## MATING DISRUPTION

# OF FRUITTREE LEAFROLLER, Archips argyrospilus, AND EFFECTS ON OTHER LEAFROLLER SPECIES

by

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# MATING DISRUPTION OF FRUITTREE LEAFROLLER (ARCHIPS ARGYROSPILUS) AND EFFECTS ON OTHER LEAFROLLER SPECIES

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MATING DISRUPTION OF FRUITTREE LEAFROLLER, Archips argyrospilus,

AND EFFECTS ON OTHER LEAFROLLER SPECIES

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### ABSTRACT

Mating disruption technologies for control of fruittree leafroller (FTLR), Archips argyrospilus (Walker), and other sympatric leafroller species were assessed. Identification of the sex pheromone of FTLR was also investigated. In small plot trials (0.09 ha), 2 pheromone blends released from Shin-Etsu rope-type dispensers were evaluated for their ability to decrease the number of leafrollers caught in pheromone- or virgin female-baited traps. The first blend, a 7:93 ratio of E11-tetradecen-1-ol and Z11-tetradecen-1-ol acetate (E11-14:OAc/Z11-14:OAc), released by Hamaki-con® dispensers was tested at concentrations of 1,000 and 2,000 disp./ha. The second blend, a 15:10:1:88 ratio of Z11-14:OAc. E11-14:OAc, Z9-tetradecen-1-ol acetate (Z9-14:OAc) and dodecan-1-ol (12:OH), released by 4-component dispensers was tested at a concentration of 1,000 disp./ha. Reductions in male catches in pheromone-baited traps in the different pheromonedisruption treatments were over 99% for FTLR, and 86-91% for obliguebanded leafroller, Choristoneura rosaceana (Harris), and European leafroller, Archips rosanus (L.). There were no significant differences between the pheromone-disruption treatments. Using virgin female-baited traps, reductions in catches of male FTLR were over 99% in the Hamakicon disruption-treatments, but only 80% in the 4-component disruption-treatment. Analysis of the effect of dispenser (Hamaki-con) density on reductions in pheromone trap catches of FTLR revealed no significant differences between treatments of 250, 500, and 1,000 disp./ha. However, the reductions in trap catches of FTLR were 97.9-99.8%. Mating disruption of FTLR was assessed in 1 ha plots using tethered virgin females. An 82%

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reduction in mating was achieved in the treated areas (1,000 Hamaki-con disp./ha). If this level of mating reduction were maintained over the entire flight period, a reduction in populations of FTLR would be expected in organic orchards.

A newly identified pheromone blend for FTLR, expected to be 6 times more attractive than the published blend, is made up of 5 components: *Z*11-14:OAc, *E*11-14:OAc, *Z*9-14:OAc, dodecan-1-ol acetate (12:OAc) and *Z*11-tetradecen-1-ol (*Z*11-14:OH) mixed in a ratio of 100:64:2:1:4. This new pheromone blend could increase the efficacy of mating disruption in FTLR when used to permeate an orchard environment.

# DEDICATION

To my wife Maria, my daughter Helena, and my parents Annette and Jacques for their understanding and encouragement.

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## **CHAPTER 1. INTRODUCTION**

### 1.1 Biology and control

The fruittree leafroller (FTLR), *Archips argyrospilus* (Walker), (Lepidoptera: Tortricidae) is a widespread and important pest of several fruit crops in the Okanagan Valley, B.C. The main cultivated hosts of FTLR include apple, *Pyrus malus* L., pear, *Pyrus communis* L., cherry, *Prunus* spp., and other stone fruits. Several native and ornamental trees and shrubs including birch, *Betula* spp., poplar, *Populus* spp., willow, *Salix* spp., rose, *Rosa* spp., and antelope bush, *Purshia tridentata* (Pursh) are also hosts (Paradis and Leroux 1965; Mayer 1973). FTLR causes direct fruit damage when larvae feed on the developing fruits. On apples, small wounds inflicted early in the season result in large russetted holes when the fruit matures. Damage levels as high as 20% have been recorded in orchards where no effective method of control was employed (Madsen 1970).

The FTLR is univoltine. Overwintering eggs hatch throughout April and May, usually between the 15 mm green and pink stages of apple-bud development (Madsen 1970). There are 5 larval instars. First, and second instars feed inside the newly opened buds, and are well protected from contact insecticides. Third, fourth and fifth instars feed on leaves and fruit, and build shelters by rolling leaves over themselves. Larvae are often present in orchards from April until June. Adults emerge 1 to 2 weeks after pupation; they are 8 to 10 mm long with distinctive brown and beige patterns on the anterior wings. Adults are generally present from June to August in B.C., and usually live about 9 days.

FTLR is a protandric species. The sex ratio changes throughout the adult flight and is male biased overall (male:female is 1.3:1). Mating occurs at temperatures above 16°C during the scotophase and under laboratory conditions, usually within days of female emergence (Paradis and Leroux 1965). From 82 to 112 eggs are laid in 1 or 2 egg masses on the bark of the tree usually on the branches. Egg masses are initially yellow and change to grey-mauve after 5 or 6 days, then to dark brown and finally to white, after the winter (Paradis and Leroux 1965).

Some of the important parasitoids of FTLR in the Okanagan Valley are: *Itoplectis quadricingulata* (Prov.) (Hymenoptera: Ichneumonidae), *Pseudoperichaeta erecta* (Coq.) (Diptera: Tachinidae), and *Nemorilla pyste* (Walker) (Diptera: Tachinidae) (Mayer 1973). The main insect predators are: *Chrysopa* spp. (Neuroptera: Chrysopidae), *Adalia bipunctata* L. (Coleoptera: Coccinellidae), *Tapinoma sessile* (Say) (Hymenoptera: Formicidae) (Mayer 1973). The red winged blackbird, *Agelaius phoeniceus* L., the brown headed cowbird, *Molothrus ater* (Boddaert), the american robin, *Turdus migratorius* L., the grey catbird, *Dumetella carolinensis* L., the chipping sparrow, *Spizella passerina* (Bechstein) and the mourning dove, *Zenaida macroura* L. are important predators in Québec (Paradis and Leroux 1965), and are also present in the Okanagan Valley.

A life table developed in Québec for FTLR (Paradis and Leroux 1965) has shown that annual decreases of 91 to 99% in populations of FTLR can be attributed to the above factors as well as to abiotic factors such as temperature. In conventional commercial orchards, the impact of biotic factors is usually reduced by the use of chemical pesticides and natural controls are generally insufficient to prevent economic damage.

In the last decade, resistance to the organophosphate insecticides, azinphos-methyl, and diazinon, has evolved in populations of FTLR near Kelowna (Madsen and Carty 1977; Vakenti *et al.* 1984; Cossentine and Jensen 1991). Other classes of insecticides, such as carbamates and pyrethroids, are not recommended for use (B.C. Ministry of Agriculture, Fisheries, and Food 1992) because they kill predaceous mites and thus have a negative impact on the integrated management of phytophagous mites. Current recommendations for control of FTLR in apples, include spraying Supracide® (which contains the organophosphate methidathion in dormant oil) at the tight-cluster stage, and *Bacillus thuringiensis* Berliner at the pink-bud or petal-fall stages if needed (B.C. Ministry of Agriculture, Fisheries, and Food 1992). These treatments are costly and have resulted in poor control on several occasions (H. Philip, personal communication<sup>1</sup>).

# 1.2 Mating disruption

Pheromone-mediated mating disruption for insect control has been the subject of considerable research in the last three decades, because it is species-specific and non-toxic (Einhom, 1986). By permeating the air of the target crop with synthetic sex pheromones, males can be prevented from finding females. It has been successfully used to control the pink bollworm, *Pectinophora gossypiella* (Saunders), the oriental fruit moth, *Grapholita molesta* Busk., the European grape berry moth, *Eupoecilia ambiguella* Hb., the fruit tortrix moth, *Adoxophyes orana* (Fisher von Roslerstamm), the tea tortrix, *Homona* 

<sup>&</sup>lt;sup>1</sup>Regional entomologist, B.C.M.A.F.F., Kelowna, B.C.

magnanima Diakonoff, the smaller tea tortrix, Adoxophyes sp., the codling moth, Cydia pomonella (L.), the tomato pinworm, Keiferia lycopersicella (Walsingham), and the western pine shoot borer, Eucosma sonomana Kearfott (Audemard 1988; Campion et al. 1989; Ridgway et al. 1990). Experiments conducted since 1988 have shown that codling moth can be controlled by this technique in the Okanagan Valley (Judd et al. 1992).

Species specificity of sex pheromone is characteristically insured by the release of a blend of a few chemicals in a precise ratio (Tamaki 1983). The male's ability to differentiate between ratios of the same chemicals has allowed the development of two main tactics for mating disruption: permeation of the air in the target crop with the entire pheromone blend (all the pheromone components identified) of the pest species (the tactic most often used), or with a partial pheromone blend (pheromone blend in which one or more of the identified pheromone components are missing) (Minks and Cardé 1988).

Three hypotheses have been proposed to explain the results obtained with this technique when the *entire* pheromone blend is used (Bartell 1982; Cardé 1990):

**1. Neurophysiological effect,** adaptation of the antennal receptors and/or habituation of the central nervous system, resulting in desensitization of the insect to the pheromone blend;

2. False trail following, diversion of males from the calling females by upwind responses to multiple artificial point sources of pheromone; and

**3. Camouflage,** inability of the male to distinguish the female signal over a background of generally higher concentration of similar odours.

Two mechanisms (Bartell 1982; Cardé 1990) potentially responsible for the decrease

in mating in an environment permeated with a *non-attractive partial* pheromone blend are: **1. imbalance in sensory input,** inability of males to recognize a natural, species-specific signal in a precise ratio when it is overwhelmed by the massive controlled release of a single component; and

2. Alteration of optimal ratio, following prolonged exposure to a single pheromonal component, males may be maximally attracted by a blend with a high ratio of that component, rather than to the natural blend. This phenomenon is probably due to partial adaptation or habituation to the single component.

Four components of the FTLR pheromone have been identified: *Z*11-tetradecen-1-ol acetate (*Z*11-14:OAc), *E*11-tetradecen-1-ol acetate (*E*11-14:OAc), *Z*9-tetradecen-1-ol acetate (*Z*9-14:OAc) and dodecan-1-ol acetate (12:OAc). A 15:10:1:50 mixture of these compounds resulted in maximum attraction of males FTLR (Cardé *et al.* 1977a). *Z*11-14:OAc is considered to be the most active component of the natural pheromone (Roelofs *et al.* 1974). High levels of dodecyl acetate have been used as a synergist to maximize trap catches (Roelofs *et al.* 1974).

Disruption of mating in populations of FTLR should have minimal effect on the management of other orchard pests, and should be completely compatible with the sterile insect release program soon to be implemented to control codling moth in the Okanagan Valley (Dyck and Gardiner 1992). Therefore, my objective was to evaluate mating disruption as a method for controlling FTLR.

# **CHAPTER 2. PHEROMONE DISPENSER CHARACTERISTICS**

Pheromone dispensers must generally meet 3 requirements: 1) they must release pheromone uniformly over time, 2) they must release different components in a precise ratio, and 3) they must protect against adverse environmental factors such as ultraviolet radiation (Weatherston 1991).

Molecular weight, solubility, and shape, can influence the speed of movement of a chemical through a barrier (Weatherston 1991). If components are released at different rates, the ratio of compounds released over time will change.

A number of chemical reactions can affect the pheromone content and hence the efficacy of a dispenser. These include: isomerization of double bounds caused either by UV light or heat; oxidation of aldehydes, alcohols, and polyenes also triggered by heat or UV light; and hydrolysis, enolization, and polymerization caused by changes in pH (Weatherston 1990).

I used rope-type dispensers (Shin-Etsu Chemical Co., Tokyo, Japan, supplied by Biocontrol Ltd., Davis, California), composed of two 20 cm long, superimposed, sealed polyethylene tubes. One tube is filled by an aluminium wire which allows the dispenser to be twisted firmly around a branch; the other is filled with pheromone (Nagata 1989). The dispensers are attached manually to branches at a height at which most of flight of the target species occurs. Because these dispensers release relatively large amounts of pheromone for several weeks, one application should be sufficient to control most univoltine species.

For the rope-type release device, the main intrinsic characteristics influencing the release rate are: 1) molecular weight and functional group of the pheromone molecule, 2) pheromone concentration, 3) thickness of the tube wall, 4) type, and stiffness, of the polymer used in the fabrication, and 5) presence of codiffusants (Weatherston 1991). The main extrinsic factor influencing the amount of pheromone released is likely to be the temperature.

Two rope-type dispensers were used. One was the Hamaki-con® dispenser manufactured to release only *Z*11-14:OAc for control of tea tortrix and smaller tea tortrix in Japan (Nagata 1989). A similar but white dispenser was custom-manufactured by Shin-Etsu to release the 4-component FTLR pheromone blend (Cardé *et al.* 1977a).

My objectives were to describe the chemical and release-rate properties of the 2 aforementioned dispensers, and to evaluate their suitability for mating disruption of FTLR.

# 2.1 Materials and Methods

#### 2.1.1 Exposure to sun

Ten Hamaki-con dispensers and 10, 4-component white dispensers were suspended on metal wires inside a white Stevensen screen at the Agriculture Canada Substation in Kelowna. Another 10 of each were suspended outside the Stevensen screen exposed to the sun. Five control dispensers of each type were held in glass jars at -15°C. At 4 and 8 weeks, 5 dispensers of each type from both the sun and shade treatments were

collected and stored at -15°C.

Twenty to 30 mg of pheromone solution was removed from each dispenser and diluted in HPLC-grade hexane to give a total volume of 25 ml. These solutions were analyzed<sup>1</sup> by coupled gas chromatography-mass spectrometry using a DB 5 capillary column, a nitrogen gas flow rate of 0.9 ml/min, and a temperature program of 50 to 260°C at 5°C/min. The mass ion detector of the mass spectrometer scanned a range of 45 to 200 atomic mass units. The weight of each pheromonal component was determined, and the ratios between components calculated. The relative proportions of each pheromone component over time were subjected to analysis of variance (ANOVA), on arcsin transformed proportions, followed by comparison of means by the Student-Newman-Keuls' (SNK) test ( $\alpha$ =0.05).

### 2.1.2 Influence of temperature on release rate

To assess the effect of temperature on release rate, 5 dispensers of each type were suspended on wires in each of seven controlled-environment chambers at temperatures of 6, 10, 15, 20, 25, 30, and 35°C. Once a week for 8 weeks, at approximately the same time of the day, the dispensers were weighed on a Mettler AE240 electronic balance. The velocity of air movement in the chambers was measured using a Kurtz hot wire anemometer (Series 490). Temperature was stable ( $\pm$  1°C) as verified with a Belfort

<sup>&</sup>lt;sup>1</sup> All chemical analyses were performed by Dr. T. Cottrell, Agriculture Canada, Research Station, Summerland, B.C.

thermohygrograph. During the fourth week the chamber maintained at 15°C malfunctioned, and its temperature rose to 24°C. Weight loss for that week was not included in the analyses. Release rate was regressed on time and temperature using SAS (SAS Institute Inc., Cary, NC, USA).

### 2.2 Results and Discussion

## 2.2.1 Exposure to sun

Neither type of control dispenser contained pheromone components in the expected ratios. The Hamaki-con dispensers contained approximately 93% *Z*11-14:OAc and 7% *E*11-14:OAc rather than 100% *Z*11-14:OAc as specified by Biocontrol Ltd., Davis, CA. The *E*11-14:OAc contaminant likely originated from the commercial synthesis of *Z*11-14:OAc. The 4-component dispensers contained 3 of the 4 known components of FTLR pheromone (Cardé *et al.* 1977a), but had dodecan-1-ol (12:OH) instead of dodecan-1-ol acetate (12:OAc). The ratio of the 3 valid components was similar to that described by Cardé *et al.* (1977a). The proportion of 12:OH (76.8%) was slightly higher than that reported for 12:OAc (65.7%) in the natural blend.

### 2.2.1.1 Four-component dispensers

Although only small changes in the relative proportions of each component of the 4-

component dispensers occurred under shaded conditions, the ratios were significantly different from controls after 8 weeks (12:OH, df=2,12, F=23.61, P<0.001; Z9-14:OAc, df=2,12, F=7.06, P<0.01; E11-14:OAc, df=2,12, F=16.44, P<0.001; Z11-14:OAc, df=2,12, F=30.15, P<0.001). Most of these differences are probably attributable to an increase of 3% in the proportion of 12:OH after 8 weeks. This phenomenon may indicate different rates of release among components (Fig. 1a). The ratio of E11-14:OAc/Z11-14:OAc increased only slightly but significantly (df=2,12, F=4.76, P<0.05) during this period, changing from 0.67 to 0.70 (Fig. 2). The pheromone content (% of total dispenser contents) did not change significantly (df=2,12, F=0.85, P>0.1) during 8 weeks in the shade, remaining near 91% (Fig. 3). The other contents in the dispenser include anti-oxidants and UV stabilizers.

After 8 weeks in the sun, differences in the relative proportions of pheromone components were larger than in the shade (Fig. 1). The changes overtime were statistically significant (12:OH, df=2,12, F=16.24, P<0.001; Z9-14:OAc, df=2,12, F=9.90, P<0.01; E11-14:OAc, df=2,12, F=8.82 P<0.01; Z11-14:OAc, df=2,12, F=37.37, P<0.001). The observed changes in relative proportions are most likely due to different rates of release among components resulting in a 3.3% increase in the proportion of 12:OH, and a large, significant (df=2,12, F=117.6, P<0.001), increase from 0.67 to 0.90 in the E11-14:OAc/Z11-14:OAc ratio (Fig. 2). The increase in E11-14:OAc is most likely the result of UV-caused isomerization (Weatherston 1991). Other degradation reactions were probably occurring, as the relative percentage of pheromone in the total contents of the sun-aged dispensers decreased significantly (df=2,12, F=10.21, P<0.01) from 92% to 79%

Fig. 1. Mean percentage of each pheromone component in 4-component dispensers after exposure to shade (a) or sun (b) for 4 or 8 weeks (n=5).

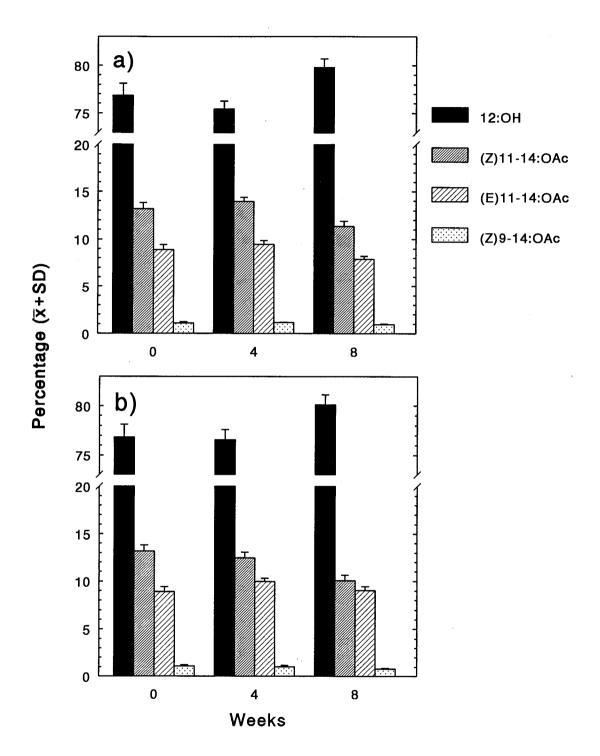
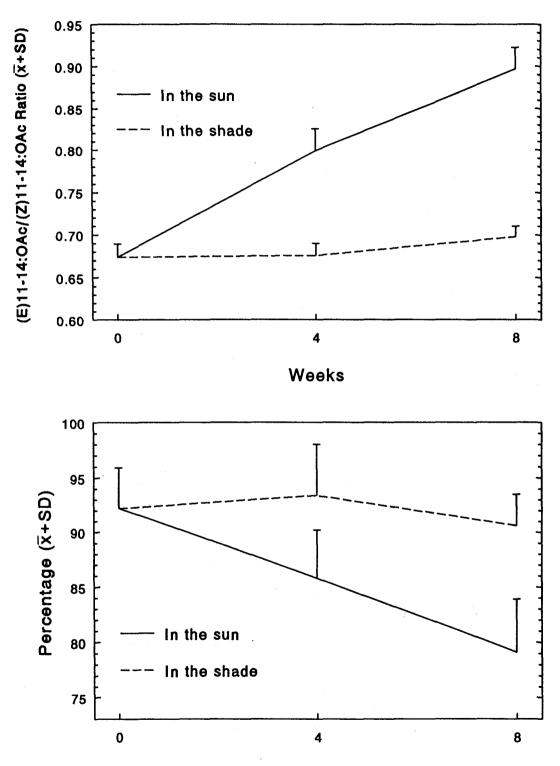


Fig. 2. Mean *E*11-14:OAc/*Z*11-14:OAc ratio in 4-component dispensers after exposure to sun or shade for 4 or 8 weeks (n=5).

Fig. 3. Mean percentage of pheromone in total dispenser content for 4-component dispensers exposed to sun or shade for 4 or 8 weeks (n=5).



Weeks

1.4

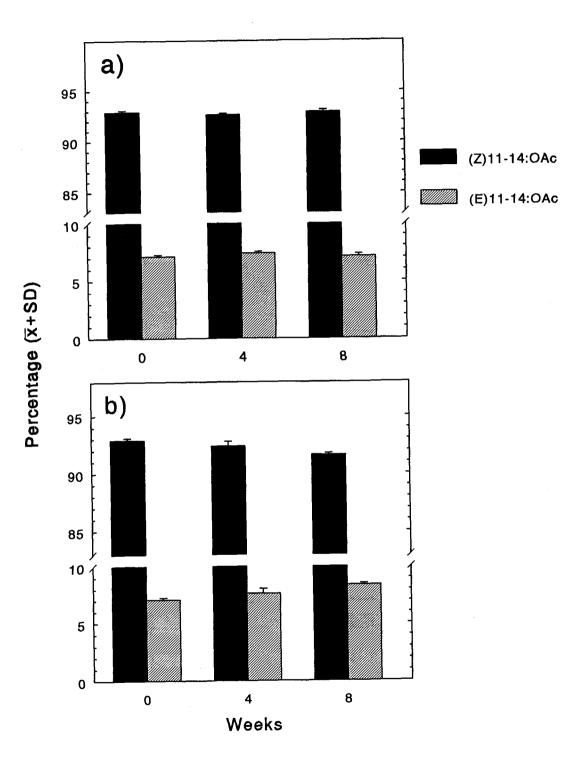
(Fig. 3).

### 2.2.1.2 Hamaki-con dispensers

In Hamaki-con dispensers, very small but significant (*E*11-14:OAc, df=2,12, F=4.32, P<0.05; *Z*11-14:OAc, df=2,12, F=4.32, P<0.05) changes occurred in the proportion of each compound after 8 weeks under shaded conditions (Fig. 4a). As in sun-exposed 4-component dispensers significant isomerization of *Z*11-14:OAc to *E*11-14:OAc probably occurred (df=2,12, F=26.36, P<0.001). The *E*11-14:OAc/*Z*11-14:OAc changed from .076 to .092 (Fig. 5). There was no significant effect on the percentage of the dispenser contents (sun exposure, df=2,12, F=3.56, *P*>0.05; shade exposure, df=2,12, F=1.38, *P*>0.1) (Fig. 6).

The major concern regarding the suitability of these dispensers for mating disruption of FTLR is their original content. 12:OH was not identified as a component of female FTLR pheromone glands eliciting a response from the male antenna in a gas chromatographic-electroantennographic detector (GC-EAD) analysis (see chapter 4). Thus, it probably will not affect the bioactivity of the other materials in the ropes. However, the absence of 12:OAc, which accounts for a 40% increase in the attractiveness of the FTLR blend (Cardé *et al.* 1977a), could lower the effectiveness of a disruption trial, if mechanisms such as false trail following or camouflage were operating. A new EAD test on FTLR using purified 12:OH is required to be certain of male response to this component.

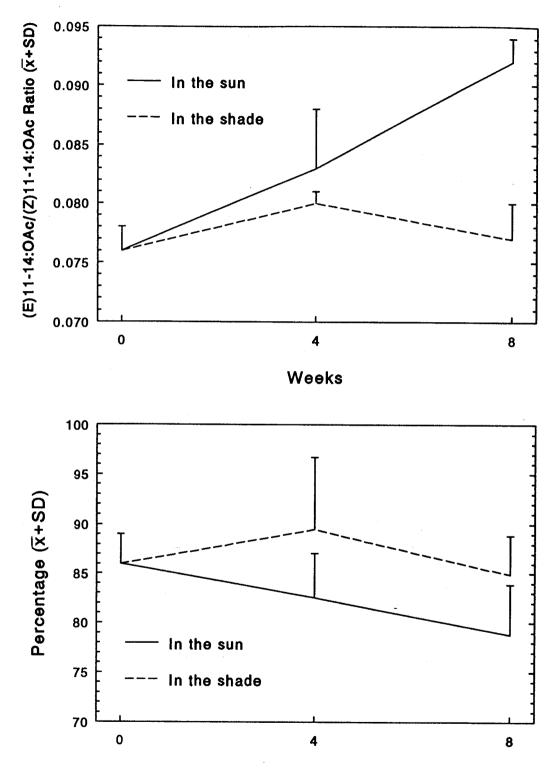
Fig. 4. Mean percentage of each pheromone component in Hamaki-con dispensers after exposure to shade (a) or sun (b) for 4 or 8 weeks (n=5).



1.7

Fig. 5. Mean *E*11-14:OAc/*Z*11-14:OAc ratio in Hamaki-con dispensers after exposure to sun or shade for 4 or 8 weeks (n=5).

Fig. 6. Mean percentage of pheromone in total dispenser content for Hamaki-con dispensers exposed to sun or shade for 4 or 8 weeks (n=5).





The presence of E11-14:OAc, a known FTLR pheromone component, in the Hamakicon dispensers could also decrease the disruptive effect if disruption were dependent on an imbalance in sensory input. However, because the concentration of E11-14:OAc in the Hamaki-con dispenser is small in comparison to that found in the natural pheromone, the loss in efficacy might be minimal. The extent of isomerization of Z11-14:OAc would not likely be as high under field conditions, as in the sun treatment, because dispensers suspended in trees are partially shaded.

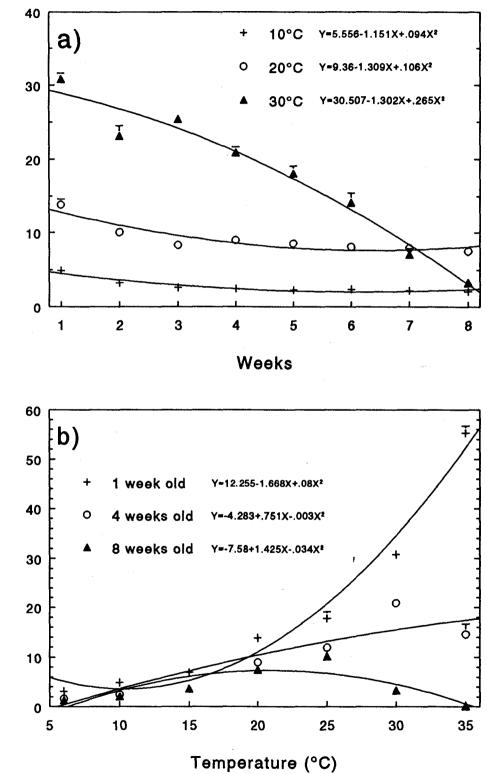
# 2.2.2 Influence of temperature on release rate

Air speeds in the controlled-environment chambers varied from 0.15 to 0.25 m/sec, not high enough to affect release rates from similar dispensers (Brown *et al.* 1992).

Release rates typically decreased initially and later stabilized. This pattern was observed for both dispensers at all temperatures, with the exception of the 4-component dispensers at 30 and 35°C (Fig. 7,8). The 4-component dispenser released pheromone at approximately twice the rate of the Hamaki-con dispenser, probably due to different constituent polymers (the 4-component dispenser was much more flexible than the Hamaki-con dispenser).

Release rates generally increased with increasing temperature (Fig. 7,8). However, as the 4-component dispensers aged, the release rates began to decline before the 35°C maximum temperature was reached. Release rates were maximal at 30 and 25°C for the 4- and 8-week-old dispensers, respectively. This may indicate that at temperatures above

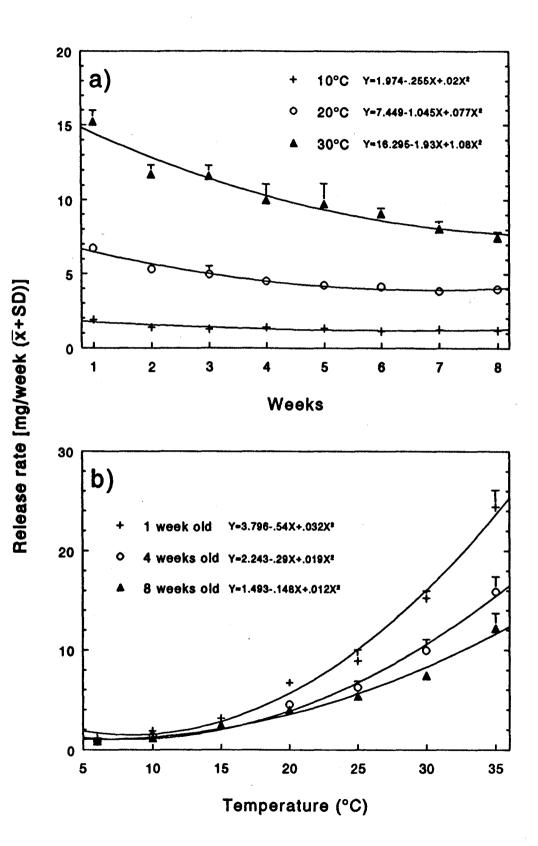
Fig. 7. Mean (n=5) pheromone release rates from 4-component dispensers held at 3 different temperatures as a function of time (a), and at 3 different ages as a function of temperature (b). Regression analyses for (a): 10°C, df=2,37 F=98.21, r<sup>2</sup>=0.84, P<0.001; 20°C, df=2,37, F=73.44, r<sup>2</sup>=0.80, P<0.001; 30°C, df=2,37, F=392.15, r<sup>2</sup>=0.95, P<0.001. Regression analyses for (b): week 1, df=2,32, F=625.56, r<sup>2</sup>=0.98, P<0.001; week 4, df=2,32, F=71.42, r<sup>2</sup>=0.82, P<0.001; week 8, df=2,32, F=37.78, r<sup>2</sup>=0.69, P<0.001.</li>



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Release rate [mg/week (x+SD)]

Fig. 8. Mean (n=5) pheromone release rates from Hamaki-con dispensers held at 3 different temperatures as a function of time (a), and at 3 different ages as a function of temperature (b). Regression analyses for (a): 10°C, df=2,37, F=17.64, r<sup>2</sup>=0.49, *P*<0.01; 20°C, df=2,37, F=181.60, r<sup>2</sup>=0.91, *P*<0.001; 30°C, df=2,37, F=115.36, r<sup>2</sup>=0.86, *P*<0.001. Regression analyses for (b): week 1, df=2,32, F=816.67, r<sup>2</sup>=0.98, *P*<0.001; week 4, df=2,32, F=556.34, r<sup>2</sup>=0.97, *P*<0.001; week 8, df=2,32, F=410.76, r<sup>2</sup>=0.96, *P*<0.001.</li>



25°C the 4-component dispensers contain insufficient pheromone to ensure normal release for 8 weeks. This was not observed with the slower-releasing Hamaki-con dispensers. Based on hourly temperatures as measured (DP-212 datapod II, Omnidata International, Inc., Logan, Utah, USA) in a Stevensen screen in a Kelowna orchard, the mean temperatures during the flight period of FTLR in 1991 and 1992 were 18.3 and 19.0°C, respectively. Under these conditions, unless high winds increase the release rates, both types of dispensers should release a high level of pheromone for the usual, 8 weeks flight period of FTLR.

These 2 types of dispenser have several qualities which make them suitable for mating disruption of FTLR. Very few chemical transformations of the pheromone components in the dispensers are expected to occur when the dispensers are used in an orchard environment. The release rates of the different pheromone components in each of the 2 dispenser types are very similar. Manual application allows the dispensers to be placed in the upper third of the canopy where the pheromone is needed most (Madsen and Madsen 1982). Finally, they are filled with enough pheromone to ensure adequate release (2.5 to 17.8 mg/week/disp.) throughout the FTLR flight season. The only problems detected were the differences in actual versus advertised content, which could affect the efficacy of disruption. As alternative dispensers were not available, I analyzed the efficacy of these 2 dispensers for mating disruption of FTLR.

### CHAPTER 3. MATING DISRUPTION OF FRUITTREE LEAFROLLER

Several methods can be used to assess the success of mating disruption trials. Comparison of the number of moths captured in pheromone-baited traps in treated and control plots gives an estimate of reduced ability of a target insect to orient toward a synthetic pheromone source, and presumably toward a live female as well. This type of evaluation depends upon the extent to which the synthetic lure mimics the natural pheromone. It does not consider the possible use of visual or tactile cues by searching males, nor does it consider the possible short range orientation to additional pheromone components when males are close to females. Inhibition of response to traps has rarely been an accurate indication of the level of mating reduction (Rothschild 1981), but this method of assessment has often been used in screening of different pheromone blends (Flint and Merkle 1984b; Sanders 1981) or determining optimal concentrations for mating disruption (Webb *et al.* 1990).

Traps baited with virgin female have limitations similar to those baited with synthetic pheromones. Although virgin females do emit the complete pheromone blend, they may release it at low rates or not at all. Traps baited with feeding lures, have occasionally been used to attract females, which are then dissected to determine if they contain spermatophores in the bursa copulatrix, thus disclosing their mating status. However, females caught in these traps are often old and mated, thus complicating the interpretation of mating disruption (Audemard 1988).

One direct method of assessing mating disruption is through the use of virgin females

tethered to a mating table or directly to the host plant for a set duration (Alford and Silk 1983). The insects are then dissected to determine if they are mated. If virgin females are tethered where wild females are most likely to be found (e.g. the upper third of the tree canopy for FTLR) (Madsen and Madsen 1982), this method gives a direct measure of mating disruption. However, insemination of females without transfer of a spermatophore has been observed in oriental fruit moths following several consecutive matings (George and Howard 1968). Secondly, gradual absorption of the spermatophore by the female has been observed in several lepidopteran families (Drummond 1984), particularly in older females (Outram 1968). If either of these phenomena occur, the frequency of mating would be underestimated. However, a comparison is generally made between the numbers of mated females found in control and treated plots. Insemination without transfer of a spermatophore is most likely to occur in control plots where multiple matings are more probable. This would result in underestimating the efficacy of disruption. Since the possibility of consecutive matings is highest in densily populated areas, the accuracy of this method of assessment could be lowest at high population densities. Concern about absorption of spermatophores can be reduced considerably when young virgin females are left in the field for no more than 2 days.

Assessment of the level of infestation and damage to the crop is often the best way to evaluate the effectiveness of mating disruption. These assessments incorporate the mating success of the target insect, and the effect of immigration of mated females or larvae from outside the treated area. If immigration is important, a high level of damage can occur even if no mating takes place in a pheromone-permeated plot. Infestation and

damage assessments are difficult to use with FTLR for two reasons. Damage caused by FTLR cannot be differentiated from that caused by the first generation of three other sympatric leafrollers, European leafroller, *Archips rosanus* (L.), obliquebanded leafroller, *Choristoneura rosaceana* (Harr.), and threelined leafroller, *Pandemis limitata* (Rob.). Secondly, the egg masses and young larvae of FTLR cannot be discriminated morphologically from those of the European leafroller (Madsen *et al.* 1977).

Another factor that must be considered is plot size. This choice is influenced by financial concerns as well as by experimental requirements. Small plots are useful for initial screening of pheromone blends and concentrations, because experiments are more easily replicated, and it is possible to apply different treatments to plants with similar characteristics. However, small plots are not suitable for assessing damage or infestation levels because of the great potential for immigration of larvae or mated females.

Large plots better represent the situation in which mating disruption would be operationally applied. They also ensure that the insect is exposed to the pheromonal treatment for a long period of time before reaching the plot centre, allowing for any habituation to occur. The magnitude of the reduction in responsiveness to pheromone caused by habituation has been related to the duration of exposure to pheromone in the light brown apple moth, *Epiphyas postvittana* (Walker) (Bartell and Lawrence 1973). Plots as small as 0.01 ha (Suckling and Clearwater 1990; Alford and Silk 1983) and as big as several ha (Sanders 1979) have been used in mating-disruption experiments.

In orchards, topography of the plot and homogeneity of crown sizes of the trees are responsible for some of the variability observed in the level of mating disruption (Judd *et* 

al. 1992; Charmillot 1990).

The experiments reported in this chapter aimed at finding a pheromone treatment that could be used to control FTLR in commercial orchards. In the first experiment, the effect of different pheromone blends in disorienting FTLR to pheromone sources was determined. This was followed by a dose-response trial using the blend which gave the highest efficacy in the first experiment. The third experiment evaluated mating disruption more directly using tethered virgin females.

# <u>3.1 Effect of permeation of the environment with different pheromone blends on</u> pheromone trap catches of three sympatric leafroller species

It is generally believed that the complete pheromone blend will be the most effective mating-disruption treatment (Roelofs 1978). Yet, Minks and Cardé (1988) reported some cases for which a partial blend was the most successful treatment, provided that the pheromone concentration was higher when a partial blend was used instead of the complete blend. Having, at my disposal, only the 2 types of dispenser described above, my objective was to test the efficacy of these 2 partial FTLR pheromone blends for mating disruption.

Four pest species of leafrollers (Tortricidae) in the Okanagan Valley are the FTLR, European leafroller (ELR), obliquebanded leafroller (OBLR), and threelined leafroller (TLLR). All share a principal pheromone component *Z*11-14:OAc (Table 1). Thus, mating disruption against FTLR might also reduce damage by 3 other leafroller species as does

Table 1. Pheromone composition (ratios by weight) of four sympatric leafroller species in the Okanagan Valley of British Columbia.

Fruittree leafroller <sup>e</sup>	Obliquebanded leafroller <sup>6</sup>	European leafroller <sup>c</sup>	Threelined leafroller <sup>d</sup>
15	98	85	94
10	2	0.1	
1			6
50			
	0.75	15	
	3		
	15 10 1	15 98 10 2 1 50 0.75	15 98 85   10 2 0.1   1 50 50   0.75 15

<sup>a</sup>Cardé *et al.* (1977a). <sup>b</sup>Vakenti *et al.* (1988); Thomson *et al.* (1991). <sup>c</sup>Roelofs *et al.* (1976b); Guerin *et al.* (1985). <sup>d</sup>Roelofs *et al.* (1976a). the blend used commercially in Japan to control both the tea tortrix and smaller tea tortrix (Tamaki *et al.* 1983). The FTLR, ELR and OBLR were common in the Kelowna area where my experiment was conducted.

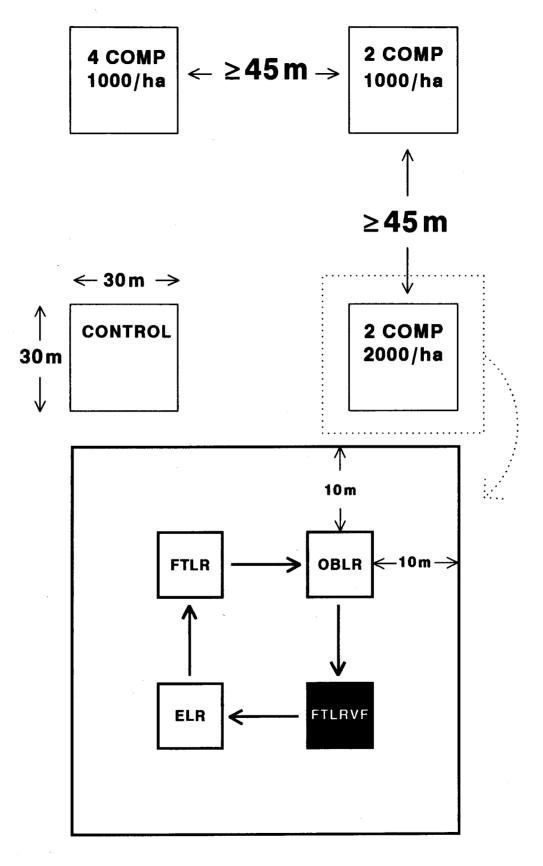
# 3.1.1 Materials and Methods

The effects of 3 pheromone-disruption treatments on the response of FTLR, ELR, and OBLR to traps baited with virgin females or synthetic pheromone were compared in a 4-replicate, randomized, complete block experiment. The first treatment employed a 4-component dispenser applied at a density of 1000/ha; the second and third treatments used the Hamaki-con dispenser applied at densities of 1000 and 2000/ha, respectively. An untreated control served as a fourth treatment.

Application rates were estimated by calculating the mean dispenser release rates over 8 weeks at 18.3°C (mean temperature of the flight period), using the quadratic equations describing the relationship between release rate and temperature (Chapter 2). The calculated mean application rates for each dispenser density were: 45 mg/h/ha for 1000 4-component dispensers, 20 mg/h/ha for 1000 Hamaki-con dispensers, and 39 mg/h/ha for 2000 Hamaki-con dispensers.

The 4 treatments were assigned to 30 X 30 m plots in two large commercial apple orchards near Kelowna, B.C. All dispensers were affixed in the upper third of the tree canopy, 7 days before the beginning of FTLR flight period. The plots in each replicate were at least 45 m apart in a square (Fig. 9). Both orchards were treated with

Fig. 9. Schematic representation of the experimental design of one replicate of Exp.1 showing layout of treatment plots and positions of baited traps within one plot. 2 comp and 4 comp represent Hamaki-con dispensers and 4-component dispensers, respectively. FTLR, OBLR and ELR represent fruittree leafroller, obliquebanded leafroller and European leafroller pheromone traps, respectively; FTLRVF represents fruittree leafroller virgin female trap.



Supracide®, *Bacillus thuringiensis*, and azinphos-methyl as needed, but due to the resistance of FTLR to some organophosphates, and to the difficulty in obtaining good coverage in the interior and upper part of the trees, relatively high populations of FTLR remained.

Within each plot were placed 4 traps baited respectively with synthetic pheromones for FTLR, OBLR, and ELR, and with 2 virgin female FTLR (Fig. 9). Each trap was suspended from a branch at a height of approximately 2 m. The traps were rotated in a clockwise direction, one position every week, so that each trap occupied each location twice in the 8-week experimental duration. Catches were counted 3 and 2 times/week for pheromone- and virgin female-baited traps, respectively.

The pheromone baits were prepared as in Table 1 using 97% pure Z11-14:OAc and Z9-14:OAc (Albany International, Needham Heights, Massachusetts), 98.2% pure E11-14:OAc and 93.9% pure Z11-14:Ald (Scentry Inc., Buckeye, Arizona), 97% pure 12:OAc (Aldrich Chemical Co. Inc., Milwaukee, Wisconsin), and 97% pure Z11-14:OH (Sigma Chemical Co., St-Louis, Missouri). Pherocon 1-C wing traps (Phero Tech Inc., Delta, B.C.) were baited with rubber septa loaded with 3 mg of a particular pheromone blend. All rubber septa were replaced after 4 weeks.

The virgin female FTLR trap was a yellow cylindrical trap (Proverbs *et al.* 1966). Twice each week, 2 virgin females  $\leq$  72 h old were placed in each trap. The number of males caught were counted 2 days later. Due to difficulty in collecting larvae, virgin females traps were used for only 2.5 weeks.

Statistical analysis was done on the total numbers of moths caught per trap type,

treatment and replicate. An Analysis of Variance (ANOVA) was performed on transformed data (log x+1) followed by a SNK test ( $\alpha$ =0.05).

### 3.1.2 Results and Discussion

For each of the 3 leafroller species the number of males caught in the control plots was significantly greater than those in pheromone-treated plots, which were not significantly different from each other (Table 2). However, significantly more male FTLR were caught in traps baited with virgin females than in traps baited with synthetic pheromone in plots treated with the 4-component dispensers (Table 3), even though the greatest quantity of pheromone was released in this treatment.

The less effective reduction in male FTLR captures in virgin female traps brought about by the 4-component blend might be related to the pheromone composition of the blend. The absence of 12:OAc, one of the 4 known pheromone components of FTLR (Cardé *et al.* 1977a) may have decreased the efficacy of the 4-component treatment by reducing false trail following and/or camouflage. Secondly, the presence of Z11-14:OAc, E11-14:OAc and Z9-14:OAc, in the natural ratio in the 4-component blend, may have decreased any disruptive effects of imbalanced sensory input that could have occurred with the Hamaki-con treatments.

The difference between the catches obtained with pheromone traps and virgin female traps in the 4-component treatment, might indicate that the known FTLR pheromone blend (Cardé *et al.* 1977a) is not the blend released by FTLR females in westem

Table 2. Response of male leafrollers to traps baited with synthetic pheromones, for their own species, in plots treated with different pheromone blends and dispenser densities over an 8 week flight period (n=4).

		Number of	males caught	(x ± SE) <sup>▶</sup>
Treatment <sup>#</sup>	Dispensers/ha	Fruittree ( leafroller	Obliquebanded leafroller	European leafroller
4-Component	1000	3.3 ± 1.0a	2.5 ± 0.9a	6.5±1.8a
Hamaki-con	1000	3.8 ± 0.9a	1.8 ± 0.5a	5.3 ± 1.1a
Hamaki-con	2000	2.8 ± 1.6a	2.0 ± 0.8a	1.8 ± 0.5a
Control	0	504.0 ± 104.4b	19.0 ± 5.3b	45.0 ± 16.5b

<sup>4</sup>4-Component treatment= *Z*11-14:OAc, *E*11-14:OAc, *Z*9-14:OAc, 12:OH, in a ratio of 15:10:1:88.

Hamaki-con treatment= Z11-14:OAc, E11-14:OAc, in a ratio of 93:7.

<sup>b</sup>Column means followed by the same letter are not significantly different (SNK test, P<0.05).

Table 3. Response of male fruittree leafroller to traps baited with synthetic pheromone or virgin females in plots treated with different pheromone blends and dispenser densities during the first two and half weeks of the flight (n=4).

		Number of males of	aught (x ± SE)
Treatment <sup>e</sup>	Dispensers/ha	Pheromone traps	Virgin female traps
4-Component	1000	$1.0 \pm 0.4a_1$	30.5 ± 12.1b <sub>2</sub>
Hamaki-con	1000	1.3 ± 0.5a <sub>1</sub>	1.0 ± 0.7a <sub>1</sub>
Hamaki-con	2000	2.0 ± 0.7a <sub>1</sub>	$0.3 \pm 0.3a_1$
Control	0	$163.3 \pm 44.9b_1$	153.5 ± 29.5c

"Treatments as in Table 2.

<sup>b</sup>Column means followed by the same letter, or row means subscripted by the same number are not significantly different (SNK test, *P*<0.05).

populations. The rate of pheromone release by the rubber septa was likely higher than by the 2 virgin females. The pheromone emission rate of FTLR females is unknown. However, oriental fruit moths and spruce budworms, Choristoneura fumiferana (Clem), release their main pheromone components at 3.2 to 8.5 ng/h (Baker et al. 1980; Lacey and Sanders 1992), and 4 to 20 ng/h (Ramaswamy and Cardé 1984), respectively. For both insects, a rate of release similar to that from one virgin female was obtained by loading rubber septa with 10 to 100 µg of pheromone (Baker et al. 1980; Ramaswamy and Cardé 1984). The rubber septa used in my experiment were loaded with 3 mg of pheromone. Assuming that females FTLR release their pheromone components at rates similar to those of oriental fruit moths and spruce budworms ca. 15 times more pheromone was released from each septum than would have been released by 2 virgin females. This release rate is not high enough to repel FTLR (personal observations). These observations support the hypothesis of a missing component, and suggest that the known pheromone blend (Cardé et al. 1977a) could be improved upon to disrupt western populations.

The high decrease (over 99%) in trap catches of FTLR in both kind of traps in the Hamaki-con treated plots (Table 4) indicates the potential of this treatment for operational mating disruption possibly at the low dispenser density of 1000/ha. The lower percentage reduction in trap catches of OBLR and ELR compared to FTLR in the Hamaki-con treated plots (Table 4) could be related to the high concentration of *Z*11-14:OAc in their natural pheromone blends (Table 1). If imbalance in sensory input or alteration of the optimal ratio were occurring, more disruption would theoretically be obtained when the pheromone

Table 4. Percentage reduction in captures of three leafroller species in traps baited with their own synthetic pheromone or virgin female fruittree leafroller in pheromone treated plots relative to untreated control plots.

		Per	cent reduction	on from cont	rols
		<u>Traps baited wi</u>	th synthetic	pheromone	Traps baited with 2 virgin
Treatment <sup>e</sup>	Disp./ha	Obliquebanded leafroller	European leafroller	Fruittree leafroller	female fruittree leafroller
4-Component	1000	86.8	85.6	99.4	80.2
Hamaki-con	1000	90.8	88.3	99.3	99.4
Hamaki-con	2000	89.5	96.1	99.5	99.8

"Treatments as in Table 2.

<sup>b</sup>The percentages have been calculated on the average number of moths caught (average catch for a given treatment X 100 / average catch in the control).

component released in the background represents a lower proportion of the target insect's natural pheromone blend.

The relative responses (mean catches) of male FTLR to different trap-types in each disruption treatment (Table 5) were significantly different from their response in the control plots, as indicated by the trap by treatment interaction (3 way ANOVA, df=6,18, F=22.06, P<0.001). Many FTLR males were caught in OBLR and ELR pheromone traps in treated plots (Table 5), causing differences in the distributions of males caught by trap type. This phenomenon could be explained in part by the fact that OBLR and ELR traps have a high proportion of Z11-14:OAc to which the male FTLR were exposed in the 3 disruption treatments. A change in the pheromone blend eliciting the strongest response, towards a blend having a higher proportion of the component to which the insect was exposed previously, has been documented in the laboratory (Linn and Roelofs 1981), and in the field (Flint and Merkle 1984a). Thus, the mechanism of disruption in the Hamaki-con treatments can at least partially be described as alteration of the optimal ratio (Cardé 1990).

# 3.2 Pheromone dispenser density trials

The number of dispensers/ha may influence the efficacy of disruption by regulating the concentration of pheromone released over a given area and by determining the number of point sources emitting pheromone. While habituation is likely to be influenced by the overall concentration of pheromone, false trail following is more probably affected

Table 5. Response of male fruittree leafroller to traps baited with its own synthetic pheromone or that of two sympatric leafrollers, in plots treated with different pheromone blends and dispenser densities during an 8 week flight period (n=4).

		Number c	f fruittree leafroller (x ± SE) <sup>6</sup>	males caught
Treatment*	Dispensers/ha	Fruittree leafroller traps	Obliquebanded leafroller traps	European leafroller traps
4-Component	1000	3.3 ± 1.0	$a_1  34.0 \pm 12.2a_2$	55.0 ± 17.1b <sub>2</sub>
Hamaki-con	1000	3.8 ± 0.9	$a_1 57.8 \pm 12.1a_2$	73.5 ± 19.2c <sub>2</sub>
Hamaki-con	2000	2.8 ± 1.5	$a_1 55.0 \pm 15.0a_2$	$33.3 \pm 6.5b_2$
Control	0	504.0 ± 104.4	$b_3 102.0 \pm 49.4a_2$	10.8 ± 3.4a

Treatments as in Table 2.

<sup>b</sup>Column means followed by the same letter or row means subscripted by the same number are not significantly different (SNK test, *P*<0.05).

by the number of point sources (Cardé 1990), and camouflage and imbalance in sensory input should be influenced by both factors.

The objective of this experiment was to determine the functional relationship between dispenser density and the level of reduction in trap catch.

# 3.2.1 Materials and Methods

As in the first experiment, 30 X 30 m plots were laid out in a randomized, complete block design in 3 commercial orchards near Kelowna, B.C. Three treatments of 250, 500, and 1000 Hamaki-con disp./ha, releasing an estimated 5, 11, and 21 mg/h/ha, respectively, were compared to an untreated control.

All dispensers were applied in the upper third of the tree canopy, after the beginning of the FTLR flight, but 4 days before any assessment of the treatments with traps baited with synthetic pheromone. Four Pherocon 1-C wing traps, baited with rubber septa loaded with 3 mg of FTLR, OBLR, ELR, or TLLR pheromone blends were used in each plot. All septa were changed after 2 weeks. Leafroller catches were recorded 3 times each week. Traps were placed as in Fig. 9, attached to branches at a height of approximately 2 m, and were rotated in a clockwise direction every week. To decrease the effect of an uneven distribution of FTLR in these orchards, all disruption treatments were rotated one position in a clockwise direction after 2 weeks by removing and redistributing all dispensers. With the exception of Z11-14:OAc (98.5% pure, Farchan Chemical Co.) all pheromone components used to prepare the pheromone trap baits, were as described

in Exp. 1 (section 3.1).

Total catches over the last 3 weeks of this 4-week experiment were transformed by  $\log_{10}(x+1)$  and subjected to ANOVA and the SNK test ( $\alpha=0.05$ ).

### 3.2.2 Results and Discussion

At each dispenser density, the number of FTLR males caught in pheromone traps during the first week was higher than in any other week (Table 6), although the time by treatment interaction (ANOVA) was not significant (df=9,27, F=2.04, P>0.05). This trend could possibly be related to a high population of male FTLR in the first week (accentuated by protandry). In addition, the ambient pheromone concentrations could have been low in the first week, prior to the establishment of an equilibrium between the pheromone released by the dispensers, and the pheromone adsorbed and re-released by the vegetation (Wall, et al. 1981). It is also possible that there was a delayed emigration of males from the treated plots. If a change in sensory input or an alteration of the optimal ratio are the operative mechanisms in mating disruption by partial blends, males of the target species should not perceive the presence of conspecific females in the permeated area. It is then likely that after a certain period of unsuccessful searching, they would migrate to other areas to find mates. Such a phenomenon could have caused decreased trap catches in the treated areas after the first week. In any case, dispensers should be applied in the orchard at least a week before the occurrence of high population of the target insect.

Treatments disp./ha	Ź	umber o cau	Number of fruittree lea caught by week	) leafroller <del>eek</del>	er	<u>leafn</u>	Perce <u>oller cau</u>	Percent reduction in fruittree	ction in 1 ive to co	Percent reduction in fruittree leafroller caught relative to control, by week	<b>X00</b> k
	-	2	ო	4	Grand total	-	2	e	4	All weeks	All Last 3 weeks weeks
0	776	689	255	45	1765						
250	47	16	ო	2	68	93.9	97.7	98.8	95.6	96.1	97.9
500	115	თ	2	-	127	85.2	98.7	99.2	97.8	92.8	98.8
1000	48	-		0	50	93.8	<u>99.9</u>	<b>9</b> .66	100	97.2	<b>99.8</b>

•

Table 6: Weekly catches of fruittree leafroller in plots treated with different densities of Hamaki-con dispensers (pooled catches from 4 replicates).

Catches of FTLR in the pheromone-treated plots in the 3 last weeks were different than in the controls (df=3,12, F=18.70, P<0.001), but there was no difference between pheromone treatments (SNK test, P>0.05). Decrease in the trap catch in the last 3 weeks relative to the control, were >97% in the 250 and 500 disp./ha treatment, and >99% in the 1000 disp./ha treatment (Table 6).

Results for OBLR, ELR, and TLLR (Table 7) were similar to those for FTLR. However, the very low numbers of moths caught in the control plots, does not allow a conclusion to be made about the effect of dispenser density on OBLR, ELR, and TLLR.

# 3.3 Assessment of fruittree leafroller mating disruption efficacy using tethered virgin females

Pheromone-baited traps provide an indirect and often inaccurate assessment of mating disruption (Rothschild 1981), because other cues excluded by trap design, could be utilized in mate searching. In a few lepidopteran species, visual stimuli are known to be important in short-range mate location (Carpenter and Sparks 1982; Castrovillo and Cardé 1980). The objective of this experiment was to assess the success of mating disruption in FTLR using tethered virgin females.

Table 7: Catches of European (ELR), obliquebanded (OBLR), and threelined leafrollers (TLLR) in pheromone baited traps, for the first and three subsequent weeks, in plots treated with different densities of Hamaki-con dispensers (pooled catches, from 4 replicates).

Treatment	Cat	ches in wee	<u>k 1</u>	Catches in weeks 2-4			
(disp./ha)	ELR	OBLR	TLLR	ELR	OBLR	TLLR	
0	29	17	3	11	20	16	
250	6	7	0	2	1	3	
500	4	3	0	2	1	1	
1000	4	0	1	0	0	0	

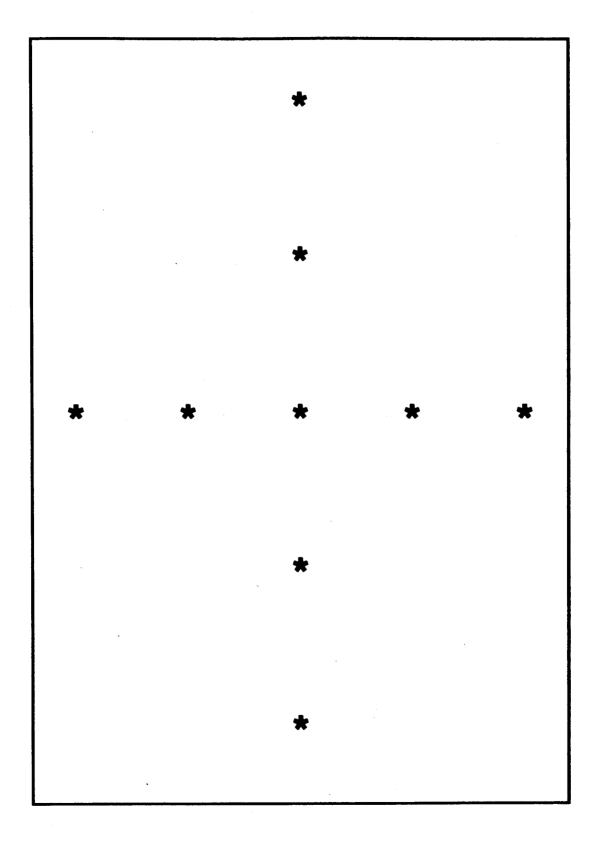
### 3.3.1 Materials and Methods

One ha, square or rectangular, treatment and control plots situated in the same orchard were separated by at least 50 m. Approximately 12 days before the beginning of the FTLR flight, Hamaki-con dispensers were applied uniformly in the upper third of the tree canopy at a density of 1000/ha providing an estimated application rate of 21 mg/h/ha.

In each of 4 replicates, 9 tethered virgin females per plot were placed individually in the upper third of the tree canopy. Threads used to tether females were looped over one forewing and the other end was attached to a branch. Females ≤72 h old were positioned along 2 perpendicular lines crossing in the plot centre (Fig. 10), with the outermost females at least 6 m inside the plot. Females were left in the field for 48 h during the first week of the experiment, but due to high mortality apparently related to hot weather, they were left for only 24 h during 3 subsequent weeks. Females were returned to the laboratory and examined for the presence of spermatophores in the bursa copulatrix. Tethered females were deployed in the first and third weeks in orchards A and B, and in the second and fourth weeks in orchards C and D. In the first and second weeks, 3 trials of 9 females per plot were conducted each week; 2 trials were conducted per plot per week in the third and fourth weeks. In each trial new females were tethered to a fresh branch on the tree used previously.

The data were analyzed by  $\chi^2$  and paired *t*-tests. An arcsin transformation was used on proportion data. Females found dead after exposure were excluded from analysis.

Fig. 10. Schematic representation of the placement of 9 tethered virgin females in 1 ha rectangular plot.



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### 3.3.2 Results and Discussion

Only 6% of the tethered virgin females were lost due to predation or escape, much less than in similar studies (Alford and Silk 1983; Cardé *et al.* 1977b). The high female recovery was probably because the orchards in which the virgin females were tethered, were treated with pesticides and likely had low predator populations.

Mating of tethered virgin female FTLR was reduced by >80% in the treated plots relative to the controls (Table 8). The reduction in mating was significantly higher in the centre of the treated plots than on the edge (Table 9). Because of this problem, a high density of pheromone dispensers is generally recommended on the edge of treated areas (Audemard 1988). If target males are not attracted into plots treated with a partial pheromone blend, the high mating occurrence on the periphery is probably mainly due to wind. Under a strong wind, the pheromone plume is narrow close to the dispenser, resulting in areas between dispensers that are not permeated with pheromone on the upwind edge. A female calling in one of these pheromone-free pockets would have a good chance of being located by males.

Based on pheromone trap catches in orchards located in the same general area as the experimental sites, there was an inverse relationship between the success of disruption and size of the FTLR population (Fig. 11). This relationship between efficacy of mating disruption and population level is common (Audemard 1988; Charmillot and Bloesch 1987), and has been attributed to the high probability of mating through random encounters, when populations are dense. The lower reduction in mating estimated by

Drchards"		Treated	·	Control	% Mating
	n	% Mating	n	% Mating	reduction
A	31	16	34	76	76
В	35	6	33	79	93
С	42	14	39	82	83
D	34	21	33	79	74
Mean <sup>b</sup>		14		79	82

Table 8: Mating of tethered virgin female fruittree leafroller in untreated and pheromonetreated plots (1000 Hamaki-con dispensers/ha). Data from five trials-per-orchard, were pooled.

<sup>e</sup>In orchards A and B, tethered virgin females were used from 15-22 June and 29 June-6 July. In sites C and D, tethered virgin females were used from 22-29 June and 6-13 July.

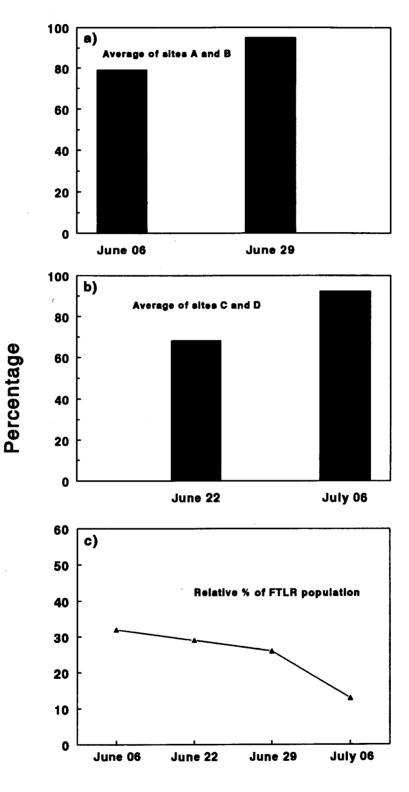
<sup>b</sup>The paired difference in mean percent mating is significant, t=18.75, P<0.001

Table 9: Comparison between the percentage mating reduction relative to the control in tethered virgin female fruittree leafroller in the periphery and in the centre of plots treated with 1000 Hamaki-con dispensers/ha.

	Percentage mating reduction		
Orchards	Edge	Centre	
Α	77	89	
В	95	94	
С	78	92	
D	67	89	
Mean"	80	91	

<sup>a</sup>Paired difference in mean percent mating reduction is significant, t=2.42, P<0.05.

Fig. 11. Percentage mating reduction in tethered virgin female fruittree leafroller in pheromone-treated (1000 Hamaki-con dispensers/ha) plots relative to the untreated control plots, average of sites A and B (a) and C and D (b), for 2 periods of one week, and relative percentage of the fruittree leafroller population in each of the 4 weeks of the tethering experiment (based on pheromone trap catches from orchards located in the same general area of the experimental sites) (c).



Date (beginning of the week)

tethering than by trap catch suggests that in addition to the published pheromone components male FTLR also use other cues to find females. My results are consistent with the onclusion that trap "shut down" is not a good indicator of the efficacy of mating disruption (Rothschild 1981).

On its own, a reduction in mating of 82%, would not bring about a decrease in the population of FTLR. However, Paradis and Leroux (1965) have shown that in organic orchards in Québec an annual reduction in the population of FTLR of 91.3 to 99% can be attributed to natural factors. If one assumes that similar biotic and abiotic pressures occur in the Okanagan Valley, one can calculate the level of mating disruption needed to keep the population constant. At the lowest annual population reduction observed in Québec (91.3%), a female fecundity of 102 eggs, and a sex ratio of 1.3 males/female, a single mated female would produce 3.9 females in the next generation [(102 X 8.7 /100) / 2.3]. Therefore, an average mating reduction of 75% would be necessary to keep the population constant, and a reduction of 82% would cause a decrease in the population of FTLR in an organic orchard. A smaller reduction in the population could be expected in conventional orchards as the biotic pressures on populations of FTLR would probably be smaller. This treatment would probably not be very efficient in orchards with very high populations of FTLR where numerous random encounters and consequently mating, would occur. Due to the relatively low number of tethered virgin females tested, and variability of 42-100% mating reduction in individual trials, a more extensive assessment of mating disruption should be conducted before it is implemented against FTLR in the Okanagan Valley. However, with the information gathered to date, this treatment has

great promise.

Mating disruption for FTLR, may also control other sympatric leafroller species, at least in part. Assessment of the effect on OBLR, ELR and TLLR must be done with tethered females, and some adjustments in components, release rates and dispenser density may be needed to achieve integrated control.

# CHAPTER 4. RE-ANALYSIS OF FRUITTREE LEAFROLLER PHEROMONE

Higher catches of FTLR in traps baited with virgin females than in synthetic pheromone-baited traps in disruption plots treated with the 4-component dispensers (Table 3), suggest that the pheromone blend identified by Cardé *et al.* (1977a) is incomplete for FTLR in the Okanagan Valley.

The identity of FTLR pheromone is based upon 3 studies. Madsen *et al.* (1973) found that a mixture of Z11-14:OAc and 12:OAc, attracted male FTLR to traps in British Columbia. Roelofs *et al.* (1974) identified *E*11-14:OAc as a third component of the FTLR pheromone. A 70:30 blend of Z11-14:OAc/*E*11-14:OAc was attractive to FTLR alone in B.C. and was synergized by 12:OAc. New-York males did not respond until 12:OAc was added to a blend of the 2, 11-14:OAc isomers. A fourth component, *Z*9-14:OAc was discovered by Cardé *et al.* (1977a). A blend of *Z*11-14:OAc, *E*11-14:OAc, *Z*9-14:OAc, and 12:OAc in a 15:10:1:50 ratio was the most attractive pheromone blend tested for both eastern and western populations of FTLR.

This chapter describes a re-analysis of the sex pheromone of western FTLR.

## 4.1 Materials and Methods

### 4.1.1 Laboratory analysis

FTLR larvae were field collected near Kelowna and reared to adults on apple leaves

at a photoperiod of 16:8. Two-to-three h into the scotophase, abdominal tips of 2- to 3day-old virgin females were removed and extracted for approximately 5 min in hexane. Aliquots of one female equivalent of pheromone gland extract were subjected to analysis by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn *et al.* 1975) and coupled GC-mass spectroscopy (MS), on 2 fused silica columns (each 30 m X 0.25 mm ID) coated with either DB-23 (J&W Scientific, CA 95630) or SP-1000 (Supelco, PA 16823)<sup>1</sup>.

# 4.1.2 Field experiments

Six field experiments (Exp.1-6), were conducted in 3 apple orchards near Kelowna using randomized, complete block design. Pheromone blends were compared by recording the number of male FTLR caught in either virgin female traps, or Pherocon 1-C wing traps loaded with pheromone. Traps were suspended 1.5 m above ground in outer tree foliage, at 15 m intervals. Each treatment was replicated 10 to 17 times, 5 to 6 times per orchard. Pheromone traps were baited with rubber septa (Sigma Chemical Co., St-Louis, Missouri) impregnated with candidate pheromone components (167-182  $\mu$ g in HPLC grade hexane) and pinned to the inner side of the trap top.

Exp.1 evaluated 10 of 12 chemicals identified in FTLR pheromone glands by GC-EAD analysis. All 10 had been identified as pheromone constituents of other tortricid species

<sup>&</sup>lt;sup>1</sup>All the chemical analyses were done by Dr. Gerhard Gries and Ms. Regina Gries, Assistant Professor and Research Technician, respectively, Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.

(Mayer and McLaughlin 1991). In one treatment these chemicals were reconstituted in the ratio found in pheromone gland extracts. This treatment was compared to the 4component FTLR pheromone blend described by Cardé *et al.* (1977a), to a mixture of the same 4 components in the ratio found in the western FTLR pheromone gland extracts (western blend), and to virgin females.

Exp.2, compared the "western blend", to different ratios of Z11-14:OAc/E11-14:OAc (Cardé *et al.* 1977a). This experiment was based on the findings of Roelofs *et al.* (1974) that a 70:30 ratio of Z11-14:OAc/E11-14:OAc, when tested as binary blend, was significantly more attractive than a 60:40 ratio for B.C. populations of FTLR.

Exp.3, tested whether any additional chemical in the 10-chemical blend (Exp.1) could increase the attractiveness of the "western blend". In all blends tested, the 4 previously published pheromone components (Cardé *et al.* 1977a) were present. The 6 other chemicals were separated into 3 groups, 3 alcohols [*Z*11-tetradecen-1-ol (*Z*11-14:OH), *E*11-tetradecen-1-ol (*E*11-14:OH), and *Z*9-tetradecen-1-ol (*Z*9-14:OH)], the *Z* and *E* isomers of dodecen-1-ol acetate (*Z*9-12:OAc and *E*9-12:OAc), and tetradecan-1-ol acetate (14:OAc). All possible combinations of these 3 groups were tested.

Exp.4 examined the role of the alcohol components alone. The 3 alcohols alone and in all possible combinations were added to the 4-component "western blend".

Two chemicals new to the Tortricidae identified by GC-EAD analysis were tested in Exp.5. I also re-tested *Z*11-14:OH and the complete group of alcohols added to the "western blend".

Exp.6 compared the "western blend", to the "western blend" plus Z11-14:OH.

In Exp.1, 2 and 4 captured FTLR were counted one day after traps were placed in the field. Catches in Exp.3 were counted after 1 day, the trap positions were re-randomized and a second count taken after the second day. The counts were pooled for statistical analysis. Exp.5 and 6 were conducted late in the season when FTLR numbers were low, so counts were taken after 2 days. Data were transformed by  $\sqrt{x+3/8}$  or ln(x + 1) and subjected to ANOVA and the SNK test ( $\alpha$ =0.05).

## 4.2 Results and Discussion

#### 4.2.1 Laboratory analysis

GC-EAD analysis of female pheromone gland extracts revealed 12 compounds that elicited responses by male moth antennae. Identical mass spectroscopic characteristics, identical retention times on DB-23 and SP-1000 columns, and comparable EAD-activity of authentic standards and female-produced compounds identified the following 8 candidate pheromone components: *Z*11-14:OAc, *E*11-14:OAc, *Z*9-14:OAc, 14:OAc, 12:OAc, *Z*11-14:OH, *E*11-14:OH, and *Z*9-14:OH. Four compounds were tentatively identified by comparing their retention indices on one fused silica column with those of authentic standards, as follows: *Z*9-12:OAc, *E*9-12:OAc, *E*11-tridecen-1-ol acetate (*E*11-13:OAc), and *E*4,*E*11-tetradecadien-1-ol acetate (*E*4,*E*11-14:OAc).

# 4.2.2 Field experiments

In Exp.1 the 10-chemical blend [treatment (trt) 3] and the 4-component "western blend" (trt 2) were significantly more attractive than the 4-component blend described by Cardé *et al.* (1977a) (trt 4) (Table 10). The lack of a significant difference between treatments 2 and 3 indicates that either none of the 6 additional chemicals in treatment 3 increased the attractiveness of treatment 2, or that one or more components were repellent for FTLR males, offsetting any enhanced attractiveness. The results clearly showed that the published pheromone blend for FTLR could be improved on the basis of laboratory analyses.

The results of experiment 2 showed that the ratio of the 4 components used in the "western blend" imparted significantly more attractiveness than occurred when the same components were mixed in different ratios (Table 11).

Exp.3 demonstrated that the combination of Z9-12:OAc and E9-12:OAc is repellent; the 4 least attractive blends contained these compounds (Table 12). Whenever the alcohol group was added to the "western blend" (trt.1 and 2) it increased the attractiveness of the "western blend". Adding the 14:OAc (trt.2 and 3) had no significant impact. Thus, the alcohol group was most promising.

Differences in the attractiveness of blends tested in experiment 4 were mostly not significant (Table 13). However, there was a trend towards higher attractiveness in chemical blends containing Z11-14:OH.

The "western blend" plus Z11-14:OH caught significantly more moths than any other

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Table 10: Evaluation of the attractiveness to male fruittree leafroller (FTLR) of pheromone traps baited with virgin females, published pheromone components, or a mixture of compounds identified from the pheromone gland extracts of female FTLR (n=10).

Trea	atment	Ratio of compounds (by weight)	Number of male FTLR caught (x ± SE) <sup>e</sup>
1:	FTLR virgin females (2)		103.3 ±8.6a
2:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc (western blend)	109:70:2:1	78.6 ±7.4ab
3:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 14:OAc, Z9-12:OAc, E9-12:OAc, 12:OAc, Z11-14:OH, E11-14:OH, Z9-14:OH	100:64:2:7:2:0.2:1:4:1:0.8	74.1 ±13.4b
4:	Z11-14:OAc, E11-14:OAc, <i>2</i> 9-14:OAc, 12:OAc (blend of Cardé <i>et al.</i> 1977a)	36:24:2:120	46.3 ±9.4c

<sup>4</sup>Means followed by the same letter are not significantly different (SNK test, P<0.05).

Table 11: Comparison of the attractiveness of different ratios of the 4 published fruittree leafroller (FTLR) pheromone components, to male FTLR (n=10).

Trea	atment	Ratio of compounds (by weight)	Number of male FTLR caught (x ± SE)*
1:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc (western blend)	109:70:2:1	49.4 <del>±9</del> .6a
2:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc	48:12:2:120	11.3 ±1.9b
3:	Z11-14:OAc, <i>E</i> 11-14:OAc, <i>Z</i> 9-14:OAc, 12:OAc (blend of Cardé <i>et al.</i> 1977a)	36:24:2:120	10.7 ±3.8b
4:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc	42:18:2:120	9.7 ±2.0b
5:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc	54:6:2:120	6.8 ±1.8b

<sup>e</sup>Means followed by the same letter are not significantly different (SNK test, P<=0.05).

Treatment Ratio of compounds Number of male FTLR (by weight) caught (x ± SE)\* Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc, 100:64:2:1:4:1:0.8 1: 48.9 ±11.2a Z11-14:OH, E11-14:OH, Z9-14:OH Trt. 1 + 14:OAc 100:64:2:1:4:1:0.8:7 2: 46.8 ±8.23a Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc, 100:64:2:1:7 30.9 ±7.21ab 3: 14:OAc Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc 100:64:2:1 20.2 ±3.6abc 4: (western blend) 5: Trt. 1 + 14:OAc. Z9-12:OAc. E9-12:OAc 100:64:2:1:4:1:0.8:7:2:0.2 13.5 ±2.4bc Trt. 1 + Z9-12:OAc, E9-12:OAc 100:64:2:1:4:1:0.8:2:0.2 11.1 ±2.5c 6: Trt. 4 + 14:OAc, Z9-12:OAc, E9-12:OAc 100:64:2:1:7:2:0.2 5.8 ±3.0d 7: 100:64:2:1:2:0.2 5.1 ±2.1d Trt. 4 + 29-12:OAc, E9-12:OAc 8:

Table 12: Effect of adding different components identified in female fruittree leafroller (FTLR) pheromone gland extracts to the 4 published FTLR pheromone components, on the attractiveness to male FTLR (n=10).

<sup>a</sup>Means followed by the same letter are not significantly different (SNK test, P<0.05).

Treatment		Ratio of compounds (by weight)	Number of male FTLR caught (x ± SE) <sup>e</sup>
1:	Z11-14:OAc, <i>E</i> 11-14:OAc, <i>Z</i> 9-14:OAc, 12:OAc, Z11-14:OH, <i>E</i> 11-14:OH, <i>Z</i> 9-14:OH	100:64:2:1:4:1:0.8	12.8 ±2.5a
2:	Z11-14:OAc, <i>E</i> 11-14:OAc, Z9-14:OAc, 12:OAc, Z11-14:OH, Z9-14:OH	100:64:2:1:4:0.8	11.7 ±3.4ab
3:	<i>Z</i> 11-14:OAc, <i>E</i> 11-14:OAc, <i>Z</i> 9-14:OAc, 12:OAc, <i>Z</i> 11-14:OH	100:64:2:1:4	11.5 ±1.9ab
4:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc, E11-14:OH	100:64:2:1:1	8.9 ±3.2ab
5:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc (western blend)	100:64:2:1	8.3 ±1.48b
6:	Trt. 5 + 211-14:0H, E11-14:0H	100:64:2:1:4:1	7.9 ±1.5ab
7:	Trt. 5 + 29-14:OH	100:64:2:1:0.8	6.1 ±1.3ab
8:	Trt. 5 + <i>E</i> 11-14:OH, <i>2</i> 9-14:OH	100:64:2:1:1:0.8	5.9 ±1.4b

Table 13: Effect of adding alcohols to the 4 published fruittree leafroller (FTLR) pheromone components on the attractiveness to male FTLR (n=15).

<sup>4</sup>Means followed by the same letter are not significantly different (SNK test, P<0.05).

treatment in Exp.5 (Table 14). The newly discovered acetates, *E*4,*E*11-14:OAc and *E*11-13:OAc, did not significantly increase the attractiveness of the "western blend".

In Exp.6 adding Z11-14:OH to the "western blend" increased the number of male FTLR caught by 3.4 times (Table 15) confirming that Z11-14:OH is a new pheromone component for FTLR in the Okanagan Valley. The proportion of the alcohol component in the blend seems to be critical. The addition of Z and E11-14:OH to Z and E11-14:OAc had previously been tested (Roelofs *et al.* 1974) but at the ratio tested, the alcohol-containing blend was less attractive than a blend composed of the 2 acetates alone.

The new, 5-component pheromone blend for western populations of FTLR comprises *Z*11-14:OAc, *E*11-14:OAc, *Z*9-14:OAc, 12:OAc and *Z*11-14:OH, in a ratio of 100:64:2:1:4. Shifting the ratio of the 4 previously published pheromone components (Cardé *et al.* 1977a) to the natural ratio found in the Okanagan Valley caused a mean increase in trap catch of 3.2 times (Tables 10-11). The addition of *Z*11-14:OH increased the mean trap catch by an additional 3.2 times (Tables 13-14-15). Therefore, this new pheromone blend is expected to be 6 times more attractive than the blend described by Cardé *et al.* (1977a). Based on the results obtained in Exp.1 (Table 10) the new pheromone blend, would be expected to capture over 2 times more FTLR males in traps baited with 200 µg of pheromone, than a trap baited with 2 FTLR virgin females. The new blend should be evaluated as a disruptant in comparison with the blend released by the Hamaki-con dispensers.

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Table 14: Effect of adding different alcohols or newly identified acetates from female fruittree leafroller (FTLR) pheromone gland extracts, to the 4 published FTLR pheromone components on the attractiveness to male FTLR (n=10).

Treatment		Ratio of compounds (by weight)	Number of male FTLR caught (X ± SE) <sup>#</sup>
1:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc, Z11-14:OH	100:64:2:1:4	3.3 ±0.7a
2:	Trt. 1 + E11-14:OH, Z9-14:OH	100:64:2:1:4:1:0.8	1.6 ±0.6b
3:	Z11-14:OAc, <i>E</i> 11-14:OAc, <i>Z</i> 9-14:OAc, 12:OAc, <i>E</i> 11-13:OAc	100:64:2:1:1	1.2 ±0.5b
4:	Z11-14:OAc, <i>E</i> 11-14:OAc, <i>Z</i> 9-14:OAc, 12:OAc, <i>E</i> 4, <i>E</i> 11-14:OAc	100:64:2:1:1	0.7 ±0.3b
5:	Z11-14:OAc, <i>E</i> 11-14:OAc, <i>Z</i> 9-14:OAc, 12:OAc (western blend)	100:64:2:1	0.6 ±0.3b

<sup>a</sup>Means followed by the same letter are not significantly different (SNK test, P<0.05).

Table 15: Effect of adding Z11-14:OH to the 4 published fruittree leafroller (FTLR) pheromone components on the attractiveness to male FTLR (n=17).

Treatment		Ratio of compounds (by weight)	Number of male FTLR caught (x ± SE) <sup>4</sup>
1:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc, Z11-14:OH	100:64:2:1:4	4.8 ±1.2a
2:	Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc (western blend)	100:64:2:1	1.4 ±0.4b

Means followed by the same letter are not significantly different (SNK test, P<0.05).

## CHAPTER 5. CONCLUDING DISCUSSION

My results demonstrate the potential of mating disruption for the control of FTLR. The average reduction of 82% in mating in highly infested areas treated with 1,000 Hamakicon dispensers/ha should result in a decrease of the FTLR population in orchards in which the biotic component of the natural control of FTLR has been preserved (such as in an organic orchard). The pheromone blend released by the Hamaki-con dispensers, *Z*11-14:OAc and *E*11-14:OAc in a ratio of 93:7, provided the best disruption, apparently involving the alteration of the optimal ratio mechanism.

I hypothesize that permeation of an orchard with pure Z11-14:OAc would be more effective than using a mixture of Z and E11-14:OAc. Unfortunately, because of spontaneous isomerization and high cost of achieving absolute purity of Z11-14:OAc, this treatment could probably not be commercialized. Disruption trials with the new 5-component pheromone blend composed of Z11-14:OAc, E11-14:OAc, Z9-14:OAc, 12:OAc and Z11-14:OH in a ratio of 100:64:2:1:4, might be more promising. It is expected to be approximately 6 times more attractive than the blend described by Cardé *et al.* (1977a) and will surely be more efficient for mating disruption if mechanisms such as false trail following or camouflage are involved.

Yet to be evaluated for its role in the outcome of mating disruption of FTLR is the migration capability of mated females. If gravid females can migrate over long distances immigration of mated females could negate the effect of a disruption treatment. Indirect evidence that migration over large areas does not occur comes from the observation that

the resistance to organophosphate insecticides which appeared in the Kelowna area several years ago (Madsen and Carty, 1977; Vakenti *et al.*, 1984) does not seem to have reached other areas of the Okanagan Valley. To evaluate the short range dispersal capability of mated females, I suggest employing an elemental, multigenerational marking technique. Rubidium has been used as marker for several lepidoptera species (Hayes 1991), including the tufted apple bud moth, *Platynota idaeusalis* (Walker), allowing identification of egg masses oviposited by female that were treated as larvae (Knight *et al.* 1990). The release of marked mated females followed by detection of rubidium in egg masses at different distances from the release point, would allow evaluation of migration capacity of mated female FTLR. This information could then be used to determine if infestations outside of treated areas constitute a reinfestation threat.

Several native plant species are hosts of FTLR (Mayer 1973). If the preferred oviposition site of an adult female or the preferred host of the ensuing larvae are influenced by the host on which the female has developed a relatively low amount of population transfer from one host species to another could occur (Carrière 1991). This would decrease the threat represented by the presence of alternative hosts beyond the boundaries of treated areas.

It should soon be possible to differentiate egg masses of FTLR from those of ELR based on a polymerase chain reaction analysis (M.J. Smirle, personal communication)<sup>1</sup>. This would allow egg counts to be used as a direct assessment of FTLR mating disruption. Such counts would incorporate the effect of migrating mated females.

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Pheromone-based disruption of FTLR could also control other sympatric leafrollers (e.g. OBLR, ELR and TLLR) sharing the principal pheromone component, *Z*11-14:OAc. This possibility has been suggested for OBLR and ELR by the reduction in catches in traps baited with synthetic pheromone in treated plots (Table 2). An experiment in which the effects of different pheromone treatments are assessed by tethered virgin females for OBLR, ELR and TLLR is needed.

It is critical that pheromone blend(s) which would efficiently control the leafroller complex present in different areas of the Okanagan Valley be characterized. Implementation of the sterile insect release program for the control of codling moth in the Okanagan Valley, should allow growers to eliminate 2 to 3 annual sprays of organophosphate insecticide used previously to control this insect. Deletion of these sprays could result in an increase in importance of leafroller species that were until now, considered secondary pests. If pheromone-based disruption could be used instead of separate sprays directed at these "secondary" pests, many growers would have the opportunity to convert to truly organic production.

Two approaches could be used to identify the most efficacious pheromone blend for mating disruption of an entire leafroller complex. The first would be to identify the best pheromone blend for mating disruption of every species, and then to examine the effect of different mixtures of these blends on the entire group of species. The second approach would be to determine the common pheromone components between species, and to estimate the effect of either these components alone or in blends on mating disruption of the group of leafroller species. This second approach is not as comprehensive as the

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first but could achieve results in a short period of time.

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