SEMIOCHEMICAL-MEDIATED OVIPOSITION BEHAVIOUR BY PEACHTREE BORER, *SYNANTHEDON EXITIOSA* (LEPIDOPTERA: SESIIDAE)

By

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B.Sc., Malaspina University-College, 2001

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PEST MANAGEMENT

In the

Department

Of

Biological Sciences

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SIMON FRASER UNIVERSITY

Summer 2006

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ABSTRACT

I tested the hypotheses that gum-frass semiochemicals induce oviposition by mated female peachtree borers, *Synanthedon exitiosa* (Say) and attract *S. exitiosa* larvae. In coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses of Porapak Q-collected gum-frass volatiles, 21 compounds elicited responses from male or female antennae. These compounds included four acids, four hydrocarbons, four ketones, three acetates, two aldehydes, γ-decalactone, conophthorin, 2-phenylethanol, and 2-isopropyl-3-methoxypyrazine. All groups of compounds, except the acetates, were needed to induce oviposition by females. Two of these components, (9Z,12Z)-octadecadienyl acetate and (9Z)-octadecenyl acetate, are larvae-produced. When they were tested as candidate pheromone components, they attracted larvae in Y-tube olfactometers. My data suggests that gum-frass semiochemicals play a significant role in oviposition decisions by female *S. exitiosa*, and that larvae produce a pheromone of as yet unknown biological significance.

Keywords

Lepidoptera, host-selection mechanisms, semiochemicals, phytophagous insects -- oviposition behaviour, trees -- British Columbia -- Okanagan Valley -- pests.
DEDICATION

For Jasmine
ACKNOWLEDGEMENTS

I thank orchardists in Osoyoos, Oliver, Okanagan Falls, Summerland, Penticton, Cawston, and Kelowna for access to orchards for insect and gum-frass collections; private consultants H. Sampson and C. Leaming for information about potential insect collection sites; S. Fairhurst, C. Williamson, W. Taylor, and H. Pierce, Jr., for technical assistance; M. Chatterton for her insight and dedication; and J. Fuest and C. McNair for contributions in an earlier phase of the project.

I thank my supervisor G. Gries for his enthusiasm, guidance, and support throughout this project; supervisory committee members G. Judd and A. Harestad for their support and insightful comments; R. Gries for support and technical assistance; fellow students in the Gries-lab for their friendship and discussion; and D. Hart, D. Hurd, M. Brar, and C. and D. Derksen for their support and encouragement.

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada, and the B.C. Fruit Growers’ Association to G.G. and G.J.R. I received additional financial support from an H. R. MacCarthy Graduate Bursary (2004), a Professor Thelma Finlayson Fellowship (2003), a Simon Fraser University Graduate Fellowship (2002), a B.C. Council of Garden Clubs Scholarship (2002), and a Dr. John Yorston Memorial Scholarship in Pest Management (2002).
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1 INTRODUCTION

There are two main approaches to studying host-selection behaviour in insects: 1) functional, whereby researchers try to understand the adaptive value of an insect’s choice of host, and 2) mechanistic, whereby researchers try to understand how an insect selects its host (Visser 1986; Dicke 2000). Studies on host selection in phytophagous insects have focused on the mechanisms by which insects find and recognize their host plants (Renwick and Chew 1994). In particular, research on the oviposition behaviour of moths and butterflies has dominated the literature (Thompson and Pellmyr 1991). This is because larvae of many lepidopterous species are relatively immobile and susceptible to desiccation and drowning. Thus, the adult female’s choice of oviposition site is critical for her offspring’s survival (Feeny et al. 1983; Renwick 1989; Renwick and Chew 1994). Furthermore, adult females often have only a limited time period in which to lay their eggs and make their genetic contribution to the next generation. As a result, female moths and butterflies require and possess accurate and efficient behavioural and physiological mechanisms of host selection (Feeny et al. 1983; Bell 1990). The objectives of this introduction are twofold: 1) to review the mechanisms of host selection in phytophagous lepidopterous insects including key examples that illustrate the diversity of host-selection mechanisms, and 2) to discuss the possible contributions of these mechanisms to the insects’ fitness (host-selection fitness). When examples for lepidopterous insects are unavailable, studies of other phytophagous insects will be referenced.
1.1 Host Selection

During host selection, insects gather information and make decisions at different levels including: 1) the habitat to search in; 2) the host plant to land on; and 3) to accept or reject that host plant for oviposition (Feeny et al. 1983; Ramaswamy 1988; Bell 1990; Thompson and Pellmyr 1991). Renwick and Chew (1994) identify several behavioural stages of host selection including search, orientation, encounter, landing, surface evaluation, and acceptance or rejection for oviposition. The first four and last two stages of host selection are more broadly referred to as host finding and host recognition, respectively (Visser 1988). During host selection, an insect uses both internal and external information, and it is the interaction and balance between them that dictates the insect's behaviour (Shorey 1977; Miller and Strickler 1984; Visser 1986, 1988; Ramaswamy 1988; Renwick 1989; Bell 1990; Renwick and Chew 1994). Both types of information can function simultaneously at more than one point in the host-selection process. An insect's internal information is characterized by its genotype, ability to learn, and physiological state, while the insect's external information comprises a suite of sensory signals, including visual, mechanical, olfactory and gustatory cues (Thompson and Pellmyr 1991). Host and non-host plants, as well as non-plant sources (e.g. sex pheromone), may provide sensory cues used by phytophagous insects during host selection (Visser 1986; Renwick and Chew 1994).

1.1.1 Internal information and host selection

According to Bell (1990), internal information an insect relies on during host selection can be derived from proprioceptors, stored, and then recalled when needed. For example, ants that orient along a path 30° to the left of the sun can reorient to this
path via proprioceptive information (internally-derived), and in the absence of the sun’s light (externally-derived), when displaced 10° further left. Thompson and Pellmyr (1991) cite several families in the Lepidoptera including Pieridae, Nymphalidae, and Papilionidae, for which a genetic basis for host preference has been found. Physiological factors, or “internal stochastic cues” (Visser 1988) such as motivation (Bell 1990) and age (Feeny et al. 1983; Renwick and Chew 1994), can influence an insect’s decision to accept or reject a host for oviposition. For example, attraction of diamondback moths, *Plutella xylostella*, to volatiles from oriental mustard, *Sisymbrium orientale*, increased with the insect’s age (Pivnick et al. 1990). During aging, their internal motivation to lay eggs and prior experience with hosts also increased. As a result of this internal excitatory input, the probability of host acceptance increases. Learning (experience) further impacts an insect’s choice of host (Feeny et al. 1983; Thompson and Pellmyr 1991; Renwick and Chew 1994). Cunningham et al. (1998) found that learning in cotton bollworm moths, *Helicoverpa armigera*, increased the frequency of pre-alighting (landing) and post-alighting (oviposition) behaviour on those hosts with which they had prior experience. Furthermore, an insect’s mating status and sex may also influence host selection behaviour. For example, gravid, unlike virgin females of the naval orangeworm, *Amyelois transitella*, flew upwind in a windtunnel towards volatiles from almond oil (Phelan and Baker 1987).

1.1.2 External information and host selection

For many diurnal lepidopterans, visual cues are very important during host finding (Feeny et al. 1983; Renwick and Chew 1994). For example, female pipevine swallowtails, *Battus philenor*, are likely to find host plants on the basis of leaf shape
Tree-feeding *Rhagoletis* spp. flies are attracted to large vertical, tree-like objects (Moericke et al. 1975). Lime butterflies, *Papilio demoleus*, are attracted to the yellow-green colour associated with their host’s foliage; however, colour alone elicited a much weaker response than colour combined with moisture and chemical cues (Saxena and Goyal 1978). A host-seeking insect may also hone in on the relative apparency (size, conspicuousness and persistence) of their host plant (Thompson and Pellmyr 1991; Renwick and Chew 1994). Female orange tip butterflies, *Anthocharis cardamines*, preferred to oviposit on young, large and relatively persistent inflorescences of their cruciferous host plants (Courtney 1982).

After landing, mechanical cues may become important (Renwick and Chew 1994). Many moth species prefer hairy and/or rough surfaces upon which to lay their eggs (Ramaswamy 1988). For example, female corn earworms, *Helicoverpa zea*, oviposited more eggs on hirsute surfaces than glabrous ones (Callahan 1957). However, female spotted stalk borers, *Chilo partellus*, preferred to lay their eggs on glabrous surfaces (Ramaswamy 1988).

Olfactory (volatile) and gustatory (nonvolatile) chemical cues play a much larger role during host finding and host recognition than previously thought (Thompson and Pellmyr 1991; Renwick and Chew 1994). In general, a plant’s odour profile increases in complexity (detectability) as the insect draws nearer to it (Schoonhoven 1968; Visser 1986; Bernays and Chapman 1994). An important corollary to this statement is that increased plant semiochemical complexity translates into more information for the insect’s sensory system (Miller and Strickler 1984; Metcalf and Metcalf 1992). Honda (1990) identified seven synergistic oviposition stimulants from the leaves and epicarp of

Ramaswamy (1988) suggests that in moths volatile chemical cues “are probably not involved” during the host-recognition phase (i.e. acceptance or rejection for oviposition). However, volatile semiochemicals can mediate both host finding and host recognition (Tumlinson 1988). In laboratory olfactometer bioassays, volatile extracts of white mustard, *Brassica (=Sinapsis) alba*, attracted and stimulated oviposition by female *P. xylostella* (Palaniswamy et al. 1986). However, because the moths could contact filter paper discs impregnated with volatile extracts of white mustard, it is not possible to determine the relative importance of gustatory and olfactory semiochemical cues during oviposition by *P. xylostella*. Nonanal, decanal, and tetradecanal, identified from steam distillation extract of corn leaves, individually deterred oviposition by female corn stalk borers, *Sesamia nonagrioides* (Konstantopoulou et al. 2004). Alkyl sulphides containing a propyl thiol moiety both attracted and stimulated oviposition by onion flies, *Delia (= Hylema) antiqua* (Vernon et al. 1981; Miller and Strickler 1984).

Some semiochemical parsimony may be expected between conspecific adults and larvae (Feeny et al. 1983). For example, α-farnesene attracts neonate larvae of the
codling moth, *Cydia pomonella*, and stimulates adult females to oviposit (Wearing and Hutchings 1973).

Nonvolatile (contact) semiochemicals are very important sources of information to ovipositing lepidopterans (Feeny et al. 1983; Ramaswamy 1988), but their molecular structure may be difficult to identify (Renwick and Chew 1994). For example, leaf extract of sweet potato, *Ipomoea batatus*, stimulated oviposition by female sweet potato hornworms, *Agrius convolvuli*, due to synergistic activity of volatile and nonvolatile semiochemicals (Shimoda and Kiuchi 1998), but the chemical identify of the latter remained unknown. Similarly, nonvolatile semiochemicals extracted from the European spindle tree, *Euonymus europaeus*, stimulated oviposition by female small ermine moths, *Yponomeuta cagnagellus* (Hora and Roessingh 1999), but their molecular structures were not identified.

Host selection may entail recognition of both suitable host and unsuitable nonhost plants (Feeny et al. 1983; Ramaswamy 1988; Renwick and Chew 1994). Host-foraging lepidopterous insects may respond to both positive cues (attractants and stimulants) and negative cues (repellents and deterrents) (Renwick and Radke 1987; Renwick 1989; Renwick and Chew 1994). For example, female cabbage white butterflies, *Pieris rapae*, recognize nonhost plants either by a greater abundance of chemical deterrents than stimulants, or by the presence of a deterrent in the absence of a stimulant (Renwick and Rake 1987). Renwick and Chew (1994) maintain that chemical stimulants are generally less important than deterrents in determining host acceptance by female insects.

More commonly, it is a combination of sensory cues (visual, mechanical, olfactory, and gustatory) that governs the entire sequence of host-selection behaviour.
Shorey 1977; Feeny et al. 1983; Miller and Strickler 1984; Visser 1986; Renwick 1989; Bell 1990). For example, oviposition by female pipevine swallowtail, \textit{Papilio demoleus} on citrus host plants requires a combination of visual (leaf colour), olfactory (leaf extract) and moisture cues (Feeny et al. 1983).

1.1.3 Sources of chemical cues used during host selection

During host selection, insects may also rely on intraspecific chemical communication (pheromones). Some female lepidopterans produce oviposition-marking pheromones that deter or attract conspecific gravid females (Bernays and Chapman 1994). For example, female cotton bollworm moths, \textit{Helicoverpa armigera}, produce an oviposition deterring pheromone composed of hexadecanoic acid and (9Z)-octadecenoic acid (Guoqing et al. 2001). Feeding conspecific larvae are another possible source of semiochemicals for host-seeking lepidopterans. Acetone extracts of tree sap (gum) mixed with the frass of larval peachtree borers, \textit{Synanthedon exitiosa}, stimulated oviposition by female \textit{S. exitiosa} (Gentry and Wells 1982). In contrast, females of the noctuid moth \textit{Spodoptera littoralis} were deterred from ovipositing on cotton leaves coated with a water suspension of larval frass (Hilker and Klein 1989). Tent caterpillars, \textit{Malacosoma neustria}, produce and are attracted to trail pheromones leading to good feeding sites (Bernays and Chapman 1994).

Host-foraging insects may also exploit chemical cues produced by microorganisms. For example, yellow peach moths, \textit{Conogethes punctiferalis}, were attracted to, and stimulated to oviposit on, artificial substrates that emit fungal volatiles (Honda et al. 1988). By feeding on host plants, some insect species may cause changes in the plant's chemistry that render the plant more or less attractive to other host-seeking
insects (Bernays and Chapman 1994). For example, female tobacco budworms, *Heliothis virescens*, avoid ovipositing on tobacco plants, *Nicotiana tabacum*, that have been damaged by conspecific larvae; feeding *H. virescens* larvae induced volatile production in tobacco that was quantitatively and qualitatively the same as that produced by feeding tobacco hornworm, *Manduca sexta*, and feeding *H. zea* (De Moraes et al. 2001).

Secondary plant metabolites are an important source of chemical signals (stimulants/deterrents) for host-seeking insects (Feeny et al. 1983; Bernays and Chapman 1994). Each plant has a unique complement of secondary metabolites that some insects use to locate or recognize a potential host plant. For example, female buckeye butterflies, *Junonia coenia*, are stimulated to oviposit by the iridoid glycosides aucubin and catapol, secondary metabolites that are prevalent in the plant families Scrophulariaceae and Plantaginaceae (Pereyra and Bowers 1988). Similarly, the cyanogenic glycoside amygdalin is a characteristic secondary metabolite in *Prunus* spp. When plant tissue is damaged, amygdalin is hydrolyzed to hydrogen cyanide and benzaldehyde (Bernays and Chapman 1994; Han and Chen 2002), the latter of which elicits oviposition by female *S. exitiosa* (see section 2.3). Plant surfaces covered in wax are composed of complex mixtures of fatty acids, esters, n-alkanes, and secondary metabolites, which are used by some host-seeking insects. For example, n-alkanes in the epicuticle of corn leaves stimulate oviposition by female *O. nubilalis* (Udayagiri and Mason 1997). Finally, plants emit diverse (semio)chemicals including short chain alcohols, aldehydes, ketones, esters, aromatic phenols, lactones and mono- and sesquiterpenes. Compounds with low molecular weight and high volatility may be used during host finding, whereas less volatile chemicals with high molecular weight could play a role during host recognition.
1.1.4 Host-selection fitness

Many studies on host selection in phytophagous insects determine the mechanisms but speculate on the adaptive significance of the insects' behaviour (Renwick and Chew 1994). Fitness components such as egg survival, larval growth and developmental rate, survivorship and/or fecundity of the adults and their offspring, as well as the timing of adult (F$_2$ generation) emergence, should be measured in order to determine the adaptive value of an insect's choice of host (Singer 1984). Such an undertaking is time-consuming and complicated, especially if conducted in the field. Thus, it is not surprising that many studies on host selection in phytophagous insects focus on mechanisms. Studies on host selection by phytophagous moths and butterflies lead to the conclusion that the oviposition site an adult female chooses will have an enormous impact on her relatively vulnerable offspring (Feeny et al. 1983; Renwick 1989; Renwick and Chew 1994), and that females should select oviposition sites that maximize the above-mentioned fitness components (optimal oviposition theory) (Scheirs 2002).

For many lepidopterous species a plant’s wound is an ideal oviposition site as it provides decreased structural and chemical barriers to young larvae seeking entry into their host. Larvae provided with ready access to food via a wound could experience higher survivorship and growth rate. There is evidence that pathogenic microbes play an important role in creating these attractive wounds. For example, Swift (1986) found an association between larvae of the lesser peachtree borer, Synanthedon pictipes, and Cytospora cankers, and hypothesized that gravid females are attracted to cankers because they present minimal structural and chemical barriers to larval entry into host trees.
Honda et al. (1988) demonstrated that fungal volatiles attract and stimulate oviposition in *C. punctiferalis*. The authors suggest that physical damage, by insect pests or plant diseases, which removed the fruit’s protective wax layer, may facilitate fungal establishment. Selection of oviposition sites by female *C. punctiferalis* in response to fungal volatiles associated with areas of reduced defences on the fruit may be adaptive for offspring development. Rocchini et al. (1999) found an association between stalactiform blister rust, *Cronartium coleosporioides*, and the wood-boring western pine moth, *Dioryctria cambiicola*, and Douglas-fir pitch moth, *Synanthedon novaroensis*. The authors hypothesized that gravid females were attracted to wounds caused by blister rust because such wounds offered little resistance to larval entry.

Damage caused by abiotic forces can also create an attractive wound. Smith and Harris (1952) found that peach trees with more winter injury produced more gum and had bigger infestations by *S. pictipes* larvae. They noticed that gravid females seemed to be attracted to the gum, and similar to Swift (1986), hypothesized that females selected oviposition sites favourable for larval survival.

Feeding lepidopterous larvae themselves create wounds on their host plants. Gentry and Wells (1982) reasoned that gravid female *S. exitiosa* were stimulated to oviposit by gum-frass, which is indicative of larval development in a prospective host tree and signals suitable entry points for neonate larvae. Alternatively, semiochemicals produced by feeding conspecific larvae may signal the presence of competitors and potentially limited resources (Thompson and Pellmyr 1991). Gravid females may be deterred by larval frass (e.g. Hilker and Klein 1989), and thus avoid laying their eggs on infested hosts that may not provide sufficient resources for their progeny.
Relationships between host plant quality, the insects' host choice and larval success have been well studied with butterflies. *Battus philenor* larvae are often forced to disperse soon after hatching in search of supplemental food, with larger larvae having a better chance of surviving this dispersal. Accordingly, females lay most eggs on host plants on which larvae can attain large size, thereby increasing their reproductive fitness (Singer 1984). However, several studies have found that female butterflies lay their eggs on hosts suboptimal for larval performance (growth rate, survivorship, fecundity, etc.) For example, female checkerspot butterflies, *Euphydryas editha*, laid their eggs on host plants that often senesced before the eggs hatched, resulting in 80% larval mortality (Singer 1984). There are three possible explanations for this seemingly maladaptive behaviour (Singer 1984).

First, the females’ host choice may be outright maladaptive for larval performance if the host plant is a recent addition to the habitat, and natural selection has not had time to select against those females that oviposit on that plant species (Thompson and Pellmyr 1991). Second, larval performance may not have been accurately assessed. For example, a gravid lepidopterous female may select a plant with secondary metabolites that can be sequestered and used for defence by their offspring (Thompson and Pellmyr 1991). Pereyra and Bowers (1988) showed that female *Junonia coenia* detected and laid most of their eggs on artificial oviposition substrates treated with catapol, a secondary metabolite found in their plantain host plant *Plantago lanceolata*. The benefit larvae accrue from the sequestered toxins may outweigh the disadvantage of slower growth and development due to the secondary metabolites. Third, the host-selection behaviour that maximizes adult fitness may not involve selecting those hosts on which larvae do best. Adults and
larvae may feed on different plants species; consequently, selection of oviposition sites in some insects may be explained in terms of resource proximity for adults (Scheirs 2002).

1.2 Peachtree borer biology

1.2.1 Hosts and distribution

The peachtree borer (PTB) is a phloephagous herbivore that specializes on ornamental shrubs and stone fruit trees in the family Rosaceae. Larvae infest apricot, *Prunus armeniaca*; cherry, *Prunus* spp.; nectarine, *P. persicae* var. *nectarina*; plum, *P. domestica*; and almond, *P. amygdalus* (Slingerland and Crosby 1914; Essig 1915; Gossard and King 1918; Armstrong 1940; Boyce 1962; Johnson and Mayes 1984; Davidson and Lyon 1987). The native hosts of PTB are purported to be chokecherry, *P. virginiana*, and wild plum, *P. americana*. However, PTB is economically most important on its preferred peach host, *Prunus persica* (Essig 1915; Gossard and King 1918; Davidson and Lyon 1987). PTB is indigenous to North and South America. In the United States, it occurs in both eastern and western peach-growing areas, but is more important in the east (Armstrong 1940). In Canada in the early twentieth century, PTB was said to occur from the Maritime Provinces in eastern Canada to Manitoba (Boyce 1962). Today, PTB is thriving in peach-growing areas of British Columbia, particularly in the southern Okanagan Valley (personal observation). Larvae develop in the phloem and vascular cambium of the roots and lower trunk of peach trees. Feeding larvae form an irregular network of tunnels in the tree, and provoke the production of gum frass, a mixture of tree phloem particles, tree sap (gum), and larval faeces (frass) (Slingerland and Crosby 1914; Gossard and King 1918). Larvae attack all ages of trees but do most damage to younger
trees, which they can girdle and kill (Slingerland and Crosby 1914; Gossard and King 1918; Armstrong 1940).

In Ontario, first instar larvae (neonates) hatched between 27 July and late September (Armstrong 1940). Negatively phototactic neonates immediately search for entry into the tree on the lower part of the trunk at or below soil surface (Gossard and King 1918). Areas on a potential host tree exuding gum frass have abridged structural barriers, and thus are ideal points of entry for neonates (Gossard and King 1918). Larvae grow slowly and go through six or seven instars (Armstrong 1940; King and Morris 1956; Smith 1965). They feed until the temperature drops below some threshold at which time they enter obligatory diapause (Slingerland and Crosby 1914; Essig 1915; Armstrong 1940; Smith 1965). In early spring, feeding resumes in existing tunnels or untouched cambium. Most populations have a two-year life cycle, but some may complete development within one year (Armstrong 1940; personal observation).

1.2.2 Pupae

Larvae pupate inside cocoons made from their silk and frass, and from particles of wood (Slingerland and Crosby 1914; Gossard and King 1918; Armstrong 1940). They build their cocoons inside or nearby host trees, adjacent to the trunk or several centimetres away from it in the soil (Slingerland and Crosby 1914). In one study in Arkansas, most pupae (92%) were located in the soil within 8 cm from tree trunks (Becker 1917). In B.C., pupation begins in May (personal observation). In Ontario, the cocooning period lasts 18 – 43 days, with an average of 28 days (Armstrong 1940).
Adults generally eclose from their cocoons between 06:00 and 10:00 hours (Becker 1917; Gossard and King 1918; Armstrong 1940). Eclosion is largely triggered by changes in photoperiod or barometric pressure (Becker 1917; Jacklin and Yonce 1969). Extreme temperatures delay eclosion, and latitude and elevation may be negatively correlated with the rate of eclosion (Becker 1917; Armstrong 1940; Johnson and Mayes 1984). Slingerland and Crosby (1914), in contrast, infer from their data that adults do not eclose earlier in warmer climates of the southern states (lower latitude) than in the cooler climates of New York and New Jersey (higher latitude). Johnson and Mayes (1984) note that similar patterns of adult eclosion occur in Arkansas, north central Florida, Georgia, New York and South Carolina, whereas in Ontario, adults eclosed earlier than those in U.S. populations. Local microclimatic conditions may modify the time periods of adult eclosion. As well, limited food resources for larvae may provoke early pupation and eclosion of undersized adults (Smith 1965). Males generally eclose earlier than females (Jacklin and Yonce 1969; personal observation). An equal number of males and females emerged in El Dorado (Arkansas) in 1913 and 1914; however in Abbott in 1914, 36.2% of the insects that eclosed were males and 63.8% were females (Becker 1917).

1.2.3 Adults

1.2.3.1 Description

Perhaps the most striking feature of females is the bright orange band of scales around the fourth, or fourth and fifth, abdominal segments. Females have an iridescent, steel blue body (19–25 mm long) (Essig 1915; Davidson and Lyon 1987) and forewings (ca. 32–38 mm wing span) (Essig 1915; Gossard and King 1918; Armstrong 1940), whereas hindwings are transparent. Males also have a steel blue body but the abdominal
segments of males are fringed with thin bands of yellow scales, as are parts of the thorax and legs. Males are noticeably smaller than females, and their fore- and hindwings (ca. 25–26 mm wing span) are transparent (Gossard and King 1918; Armstrong 1940). The wing veins are outlined with scales in both sexes.

1.2.3.2 Feeding and longevity

The average life span of laboratory-reared, unfed females and males was 6.2 and 8 days, respectively (Armstrong 1940). Under laboratory conditions, unmated females lived for 13.8 days (Armstrong 1940). Provisioned with a sugar solution, females lived 15.8 days (Becker 1917; Armstrong 1940). There are no reports of adults feeding in nature.

1.2.3.3 Mating system

The females produce a 2-component sex pheromone consisting of (3Z,13Z)- and (3E, 13Z)-octadecadienyl acetate (96:4 v:v) to attract mates (Tumlinson 1974). Adults are diurnal and most active during high temperatures (Slingerland and Crosby 1914; Becker 1917; Gossard and King 1918). They become relatively inactive after 16:00 or 17:00 hours (Becker 1917). Copulations have been observed to occur between 08:00 to 14:00 hours; however most copulations seemed to occur around 10:00 hours (Becker 1917). In the laboratory, adults usually mated soon after eclosion. Healthy females mated up to 4 days post-eclosion with no adverse effect on their fertility (Slingerland and Crosby 1914; Armstrong 1940; Smith 1970). In laboratory experiments, females that mated once or repeatedly were equally fertile. Male PTB can mate multiple times.
1.2.3.4 Oviposition

Before laying an egg, females tap their antennae on the surface and use their ovipositor to probe the potential oviposition site (Becker 1917; personal observation). Females lay 200 – 800 eggs (Slingerland and Crosby 1914; Essig 1915) among cracks and crevices on the tree trunk (Slingerland and Crosby 1914; Essig 1915; Becker 1917; Gossard and King 1918; Boyce 1962; Davidson and Lyon 1987), leaves (Becker 1917; Armstrong 1940; Boyce 1962), and twigs (Becker 1917). They also oviposit on nearby weeds (Essig 1915; Becker 1917; personal observation), and on soil (Essig 1915; Becker 1917; Gossard and King 1918; Armstrong 1940; Boyce 1962). However, Gossard and King (1918) claim that eggs are laid on leaves and twigs only in field cages and not in nature. Becker (1917) calculated the percent eggs laid by females on tree trunks (84%), leaves (10%), twigs (3%), weeds (2%), and soil (1%). Females may deposit eggs on soil next (15-17 cm) to tree trunks (Gossard and King 1918; Armstrong 1940). Eggs may also be deposited on the leaves of suckers (sprouts) at the base of trees (Davidson and Lyon 1987; personal observation). In nature, more than 99% of neonates don’t survive due to desiccation and hatching too far from host trees (Becker 1917; Armstrong 1940; Smith 1965).

Females begin to oviposit and lay most eggs soon after copulation but continue to lay eggs for an average of 4 days (Slingerland and Crosby 1914; Armstrong 1940). Eggs are deposited singly or more commonly in cluster (Slingerland and Crosby 1914; Becker 1917; Gossard and King 1918; Armstrong 1940). Becker (1917) notes that females usually deposit clusters of eggs on tree trunks but single eggs on leaves. Females oviposit on more than one tree. For example, Gossard and King (1918) observed one female
oviposit on five different trees. Females also deposit clusters of eggs in different places on the same tree (Becker 1917). They may lay eggs in small clusters in many different locations in order to increase outbreeding and the overall survival of their offspring (Stamp 1980). Peak oviposition seems to occur from 10:00 to 16:00 hours. The rate of oviposition is temperature-dependant. On sunny days, females can oviposit hundreds of eggs in a few hours (Armstrong 1940). The rate of oviposition also depends on how long the female requires to locate suitable oviposition sites (Becker 1917). The insect’s internal state (e.g. time since mating or last oviposition) and external environment (e.g. humidity) is not constant, and thus oviposition rates vary.

In field cages, females laid large numbers of eggs in single locations (Gossard and King 1918). In such a cage, there may be fewer disturbances, less movement by the female, longer oviposition bouts, and thus more eggs oviposited in a single location. Dissection of captive females indicates that there are a number of eggs that are never oviposited (Becker 1917). Gossard and King (1918) counted the number of eggs oviposited by each of 10 different females, as well as the number of eggs remaining in their body after they died. Caged females oviposited an average of 405 eggs but developed an average of 605 eggs (204 eggs not laid prior to death). Natural oviposition stimulants and microclimatic conditions that provoke oviposition may be absent in confined settings, such as field cages, and provoke females to retain some of their eggs until ‘conditions become favourable’.

In Ontario, eggs required an average of 12 days (range 9-25 days) to mature and hatch depending on temperature (Armstrong 1940). Larvae probably hatch at night, because hatching was rarely observed during the day (Armstrong 1940). Similarly, eggs
hatched around sunrise or after sunset in the laboratory (Gossard and King 1918). Because females sometimes lay their eggs far from host trees, neonates must be capable of locating host plants. Gentry and Wells (1982) determined that extracts of tree sap and larval faeces (frass) stimulate oviposition by females. To the best of my knowledge, the semiochemicals in these extracts were not identified.

1.3 Research objectives

My overall objective is to contribute to the understanding of host selection in insects, especially phytophagous Lepidoptera. Semiochemicals are involved in a range of behaviours and ecological processes (see Section 1.1). I investigated aspects of semiochemical-mediated oviposition behaviour by peachtree borer, Synanthedon exitiosa, and identified chemical components in their frass. I relate these findings to adaptive host-selection theory and management of peachtree borers as pests of orchards. Specifically, my objectives were:

1) To test the hypothesis that gum frass, or volatile semiochemicals thereof, induce(s) oviposition by females;

2) To identify the gum-frass semiochemicals;

3) To test the hypothesis that gum frass, or semiochemicals thereof, attract larvae; and

4) To test the hypothesis that gum-frass volatiles contain a larval pheromone.
1.4 References


2 SEMIOCHEMICAL-MEDIATED OVIPOSITION BEHAVIOUR

2.1 Introduction

Selection of potential oviposition sites is critical for mono- and oligophagous insect herbivores, particularly when neonate larvae are relatively immobile and subject to desiccation (Feeny et al. 1983; Renwick 1989; Renwick and Chew 1994). Short-lived adult females of such species require and often possess accurate and efficient behavioural and physiological mechanisms of host selection (Feeny et al. 1983; Bell 1990).

Plant-derived semiochemicals play a role in host finding and host recognition behaviours of Lepidoptera (Thompson and Pellmyr 1991; Renwick and Chew 1994). As foraging insects approach prospective host plants, the plants’ detectable semiochemical profile likely increases in chemical complexity (Schoonhoven 1968; Visser 1986) and information content (Miller and Strickler 1984). Female Acrolepiopsis assectella select plants that emit both sulphur-containing and green leaf semiochemicals (Thibout and Auger 1996). Female *H. armigera* oviposit in the presence of complex host plant volatile blends, but not in the presence of individual components (Jallow et al. 1999). Finally, female giant danaine butterflies, *Idea leuconoe*, are stimulated to oviposit by host-specific macrocyclic pyrrolizidine alkaloids, including parsonsianine, parsonsianidine, and 17-methylparsonsianidine (Honda et al. 1997).
In a non-replicated experiment, gum frass [a mixture of host-tree wood particles, tree sap (gum), and larval faeces (frass)] stimulated oviposition by gravid female PTB (Gentry and Wells 1982).

My objectives in chapter 2 were to test the hypothesis that gum frass, or semiochemicals thereof, induce oviposition by female PTB, and to identify the gum-frass semiochemicals.

2.2 Methods and materials

2.2.1 Experimental insects

Pupae of PTB were collected from conventional fruit tree orchards in Osoyoos (B.C., Canada), transported to Simon Fraser University, and kept in Petri dishes (8.5 cm diam.) with moist sand, which were maintained in an environmental chamber (20-22°C, 30-70% RH, 16L:8D). Eclosed adult moths were transferred to new Petri dishes each provisioned with a moist filter paper (Whatman No. 1; 7 cm diam) and a cotton wick (1 x 1.2 cm diam; Richmond Dental Company, Charlotte, NC, U.S.A.) soaked in a 10% honey water solution. To facilitate mating of moths, 0- to 7-d-old virgin females (1–4 females per cage) were placed on the wall of a mesh cage (1 x 1 x 1 m) facing a stationary fan (Windmere Model No. DF121M) for dissemination of pheromone from calling females. Then 0- to 16-d-old males (1-8 males per cage) were released into the cage, and pairs in copula were carefully transferred to Petri dishes where they remained until they
turned back on (Weaver and Boyce 1965). Presumed mated females were bioassayed the same day.

### 2.2.2 Collection and identification of gum-frass semiochemicals

To acquire gum-frass volatiles for oviposition experiments, 50 g of gum frass was collected in May 2001 near Osoyoos, B.C., from the rootstalk of various peach varieties (Whiteflush, Glohaven, Redhaven, Cresthaven, and Early Redhaven). The material was kept in a sealed container, stored on ice or in a refrigerator (4-5°C), and then placed in a Pyrex glass chamber (15.5 diam x 20 cm) and aerated. Aeration of an empty chamber served as a control. A water aspirator drew charcoal-filled air at 0.7 L/min through each chamber and through a glass column (14 x 1.3 cm OD) containing 0.5 g of Porapak Q (50-80 mesh, Waters Associates, Inc. Milford, Massachusetts 01757). After 24 hr, gum frass was replenished with new material and volatile collection continued for 24 hr with the same Porapak Q trap. Trapped volatiles were desorbed from Porapak Q with 3 ml of redistilled pentane and ether (1:1 v:v). This procedure was repeated in August 2002 with different samples of gum frass, following the same protocol. Extracted volatiles were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al. 1975; Gries et al. 2002), employing a Hewlett Packard 5890A gas chromatograph equipped with a GC column (30 m x 0.32 mm ID) coated with DB-5 (J & W Scientific, Folsom, CA, U.S.A.). Compounds eliciting responses from male or female antennae* were identified by calculating their respective retention indices relative to straight chain alkanes (van den Dool and Kratz 1963), and by comparative GC, GC-mass

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* Male and female antennae were used because male antennae, in general, responded more strongly to volatiles during GC-EAD analysis than did female antennae.
spectrometry (MS), and GC-EAD of natural gum frass volatiles and authentic standards (Table 1). GC-MS analyses in full scan electron impact mode employed a Varian Saturn 2000 Ion Trap GC-MS fitted with the same GC column used for GC-EAD analyses.

2.2.3 General design for two-choice oviposition experiments

One cylinder (7 x 4 cm diam) with a plastic mesh (10 ml, 32 mesh; Synthetic Industries, Lumite Division 10367, Surrey, B.C., Canada) wall and Petri dish top and bottom was placed on each of two filter paper (Whatman No. 1) disks (7 cm diam.) in a Plexiglas cage (31.5 x 26 x 21 cm) with a round mesh window (11.5 cm diam.) in the front (Figure 2.1). Each cylinder was provisioned with an inner black broadcloth insert (35% cotton, 65% polyester; W778-99, Wal-Mart stores) to obscure visual and tactile cues associated with test stimuli placed inside the cylinder. A moist dental cotton wick (3 x 1.2 cm) was suspended from the top of the cylinder to increase the relative humidity of the artificial oviposition site. An experimental replicate was initiated by assigning randomly a treatment or control stimulus to one of the two cylinders, after which a single gravid female was placed in each cage. One female per cage was used to avoid possible interaction between the oviposition responses of more than one female. Twenty-four hr later the female was removed and the number of eggs oviposited on each cylinder and each filter paper disk was recorded.

Experiment 1 tested the hypothesis that visual, tactile, contact or volatile semiochemical cues associated with gum frass induce oviposition by females. Gum frass was collected in June or July 2002 from peach trees naturally infested with S. exitiosa larvae, and 4-g aliquots were administered in form of two vertical strips (ca. 1 x 5 cm) on opposite sites of treatment cylinders. Control cylinders received no gum frass.
Experiment 2 tested the hypothesis that volatile semiochemicals from gum frass, in the absence of visual, tactile or gustatory contact cues induce oviposition by females. Gum frass in 4-g aliquots was spread on a mesh rectangle (16 x 6 mm) suspended inside the cylinder from the Petri dish top. Great care was taken to ensure gum frass did not come into contact with the black cloth insert (Figure 2.1). Control cylinders did not receive any gum frass.

Experiment 3 tested the hypothesis that a synthetic blend (SB) of candidate gum-frass semiochemicals induces oviposition by female PTB. Semiochemicals were released from filter paper- (Whatman No. 1; 4.25 cm diam) or rubber septa- (West Company, Phoenixville, PA, U.S.A.) type dispensers, adjusting the semiochemical loading (Table 2.2) such that the release rate* and ratio of candidate semiochemicals approximated those of natural semiochemicals released from 4.8 g of gum frass during 24 hr of volatile collection on Porapak Q (Table 2.1). Control baits received an equivalent amount of solvent (pentane, 99% pure).

To determine essential semiochemicals in the synthetic gum-frass blend, experiments 4-10 tested blends lacking certain classes of organic chemicals, such as acetates (Experiment 4), acids (Experiment 5), ketones and aldehydes (Experiment 6), γ-decalactone and conophthorin (Experiment 7), 2-phenylethanol (Experiment 8), 2-isopropyl-3-methoxypyrazine (Experiment 9), and hydrocarbons (Experiment 10).

The 21 candidate semiochemicals comprising SB in experiment 3 were identified in Porapak extracts of various gum-frass collections made between 2001-2002. No single

* Determined by Porapak Q capture of dispenser-emitted semiochemicals, and by quantitative GC analysis of Porapak Q extracts.
extract contained detectable quantities of all 21-candidate semiochemicals, or was available at any one time to be combined with other extracts. Thus, direct comparison of attractiveness of natural vs. synthetic gum-frass semiochemicals in a final experiment was not possible.

Paired oviposition data from experiments 1-10 were analyzed by Wilcoxin’s signed-rank test, $\alpha = 0.05$ (Zar 1984; SAS 1999-2001).

### 2.3 Results

In GC-EAD analyses of various Porapak Q extracts of gum-frass volatiles (Figure 2.2), 21 compounds including four acids [butanoic, heptanoic, nonanoic and decanoic acid], four hydrocarbons [heptadecane, octadecane, heneicosane, docosane], four ketones [4-heptanone, 3-octanone, camphor, geranyl acetone], three acetates [hexadecyl acetate, (9Z)-octadecenyl acetate, (9Z,12Z)-octadecadienyl acetate], two aldehydes [nonanal, benzaldehyde], $\gamma$-decalactone, conophthorin, 2-phenylethanol, and 2-isopropyl-3-methyoxypyrazine elicited responses from male and female antennae. The molecular structure of these candidate semiochemicals was assigned and confirmed by comparing their GC retention times, mass spectra and EAD-activity with those of authentic standards (Figure 2.2).

In two-choice oviposition experiments, gravid females laid significantly more eggs on mesh cylinders (Figure 2.1) with accessible or inaccessible gum frass than on those without gum frass (Experiment 1, 2; Figure 2.3). Similarly, females laid significantly more eggs on mesh cylinders baited with the SB of all candidate semiochemicals than on cylinders baited with solvent (Experiment 3; Figure 2.3). The SB
lacking acetates induced oviposition by females (Experiment 4; Figure 2.3), whereas SBs lacking acids (Experiment 5; Figure 2.3), ketones and aldehydes (Experiment 6; Figure 2.3), \( \gamma \)-decalactone and conophthorin (Experiment 7; Figure 2.3), 2-phenylethanol (Experiment 8; Figure 2.3), 2-isopropyl-3-methoxypyrazine (Experiment 9; Figure 2.3), or hydrocarbons (Experiment 10; Figure 2.3) were no more effective than solvent controls in inducing oviposition.

2.4 Discussion

My data support the hypothesis that gum frass, and volatile semiochemicals therein, induce(s) oviposition by mated female *S. exitiosa*. Gum frass, produced by specific peach tree varieties in response to feeding larvae, may signal the presence of a tree that sustains larval development, and thus may induce oviposition by females (Figure 2.3, Experiment 1-3). Moreover, gum frass may demark a microlocation with decreased structural and chemical barriers for neonate larvae that seek entry into host trees (Gentry and Wells 1982; Swift 1986). Plant wounds are also preferred oviposition sites for congeneric female lesser peachtree borers, *S. pictipes* (Swift 1986), and Douglas fir pitch moths, *S. novaroensis* (Rocchini et al. 1999), as well as for yellow peach moths, *Conogethes punctiferalis* (Honda et al. 1988). Larvae hatching from eggs near plant wounds may find ready access to food, and may experience greater growth rates and survivorship, thus maximizing fitness of ovipositing females (Scheirs 2002).

Selection of oviposition sites by female moths and butterflies relies on complex physical, visual, gustatory, and olfactory cues from prospective host plants (Ramaswamy 1988, Feeny et al. 1983). That female *S. exitiosa* oviposited on mesh cylinders, stimulated merely by olfactory cues from invisible and physically inaccessible gum frass (Figure
2.3; Experiment 2) underlines the importance of airborne semiochemicals in oviposition decisions. Similarly, female *P. xylostella* laid more eggs on their *Brassica napus* host plants in the presence of gustatory and olfactory chemical cues than in the presence of gustatory cues alone (Justus and Mitchell 1996). Finally, when female sweet potato hornworms, *A. convolvuli*, oviposited in response to leaf extract of sweet potato, *I. batatus*, volatile and nonvolatile semiochemicals had a synergistic effect (Shimoda and Kiuchi 1998).

The semiochemical signal that induced oviposition by female PTB is complex, comprising many components with synergistic activity. One or more representatives of various classes of organic chemicals needed to be present to provoke oviposition (Figure 2.3; Experiments 4-10). Similarly selective oviposition decisions have been reported for *P. xuthus*. Here, oviposition required the presence of 10 different constituents in the foliage of citrus mandarin (Rcnwick and Chew 1994).

The diversity of semiochemicals emanating from gum frass suggested that components originated from different sources. (9Z)-Octadecenyl acetate and (9Z,12Z)-octadecadienyl acetate are attractive pheromone components for communication among larvae (see section 3.3), but apparently not among larvae and female adults in an ovipositional context (Figure 2.3; Experiment 4). Butanoic acid inhibits the growth of some soil-borne bacteria and fungi; thus, trees may release the acids to curtail the invasion of pathogenic microbes (Walker et al. 2003). Fungi that have been noticed to grow on gum frass may produce 3-octanone. Beet armyworm, *Spodoptera exigua*, larvae were attracted to, or stimulated to feed on, host plants infected with white mould, *Sclerotium rolfsii*, and emitting 3-octanone (Cardoza et al. 2002). 4-Heptanone is a
common green leaf volatile (Bernays and Chapman 1994). Benzaldehyde is formed during hydrolysis of prunasin, a cyanogenic glucoside found in peach trees (Han and Chen 2002). Benzaldehyde, nonanal, 2-phenylethanol and/or camphor are semiochemicals in various plant volatile blends shown to attract *M. sexta*, cabbage moth, *Mamestra brassicae*, *C. pomonella*, and several species of noctuid moths (Rojas 1999; Hern and Dorn 2002; Meagher 2002; Fraser et al. 2003). Alkylmethoxypyrazines signal plant toxicity and host suitability to many herbivorous insects (Aldrich et al. 1997). Female monarch butterflies, *Danaus plexippus*, oviposited significantly more eggs on host plants that contained relatively high concentrations of alkylmethoxypyrazines (Rothschild et al. 1984). Finally, several long chain hydrocarbons identified from corn leaf extracts elicited oviposition by female *O. nubilalis* (Udayagiri and Mason 1997).

Current tactics for *S. exitiosa* control include repeated trunk drenches with organophosphate pesticides, targeting neonate larvae before they enter the tree, and sex pheromone-based mating disruption of adult moths. My research has revealed semiochemicals that play a role in oviposition decisions by female *S. exitiosa*. Eventually, these semiochemicals could become part of an insecticide-laced, attract-and-kill formulation to attract and kill gravid moths. Such systems have been developed for other insect species, including sap beetles, *Carpophilus* spp. (Coleoptera: Nitidulidae) (Hossain et al. 2006), fruit flies, *Bactrocera* spp. (Diptera; Tephritidae) (Vargas et al. 2005), and *Rhagoletis* spp. (Barry et al. 2004).

Future studies should determine the semiochemicals within each class of organic compounds that are essential to induce oviposition. These compounds could then be tested for their ability to attract gravid females, so they can be killed, or to enhance the
effectiveness of the sex pheromone. For example, male forest cockchafers, *Melolontha hippocastani*, are more strongly attracted to traps baited with green leaf volatiles plus female sex pheromone than to traps baited with sex pheromone alone (Joachim and Hilker 2003).

In conclusion, gum-frass semiochemicals appear to play a significant role in host-selection and oviposition decisions by female *S. exitiosa*. Blend components are abundant and thus detectable by foraging insects. They may also be indicative of host suitability, because only trees infested with developing larvae seem to produce the characteristic gum frass. A larval pheromone (see section 3.3) is potentially the most reliable, but also least detectable, signal of successful larval development in a suitable tree. By responding to gum-frass semiochemicals, rather than to larval pheromone, females seem to exploit signals that are more detectable than larval pheromone, but still reliably indicate host suitability for larval development.

2.5 References


Table 2.1  Relative amounts of candidate semiochemicals released from 4.8 g of gum frass and captured on Porapak Q during 24 hours of volatile collection.

<table>
<thead>
<tr>
<th>Semiochemical</th>
<th>Amount (ng)</th>
<th>Chemical class</th>
<th>% in class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecyl acetate</td>
<td>1.4</td>
<td>Acetate</td>
<td>42.3</td>
</tr>
<tr>
<td>(9Z)-octadecenyl acetate</td>
<td>1.0</td>
<td>Acetate</td>
<td>28.9</td>
</tr>
<tr>
<td>(9Z,12Z)-octadecadienyl acetate</td>
<td>1.0</td>
<td>Acetate</td>
<td>28.9</td>
</tr>
<tr>
<td>Butanoic acid*</td>
<td>300.0</td>
<td>Acid</td>
<td>65.9</td>
</tr>
<tr>
<td>Heptanoic acid*</td>
<td>30.0</td>
<td>Acid</td>
<td>6.6</td>
</tr>
<tr>
<td>Nonanoic acid*</td>
<td>90.0</td>
<td>Acid</td>
<td>19.8</td>
</tr>
<tr>
<td>Decanoic acid*</td>
<td>35.0</td>
<td>Acid</td>
<td>7.7</td>
</tr>
<tr>
<td>4-Heptanone</td>
<td>5.0</td>
<td>Ketone</td>
<td>8.1</td>
</tr>
<tr>
<td>3-Octanone</td>
<td>11.0</td>
<td>Ketone</td>
<td>17.9</td>
</tr>
<tr>
<td>Camphor</td>
<td>43.0</td>
<td>Ketone</td>
<td>69.6</td>
</tr>
<tr>
<td>Geranyl acetone</td>
<td>2.7</td>
<td>Ketone</td>
<td>4.4</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>3.5</td>
<td>Aldehyde</td>
<td>14.3</td>
</tr>
<tr>
<td>Nonanal</td>
<td>21.0</td>
<td>Aldehyde</td>
<td>85.7</td>
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<tr>
<td>γ-Decalactone*</td>
<td>80.0</td>
<td>Ester</td>
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</tr>
<tr>
<td>Conophthorin</td>
<td>0.5</td>
<td>Spiroacetal</td>
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<tr>
<td>2-Phenylethanol</td>
<td>2.0</td>
<td>Alcohol</td>
<td>100.0</td>
</tr>
<tr>
<td>2-Isopropyl-3-methoxy-pyrazine*</td>
<td>1.0</td>
<td>Pyrazine</td>
<td>100.0</td>
</tr>
<tr>
<td>Heptadecane</td>
<td>8.5</td>
<td>Hydrocarbon</td>
<td>1.9</td>
</tr>
<tr>
<td>Octadecane</td>
<td>7.0</td>
<td>Hydrocarbon</td>
<td>8.3</td>
</tr>
<tr>
<td>Heneicosane</td>
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<td>Hydrocarbon</td>
<td>40.6</td>
</tr>
<tr>
<td>Docosane*</td>
<td>0.3</td>
<td>Hydrocarbon</td>
<td>49.3</td>
</tr>
</tbody>
</table>

*Identified in gum frass volatile sample(s) other than that depicted in Figure 2.2
Table 2.2  Candidate semiochemicals used in two-choice oviposition experiments with mated female *Synanthedon exitiosa*.

<table>
<thead>
<tr>
<th>Semiochemical</th>
<th>Dispenser type</th>
<th>Loading (μg)</th>
<th>Supplier</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecyl acetate</td>
<td>FP</td>
<td>0.2</td>
<td>Aldrich</td>
<td>95</td>
</tr>
<tr>
<td>(9Z)-octadecenyl acetate</td>
<td>FP</td>
<td>20.0</td>
<td>Sigma</td>
<td>97</td>
</tr>
<tr>
<td>(9Z,12Z)-octadecadienyl acetate</td>
<td>FP</td>
<td>20.0</td>
<td>Sigma</td>
<td>99</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>RS</td>
<td>20.0</td>
<td>Aldrich</td>
<td>95</td>
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*Abbreviations: RS = rubber septum, FP = filter paper

*Purchased as alcohol and acetylated

*Available from previous work
Figure 2.1  Artificial oviposition site consisting of: filter paper disk (1; 7 cm diam.); mesh cylinder (2; 7 x 4 cm diam.), Petri dish top (3) and bottom (4); cloth insert (5; 7 x 4 cm diam.) to obscure visual cues associated with test stimuli; moist cotton wick (6; 3 x 1.5 cm diam.); and various types of dispensers [mesh rectangle (7), rubber septum (8), filter paper (9)] for natural gum frass or synthetic semiochemicals.
Figure 2.2  Representative recording of flame ionization detector (FID) and electroantennographic detector (EAD: male *Synanthedon exitiosa* antenna) responses to natural gum frass volatiles (top) or to synthetic standards (bottom). Chromatography: Hewlett Packard 5890A equipped with a DB-5 GC column (30 m x 32 mm ID); linear flow velocity of carrier gas: 35 cm per sec; injector and FID detector temperature: 240°C; temperature program: 50°C (2 min), then 10°C per min to 280°C (5 min). Antennally active compounds: 4-heptanone (1), benzaldehyde (2), 3-octanone (3), conophthorin (4), nonanal (5), 2-phenylethanol (6), camphor (7), geranyl acetone (8), heptadecane (9), octadecane (10), hexadecyl acetate (11), heneicosane (12), (9Z)-Octadecenyl acetate, (9Z,12Z)-octadecadienyl acetate, docosane, 2-isopropyl-3-methoxypyrazine, γ-decalactone, butanoic acid, heptanoic acid, nonanoic acid, and decanoic acid were identified in different gum-frass collections; 4.8 gram-hour equivalents = volatiles released from 4.8 g of gum frass during 1 hr of volatile collection.
FID: Gum-frass volatiles

EAD: \( \delta^+ S. \text{exitiosa} \) antenna

FID: Synthetic standards

EAD: \( \delta^+ S. \text{exitiosa} \) antenna
Figure 2.3  Mean (+ SE) numbers of eggs laid in two-choice experiments by mated female *Synanthedon exitiosa* on oviposition sites (Figure 2.1) baited with test stimuli or left unbaited. SB = synthetic blend of 21 candidate semiochemicals at 4.8 gram-hour equivalents (see Table 2.1). In each experiment, an asterisk (*) indicates a significant response to a particular treatment; *P < 0.05; **P < 0.01, Wilcoxin's signed-rank test, α = 0.05.
| EXP. 1 | Accessible gum-frass | ** | No gum-frass | n = 10 |
| EXP. 2 | Inaccessible gum-frass | ** | No gum-frass | n = 8 |
| EXP. 3 | Synthetic blend (SB) | * | Solvent | n = 32 |
| EXP. 4 | SB minus acetates | * | Solvent | n = 8 |
| EXP. 5 | SB minus acids | | Solvent | n = 10 |
| EXP. 6 | SB minus ketones & aldehydes | | Solvent | n = 8 |
| EXP. 7 | SB minus ester & spiroacetal | | Solvent | n = 8 |
| EXP. 8 | SB minus alcohols | | Solvent | n = 8 |
| EXP. 9 | SB minus pyrazine | | Solvent | n = 8 |
| EXP. 10 | SB minus hydrocarbons | | Solvent | n = 17 |

Test stimuli | Mean (+ SD) number of eggs laid
APPENDIX: LARVAE PRODUCE AND RESPOND TO PHEROMONE

Introduction

The function and structure of pheromones have been studied in many adult insects (Landolt 1997), but little is known about larval pheromones in holometabolous insects. Male and female *Cydia pomonella* larvae produce and respond to aggregation pheromone likely as part of a mating strategy (Duthie et al. 2003; Jumean et al. 2004a, 2004b, 2005). Larvae of the great spruce bark beetle, *Dendroctonus micans*, produce an aggregation pheromone, and thus feed in large groups which may help minimize adverse effects of constitutive or induced host tree defences (Storer et al. 1997).

*Synanthedon exitiosa* larvae develop in the vascular cambium and phloem of peach trees. Larval feeding provokes the production of gum frass, a mixture of tree phloem particles, tree resin (gum), and larval faeces (frass). Gum-frass semiochemicals induce oviposition by gravid females (Chapter 2), and may also demark potential sites of entry for resource-seeking larvae. Conceivably, gum frass semiochemicals may even contain a larval pheromone, as in *D. micans*, for communication among larvae.

My objective was to test the hypothesis that *S. exitiosa* larvae produce and respond to pheromone.
Materials and methods

Experimental insects

Larvae were reared from eggs laid by females mated under laboratory conditions. Eggs were transferred onto new filter paper discs, which were placed inside Ziploc® bags, and kept in an environmental chamber (20-22°C, 30-70% RH, 16L: 8D) until eggs hatched. Neonates were transferred from these bags to artificial diet (Smith 1965) and kept in an environmental chamber (22-26°C, 70-80% RH, 0L: 24D). Diet was replaced every 30 d, or as needed. Larvae were removed from the diet 12-24 hr prior to bioassays.

Acquisition of larvae-produced volatiles

To determine whether larvae produce a pheromone, they were fed an artificial diet (Smith 1965). Their frass (198.9 g) was collected, placed in a Pyrex glass chamber (15.5 x 20 cm), and aerated. Aeration of artificial diet in the absence of larvae served as a control. A water aspirator drew charcoal-filtered air at 0.8-0.9 L/min through each chamber and through a glass column (14 x 1.3 cm OD) containing 0.1 g of Porapak Q (50-80 mesh, Waters Associates, Inc. Milford, Massachusetts 01757). After 207 hr, trapped volatiles were desorbed from Porapak Q with 2 ml of redistilled pentane. Aliquots of Porapak Q extract were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al. 1975; Gries et al. 2002), employing a Hewlett Packard 5890A gas chromatograph equipped with a GC column (30 m x 0.25 or 0.32 mm ID) coated with DB-5, DB-23 or DB-210 (J&W Scientific, Folsom, CA, U.S.A.). Antennae of adult males or females were used as electroantennographic detector because larval antennae were deemed too small for optimal recordings.
Olfactometer experiments with gum-frass semiochemicals and candidate larval pheromone

Olfactometers were housed in a Styrofoam box such that there was a light to dark gradient from the stem to the arms of the Y-tube (Figure 1), thus encouraging a response from negatively phototactic larvae. A water aspirator drew humidified, charcoal-filtered air at 0.7 L/min through the Y-tubes. For each replicate, treatment and control stimuli were randomly assigned and applied to a filter paper disc, which was inserted into Nalgene® tubing connected to the side arms of the Y-tube. Larvae traversing 6 cm into a side arm within 15 min were considered responders, and were included in statistical analyses. Numbers of responding larvae were analyzed with the $\chi^2$ test with Yates' correction for continuity ($\alpha = 0.05$) (Zar 1984; SAS 1999-2001).

Experiments 1 - 3 tested the candidate larval pheromone components (9Z)-octadecenyl acetate (9Z-18:OAc) and (9Z,12Z)-octadecadienyl acetate (9Z,12Z-18:OAc) in combination at 1 ng each (Experiment 1), 10 ng each (Experiment 2) and 100 ng each (Experiment 3).

Results and discussion

Comparative GC-MS analyses of Porapak Q volatile extracts of larval frass and artificial diet revealed two larva-specific compounds, which were identified by comparative GC-MS with authentic standards as 9Z-18:OAc and 9Z,12Z-18:OAc.

In olfactometer bioassays, these two compounds at a 1:1 ratio and 2-ng dose significantly attracted larvae (Figure 2, Experiment 2). The same trend was observed for lower (2 ng) and higher (200 ng) doses but data were not statistically significant (Figure 2, Experiments 1, 3).
Future experiments should determine whether gum-frass semiochemicals also attract neonates, whether both 9Z-18:OAc and 9Z,12Z-18:OAc are necessary for attraction of larvae, and whether production and response to these compounds confers fitness to developing larvae.
Figure 1  Y-tube olfactometer: Styrofoam box (1; 40 x 24 x 13.5 cm, FedEx); Y-tube (2; stem 7.5 cm, arm 7.0 cm, 1 cm inner diam.); Nalgene® tubes (3; thin: 600 x 8.5 mm inner diam.; thick: 400 x 13.5 mm inner diam.); water bottle humidifiers (4; 23.5 x 5.7 cm inner diam.); activated-charcoal (5; 50-80 mesh, Fisher Scientific); water aspirator (6; 0.7 L/min); filter paper discs (7; 1.27 cm diam.) with test stimuli.
Figure 2  Number of *Synanthedon exitiosa* larvae responding in Y-tube
olfactometers (Figure 1) to a 2-component blend of (9Z)-octadecenyl acetate
(9Z-18:OAc) and (9Z,12Z)-octadecadienyl acetate (9Z,12Z-18:OAc) at 1 ng
each (Experiment 1), 10 ng each (Experiment 2), and 100 ng each
(Experiment 3). In each experiment, an asterisk (*) indicates a significant
preference for a particular treatment; \*P < 0.05, \( \chi^2 \) test with Yates' correction
for continuity, \( \alpha = 0.05 \).
Exp. 1
9Z-18:OAc (1 ng)
9Z12Z-18:OAc (1 ng)
Solvent
n = 20

Exp. 2
9Z-18:OAc (10 ng)
9Z12Z-18:OAc (10 ng)
Solvent
n = 20

Exp. 3
9Z-18:OAc (100 ng)
9Z12Z-18:OAc (100 ng)
Solvent
n = 20

Test stimuli
Number of responding larvae
References


