candidate almond semiochemicals

Anemotactic responses of neonate larvae were assessed in a vertical Y-shaped Pyrex® glass olfactometer (Y-tube) (Figure 4.1) at 20 ± 3°C and 35 ± 5% relative humidity. The olfactometer was placed vertically and illuminated from above with tubes of fluorescent “daylight” and “wide spectrum grow light” (Osram Sylvania Ltd., Mississauga, ON) because *A. lineatella* larvae are both negatively geotactic and positively phototactic (personal observation). Two pieces of 20 gauge steel wire were suspended inside Y-tubes to facilitate movement of larvae (Landolt et al. 1998), with one piece of V-shaped wire connecting the opening of each side arm and a linear piece suspended from it after insertion from below. Visual cues were standardized by enclosing the olfactometer on three sides with black poster board.

Treatment and control test stimuli were micropipetted onto Whatman No. 2 filter papers (1.27 cm diam.), inserted 1 cm into the orifice of each side arm. Pipetting was done in a separate room to avoid contamination. For each replicate, a new (cleaned and oven-dried) Y-tube, steel wire, insect and filter paper were used, with test stimuli randomly assigned to side arms. Air drawn through the apparatus at 0.1-0.2 L/min with a water aspirator was humidified before entering the side arms. Nalgene tubes running from the humidifiers to the side arms were dedicated treatment or control tubes to avoid contamination. Thirty seconds after placement of stimuli, the linear piece of wire, with a neonate on it, was connected to the V-shaped wire. All neonates were less than 5 hours old at the time of bioassay. Neonates that travelled more than 2 cm up a side arm within 10 min were classed as responders; all others were classed as non-responders and were not included in statistical analyses.

Stimuli tested in Exps. 4.1-4.11 are listed in Table 4.2. Exp. 4.1-4.2 tested Porapak Q extract of peach and almond shoots, respectively, and Exps. 4.3-4.11 tested five synthetic or plant-derived almond volatiles that were antennally-active to the adult antennae (Chapter II, 2.2.4.2). Exps. 4.3-4.6 tested three of those five components singly and nonanal and decanal in combination. To determine whether β-bourbonene, as the most attractive of the five components, was solely responsible for attraction of neonates to Porapak Q almond extract, Exp. 4.7 tested β-bourbonene versus Porapak Q almond extract. Because Porapak Q extract was more attractive than β-bourbonene, Exps. 4.8-4.10 tested β-bourbonene singly versus binary combinations of β-bourbonene with (*E,E*)-α-farnesene (Exp. 4.8), (*E*)-β-ocimene (Exp. 4.9), or with nonanal plus decanal (Exp. 4.10). Exp. 4.11 tested β-bourbonene plus (*E,E*)-α-farnesene, the only binary combination more attractive than β-bourbonene alone, versus Porapak Q almond extract.

Data were analyzed with the χ^2 goodness-of-fit test using Yates correction for continuity to determine whether observed frequencies deviated significantly from expected frequencies, under the null hypothesis that *A. lineatella* neonate larvae did not prefer either treatment or control stimuli (Zar 1996).

3. RESULTS AND DISCUSSION

In Y-tube olfactometer bioassay experiments, more larvae responded to Porapak Q extracts of peach shoots ($\chi^2 = 8.2$, $P < 0.005$) or almond shoots and fruits ($\chi^2 = 18.9$, $P < 0.001$) than to solvent controls (Figure 4.2, Exps. 4.1-4.2) demonstrating that *A. lineatella* neonate larvae orient chemoanemotactically to host volatiles. Almond volatiles were further bioassayed because β-bourbonene, the most abundant component, was present only in almonds, rendering it potentially useful for attraction of *A. lineatella* in peach orchards with no naturally occurring competing sources of the compound.

Of the five almond volatiles bioassayed, only β-bourbonene was attractive alone to neonate larvae ($\chi^2 = 22.1$, $P < 0.001$) (Figure 4.3, Exps. 4.3-4.6). However, β-bourbonene was not as attractive as Porapak Q extracts of almond shoots and fruits ($\chi^2 = 5.4$, $P < 0.05$) (Figure 4.4, Exp. 4.7), suggesting that the almond volatiles contained at least one additional semiochemical. Of the candidate components only (*E,E*)-α-farnesene enhanced attractiveness of β-bourbonene ($\chi^2 = 5.3$, $P < 0.05$) (Figure 4.4, Exps. 4.8-4.10). No difference in attractiveness between almond Porapak Q extract and β-bourbonene plus (*E,E*)-α-farnesene (Figure 4.4, Exp. 4.11) indicated that the latter two components mediate attraction of neonate larvae to almond extract.

(*E,E*)-α-Farnesene is also a component of (Granny Smith) apples, and attracted codling moth larvae in Petri dish bioassays (Bradley and Suckling 1995). The (*E,E*)-isomer in 'Granny Smith' apples accounts for 99.5% of the total α-farnesene content (Bradley and Suckling 1995) and it is also the predominant isomer in Porapak Q extracts of almond volatiles.

Because neonates are susceptible to predation and poor weather, effective foraging through orientated movement toward β-bourbonene and (*E,E*)-α-farnesene would most likely increase the chances of successful shoot or fruit location, host penetration, and survival (Jackson and Harwood 1980). In integrated pest management programs, neonate *A. lineatella* could possibly be controlled by depositing bait droplets impregnated with attractive β-bourbonene and (*E,E*)-α-farnesene and laced with insecticide on tree twigs. However, this "attract and kill" tactic would be effective only if larvae were to be attracted over a considerable distance, if both sesquiterpenes would become readily available, and if air oxidation of (*E,E*)-α-farnesene (Anet 1969) could be prevented. Alternatively, spraying β-bourbonene into an orchard canopy could increase the time larvae spend foraging for suitable feeding sites. This would increase the natural mortality of larvae or mortality due to exposure to biological control agents or microbial insecticide residues.

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Table 4.1 Name, amount, chemical purity and source of larval bioassay synthetic test stimuli used in experiments 4.1 – 4.11.

Compound	Amount (ng / lure)	Chemical purity (%)	Source[a]
Porapak Q Peach twig extract[b]	85.0		
Porapak Q Almond twig + fruit extract[b]	39.4		
Almond twig + fruit volatiles[c]			
β-Bourbonene	12.0	99	Saje[de]
(*E*,*E*)-α-Farnesene	3.0	99	TCI[e]
(*E*)-β-Ocimene	3.0	99	IFF[e]
Nonanal	6.4[f]	95	Aldrich
Decanal	6.4[f]	95	Aldrich

[a] Saje, Delta, BC (Geranium essential oil); TCI = Tokyo Chemical Industry, Portland, OR; IFF = International Flavours and Fragrances, New York, NY.

[b] Amount is the sum of all antennally-active compounds present in 5μL Porapak Q extract

[c] Amounts of almond volatiles used was equivalent to the amounts present in 5μL Porapak Q almond twig extracts.

[d] Purified using High Performance Liquid Chromatography (Regine Gries S.F.U.).

[e] Purified to 99% by preparative Gas Chromatography (Regine Gries S.F.U.).

[f] 3.2 ng of both nonanal and decanal were used for a total of 6.4 ng.

Table 4.2 Stimuli tested in Y-shaped Pyrex® glass olfactometer experiments and number of neonate larvae responding.

Experiment No.	Test Stimuli		Larvae Tested
	Treatment	Control	(n)[a]
4.1	Porapak Q Peach twig extract	Pentane	175 (106)
4.2	Porapak Q Almond twig/fruit extract	Pentane	170 (105)
4.3	β-Bourbonene	Pentane	260 (155)
4.4	(*E*,*E*)-α-Farnesene	Pentane	60 (37)
4.5	(*E*)-β-Ocimene	Pentane	60 (38)
4.6	Nonanal + decanal	Pentane	90 (59)
4.7	β-Bourbonene	Porapak Q extract	60 (34)
4.8	β-Bourbonene + (*E*,*E*)-α-farnesene	β-Bourbonene	80 (58)
4.9	β-Bourbonene + (*E*)-β-ocimene	β-Bourbonene	60 (45)
4.10	β-Bourbonene + nonanal + decanal	β-Bourbonene	60 (37)
4.11	β-Bourbonene + (*E*,*E*)-α-farnesene	Porapak Q extract	150 (115)

[a] Number of responding insects given in parenthesis

Figure 4.1 Vertical Y-tube olfactometer used for testing chemoanemotactic responses of neonate *Anarsia lineatella* larvae to test stimuli in experiments 4.1 – 4.11. Neonates were placed on the steel wire and classed as responders when they crawled >2 cm up a side arm within 10 min; all others were classed as non-responders and were not included in statistical analyses.

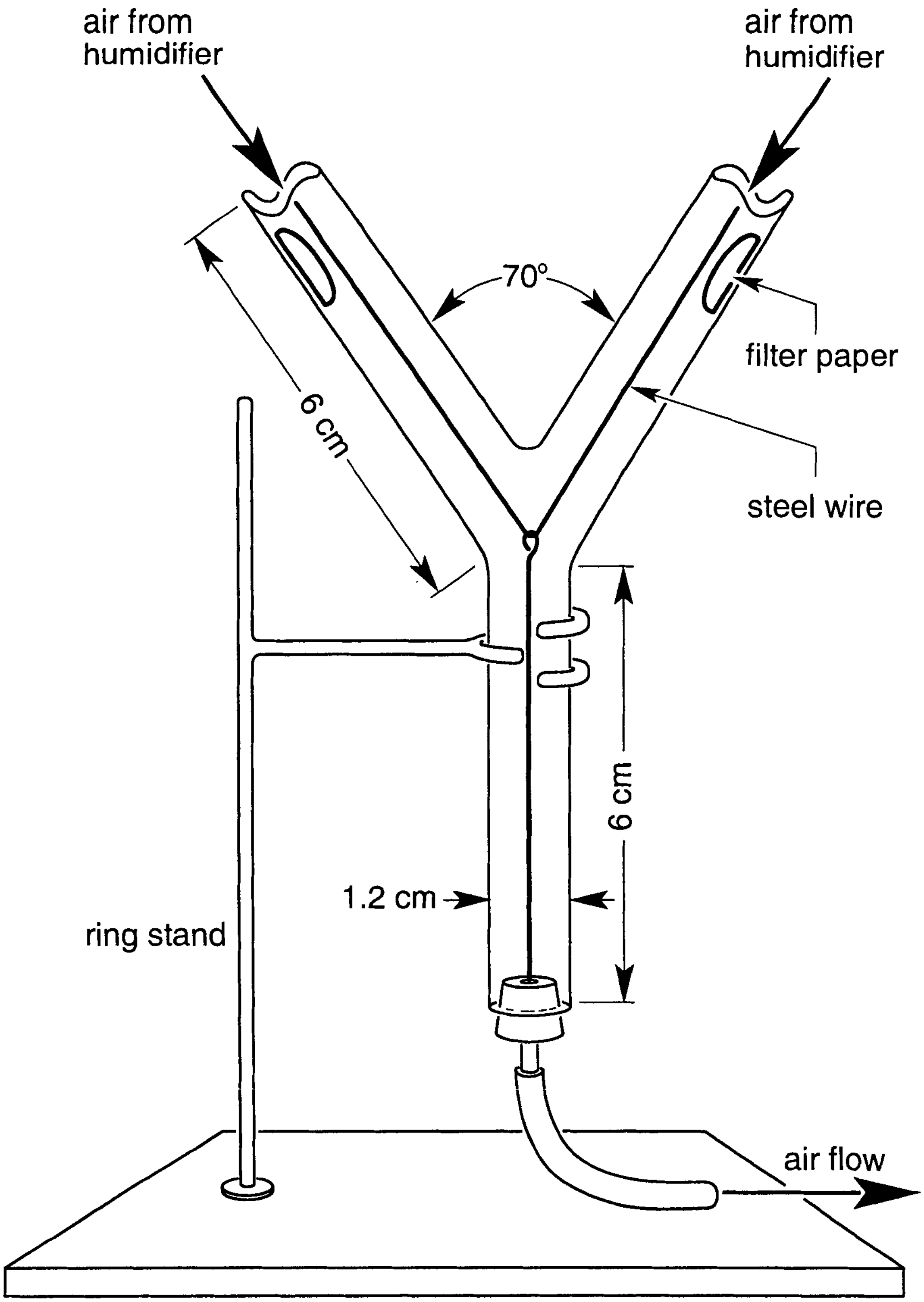

air from humidifier
air from humidifier
70°
6 cm
filter paper
steel wire
6 cm
1.2 cm
ring stand
air flow

Figure 4.2 Anemotactic response of neonate *Anarsia lineatella* larvae in Y-tube olfactometer (Fig. 4.1) to Porapak Q extracts of peach shoots (Experiment 4.1) or almond shoots and fruits (Experiment 4.2). For each experiment, bars with asterisks (*) indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.005$; ** $P < 0.001$.

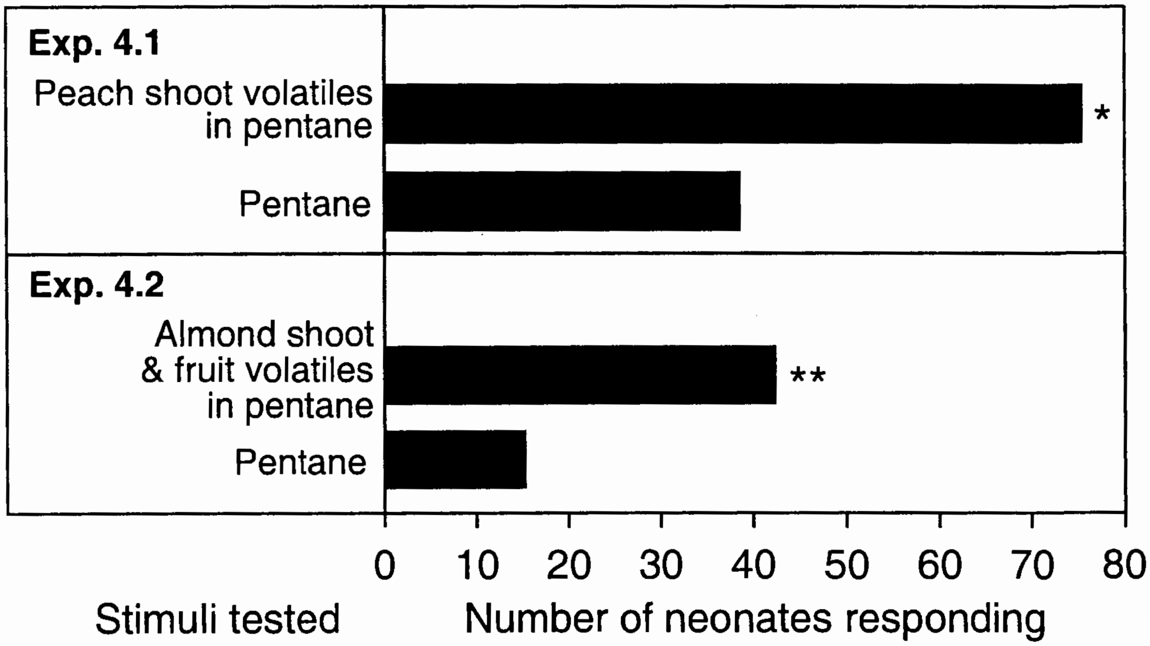

Exp. 4.1
Peach shoot volatiles in pentane
*
Pentane
Exp. 4.2
Almond shoot & fruit volatiles in pentane
**
Pentane
0
10
20
30
40
50
60
70
80
Stimuli tested
Number of neonates responding

Figure 4.3 Anemotactic response of neonate *Anarsia lineatella* larvae in Y-tube olfactometer (Fig. 4.1) experiments 4.3 – 4.6 to one of five synthetic candidate semiochemicals identified in Porapak Q extracts of almond shoots and fruits. For each experiment, bars with asterisks (*) indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.001$.

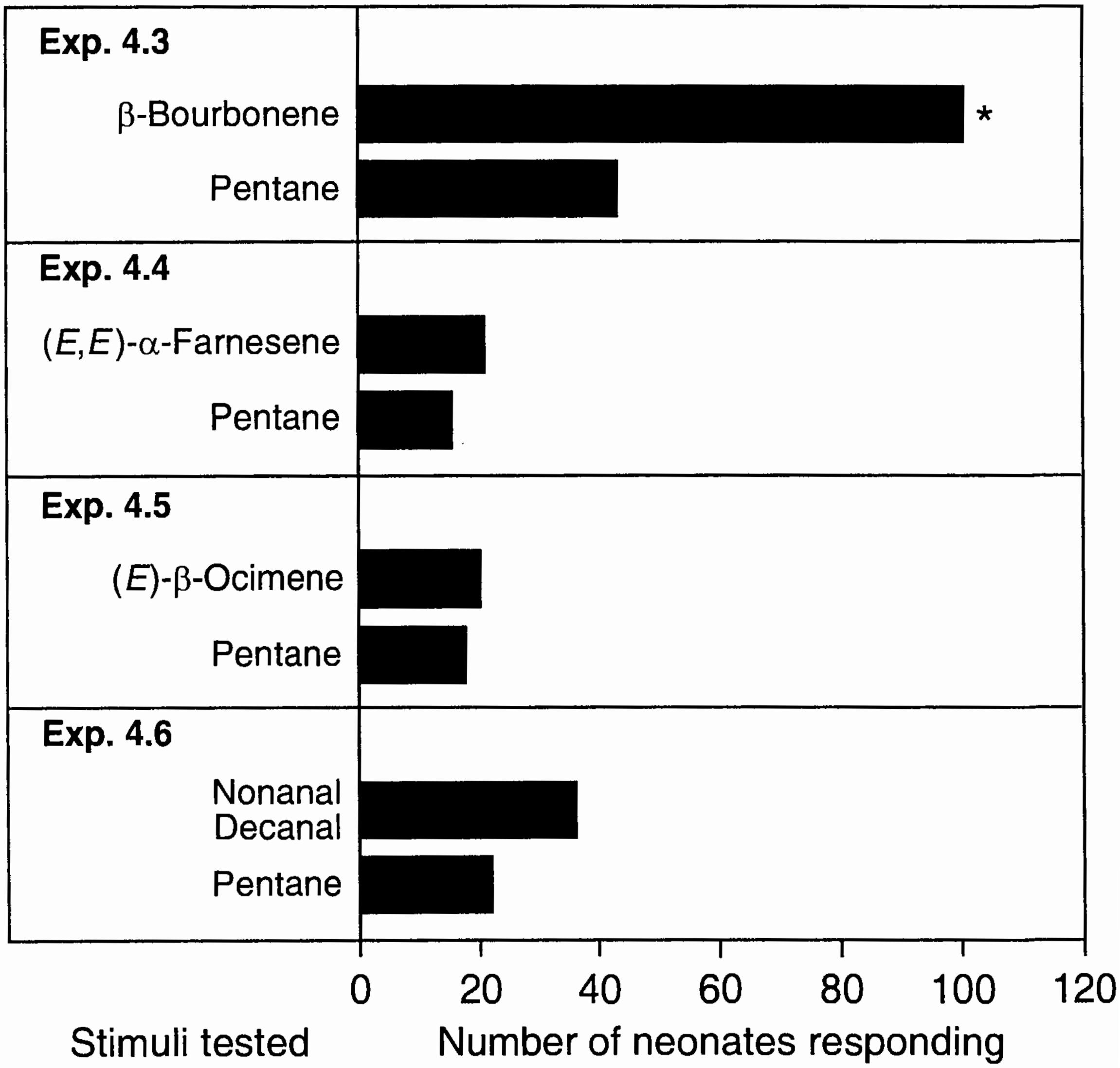
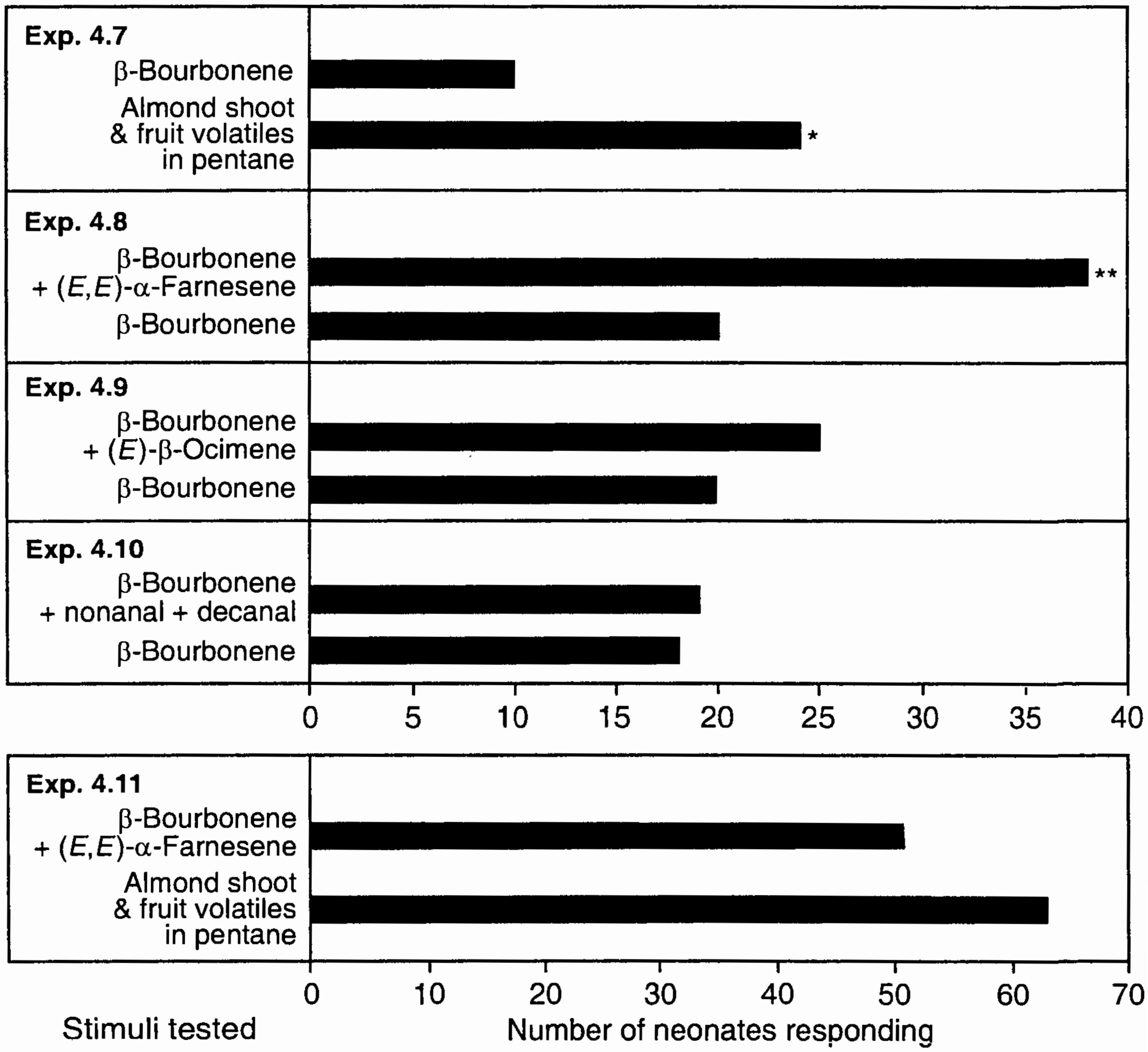

Exp. 4.3
β-Bourbonene
Pentane
*
Exp. 4.4
(E,E)-α-Farnesene
Pentane
Exp. 4.5
(E)-β-Ocimene
Pentane
Exp. 4.6
Nonanal
Decanal
Pentane
0
20
40
60
80
100
120
Stimuli tested
Number of neonates responding

Figure 4.4 Anemotactic response of neonate *Anarsia lineatella* larvae in Y-tube olfactometer (Fig. 4.1) experiments 4.7 – 4.11 to Porapak Q extracts of almond shoot and fruit volatiles or to synthetic candidate semiochemicals identified in those extracts. For each experiment, bars with an asterisk (*) indicate a significant preference for a particular treatment; χ^2 test with Yates correction for continuity, treatment *versus* control; * $P < 0.025$, ** $P < 0.05$.


Exp. 4.7
β-Bourbonene
Almond shoot & fruit volatiles in pentane
*
Exp. 4.8
β-Bourbonene + (*E*,*E*)-α-Farnesene
**
β-Bourbonene
Exp. 4.9
β-Bourbonene + (*E*)-β-Ocimene
β-Bourbonene
Exp. 4.10
β-Bourbonene + nonanal + decanal
β-Bourbonene
0
5
10
15
20
25
30
35
40
Exp. 4.11
β-Bourbonene + (*E*,*E*)-α-Farnesene
Almond shoot & fruit volatiles in pentane
0
10
20
30
40
50
60
70
Stimuli tested
Number of neonates responding

V CONCLUDING SUMMARY

Host selection

- Male and female moths discriminate between semiochemicals from host *Prunus persica* and non-host *Malus domestica* trees, showing a preference for host trees.
- No immature or mature peach fruits attract adult moths. Soft-ripe peach fruits are repellent.
- A blend of 22 synthetic volatiles associated with soft-ripe peach fruits are also repellent.

Oviposition decisions

- Oviposition is stimulated by:
    - Hairs or crevices on the substrate.
    - Semiochemicals from peach and almond shoots.
    - Semiochemicals from immature, green mature, and hard-ripe peach fruits, but not soft-ripe peach fruits.

Larval foraging

- Larvae are attracted chemotactically to Porapak Q extracts of peach shoots and almond fruits and shoots.
- β-Bourbonene and (*E,E*)-α-farnesene account for the attraction of larvae toward almond fruits and shoots.