SEMIOCHEMICAL-BASED DISRUPTION OF MATE-FINDING BEHAVIOUR IN *Choristoneura rosaceana* (HARRIS) AND *Pandemis limitata* (ROBINSON) (LEPIDOPTERA: TORTRICIDAE) IN BRITISH COLUMBIA APPLE ORCHARDS

by

Maya L. Evenden

B.Sc., University of Victoria, 1991

M.P.M., Simon Fraser University, 1994

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department of Biological Sciences

SIMON FRASER UNIVERSITY

August, 1998

ABSTRACT

Mate finding by male *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) was equally disrupted when the atmosphere was treated with an attractive pheromone blend, less attractive partial blends and an unattractive blend containing a behavioural antagonist. Similarly, for the sympatric tortricid species, *Pandemis limitata* (Robinson), mate finding was disrupted with an attractive pheromone blend, an off-ratio blend and *Z*11-tetradecenyl acetate which is an unattractive, shared main component of both species' pheromones. Mating disruption of both *C. rosaceana* and *P. limitata* was achieved simultaneously by a blend containing mostly the shared major component, or a 1:1 ratio of *Z*11- and *Z*9-tetradecenyl acetate, the minor component of the *P. limitata* pheromone. Pre-exposure of *C. rosaceana* males to their pheromone or to a partial pheromone blend did not alter their response to calling virgin females in a wind tunnel. However, placement of sources of the complete blend or the partial blend upwind of the calling female significantly lowered the proportion of males contacting the female. The more attractive blend caused greater disorientation of males than the partial blend. Atmospheric permeation with *Z*9-tetradecenyl acetate induced male *C. rosaceana* to respond to normally unattractive synthetic pheromone sources containing this behavioural antagonist. The importance of *Z*9-tetradecenyl acetate as a synomone imparting distinct communication channels between *C. rosaceana* and *P. limitata* was demonstrated by induction of cross-species attraction in an atmosphere treated with this component. Female *C. rosaceana* delayed the commencement of calling in plots treated with pheromone and in one of two experiments reduced the total time spent calling. However, female *C. rosaceana* showed no alteration in mate preference for

virgin or mated males in an atmosphere treated with pheromone. A physiological time scale based on thermal sums was used to model the timing of eclosion and oviposition by female *C. rosaceana* on apple in B.C., and hence when mating disruption formulations need to be applied. These results elucidate the mechanisms of mating disruption in *C. rosaceana* and *P. limitata*, and indicate that formulations containing *Z*11-tetradecenyl acetate alone or combined with *Z*9-tetradecenyl acetate are adequate to reduce mating of both species simultaneously.

ACKNOWLEDGEMENTS

This work was made possible by the contributions of many people and organizations. I would firstly like to thank my co-senior supervisors, Drs. J.H. Borden and G.J.R. Judd for their tremendous interest, support and active participation in this work from its inception to completion. The encouragement and guidance provided by Dr. Borden throughout my six year tenure at Simon Fraser University has made my graduate studies a stimulating and enjoyable learning experience. Dr. Judd welcomed me into his laboratory, and taught, encouraged and inspired me throughout my stay in Summerland. I would also like to thank Dr. G. Gries for his advice and interest in this work. Dr. H.L. McBrien assisted me greatly in all aspects of my work in Summerland. This work would not have been possible without the excellent technical assistance of Mr. M.G.T. Gardiner. The dedication and contribution of ideas of three superb student assistants, Ms. N.C. Delury, Ms. N.L. Weremy and Ms. L.E. Delury is greatly appreciated. Ms. L.J. Chong and Ms. N. Verpaelst provided technical assistance throughout the course of this study. Ms. R. Gries and Dr. H.D. Pierce Jr. conducted chemical analyses for all semiochemical formulations used in this work. Dr. C.A. Levesque was instrumental in teaching me about logistic regression analysis and Dr. J. Hull assisted me in other statistical analyses. Dr. M. Weiss created Figure 1. I would like to thank Mr. F. Nelson, Mr. T. Wills, Mr. J. Hutchison, Mr. B. Potash, Mr. and Mrs. R.E. Stewart, Ms. D. Gregoire, Mr. R. Mennell, Mr. D. Taylor, Mr. W. Still, and Mr. B. Harker for welcoming my research in their orchards. I am especially grateful to Mr. R. Schneider and Ms. A. Turner for their willingness to accommodate my work in their orchard, their interest in and support of pheromone-based mating disruption, and the

generous supply of fresh fruit. I would also like to thank Ms. L.B.M. Jensen, and Drs. S. and K.A. Bloem whose friendship and assistance made my stay in Summerland most enjoyable. Finally, I would like to thank my family: Len, Polly, Kirstin, Matthew, Arlan and Charlene, for their interest, assistance and encouragement throughout the course of my graduate studies. I am especially grateful to my loving husband Steven, whose support, encouragement and belief in me made completion of this work possible. This work was financially supported by: the Similkameen Okanagan Organic Producers' Association, the British Columbia Fruit Growers' Association, Phero Tech Inc., the British Columbia Science Council, the Natural Science and Engineering Research Council of Canada, Simon Fraser University and Agriculture and Agri-food Canada.

TABLE OF CONTENTS

Approval....ii

Abstract....iii

Acknowledgements....v

Table of Contents....vii

List of Tables....xii

List of Figures....xiii

1.0 GENERAL INTRODUCTION....1

1.1 Mating disruption of Lepidopterous Pests....2

1.2 Orchard-Inhabiting Tortricines....5

1.3 Thesis Objectives....7

2.0 GENERAL METHODOLOGY....9

2.1 Insect Colonies....9

2.2 Wind-Tunnel Studies....10

2.2.1 Wind Tunnel Description....10

2.2.2 Insect Handling Procedure....10

2.2.3 Synthetic Semiochemical Sources....14

2.3 Small-Plot Protocol for Mating-disruption Trials....15

2.3.1 Plot Description....15

2.3.2 Treatment Assessment....18

2.4 Sticky Traps....18

2.5 Statistical Analysis of Proportional Data....19

3.0 PHENOLOGY OF *Choristoneura rosaceana* ON APPLE IN BRITISH COLUMBIA.....22

3.1 Introduction.....22

3.2 Methods and Materials.....24

3.2.1 Larval Development.....24

3.2.2 Adult Eclosion.....25

3.2.3 Male Moth Flight.....26

3.2.4 Oviposition.....26

3.2.5 Weather Data.....27

3.2.6 Statistical Analyses.....28

3.2.7 Determination of Male *C. rosaceana* Mating Status.....29

3.3 Results.....31

3.3.1 Larval Development.....31

3.3.2 Adult Eclosion.....31

3.3.3 Male Moth Flight.....34

3.3.4 Oviposition.....42

3.3.5 Determination of Male *C. rosaceana* Mating Status.....42

3.4 Discussion.....46

4.0 MATING DISRUPTION OF *C. rosaceana* : IS THE MOST ATTRACTIVE BLEND REALLY MOST EFFECTIVE?.....55

4.1 Introduction.....55

4.2 Methods and Materials.....56

4.2.1 Field Experiments.....56

4.2.2 Wind-Tunnel Experiments.....62

4.3 Results....65

4.3.1 Field Experiments....65

4.3.2 Wind-Tunnel Experiments....72

4.4 Discussion....79

5.0 COMMUNICATION DISRUPTION OF *C. rosaceana* AND *P. limitata* WITH ATTRACTIVE AND INHIBITORY SEMIOCHEMICAL BLENDS....92

5.1 Introduction....92

5.2 Methods and Materials....94

5.2.1 Wind-Tunnel Experiments....94

5.2.2 Field Experiments....96

5.2.3 Statistical Analyses....97

5.3 Results....99

5.3.1 Wind-Tunnel Experiments....99

5.3.2 Field Experiments....105

5.4 Discussion....105

6.0 SIMULTANEOUS MATING DISRUPTION OF *C. rosaceana* AND *P. limitata*....113

6.1 Introduction....113

6.2 Methods and Materials....114

6.2.1 General Protocol....114

6.2.2 Experiments....116

6.2.3 Statistical Analyses....117

6.3 Results....118

6.4 Discussion....125

7.0 *Z*9-14:OAc: A SYNOMONE IMPARTING DISTINCT SEX PHEROMONE COMMUNICATION CHANNELS FOR *C. rosaceana* AND *P. limitata*.....134

7.1 Introduction.....134

7.2 Methods and Materials.....137

7.2.1 Wind-Tunnel Experiments.....137

7.2.2 Field Experiments.....140

7.3 Results.....143

7.3.1 Wind-Tunnel Experiments.....143

7.3.2 Field Experiments.....151

7.4 Discussion.....156

8.0 BEHAVIOUR OF FEMALE *C. rosaceana* IN PHEROMONE-TREATED PLOTS.....161

8.1 Introduction.....161

8.2 Methods and Materials.....163

8.2.1 Calling behaviour of female *C.rosaceana* in control and pheromone-treated plots.....163

8.2.2 Properties of spermatophores transferred to females by virgin and mated males.....164

8.2.3 Mating status of males that provided spermatophores to tethered females in control and pheromone-treated plots.....166

8.3 Results.....167

8.3.1 Calling behaviour of female *C.rosaceana* in control and pheromone-treated plots.....167

8.3.2 Properties of spermatophores transferred to females by virgin and mated males.....170

8.3.3 Mating status of males that provided spermatophores to tethered females in control and pheromone-treated plots.....170

8.4 Discussion.....172

9.0 CONCLUDING DISCUSSION.....175

APPENDIX 1 Pintobean-based diet for *C. rosaceana* and *P. limitata*.....183

APPENDIX 2 Contents of pheromone dispensers (Ecogen Inc., Billings, MT) used in field and lab experiments, as determined by gas chromatographic analysis.....184

APPENDIX 3 Descriptions of experimental plots used in communication and mating-disruption experiments in the Similkameen Valley, B.C.....186

APPENDIX 4 Comparison of number of male *P. limitata* attracted to tethered *vs.* not tethered females in traps.....188

APPENDIX 5 Male *C. rosaceana* and *P. limitata* captured in pheromone-baited traps adjacent to mating-disruption experiments.....189

APPENDIX 6 Mean numbers of male *C. rosaceana* captured in synthetic pheromone-baited traps (3 mg, 4-component blend) in each replicate of Exp. 1, Chapter 4.....191

10.0 REFERENCES CITED.....192

LIST OF TABLES

Table 1. Proportion of larvae in each instar as determined by head capsule measurements (J. Cossentine, pers. comm., Agriculture and Agri-food Canada, PARC, Summerland, B.C.,) at various collection times in 1997.....32

Table 2. Eclosion and trapping events for adult *C. rosaceana* in 1996 and 1997.....33

Table 3. Estimated values for parameters of Weibull functions and their goodness of fit to cumulative percent distributions of various phenological events at PARC (1996-1997) and trap catch in Cawston (1994-1997).....35

Table 4. Assortment of virgin and mated *C. rosaceana* males in relation to the condition of segment 7 of the primary simplex of the *ductus ejaculatorius*.....45

Table 5. Pheromone treatments, release rates and dispenser densities used in mating-disruption experiments (Chapter 4).....58

Table 6. Response by male *C. rosaceana* to pheromone of *C. rosaceana* alone and with *Z*9-14:OAc added in various quantities in Exp. 1 and 2, Chapter 5, in a wind tunnel. Seven replications per experiment with 5-7 males tested per treatment, per replicate.....100

Table 7. Response by male *C. rosaceana* to pheromone of *C. rosaceana* alone or with *Z*9-14:OAc, *Z*9-14:OAc alone, and hexane alone in Exp. 3, Chapter 5, in a wind tunnel. Seven replications, 5-7 males tested per treatment, per replicate.....104

Table 8. Composition of blends used in mating-disruption experiments against *C. rosaceana* and *P. limitata* (Chapter 6).....115

Table 9. Synthetic pheromone treatments used in trapping experiment (Exp. 5, Chapter 7).....142

Table 10. Size and description of spermatophores produced by previously mated and virgin male *C. rosaceana*.....171

LIST OF FIGURES

Figure 1. Wind tunnel used in all laboratory bioassay experiments. A. Cage for release of individual males into plume at the centre of the wind tunnel. B. Cage used to house calling virgin females as semiochemical sources.....11

Figure 2. Plot design used to test mating disruption in 0.1 ha plots located in organically-managed apple orchards in Cawston, B.C. Plots were spaced ≥ 40 m apart and ≥ 10 m from the edge of each orchard. PC = plot centre. ♀ = position of two tethered females (except where otherwise mentioned), one female was placed in the upper third of the canopy and the other at head height (ca. 1.5 m).....16

Figure 3. Observed cumulative first-generation *C. rosaceana* adult eclosion (◦) in 1996 and 1997 alone and combined, plotted against $DD_{10°C}$ air temperature after 1 January compared with curves (solid lines) modelled by Weibull functions (Table 3).....36

Figure 4. Cumulative trap captures of first-generation male *C. rosaceana* (◦) in pheromone traps at PARC during 1996 and 1997 alone and combined, plotted against $DD_{10°C}$ air temperature after 1 January, compared with curves (solid lines) modelled by Weibull functions (Table 3).....38

Figure 5. Cumulative trap captures of first (G1)- and second (G2)-generation *C. rosaceana* (◦) in pheromone traps in Cawston, 1994-1997 combined, plotted against $DD_{10°C}$ air temperature after 1 January, compared with curves (solid lines) modelled by Weibull functions (Table 3).....40

Figure 6. Observed cumulative oviposition (◦) for first-generation (G1) *C. rosaceana* in 1996 and for first (G1)- and second (G2)-generations in 1997 plotted against $DD_{10°C}$ air temperature after first female eclosion, compared with curves (solid lines) modelled by Weibull functions (Table 3).....43

Figure 7. Relationships between percent mated males captured in monitoring traps located at PARC in 1996 and 1997 during the first-generation *C. rosaceana* flight and $DD_{10°C}$ air temperature after 1 January. Regression equations are $\hat{y} = 0.18x-37.59$ (1996) and $\hat{y} = 0.12x-16.26$ (1997).....47

Figure 8. Percent cumulative phenological events and total number of males captured in pheromone-baited traps for the first- and second-

generation *C. rosaceana* in 1997, plotted against $DD_{10°C}$ air temperature after 1 January. Cumulative percent distributions of male and female eclosion and oviposition obtained from data collected at PARC. Cumulative percent distribution of trap catch and total number of males captured obtained from data collected in Cawston.....53

Figure 9. Proportions of tethered, female *C. rosaceana* mating in nontreated plots or plots treated atmospherically with the four-component blend and two partial pheromone blends in Exp. 1, Chapter 4, 6 July-27 August, 1994, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.....66

Figure 10. Relative proportions (proportion of mated, tethered female *C. rosaceana* in no-pheromone control plots = 1.0) of mated, tethered females in plots treated atmospherically with the four-component blend at various release rates and a dispenser density of 1000 ha^{-1} in Exp. 2, Chapter 4, 7 June-4 August, 1995, Exp. 4, 10 August-1 September, 1995, and Exp. 3, 20 June-28 July, 1996, Cawston, B.C. Within each experiment, bars marked with * are significantly different from the control and bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4, in all experiments.....68

Figure 11. Proportions of tethered, female *C. rosaceana* mating in plots treated atmospherically with the four-component blend and two-component blend, at reduced release rates and a dispenser density of 1000 ha^{-1} in Exp. 4, Chapter 4, 10 August-1 September, 1995, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.....70

Figure 12. Proportions of tethered, female *C. rosaceana* mating in plots treated atmospherically with the four-component blend and two-component blend at a release rate of 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ and a reduced dispenser density in Exp. 5, Chapter 4, 13 June-22 July, 1997, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.....73

Figure 13. Total number of male *C. rosaceana* captured in two blocks, over three, two-night periods in wing traps baited with monitoring lures, mating-disruption dispensers, or virgin females in Exp. 6, Chapter 4, 5-13 July, 1994, Cawston, B.C. Bars with the same letter superscript are not significantly different χ^2 -test $P>0.05$.....75

Figure 14. Mean proportions of male *C. rosaceana* flying upwind and

contacting a calling female *C. rosaceana* in the wind tunnel. A. Males placed in female's plume for 10 s prior to release or with background treatment with the two- or four-component blend in addition to the female in Exp. 7, Chapter 4. B. Males placed in female's plume for 10 s (control 1) or 1 min (control 2) or for 1 min with female plus background treatment with the two- or four-component blend in Exp. 8, Chapter 4. Pie graph inserts indicate the proportion and end destination of males that did not contact calling virgin females. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=5 in both experiments.....77

Figure 15. Mean proportions of male *C. rosaceana* captured in *C. rosaceana* female-baited trap in the wind tunnel following pre-exposure to the four-component pheromone for 1 h followed by 10-30 min in clean air before bioassay in Exp. 9, Chapter 4, or pre-exposure to the two-component blend for 1 h followed by 10-30 min in clean air prior to bioassay in Exp. 10, Chapter 4. N=5 in both experiments.....80

Figure 16. Relative proportions (proportion of *C. rosaceana* responding to *C. rosaceana* pheromone = 1.0) of male *C. rosaceana* responding to 100 µg of its sex pheromone and 100 µg of pheromone with increasing quantities of *Z*9-14:OAc added, in a wind tunnel in Exp. 1 and 2, Chapter 5. N=7 in both experiments.....102

Figure 17. Mean numbers of male *C. rosaceana* and *P. limitata* captured in wing traps baited with pheromone of *C. rosaceana* alone or in combination with *Z*9-14:OAc and *Z*9-14:OAc alone, in Exp. 4, Chapter 5, 10-27 August, 1994, Cawston, B.C. N=12.....106

Figure 18. Proportions of wing traps baited with virgin female *C. rosaceana* or *P. limitata* attracting at least one conspecific male in various atmospheric semiochemical treatments in Exp. 5, Chapter 5, 18 August-6 September, 1995. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.....108

Figure 19. Proportions of tethered, female *P. limitata* mating in nontreated plots or plots treated with the four-component blend (blend 1) and two partial blends (2 and 3) in Exp. 1, Chapter 6, 18 July-15 September, 1994, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.....119

Figure 20. Relative proportions (proportion of mated, tethered female *P. limitata* mating in no-pheromone control plots = 1.0) of mated, tethered females in nontreated plots or plots treated with the

four-component blend (blend 1) at various release rates in Exp. 2, Chapter 6, 7 June-4 August, 1995 and Exp. 3, 20 June-28 July, 1996, Cawston, B.C. Within each experiment bars with the same letter superscript are not significantly different, *Z*-tests *P*>0.05. N=4 in both experiments.....121

Figure 21. Proportions of tethered, female *C. rosaceana* and *P. limitata* mating in nontreated plots or plots treated with blends 1-3 in combination with *Z*9-14:OAc (blend 5) in a 1:1 ratio in Exp. 4, Chapter 6, 21 June-24 July, 1996, Cawston, B.C. Within each species, bars with the same letter superscript are not significantly different, *Z*-tests, *P*>0.05. Asterisks indicate proportions that approached 0 and for which S.E.'s could not be estimated by the logistic regression function. N=4.....123

Figure 22. Proportions of tethered, female *C. rosaceana* and *P. limitata* mating in nontreated plots or plots treated with the *P. limitata* pheromone (blend 4) or blend 3 in combination with *Z*9-14:OAc (blend 5) in a 1:1 ratio, in Exp. 5, Chapter 6, 12 August-30 August, 1996, Cawston, B.C. Within each species, bars with the same letter superscript are not significantly different, *Z*-tests *P*>0.05. N=3.....126

Figure 23. Proportions of tethered, female *C. rosaceana* and *P. limitata* mating in nontreated plots or plots treated with *Z*9-14:OAc alone, blend 3 alone, or blend 3 in combination with *Z*9-14:OAc (blend 5) in a 1:1 ratio, in Exp. 6, Chapter 6, 14 June-19 July, 1997, Cawston, B.C. Within each species, bars with the same letter superscript are not significantly different *Z*-tests *P*>0.05. N=4.....128

Figure 24. Mean proportions of male *C. rosaceana* responding to synthetic semiochemical sources in clean air and with *Z*9-14:OAc released at approximately 5 $\mu g \cdot h^{-1}$ upwind of the source in a wind tunnel in Exp. 1, Chapter 7. Bars with the same letter superscript are not significantly different *Z*-tests *P*>0.05. N=7.....144

Figure 25. Mean proportions of male *C. rosaceana* and *P. limitata* responding to calling females of the opposite species in clean air and with *Z*9-14:OAc released at approximately 5 $\mu g \cdot h^{-1}$ upwind of the source in a wind tunnel in Exp. 2 and Exp. 3, Chapter 7. Asterisks indicate significant differences (Logistic regression analysis) between treatments within each behavioural response. N=5 in both experiments.....147

Figure 26. Mean proportions of male *P. limitata* responding to calling

female *P. limitata* in clean air and with *Z*9-14:OAc released at approximately 5 $\mu g \cdot h^{-1}$ upwind of the source in a wind tunnel in Exp. 4, Chapter 7. Asterisks indicate significant differences (Logistic regression analysis) between treatments within each behavioural response. N=5.....149

Figure 27. Mean numbers of male *C. rosaceana* and *P. limitata* captured in synthetic semiochemical-baited traps in 0.1 ha control and *Z*9-14:OAc-treated plots in Exp. 5, Chapter 7, Cawston, B.C. Within each trap type, asterisks indicate a significant shift in male response, LSD test $P<0.05$. N=3.....152

Figure 28. Comparison of numbers of male *C. rosaceana* and *P. limitata* captured by con- and heterospecific females in 0.1 ha control or *Z*9-14:OAc-treated plots in Exp. 6, Chapter 7. N=6.....154

Figure 29. Comparison of mean times to onset to calling and duration of calling by caged female *C. rosaceana* in pheromone-treated (four-component blend) and control 0.1 ha plots on two different nights in August, 1997. Probabilities of differences occurring by chance (Wilcoxon sum rank test) are shown for each set of paired bars.....168

1.0 GENERAL INTRODUCTION

The first step in the mating sequence of sexual animals with internal fertilization is pair formation or rapprochement (Alexander et al. 1997). Methods of pair formation in insects have evolved in response to signals or noise from external sources (Linn and Roelofs 1995; Alexander et al. 1997), predators and parasites attracted to signals (Alexander et al. 1997), sexual selection, and changes in patterns of parental investment (West-Eberhard 1984; Alexander et al. 1997; Phelan 1992, 1997a,b). The use of pheromones by moths for attraction of mates leading to pair formation, is a primitive trait in this group of insects (Linn and Roelofs 1995; Phelan 1997a). In most cases females produce small amounts of pheromone to which males respond over long distances. Female-based pheromone systems in moths appear to have had a single origin and have undergone little change in chemistry of pheromone components or glandular structure for release of pheromone (Phelan 1997a). Response by males to female-produced pheromones is not known to be genetically linked to production of pheromone by females (Phelan 1992; Löfstedt 1993). Therefore, changes in the pheromone blend released by the female would require an alteration or broadening of male response in order to signal mate-attraction. The strict reliance of most species of moths on female-produced pheromones for long-range attraction of mates, and the relative stability of the signalling system makes production of and response to pheromones ideal targets for management of lepidopteran pests.

1.1 Mating Disruption of Lepidopterous Pests

The idea of using synthetic sex pheromone as an atmospheric treatment to control insect pests was put forward by Babson (1963) as a method to eradicate the gypsy moth, *Lymantria dispar* (L.), and by Wright (1963) as a selective method of controlling insect pests. Field trials were first conducted unsuccessfully against the gypsy moth (Burgess 1964) using the wrongly identified "gyplure", and successfully against the cabbage looper *Trichoplusia ni* (Hübner) (Gaston *et al.* 1967; Shorey et al. 1967). Terms such as pheromone-mediated communication disruption have been used to refer to this pest management strategy (Shorey 1977; Cardé 1990), but the term mating disruption is most commonly used, because the ultimate aim is to prevent insects from mating by disabling the pair formation process.

Pheromone-based mating disruption has been investigated against many lepidopteran insect pests in different cropping systems. The most well-studied species is the pink bollworm, *Pectinophora gossypiella* (Saunders) (Gaston et al. 1977; Flint and Merkle 1983, 1984a,b; Critchley et al. 1985; Or et al. 1986; El-Adl et al. 1988; Miller et al. 1990; Cardé et al. 1993; Cardé et al. 1997; Staten et al. 1997), for which mating disruption in cotton is now used commercially (Baker et al. 1990). Mating-disruption techniques have also been developed for other field crop pests, including the tobacco budworm, *Heliothis virescens* (F.) (Zvirgzdins et al. 1984), and the tomato pinworm, *Keiferia lycopersicella* (Walsingham) (Van Steenwyck and Oatman 1983; Jiménez et al. 1988; Jenkins et al. 1990). Important fruit pests for which this technique has been developed and is now available commercially (Thomson 1991) include the codling moth, *Cydia pomonella* (L.) (Cardé et al. 1977a; Moffit and Westigard 1984; Charmillot

1990; Weinzierl 1991; Barnes et al. 1992; Howell et al. 1992; Pfeiffer et al. 1993a; Judd et al. 1996a, 1997; Vickers et al. 1998), the Oriental fruit moth, *Grapholita molesta* (Busck) (Rothschild 1975; Cardé et al. 1977a; Weakley et al. 1987; Rice and Kirsch 1990), and the grape berry moth, *Endopiza viteana* Clemens (Taschenberg and Roelofs 1977; Dennehy et al. 1990; Trimble et al. 1991; Trimble 1993) . The most important forest lepidopteran pest for which this technique has been examined is the gypsy moth, (Granett and Doane, 1975; Schwalbe et al. 1974; Schwalbe and Mastro 1988; Webb et al. 1988; Kolodny-Hirsch et al. 1990; Kolodny-Hirsch and Webb 1993).

Mating-disruption field trials have illuminated limitations to this tactic. For example, one weakness of mating disruption involves the migration of mated females into the treated area (Cardé and Minks 1995). As a result mating disruption will likely work best as an area-wide management tool (Ogawa 1990; Staten et al. 1997). A high population density of the target insect can also thwart control efforts (Cardé and Minks 1995), especially if the disruption mechanism is based on competition between synthetic and female-produced pheromone plumes. Variable crop canopies, wind direction and wind speed could interact to alter plume structure (Cardé and Minks 1995) and thus potential for adsorption and re-release of pheromone from the crop canopy (Wall et al. 1981; Wall and Perry 1983; Noldus et al. 1991; Karg et al. 1994; Suckling et al. 1996; Schmitz et al. 1997; Sauer and Karg 1998). Different pheromone formulations can result in different mating-disruption mechanisms that could vary in effectiveness over time due to release-rate characteristics (Weatherston 1990) and population pressure. Finally, selection imposed by continuous application of synthetic pheromone could lead to the development of resistance against mating disruption through an

alteration in the chemical communication system of the target pest (Haynes and Baker 1988).

Limitations may be best overcome with an increased knowledge of how a given tactic alters mate-finding behaviour. Surprisingly, little is known about how mating is disrupted when synthetic pheromone is released. Shorey (1977) suggested three mechanisms by which pair formation could be impeded by atmospheric treatment with synthetic pheromone: 1) sensory adaptation of chemoreceptors on the responding insects' antennae; 2) habituation of the central nervous system; and 3) confusion due to competition among sources of synthetic and natural pheromone. Bartell (1982) discussed four possible mechanisms: 1) neural effects including both sensory adaptation and habituation of the central nervous system; 2) false-trail following, similar to Shorey's (1977) confusion mechanism, whereby males pursue plumes generated by synthetic pheromone and not female-produced plumes; 3) inability of responding males downwind of a calling female to distinguish her naturally-produced plume from a synthetic pheromone-laden background, later termed camouflage (Cardé 1981); and 4) alteration of behaviour caused by an imbalance in the pattern of sensory input, eg. when only one component of a multicomponent pheromone is released. Cardé (1990) suggested that an imbalance in sensory input was caused by either adaptation of antennal neurons or long term habituation to one component of the insect's pheromone blend. Other possible mechanisms of pheromone-mediated disruption include: 1) arrestment of male orientation behaviour in plumes of synthetic pheromone released at high rates (Cardé et al. 1997); 2) shifted rhythms of response to pheromone (Cardé et al. 1993; Cardé and Minks 1995; Cardé et al. 1997) so that periods of receptivity by

males and calling by females are offset; 3) a delay in the age at which mating occurs which could result in a reduction in the reproductive output of an insect (Knight 1997); and 4) release of synthetic pheromone filaments into the atmosphere so that the fine-scale structure of the natural plume is distorted (Cardé and Minks 1995), eliminating the organized plume (Murlis and Jones 1981; Murlis et al. 1992) that is critical for oriented flight in male moths (Kennedy et al. 1980; Kennedy et al. 1981; Willis and Baker 1984; Baker et al. 1985). These possible mechanisms of mating disruption are not mutually exclusive (Cardé and Minks 1995) and are dependent on the pheromone formulation (Minks and Cardé 1988), the method of pheromone release into the crop (Bartell 1982; Cardé and Minks 1995) and the insects' behaviour (Cardé and Minks 1995).

1.2 Orchard-Inhabiting Tortricines

The family Tortricidae has over 5000 described species worldwide, three subfamilies, Oleuthreutinae, Tortricinae and Chlidanotinae (Horak and Brown 1991). The subfamily Tortricinae comprises ca. 1500 species of polyphagous folivores found world-wide (Roelofs and Brown 1982), and is thought to have diverged early from the ancestral oleuthreutid form (Chapman 1973). In North America, native and exotic tortricids have successfully colonized and become major pests of apple, *Malus domesticus* (Borkh.) a crop introduced by early European settlers (Chapman and Lienk 1971). Indigenous members of these multi-species feeding guilds, that now occur on apple in eastern and western North America, previously infested host plants from the genera *Malus*, *Crataegus*, and *Amelanchier* (Chapman 1973).

The physiological constraints of pheromone production have apparently resulted

in pheromone blends of many tortricine species that consist of the same or closely related 14-carbon components (Roelofs and Brown 1982; Roelofs and Bjostad 1984; Horak and Brown 1991). Species specificity in pheromone communication appears to be achieved by response synergism to the conspecific pheromone blend and response antagonism to heterospecific pheromone components (Cardé and Baker 1984; Linn and Roelofs 1995; Phelan 1992).

In British Columbia (B.C.), the tortricine guild feeding on apple was first reported to consist of four economically important species (Venables 1924). Mayer and Beirne (1974a) found seven species on apple in the Okanagan Valley, two of which had previously been mentioned by Venables (1924). The tortricine guild presently consists of four primary species (Madsen and Madsen 1980; Madsen and Proctor 1985): the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), the threelined leafroller, *Pandemis limitata* (Robinson), the fruittree leafroller, *Archips argyrospilus* (Walker), and the introduced species the European leafroller, *Archips rosanus* L..

Choristoneura rosaceana was the major pest species in the Okanagan Valley until the early 1920's (Venables 1924), was rarely found in 1972 (Mayer and Beirne 1974a), but it has since then again become a major pest (Madsen and Madsen 1980). *Archips argyrospilus* was recorded as the most important leafroller species in the early 1920s (Venables 1924) and has continued to be a major pest (Madsen and Madsen 1980). *Pandemis limitata* has long been a pest of apples in eastern North America (Chapman and Lienk 1971) but was not recorded on apple in the Okanagan Valley until 1972 (Mayer and Beirne 1974a). *Archips rosanus* was first found on common privet, *Ligustrum vulgare* (Cheyenne), in the Okanagan Valley in 1968, but was not found

feeding on apple until 1972 (Mayer and Beirne 1974a). All four species also infest other hosts in the family Rosaceae (Mayer and Beirne 1974b). In the interior of B.C. *A. argyrospilus* and *A. rosanus* are univoltine and overwinter as eggs, whereas *C. rosaceana* and *P. limitata* are bivoltine and overwinter as larvae (Madsen and Proctor 1985). Adults of all species are present in the orchard at the same time (Madsen and Madsen 1980), and share *Z*11-tetradecenyl acetate as a major pheromone component and all have several minor components in common (Roelofs et al. 1976a,b; Vakenti et al. 1988; Deland et al. 1993).

Because of the sympatric ranges, synchronous habits of adults and the similarity in pheromone blend components, this feeding guild is an ideal group of insects to study with respect to inter- and intraspecific chemical communication, and control by semiochemical-mediated mating disruption.

1.3 Thesis Objectives

This thesis reports the results of investigations on sexual chemical communication in the two bivoltine species, *C. rosaceana* and *P. limitata*, and the potential for use of semiochemical-mediated mating disruption to control these species on apple in B.C. Emphasis is given to discriminating the mechanisms by which mating disruption may occur and the potential for developing a semiochemical treatment that can be used in a multi-species mating-disruption programme.

My specific objectives were:

1) to determine when adult emergence, mating and oviposition of *C. rosaceana* occurs on a physiological scale so that mating disruption can be appropriately timed;

2) to determine whether attractiveness of semiochemical formulations is correlated with disruptive efficacy, using *C. rosaceana* and *P. limitata* as target species;

3) to test whether atmospheric treatment with components common to both species pheromones would disrupt pheromone-mediated communication and mating in both species;

4) to determine if the pheromonal response of *C. rosaceana* males could be altered in the presence of a unique minor component of the *P. limitata* pheromone, and if heterospecific mate location would occur in an atmosphere treated with this component;

5) to evaluate the effect of atmospheric pheromone treatment on aspects of the behaviour of female *C. rosaceana*.

2.0 GENERAL METHODOLOGY

2.1 Insect Colonies

Choristoneura rosaceana used in 1994 and 1995-1998 experiments came from laboratory colonies respectively started from individuals collected from orchards in the Okanagan Valley and from organic orchards in the Similkameen Valley in 1995. The latter colony was started from 100 mating pairs housed individually in 150 mL cups to ensure high genetic variability. *Pandemis limitata* used in all experiments originated from a laboratory colony started from individuals collected in the Similkameen Valley in 1992. Because of the possibility that *P. limitata* could be intermixed with *P. pyrusana* Kearfott, which occurs in Washington State, identity of the species was confirmed by P.T. Dang, Biosystematics Research Centre, Ottawa, ON before establishing the colony.

Colonies of both species were housed in a walk-in Conviron® growth chamber equipped with fluorescent lighting and maintained at a 16:8 h L:D photoregime and 24°C at Agriculture and Agri-food Canada's, Pacific Agriculture Research Centre (PARC), in Summerland, B.C. Adult moths were held in a 22.7 L bucket, lined with fibreglass mesh on which they do not normally oviposit, and provided with four 10 x 30 cm pieces of wax paper, suspended from the lid of the bucket, as oviposition substrates. Water was provided to adults by a dental cotton wick that extended into the bucket from a plastic bottle filled with distilled water on the outside of the bucket. Every week individual egg masses were cut from the wax paper substrates and placed individually on artificial diet (Shorey and Hale 1965; Appendix 1) in 29 mL plastic Solo

cups (Rap-id paper, Kelowna, B.C.). First-instar larvae were transferred with a fine camel hair brush to fresh diet at a density of two larvae per cup; larvae remained in the same cup until they pupated. Pupae were removed 1-2 times per week, sexed, counted and rinsed with a 5% sodium hypochlorite solution prior to use in experiments or placement in the 22.7 L bucket for the continuation of the colony. Buckets housing adult moths were cleaned with bleach each month.

2.2 Wind-Tunnel Studies

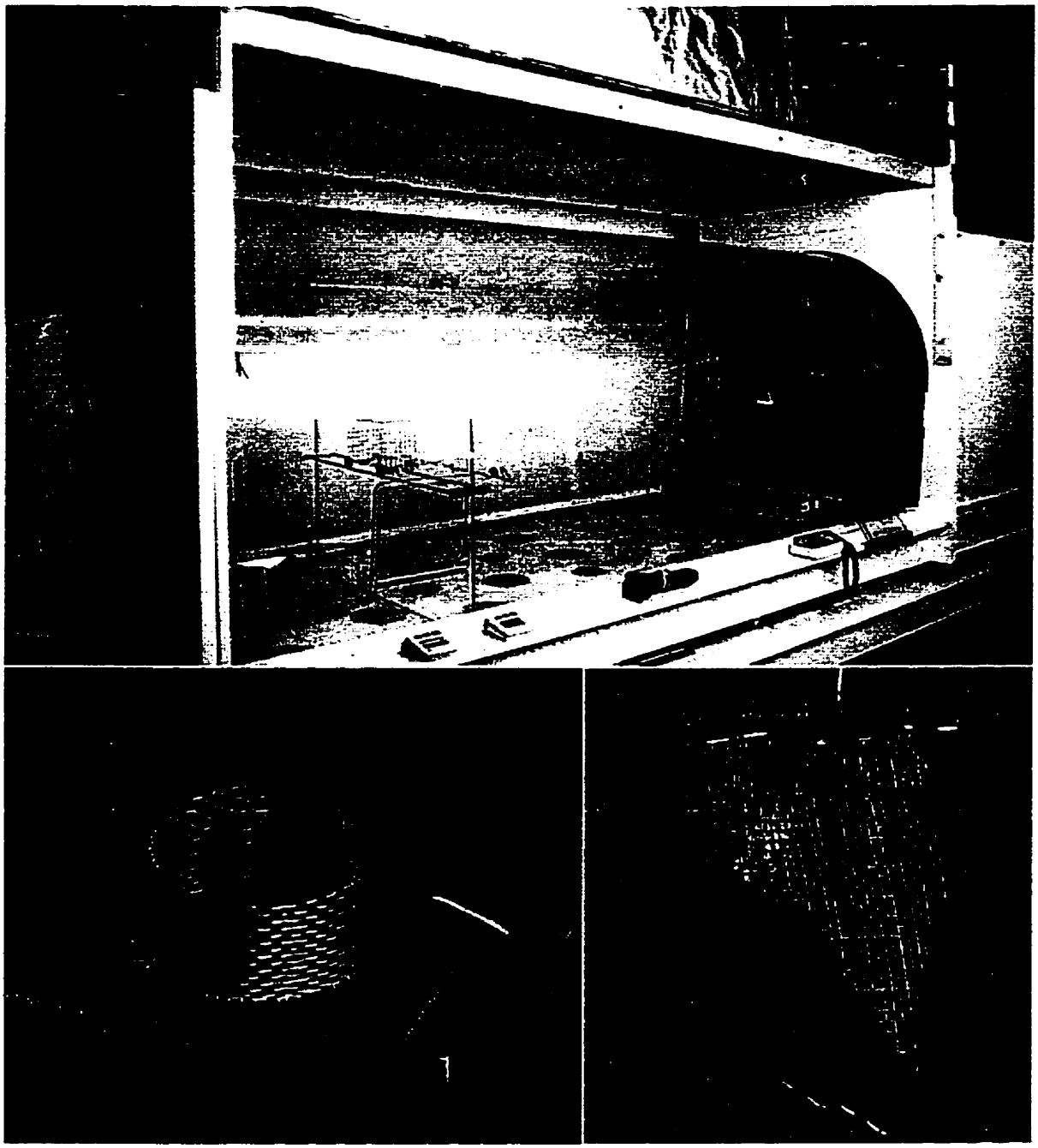
2.2.1 Wind Tunnel Description

The wind tunnel used in laboratory bioassay experiments was built using the design of Miller and Roelofs (1978). Air was pushed through the tunnel with a variable speed fan at a speed of 0.35-0.40 $m \cdot s^{-1}$ and the pheromone plume was exhausted outside the building by a centrally located, variable speed fan. The flight section of the tunnel was 2.45 m long and 1 m high at its centre (Fig. 1). Uniform, dim white light (0.5 lux) was provided by six, 25 watt incandescent bulbs, shielded by sheet metal and held 17 cm above three layers of fine black fibreglass mesh (Ace Distribution, Calgary Alta) and 2 layers of white Templast® plastic (Boyds, Kelowna B.C.). Tunnel temperatures varied between 21.5 and 26.5°C.

2.2.2 Insect Handling Procedure

Male pupae from the laboratory colonies were placed individually in 150 mL clear, plastic cups, provided with water in a dental cotton wick, and held in a Conviron®

Figure 1. A. Wind tunnel used in all laboratory bioassay experiments. B. Cage for release of individual males into plume at the centre of the wind tunnel. C. Cage used to house calling virgin females as semiochemical sources.



growth chamber maintained at 24°C under a reversed 8:16 h D:L photoregime, isolated from any adult females to ensure no exposure to pheromone. Adult males (24-72 h old) were collected during the last half hour of the photophase, immobilized for 10-30 min at 0.5°C, and placed in cylindrical (3-4 cm diam. x 1.5 cm height) wire mesh release cages fitted with removable sheet metal lids and attached to 5 x 5 cm wire mesh bases (Fig.1). To remove residual pheromone between replicates, both cages and lids were heated for at least 4 h at 200°C; lids were also rinsed in acetone or 95% ethanol. Males were evenly distributed among treatments by age to avoid any age-related behavioural bias. Before testing, all males in release cages were allowed to acclimate in the wind tunnel room under ambient conditions during the first 15-30 min of scotophase. Unless otherwise mentioned, males were placed on a small table adjacent to the wind tunnel during acclimatization. Bioassays were conducted within the first 3 h of the scotophase which corresponds to the peak activity period of *C. rosaceana* (Knight et al. 1994). Except for two experiments, males were presented individually to semiochemical sources. Each screen cage was placed on a release device through a port in the side of the tunnel and positioned into the odour plume by a lever operated on the outside of the tunnel, so as not to disrupt the plume and contaminate the tunnel walls. When in the plume, moths were 43 cm above the tunnel floor, 56 cm from the downwind end, and 1.5 m from the test odour source. Semiochemical sources (synthetic or natural) were hung from a wire extending below a metal ring stand, 44 cm above the tunnel floor and 34 cm from its upwind end. Once the release cage was positioned in the plume the sheet metal lid was removed by pulling on an attached line, also operated outside the tunnel. Male response to semiochemical treatments was

graded as + or - for wing fanning, take-off, locking-on to the plume, oriented upwind flight and source contact.

To obtain adult females for use as semiochemical sources, female pupae were collected from the laboratory colonies, placed individually in 150 mL clear, plastic cups, provided with water in a dental cotton wick, and held in a Conviron® growth chamber at 24°C under a reversed 8:16 h D:L photoregime, isolated from any adult males. Adult females (24 h old) were collected during the last half hour of the photophase, immobilized for 10-30 min at 0.5°C, and placed individually in fibreglass mesh bags (9 cm x 6.5 cm) (Fig. 1). Bags were secured with staples and placed in 150 mL cups sealed with a snap lid. Females were acclimatized to the tunnel conditions during the first 15-30 min of scotophase. One mesh bag containing a female was removed from the plastic cup and hung from a metal rod extending from the ring stand positioned at the upwind end of the tunnel. Other females were held in the plastic cups at the downwind end of the tunnel during acclimatization and were used only if the female positioned in the tunnel did not call. Only one calling female was used in each replicate of each experiment. Prior to the flight of each individual male, the female was examined to ensure she was calling if necessary illuminating her with a flashlight covered with a B+W 62E coated dark red filter (Beau Photo Supplies Inc., Vancouver, B.C.).

2.2.3 Synthetic Semiochemical Sources

In several wind tunnel experiments synthetic pheromone sources were used as lures. Red rubber septa (The West Company, Linville PA) were loaded with pheromone

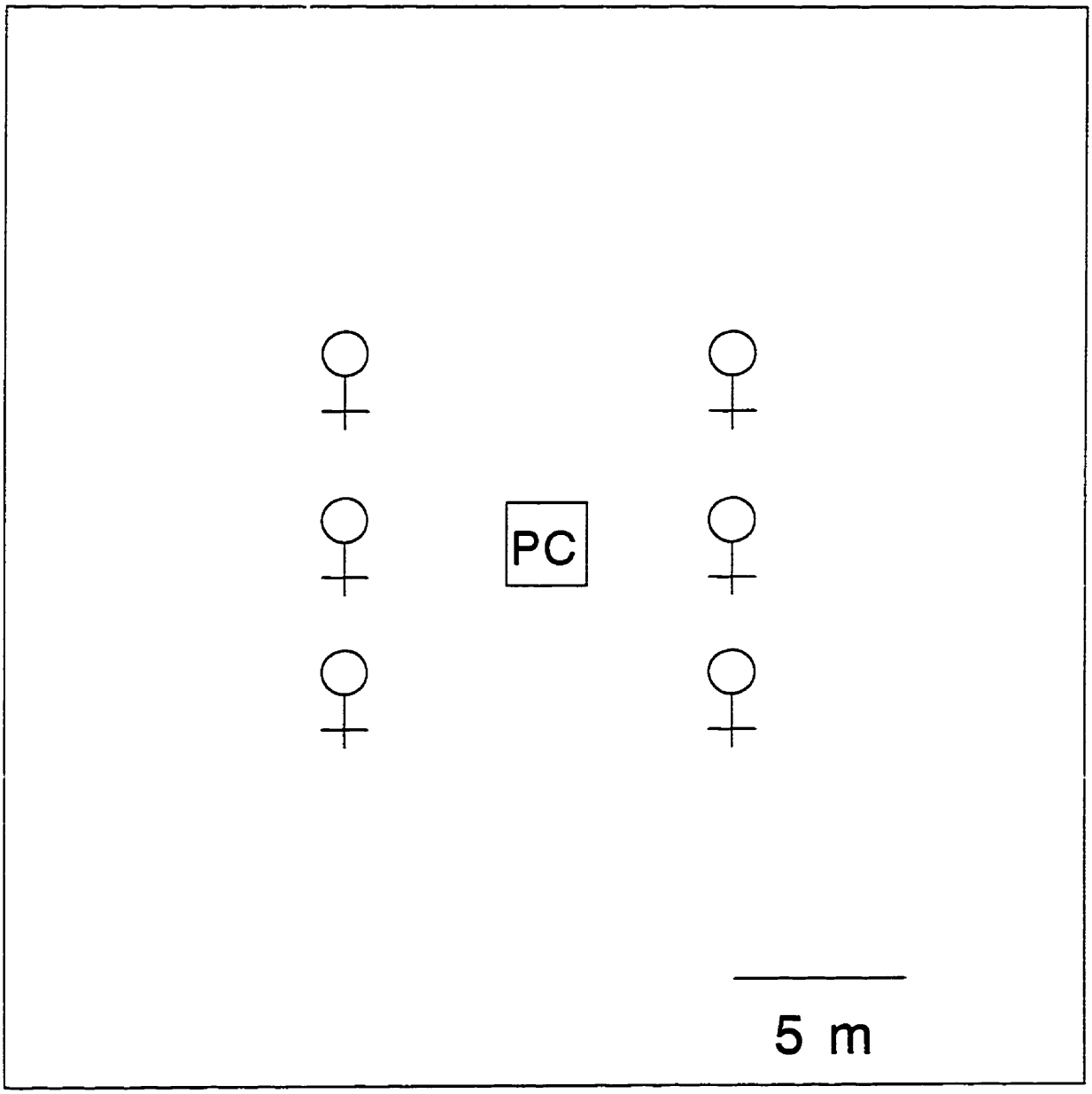
components in 100 µL HPLC-grade hexane and aged in a fumehood at 22°C for 24 h prior to use. Septa that were not used immediately thereafter were stored at -15°C. Stored septa were warmed at room temperature for 1 h before use in bioassays. Purities of compounds used were: (*Z*)-11-tetradecenyl acetate (*Z*11-14:OAc), 98% with 2% *E* isomer; (*Z*)-11-tetradecenyl aldehyde (*Z*11-14:Ald), 96%; (*Z*)-11-tetradecenyl alcohol (*Z*11-14:OH), 97%; and (*Z*9)-tetradecenyl acetate (*Z*9-14:OAc), 96%. Dilutions and blend compositions used in each experiment were verified by gas chromatographic analyses (R. Gries, Dept. Biological Sciences, Simon Fraser University, Burnaby, B.C.).

2.3 Small-Plot Protocol for Mating-disruption Trials

2.3.1 Plot Description

Unless otherwise mentioned, all mating-disruption experiments were set up following a Latin Square design. Plots (Fig. 2) were 0.1 ha in size within the same orchard, or two adjacent orchards, at least 10 m from the edge of the orchard and separated by at least 40 m. Treatments were randomly assigned to plots and re-randomized over time so that each treatment occupied each plot for one time period. Pheromone was applied using Conrel® fibre-tape dispensers (Ecogen Inc., Billings MT), hung in the upper third of the tree canopy, on the northeast side of the tree. Contents of pheromone dispensers used in all mating-disruption experiments (Appendix 2) were confirmed by gas chromatographic analyses (H.D. Pierce Jr., Dept. Biological Sciences, Simon Fraser University, Burnaby, B.C.). New dispensers were used at the beginning of each replicate and were deployed at a dispenser density of 1000 ha^{-1}, at least 48 h

Figure 2. Plot design used to test mating disruption in 0.1 ha plots located in organically-managed apple orchards in Cawston, B.C. Plots were spaced ≥ 40 m apart and ≥10 m from the edge of each orchard. PC = plot centre. ♀ = position of two tethered females (except where otherwise mentioned), one female was placed in the upper third of the canopy and the other at head height (ca. 1.5 m).


PC
5 m

before treatment assessment, unless otherwise specified. Tree spacings, canopy measurements and apple varieties for each plot in each experiment are listed in Appendix 3.

2.3.2 Treatment Assessment

In all but one mating-disruption experiment, pheromone treatments were assessed using tethered, virgin females. Six to 96-h-old female *C. rosaceana* and *P. limitata* were obtained as described in section 2.2.2. Females were immobilized and tethered at 0.5 °C by tying a polyester thread around the base of one forewing. Moths were transported to field sites in refrigerated containers and in late afternoon secured in trees arrayed around the centre of the plots (Fig. 2) by taping the tethering thread to a branch. In most cases, females were placed at two levels, at head height (~1.5 m above ground) and in the upper third of the canopy. Placement location was marked with flagging tape and maintained throughout an experiment. Females were distributed evenly among treatments by age. To determine mating status, they were collected the following morning and dissected and examined for the presence of at least one spermatophore in the *bursa copulatrix*. The tethering procedure did not alter the females' ability to attract mates (Appendix 4).

2.4 Sticky Traps

Traps were used to monitor populations of *C. rosaceana* and *P. limitata* throughout the flight season, and in several experiments. Traps were constructed from opposing wing-trap tops (Phero Tech Inc., Delta, B.C.) held 5 cm apart with pieces of

drinking straw. Stickem Special (Phero Tech Inc., Delta, B.C.) was thinly applied to the inside surface of the trap bottom (an inverted top) to capture attracted moths. All traps were baited at the study site. Fresh, disposable plastic gloves were worn while handling lures, which were suspended from the inside top of traps using a straight pin. Traps were hung by a wire hanger from trees approximately 1.5 m above ground.

Lures used to monitor *C. rosaceana* and *P. limitata* consisted of 3 mg (unless otherwise mentioned) of each of their respective pheromones in 200 µL of HPLC-grade hexane. Blend compositions were a 100:2:1.5:1 ratio of *Z*11-14:OAc, *E*11-14:OAc, *Z*11-14:OH, and *Z*11-14:Ald, and a 94:6 ratio of *Z*11-14:OAc and *Z*9-14:OAc for *C. rosaceana* and *P. limitata* blends, respectively. Blend compositions and dilutions were verified by gas chromatographic analysis (R. Gries, Dept. Biological Sciences, Simon Fraser University, Burnaby, B.C.). Purities of compounds used were as in section 2.2.3.

2.5 Statistical Analysis of Proportional Data

The majority of data generated in wind-tunnel and mating-disruption experiments were proportional, and analyzed by logistic regression, a more powerful method of analyzing proportional data (Levesque 1990) than either standard Chi-square (χ^2) tests or Analysis of Variance (ANOVA) on arcsin-squareroot-transformed data (Zar 1984). Selecting the most powerful method reduces the possibility of committing a type II error, i.e. not rejecting the null hypothesis when it is false.

The linear logistic model is part of a class of general linear models (McCullagh and Nelder 1983), for which there are three components (Gilchrist and Green 1993):

1) RANDOM COMPONENT: a description of the assumed underlying probability distribution of the variable of interest. It is assumed that the observed values of the random variable are independently distributed.

2) SYSTEMATIC COMPONENT: a linear combination of explanatory variables; and

3) LINK COMPONENT: a link between the random and systematic components.

In logistic regression the response variable (p_i) is binomially distributed and has the form y/n, where y = number of successes, eg. the number of insects mating out of n = total number tested

equation (1): $p_i = \exp(\beta_0 + \beta_{1x1i} + \ldots \beta_{kxki}) / 1 + \exp(\beta_0 + \beta_{1x1i} + \ldots \beta_{kxki})$

where the relationship between p_i and x_i is sigmoidal, but it can be shown (Collet 1989) that the logit (p_i) is linearly related to x_i,

equation (2): logit $(p_i) = \log(p_i/(1-p_i)) = \beta_0 + \beta_{1x1i} + \ldots \beta_{kxki}$.

The logit is the link function, a linear transform used in the logistic regression model. GLIM (1985), the software used to conduct the logistic regression analysis, carries out weighted regression, in which individual sample sizes are used as weights (Crawley 1993).

In order to fit a linear logistic model the unknown parameters (βs in equation 2) must be estimated (Collet 1989). These parameters are estimated by the method of maximum likelihood. The scaled deviance is the likelihood-ratio statistic used to compare a component of a particular model with the full model (Collet 1989), and can be thought of as the measure of discrepancy between the observed data and corresponding fitted values (Gilchrest 1993). The non-negative difference in scaled deviances of two models is asymptotically distributed as χ^2 distribution.

Fitting of a significant logistic regression model was followed by *Z*-tests to compare individual proportions. The α-value for these individual comparisons was adjusted using the Bonferroni inequality to hold the experiment-wise error rate to 5%, thus controlling for type I errors which depend on the number of simultaneous comparisons being made (Zar 1984).

3.0 PHENOLOGY OF *Choristoneura rosaceana* ON APPLE IN BRITISH COLUMBIA

3.1 Introduction

Choristoneura rosaceana overwinters as diapausing third- or fourth-instar larvae (Gangavalli and AliNiazee 1985a) in protective hibernacula on woody host plants (Chapman et al. 1968). Diapause is facultative (Chapman et al. 1968), allowing for additional generations if conditions are favourable. In B.C., overwintering larvae break diapause early in the spring and resume development through the remainder of six instars. Adult flight usually starts in early June and mating and oviposition are presumed to occur from mid-June to August; a second flight begins in August and continues until October (Madsen and Proctor 1985). Eggs are laid during this period and larvae that emerge in September and October overwinter in diapause.

The number of generations per year varies by location: two in New York (Chapman et al. 1968), southern Québec (Delisle 1992a; Hunter and McNeil 1997) and Oregon (AliNiazee 1986), but only one in northern Québec (Hunter and McNeil 1997), Nova Scotia (Sanders and Dustan 1919) and Utah (Knowlton and Allen 1937). In B.C., *C. rosaceana* has been reported to be univoltine (Venables 1924) but Madsen et al. (1984) suggested that it is univoltine in the northern Okanagan Valley and at high elevations, and is bivoltine in the southern Okanagan and Similkameen Valleys. The quality of the host plant influences the proportion of *C. rosaceana* that enter diapause (Hunter and McNeil 1997), which may contribute to variable voltinism in the same area.

Emergent overwintering larvae can cause premature fruit drop, or deep russetted scarring of the apple (Reissig 1978). Summer-generation larvae cause damage by

tying leaves to the surface of fruit, resulting in an irregular scarring of the fruit (Madsen and Proctor 1985). This damage can be more serious than that from overwintering larvae, because most damaged apples remain on the tree at harvest (Reissig 1978).

Control of the codling moth, *C. pomonella* (L.) by the Sterile Insect Release (SIR) programme (Dyck et al. 1993) or by pheromone-based mating disruption (Judd et al. 1996a, 1997) in the Okanagan, Similkameen and Creston Valleys of B.C. will reduce insecticide application in orchards and may elevate the pest status of *C. rosaceana* and other leafroller species. In an effort to benefit from organic control of *C. pomonella* and produce insecticide-free fruit, growers would like to develop organic methods of managing *C. rosaceana* such as pheromone-based mating disruption and/or applications of *Bacillus thuringiensis* Berliner (*B.t.*). In order to schedule pheromone applications so that they are effective at the time of mating and oviposition, information on the developmental physiology of this insect is required.

AliNiazee (1986) developed a degree-day (DD) model for predicting male *C. rosaceana* flight as measured by moth capture in pheromone-baited traps. In Oregon, the first adult flight lasted 1172 DD above 10°C ($DD_{10°C}$) from first to last moth capture, but the second generation flight was much shorter, lasting only 519 $DD_{10°C}$. In a laboratory study, Gangavalli and AliNiazee (1985b) demonstrated that after eclosion female *C. rosaceana* preoviposition required 35.2 $DD_{11.9\,°C}$, egg development from oviposition to hatch took 111.9 $DD_{10°C}$, larvae needed 435.6 $DD_{10°C}$ to complete development, and pupae eclosed after 117.4 $DD_{11.9°C}$. Reissig (1978) developed a temperature-driven model to predict egg hatch based on when the first male moth was captured in pheromone-baited traps; this biological indicator is referred to as biofix.

Onstad et al. (1985) developed a model to predict the number of larvae hatching at a given time in order to time spray applications. The crucial developmental information required for implementation of a pheromone-based mating-disruption programme is eclosion of female moths and timing and duration of mating and oviposition in the field.

My objectives were to: 1) determine adult eclosion of both sexes from both generations of *C. rosaceana* on a physiological scale (DD); 2) determine the onset and duration of oviposition on a physiological scale; and 3) relate developmental events to trap capture and mating status of male moths in pheromone-baited traps on a physiological scale.

3.2 Methods and Materials

3.2.1 Larval Development

Larvae were collected in 1996 from an organic apple orchard in Cawston, B.C., from 5-8 May, corresponding to 86.9-89.1 degree days above 10°C ($DD_{10°C}$) accumulated from 1 January. Larvae were taken from throughout the orchard, placed in paper bags and transported to the laboratory in refrigerated containers, and transferred in groups of 5-10 into mesh cylindrical sleeve cages (50 cm long x 20 cm diam.) made from white nylon organza. Each cage was secured at both ends over a leaf-bearing branch ca. 2 m above ground on apple trees in an experimental orchard at PARC for the duration of larval development. Caged larvae were moved to a fresh branch if necessary to ensure an adequate supply of apple leaves on which to feed.

Larvae were collected in 1997 from the same orchard as in 1996. Sixty apple

trees were sampled weekly while overwintering larvae were emerging from hibernaculae from 6 May-3 June, corresponding to 76.1-258.7 $DD_{10\,°C}$ accumulated from 1 January. Sampled trees were spaced evenly throughout the orchard and both edge and interior trees were included. A sample consisted of a 5 min full-tree search for larval nests, leaves or petals webbed together, which were removed during the sample. Collected larvae were stored, transported, and reared in groups of 10-20 in mesh cylindrical sleeve cages as in 1996. Every 10th larva was preserved in 70% ethanol for head capsule measurement.

3.2.2 Adult Eclosion

Once pupae were detected, caged branches were cut from the tree and sleeve cages and leaves were carefully inspected for pupae and larvae. Searches for pupae were repeated weekly after the onset of pupation from 29 May-24 July, 1996 and from 21 May-9 July, 1997, until all larvae had pupated. The incidence of parasitism or disease was noted during each inspection.

In 1996, pupae were sexed and transferred to a mesh emergence cage with a wooden frame (28 x 28 x 37 cm), located on a platform 1.4 m above ground within the tree canopy in the PARC orchard. The cage was checked daily for newly-eclosed adults. Adults were sexed and two out of every three males and females were transferred to a mesh oviposition cage with an aluminium frame (44 x 41 x 41 cm) placed on a platform 1.4 m above ground within the tree canopy in the orchard. In the oviposition cage, moths were provided with 3-4 freshly excised apple branches placed in water as an oviposition substrate and dental cotton wicks in flasks of distilled water

as a water source. The remaining adults were transferred to a field cage (3.6 x 3.6 x 2.4 m) placed over a small apple tree, which had previously been stripped of all leafroller larvae, to mate and produce larvae as a collection source for the second generation.

In 1997, pupae were sexed and transferred to emergence cages as in 1996, but were segregated into five cages by larval collection date. Newly-eclosed adults were collected daily, sexed, transferred to a communal oviposition cage (44 x 41 x 41 cm) placed on a platform 1.4 m above ground, within the tree canopy in the same orchard, and provided with apple branches and water as in 1996.

3.2.3 Male Moth Flight

Two monitoring traps were hung in the PARC orchard on 31 May 1996 and 7 June 1997. Traps were assembled and baited as in section 2.4 and lures were replaced every four weeks throughout the flight period. Traps were checked daily for the duration of the first-generation flight in 1996 and throughout the entire flight period in 1997. Each day, trap bottoms with captured males were removed and replaced. Monitoring traps were also placed in three orchards in Cawston, from 1994-1997 and checked at 2-3 d intervals throughout the flight period. Trap catches were pooled at each site prior to analysis.

3.2.4 Oviposition

Excised apple branches in the oviposition cages were checked daily, inspected for egg masses and replaced by freshly cut branches. In 1996, egg masses were

counted, labelled, transported to the laboratory for enumeration of eggs and discarded. In 1997, egg masses were counted, labelled and transferred to a (3.6 x 3.6 x 2.4 m) field cage covering a small apple tree which had previously been stripped of all leafroller larvae. Larvae emerging from these egg masses were used as a collection source for the second generation.

3.2.5 Weather Data

Hourly air temperatures were recorded year-round in Cawston at an orchard located approximately 1 km from the orchard in which larvae were collected, and in the PARC orchard using DP-212 datapods (Omnidata, Logan, UT) housed in standard-height Stevenson screens placed in the centre of each orchard. When temperature data were missing due to malfunction of equipment, replacement data were obtained from Cawston (Integrated Crop Management) or Keremeos (Environment Canada), and from Summerland (PARC, Agriculture and Agri-food Canada). Daily DD summations for each location and year were calculated by fitting a sine wave (Allen 1976) to daily temperature minima and maxima using the computer programme described by Higley et al. (1986). A lower base temperature of 10°C and upper threshold temperature of 31°C were chosen, based on developmental data for *C. rosaceana* (Gangavalli and AliNiazee 1985b). A lower base temperature of 10°C is used by growers and extension workers in management of codling moth (Riedl et al. 1976) and could easily be incorporated into an integrated pest management programme for *C. rosaceana*. DD accumulations were started on 1 January of each year instead of after the first trap catch (biofix) because larvae originated in a different location from where adults

emerged. The difference in DD accumulations between Cawston and PARC was added to the DD accumulations at PARC, when larvae were moved to this more northerly location.

3.2.6 Statistical Analyses

Daily trap catches, and eclosion of males and females at PARC in both years were converted to cumulative percentages of total generational trap catch, or eclosion, respectively, and plotted against $DD_{10°C}$ accumulated from 1 January. Cumulative percentages could not be calculated for the second generation trap catch or eclosion in 1996 or for trap catch in 1997. A non-parametric, two-sample Kolmogorov-Smirnov test was used to test the hypothesis that the cumulative distributions for eclosed adult males and females were equivalent (Conover 1971). The test statistic, T is defined as the greatest vertical distance between the two empirical distribution functions S_1 and S_2, which were obtained by a random sample (Conover 1971).

To determine if the number of egg masses deposited was an adequate measure of total eggs laid, the number of $DD_{10°C}$ accumulated after first female emergence in 1996 was regressed against the number of eggs per sampled egg mass (SAS 1996). The number of eggs per mass did not change throughout the oviposition period (r^2=0.0650) so all calculations in 1996 and 1997 were conducted on the number of egg masses laid. The numbers of egg masses produced daily were converted to cumulative percentages of the total number of egg masses produced per generation and plotted against $DD_{10°C}$ accumulated from the first female emergence. Cumulative percentages were not calculated for second-generation oviposition in 1996.

The nonlinear relationship between female eclosion, male eclosion, oviposition or trap catch and temperature was modelled with cumulative Weibull functions (Wagner et al. 1984). This technique has been used to describe the relationship between temperature and insect development and eclosion in other species (Wagner et al. 1984; Cockfield et al. 1994; Judd et al. 1996b; Judd and Gardiner 1997; McBrien and Judd 1998). A cumulative Weibull function of the form:

$$f(x)=100\left[\,1-\exp^{-(x/a)^b}\,\right]$$

in which, f(x) is the cumulative percentage emergence or trap catch, x is the predictor variable (time or degree days), and a and b are parameters to be estimated. Estimated values for parameters were determined using the nonlinear regression procedure in SigmaStat™ (1994).

3.2.7 Determination of Male *C. rosaceana* Mating Status

To determine if the proportion of male *C. rosaceana* mated at any given time is related to the progression of adult flight, a simple technique to assess mating status of males trapped in pheromone-baited traps is needed. Bergh and Seabrook (1986a) developed a technique to determine mating status of male eastern spruce budworms, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) based on colouration of the 7th segment of the primary simplex of the *ductus ejaculatorius*. Several experiments were conducted to determine if the technique could be adapted for *C. rosaceana*.

In Experiment (Exp.) 1, male pupae were placed alone or with a female pupa in

150 mL cups and provided with a water source. Male and female pupae were obtained from the laboratory colony. After eclosion, females were dissected to ensure that pairs had copulated. The precise time since mating was not known when males were dissected. Mated and virgin males ranged in age from 24-168 h and 6-144 h, respectively, at the time of dissection. Dissections of males followed the protocol of Bergh and Seabrook (1986a). The male reproductive tract was removed by securing the moth at the thorax and gently pulling on the claspers at the tip of the abdomen. The colour and transparency of the 7th segment of the primary simplex of the *ductus ejaculatorius* was recorded. This segment of the primary simplex is easily identified by the loop in the cuticular simplex directly to the posterior (Outram 1970).

Exp. 2 examined the effect of time since mating on the appearance of segment 7 of the primary simplex of mated males. Male and female pupae were set up in cups as in Exp. 1, but were held at 24°C under a reversed photoregime of 8:16 h D:L. After adult eclosion, cups containing moth pairs were observed 3 h into the scotophase and recorded as *in copulo* if they were mating. Females were removed at the onset of photophase and males were either dissected at that point or aged for up to 6 d before dissection. Dissection of males was conducted as in Exp. 1.

In addition to these laboratory experiments, 20 (1996) or 10 (1997) males from daily trap captures in the PARC orchard were dissected to determine mating status as above. Moths were dissected immediately after removal of trap bottom from traps or trap bottoms were wrapped in plastic and stored at 4°C for 1-2 d before dissection. All moths on the trap bottom were dissected if the number of moths captured was <20 (1996) and <10 (1997). DD accumulations were regressed against the percent of

mated first-generation males captured in traps (SAS 1996).

3.3 Results

3.3.1 Larval Development

Head capsule measurement of larvae in 1997 indicated that the first collection period, conducted 76.1 $DD_{10°C}$ after 1 January, coincided with the end of larval diapause as all of the larvae measured were 2nd-4th instars (Table 1). Only one individual diapaused as a 2nd instar. All larvae were in the 4th-6th instar by the second collection period, conducted 130.1 $DD_{10°C}$ after 1 January.

3.3.2 Adult Eclosion

Similar numbers of male and female *C. rosaceana* eclosed in 1996 and 1997 (Table 2), suggesting a 1:1 sex ratio in nature. In both years protandrous males emerged before females. The cumulative distributions of percent male and female eclosion were significantly different for first-generation moths in 1996 ($T_{60,60} = 0.2483$, $P < 0.05$) and in 1997 ($T_{114,130} = 0.15526$, $P<0.05$). In 1996 and 1997 the first male moths of the first generation eclosed at 220.7 and 254.6 $DD_{10°C}$, respectively, 18.2 and 7.7 $DD_{10°C}$ before first female moths. Fifty percent of first-generation males in 1996 and 1997 eclosed 26.2 and 18.6 $DD_{10°C}$, respectively, before 50% females eclosed, indicating protandry throughout the eclosion period. Second-generation eclosion could only be followed in 1997 (Table 2), as too few individuals were recovered in 1996. As in the first generation, males eclosed before females ($T_{12,19} = 0.4722$, $P<0.05$). Few

Table 1. Proportion of larvae in each instar as determined by head capsule measurements (J. Cossentine, pers. comm., Agriculture and Agri-food Canada, PARC, Summerland, B.C.) at various collection times in 1997.

$DD_{10°C}$ at collection	Collection date	No. larvae measured	Proportion of larvae measured				
			2nd instar	3rd instar	4th instar	5th instar	6th instar
76.1	6 May	9	0.11	0.33	0.56	-	-
130.1	13 May	18	-	-	0.22	0.56	0.22
185.3	20 May	16	-	-	0.06	0.25	0.69
216.4	27 May	10	-	-	-	0.30	0.70
258.7	3 June	3	-	-	-	-	1

Table 2. Eclosion and trapping events for adult *C. rosaceana* in 1996 and 1997.

					Observed $DD_{10°C}$ from January 1, 1996 & 1997 for various eclosion (Ec) and trapping (Tr) events							
			No. of insects		First occurrence		5th percentile		50th percentile		95th percentile	
Year	Generation	Sex	Ec	Tr	Ec	Tr	Ec	Tr	Ec	Tr	Ec	Tr
1996	1	♀♀	60		238.9		246.4		290.1		428.3	
		♂♂	60	3714	220.7	214	220.7	306	263.9	452.3	335.1	634.8
1997	1	♀♀	130		262.3		285.4		346.8		472.5	
		♂♂	114	4169	254.6	248.8	278.7	332.3	328.2	530.3	481.7	690.7
	2	♀♀	16		842.8		842.8		908.7		100.4	
		♂♂	12		740.9		740.9		842.8		929	

larvae that emerged from egg masses deposited by the collected individuals developed through to second-generation adults, suggesting that most summer-generation larvae entered diapause. This is supported by the collection of 288 larvae that emerged on this same caged tree in the spring of 1998.

Adult male and female eclosion in the first generation modelled using Weibull functions described the within year eclosion accurately for both sexes. However, Weibull functions did not fit the combined data well (Table 3, Fig. 3).

3.3.3 Male Moth Flight

First trap catches of males in the PARC orchard occurred at 214 and 248.8 $DD_{10°C}$ in 1996 and 1997, respectively (Table 2). The first male trap catch preceded first male eclosion by 6.7 and 5.8 $DD_{10°C}$ in 1996 and 1997, respectively, which corresponds to only one calender day in both years. Despite the congruence between first trap capture and first male eclosion, cumulative percentages of males captured in traps lagged behind cumulative percentages of male eclosion in 1996 and 1997 (Table 2). First male trap catch preceded first female eclosion by 24.9 and 13.5 $DD_{10°C}$, corresponding to five and two calender days in 1996 and 1997, respectively (Table 2). Cumulative percentage curves of male trap captures in 1996 and 1997 also lagged behind female eclosion.

Weibull functions accurately described cumulative percentages of first-generation trap capture at PARC in 1996 and 1997 and for both years combined (Table 3, Fig. 4). Similarly, a Weibull function fit the cumulative percentages of first-generation trap capture in Cawston over a four-year period (Table 3, Fig. 5). The functions of trap

Table 3. Estimated values for parameters of Weibull functions and their goodness of fit to cumulative percent distributions of various phenological events at PARC (1996-97) and trap catch in Cawston (1994-97).

Phenological event[a]	Year	Generation	Sex	Weibull parameter	Estimated values (± S.E.)	Model S.E.	r^2 (*P* value)
Eclosion	1996	1	♀♀	*a*	309.65 ± 2.98	8.09	0.93 (0.0001)
				b	6.24 ± 0.58		
Eclosion	1996	1	♂♂	*a*	274.70 ± 1.34	5.18	0.97 (0.0001)
				b	10.10 ± 0.68		
Eclosion	1997	1	♀♀	*a*	370.57 ± 1.23	4.96	0.98 (0.0001)
				b	7.52 ± 0.28		
Eclosion	1997	1	♂♂	*a*	359.06 ± 1.63	5.46	0.97 (0.0001)
				b	6.22 ± 0.24		
Eclosion	1996-97	1	♀♀	*a*	357.76 ± 3.67	14.56	0.78 (0.0001)
				b	5.29 ± 0.45		
Eclosion	1996-97	1	♂♂	*a*	333.35 ± 5.01	18.65	0.68 (0.0001)
				b	4.68 ± 0.52		
Trap Catch PARC	1996	1	♂♂	*a*	486.18 ± 1.68	2.71	0.99 (0.0001)
				b	5.31 ± 0.13		
Trap Catch PARC	1997	1	♂♂	*a*	555.34 ± 0.80	1.25	0.99 (0.0001)
				b	5.37 ± 0.05		
Trap Catch PARC	1996-97	1	♂♂	*a*	486.18 ± 1.68	2.71	0.99 (0.0001)
				b	5.31 ± 0.13		
Trap Catch Cawston	1994-97	1	♂♂	*a*	478.53 ± 3.15	6.11	0.97 (0.0001)
				b	4.24 ± 0.15		
Trap Catch Cawston	1994-97	2	♂♂	*a*	1211.22 ± 8.96	15.05	0.84 (0.0001)
				b	8.99 ± 0.80		
Oviposition	1996	1		*a*	83.40 ± 0.85	3.44	0.99 (0.0001)
				b	3.52 ± 0.17		
Oviposition	1997	1		*a*	131.62 ± 0.74	1.98	0.99 (0.0001)
				b	2.55 ± 0.06		
Oviposition	1997	2		*a*	113.53 ± 2.59	6.96	0.95 (0.0001)
				b	2.74 ± 0.28		
Oviposition	1996-97	1(1996) 1, 2 (1997)		*a*	110.66 ± 2.89	12.13	0.87 (0.0001)
				b	2.16 ± 0.19		

[a]For eclosion and trap catch models, the predictor variable was degree days above 10°C starting 1 January. For oviposition models, the predictor variable was degree days above 10°C starting at first female emergence.

Figure 3. Observed cumulative first-generation *C. rosaceana* adult eclosion (o) in 1996 and 1997 alone and combined, plotted against $DD_{10°C}$ air temperature after 1 January compared with curves (solid lines) modelled by Weibull functions (Table 3).

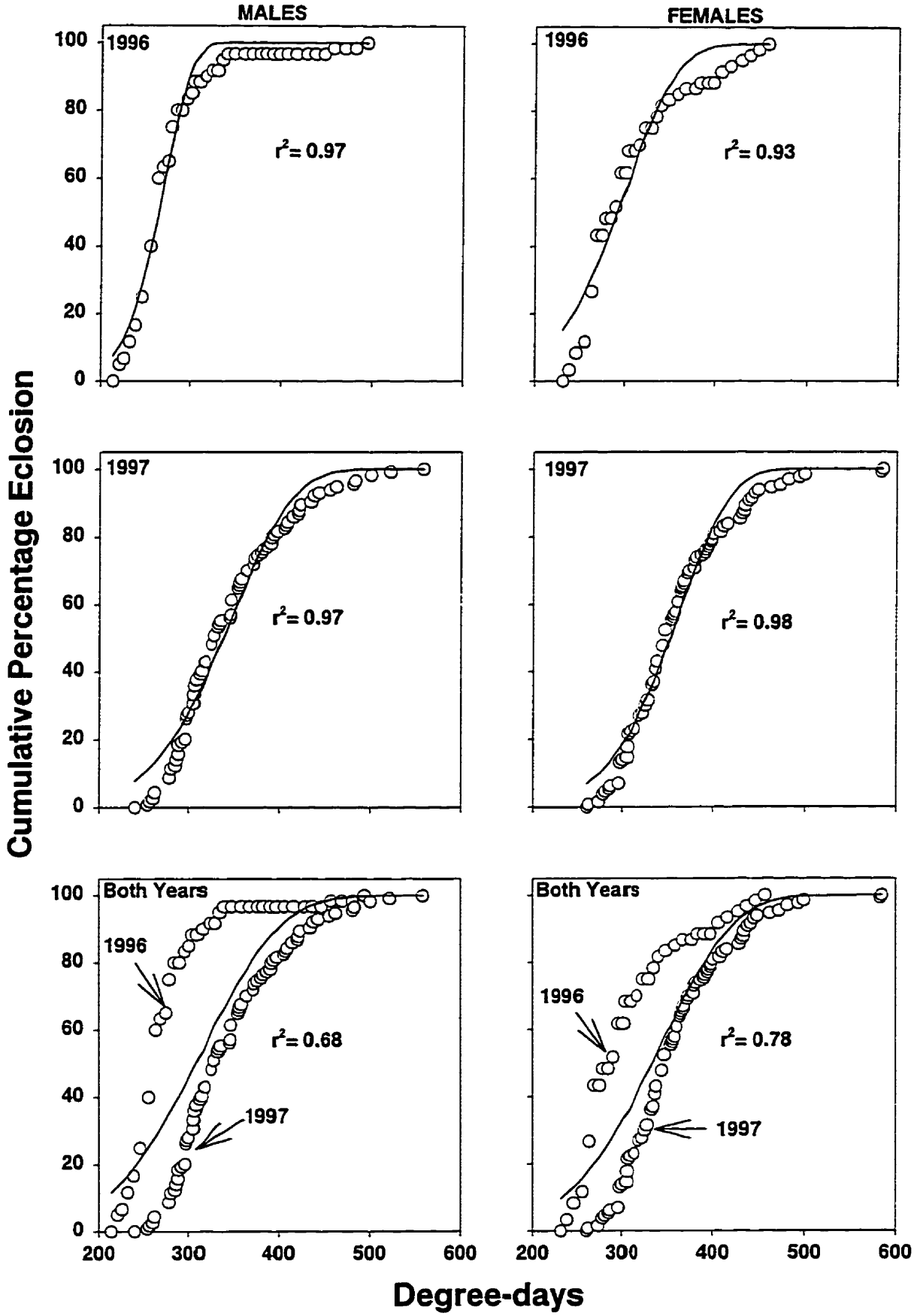

MALES
FEMALES
1996
1997
Both Years
r^2= 0.97
r^2= 0.93
r^2= 0.97
r^2= 0.98
r^2= 0.68
r^2= 0.78
Cumulative Percentage Eclosion
Degree-days
0
20
40
60
80
100
200
300
400
500
600

Figure 4. Cumulative trap captures of first-generation male *C. rosaceana* (o) in pheromone traps at PARC during 1996 and 1997 alone and combined, plotted against $DD_{10°C}$ air temperature after 1 January, compared with curves (solid lines) modelled by Weibull functions (Table 3).

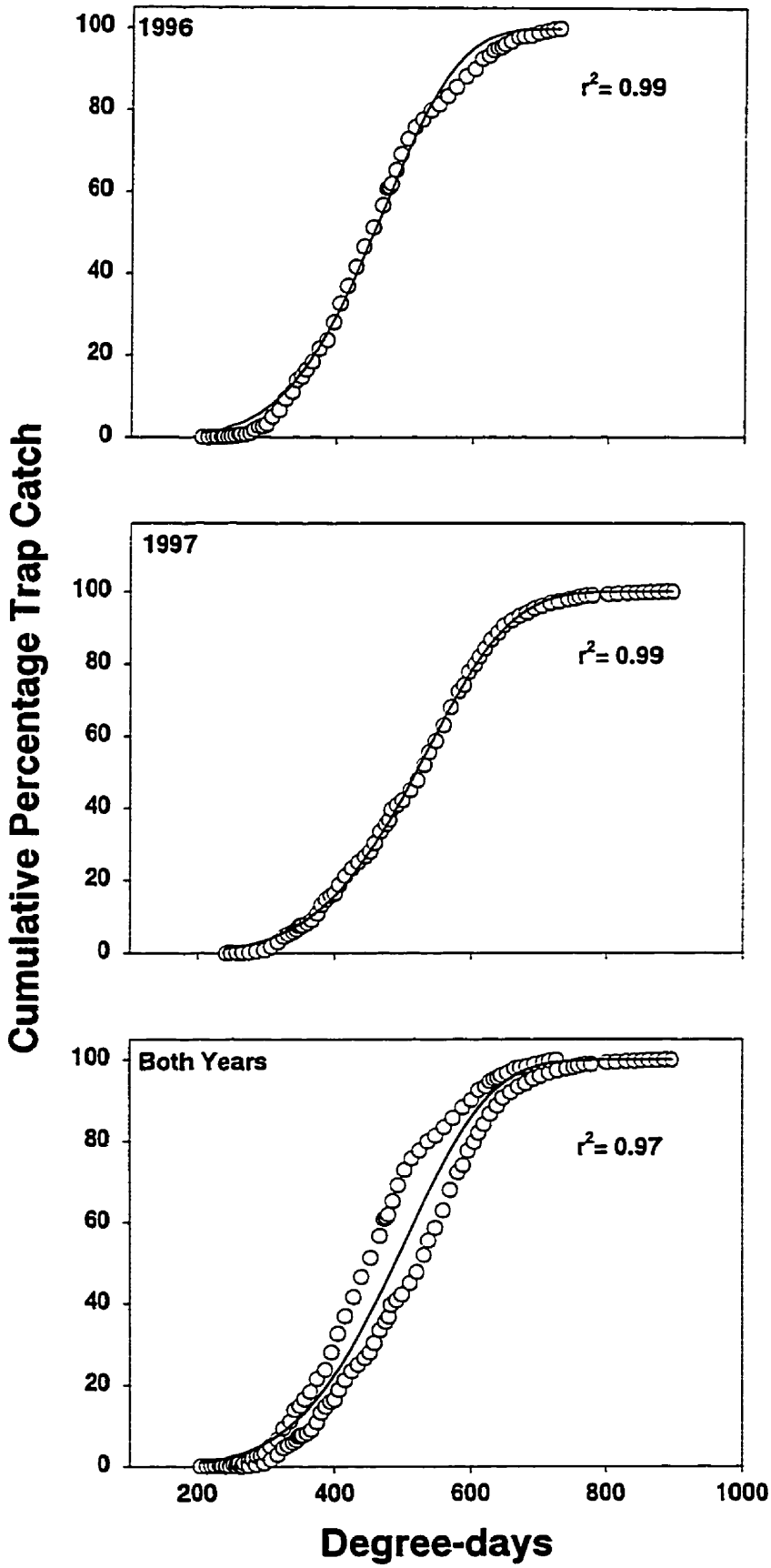
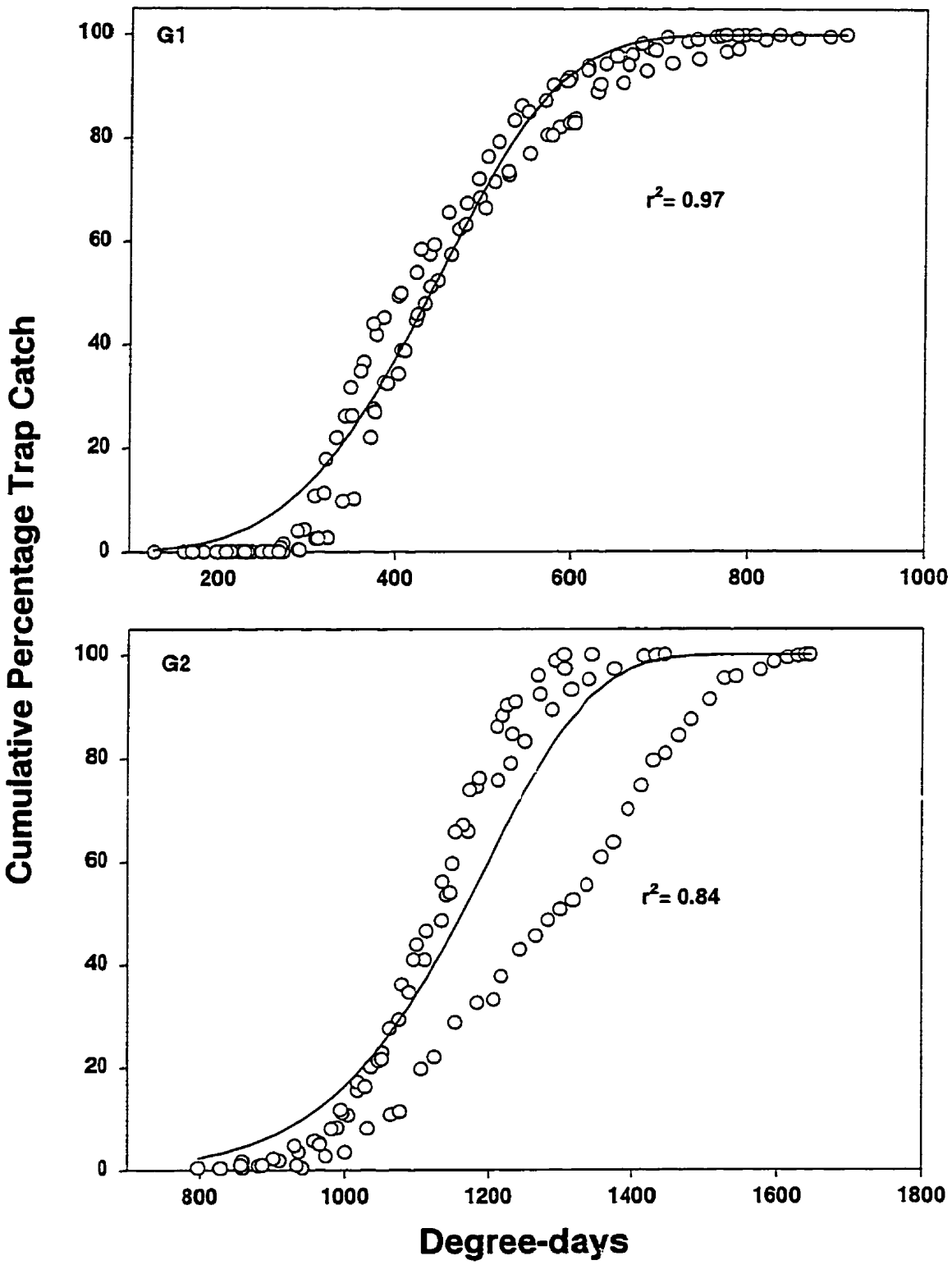

1996
r^2= 0.99
1997
r^2= 0.99
Both Years
r^2= 0.97
Cumulative Percentage Trap Catch
Degree-days
0
20
40
60
80
100
200
400
600
800
1000

Figure 5. Cumulative trap captures of first (G1)- and second (G2)-generation *C. rosaceana* (o) in pheromone traps in Cawston, 1994-1997 combined, plotted against $DD_{10°C}$ air temperature after 1 January, compared with curves (solid lines) modelled by Weibull functions (Table 3).


G1
r^2= 0.97
G2
r^2= 0.84
Cumulative Percentage Trap Catch
Degree-days
0
20
40
60
80
100
200
400
600
800
1000
1200
1400
1600
1800

catch from the two areas were similar and predicted 50% flight at 438 (Cawston) and 484.5 (Summerland) $DD_{10°C}$ after 1 January. Second-generation cumulative trap captures in Cawston were not as accurately described by a Weibull function (Table 3, Fig. 5). First- and second-generation male flight had similar durations in Cawston, varying from 480.9-636.2 and 475.8-779 $DD_{10°C}$, respectively.

3.3.4 Oviposition

Oviposition commenced 29.3 ± 2.7 $DD_{10°C}$ after first female eclosion (Fig. 6). For the first generation in 1996, and the first and second generations in 1997, and for all generations combined, Weibull functions predicted 50% oviposition to occur 77.6, 112.9, 98.5 and 91.1 $DD_{10°C}$ after first female eclosion, respectively. However, duration of the oviposition period is probably best estimated by the first-generation oviposition in 1997, because the adults producing the eggs were collected throughout the eclosion period and not at one time only, as in 1996. The oviposition period for the first generation in 1997 lasted 303.4 $DD_{10°C}$ after first female eclosion.

3.3.5 Determination of Male *C. rosaceana* Mating Status

Examination of dissected males demonstrated that the technique developed by Bergh and Seabrook (1986a) for *C. fumiferana* can be adapted for *C. rosaceana*. Segment seven of the primary simplex of the *ductus ejaculatorius* of *C. rosaceana* was opaque in 93.8% of the 80 virgin males dissected and clear in 93.3% of 60 mated males (Table 4). However, unlike *C. fumiferana*, the colour of the segment varied and appeared either yellow or white.

Figure 6. Observed cumulative oviposition (o) for first-generation (G1) *C. rosaceana* in 1996 and for first (G1)- and second (G2)-generations in 1997 plotted against $DD_{10°C}$ air temperature after first female eclosion, compared with curves (solid lines) modelled by Weibull functions (Table 3).

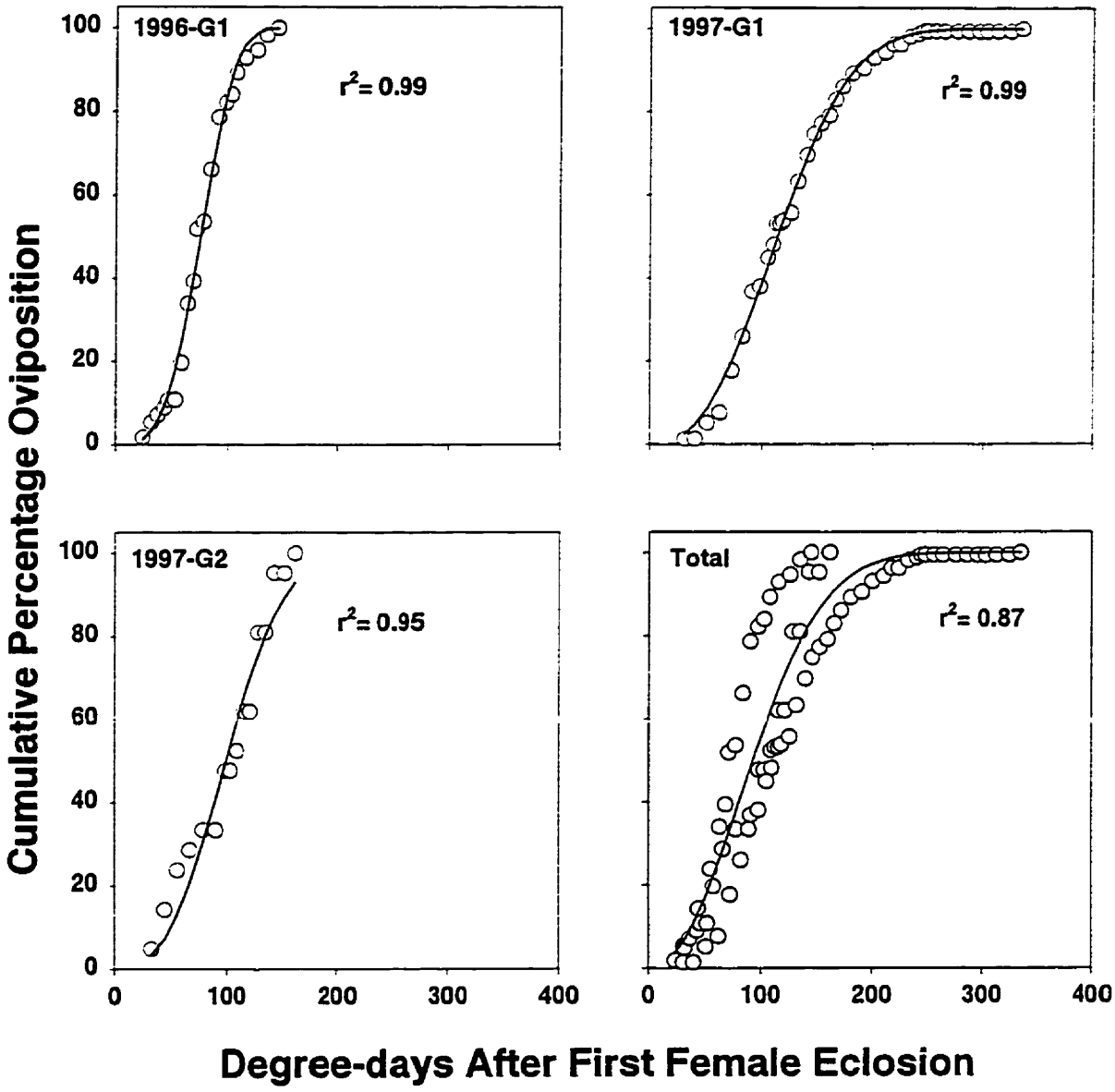

1996-G1
r^2= 0.99
1997-G1
r^2= 0.99
1997-G2
r^2= 0.95
Total
r^2= 0.87
Cumulative Percentage Oviposition
0
20
40
60
80
100
0
100
200
300
400
Degree-days After First Female Eclosion

Table 4. Assortment of virgin and mated *C. rosaceana* males in relation to the condition of segment 7 of the primary simplex of the *ductus ejaculatorius*.

		Percent of *C. rosaceana* sampled					
		Segment 7 opaque		Segment 7 clear		Segment 7 grainy	
No. dissected	Mating status	White	Yellow	Colourless	Yellow	White	Yellow
80	virgin	52.5	41.3	1.3	0	1.3	3.8
60	mated	1.7	0	93.3	0	5.0	0

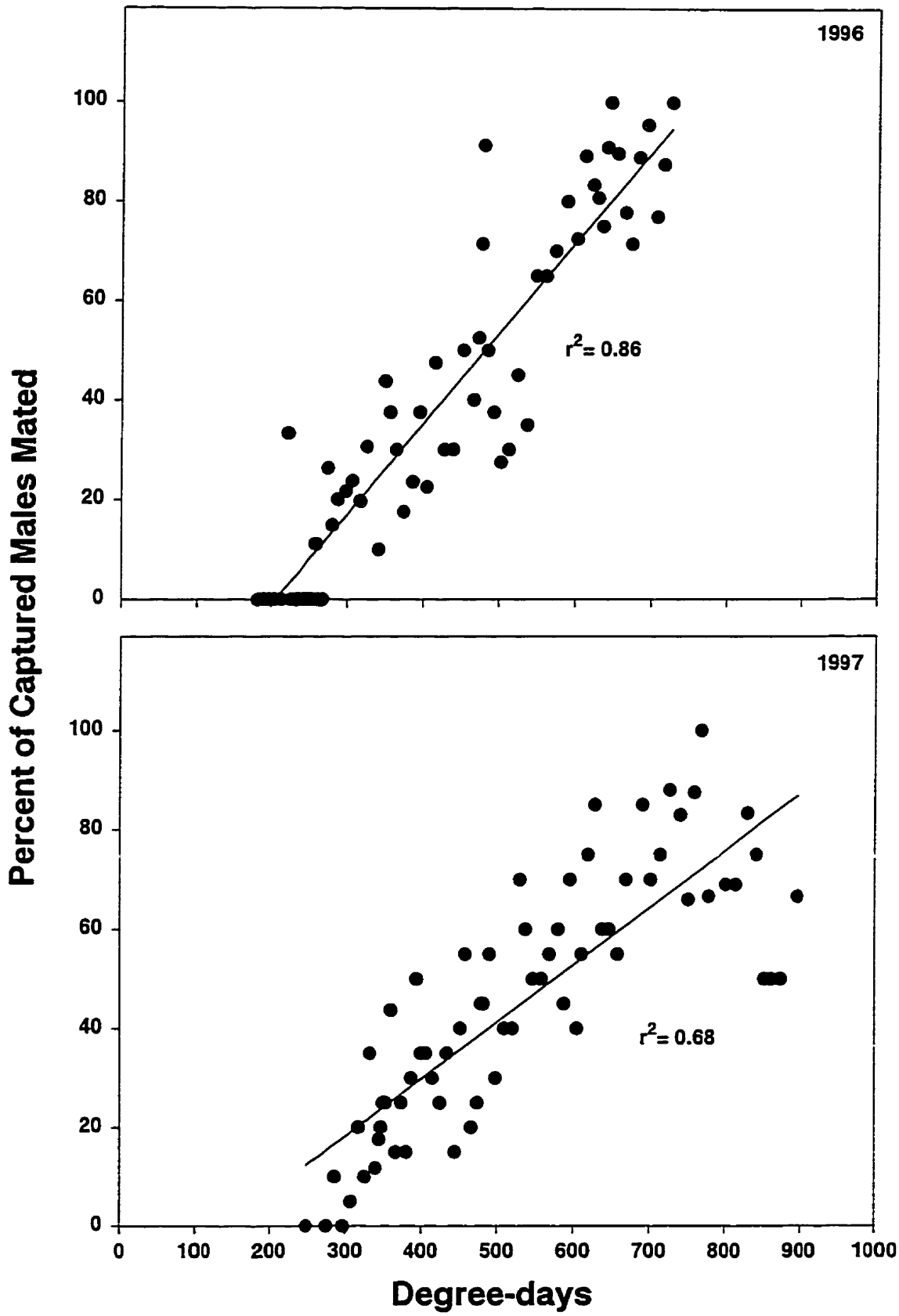
Lack of colouration in segment seven of the primary simplex in mated males appeared to persist several days after mating. Seven of 10 mated males showed no colouration 3-4 d after mating, whereas three showed some colouration but the segment was not opaque. Eight males dissected 5-6 d after mating showed no colouration.

The percentage of dissected males in pheromone-baited traps that were mated increased linearly over the duration of the first flight in both 1996 and 1997 (Fig. 7). In both years, 50% mating in trapped males corresponded to 100% female and male eclosion. Fifty percent male and female eclosion, as predicted by Weibull functions, corresponded to 10 and 24% mated males and 15 and 30% mated males captured in pheromone-baited traps in 1996 and 1997, respectively.

3.4 Discussion

The discovery that eclosion of *C. rosaceana* is protandrous in B.C., agrees with reports elsewhere in its range (Onstad et al. 1985; Delisle and Bouchard 1995). Protandry may be adaptive for *C. rosaceana*, as mating success of 2- to 4-d-old males is significantly greater than 0- to 2-d-old males (Delisle 1995). Weibull functions did not fit the combined eclosion data for either sex well (Table 3, Fig. 3), suggesting inter-year variation in adult eclosion patterns. In 1996, larvae were collected between 86.9-89.1 $DD_{10°C}$ after 1 January, instead of throughout the larval activity period as in 1997. Eclosion percentiles in 1996 always preceded those in 1997 (Table 2), suggesting that larvae that broke diapause late may have been missed in the 1996 sample. Larval

Figure 7. Relationships between percent mated males captured in monitoring traps located at PARC in 1996 and 1997 during the first-generation *C. rosaceana* flight and $DD_{10°C}$ air temperature after 1 January. Regression equations are $\hat{y} = 0.18x - 37.59$ (1996) and $\hat{y} = 0.12x - 16.26$ (1997).


1996
1997
r^2= 0.86
r^2= 0.68
Percent of Captured Males Mated
Degree-days
0
20
40
60
80
100
0
100
200
300
400
500
600
700
800
900
1000

sampling should be conducted throughout the eclosion period or after 185.3 $DD_{10°C}$ to ensure that all individuals have broken diapause (Table 1).

The capture of males in pheromone-baited traps prior to the first observed male eclosion may indicate that the active range of the pheromone-baited trap may be large enough to attract males from slightly warmer microclimates than the collected larvae had experienced. However, because the first trap catch in both years preceded eclosion of both sexes by a small and consistent margin, trap catches can be used as an indicator of adult eclosion.

The relationships between cumulative percent of trap catch and accumulated $DD_{10°C}$ in the first generation were similar in the four field seasons in Cawston as well as the two seasons at PARC (Figs. 4 and 5). The first male trap catch at 214-292.2 $DD_{10°C}$ after 1 January in the six site-years is earlier than male trap catch in filbert orchards in Oregon (356-409 $DD_{10°C}$ after 1 March) (AliNiazee 1986) but similar to first trap catch in trapping studies conducted in apple, raspberry and filbert in Oregon ($\bar{x}$=203 $DD_{10°C}$ after 1 March) (Gangavalli 1985). Any difference between January and March start dates for $DD_{10°C}$ accumulation would be negligible as very few DD accumulated between 1 January and 1 March in all four years of my study. The first male captured in the second-generation flight could only be measured accurately from the Cawston flight data (Fig. 5). The first male captured in pheromone-baited traps 797-941.5 $DD_{10°C}$ after 1 January preceded the observed second generation in filbert orchards in Oregon (1509-1663 $DD_{10°C}$ after 1 March) (AliNiazee 1986), but was similar to the second flight data in trapping studies in apple, raspberry and filbert in Oregon ($\bar{x}$: 713 $DD_{10°C}$ after 1 March) (Gangavalli 1985). Differences in initiation of flight may be

due to variation in larval development on different hosts (Onstad et al. 1985; Carrière 1992) as filbert is known to be a poor quality host (Delisle and Bouchard 1995).

The durations of the first and second flights were similar as indicated by $DD_{10°C}$ accumulations from first to last moth capture in pheromone-baited traps in Cawston which ranged from: 480.9-636.2 $DD_{10°C}$ and 475.8-779 $DD_{10°C}$ for first and second flights, respectively. First flight in filbert orchards in Oregon lasted much longer (1172 $DD_{10°C}$) but duration of the second generation was similar to my findings (519 $DD_{10°C}$) (AliNiazee 1986). Duration of the second flight will vary depending on environmental conditions. For example, constant high temperatures of 32°C may cause development of *C. rosaceana* to slow or cease and temperatures between 28-32°C can induce diapause in *C. rosaceana* larvae despite summer photoperiod conditions (Gangavalli and AliNiazee 1985a,b). High summer temperatures in Oregon prolonged development of the first generation and resulted in a long flight duration (AliNiazee 1986). Low numbers of second (summer) generation adults may be the result of several factors. A late spring eclosion due to cool temperatures could cause first- and second-instar larvae of the summer generation to be exposed to diapause-inducing conditions (short day length, cool temperatures) and cease development. High summer temperatures may also induce summer-generation larvae to enter diapause. Larval hosts can influence diapause induction (Carrière 1992; Hunter and McNeil 1997) and larval development (Onstad et al. 1986; Carrière 1992). For example, summer-generation larvae that feed on old apple leaves due to a delay in spring eclosion of overwintering larvae will develop slowly (Onstad et al. 1986) and may enter diapause before completing development. A very small second-generation flight was observed in 1997

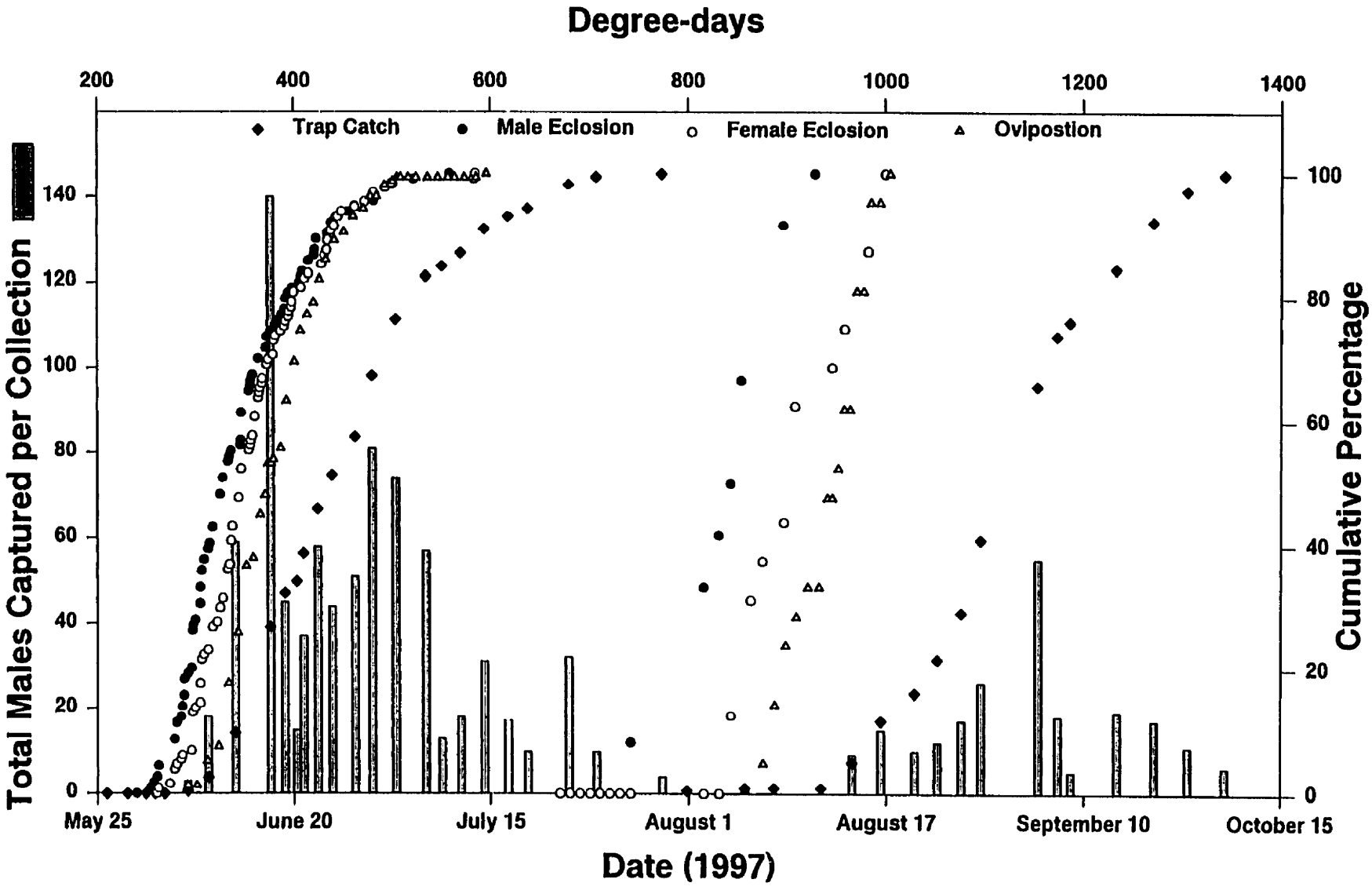
at PARC, probably because a cool spring delayed adult eclosion, and most summer-generation larvae entered diapause.

Oviposition started 24, 31.4 and 32.6 $DD_{10°C}$ after first female eclosion in the first generation in 1996 and the first and second generations in 1997, respectively (Fig. 6). In comparison, Gangavalli and AliNiazee (1985b) observed a pre-oviposition period of 35.2 $DD_{11.9°C}$ while Onstad et al. (1985) estimated it to be 14 $DD_{6°C}$. Older studies (Schuh and Mote 1948; Chapman and Lienk 1971) showed that no oviposition occurred within the first 24 h of male-female interaction.

Although the results show that the technique to asses mating of male *C. fumiferana* (Bergh and Seabrook 1986a) can be adapted for *C. rosaceana* (Table 4), the technique may be of limited use in predicting developmental events. When 50% of trapped males were observed to be mated, 100% of female and male moths had eclosed. This could indicate that males prefer to mate at an older age. Delisle (1995) demonstrated that mating success of male *C. rosaceana* increased with age over three days and then decreased. However, female mating success decreased linearly with age (Delisle 1995), suggesting that when 100% female eclosion had occurred more than 50% of males should be mated. These results may also demonstrate that only a portion of male *C. rosaceana* mate in nature. Alternatively, virgin males may be preferentially drawn to synthetic pheromone traps or mated males could have regenerated the secretion in the ejaculatory tract and appear to be virgins. Bergh and Seabrook (1986b) observed such regeneration in *C. fumiferana* three days after mating, but I saw no regeneration of material up to six days after mating in a sample of eight male *C. rosaceana*.

Capture of the first male moth in pheromone-baited traps preceded the first observed eclosion of adult males and females of the overwintering generation by a consistent margin in both 1996 and 1997, and therefore could be used as a reliable indicator of adult eclosion (Fig. 8). Pheromone dispensers, for the purposes of mating disruption, could be positioned in the orchard immediately after biofix and effectively disrupt mating of even the earliest eclosing moths. Pheromone dispensers should release enough pheromone to disrupt adult mate-finding behaviour throughout both flight periods, until early October, as the size of the summer generation is difficult to predict. Alternatively, growers may be able to disrupt the adults that emerge from the overwintering generation and add additional dispensers later in the season if conditions indicate a large second generation. If the second approach is taken, pheromone dispensers to disrupt the first generation should be effective throughout the first oviposition period. Oviposition started approximately 30 $DD_{10°C}$ after the first female eclosed, approximately 45 $DD_{10°C}$ after first moth capture and 50% of oviposition was predicted to occur ca. 113 $DD_{10°C}$ after first female eclosion, approximately 130 $DD_{10°C}$ after first moth capture (Fig. 8). However, direct correlations between biofix and developmental stages of *C. rosaceana* were not obtained in this study, and should be conducted before recommendations of mating disruption of the overwintering generation alone are made.

Figure 8. Percent cumulative phenological events and total number of males captured in pheromone-baited traps for the first- and second-generation *C. rosaceana* in 1997, plotted against $DD_{10°C}$ air temperature after 1 January. Cumulative percent distributions of male and female eclosion and oviposition obtained from data collected at PARC. Cumulative percent distribution of trap catch and total number of males captured obtained from data collected in Cawston.


Degree-days
200
400
600
800
1000
1200
1400
Trap Catch
Male Eclosion
Female Eclosion
Ovipostion
Total Males Captured per Collection
0
20
40
60
80
100
120
140
Cumulative Percentage
0
20
40
60
80
100
May 25
June 20
July 15
August 1
August 17
September 10
October 15
Date (1997)

4.0 MATING DISRUPTION OF *C. rosaceana* : IS THE MOST ATTRACTIVE BLEND REALLY MOST EFFECTIVE?

4.1 Introduction

As an extension of Roelofs' (1978) "Threshold Hypothesis for Pheromone Perception", Minks and Cardé (1988) suggested that an insects' 'natural' pheromone blend should be the best disruptant of mating at the lowest application rate, because it would probably elicit the greatest number of disruptive mechanisms (see section 1.1) (Bartell 1982; Cardé 1990). However, because the complete 'natural' pheromone blend can probably never be known with certainty, the Minks and Cardé (1988) hypothesis cannot be tested with certainty. However, a related hypothesis "that the most attractive pheromone blend in a series of blends should be the most effective disruptant of pheromone communication and mating", can be tested.

In eastern North America, *C. rosaceana* pheromone lures containing three components, *Z*11-14:OAc, *E*11-14:OAc and *Z*11-14:OH in a 90:5:5 ratio caught the greatest number of moths (Hill and Roelofs 1979). In orchards treated with this three-component blend, Agnello et al. (1996) demonstrated a significant reduction in catches of male *C. rosaceana* in synthetic pheromone-baited traps (56-97%), and Lawson et al. (1996) found a significant reduction in mating among tethered, virgin females. Reissig et al. (1978) showed that a blend containing *Z*11-14:OAc and *Z*11-14:OH was a more efficacious disruptant of orientation to pheromone traps in eastern populations of *C. rosaceana* than either component alone. Roelofs and Novak (1981) found that a three-component pheromone blend was more efficacious in reducing orientation of male *C. rosaceana* to pheromone traps than the individual components tested alone and in

partial or off-ratio blends.

In western North America, the most attractive pheromone lure for *C. rosaceana* consists of *Z*11-14:OAc, *E*11-14:OAc, *Z*11-14:OH and Z11-14:Ald in a ratio of 100:2:1.5:1 (Vakenti et al. 1988; Thomson et al. 1991). Deland et al. (1994) showed that atmospheric treatment with a 93:7 blend of *Z*11-14:OAc and *E*11-14:OAc alone disrupted orientation to synthetic pheromone-baited traps by 89-91%. However, there has been no direct comparison of the efficacy of partial or off-ratio pheromone blends and the most attractive pheromone blend as mating disruptants in western populations of *C. rosaceana*. I tested the hypothesis that the more attractive four-component pheromone blend (Vakenti et al. 1988) is a more effective mating disruptant in western populations of *C. rosaceana* than less attractive partial blends containing the major component *Z*11-14:OAc.

4.2 Methods and Materials

4.2.1 Field Experiments

All mating-disruption experiments were conducted in the Similkameen Valley, at Cawston, B.C., during 1994-1997 using the small-plot protocol (Roelofs and Novak 1981) described in section 2.3.1.

All atmospheric pheromone treatments were applied following a Latin Square design (see section 2.3.1), using Conrel® fibre-tape disruption dispensers (Ecogen Inc., Billings, MT). Disruption dispensers contained one of three different pheromone blends: 1) a **four-component blend** that contained *Z*11-14:OAc, *E*11-14:OAc, *Z*11-

14:OH, and *Z*11-14:Ald in a 100:2:1.5:1 ratio (Vakenti et al. 1988; Thomson et al. 1991), 2) a **three-component partial blend**, containing *Z*11-14:OAc, *E*11-14:OAc, and *Z*11-14:OH in a 100:2:1.5 ratio and 3) a **two-component partial blend**, containing *Z*11-14:OAc and *E*11-14:OAc in a 100:2 ratio. Actual blend ratios, as verified by gas chromatography (H.D. Pierce Jr., Dept. Biological Sciences, Simon Fraser University, Burnaby, B.C.), are listed in Appendix 2. Different pheromone release rates, based on approximate release rates at 20°C (Ecogen Inc., Billings MT) were provided by varying the numbers of fibres per tape and different dispenser densities were used in each experiment (Table 5). Dispenser placement followed the standard protocol outlined in section 2.3.1.

Disruption caused by pheromone treatments was assessed using tethered, virgin females placed in the canopy of trees at plot centre (Fig. 2) at both high and low locations in the late afternoon and collected early the following morning. Females were placed directly on the leaf surface (except in Exp. 1, see below) and secured by taping the thread to a branch. The mating status of females recovered from experimental plots was determined by dissection (section 2.3.2). The number of females used per treatment in each experiment ranged from 55-118.

Exp. 1 tested the hypothesis that atmospheric treatment with the two-, three-, and four-component blends would disrupt mating of tethered *C. rosaceana* equally. Disruption dispensers with 100 fibres per tape were hung in the upper third of the canopy at a dispenser density of 100 per plot (1000 ha^{-1}) providing an approximate release rate of 10 $mg \cdot ha^{-1} \cdot h^{-1}$ (Table 5). The experiment ran from 6 July-27 August, 1994. Females were placed only at the upper location in the canopy on open platforms

Table 5. Pheromone treatments, release rates and dispenser densities used in mating-disruption experiments (Chapter 4).

Experiment	Pheromone treatment	Release rate $mg \cdot ha^{-1} \cdot h^{-1}$	Dispenser density ha^{-1}
Exp. 1	None	-	-
	4-Component blend	10	1000
	3-Component blend	10	1000
	2-Component blend	10	1000
Exp. 2	None	-	-
	4-Component blend	10	1000
	4-Component blend	5	1000
	4-Component blend	2.5	1000
Exp. 3	None	-	-
	4-Component blend	1.3	1000
	4-Component blend	0.6	1000
	4-Component blend	0.1	1000
Exp. 4	None	-	-
	4-Component blend	2.5	1000
	4-Component blend	1.3	1000
	2-Component blend	1.3	1000
Exp. 5	None	-	-
	4-Component blend	1.3	250
	2-Component blend	1.3	250
	2-Component blend	1.3	500

with a square cardboard base of 42 cm^2 and a circular roof of 64 cm^2.

A dose-response test, conducted to determine the lowest release rate at which atmospheric treatment with the four-component blend would provide a significant reduction in mating of tethered females, was split into two experiments to accommodate all tested release rates. Exp. 2, from 7 June-4 August, 1995, compared three different release rates of the four-component blend. Disruption dispensers with 25, 50 and 100 fibres per dispenser, providing approximate release rates of 2.5, 5 and 10 $mg \cdot ha^{-1} \cdot h^{-1}$, respectively, were hung in the upper third of the canopy at a density of 100 per plot (1000 ha^{-1}) (Table 5). Exp. 3, from 20 June-28 July, 1996, tested the four-component blend at release rates of approximately 0.1, 0.6 and 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ from disruption dispensers with 1, 6 and 13 fibres per dispenser, respectively, hung in the upper third of the canopy at a density of 100 per plot (1000 ha^{-1}) (Table 5).

In Exp. 4, from 10 August-1 September, 1995, I tested two atmospheric treatments of the four-component blend and one treatment of the two-component blend all at a low release rate selected to reveal any differences between the efficacy of the most attractive pheromone blend and the simplest partial blend. A higher release rate of the four-component blend was also included as a treatment in this experiment so that I could compare results of this experiment with those of Exp. 2. Disruption dispensers loaded with the four-component blend were prepared with 25 and 13 fibres per dispenser and dispensers loaded with the two-component blend had 13 fibres per dispenser. All dispensers were hung in the upper third of the canopy at a density of 100 per plot (1000 ha^{-1}) providing approximate release rates of 2.5 (four-component blend) and 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ (four- and two-component blends) (Table 5).

Exp. 5, from 13 June-22 July, 1997, tested one atmospheric treatment of the four-component blend and two treatments of the two-component blend at the same low release rate but with differing dispenser densities to determine if at both a low release rate and dispenser density the more attractive four-component blend would reduce the incidence of mating more than the partial two-component blend. Treatments 1 and 2 consisted of disruption dispensers containing the four- and two-component blends, respectively, both with 50 fibres per dispenser, hung in the upper third of the canopy at a density of 25 per plot (250 ha^{-1}), providing an approximate release rate of 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ (Table 5). Treatment 3 consisted of disruption dispensers containing the two-component blend with 25 fibres per dispenser, hung in the upper third of the canopy at a density of 50 per plot (500 ha^{-1}) providing an approximate release rate of 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ (Table 5). Treatment 3 was included to determine if at the same release rate a partial blend at double the number of point sources would reduce mating as well as the most attractive blend. In this experiment females were placed only in the upper tree canopy.

An eleven-treatment trapping experiment (Exp. 6) was designed to compare the relative attractiveness of the disruption dispensers used in Exp. 1-5. Disruption dispensers contained two-, three-, and four-component blends, each loaded at 25, 50, and 100 fibres per dispenser, releasing 2.5, 5, and 10 $\mu g \cdot h^{-1}$, respectively (Ecogen Inc., Billings MT). Two additional treatments were traps baited with red rubber septa lures loaded with 3 mg of blend 1 in 200 µL of hexane, and traps baited with a single <6- to 96-h-old virgin female in a cylindrical mesh canister (3 cm diam. x 5 cm height). Females were obtained from the laboratory colony. Lures were separated by treatment

and transported to field sites in glass jars (except virgin females), housed in refrigerated containers. Traps constructed and baited as in section 2.4 were hung ~1.5 m high and 25 m apart on the north side of apple trees, in two randomized complete blocks, 40 m apart in an orchard growing Red and Golden Delicious cvs. Traps were run in each block on each of three two-night periods between 5-13 July, 1994. Trap positions were re-randomized between each trapping interval and lures were replaced.

Proportions of tethered, female *C. rosaceana* that were mated after being exposed for one night in pheromone-treated or control plots, were compared by a linear logistic regression model (see section 2.5) in which disruption treatment was the explanatory variable, and replicates over time, and plot position were treated as dummy variables. In Exp. 2, 3, and 4 the dose of the four-component blend could be considered as a continuous variable but a categorical model was used, because it allowed the dose at which mating disruption became ineffective to be identified. Fitting of the logistic regression model was followed by *Z*-tests to compare individual proportions. The α-value for each comparison was adjusted using the Bonferroni inequality to control the experiment-wise type I error rate which depends on the number of comparisons being made (Zar 1984).

The total numbers of male moths captured in traps baited with disruption dispensers in Exp. 6 in each block and over each trapping period were pooled and compared using a 2 x 3 contingency table (two blends, four- and three-component blends, each at three release rates) to test the hypothesis that male attraction to pheromone blends was independent of release rate. Two marginal χ^2 tests were also conducted to test the hypotheses that attraction of males to traps differed with

pheromone blend and with dispenser release rate, respectively. As no males were attracted to traps baited with the two-component blend at any release rate, these treatments were excluded from the analysis. Males captured in traps baited with rubber septa lures and virgin females were not included in the analysis.

In all cases α=0.05, unless otherwise indicated.

4.2.2 Wind-Tunnel Experiments

Exp. 7 and 8, conducted in the wind tunnel (Fig. 1), tested the hypothesis that treatment of the atmosphere upwind of a calling female with synthetic *C. rosaceana* pheromone would result in greater disorientation away from the calling female than treatment with the less attractive main component alone (*Z*11-14:OAc + 2% *E*11-14:OAc). Moths were handled as in section 2.2.2. "Background" treatment of the atmosphere was achieved by vertically suspending a piece of aluminium mesh hardware cloth (5 x 4.5 cm) from a metal rod 10 cm directly upwind of the positioned female with three rubber septa inserted in centrally-located holes in the mesh separated by 2.5 cm. Rubber septa, loaded and aged as in section 2.2.3, contained either the four-component pheromone (Vakenti et al. 1988; Thomson et al. 1991) or a 98:2 ratio of *Z:E*11-14:OAc, at a dose of 100 µg of the main pheromone component *Z*11-14:OAc. In Exp. 7, males were flown individually to one of the following three treatments: 1) a calling female *C. rosaceana*; 2) the same calling female with a background treatment of the two-component blend; and 3) the same calling female with a background treatment of the four-component pheromone. All three treatments were presented in each replicate (day of flight) in the above order to avoid contamination of the fibreglass mesh

bag housing the calling female. Between treatments the release stand was cleaned and a new piece of hardware cloth was suspended from a new metal rod. Septa were placed in the hardware cloth in the tunnel 5 min before introducing males. Six to eight males were presented to each treatment in each of six replicates. Males were placed individually in the centre of the tunnel for 10 s before release. Male response was graded as + or - for contacting the mesh bag containing the calling female. Exp. 8 tested the same treatments as in Exp. 7 except that males remained in the centre of the tunnel for 1 min before release. In addition, two control treatments were used in which males were flown to calling virgin females in clean air after placement in the plume for 10 s (control 1) or 1 min (control 2) before release. Four to seven males were presented individually to each treatment in each of seven replicates.

Exp. 9 and 10 tested the hypothesis that pre-exposure to pheromone (Exp. 9) or the main pheromone component (Exp. 10) would alter the subsequent response of male *C. rosaceana* to calling virgin females. Males were handled as outlined in section 2.2.2 except that they were placed in two groups of 10 moths, each in large cylindrical (9 cm diam. x 2 cm height) wire mesh cages equipped with removable wire mesh lids, and attached to 11 x 11 cm wire mesh bases. Both groups of males were transported to the wind tunnel 30 min prior to the onset of scotophase. The treatment group was placed in the centre of the wind tunnel where they were exposed to pheromone (Exp. 9) or the main component (Exp. 10) released from three rubber septa as above. The control group was placed on the wind tunnel floor, close to the side, so that males were out of the pheromone plumes but experienced the same light and wind conditions as the treated group. Moths remained in release containers in the tunnel for 30 min under

ambient room light conditions and 30 min under the light conditions of the bioassay (0.5 lux). After 1 h the hardware cloth holding the septa was removed from the tunnel and males were exposed to clean air for 10-30 min prior to bioassay.

Females were handled as in section 2.2.2 except that fibreglass mesh bags containing the individual females were suspended in cylindrical (12 cm diam. x 13 cm length) sticky traps aligned with the open ends parallel to the wind direction. Traps were suspended from a metal rod attached to a ring stand so that females were located 44 cm above the tunnel floor. Traps were constructed from transparent acetate (Commercial Plastics, Vancouver, B.C.) and lined with a piece of acetate coated with STP® oil treatment, as an adhesive to capture responding moths. New traps were used in each replicate. The female-baited trap was positioned in the tunnel after removal of the pre-exposure treatment. Females were observed to be calling before placement of males in the female-produced plume.

In Exp. 9 and 10, males within a randomly selected release cage were maintained in the female-produced plume for 10 s before release, and those captured in the female-baited trap were counted 5 min later. Males that were not captured were aspirated out of the tunnel. The second release cage was then placed in the plume of the same calling female and the same release and capture procedure was repeated. Groups of 9-10 males were flown to female-baited traps on each of five test days.

The proportions of released males that contacted the bag housing the calling virgin female (Exp. 7 and 8) or were captured in female-baited traps (Exp. 9 and 10) were analyzed by a logistic regression model (see section 2.5) in which background or pre-exposure treatment was the explanatory variable, and replicates over time were

treated as dummy variables. Individual treatments were compared by *Z*-tests as above.

4.3 Results

4.3.1 Field Experiments

In Exp. 1, atmospheric treatment with the four-, three-, and two-component blends at an approximate release rate of 10 mg·ha^{-1}·h^{-1} using a dispenser density of 1000 ha^{-1}, provided equally effective (85-90 %) reductions in the proportions of tethered female *C. rosaceana* mating, compared to mating females in nontreated control plots (Fig. 9).

Levels of mating disruption achieved at a constant dispenser density of 1000 ha^{-1} with the four-component blend, the most complex blend tested, varied as a function of release rate (Fig. 10). In Exp. 2, atmospheric treatment with the four-component blend at release rates of 2.5, 5 and 10 mg·ha^{-1}·h^{-1} equally reduced the proportion of mating among tethered females by 70-86 % as compared to a nontreated control. However, mating disruption using the four-component blend appeared to falter at a release rate of ca. 1.3 mg·ha^{-1}·h^{-1} (Fig. 10). In Exp. 3, the four-component blend released at 0.1, 0.6 and 1.3 mg·ha^{-1}·h^{-1} caused no reduction in the proportion of tethered females mating, but in Exp. 4, the four-component blend released at 1.3 mg·ha^{-1}·h^{-1} significantly reduced the proportion of tethered females mating and was as effective as the four-component blend released at 2.5 mg·ha^{-1}·h^{-1} (Fig. 11).

In Exp. 4, the four- and two-component blends applied at a release rate of 1.3 mg·ha^{-1}·h^{-1} and a high dispenser density of 1000 ha^{-1} were equally effective disruptants,

Figure 9. Proportions of tethered, female *C. rosaceana* mating in nontreated plots or plots treated atmospherically with the four-component blend and two partial pheromone blends in Exp. 1, Chapter 4, 6 July-27 August, 1994, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.

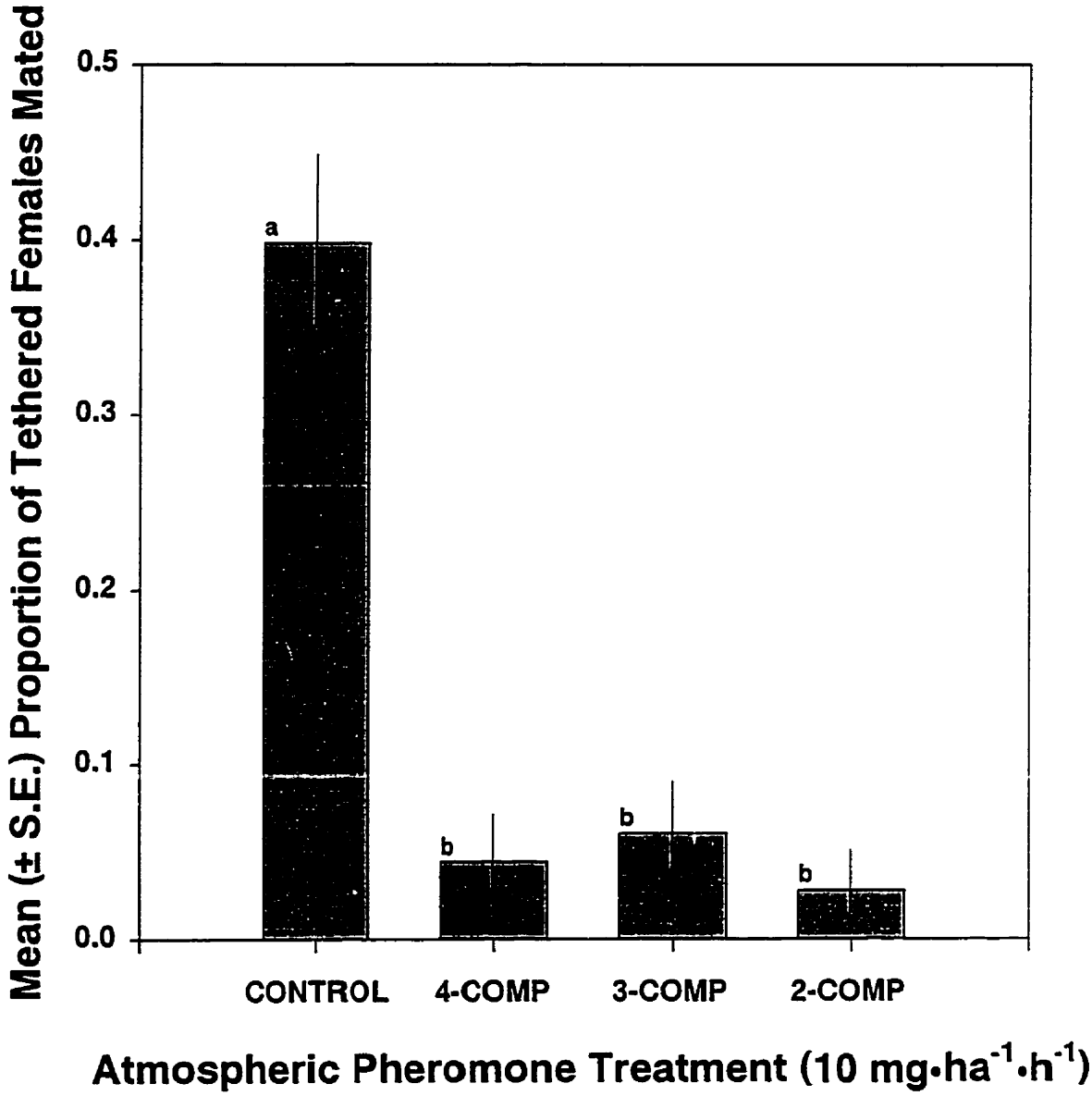

Mean (± S.E.) Proportion of Tethered Females Mated
0.5
0.4
0.3
0.2
0.1
0.0
a
b
b
b
CONTROL
4-COMP
3-COMP
2-COMP
Atmospheric Pheromone Treatment (10 mg•ha-1•h-1)

Figure 10. Relative proportions (proportion of mated, tethered female *C. rosaceana* in no-pheromone control plots = 1.0) of mated, tethered females in plots treated atmospherically with the four-component blend at various release rates and a dispenser density of 1000 ha^{-1} in Exp. 2, Chapter 4, 7 June-4 August, 1995, Exp. 4, 10 August-1 September, 1995, and Exp. 3, 20 June-28 July, 1996, Cawston, B.C. Within each experiment, bars marked with * are significantly different from the control and bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4, in all experiments.

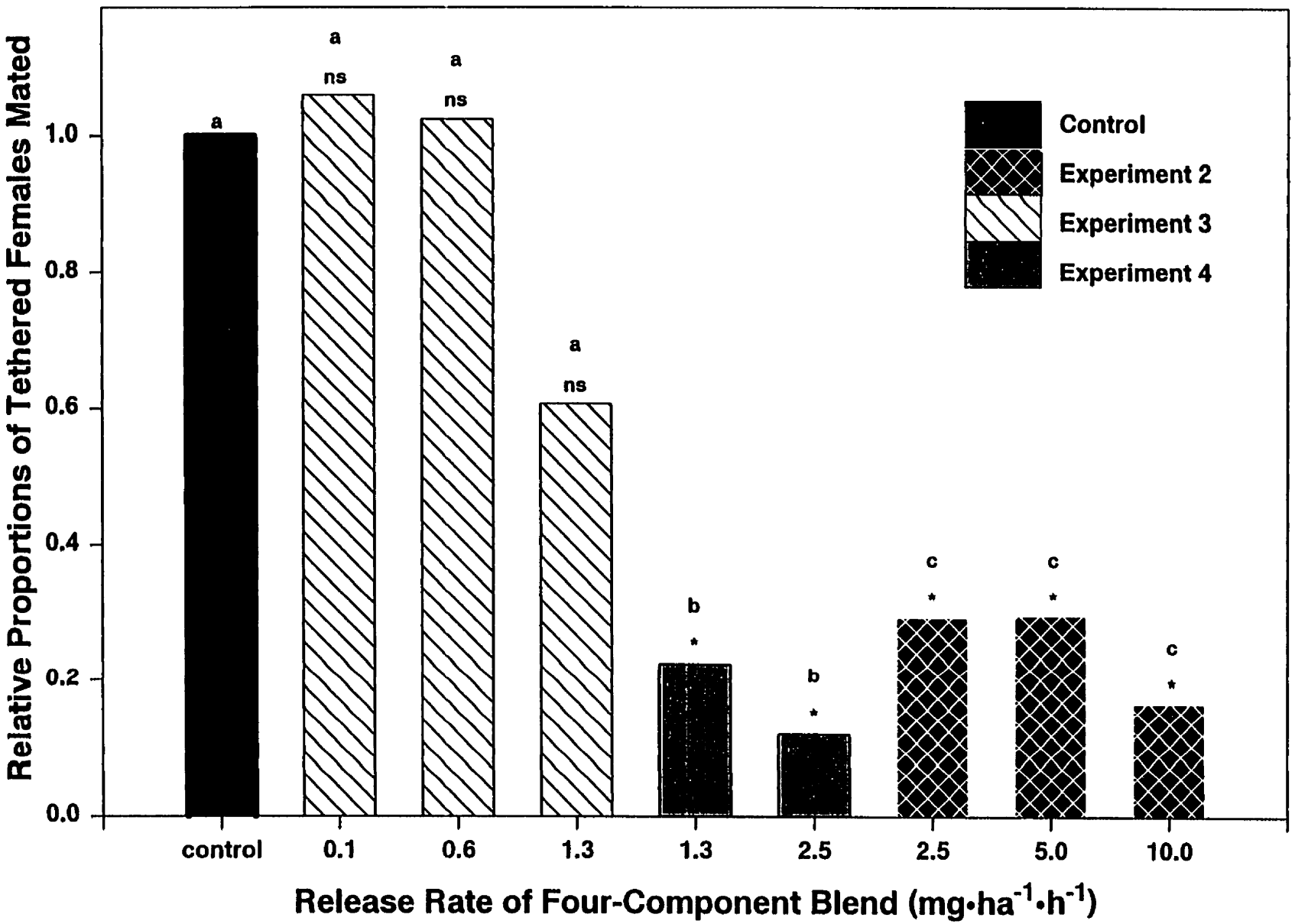
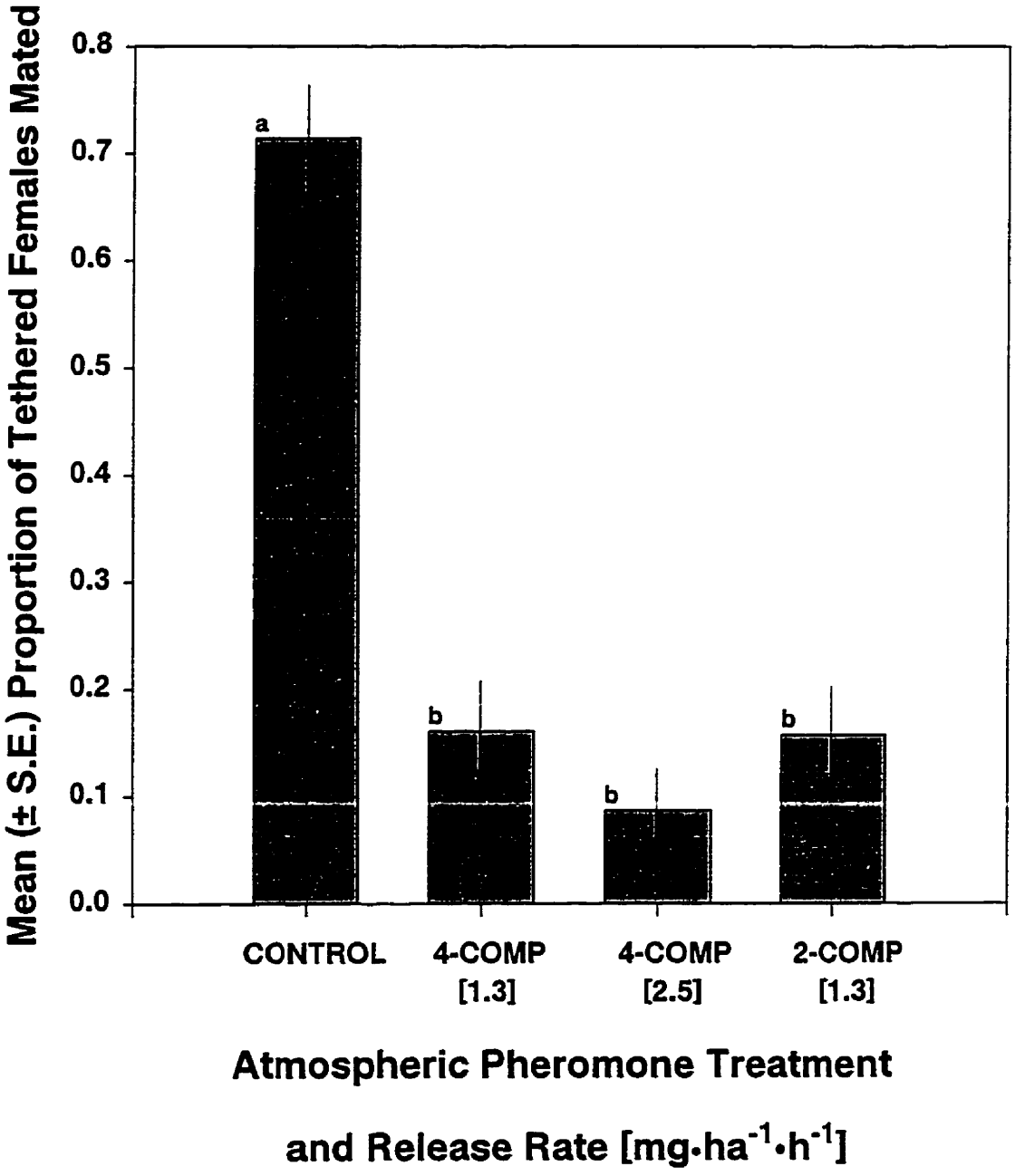

Relative Proportions of Tethered Females Mated
0.0
0.2
0.4
0.6
0.8
1.0
a
a
ns
a
ns
a
ns
b
*
b
*
c
*
c
*
c
*
Control
Experiment 2
Experiment 3
Experiment 4
control
0.1
0.6
1.3
1.3
2.5
2.5
5.0
10.0
Release Rate of Four-Component Blend (mg•ha-1•h-1)

Figure 11. Proportions of tethered, female *C. rosaceana* mating in plots treated atmospherically with the four-component blend and the two-component blend, at reduced release rates and a dispenser density of 1000 ha^{-1} in Exp. 4, Chapter 4, 10 August-1 September, 1995, Cawston B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.


Mean (± S.E.) Proportion of Tethered Females Mated
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
b
b
b
CONTROL
4-COMP [1.3]
4-COMP [2.5]
2-COMP [1.3]
Atmospheric Pheromone Treatment and Release Rate [mg·ha-1·h-1]

and no different from the four-component blend applied at 2.5 $mg \cdot ha^{-1} \cdot h^{-1}$ at the same dispenser density (Fig. 11). In Exp. 5, the four- and two-component blends applied at an apparent threshold release rate of 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ and at a low dispenser density of 250 dispensers ha^{-1} and the two-component blend applied at the threshold release rate but at a dispenser density of 500 ha^{-1} all significantly and equally reduced the proportion of tethered females mating as compared to the nontreated control (Fig. 12).

Attraction of male *C. rosaceana* to traps baited with disruption dispensers containing the four- and three-component blends was independent of release rate ($\chi^2_{0.05,3}$=2.32, P>0.5). Males were equally attracted to traps regardless of dispenser release rate ($\chi^2_{0.05,2}$=0.25, P>0.75) but attraction to traps differed with chemical composition of the dispenser bait ($\chi^2_{0.05,1}$=39.11, P<0.001) (Fig. 13). Males were not attracted to any of the 18 traps baited with the two-component blend at any dose level.

4.3.2 Wind-Tunnel Experiments

The proportion of males contacting the calling female in the wind tunnel was significantly reduced when septa containing either the main component or the complete pheromone were positioned upwind of the female (Fig. 14). Background treatment in Exp. 7 with the four-component blend resulted in a significantly greater reduction of orientation to females than treatment with the main component (two-component blend) (Fig 14A). Of the males that did not contact the calling females, 14% contacted background septa containing the main component, whereas 39% contacted background septa containing the four-component blend (Fig. 14A).

In Exp. 8 there was no difference in orientation to calling females if males

Figure 12. Proportions of tethered, female *C. rosaceana* mating in plots treated atmospherically with the four-component blend and the two-component blend at a release rate of 1.3 mg·ha^{-1}·h^{-1} and a reduced dispenser density in Exp. 5, Chapter 4, 13 June-22 July, 1997, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests, $P>0.05$. N=4.

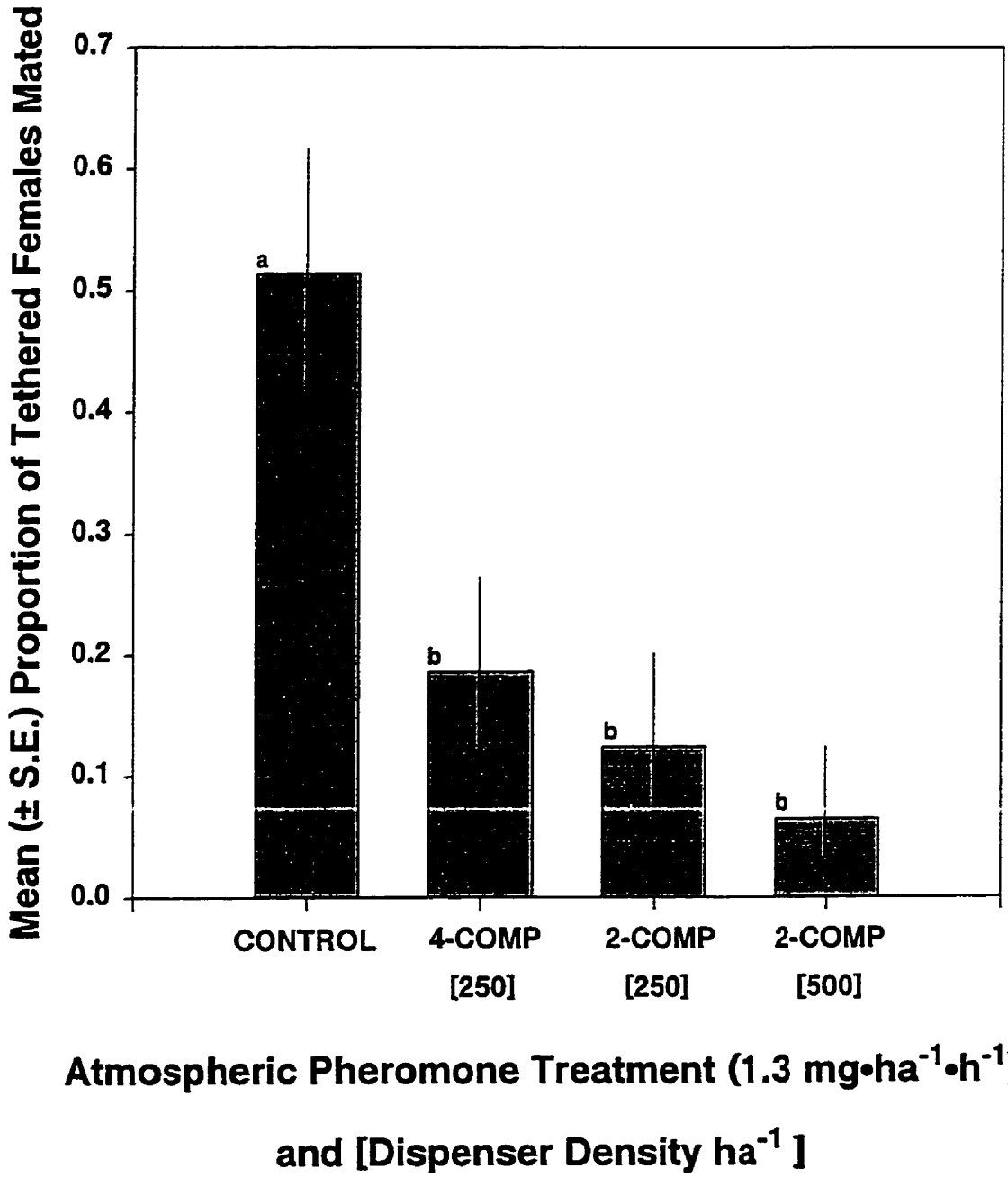

Mean (± S.E.) Proportion of Tethered Females Mated
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
b
b
b
CONTROL
4-COMP [250]
2-COMP [250]
2-COMP [500]
Atmospheric Pheromone Treatment (1.3 mg•ha-1•h-1) and [Dispenser Density ha-1]

Figure 13. Total number of male *C. rosaceana* captured in two blocks, over three, two-night periods in wing traps baited with monitoring lures, mating-disruption dispensers, or virgin females in Exp. 6, Chapter 4, 5-13 July, 1994, Cawston, B.C. Bars with the same letter superscript are not significantly different, χ^2-test $P>0.05$.

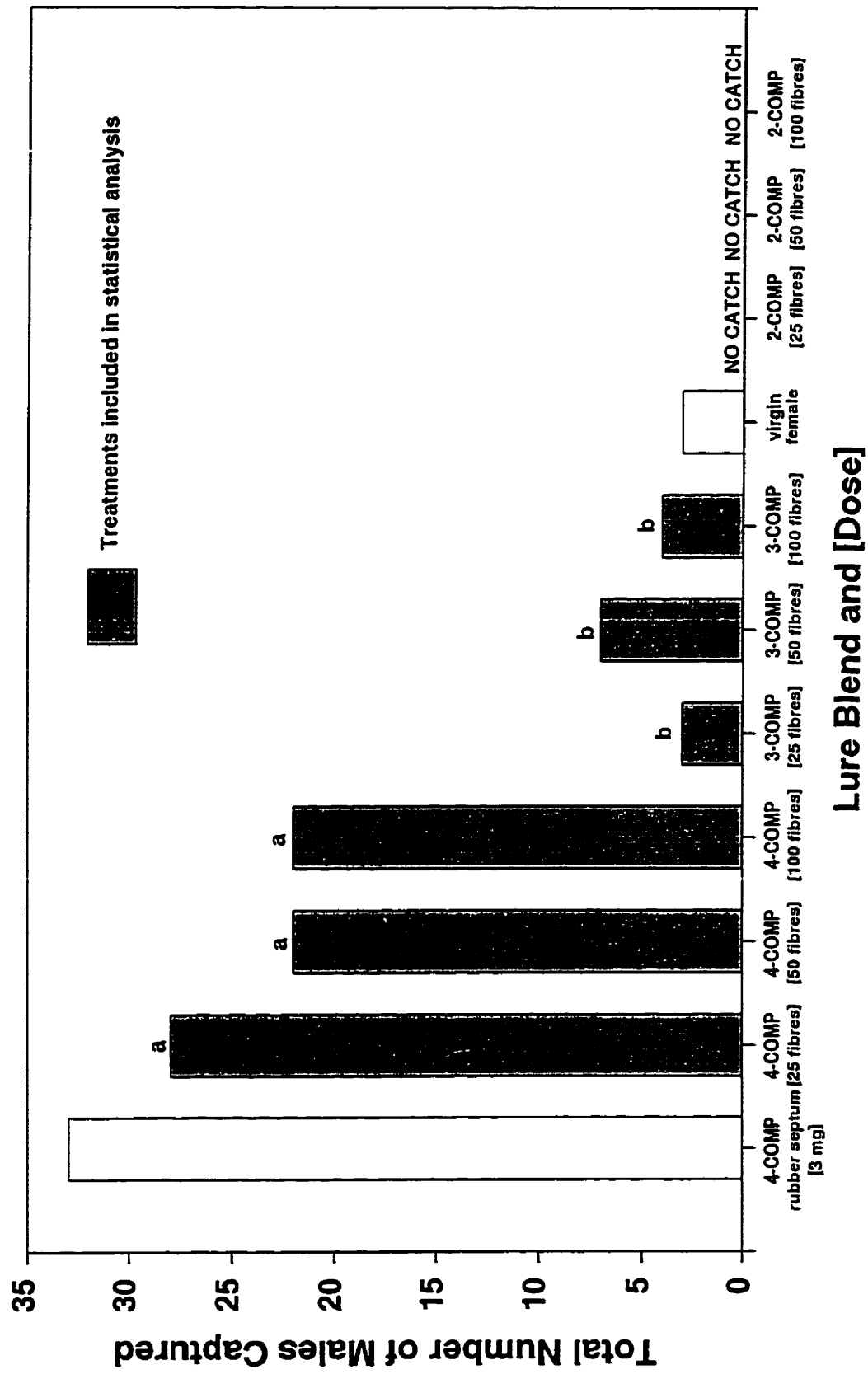
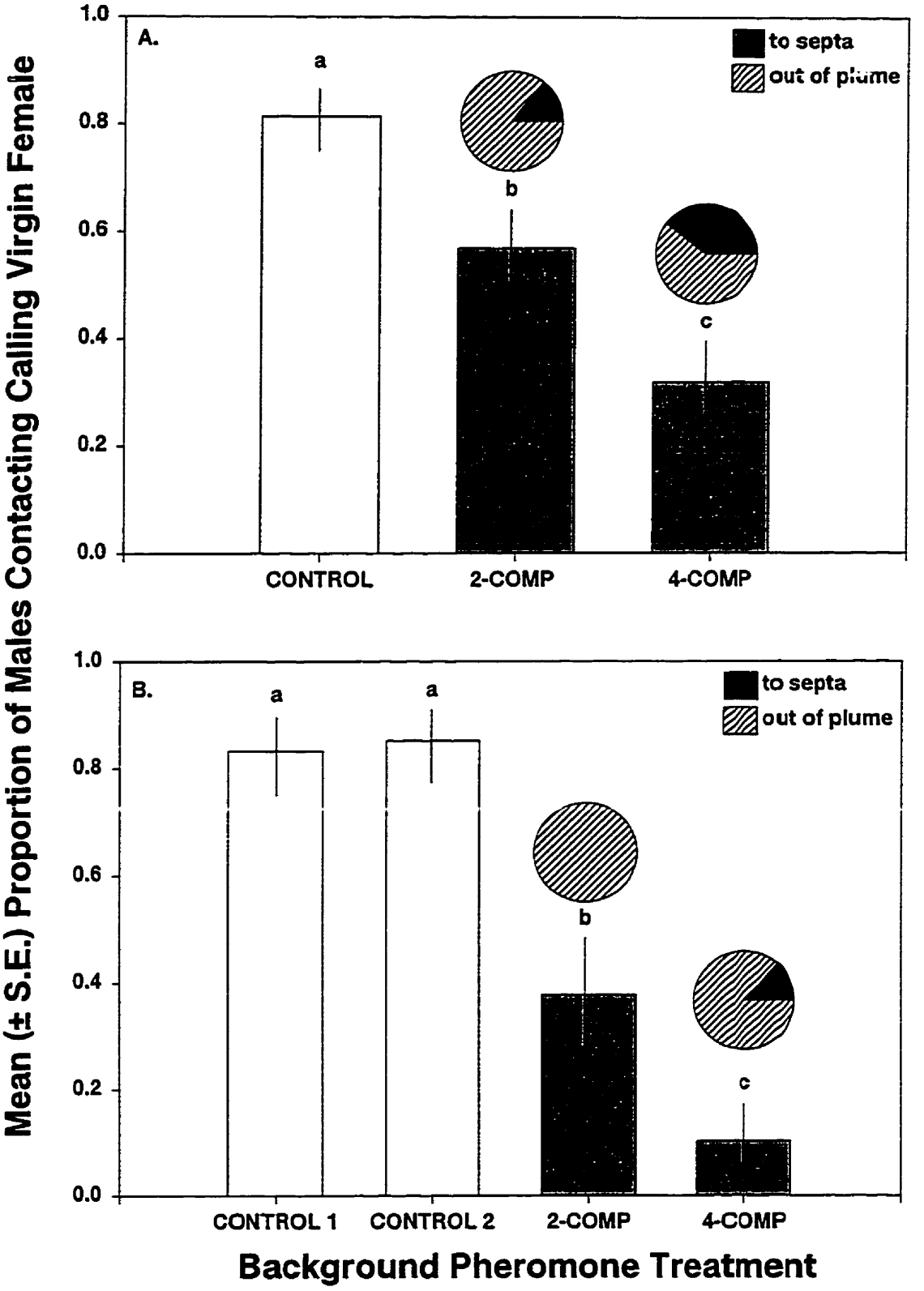

Total Number of Males Captured
0
5
10
15
20
25
30
35
Treatments included in statistical analysis
a
a
a
b
b
b
4-COMP rubber septum [3 mg]
4-COMP [25 fibres]
4-COMP [50 fibres]
4-COMP [100 fibres]
3-COMP [25 fibres]
3-COMP [50 fibres]
3-COMP [100 fibres]
virgin female
NO CATCH
2-COMP [25 fibres]
NO CATCH
2-COMP [50 fibres]
NO CATCH
2-COMP [100 fibres]
Lure Blend and [Dose]

Figure 14. Mean proportions of male *C. rosaceana* flying upwind and contacting a calling female *C. rosaceana* in the wind tunnel. A. Males placed in female's plume for 10 s prior to release or with background treatment with the two- or four-component blend in addition to the female in Exp. 7, Chapter 4. B. Males placed in female's plume for 10 s (control 1) or 1 min (control 2) or for 1 min with female plus background treatment with the two- or four-component blend in Exp. 8, Chapter 4. Pie graph inserts indicate the proportion and end destination of males that did not contact calling, virgin females. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=5 in both experiments.


A.
to septa
out of plume
a
b
c
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL
2-COMP
4-COMP
B.
to septa
out of plume
a
a
b
c
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL 1
CONTROL 2
2-COMP
4-COMP
Mean (± S.E.) Proportion of Males Contacting Calling Virgin Female
Background Pheromone Treatment

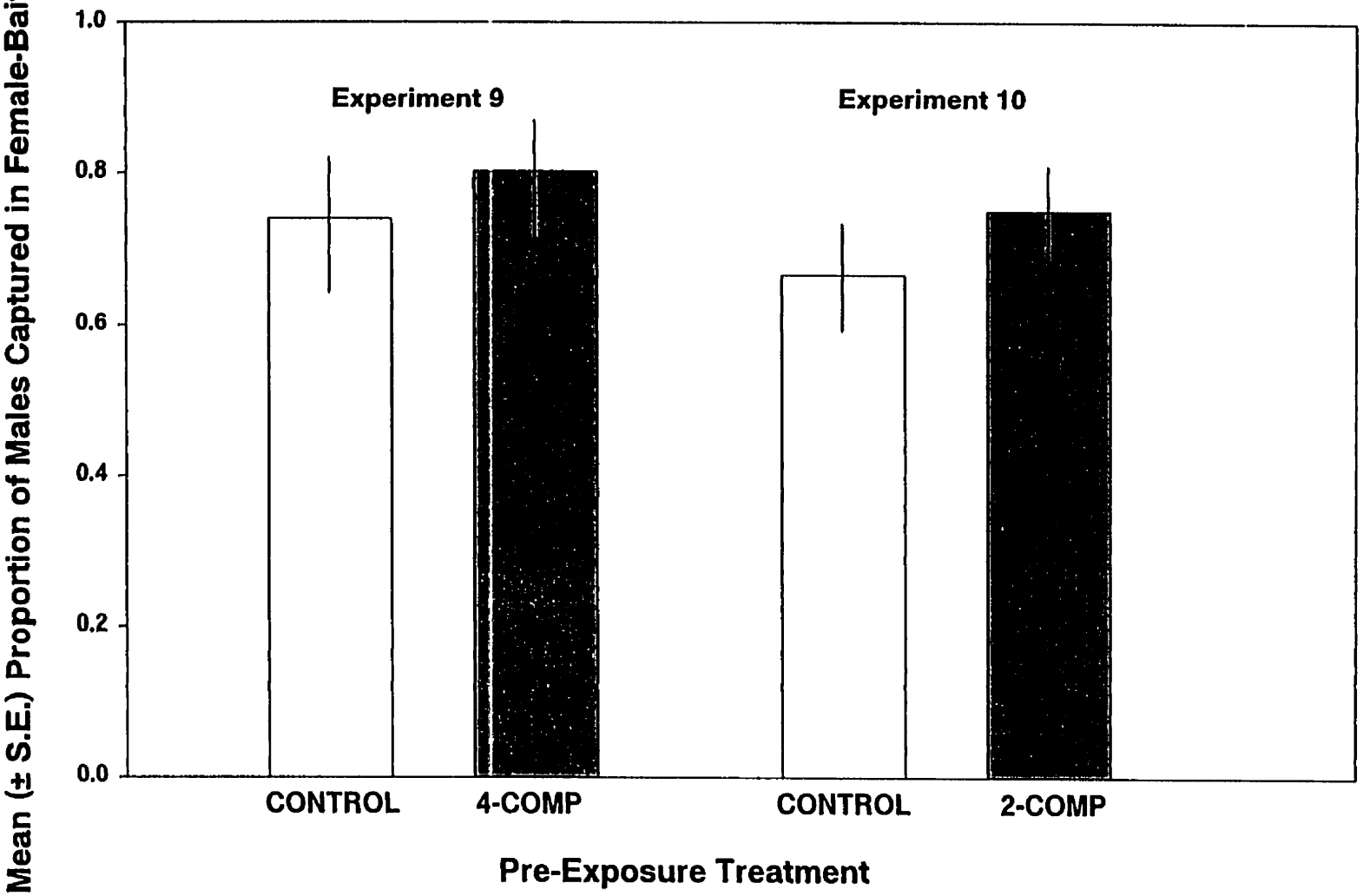
remained in the female-produced plume for 10 s or 1 min (Fig. 14B). When males were left in the plume for 1 min, contact with the female was again reduced to a greater extent in a background treatment with the four-component blend compared to the main component (two-component blend) (Fig. 14B), and the overall effect was greater than after a 10 s exposure (Fig. 14A). In both experiments there were fewer males that did not contact the female but also did not fly upwind to the septa in a background of the two-component blend than in a background of the four-component blend.

Pre-exposure of male *C. rosaceana* to either the two-component blend or the four-component pheromone did not reduce subsequent upwind flight to calling virgin females. Equal numbers of control or pre-exposed males were captured in female-baited traps in both Exps. 9 and 10 (Fig. 15).

4.4 Discussion

My data demonstrate that disruption of mating in western Canadian populations of *C. rosaceana* can be achieved equally well with attractive and less attractive pheromone blends containing the major pheromone component *Z*11-14:OAc, over a broad range of release rates and dispenser densities. At a release rate of 10 $mg \cdot ha^{-1} \cdot h^{-1}$, a dose that has provided control in trials for codling moth (Judd et al. 1996a,1997) and other tortricids (Alford and Silk 1983; Trimble 1993) all blends significantly and equally reduced mating of tethered females compared to the nontreated control. Among the three pheromone blends tested at this release rate, there was no correlation between their relative attractiveness as lures and their

Figure 15. Mean proportions of male *C. rosaceana* captured in *C. rosaceana* female-baited trap in the wind tunnel following pre-exposure to the four-component pheromone for 1 h followed by 10-30 min in clean air before bioassay in Exp. 9, Chapter 4, or pre-exposure to the two-component blend for 1 h followed by 10-30 min in clean air prior to bioassay in Exp. 10, Chapter 4. N=5 in both experiments.


Mean (± S.E.) Proportion of Males Captured in Female-Baited Trap
1.0
0.8
0.6
0.4
0.2
0.0
Experiment 9
Experiment 10
CONTROL
4-COMP
CONTROL
2-COMP
Pre-Exposure Treatment

effectiveness as mating disruptants. Similarly, Fitzpatrick et al. (1995) demonstrated that the major pheromone component of the blackheaded fireworm, *Rhopobota naevana* (Hübner), disrupted mating as effectively as a blend close to the natural pheromone blend. However, in another tortricid, the Oriental fruit moth, a more attractive three-component blend was demonstrated to be a more efficacious disruptant at a lower release rate than a two-component partial blend (Charlton and Cardé 1981).

Other authors have observed that atmospheric treatment with partial pheromone blends is as effective as treatment with more complex pheromone blends in reducing orientation to a pheromone source in a wind tunnel (Hiyori et al. 1986; Palaniswamy and Underhill 1988) or the field (Flint and Merkle 1983; Palaniswamy and Underhill 1988). However, each of these authors have suggested different mechanisms as the cause of communication disruption in each of these situations (Flint and Merkle 1983; Hiyori et al. 1986; Palaniswamy and Underhill 1988). Hiyori et al. (1986) demonstrated that attraction of male *Adoxphyes* spp. to its four-component pheromone in a wind tunnel was reduced equally by treatment with the single major pheromone component or the four-component blend. In the atmosphere treated with the unattractive major component, males remained inactive at the downwind end of the tunnel, suggesting that because of neurophysiological effects caused by the atmospheric treatment, they could not detect the target pheromone source. In an atmosphere treated with the attractive four-component pheromone blend, males flew to the upwind end of the tunnel but did not locate the target pheromone source, suggesting that camouflage of the pheromone plume or false-trail following caused disorientation (Hiyori et al. 1986).

In my wind tunnel studies, background treatment with the four-component

pheromone resulted in a greater reduction in the proportion of males contacting the virgin female source than treatment with the less attractive two-component blend (Fig. 14). These experiments provided evidence for several mechanisms which may have contributed to male disorientation in the wind tunnel. Males that did not orient to the calling female were observed to fly upwind to background septa, fly upwind and become arrested, or never initiate upwind flight and exit from the pheromone plume (Fig. 14). When males were positioned in the plumes produced from the female and background septa for 10 s prior to release, 39% of males that did not contact a female, flew upwind to and contacted the background septa containing the four-component blend, but only 14% of disoriented males flew to septa containing the two-component blend. False-trail following contributed to the additive disruption effect observed with the more attractive blend. However, in a similar experiment Sanders (1995) demonstrated that even with high levels of false-trail following, male eastern spruce budworms, *C. fumiferana*, left in the wind tunnel would eventually locate a female, suggesting that false-trail following alone is not an effective disruption mechanism. When male *C. rosaceana* were positioned in the plumes produced from the female and background septa for 1 min in Exp. 8, 13% of males that did not contact the female flew to background septa containing the four-component blend, and no males flew to septa containing the two-component blend. Although not compared directly, the reduction in the ability of males to fly upwind to females or septa after remaining in the plumes for 1 min suggests a major role of neurophysiological effects in the disorientation observed in Exp. 8. Flight arrestment was observed when background septa were treated with either the two or four-component pheromone blend. Baker et

al. (1989) demonstrated that flight arrestment of the Oriental fruit moth, *G. molesta*, was correlated with adaptation of receptors on the antennae. It is possible that receptors for *Z*11-14:OAc became adapted in both background treatments. The superior efficacy of the four-component blend in Exp. 8 can not therefore be attributed to adaptation alone. False-trail following and camouflage may have additive roles as well as blend-specific habituation of neurons in the central nervous system (Valeur and Löfstedt 1996).

Atmospheric treatment in the field with one of the two components of the pheromone of the pink bollworm, *P. gossypiella*, apparently caused a neurophysiological alteration resulting in an imbalance in sensory input such that male moths were more attracted to off-ratio blends than to the normally attractive natural-ratio blend (Flint and Merkle 1984a). Attraction to off-ratio blends was not observed when the complete blend was used in communication disruption. Although communication was disrupted in both situations (Flint and Merkle 1984b), the neurophysiological alteration of the antennal receptors and/or insects' brain appear to differ when different blends are used.

Disruption of mating in Exp. 1 (Fig. 9) using the attractive three- and four-component blends could have been achieved through false-trail following, camouflage of the female-produced plumes or neurophysiological effects. The major pheromone component, *Z*11-14:OAc, has been shown to be attractive to eastern populations of *C. rosaceana* (Roelofs and Tette 1970) and a blend containing *Z*11-14:OAc and *E*11-14:OAc in a 94.3:5.7 ratio was weakly attractive to western *C. rosaceana* males (Vakenti et al. 1988), but the two-component blend released from disruption dispensers was not attractive to male *C. rosaceana* in traps (Fig. 13), so it is unlikely that it caused

false-trail following. However, it is possible that upwind orientation occurred to plumes of the two-component blend downwind of dispensers, but that male moths did not follow the plumes to the source as was the case for the three- and four-component blends (Fig. 13). If false-trail following was elicited in plots treated with the attractive three- and four-component blends at 10 $mg \cdot ha^{-1} \cdot h^{-1}$, it does not appear to enhance or exceed any neurophysiological or camouflage effects (Minks and Cardé 1988), that could have been produced by the two-component blend applied at this release rate, as all three treatments equally reduced mating of tethered females by 85-92% (Fig. 9). In a further test of the false-trail following hypothesis, a behavioural antagonist was added to the attractive four-component blend (rendering it unattractive) and the effectiveness of this blend was compared to that of the attractive blend alone as an orientation disruptant of western *C. rosaceana* (Chapter 5). The blends were found to be equally effective, suggesting that false-trail following did not contribute to communication disruption of this species at a release rate of 10 $mg \cdot ha^{-1} \cdot h^{-1}$.

An alternative hypothesis is that both single- and multiple-component pheromone disruptants caused neurophysiological effects at the release rates tested. Exposure of moths to single pheromone components in the laboratory (Linn and Roelofs 1981; Liu and Haynes 1993a), and exposure of moths (Flint and Merkle 1984a) and bugs (Judd et al. 1995) in the field can apparently cause neurophysiological adaptation or habituation resulting in upwind flight of males to off-ratio pheromone blends. Whether the complete pheromone blend is required to achieve habituation and subsequent reduction in response to a pheromone source appears to vary among species. Liu and Haynes (1993a) showed that a reduction in response by male cabbage loopers, *T. ni*, to

the full pheromone blend in a wind tunnel was just as great after pre-exposure to the major pheromone component alone as to the full pheromone blend. In contrast, Bartell and Lawrence (1977a) demonstrated that pre-exposure of male *Epiphyas postvittana* (Walker) to either of its two pheromone components, which are not attractive alone, did not alter the subsequent behavioural response to the complete pheromone blend, but pre-exposure to the two components together resulted in a reduction in subsequent response to pheromone. Habituation of the central nervous system may be of little importance in disruption of pheromone communication in *C. rosaceana* as pre-exposure to the four-component pheromone or the two-component blend caused no reduction in subsequent orientation to calling virgin females in Exp. 9 and 10 (Fig. 15). In the congeneric species, *C. fumiferana*, Sanders (1996) was also unable to demonstrate any effect of pre-exposure to pheromone (for up to 4 days) on the subsequent upwind flight to calling virgin females. Exposure of male Oriental fruit moth to pheromone for 3 h prior to bioassay in a wind tunnel had no significant effect on the number of disoriented males (Sanders and Lucuik 1996). Pre-exposed males exhibited some habituation, however, because they remained less active than naive males when presented with high background levels of pheromone during the bioassay (Sanders and Lucuik 1996). Rumbo and Vickers (1997) showed that Oriental fruit moth males had to be exposed to high levels (3200 female equivalents) of pheromone to reduce subsequent behavioural response in wind tunnel and field studies. Liu and Haynes (1993a) demonstrated a reduction in upwind flight to a synthetic pheromone source after a three-day exposure to the main component or full pheromone of *T. ni*. However,

the effect of pre-exposure was only moderate as compared to the effect of background pheromone placed upwind of the pheromone source (Liu and Haynes 1993a). Similarly, Cardé et al. (1997) demonstrated a 50% reduction in orientation to traps after pre-exposure of male pink bollworm to pheromone for 24 h. However, Cardé et al. (1997) observed that some males took flight 10-60 s after removal from the pre-exposure treatment and many males oriented directly to the pheromone-baited traps, suggesting a limited habituation effect.

My findings contradict the results of Roelofs and Novak (1981) who found that the complete pheromone blend of eastern *C. rosaceana* reduced orientation to traps to a greater extent than either partial or off-ratio blends at the same release rate of 10 $mg \cdot ha^{-1} \cdot h^{-1}$. It is likely that population pressures and canopy structure differed between the two experiments and may have affected the efficacy of the tested formulations. Further, I assessed reduction in mating of tethered virgin females, while Roelofs and Novak (1981) assessed efficacy by the reduction of orientation to traps baited with synthetic pheromone. Although suppression of response to traps baited with synthetic pheromone traps can be useful in screening potential mating disruptants, it is often not well correlated with the actual level of mating disruption (Rothschild 1981).

The release rate of pheromone is an important factor in preventing mating and providing crop protection in several mating disruption systems (Schwalbe and Mastro 1988; Webb et al. 1990; Suckling and Shaw 1992) but the dispenser density and total pheromone release rate are often confounded (i.e. Webb et al.1990; Agnello et al. 1996; Lawson et al. 1996). Lawson et al. (1996) found no difference in efficacy among

three release rates of the eastern *C. rosaceana* pheromone blend when tested in large plots. However, the release rates tested by these authors (24.8-74.3 $mg \cdot ha^{-1} \cdot h^{-1}$) were well above the 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ threshold rate at which I observed a reduction in mating-disruption efficacy (Fig. 10). Furthermore, Lawson et al. (1996) did not discriminate between the effect of increased release rate on orientation and mating and an increased number of pheromone dispensers ha^{-1}. Roelofs and Novak (1981) found no difference among three release rates (5, 10 and 20 $mg \cdot ha^{-1} \cdot h^{-1}$) of the eastern *C. rosaceana* pheromone blend when the number of dispensers used was held constant. However, these release rates also well exceeded my threshold release rate of 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ at a dispenser density of 1000 dispensers ha^{-1}.

The discrepancy between Exp. 3 and 4 in the disruptive efficacy of the four-component blend (Fig. 10) may be partially attributed to a lesser population pressure in Exp. 4 than Exp. 3 (Appendix 5). Population density of insect pests may influence the success of mating-disruption trials (Cardé and Minks 1995). Howell et al. (1992) found slightly higher damage levels in pheromone-treated plots in which they had raised the population density by releasing laboratory-reared codling moths, *C. pomonella*. High population densities of gypsy moths, *L. dispar*, reduced the efficacy of pheromone treatments in suppressing male orientation to traps and mating of sentinel females (Webb et al. 1988, 1990).

Minks and Cardé (1988) hypothesized that the natural pheromone should be a better mating disruptant at a lower release rate than either partial or off-ratio blends, primarily because the natural pheromone should be more attractive than less complete

or partial blends. One problem with attempting to test this hypothesis is that it does not differentiate between low release rates achieved by reducing amounts of pheromone released per dispenser or reducing the number of dispensers ha^{-1}. The latter could be very important as it influences the amount of false-trail following that is likely to occur. In Exp. 4 (Fig. 11), the two-component blend, which is unattractive (Fig. 13), was as effective a disruptant as the attractive four-component blend, when compared at the threshold release rate of 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ and a dispenser density of 1000 dispensers ha^{-1}. These data do not support the Minks and Cardé (1988) hypothesis. However, if false-trail following were evoked by the four-component blend, it should be most effective when the dispenser density is low, providing discrete plumes and a low chance of camouflage and adaptation or habituation to pheromone caused by uniformly dispersed pheromone plumes. In Exp. 5, when the total pheromone release rate was maintained at 1.3 $mg \cdot ha^{-1} \cdot h^{-1}$ but the dispenser density of the two- and four-component blends was reduced to 250 ha^{-1} and compared to the two-component blend at a dispenser density of 500 ha^{-1}, all three treatments equally reduced the proportion of tethered females mating (Fig. 12). This finding indicates that if any additional mating-disruption mechanisms were evoked by the attractive blend they did not enhance or add to those provided by the two-component blend, even at a reduced release rate and dispenser density. It could be argued that the most attractive four-component blend induces immigration of males into treated plots, especially if the plots are relatively small, and therefore it may have been difficult to demonstrate a difference among the treatments because of treatment-imposed variation in population density. However,

synthetic pheromone traps placed in treated plots on nights that tethered females were not present did not show any detectable difference in population density (Appendix 6).

Suckling and Angerilli (1996) found that disorientation to pheromone-baited traps by *E. postvittana* increased with an increased dispenser density. However, they tested lower dispenser densities at a much wider range (2, 18, and 200 dispensers ha^{-1}) than I did. Perhaps a dispenser density < 250 ha^{-1} is required to detect a difference in efficacy among attractive and unattractive blends. However, other studies have demonstrated that a few dispensers releasing high levels of pheromone adequately reduce both mating (Charmillot et al. 1995; Mafro-Neto and Baker 1996a) and orientation to traps baited with synthetic pheromone or virgin females (Shorey and Gerber 1996 a,b). Pheromone released from a small number of sources under average wind conditions could result in multiple pulses of pheromone (Murlis and Jones 1981), which have been shown to habituate male moths to a greater extent than a continuous exposure (Bartell and Lawrence 1977b; Kuenen and Baker 1981). Alternatively, pheromone released from a few dispensers in large amounts could be adsorbed by foliage and re-released at high enough rates to elicit a behavioural response by male moths, mimicking the effect of many small point sources of pheromone (Wall et al. 1981; Wall and Perry 1983; Noldus et al. 1991; Karg et al. 1994; Suckling et al. 1996; Schmitz et al. 1997; Sauer and Karg 1998). A dispenser density of 250 ha^{-1} appears to provide pulses of pheromone or adequate coverage from point sources and re-release of pheromone from the orchard canopy to reduce the proportion of mated females by 61-64% for the four- and two-component blends respectively, as compared to a

nontreated control.

My data obtained from small-plot field trials provide no evidence to support the hypothesis that an attractive, complete pheromone blend is more effective at reducing mating of western Canadian populations of *C. rosaceana* at a lower release rate than partial blends. Although a complex pheromone blend of western populations of *C. rosaceana* (Vakenti et al. 1988) was more effective at disorienting males in a wind tunnel than a partial pheromone blend containing the major component (Fig. 14), this was not the case in the field. The most attractive four-component blend and partial blends containing the major component but with fewer total components were equally as effective at reducing mating in tethered virgin females over a range of release rates and dispenser densities. If anything, the efficacy of disruption was related more to the release rate and dispenser density than the attractiveness and complexity of the pheromone blend. Therefore, any additional mechanisms that may be evoked by using attractive, complete blends do not necessarily enhance the effects provided by partial blends in a small-plot field situation. It is probably most cost-effective to develop a partial pheromone blend for control of western populations of *C. rosaceana* on a commercial scale.

5.0 COMMUNICATION DISRUPTION OF *C. rosaceana* AND *P. limitata* WITH ATTRACTIVE AND INHIBITORY SEMIOCHEMICAL BLENDS

5.1 Introduction

Choristoneura rosaceana, and *P. limitata*, occur sympatrically in the Okanagan and Similkameen Valleys of B.C. Both species share the same major pheromone component, *Z*11-14:OAc (Roelofs et al. 1976a; Vakenti et al. 1988). *Z*9-14:OAc is a minor component for *P. limitata* (Roelofs et al. 1976a).

Sympatry of these and other leafroller species in B.C. demands an integrated approach to the development of a multiple-species mating-disruption system (Mitchell 1975). One approach to achieving such disruption is the use of common pheromone components (Van Deventer and Blommers 1992; Pfeiffer et al. 1993b; Deland et al. 1994) but another approach might be the use of pheromone components that act interspecifically as inhibitors or antagonists of pheromonal communication (Roelofs and Comeau 1971).

Semiochemicals used in communication between species are called allelochemics (Whittaker 1970). Many chemical signals that evolved primarily as pheromone components have been exploited by other species (Blum 1996), and act as kairomones to induce behaviours that are adaptively favourable to the receiver of the signal (Brown et al. 1970) or as synomones that favour both the emitter and receiver of the signal (Nordlund and Lewis 1976). Many bark beetles perceive components of the aggregation pheromone of sympatric species that are thought to function as synomones in resource partitioning (Blum 1996). For example, (-)-ipsdienol is used as an aggregation pheromone by the pine engraver, *Ips pini* (Say), but it inhibits

aggregation by the California fivespined ips, *I. paraconfusus* Lanier (Birch et al. 1980). Aggregation of *I. pini* is also inhibited by verbenone and ipsenol, compounds produced by the sympatric mountain pine beetle, *Dendroctonus ponderosae* Hopkins and by *I. latidens* (LeConte), respectively (Borden et al. 1991). In the Lepidoptera, pheromone components of one species incorporated in the pheromonal filament (Witzgall and Priesner 1991; Liu and Haynes 1992; Rumbo et al. 1993; Fadamiro and Baker 1997) of another species can inhibit response by the latter species. In male *H. virescens*, incorporation of an interspecific compound into the pheromone blend resulted in stunted upwind surges and increased casting behaviour (Vickers and Baker 1997). Course angles, air and groundspeeds of male *T. ni* flight were reduced when a behavioural antagonist was positioned upwind of a pheromone source producing overlapping plumes (Liu and Haynes 1993b). Linn and Roelofs (1995) suggested that use of these compounds was adaptive to refining an already functional species-specific pheromone signal. The fact that inhibitory synomones are used by closely and distantly related species that occur sympatrically and share some pheromone components suggests that these compounds are important in partitioning the chemical communication channel (Greenfield and Karandinos 1979; Linn and Roelofs 1995).

The strong inhibitory action of these naturally-occurring synomones prompted many researchers to attempt mating disruption with them on the premise that males would be repelled from treated areas (McLaughlin et al. 1972; Kaae et al. 1974; Rothschild 1974; Daterman et al. 1975; Minks et al. 1976; Mitchell 1976). However, behavioural antagonists alone have not been effective mating disruptants (i.e. McLaughlin et al. 1972; Kaae et al. 1974; Rothschild 1974; Daterman et al. 1975;

Mitchell 1976), but they have been demonstrated to stimulate interspecific attraction (Stadelbacher et al. 1983). A combination of pheromone components and antagonists could result in mating disruption by causing species to emigrate from treated areas (Bengtsson et al. 1994). Integration of behavioural antagonists into attractive pheromone formulations also permits testing the mechanisms of mating disruption. One way to test if false-trail following is important in a mating-disruption system is to compare an attractive pheromone formulation alone with a formulation containing the pheromone and the antagonist.

Dodecyl acetate was shown to reduce pheromone trap catches of male *C. rosaceana* in New York (Roelofs and Comeau 1971). Cardé and Baker (1984) cite evidence from eastern North America that *Z*9-14:OAc, the minor pheromone component in *P. limitata*, can inhibit attraction of *C. rosaceana* to its pheromone.

I tested the hypothesis that *Z*9-14:OAc is a behavioural antagonist for a western Canadian population of *C. rosaceana* and compared the efficacy of *Z*9-14:OAc alone and a blend of *Z*9-14:OAc with the sex pheromone of *C. rosaceana* as disruptants of pheromone communication in both *C. rosaceana* and *P. limitata*.

5.2 Methods and Materials

5.2.1 Wind-Tunnel Experiments

Three experiments in a wind tunnel (Fig. 1) were conducted to investigate the hypothesis that addition of *Z*9-14:OAc to the *C. rosaceana* pheromone would inhibit the pheromone-positive response of male *C. rosaceana* obtained from the laboratory

colony. Exp. 1 compared the attractiveness of rubber septa lures containing 100 µg of the pheromone in 37 µL of HPLC-grade hexane to lures containing 100 µg of the same blend plus 50, 6 and 1 µg of *Z*9-14:OAc added in 10 µL of HPLC-grade hexane. The pheromone of *C. rosaceana* contained a 100:2:1.5:1 ratio [confirmed by gas chromatographic (GC) analysis] of *Z*11-14:OAc, *E*11-14:OAc, *Z*11-14:OH, and *Z*11-14:Ald (Thomson et al. 1991; Vakenti et al. 1988). Septa were loaded and aged as outlined in section 2.2.3.

Exp. 2 was identical to Exp. 1 except that 100 µg lures of the complete pheromone were compared to lures containing 100 µg of the same blend plus 1, 0.1, and 0.01 µg of *Z*9-14:OAc added in 10µL of HPLC-grade hexane. Exp. 3 tested the positive control, 100 µg of the *C. rosaceana* pheromone, septa containing 100 µg of the same blend plus 1 µg *Z*9-14:OAc, septa loaded with 1 µg of *Z*9-14:OAc alone, and negative control septa loaded with HPLC grade hexane alone. All treatments in Exp. 3 were diluted in 47 µL of HPLC-grade hexane.

Bioassays were conducted during the first 2 h of scotophase, the peak period of male activity (Knight et al. 1994). The order of presentation of pheromone treatments was randomly determined. Five to seven moths were presented individually to each pheromone treatment on each of seven days. Individual male response to each pheromone treatment was graded as + or - for the following behaviours: wing fanning, take-off, locking-on to the plume, oriented upwind flight and source contact. The release device and ring stand were rinsed with hexane between each treatment.

5.2.2 Field Experiments

Exp. 4 was designed to test if *Z*9-14:OAc could prevent male western *C. rosaceana* from locating traps baited with synthetic pheromone. Pheromone blends tested included: 1) the four-component *C. rosaceana* pheromone 2) *Z*9-14:OAc alone; and 3) a 94:6 blend of the four-component pheromone plus *Z*9-14:OAc. The blends for treatments 1 and 3 were loaded onto red rubber septa lures at a dose of 3 mg in 200 µL of HPLC-grade hexane. *Z*9-14:OAc (treatments 2 and 3) was loaded at a dose of 0.18 mg. Septa were separated by treatment and transported to field sites in glass jars, housed in refrigerated containers.

Traps were constructed and baited as in section 2.4. At the study sites, traps were hung by a wire hanger approximately 1.5 m high on the north side of apple trees. Traps were placed 25 m apart in three randomized complete blocks, two in an orchard growing McIntosh cv., and one in an orchard approximately 1 km distant growing Red and Golden Delicious cvs. Traps in each block were run on four, two-night periods between 10 and 27 August, 1994. Trap positions were re-randomized between trapping periods and lures were replaced.

The efficacy of using *Z*9-14:OAc alone and in combination with the *C. rosaceana* pheromone as an atmospheric treatment to disrupt pheromone communication of *C. rosaceana* and *P. limitata* in the field was tested using a small-plot protocol (section 2.3) in Exp. 5 during 18 August to 6 September, 1995. Four 0.1 ha plots (Fig. 2) were established in two orchards in Cawston, B.C. (Appendix 3). Atmospheric semiochemical treatments (Appendix 2) assigned included: 1) the four-component *C. rosaceana* sex pheromone ; 2) *Z*9-14:OAc alone; 3) treatments 1 + 2 in a 1:1 ratio; and

4) a nontreated control. Treatments were applied using Conrel® fibre-tape dispensers (Ecogen Inc., Billings, MT) at an approximate release rate of 10 $mg \cdot ha^{-1} \cdot h^{-1}$, at 20°C (Ecogen Inc., Billings, MT).

Disruption caused by semiochemical treatments was assessed using wing traps baited with virgin females of either species obtained from laboratory colonies. Cylindrical (3 cm diam. x 5 cm height) mesh cages with a water source, each containing one 6- to 96-h-old virgin female, were transported to the field in refrigerated containers and suspended inside wing traps with push pins. Traps for each species were hung in the tree canopy at both high (2.8-2.9 m) and low (1.8 m) positions. Traps were placed in the plot centre in six groups of four traps with one female-baited trap for each of the two species at each of the two heights on opposing sides of each tree. On each of two days for each replicate, traps were baited in late afternoon and catches were recorded the following morning. The proportion of female-baited traps catching at least one conspecific male in each atmospheric treatment was calculated as a measure of the disruptive effect relative to the nontreated control. To avoid any possible contamination, trap wires and traps were used with only one semiochemical treatment, and were moved between replicates.

5.2.3 Statistical Analyses

For Exp. 1-3, proportions of male *C. rosaceana* responding in the wind tunnel to its sex pheromone alone and with varying quantities of *Z*9-14:OAc added, were compared by a linear logistic regression model (section 2.5) in which blocks of time or replicate was treated as a dummy variable and quantity of *Z*9-14:OAc was treated as

an explanatory variable. The quantity of *Z*9-14:OAc added to the *C. rosaceana* pheromone in Exp. 1 and 2 could be considered as a continuous variable but the dose of *Z*9-14:OAc required to inhibit *C. rosaceana* response was identified using a categorical model. Fitting of a significant ($P<0.05$) logistic regression model was followed by *Z*-tests to compare individual proportions. The α-value for each comparison was adjusted using the Bonferroni inequality to control the experiment-wise type I error rate which depends on the number of comparisons being made (Zar 1984).

Exp. 4, which examined the effect of adding *Z*9-14:OAc to synthetic sex pheromone of *C. rosaceana* in wing traps in the field, provided such unequivocal results that no statistical analysis was necessary.

In Exp. 5, the efficacy of atmospheric semiochemical treatments as communication disruptants was assessed by comparing the proportion of virgin-female-baited traps capturing at least one conspecific male. These proportions were compared using a linear logistic regression model with disruption treatment as explanatory variable and replicate and plot coded as dummy variables (GLIM 1985) followed as above by *Z*-tests to compare the treatment proportions using adjusted α-values (Zar 1984).

The effect of trap position on the capture of male moths within disruption plots, was analyzed using a split-plot ANOVA, with pheromone treatment, plot and replicate as whole-plot factors, and trap height as the subplot factor (SAS 1996). Replicate and plot were specified as random factors. Numbers of males captured were transformed by $\ln(x+1)$. Except where otherwise indicated, $\alpha = 0.05$.

5.3 Results

5.3.1 Wind-Tunnel Experiments

In Exp. 1, in the wind tunnel, departure from the release device was not influenced by the addition of *Z*9-14:OAc (Table 6, Fig. 16), but in the presence of 1-50 µg of *Z*9-14:OAc, more males generally flew out of the plume immediately after take-off than normally did in the presence of the pheromone blend. Male *C. rosaceana* were completely deterred from contacting a 100 µg source of its normally attractive pheromone when *Z*9-14:OAc was added at rates of 6 and 50 µg (Fig. 16). The addition of 1, 6 and 50 µg of *Z*9-14:OAc to 100 µg of the sex pheromone also resulted in a significant reduction in the proportion of males wing fanning, locking-on to the plume, achieving oriented upwind flight and source contact (Table 6, Fig. 16).

In Exp. 2, the addition of 1 µg *Z*9-14:OAc significantly reduced the proportion of males that exhibited all behavioural responses. Not until the quantity of *Z*9-14:OAc was $\leq$ 0.1 µg did its inhibition of pheromone response disappear (Table 6, Fig. 16). As in Exp. 1, additions of 0.01, 0.1 or 1 µg of *Z*9-14:OAc had no influence on take-off from the release device.

An equal proportion of males in Exp. 3 presented with 1 µg of *Z*9-14:OAc alone or with hexane alone, departed from the release device (Table 7). A greater proportion of males presented with the sex pheromone alone or with 1 µg of *Z*9-14:OAc incorporated into the pheromone departed from the release device than males presented with 1 µg of *Z*9-14:OAc alone or hexane alone (Table 7).

Table 6. Response by male *C. rosaceana* to pheromne of *C. rosaceana* alone and with Z9-14:OAc added in various quantities in Exp. 1 and 2, Chapter 5, in a wind tunnel. Seven replications per experiment with 5-7 males tested per treatment, per replicate.

Experiment number	Behaviour	Additions (µg) of Z9-14:OAc added to 100 µg of *C. rosaceana* pheromone[a]	Mean Proportion of male *C. rosaceana* exhibiting response[b]	Logistic Regression Standard Errors [c]	
				Mean Proportion + S.E.	-S.E.
1	wing fanning	0	0.80a	0.86	0.74
		1	0.41b	0.49	0.34
		6	0.15c	0.21	0.10
		50	0.13c	0.19	0.09
1	take-off	0	no treatment effect		
		1			
		6			
		50			
1	lock-on	0	0.52a	0.60	0.44
		1	0.16b	0.23	0.11
		6	0	*	*
		50	0.04b	0.09	0.02
1	upwind flight	0	0.37a	0.44	0.30
		1	0.07b	0.12	0.04
		6	0	*	*
		50	0	*	*
1	source contact	0	0.35a	0.42	0.28
		1	0.05b	0.09	0.02
		6	0	*	*
		50	0	*	*

2	wing fanning	0	0.93a	0.96	0.88
		0.01	0.64b	0.72	0.55
		0.1	0.89a	0.93	0.83
		1	0.30c	0.39	0.23
2	take-off	0			
		0.01			
		0.1	no treatment effect		
		1			
2	lock-on	0	0.49a	0.57	0.41
		0.01	0.53a	0.61	0.45
		0.1	0.55a	0.62	0.46
		1	0.02b	0.06	0.01
2	upwind flight	0	0.40a	0.48	0.33
		0.01	0.43a	0.50	0.35
		0.1	0.41a	0.49	0.34
		1	0.02b	0.06	0.01
2	source contact	0	0.36a	0.43	0.29
		0.01	0.43a	0.50	0.36
		0.1	0.39a	0.46	0.32
		1	0.02b	0.06	0.01

[a] *C. rosaceana* pheromone source was a 100 µg lure containing a 100:2:1.5:1 ratio of *Z*11-14:OAc : *E*11-14:OAc : *Z*11-14:OH : *Z*11-14:Ald.

[b] Proportions followed by the same letter within the same experiment and behaviour are not significantly different ($P>0.05$, *Z*-tests).

[c] Asterisks indicate that standard errors could not be estimated by the logistic regression function.

Figure 16. Relative proportions (proportion of *C. rosaceana* responding to *C. rosaceana* pheromone = 1.0) of male *C. rosaceana* responding to 100 µg of its sex pheromone and 100 µg of pheromone with increasing quantities of *Z*9-14:OAc added, in a wind tunnel in Exp. 1 and 2, Chapter 5. N=7 in both experiments.

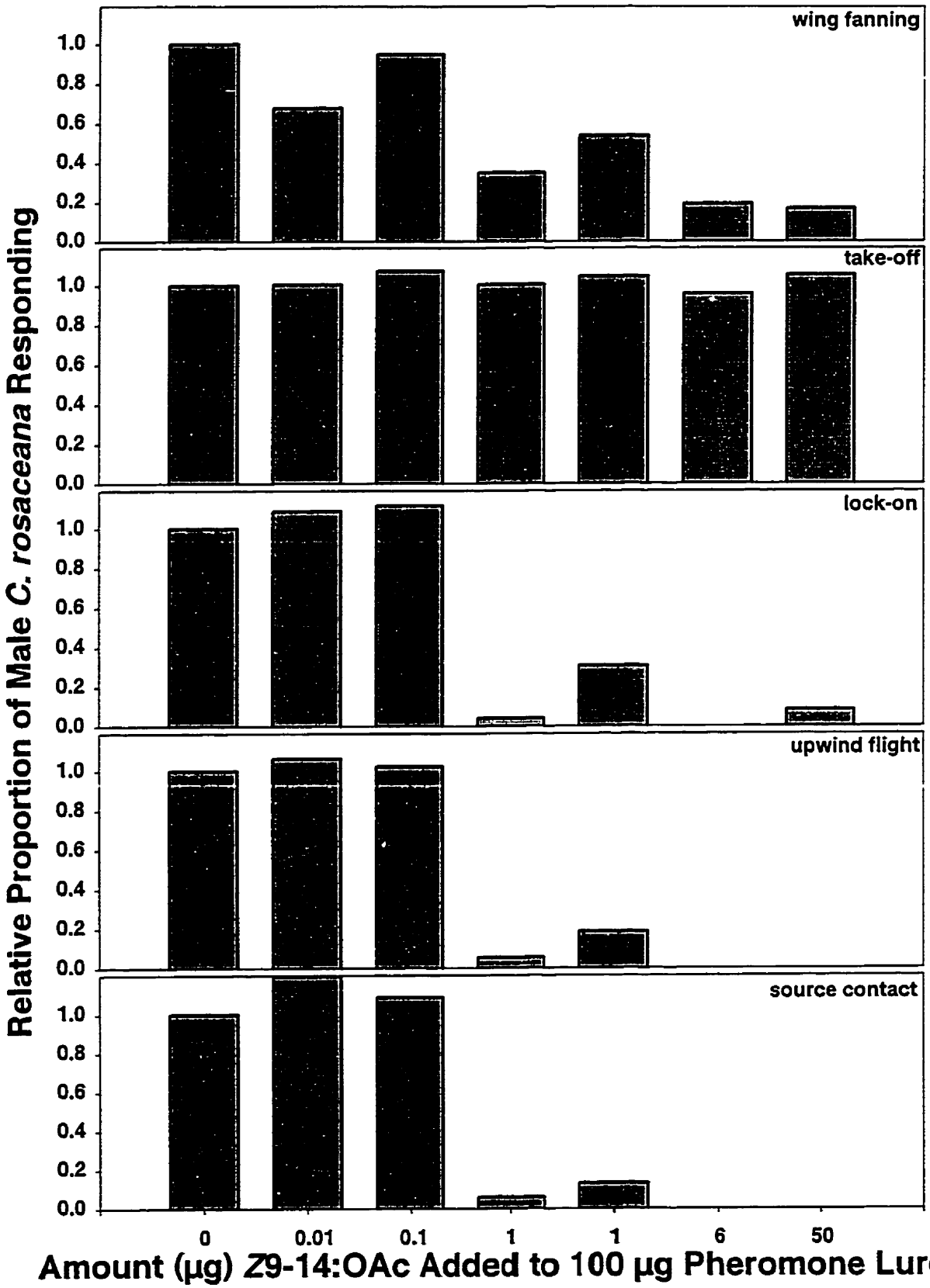

wing fanning
take-off
lock-on
upwind flight
source contact
Relative Proportion of Male C. rosaceana Responding
1.0
0.8
0.6
0.4
0.2
0.0
0
0.01
0.1
1
1
6
50
Amount (µg) Z9-14:OAc Added to 100 µg Pheromone Lure

Table 7. Response by male *C. rosaceana* to pheromone of *C. rosaceana* alone or with *Z*9-14:OAc, *Z*9-14:OAc alone, and hexane alone in Exp. 3, Chapter 5, in a wind tunnel. Seven replications, 5-7 males tested per treatment, per replicate.

			Logistic Regression Standard Errors[c]	
Behaviour	Stimulus[a]	Proportion of male *C. rosaceana* exhibiting response[b]	Mean Proportion +S.E.	-S.E.
wing fanning	*C. rosaceana* pheromone	0.89a	0.93	0.83
	C. rosaceana pheromone +*Z*9-14:OAc	0.48b	0.55	0.40
	*Z*9-14:OAc	0.09c	0.15	0.06
	Hexane	0	*	*
take-off	*C. rosaceana* pheromone	0.86a	0.92	0.78
	C. rosaceana pheromone +*Z*9-14:OAc	0.93a	0.97	0.86
	*Z*9-14:OAc	0.52b	0.62	0.43
	Hexane	0.51b	0.61	0.42
lock-on	*C. rosaceana* pheromone	0.41a	0.48	0.34
	C. rosaceana pheromone +*Z*9-14:OAc	0.07b	0.12	0.04
	*Z*9-14:OAc	0	*	*
	Hexane	0	*	*
upwind	*C. rosaceana* pheromone	0.27a	0.34	0.21
flight	*C. rosaceana* pheromone +*Z*9-14:OAc	0.02b	0.06	0.01
	*Z*9-14:OAc	0	*	*
	Hexane	0	*	*
source	*C. rosaceana* pheromone	0.20a	0.27	0.15
contact	*C. rosaceana* pheromone +*Z*9-14:OAc	0.02b	0.06	0.01
	*Z*9-14:OAc	0	*	*
	Hexane	0	*	*

[a]*C.rosaceana* pheromone source was a 100 µg lure containing a 100:2:1.5:1 ratio of *Z*11-14:OAc:*E*11-14:OAc: *Z*11-14:OH: *Z*11-14:Ald. *Z*9-14:OAc was tested at a dose of 1 µg.

[b]Proportions followed by the same letter within the same behaviour are not significantly different ($P>0.05$, *Z*-tests).

[c]Asterisks indicate that standard errors could not be estimated by the logistic regression function.

5.3.2 Field Experiments

The addition of 6% *Z*9-14:OAc to the sex pheromone of *C. rosaceana* resulted in a complete inhibition of conspecific male catches (Fig. 17). In turn, male *P. limitata* responded exclusively to this altered blend. As expected, *Z*9-14:OAc alone did not attract males of either leafroller species.

In an orchard atmospherically-treated with *Z*9-14:OAc alone, traps baited with virgin females of either species were as effective at capturing conspecific males as female-baited traps in untreated plots (Fig. 18). In contrast, treatment of the atmosphere with the four-component sex pheromone of *C. rosaceana* resulted in 88 and 83% reductions in catches of male *C. rosaceana* and *P. limitata*, respectively. A mixture of *Z*9-14:OAc + the four-component blend in a 1:1 ratio, further reduced the trap catches for both species, although these added effects were not significant. Catches of heterospecific males by traps baited with either *C. rosaceana* or *P. limitata* females occurred most frequently in plots treated with *Z*9-14:OAc.

Trap height had no effect on the trap captures of either species: *C. rosaceana* ($F_{1,12}$=0.3246, P=0.5794); *P. limitata* ($F_{1,12}$=0.0349, P=0.8550).

5.4 Discussion

My results uphold the hypothesis that *Z*9-14:OAc acts as a behavioural antagonist for western Canadian populations of *C. rosaceana* (Figs. 16, 17). Despite the fact that eastern and western populations of *C. rosaceana* appear to use different pheromone blends (Thomson et al. 1991; Vakenti et al. 1988), *Z*9-14:OAc appears to

Figure 17. Mean numbers of male *C. rosaceana* and *P. limitata* captured in wing traps baited with pheromone of *C. rosaceana* alone or in combination with *Z*9-14:OAc and *Z*9-14:OAc alone, in Exp. 4, Chapter 5, 10-27 August, 1994, Cawston, B.C. N=12.

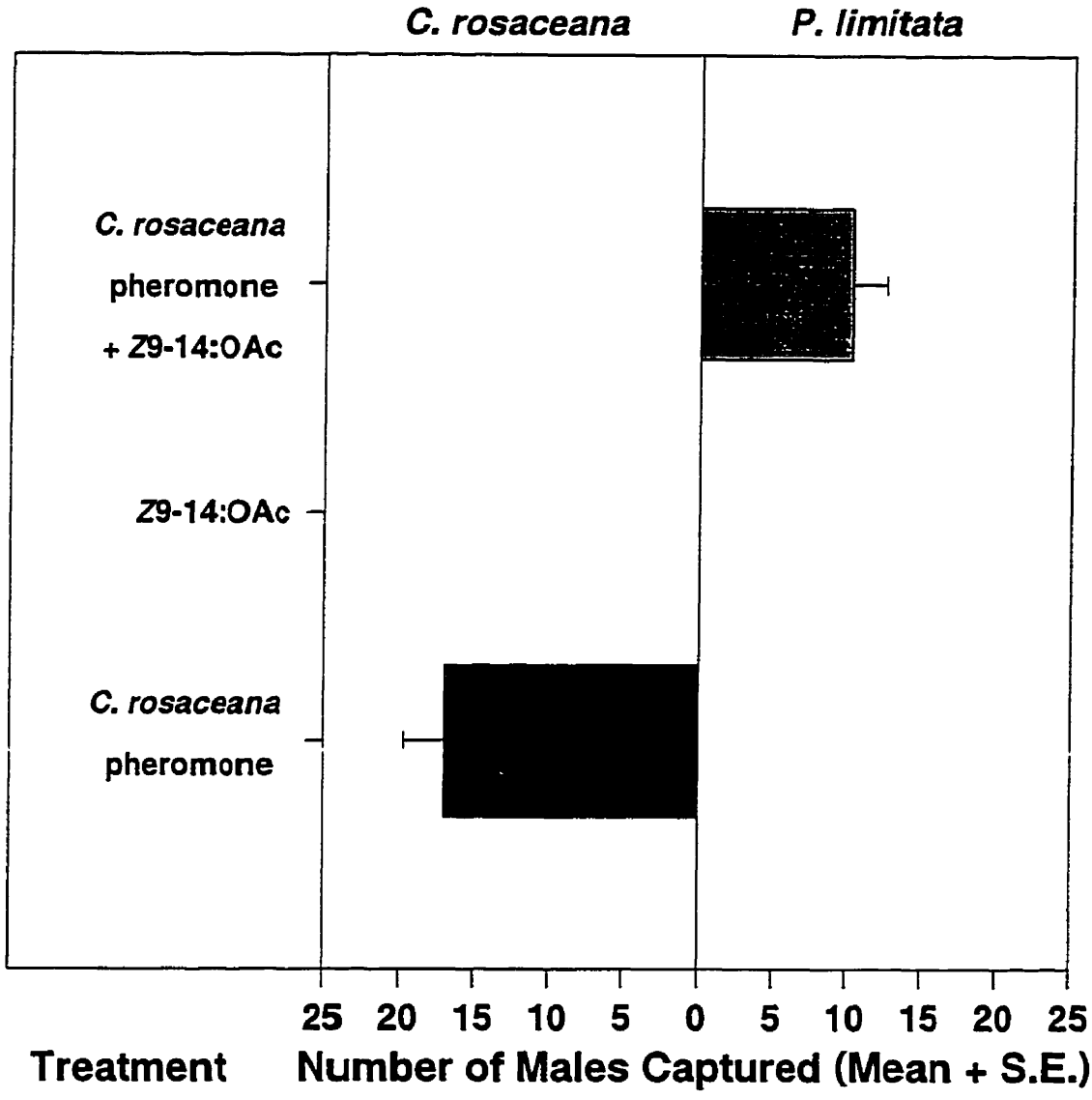
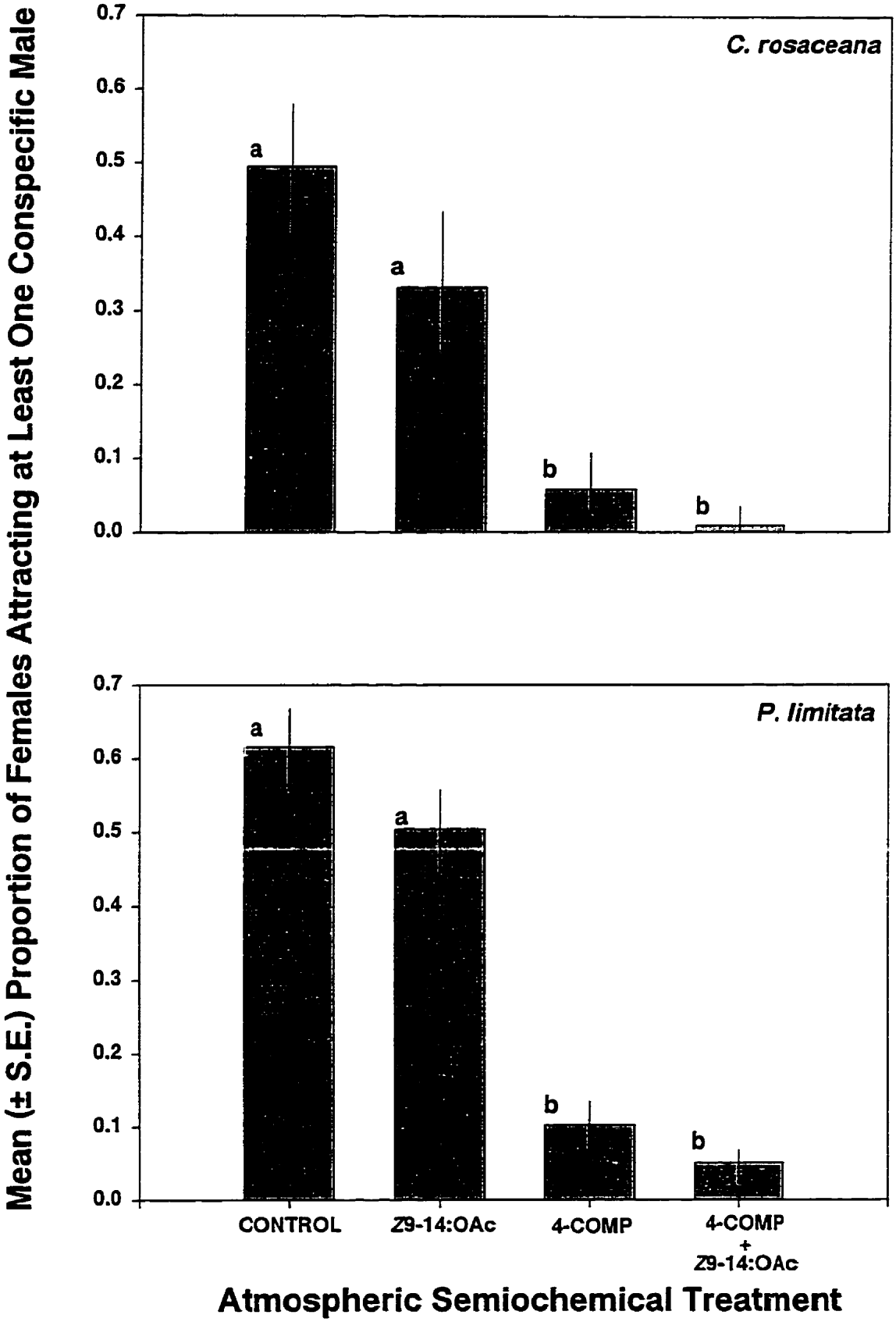

C. rosaceana
P. limitata
C. rosaceana pheromone + Z9-14:OAc
Z9-14:OAc
C. rosaceana pheromone
25 20 15 10 5 0 5 10 15 20 25
Treatment
Number of Males Captured (Mean + S.E.)

Figure 18. Proportions of wing traps baited with virgin female *C. rosaceana* or *P. limitata* attracting at least one conspecific male in various atmospheric semiochemical treatments in Exp. 5, Chapter 5, 18 August-6 September, 1995. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.


Mean (± S.E.) Proportion of Females Attracting at Least One Conspecific Male
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C. rosaceana
a
a
b
b
P. limitata
a
a
b
b
CONTROL
Z9-14:OAc
4-COMP
4-COMP + Z9-14:OAc
Atmospheric Semiochemical Treatment

act without geographic specificity throughout North America. This is possibly due to the sympatry of *C. rosaceana* and *P. limitata*, as well as other orchard-inhabiting tortricid leafrollers, for which *Z*9-14:OAc also serves as a pheromone component (Cardé and Baker 1984). Linn and Roelofs (1995) suggested that the response to behavioural antagonists has evolved to prevent interspecific matings and increase the uniqueness of the already functional pheromone signal. In fact, research has shown that in some species there are antennal receptors specifically tuned to behavioural antagonists (Christensen et al. 1990; Den Otter and Van Der Haagen 1989; Grant et al. 1988) and glomeruli specific for processing information about behavioural antagonists in the macroglomerular complex (Mustaparta 1996). The low concentration (approximately 1%) at which *Z*9-14:OAc retains behavioural activity supports the hypothesis that it is an important natural semiochemical.

It has been shown for other insects (Witzgall and Priesner 1991; Liu and Haynes 1992; Rumbo et al. 1993; Fadamiro and Baker 1997) that behavioural antagonists are effective inhibitors of pheromonal response only when incorporated into the pheromone filament. My results support this conclusion. When added to the pheromone of *C. rosaceana*, *Z*9-14:OAc caused a significant reduction in pheromone-mediated flight behaviours and completely inhibited trap catches. However, when *Z*9-14:OAc was released as a background atmospheric treatment it had almost no effect on capture of males in female-baited traps. This latter finding is in agreement with other studies on mating-disruption attempts using behavioural antagonists (McLaughlin et al. 1972; Kaae et al., 1974; Rothschild 1974; Daterman et al., 1975; Mitchell 1976).

Interestingly, the disruptive effect of the attractive pheromone of *C. rosaceana*

was no greater than that of a 1:1 mixture of this pheromone and *Z*9-14:OAc against male *C. rosaceana* (Fig. 18). Suckling and Burnip (1996) also found no difference in disruption of mate location of *Planotortrix octc* (Dugdale) (Lepidoptera: Tortricidae) when an attractive pheromone disruptant treatment and an unattractive partial pheromone containing an antagonist were compared. Flint and Merkle (1983) also demonstrated that disruption of mating in the pink bollworm, *P. gossypiella*, was equivalent when using unattractive single pheromone components or the complete pheromone blend. These authors suggested that different mechanisms of communication disruption (Bartell 1982) were evoked by the different treatments. False-trail following by male *C. rosaceana* may or may not occur in the presence of its four-component pheromone released at 10 $mg \cdot ha^{-1} \cdot h^{-1}$ from 100 point sources as it was in Exp. 5. However, it is highly unlikely that disruption was achieved through false-trail following when *Z*9-14:OAc, a behavioural antagonist (Figs. 16, 17), was added to the attractive pheromone. Reduction in capture of male *C. rosaceana* by conspecific female-baited traps when a 1:1 mixture of *Z*9-14:OAc and *C. rosaceana* pheromone was used as a disruptant could have occurred by neurophysiological mechanisms or by alternative mechanisms, such as inhibition of female calling, induction of male dispersal out of the treated plots, or some combination thereof. The observation that males in wind-tunnel experiments immediately flew out of an otherwise attractive odour plume when *Z*9-14:OAc was added, suggests that the latter mechanism may be invoked. In support of this hypothesis, male pea moths, *Cydia nigricana* F., were repelled from pheromone-treated fields after isomerization of pheromone components to inhibitory isomers (Bengtsson et al. 1994). The effect of *Z*9-14:OAc on the behaviour of female

C. rosaceana remains to be investigated.

Although minor components alone may be effective mating disruptants (Yashima et al. 1975; Miyashita et al. 1976; Kanno et al. 1978; Stadelbacher et al. 1983), *Z*9-14:OAc had no such effect on male *P. limitata* (Fig.18). As is the case with male *C. rosaceana* exposed to *Z*9-14:OAc alone, *P. limitata* males may have become habituated to this compound, but response to pheromone plumes produced by female *P. limitata* was not altered. It appears that the major pheromone component of both species, *Z*11-14:OAc, is required to provide adequate disruption of *P. limitata*, as mate location was reduced by 83 and 90% in plots treated atmospherically with the *C. rosaceana* pheromone and a 1:1 mixture of the pheromone and *Z*9-14:OAc.

From a practical standpoint it would be useful to suppress several insect species using one pheromone formulation. This may be possible in B.C. orchards, as both the *C. rosaceana* pheromone alone and combined with *Z*9-14:OAc provide >83% disruption of mate location for both species. The effect of *Z*9-14:OAc on other leafroller species in the Okanagan Valley needs to be determined so that a blend suitable for all four sympatric species can be developed.

6.0 SIMULTANEOUS MATING DISRUPTION OF *C. rosaceana* AND *P. limitata*

6.1 Introduction

Pheromone-based mating disruption has been used successfully to control several species of insect pests (Cardé and Minks 1995), but there are few examples of multiple-species mating-disruption systems (Ridgway et al. 1990). Sympatry of *C. rosaceana*, *P. limitata* and other leafroller species in B.C. requires an integrated approach to the development of a multiple-species mating-disruption system (Judd and McBrien 1994). One approach to achieving this goal is the use of common pheromone components (Deland et al. 1994, Van Deventer and Blommers 1992, Pfeiffer et al. 1993b), but another might be the use of interspecific antagonists (Bengtsson et al. 1994).

*Z*9-14:OAc, a minor pheromone component of the *P. limitata* pheromone, was demonstrated to be a behavioural antagonist for populations of *C. rosaceana* in western Canada (Chapter 5) and has also been cited as a behavioural antagonist for eastern North American populations (Cardé and Baker 1984). Although behavioural antagonists alone have not been effective mating disruptants (Chapter 5; McLaughlin et al. 1972; Kaae et al. 1974; Rothschild 1974; Daterman et al. 1975; Mitchell 1976), they sometimes stimulate cross-species attraction (Stadelbacher et al. 1983). In combination with pheromone components released from the same dispenser they could cause mating disruption (Bengtsson et al. 1994; Suckling and Shaw 1995; Suckling and Burnip 1996; Witzgall et al. 1996), eg. by causing emigration from treated areas (Bengtsson et al. 1994). Attractive and partial pheromone blends were equally effective

as mating disruptants against *C. rosaceana*, indicating that false-trail following did not augment other mechanisms provided by less attractive formulations (Chapter 4). Addition of *Z*9-14:OAc to the pheromone blend of *C. rosaceana* disrupted the response of males of both *C. rosaceana* and *P. limitata* to traps baited with virgin females (Chapter 5), indicating the potential of combining pheromone components from both species as a mating disruptant for both species.

I tested *Z*9-14:OAc alone, and in combination with the main pheromone component of both species, *Z*11-14:OAc, and minor pheromone components for *C. rosaceana,* to determine whether a blend could be developed that would provide satisfactory disruption of mating in both species.

6.2 Methods and Materials

6.2.1 General Protocol

All experiments were conducted in Cawston, B.C. during 1994-1997 using a small-plot protocol (Fig. 2).

Five atmospheric semiochemical treatments (Table 8) were applied using Conrel® fibre-tape dispensers (Ecogen Inc., Billings, MT). The small percentages of *E*11-14:OAc and *E*9-14:OAc in blends 4 and 5 (Table 8; Appendix 2) were inescapable by-products of synthesis of the *Z* isomers. Assignment of treatments to plots and dispenser placement followed the standard protocol outlined in section 2.3.1. Dispenser density was maintained at 1000 ha^{-1} in all experiments. Except where otherwise mentioned, semiochemical release rates were 10 $mg \cdot ha^{-1} \cdot h^{-1}$ (Ecogen Inc.,

Table 8. Composition of blends used in mating-disruption experiments against *C. rosaceana* and *P. limitata* (Chapter 6).

Experiment number	Blend number	Percent component in blend[a]					
		*Z*11-14:OAc	*E*11-14:OAc	*Z*11-14:OH	*Z*11-14:Ald	*Z*9-14:OAc	*E*9-14:OAc
1, 2, 3, 4	1	95.7	1.9	1.4	1.0	-	
1, 4	2	96.7	1.9	1.4	-	-	
1, 4, 5, 6	3	98.0	2.0	-	-	-	
5	4	91.4	2.4	-	-	6.1	0.2
4, 5, 6	5	-	-	-	-	97.8	2.2

[a] Compositions of blends 1-3 were as determined by Ecogen Inc. and verified by GC analysis (Appendix 2). Contents of blends 4 and 5 were as determined by GC analysis.

Billings, MT).

Disruption of mating was assessed using tethered, virgin female *C. rosaceana* and *P. limitata* obtained from laboratory colonies described in section 2.1. Females 6 to 96 h old were prepared, transported and distributed in field sites as in section 2.3.2. Females recovered from experimental plots were dissected to determine mating status (see 2.3.2).

6.2.2 Experiments

Exp. 1-3 were conducted to test the hypothesis that blends containing the major pheromone component of both species and minor components of the *C. rosaceana* pheromone, blends which are all effective mating disruptants for *C. rosaceana* (Chapter 4), would disrupt mating by *P. limitata*. Exp. 1, conducted from 18 July to 15 September, 1994, tested blend 1, the four-component pheromone of *C. rosaceana*, and simpler partial blends lacking *Z*11-14:Ald (blend 2) and containing only *Z*11-14:OAc + *E*11-14:OAc (blend 3) (Table 8). In this experiment, females were placed only at high levels in the canopy on open platforms with a square cardboard base of 42 cm^2 and a circular roof of 64 cm^2.

Exp. 2 and 3 tested blend 1 at various release rates to determine the optimum dose required to disrupt mating by *P. limitata*. In Exp. 2, conducted from 7 June to 4 August, 1995, dispensers with 25, 50 and 100 fibres per dispenser provided approximate release rates of 2.5, 5 and 10 $mg \cdot ha^{-1} \cdot h^{-1}$, respectively, at 20°C (Ecogen Inc., Billings MT). In Exp. 3, conducted from 20 June to 28 July, 1996, disruption dispensers with 1, 6 and 13 fibres per dispenser produced approximate release rates of

0.1, 0.6 and 1.3 mg·ha^{-1}·h^{-1}, respectively.

Exp. 4-6 were conducted to determine the minimum number of components in the pheromone of *C. rosaceana*, that alone, or in combination with *Z*9-14:OAc, would provide simultaneous mating disruption of both *C. rosaceana* and *P. limitata*. Exp. 4, conducted from 21 June to 24 July, 1996, compared atmospheric treatments of blends 1, 2, and 3 in combination with *Z*9-14:OAc in a 1:1 ratio. Dispensers with 100 fibres of blends 1, 2, or 3 were attached to disruption dispensers containing 100 fibres of *Z*9-14:OAc (blend 5) producing an approximate combined release rate of 20 mg·ha^{-1}·h^{-1}, 10 mg from each dispenser type.

In Exp. 5, conducted from 12 August to 30 August, 1996, treatments included the major component of both species with *E*11-14:OAc (blend 3) plus *Z*9-14:OAc with its minor contaminant *E*9-14:OAc (blend 5) in a 1:1 ratio, and blend 4, an attractive blend to *P. limitata* containing *Z*11-14:OAc, *E*11-14:OAc, *Z*9-14:OAc and *E*9-14:OAc in a 91.4:2.4:6.1:0.2 ratio (Table 8). In treatment 1, fibre-tape disruption dispensers with 100 fibres of blend 3 were attached to dispensers containing 100 fibres of blend 5 with a release rate of ca. 10 mg·ha^{-1}·h^{-1} from each dispenser.

In Exp. 6, conducted from 14 June to 19 July, 1997, the three treatments were: 100 fibres of blend 3, 100 fibres of blend 5, and 100 fibres each of blends 3 and 5 in a 1:1 ratio. Release rates were ca. 10 mg·ha^{-1}·h^{-1} for each blend.

6.2.3 Statistical Analyses

Proportions of tethered female *C. rosaceana* that were mated after being exposed for one night in pheromone-treated or control plots, were compared by a linear

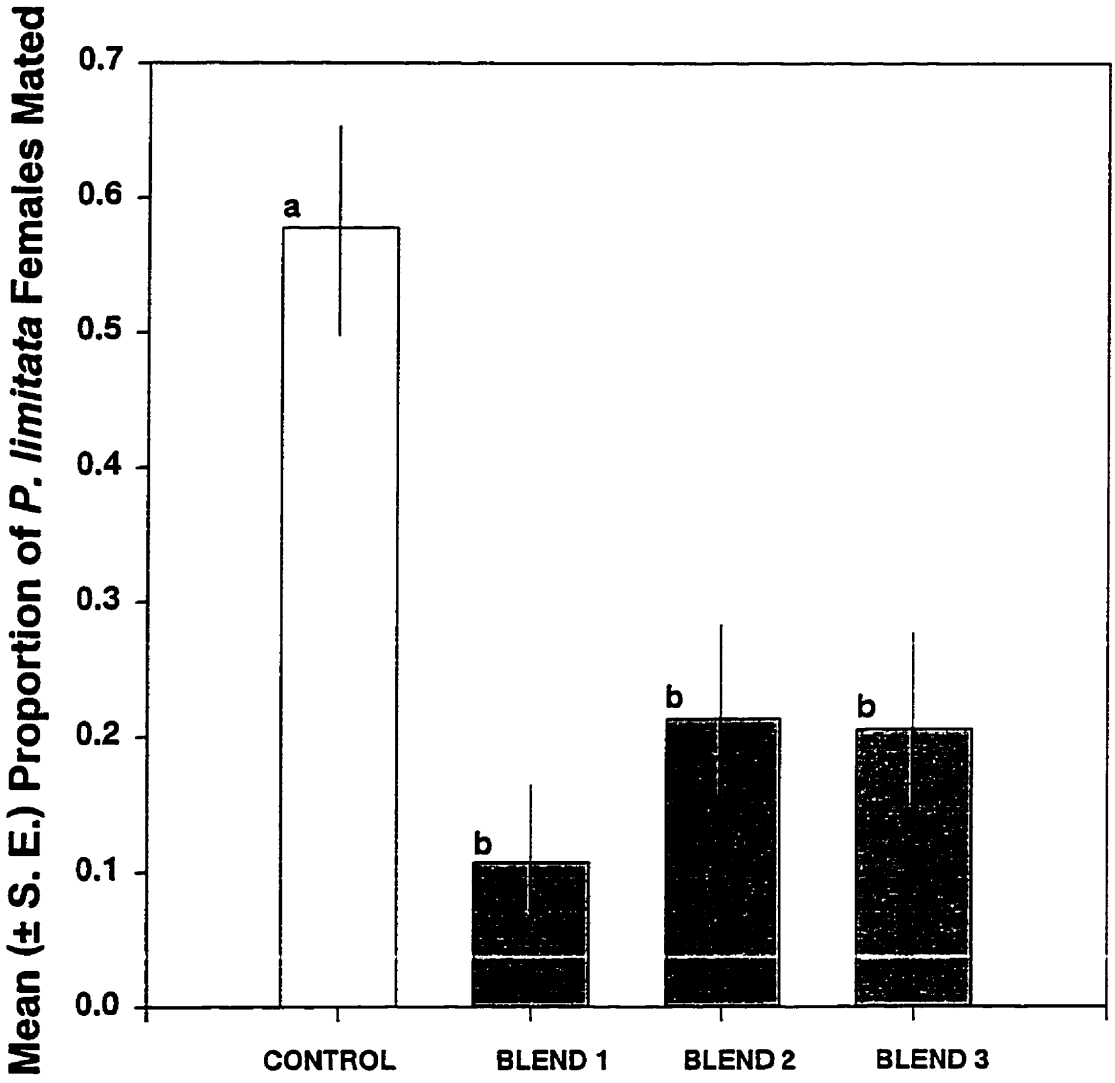
logistic regression model (section 2.5) (GLIM 1985) in which disruption treatment was the explanatory variable, and replicates over time, and plot position were treated as dummy variables. In Exp. 2-4 the dose of blend 1 could be considered a continuous variable but was treated as a categorical variable because it allowed the dose at which mating disruption became ineffective to be identified. Fitting of the logistic regression model was followed by *Z*-tests to compare individual proportions. The α-values for each comparison were adjusted using the Bonferroni inequality to control the experiment-wise type I error rate which depends on the number of comparisons being made (Zar 1984).

6.3 Results

Atmospheric treatment in Exp. 1 with pheromone blends 1-3 containing *Z*11-14:OAc, the major component of both species, and minor components of *C. rosaceana*, resulted in a 63-81% reduction in the proportion of mating among tethered, female *P. limitata* (Fig. 19). However, all three blends were statistically indistinguishable in effectiveness. Mating disruption of *P. limitata* provided in Exp. 2 and 3 by blend 1, the four-component pheromone of *C. rosaceana*, was significant and equal at release rates ranging from 0.6 to 10.0 $mg \cdot ha^{-1} \cdot h^{-1}$ but not at 0.1 $mg \cdot ha^{-1} \cdot h^{-1}$ (Fig. 20).

Atmospheric treatment in Exp. 4 with blends 1-3 in combination with *Z*9-14:OAc (blend 5), strongly reduced the proportion of *P. limitata* that mated, but only the combination of blends 2 + 5 resulted in a significant reduction in mating by *C. rosaceana* (Fig. 21). No treatment differences were detected for the three

Figure 19. Proportions of tethered, female *P. limitata* mating in nontreated plots or plots treated with the four-component blend (blend 1) and two partial blends (2 and 3) in Exp. 1, Chapter 6, 18 July-15 September, 1994, Cawston, B.C. Bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.



*Z*11-14:OAc	0	9.6	9.7	9.8
*E*11-14:OAc	0	0.2	0.2	0.2
*Z*11-14:OH	0	0.1	0.1	0
*Z*11-14:Ald	0	0.1	0	0

Approximate Release Rates ($mg \cdot ha^{-1} \cdot h^{-1}$) of Semiochemical Components

Figure 20. Relative proportions (proportion of mated, tethered female *P. limitata* mating in no-pheromone control plots = 1.0) of mated, tethered females in nontreated plots or plots treated with the four-component blend (blend 1) at various release rates in Exp. 2, Chapter 6, 7 June-4 August, 1995 and Exp. 3, 20 June-28 July, 1996, Cawston, B.C. Within each experiment bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4 in both experiments.

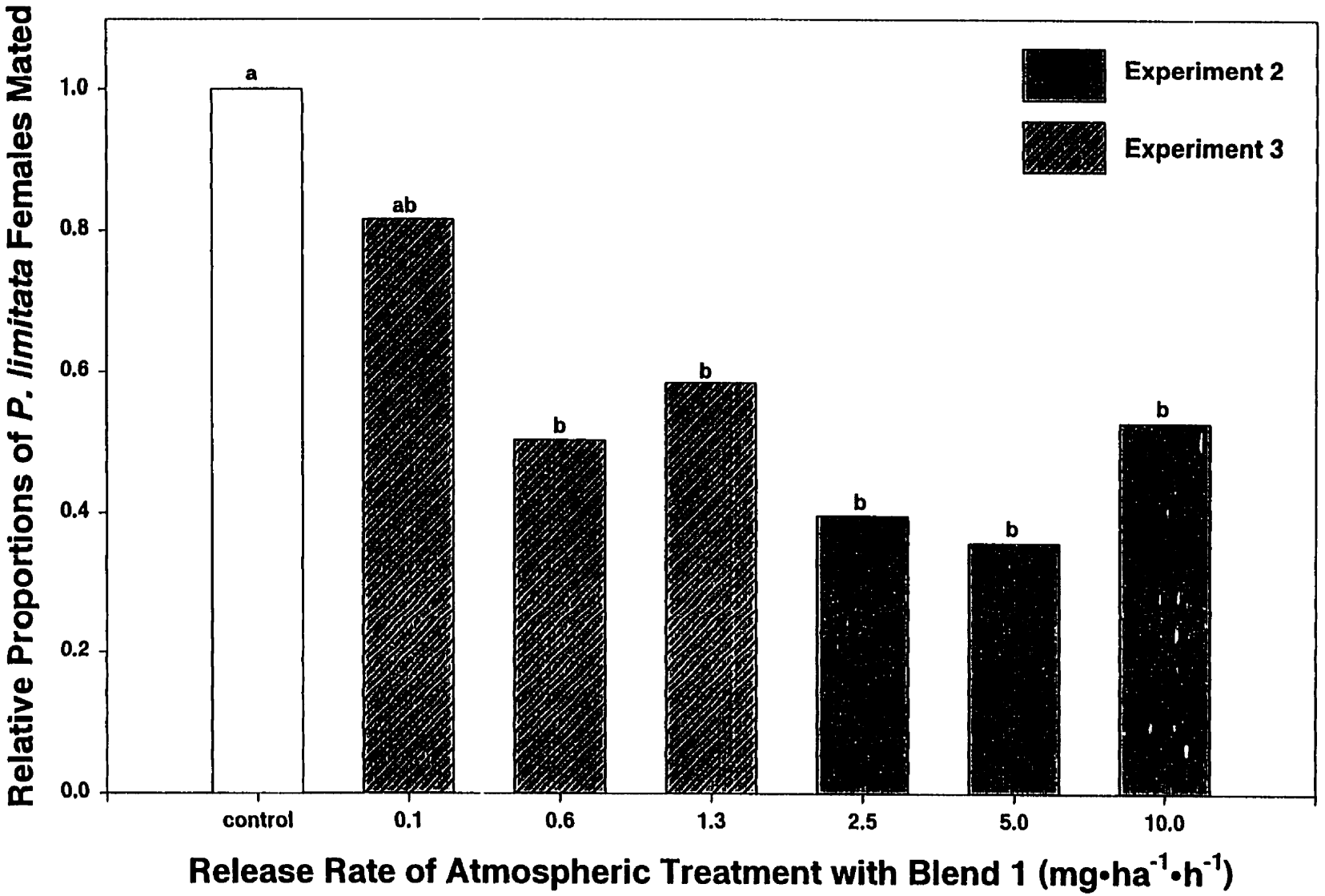
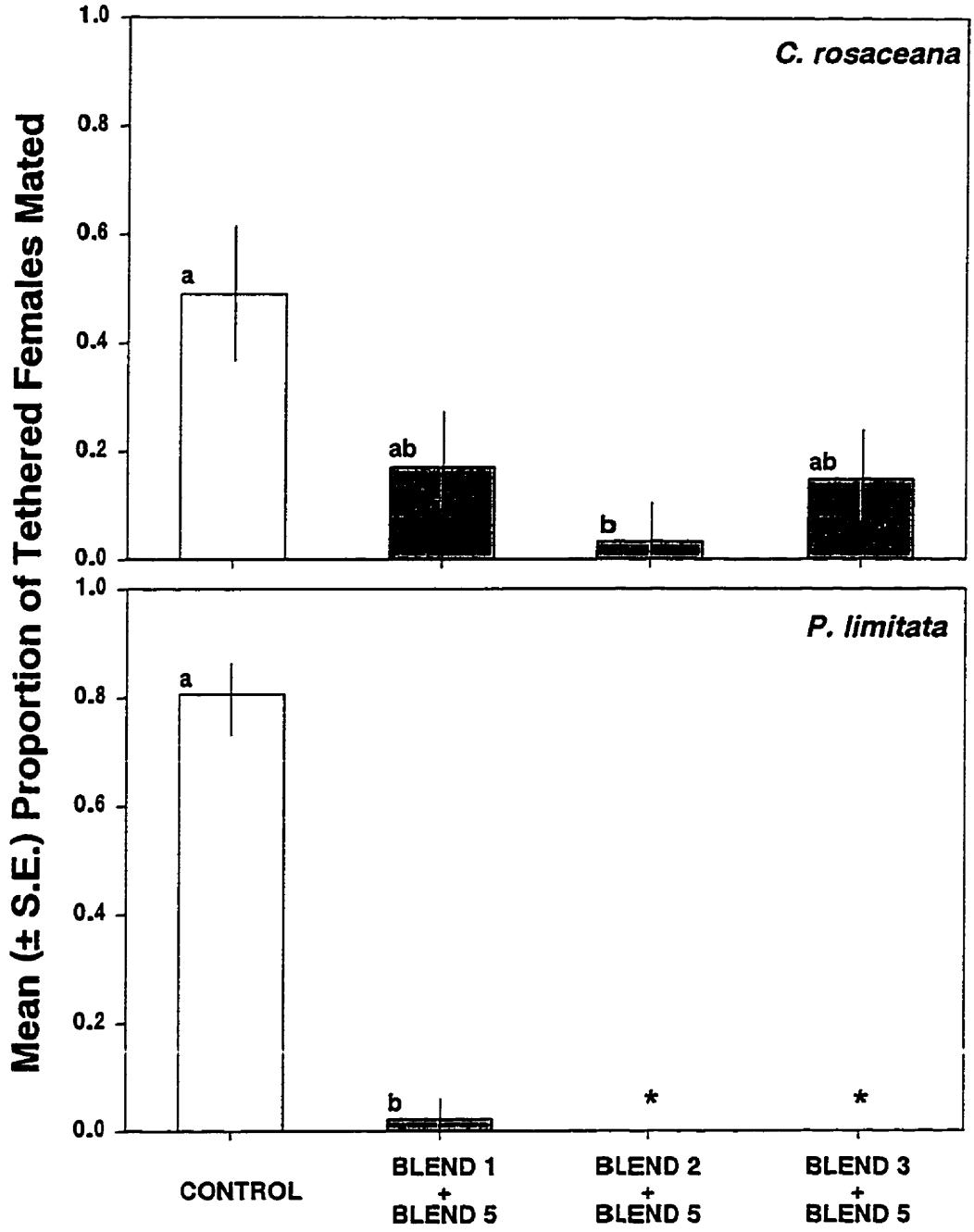

Relative Proportions of P. limitata Females Mated
1.0
0.8
0.6
0.4
0.2
0.0
a
ab
b
b
b
b
b
Experiment 2
Experiment 3
control
0.1
0.6
1.3
2.5
5.0
10.0
Release Rate of Atmospheric Treatment with Blend 1 (mg•ha-1•h-1)

Figure 21. Proportions of tethered, female *C. rosaceana* and *P. limitata* mating in nontreated plots or plots treated with blends 1-3 in combination with *Z*9-14:OAc (blend 5) in a 1:1 ratio in Exp. 4, Chapter 6, 21 June-24 July, 1996, Cawston, B.C. Within each species, bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. Asterisks indicate proportions that approached 0 and for which S.E.'s could not be estimated by the logistic regression function. N=4.



*Z*11-14:OAc	0	9.6	9.7	9.8
*E*11-14:OAc	0	0.2	0.2	0.2
*Z*11-14:OH	0	0.1	0.1	0
*Z*11-14:Ald	0	0.1	0	0
*Z*9-14:OAc	0	9.8	9.8	9.8
*E*9-14:OAc	0	0.2	0.2	0.2

Approximate Release Rates ($mg \cdot ha^{-1} \cdot h^{-1}$) of Semiochemical Components

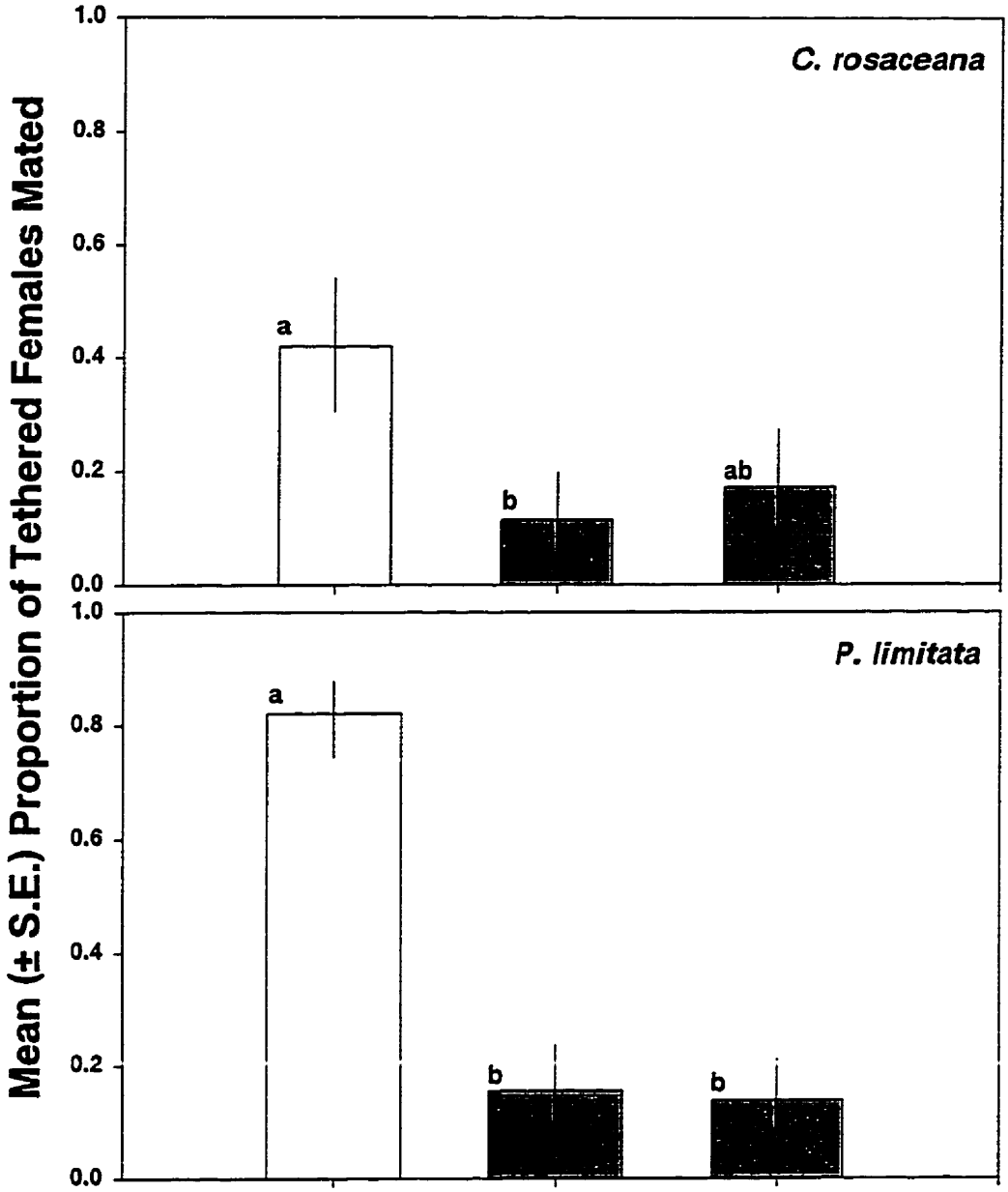
semiochemical blends tested in Exp. 4 for either species. In Exp. 5, blends 3 + 5, an off-ratio *P. limitata* pheromone blend, was tested against blend 4, an attractive blend to *P. limitata* . As in Exp. 4, treatments containing *Z*9-14:OAc produced a greater reduction of mating in *P. limitata* (81-83%) than *C. rosaceana* (Fig. 22). For *C. rosaceana*, only blends 3 + 5 resulted in a significant reduction (73%) in the proportion of females that mated.

In Exp. 6, *Z*9-14:OAc alone (blend 5) the minor component of the *P. limitata* pheromone did not reduce mating in either species, whereas *Z*11-14:OAc (blend 3), the major component of both species did (Fig. 23). Blends 3 + 5, the off-ratio blend of the *P. limitata* pheromone, was no more effective as a mating disruptant against *P. limitata* than *Z*11-14:OAc (blend 3) the major component alone. For *C. rosaceana*, treatment with blends 3 + 5 significantly reduced mating, but was no more effective than *Z*11-14:OAc alone (blend 3).

6.4 Discussion

Data presented in this chapter suggest that simultaneous disruption of mating in *C. rosaceana* and *P. limitata* can be achieved by treating the atmosphere with *Z*11-14:OAc, the compound that comprises the majority of the pheromone in both species, or with a blend containing both *Z*11-14:OAc and *Z*9-14:OAc, the minor component for *P. limitata* and antagonist to *C. rosaceana*. Furthermore, successful mating disruption of *P. limitata* using a highly attractive formulation, a moderately attractive off-ratio formulation and an unattractive partial blend support the results obtained for *C.*

Figure 22. Proportions of tethered, female *C. rosaceana* and *P. limitata* mating in nontreated plots or plots treated with the *P. limitata* pheromone (blend 4) or blend 3 in combination with *Z*9-14:OAc (blend 5) in a 1:1 ratio in Exp. 5, Chapter 6, 12 August-30 August, 1996, Cawston, B.C. Within each species, bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=3.

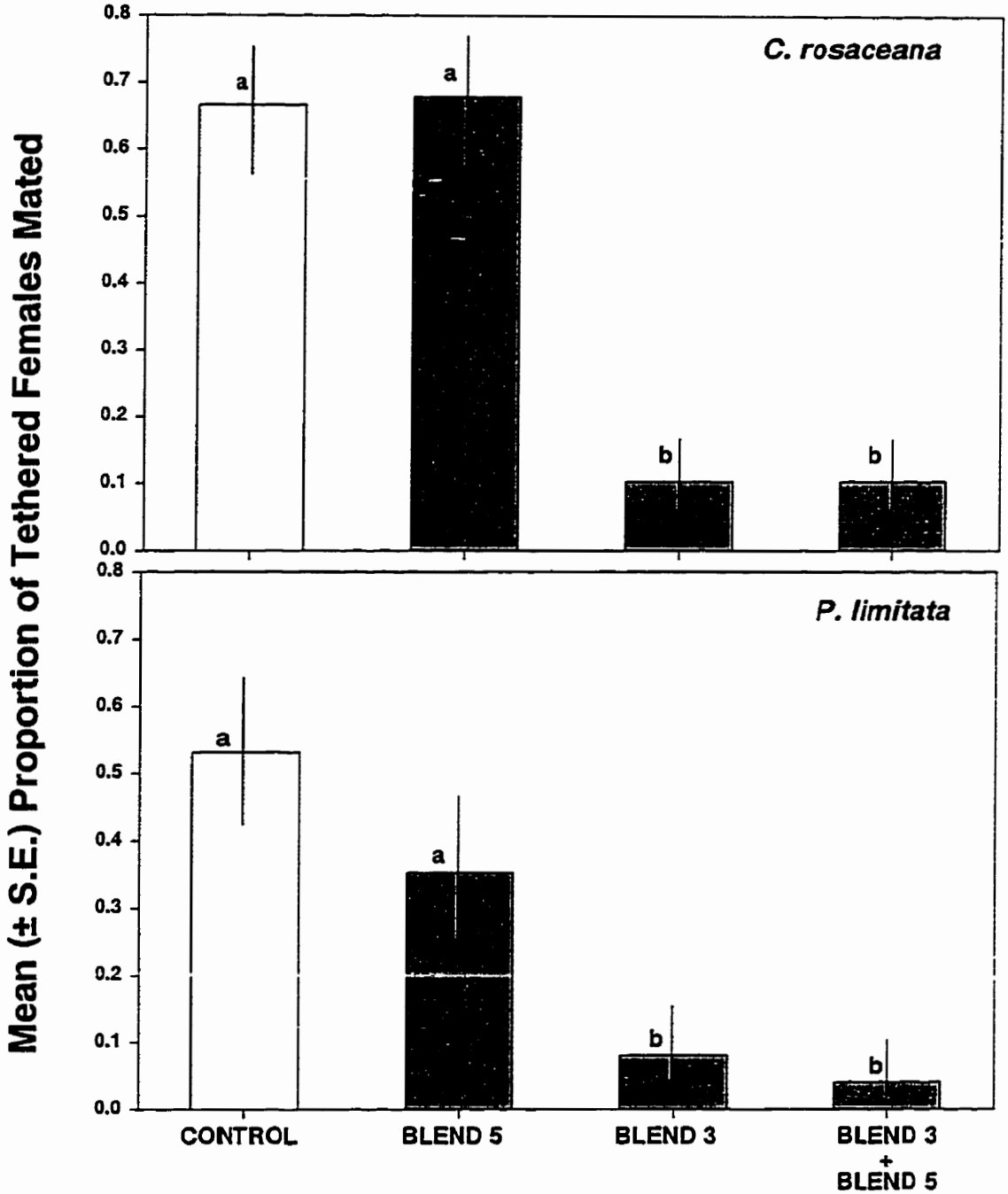


*Z*11-14:OAc	0	9.8	91.4
*E*11-14:OAc	0	0.2	2.4
*Z*11-14:OH	0	0	0
*Z*11-14:Ald	0	0	0
*Z*9-14:OAc	0	9.8	6.1
*E*9-14:OAc	0	0.2	0.2

Approximate release rates ($mg \cdot ha^{-1} \cdot h^{-1}$)

of Semiochemical Components

Figure 23. Proportions of tethered, female *C. rosaceana* and *P. limitata* mating in nontreated plots or plots treated with *Z*9-14:OAc alone, blend 3 alone, or blend 3 in combination with *Z*9-14:OAc (blend 5) in a 1:1 ratio, in Exp. 6, Chapter 6, 14 June-19 July, 1997, Cawston, B.C. Within each species, bars with the same letter superscript are not significantly different, *Z*-tests $P>0.05$. N=4.



*Z*11-14:OAc	0	0	9.8	9.8
*E*11-14:OAc	0	0	0.2	0.2
*Z*11-14:OH	0	0	0	0
*Z*11-14:Ald	0	0	0	0
*Z*9-14:OAc	0	9.8	0	9.8
*E*9-14:OAc	0	0.2	0	0.2

Approximate Release Rates ($mg \cdot ha^{-1} \cdot h^{-1}$) of Semiochemical Components

rosaceana in chapters 4 and 5 that attractiveness of the pheromone formulation is not correlated with effectiveness as a mating disruptant.

In Exp. 1-3, reductions in the proportions of tethered, female *P. limitata* that mated was most likely due to neurophysiological effects resulting from exposure to *Z*11-14:OAc. Blends 1 and 2, containing *Z*11-14:OH and *Z*11-14:Ald were no more effective at reducing mating in *P. limitata* than blend 3 which contained 98% *Z*11-14:OAc with 2% *E*11-14:OAc. Trap capture of *P. limitata* males is inhibited by the addition of 6% *E*11-14:OAc to its pheromone blend (Roelofs et al. 1976a). *Z*11-14:OH is present in the effluvia of female *P. limitata* (G. Gries, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., pers. comm.) but it is not known if the *Z*11-14:Ald is perceived by *P. limitata* males and acts interspecifically in some manner. As *Z*9-14:OAc, the minor component of the *P. limitata* blend is required for attraction of males of this species (Roelofs et al. 1976a) it is highly unlikely that disruption using any of blends 1-3 (Table 8) was the result of false-trail following. Although it may be possible to camouflage a plume with an incomplete pheromone blend (Minks and Cardé 1988), it is most likely that neurophysiological effects caused the observed reduction in mating of *P. limitata* in Exp. 1-3. Pre-exposure or constant exposure to single pheromone components in the laboratory (Linn and Roelofs 1981, Liu and Haynes 1993a) and in the field (Flint and Merkle 1984a, Judd et al. 1995) has resulted in upwind flight of males to off-ratio pheromone blends that is best explained by neurophysiological adaptation or habituation.

The significant reduction in the proportion of female *P. limitata* that mated over a wide range of release rates of blend 1 in Exp. 2 and 3, except at the lowest release rate

of 0.1 mg·ha^{-1}·h^{-1} (Fig. 20), is in contrast to observations that release rate of pheromone is important in preventing mating and providing crop protection in several mating-disruption systems (Schwalbe and Mastro 1988, Webb et al. 1990, Suckling and Shaw 1992). Blend 1 also reduced mating by tethered *C. rosaceana* females at pheromone release rates >1.3 mg·ha^{-1}·h^{-1} (Chapter 4).

Atmospheric treatments comprised of components from both species' pheromones disrupted mating in both species to varying degrees. In Exp. 4 and 5 blends incorporating *Z*9-14:OAc disrupted mating of *P. limitata* but were less effective against *C. rosaceana* (Figs. 21, 22). Mechanisms that may be acting to prevent *P. limitata* from mating in these experiments include camouflage of female-produced plumes, adaptation and habituation. In Exp. 5, the blends tested contained the same components in different ratios. Blend 4, a 91.4: 6.1 ratio of *Z*11-14:OAc to *Z*9-14:OAc is an attractive blend to *P. limitata*, and could therefore have invoked false-trail following. The other treatment, blends 3 + 5, represents an off-ratio *P. limitata* blend with an approximate 1:1 ratio of these components. Exp. 5 (Fig. 22) shows that at the tested release rate, the attractive blend 4 was no more effective as a mating disruptant of *P. limitata* than the less attractive off-ratio blend. This observation is consistent with the results of chapter 4 in which the most attractive blend was no more effective than less attractive blends in disrupting *C. rosaceana*. Due to the presence of the antagonist *Z*9-14:OAc, the reduction in mating of *C. rosaceana* in these same plots, could only be the result of neurophysiological effects or emigration from treated areas. In support of this finding, partial pheromone blends (blends 2 and 3) were equally effective as mating disruptants of *C. rosaceana* as the more attractive blend 1 (Chapter 4). Blends

containing behavioural antagonists and pheromone components have been used as mating disruptants for several species. Large-scale mating disruption of the light brown apple moth, *E. postvittana*, was achieved using a formulation that contained a behavioural antagonist, produced as a by-product of pheromone synthesis, that rendered the formulation unattractive (Suckling and Shaw 1995). Suckling and Burnip (1996) found no difference in disruption of mate location of *P. octo* when an attractive pheromone disruptant treatment and an unattractive partial pheromone containing an antagonist were compared. Bengsston et al. (1994) demonstrated that male pea moths emigrated from treated areas when isomerization of a pheromone component resulted in the production of an inhibitory isomer in the disruptant formulation.

In Exp. 6, unlike in Exp. 4 and 5, mating in both species was reduced significantly when the atmosphere was treated with blend 3 alone and blend 3 + 5 in a 1:1 ratio (Fig. 23). Blends 3 + 5 reduced mating of *C. rosaceana* females by 85% in Exp. 6, which is greater than the 65% and 73% reductions in Exp. 4 and 5, respectively. The latter two experiments were conducted the year before Exp. 6 in different orchards with differing canopy structures, and with greater population pressure as measured by monitoring traps adjacent to experimental plots (Appendix 5). The relatively low proportion of females that mated in the control plots in Exp. 4 and 5 may thus reflect a high level of competition from feral females.

Treatment with *Z*9-14:OAc (blend 5) alone had no effect on the proportion of females of either species that mated. For *C. rosaceana* this was expected because *Z*9-14:OAc is a known behavioural antagonist (Chapter 5; Cardé and Baker 1984), and treatment with behavioural antagonists alone have not provided effective mating

disruption in other studies (Chapter 5; McLaughlin et al. 1972; Kaae et al. 1974; Rothschild 1974; Daterman et al. 1975; Mitchell 1976). Behavioural antagonists do not provoke an antagonistic response unless they are present in the same plume as the pheromone components (Witzgall and Preisner 1991; Liu and Haynes 1992; Rumbo et al. 1993; Fadamiro and Baker 1997). Furthermore, there are glomeruli in the macroglomerular complex in the antennal lobe of some insects that process response to behavioural antagonists (Mustaparta 1996), and over stimulation of this pathway does not appear to alter pheromone blend integration and upwind flight to females. For *P. limitata*, however, *Z*9-14:OAc is a minor pheromone component (Roelofs et al. 1976a). There was a slight, non-significant reduction in mating of *P. limitata* in plots treated with *Z*9-14:OAc alone (Fig. 23). In other species minor components alone may be effective as mating disruptants (Yashima et al. 1975; Miyashita et al. 1976; Kanno et al. 1978; Stadelbacher et al. 1983). I previously demonstrated (Chapter 5) that *Z*9-14:OAc does not alter capture of male *P. limitata* in female-baited traps. It appears that the major pheromone component, *Z*11-14:OAc, is required to provide adequate mating disruption of both *P. limitata* and *C. rosaceana*.

7.0 *Z*9-14:OAc: A SYNOMONE IMPARTING DISTINCT SEX PHEROMONE COMMUNICATION CHANNELS FOR *C. rosaceana* AND *P. limitata*.

7.1 Introduction

Synomones are compounds produced or acquired by an organism, which, when it contacts an individual of another species, evokes a response that is adaptively favourable to both the emitter and receiver (Nordlund and Lewis 1976). In the Lepidoptera, synomones have apparently evolved primarily as species-specific sex pheromone components that also function as inhibitors of pheromone response to heterospecific males (Linn and Roelofs 1995). They appear to function to enhance the already species-specific pheromone signal by reducing competition in the sex pheromone communication channel (Greenfield and Karandinos 1979; Cardé 1986) and preventing heterospecific mating attempts (Linn and Roelofs 1995; Cardé 1986).

Some authors argue that divergence in moth pheromone structure in general, including the occurrence of synomonal components, has evolved partially in response to the selective pressure from the presence of closely related heterospecifics which may share common pheromone components (Cardé et al. 1977b; Cardé 1986; Linn and Roelofs 1995; Löfstedt 1993). Other authors postulate that mate-signalling systems in general have evolved in response to environmental factors simply as part of a species-specific mate recognition system (Paterson 1985, Paterson 1993). Alternatively, it has been suggested that pheromone diversity is the result of secondary characteristics that have evolved after divergence of species under sexual selection (Thornhill and Alcock 1983; West-Eberhard 1984). The fact that some insects, that are not closely related but share common pheromone components and occur sympatrically

and synchronically, often use antagonistic synomones suggests that response to such compounds is an adaptive strategy that has evolved to avoid loss of time and energy in mating attempts with heterospecifics (Cardé 1986; Linn and Roelofs 1995).

Regardless of the origin of these signals, synomones, which inhibit pheromonal response of sympatric species, can be found in closely related lepidopteran species and also in more distantly related species that occur sympatrically and synchronically. For two closely related species of gelechiid moths, *Bryotopha* spp., Roelofs and Comeau (1969) found that each species was attracted to a different geometrical isomer of the same compound, but when the isomers were combined neither species was attracted. For two noctuids, the cabbage looper, *T. ni* and the soybean looper, *Pseudoplusia includens* (Walker), *Z*-7-dodecen-1-ol acetate is a common major pheromone component (Bjostad et al. 1984; Linn et al. 1987a), but *Z*-5-dodecen-1-ol acetate, a minor pheromone component produced only by female cabbage loopers inhibits male soybean looper response (Landolt and Heath 1987; Linn and Roelofs 1995). These two species have overlapping temporal activities and geographic distributions but interspecific mating attempts are avoided by males being optimally tuned to the multi-component pheromone plume produced by conspecific females (Linn et al. 1988), and by soybean looper males being inhibited by a minor component in the cabbage looper blend (Landolt and Heath 1987; Linn et al. 1988; Linn and Roelofs 1995). All *Yponomeuta spp.*, share *Z*11-14:OAc as their major pheromone component. Pheromone blends of different species differ by the amount of *E*11-14:OAc present in the blend and the presence of minor pheromone components. Females of the ermine moth, *Yponomeuta padella* (L.) produce *Z*11-16:OAc as a minor component and this

compound is inhibitory to sympatric species (Löfstedt et al. 1991).

Among tortricines feeding on apple, many species possess pheromone components that also act as inhibitory synomones to other species (Cardé and Baker 1984). The fruittree leafroller, *Archips aryrospilus* , *Archips mortuanus* Kearfoot and the redbanded leafroller, *Argyrotaenia velutinana* (Walker), females all produce dodecyl acetate as a constituent of their pheromone blend (Roelofs et al. 1975; Cardé et al. 1977b; Deland et al. 1993), this compound inhibits pheromonal response in *C. rosaceana* (Roelofs and Comeau 1971). Cardé et al. (1977b) and Cardé and Baker (1984) cite results that *Z*9-14:OAc, which is part of the pheromone blend of *A. aryrospilus* , *A. mortuanus* and *P. limitata* (Roelofs et al. 1976a; Cardé et al. 1977b; Deland et al. 1993), is a behavioural antagonist to *C. rosaceana* in eastern North America. I found *Z*9-14:OAc to be an antagonist of pheromonal response to *C. rosaceana* in western Canada (Chapter 5).

One way of testing the hypothesis that inhibitory synomones are important semiochemicals for minimizing competition in an overlapping sexual communication channel is to permeate the atmosphere with the synomone alone and to determine if adaptation and/or habituation to it results in interspecific mate location. Stadelbacher et al. (1983) demonstrated in field and wind-tunnel studies that atmospheric treatment with a component produced by *H. virescens* females, but which is inhibitory to male *H. zea*, resulted in *H. zea* males flying upwind to and mating with tethered female *H. virescens*. Interestingly, a small but significant number of *H. virescens* males were also captured in traps baited with *H. zea* females (Stadelbacher et al. 1983).

My first objective was to test the hypothesis that atmospheric permeation with

*Z*9-14:OAc would result in male *C. rosaceana* conducting upwind flight and source contact to normally unattractive sources containing *Z*9-14:OAc (Chapter 5). My second objective was to determine the importance of *Z*9-14:OAc in separating the overlapping sex pheromone-communication channels of *C. rosaceana* and *P. limitata*, by treating the atmosphere with this compound and determining if interspecific mate-finding occurred.

7.2 Methods and Materials

7.2.1 Wind-Tunnel Experiments

Exp. 1 tested the hypothesis that placement of a source releasing *Z*9-14:OAc upwind of a septum containing the four-component *C. rosaceana* pheromone and the behavioural antagonist, *Z*9-14:OAc, would induce male *C. rosaceana* to fly to the normally unattractive source. Males were obtained from the laboratory colony, prepared for wind-tunnel bioassays and presented to septa following the protocol established in section 2.2.2. Septa (section 2.2.3) were loaded with either: 1) 100 µg of the *C. rosaceana* pheromone, or 2) 100 µg of the *C. rosaceana* pheromone plus 6 µg of *Z*9-14:OAc. They were presented to males in clean air or with 50 fibres (Ecogen Inc., Billings MT) of *Z*9-14:OAc (Appendix 2), releasing approximately 5 $\mu g \cdot h^{-1}$, placed 10 cm upwind of the septa. Five to seven males (section 2.2.2) were presented individually to each treatment in each background during the first 2 h of scotophase on each of seven test days. Treatment order was random but septa were always presented to males first in clean air to avoid contamination of the test septa. Behavioural response to each

treatment and background combination was marked as + or - for wing fanning, take-off from the release stand, locking-on to the plume and source contact.

Exp. 2 and 3 tested the hypothesis that placement of a source releasing *Z*9-14:OAc upwind of a calling female would result in interspecific mate location. In Exp. 2, *C. rosaceana* males were presented individually to a single calling *P. limitata* female, and in Exp. 3, *P. limitata* males were presented to a calling *C. rosaceana* female (section 2.2.2). Males were placed in the female-produced plume as above in either clean air (control) or with 50 fibres of *Z*9-14:OAc, releasing approximately 5 $\mu g \cdot h^{-1}$, placed 10 cm upwind of the calling female. In Exp. 2, seven to 10 male *C. rosaceana* were tested individually to a female *P. limitata* in either the control or experimental treatment and in Exp. 3, eight or nine male *P. limitata* were presented individually to a female *C. rosaceana*, in each treatment during the first 2 h of scotophase on each of five test days. On each test day a single female was used in both background treatments. Females were always presented in clean air first to avoid the possibility of *Z*9-14:OAc residue remaining on the mesh bag containing the female. Behavioural response to the calling female and background treatment was scored as + or - for wing fanning, take-off from the release stand, locking-on to the plume and source contact. To confirm the inactivity of the background treatment alone in Exp. 1-3, 2-5 males were presented to *Z*9-14:OAc alone at the end of each replicate.

Exp. 4 was conducted to determine the effect of background treatment with *Z*9-14:OAc on the oriented upwind flight of male *P. limitata* to conspecific females. Seven to 10 males were presented individually (section 2.2.2) to a single, calling *P. limitata* female in clean air or with 50 fibres of *Z*9-14:OAc, releasing approximately 5 $\mu g \cdot h^{-1}$,

placed 10 cm upwind of the calling female. Replications were completed in each background during the first 2 h of scotophase on each of five test days. On each day a single female (section 2.2.2) was presented in clean air first and then used in the background treatment. Behavioural response to the calling female and background treatment was marked as + or - for wing fanning, take-off from the release stand, locking-on to the plume and source contact.

All wind-tunnel experiments were analyzed using linear logistic regression as outlined in section 2.5 (GLIM 1985). In Exp. 1, a split-plot model was analyzed with day of test specified as whole plot factors and background treatments specified as sub plot factors. The model tested had the following form:

$$\text{logit}(y/n)=\beta_0 + (\text{septa treatment})x + (\text{day of test})x + (\text{background treatment})x + (\text{day of test}*\text{background treatment})x + (\text{septa treatment}*\text{background treatment})x,$$

where y is the number of males performing the appropriate behaviour and n is the number of males presented to the female. If removal of the septa treatment * background treatment term from the whole model resulted in a significant increase in deviance (see section 2.5), the effect of *Z*9-14:OAc on the type of septa approached by the male was considered significant. If the septa treatment * background treatment term was determined to be significant and removal of the day of test * background treatment term did not result in a significant increase in deviance, the model was simplified to a randomized block design with each septa treatment * background treatment combination coded individually, so that treatment estimates could be

obtained. If the septa treatment * background treatment term was determined to be significant and removal of the day of test * background treatment term also resulted in a significant increase in deviance it was not possible to simplify the model and the individual septa treatment * background treatment combinations could not be estimated. If septa treatment * background treatment combinations could be obtained, individual proportions were compared using *Z*-tests. The α-value for each comparison was adjusted using the Bonferroni inequality to control the experiment-wise type I error rate which depends on the number of comparisons being made (Zar 1984).

In Exp. 2-4, the proportions of males conducting each behavioural response to females in clean air *vs.* air treated with *Z*9-14:OAc were analyzed using linear logistic regression following a randomized block design, with day of test treated as the blocking factor and coded as a dummy variable.

7.2.2. Field Experiments

Two field experiments (Exp. 5 and 6) were conducted to determine if atmospheric treatment with *Z*9-14:OAc would shift the response of *C. rosaceana* and *P. limitata* males to synthetic or female-produced pheromone plumes. In each of three organic orchards in Cawston, B.C., two 0.1 ha plots were established ≥ 40 m apart and ≥10 m from the edge of the orchard. One hundred dispensers releasing *Z*9-14:OAc at approximately 10 $mg \cdot ha^{-1} \cdot h^{-1}$ (Appendix 4) were hung in the orchard canopy of one randomly chosen plot in each orchard, following the protocol outlined in section 2.3.

In Exp. 5, five traps containing septa loaded with one of five synthetic

pheromone treatments (Table 9) were placed in both plots in each orchard (section 2.4). Traps were hung in trees ca. 1.5 m above the ground randomly placed in a grid formation separated by 5 m intervals at plot centre. After seven days, captured males of both species were counted.

In Exp. 6, 6- to 96-h-old virgin female *C. rosaceana* or *P. limitata* were cooled at 0.5°C for 10-20 min and placed in black fibreglass mesh bags (9 x 6 cm) secured inside the tops of traps with velcro®. Traps were hung at ca. 1.5 m above the ground and in the upper third of the canopy at plot centre in four groups of four traps with one female-baited trap for each of the two species at each height on opposing sides of each tree. Groups of traps were separated by at least 5 m. On each of two days, traps were baited in late afternoon and catches were recorded after two nights in the field. Males captured in traps baited with females that had died by the time of enumeration were excluded.

A split-plot ANOVA (SAS 1996) with orchards specified as whole-plot factors and atmospheric disruption treatment (treated or control) specified as sub-plot factors was used to analyze male trap captures in Exp. 5 and 6. Orchard and orchard * mating disruption treatment terms were specified as random effects. Numbers of males captured in the eight female-baited traps for each species and within each plot in Exp. 6 were pooled prior to analysis. Numbers of males captured in traps were transformed by ln(x+1) to ensure homogeneity of variances. If the mating disruption * trap bait term was significant, *Z*9-14:OAc was considered to have affected a shift in the pheromone source approached by males. In Exp. 5, a significant split plot ANOVA ($P<0.05$) was followed by comparison of mean number of males captured in each trap type in both

Table 9. Synthetic pheromone treatments used in trapping experiment (Exp. 5, Chapter 7).

	Semiochemical components (µg)				
Treatment	*Z*11-14:OAc	*E*11-14:OAc	*Z*11-14:OH	*Z*11-14:Ald	*Z*9-14:OAc
1	957	19	0	0	0
2	957	19	14	10	0
3	957	19	14	10	10
4	957	19	14	10	100
5	957	19	0	0	61

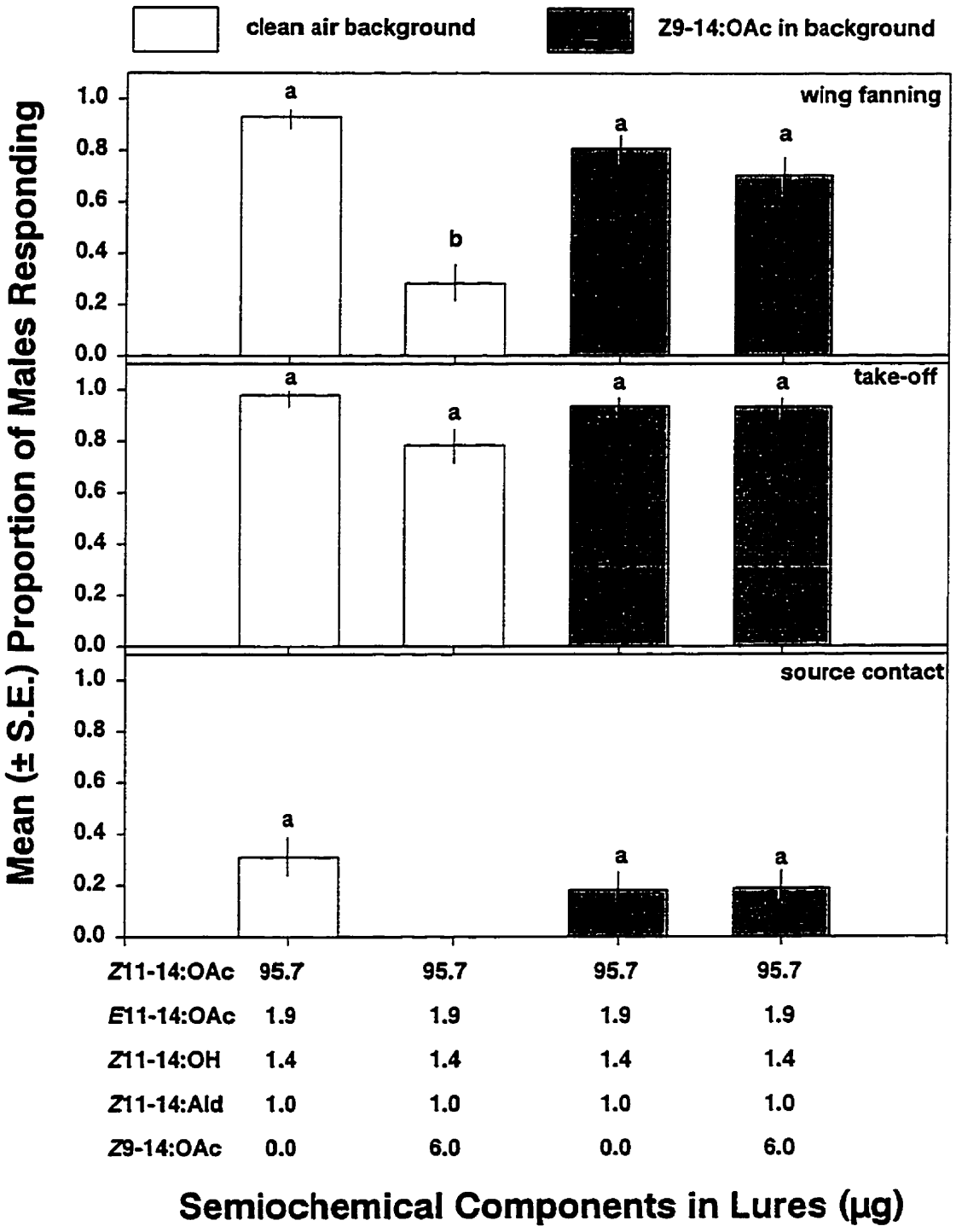
background treatments by a protected Least Significance Difference (LSD) test (SAS 1996).

7.3 Results

7.3.1 Wind-Tunnel Experiments

In Exp. 1, *Z*9-14:OAc placed upwind of septa caused significant behavioural shifts in male *C. rosaceana*. The semiochemical source most often approached by males differed in the two background treatments as indicated by a significant increase in scale deviance with the removal of the septa treatment * background treatment term from the model. Treatment estimates could only be obtained for wing-fanning, take-off and source-contact behaviours (Fig. 24) as the day of test * background treatment term was significant in the models for locking-on to the plume and upwind oriented flight. In a clean air background, a significantly greater proportion of males wing fanned in response to the *C. rosaceana* pheromone alone compared to the pheromone in combination with *Z*9-14:OAc (Fig. 24). With background treatment of *Z*9-14:OAc, however, equal proportions of males wing fanned to septa containing the *C. rosaceana* pheromone alone and the pheromone plus *Z*9-14:OAc, and this proportion did not differ from the response to pheromone alone in clean air (Fig. 24). The increase in scale deviance due to the removal of the septa treatment * background treatment term was small but significant for the take-off from release device behaviour. However, comparison of treatment estimates, a less powerful analysis, did not reveal any significant differences among septa treatment * background treatment terms for this

Figure 24. Mean proportions of male *C. rosaceana* responding to synthetic semiochemical sources in clean air and with *Z*9-14:OAc released at approximately 5 $\mu g \cdot h^{-1}$ upwind of the source in a wind tunnel in Exp. 1, Chapter 7. Bars with the same letter superscript are not significantly different *Z*-tests $P>0.05$. N=7.


clean air background
Z9-14:OAc in background
wing fanning
take-off
source contact
Mean (± S.E.) Proportion of Males Responding
1.0
0.8
0.6
0.4
0.2
0.0
a
b
a
a
a
a
a
a
a
a
a
a
Z11-14:OAc 95.7 95.7 95.7 95.7
E11-14:OAc 1.9 1.9 1.9 1.9
Z11-14:OH 1.4 1.4 1.4 1.4
Z11-14:Ald 1.0 1.0 1.0 1.0
Z9-14:OAc 0.0 6.0 0.0 6.0
Semiochemical Components in Lures (µg)

behaviour (Fig. 24). Equal proportions of males made contact with the septa when the source contained the *C. rosaceana* pheromone alone in clean air or in a treated background of *Z*9-14:OAc and the pheromone plus *Z*9-14:OAc in a background of *Z*9-14:OAc. No males contacted the source when it contained the *C. rosaceana* pheromone plus the antagonist, *Z*9-14:OAc in clean air.

In Exp. 2, no male *C. rosaceana* locked-on to the plume, conducted upwind flight or contacted the mesh bag containing the *P. limitata* female in clean air. However, when the background was treated with *Z*9-14:OAc (Fig. 25) a significantly greater proportion of male *C. rosaceana* responded to calling female *P. limitata* in all behavioural categories. In a background of *Z*9-14:OAc, 23% of the tested males contacted the bag containing the heterospecific female.

The response of male *P. limitata* to calling female *C. rosaceana* was more variable than that of male *C. rosaceana* to female *P. limitata* in both background treatments (Fig. 25). However, a greater proportion of *P. limitata* males locked-on and flew upwind to the *C. rosaceana* female-produced plume in a background of *Z*9-14:OAc as compared to a clean air situation when no *P. limitata* males conducted these behaviours (Fig. 25).

In Exp. 4, the presence of *Z*9-14:OAc placed upwind of a calling *P. limitata* female dampened the response of *P. limitata* males to the conspecific plume (Fig. 26). In all behavioural responses except take-off from the release device, a greater proportion of males responded in clean air than in air treated with *Z*9-14:OAc.

Figure 25. Mean proportions of male *C. rosaceana* and *P. limitata* responding to calling females of the opposite species in clean air and with *Z*9-14:OAc released at approximately 5 $\mu g \cdot h^{-1}$ upwind of the source in a wind tunnel in Exp. 2 and Exp. 3, Chapter 7. Asterisks indicate significant differences (Logistic regression analysis) between treatments within each behavioural response. N=5 in both experiments.

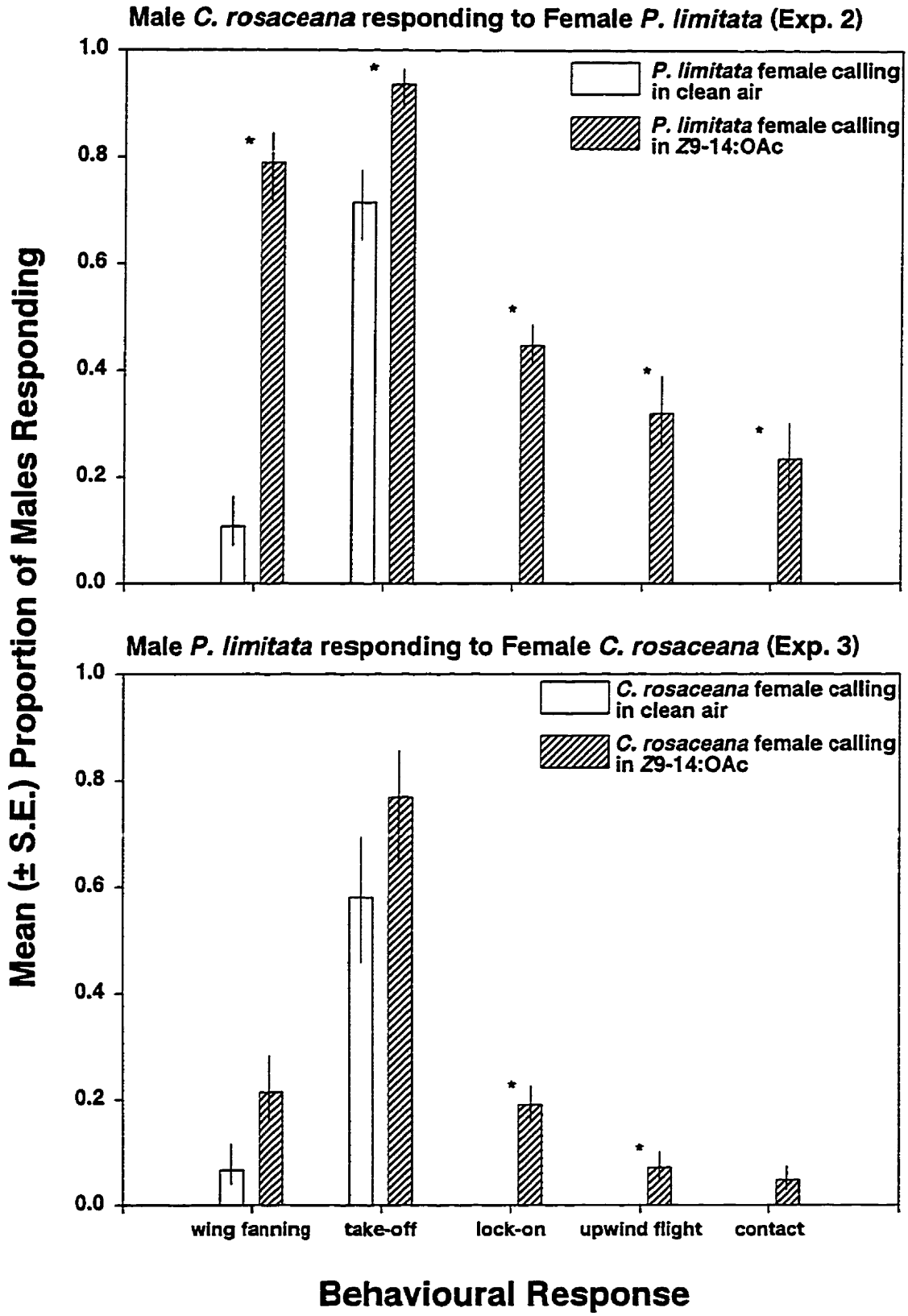
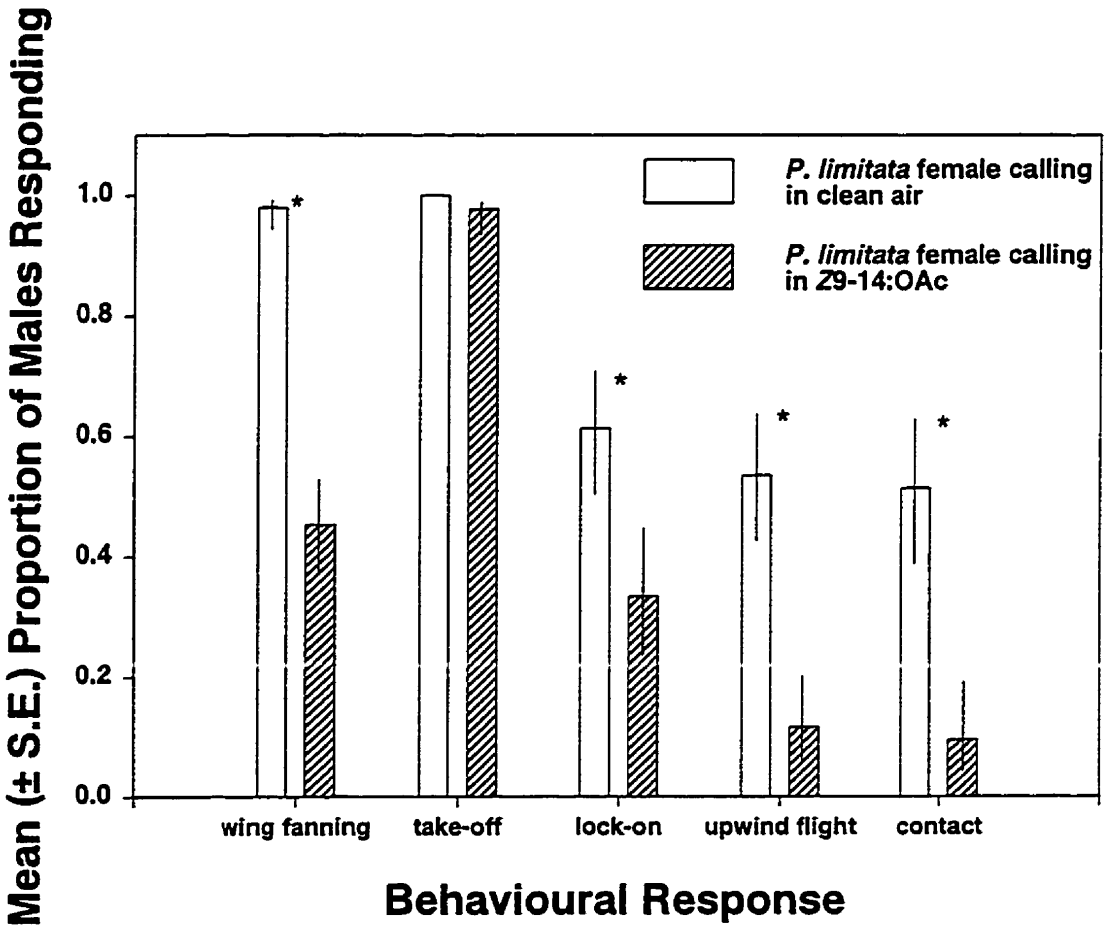

Male C. rosaceana responding to Female P. limitata (Exp. 2)
P. limitata female calling in clean air
P. limitata female calling in Z9-14:OAc
Male P. limitata responding to Female C. rosaceana (Exp. 3)
C. rosaceana female calling in clean air
C. rosaceana female calling in Z9-14:OAc
Mean (± S.E.) Proportion of Males Responding
1.0
0.8
0.6
0.4
0.2
0.0
wing fanning
take-off
lock-on
upwind flight
contact
Behavioural Response

Figure 26. Mean proportions of male *P. limitata* responding to calling female *P. limitata* in clean air and with *Z*9-14:OAc released at approximately 5 $\mu g \cdot h^{-1}$ upwind of the source in a wind tunnel in Exp. 4, Chapter 7. Asterisks indicate significant differences (Logistic regression analysis) between treatments within each behavioural response. N=5.


Mean (± S.E.) Proportion of Males Responding
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
*
P. limitata female calling in clean air
P. limitata female calling in Z9-14:OAc
wing fanning
take-off
lock-on
upwind flight
contact
Behavioural Response

7.3.2 Field Experiments

Atmospheric treatment with *Z*9-14:OAc had a significant effect on the synthetic semiochemical source approached by males of both species (Fig. 27). More male *C. rosaceana* were captured in traps baited with septa containing the four-component pheromone plus *Z*9-14:OAc when the atmosphere was treated with *Z*9-14:OAc. The numbers of male *C. rosaceana* captured in traps baited with the major pheromone component, the *C. rosaceana* pheromone alone and the *P. limitata* pheromone did not differ between the control and *Z*9-14:OAc-treated plots. Atmospheric treatment with *Z*9-14:OAc resulted in significantly lower captures of *P. limitata* males in traps baited with the *P. limitata* pheromone, and in traps baited with the *C. rosaceana* pheromone + 10% *Z*9-14:OAc (Fig. 27). All other synthetic pheromone treatments attracted very few male *P. limitata* in either the control or *Z*9-14:OAc-treated plots.

In Exp. 6, cross-attraction of male *P. limitata* to female *C. rosaceana* and male *C. rosaceana* to female *P. limitata* occurred only in plots treated with *Z*9-14:OAc (Fig. 28). The mating disruption * female bait term was significant only in the case of male *P. limitata*, as fewer males approached conspecific females and more males approached heterospecific females in the *Z*9-14:OAc-treated plots than in control plots. Although some male *C. rosaceana* did approach female *P. limitata* in the *Z*9-14:OAc-treated plots, an equal number of male *C. rosaceana* approached conspecific females in control and *Z*9-14:OAc-treated plots, rendering the mating disruption * female bait term non significant.

Figure 27. Mean numbers of male *C. rosaceana* and *P. limitata* captured in synthetic semiochemical-baited traps in 0.1 ha control and *Z*9-14:OAc-treated plots in Exp. 5, Chapter 7, Cawston, B.C. Within each trap type, asterisks indicate a significant shift in male response, LSD test $P<0.05$. N=3.

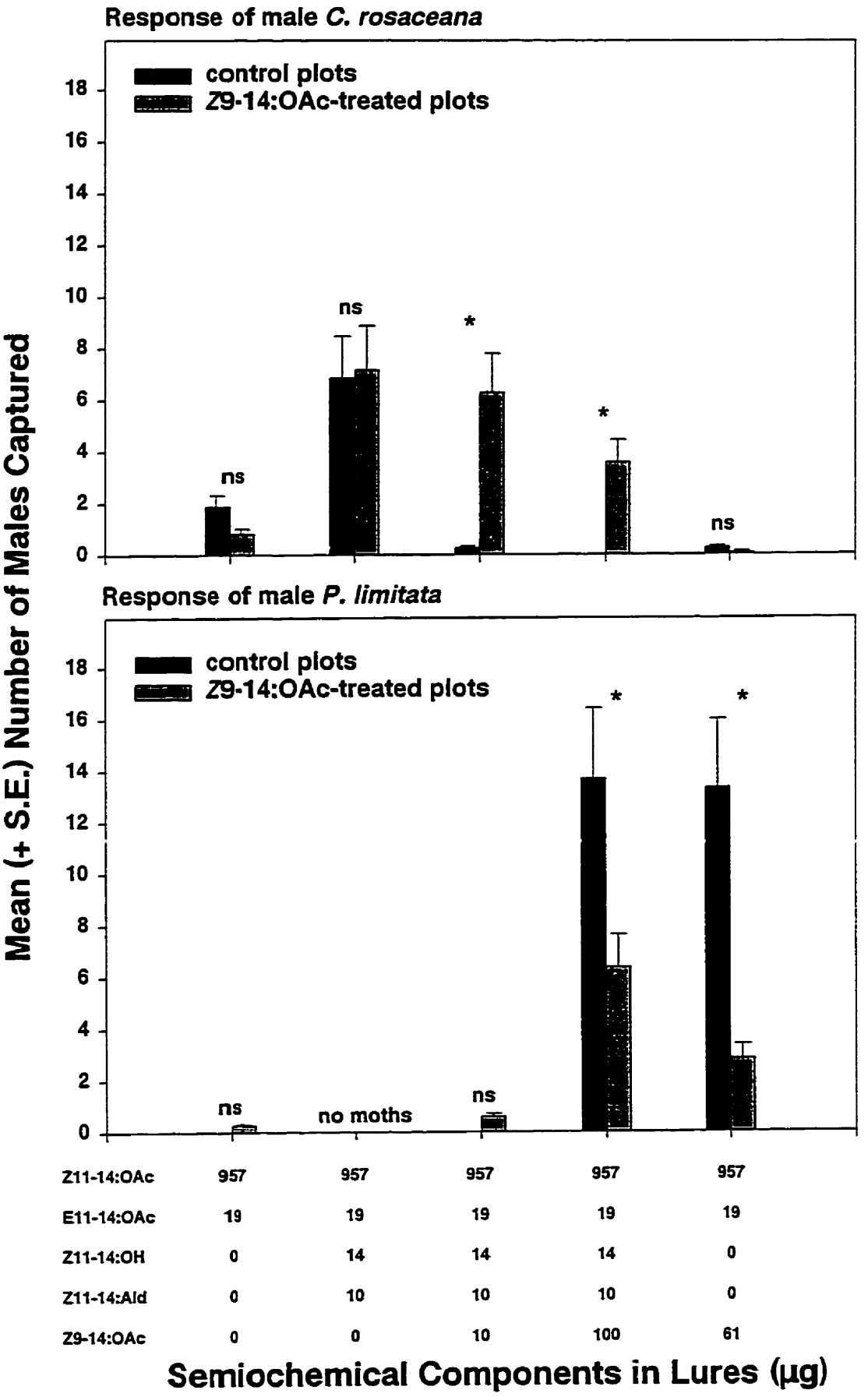
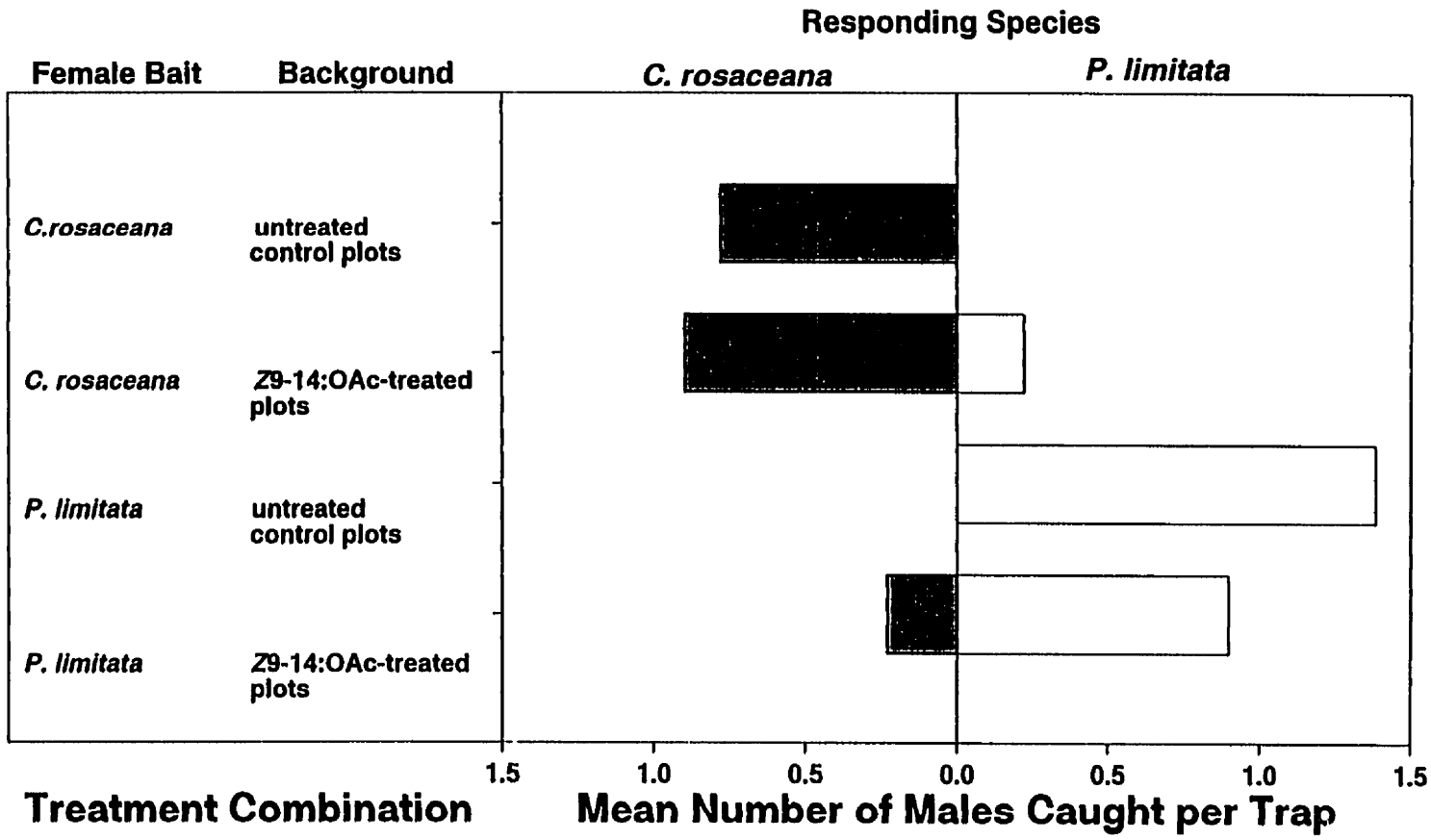

Response of male C. rosaceana
control plots
Z9-14:OAc-treated plots
Response of male P. limitata
control plots
Z9-14:OAc-treated plots
Mean (+ S.E.) Number of Males Captured
ns
ns
*
*
ns
ns
no moths
ns
*
*
Z11-14:OAc 957 957 957 957 957
E11-14:OAc 19 19 19 19 19
Z11-14:OH 0 14 14 14 0
Z11-14:Ald 0 10 10 10 0
Z9-14:OAc 0 0 10 100 61
Semiochemical Components in Lures (µg)

Figure 28. Comparison of numbers of male *C. rosaceana* and *P. limitata* captured by con- and heterospecific females in 0.1 ha control or *Z*9-14:OAc-treated plots in Exp. 6, Chapter 7. N=6.


Responding Species
Female Bait
Background
C. rosaceana
P. limitata
C.rosaceana
untreated control plots
C. rosaceana
Z9-14:OAc-treated plots
P. limitata
untreated control plots
P. limitata
Z9-14:OAc-treated plots
1.5
1.0
0.5
0.0
0.5
1.0
1.5
Treatment Combination
Mean Number of Males Caught per Trap

7.4 Discussion

My data reveal that treatment of the atmosphere with *Z*9-14:OAc in both the wind tunnel and the field induced male *C. rosaceana* to contact normally unattractive synthetic and natural semiochemical sources containing the antagonist, *Z*9-14:OAc. When a background source of *Z*9-14:OAc was placed upwind of septa, equal proportions of males made contact with the normally unattractive septa, emitting their pheromone and 6% of the antagonist *Z*9-14:OAc and with septa containing the pheromone alone (Fig. 24). Male *C. rosaceana* most likely became adapted to the *Z*9-14:OAc and responded to the plume as if it only contained the *C. rosaceana* pheromone. This result is similar to that found by Liu and Haynes (1993a) when male *T. ni* flew upwind to sources containing a behavioural antagonist, *Z*-7-dodecenol, when a cloud of this compound was generated upwind of the pheromone plus antagonist source. Neurons responsive to *Z*-7-dodecenol can become adapted to it in 4 s (Borroni and O'Connell 1992).

In the field when the atmosphere was treated with *Z*9-14:OAc, male *C. rosaceana* were captured in traps baited with septa containing only their pheromone or to the normally unattractive septa containing pheromone plus the antagonist (Fig. 27). Orientation to pheromone blends when the atmosphere is treated with a behavioural antagonist has also been demonstrated in several other species of Lepidoptera (McLaughlin et al. 1972; Kaae et al. 1974; Rothschild 1974; Daterman et al. 1975; Mitchell 1976), suggesting that specific receptors tuned to antagonists (Grant et al. 1988; Den Otter and Van Der Haagen 1989; Christensen et al. 1990; Borroni and

O'Connell 1992) became adapted, or that an antagonist-specific neural pathway (Mustaparta 1996) became habituated without affecting the pheromone blend integration pathway. The shift in male response to sources emitting pheromone plus an antagonist in an atmosphere treated with the antagonist alone is similar to shifts in pheromonal response observed in atmospheres treated with a single pheromone component (Flint and Merkle 1984a; Judd et al. 1995). Pink bollworm males respond optimally to a pheromone blend consisting of a 1:1 ratio of the two isomers *Z,Z* and *Z,E*-7,11-hexadecadienyl acetates. However, in an atmosphere treated with the *Z,Z*-isomer alone male response was shifted to respond to blends rich in that component (Flint and Merkle 1984a). Similarly, in the mullein bug *Campylomma verbasci* (Meyer), atmospheric treatment with the minor component crotyl-butyrate resulted in a shift of male response to blends enriched with this component (Judd et al. 1995). In clean air, few male *C. rosaceana* flew upwind to septa containing as little as 1% *Z*9-14:OAc and no males were trapped when 10% *Z*9-14:OAc was present. However, in an atmosphere treated with *Z*9-14:OAc male response was shifted to accept these unattractive plumes probably due to adaptation or habituation to *Z*9-14:OAc (Fig. 27).

Similar to the response observed with synthetic pheromone, male *C. rosaceana* followed plumes emitted by female *P. limitata* in an atmosphere treated with *Z*9-14:OAc in both the wind tunnel (Fig. 25) and the field (Fig. 28). The lack of a significant effect of atmospheric treatment on the species of female approached by male *C. rosaceana* in the field was due to equivalent numbers of males captured in conspecific female-baited traps in control and *Z*9-14:OAc-treated plots, not the lack of interspecific-attraction in *Z*9-14:OAc-treated plots. Similarly, *H. zea* males flew upwind and copulated with *H.*

virescens females when the atmosphere was treated with *Z*9-tetradecenal, a minor pheromone component for *H. virescens* and an antagonist for *H. zea* (Stadelbacher et al. 1983). Although interspecific mating was never observed between *C. rosaceana* males and tethered *P. limitata* females in plots treated with *Z*9-14:OAc (Chapter 6), males that contacted heterospecific females in mesh bags in the wind tunnel displayed courtship behaviour. Following the above hypothesis, male *C. rosaceana* presumably became adapted to *Z*9-14:OAc and responded to the *Z*11-14:OAc produced by the female *P. limitata*. Although male *C. rosaceana* are attracted in low numbers to *Z*11-14:OAc containing small amounts of *E*11-14:OAc (Vakenti et al. 1988) they did not fly upwind to synthetic lures of the *P. limitata* pheromone in *Z*9-14:OAc-treated plots and few flew to septa containing *Z*11-14:OAc + 2% *E*11-14:OAc in control or *Z*9-14:OAc-treated plots (Fig. 27). This suggests either that the release rate of the *Z*11-14:OAc from the synthetic lure is too great and causes arrestment to the weakly attractive blend (Roelofs 1978; Linn et al. 1987b; Baker et al. 1989) or that there are other components emitted by *P. limitata* females, eg. the *Z*11-14:OH found in female gland extracts (G. Gries , Dept. Biological Sciences, Burnaby, B.C. pers. comm.) that make them attractive to *C. rosaceana* in an atmosphere treated with *Z*9-14:OAc. However, 23% of male *C. rosaceana* tested contacted female *P. limitata* in a wind tunnel with *Z*9-14:OAc placed upwind of the female (Fig. 25) and far fewer male *C. rosaceana* were captured in traps baited with female *P. limitata* than with female *C. rosaceana* in plots treated with *Z*9-14:OAc (Fig. 28).

A significant proportion of male *P. limitata* oriented to female *C. rosaceana* in the wind tunnel when a source of *Z*9-14:OAc was placed upwind of the calling female (Fig.

25). In the field, there was an increased number of male *P. limitata* attracted to female *C. rosaceana* in *Z*9-14:OAc-treated plots along with a slight reduction in the number of male *P. limitata* attracted to conspecific females (Fig. 28). It is difficult to explain why male *P. limitata* follow plumes that do not contain *Z*9-14:OAc when they are adapted or habituated to it. *Z*11-14:OAc alone is not attractive to male *P. limitata* (Roelofs et al. 1976a). Possibly, there is some blend integration occurring in the insect's brain when its antennal receptors are receiving stimuli from *Z*9-14:OAc (background treatment) and *Z*11-14:OAc (heterospecific female plume). Bartell (1985) presented male light brown apple moths, *E. postvittana*, with a two-component pheromone blend that induced wing-fanning, hair pencilling and homosexual mating attempts. These behaviours persisted when the secondary component was removed from the stimulus even though this component is essential in eliciting the behaviour. It was hypothesized that stimulation from the major component was integrated with a "memory" of the secondary component, thus maintaining the behaviour (Bartell 1985). Furthermore, after initial stimulation with both compounds, males responded to the normally unattractive major component alone (Bartell 1985). Antennal receptors for the secondary component were slow to disadapt and the "memory" phenomenon seemed to parallel changes in the receptors. Bartell (1985) maintained that it was not exclusively a peripheral nervous system phenomenon, because alternating pulses of the two components did not elicit a behavioural response (Bartell 1985). Similarly, male *C. fumiferana* will fly upwind to an off-ratio blend after exposure to the natural female-produced blend which was hypothesized to be due to adaptation of antennal receptors to the minor pheromone component (Sanders 1997). Like male *P. limitata* responding to *C. rosaceana*, male *H.*

virescens flew upwind to female *H. zea* in small numbers when the background was treated with a component that only occurred in *H. virescens* (Stadelbacher et al. 1983). However, although male *P. limitata* flew to female *C. rosaceana* in an atmosphere treated with *Z*9-14:OAc they did not respond to synthetic sources of this blend or to synthetic sources of *Z*11-14:OAc (Fig. 27). This may be due to the high release rate of the septa containing off-ratio blends.

The interspecific mate location that was induced in male *C. rosaceana* and *P. limitata* when the atmosphere was treated with *Z*9-14:OAc supports the hypothesis that this compound is important in reducing competition in the sex pheromone communication channel (Greenfield and Karandinos 1979). However, it is probable that synomonal activity is secondary to pheromonal response to a conspecific plume in maintaining behavioural reproductive isolation, because most males approached conspecific females even in plots treated with *Z*9-14:OAc.

8.0 BEHAVIOUR OF FEMALE *C. rosaceana* IN PHEROMONE-TREATED PLOTS

8.1 Introduction

Some female moths can perceive the sex pheromones that they produce (Mitchell et al. 1972; Birch 1977; Palaniswamy and Seabrook 1978; Light and Birch 1979), an ability that is also found in *C. rosaceana* (R. Gries, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C. pers. comm.). Behaviour of females exposed to their own sex pheromone appears to differ among species. Female *T. ni* were captured in traps baited with either synthetic pheromone or virgin females, implying that they actually oriented upwind to the pheromone (Mitchell et al. 1972; Birch 1977). However, female moths do not possess a macroglomerular complex in the antennal lobe of the brain (Mustaparta 1984); thus integration of pheromone components could not occur in the female brain as it does in males. Behavioural responses to pheromone by female *C. fumiferana* include: increased walking, extension of the ovipositor, antennal grooming (Palaniswamy and Seabrook 1978) and heightened flight activity (Sanders 1987). Such behaviours suggest that females could sense a high level of sex pheromone during outbreaks, and might be induced to oviposit and then depart in mass dispersal flights that are characteristic of *C. fumiferana* during outbreaks (Palaniswamy et al. 1979). The onset of calling by female *C. fumiferana* was advanced when females were exposed to pheromone and more females called in the presence of sex pheromone than in its absence (Palaniswamy and Seabrook 1985). The temporal pattern of calling by female codling moths was

unaffected by exposure to codlemone, the major component of their sex pheromone, but more females called in its presence than in an uncontaminated environment (Weissling and Knight 1996). Female *C. rosaceana* alter their calling behaviour in response to temperature, and older females call earlier than younger females (Delisle 1992b; Delisle and Royer 1994), but it is not known if they alter their calling behaviour in the presence of sex pheromone.

Female *C. rosaceana* mated most readily with virgin males, from whom they received larger spermatophores than from mated males, imparting an hypothesized adaptive advantage in female mate choice (Delisle and Bouchard 1995). However, there was no direct relationship between spermatophore size and male investment as a five-fold decrease in spermatophore size from first to second matings resulted in only a 25% reduction in reproductive output by females (Delisle and Bouchard 1995). In other tortricid species, females mated to previously mated males, that transferred small spermatophores, produced normal numbers of fertile eggs (Outram 1971; Carroll 1994). Delisle and Bouchard (1995) suggested that the quality of the male transfer is more important than its quantity, because males produced slightly smaller spermatophores when reared on a poor quality host than on a high quality host, but the subsequent reduction in reproductive output of the female declined by 40%. Other authors have suggested that substances transferred from the male may influence the ability of the female to remate, and that this substance is depleted with consecutive copulations (Carroll 1994; Foster and Ayers 1996). This may be the case for *C. rosaceana* as the incidence of polyandry increased at the end of the flight when most males in the population would have already mated (Delisle and Bouchard 1995). If female *C.*

rosaceana do exhibit mate-choice behaviour, they may alter this behaviour in a competitive situation when many females are calling, or when the atmosphere is treated with synthetic pheromone.

My first objective was to determine if the presence of synthetic pheromone in mating-disruption trials altered the calling behaviour of female *C. rosaceana*. Secondly, I tested the hypothesis that atmospheric treatment with the complete pheromone or pheromone components of *C. rosaceana* influenced the proportion of females that received spermatophores from previously mated males.

8.2 Methods and Materials

8.2.1 Calling behaviour of female *C. rosaceana* in control and pheromone-treated plots

Observations of female *C. rosaceana* in pheromone-treated and control plots were conducted on the nights of 7 and 11 August, 1997. Two 0.1 ha plots were established in a conventionally-managed orchard in Summerland, B.C. One of the two plots was randomly chosen to be treated with the four-component *C. rosaceana* blend at a release rate of 10 $mg \cdot ha^{-1} \cdot h^{-1}$ and dispenser density of 1000 ha^{-1} as outlined in section 2.3.1. Plots were treated 48 h before the first observation night.

Six- to 96-h-old female *C. rosaceana* were obtained from the laboratory colony, chilled at 0.5°C for 10-15 min and placed in cylindrical aluminium mesh cages (6 cm height x 4 cm diam.). Cages were stapled shut and transported to the field in refrigerated containers. One h before the first observation on each night 24 cages,

each containing a female, were wired to branches of 12 trees in the centre of each plot. Cages were placed ~1.5 m above ground on the northwest and southwest side of each tree. Observations of the females were taken at 15 min intervals, beginning at 2000 h Pacific Daylight Time (PDT), approximately 30 min after sunset (Environment Canada, Kelowna, B.C.) and ending at 1 h before sunrise or until all females were observed to be resting. Females were observed in red light provided by a flashlight covered with a B+W 62E coated dark red filter (Beau Photo Supplies Inc., Vancouver, B.C.) and recorded as not calling or calling (pheromone gland extruded). Temperature was recorded at hourly intervals in the control plot. Between nights of observation the cages were rinsed in acetone and heated to 200°C for at least 4 h.

The onset time and duration of calling for females located in control and pheromone-treated plots were compared using Wilcoxon rank sum tests (Zar 1984). Females that did not call throughout the observation period were excluded from analysis (N=2,4,1,0 females not calling in the pheromone-treated plots on the first and second night of observation and in the control plots on the first and second night of observation, respectively).

8.2.2 Properties of spermatophores transferred to females by virgin and mated males

Two laboratory experiments were conducted which tested the hypothesis that spermatophores transferred to laboratory-colony females from virgin males are larger than spermatophores transferred from mated males, as has been demonstrated for *C. rosaceana* in eastern North America (Delisle and Bouchard 1995). In Exp. 1 males

were obtained from the laboratory colony whereas in Exp. 2, they were collected as larvae on apple hosts.

In Exp. 1, male and female pupae were placed in 43 pairs in 150 mL cups, provided with water, and maintained under the same conditions as the colony (section 2.1). Adults were allowed to remain together for 1-2 nights after eclosion before females were dissected. Mated males were then transferred to another container that held another virgin female and the procedure was repeated. The mating history of each male was followed and measurements of the first spermatophore, from males that provided two spermatophores (N=14), were excluded from analysis to ensure independent samples. At the time of mating virgin and mated males providing spermatophores were 6-72 and 24-96 h old, respectively, and females were 6-120 h old. The second mating occurred 24-120 h after the first. *Bursae copulatrices* were excised from females as in section 2.3.2 and stored in 70% ethanol prior to further dissection to remove the spermatophore. Spermatophores had a spherical corpus and long collum which extended through the *ductus bursae*. Spermatophore diameters were measured perpendicular and parallel to the collum under a dissecting microscope using a micrometer eyepiece. The latter measurements were the consistently larger of the two, remained constant even when the spermatophores were empty, and were used in analysis.

For Exp. 2 larvae were collected from 6-20 May, 1997, in an organically-managed apple orchard in Cawston, B.C. , held in 58 mL cups under the same conditions as the laboratory colony (section 2.1), and fed apple leaves until pupation. Forty-four pairs of field-collected male and female pupae from the laboratory colony

were placed in 150 mL cups and the protocol used in Exp. 1 was repeated. Mated male moths were exposed to a second virgin female. The mating history of each male was followed and measurements of the first spermatophore from males that provided two spermatophores (N=13) were excluded from analysis to ensure independent samples. At the time of mating, virgin and mated males were 6-120 and 24-144 h old, respectively, and females were 6-96 h old. The time between matings ranged from 24-144 h. Spermatophores were measured directly after dissection of the females.

During Exp. 1 it became apparent that spermatophores from virgin males had surficial scleritized bars, which were possibly the cornuti from the end of the male aedeagus that had been dislodged during mating, and no bars were present on spermatophores produced by mated males. The presence or absence of scleritized bars was noted for all spermatophores after that point.

Diameter measurements of spermatophores from virgin and mated males in both Exp. 1 and 2 were compared using a two-sample t-test. Prior to analysis data were checked for normality and homoscedasticity using the UNIVARIATE procedure in SAS (SAS 1996).

8.2.3 Mating status of males that provided spermatophores to tethered females in control and pheromone-treated plots

I tested the hypothesis that the size of spermatophores collected from females, and therefore the mating status of males producing the spermatophores, would differ in pheromone-treated and control plots. Spermatophores were collected from tethered females that were used to assess the effectiveness of mating disruption in small plots in

Exp. 3 (Chapter 4) and Exp. 5 (Chapter 4) and in one other experiment that had the same treatments as Exp. 5 (Chapter 4) (Appendix 2) but was not reported on previously. At the time of collection of spermatophores, the ratio of mated:virgin males in the area was determined by dissecting males (section 3.2.7) captured in pheromone-baited monitoring traps (section 2.4) located in the same orchard but outside the experimental area, and the ratio of mated:virgin males was also determined for males captured in monitoring traps placed in control (3 mg lure) and treated plots (10 mg lure) on different nights than females but within the same replicate. *Bursae copulatrices* were stored in 70% ethanol until measurements of spermatophores were made as in Exp.1 and 2. Presence or absence of scleritized bars on the surface of spermatophores was also recorded.

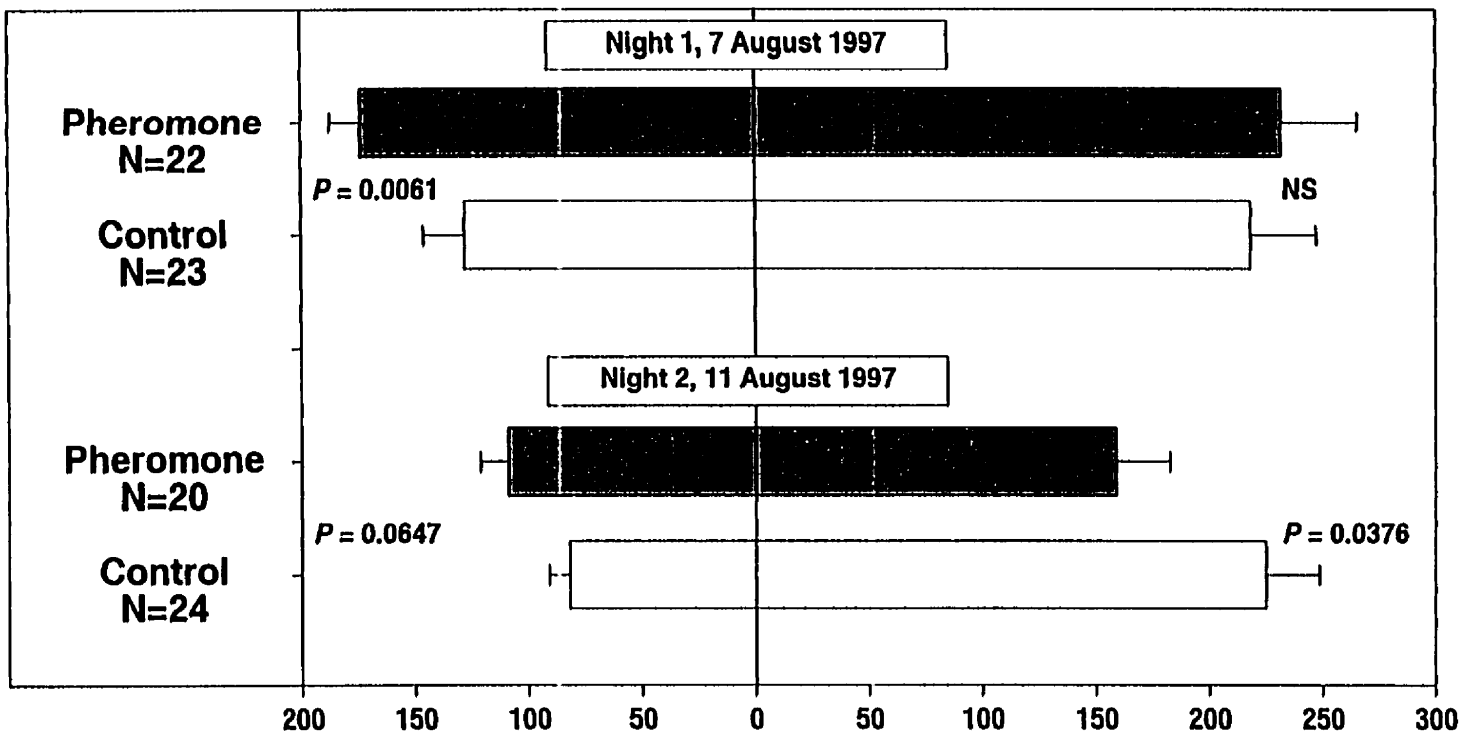
The hypothesis that mating status of males was independent of the atmospheric treatment in which females were located was evaluated by a χ^2 test using pooled data from all three experiments (α=0.05).

8.3 Results

8.3.1 Calling behaviour of female *C. rosaceana* in control and pheromone-treated plots

Female *C. rosaceana* in the pheromone-treated plot started calling significantly later than females located in the control plot on the first night of observation and on the second night of observation the difference in mean onset time to calling between females approached significance (Fig. 29).

Figure 29. Comparison of mean times to onset to calling and duration of calling by caged female *C. rosaceana* in pheromone-treated (four-component blend) and control 0.1 ha plots on two different nights in August, 1997. Probabilities of differences occurring by chance (Wilcoxon sum rank test) are shown for each set of paired bars.


Night 1, 7 August 1997
Pheromone N=22
Control N=23
P = 0.0061
NS
Night 2, 11 August 1997
Pheromone N=20
Control N=24
P = 0.0647
P = 0.0376
200
150
100
50
0
50
100
150
200
250
300
Atmospheric Treatment
Mean(+S.E.) Minutes to Onset of Calling
Mean (+S.E.) Minutes Duration of Calling

Females spent less time calling in the pheromone-treated plot than in the control plot on the second night of observation, but not on the first (Fig. 29).

8.3.2 Properties of spermatophores transferred to females by virgin and mated males

Spermatophores produced by either laboratory or wild males that had mated once were significantly smaller than spermatophores produced by virgin males, and the range in diameters of spermatophores produced by virgin and mated males overlapped by only 0.1 mm in each experiment (Table 10). Spermatophores produced by all wild, virgin males and 94.7 % (all except one individual) of laboratory-reared, virgin males exhibited surficial scleritized barring. None of the previously-mated males produced spermatophores with barring (Table 10).

8.3.3 Mating status of males that provided spermatophores to tethered females in control and pheromone-treated plots

In pooled data from the three experiments there was no difference in the percentages of mated males that provided spermatophores to virgin females in control plots (49.5 % of 93 males) and those that provided spermatophores to virgin females in pheromone-treated plots (57.5 % of 153 males) (χ^2 =1.514, P >0.10). Similarly the percentage of mated males trapped outside of the experimental area (46.7% of 90 males) was not different from the 57.5% mated males that provided spermatophores to virgin females in pheromone-treated plots (χ^2 =2.639, P >0.10). Finally, there was no difference in the percentages of mated males trapped in control plots (46.3 % of 82 males) and those trapped in pheromone-treated plots (42.9% of 268 males) (χ^2 =0.313,

Table 10. Size and description of spermatophores produced by previously mated and virgin male *C. rosaceana*.

Exp.	N	Male mating status	Male source	Mean ± S.E.[a] spermatophore diam. (mm)	Spermatophore diam. (mm) range	Spermatophores with sclerotized bars	
						N	%
1	29	Virgin	Lab	1.61 ± 0.04 a	1.4 - 2.0	19	94.7
1	14	Mated	Lab	1.08 ± 0.04 b	0.8 - 1.4	11	0
2	22	Virgin	Wild	1.78 ± 0.06 a	1.5 - 2.2	31	100
2	13	Mated	Wild	1.20 ±0.05 b	0.7 -1.6	13	0

[a] Within each experiment, means followed by different letters are significantly different , $P<0.05$, Two-sample *t*-test.

$P>0.50$).

8.4 Discussion

My data indicate that female *C. rosaceana* delayed the onset of calling behaviour in pheromone-treated plots (Fig. 29). This is contrary to results for *C. fumiferana* females which initiated calling earlier when exposed to synthetic pheromone than females in clean air (Palaniswamy and Seabrook 1985), and for *C. pomonella* females which did not alter the onset of calling in pheromone-treated air (Weissling and Knight 1996). Some females were observed to be walking within the mesh cages at the beginning of the experiment and it may be that exposure to high levels of pheromone induces flight in *C. rosaceana* females, as has been observed for *C. fumiferana* (Sanders 1985,1987). A delay in the commencement of calling could also indicate adaptation or habituation of female moths to pheromone followed by resumption of normal calling behaviour. Female *C. pomonella* exposed to codlemone in still air showed no alteration in calling behaviour, which may mean that antennal receptors become adapted quickly in a pheromone-saturated environment (Weissling and Knight 1996). Females called for the same period of time in pheromone-treated and control plots on the first night of observation, but the duration of calling was significantly reduced in pheromone-treated plots on the second night. A reduction in duration of calling would be closely associated with delayed onset time. Females which start calling are probably not deterred thereafter by a high level of synthetic pheromone.

In agreement with the results of Delisle and Bouchard (1995) for *C. rosaceana* in

eastern North America, spermatophores transferred to females from once-mated males were significantly smaller than those transferred by virgin males (Table 10). Both diets used in this study are considered to be high quality for *C. rosaceana* (Carrière 199?) and the ranges of spermatophore size produced by males on both diets overlapped (Table 10). Thus the reduction in spermatophore size observed on poor quality hosts observed by Delisle and Bouchard (1995) would not have been expected.

Virtually all spermatophores transferred by virgin males had visible scleritized barring on their outside, and none of the spermatophores from previously-mated males displayed this trait (Table 10). Male *C. rosaceana* have spines called cornuti at the end of the aedeagus (Dang 1992). In other tortricids, cornuti interlock with a scleritized plate in the *bursa copulatrix* of the female during mating, presumably augmenting the male claspers' hold on the female (Ferro and Akre 1975). In some tortricids cornuti can remain in the *bursa copulatrix* after copulation (Horak and Brown 1991). In the case of *C. rosaceana* it appears that they not only remain but become imbedded in the surface of the first spermatophore. In support of this finding, <10% of the 246 spermatophores dissected from females in field experiments appeared to be from mated or virgin males with respect to size and the opposite status with respect to presence or absence of cornuti.

There was no evidence that atmospheric treatment with pheromone influenced mate choice by tethered females, nor was there any evidence that females in control plots exercised any selection for either virgin or mated males. This finding is in agreement with that of Delisle and Bouchard (1995) who found that tethered-virgin females mated with mated males with increasing frequency as the numbers of

previously-mated males increased at the end of the flight period. An increase in female flight activity (Sanders 1985, 1987) and possibly dispersal (Palaniswamy et al. 1979) at high concentrations of pheromone in the atmosphere would be an alternative adaptive mechanism that might increase a female's chances of finding males in a new habitat. However, because there is only a 25% reduction in reproductive output by *C. rosaceana* females mated to mated males (Delisle and Bouchard 1995), there would be only weak selection pressure to either choose virgin males or to disperse in the presence of high pheromone levels. Acceptance of mated males and a reluctance to disperse by female *C. rosaceana* would benefit individuals that persist at low population levels in patchy habitats, where mate attraction may be relatively uncertain and dispersal risky.

A delayed onset and reduced duration of calling (Fig. 29) would occur for tethered females, supporting the accuracy of this method of evaluating the efficacy of mating-disruption treatments. An increased tendency to disperse (if it does occur) would mean that assessing efficacy of disruption by mating of tethered females would lead to an undervaluation of efficacy. Further testing of female flight behaviour in the presence of pheromone, as done for *C. fumiferana* (Sanders 1987), is needed for orchard tortricids.

9.0 CONCLUDING DISCUSSION

Moths have evolved to depend on chemical communication for mate finding. Mating disruption aims to exploit this essential behaviour by treatment of the atmosphere with synthetic semiochemicals. Illumination of the mechanisms by which mate finding is altered in a semiochemical-treated atmosphere would aid in the development of mating-disruption programmes. For a given species, mechanisms of mating disruption will change with pheromone formulation and concentration, point source distribution, structure and condition of the crop canopy, as well as pest biology and population density. Based on the behaviour of male moths in the presence of conspecific pheromone plumes (Roelofs 1978), it was predicted that the natural pheromone blend should be the most effective disruptant of mate-finding behaviour at the lowest release rate (Minks and Cardé 1988). The data presented in my thesis for the pheromone-based mating disruption of *C. rosaceana* and *P. limitata* do not support the Minks and Cardé (1988) hypothesis.

Attractiveness of the semiochemical treatment was not correlated with effectiveness as a mating disruptant for either species of leafroller. Mating disruption of *C. rosaceana* was achieved equally well with an attractive pheromone blend, suboptimally attractive and unattractive partial blends, and an unattractive blend containing a pheromone antagonist. Similarly, mating of *P. limitata* was equally disrupted by an attractive pheromone blend, a less attractive off-ratio blend, and unattractive partial blends containing the major pheromone component. These findings suggest that the mechanism of false-trail following was not essential to achieve

disruption of mating in these species under the experimental conditions I employed. Certain mating-disruption systems may require an attractive formulation to be optimally effective (Mafra-Neto and Baker 1996a). However, reliance on false-trail following as the sole source of disruption means that as a population increases, the increased number of calling females will present more competition for the synthetic pheromone dispensers. If instead of an attractive formulation, an unattractive off-ratio blend is used in high enough concentrations it may alter the response of males so that they optimally respond to a blend not produced by the female (Flint and Merkle 1984a; Judd et al. 1995). For polyphagous species, like many tortricine leafrollers, it may be best to use an unattractive formulation that does not attract immigrant males from other host plants.

Based on field results (Chapters 4-6), I hypothesized that neurophysiological effects of adaptation and habituation were probably the most important mechanisms of mating disruption in both *C. rosaceana* and *P. limitata*. However, all attempts to habituate male *C. rosaceana* to the main pheromone component, to the complete pheromone blend or to the antagonist alone (data not shown) in the laboratory failed. It appears that male *C. rosaceana*, like its congener *C. fumiferana* (Sanders 1996) have evolved to resist habituation of the central nervous system. Complete adaptation of pheromone receptors on the antennae may protect the central nervous system from habituation in these species. Avoidance of habituation may have evolved within the tortricines that lay eggs in masses and could potentially, as is the case of *C. fumiferana*, become outbreak species. It would be adaptive for males to be protected from central nervous system habituation in order to locate females at outbreak population levels when the ambient pheromone level would be high. The ability of male *C. fumiferana* to

withstand habituation of the central nervous system may be one reason attempts of mating disruption to control this species have not been as successful as mating disruption of oleuthreutines such as the Oriental fruit moth (Sanders and Lucuik 1996). Selection for maintenance of this trait in *C. rosaceana* may result from the high probability of encountering heterospecific plumes which, due to physiological constraints, contain common pheromone components. Protection of central nervous system habituation would permit male *C. rosaceana* to orient to a conspecific plume when it is encountered.

Sympatry of closely related species that share common sex pheromone components permits the testing of several hypotheses about intra- and interspecific chemical communication. If sympatric species are pests in a common cropping system, shared pheromone components and components that act interspecifically could be used to disrupt mate-finding behaviour in both (or many) species simultaneously. However, because sex pheromones have evolved as species-specific signals in most lepidoptera, use of partial or off-ratio blends, or blends containing components of other species' pheromones that act as behavioural antagonists, will alter the behaviour of males (and perhaps females) of each species and in turn affect the mechanisms involved in mating disruption. The results of my thesis reveal that mating disruption of the sympatric species *C. rosaceana* and *P. limitata* can be achieved simultaneously with a single formulation. Either the main component of both species alone, *Z*11-14:OAc, or *Z*11-14:OAc plus the minor component of *P. limitata*, and behavioural antagonist to *C. rosaceana*, *Z*9-14:OAc, could be employed as mating disruptants against both species. The main effects of pheromone treatment on males of these

species appears to be neurophysiological, and in the case of *C. rosaceana* perhaps mainly at the peripheral nervous system level. A relatively even distribution of pheromone provided by many point sources, as would be achieved by a sprayable formulation, may be the best approach against these species, to ensure high levels of receptor adaptation. Because there was no difference in reduction of mating of *C. rosaceana* with a reduction in dispenser density, it is possible that evenly spaced dispensers with a high release rate, emit enough pheromone to be adsorbed on the surrounding foliage (Wall et al. 1981; Wall and Perry 1983; Noldus et al. 1991; Karg et al. 1994; Suckling et al. 1996; Sauer and Karg 1998). Pheromone re-emission from apple leaves provides a continuous low level of atmospheric pheromone (Suckling et al. 1996; Suckling and Karg 1997) that may be sufficient to raise the threshold level of male response (Mafra-Neto and Baker 1996b) above that of a calling female or to provide persistent adaptation of sensory receptors.

Members of the tortricine feeding guild found on apple in eastern and western North America appear to have evolved the ability to detect heterospecific pheromone components (Cardé and Baker 1984) which may act to partition the chemical communication channel. Signals should arise to minimize the background noise and interfering signals from other species (Endler 1993). Because the range of structures of pheromone components produced by tortricines is restricted, many species utilize common pheromone components (Roelofs and Brown 1982). It would therefore be adaptive for males to recognize heterospecific signals in order to avoid wasting time and energy and increased risk of predation following plumes produced by females of other species. When the atmosphere was treated with *Z*9-14:OAc alone, male *C.*

rosaceana shifted their response to accept plumes containing this antagonist that were unattractive in clean air. Rapid adaptation of antennal receptor neurons to these compounds permits filtering out of background noise that is not pertinent to mate-finding and as a result behavioural antagonists applied alone do not provide mating disruption.

Atmospheric treatment with *Z*9-14:OAc alone in the field and in the wind tunnel also induced interspecific mate location between *C. rosaceana* and *P. limitata*. It is evident that *Z*9-14:OAc is an important semiochemical for both species. Its presence in the *P. limitata* plume is imperative for response by conspecific males and prevents costly orientation attempts by heterospecific males (*C. rosaceana*). It is probable that the inhibitory response to *Z*9-14:OAc by male *C. rosaceana* evolved in response to heterospecific signals containing this compound (Cardé 1986; Löfstedt 1993; Linn and Roelofs 1995), but there is no evidence that it evolved as a pre-mating reproductive isolating mechanism. Although cross-attraction between species occurred when the atmosphere was treated with *Z*9-14:OAc, it always occurred at low levels in comparison to attraction to conspecifics. Furthermore, no interspecific mating was observed when tethered females were positioned in plots treated with *Z*9-14:OAc. I hypothesize that other pre- and post-mating reproductive isolating mechanisms occurred in the past and continue to occur between these two species, and that male *C. rosaceana* have evolved to perceive this compound as a mechanism of increasing the effectiveness of reception of their conspecific signal.

Mating disruption is a control technique which targets the response of male moths to the female-produced pheromone. However, some female moths, including *C.*

rosaceana, are able to perceive the pheromone that they emit to attract conspecific males. It could be hypothesized that females perceive their own pheromone in order to assess the population density and likelihood of attracting a mate. If there are many calling females in a given area, a female may alter her behaviour to disperse from that area or alter her calling behaviour so that she is more competitive than other females. There is evidence that old female *C. rosaceana* call earlier in the scotophase in order to compete with young females that have a higher pheromone titer (Delisle 1992b; Delisle and Royer 1994). If females do alter their behaviour in response to stimulation with their own pheromone they may also behave differently in synthetic pheromone-treated plots. I demonstrated that the onset to calling time is delayed for females located in pheromone-treated plots. A delay in the commencement of calling could indicate a propensity to disperse as some caged females were observed to be walking in an agitated manner. Alteration of female behaviour in response to atmospheric treatment with synthetic pheromone may contribute to the mechanisms by which mate finding is disrupted and warrants further investigation.

The data presented in my thesis provide information for the development of a semiochemical-based mating-disruption programme for *C. rosaceana* and *P. limitata*. The biology of tortricine leafrollers dictates the way in which such a programme should be designed. An attractive formulation is not required to disrupt mate-finding behaviour in *C. rosaceana* or *P. limitata* nor in other orchard-inhabiting tortricids (Deland et al. 1994). Therefore, simultaneous mating disruption of these species may be best achieved by an unattractive formulation which eliminates the danger of attracting males of these polyphagous species to the orchard from other host plants. Formulations

containing *Z*11-14:OAc alone or combined with *Z*9-14:OAc in a 1:1 ratio are adequate to reduce mating significantly. Adaptation of antennal neurons should be exploited in a mating-disruption programme by even dissemination of pheromone throughout the orchard possibly by sprayable formulations.

A physiological time scale, timed to first male moth capture in pheromone-baited traps, can be used to indicate the timing of female eclosion and oviposition of *C. rosaceana* on apple in B.C. and therefore when mating-disruption dispensers should be disseminated in the orchard. As *Z*11-14:OAc also constitutes the major pheromone component of the other two tortricine leafroller species found on apple in B.C., *A. argyrospilus* (Deland et al. 1993) and *A. rosanus* (Roelofs et al. 1976b), it is likely that treatment with this component will reduce mating in all four species. Deland et al. (1994) demonstrated 99.3 and 88.3% reductions in synthetic pheromone-baited trap captures of male *A. argyrospilus* and *A. rosanus*, respectively, in an atmosphere treated with a 93:7 ratio of *Z*11-14:OAc: *E*11-14:OAc at a release rate of ca. 20 $mg \cdot ha^{-1} \cdot h^{-1}$. Mating of tethered female *A. argyrospilus* was reduced by 82% by this same treatment (Deland et al. 1994). The addition of *Z*9-14:OAc to *Z*11-14:OAc may increase the disruptive efficacy of *Z*11-14:OAc alone against *A. argyrospilus,* as *Z*9-14:OAc is a minor pheromone component in this species (Cardé et al. 1977b; Deland et al. 1993). The effect of *Z*9-14:OAc on *A. rosanus* needs to be determined but preliminary evidence indicates that *Z*9-14:OAc is a behavioural antagonist to *A. rosanus* (H. McBrien, Dept. of Entomology, University of California-Riverside, pers. comm.) as it is to *C. rosaceana* (Chapter 5).

Organic control of *C. pomonella* by the sterile insect release technique (Dyck et al. 1993) or by pheromone-based mating disruption (Judd et al. 1996a) in the Okanagan and Similkameen Valleys of B.C. will permit widespread insecticide-free fruit production only if organic control of secondary pests is achieved. The tortricine leafroller guild, in association with the eyespotted budmoth, *Spilonota ocellana* (Dennis and Schiffermüller) in the northern Okanagan Valley, represent a complex of secondary pests that will be released from insecticidal control with reduction of organophosphate sprays targeted at *C. pomonella*. A pheromone-based mating-disruption programme based on a formulation emitting *Z*11-14:OAc or *Z*11-14:OAc and *Z*9-14:OAc should reduce mating in all four species of tortricine leafrollers but it remains to be determined if population control can be achieved and if damage will also be reduced. Large-plot trials in which efficacy is assessed using the mating status of feral females and crop damage need to be conducted. High population densities or a clumped distribution of emerging adults may promote mate finding by non-chemical means which could undermine population control by mating disruption (Barclay and Judd 1995). In areas with high leafroller populations, growers may have to implement other control measures prior to using a semiochemical-based mating-disruption programme. In northern parts of the Okanagan Valley, treatment with the *S. ocellana* pheromone (McBrien et al. 1998) will be required in addition to a formulation aimed at the tortricine leafrollers to impart organic control of this secondary pest complex.

APPENDIX 1- Pintobean-based diet for *C. rosaceana* and *P. limitata*

Ingredients:

80.0 g agar
1440 ml dH_20

3400 ml dH_20

852 g dried ground pinto beans
128 g brewers yeast
12.8 g ascprboc acod
8.0 g methyl-p-hydroxybenzoate
4.0 g sorbic acid
16.0 g vitamin mix

Procedure:

Agar and water were heated in a large kettle until the mixture had boiled, thickened and turned golden brown in colour. Additional water and dry ingredients were added to the agar mixture and stirred. Diet was poured while hot into plastic condiment dispensers and dispensed into approximately 800 individual 29 ml Solo plastic cups (Rap-id paper, Kelowna, B.C.). Cups containing diet were stored on trays and covered in plastic at 2.5°C until use.

APPENDIX 2- Contents of pheromone dispensers (Ecogen Inc., Billings, MT) used in field and lab experiments, as determined by gas chromatographic analysis.

			Percent Component in Blend					
Chapter	Exp.	Blend	*Z*11-14:OAc	*E*11-14:OAc	*Z*11-14:OH	*Z*11-14:Ald	*Z*9-14:OAc	*E*9-14:OAc
4	1	2-COMP	95.4	4.6	-	-	-	-
		3-COMP	95.4	3.6	1.0	-	-	-
		4-COMP	91.4	3.4	3.3	1.9	-	-
	2	4-COMP	91.4	3.4	3.3	1.9	-	-
	3	4-COMP	90.8	4.2	3.3	1.7	-	-
	4	2-COMP	95.4	4.6	-	-	-	-
		4-COMP	91.4	3.4	3.3	1.9	-	-
	5	2-COMP	98	2	-	-	-	-
		4-COMP	93.8	2.8	2.0	1.4	-	-
	6	2-COMP	95.4	4.6	-	-	-	-
		3-COMP	95.4	3.6	3.4	-	-	-
		4-COMP	91.4	3.4	3.3	1.9	-	-

APPENDIX 2 (Cont'd) - Contents of pheromone dispensers used in field and lab experiments.

			Percent Component in Blend					
Chapter	Exp.	Blend	*Z*11-14:OAc	*E*11-14:OAc	*Z*11-14:OH	*Z*11-14:Ald	*Z*9-14:OAc	*E*9-14:OAc
5	5	4-COMP	90.8	4.2	3.3	1.7	-	-
		*Z*9-14:OAc	-	-	-	-	97.8	2.2
6	1	2-COMP	95.4	4.6	-	-	-	-
		3-COMP	95.4	3.6	3.4	-	-	-
		4-COMP	91.4	3.4	3.3	1.9	-	-
	2	4-COMP	91.4	3.4	3.3	1.9	-	-
	3	4-COMP	90.8	4.2	3.3	1.7	-	-
	4	2-COMP	95.4	4.6	-	-	-	-
		3-COMP	95.4	3.6	1.0	-	-	-
		4-COMP	91.4	3.4	3.3	1.9	-	-
	5	*Z*9-14:OAc	-	-	-	-	97.8	2.2
		2-COMP	98	2	-	-	-	-
		P. limitata	91.4	2.4	-	-	6.1	0.2
	6	*Z*9-14:OAc	-	-	-	-	97.8	2.2
		2-COMP	98	2	-	-	-	-
7	1-6	*Z*9-14:OAc	-	-	-	-	97.8	2.2

APPENDIX 3- Descriptions of experimental plots used in communication and mating-disruption experiments in the Similkameen Valley, B.C.

Chapter	Exp.	Plot	Apple Varieties[a]	Spacing (m) tree x row	Tree height (m) ($\bar{x}$±SE), n=10	Tree width (m)[b] ($\bar{x}$±SE), n=10
4	1	1	R,S	3.8 x 5.0	4.0 ± 0.8	2.9 ± 0.8
		2	R,S	3.8 x 5.0	4.2 ± 0.6	3.8 ± 0.8
		3	M,S	3.0 x 5.5	3.9 ± 0.5	3.1 ± 0.5
		4	W, T-G, M	3.0 x 5.5	3.4 ± 0.3	3.0 ± 0.4
	2,4	1	R,S	3.8 x 5.0	3.9 ± 0.5	3.2 ± 0.6
		2	R,S	3.8 x 5.0	3.8 ± 0.5	3.2 ± 0.6
		3	M,S	3.0 x 5.5	4.1 ± 0.5	3.2 ± 0.5
		4	W, T-G, M	3.0 x 5.5	3.6 ± 0.4	3.6 ± 0.3
	3	1	R,S	3.8 x 5.0	4.0 ± 0.7	3.7 ± 1.0
		2	R,S	3.8 x 5.0	3.9 ± 0.5	3.9 ± 0.5
		3	M,S	3.0 x 5.5	4.5 ± 0.5	3.3 ± 0.3
		4	W, T-G, M	3.0 x 5.5	3.3 ± 0.4	3.1 ± 0.4
	5	1	J, M, Ro	4.0 x 2.0	3.0 ± 0.7	2.0 ± 0.2
		2	Gr, J	4.0 x 2.0	3.4 ± 0.4	1.9 ± 0.3
		3	Ga, J	4.0 x 2.0	3.6 ± 0.4	1.9 ± 0.3
		4	J, Su	4.0 x 2.0	3.7 ± 0.4	1.9 ± 0.3
5	5	1	R	4.0 x 5.7	4.2 ± 0.2	3.9 ± 0.1
		2	R	4.0 x 5.7	4.5 ± 0.2	4.1 ± 0.1
		3	Ga	3.4 x 5.6	3.3 ± 0.2	2.9 ± 0.3
		4	S	5.6 x 6.2	4.7 ± 0.2	4.9 ± 0.2
6	1	1	R,S	3.8 x 5.0	4.0 ± 0.8	2.9 ± 0.8
		2	R,S	3.8 x 5.0	4.2 ± 0.6	3.8 ± 0.8
		3	M,S	3.0 x 5.5	3.9 ± 0.5	3.1 ± 0.5
		4	W, T-G, M	3.0 x 5.5	3.4 ± 0.3	3.0 ± 0.4
	2	1	R,S	3.8 x 5.0	3.9 ± 0.5	3.2 ± 0.6
		2	R,S	3.8 x 5.0	3.8 ± 0.5	3.2 ± 0.6
		3	M,S	3.0 x 5.5	4.1 ± 0.5	3.2 ± 0.5
		4	W, T-G, M	3.0 x 5.5	6.0 ± 0.4	3.6 ± 0.3

APPENDIX 3 (Cont'd) - Descriptions of experimental plots used in communication and mating-disruption experiments in the Similkameen Valley, B.C.

Chapter	Exp.	Plot	Apple Varieties[a]	Spacing (m) tree x row	Tree height (m) ($\bar{x}$±SE), n=10	Tree width (m) [b] ($\bar{x}$±SE), n=10
6	3	1	R,S	3.8 x 5.0	4.0 ± 0.7	3.7 ± 1.0
		2	R,S	3.8 x 5.0	3.9 ± 0.5	3.9 ± 0.5
		3	M,S	3.0 x 5.5	4.5 ± 0.5	3.3 ± 0.3
		4	W, T-G, M	3.0 x 5.5	3.3 ± 0.4	3.1 ± 0.4
	4	1	Ga, E, Su, S	2.0 x 4.0	3.0 ± 0.5	2.1 ± 0.3
		2	S, G, F	2.0 x 4.0	2.7 ± 0.6	2.5 ± 1.3
		3	Sm, G, Su	2.0 x 4.0	2.6 ± 0.3	2.3 ± 0.3
		4	J	2.0 x 4.0	2.5 ± 0.4	2.1 ± 0.4
	5	1	Ga, E, Su, S	2.0 x 4.0	3.0 ± 0.5	2.1 ± 0.3
		2	S, G, F	2.0 x 4.0	2.7 ± 0.6	2.5 ± 1.3
		3	Sm, G, Su	2.0 x 4.0	2.6 ± 0.3	2.3 ± 0.3
	6	1	S	4.0 x 6.0	4.8 ± 0.8	3.6 ± 0.4
		2	S	4.0 x 6.0	3.9 ± 0.2	3.6 ± 0.3
		3	S	6.0 x 6.0	4.6 ± 0.5	5.5 ± 0.9
		4	J	4.0 x 6.0	4.0 ± 0.5	4.1 ± 0.6

[a]Abbreviations for apple varieties are: Elstar (E), Fuji (F), Golden Delicious (G), Gala (Ga), Granny Smith (Gr), Jonagold (J), McIntosh (M), Red Delicious (R), Romes (Ro), Spartan (S), Smoothie (Sm), Sunrise (Su), Transparent-Gold (T-G), Winesap (W).

[b]Distance between longest branch tips.

APPENDIX 4- Comparison of number of male *P. limitata* attracted to tethered *vs.* not tethered females in traps.

A trapping study was conducted to determine if the act of tethering females had any effect on a female's ability to attract males.

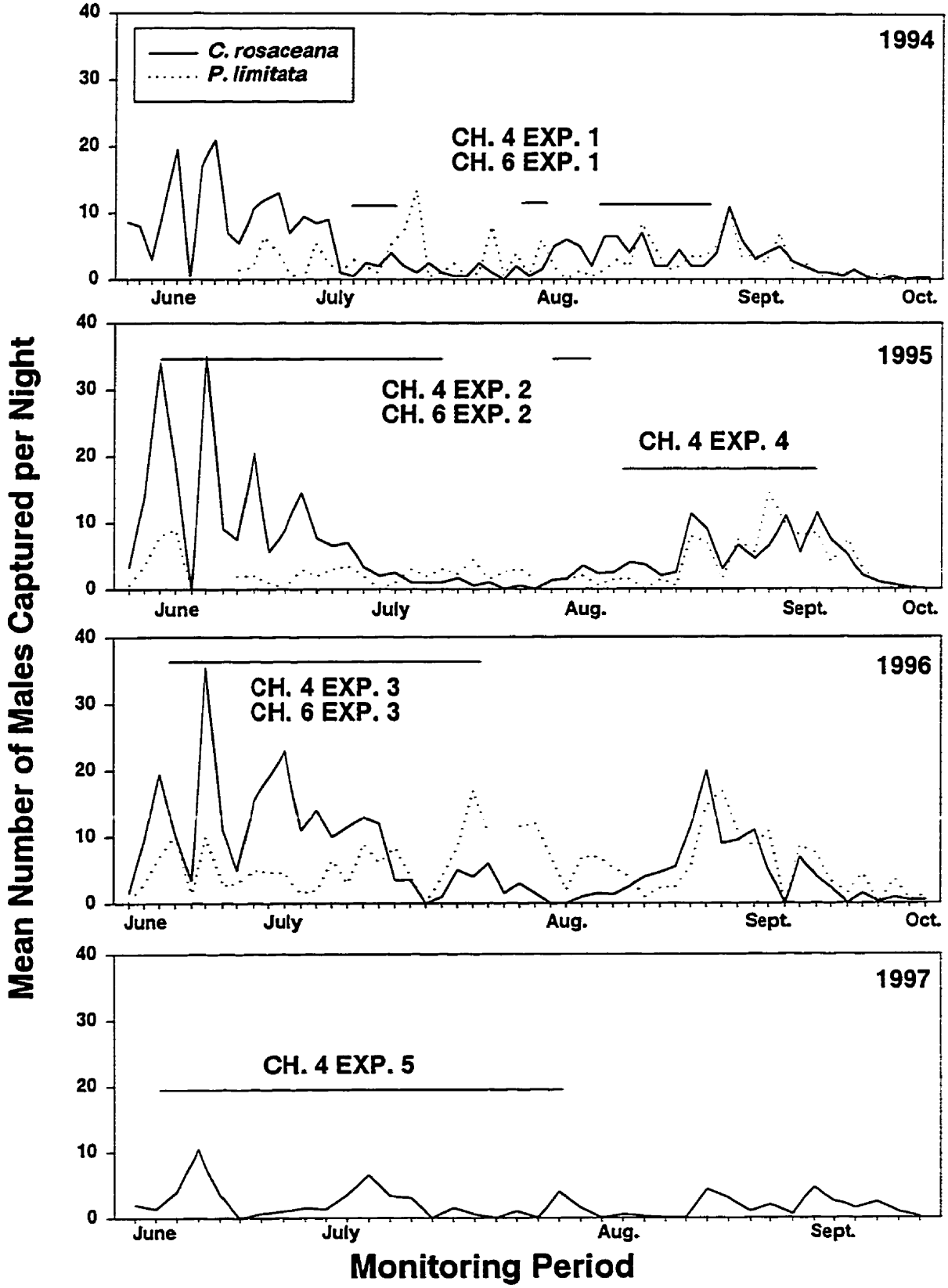
Methods and Materials

Female *P. limitata* pupae were obtained from the laboratory colony (section 2.1), placed individually in 150 mL cups and provided with a water source. Female *P. limitata* moths aged six-96 h were chilled at 0.5°C for 10 min. Twenty-one females were tethered (section 2.3.2) while 21 other females remained not tethered. All females were then placed in cylindrical (3 cm diam. x 5 cm height) mesh cages and provided with a water source. Females were transferred to field sites in a refrigerated container. At the field sites (N=3 orchards), 14 wing traps (section 2.4) were baited with females in cages, 7 with tethered females and 7 with females that were non-tethered. Traps were hung 1.5 m above the ground and were separated by 10 m. Traps remained in the field for 2 nights at which point males captured were enumerated. The procedure was repeated for a total of six site-trapping periods. Numbers of male moths captured were transformed by ln(x+1) prior to analysis by a randomized block ANOVA (SAS, 1996).

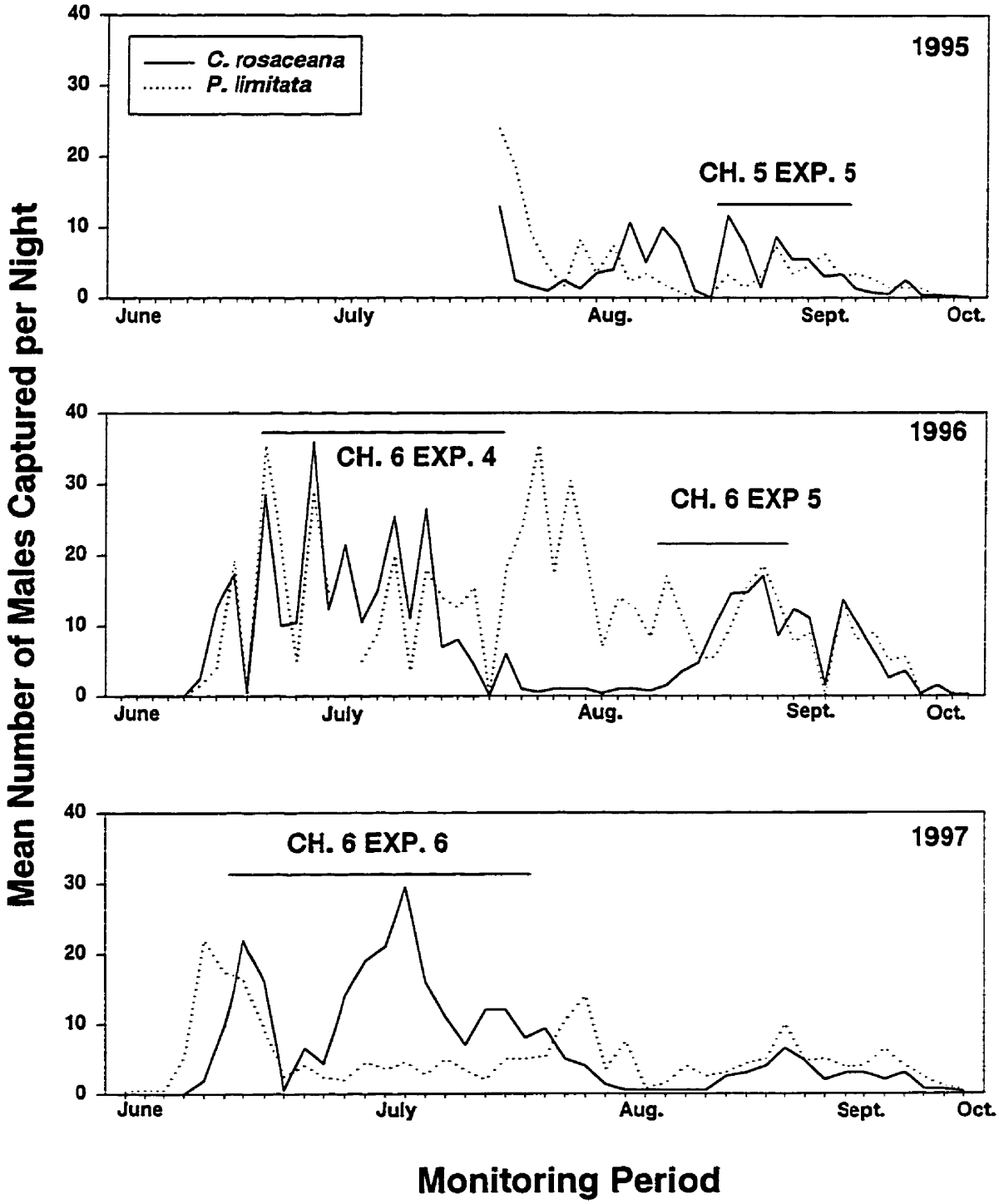
Results

An equal number of males were attracted to both tethered females ($\bar{x}$ =1.591 ± 0.18) and non-tethered females ($\bar{x}$ =1.15 ± 0.14) ($F_{1,72}$=1.37, P=0.2453).

APPENDIX 5- Male *C. rosaceana* and *P. limitata* captured in pheromone-baited traps adjacent to mating-disruption experiments.



APPENDIX 5 (Cont'd) - Male *C. rosaceana* and *P. limitata* captured in pheromone-baited traps adjacent to mating-disruption experiments.



APPENDIX 6. Mean numbers of male C. *rosaceana* captured in synthetic pheromone-baited traps (3mg, 4-component blend) in each replicate of Exp. 1, Chapter 4.

Replicate	Mean number of males captured per night			
	Atmospheric Treatment			
	Control	2-component blend	3-component blend	4-component blend
1	5.9	0.4	0.3	0.1
2	2.6	0.4	1	0.4
3	8.8	3.6	0.8	2.2
4	7.5	0	0.75	0.3

10.0 REFERENCES CITED

Agnello, A.M., W.H. Reissig, S.M. Spangler, R.E. Charlton and D.P. Kain. 1996. Trap response and fruit damage by obliquebanded leafroller (Lepidoptera: Tortricidae) in pheromone-treated apple orchards in New York. Environ. Entomol. 25: 268-282.

Alexander, R.D., D.C. Marshall, and J.R. Cooley. 1997. Evolutionary perspectives on insect mating. In: J. C. Choe and B.J. Crespi (eds.). The Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge. pp. 4-31.

Alford, A.R. and P.J. Silk. 1983. Effect of pheromone-releaser distribution and release rate on the mating success of spruce budworm (Lepidoptera: Tortricidae). J. Econ. Entomol. 76: 774-778.

AliNiazee, M.T. 1986. Seasonal history, adult flight activity, and damage of the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae), in filbert orchards. Can. Entomol. 118: 353-361.

Allen, J. C. 1976. A modified sine wave method for calculating degree days. Environ. Entomol. 5: 388-396.

Babson, A.L. 1963. Eradicating the gypsy moth. Science 142: 447-448.

Baker, T.C., M.A. Willis, K.F. Haynes and P.L. Phelan. 1985. A pulsed cloud of sex pheromone elicits upwind flight in male moths. Physiol. Entomol. 10: 367-265.

Baker, T.C., B.S. Hansson, C. Löfstedt and J. Löfqvist. 1989. Adaptation of male moth antennal neurons in a pheromone plume is associated with cessation of pheromone-mediated flight. Chem. Senses 14: 439-448.

Baker, T.C., R.T. Staten and H.M. Flint. 1990. Use of pink bollworm pheromone in the southwestern United States. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds.). Behavior-Modifying Chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker, Inc. New York, pp. 417-436.

Barclay, H.J. and G.J.R. Judd. 1995. Models for mating disruption by means of pheromone for insect pest control. Res. Popul. Ecol. 37: 239-247.

Barnes, M.M., J.G. Millar, P.A. Kirsch and D.C. Hawks. 1992. Codling moth (Lepidoptera: Tortricidae) control by dissemination of synthetic female sex pheromone. J. Econ. Entomol. 85: 1274-1277.

Bartell, R. J. 1982. Mechanisms of communication disruption by pheromone in the

control of Lepidoptera: a review. Physiol. Entomol. 7: 353-364.

Bartell, R.J. 1985. Pheromone-mediated behaviour of male lightbrown apple moth, *Epiphyas postvittana*, correlated with adaptation of pheromone receptors Physiol. Entomol. 10: 121-126.

Bartell, R.J. and L.A. Lawrence. 1977a. Reduction in responsiveness of male light-brown apple moths, *Epiphyas postvittana*, to sex pheromone following pulsed pre-exposure to pheromone components. Physiol. Entomol. 2: 89-95.

Bartell, R.J. and L.A. Lawrence. 1977b. Reduction in responsiveness of male apple moths, *Epiphyas postvittana*, to sex pheromone following pulsed pheromonal exposure. Physiol. Entomol. 2: 1-6.

Bergh, J.C. and W.D. Seabrook. 1986a. A simple technique for indexing the mating status of male spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) Can. Entomol. 118: 37-41.

Bergh, J.C. and W.D. Seabrook. 1986b. The mating status of spruce budworm males, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), caught in pheromone-baited traps. J. Entomol. Sci. 21: 254-262.

Bengtsson, M., G. Karg, P.A. Kirsch, J. Löfqvist, A. Sauer and P. Witzgall. 1994. Mating disruption of pea moth *Cydia nigricana* F. (Lepidoptera: Tortricidae) by a repellent blend of sex pheromone and attraction inhibitors. J. Chem. Ecol. 20: 871-887.

Birch, M.C. 1977. Response of both sexes of *Trichoplusia ni* (Lepidoptera: Noctuidae) to virgin females and to synthetic pheromone. Ecol. Entomol. 2: 99-104.

Birch, M.C., D.M. Light, D.L. Wood, L.E. Browne, R.M. Silverstein, B.J. Bergot, G. Ohloff, J.R. West, and J.C. Young. 1980. Pheromonal attraction and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. J. Chem. Ecol. 6: 703-717.

Bjostad, L.B., C.E. Linn, and J.W. Du. 1984. Identification of new sex pheromone components in *Trichoplusia ni*, predicted from biosynthetic precursors. J. Chem. Ecol. 10: 1309-1323.

Blum, M.S. 1996. Semiochemical parsimony in the Arthropoda. Annu. Rev. Entomol. 41: 353-374.

Borden, J.H., D.R. Devlin, and D.R. Miller. 1991. Synomones of two sympatric species deter attack by the pine engraver, *Ips pini* (Coleoptera: Scolytidae). Can. J. For. Res. 22: 381-387.

Borroni, P.F. and R.J. O'Connell. 1992. Temporal analysis of adaptation in moth (*Trichoplusia ni*) pheromone receptor neurons. J. Comp. Physiol. A 170: 691-700.

Brown, W.L., Jr., T. Eisner and R.H. Whittaker. 1970. Allomones and kairomones: transspecific chemical messengers. BioScience 20: 21-22.

Burgess, E.D. 1964. Gypsy moth control. Science 143: 526.

Cardé, R.T. 1981. Disruption of long-distance pheromone communication in the Oriental fruit moth: camouflaging the natural aerial trails from females? In: E.R. Mitchell (ed.). Management of Insect Pests with Semiochemicals. Plenum Press, New York, pp. 385-398.

Cardé, R.T. 1986. The role of pheromones in reproductive isolation and speciation of insects. In: M.D. Huettel (ed.). Evolutionary Genetics of Invertebrate Behavior: Progress and Prospects. Plenum Press, New York and London, pp. 303-317.

Cardé, R.T. 1990. Principles of mating disruption. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds.). Behavior-Modifying Chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York, pp. 47-71.

Cardé, R.T. and T.C. Baker. 1984. Sexual communication with pheromones. In: W.J. Bell and R.T. Cardé (eds.). Chemical Ecology of Insects. Sinauer Associates Inc., Sunderland, Massachusetts, pp. 355-383.

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

Cardé, R.T., T.C. Baker and P. J. Castrovillo. 1977a. Disruption of sexual communication in *Laspeyresia pomonella* (codling moth), *Graphotitha molesta* (Oriental fruit moth) and *G. prunivora* (Lesser appleworm) with hollow fiber attractant sources. Entomol. Exp. and Appl. 22: 280-288.

Cardé, R.T., A.M. Cardé, A.S. Hill and W.L. Roelofs. 1977b. Sex pheromone specificity as a reproductive isolating mechanism among the sibling species *Archips argyrospilus* and *A. mortuanus* and other sympatric tortricine moths (Lepidoptera: Tortricidae). J. Chem. Ecol. 3: 747-751.

Cardé, R.T., A. Mafra-Neto, R.T. Staten, U. Koch, P. Färbert. 1993. Evaluation of communication disruption in the pink bollworm in field wind tunnels. IOBC wprs Bull.16: 23-28.

Cardé, R.T., A. Mafra-Neto, R.T. Staten and L.P.S. Kuenen. 1997. Understanding

mating disruption in the pink bollworm moth. IOBC wprs Bull. 20: 191-201.

Carrière, Y. 1992. Host plant exploitation within a population of a generalist herbivore, *Choristoneura rosaceana*. Entomol. Exp. and Appl. 65: 1-10.

Carroll, A.L. 1994. Interactions between body size and mating history influence the reproductive success of males of a tortricid moth, *Zeriaphera canadensis*. Can. J. Zool. 72: 2124-2132.

Chapman, P.J. 1973. Bionomics of apple-feeding Tortricidae. Ann. Rev. Entomol. 18: 73-96.

Chapman, P.J. and S.E. Lienk 1971. Tortricid Fauna of Apple in New York. New York State Agricultural Experiment Station, Division of the College of Agriculture, Cornell University, Geneva, New York.

Chapman, P.J., S.E. Lienk and R.W. Dean. 1968. Bionomics of *Choristoneura rosaceana*. Ann. Entomol. Soc. Am. 61: 285-290.

Charlton, R.E. and R.T. Cardé. 1981. Comparing the effectiveness of sexual communication disruption in the Oriental fruit moth (*Grapholitha molesta*) using different combinations and dosages of its pheromone blend. J. Chem. Ecol. 7: 501-508.

Charmillot, P.J. 1990. Mating disruption technique to control codling moth in western Switzerland. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds). Behavior-Modifying Chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York, pp. 165-182.

Charmillot, P.J., D. Pasquier, N.J. Alipaz and U. Neumann. 1995. Effect of pheromone dispensers placed as a belt on the distribution of male catches, tethered female matings and damage of *Lobesia botrana* Den. & Schiff. in vineyards. J. Appl. Entomol. 119: 201-206.

Christensen, T.A., S.C. Geoffrion and J.G. Hildebrand. 1990. Physiology of interspecific chemical communication in *Heliothis* moths. Physiol. Entomol. 15: 275-283.

Cockfield, S.D, S.L. Butkewich, K.S. Smoil and D.L. Mahr. 1994. Forecasting flight activity of *Sparganothis sulfereana* (Lepidoptera: Tortricidae) in cranberries. J. Econ. Entomol. 87: 193-196.

Collett, D. 1989. Modelling Binary Data. Chapman and Hall, New York.

Conover, W.J. 1971. Non Parametric Statistics. 1971. John Wiley and Sons Inc.,

London.

Crawley, M.J. 1993. GLIM for ecologists. In: J.H. Lawton and G.E. Likens (eds.). Methods in Ecology. Blackwell Science, Oxford.

Critchley, B.R., D.G. Campion, L.J. Mcveigh, E.M. McVeigh, G.G. Cavanagh, M.M. Hosny, E.S. Nasr, A.A. Khidr and M. Naguib. 1985. Control of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in Egypt by mating disruption using hollow-fibre, laminate-flake and microencapsulated formulations of synthetic pheromone. Bull. Entomol. Res. 75: 329-345.

Dang, P.T. 1992. Morphological study of male genitalia with phylogenetic inference of *Choristoneura* Lederer (Lepidoptera: Tortricidae). Can. Entomol. 124: 7-48.

Daterman, G.E., G. Doyle Daves, Jr., and R.G. Smith. 1975. Comparison of sex pheromone versus an inhibitor for disruption of pheromone communication in *Rhyacionia buoliana*. Environ. Entomol. 4: 944-946.

Deland, J.P., R. Gries, G. Gries, G.J.R. Judd, and B.D. Roitberg. 1993. Sex pheromone components of the fruit-tree leaf roller, *Archips argyrospilus* (Walker) (Lepidoptera: Tortricidae), in British Columbia. J. Chem. Ecol. 19: 2855-2864.

Deland, J.P., G.J.R. Judd, and B.D. Roitberg. 1994. Disruption of pheromone communication in three sympatric leafroller (Lepidoptera: Tortricidae) pests of apple in British Columbia. Environ. Entomol. 23: 1084-1090.

Delisle, J. 1992a. Monitoring the seasonal male flight activity of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) in eastern Canada using virgin females and several different pheromone blends. Environ. Entomol. 21: 1007-1012.

Delisle, J. 1992b. Age related changes in the calling behaviour and the attractiveness of obliquebanded leafroller virgin females, *Choristoneura rosaceana*, under different constant and fluctuating temperature conditions. Entomol. Exp. and Appl. 63: 55-62.

Delisle, J. 1995. Effect of male and female age on the mating success of the obliquebanded leafroller *Choristoneura rosaceana* (Lepidoptera: Tortricidae) under different ecological conditions. J. Insect Behav. 8: 781-799.

Delisle, J. and L. Royer. 1994. Changes in pheromone titer of obliquebanded leafroller, *Choristoneura rosaceana*, virgin females as a function of time of day, age, and temperature. J. Chem. Ecol. 20: 45-69.

Delisle, J. and A. Bouchard. 1995. Male larval nutrition in *Choristoneura rosaceana* (Lepidoptera: Tortricidae): an important factor in reproductive success.

Oecologia 104: 508-517.

Den Otter, C. J., and M. M. van Der Haagen. 1989. Sex pheromone attractants and inhibitors in the cabbage armyworm, *Mamestra brassicae* L. (Lep.: Noctuidae): electrophysiological discrimination. Insect Science Appl. 10: 235-242.

Dennehy, T.J., W.L. Roelofs, E.F. Taschenberg and T.N. Taft. 1990. Mating disruption for control of grape berry moth in New York Vineyards. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds). Behavior-Modifying Chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York, pp. 223-240.

Dyck, V. A., S. H. Graham & K. A. Bloem. 1993. Implementation of the sterile insect release programme to eradicate the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae), in British Columbia, Canada. In: Proceedings of International Symposium on Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. International Atomic Energy Agency, Vienna, pp. 285-297.

El-Adl, M.A., M.M. Hosny and D.G. Campion. 1988. Mating disruption for the control of pink bollworm *Pectinophora gossypiella* (Saunders) in the delta cotton growing area of Egypt. Trop. Pest Mgt. 34: 210-214.

Endler, J.A. 1993. Some general comments on the evolution and design of animal communication systems. Phil. Trans. R. Soc. Lond. B. 340: 215-225.

Fadamiro, H.Y. and T.C. Baker. 1997. *Helicoverpa zea* males (Lepidoptera: Noctuidae) respond to the intermittent fine structure of their sex pheromone plume and an antagonist in a flight tunnel. Physiol. Entomol. 22: 316-324.

Ferro, D.N. and R.D. Akre. 1975. Reproductive morphology and mechanics of mating of the codling moth, *Laspeyresia pomonella*. Ann. Entomol. Soc. Am. 68: 417-424.

Fitzpatrick, S.M., J.T. Troubridge, C. Maurice and J. White. 1995. Initial studies of mating disruption of the blackheaded fireworm of cranberries, *Rhopobota naevana* (Hbn.) (Lepidoptera: Tortricidae). J. Econ. Entomol. 88: 1017-1023.

Flint, H.M. and J.R. Merkle. 1983. Pink bollworm (Lepidoptera: Gelechiidae): communication disruption by pheromone composition imbalance. J. Econ. Entomol. 76: 40-46.

Flint, H.M. and J.R. Merkle. 1984a. The pink bollworm (Lepidoptera: Gelechiidae): alteration of male response to gossyplure by release of its component Z,Z-isomer. J. Econ. Entomol. 77: 1099-1104.

Flint, H.M. and J.R. Merkle. 1984b. Pink bollworm: disruption of sexual communication by the release of the Z,Z-isomer of gossyplure. Southwest. Entomol. 9: 58-61.

Foster, S.P. and R.H. Ayers. 1996. Multiple mating and its effects in the lightbrown apple moth, *Epiphyas postvittana* (Walker). J. Insect Physiol. 42: 657-667.

Gangavalli, R.R. 1985. Influence of temperature and photoperiod on the developmental biology of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae). PhD. thesis, Oregon State University, Corvalis.

Gangavalli, R.R. and M.T. AliNiazee. 1985a. Diapause induction in the oblique-banded leafroller *Choristoneura rosaceana* (Lepidoptera: Tortricidae): role of photoperiod and temperature. J. Insect Physiol. 31: 831-835.

Gangavalli, R.R. and M.T. AliNiazee. 1985b. Temperature requirements for development of the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae). Environ. Entomol. 14: 17-19.

Gaston, L.K., H.H. Shorey, C.A. Saario. 1967. Insect population control by the use of sex pheromones to inhibit orientation between the sexes. Nature 213: 1155.

Gaston, L.K., R.S. Kaae, H.H. Shorey and D. Sellers. 1977. Controlling the pink bollworm by disruption of sex pheromone communication between adult moths. Science 196: 904-905.

Gilchrest, R. 1993. Guide to statistical modelling with GLIM. In: B. Francis, M. Green, C. Payne (eds.). The Glim System, Release 4 Manual, Claredon Press, Oxford. pp. 225-257.

Gilchrest, R. and P. Green. 1993. The theory of generalized linear models. In: B. Francis, M. Green, C. Payne (eds.). The Glim System, Release 4 Manual, Claredon Press, Oxford. pp. 259-305.

GLIM, 1985. The Statistical System for Generalized Linear Interactive Modelling, 3.77. Numerical Algorithms Group, Oxford Science Publications, Oxford.

Granett, J. and C.C. Doane. 1975. Reduction of gypsy moth male mating potential in dense populations by mistblower sprays of micro-encapsulated disparlure. J. Econ. Entomol. 68: 435-437.

Grant, A.J., R.J. O'Connell, and A.M. Hammond, Jr. 1988. A comparative study of pheromone perception in two species of noctuid moths. J. Insect Behav. 1: 75-96.

Greenfield, M.D. and M.G. Karandinos. 1979. Resource partitioning of the sex communication channel in clearwing moths (Lepidoptera: Sesiidae) of Wisconsin. Ecol. Mon. 49: 403-426.

Haynes, K.F. and T.C. Baker. 1988. Potential for evolution of resistance to pheromones worldwide and local variation in chemical communication system of pink bollworm moth, *Pectinophora gossypiella*. J. Chem. Ecol. 14: 1547-1560.

Higley, L.G., L.P. Pedigo and K.R. Ostlie. 1986. DEGDAY: a program for calculating degree-days, and assumptions behind the degree-day approach. Environ. Entomol. 15: 999-1016.

Hill, A.S. and W.L. Roelofs. 1979. Sex pheromone components of the obliquebanded leafroller moth, *Choristoneura rosaceana*. J. Chem. Ecol. 5: 3-11.

Hiyori, T., Y. Kainoh and Y. Ninomiya. 1986. Wind tunnel tests on the disruption of pheromonal orientation of the male smaller tea tortrix moth, *Adoxophyes sp.* (Lepidoptera: Tortricidae) 1. Disruptive effect of sex pheromone components. Appl. Entomol. Zool. 21: 153-158.

Horak, M. and R.L. Brown. 1991. Morphology, taxonomy and phylogeny. In: W. Helle, L.P.S. van der Geest and H.H. Evenhuis (eds.). World Crop Pests vol. 5. Tortricid Pests Their Biology, Natural Enemies and Control. Elsevier, Amsterdam. pp. 23-48.

Howell, J.F., A.L. Knight, T.R. Unruh, D.F. Brown, J.L. Krysan, C.R. Sell and P.A. Kirsch. 1992. Control of codling moth in apple and pear with sex pheromone-mediated mating disruption. J. Econ. Entomol. 83: 918-925.

Hunter, M.D. and J.N. McNeil. 1997. Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. Ecology 78: 977-986.

Jenkins, J.W., C.C. Doane, D.J. Schuster, J.R. McLaughlin, and M.J. Jimenez. 1990. Development and commercial application of sex pheromone for control of the tomato pinworm. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds). Behavior-Modifying Chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York, pp. 269-280.

Jiménez, M.J., N.C. Toscano, D.L. Flaherty, P. Ilic, F.G. Zalom, and K. Kido. 1988. Controlling tomato pinworm by mating disruption. California Agric. Nov.-Dec.: 10-12.

Judd, G.J.R. and H.L. McBrien. 1994. Pheromone-based mating disruption of pome fruit pests in British Columbia: a multiple-species approach. Proceedings of a Workshop " Research, development and commercialization of semiochemicals in

insect pest management in Canada," Winnipeg, 19 October 1994. Pest Management Alternatives Office, Natural Resources, Ottawa, Canada. pp. 9-17.

Judd, G.J.R. and M.G.T. Gardiner. 1997. Forecasting phenology of *Orthosia hibisci* Guenée (Lepidoptera: Noctuidae) in British Columbia using sex-attractant traps and degree-day models. Can. Entomol. 129: 815-825.

Judd, G.J.R., H.L. McBrien, and J.H. Borden. 1995. Modification of responses by *Campylomma verbasci* (Heteroptrea: Miridae) to pheromone blends in atmospheres permeated with synthetic sex pheromone or individual components. J. Chem. Ecol. 21: 1991-2002.

Judd, G.J.R., M.G.T. Gardiner and D.R. Thomson. 1996a. Commercial trials of pheromone-mediated mating disruption with Isomate-C® to control codling moth in British Columbia apple and pear orchards. J. Entomol. Soc. Brit. Columbia 93: 23-34.

Judd, G.J.R., M.G.T. Gardiner and D.R. Thomson. 1996b. Monitoring and predicting seasonal flight of *Orthosia hibisci* (Lepidoptera: Noctuidae) in the Okanagan and Similkameen Valleys of British Columbia. J. Entomol. Soc. Brit. Columbia 93: 11-22.

Judd, G.J.R., M.G.T. Gardiner, and D.R. Thomson. 1997. Control of codling moth in organically-managed apple orchards by combining pheromone-mediated mating disruption, post-harvest fruit removal and tree banding. Entomol. Exp. and Appl. 83: 137-146.

Kaae, R.S., H.H. Shorey, L.K. Gaston and H.H. Hummel. 1974. Sex pheromones of Lepidoptera: disruption of pheromone communication in *Trichoplusia ni* and *Pectinophora gossypiella* by permeation of the air with nonpheromone chemicals Environ. Entomol. 3: 87-89.

Kanno, H., S.Tatsuki, K. Uchiumi, M. Kurihara, J-i Fukami, Y. Fujimoto and T.Tatsuno. 1978. Disruption of sex attraction in the rice stem borer moth, *Chilo suppressalis* Walker, with components of the sex pheromone and related chemicals. Appl. Entomol. Zool. 13: 321-323.

Karg, G., D.M. Suckling, and S.J. Bradley. 1994. Absorption and release of pheromone of *Epiphyas postvittana* (Lepidoptera: Tortricidae) by apple leaves. J. Chem. Ecol. 20: 1825-1841.

Kennedy, J.S., A.R. Ludlow, and C.J. Sanders. 1980. Guidance system used in moth attraction. Nature 295: 475-477.

Kennedy, J.S., A.R. Ludlow and C.J. Sanders. 1981. Guidance of flying male moths by

wind-borne sex pheromone. Physiol. Entomol. 6: 395-412.

Knight, A.L. 1997. Delay of mating of codling moth in pheromone disrupted orchards. IOBC wprs Bull. 20: 203-206.

Knight, A.L., M. Weiss and T. Weissling. 1994. Diurnal patterns of adult activity of four orchard pests (Lepidoptera: Tortricidae) measured by timing trap and actograph. J. Agric. Entomol. 11: 125-136.

Knowlton, G.F. and M.W. Allen. 1937. Oblique-banded leaf roller, a dewberry pest in Utah. J. Econ. Entomol. 30: 780-785.

Kolodny-Hirsch, D.M. and R.E. Webb. 1993. Mating disruption of gypsy moth (Lepidoptera: Lymantriidae) following ground application of high rates of racemic disparlure. J. Econ. Entomol. 86: 815-820.

Kolodny-Hirsch, D.M., R.E. Webb, R. Olson and L. Venables. 1990. Mating disruption of gypsy moth (Lepidoptera: Lymantriidae) following repeated ground application of racemic disparlure. J. Econ. Entomol. 83: 1972-1976.

Kuenen, L.P.S. and T.C. Baker. 1981. Habituation versus sensory adaptation as the cause of reduced attraction following pulsed and constant sex pheromone pre-exposure in *Trichoplusia ni*. J. Insect Physiol. 27: 721-726.

Landolt, P.J. and R.R. Heath. 1987. Role of female-produced sex pheromone in behavioral reproductive isolation between *Trichoplusia ni* (Hübner) and *Pseudoplusia includens* (Walker) (Lepidptera: Noctuidae, Plusiinae).

Lawson, D.S., W.H. Reissig, A.M. Agnello, J.P. Nyrop and W.L. Roelofs. 1996. Interference with the mate-finding communication system of the obliquebanded leafroller (Lepidoptera: Tortricidae) using synthetic sex pheromones. Environ. Entomol. 25: 895-905.

Levesque, C.A. 1990. The nature and significance of fungal colonizers in the herbicidal effect of glyphosate. PhD. thesis, Simon Fraser University, Burnaby, B.C., Canada.

Light, D.M., and M.C. Birch. 1979. Electrophysiological basis for the behavioural response of male and female *Trichoplusia ni* to synthetic female pheromone. J. Insect Physiol. 25: 161-167.

Linn, C.E. Jr. and W.L. Roelofs. 1981. Modification of sex pheromone blend discrimination in male Oriental fruit moths by pre-exposure to (*E*)-8-dodecenyl acetate. Physiol. Entomol. 6: 421-429.

Linn, C.E., Jr. and W.L. Roelofs. 1995. Pheromone communication in moths and its role in the speciation process. In: D.M. Lambert and H. G. Spencer (eds). Speciation and the Recognition Concept: Theory and Application. The Johns Hopkins University Press, Baltimore. pp. 263-300.

Linn, C.E., Jr., J.W. Du, A. Hammond, and W.L. Roelofs. 1987a. Identification of unique pheromone components for the soybean looper moth *Pseudoplusia includens*. J. Chem. Ecol. 13: 1351-1360.

Linn, C.E., Jr., M.G. Campbell, and W.L. Roelofs. 1987b. Pheromone components and active spaces: what do moths smell and where do they smell it? Science. 237: 650-652.

Linn, C.E., Jr., A. Hammond, J. Du, W.L. Roelofs. 1988. Specificity of male response to multicomponent pheromones in noctuid moths *Trichoplusia ni* and *Pseudoplusia includens*. J. Chem. Ecol. 14: 47-57.

Liu, Y-B and K.F. Haynes. 1992. Filamentous nature of pheromone plumes protects integrity of signal from background chemical noise in cabbage looper moth, *Trichoplusia ni*. J. Chem. Ecol. 18: 299-306.

Liu, Y.-B. and K.F. Haynes. 1993a. Pheromone-mediated responses of male cabbage looper moths, *Trichoplusia ni*, following various exposures to sex pheromone or (*Z*)-7-Dodecenol. J. Chem. Ecol. 19: 203-515.

Liu, Y-B and K.F. Haynes. 1993b. Impact of (Z)-7-dodecenol and turbulence on pheromone-mediated flight manoeuvres of male *Trichoplusia ni*. Physiol. Entomol. 18: 363-371.

Löfstedt, C. 1993. Moth pheromone genetics and evolution. Phil. Trans. R. Soc. Lond. B. 340: 167-177.

Löfstedt, C., W.M. Herrebout and S.B.J. Menken. 1991. Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). Chemoecology 2: 20-28.

Madsen, H.F. and B.J. Madsen. 1980. Response of four leafroller species (Lepidoptera: Tortricidae) to sex attractants in British Columbia orchards. Can. Entomol. 112: 427-430.

Madsen, H.F. and P.J. Procter. 1985. Insects and mites of tree fruits in British Columbia. Ministry of Agriculture and Food, Victoria, B.C.

Madsen, H.F., J.M. Vakenti, and A.P. Gaunce. 1984. Distribution and flight activity of obliquebanded and threelined leafrollers (Lepidoptera: Tortricidae) in the

Okanagan and Similkameen valleys of British Columbia. Can. Entomol. 116: 1659-1664.

Mafra-Neto, A. and T.C. Baker. 1996a. Timed, metered sprays of pheromone disrupt mating of *Cadra cautella* (Lepidoptera: Pyralidae). J. Agric. Entomol. 13: 149-168.

Mafra-Neto, A. and T.C. Baker. 1996b. Elevation of pheromone response threshold in almond moth males pre-exposed to pheromone spray. Physiol. Entomol. 21: 217-222.

Mayer, D.F. and B.P. Beirne. 1974a. Aspects of the ecology of apple leaf rollers (Lepidoptera: Tortricidae) in the Okanagan Valley, British Columbia. Can. Entomol. 106: 349-352.

Mayer, D.F. and B.P. Beirne 1974b. Occurrence of apple leaf rollers (Lepidoptera: Tortricidae) and their parasites in the Okanagan Valley, British Columbia. J. Entomol. Soc. Brit. Columbia 71: 22-25.

McBrien, H.L. and G.J.R. Judd. 1998. Forecasting emergence, flight and oviposition of the eye-spotted bud moth, *Spilonota ocellana* (Lepidoptera: Tortricidae), in British Columbia. Environ. Entomol. In press.

McBrien, H.L., G.J.R. Judd, and J.H. Borden. 1998. Development of pheromone-based mating disruption for control of the eye-spotted bud moth, *Spilonota ocellana*. Entomol. Exp. Appl. In press.

McCullagh, P. and J.A. Nelder. 1983. Generalized linear models. In: D.R. Cox and D.V. Hinkley (eds.) Monographs on Statistics and Applied Probability. Chapman and Hall, New York.

McLaughlin, J.R., L.K. Gaston, H.H. Shorey, H.E. Hummel and F.D. Stewart. 1972. Sex pheromones of Lepidoptera XXXIII. Evaluation of the disruptive effect of tetradecyl acetate on sex pheromone communication in *Pectinophora gossypiella*. J. Econ. Entomol. 65: 1592-1593.

Miller, E., R.T. Staten, C. Nowell and J. Gourd. 1990. Pink bollworm (Lepidoptera: Gelechiidae): point source density and its relationship to efficacy in attracticide formulations of gossyplure. J. Econ. Entomol. 83: 1321-1325.

Miller, J.R. and W.L. Roelofs. 1978. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. J. Chem. Ecol. 4: 187-198.

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

Minks, A.K., S. Voerman, J.A. Klun. 1976. Disruption of pheromone communication with micro-encapsulated antipheromones against *Adoxophyes orana*. Entomol. Exp. and Appl. 20: 163-169.

Mitchell, E.R. 1975. Disruption of pheromonal communication among coexistent pest insects with multichemical formulations. BioScience 25: 493-499.

Mitchell, E.R. 1976. Inhibition of pheromone perception by male cabbage loopers and beet armyworms: proximity vs. atmospheric permation. Environ. Entomol. 5: 770-772.

Mitchell, E.R., J.R. Webb, and R.W. Hines. 1972. Capture of male and female cabbage loopers in field traps baited with synthetic sex pheromone. Environ. Entomol. 1: 525-526.

Miyashita, K, K, Kawasaki, K. Nakamura, Y. Uesumi and T. Sugiura. 1976. Mating suppression of *Spodoptera litura* F. (Lepidoptera: Noctuidae) in greenhosuses by a component of its sex pheromone. Appl. Entomol. Zool. 11: 364-367.

Moffit, H.R. and P.H. Westigard. 1984. Suppression of the codling moth (Lepidoptera: Tortricidae) population on pears in southern Oregon through mating disruption with sex pheromone. J. Econ. Entomol. 77: 1513-1519.

Murlis, J. and C.D. Jones. 1981. Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant sources. Physiol. Entomol. 6: 71-86.

Murlis, J., J.S. Elkinton and R.T. Cardé. 1992. Odor plumes and how insects use them Annu. Rev. Entomol. 37: 505-532.

Mustaparta, H. 1984. Olfaction. In: W.J. Bell and R.T. Cardé (eds.). Chemical Ecology of Insects. Sinauer Associates Inc., Sunderland, Massachusetts. pp. 37-70.

Mustaparta, H. 1996. Central mechanisms of pheromone information processing Chem. Senses 21: 269-276.

Noldus, L.P.J.J., R.P.J. Potting and H.E. Barendregt. 1991. Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. Physiol. Entomol. 16: 329-344.

Nordlund, D.A. and W.J. Lewis. 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. J. Chem. Ecol. 2: 211-220.

Ogawa, K. 1990. Commercial development: mating disruption of tea tortrix moths. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds). Behavior-Modifying

Chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York. pp. 547-551.

Onstad, D.W., W.H. Reissig and C.A. Shoemaker. 1985. Phenology and management of the obliquebanded leafroller (Lepidoptera: Tortricidae) in apple orchards. J. Econ. Entomol. 78: 1455-1462.

Onstad, D.W., W.H. Reissig and C.A. Shoemaker. 1986. Influence of apple cultivar, tree phenology, and leaf quality on the development and mortality of *Choristoneura rosaceana* (Lepidoptera: Tortricidae). Can. Entomol. 118: 123-132.

Or, R., M. Kehat, Ch. Chen, Z. Klein and G. Fishler. 1986. Determining the efficiency of sex pheromones in controlling the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in cotton fields in Israel. Israel J. Entomol. 20: 25-35.

Outram, I. 1970. Morphology and histology of the reproductive system of the male spruce budworm, *Choristoneura fumiferana*. Can. Entomol. 102: 404-414.

Outram, I. 1971. Aspects of mating in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Entomol. 103: 1121-1128.

Palaniswamy, P. and W.D. Seabrook. 1978. Behavioral responses of the female eastern spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) to the sex pheromone of her own species. J. Chem. Ecol. 4: 649-655.

Palaniswamy, P. and W.D. Seabrook. 1985. The alteration of calling behaviour by female *Choristoneura fumiferana* when exposed to synthetic sex pheromone. Entomol. Exp. Appl. 37: 13-16.

Palaniswamy, P. and E.W. Underhill. 1988. Mechanisms of orientation disruption by pheromone components in the redbacked cutworm *Euxoa ochrogaster* (Guenée) (Lepidoptera: Noctuidae). Environ. Entomol. 17: 432-441.

Palaniswamy, P., P. Sivasubramanian, and W.D. Seabrook. 1979. Modulation of sex pheromone perception in female moths of eastern spruce budworm, *Choristoneura fumiferana* by altosid. J. Insect Physiol. 25: 571-574.

Paterson, H.E.H. 1985. The recognition concept of species. In: E.S. Vrba (ed.). Species and Speciation, Transvall Museum, Pretoria. pp. 21-29.

Paterson, H.E.H. 1993. Variation and the specific-mate recognition system. Perspectives in Ethology, Behavior and Evolution. 10: 209-227.

Pfeiffer, D.G., W. Kaakeh, J.C. Killian, M.W. Lachance and P. Kirsch. 1993a. Mating disruption for control of damage by codling moth in Virginia apple orchards. Entomol. Exp. and Appl. 67: 57-64.

Pfeiffer, D.G., W.Kaakeh, J.C. Killian, M.W. Lachance and P. Kirsch. 1993b. Mating disruption to control damage by leafrollers in Virginia apple orchards. Entomol. Exp. and Appl. 67: 47-56.

Phelan, P.L. 1992. Evolution of sex pheromones and the role of asymmetric tracking. In: B.D. Roitberg and M.B. Isman (eds.). Insect Chemical Ecology an Evolutionary Approach. Chapman and Hall, New York. pp. 265-314.

Phelan, P.L. 1997a. Evolution of mate-signaling in moths: phylogenetic considerations and predictions from the asymmetric tracking hypothesis. In: J. C. Choe and B.J. Crespi (eds.). The Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge. pp. 240-256.

Phelan, P.L. 1997b. Genetics and phylogenetics in the evolution of sex pheromones. In: R.T. Cardé and A.K. Minks (eds.). Insect Pheromone Research New Directions. Chapman and Hall, New York. pp. 563-579.

Reissig, W.H. 1978. Biology and control of the obliquebanded leafroller on apples J. Econ. Entomol. 71: 804-809.

Reissig, W.H., M. Novak and W.L. Roelofs. 1978. Orientation disruption of *Argyrotaenia velutinana* and *Choristoneura rosaceana* male moths. Environ. Entomol. 7: 631-632.

Rice, R.E. and P. Kirsch. 1990. Mating disruption of Oriental fruit moth in the United States. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds). Behavior-Modifying chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York. pp. 193-211.

Ridgway, R. L., R. M. Silverstein and M. N. Inscoe. 1990. Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants. Marcel Dekker, Inc., New York.

Riedl, H., B.A. Croft and A.J. Howitt. 1976. Forecasting codling moth phenology based on pheromone trap catches and physiological-time models. Can. Entomol. 108: 449-460.

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

Roelofs, W.L. and A. Comeau. 1969. Sex pheromone specificity: taxonomic and

evolutionary aspects in Lepidoptera. Science 165: 398-400.

Roelofs, W.L. and J. P. Tette. 1970. Sex pheromone of the oblique-banded leafroller moth. Nature 226: 1172.

Roelofs, W.L. and A. Comeau. 1971. Sex pheromone perception: synergists and inhibitors for the red-banded leafroller attractant. J. Insect Physiol. 17: 435-448.

Roelofs, W.L. and M. A. Novak. 1981. Small-plot disorientation tests for screening potential mating disruptants. In: E.R. Mitchell (ed.), Management of Insect Pests with Semiochemicals. Plenum, New York, pp. 229-242.

Roelofs, W.L. and R.L. Brown. 1982. Pheromones and evolutionary relationships of Tortricidae. Ann. Rev. Ecol. and Syst. 13: 395-422.

Roelofs, W.L. and L. Bjostad. 1984. Biosynthesis of lepidopteran pheromones. Bioorgan. Chem. 12: 279-298.

Roelofs, W.L., A. Hill, and R.T. Cardé. 1975. Sex pheromone components of the redbanded leafroller, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae). J. Chem. Ecol. 1: 83.

Roelofs, W.L., A. Cardé, A. Hill and R.T. Cardé. 1976a. Sex pheromone of the threelined leafroller, *Pandemis limitata*. Environ. Entomol. 5: 649-652.

Roelofs, W.L., A. Cardé, R.T. Cardé, H.F. Madsen and J.M. Vakenti. 1976b. Sex pheromone of the European leafroller, *Archips rosanus*. Environ. Entomol. 5: 362-364.

Rothschild, G.H.L. 1974. Problems in defining synergists and inhibitors of the Oriental fruit moth pheromone by field experimentation. Entomol. Exp. and Appl. 17: 294-302.

Rothschild, G.H.L. 1975. Control of Oriental fruit moth *Cydia molesta* (Busck) (Lepidoptera: Tortricidae) with synthetic female pheromone. Bull. Entomol. Res. 65: 473-490.

Rothschild, G.H.L. 1981. Mating disruption of lepidopterous pests: current status and future prospects. In: E.R. Mitchell (ed.). Management of Insect Pests with Semiochemicals. Plenum, New York. pp. 207-228.

Rumbo, E.R. and R.A. Vickers. 1997. Prolonged adaptation as possible mating disruption mechanism in Oriental fruit moth, *Cydia* (=*Grapholita*) *molesta*. J. Chem. Ecol. 23: 445-457.

Rumbo, E.R., S.M. Deacon and L.P. Regan. 1993. Spatial discrimination between sources of pheromone and an inhibitor by the light-brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). J. Chem. Ecol. 19: 953-962.

Sanders, C.J. 1985. Sex pheromone as a trigger of female moth dispersal. In: C.J. Sanders, R.W. Stark, E.J. Mullins and J. Murphy (eds.). Recent Advances in Spruce Budworms Research. Proceedings of CANUSA Spruce Budworms Research. Minister of Supply and Services Canada, Ottawa.

Sanders, C.J. 1987. Flight and copulation of female spruce budworm in pheromone-permeated air. J. Chem. Ecol. 13: 1749-1758.

Sanders, C.J. 1995. Disruption of male spruce budworm orientation to female moths by pheromone and pheromone analogues in a wind tunnel. Physiol. Entomol. 89: 71-80.

Sanders, C.J. 1996. Effects of prolonged exposure to different concentrations of synthetic pheromone on mating disruption of spruce budworm moths in a wind tunnel. Can. Entomol. 128: 57-66.

Sanders, C.J. 1997. Mechanisms of mating disruption in moths. In: R.T. Cardé and A.K. Minks (eds.). Insect Pheromone Research New Directions. Chapman and Hall, New York. pp. 333-346.

Sanders, C.J. and G.S. Lucuik. 1996. Disruption of male Oriental fruit moth to calling females in a wind tunnel by different concentrations of synthetic pheromone. J. Chem. Ecol. 22: 1971-1986.

Sanders, G.E. and A.G. Dustan. 1919. The apple bud-moths and their control in Nova Scotia. Bull. 16. Dominion of Canada, Department of Agriculture, Ottawa.

SAS, 1996. SAS User's Guide: Basics, 6.03 ed., SAS Institute Inc., Cary, North Carolina.

Sauer, A.E. and G. Karg. 1998. Variables affecting pheromone concentration in vineyards treated for mating disruption of grape vine moth *Lobesia botrana*. J. Chem. Ecol. 24: 289-302.

Schmitz, V., L. Charlier, R. Roehrich, and J. Stockel. 1997. Disruption mechanisms of pheromone communication in the European grape moth, *Lobesia botrana* Den. et Schiff. IV-What is the part of absorption of pheromone by foliage? J. Appl. Entomol. 121: 41-46.

Schwalbe, C.P. and V.C. Mastro. 1988. Gypsy moth mating disruption: dosage effects. J. Chem. Ecol. 14: 581-588.

Schwalbe, C.P., E.A. Cameron, D.J. Hall, J. V. Richerson, M. Beroza, and L.J. Stevens. 1974. Field tests of microencapsulated disparlure for suppression of mating among wild and laboratory-reared gypsy moths. Environ. Entomol. 3: 589-592.

Schuh, J. and D.C. Mote. 1948. The oblique-banded leaf roller on red raspberries. Tech. Bull. 13. Oregon Agricultural Experiment Station, Oregon State College, Corvallis.

Shorey, H.H. 1977. Manipulation of insect pests of agricultural crops. In: H.H. Shorey and J. J. McKelvey, Jr. (eds.). Chemical Control of Insect Behavior Theory and Application. John Wilely and Sons, Toronto, pp. 353-367.

Shorey, H.H. and R.L. Hale. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol. 58: 522-524.

Shorey, H.H. and R.G. Gerber, 1996a. Use of puffers for disruption of sex pheromone communication of codling moths (Lepidoptera: Tortricidae) in walnut orchards. Environ. Entomol. 25: 1398-1400.

Shorey, H.H. and R.G. Gerber, 1996b. Disruption of pheromone communication through the use of puffers for control of beet armyworm (Lepidoptera: Noctuidae) in tomatoes. Environ. Entomol. 25: 1401-1405.

Shorey, H.H., L.K. Gaston and C.A. Saario. 1967. Sex pheromones of noctuid moths. XIV. Feasibility of behavioral control by disruption of pheromone communication in cabbage loopers. J. Econ. Entomol. 60: 1541-1545.

SigmaStat. 1994. SigmaStat Statistical Software for Windows, User's Manual. Jandel Scientific, San Rafael, CA.

Stadelbacher, E.A., M.W. Barry, A.K. Raina and J.R. Plimmer. 1983. Fatal interspecific mating of two *Heliothis* species induced by synthetic sex pheromone. Experientia 39: 1174-1176.

Staten, R.T., O. El-Lissy, and L. Antilla. 1997. Successful area-wide program to control pink bollworm by mating disruption. In: R.T. Cardé and A.K. Minks (eds.). Insect Pheromone Research New Directions. Chapman and Hall, New York. pp. 383-396.

Suckling, D.M. and P.W. Shaw. 1992. Conditions that favor mating disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae). Environ. Entomol. 21: 949-956.

Suckling, D.M. and P.W. Shaw. 1995. Large-scale trials of mating disruption of lightbrown apple moth in Nelson, New Zealand. N. Z. J. of Crop and Hort.

Science. 23: 123-137.

Suckling, D.M. and N.P.D. Angerilli. 1996. Point source distribution affects pheromone spike frequency and communication disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae). Environ. Entomol. 25: 101-108.

Suckling, D.M. and G.M. Burnip. 1996. Orientation disruption of *Planotortrix octo* using pheromone or inhibitor blends. Entomol. Exp. and Appl. 78: 149-158.

Suckling, D.M. and G. Karg. 1997. The role of foliage in mating disruption in apple orchards. IOBC wprs Bull. 20: 169-173.

Suckling, D.M., G. Karg, and S.J. Bradley. 1996. Apple foliage enhances mating disruption of light-brown apple moth. J. Chem. Ecol. 22: 325-341.

Taschenberg, E.F. and W.L. Roelofs. 1977. Mating disruption of grape berry moth, *Paralobesia viteana*, with pheromone released from hollow fibers. Environ. Entomol. 6: 761-763.

Thomson, D.R. 1991. The science and application of mating disruption. Proc. Wash. State Hort. Assoc. 86: 202-203.

Thomson, D.R., N.P.D. Angerilli, C. Vincent and A.P. Gaunce. 1991. Evidence for regional difference in the response of obliquebanded leafroller (Lepidoptera: Tortricidae) to sex pheromone blends. Environ. Entomol. 20: 935-938.

Thornhill, R. and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Massachusetts.

Trimble, R.M. 1993. Efficacy of mating disruption for controlling the grape berry moth, *Endopiza viteana* (Clemens) (Lepidoptera: Tortricidae), a case study over three consecutive growing seasons. Can. Entomol. 125: 1-9.

Trimble, R.M., D.J. Pree, P.M. Vickers and K.W. Ker. 1991. Potential of mating disruption using sex pheromone for controlling the grape berry moth, *Enopiza viteana* (Clemens) (Lepidoptera: Tortricidae), in Niagara Penninsula, Ontario vineyards. Can. Entomol. 123: 451-460.

Vakenti, J.M., A.P. Gaunce, K.N. Slessor, G.G.S. King, S.A. Allan, H.F. Madsen, and J.H. Borden. 1988. Sex pheromone components of the oblique-banded leafroller, *Choristoneura rosaceana* in the Okanagan Valley of British Columbia. J. Chem. Ecol. 14: 605-621.

Valeur, P.G. and C. Löfstedt. 1996. Behaviour of male oriental fruit moth, *Grapholita molesta*, in overlapping sex pheromone plumes in a wind tunnel. Entomol. Exp.

and Appl. 79: 51-59.

Van Deventer, P. and L.H.M. Blommers. 1992. Mating disruption of several leaf-feeding orchard leaf-roller species with a single sex pheromone component. Acta Phytopath. et Entomol. Hung. 24: 615-620.

Van Steenwyk, R.A. and E.R. Oatman. 1983. Mating disruption of tomato pinworm (Lepidoptera: Gelechiidae) as measured by pheromone trap, foliage, and fruit infestation. J. Econ. Entomol. 76: 80-84.

Venables, E.P. 1924. Leaf-rollers attacking orchard trees in the Okanagan Valley. Proc. Entomol. Soc. B.C. 21: 22-27.

Vickers, N.J. and T.C. Baker. 1997. Chemical communication in heliothis moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioural antagonist. J. Comp. Physiol. A. 180: 523-536.

Vickers, R.A., W.G. Thwaite, D.G. Williams and A.H. Nicholas. 1998. Control of codling moth in small plots by mating disruption: alone and with limited insecticide. Entomol. Exp. Appl. 86: 229-239.

Wagner, T.L., H. Wu, P.J.H. Sharpe and R.N. Coulson. 1984. Modeling distributions of insect development time: a literature review and application of a Weibull function. Ann. Entomol. Soc. Am. 77: 475-487.

Wall, C. and J.N. Perry. 1983. Further observations on the responses of male pea moth, *Cydia nigricana*, to vegetation previously exposed to sex-attractant Entomol. Exp. and Appl. 33: 112-116.

Wall, C., D.M. Sturgeon, A.R. Greenway and J.N. Perry. 1981. Contamination of vegetation with synthetic sex-attractant released from traps for the pea moth, *Cydia nigricana*. Entomol. Exp. and Appl. 30: 111-115.

Weakley, C.V., P. Kirsch, R.E. Rice. 1987. Control of Oriental fruit moth by mating disruption. California Agric. May-June: 7-8.

Weatherston, I. 1990. Principles of design of controlled-release formulations. In: R.L. Ridgway, R.M. Silverstein and M.N. Inscoe (eds). Behavior-Modifying chemicals for Insect Management: Applications of Pheromone and other Attractants. Marcel Dekker Inc., New York, pp. 93-112.

Webb, R.E., K.M. Tatman, B.A. Leonhardt, J.R. Plimmer, V, Keven Boyd, P.G. Bystrak, C.P. Schwalbe and L. W. Douglass. 1988. Effect of aerial application of racemic disparlure on male trap catch and female mating success of gypsy moth

(Lepidoptera: Lymantriidae). J. Econ. Entomol. 81: 268-273.

Webb, R.E., B.A. Leonhardt, J.R. Plimmer, K.M. Tatman, V.K. Boyd, D.L. Cohen, C.P. Schwalbe, and L.W. Douglass. 1990. Effect of racemic disparlure released from grids of plastic ropes on mating success of gypsy moth (Lepidoptera: Lymantriidae) as influenced by dose and by population density. J. Econ. Entomol. 83: 910-916.

Weinzierl, R. 1991. What are the alternatives in apple insect control? Transactions of Illinois State Hort. Soc. 125: 38-42.

Weissling, T.J. and A.L. Knight. 1996. Oviposition and calling behavior of codling moth (Lepidoptera: Tortricidae) in the presence of codlemone. Ann. Entomol. Soc. Am. 89: 142-147.

West-Eberhard, M.J. 1984. Sexual selection, competitive communication and species-specific signals in insects. In: T. Lewis (ed.). Insect Communication. Royal Entomol. Soc., London. pp. 283-324.

Whittaker, R.H. 1970. Plants and the chemical environment. In: E. Sondheimer and J.B. Simeone (eds.). Chemical Ecology. Academic Press, New York. pp. 71-82.

Willis, M.A. and T.C. Baker. 1984. Effects of intermittent and continuous pheromone stimulation on the flight behaviour of the Oriental fruit moth, *Grapholita molesta* Physiol. Entomol. 9: 341-358.

Witzgall, P. and E. Priesner. 1991. Wind-tunnel study on attraction inhibitor in male *Coleophora laricella* Hbn. (Lepidoptera: Coleophoridae) J. Chem. Ecol. 17: 1355-1362.

Witzgall, P., M. Bengtsson, G. Karg, A-C Backman, L. Streinz, P.A. Kirsch, Z. Blum and J. Löfqvist. 1996. Behavioral observations and measurements of aerial pheromone in a mating disruption trial against pea moth *Cydia nigricana* F. (Lepidoptera: Tortricidae). J. Chem. Ecol. 22: 191-206.

Wright, R.H. 1963. Chemical control of chosen insects. New Scientist 20: 598-600.

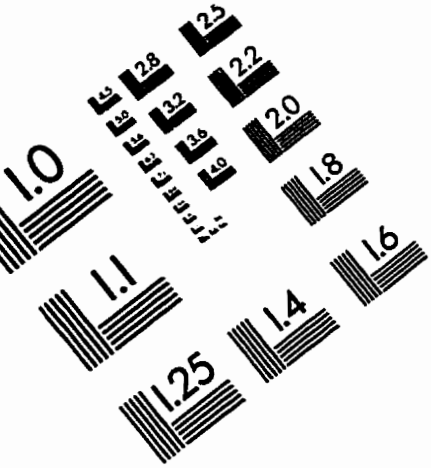
Yashima, T., Y. Tamaki, S. Kamano and M. Oyama. 1975. Suppression of mating of the armyworm moth, *Spodoptera litura* (F.), by a component of its sex pheromone. Appl. Entomol. Zool. 10: 237-239.

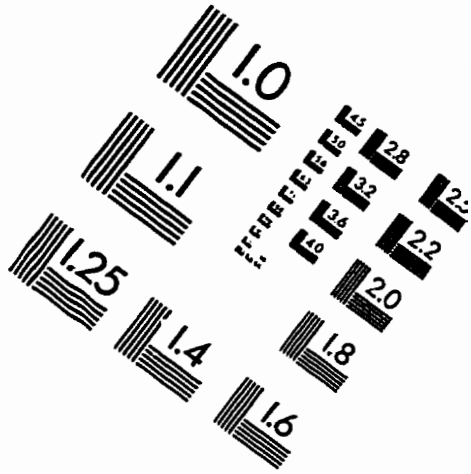
Zar, J.H. 1984. Biostatistical Analysis, 2nd ed. Prentice Hall, Englewood Cliffs.

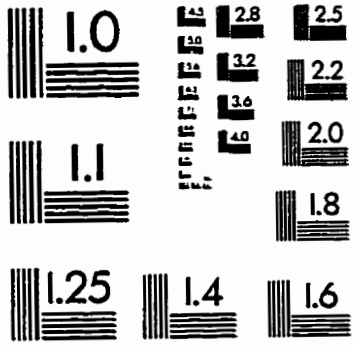
Zvirgzdins, A., P.D. Lingren, T.J. Henneberry, C.E. Nowell, and J.M. Gillespie. 1984. Mating disruption of a wild population of tobacco budworm (Lepidoptera:

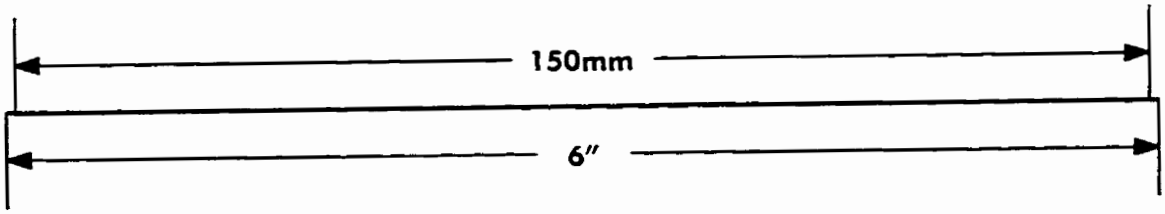
Noctuidae) with virelure. J. Econ. Entomol. 77: 1464-1469.

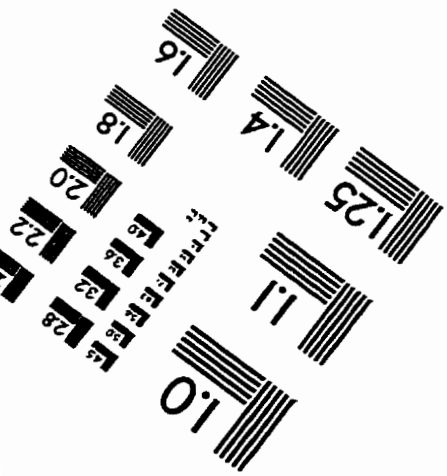
IMAGE EVALUATION
TEST TARGET (QA-3)

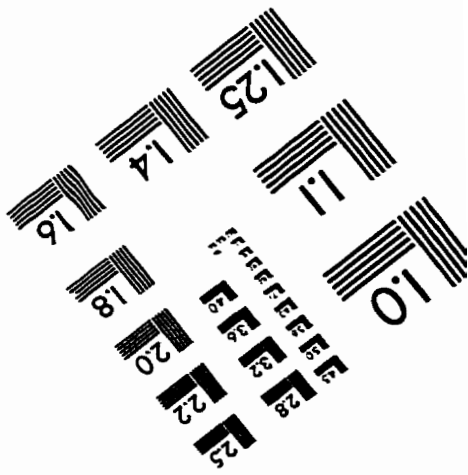












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989