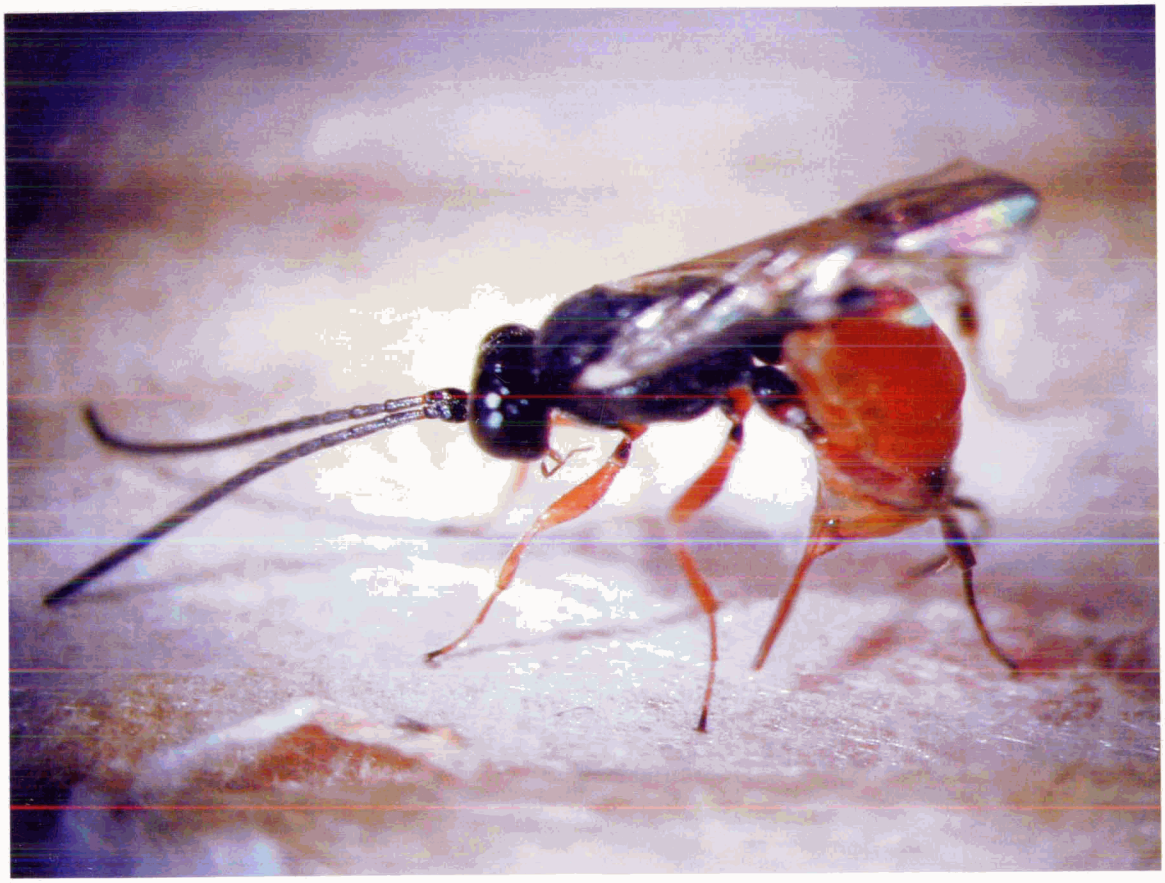


PREPUPAL PARASITOID *Mastrus ridibundus* EAVESDROP ON PHEROMONAL COMMUNICATION OF COCOON-SPINNING *Cydia pomonella* LARVAE

by

Zaid Jumean
B.Sc. (Hons. Zoology), University of Western Ontario, 2000

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

MASTER OF PEST MANAGEMENT

In the
Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

April 2004

APPROVAL

Name: Zaid Jumean

Degree: Master of Pest Management

Title of Thesis:

Prepupal parasitoid *Mastrus ridibundus* eavesdrop on pheromonal communication of cocoon-spinning *Cydia pomonella* larvae

Examining Committee:

Chair: Dr. J.E. Rahe

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

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

Dr. T.R. Unruh, Research Scientist
United States Department of Agriculture

Dr. B.D. Roitberg, Professor
Department of Biological Sciences, S.F.U.

Dr. C. Lowenberger, Assistant Professor
Department of Biological Sciences, S.F.U.
Public Examiner

April 13, 2004
Date Approved

SIMON FRASER UNIVERSITY

Partial Copyright Licence

ABSTRACT

As shown in a recent study, cocoon-spinning larvae of the codling moth, *Cydia pomonella* L. (Lepidoptera: Olethreutidae) employ a pheromone that attracts or arrests pupation site seeking conspecific larvae. Such intraspecific communication signals are important cues for illicit receivers such as parasitoids to exploit. I tested the hypothesis that the specialist prepupal *C. pomonella* parasitoid *Mastrus ridibundus* Gravenhorst (Hymenoptera: Ichneumonidae) exploit the larval aggregation pheromone to locate host prepupae. In laboratory olfactometer experiments, female *M. ridibundus* were attracted to 3-day-old cocoons containing *C. pomonella* larvae or prepupae. Older cocoons containing *C. pomonella* pupae, or larvae and prepupae excised from cocoons, were not attractive. In coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses of bioactive Porapak Q extract of cocoon-derived airborne semiochemicals, ten compounds elicited responses from the antennae of female *M. ridibundus*. Comparative GC-mass spectrometry of authentic standards and cocoon-derived volatiles determined that compounds were 3-carene, myrcene, heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-nonenal, sulcatone, and geranylacetone. A synthetic 11-component blend consisting of these ten EAD-active compounds plus EAD-inactive (+)-limonene (the most abundant cocoon-derived volatile) was as effective as Porapak Q cocoon extract in attracting both female *M. ridibundus* and *C. pomonella* larvae seeking pupation sites. Only three components [myrcene, (*E*)-2-nonenal, (+)-limonene] could be deleted from the 11-component blend without diminishing its attractiveness for *M. ridibundus* which

underlines the complexity of information conveyance during host foraging. *Mastrus ridibundus* obviously eavesdrop on the pheromonal communication signals of *C. pomonella* larvae that reliably indicate host presence. Whether the larval aggregation pheromone is as complex as the semiochemical blend attracting *M. ridibundus* is currently under investigation.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks and appreciation to my senior supervisor, Gerhard Gries. I am grateful for his thorough supervision, for his unending encouragement, and for allowing me the opportunity to work on such a fascinating system. I also wish to thank: Tom Unruh for graciously supplying me with *Mastrus ridibundus* and for his guidance and continual encouragement throughout the research; Regine Gries for patiently sharing with me her expertise in electrophysiology and electrophysiological techniques; Bernie Roitberg and Manfred Mackauer for their input and critical review of my thesis; Jeff Upton, Valerie Pleasance, and Coralee Harrison for shipment of insects; Bob Birtch and Elizabeth Carefoot for preparation of the figures.

I thank my colleagues in the Gries and Roitberg labs and also my friends and family for their support and encouragement throughout the duration of this research.

I would finally like to thank those who made this research possible through funding: Natural Sciences and Engineering Research Council of Canada (NSERC) [GG], Washington Tree Fruit Research Commission [TU], Professor Thelma Finlayson Fellowship [ZJ].

TABLE OF CONTENTS

Mastrus ridibundus parasitizing _Cydia pomonella_ host ..... frontispiece

Approval ii

Abstract iii

Acknowledgements v

Table of Contents vi

List of Tables vii

List of Figures viii

Glossary xi

1.0. INTRODUCTION 1

2.0. LIFE HISTORIES 4

2.1. _Cydia pomonella_ 4
2.2. _Mastrus ridibundus_ 8

3.0. METHODS AND MATERIALS 10

3.1. Experimental Insects 10
3.2. Acquisition of Volatiles 11
3.3. Analyses of Volatiles 12
3.4. Olfactometer Bioassays 12
3.4.1. Y-tube Olfactometer Bioassays 12
3.4.2. Petri-dish Pitfall Olfactometer Bioassay 14
3.5. Statistical Analyses 19

4.0. RESULTS 20

4.1. Identification of Semiochemicals 20
4.2. Bioassay Experiments with Natural and Synthetic Semiochemicals 20

5.0. DISCUSSION 39

6.0. REFERENCES 43

LIST OF TABLES

Table 1 Details on experimental insects and stimuli tested in olfactometer bioassays. 15

LIST OF FIGURES

Figure 1 Life cycle of (a) the codling moth, *Cydia pomonella*, and (b) its prepupal parasitoid, *Mastrus ridibundus*.....5

Figure 2 Flame ionization detector (FID) and electroantennographic detector (EAD: female *Mastrus ridibundus* antenna) responses to aliquots of 10 CSLHE of Porapak Q extract of airborne volatiles from *Cydia pomonella* cocoons (1-3-d-old). Note: 1) 10 CSLHE (cocoon-spinning larvae hour equivalents = volatiles released from 10 cocoon-spinning *C. pomonella* larvae during 1 hr of cocoon-spinning activity); 2) antennal responses to 3-carene and myrcene (two additional candidate semiochemicals) not visible in the depicted trace; 3) although (+)-limonene was not EAD-active, it was the most abundant chemical in Porapak Q extracts and as such was included in behavioural experiments. 21

Figure 3 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 1-4 to test stimuli consisting of either five *Cydia pomonella* cocoons (1-3-d-old), five larvae/prepupae excised from cocoons or both. Strips of corrugated cardboard (CB) served as pupation site. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.005. 24

Figure 4 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 5-9 to test stimuli consisting of five *Cydia pomonella* cocoons each containing either a larva/prepupa or pupa, with or without corrugated cardboard strips (CB) that had served as pupation site. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.025. 26

Figure 5 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 10-15 to MeOH cocoon extracts, MeOH extracted cocoons, or Porapak Q extracts of airborne cocoon-derived volatiles. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular

treatment; χ^2 test with Yates correction for continuity; *P<0.05; **P<0.01; ***P<0.005. Note: 1) cocoons were 1-3 days old at the time of extraction or aeration but MeOH cocoon extracts or Porapak Q extracts were tested before and after aging to determine stability of semiochemicals; 2) the amount of stimulus tested was equivalent to 20 CEE (cocoon-extract equivalents = MeOH extract of 20 *Cydia pomonella* cocoons), and 1 or 50 CSLHE (cocoon-spinning larvae hour equivalents = semiochemicals produced by 1 or 50 cocoon-spinning *C. pomonella* larva(e) during 1 hr of cocoon-spinning activity); 3) the same amount of solvent was applied to treatment and control stimuli. 28

Figure 6 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 16-21 to a 11-component blend of synthetic candidate semiochemicals (SB), or SB lacking groups of organic chemicals. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.01. Note: 1) SB consisted of 3 monoterpenes [(+)-limonene, 3-carene, myrcene], 4 saturated aldehydes [heptanal, octanal, nonanal, decanal], 2 unsaturated aldehydes [(*E*)-2-octenal, (*E*)-2-nonenal], and 2 ketones [sulcatone, geranylacetone]; 2) aliquots of 1 CSLHE (see caption of figure 2) were tested; 3) the same amount (5 µl) of pentane was applied to treatment and control stimuli. 30

Figure 7 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 22-32 to a 11-component blend of synthetic candidate semiochemicals (SB; see also caption of figure 6) or SB lacking individual components. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.025; **P<0.01. Note: 1) Aliquots of 5 CSLHE (see caption of figure 2) were tested; 2) the same amount (5 µl) of pentane was applied to treatment and control stimuli. 32

Figure 8 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 33-34 to test stimuli consisting of either the 11-component blend of synthetic candidate semiochemicals (SB; see also caption of figure 6), Porapak Q extracts of airborne cocoon-derived volatiles, or the 8-component blend of semiochemicals. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses. Note: 1) the 8-component blend consisted of one monoterpene [3-carene], four saturated aldehydes [heptanal, octanal,

nonanal, decanal], one unsaturated aldehyde [(*E*)-2-octenal], and two ketones [sulcatone and geranylacetone]; 2) aliquots of 5 CSLHE (see caption of figure 2) were tested.....35

Figure 9 Response of 5th instar *Cydia pomonella* larvae in Petri dish pitfall olfactometer (Duthie *et al.* 2003) experiment 35 to either a 11-component blend of semiochemicals (SB; see caption of figure 6) or a solvent (pentane) control stimulus. Number of larvae responding to each stimulus given within bars; number of larvae not responding in experiment 35 given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.001. Note: the same amount (25 µl) of pentane was applied to treatment and control stimuli.....37

GLOSSARY

Communication. The process in which one individual uses specially designed signals or displays to modify the behaviour of another (Krebs and Davies 1998).

Eavesdrop. The interception and exploitation by a parasitoid of semiochemical signals from its host (Stowe *et al.* 1995).

Fresh cocoon. Cocoon material collected within three days from the onset of cocoon-spinning activity by larvae.

Gregarious parasitoid. A parasitoid species with one or more individuals developing in or on the same host (Godfray 1994).

Illicit receiver. A parasitoid which exploits the communication signals produced by other organisms to find resources (Haynes and Yeargan 1998).

Naïve parasitoid. A parasitoid that has not had previous experience with a particular test stimulus.

Non-responding insects. Experimental insects which did not show a positive response to either the treatment or the control stimulus.

Parasitoid. The larva of an arthropod that feeds exclusively on the body of another arthropod, its host, eventually killing it (Godfray 1994).

Semiochemical. Any chemical involved in communications among organisms (Pedigo 1999).

1.0. INTRODUCTION

Host location by hymenopterous parasitoids has been reported to proceed in four steps: location of host habitat, location of host, acceptance of host, and determination of host suitability (Doutt 1959; Vinson and Iwantsch 1980; Vinson 1976, 1984a,b, 1998). In many parasitoids, host habitat location seems mediated by semiochemicals from the food of the host (Vinson 1981; Vet *et al.* 1991; Vet and Dicke 1992), whereas host location often is mediated by semiochemicals directly from the host insect (Stowe *et al.* 1995, Haynes and Yeargan 1999). In many tritrophic communication systems, parasitoids respond to qualitative and quantitative changes in semiochemicals emanating from food plants fed on by host larvae (Turlings *et al.* 1991; Tumlinson *et al.* 1993; Alborn *et al.* 1997; De Moraes *et al.* 1998; Mattiacci *et al.* 1999). To locate their hosts, parasitoids exploit diverse chemical cues that are produced directly or indirectly by potential hosts and reliably indicate their presence (Vet *et al.* 1995). These semiochemicals emanate from the host itself (moth scales; DeLury *et al.* 1999) or its metabolites (excrement, silk; Lewis and Jones 1971; Weseloh 1976; Mattiacci *et al.* 1999).

In many systems, parasitoids are attracted to their host by semiochemicals emitted by their future "victims" (Stowe *et al.* 1995). Host foraging parasitoids are faced with a reliability-detectability problem. Semiochemicals from the first trophic level are highly detectable and direct parasitoids to host habitat but are poor indicators of host presence. Semiochemicals from the host itself, in contrast, are reliable indicators of host presence, but are scarcely detectable to parasitoids because host insects express a low

semiochemical profile (Vet *et al.* 1991; Vet and Dicke 1992; Stowe *et al.* 1995). Parasitoids that learn to associate these highly detectable host-habitat derived semiochemicals with highly reliable host-produced semiochemicals enhance their foraging effectiveness and increase their Darwinian fitness (Vet and Dicke 1992; Hoffmeister and Roitberg 1997a,b; Geervliet *et al.* 1998; Hoffmeister *et al.* 2000).

While maintaining a low semiochemical profile is generally advantageous to hosts, they cannot completely avoid emitting semiochemicals such as those that may serve as intraspecific signals to attract mates, mark oviposition sites, or defend territories (Stowe *et al.* 1995, Hedlund *et al.* 1996). As such, these intraspecific communication signals may become important cues for illicit receivers to exploit (Stowe *et al.* 1995; Haynes and Yeargan 1999).

Mastrus ridibundus (Gravenhorst) (Hymenoptera: Ichneumonidae) is an introduced gregarious ectoparasitoid specialising on late instar/prepupal codling moth, *Cydia pomonella* L. (Lepidoptera: Olethreutidae), an exotic pest of apples and other pome fruits in North America (Pedigo 1999; Bezemer and Mills 2001). In its native Kazakhstan, *M. ridibundus* may parasitize ≥ 60% of overwintering *C. pomonella* prepupae.

Observations (Tom Unruh, USDA-Agricultural Research Station, Wapato, Washington; personal communication) suggested that female *M. ridibundus* are attracted to semiochemicals emanating from cocoon-spinning *C. pomonella* larvae and prepupae. Moreover, Duthie *et al.* (2003) provided evidence that cocoon-spinning *C. pomonella* larvae produce an aggregation pheromone that mediates aggregation/arrestment of pupation site seeking larvae in a microlocation. This aggregation pheromone is produced

and detectable, respectively, in the cocoon-spinning larval and prepupal stage but not in the pupal stage (Duthie *et al.* 2003). Conceivably, *M. ridibundus* may eavesdrop on the intraspecific communication of *C. pomonella* larvae by exploiting the larval aggregation pheromone as a reliable and detectable host derived semiochemical.

My objectives were: 1) to test the hypothesis that semiochemicals from cocoon-spinning *C. pomonella* larvae attract host foraging female *M. ridibundus*; 2) to determine the semiochemical source and longevity; 3) to identify the semiochemicals; and 4) to investigate whether *M. ridibundus* eavesdrop on pheromonal communication by cocoon-spinning *C. pomonella* larvae.

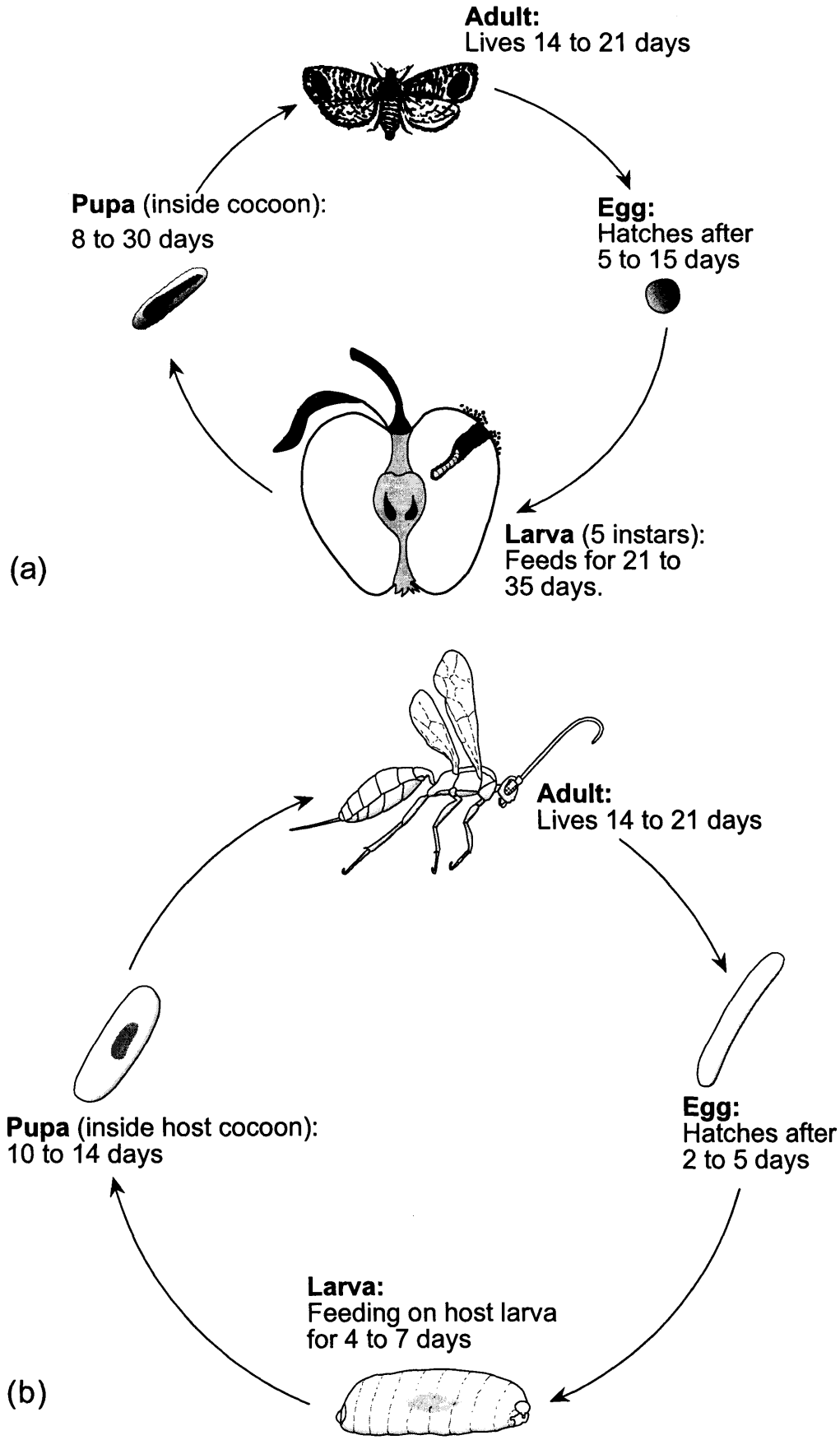
2.0. LIFE HISTORIES

2.1. Cydia pomonella

Cydia pomonella (Figure 1a) peak flight occurs just prior to and after sunset and, under favourable conditions, may last for up to 2 hr (Dolstad 1985; Howell *et al.* 1990). Optimal temperatures for flight range from 12-33°C, above and below which flight activity ceases (Dolstad 1985). At dusk, females call potential mates by releasing a long-range sex pheromone blend comprising (*E,E*)-8,10-dodecadienol (Roelofs *et al.* 1971), the primary component, and secondary components (*E,Z*)-8,10-dodecadienol, (*E*)-9-dodecanol, dodecanol, and tetradecanol (El-Sayed 1999). At close range, females may rely on visual stimuli in accepting a mate (Weissling and Knight 1994). Gravid and mated females oviposit eggs on or near fruit during sunset and may deposit an epideictic pheromone after oviposition (Thiery *et al* 1995).

Mated females deposit 30-50 eggs (Geier 1963; Pedigo 1999; Unruh and Lacey 2000) singly directly on the apple, pear, or walnut fruit or near the fruit on host foliage (Summerland and Steiner 1943; Thiery *et al.* 1995; Landolt *et al.* 1999). The freshly laid egg is white and convex, and appressed closed to the substrate (Dolstad 1985). As the embryo develops, the egg darkens following melanization and a red ring surrounds the developing insect. Finally, 1-2 days prior to hatching, the head capsule of the larva becomes visible through the egg as a black spot (Dolstad 1985). Neonate larvae hatch 5-15 days after oviposition and commence foraging for fruit within a few hours (Pedigo 1999).

Figure 1 Life cycle of (a) the codling moth, *Cydia pomonella*, and (b) its prepupal parasitoid, *Mastrus ridibundus*.


Adult:
Lives 14 to 21 days
Egg:
Hatches after
5 to 15 days
Larva (5 instars):
Feeds for 21 to
35 days.
Pupa (inside cocoon):
8 to 30 days
(a)
Adult:
Lives 14 to 21 days
Egg:
Hatches after
2 to 5 days
Larva:
Feeding on host larva
for 4 to 7 days
Pupa (inside host cocoon):
10 to 14 days
(b)

Larvae complete five instars before pupating. The white first instar larva is ca. 1.5 mm in length with a black head capsule, whereas the mature fifth instar is ca. 20 mm in length with a brown head capsule and cervical shield, and a pinkish body (Dolstad 1985).

Neonate larvae rely partially on semiochemicals from apple skin to guide them to the fruit within a few hours post-hatching (Dolstad 1985; Landolt *et al.* 1999). Once an apple fruit is located, larvae burrow in through the calyx leaving a sting mark, and continue to feed on the flesh of the fruit until they reach the core within which they attack the seeds (Dolstad 1985; Unruh and Lacey 2000, Higbee *et al.* 2001). The number of larvae per fruit is limited due to aggressive interactions between third instar larvae. Mature fifth instars burrow out of the apple, usually causing a second sting mark, and seek cryptic and protective microhabitats, such as cracks or crevices within the tree's bark, ground litter, or in other shelters, where they will spin a cocoon and pupate in aggregations (Geier 1963; Unruh and Lacey 2000; Duthie *et al.* 2003). The time from larval hatching to pupation takes 14-35 days (Geier 1963). Pupae are dark brown in colour, and the pupal period lasts 8-30 days (DeLury 1998).

Cydia pomonella are protandrous. Adult males eclose up to 2 days prior to females (Duthie *et al.* 2003). The moths are 6-7 mm long with an average wingspan of 19 mm (Dolstad 1985). They are mostly grey with copper coloured spots lying distally on the forewing. A buff coloured variant is light brown in colour (Dolstad 1985; Pedigo 1999). At rest, the wings are held roof-like over the body (Dolstad 1985). Adult moths live for 14-21 days in the wild (Pedigo 1999).

2.2. *Mastrus ridibundus*

Mastrus ridibundus (Figure 1b) was originally collected in Kazakhstan in 1994 and released into North America in 1995 (Unruh 1997; Kuhlmann and Mills 1999). *Mastrus ridibundus* is a gregarious ectoparasitoid specialising on cocooning late instar/prepupal *C. pomonella* larvae. Female *M. ridibundus* primarily attack *C. pomonella*, and only rarely attack congeneric hosts (Kuhlmann and Mills 1999) or *Grapholita prunivora* (Walsh) (Dhalsten 1994), which is also a minor pest in apple orchards.

Female *M. ridibundus* deposit 1-10 eggs on the integument of the host insect after stinging and paralyzing it (Abdullaev 1974; Makarov 1983; Dhalsten 1994). Parasitoid larvae hatch 1-3 days after oviposition (personal observation) and continue for 4-7 days to feed on the *C. pomonella* host until only the host's head capsule and integument remains (personal observation). Final fifth instar *M. ridibundus* proceed to spin their cocoons within the cocoon of their *C. pomonella* host before pupating. Depending upon whether host larvae are overwintering (diapausing) or not, *M. ridibundus* will spin light-dark brown cocoons and thin white cocoons, respectively (Abdullaev 1974; Dhalsten 1994). Non-diapausing adults eclose within 10-14 days after pupating, whereas diapausing adults eclose the following spring. The sex ratio of adult parasitoids is typically 1:1 (Tom Unruh, personal communication).

Male *M. ridibundus* eclose 2-3 days before females. Adults are 5-6 mm in length with a black head and thorax, and orange legs and abdomen. The antennae are ca. 3 mm long, and the wings are transparent and shaded light brown/grey. Females with their protruding 3 mm long ovipositor can easily be distinguished from males. Although there

is much variation in adult size, most likely reflecting the number of larvae per host, (Horstmann 1990), adult females generally tended to be larger than adult males (personal observation).

Females and males live for 14-21 days at 22-24°C (Isankalova 1972; Abdullaev 1974) and may mate shortly after emergence from cocoons. Females do not exhibit any kind of calling behaviour prior to mating (personal observation). *Mastrus ridibundus* are arrhenotokous; impaternate males develop from unfertilized eggs, whereas females develop from fertilized eggs.

3.0. METHODS AND MATERIALS

3.1. Experimental Insects

Cydia pomonella. Larvae reared on artificial diet were shipped from the Sterile Insect Release Program in Osoyoos, British Columbia, Canada. The colony has been maintained since 1991 with periodic introductions of wild individuals, the last introduction occurring in 2001. Trays containing ca. 1000 larvae were kept in a glass aquarium (60 × 31 × 31 cm) and stored at 15°C under a photoregime of 16L:8D. To generate a test stimulus for bioassay experiments, five final instars were removed from the diet and allowed to cocoon on a corrugated cardboard strip (2.5 × 2.5 cm) (0.46 m × 76 m single face corrugated cardboard; Shippers Supply Inc., British Columbia, Canada) placed in a 35 mm plastic Petri dish. If the test stimulus consisted of cocoons, larvae were excised from their cocoons ~1.5 hr prior to bioassays.

Mastrus ridibundus. *Cydia pomonella* hosts parasitized with *M. ridibundus* were shipped in corrugated cardboard rolls from the USDA-Agricultural Research Station, Wapato, Washington, USA. The colony has been maintained since 1996. Shipments included both diapausing and non-diapausing *M. ridibundus*.

Rolls with non-diapausing *M. ridibundus* were kept in a partially meshed Plexiglass cage (40 × 30 × 30 cm) maintained at 20-23°C, 30-50% RH, and a 16L:8D photoperiod. Adult parasitoids emerged from the rolls during a 5-d period, and were sustained with a 10% honey-water solution and a water-soaked cotton wick *ad libitum*. All insects used in bioassays were allowed a minimum two-day pre-oviposition period (Tom Unruh; personal communication) and were between 3-14 days old at the time of the

experiment. Rolls with diapausing *M. ridibundus* were stored at 3°C in complete darkness. Diapause was broken by exposing insects to a 16L:8D photoperiod and 20-23°C. Parasitoids began to emerge after 17-20 d.

Female parasitoids were transferred to a small wooden cage (13 × 18 × 18 cm) with a sliding Plexiglass front and a meshed back one day prior to experiments, and transferred singly to plastic cups (4 × 4 cm) 1 hr prior to experiments to allow acclimation. Naïve parasitoids were used in all bioassay experiments and each insect was tested only once. Although mating status was not confirmed, females spent three or more days with males and were very likely mated (personal observation).

3.2. Acquisition of Volatiles

To solvent-extract *C. pomonella* cocoons, 460 late instar larvae were allowed to cocoon in a Sparkleen™ washed glass aquarium (60 × 31 × 31 cm) for three days. Three hr before bioassays, larvae were excised from cocoons, and cocoons removed from the aquarium and extracted in methanol (MeOH). Twenty cocoon extract equivalents (20 CEE = 230 µl of methanol extract from 20 *C. pomonella* cocoons) were tested per replicate.

To collect airborne volatiles from cocoon-spinning larvae, three-hundred 5th instar larvae were removed from the diet, placed in a cylindrical Pyrex® glass chamber (15.5 ID × 20 cm high), and aerated for ~72 hr. A water aspirator drew charcoal-filtered air at 2 litres/min through the chamber and a downstream glass column (1400 × 10.1 mm ID) filled with Porapak Q (50-80 mesh, Waters Associates, Inc., Milford, Massachusetts 01757). Volatiles were eluted from the Porapak Q with 3 ml of solvent [redistilled pentane:ether (95:5)]. The eluent was concentrated to 1 ml adjusting the volatile extract

so that 1 µl was equivalent to 10 cocoon-spinning larvae hour equivalents (10 CSLHE = volatiles released from 10 cocoon-spinning *C. pomonella* larvae during 1 hr).

3.3. Analyses of Volatiles

Aliquots of 20 CSLHE of Porapak Q extract were subjected to analysis by coupled gas chromatographic-electroantennagraphic detection (GC-EAD) (Arn *et al.* 1975) employing a Hewlett Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m × 0.25 or 0.32 mm ID) coated with DB-5, DB-210, or DB-23 (J & W Scientific, Folsom, California, USA). For GC-EAD recordings, the proximal end of a severed female *M. ridibundus* antenna was placed into the opening of a glass capillary electrode (1.0 × 0.58 × 100 mm) (A-M Systems, Inc., Carlsborg, Washington, USA) filled with saline solution. The distal end with its tip removed by spring microscissors (Fine Science Tools Inc., North Vancouver, British Columbia, Canada) was placed into the recording capillary electrode (Gries *et al.* 2002).

Full scan electron impact (EI) mass spectra of EAD-active compounds were obtained by GC-mass spectrometry (MS), using a Varian Saturn 2000 Ion Trap GC-MS, and a HP 5985B GC-MS, respectively, each fitted with the DB-210 or DB-5 column referred to above.

3.4. Olfactometer Bioassays

3.4.1. Y-tube Olfactometer Bioassays

Anemotactic responses of naïve female *M. ridibundus* to test stimuli were bioassayed in a vertically oriented Y-tube Pyrex® glass olfactometer (ID 23 mm; stem 25 cm; arms 20 cm at a 120° angle) at 20-25°C and 40-70% RH. Air drawn through the apparatus at ~1.2 L/min with a water aspirator carried volatiles from each arm of the Y-

tube olfactometer toward upwind traveling parasitoids released individually into the stem. For each replicate, the position of the odour source was randomly assigned to Y-tube arms. A parasitoid that penetrated ≥10 cm into one arm within 15 min was classified as a responder. All non-responding insects were excluded from statistical analyses. Each replicate employed a new odour source, Y-tube, and parasitoid. During bioassays, a single light source composed of one fluorescent "daylight" tube (F40D Hb58; Osram Sylvania Ltd., Ontario, Canada) and one "wide spectrum" grow light (F40GRO WS6 H568; Osram Sylvania Ltd., Ontario, Canada) was centered ~20 cm above the olfactometer. Y-tubes were washed between replicates with Sparkleen™ and dried at 125°C for ≥ 30 min.

Experiments 1-3 (Table 1) tested potential sources of semiochemicals (larvae/prepupae, cocoon) derived from *C. pomonella* working under the hypothesis that *M. ridibundus* females are attracted to semiochemicals emanating from *C. pomonella* cocoons. Taking into account that semiochemical attractiveness resided with the cocoons, experiments 4-9 (Table 1) explored whether the age of cocoons affects their semiochemical attractiveness hypothesizing that aged cocoons will be less attractive than fresh cocoons due to cessation of production and degradation/dissipation of the semiochemical respectively. Experiments 10-11 (Table 1) then tested whether semiochemicals could be solvent extracted from cocoons (experiment 10), whether aging of extracts affected their attractiveness (experiment 11) and whether solvent washed cocoons still maintained attractiveness (experiment 12). Experiments 13-15 (Table 1) explored whether Porapak Q extracts of airborne cocoon-derived semiochemicals are attractive (experiment 13), and whether dose (experiment 14) or age (experiment 15) of

extracts affected their attractiveness. Considering the strong attraction of *M. ridibundus* to Porapak Q extract of cocoon semiochemicals (experiment 13), experiments 16-21 (Table 1) explored which semiochemicals were essential for blend attractiveness by testing specific blends lacking classes of organic chemicals (saturated aldehydes, unsaturated aldehydes, ketones, or monoterpenes). Follow-up experiments 22-32 (Table 1) determined which individual compounds were essential components of the semiochemical blend. Experiments 33-34 (Table 1) tested the 8-component semiochemical blend lacking all non-essential components [(*E*)-2-nonenal, myrcene, and (+)-limonene] *versus* Porapak Q cocoon extracts and *versus* the 11-component semiochemical blend respectively.

3.4.2. Petri-dish Pitfall Olfactometer Bioassay

Responses of pupation site seeking 5th instar *C. pomonella* larvae were tested in binary choice Petri dish pitfall olfactometers (Duthie *et al.* 2003). Test stimuli were randomly assigned to one of two 4 mL "pitfall" vials (Table 1) equipped with modified Eppendorf tubes to prevent contact of the experimental insects with a test stimulus. For each replicate, one fifth-instar larva was placed in the centre of the olfactometer, and its pupation site was recorded 18-24 hr later. All non-responding insects were excluded from statistical analyses. Olfactometers were kept at 21-26°C in complete darkness.

Experiment 35 (Table 1) tested attractiveness/arrestment of 200 CSLHE (see above) of a complete blend of synthetic semiochemicals *versus* that of a pentane control to determine if the same semiochemicals utilised by *M. ridibundus* females in host location also serve as an aggregation pheromone for *C. pomonella* larvae seeking pupation sites.

Table 1 Details on experimental insects and stimuli tested in olfactometer bioassays.

Experiment No.	♀ *M. ridibundus* (n)	Y-tube olfactometer Arm 1	Arm 2
1	36	Cocoon	No stimulus
2	29	Cocoon + cardboard (CB[a])	CB
3	35	Prepupa + CB	CB
4	32	Cocoon + prepupa + CB	Cocoon + CB
5	36	3-d-old cocoon + larva/ prepupa + CB	CB
6	34	7-d-old cocoon + pupa + CB	CB
7	31	13-d-old cocoon + pupa + CB	CB
8	31	3-d-old cocoon + larva/ prepupa + CB	7-d-old cocoon + pupa + CB
9	32	3-d-old cocoon + larva/ prepupa + CB	13-d-old cocoon + pupa + CB
10	18	MeOH cocoon extract at 20 CEE[b] (1-hr-old)	MeOH
11	18	MeOH cocoon extract at 20 CEE (8-d-old)	MeOH
12	17	20 MeOH washed cocoons	No stimulus
13	19	Porapak Q extract (1 CSLHE[c], 5-d-old)	Solvent[d]
14	19	Porapak Q extract (1 CSLHE, 17-d-old)	Solvent
15	13	Porapak Q extract (50 CSLHE)	Solvent
16	18	Synthetic blend (SB[e])	Solvent

Table 1 continued

Experiment No.	♀ *M. ridibundus* (n)	Y-tube olfactometer	
		Arm 1	Arm 2
17	20	SB *minus* all aldehydes	Solvent
18	18	SB *minus* unsaturated aldehydes	Solvent
19	17	SB *minus* saturated aldehydes	Solvent
20	17	SB *minus* ketones	Solvent
21	17	SB *minus* monoterpenes	Solvent
22	17	SB *minus* decanal	Solvent
23	17	SB *minus* nonanal	Solvent
24	19	SB *minus* octanal	Solvent
25	18	SB *minus* heptanal	Solvent
26	19	SB *minus* (*E*)-2-nonenal	Solvent
27	18	SB *minus* (*E*)-2-octenal	Solvent
28	18	SB *minus* geranylacetone	Solvent
29	20	SB *minus* sulcatone	Solvent
30	18	SB *minus* 3-carene	Solvent
31	17	SB *minus* myrcene	Solvent
32	19	SB *minus* (+)-limonene	Solvent
33	16	8-component blend[f]	Porapak Q extract (5 CSLHE)
34	19	SB	8-component blend

Table 1 continued

Experiment No.	*C. pomonella*[g] (n)	Petri Dish Pitfall Olfactometer	
		Pitfall 1	Pitfall 2
35	35	SB	Solvent

[a] insects were allowed to cocoon on an open fluted cardboard (CB) strip 2.5 cm^2;
[b] CEE = cocoon extract equivalents;
[c] CSLHE = cocoon-spinning larvae hour equivalents;
[d] solvent consisted of redistilled pentane:ether (95:5) or pentane;
[e] SB = decanal, nonanal, octanal, heptanal, (*E*)-2-nonenal, (*E*)-2-octenal, geranylacetone, sulcatone, 3-carene, myrcene, (+)-limonene;
[f] 8-component blend consisted of SB *minus* (*E*)-2-nonenal, myrcene, (+)-limonene;
[g] male and female 5th instar larvae

3.5. Statistical Analyses

Proportions of *M. ridibundus* adults and *C. pomonella* larvae responding to treatment and control stimuli in olfactometer bioassays were compared using the Chi-square goodness of fit test using Yates correction for continuity ($\alpha = 0.05$) to determine whether observed frequencies deviated significantly from expected frequencies under the null hypothesis that experimental insects did not have a preference for either treatment or control stimuli (Zar 1996).

4.0. RESULTS

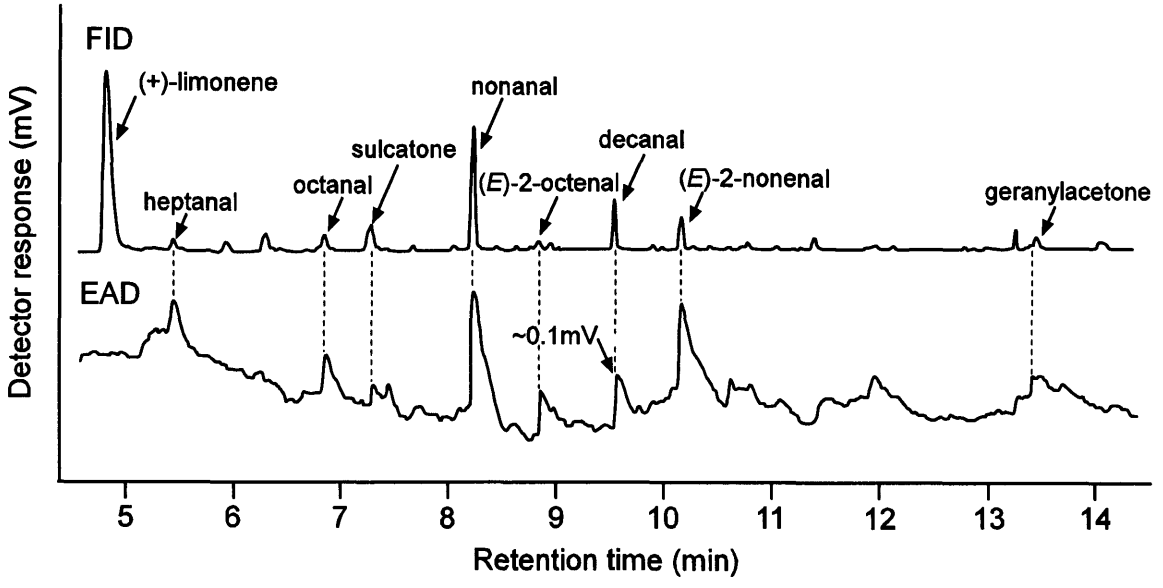
4.1. Identification of Semiochemicals

GC-EAD analyses of Porapak Q extracts of airborne cocoon-derived semiochemicals revealed 10 volatiles that elicited responses from female *M. ridibundus* (Figure 2). EAD active compounds, plus an 11th compound which was most abundant in the Porapak Q extract, were further subjected to GC-MS, and their identification confirmed by comparative GC, GC-MS, and GC-EAD analyses of *C. pomonella* produced and authentic standards. These 11 compounds were identified as: heptanal, 6-methyl-5-hepten-2-one [sulcatone], 7-methyl-3-methylene-1,6-octadiene [myrcene], octanal, 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene [3-carene], 1-methyl-4-(1-methylethenyl)-cyclohexene [(+)-limonene], (*E*)-2-octenal, nonanal, (*E*)-2-nonenal, decanal, and trans-6,10-dimethyl-5,9-undecadien-2-one [geranylacetone].

4.2. Bioassay Experiments with Natural and Synthetic Semiochemicals

Observations by Tom Unruh (personal communication) suggested the possibility that *M. ridibundus* may become entrained to cardboard odours upon emergence from codling moth cocoons in cardboard rolls. Unpublished experiments (Jumean) tested the attractiveness of cardboard *versus* no stimulus in Y-tube olfactometers revealing that naïve *M. ridibundus* females are not preferentially attracted to clean cardboard. This indicated that cardboard odours did not affect the parasitoids' decision and that cardboard could be used as a control stimulus.

Figure 2 Flame ionization detector (FID) and electroantennographic detector (EAD: female *Mastrus ridibundus* antenna) responses to aliquots of 10 CSLHE of Porapak Q extract of airborne volatiles from *Cydia pomonella* cocoons (1-3-d-old). Note: 1) 10 CSLHE (cocoon-spinning larvae hour equivalents = volatiles released from 10 cocoon-spinning *C. pomonella* larvae during 1 hr of cocoon-spinning activity); 2) antennal responses to 3-carene and myrcene (two additional candidate semiochemicals) not visible in the depicted trace; 3) although (+)-limonene was not EAD-active, it was the most abundant chemical in Porapak Q extracts and as such was included in behavioural experiments.


FID
(+)-limonene
heptanal
octanal
sulcatone
nonanal
(E)-2-octenal
decanal
(E)-2-nonenal
geranylacetone
EAD
~0.1mV
Detector response (mV)
5
6
7
8
9
10
11
12
13
14
Retention time (min)

In separate experiments, cocoons (3-d-old) but not larvae or prepupae of *C. pomonella* attracted female *M. ridibundus* (Figure 3; experiments 1-3). Attractiveness of cocoons was not enhanced by the presence of prepupae (Figure 3; experiment 4), and depended upon the age of cocoons. Three-day-old cocoons containing late instar/prepupal *C. pomonella* were attractive (Figure 4; experiment 5) but 7- or 13-day-old cocoons containing *C. pomonella* pupae were not (Figure 4; experiments 6, 7). Similarly, 3-day-old cocoons were more attractive than 7- and 13-day-old cocoons (Figure 4, experiments 8, 9) in separate experiments although the former was not statistically significant.

Fresh (2 hr-old), unlike aged (8-d-old) MeOH extracts of 3-day-old cocoons attracted *M. ridibundus* (Figure 5; experiments 10, 11). Methanol washed cocoons were not attractive to *M. ridibundus* (Figure 5; experiment 12). Both 5- and 17-day-old Porapak Q extracts of airborne cocoon volatiles at aliquots of 1 CSLHE attracted *M. ridibundus*, whereas aliquots of 50 CSLHE did not (Figure 5; experiments 13-15).

The complete blend of 11 synthetic candidate semiochemicals (highlighted on figure 2) at 1 CSLHE strongly attracted *M. ridibundus* (Figure 6; experiment 16). The blend lacking either all aldehydes (experiment 17), unsaturated aldehydes (experiment 18), saturated aldehydes (experiment 19), ketones (experiment 20), or monoterpenes (experiment 21) was as unattractive as a pentane control. Deleting a single component from the 11-component blend in experiments 22-32 (Figure 7) determined that an 8-component blend (heptanal, octanal, nonanal, decanal, (*E*)-2-octenal, sulcatone, geranylacetone, and 3-carene) is required to strongly attract *M. ridibundus*. Neither Porapak Q cocoon extracts nor the 8-component semiochemical blend differentially

Figure 3 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 1-4 to test stimuli consisting of either five *Cydia pomonella* cocoons (1-3-d-old), five larvae/prepupae excised from cocoons or both. Strips of corrugated cardboard (CB) served as pupation site. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.005.

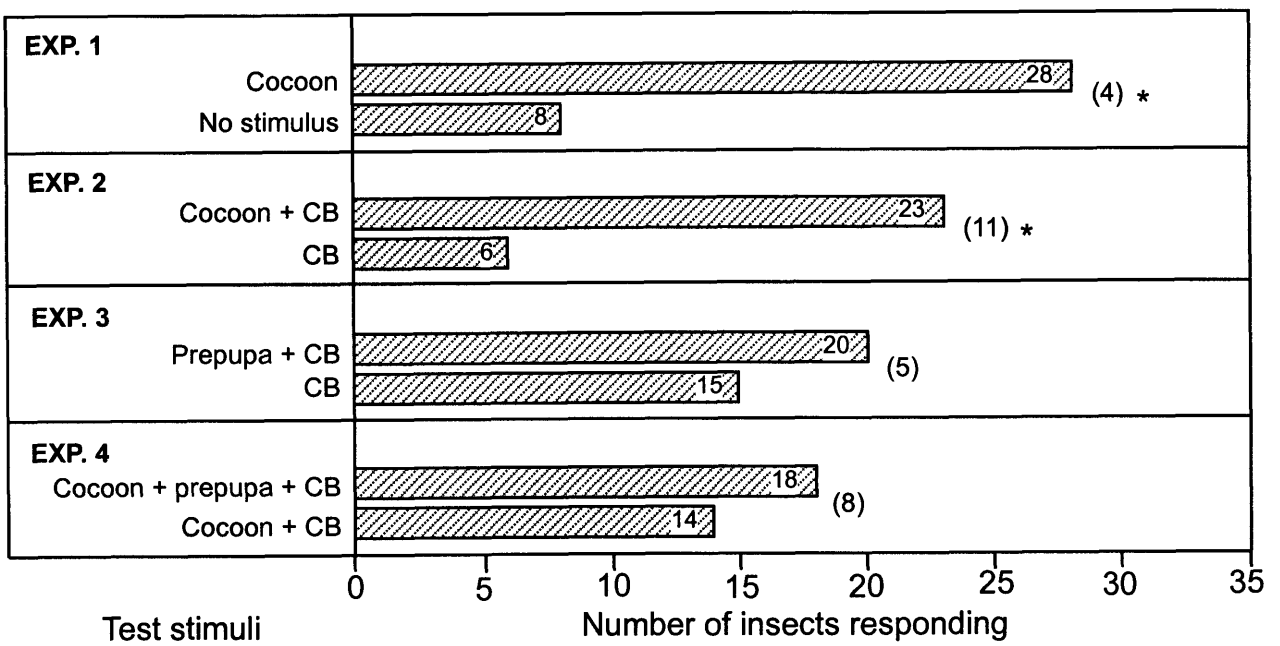

EXP. 1
Cocoon
No stimulus
28
8
(4) *
EXP. 2
Cocoon + CB
CB
23
6
(11) *
EXP. 3
Prepupa + CB
CB
20
15
(5)
EXP. 4
Cocoon + prepupa + CB
Cocoon + CB
18
14
(8)
0
5
10
15
20
25
30
35
Test stimuli
Number of insects responding

Figure 4 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 5-9 to test stimuli consisting of five *Cydia pomonella* cocoons each containing either a larva/prepupa or pupa, with or without corrugated cardboard strips (CB) that had served as pupation site. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.025.

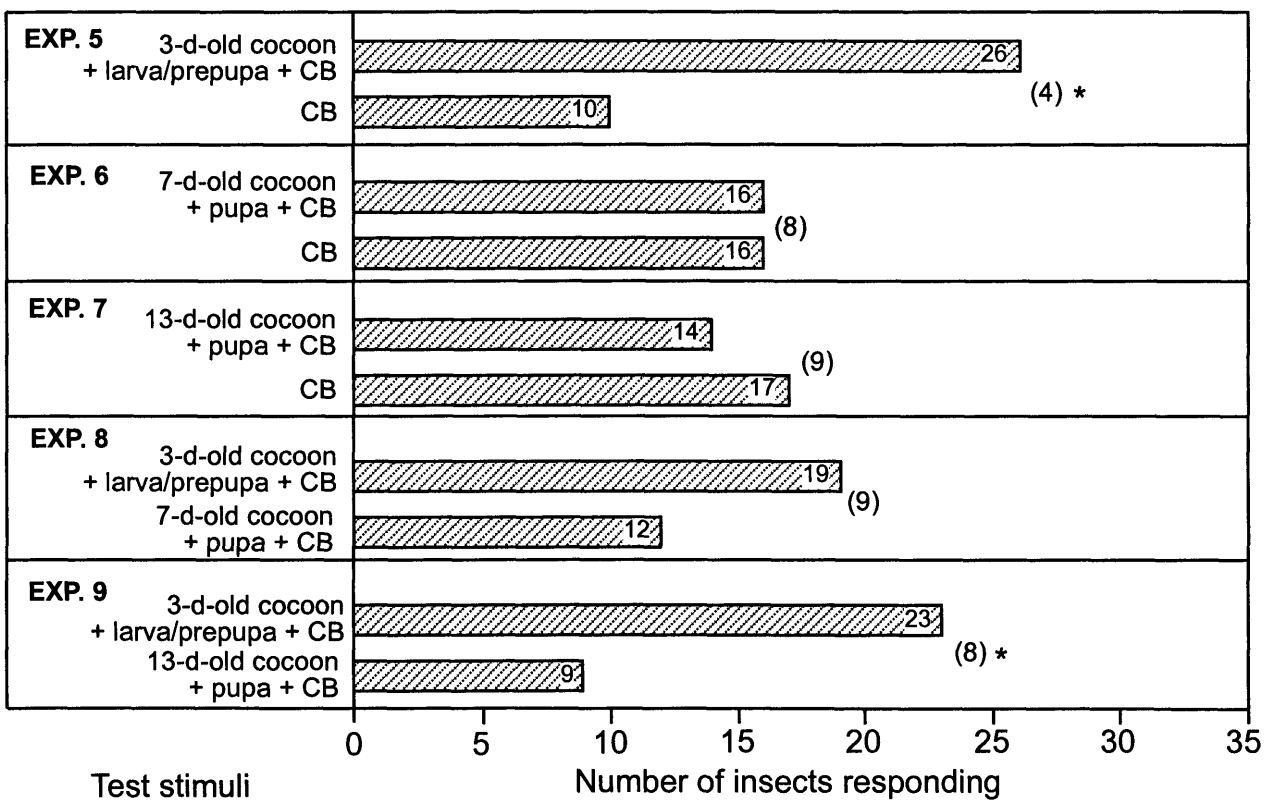

EXP. 5
3-d-old cocoon + larva/prepupa + CB
CB
26
10
(4) *
EXP. 6
7-d-old cocoon + pupa + CB
CB
16
16
(8)
EXP. 7
13-d-old cocoon + pupa + CB
CB
14
17
(9)
EXP. 8
3-d-old cocoon + larva/prepupa + CB
7-d-old cocoon + pupa + CB
19
12
(9)
EXP. 9
3-d-old cocoon + larva/prepupa + CB
13-d-old cocoon + pupa + CB
23
9
(8) *
0
5
10
15
20
25
30
35
Test stimuli
Number of insects responding

Figure 5 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 10-15 to MeOH cocoon extracts, MeOH extracted cocoons, or Porapak Q extracts of airborne cocoon-derived volatiles. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.05; **P<0.01; ***P<0.005. Note: 1) cocoons were 1-3 days old at the time of extraction or aeration but MeOH cocoon extracts or Porapak Q extracts were tested before and after aging to determine stability of semiochemicals; 2) the amount of stimulus tested was equivalent to 20 CEE (cocoon-extract equivalents = MeOH extract of 20 *Cydia pomonella* cocoons), and 1 or 50 CSLHE (cocoon-spinning larvae hour equivalents = semiochemicals produced by 1 or 50 cocoon-spinning *C. pomonella* larva(e) during 1 hr of cocoon-spinning activity); 3) the same amount of solvent was applied to treatment and control stimuli.

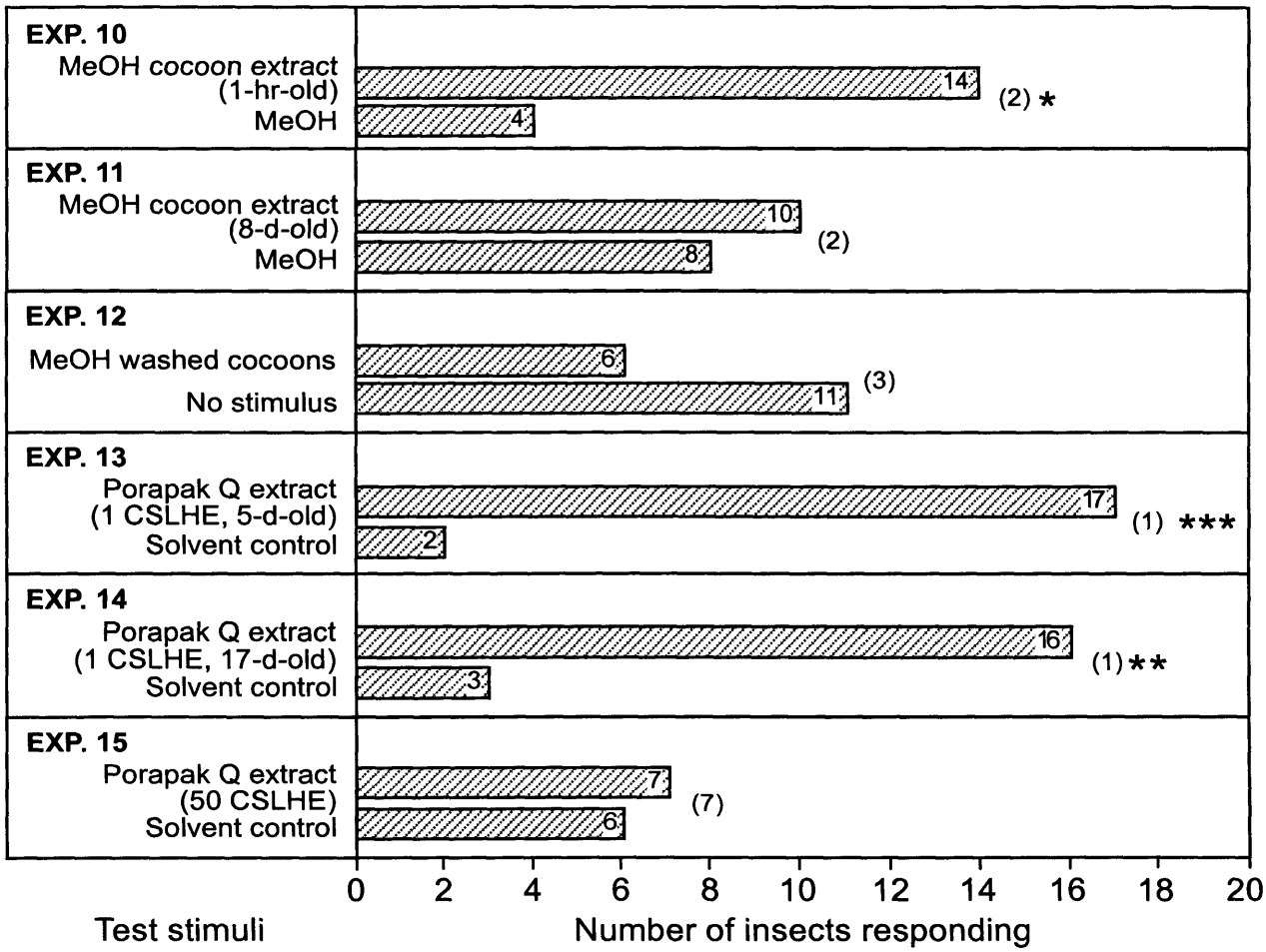

EXP. 10
MeOH cocoon extract (1-hr-old)
MeOH
14
4
(2) *
EXP. 11
MeOH cocoon extract (8-d-old)
MeOH
10
8
(2)
EXP. 12
MeOH washed cocoons
No stimulus
6
11
(3)
EXP. 13
Porapak Q extract (1 CSLHE, 5-d-old)
Solvent control
17
2
(1) ***
EXP. 14
Porapak Q extract (1 CSLHE, 17-d-old)
Solvent control
16
3
(1) **
EXP. 15
Porapak Q extract (50 CSLHE)
Solvent control
7
6
(7)
0 2 4 6 8 10 12 14 16 18 20
Test stimuli
Number of insects responding

Figure 6 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 16-21 to a 11-component blend of synthetic candidate semiochemicals (SB), or SB lacking groups of organic chemicals. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.01. Note: 1) SB consisted of 3 monoterpenes [(+)-limonene, 3-carene, myrcene], 4 saturated aldehydes [heptanal, octanal, nonanal, decanal], 2 unsaturated aldehydes [(*E*)-2-octenal, (*E*)-2-nonenal], and 2 ketones [sulcatone, geranylacetone]; 2) aliquots of 1 CSLHE (see caption of figure 2) were tested; 3) the same amount (5 µl) of pentane was applied to treatment and control stimuli.

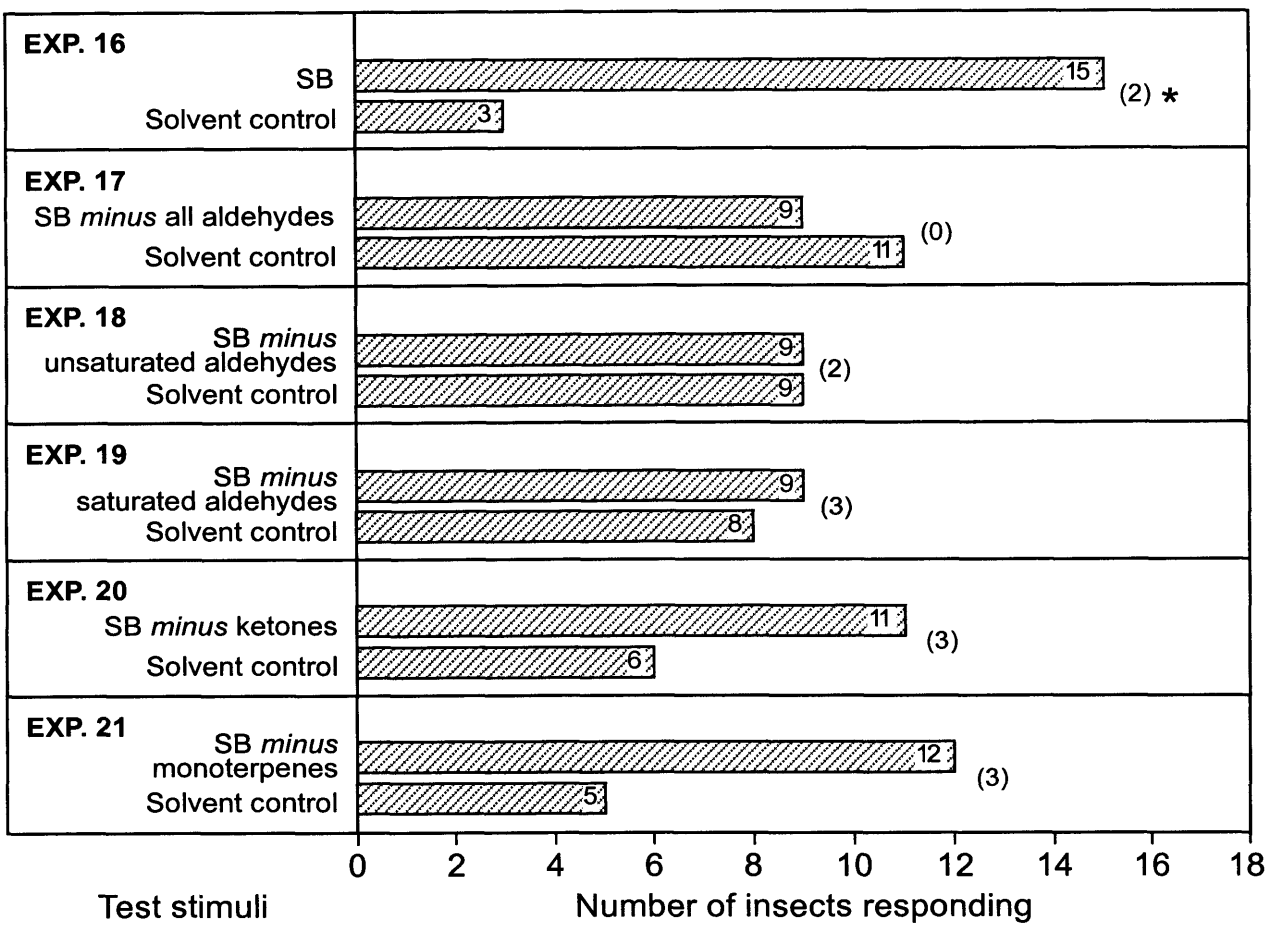
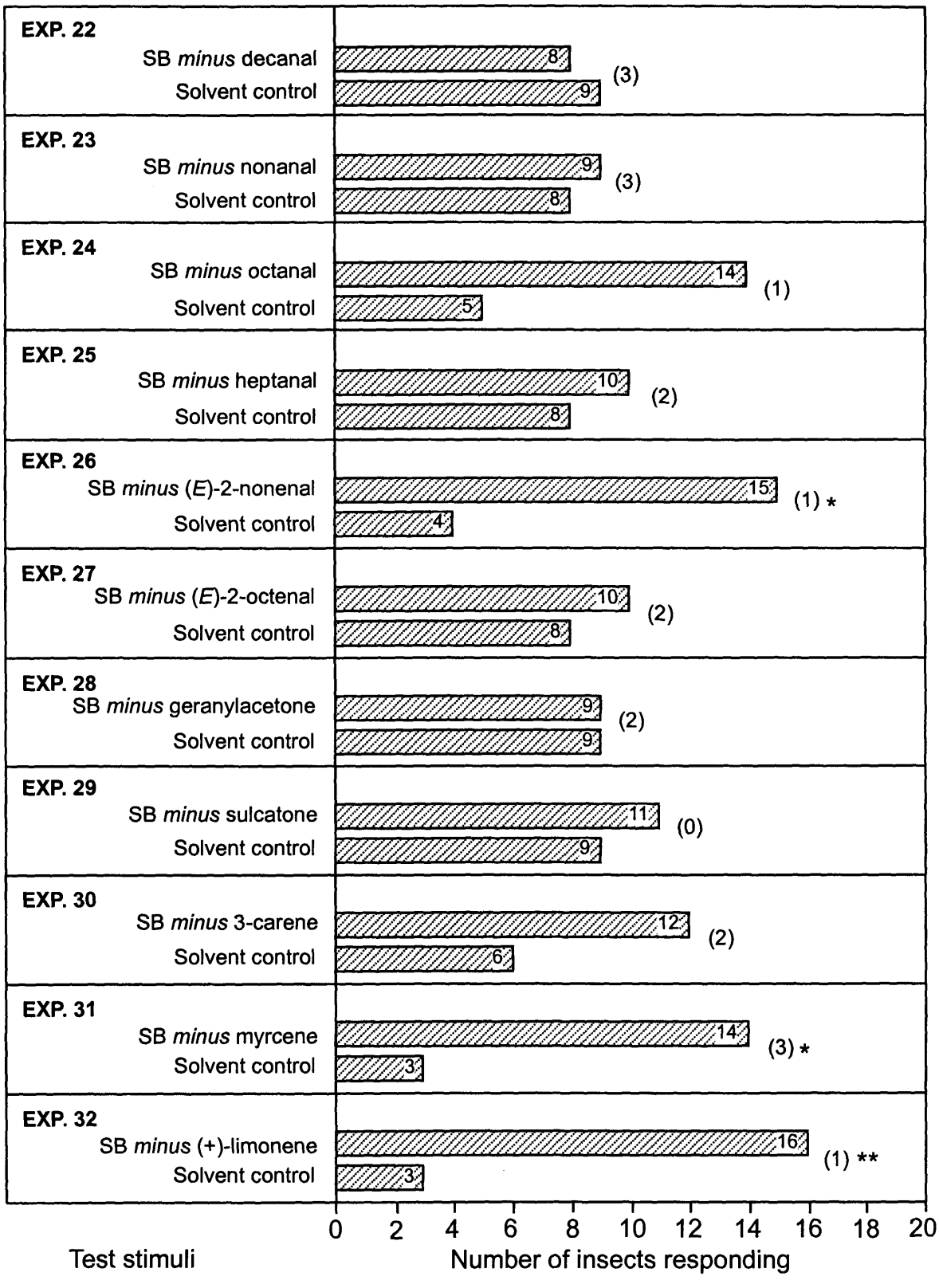

EXP. 16
SB
Solvent control
15
3
(2) *
EXP. 17
SB minus all aldehydes
Solvent control
9
11
(0)
EXP. 18
SB minus unsaturated aldehydes
Solvent control
9
9
(2)
EXP. 19
SB minus saturated aldehydes
Solvent control
9
8
(3)
EXP. 20
SB minus ketones
Solvent control
11
6
(3)
EXP. 21
SB minus monoterpenes
Solvent control
12
5
(3)
0 2 4 6 8 10 12 14 16 18
Test stimuli
Number of insects responding

Figure 7 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 22-32 to a 11-component blend of synthetic candidate semiochemicals (SB; see also caption of figure 6) or SB lacking individual components. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.025; **P<0.01. Note: 1) Aliquots of 5 CSLHE (see caption of figure 2) were tested; 2) the same amount (5 µl) of pentane was applied to treatment and control stimuli.


EXP. 22
SB minus decanal
Solvent control
8
9
(3)
EXP. 23
SB minus nonanal
Solvent control
9
8
(3)
EXP. 24
SB minus octanal
Solvent control
14
5
(1)
EXP. 25
SB minus heptanal
Solvent control
10
8
(2)
EXP. 26
SB minus (E)-2-nonenal
Solvent control
15
4
(1) *
EXP. 27
SB minus (E)-2-octenal
Solvent control
10
8
(2)
EXP. 28
SB minus geranylacetone
Solvent control
9
9
(2)
EXP. 29
SB minus sulcatone
Solvent control
11
9
(0)
EXP. 30
SB minus 3-carene
Solvent control
12
6
(2)
EXP. 31
SB minus myrcene
Solvent control
14
3
(3) *
EXP. 32
SB minus (+)-limonene
Solvent control
16
3
(1) **
0 2 4 6 8 10 12 14 16 18 20
Test stimuli
Number of insects responding

attracted *M. ridibundus* over the complete 11-component semiochemical blend (Figure 8; experiments 33, 34).

The 11-component synthetic semiochemical blend tested at 200 CSLHE attracted/arrested pupation site seeking fifth instar *C. pomonella* (Figure 9; experiment 35).

Figure 8 Anemotactic response of female *Mastrus ridibundus* in Y-tube olfactometer experiments 33-34 to test stimuli consisting of either the 11-component blend of synthetic candidate semiochemicals (SB; see also caption of figure 6), Porapak Q extracts of airborne cocoon-derived volatiles, or the 8-component blend of semiochemicals. Number of insects responding to each stimulus given within bars; number of insects not responding in each experiment given in parentheses. Note: 1) the 8-component blend consisted of one monoterpene [3-carene], four saturated aldehydes [heptanal, octanal, nonanal, decanal], one unsaturated aldehyde [(*E*)-2-octenal], and two ketones [sulcatone and geranylacetone]; 2) aliquots of 5 CSLHE (see caption of figure 2) were tested.

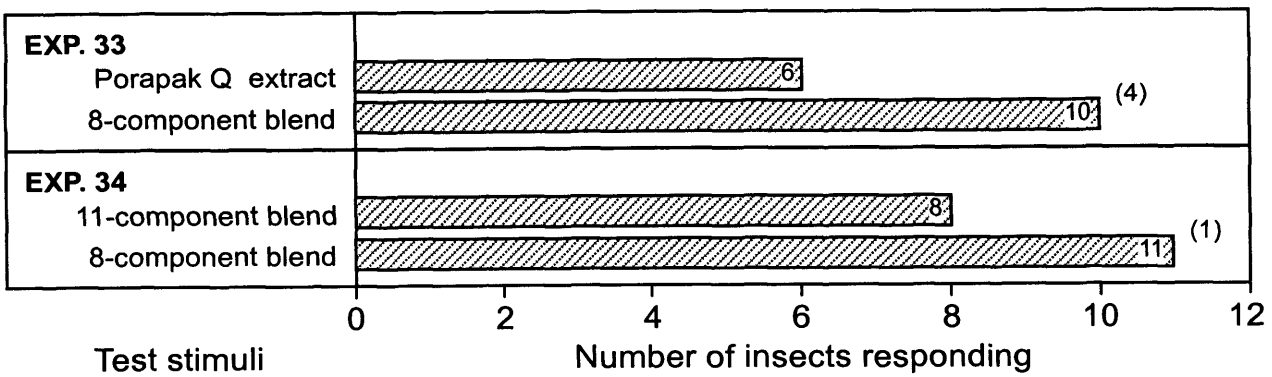
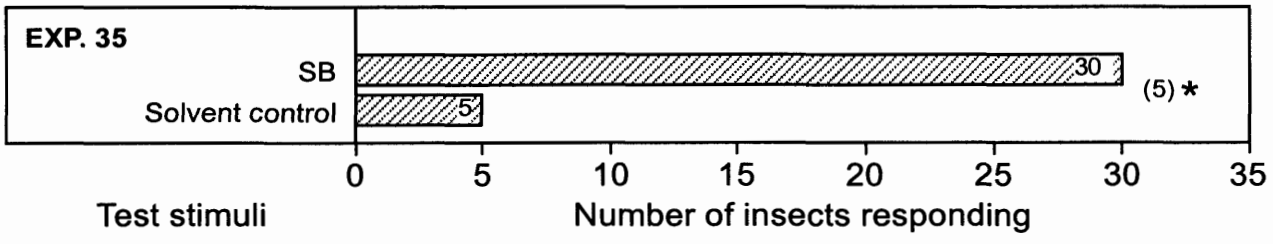

EXP. 33
Porapak Q extract
8-component blend
6
10
(4)
EXP. 34
11-component blend
8-component blend
8
11
(1)
0
2
4
6
8
10
12
Test stimuli
Number of insects responding

Figure 9 Response of 5th instar *Cydia pomonella* larvae in Petri dish pitfall olfactometer (Duthie *et al.* 2003) experiment 35 to either a 11-component blend of semiochemicals (SB; see caption of figure 6) or a solvent (pentane) control stimulus. Number of larvae responding to each stimulus given within bars; number of larvae not responding in experiment 35 given in parentheses; asterisks indicate a significant response to a particular treatment; χ^2 test with Yates correction for continuity; *P<0.001. Note: the same amount (25 µl) of pentane was applied to treatment and control stimuli.


EXP. 35
SB
Solvent control
30
5
(5) *
0
5
10
15
20
25
30
35
Test stimuli
Number of insects responding

5.0. DISCUSSION

My data support the hypothesis that semiochemicals from cocoon-spinning *C. pomonella* larvae attract host-foraging *M. ridibundus*. This conclusion is based on the findings that *M. ridibundus* were attracted to a) cocoons containing *C. pomonella* late instar larvae or prepupae (Figure 3; experiment 1); b) fresh MeOH cocoon extract (Figure 5; experiment 10); and c) Porapak Q extract of cocoon-derived airborne semiochemicals (Figure 5; experiments 13, 14).

Moreover, attraction of *M. ridibundus* to *C. pomonella* cocoons (Figure 3; experiments 1, 3), equal attraction to cocoons with or without insects inside, and no attraction to excised *C. pomonella* prepupae (Figure 3, experiments 2, 4), all strongly suggest that the semiochemicals are associated with the cocoon.

Attractiveness of cocoons containing *C. pomonella* late instar larvae or prepupae, and lack of attractiveness of cocoons containing pupae (Figure 4; experiments 5-7) further indicate that the prepupal parasitoid *M. ridibundus* exploit host semiochemicals not only to locate hosts, but also to locate hosts in the stage of development suitable for parasitism. While cocoon-derived semiochemicals have been implied as contact host recognition cues for parasitoids (Weseloh 1977, 1981; Bekkaoui and Thibout 1993; Benedet *et al.* 1999), my data show that cocoon-derived airborne semiochemicals serve as long-range attractants to *M. ridibundus*.

Some constituents of this semiochemical blend have also been reported to elicit antennal or behavioural responses by other parasitic wasps. Heptanal elicits antennal

activity by the braconid parasitoid *Microplitis croceipes* (Cresson) (Li *et al.* 1992), and nonanal and geranylacetone attract the clothes moth parasitoid *Apantales carpatus* to host habitat (Takács *et al.* 1997). Further, octanal, nonanal, and decanal derived from stink bug abdominal glands may serve as semiochemicals for parasitic Tachinids (Diptera) and Scelionids (Hymenoptera) (Aldrich 1995). Finally, heptanal, octanal, nonanal, and decanal emanating from scales of *C. pomonella* serve as semiochemicals to *A. quadridentata*, a braconid egg parasitoid of *C. pomonella* (DeLury *et al.* 1999).

Response of *C. pomonella* larvae seeking pupation sites to the 11-component semiochemical blend (Figure 9; experiment 35) reveals that one or more of these semiochemicals serve as an aggregation pheromone (Duthie *et al.* 2003) for *C. pomonella* larvae. Obviously, female *M. ridibundus* eavesdrop on the pheromonal communication of cocoon-spinning *C. pomonella* larvae to locate host prepupae. The pheromone is effective, and most attractive to *M. ridibundus*, during the larval cocoon-spinning stage (Duthie *et al.* 2003; this study) but remains detectable in the prepupal stage (Figure 4; experiments 8, 9). Degradation or dissipation of the pheromone within 3 days after cocoon-spinning activity has ceased appears adaptive to both *C. pomonella* larvae and female *M. ridibundus*. If pheromone-based aggregation of *C. pomonella* larvae is a strategy to procure and synchronize development of future mates (Duthie *et al.* 2003), fading attractiveness of aging cocoons prevents continued attraction of larvae and asynchronous eclosion of potential mates. This characteristic of the semiochemical signal also makes it a reliable indicator of host presence. *Mastrus ridibundus* as a specialist of cocoon-spinning *C. pomonella* larvae (Isankalova 1972; Abdullaev 1974;

Kuhlman and Mills 1999) seem to use these temporal dynamics of the semiochemicals to determine the proper late instar/prepupal stage of the host.

Semiochemicals also facilitate foraging in patches with high probability of host encounter (Chiri and Legner 1982, 1986). If the semiochemical concentration is proportional to host density, *M. ridibundus* may estimate host density, and thus patch profitability, even before encountering a host insect (van Alphen and Vet 1986; Geervliet *et al.* 1998; Wertheim *et al.* 2003). Moreover, if foraging females can detect variation in host density on trees, hosts on trees supporting high densities of *C. pomonella* larvae are at a greater risk of being discovered and parasitized by *M. ridibundus* (Bezemer and Mills 2001).

For *C. pomonella* larvae, pheromone-based aggregation may represent a trade-off between procuring future mates (Duthie *et al.* 2003), and the risk of attracting parasitoids. For example, Bezemer and Mills (2001) provided evidence that female *M. ridibundus* search and oviposit more frequently on trees with high rather than low host densities. Similarly, Roitberg and Lalonde (1991) demonstrated that female rosehip flies, *Rhagoletis basiola*, oviposit on and mark the rosehips with semiochemicals which in turn facilitates egg location and increases the risk of egg parasitism by the pteromalid wasp *Halticoptera rosae*. However, if *M. ridibundus* females are egg-limited (Bezemer and Mills 2001), then the probability of parasitism may be lower for *C. pomonella* larvae cocooning in aggregates than for those cocooning singly.

Information conveyance plays an important role in host-parasitoid interactions. Female *M. ridibundus* seem to have solved the reliability-detectability challenge of host signals by eavesdropping on the pheromonal communication of host larvae/prepupae.

Host pheromones are most suitable signals for foraging parasitoids because pheromones tend to be detectable and reliable indicators of host presence (Wiskerke *et al.* 1993; Wertheim *et al.* 2003). The reliability of the cocoon-derived signal seems to be based on its complexity. Eight compounds from four classes of organic chemicals were required to elicit a strong behavioural response of *M. ridibundus* (Figure 6, experiments 17-21). It will now be intriguing to investigate whether the aggregation pheromone of *C. pomonella* larvae comprises an equally complex signal.

6.0. REFERENCES

Abullaev, E.N. 1974. Insect enemies of codling moth. *Zashchita Rastenii.* 8: 27-

Alborn, H.T., Turlings, C.J., Jones, T.H., Stenhagen, G., Loughrin, J.H., and Tumlinson, J.H. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276: 945-949.

Aldrich, J.R., Rosi, M.C., and Bin, F. 1995. Behavioral correlates for minor volatile compounds from stink bugs (Heteroptera: Pentatomidae). *Journal of Chemical Ecology* 21: 1907-1920.

van Alphen, J.J.M. and Vet, L.E.M. 1986. An evolutionary approach to host finding and selection, pp. 23-62, *in* J. Waage and D. Greathead (eds.). Insect Parasitoids. Academic Press, London.

Arn, H., Städler, E., and Rauscher, S. 1975. The electroantennographic detector – a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Zeitschrift fur Naturforschung* 30: 722-25.

Bekkaoui, A. and Thibout, E. 1993. Role of the cocoon of *Acrolepiopsis assectella* (Lep., Hyponomeutidae) in host recognition by the parasitoid *Diadromus pulchellus* (Hym., Ichneumonidae). *Entomophaga* 38: 101-113.

Benedet, F., Bigot, Y., Renault, S., Pouzat, E., and Thibout, E. 1999. Polypeptides of *Acrolepiopsis assectella* cocoon (Lepidoptera: Yponomeutidae): an external host-acceptance kairomone for the parasitoid *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). *Journal of Insect Physiology* 45: 375-384.

Bezemer, T.M. and Mills, N.J. 2001. Host density responses of *Mastrus ridibundus*, a parasitoid of the codling moth, *Cydia pomonella*. *Biological Control* 22: 169-175.

Chiri, A.A. and Legner, E.F. 1982. Host-searching kairomones alter behavior of *Chelonus* sp. nr. *curvimaculatus*, a hymenopterous parasite of the pink bollworm, *Pectinophora gossypiella* (Saunders). *Environmental Entomology* 11: 452-455.

Chiri, A.A. and Legner, E.F. 1986. Response of three *Chelonus* (Hymenoptera: Braconidae) species to kairomones in scales of six lepidoptera. *Canadian Entomologist* 118: 329-333.

DeLury, N.C. 1998. Pheromonal and kairomonal attraction of *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of *Cydia pomonella* L. (Lepidoptera: Tortricidae). M.Sc. Thesis. Simon Fraser University, Burnaby.

DeLury, N.C., Gries, R., Gries, G., Judd, G.J.R., and Khaskin, G. 1999. Moth scales-derived kairomones used by egg-larval parasitoid *Ascogaster quadridentata* to locate eggs of its host, *Cydia pomonella*. *Journal of Chemical Ecology* 25: 2419-2431.

DeMoraes, C.M., Lewis, W.J., Pare, P.W., Alborn, H.T., and Tumlinson, J.H. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393: 570-573.

Dhalsten, D.L. 1994. Background information in support of the application for the release in California of *Mastrus ridibundus* (Gravenhorst) (Hymenoptera: Ichneumonidae), collected in Kazakhstan, for the classical biological control of the codling moth, *Cydia pomonella* (L.). *PPQ 526 Permit 940857 of 03/22/94.*

Dolstad, K.D. 1985. Biology and control of the codling moth in the Pacific Northwest. M.P.M. Professional Paper. Simon Fraser University, Burnaby.

Doutt, R.L. 1959. The biology of parasitic Hymenoptera. *Annual Review of Entomology* 4: 161-182.

Duthie, B., Gries, G., Gries, R., Krupke, C and Derksen, S. 2003. Does pheromone based aggregation of codling moth larvae help procure future mates? *Journal of Chemical Ecology* 29: 425-436.

El-Sayed, A., Bengtsson, M., Rauscher, S., Lofqvist, J., and Witzgall, P. 1999. Multicomponent sex pheromone in codling moth (Lepidoptera: Tortricidae). *Environmental Entomology* 28:775-779.

Geervliet, J.B.F., Ariens, S., Dicke, M., and Vet, L.E.M. 1998. Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). *Biological Control* 11: 113-121.

Geier, P.W. 1963. The life history of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the Australian Capital Territory. *Australian Journal of Zoology* 11: 323-367.

Godfray, H.C.J. 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, New Jersey.

Gries, R., Khaskin, G., Gries, G., Bennett, R.G., King, G.G.S., Morewood, P., Slessor, K. and Morewood, W.D. 2002. (*Z*,*Z*)-4,7-Tridecadien-(*S*)-2-yl acetate: sex

pheromone of Douglas-fir cone gall midge. *Journal of Chemical Ecology* 28: 2283-2297.

Haynes, K.F. and Yeargan, K.V. 1999. Exploitation of intraspecific communication systems: Illicit signalers and receivers. *Annals of the Entomological Society of America* 92: 960-970.

Hedlund, K., Vet, L.E.M., and Dicke, M. 1996. Generalist and specialist parasitoid strategies of using odours of adult drosophilid flies when searching for larval hosts. *Oikos* 77: 390-398.

Higbee, B.S., Calkins, C.O., and Temple, C.A. 2001. Overwintering of codling moth (Lepidoptera: Tortricidae) larvae in apple harvest bins and subsequent moth emergence. *Journal of Economic Entomology* 94: 1511-1517.

Hoffmeister, T.S. and Roitberg, B.D. 1997a. Counterespionage in an insect herbivore-parasitoid system. *Naturwissenschaften* 84: 117-119.

Hoffmeister, T.S. and Roitberg, B.D. 1997b. To mark the host or the patch: Decisions of a parasitoid searching for concealed host larvae. *Evolutionary Ecology* 11: 145-168.

Hoffmeister, T.S., Roitberg, B.D., and Lalonde, R.G. 2000. Catching Ariadne by her thread: How a parasitoid exploits the herbivore's marking trails to locate its host. *Entomologia Experimentalis et Applicata* 95: 77-85.

Horstmann, K. 1990. Neubeschreibungen einiger Sclupfwespen – Arten aus den Gattungen *Mastrus* FÖRSTER, *Odontoneura* FÖRSTER und *Zoophthorus* FÖRSTER

(Hymenoptera, Ichneumonidae, Cryptinae). *Zeitschrift der Arbeitsgemeinschaft Österr. Entomologen* 42: 1-14.

Howell, J.F., Schmidt, R.S., Horton, D.R., Khattak, S.U.K., and White, L.D. 1990. Codling moth: male moth activity in response to pheromone lures and pheromone-baited traps at different elevations within and between trees. *Environmental Entomology* 19: 573-577.

Isankalova, N.M. 1972. Biology of *Mastrus*-sp., a parasite of the codling moth. *Samarkandskifi Gosudarstyen Universitet* 211: 96-99.

Krebs, J.R. and Davies, N.B. 1998. An Introduction to Behavioural Ecology. Blackwell Science Ltd., Oxford.

Kuhlman, U., and Mills, N.J. 1999. Exploring the biodiversity of Central Asia to assess specialized parasitoids for biological control of apple pests in Europe and North America. Integrated Plant Protection In Orchards. IOBC wprs Bulletin 22: 1-6.

Landolt, P.J., Hofstetter, R.W., and Biddick, L.L. 1999. Plant essential oils as arrestants and repellents for neonate larvae of the codling moth (Lepidoptera: Tortricidae). *Environmental Entomology* 28: 954-960.

Lewis, W.J., and Jones, R.L. 1971. Substance that stimulates host-seeking by *Microplitis croceipes* (Hymenoptera: Braconidae), a parasite of *Heliothis* species. *Annals ofthe Entomological Society of America* 64: 471-473.

Li, Y., Dickens, J.C., and Steiner, W.W.M. 1992. Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera: Braconidae) to cotton plant volatiles. *Journal of Chemical Ecology* 18: 1761-1773.

Makarov, E.M. 1983. The parasites of the codling moth in the mountain orchards of Alma Ata region and their use, pp. 117-126, *in* Publication of the Kazakh Institute of Plant Protection.

Mattiacci, L., Hutter, E., and Dorn, S. 1999. Host location of *Hyssopus pallidus*, a larval parasitoid of the codling moth, *Cydia pomonella*. *Biological Control* 15: 241-251.

Pedigo, L.P. 1999. Entomology and pest management. Prentice-Hall, Inc., New Jersey.

Roelofs, W.L., Comeau, A., Hill, A., and Milicevic, G. 1971. Sex attractant of the codling moth: Characterization with eletroantennogram technique. *Science (Washington)* 174:297-299.

Roitberg, B.R. and Lalonde, R.G. 1991. Host marking enhances parasitism risk for a fruit-infesting fly *Rhagoletis basiola*. *Oikos* 61: 389-393.

Stowe, M.K., Turlings, T.C.J., Loughrin, J.H., Lewis, W.J., and Tumlinson, J.H. 1995. The chemistry of eavesdropping, alarm, and deceit. *Proceedings of the National Academy of Sciences of the United States of America* 92: 23-28.

Summerland, S.A., and Steiner, L.F. 1943. Codling moth oviposition and fate of eggs. *Journal of Economic Entomology* 36: 72-75.

Takács, S., Gries, G., and Gries, R. 1997. Semiochemical-mediated location of host habitat by *Apantales carpatus* (Say) (Hymenoptera: Braconidae), a parasitoid of clothes moth larvae. *Journal of Chemical Ecology* 23: 459-472.

Thiery, D., Gabel, B., Farkas, P., and Jarry, M. 1995. Egg dispersion in codling moth: influence of egg extract and of its fatty acid constituents. *Journal of Chemical Ecology* 21: 2015-2026.

Tumlinson, J.H., Turlings, T.C.J., and Lewis, W.J. 1993. Semiochemically mediated foraging behavior in beneficial parasitic insects. *Archives of Insect Biochemistry and Physiology* 22: 385-391.

Turlings, T.C.J., Tumlinson, J.H., Eller, F.J., and Lewis, W.J. 1991. Larval-damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. *Entomologia Experimentalis et Applicata* 58: 75-82.

Unruh, T.R. 1997. From Russia with love: new predators and parasites for control of tree fruit insect pests. *Proceedings of the 93rd Annual Meeting of the Washington State Horticultural Association*. pp. 42-49.

Unruh, T.R. and Lacey, L.A. 2001. Control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), with *Steinernema carpocapsae*: Effects of supplemental wetting and pupation site on infection rate. *Biological Control* 20: 48-56.

Vet, L.E.M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*. 37: 141-172.

Vet, L.E.M., Lewis, W.J., and Carde, R.T. 1995. Parasitoid foraging and learning, pp. 65-101, *in* R.T. Carde and W.J. Bell (eds.). Chemical Ecology of Insects 2. Chapman and Hall, Toronto.

Vet, L.E.M., Wäckers, F.L., and Dicke, M. 1991. How to hunt for hiding hosts: The reliabilty-detecability problem in foraging parasitoids. *Netherlands Journal of Zoology* 41:202-213.

Vinson, S.B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21:109-133.

Vinson, S.B. 1981. Habitat location, pp. 51-78, *in* D.A. Nordlund, R.L. Jones, and W.J. Lewis (eds.). Semiochemicals: Their Role in Pest Control. Wiley, New York.

Vinson, S.B. 1984a. Parasitoid-host relationship, pp. 205-233, *in* W.J. Bell and R.T. Carde (eds.). Chemical Ecology of Insects. Sinauer Associates, Sunderland, Massachusetts.

Vinson, S.B. 1984b. How parasitoids locate their hosts: a case of insect espionage, pp. 325-348, *in* W.J. Lewis (ed.). Insect Communication. Academic Press, London.

Vinson, S.B. 1998. The general host selection behaviour of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control* 11:79-96.

Vinson, S.B. and Iwantsch, G.F. 1980. Host suitability for insect parasitoids. *Annual Review of Entomology* 25: 397-419.

Weissling, T.J., and Knight, A.L. 1994. Passive trap for monitoring codling moth (Lepidoptera: Tortricidae) flight activity. *Journal of Economic Entomology* 87: 103-107.

Wertheim, B., Vet, L.E.M., and Dicke, M. 2003. Increased risk of parasitism as ecological costs of using aggregation pheromones: laboratory and field study of *Drosophila-Leptopilina* interaction. *Oikos* 100: 269-282.

Weseloh, R.M. 1976. Behavioural response of the parasite, *Apanteles melanoscelus*, to gypsy moth silk. *Environmental Entomology* 5: 1128-1132.

Weseloh, R.M. 1977. Effects of behavior of *Apantales melanoscelus* females caused by modifications in extraction, storage, and presentation of gypsy moth silk kairomone. *Journal of Chemical Ecology* 3: 723-735.

Weseloh, R.M. 1981. Host location by parasitoids, pp. 79-95, *in* D.A. Nordlund, R.L. Jones, and W.J. Lewis (eds.). Semiochemicals: their Role in Pest Control. Wiley, New York.

Wiskerke, J.S.C., Dicke, M., and Vet, L.E.M. 1993. Larval parasitoid uses aggregation pheromone of adult hosts in foraging behaviour: a solution to the reliability-detectability problem. *Oecologia* 93: 145-148.

Zar, J.H. 1996. Biostatistical Analysis. Prentice-Hall, Inc., New Jersey.