SEX PHEROMONE OF THE APPLE BLOTCH LEAFMINER, *Phyllonorycter crataegella* (Clemens) (Lepidoptera: Gracillariidae) AND ITS EFFECT ON *P. mespilella* (Hübner) PHEROMONE COMMUNICATION

by

Patricia N. Ferrao

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Name: **PATRICIA FERRAO**

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Examining Committee:

Chair: Dr. L. Albright, Professor.

Dr. G. Gries, Assistant Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. J. Borden, Professor
Department of Biological Sciences, SFU

Dr. G. Judd, Research Scientist
Agriculture Canada

Dr. S. Fitzpatrick, Research Scientist
Agriculture Canada, Vancouver, B.C.
Public Examiner

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Title of Thesis/Project/Extended Essay

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Phyllonorycter crataegella (Clemens) (Lepidoptera:

Gracillariidae) AND ITS EFFECT ON P. mespilella

(Hübner) PHEROMONE COMMUNICATION

Author:
(signature)

(name)

AUGUST 6, 1996
(date)

ABSTRACT

*Z*10,*Z*12-tetradecadienyl acetate (*Z*10,*Z*12-14:OAc) and *E*10,*E*12-tetradecadienyl acetate (*E*10,*E*12-14:OAc) are sex pheromone components of the apple blotch leafminer (ABLM), *Phyllonorycter crataegella* (Clemens). Compounds extracted from female pheromone glands were identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses on 3 fused silica columns coated with DB-5, DB-23 or DB-210, retention index calculations of EAD-active compounds, and by comparative GC-EAD analyses of female ABLM-produced and authentic (synthetic) compounds. In field trapping experiments in Connecticut, *Z*10,*Z*12-14:OAc alone attracted ABLM males. Addition of *E*10,*E*12-14:OAc to *Z*10,*Z*12-14:OAc at 0.1:10 or 1:10 ratios enhanced attractiveness of the lure, whereas geometrical isomers *E*10,*Z*12- or *Z*10,*E*12-14:OAc at equivalent ratios were behaviorally benign. In field trapping experiments in British Columbia, *Z*10,*Z*12-14:OAc plus *E*10,*E*12-14:OAc did not attract any *Phyllonorycter* moths, confirming that ABLM is not present in the fruit growing regions of British Columbia. *Z*10,*Z*12-14:OAc added to *P. mespilella* pheromone *E*4,*E*10-dodecadienyl acetate strongly inhibited response by *P. mespilella* males. Recognition of the ABLM pheromone blend by allopatric *P. mespilella* males suggests a phylogenetic relationship and previous sympatry of these two *Phyllonorycter* spp. If pheromonal attraction of ABLM males were reciprocally inhibited by *P. mespilella* pheromone, a generic *Phyllonorycter* pheromone blend could be tested for pheromone-based mating disruption of the apple-leafmining *Phyllonorycter* guild in North America.

ACKNOWLEDGMENTS

This research would not have been possible without the support, advice and assistance of many people. I am especially grateful to

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- Dr. Chris Maier, for providing me with the insects and carrying out all the field experiments in Connecticut.
- Mr. Marc Rhainds, for his assistance in the experimental setup in Summerland.

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1.0 INTRODUCTION

1.1 General Importance of Leafminer Moths

Leafminer moths of economic importance have world wide distributions (Table 1). With the onset of insecticide resistance some leafminer moths have emerged as serious crop pests. In North America, leafminer moths of economic importance in apple orchards (Table 2) are limited mostly to the family Gracillariidae (Brown, 1990), with the apple blotch leafminer (ABLM), *P. crataegella* (Clemens), and the spotted tentiform leafminer, *P. blancardella* (F.), being particularly important.

1.2 Biology of *P. crataegella*

The ABLM, is a major pest of apple, *Malus domestica* Borkh., in New England and Eastern New York. In New England, larvae mine in at least 17 hosts of 7 plant genera in the family Rosaceae. The density of mines is highest in *M. domestica* (Maier, 1985).

ABLM is trivoltine. Adults emerge in early spring from pupae present in leaves from the previous season. Each generation has five larval instars which spend their entire life within one mine. Sap feeding first to third instars with dorsoventrally flattened heads make blotches on the underside of leaves. Fourth instar larvae undergo hypermetamorphosis to acquire characteristic lepidopteran larval morphology. Tissue feeding fourth and fifth instars chew holes in the roof of the mine and cause visible spots on the upper side of leaves. Larvae pupate within the mine and pupae force their head region through the bottom of the mine possibly to facilitate adult emergence.

Table 1 Leafminer moths of economic importance worldwide.

Species	**Family**	**Host**	**Geographic Distribution**	**References**
Phyllocnistis citrella, Citrus leafminer	Gracillariidae : Phyllocnistinae	citrus	Southern Asia, Australia, East Africa, Southern Florida (U.S.A)	Heppner (1993)
Phyllonorycter blancardella, Spotted tentiform leafminer	Gracillariidae	apple	Central Europe, North America	Brown (1990), Maier & Davis (1989)
Phyllonorycter ringoniella	Gracillariidae	apple and pear	Japan	Sekita and Yamada (1979)
Lyonetia clerkella, Peach leafminer	Lyonetiidae	peach, apple and pear	Japan, Central Asia and Europe	Sekita and Yamada (1979)
Lyonetia prunifoliella subsp. malinella	Lyonetiidae	apple and pear	Japan	Sekita and Yamada (1979)
Leucoptera scitella	Lyonetiidae	apple and pear	Europe and Central Asia	Sekita and Yamada (1979)
Leucoptera coffeella, Coffee leafminer	Lyonetiidae	coffee	Brazil to Mexico	Sanchez Deleon (1984)

Table 2 Geographical distribution of economically important leafminer moths on apple in North America.

Species	**Geographic distribution**	**References**
Phyllonorycter blancardella, Spotted tentiform leafminer	Eastern North America (Western New York, Champlain Valley and Vermont, Ontario, Quebec)	Weires et al., (1980), Brown (1990)
Phyllonorycter crataegella, Apple blotch leafminer	Eastern North America (Massachusetts, Connecticut and the area east of the Hudson River in New York State)	Weires et al., (1980), Brown (1990)
Phyllonorycter mespilella Tentiform leafminer	Western North America (The Okanagan and Similkameen Valleys, British Columbia)	Cossentine and Jensen (1992)
Phyllonorycter elamaella, Western tentiform leafminer	Western North America (Washington and Oregon)	Barrett and Brunner (1990)
Lyonetia speculella	Mid-Atlantic States of North America	Brown (1989)

Leafminers of the first and second generation develop from egg to adult in 1.25 - 2 months. Third generation specimens require nearly 9 months to complete development because pupae enter diapause in autumn and remain in larval mines until spring (Maier, 1981).

Diel ABLM flight shows 2 distinct 3-4 hour activity periods (Green and Prokopy, 1984). The first commences at sunrise and involves males foraging for females. Lower wind speeds early in the morning may facilitate orientation toward and location of pheromone-emitting females. The second flight before sunset, involves gravid females foraging for oviposition sites. Throughout the season, females preferentially oviposit on the mid-section of leaves within the interior tree canopy, and in the lower tree canopy during the first generation (Beckham et al., 1950; Green and Prokopy, 1991). This oviposition preference may have evolved in response to little wind interference with oviposition and a reduced tendency for interior leaves to abscise prior to completion of larval development (Bultman and Faeth, 1986).

1.3 Effect of Leaf Mining *P. crataegella* Larvae on Host Trees

Gracillariid leafminers are known to destroy photosynthetically-active tissue of apple leaves (Dutcher and Howitt, 1978; Maier, 1981). Both sap feeding first to third instars and tissue feeding fourth and fifth instars of *Phyllonorycter* spp. injure leaves, but the latter inflict the most damage (Maier, 1981). Large infestations of various apple leafminers can cause an early abscission of leaves and fruit, decrease in branch and fruit growth or reduction in fruit set the following year (Hill and Hough 1957, Kremer 1963, and Pottinger and LeRoux, 1971). Based on quantitative analyses, ABLM larval densities >1.6 - 2.6 mines per leaf in the second generation can be associated with premature fruit drop and reduced fruit set, size and production (Reissig et al., 1982). Injury from apple leafminers may cause early abscission of leaves when 20 percent of the leaf surface (>5 mines per leaf) is destroyed (Maier et al., 1983). First generation ABLM larvae have not been shown to cause economic losses, but mine densities >0.13 -0.25 per leaf may result in injurious second generation densities if not controlled (Coli and Prokopy, 1982).

1.4 Monitoring and Control of *P. crataegella* and Role of Natural Enemies

Until recently, ABLM was considered a minor pest. Since 1973 however, numerous serious outbreaks have occurred (Johnson et al., 1976; Maier, 1981; Coli and Prokopy, 1982) largely due to resistance against organophosphate and carbamate insecticides used for ABLM control (Weires, 1977; Coli and Prokopy, 1982).

To monitor ABLM emergence and flight activity before bloom, red-painted, Tangle trap^R covered cardboard traps (1 per 2-3 acres) are suspended at chest height from apple trees (Green and Prokopy, 1986). Control is advised when at the pink stage of bud development ≥13 ABLM are captured. Growers then apply Thiodan or synthetic pyrethroids, such as Ambush, Pounce and Pydrin, to control the spring generation of ABLM adults. If control of the spring generation ABLM is not carried out Vydate (extremely toxic compound to humans if inhaled) and Lannate are applied in midsummer against larvae and adults (Coli and Prokopy, 1982). ABLM control prior to bloom is encouraged in order to achieve season long control with little leaf injury, reduce pesticide-associated phytotoxicity and to preserve the principle mite predator, *Amblyseius* *fallacis* (Qarman) (Green et al., 1985) which is highly susceptible to most ABLM insecticides.

Applications of insecticides are detrimental to natural ABLM enemies which are important in many existing and developing Integrated Pest Management (IPM) programs for apple pests. Decisions to apply insecticides are usually based on the density of larval mines or on adult counts, without considering the abundance or future impact of parasites. Continuous and sometimes unjustified use of insecticides prevents parasitoids from expressing their full potential for ABLM control. Apple leafminers are excellent candidates for biological control by parasitic wasps. Sedentary larvae are easily located by searching parasitoids, and at low densities cause no excessive damage (Maier, 1990). In the absence of insecticides, parasitic wasps appear to be principal regulators of *Phyllonorycter* spp. during spring and summer (Askew and Shaw, 1979; Maier, 1988). In Connecticut, ABLM parasitism was higher in unsprayed than sprayed orchards (Maier, 1982).

ABLM is parasitized by at least 16 species of parasitic wasps. Female parasitoids oviposit eggs singly near or into ABLM larvae within mines. After hatching, wasp larvae feed externally or internally on tissue feeders causing their death (Maier, 1982; Maier, 1984). Among the diverse parasitic guild that attacks ABLM larvae, native *Sympiesis marylandensis* (Girault) (Eulophidae) is most abundant in commercial orchards in Connecticut (Maier, 1988). Its potential to regulate leafminer populations is, however, limited due to extensive use of insecticides (Maier, 1990).

1.5 Pheromone-Based Control of Lepidopteran Pests

Identification and synthesis of lepidopteran female sex pheromones has provoked interest in their use as trap lures and mating disruptants. Pheromone-based ABLM sampling would greatly help to determine the presence, distribution and density of ABLM populations. Pheromone-mediated mating disruption already successfully controls lepidopteran pests, such as pink bollworm, *Pectinophora gossypiella* (Saunders), oriental fruit moth, *Grapholita molesta* (Busk), European grape berry moth, *Eupoecilia ambiguella* (Hb.), fruit tortrix, *Adoxophyes orana* (Fisher Von Roslerstamm), tea tortrix, *Homona magnanima* (Diakonoff), smaller tea tortrix, *Adoxophyes sp.*, codling moth, *Cydia pomonella* (L.), tomato pinworm, *Keiferia lycopersicella* (Walsingham) and western pine shoot borer, *Eucosma sonomana* (Kearfott) (Cardé and Minks, 1995), and may also be a viable management alternative for ABLM populations.

Although highest efficacy in mating disruption is achieved when the disruptants closely resembles the pheromone blend emitted by the female moths (Roelofs, 1978; Mink and Cardé, 1988), release of pheromone components from sympatric, co-seasonal moths may also result in successful disorientation of mate seeking males. For example, 2% of *E*11-tetradecenyl acetate (*E*11-14:OAc), a synergistic pheromone component in the fruit-tree leaf roller, *Archips argyrospilus* (Walker) (Roelofs et al., 1974), added to pheromone blend of the European leaf roller, *Archips rosanus* (L.), disrupt the response of *A. rosanus* males (Vakenti et al., 1988). Such use of pheromone components from congeners may also be applicable for the control of ABLM populations.

1.1 Objectives

The research objectives of this study therefore were to:

1. identify and field test the female ABLM sex pheromone blend, and
2. determine interspecific effects of the ABLM sex pheromone on congeneric *P. mespilella* (Hübner).

2.0 MATERIALS AND METHODS

2.1 Experimental Insects

In April 1994, leaves infested with overwintering ABLM pupae were collected by Dr. Chris Maier[1] from Connecticut apple orchards and sent to Simon Fraser University. Male and female pupae were removed from leaves and kept separately in filter-paper lined Petri dishes at 20°C and a photoperiod of 14:10 (Light:Dark).

2.2 Analysis of *P. crataegella* Pheromone

At the onset of the photophase, during peak calling activity, pheromone glands of 1-2 day old virgin females were removed and extracted for about 5 minutes in hexane. Aliquots of 1-2 female equivalents (FE) of pheromone extract were subjected to gas chromatographic-electroantennographic detection (GC-EAD) analyses (Arn et al., 1975) on three fused silica columns (30m X 0.25mm ID) coated with DB-5, DB-210 or DB-23 (J&W Scientific, Folsom, California, 95630). For Kovats retention index calculations (Kovats, 1965) of EAD-active compounds, standards of synthetic straight C_{14}- C_{23} hydrocarbons were chromatographed under identical conditions. To determine double bond positions in candidate pheromone components, series of synthetic *E*3- to *E*12- and *Z*3- to *Z*12-tetradecenyl acetates plus Δ13-tetradecenyl acetate were analyzed by GC-EAD.

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[1] Department of Entomology, The Connecticut Agricultural Experimental Station, New Haven, Connecticut.

2.3 Syntheses of Candidate Pheromone Components

Syntheses of Z10,Z12-14:OAc was conducted by Dr. Jianxiong Li and Mr. P.D.C. Wimalaratne[2]. Geometrical isomers of Z10,Z12-14:OAc were synthesized by Mr. P.D.C. Wimalaratne. Field-tested compounds were >97% chemically and ≥98% geometrically pure. Syntheses of these compounds will be reported elsewhere.

2.4 Field Experiments

2.4.1 Test of Candidate *P. crataegella* Pheromone Components

Eight field experiments were conducted from May-August 1995 in apple orchards in Middlesex County, Connecticut. Wing traps (Pherocon 1C. Trècè Corp. Salinas, California) were suspended 1.5 - 2 m above ground 1 m apart from the main trunk in randomized complete blocks with traps and blocks at 20 m intervals. Traps were baited with gray rubber septa (Aldrich Chem. Comp., Milwaukee, Wisconsin 53233) impregnated with candidate pheromone components in HPLC grade hexane. The first experiment tested *Z*10,*Z*12-14:OAc (10 µg) and *E*10-12:OAc (10 µg) singly and in combinations at 10:1 and 10:0.1 ratios. The second experiment tested Z10,Z12-14:OAc at increasing doses of 0.01, 0.1, 1 and 10 µg. Experiment 3 tested attractiveness of Z10,*Z*12-14:OAc (10 µg) singly and in quartenary combinations with *E*10,*E*12-14:OAc, Z10,*E*12-14:OAc and *E*10,Z12-14:OAc at 10:0.1, 10:1 and 1:1 ratios. Experiment 4 tested Z10,*Z*12-14:OAc (10 µg) singly and in all ternary and quartenary combinations with *E*10,*E*12-14:OAc, Z10,*E*12-14:OAc and *E*10,Z12-14:OAc at 1 µg each.

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[2] Department of Biology and Chemistry, respectively, Simon Fraser University, Burnaby, British Columbia V3J 1C5.

Experiment 5 tested *Z*10,*Z*12-14:OAc (10 μg) singly and in all binary and quartenary combinations with *E*10,*E*12-14:OAc, *E*10,*Z*12-14:OAc, and *Z*10,*E*12-14:OAc at 1 μg each.

In experiments 1-4, captured ABLM males were counted 24 hours after trap placement. In experiment 5, trap positions were rerandomized after 24 hours and captured males counted before 24 hours and 48 hours after experiment initiation.

Statistical Analysis were conducted with SAS statistical package (SAS Institute Inc., Cary, North Carolina 27513). To ensure homogeneity of variance, data in experiment 4 were transformed by taking their square root and subjected to analysis of variance (ANOVA) followed by a Student-Newman-Keuls' means separation procedure (α=0.05). Despite transformation, data from all other field experiments were not normally distributed and were therefore, subjected to non parametric analysis of variance by ranks (Friedman's test) (Zar, 1984; SAS/STAT User's guide, 1988, release 6.03 edition, SAS Institute Inc., Cary, NC 27513).

2.4.2 Effect of *P. crataegella* Pheromone Components on *P. mespilella* Pheromone Communication

Three field experiments were conducted in September 1995 in the Okanagan Valley, British Columbia. Delta milk carton traps (Gray, 1984) were suspended from apple trees 1.5 - 2 m above ground 1 m apart from the main trunk in randomized complete blocks with traps and blocks at 20 m and 40 m intervals, respectively. Traps were baited with gray rubber septa impregnated with candidate pheromone components in HPLC grade hexane. The first experiment (1a) tested *P. crataegella* pheromone blend

*Z*10,*Z*12-14:OAc (10 µg) plus *E*10,*E*12-14:OAc (1 µg) versus *P. mespilella* pheromone component, *E*4,*E*10-12:OAc (10 µg) (Gries et al., 1993). The second experiment (2a) tested *E*4,*E*10-12:OAc (10 µg) singly and in ternary combinations with *Z*10,*Z*12-14:OAc and *E*10,*E*12-14:OAc at ratios of 10:10:1, 10:1:0.1 and 10:0.1:0.01 (µg:µg:µg). Experiment 3a tested *E*4,*E*10-12:OAc (10 µg) singly and in binary and ternary combinations with *Z*10,*Z*12-14:OAc (10 µg) and *E*10,*E*12-14:OAc (1 or 10 µg).

In all three experiments captured *P. mespilella* males were counted 24 hours after trap placement.

Despite transformation, data of all 3 field experiments were not normally distributed and were therefore subjected to non parametric analysis of variance by ranks (Friedman's test) (Zar, 1984; SAS/STAT User's Guide, 1988, release 6.03 edition, SAS Institute Inc., Cary, NC 27513).

3.0 RESULTS

3.1 Analysis of *P. crataegella* pheromone

GC-EAD analysis of female ABLM pheromone extracts revealed 6 antennal responses with FID-detectable compound 5 being most EAD-active (Fig. 1). Based on its retention index 1870 and previously reported *Phyllonorycter* sex attractants the first EAD-active compound was hypothesized to be *E*10-dodecenyl acetate (*E*10-12:OAc). Retention indices of EAD-active compounds 3-6 were indicative of isomeric, conjugated tetradecadienyl acetates. In GC-EAD analyses of monoene *E* or *Z* tetradecenyl acetates (Fig. 2), *Z*10- and *Z*12-tetradecenyl acetates (Fig. 3) and *E*4-, *E*10- and *E*12-tetradecenyl acetates (Fig. 4) elicited the strongest antennal responses. Double bond positions in compounds 3-6 were therefore hypothesized to be at C_{10} and C_{12}. Because geometrical isomers of homologous 8,10-dodecadienyl acetate eluted in the order of *Z*8,*E*10-, *E*8,*E*10-, *Z*8,*Z*10- and *E*8,*Z*10-dodecadienyl acetate, EAD-active compounds 3-6 were hypothesized to be corresponding *Z*10,*E*12-, *E*10,*E*12-, *Z*10,*Z*12- and *E*10,*Z*12-tetradecadienyl acetates, respectively (Fig. 5). Identical retention times of synthetic and female-produced compounds 1 and 3-6 on the DB-210 column, confirmed structural assignments of the candidate pheromone components.

Fig. 1 Flame ionization detector (FID) and electroantennographic detector (EAD: male *P. crataegella* antenna) responses to one female equivalent (FE) of *P. crataegella* pheromone gland extract chromatographed on a DB-210 column (70°C for 1 min, 20°C/min to 140°C, 2°C/min to 220°C). RI = Retention Index.

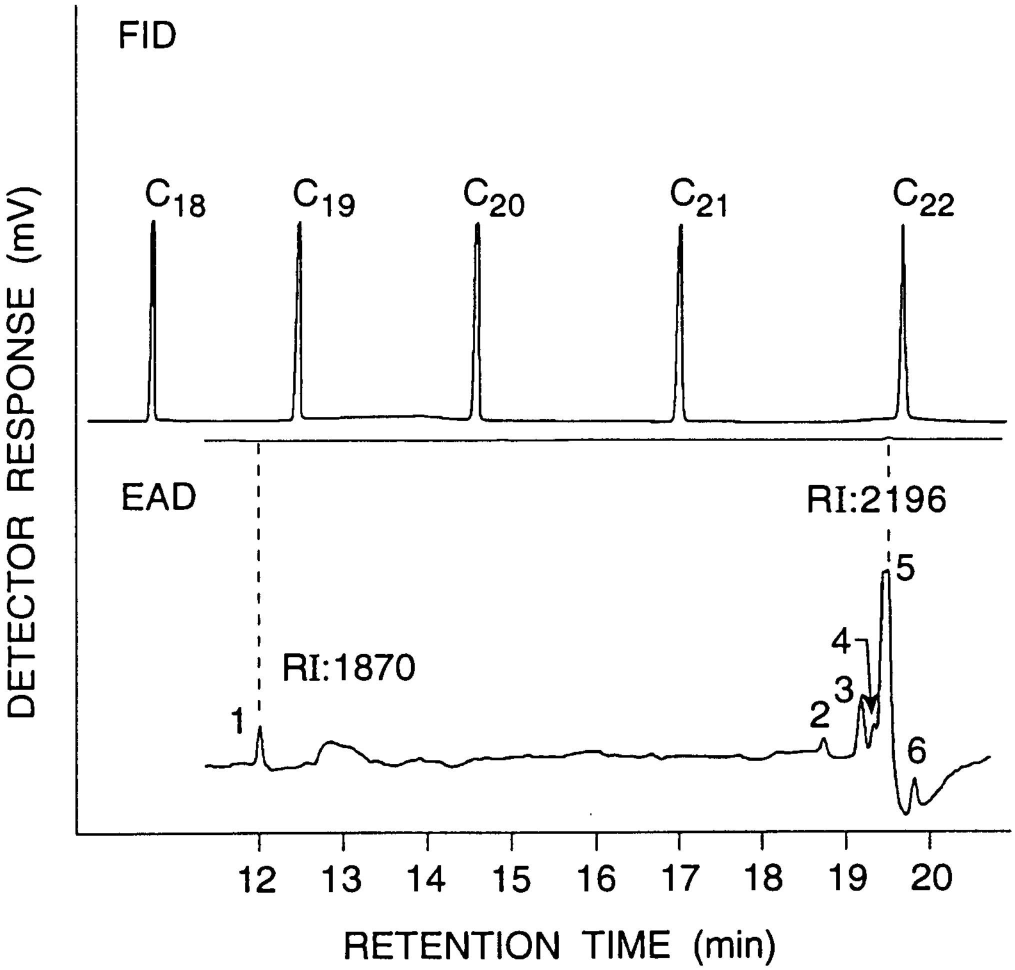
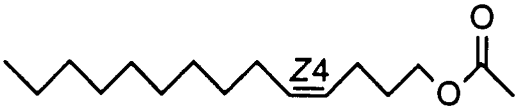
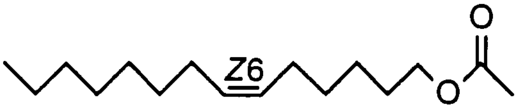

FID
C18
C19
C20
C21
C22
EAD
RI:2196
5
4
3
2
RI:1870
1
6
DETECTOR RESPONSE (mV)
12
13
14
15
16
17
18
19
20
RETENTION TIME (min)

Fig. 2 Four series of synthetic, tetradecenyl acetates used in GC-EAD analyses (Figure 3,4) to determine double bond positions of the candidate diene pheromone component **5** in female *P. crataegella* pheromone gland extract (Figure 1).


Z4
O
O

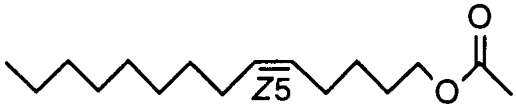

Z6
O
O

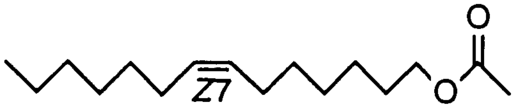

Z8
O
O


Z10
O
O


Z12
O
O


Z3
O
O

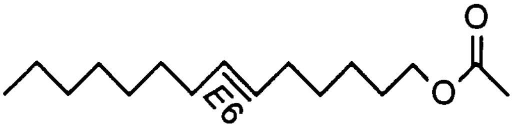

Z5
O
O

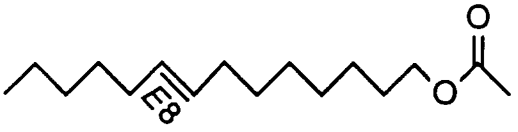

Z7
O
O


Z9
O
O


Z11
O
O


E4
O
O


E6
O
O

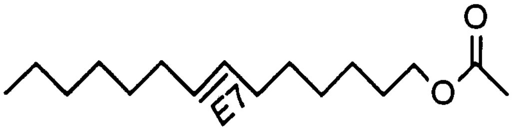

E8
O
O


E10
O
O


E12
O
O


E3
O
O


E5
O
O


E7
O
O


E9
O
O


E11
O
O

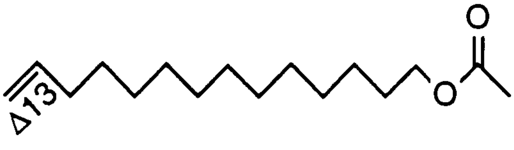

Δ13
O
O

Fig. 3 Flame ionization detector (FID) and electroantennographic detector (EAD: male *P. crataegella* antenna) responses to synthetic tetradecenyl acetates chromatographed on a DB-23 column (50°C for 1 min, 20°C/min to 100°C, 5°C/min to 200°C).

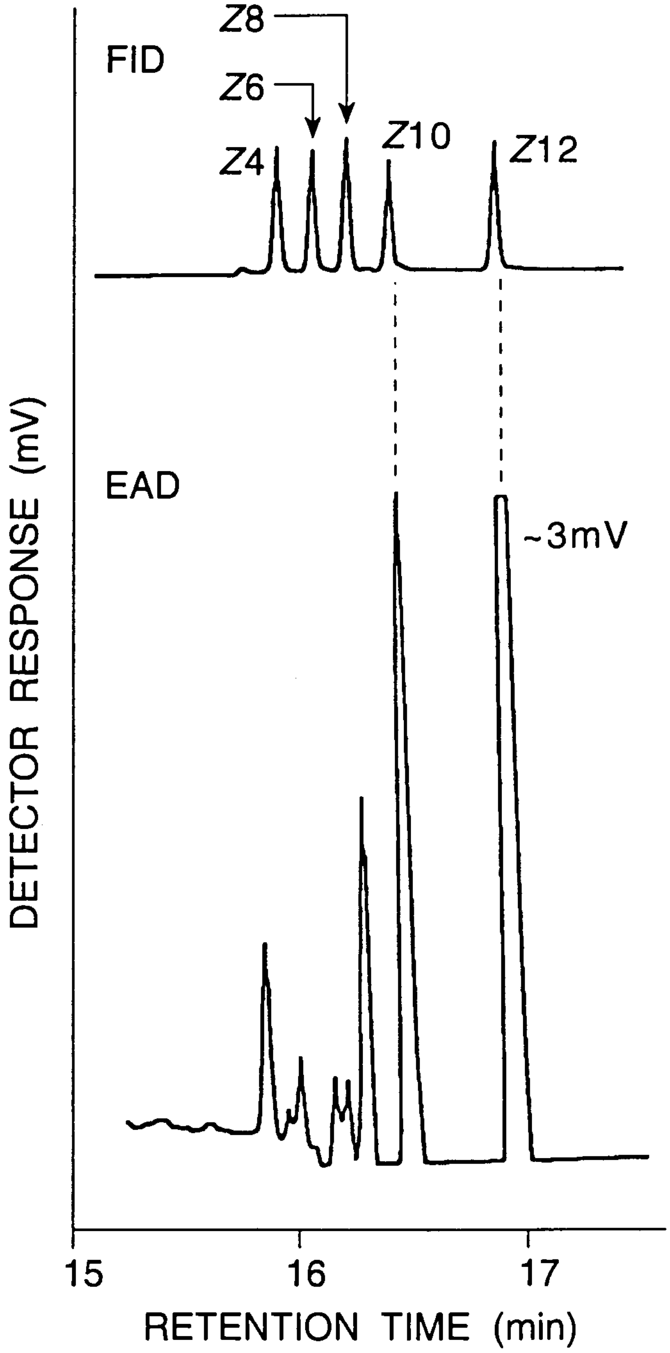

FID
Z8
Z6
Z4
Z10
Z12
EAD
~3mV
DETECTOR RESPONSE (mV)
15
16
17
RETENTION TIME (min)

Fig. 4 Flame ionization detector (FID) and electroantennographic detector (EAD: male *P. crataegella* antenna) responses to synthetic tetradecenyl acetates. Chromatography as in figure 3.

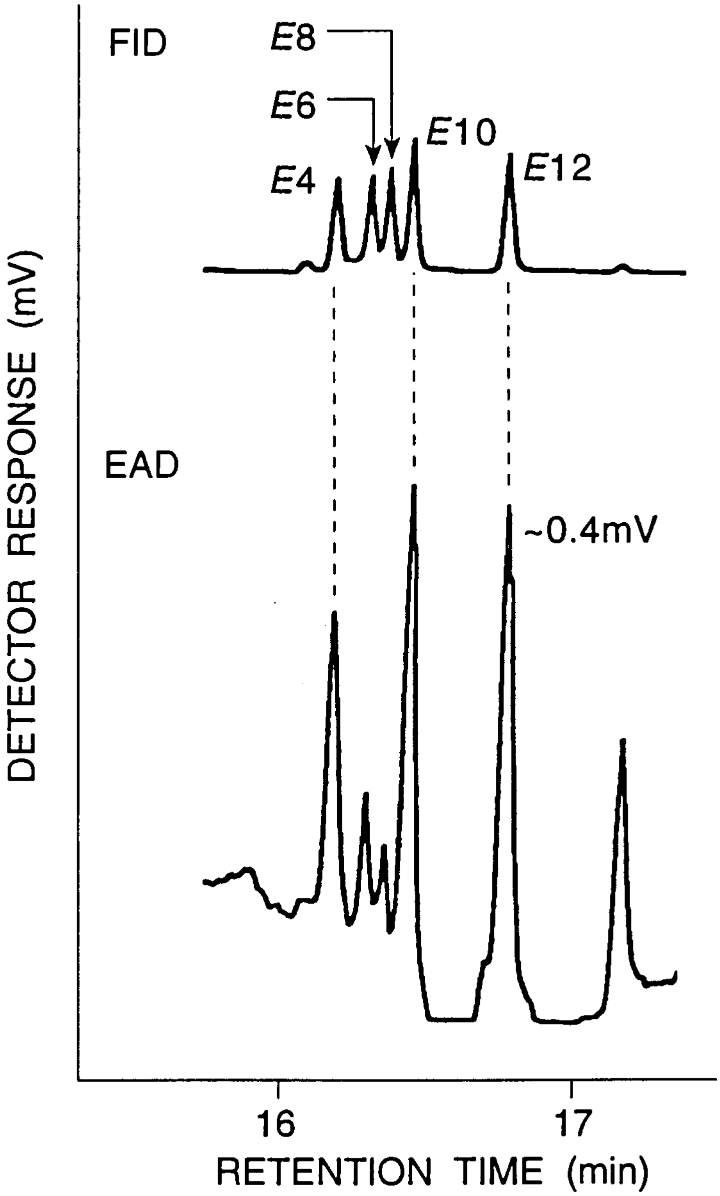
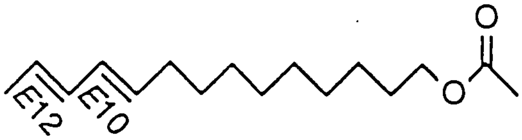
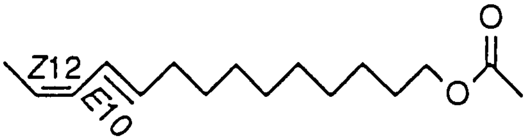

FID
E8
E6
E10
E4
E12
EAD
~0.4mV
DETECTOR RESPONSE (mV)
16
17
RETENTION TIME (min)

Fig. 5 Geometrical isomers of 10,12-tetradecadienyl acetate.

*E*10,*E*12-14:OAc

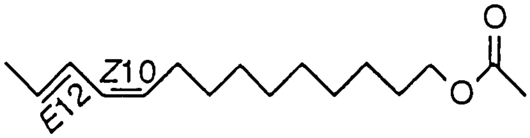


*E*10,*Z*12-14:OAc



*Z*10,*Z*12-14:OAc

*Z*10,*E*12-14:OAc



3.2 Field Experiments

3.2.1 Test of Candidate *P. crataegella* Pheromone Components

Major candidate pheromone component *Z*10,*Z*12-14:OAc at 10 µg was highly attractive to ABLM males (Exp.1, Fig. 6), whereas *E*10-12:OAc was behaviorally benign. Addition of *E*10-12:OAc to *Z*10,*Z*12-14:OAc neither enhanced or reduced attractiveness of the lure (Exp.1, Fig. 6). At least a ten micrograms of *Z*10,*Z*12-14:OAc were required to attract ABLM males (Exp. 2, Fig. 7). *Z*10,*Z*12-14:OAc (10 µg) in quartenary combinations with all 3 geometrical isomers at 1 or 0.1 µg each was significantly more attractive than by itself (Exp. 3, Fig. 8). Testing *Z*10,*Z*12-14:OAc (10 µg) singly and in all ternary (Exp. 4, Fig. 9), binary (Exp. 5, Fig. 10) and quartenary combinations (Exp. 4,5 Figs. 9,10) with geometrical isomers revealed synergistic behavioral activity of *E*10,*E*12-14:OAc.

3.2.2 Effect of *P. crataegella* Sex Pheromone on *P. mespilella* Pheromone Communication

In the Okanagan Valley, *P. mespilella* pheromone component *E*4,*E*10-12:OAc (10 µg) was highly attractive to *P. mespilella* males, whereas ABLM pheromone blend *Z*10,*Z*12-14:OAc (10 µg) plus *E*10,*E*12-14:OAc (1 µg) was not (Exp. 1a, Fig. 11). Attractiveness of *E*4,*E*10-12:OAc (10 µg) was greatly reduced by the addition of *Z*10,*Z*12-14:OAc (1 µg) and *E*10,*E*12-14:OAc (0.1 µg) (Exp. 2a, Fig. 12). *Z*10,*Z*12-14:OAc (10 µg) was significantly more effective than *E*10,*E*12-14:OAc (10 µg) in reducing attraction of *P. mespilella* males to *E*4,*E*10-12:OAc (10 µg) (Exp. 3a, Fig. 13). *Z*10,*Z*12-14:OAc (10 µg) singly or in combination with *E*10,*E*12-14:OAc (1 µg) were equally inhibitory (Exps. 3a, Fig. 13)

Fig. 6 Mean (+SE) numbers of *P. crataegella* males captured in wing traps (Exp. 1) baited with $Z10,Z12$-14:OAc and $E10$-12:OAc singly at 10 μg and in combinations at 10:1 and 10:0.1 ratios. Middlesex County, Connecticut, May 3-5, 1995; N=10. Bars with the same superscript are not significantly different, $P<0.05$.

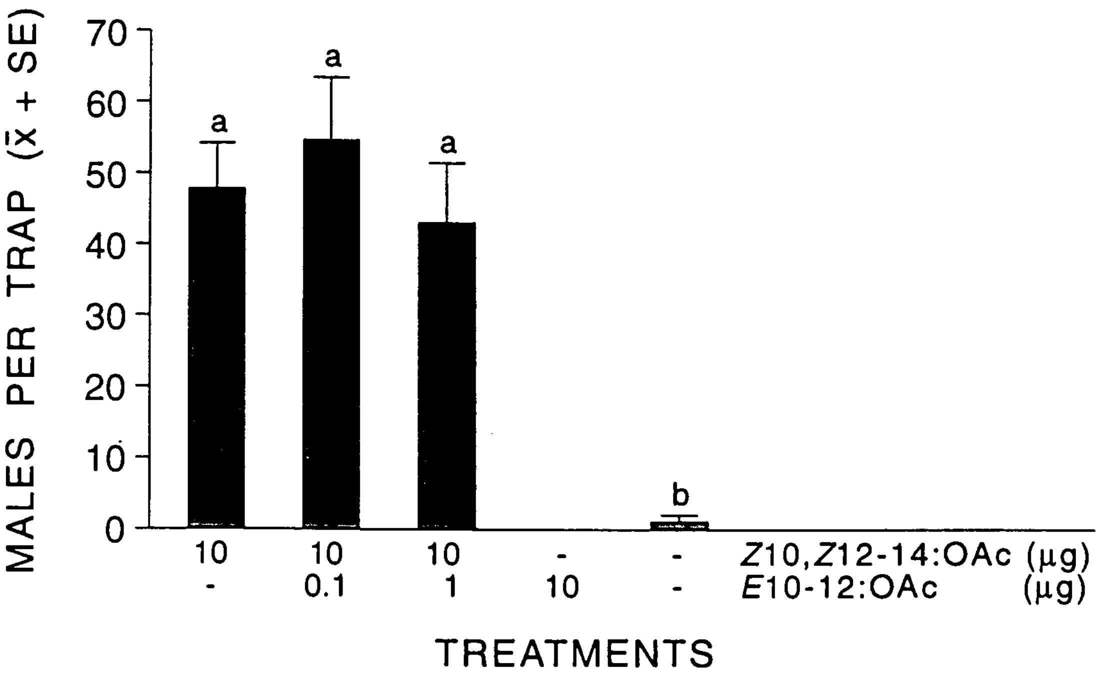

MALES PER TRAP ($\bar{x}$ + SE)
70
60
50
40
30
20
10
0
a
a
a
b
10 10 10 - - Z10,Z12-14:OAc (µg)
- 0.1 1 10 - E10-12:OAc (µg)
TREATMENTS

Fig. 7 Dose-dependent attraction of *P. crataegella* males to wing traps (Exp. 2) baited with Z10,Z12-14:OAc at 0.01, 0.1, 1 and 10 µg. Middlesex County, Connecticut, May 9-12, 1995; N=10. Bars with the same superscript are not significantly different, $P<0.05$.

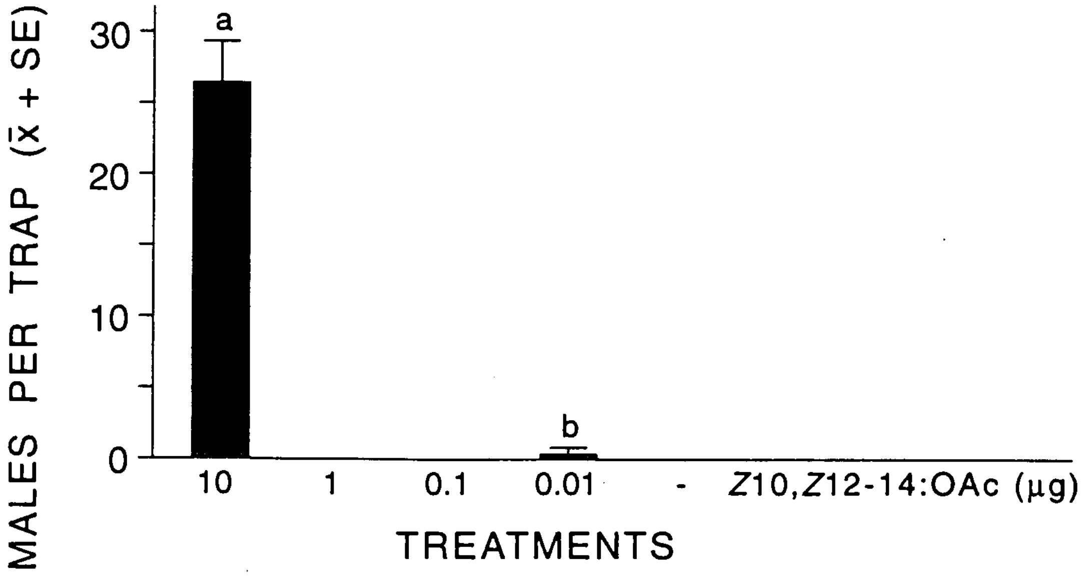

MALES PER TRAP ($\bar{x}$ + SE)
30
20
10
0
a
b
10
1
0.1
0.01
-
Z10,Z12-14:OAc (µg)
TREATMENTS

Fig. 8 Mean (+SE) numbers of *P. crataegella* males captured in wing traps (Exp. 3) baited with $Z10,Z12$-14:OAc singly at 10 µg and in quartenary combinations with $E10,E12$-14:OAc, $E10,Z12$-14:OAc and $Z10,E12$-14:OAc at a 10:0.1, 10:1 and 10:10 ratios. Middlesex County, Connecticut, August 8-9, 1995; N=10. Bars with the same superscript are not significantly different, $P<0.05$.

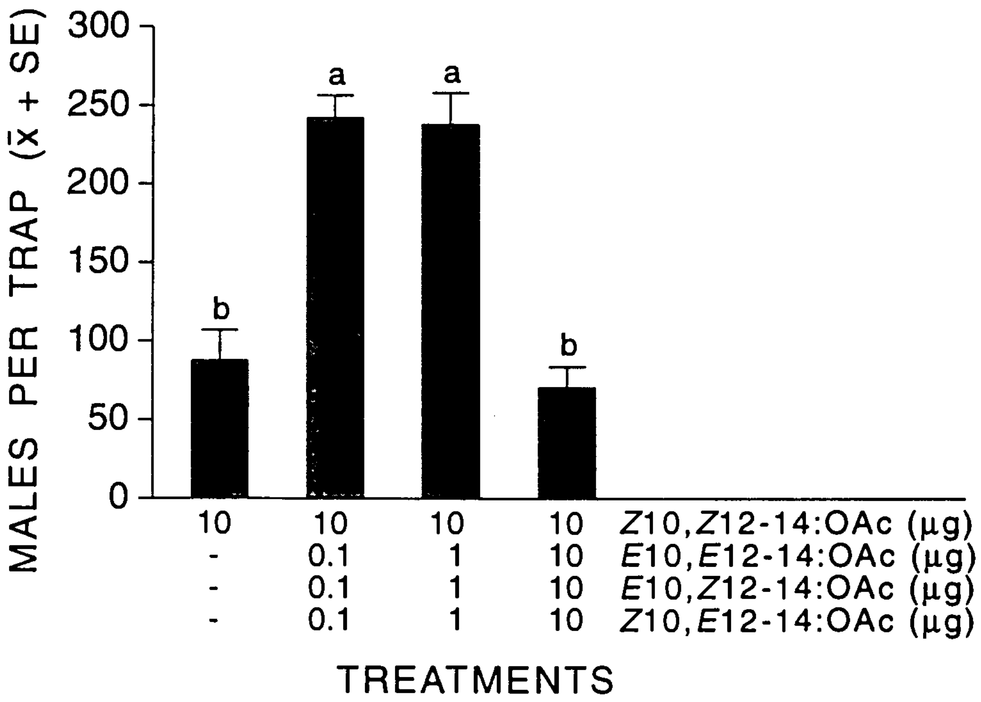

MALES PER TRAP ($\bar{x}$ + SE)
300
250
200
150
100
50
0
b
a
a
b
10 10 10 10 Z10,Z12-14:OAc (µg)
- 0.1 1 10 E10,E12-14:OAc (µg)
- 0.1 1 10 E10,Z12-14:OAc (µg)
- 0.1 1 10 Z10,E12-14:OAc (µg)
TREATMENTS

Fig. 9 Mean (+SE) numbers of *P. crataegella* males captured in wing traps (Exp. 4) baited with *Z*10,*Z*12-14:OAc singly at 10 µg and in ternary and quartenary combinations with *E*10,*E*12-14:OAc, *E*10,*Z*12-14:OAc and *Z*10,*E*12-14:OAc at 1 µg each. Middlesex County, Connecticut, August 23-24, 1995; N=8. Bars with the same superscript are not significantly different, $P<0.05$.

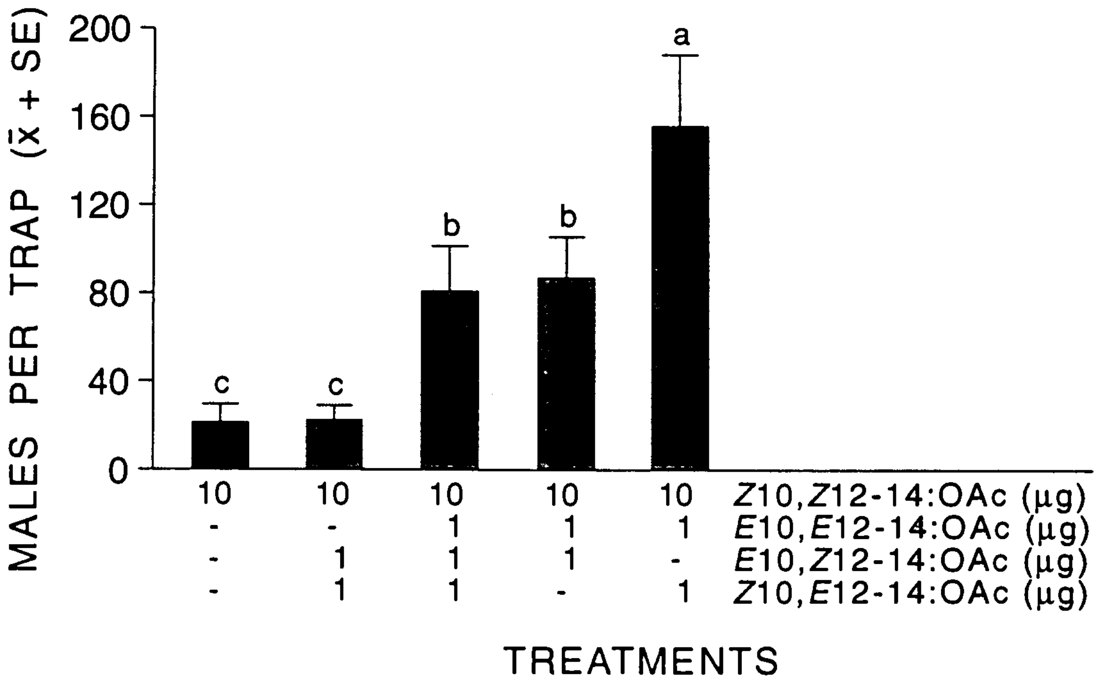

MALES PER TRAP ($\bar{x}$ + SE)
200
160
120
80
40
0
c
c
b
b
a
10 10 10 10 10 $Z10,Z12$-14:OAc (µg)
- - 1 1 1 $E10,E12$-14:OAc (µg)
- 1 1 1 - $E10,Z12$-14:OAc (µg)
- 1 1 - 1 $Z10,E12$-14:OAc (µg)
TREATMENTS

Fig. 10 Mean (+SE) numbers of *P. crataegella* males captured in wing traps (Exp. 5) baited with *Z*10,*Z*12-14:OAc singly at 10 μg and in binary and quartenary combinations with *E*10,*E*12-14:OAc, *E*10,*Z*12-14:OAc and *Z*10,*E*12-14:OAc at 1 μg each. Middlesex County, Connecticut, August 28-29,1995; N=16. Bars with the same superscript are not significantly different, *P*<0.05.

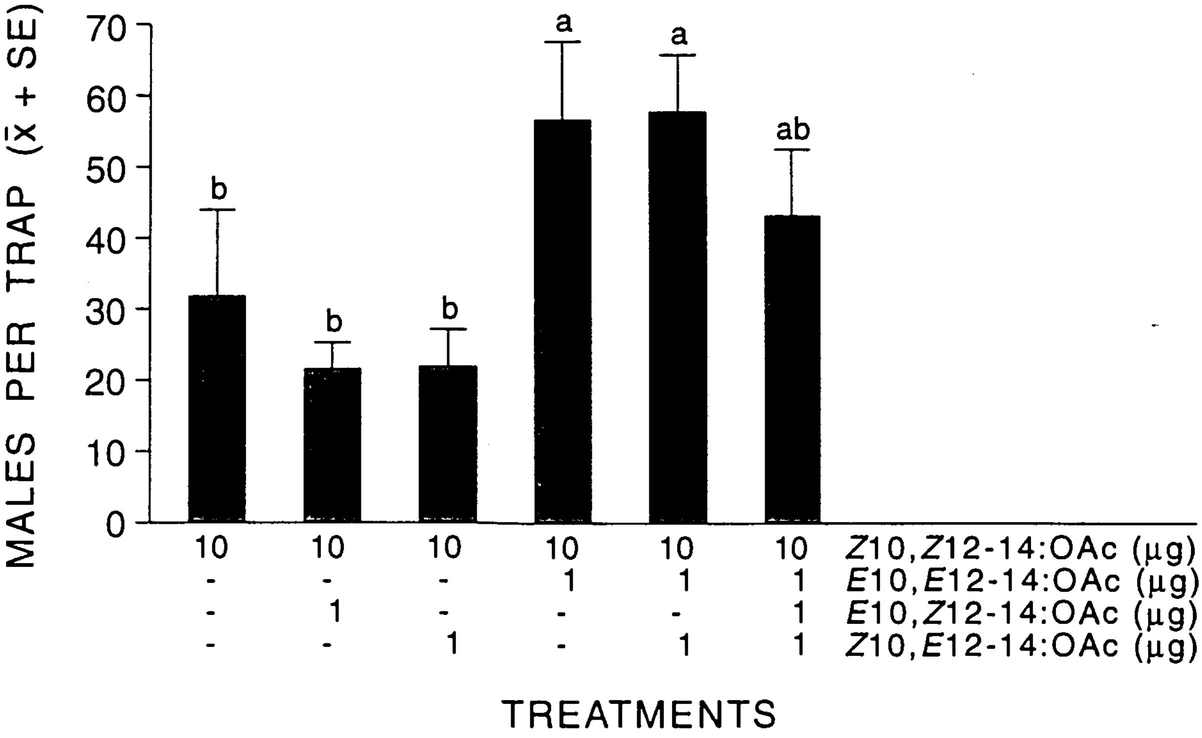

MALES PER TRAP ($\bar{x}$ + SE)
70
60
50
40
30
20
10
0
b
b
b
a
a
ab
10 10 10 10 10 10 *Z*10,*Z*12-14:OAc (μg)
- - - 1 1 1 *E*10,*E*12-14:OAc (μg)
- 1 - - - 1 *E*10,*Z*12-14:OAc (μg)
- - 1 - 1 1 *Z*10,*E*12-14:OAc (μg)
TREATMENTS

Fig. 11 Mean (+SE) numbers of *P. mespilella* males captured in wing traps (Exp. 1a) baited with *Z*10,*Z*12-14:OAc (10 µg) plus *E*10,*E*12-14:OAc (1 µg) versus *E*4,*E*10-12:OAc (10 µg). Summerland, Okanagan Valley, British Columbia, September 13-14, 1995; N=10. Bars with the same superscript are not significantly different, $P<0.05$.

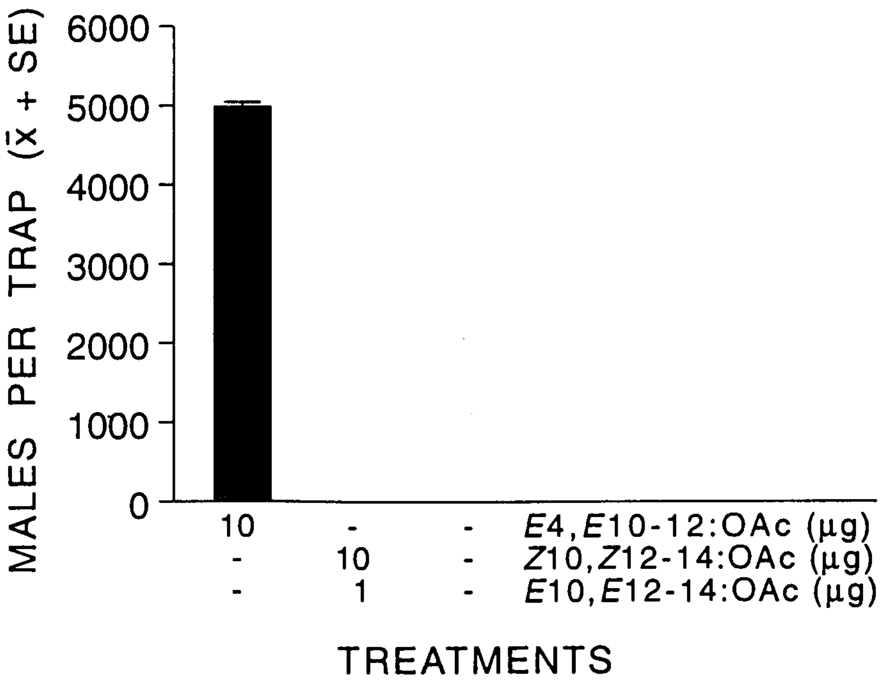

MALES PER TRAP (x̄ + SE)
6000
5000
4000
3000
2000
1000
0
10
-
-
E4,E10-12:OAc (µg)
-
10
-
Z10,Z12-14:OAc (µg)
-
1
-
E10,E12-14:OAc (µg)
TREATMENTS

Fig. 12 Mean (+SE) numbers of *P. mespilella* males captured in wing traps (Exp. 2a) baited with *E*4,*E*10-12:OAc singly at 10 µg and in ternary combinations with *Z*10,*Z*12-14:OAc and *E*10,*E*12-14:OAc at 10:10:1, 10:1:0.1 and 10:0.1:0.01 ratios. Summerland, Okanagan Valley, British Columbia, September 14-15, 1995; N=10. Bars with the same superscript are not significantly different, $P<0.05$.

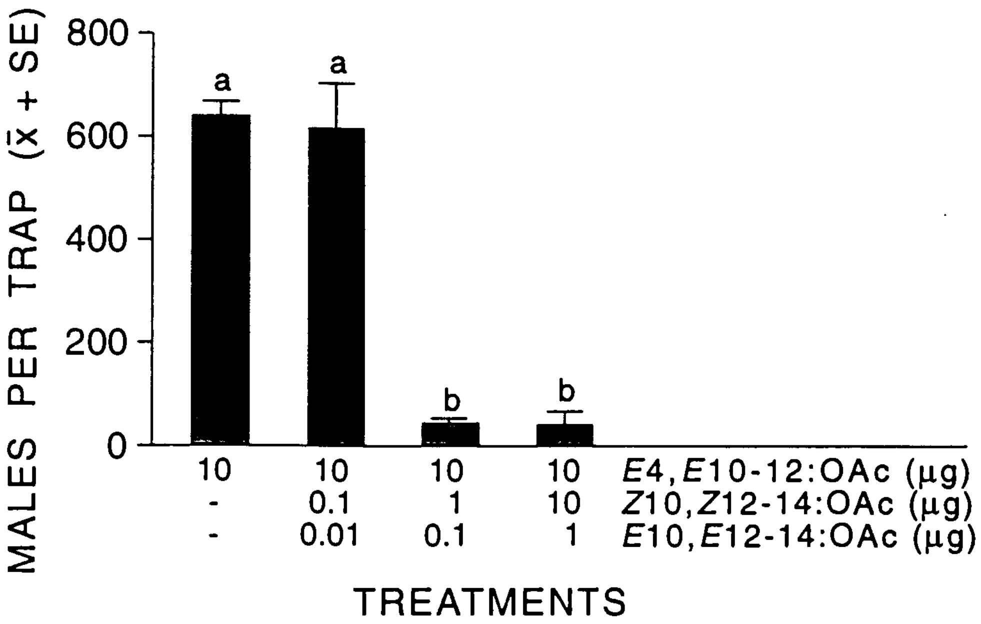
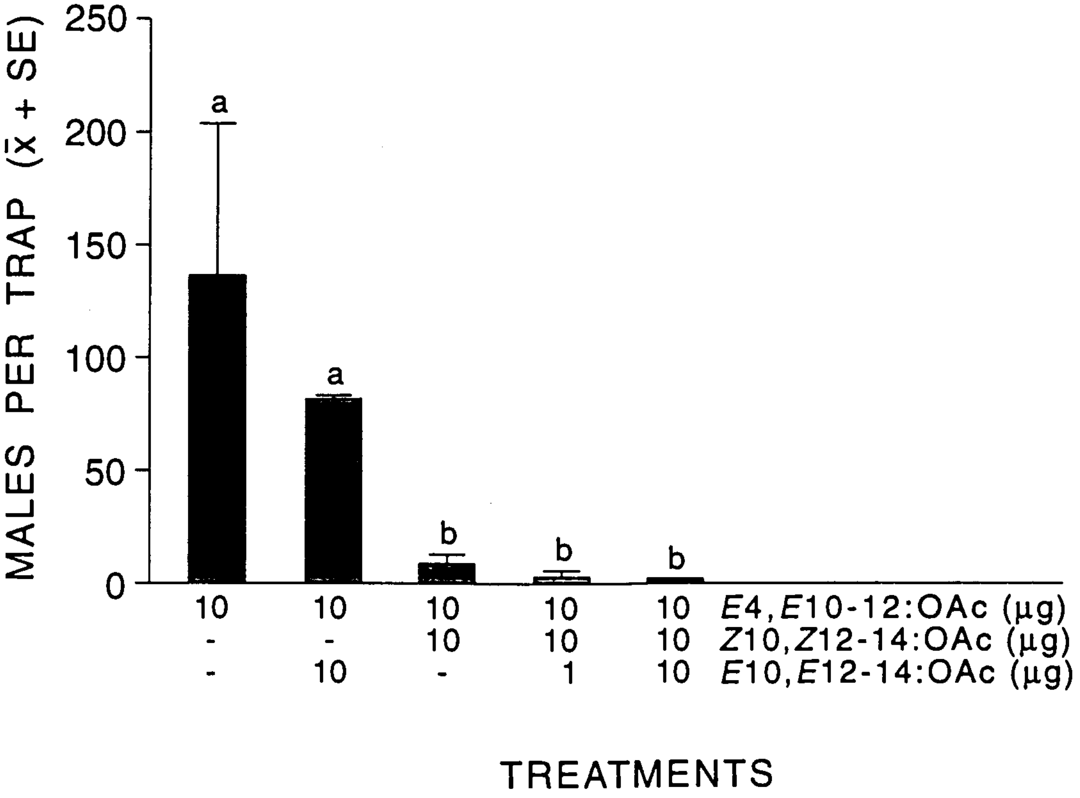

MALES PER TRAP ($\bar{x}$ + SE)
800
600
400
200
0
a
a
b
b
10 10 10 10 E4,E10-12:OAc (µg)
- 0.1 1 10 Z10,Z12-14:OAc (µg)
- 0.01 0.1 1 E10,E12-14:OAc (µg)
TREATMENTS

Fig. 13 Mean (+SE) numbers of *P. mespilella* males captured in wing traps (Exp. 3a) baited with *E*4,*E*10-12:OAc singly at 10 µg and in binary and ternary combinations with *Z*10,*Z*12-14:OAc (10 µg) and *E*10,*E*12-14:OAc (1 or 10 µg). Summerland, Okanagan Valley, British Columbia, September 15-16, 1995; N=10. Bars with the same superscript are not significantly different, $P<0.05$.


MALES PER TRAP (x̄ + SE)
250
200
150
100
50
0
a
a
b
b
b
10 10 10 10 10 E4,E10-12:OAc (µg)
- - 10 10 10 Z10,Z12-14:OAc (µg)
- 10 - 1 10 E10,E12-14:OAc (µg)
TREATMENTS

4.0 DISCUSSION

4.1 Sex Pheromone of *P. crataegella* and Other *Phyllonorycter* Moths

Evidence that *Z*10,*Z*12-14:OAc is the major ABLM pheromone component includes: 1) strong EAD-activity of both synthetic *Z*10- and *Z*12-14:OAc (Fig. 3); 2) identical retention characteristic of female-produced compound 5 (Fig. 1) and synthetic *Z*10,*Z*12-14:OAc on DB-5, DB-210 and DB-23 columns, respectively; 3) comparable EAD-activity of synthetic *Z*10,*Z*12-14:OAc and female-produced compound 5, and 4) attractiveness of *Z*10,*Z*12-14:OAc to ABLM males in field experiments (Figs. 6-10).

*Z*10,*E*12-, *E*10,*E*12-, and *E*10,*Z*12-14:OAc co-chromatographing with EAD-active compounds 3, 4 and 6, respectively, in female ABLM pheromone extract (Fig. 1) may be produced by female ABLM or may result from partial isomerization of *Z*10,*Z*12-14:OAc in the injection port (>240°C) of the gas chromatograph. Strong EAD-activity of *E*10- and *E*12-14:OAc (Fig. 4), and of *E*10,*E*12-14:OAc, and synergistic behavioral activity of *E*10,*E*12-14:OAc in field experiments (Fig. 10) provide compelling evidence that *E*10,*E*12-14:OAc could indeed be produced by and be a pheromone component of female ABLM.

Equal attractiveness of 0.1:10 or 1:10 ratios of *E*10,*E*12- and *Z*10,*Z*12-14:OAc (Figs. 8-10) is indicative that “correct” relative proportions of these two pheromone components are not critical for optimal attraction of ABLM males. The western avocado leafroller, *Amorbia cuneana* (Walsingham), in contrast, responds best to a specific 1:9 ratio of *E*10,*E*12- and *E*10,*Z*12-14:OAc (Bailey et al., 1986). Because attraction of ABLM males to crude, non-HPLC purified *Z*10,*Z*12-14:OAc with 1-2% of *E*10,*E*12-

14:OAc could not be improved by further addition of *E*10,*E*12-14:OAc (data not shown), trace amounts (≤ 1%) of the *EE*- isomer seems sufficient to express synergistic behavioral activity.

Other geometrical isomers of *Z*10,*Z*12-14:OAc at ≤ 10% of the volatile blend neither enhanced nor reduced attractiveness of the volatile blend (Figs. 9,10) and are obviously not part of the ABLM sex pheromone. Similarly, *E*10-12:OAc was present in female ABLM pheromone extracts (Fig. 1) but in field experiments singly or in combination with *Z*10,*Z*12-14:OAc failed to attract ABLM males or to enhance attractiveness of the major pheromone component (Fig. 6).

Following the identification of *Z*10,*Z*12-14:OAc as the major pheromone component in *Notocelia uddamanniana* (L.) (Witzgall et al., 1991), this study reveals that *Z*10,*Z*12-14:OAc is reported for the second time as the major pheromone component produced by ABLM females. This conjugated diene acetate as well as isomeric and synergistic *E*10,*E*12-14:OAc contrast with other monoene or non-conjugated diene acetate pheromone components reported for *Phyllonorycter* moths (Table 3). Behaviorally benign in ABLM, *E*10-12:OAc attracts *P. blancardella* (Roelofs et al., 1977) and *P. mespilella* (Gries et al., 1993). *P. mespilella*-produced *E*10-12:OAc and *E*4,*E*10-12:OAc are attractive by themselves, but unlike the ABLM pheromone components, are not synergistically attractive. Whether *P. ringoniella*-produced *Z*10-14:OAc and *E*4,*Z*10-14:OAc (Sugie et al., 1986) are singly or synergistically attractive is not apparent from the data reported.

Table 3 Sex attractants/pheromone components reported for *Phyllonorycter* spp. and other leafminer moths.

Lepidoptera : Gracillariidae

Species	Sex attractants[3]	Pheromone Components
Phyllocnistis citrella	(*Z*,*Z*)-7,11-Hexadecadienal (Ando et al., 1985)	
Phyllonorycter orientalis	(Z)-10-Tetradecenyl acetate (Ando et al., 1977)	
Phyllonorycter blancardella	(*E*)-10-Dodecenyl acetate (Roelofs et al., 1977)	
Phyllonorycter corylifoliella	(*E*,*Z*)-4,7-Tridecadienyl acetate (Voerman and Herrebout, 1978)	
Phyllonorycter froelichiella	(*Z*)-8-Dodecenyl acetate (*E*)-8-Dodecenyl acetate (Beauvais et al., 1977)	
Phyllonorycter kleemanella	(*Z*)-10-Tetradecenyl acetate (*E*)-10-Tetradecenyl acetate (Booij et al., 1984)	
Phyllonorycter ringoniella		(*Z*)-10-Tetradecenyl acetate (*E*,*Z*)-4,10-Tetradecadienyl acetate (Sugie et al., 1986)
Phyllonorycter watanabei	(*Z*)-10-Tridecenyl acetate (Ando et al., 1977)	
Phyllonorycter pulchrum	(*E*,*E*)-8,10-Tetradecadienyl acetate (Ando et al., 1987)	
Phyllonorycter pygmaea	(*Z*)-8-Tetradecenyl acetate (Ando et al., 1977)	
Phyllonorycter mespilella		(*E*,*E*)-4,10-Dodecadienyl acetate (*E*)-10-Dodecenyl acetate (Gries et al., 1993)

Lepidoptera : Lyonetiidae

Species	Sex attractants	Pheromone Components
Lyonetia clerkella		(*S*)-14-methyl-1-octadene (Sugie et al., 1984)
Leucoptera mallifoliella		5,9-dimethyl-heptadecane (Francke et al., 1987) 5,9-dimethyl-octadecane (Riba et al., 1990)

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[3] Sex attractants have demonstrated bioactivity in the field, but have not been demonstrated to be female-produced, usually because experiments have not been conducted.

4.2 Effect of *P. crataegella* Sex Pheromone on *P. mespilella* Pheromone Communication

Failure of the ABLM pheromone blend to attract any *Phyllonorycter* moths in the Okanagan Valley (Fig. 11) confirms that ABLM is not present in the fruit growing region of British Columbia. Strong attraction of *P. mespilella* males to synthetic *P. mespilella* pheromone *E*4,*E*10-12:OAc (Fig. 11,12) and inhibition of pheromonal attraction in the presence of the ABLM pheromone blend (Fig. 12) clearly indicates recognition of the heterospecific ABLM pheromone blend by *P. mespilella* males. Similarly, response of male obliquebanded leafrollers (OBLR), *Choristoneura rosaceana* (Harris), to the OBLR pheromone blend is inhibited in the presence of *Z*9-tetradecenyl acetate (*Z*9-14:OAc), a minor pheromone component of the sympatric three-lined leafroller, *Pandemis limitata* (Robinson) (Maya Evenden[4], unpublished data). Testing the effect of ABLM components *Z*10,*Z*12-14:OAc and *E*10,*E*12-14:OAc singly indicated that inhibition of *P. mespilella* males is associated with *Z*10,*Z*12-14:OAc (Fig. 13). This inhibition supports the hypothesis of a phylogenetic relationship and previous sympatry of ABLM and *P. mespilella*. It would now be of great interest to test possible reciprocal inhibition of ABLM males by *P. mespilella* pheromone components.

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[4] Ph.D candidate, Department of Biological Sciences, Simon Fraser University, Burnaby, B. C. V5A 1S6.

instance, in mating disruption trails with the codling moth, *Cydia pomonella* (L.), an isomeric mixture of *E*8,*E*10(61%)-, *E*8,*Z*10(20%)-, *Z*8,*E*10(14%)- and *Z*8,*Z*10(5%)-dodecadienol or *EZ*- and *ZE*-dodecadienol isomers individually disrupted *C. pomonella* sexual communication more effectively than the pure *E*8,*E*10-dodecadienol pheromone component (McDonough et al., 1994). In contrast, pheromonal attraction of male ABLMs was not strongly effected in the presence of non pheromone isomers *Z*10,*E*12 or *E*10,*Z*12-14:OAc (Figs. 9,10), suggesting that both compounds should be ineffective as ABLM mating disruptants. However, ABLM pheromone component *Z*10,*Z*12-14:OAc inhibited response of *P. mespilella* males to *P. mespilella* pheromone (Fig. 13). If ABLM males were reciprocally inhibited by *P. mespilella* pheromone, a "disruptive" blend containing ABLM, *P. mespilella* and possibly even *P. blancardella* pheromone could be developed for pheromone-based control of the apple leafmining *Phyllonorycter* guild in North America.

References

1. **Ando, T., Koike, M., Uchiyama, M., and Kuroko, H. 1987.** Lepidopterous sex attractants with a conjugated diene system. *Agric. Biol. Chem.* 51: 2691-2694.

2. **Ando, T., Taguchi, K. Y., Uchiyama, M., Ujiye, T., and Kuroko, H. 1985.** (7*Z*,11*Z*)-Hexadecadienal: sex attractant of the citrus leafminer moth, *Phyllocnistis citrella* (Stainton). *Agric. Biol. Chem.* 49: 3633-3635.

3. **Ando, T., Yoshida, S., Tatsuki, S., and Takahashi, N. 1977.** Sex attractants for male lepidoptera. *Agric. Biol. Chem.* 41: 1485-1492.

4. **Arn, H., Staudler, E., and Rauscher, S. 1975**. The electroantennographic detector-a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch* 30c: 722-725.

5. **Askew, R. R., and Shaw, M. R. 1979**. Mortality factors affecting leaf-mining stages of *Phyllonorycter* (Lepidoptera: Gracillariidae) on oak and birch. 1. Analysis of the mortality factors. *Zool. J. Linn. Soc.* 67:31-50.

6. **Bailey, J. B., McDonough, L. M., and Hoffman, M. P. 1986.** Western avocado leafroller, *Amorbia cuneana* (Walsingham) (Lepidoptera: Tortricidae) - Discovery of populations using different ratios of sex pheromone components. *J. Chem. Ecol.* 12: 1239-1245.

7. **Barrett, B. A. and Brunner, J. F., 1990.** Temporal distribution of *Phyllonorycter elmaella* (Lepidoptera: Gracillariidae) and its major parasitoid, *Pnigalio flavipes* (Hymenoptera: Eulophidae), in Washington apple orchards. *Environ. Entomol.* 19: 362-369.

8. **Beauvais, F., Biwer, G., and Charmillot, P. J. 1977.** Essais de piégeage sexuel de *Grapholitha janthinana* (Dup.) par un mélange d'acetoxy-1-dodècène 8*Z* et 8*E* (8*Z*DDA et 8*E*DDA). *Ann. Zool. Ecol. Am.* 9: 457.

9. **Beckham, C. M., Hough, W. S., and Hill, C. H. 1950**. Biology and control of the spotted tentiform leafminer on apple trees. *Va. Agric. Exp. Stn. Tech. Bull.* 114: 1-19.

10. **Booij, C. J. H., and Voerman, S. 1984.** New sex attractant for 35 Totricids and 4 other lepidopterous species found by systematic field screening in the Netherlands. *J. Chem. Ecol.* 10: 135-144.

11. **Brown, M. W. 1989.** Spatial dynamics and sampling of *Lyonetia speculella* (Lepidoptera: Lyonetiidae), a leafminer of apple. *Environ. Entomol.* 18: 875-880.

12. **Brown, M. W. 1990**. Abundance and identification of the leafmining guild on apple in the mid-Atlantic states. *Great Lakes Entomol.* 23: 179-188.

13. **Bultman, T. L., and Faeth, S. H. 1986**. Selective oviposition by a leafminer in response to temporal variation in abscission. *Oecologia* 69: 117-120.

14. **Cardé, R. T., and Minks, A. K. 1995**. Control of moth pests by mating disruption: Success and constraints. *Ann. Rev. Entomol.* 40: 559-85.

15. **Coli, W. M., and Prokopy, R. J. 1982**. Biology, monitoring and control of leafminers in Massachusetts. *Proc. Mass. Fruit Growers Assoc. Annu. Mtg.* 86: 30-44.

16. **Cossentine, J. E., and Jessen, L. B. 1992**. Establishment of *Phyllonorycter mespilella* (Hübner) (Lepidoptera: Gracillariidae) and its parasitoid, *Pnigalio flavipes* (Hymenoptera: Eulophidae), in fruit orchards in the Okanagan and Similkameen valleys of British Columbia. *J. Entomol. Soc. B. C.* 89: 18-24.

17. **Dutcher, J. D., and Howitt, A. J. 1978**. Bionomics and control of *Lithocolletis blancardella* in Michigan. *J. Econ. Entomol.* 71: 736-738.

18. **Francke, W., Francke, S., Toth, M., Szöcs, g., Guerin, P., and Arn, H. 1987.** Identification of 5,9-dimethyl heptadecane as the sex pheromone of the moth *Leucoptera scitella. Naturwissenschaften* 74: 143-144.

19. **Gray, T. G., Slessor, K. N., Shepherd, R. F., Grant, G. G., and Manville, J. F. 1984.** European pine shoot moth, *Rhyacionia buoliana* (Lepidoptera: Tortricidae): Identification of additional pheromone components resulting in an improved lure. *Can. Entomol.* 116: 1525-1532.

20. **Green, T. A., and Prokopy, R. J. 1984**. Daily activity of apple blotch leafminer adults. *Mass. Fruit Notes* 49: 19-22.

21. **Green, T. A., and Prokopy, R. J. 1986.** Visual monitoring trap for the ABLM moth, *Phyllonorycter crataegella* (Lepidoptera: Gracillariidae). *Environ. Entomol.* 15: 562-566.

22. **Green, T. A., and Prokopy, R. J. 1991**. Oviposition behavior of the apple blotch leafminer, *P. crataegella* (Clemens) (Lepidoptera: Gracillariidae). *J. New York Entomol. Soc.* 99: 654-663.

23. **Green, T. A., Prokopy, R. J., and Coli, W. A. 1985**. Behavior and monitoring of adult apple blotch leafminers. *New England Fruit Meetings* 91: 74-82.

24. **Gries, G., McBrien, H. L., Gries, R., Li, J., Judd, G. J. R., Slessor, K. N., Borden, J. H., Smith, R. F., Christie, M., Troubridge, J. T., Wimalaratne, P. D. C., and Underhill, E. W. 1993.** (*E*4,*E*10)-Dodecadienyl acetate: Novel sex pheromone component of tentiform leafminer, *Phyllonorycter mespilella* (Hübner) (Lepidoptera: Gracillariidae). *J. Chem. Ecol.* 19: 1789-1798.

25. **Heppner, J. B. 1993**. Citrus leafminer, *Phyllocnistis citrella*, in Florida. (Lepidoptera: Gracillariidae: Phyllocnistinae). *Tropical Lepidoptera* 4: 49-63.

26. **Hill, C. H., and Hough, W. S. 1957**. The unspotted tentiform leafminer on apple trees. *Va. Agric. Exp. Stn. Tech. Bull.* 130: 23.

27. **Johnson, E. F., Laing, J. E., and Trottier, R. 1976.** The seasonal occurrence of *Lithocolletis blancardella* (Gracillariidae) and its major natural enemies in Ontario apple orchards. *Proc. Entomol. Soc. Ont.* 107: 31-45.

28. **Kovats, E. S. 1965.** Gas chromatographic characterization of organic substances in retention index system, p. 229, *in* J. C. Giddings & R. A. Keller (eds.). Advances in chromatography, Volume I. Marcel Dekker, New York.

29. **Kremer, F. W. 1963**. Major leafminer species occurring in the South Tirolean fruit-farming region and their control. *Pflanzenschutz-Nachr. (Bayer)* 16: 1-16.

30. **Maier, C. T. 1981**. Seasonal occurrence, abundance and leaf damage of the apple blotch leafminer, *Phyllonorycter crataegella* (Lepidoptera: Gracillariidae), in Connecticut apple orchards. *Environ. Entomol.* 10: 645-649.

31. **Maier, C. T. 1982**. Parasitism of the apple blotch leafminer, *Phyllonorycter crataegella*, on sprayed and unsprayed apple trees in Connecticut. *Environ. Entomol.* 11: 603-610.

32. **Maier, C. T. 1983**. Effect of the apple blotch leafminer (Lepidoptera: Gracillariidae) on apple leaf abscission. *J. Econ. Entomol.* 76: 1265-1268.

33. **Maier, C. T. 1984.** Abundance and phenology of parasitoids of the spotted tentiform leafminer, *Phyllonorycter blancardella* (Lepidoptera: Gracillariidae) in Connecticut. *Can Entomol.* 116: 443-449.

34. **Maier, C. T. 1985**. Rosaceous hosts of *Phyllonorycter* species (Lepidoptera: Gracillariidae) in New England. *Ann. Entomol. Soc. Am.* 78: 826-830.

35. **Maier, C. T. 1988.** Comparison of parasitoid complexes of *Phyllonorycter* species (Lepidoptera: Gracillariidae) in Connecticut apple orchards. *Proc XVIII Int. Congr. Entomol.* 1988: 3663.

36. **Maier, C. T. 1990**. Potential for the biological control of apple leafminers by parasitic wasps. *Proc. New England Fruit Meeting* 96: 60-74.

37. **Maier, C. T., and Davis, D. R. 1989**. Southern New England host and distributional records of Lithocolletine Gracillariidae (Lepidoptera). With comparison of host specificity in temperate regions. Miscellaneous Publication, *Entomol. Soc. Am.* 70: 1-21.

38. **McDonough, L. M., Davis, H. G., Chapman, P. S., and Smithhisler, C. L. 1994**. Codling moth (*Cydia pomonella*): Disruptants of sex pheromonal communication. *J. Chem. Ecol.* 20: 171-181.

39. **Minks, A. K., and Cardé, R. T. 1988**. Disruption of pheromone communication in moths: Is the natural blend really most efficacious? *Entomol. Exp. Appl.* 49: 25-36.

40. **Pottinger, R. P., and LeRoux, E. J. 1971**. The biology and dynamics of *Lithocolletis blancardella* (Lepidoptera: Gracillariidae) on apple in Quebec. *Mem. Entomol. Soc. Can.* 77: 431-437.

41. **Reissig, W. H., Weires, R. W., and Forshey, C. G. 1982**. Effects of gracillariid leafminers on apple growth and production. *Environ. Entomol.* 11: 958-963.

42. **Riba, M., Rosell, J. A., Eizaguirre, M., Canela, R., and Guerrero, A. 1990.** Identification of a minor component of the sex pheromone of *Leucoptera malifoliella* (Lepidoptera: Lyonetiidae). *J. Chem. Ecol.* 16:1471-1483.

43. **Roelofs, W. L. 1978.** Threshold hypothesis for pheromone perception. *J. Chem. Ecol.* 4: 685-699.

44. **Roelofs, W. L., Hill, A., Cardé, R., Tette, J., Madsen, H., and Vakenti, J. 1974.** Sex pheromones of fruit-tree leafroller moth, *Archips argyrospilus*. *Environ. Entomol.* 3: 747-751.

45. **Roelofs, W. L., Reissig, W.H., and Weires, R. W. 1977.** Sex attractant for the spotted tentiform leafminer moth *Lithocolletis blancardella*. *Environ. Entomol.* 6: 373-374.

46. **Sanchez-De Leon, A. 1984.** El minador de al hoja del cafe. *Revista cafetalera (Gautemala)* 244: 6-10.

47. **Sekita, N., and Yamada, M. 1979**. Life history of *Lyonetia prunifoliella* (Hübner) subsp. *malinella* (Matsumura) (Lepidoptera: Lyonetiidae). *Appl. Entomol. Zool.* 14: 285-292.

48. **Sugie, H., Tamaki, Y., Kawasaki, K., Wakou, M., Oku, T., Hirano, C., and Horiike, M. 1986.** Sex pheromone of the apple leafminer moth, *Phyllonorycter ringioniella* (Matsumura) (Lepidoptera: Gracillariidae): Activity of geometrical isomers of tetradecadienyl acetates. *Appl. Entomol. Zool.* 21: 578-581.

49. **Sugie, R., and Kumakura, M. 1984.** Sex pheromone of the peach leafminer moth, *Lyonetia clerkella* (Linne): isolation and identification. *Appl. Entomol. Zool.* 19: 323-330.

50. **Vakenti, J. M., Gaunce, A. P., Slessor, K. N., King, G. G. S., Allan, S. A., Madsen, H.F., and Borden, J. H. 1988**. Sex pheromone components of the

oblique-banded leafroller, *Choristoneura rosaceana* in the Okanagan valley of British Columbia. *J. Chem. Ecol.* 14: 605-621.

51. **Voerman, S., and Herrebout, W. M. 1978.** A sex attractant for the leafminer moth *Lithocolletis corylifoliella* and its influence on that of *L. blancardella*. *Entomol. Exp. Appl.* 23: 96-98.

52. **Weires, R. W. 1977**. Control of *Phyllonorycter crataegella* in Eastern New York. *J. Econ. Entomol.* 70: 521-523.

53. **Weires, R. W., Davis, D. R., Leeper, J. R., and Reissig, W. H. 1980.** Distribution and parasitism of Gracillariid leafminers on apple in the Northeast. *Ann. Entomol. Soc. Am.* 73: 541-546

54. **Witzgall, P. P., Frérot, B., and Malosse, C. 1991.** Identification du composé majoritaire de la sécrétion phéromonale de *Notocelia uddmanniana* L. (Lepidoptera: Tortricidae). *J. Appl. Entomol.* 112: 71-75.

55. **Zar, Jerold H. 1984.** Biostatistical Analysis, 2nd edition.