SEX PHEROMONE COMPONENTS AND PHEROMONE-BASED MALE DISORIENTATION OF THE CHERRY BARK TORTRIX, Enarmonia formosana (SCOPOLI) (LEPIDOPTERA: TORTRICIDAE)

by

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PEST MANAGEMENT

in the Department

of

Biological Sciences

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SIMON FRASER UNIVERSITY

November 1997

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0-612-24198-X



APPROVAL

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SEX PHEROMONE COMPONENTS AND PHEROMONE-BASED MALE DISORIENTATION OF THE CHERRY BARK TORTRIX, ENARMONIA FORMOSANA (SCOPOLI) (LEPIDOPTERA: TORTRICIDAE).

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ABSTRACT

My objectives were to identify sex pheromone components of the cherry bark tortrix (CBT), Enarmonia formosana (Scopoli), and to investigate the potential for disrupting pheromonal communication of CBT. (E)-9-Dodecenyl acetate (E9-12:OAc) and (Z)-9dodecenyl acetate (Z9-12:OAc) were identified as major components of the sex pheromone of the CBT. The compounds were identified in extracts of the female's pheromone gland by coupled gas chromatographic-electroantennographic detection (GC-EAD) and by coupled GC-mass spectrometry. In field experiments, traps baited with E9-12:OAc or Z9-12:OAc singly were unattractive to male CBT, but in combination at ratios of 50:50 or 40:60 attracted numerous males. Increasing quantities of this two-component pheromone blend resulted in increasing numbers of male CBT captured. Attractiveness of a 10 mg dose strongly outcompeted that of two unmated female CBT moths. Six other EAD-active acetates identified in extracts of pheromone glands failed to enhance attractiveness of the two-component pheromone blend. These compounds may serve to reduce cross-attraction of heterospecific male moths or may play a role in courtship behaviour. (Z)-7-Decenyl acetate (Z7-10:OAc) at 1% was the only compound that initially appeared to enhance attractiveness of the E9- and Z9-12:OAc pheromone blend, and thus was included in the pheromone blend of the mating disruption experiment.

The potential for pheromone-mediated mating disruption of CBT was evaluated in mature (>30-year-old) ornamental cherry trees, *Prunus* cvs., spaced >15 m apart in suburban streets of New Westminster and Vancouver. In each of four experiments,

pheromone-treated trees received 1-4 polyvinyl chloride tube-type dispensers attached to their trunks. In experiment (Exp.) 1, dispensers released a blend of *E*9-12:OAc (49.5%), *Z*9-12:OAc (49.5%) and *Z*7-10:OAc (1%) at a rate of 29 mg/24h. In Exp. 2-4, dispensers released *E*9-12:OAc alone at 27 mg/24h (Exp. 2), 4 mg/24h (Exp. 3) and 0.5 mg/24h (Exp. 4). Each experimental tree received a sticky delta trap baited with a grey rubber septum impregnated with CBT pheromone [*E*9-12:OAc (40 μ g), *Z*9-12:OAc (60 μ g), and *Z*7-10:OAc (1 μ g)]. In all four experiments, captures of male CBT in treatment trees were significantly reduced compared to control trees. Pheromone-based disruption of communication in CBT on individual cherry trees appears to be feasible and may become part of an integrated management strategy for CBT in urban environments.

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ACKNOWLEDGMENTS

I thank D. Holden for collection of pupae; Dr. E. Underhill of the National Research Council for reference samples of chemicals; M. Sidney, K. Vickars and T. Mast for field assistance; E. Carefoot for the preparation of figures; and D. Wakarchuk of Phero Tech Inc. for preparation of custom-made release devices and measurement of release rates. The Parks and Recreation Departments of New Westminster, Vancouver, Surrey and Burnaby provided CBT-infested cherry logs and field sites for experiments.

The studies reported in this thesis received initial funding from Robert and Judy Hager of Vancouver. Major funding was provided by Cannor Nurseries, Ltd. of Chilliwack, with a matching grant from the B.C. Ministry of Agriculture, Fisheries and Food under the Applied Research Program (1994-6). I received additional support from Simon Fraser University Graduate Fellowships (1994, 1995, 1997), a B. C. Council of Garden Clubs Scholarship (1995), and a B.C. Science Council Graduate Research Engineering and Technology Award (1996-7).

I wish to express my appreciation to my senior supervisor Dr. Gerhard Gries for unflagging supervisory enthusiasm and guidance, Regine Gries for sharing her technical expertise in electrophysiology, and my committee member, Dr. Gary Judd, for helpful discussions and review of two manuscripts that were submitted to refereed journals. I am profoundly grateful to my wife, Karin, for all her support and encouragement.

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1.0. INTRODUCTION

1.1. THE CHERRY BARK TORTRIX (CBT) IN BRITISH COLUMBIA

The cherry bark tortrix (CBT), *Enarmonia formosana* (Scopoli), has a long history (Köllar 1837) as a fruit tree pest in the Palaearctic region, where its range extends from Ireland (Beirne 1941) to Siberia (Kuznetsov 1988). Trees within the genus *Prunus* serve as primary hosts, but CBT also attacks other rosacean genera, including *Malus*, *Pyrus*. *Sorbus*, *Cydonia* and *Pyracantha* (Dickler and Zimmerman 1972, Dirimanov and Sengalevich 1962, Höbaus 1982, Ivinskis and Pakal'nishkis 1983, Lagunov 1979, Meyrick 1928, Savkovskii 1979). Species that form the most locally abundant crop or are most susceptible to attack are targeted (Crémoux and Lambert 1964, Minks *et_al.* 1976, Rozsypal 1942, Winfield 1964). CBT causes three main types of damage to fruit trees (Dickler and Zimmerman 1972): (1) direct damage by larval feeding, between the bark and the cambium; (2) indirect damage by secondary pests such as scolytids that are attracted to and invade wounded and weakened trees; and (3) indirect damage through increased susceptibility of infested trees to frost and other unfavourable weather conditions.

Introduced and established in North America (Dang and Parker 1990), CBT is currently found in south-western British Columbia and north-western Washington State (Klaus 1992), where it has become the key pest of top-grafted ornamental cherry trees (*Prunus* cvs.). Differential growth rates of rootstock and scion, and partial graft rejection result in cracks in the graft union area which facilitate entry of neonate CBT larvae. Larval tunnelling in the phloem weakens and eventually kills trees. Accelerated decline and death of infested trees has created a serious problem for Lower Mainland municipalities, which planted over 100,000 ornamental cherry trees as boulevard trees between the 1940's and 1980's (Vancouver-area municipal arborists, personal communications). Because infested trees are replaced by different tree species, sales of valuable cherry trees have declined seriously (John Matthies, Cannor Nurseries, Chilliwack; personal communication). CBT also poses a threat to the orchard industry, when it reaches interior, fruit-growing areas of British Columbia and Washington State.

1.2. LIFE CYCLE OF CBT

CBT is univoltine. Throughout the May to August flight season, females oviposit 14-84 eggs, singly or in groups of two or three, near wounds in the bark of tree trunks (Roediger 1956, Winfield 1964). Eggs are attached to the bark with a gummy secretion (Winfield 1964). Embryonic development times are inversely related to temperature. At average temperatures of 12, 20-22.2 and 24°C, eggs hatch in 24, 12-14 and 9-10 days, respectively (Dirimanov and Sengalevich 1962). Unable to penetrate intact bark, neonate, larvae immediately crawl into cracks. They overwinter in shallow tunnels in the phloem, passing through five instars (Roediger 1956). Without diapausing, larvae feed whenever the temperature rises above 7 or 8°C (Sermann and Zahn 1986). Beginning in late spring, mature larvae web together larval frass to form pupation ehambers which often protrude ~1 cm from the trunk. The pupal stage lasts from 10-19 d (Dirimanov and Sengalevich 1962). Cryptic adults eclose between May and August, and mate one or two days after emergence (Roediger 1956).

Adults fly mainly in the early morning, but will fly later into the day on warm, sunny

days (Dickler 1970, Dickler and Zimmerman 1972). Moths do not seem to disperse far from their natal tree, as long as it remains suitable for development of larvae (Sziraki 1984, Winfield 1964).

1.3. MANAGEMENT OF CBT: THE NEED FOR NEW APPROACHES

1.3.1. Blocking Exit Holes

This control tactic is based on coating cracked bark areas and wounds with a plethora of substances, including creosote (Winfield 1964), lime with or without casein glue (Dobroserdov 1981, Lyalyuts'ka 1965, Savskovskii and Lyalyutskaya 1976), pigmented vinyl acetate and even a paste consisting of clay, BHC dust and cow dung (Dobroserdov 1970). These tactics, however, are too labour intensive to be practical in an urban setting, apart from other considerations. In 1991, Agriculture Canada recommended spraying affected areas with an aerosol formulation of Tanglefoot® to prevent emergence of moths (O. Croy, Agriculture Canada, New Westminster; personal communication), but the Tanglefoot appeared to dry out the bark, and to lose its stickiness before the end of the season (personal observation).

1.3.2. Insecticidal Control

The bark protects CBT larvae from biorational contact insecticides, such as horticultural oils and soaps, and *Bacillus thuringiensis* Kurstaki (Btk). Among chemical insecticides tested in English apple orchards during 1957-1960 (Winfield 1964). Trichlorphon^{*} effected the best control (except for Endrin^{*}, discontinued because of its high mammalian toxicity). The recommended treatment time is the beginning of May, when the first pupation tubes are formed and overwintered larvae are close to the surface

of the bark (Winfield 1964). The Trichlorphon treatment is efficacious (personal observation), but municipal by-laws prohibit the use of chemical insecticides on park and street trees. Moreover, garden owners increasingly oppose the use of chemicals on their privately-owned cherry trees.

1.3.3. Biological Control

Roediger (1956) lists several insects parasitic on CBT in central Europe; and Boldyrev and Dobroserdov (1981) report that in the (former) USSR CBT larvae are destroyed by various insect predators, the most important being the raphidiid, *Agulla xanthostigma* (Schummel). However, natural enemies do not appear to control CBT within its palaearctic range, and the degree of predation and parasitism is low in the Vancouver area (personal observation).

1.4 OBJECTIVES

To date, the long period of adult activity of the CBT (May – August) has led pest managers to focus control efforts on the larvae. However, the ineffectiveness of such controls suggests that the potential for control of adult CBT should be re-examined. Accordingly, my research objectives were: '

1) to identify sex pheromone components for CBT; and

2) to investigate the potential for pheromone-based control of the CBT.

2.0. SEX PHEROMONE COMPONENTS

2.1. INTRODUCTION

Four chemicals, (*E*)-8- and (*Z*)-8-dodecenyl acetate (*E*8- and Z8-12:OAc) as well as (*E*)-9- and (*Z*)-9-dodecenyl acetate (*E*9- and *Z*9-12:OAc) have been reported to attract male CBT moths, but whether any of them are pheromone components of the CBT has not been investigated. Traps baited with *Z*8-12:OAc in admixture with quantitatively minor *E*8-12:OAc captured male CBT in Europe (Alford 1978, Hrdý et al. 1979). A 1:1 mixture of *E*9- and *Z*9-12:OAc also captured significant numbers of CBT males (Minks et al. 1976). *E*9- and *Z*9-12:OAc at blend ratios of 1:1 (Sziráki 1984) and 4:1 (Brewer et al. 1985) have been employed as attractants for CBT in Europe. My objective was to determine whether female CBT produce *E*8- and *Z*8-12:OAc and/or *E*9- and *Z*9-12:OAc as sex pheromone components; and whether they also produce additional compounds that enhance attractiveness of the pheromone blend.

2.2. MATERIALS AND METHODS

2.2.1. Insects and Laboratory Analysis

In early May, puparia of the CBT were excised from the bark of ornamental cherry trees in New Westminster, B.C., and held at 20°C, 70% RH, and a photo-regime of 14L:10D. Pupae were sexed (Roediger 1956) and male and female pupae were kept separately in Petri dishes to avoid mating of emergent moths. Abdominal tips with pheromone glands of 1- to 3-day-old unmated females were removed 1-2 hr into the photophase (Roediger 1956) and extracted for 5 min in HPLC grade hexane. Alfquots of

gland extract were subjected to: 1) coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al. 1975), employing a Hewlett Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m x 0.25 or 0.32 mm ID) coated with DB-5, DB-23 or DB-210 (J&W Scientific, Folsom, CA, USA); 2) GC-mass spectrometry (MS), employing a HP5985 and a Varian Saturn II ion trap, both fitted with a DB-5 column; and 3) dimethyl-disulphide (DMDS) treatment to identify positions of ' double bonds (Dunkelblum et al. 1985). Comparative analyses of authentic standards (Table 1) and EAD-active compounds in pheromone extract by GC, GC-EAD and GC-MS (before and after DMDS) facilitated the identification of candidate pheromone components.

2.2.2. Trapping Experiments

Field experiments (1994-1996) used rows of mature (>30-year-old) ornamental cherry trees spaced >15m apart along the boulevards of suburban streets in New Westminster and Vancouver, British Columbia. Sticky two-litre milk-carton Delta traps (Gray et al. 1984) were suspended from trees 2 m above ground and baited with a grey rubber septum (West Co., Lionville, PA, USA) impregnated with candidate pheromone components in 10-100 μ l of hexane (HPLC grade). In Exp. 1-4, each treatment was randomly assigned to a tree, whereas in Exp. 5-11, one baited and one unbaited (control) trap were suspended from each tree, equidistant to and in opposed directions from the tree trunk.

Exp. 1 tested E9-12:OAc (100 μ g) and Z9-12:OAc (100 μ g) singly and at respective ratios of 80:20, 60:40, 50:50, 40:60 and 20:80. Exp. 2-4 tested the blend of E9-12:OAc

Table 1. Names and sources of chemicals employed for the identification of candidate pheromone components in gland extracts, and tested in field Exps. 1-11.

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Chemical	Abbreviation	Source		
(Z)-7-decenyl acetate	Z7-10:OAc	Bedoukian In	c., Danbury	CN, USA
(E)-9-dodecenyl acetate	<i>E</i> 9-12:OAc			
(Z)-9-dodecenyl acetate	<i>Z</i> 9-12:OAc		••	
(<i>E</i>)-9-undecenyl acetate	<i>E</i> 9-11:OAc	Plant Biotechnology Institute. National Research Council. Saskatoon, SA		
(Z)-9-undecenyl acetate	Z9-11:OAc	·,		
(\tilde{E}) -9.11-dodecadienyl acetate	<i>E</i> 9,11-12:OAc			
(Z)-9.11-dodecadienyl acetate	Z9,11-12:OAc			
Dodecyl acetate	12:0Ac	Sigma Chemi ON	cal Co., Mis	ssissauga.

⁻¹ All compounds were >95% chemically pure.

(40 µg) plus Z9-12:OAc (60 µg) alone and in combination with either and both of: 12:OAc (1 µg) and Z7-12:OAc (1 µg) (Exp. 2); E9- and Z9-11:OAc (Exp. 3); and E9,11and Z9,11-12:OAc (Exp. 4). For re-evaluation of weak behavioural activity of Z7-10:OAc, Exp. 5 (n=14), 6 (n=16) and 7 (n=12) retested E9-12:OAc (40 µg) plus Z9-12:OAc (60 µg) alone and in admixture with Z7-10:OAc (1 µg). Paired comparisons (one pair per tree) of these 2- and 3-component blends were tested in Exp. 8 at quantities of respectively 1, 10, 100, 1,000 and 10,000 µg. Each of the three final Exps. 9 (n=6), 10 (n=8) and 11 (n=8) tested attractiveness of E9-12:OAc (4 mg) and Z9-12:OAc (6 mg) versus that of two virgin, one- or two-day-old female CBT. Females were confined within a cylindrical (5 x 3.5 cm) screen cage and provided with moistened cotton batten. Exps. 9-11 were conducted from ~21:00 hr to ~13:00 hr the following day.

2.2.3. Statistical Analyses

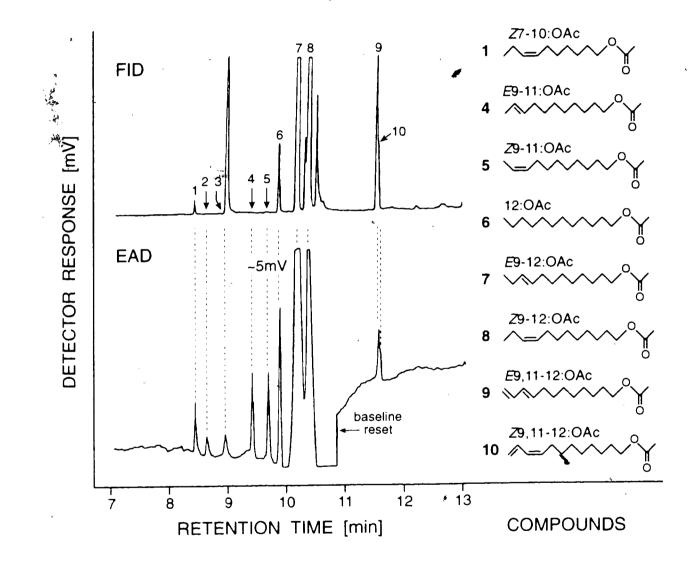
Data were analysed by analysis of variance (ANOVA), and means were separated by the Student-Newman-Keuls' multiple comparisons test (Exps. 1-7, 9-11), or by the General Linear Model followed by the Least Significant Mean test (Exp. 8). In all cases, α =0.05 (SAS Release 6.11, SAS Institute, 1989).

2.3. RESULTS

2.3.1. Pheromone Analysis

GC-EAD analysis of pheromone extracts from female CBT revealed 10 compounds that elicited antennal responses from male CBT (Fig. 1). GC-MS of compounds 6-10 in electron impact and chemical ionisation modes of operation indicated that they were dodecyl acetate (6), dodecenyl acetates (7(8), and dodecadienyl

FIG. 1. Flame ionisation detector (FID) and electroantennographic detector (EAD: male *E. formosana* antenna) responses to 1 female equivalent of pheromone gland extract. EAD-active compounds were identified as follows: 1 = (Z)-7-decenyl acetate (Z7-10:OAc); 4 = (E)-9-undecenyl acetate (E9-11:OAc); 5 = (Z)-9-undecenyl acetate (Z9-11:OAc); 6 = dodecyl acetate (12:OAc); 7 = (E)-9-dodecenyl acetate (E9-12:OAc); 8 = (Z)-9-dodecenyl acetate (Z9-12:OAc); 9 = (E)-9,11-dodecadienyl acetate (E9,11-12:OAc); and 10 = (Z)-9,11-dodecadienyl acetate (Z9,11-12:OAc). EAD-active compounds 2 and 3 are unknown. Chromatography: splitless injection, injector and FID detector: 240°C, DB-23 column (30 m x 0.32 mm ID); temperature program: 1 min at 50°C, 20°C/min to 100°C. 10°C/min to 200°C.



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acetates (9,10). DMDS-derivatisation of compounds 7 and 8, the most abundant and EAD-active compounds, indicated a double bond in the C9 position. Compounds 7 and 8 were thus hypothesised to be, and through GC-MS comparison with authentic standards, confirmed to be, E9-12:OAc (7) and Z9-12:OAc (8). Retention indices of compounds 9 and 10 were indicative of conjugated diene acetates. With E9- and Z9double bonds identified in compounds 7 and 8, double bond positions in compounds 9 and 10 were hypothesised to be E9,11-12:OAc (9) and Z9,11-12:OAc (10). Identical and mass spectrometric characteristics of female-produced and synthetic retention compounds confirmed these structural assignments. The retention indices of compounds 4 and 5 on three fused silica columns (DB-23, DB-5 and DB-210) suggested that they were lower homologues of compounds 7 and 8, respectively. However, EAD-activity and retention characteristics of E9- and Z9-11:OAc, rather than E8- and Z8-11:OAc, were consistent with candidate pheromone components 4 and 5, respectively. In contrast, comparative GC, GC-EAD and GC-MS of EAD-active compound 1 and authentic standards, confirmed 1 as a true homologue (Z7-10:OAc) of compound 8 (Z9-12:OAc). EAD-active compounds 2 and 3 are as yet unknown.

2.3.2. Trapping Experiments

*E*9- and *Z*9-12:OAc at 50:50 or 40:60 blend ratios were highly attractive to CBT males, whereas either compound singly was completely unattractive (Fig. 2, Exp. 1). Attractiveness of this blend was not further enhanced by admixture of either or both of 12:OAc and *Z*7-12:OAc (Exp. 2), *E*9- and *Z*9-11:OAc (Exp. 3), or *E*9,11- and *Z*9.11-12:OAc (Exp. 4). Admixture of *Z*7-10:OAc in Exp. 5 increased attractiveness of the blend (Fig. 3), but behavioural activity of *Z*7-10:OAc could neither be confirmed in Exps.

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FIG. 2. Mean number of male *E. formosana* captured in sticky Delta traps baited with *E*9-12:OAc or *Z*9-12:OAc alone and in various combinations. New Westminster, British Columbia, 10-23 June 1994; n=8. Bars with the same letter superscript are not significantly different. ANOVA (P<0.05) followed by Student-Newman-Keuls' test, α <0.05.

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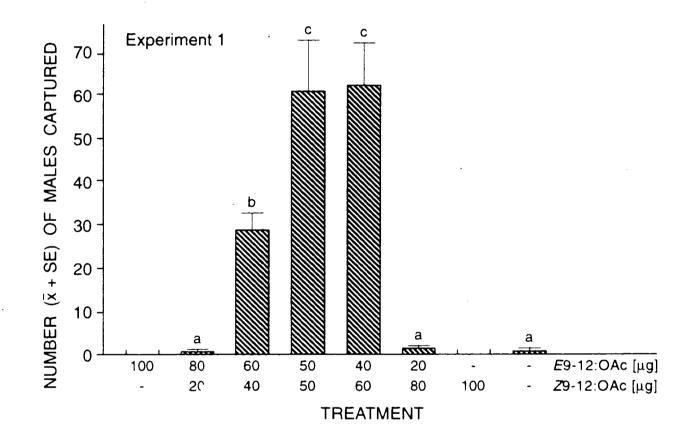


FIG. 3. Mean number of male *E. formosana* captured in sticky Delta traps baited with *E*9-12:OAc plus *Z*9-12:OAc alone and in combination with *Z*7-10:OAc (black bars). Exp. 5 (11-14 July 1994; n=14) and Exp. 6 (14-26 August 1994; n=16) conducted in New Westminster, experiment 7 (15-26 May 1995; n=12) in Vancouver, British Columbia. Within each experiment, bars with the same letter superscript are not significantly different. ANOVA (*P*<0.05) followed by Student-Newman-Keuls' test, α <0.05.

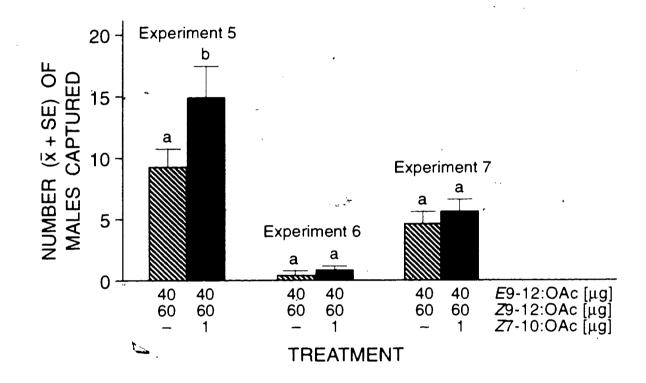
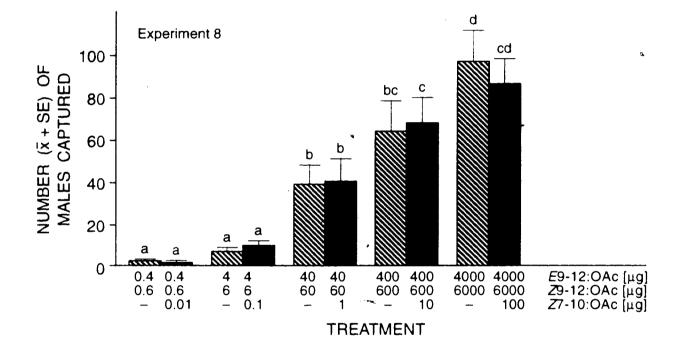


FIG. 4. Mean number of male *E. formosana* captured in sticky Delta traps baited with increasing doses of *E*9-12:OAc plus Z9-12:OAc alone and in combination with Z7-10:OAc (black bars). Vancouver, British Columbia, 26 June to 2 July 1995; n=10. Bars with the same letter superscript are not significantly different. GLM/LSMeans, α <0.05.



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6 and 7 (Fig. 3) nor in Exp. 8 (Fig. 4). Increasing the dose of *E*9- and *Z*9-12:OAc at a 40:60 blend ratio resulted in increasing numbers of male CBT moths captured (Fig. 4, Exp. 8). Attractiveness of this binary blend at the 10 mg dose outcompeted that of two caged unmated female CBT in three consecutive tests (Fig. 5; Exps. 9-11). Environmental conditions for pheromone release by females were suboptimal for Exp. 9, but ideal for Exp. 10 (sunny, 16-20°C, light breeze) and 11 (sunny, 18-24°C, light breeze). All females were alive and active at the end of each experiment.

2.4. DISCUSSION

Evidence that E9-12:OAc and Z9-12:OAc are sex pheromone components of female CBT moths includes: 1) both compounds were most abundant and most EAD-active in pheromone gland extracts of female CBT (Fig. 1); 2) identifications were based on comparative GC and GC-MS of female-produced and authentic standards before and after treatment with DMDS; and 3) in field experiments, male CBT moths were strongly attracted to rubber septa impregnated with E9- plus Z9-12:OAc at blend ratios of 50:50 or 40:60. These results are consistent with the finding by Minks et al. (1976) that the blend of E9- plus Z9-12:OAc is a potent attractant of male CBT moths in Western Europe. Attractiveness of E8- plus Z8-12:OAc (Alford 1978, Hrdy et al. 1979) may be attributed to their structural similarity with the putative sex pheromone of CBT, E9- plus Z9-12:OAc.

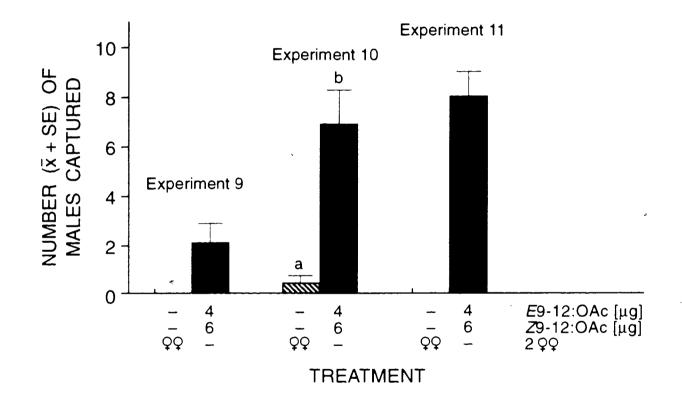
Six other EAD-active compounds were identified in pheromone gland extracts of female CBT, but none of them enhanced attractiveness of the two-component pheromone blend. Although extracted from pheromone glands and EAD-active, these additional

FIG. 5. Mean number of male *E. formosana* captured in sticky Delta traps baited with *E*9-12:OAc plus *Z*9-12:OAc (black bars) or with two caged virgin female *E. formosana*. Exp. 9 (17-18 June 1996; n=6), Exp. 10 (20-21 June 1996; n=8) and Exp. 11 (30 June to 1 July 1996; n=8) all conducted in New Westminster, British Columbia. Within each experiment, bars with the same letter superscript are not significantly different. ANOVA (P<0.05) followed by Student-Newman-Keuls' test, α <0.05.

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compounds may not be present in the effluvium of a calling female. Alternatively, they may serve to prevent or reduce cross attraction of heterospecific males, or play a role in close-range courtship behaviour. If as yet unknown compounds 2 and 3 (Fig. 1) are part of the CBT pheromone blend, their admixture to synthetic *E*9- and *Z*9-12:OAc may help to reduce the pheromone dose that outcompetes attractiveness of calling unmated female CBT (Fig. 5).

The acetates E9-12:OAc plus Z9-12:OAc are also major pheromone components of several other Qld World tortricids (Arn et al. 1992). A blend ratio of 50:50 to 60:40 strongly attracts males of the cereal tortrix, *Cnephasia pumicana* Zeller, a European pest of cereal grains (Biwer et al. 1977). Similarly, blend ratios of 50:40 and 80:20 attract males of respectively *Homona spargotis* Meyrick in Australian avocado orchards (Whittle et al. 1987) and *Ancylis sativa* (Liu) in Chinese date orchards (Lin et al. 1984). In my field trapping studies in urban areas of south-western B.C., lures baited with *E*9-12:OAc plus Z9-12:OAc at a 40:60 ratio demonstrated strong attraction of male CBT moths with insignificant cross-attraction of heterospecific male tortricids.

Because the two pheromone components of the CBT are chemically stable, readily available and formulated, and at a 10 mg dose are highly attractive to female-seeking males (Fig. 5), pheromone-based mass trapping may become a control tactic for this insect pest. In 1996, mass trapping of male CBT moths was initiated on >1,200 boulevard trees in ~70 city blocks of New Westminster (M. Clodius, Centre for Pest Management, Simon Fraser University; pers. comm.). Comparative analyses of trap captures of CBT males in consecutive years will allow investigators to assess whether this tactic suppresses populations of the CBT and reduces damage inflicted by CBT. Failure of E9-12:OAc singly to attract CBT males (Fig. 2) may be attributed to an imbalance in the pattern of sensory input perceived by mate-seeking males.

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3.0. PHEROMONE-BASED DISORIENTATION OF MALE CBT MOTHS

3.1. INTRODUCTION

Synthetic sex pheromones have been used successfully to suppress mating of several lepidopteran agricultural pests (Baker et al. 1990; Jenkins et al. 1990; Rice and Kirsch 1990; Vickers 1990; Trimble 1993; Fitzpatrick et al. 1995; Judd et al. 1997) by disrupting their pheromonal communication. Mate-seeking male moths are disrupted by natural (Cardé 1981) or "imbalanced" ratios (Bartell 1982) of synthetic pheromone components disseminated in the atmosphere of various crops. Neither tactic has been evaluated for control of lepidopteran pests of urban amenity trees.

I report disruption of pheromonal communication in CBT around urban amenity trees. using *E*9-12:OAc singly at various doses, and in ternary combination with *Z*9-12:OAc and *Z*7-10:OAc. Z7-10:OAc was included because initially it seemed to increase attractiveness of the *E*9- plus *Z*9-12:Oac pheromone blend (Fig. 3; Exp. 5).

3.2. MATERIALS AND METHODS

3.2.1 Disruption of Pheromonal Communication

Four experiments were conducted using rows of mature (>30-year-old) ornamental cherry trees, spaced >15 m apart along the boulevards of suburban streets in New Westminster and Vancouver, B.C. Within each experiment, treated and control trees were randomly selected. Experiments tested the effect of single and three-component pheromone blends (Table 2), released from polyvinyl tubing dispensers with polyurethane cores (Phero Tech Inc., Delta, B.C.), attached to tree trunks, on the capture

Exp. no.	n	Period (1995)	Release Devices ^a	Compound(s) Released ^b	Release Rate (per tree) ^c
12	10	3 May - 8 Sept.	4 polyvinyl chloride tubes, I.D. 4.2 mm, O.D. 4.7 mm, 1.95 m long	E9-12:OAc (49.5%) Z912:OAc (49.5%) Z7-10:OAc(1%)	29 mg/24 h (3 compounds combined)
13	10	3 May - 8 Sept.	4 polyvinyl chloride tubes, I.D. 4.2 mm, O.D. 4.7 mm, 1.95 m long	<i>E</i> 9-12:OAc	27 mg/24 h
14	12	7 July - 1 Sept.	1 polyvinyl chloride tube, I.D. 3.5 mm, O.D. 4.0 mm, 2.0 m long	<i>E</i> 9-12:OAc	4 mg/24 h
15	12	23 June - 1 Sept.	l polyvinyl chloride tube, I.D. 3.5 mm, O.D. 4.0 mm, 2.0 m long	<i>E</i> 9-12:OAc	0.5 mg/24 h

Table 2. Number of replicates (n), time periods, release devices, as well as chemicals and release rates employed in Exps. 12-15.

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- ^a Three holes were drilled in each tube (5, 85 and 165 cm from the top) through which copper wire (American wire gauge 19) (Exps. 12, 13) or transparent fishing line (Exps. 14, 15) were threaded to attach ropes snugly, parallel to tree trunks (Fig. 1) equidistant from each other (Exps. 12, 13).
- ^b E9-12:OAc = (E)-9-dodecenyl acetate; Z9-12:OAc = (Z)-9-uodecenyl acetate; Z7-10:OAc = (Z)-7-decenyl acetate; all compounds >97% chemically pure (Bedoukian Inc., Danbury, CT).

^c Pheromone release rates over time in each experiment were linear. At the end of experiments 12 and 13, an average of 41% (Exp. 12) and 46% (Exp. 13) of pheromone were still present in dispensers. At the end of experiments 14 and 15, an average of 55% (Exp. 14) and 51% (Exp. 15) of pheromone were still present in dispensers.

of male CBT moths in synthetic pheromone-baited traps. Pheromone release rates in these experiments were modified by adjusting the length and number of polyvinyl tubes on each tree (Table 2).

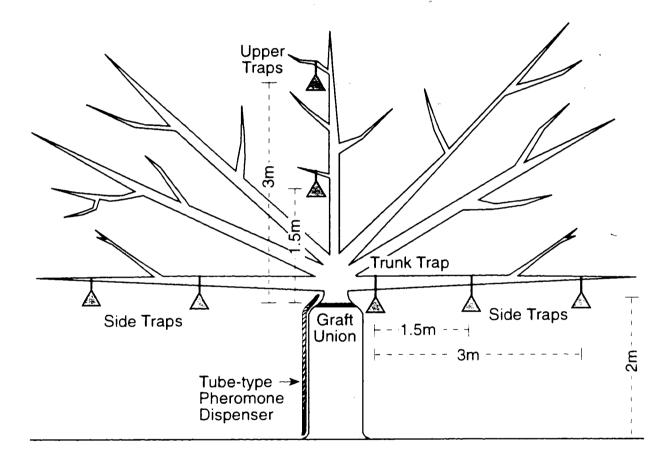
3.2.2. Assessing Disruption of Pheromonal Communication

Sticky two-litre milk-carton delta traps (Gray et al. 1984), baited with a grey rubber septum (West Co., Lionville, PA, USA) impregnated with pheromone components [*E*9-12:OAc (40 μ g), *Z*9-12:OAc (60 μ g) and *Z*7-10:OAc (1 μ g)] were used to assess disruption of pheromonal communication. Captures of male CBT moths in "trunk" traps (Fig. 6) were recorded weekly throughout the experimental period, and in "upper" and "side" traps for shorter periods. Trap lures in control and treated trees were replaced every three weeks during an experiment.

Efficacy of "disruption" treatments within each experiment was evaluated using two different methods. The first method compared mean trap catches in treated and control trees. The second method counted the number of traps in each treatment catching at least 1 male during the weekly interval (Rothschild 1981, Charlton and Cardé, 1981).

3.2.3. Estimation of Pheromone Release Rates

Release of pheromone from polyvinyl tubing dispensers was assessed by collecting, and measuring pheromone loss from, subsamples of dispensers attached to trees near experimental plots. At experiment initiation and at seven (Exps. 12, 13) and four (Exps. 14, 15) two-week intervals, the lowest 1 cm of these tubes was discarded and tubing 2 cm (Exps. 12, 13), 15 cm (Exp. 14) or 30 cm (Exp. 15) in length was removed and stored at FIG. 6. Schematic cherry tree depicting placement of tube-type pheromone "disruption" dispensers (only one shown) and placement of pheromone-baited traps at the tree trunk ("trunk" trap), upper canopy ("upper" traps) and to the north and south ("side" traps). Traps served to assess the within-tree zone of effective disorient-ation of male CBT.



 \sim -1°C. Prior to analysis the samples were thawed and weighed. Pheromone components were ether-extracted (16-20 hr) and the extracts were analysed by gas chromatography, employing a Hewlett Packard 5890 equipped with an RT-1701-coated fused silica column (30 m x 0.25 mm ID). Pheromone release rates per tube and per tree were deduced by weighing residues and calculating the percentage of pheromone weight per sample.

3.2.4. Statistical Analyses.

Reductions in trap catches of male CBT moths in treatment- and control-tree traps were subjected to analysis of variance (ANOVA) followed by Student-Newman-Keuls' test (α =0.05). The number of traps catching at least one male CBT were compared using χ^2 test, α <0.05 (SAS Institute Inc., Cary, NC).

3.3. RESULTS

All four treatments in pheromone-based disorientation Exps. 12-15 provided a 96-99% reduction in the number of male CBT moths captured in pheromone-baited "trunk" traps (Fig. 6) on pheromone-treated trees as compared to untreated control trees (Fig. 7). These reductions remained consistent over the entire flight season (Exps. 12, 13), or the duration of the experiment (Exps. 14, 15). All four treatments also reduced the number of traps that captured at least one male, but reductions were less pronounced (Fig. 8).

Release (29 mg/24h) of the three-component (*E*9-12:OAc, *Z*9-12:OAc and *Z*7-10:OAc) pheromone blend resulted in significant, seasor-long reduction (96.5%) in total captures of male CBT moths in trunk traps on pheromone-treated trees (51) relative to control trees (1.412) (Fig. 7, Exp. 12). *E*9-12:Oac released alone at 27 mg/24h also

FIG. 7. Numbers of male CBT moths captured in Exps. 12-15 in "trunk" traps (Fig. 6) baited with E9-12:OAc (40 μg), Z9-12:OAc (60 μg) and Z7-10:OAc (1 μg), and suspended from untreated control trees and trees treated with tube-type dispensers (Fig. 6. Table 2) releasing blends of the above three compounds at 29 mg/24h (Exp. 12); E9-12:OAc alone at 27 mg/24h (Exp. 13), 4 mg/24h (Exp. 14), or 0.5 mg/24h (Exp. 15).

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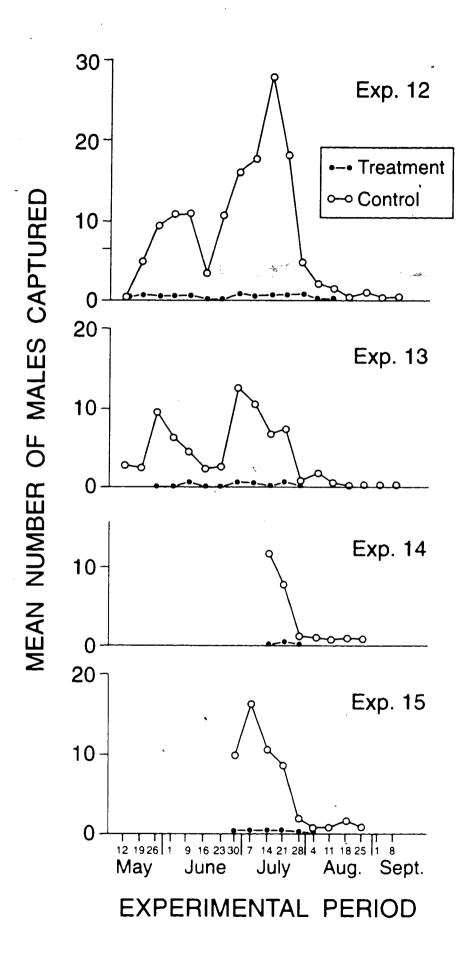
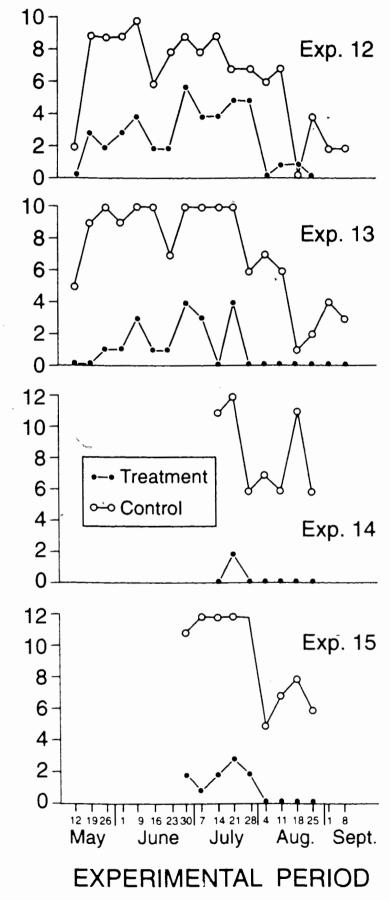


FIG. 8. Numbers of "trunk" traps (Fig. 6) in control and treatment trees capturing at least one male CBT moth per recording period in Exps. 12-15. Type and dose of pheromone released from tube-type dispensers as listed in Table 2; trap lures as described in Fig. 7 caption. For all experiments, the number of traps for treatment and control trees that captured at least one CBT male differed significantly (χ^2 -test, P<0.05).

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1. No.

NUMBER OF TRAPS THAT CAPTURED AT LEAST ONE MALE



caused a significant reduction (96.4%) in total captures of male CBT in trunk traps on pheromone-treated trees (30) compared to control trees (837) (Fig. 7, Exp. 13). When E9-12:OAc was released at the decreased rate of 4 mg/24h (Exp. 14) captures of male CBT in trunk traps on treated trees (2) were reduced by 99.3% compared to control trees (299) (Fig. 7). E9-12:OAc released at the lowest rate of 0.5 mg/24h also caused a significant reduction (98.0%) in total captures of male CBT in trunk traps on treated trees (13) compared to control trees (643) (Fig. 7).

When disruption of orientation to trunk traps was expressed as "number of traps catching at least one male CBT moth", E9-12:OAc alone (Fig. 9, Exp. 13) scored better (= fewer traps with at least one male captured) than the three-compound blend (Fig. 9, Exp. 12). Lower release rates of E9-12:OAc in Exps. 14 and 15 (Table 2) still resulted in a reduced number of traps that captured at least one CBT male in disrupted trees compared to untreated trees (Fig. 9).

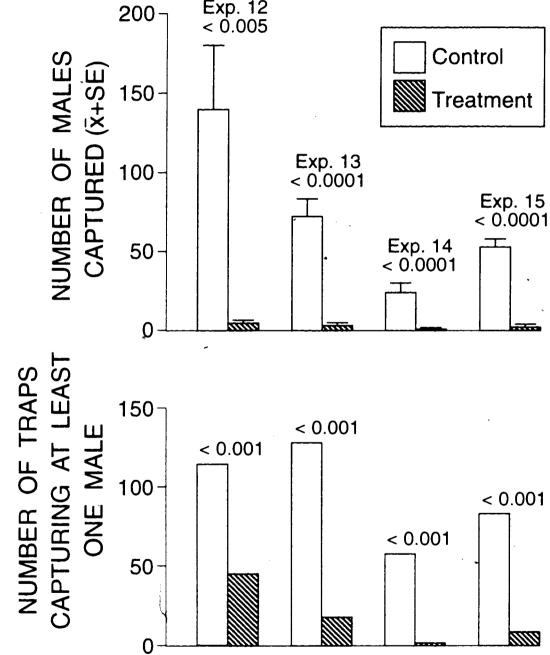
Captures of male CBT moths in "upper" and "side" traps (Fig. 6) of pheromonetreated trees were invariably lower than those in corresponding traps of control trees, but differences were not always statistically significant (Fig. 10).

3.4. DISCUSSION

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The efficacy of disorientation of male CBT in all experiments was expressed as percentage reduction in trap captures of male CBT in pheromone-treated relative to control trees. Because capture of even one male represents the possibility for a successful mating, efficacy of "disorientation treatments" was also, and more realistically, expressed as the number of traps per treatment that captured at least one male (Rothschild 1981,

FIG. 9. Number of CBT males in Exp. 12-15 captured in "trunk" traps (Fig. 6), and number of "trunk" traps capturing at least one CBT male, in control and treatment trees per recording interval. Type and dose of pheromone released from tube-type dispensers as listed in Table 2; trap lures as described in Fig. 7 caption.



Exp. 12 < 0.005

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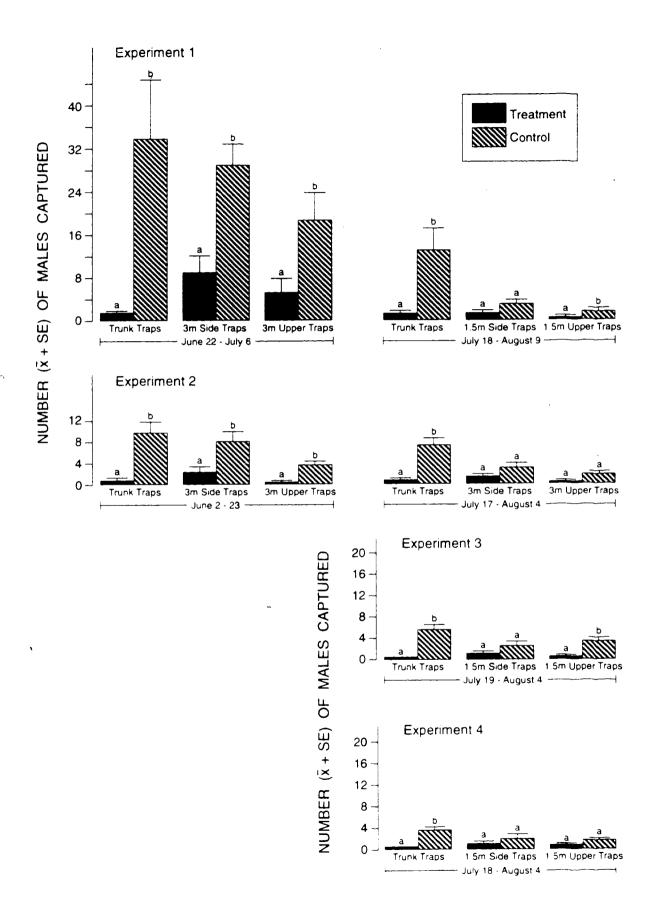
FIG. 10. Effectiveness of disorientation of male CBT moths assessed by their capture in pheromone-baited traps at the trunk (Exps. 12-15), 3 m (Exps. 12, 13) and 1.5 m (Exps. 12-15) above and on opposite sides of the tree's graft union area. Within paired comparisons, bars with the same superscript are not significantly different (P<0.05). Details about tube-type dispensers in Table 2; pheromone lures of traps as in Fig. 7 caption.

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Charlton and Cardé 1981). Based on either measure of assessment, captures of male CBT in trunk traps (Fig. 6) suspended in pheromone-treated trees were significantly reduced compared to control trees (Figs. 7-9), indicating disorientation of male CBT moths around the trees' graft union. However, disorientation of male moths may or may not have resulted in effective disruption of mating. Suppression of response by male moths to synthetic pheromone traps does not necessarily correspond with the level of mating disruption achieved (Rothschild 1981).

*E*9-12:OAc and the three-component blend at equivalent rates suppressed trap captures of male CBT moths equally effectively (Figs. 7-9, Exps. 12 and 13), although the underlying mechanisms ought to be different. Reduction of trap catches achieved by the three-component "disorientation" blend is likely based on "false trail following" by mate-seeking males or "camouflage" of the female's pheromone blend. The disorientation effect of *E*9-12:OAc, in contrast, may be associated with neurophysiological effects (Hiyori et al. 1986), such as an imbalance in sensory input (Flint and Merkle 1984).

Even though both numbers of moths captured and numbers of traps capturing at east one male were lower in "upper" and "side" traps of pheromone-treated trees than in respective traps of control trees, these differences were not always significant (Fig. 10). Disorientation of males caused in a treatment to the lower trunk would thus probably be insufficient to prevent them from locating, and mating with, females >1.5 m away from the trunk. The limited zone of effective disorientation may be attributed to the wide spacing of urban ornamental cherry trees that may effectively prevent the overlap of disorienting pheromone plumes that may characteristic of closely spaced "pheromone-

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disrupted" orchard trees. Because larvae of CBT and other small lepidopteran phloem feeders cannot penetrate intact bark (Bobb 1959, Pless and Stanley 1967), and female CBT typically "call", mate and oviposit on or near the tree's graft union, a small but effective zone of male CBT disorientation may suffice to prevent mating. However, graft-union-centred mating disruption might facilitate the development of "behavioural resistance by place of mating" because females that call from the tree's periphery are selected for.

With about 50% of pheromone retained in dispensers after 56-98 days (Table 2), pheromone release rates were likely sufficient to disorient male CBT moths throughout a four-month flight season. The lowest and still effective release rate of 0.5 mg/24h (Exp. 4; Figs. 8.9) is comparable to pheromone release rates for disorientation of other lepidopteran pests (Baker et al. 1990, Jenkins et al. 1990, Rice and Kirsch 1990, Vickers 1990, Trimble 1993, Fitzpatrick et al. 1995).

My finding that E9-12:OAc singly or with Z9-12:OAc and Z7-12:OAc (Figs. 7-9) effectively disoriented male CBT moths, may allow development of a "push-pull" management strategy for CBT (Miller and Cowles 1990). Unattractive E9-12:OAc could be used to disorient CBT males around susceptible tree trunks, while an attractive pheromone bait is employed to lure males into traps in non-host trees nearby.

4.0 CONCLUSION

The Palaearctic CBT is now established in south-western British Columbia and north-western Washington, where it has become the key pest of ornamental cherry trees. CBT also poses a threat to the orchard industry when it reaches interior, fruit-growing areas. A biorational control strategy for this insect is vitally needed.

My research objectives were to identify sex pheromone components for CBT, and to investigate the potential for one method of pheromone-based control. Analysis and identification of pheromone components in extracts of the female's pheromone gland were based upon coupled GC-EAD and coupled GC-mass spectrometry. The two most abundant and most EAD-active components in gland extracts. *E*9- and *Z*9-12:OAc, at ratios of 50:50 or 40:60 proved highly attractive to male CBT moths. Trap lures baited with 10 mg of synthetic *E*9- plus *Z*9-12:OAc strongly outcompeted attractiveness of CBT females. This result indicates the feasibility of mass trapping male CBT moths in pheromone-baited traps. In a large-scale experiment encompassing 70 city blocks of New Westminster (M. Clodius, SFU; pers. comm.), pheromone traps are being suspended from >1.200 rosacean trees, and weekly trap counts of CBT males are being maintained over three flight seasons to assess the impact of this tactic on CBT populations.

Lepidopteran pests within forestry and agriculture have been controlled by pheromone-based mating disruption, but the feasibility of this tactic has not been assessed for the protection of individual, relatively isolated urban amenity trees. I have successfully demonstrated pheromone-mediated disorientation of male CBT in four experiments, employing single and three-component pheromone blends. Attempting to assess whether oviposition by female CBT moths in these experiments had been reduced

on treated trees, trunks of both treated and untreated (control) trees were wrapped with "drain cloth" throughout the subsequent flight season. However, because predators and detritus feeders, such as spiders, ants and earwigs, left almost no remains of emergent CBT on either treated or control trees, counts of adult CBT were inconclusive.

Overall, my findings support the contention that pheromone-based tactics should become part of an integrated pest management strategy for CBT. Less easily quantified than my research findings, but equally encouraging, was the strong support given by hundreds of residents and passers-by within the experimental districts, who were first curious, then enthusiastic about this technique as an alternative to conventional pesticides.

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