University of Alberta

Behavioural Manipulation of *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) Using Microencapsulated Sex Pheromones and Horticultural Oil

by

Andreas H. Wins-Purdy 

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Environmental Biology and Ecology

Department of Biological Sciences

Edmonton, Alberta
Fall 2007

Your file Votre référence
ISBN: 978-0-494-33372-3
Our file Notre référence
ISBN: 978-0-494-33372-3

ABSTRACT

This study evaluated a microencapsulated (MEC) sex pheromone formulated with horticultural oil to control an important tree fruit pest in North America, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae). Laboratory assays showed that the addition of 2% oil to MEC pheromone marginally increased communication disruption of male *C. rosaceana* to calling virgin females. Close proximity of males to MEC treated surfaces was crucial for disruption of mate-finding behaviour that occurred mainly by nervous system habituation. In small-plot field studies, MEC formulations provided significant communication disruption for ≥ 42 days, though the addition of oil did not enhance the activity of the MEC pheromone. Residual treatments of 2% oil in water significantly reduced female reproductive output, and topical oil applications to egg masses caused 99% egg mortality. My results demonstrate the compatibility of formulating a MEC pheromone with a horticultural oil, and suggest that this strategy could fit well into an integrated management programme against tortricid pests in apple agroecosystems.

Dedication

This thesis is dedicated to my parents, Sheila and Marinus,
who have always given me their unconditional
love and support through everything.
Thanks for always being there.

ACKNOWLEDGEMENTS

This work relied on the support and encouragement from many people and organisations. First and foremost I would like to thank my supervisor Maya Evenden and my primary committee member Gary Judd for giving me the opportunity to pursue this degree and for their unwavering support from inception to completion. I feel very fortunate to have had such an excellent supervisory team who made this experience an extremely positive one. Thanks to Maya Evenden for fostering a welcoming and stimulating atmosphere for all of us in the lab; her diligence, leadership, insight, and open-door policy were all greatly appreciated. Thanks to Gary Judd for similarly welcoming me into his lab, providing me with all the tools and techniques I needed, giving sage guidance at critical times, and for his open-door policy as well. Thanks to both of you for all your considerable editorial efforts on manuscripts; they were immensely appreciated. Thanks also to my supervisory committee, Lloyd Dosdall and Andrew Keddie, who both provided ideas, enthusiasm, and support throughout the project.

This work would not have been possible without the assistance of many people. Trevor D'Arcy managed to maintain his sanity through endless hours of insect rearing, helped with experiments, and provided many a priceless moment of hijinks and hilarity. Caroline Whitehouse's hours of insect rearing were greatly appreciated as well, as was her sunny attitude and organizational ability. Mark Gardiner's unsurpassed technical abilities improved many of my experimental designs, and his ideas and help throughout were invaluable. Brent Tiffin did all of the orchard sprays for this project, Michael Weiss and Danny Shpeley provided training and equipment for digital imagery analysis, and Jack Scott made the SEM work

possible. Regine Gries conducted some chemical analyses of pheromone material. Michael Fefer of Petro-Canada graciously provided Purespray Green® oil.

Many great friends in Summerland and Edmonton alike helped me through everything. A huge thank you to Linda and Vic Jensen for being so amazingly hospitable and wonderful, without them I would have undoubtedly perished from malnutrition long ago. Nadine Hawryluk helped make the Penticton winter a blast, and Sarah Cockburn was a stabilizing force always during my stay. My fellow entomology graduate students in Edmonton enriched my experience and knowledge greatly through active discussions and friendship. I was fortunate enough to have the best labmates ever in Brad Jones and Christina Elliott, with whom I shared so many laughs and who were always there to lend a hand. Thanks always to Melissa Tesche for friendship, love, and support. I am especially grateful to my family: Sheila, Marinus, and John, for always being there and believing in me.

This research was financially supported by: the Natural Science and Engineering Research Council of Canada PGS M, the Department of Biological Sciences at the University of Alberta, the Entomological Society of Canada, and an Agriculture and Agri-Food Canada IFSP grant to G.J.R. Judd.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

EAD – Electroantennographic detection

IPM – Integrated Pest Management

MEC – Microencapsulated

PARC – Pacific Agri-Food Research Centre

RM – Repeated Measures

*Z*11-14:Ac – (*Z*)-11-tetradecenyl acetate

CHAPTER 1. INTRODUCTION

1.1 Life History of *Choristoneura rosaceana*

The obliquebanded leafroller *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) is a native, polyphagous insect that is widely distributed throughout temperate North America (Chapman and Lienk 1971). It is a generalist herbivore that can exploit a large number of woody plants, however its preferred hosts include genera in the family Rosaceae, most significantly *Malus*, *Crataegus*, *Rubus*, *Prunus*, and *Rosa* (Chapman et al. 1968). It overwinters as a third- or fourth-instar larva within a silken hibernaculum in the cracks of woody trees, and breaks diapause in the spring (Chapman et al. 1968, Gangavalli and Aliniazee 1985). Although the number of generations may vary between 1 and 3 depending on its location in North America, *C. rosaceana* is bivoltine throughout the apple growing regions of the southern Okanagan Valley in British Columbia (BC), where it is a serious pest of apples, *Malus domestica* (Borkh) (Madsen et al. 1984, Carrière 1992). Larvae from the overwintered generation break diapause to feed on developing apple shoots, and adults are typically active beginning in early June. The summer brood of larvae feed on both leaves and fruit, and generally cause the greatest amount of fruit damage (Chapman and Lienk 1971). A second adult flight occurs from about August until October. Larvae from eggs laid from this generation feed on foliage and ripening fruit in September and October, and enter diapause in response to changing daylight and temperature conditions (Gangavalli and Aliniazee 1985, Trimble and Appleby 2004).

Historically, *C. rosaceana* was considered a minor pest of apple in western North America. However, in recent years its importance has increased, and it is now considered a major secondary pest of apple in BC (Evenden et al. 1999a,b, Waldstein

et al. 2001, Trimble and Appleby 2004, Judd et al. 2005). This is partly due to the evolution of resistance to several classes of insecticides, including organophosphates (e.g. azinphos-methyl), pyrethroids (e.g. deltamethrin), and ecdysone agonists (e.g. tebufenozide) (Pree et al. 2001, Trimble and Appleby 2004). Furthermore, several area-wide pest management strategies that specifically target the key apple pest, the codling moth *Cydia pomonella* (L.), have contributed to raising the pest status of secondary moth pests such as *C. rosaceana.* Such strategies include the Sterile Insect Release programme in BC and the pheromone-based Codling Moth Area Wide Management programme in Washington State, which have decreased the use of traditional broadcast insecticides in the conventional orchards of western North America (Thomson et al. 2001). Within organic orchards in BC, a large diversity of natural enemies are present, including parasitoid Hymenoptera from the Braconidae, Ichneumonidae, Eulophidae, and Trichogrammatidae (Cossentine and Jensen 2000, Cossentine et al. 2004). Parasitoids likely play a significant role in controlling leafroller populations in organic orchards, and parasitism of the summer generation of larvae can reach levels of 68% (Cossentine et al. 2004).

Like most moths, mate-finding behaviour by *C. rosaceana* males is mediated by a female-produced sex pheromone. Females attract males by emitting a sex pheromone containing the following four compounds: the major component (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac), and three minor components (*E*)-11-tetradecenyl acetate (*E*11-14:Ac), (*Z*)-11-tetradecen-1-ol (*Z*11-14:OH), and (*Z*)-11-tetradecenal (*Z*11-14:Al) (Vakenti et al. 1998, El-Sayed et al. 2003). Although the complete natural pheromone blend emitted by the female may vary both spatially and temporally (Knight et al. 1998, El-Sayed et al. 2003), Vakenti et al. (1988) found that for western North American populations, the most attractive pheromone blend for *C.*

rosaceana on a lure consisted of *Z*11-14:Ac, *E*11-14:Ac, *Z*11-14:OH, and *Z*11-14:Al loaded in a ratio of 100:2:1.5:1.

1.2 Pheromone Perception and Processing

Male moths perceive individual pheromone molecules using specific pheromone receptor neurons located in sensilla trichodea along the antenna (Breer 1997). The sensillum trichodeum is a bristle-like structure that contains the dendrites of the sensory neurons, and the inner cavity of the sensillum is filled with an aqueous solution, the sensillar lymph (Hansson 1995, Breer 1997). The hydrophobic pheromone molecules diffuse into the interior of the sensillum, and mover through the sensillar lymph bound to a pheromone-binding protein (Breer 1997). This complex contacts the dendrites of the chemosensory neurons, and axons from sex pheromone receptor neurons project to the macroglomerular complex within the male antennal lobe. Integration of the signal occurs in the macroglomerular complex, and projection interneurons from the macroglomerular complex stimulate higher brain centres that induce mate-finding behaviours (Christensen 1997).

Pheromone plumes produced by female moths diffuse downwind in a complex, filamentous structure (Murlis et al. 1992). The pheromone blend hypothesis (*sensu* Linn et al. 1987) states that all pheromone components act as a unit to mediate attraction in males over their entire response range. Males that perceive a pheromone plume will initiate a behavioural sequence starting with wing fanning, orienting toward the source, and take off (Murlis et al. 1992). Upwind flight toward the pheromone source is maintained through optomotor anemotaxis and an internally controlled program. Optomotor anemotaxis is the process by which a male judges wind direction using visual cues to assess sideslip and maintain forward motion through the plume (Kennedy and Marsh 1974). If a male exits a plume, he arrests

forward motion and begins to cast back and forth in a zig-zag fashion, increasing the probability of re-encountering the plume. At close range, landing behaviour may be mediated by both an increase in pheromone concentration as well as appropriate visual cues that enable the male to locate the female (Murlis et al. 1992).

1.3 Pheromone-Based Mating Disruption

For many economically important lepidopteran pests, pheromone-based mating disruption has been successfully employed as a specific and effective management tool (Cardé and Minks 1995, Stelinski et al. 2003, Judd et al. 2005). Mating behaviour is disrupted through the release of synthetic pheromone into the atmosphere in sufficient quantities to interfere with one or more processes by which male moths locate females. There are several specific mechanisms by which mating disruption is thought to act, first elucidated by Bartell (1982) and later expanded upon by Cardé (1990): 1) Sensory adaptation of the antennal receptors, in which exposure to pheromone molecules reduces the firing rate of the male's antennal receptors, 2) central nervous system habituation, in which continuous exposure to pheromone molecules causes a disruption in the normal neural response to olfactory information processed in the central nervous system, 3) false trail following, a process by which males waste time and energy by following an attractive synthetic pheromone plume from a point source instead of from a calling female, and 4) camouflage of the natural source, in which atmospheric levels of a synthetic pheromone are elevated enough to render a calling female's pheromone plume undetectable by a male. Several authors (Cardé 1990, Cardé et al. 1998, Daly and Figueredo 2000, Miller et al. 2006) have pointed out that these mechanisms need not be mutually exclusive, and may act synergistically in many mating-disruption scenarios. For example, a male may follow

the pheromone plume from a high-dosage dispenser to its source, and consequently be exposed to elevated levels of pheromone that induce significant neurological effects.

Pheromone-mediated mating disruption is relatively effective for controlling some tortricid pests such as the oriental fruit moth *Grapholita molesta* (Busck) and *C. pomonella* (Cardé and Minks 1995). However, it has proven far less successful against *C. rosaceana* (Agnello et al. 1996, Lawson et al. 1996, Knight et al. 1998, Stelinski et al. 2004), although some small-plot trials have demonstrated good potential for mating disruption of *C. rosacena* in western North America (Knight et al. 1998, Evenden et al. 1999a,b). Pheromone-based mating disruption is most commonly achieved through the use of hand-applied dispensers, which usually consist of a plastic twist-tie or "rope" structure impregnated with pheromone and hung throughout the crop (Judd et al. 2005). These dispensers release large quantities of synthetic pheromone from discrete point sources throughout the crop canopy. However, sprayable, microencapsulated (MEC) pheromone formulations have been developed that may be easier and more cost-effective to apply, since they can be tank-mixed with other pesticides, foliar fertilizers, or horticultural oils. In contrast with hand-applied treatments, MEC pheromones have the advantage of thoroughly covering the crop canopy with pheromone. As male *C. rosaceana* are often found resting on leaves, close insect proximity to pheromone treated foliage may strongly impact the longevity and efficacy of MEC formulations. However, one of the disadvantages of MEC formulations is that they are generally characterized by a high initial pheromone release rate that quickly drops to low levels within several days (Hall and Marrs 1989, Polavarapu et al. 2001, Albajes et al. 2002). As well, microcapsules tend to exhibit poor retention on foliage under various environmental stresses such as rain and UV light (Knight et al. 1998, Waldstein and Gut 2004).

These two inherent shortcomings need to be improved upon for MEC formulations to become a viable and effective method to disrupt mating and achieve season-long pest control.

1.4 Horticultural Oils

Along with mating disruption, another possible integrated pest management (IPM) tool for controlling *C. rosaceana* is the use of horticultural spray oils. Various types of oils have been used for many years to directly control different orchard pests, most notably mites, scale insects, aphids, and tortricid eggs (Riedl et al. 1995). There is also some evidence that oil sprays may suppress oviposition behaviour in some insects, including *Helicoverpa punctigera* (Wallengren) (Mensah et al. 1995), *Phyllonorycter ringoniella* (Matsumura) (Sun 2002), and *Helicoverpa armigera* Hübner (Mensah et al. 2005). Benefits of horticultural oils over organophosphate insecticides include rapid biodegradation, few cases of insect resistance, low mammalian toxicity, and generally marginal negative effects on beneficial insects (Mensah et al. 1995, Agnello 2002, Fernandez et al. 2005). However, the use of horticultural oil in tree fruit IPM has been mostly limited to prebloom sprays, due to the potential for impurities in the oils to cause phytotoxic effects on both fruit and foliage (Riedl et al. 1995). In a recent review of phytotoxicity by spray oils, Hodgkinson et al. (2002) delineated between acute and chronic phytotoxicity. Acute phytotoxicity is thought to be the result of the oil causing the disruption of semipermeable foliar membranes. This damage manifests itself as burns on the plant tissue, increased sunburn damage, or premature fruit drop. Conversely, chronic phytoxicity causes stress to the plant over longer periods of time, through a disruption in plant metabolic processes such as photosynthesis, respiration, and transpiration. Both acute and chronic phytotoxicity can occur simultaneously, and are caused by the

presence of unsaturated oil molecules, namely polycyclic aromatics, phenols, and open branched alkenes (Hodgkinson et al. 2002). However, many horticultural mineral oils currently available are refined petroleum products with high standards for purity. This greatly reduces their phytotoxic effects, and makes them more suitable for summer spraying. Purified horticultural oils for summer sprays are normally combined with an emulsifying agent and sprayed in an orchard at approximately 2% active ingredient (Agnello 2002). Even with purified horticultural mineral oils, concentrations above 2% (v:v) cause phytotoxic effects in fruit trees, and are generally not suitable for post-bloom application (Riedl et al. 1995).

Spray oils are assumed to act on tortricid pests by coating eggs and suffocating the developing embryos, or by blocking the spiracular openings of the larvae and suffocating the insect (Taverner et al. 2001). As well, recent evidence suggests that certain alkanes can also influence insects at the neurological level. In another tortricid leafroller, the lightbrown apple moth *Epiphyas postvittana* (Walker), Taverner et al. (2001) found that exposing larvae to a horticultural oil caused direct nervous system disruption, bringing about rapid firing in peripheral nerves. This occurs as a result of oil penetration of nerve membranes and displacement of protective lipids, thereby increasing neural membrane permeability to ion exchange (Taverner et al. 2001). These results suggest that the potential for horticultural oils to affect leafroller behaviour through direct nervous system toxicity needs to be examined in greater detail.

1.5 Thesis Overview

This thesis explored the potential for synergy between MEC pheromones and horticultural oils for controlling *C. rosaceana*. Judd et al. (2006) found that under laboratory conditions, formulations of MEC pheromone and horticultural oil sprayed

onto metal surfaces release pheromone at a more constant rate than does MEC pheromone in water formulations. MEC pheromone formulated in oil also release more pheromone at time intervals greater than six days after application than does MEC pheromone formulated in water. As well, several studies have shown a positive effect of petroleum derived spray oils on the physical rainfastness of herbicides and insecticides on plant tissues (Taylor and Matthews 1986, Wilson 1989, Kudsk et al. 1991, Kudsk 1992). This suggests that the addition of horticultural oil to MEC formulations could enhance the mate-finding disruption efficacy of MEC formulations, with the oil further enhancing control by deterring oviposition or acting as an ovicide. My specific objectives in this thesis were:

1) To use flight-tunnel and electrophysiological assays to examine the specific mechanisms of communication disruption elicited by MEC pheromones formulated in 2% horticultural oil and water on male *C. rosaceana*, tested through direct male contact with MEC- and MEC + oil-treated surfaces.
2) To use flight-tunnel assays to test the hypothesis that MEC pheromones formulated in 2% horticultural oil and applied to metal, surrogate-leaf surfaces exhibit a stronger and more sustained disruption of male mate-finding behaviour than MEC pheromones formulated in water.
3) To use flight-tunnel and small-plot field assays to test the hypothesis that MEC pheromones formulated in 2% horticultural oil and applied to plots of apple trees exhibit a stronger and more sustained disruption of male mate-finding behaviour over time than MEC pheromones formulated in water.
4) To use laboratory bioassays to test the hypothesis that horticultural oil formulated in water affects oviposition behaviour of female *C. rosaceana* and exerts an ovicidal effect on *C. rosaceana* egg stages.

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CHAPTER 2 . MECHANISMS OF COMMUNICATION DISRUPTION IN *C. rosaceana* ELICITED BY MICROENCAPSULATED SEX PHEROMONES FORMULATED WITH AND WITHOUT HORTICULTURAL OIL

2.1 Introduction

Sprayable, microencapsulated (MEC) pheromone formulations have been investigated extensively as mating disruptants of targeted lepidopteran pests (Beroza et al. 1974, Taschenberg and Roelofs 1976, Hall and Marrs 1989, Judd et al. 2005). Several comparative studies have concluded that MEC formulations lack efficacy and longevity relative to hand-applied dispensers when applied as disruptants against several moth species, including important fruit pests like *Grapholita molesta* (Busck) (Trimble et al. 2004, Kovanci et al. 2005), *Endopiza viteana* (Clemens) (Trimble et al. 2003), and *Choristoneura rosaceana* (Harris) (Trimble and Appleby 2004). Reasons for the reduced efficacy of MEC formulations are largely unknown because few studies have specifically examined the mechanisms by which these formulations elicit communication disruption. A better understanding of how MEC pheromone formulations disrupt communication and achieve mating disruption could provide important insights for optimising this flexible technology (Knight and Larsen 2004, Stelinski et al. 2005).

The major mechanisms of mating disruption as postulated by Bartell (1982) and discussed by Cardé (1990) fall into two discrete categories, competitive vs. non-competitive (*sensu* Miller et al. 2006a). Competitive attraction, or false-plume following, occurs when males spend a portion of the female calling period orienting to and following attractive synthetic pheromone plumes from dispensers, thereby reducing the odds of successful mate location (Miller et al. 2006a). Major non-competitive mechanisms include: (1) camouflage of the female-produced pheromone plume by relatively constant atmospheric levels of synthetic pheromone; (2)

adaptation of the male peripheral nervous system via a reduction in the sensitivity of male antennal receptors; (3) habituation at any level of the male central nervous system through exposure to pheromone which leads to a decrease or cessation of appropriate sexual response; and (4) sensory imbalance of the signal processed by males as a result of the release of one or more synthetic pheromone components (Bartell 1982, Cardé 1990) released in a ratio that differs from the female-produced blend (Flint and Merkle 1983, Judd et al. 1995).

Despite precise definitions of each mechanism it is often difficult to unambiguously demonstrate the importance of one mechanism over another, as most mating-disruption technologies probably elicit more than one mechanism simultaneously (Sanders and Lucuik 1996, Cardé et al. 1998, Miller et al. 2006a,b). Under certain circumstances however, one can *a priori* eliminate the importance of competitive mechanisms and experimentally examine the importance of various non-competitive mechanisms. MEC formulations that release an incomplete or otherwise unattractive pheromone blend and are applied uniformly to crop foliage over large areas should not invoke significant competitive attraction, as a relatively diffuse cloud of unattractive pheromone is produced (Weatherston 1990, Doane 1999). In this case, disruption of male mate-finding behaviour should stem solely from non-competitive mechanisms that are concentration dependent (Miller et al. 2006a) and can be individually examined under experimental conditions.

MEC formulations exhibit characteristically high initial pheromone release rates that decline quickly, and it is this drop in release rate that is thought to reduce formulation efficacy (Hall and Marrs 1989, Polavarapu et al. 2001, Albajes et al. 2002). This observation has led to the conclusion that MEC pheromone technology fails to achieve significant disruption because it fails to produce atmospheric

concentrations of pheromone necessary to camouflage natural female plumes or invoke significant sensory effects. What has not been considered in this argument is that insects alighting or resting on foliage treated with MEC pheromone may be in continuous contact with undiluted pheromone at the leaf surface–air interface, exposing them to far greater pheromone concentrations than the average atmospheric concentration (Judd et al. 2005). It is possible the MEC pheromone formulations fail because some insects spend very little time on treated surfaces, or pheromone microcapsules are rapidly dislodged from plants even though they release adequate amounts of pheromone to cause disruption. The degree to which surface contact exposure to pheromone might induce various non-competitive mechanisms of disruption has received almost no attention (Miller et al. 2006a).

This chapter had two objectives. The first objective was to use behavioural and electrophysiological assays to examine the non-competitive mechanisms of communication disruption elicited by MEC (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac) when male *C. rosaceana* were exposed to this formulation through direct contact. *Z*11-14:Ac is the major pheromone component in the four-component blend of *C. rosaceana* (El-Sayed et al. 2003). The second objective was to examine whether adding horticultural oil as an adjuvant to a MEC formulation affected its efficacy and any communication disruption mechanisms evoked. This objective arose from the fact that the addition of horticultural oil stabilized the release rate of *Z*11-14:Ac from microcapsules over several weeks (Judd et al. 2006), potentially increasing formulation longevity. Specifically, I tested the hypothesis that formulating a MEC pheromone with a 2% horticultural oil adjuvant would result in stronger and more sustained disruption of male mate-finding behaviour over time.

2.1 Materials And Methods

2.1.1 Insects

C. rosaceana used in all assays came from a 10-year-old colony originally collected in the Okanagan and Similkameen valleys of southern British Columbia (BC), and periodically restocked with wild insects. Larvae were reared individually in 25 ml Solo® cups on a modified pinto bean-based diet (Shorey and Hale 1965) at 23°C under a LD 16:8 hr photoregime. Pupae were collected weekly, separated by sex, and placed in separate 10 litre buckets for each sex. Eclosed adults were collected each day, transferred to a different 10 litre bucket, and held under rearing conditions until assays were conducted. Care was taken to ensure that males were not exposed to pheromone before assays.

2.1.2 Treatment Formulations

3M MEC-LR® containing 20% MEC *Z*11-14:Ac by weight was obtained from 3M Canada (London, ON) and stored at 4°C between experiments. This formulation of pheromone is no longer commercially available. Purespray Green® horticultural oil (batch # 655-0602, Petro-Canada, Mississauga, ON) is a highly purified, *n*C23 mineral oil, with a molecular weight of 325, and an average boiling point of 223.9°C (ASTM D 1160) (Petro-Canada, technical data sheet). This oil is prepared with a proprietary emulsifier and approved for use by organic producers in the USA (Organic Materials Review Institute, Eugene, OR). Purespray Green® was held at room temperature before use in formulations.

2.1.3 Treatment Application and Ageing

In all flight-tunnel and electroantennographic-detection (EAD) assays, male moths were exposed to treatment formulations by resting on 8.5 cm diameter galvanized sheet metal discs (Fehling's Sheet Metal, Penticton, BC) that acted as

surrogate-leaf surfaces. Each disc received spray applications of one of the following four treatments: (1) distilled water control; (2) 2% (v:v) Purespray Green® in distilled water, prepared by adding 14 ml Purespray Green® to 686 ml distilled water; (3) MEC-LR® mixed in distilled water and applied at 1 μg a.i. cm^{-2}, prepared by adding 0.35g MEC-LR® to 700 ml distilled water; and (4) MEC-LR® applied at 1 μg a.i. cm^{-2} in a 2% (v:v) Purespray Green® plus distilled water mixture, prepared by adding 14 ml Purespray Green® to 686 ml distilled water, agitating for 10 min using a magnetic stir bar, then adding 0.35g MEC-LR®. Hereafter, these treatments are referred to as: water, oil, MEC, and MEC + oil, respectively. One hundred ml of each solution was dispensed into 160 ml glass bottles attached to hand-held, pressurized, disposable spray guns (Preval® Sprayer, Precision Valve Corporation, Yonkers, NY). For each treatment, metal discs were placed on a disposable plastic sheet within a 40 × 50 cm marked area. The area was sprayed with 20 ml of solution at a distance of 30 cm in successive horizontal and vertical transects. The resulting application rate of 1 μg a.i. cm^{-2} per disc for MEC-LR® best approximated both label-recommended field application rates of 100 g a.i. *Z*11-14:Ac ha^{-1} for MEC-LR® and 1000 L water ha^{-1} for Purespray Green®. Discs were air dried and stored in a fume hood where the sprayed formulations were allowed to age for different times before use.

2.1.4 Flight-tunnel Bioassay Protocol

All flights were conducted in a pushing-type flight tunnel based on the design of Miller and Roelofs (1978) (Fig. 2.1). The tunnel walls were constructed out of clear Lexan® bent into a horseshoe shape, with a clear Lexan floor. Below the floor was a piece of white templast (Cameron Ashley Building Products, Kelowna, BC) with an irregular pattern of dark blue dots for moth orientation during flight. The tunnel was illuminated from above by uniform, diffuse white light from six 25 W

incandescent bulbs held in a light box positioned 17 cm above the tunnel. The bottom of the light box was composed of three layers of black fibreglass screen and two layers of white templast through which the light passed. The mean light intensity in the centre of the tunnel was 1 lux. The flight section of this tunnel was 2.45 m long and 1 m high. Air was pushed uniformly through the tunnel by a variable speed fan, and exhausted directly out of the building by a downwind, centrally located exhaust fan. For all experiments, the wind speed in the centre of the tunnel was held constant at 0.3 - 0.4 m sec^{-1}, and the tunnel temperature was 22-24°C. Beside the flight tunnel was a small fume hood that drew in tunnel room air and exhausted it directly out of the building and was used to store MEC-treated surfaces before bioassays.

In each replicate of each flight-tunnel experiment, a single, calling virgin female (4- to 48-hr-old) served as the pheromone source. The female was presented in a wire mesh cage (3 cm^3), positioned on a small metal platform 45 cm above the tunnel floor and 20 cm from the upwind end. A cylindrical trap (13 cm long × 11 cm diameter) constructed from clear, 1 mm thick polyester (GE Polymershapes, Coquitlam, BC), was suspended from a ring stand directly downwind of the female's cage so that its long axis was parallel to the tunnel floor and the pheromone plume travelled directly through the centre of the cylinder. The trap was coated internally with STP Oil (First Brands Corporation, Scarborough, ON) to capture males that flew upwind in close proximity to the female.

Male moths (24- to 96-hr-old) were briefly chilled at 2°C, randomly divided into groups of 10, and each group was lightly dusted with a uniquely-coloured Day-Glo® Daylight Fluorescent Powder to identify each of the 4 different treatments (Switzer Brothers Inc., Cleveland, OH). One hr before commencing flights, a group of 10 males was placed on a treated disc in a wire mesh release cage (9 cm diameter ×

2 cm high) with a removable wire mesh lid. Males were immediately transferred into the bioassay room, placed in a small fume hood adjacent to the tunnel, and held under test conditions. This process was repeated every 15 min until all treatments were established. Flights were staggered at 15 min intervals to maintain a 1 hr treatment exposure period for each group. A clear plastic divider within the fumehood separated males on control (water- or oil-sprayed) discs from males on treatment (MEC- or MEC + oil-sprayed) discs to ensure no pheromone exposure of control moths. After each flight day for all experiments, the flight tunnel was wiped down with ethanol, and the wire-mesh release devices, female cages and female platform were rinsed with acetone and heated for ca. 12 hr at 200°C.

2.1.5 Experiment 1: Disruption of Mate-Finding Behaviour Through Continuous Exposure with a Pheromone-Treated Surface

In this experiment I tested the hypothesis that continuous male contact with surrogate leaf surfaces (i.e. metal discs) treated with MEC pheromones disrupted orientation to a calling female. This experiment also tested the hypothesis that the disruptive efficacy of the MEC treatment would decrease more rapidly over time than the MEC + oil treatment. Discs were sprayed with the 4 treatments as described above, and aged in the fume hood for 1, 6, or 13 days prior to use in the bioassay. Following the 1 hr treatment exposure, the wire mesh release cage containing the first test group of 10 males on the treated disc was introduced into the downwind end of the tunnel on a sliding cart. An externally operated line was used to lift the lid off the release device 5 sec after cage introduction. Each group of males was left to respond to the calling female for 5 min, after which the release device and trap were removed. Males caught in each trap were examined under UV light to identify their fluorescent mark, and only males bearing the appropriate mark were used in subsequent analyses.

A fresh trap was inserted after each group flight, with subsequent flights initiated every 15 min. Forty males (4 groups of 10) were tested at each treatment and disc age combination (Table 2.1).

2.1.6 Experiments 2 and 3: Disruption of Mate-Finding Behaviour After Exposure to Pheromone-Treated Discs

Experiments 2 and 3 eliminated the role of camouflage and tested the hypothesis that prolonged sensory effects from pheromone exposure caused disruption of male mate-finding behaviour. In experiment 2, males in groups of 10 were exposed for 1 hr to disc treatments as above. Discs were aged in the fumehood for increasing lengths of time (1, 6, 13, 20, 33 and 47 days) to further test the hypothesis that the disruptive efficacy of the MEC treatment would decrease more rapidly over time than the MEC + oil treatment (Table 2.1). After the 1 hr exposure, males were chilled briefly in a cooler of ice and quickly transferred to a clean release device before introduction into the flight tunnel exactly 75 sec after removal from the treated disc. A preliminary experiment showed no effect of the brief cooling protocol on pheromone response of naïve males, and the 75 sec recovery period was chosen to correspond with EAD experiments (experiments 5 and 6) that directly measured antennal receptor adaptation. Sixty to seventy males (6-7 groups of 10) were tested at each treatment and disc age combination (Table 2.1).

Experiment 3 tested the effect of a longer clean air recovery period (1 hr) after exposure to treated discs. Only discs aged in the fume hood for 6 days were used. Flight protocol was identical to experiment 2 except that after the 1 hr exposure period, chilled males were transferred to a clean release device and placed in a clear Plexiglas® box equipped with a fan that blew charcoal-filtered air over males under

bioassay conditions for 1 hr before flights. Seven groups of 10 males were tested per treatment.

2.1.7 Experiment 4: Disruption of Mate-Finding Behaviour at Increasing Times After Exposure to Pheromone-Treated Discs

In this experiment I flew males individually to examine the impact of exposure to MEC formulations on mate-finding behaviour at increasing times following exposure. Individual flights allowed for closer observation of individual moth behaviour and allowed a range of recovery periods (between 1 and 50 min) after exposure. The oil treatment was excluded from this experiment (Table 2.1) as earlier group flight assays (experiments 1-3) indicated no differences in behaviour of males exposed on oil-treated discs compared to males exposed on water-treated discs. Each replicate consisted of males (24- to 96-hr-old) exposed for 1 hr on a metal disc sprayed with either water, MEC, or MEC + oil treatments aged for 6 days. After the 1 hr exposure, the wire mesh release cages containing males on treated discs were briefly chilled in a cooler of ice and males transferred into clean, individual wire mesh release cages (3.5 cm diameter × 1.5 cm high) with a sheet metal lid. Individual release cages were positioned in the clear Plexiglas® box adjacent to the flight tunnel, where charcoal-filtered air was continually blown over them until use in the bioassay. Individual flights were initiated every 2 min, alternating males from the 3 treatments, so that males were assayed at various times between 1 and 50 min after removal from treated discs. Males were flown individually to the calling female by inserting the individual release cage into the centre of the pheromone plume on the sliding cart at the downwind end of the tunnel. Female handling and placement was identical to that of experiments 1-3, except no cylindrical trap was used. Males were given 5 sec in the plume before release. Male responses were scored as positive (+) or negative (-)

for contact with the cage housing the female (source contact). Males were quickly aspirated out of the tunnel after each flight.

2.1.8 Experiments 5 and 6: Electrophysiological Effects After Exposure to Pheromone-Treated Discs

I conducted electroantennographic-detection (EAD) assays to directly measure antennal receptor adaptation as a result of exposure to MEC- and MEC + oil-treated discs. The effect of 1- and 24-hr exposure periods to each of the four treatment formulations aged for 6 days in the fume hood on subsequent antennal sensitivity to *Z*11-14:Ac was tested in experiments 5 and 6, respectively. The EAD system consisted of an IDAC-02 data acquisition interface board, an INR-02 EAG-SSR system, and EAG 2000 software from Syntech (Hilversum, The Netherlands). Each antenna was attached to a gold-plated Syntech PRG-2 probe using a small quantity of Spectra 360 conductive gel (Parker Laboratories Inc., Orange, NJ). Pheromone loading doses consisted of neat *Z*11-14:Ac (99% purity, Pherobank, Wageningen, The Netherlands) serially diluted in HPLC-grade hexane to obtain decade solutions between 0.2 ng and 20 μg *Z*11-14:Ac μl^{-1} hexane. Fifty μl of each solution, and 50 μl of a hexane control, were pipetted individually onto 1 × 2 cm strips of folded Whatman no. 1 filter paper and allowed to evaporate in a fume hood for 5 min. As a standard, 10 μl of the plant volatile (*E*)-2-hexenal (1 μg / μl mineral oil) was also pipetted onto filter paper and allowed to evaporate. Treated strips were inserted into disposable Pasteur pipettes and allowed to equilibrate for 30 min before use. Stimulus puffs were generated with a Syntech CS-05 pulse generator with a pulse duration of 0.2 sec and flow of 10 ml sec^{-1}. EAD responses were measured as the maximum amplitude of depolarisation elicited by the stimulus applied. An initial dose-response experiment using antennae from naïve male *C. rosaceana* determined an optimal

range of antennal responsiveness to stimulus puffs between 10 and 1000 μg *Z*11-14:Ac in 50 μl hexane, therefore this stimulus range was used in subsequent experiments.

Metal discs were sprayed with 1 of the 4 treatments and aged in the fumehood for 6 days. Male moths (24- to 96-hr-old) were individually exposed in standard release devices placed in a fume hood where air was continuously drawn over them for either 1 or 24 hr (Table 2.1). After exposure, a male was removed from a treated disc, and one antenna was excised and mounted on the EAD. A stimulus puff of 10 μg *Z*11-14:Ac was administered to the antenna exactly 75 sec after exposure, and each antenna received a series of puffs delivered every 30 sec in the following order: 10 μg *Z*11-14:Ac, 10 μg (*E*)-2-hexenal, 100 μg *Z*11-14:Ac, 10 μg (*E*)-2-hexenal, 1000 μg *Z*11-14:Ac, 10 μg (*E*)-2-hexenal, hexane control, and 10 μg (*E*)-2-hexenal. The plant volatile was used to normalise antennal response over one dose series by dividing mV response to each *Z*11-14:Ac stimulus by the average mV response for (*E*)-2-hexenal for one antenna. Ten and 9 male antennae per treatment were used for the 1- and 24-hr exposure experiments, respectively.

2.1.9 Experiment 7: Microcapsule Density

Microcapsules on MEC- and MEC + oil-sprayed discs were counted to determine rates of microcapsule deposition from each formulation. The disc spraying protocol was identical to experiments 1-6, however formulations included 0.2% rose bengal dye (Aldrich Inc., Milwaukee, WI) to increase microcapsule visibility. After discs had dried, microcapsules were counted using an Olympus SZX12 stereo microscope (72× magnification, 9.1 mm^2 field of view). Six fields of view were examined per disc, with $N = 16$ discs/treatment.

2.1.10 Statistical Analyses

For both experiments 1 and 2, mean percent trap catch of males in group flights was analysed with a two-way analysis of variance (ANOVA) with formulation treatment and disc age as factors. Significant treatment differences were separated using the Student-Newman-Keuls' multiple comparison procedure. In experiment 3, mean percent trap catch of males was analysed with a one-way ANOVA followed by a Student-Newman-Keuls'test to separate significant treatment differences. Experiments 1-3 were analysed in Sigmastat® 3.0.1 (SYSTAT Software Inc., San Jose, CA). For individual flights (experiment 4), the relationship between time after exposure and male ability to make source contact was analysed separately for each treatment using logistic regression due to the binomial nature of the data (i.e. males scored as (+) or (-) for contacting the female). As both MEC-exposed and MEC + oil-exposed males showed a significant effect of time after exposure on ability to contact the female, a pooled logistic regression model with treatment (MEC and MEC + oil) as a categorical variable and time after exposure as a continuous variable was used to test the effect of treatment on male ability to contact the female. For experiments 5 and 6, differences in normalised mV antennal response between treatments at each stimulus dose were compared using a one-way ANOVA. The number of microcapsules deposited on metal discs by application of MEC and MEC + oil in experiment 7 was compared using a one-way nested ANOVA with the number of microcapsules within each sampled field of view nested within each sampled disc. Experiments 4-7 were analysed with SYSTAT 11 (SYSTAT Software Inc., San Jose, CA). All percentage data were arcsine square-root transformed prior to analysis, and significance set at $\alpha = 0.05$ for all tests.

2.2 Results

2.2.1 Experiment 1: Disruption of Mate-Finding Behaviour Through Continuous Exposure with a Pheromone-Treated Surface

In experiment 1, percent male catch in female-baited traps in the flight tunnel was significantly reduced when males were continuously exposed to pheromone while resting on MEC- or MEC + oil-treated discs as compared to control discs ($F_{3,36}$ = 74.73, $P < 0.001$). Formulation age up to 13 days after spray did not affect trap catch ($F_{2,36}$ = 1.36, P = 0.268) (Fig. 2.2). Mate finding was equally disrupted by exposure to MEC alone and MEC + oil treatments (Fig. 2.2).

2.2.2 Experiments 2 and 3: Disruption of Mate-Finding Behaviour After Exposure to Pheromone-Treated Discs

In experiment 2, when males exposed to formulations for 1 hr were introduced into the tunnel without the treated disc, the effect of different exposure treatments on mean trap catch was highly significant ($F_{3,124}$ = 95.69, $P < 0.001$) (Fig. 2.3). Mean percent trap catch over the 47-day period was not significantly different between males exposed to the water (70.4 ± 2.2%) and oil (74.4 ± 2.8%) treatments. Exposure to the MEC and MEC + oil treatments caused a significant reduction in trap catch compared to control treatments. In addition, the overall mean percent trap catch in MEC + oil-exposed males (16.8 ± 1.9%) was significantly less than MEC-exposed males (26.6 ± 3.5%) (Fig. 2.3). Experiment 2 also showed a significant effect of disc age ($F_{5,124}$ = 4.35, P = 0.001), indicating that trap catch increased across all treatments as formulations aged, but there was no significant interaction between treatment and formulation age ($F_{15,124}$ = 1.29, P = 0.215). Although pheromone release rates should have dropped considerably 47 days after applying treatments, the effect of both MEC and MEC + oil treatments on mate finding was still strong at this time (Fig. 2.3).

In experiment 3, even though males were given a 1-hr recovery period following exposure to 6-day-old formulations (Fig. 2.4), there was a significant difference in male trap catch among treatments ($F_{3,18} = 4.5$, $P = 0.016$). Mean trap catch was significantly reduced in males exposed to the MEC + oil treatment compared to males exposed to either control treatment (Fig. 2.4), whereas there was no significant difference in mean trap catch between males exposed to MEC and control treatments (Fig. 2.4).

2.2.3 Experiment 4: Disruption of Mate-Finding Behaviour at Increasing Times After Exposure to Pheromone-Treated Discs

For males exposed to water-treated discs, no significant relationship existed between flight time after exposure (1-50 min) and the probability of source contact (N = 98, slope = 0.012, $P = 0.545$). Male ability to contact the calling female increased significantly between 1-50 min after a 1-hr exposure to both MEC (N = 103, slope = 0.04, $P = 0.006$) and MEC + oil treatments (N = 95, slope = 0.04, $P = 0.010$). However, 8 of 20 MEC- and 9 of 21 MEC + oil-exposed males flown within 5 min of removal from treated discs made contact with the female. When MEC- and MEC + oil-exposed males were included in a pooled model, there was no difference in male recovery time between the two treatments (N = 198, $P = 0.349$).

2.2.4 Experiments 5 and 6: Electrophysiological Effects After Exposure to Pheromone-Treated Discs

Neither a 1- nor a 24-hr exposure period to 6-day-old formulations of MEC and MEC + oil reduced male antennal response to *Z*11-14:Ac compared to controls (Fig. 2.5A,B). Interestingly, after a 1-hr exposure, MEC-exposed males showed a small but significantly greater antennal response to the 1000 µg *Z*11-14:Ac stimulus ($F_{3,36} = 3.92$, $P = 0.016$) than did oil-exposed males (Fig. 2.5A), and after a 24-hr

exposure (Fig. 2.5B) MEC + oil-exposed males showed enhanced response to the 100 μg Z11-14:Ac stimulus ($F_{3,32} = 3.66$, $P = 0.018$).

2.2.5 Experiment 7: Microcapsule Density

Direct counts of microcapsules on sprayed discs indicated that the MEC + oil treatment deposited significantly more microcapsules on the surface of the metal disc than the MEC alone. MEC + oil-treated discs had a mean of 95.1 ± 8.2 microcapsules cm^{-2} compared to a mean of 64.8 ± 5.1 microcapsules cm^{-2} on MEC treated discs ($F_{1,30} = 4.45$, $P = 0.04$).

2.3 Discussion

My data demonstrate that surface exposure to formulations of MEC pheromones in water or oil has a strong effect on mate-finding behaviour in *C. rosaceana* over extended periods, but causes no reduction of antennal response to *Z*11-14:Ac tested immediately after exposure. Taken collectively, my behavioural and electrophysiological results suggest that habituation of the male central nervous system may be a major mechanism of disruption evoked by MEC pheromones on *C. rosaceana*, and that the disruptive effect of MEC formulations may be moderately enhanced by the addition of 2% oil.

Experiments 1 and 2 showed that exposure to MEC-LR® formulated in oil or water disrupted mate-finding behaviour compared to controls for prolonged periods, up to 47 days after application. As males in experiment 1 rested on the MEC- and MEC + oil-treated discs in the flight tunnel, reduction in mate-finding behaviour may have been due to a combination of camouflage of the female plume, peripheral nervous system adaptation, or central nervous system habituation. Sensory imbalance is probably not an important mechanism in this case as the female pheromone plume in *C. rosaceana* consists of ca. 98% *Z*11-14:Ac (El-Sayed et al. 2003), and therefore

the ratio of components would not be altered significantly by exposure to excessive amounts of Z11-14:Ac from the treated disc. Experiments 2-4 provided behavioural evidence that habituation may be an important mechanism of communication disruption for *C. rosaceana* exposed to MEC formulations, as these experiments eliminated the role of camouflage of the female plume as a potential mechanism. When males were assayed in the flight tunnel 75 sec after removal from the treated surface in experiment 2, a similar reduction in trap catch to experiment 1 was observed, indicating that the role of physical camouflage of the pheromone plume (Judd et al. 2005) may be less important once significant habituation has been induced. My finding that pheromone exposure resulted in a significant reduction in subsequent mate-finding behaviour up to one hour after exposure is consistent with previous studies on other moths species such as *G. molesta* (Figueredo and Baker 1992, Rumbo and Vickers 1997), *Trichoplusia ni* (Hübner) (Kuenen and Baker 1981, Liu and Haynes 1993) and *Heliothis virescens* (Fabricius) (Daly and Figueredo 2000). However, my results contradict those of Evenden et al. (2000), who concluded that habituation did not occur in *C. rosaceana* because a one hour exposure to atmospheric pheromone in a flight tunnel caused no reduction in subsequent male response to a calling female 10-30 min after exposure. It is possible that the method of exposure to pheromone-treated septa in a flight tunnel (Evenden et al. 2000) did not achieve sufficiently elevated pheromone concentrations to induce habituation as compared to males resting directly on a MEC-treated disc. Although Evenden et al. (2000) pre-exposed males using blends that included minor pheromone components, there is little evidence to suggest that exposure to the major component alone rather than a more complete blend should be more effective at inducing habituation.

Experiment 4 was designed to examine whether there was a distinct time after exposure at which the majority of males regained the ability to locate the female. Both MEC- and MEC + oil-exposed males exhibited a significant positive relationship between time after exposure and probability of contacting the female. This indicates that a pheromone-exposed male, given a 50 min recovery period, is more likely to locate the female than a pheromone-exposed male given a one min recovery period. However, this relationship was not as strong as expected given the results from experiments 2 and 3. This suggests that the ability to recover from pheromone exposure and the time necessary for successful recovery varies considerably among individual male *C. rosaceana*. In experiment 4, 17 of 40 males (42.5%) flown within five min of removal from MEC and MEC + oil-treated discs made source contact with the female, indicating that a significant proportion of male *C. rosaceana* are able to recover rapidly, perhaps within minutes of returning to clean air.

The results of the electrophysiological experiments also indicate that central nervous system habituation is more important as a mechanism of mating disruption in *C. rosaceana* than antennal receptor adaptation once males have returned to clean air. No significant antennal adaptation was found when EAD assays were performed 75 sec after removal of males from treated discs. This result is generally consistent with previous studies that showed a threshold concentration of 500 pg *Z*11-14:Ac ml^{-1} air was necessary to induce significant "long-lasting" antennal adaptation in male *C. rosaceana* (Stelinski et al. 2003a,b). Although it is difficult to estimate the exact concentration of *Z*11-14:Ac that a male would be exposed to while resting on a surface treated with MEC-LR®, release rates of *Z*11-14:Ac from MEC-LR® in water and oil at six days post-spray were ≈ 8 pg *Z*11-14:Ac cm^{-2} min^{-1} (Judd et al. 2006), a

rate which is well below that necessary for the induction of long-lasting adaptation in *C. rosaceana* (Stelinski et al. 2003a,b). Interestingly, my EAD experiments showed a marginal increase in antennal response to a stimulus of *Z*11-14:Ac following exposure to MEC-LR® formulations, which could be a result of antennal sensitisation similar to that described by Stelinski et al. (2003c).

The addition of oil to the MEC formulation moderately enhanced disruption over time (Fig. 2.3). Using a similar disc spraying protocol, Judd et al. (2006) found that MEC-treated discs released *Z*11-14:Ac at a rate of ≈ 50 pg cm^{-2} min^{-1} at day 1, ≈ 8 pg cm^{-2} min^{-1} at day 6, and a relatively constant rate of ≈ 2 pg cm^{-2} min^{-1} between day 13 and 30. In contrast, MEC + oil-treated discs released *Z*11-14:Ac at a rate of ≈ 5 pg cm^{-2} min^{-1} at day 1, ≈ 8 pg cm^{-2} min^{-1} at day 6, and a relatively constant rate of ≈ 8 pg cm^{-2} min^{-1} between day 13 and 30 (Judd et al. 2006). The increased efficacy of the MEC + oil treatment compared to the MEC treatment over the 47-day period (Fig. 2.3) could be due to an elevated and sustained rate of pheromone release after day 13 (Judd et al. 2006). The greater efficacy of the MEC + oil treatment could also be attributed to the increased number of microcapsules deposited on the discs found in this study. Although the reasons for this remain unclear, it could be a result of the oil increasing the deposition of spray droplets by affecting the surface tension properties and spreading coefficient of the spray (Anderson et al. 1987, Streibig and Kudsk 1992, McWhorter et al. 1993). As both pheromone treatments showed a relatively strong disruptive effect up to 47 days after application, a test of lower pheromone concentration in water and oil formulations may delineate potential differences in efficacy between the two formulations. Although the possibility exists that the oil alone had a physiological effect on males, response of males to female-produced plumes did not vary between groups of control males exposed to oil or water alone in

experiments 1-3. Although exposure to horticultural oil has been shown to cause nervous system disruption in Lepidoptera (Taverner et al. 2001), there was no evidence to indicate that exposure to 2% oil alone affected mate-finding behaviour or EAD response in *C. rosaceana.*

After a one-hour recovery period, the mate-finding ability of MEC-exposed males was no different than for control males, while the mate-finding ability of MEC + oil-exposed males was still significantly reduced (Fig. 2.4). As pheromone release rates should have been nearly equal from MEC and MEC + oil discs aged 6 days (Judd et al. 2006), disruption after the disc has been removed in this case may be affected by small amounts of pheromone absorbed into male moth tarsi as a result of direct physical contact with the treated discs. Although the difference between MEC and MEC + oil treatments in experiment 3 was small, it could potentially be attributable to the oil enhancing disruption through increased physical absorption of pheromone by the male (Krupke et al. 2002, Evenden et al. 2005).

The 47-day period of sustained treatment efficacy in this study is in sharp contrast to the results of Judd et al. (2005), who found that MEC-LR® applied as an atmospheric treatment at the upwind end of a flight tunnel became an ineffective disruptant of mate finding in *C. rosaceana* only 54 hours after application. Although Judd et al. (2005) did not incorporate a pheromone exposure period before the assay started, the degree of formulation longevity in this study highlights the role that male proximity to the treated surface may play in maintaining a significant disruptive effect even when pheromone release rates from microcapsules drop to low levels. As changes in pheromone concentration should vary with the inverse square root of distance from source (Karg et al. 1994), pheromone concentrations < 1 cm from the treated surface, where an insect may alight or rest, may be many times greater than

the average atmospheric concentration throughout a treated area. Therefore, these results reveal the importance of male exposure by close contact with the MEC-treated surfaces for communication disruption vs. a background treatment regime (Evenden et al. 2000, Judd et al. 2005).

The importance of competitive attraction has often been stressed in mating disruption regimes employing attractive dispensers (Miller et al. 2006a,b), but the potential interaction between competitive and non-competitive mechanisms of disruption is often overlooked. Using attractive dispensers, habituation may be more effectively induced in males approaching and landing on high-dosage dispensers and "bathing" themselves in unnaturally elevated levels of pheromone (Miller et al. 2006a,b), than in quiescent males resting on untreated surfaces. Using MEC pheromones, my results indicate that habituation may be effectively induced in males resting on uniformly sprayed surfaces. However, in a field situation, this habituation may dissipate within minutes in males that have taken flight and "escaped" the boundary layer of high pheromone concentration, and are subsequently exposed only to lower atmospheric concentrations of synthetic pheromone.

The reduced efficacy and longevity of MEC formulations compared to dispensers is often ascribed to a rapid initial "burst" of pheromone from microcapsules, however the mechanism by which each tactic induces habituation may also play a role. Male exposure to synthetic pheromone in a dispenser-treated crop canopy should occur in a more spatially- and temporally-intermittent fashion. Spatially, plumes will vary in concentration as a function of various factors such as distance from dispenser, wind speed and direction, canopy type, and male height in canopy. Temporally, males should be exposed to much higher concentrations of synthetic pheromone during scotophase, as they actively search for females and

approach pheromone dispensers, than during the non-active period of the diel cycle. This compares to a MEC-treated orchard, where a relatively uniform distribution of microcapsules over foliage should result in much greater spatial homogeneity of pheromone concentrations, especially in the "boundary" layer on foliage where a male may rest and be exposed to a consistently high level of pheromone during the non-active period of the diel cycle. As several studies have shown that pulsed or intermittent pheromone exposure is more effective at inducing nervous system habituation than constant exposure (Bartell and Lawrence 1977a,b, Kuenen and Baker 1981), a fundamental difference in how habituation is achieved by competitive vs. non-competitive disruption applications could partly explain why hand-applied dispensers tend to outperform MEC formulations in the field.

C. rosaceana is a difficult pest to successfully control through mating disruption alone, though the reasons for this remain unclear (Agnello et al. 1996, Lawson et al. 1996, Knight et al. 1998, Stelinski et al. 2003a, Trimble and Appleby 2004). Evenden et al. (2000) concluded that habituation did not occur in this species as a result of exposure to their atmospheric pheromone treatment, and Stelinski et al. (2004) demonstrated an increase in male responsiveness to pheromone 24 hours after pheromone exposure. Therefore, the current study is the first to demonstrate significant nervous system habituation in *C. rosaceana.* However, it is worthwhile to note that despite the strong effects of a one hour exposure to MEC- and MEC + oil-treated surfaces on the majority of males, in none of the trials did pheromone exposure completely eliminate response of all males to calling virgin females. Even with males resting on formulations aged for one day in experiment 1, 7 out of 80 males were able to detect the female-produced pheromone plume, fly upwind, and make source contact. This reinforces the suggestion that a small proportion of males

does not habituate to a significant degree and can successfully locate a female. Stelinski et al. (2003a) suggested that significant long-lasting antennal adaptation found in *C. rosaceana* could act to shield the central nervous system from the effects of habituation and allow some males to overcome mating disruption. While this seems an unlikely explanation for my results given the lack of any significant antennal adaptation after exposure, it is plausible that transient adaptation of antennal receptors while males rested on the MEC-LR®-treated surface played a role in shielding some males from significant habituation. Transient antennal adaptation was demonstrated in *T. ni* (Kuenen and Baker 1981), and would allow for a rapid recovery of sexual behaviour once the male leaves an area of high pheromone concentration, such as a treated leaf or near a high-dosage dispenser. Electrophysiological data from male moths in close proximity to a MEC-treated surface combined with flight-tunnel assays would help to elucidate the relationship between transient antennal adaptation and habituation in this species.

The results of the flight-tunnel assays suggest a moderate enhancement of disruption of *C. rosaceana* by adding 2% Purespray Green® to the MEC-LR® formulation, and show that the addition of an oil adjuvant may be a simple approach to increasing MEC formulation efficacy and longevity. In chapter 3, I examine whether adding 2% Purespray Green® to the MEC-LR® formulation similarly enhances disruption when applied to foliage.

Table 2.1 Bioassay description for flight-tunnel and electroantennographic-detection (EAD) assays conducted on male *C. rosaceana* in Experiments 1-6.

Exp	Assay	Treatment (Tmt)[a]	Formulation Age[b] (day)	♂s tested per Tmt/Age Combination	Exposure Time[c]	Recovery Time[d]
1	Group Flights	Water	1, 6, 13	40	60 min	None
		Oil	1, 6, 13	40	60 min	None
		MEC	1, 6, 13	40	60 min	None
		MEC + oil	1, 6, 13	40	60 min	None
2	Group Flights	Water	1, 6, 13, 20, 33, 47	60-70	60 min	75 sec
		Oil	1, 6, 13, 20, 33, 47	60-70	60 min	75 sec
		MEC	1, 6, 13, 20, 33, 47	60-70	60 min	75 sec
		MEC + oil	1, 6, 13, 20, 33, 47	60-70	60 min	75 sec
3	Group Flights	Water	6	70	60 min	60 min
		Oil	6	70	60 min	60 min
		MEC	6	70	60 min	60 min
		MEC + oil	6	70	60 min	60 min
4	Individual Flights	Water	6	98	60 min	1-50 min
		MEC	6	103	60 min	1-50 min
		MEC +oil	6	95	60 min	1-50 min
5	EAD	Water	6	10	60 min	75 sec
		Oil	6	10	60 min	75 sec
		MEC	6	10	60 min	75 sec
		MEC + oil	6	10	60 min	75 sec
6	EAD	Water	6	9	24 hr	75 sec
		Oil	6	9	24 hr	75 sec
		MEC	6	9	24 hr	75 sec
		MEC + oil	6	9	24 hr	75 sec

[a] Treatment formulations: 1)Water = distilled water; 2) Oil= 2% Purespray Green® in water; 3) MEC= MEC-LR® applied at 1 μg a.i.$^{-1}$ cm^{-2}; 4) MEC + oil= MEC-LR® at 1 μg a.i.$^{-1}$ cm^{-2} + 2% Purespray Green®. [b] Formulation age after spray application on disc at time of moth exposure. [c] Time spent by each group of male moths on treated discs prior to the initiation of the bioassay. [d] Time spent by each group of male moths in clean air after removal from treated discs prior to the initiation of the bioassay.

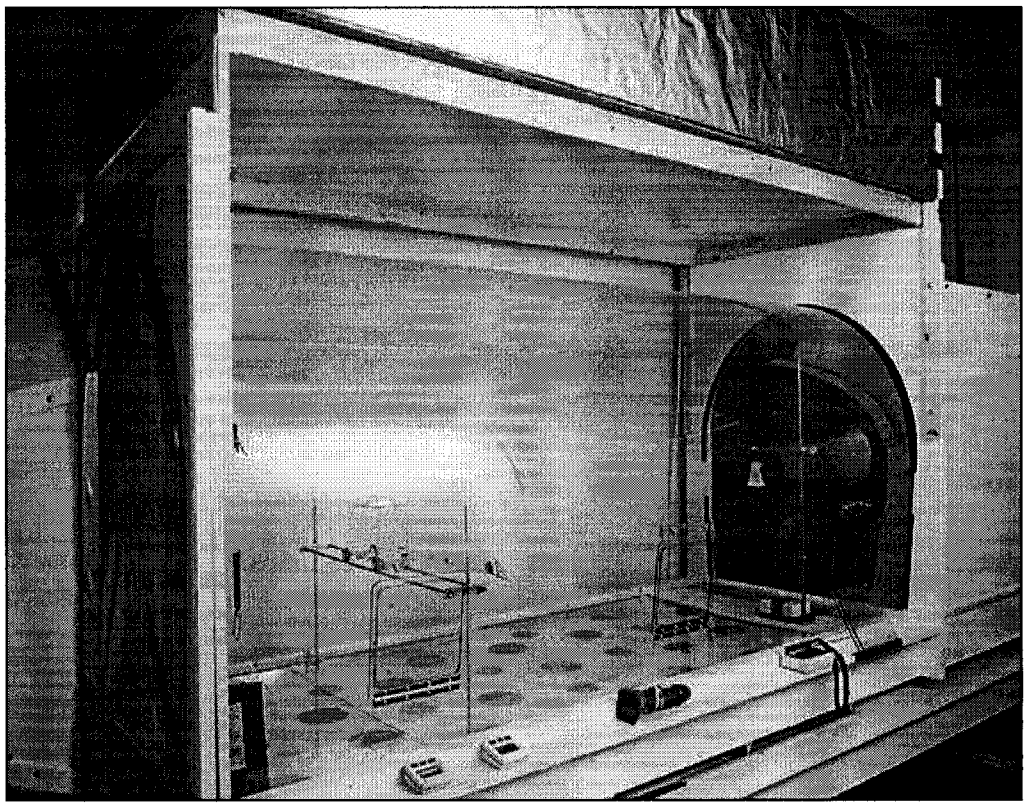

Figure 2.1 Pushing-type flight tunnel used in all flight-tunnel bioassays.

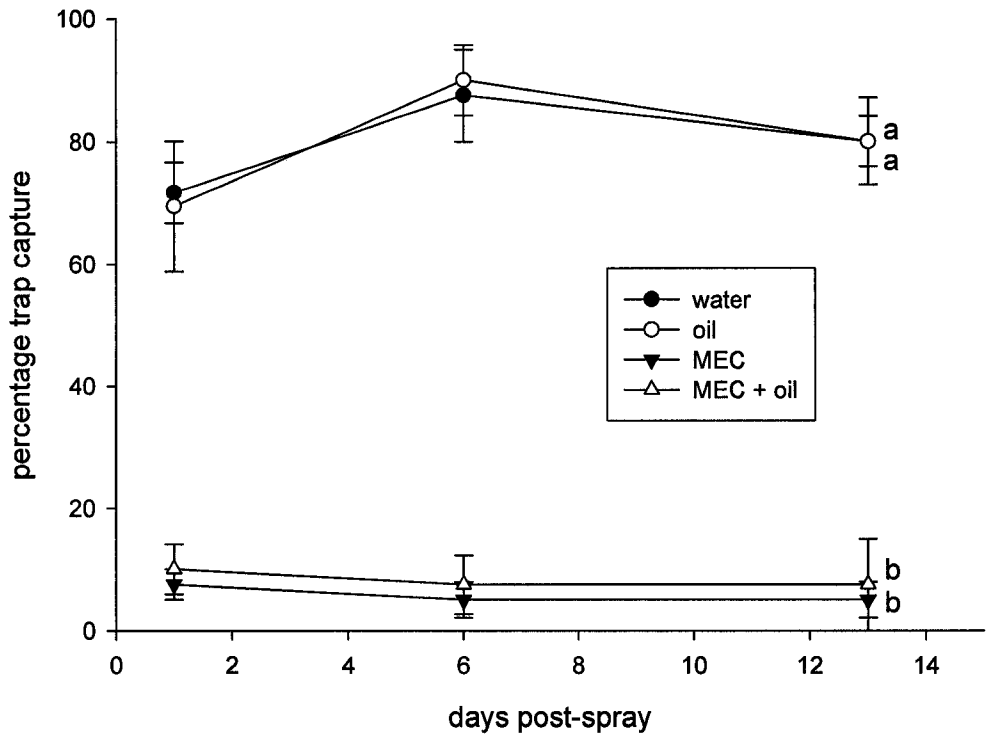

Figure 2.2 Mean (± SE) percentage of male *C. rosaceana* caught in female-baited traps in a flight tunnel in Exp. 1 following a 1 hr exposure on a metal disc sprayed with either water, 2% Purespray Green® (oil), MEC-LR® applied at 1 μg a.i.$^{-1}$ cm^{-2} (MEC), or MEC-LR® at 1 μg a.i.$^{-1}$ cm^{-2} + 2% Purespray Green® (MEC + oil). Groups of 10 males were introduced into a female-produced pheromone plume while resting on treated discs, with formulations aged on discs for 1, 6, or 13 days ($N = 4$ groups of 10 males/treatment). Treatments with different letters are significantly different by Student-Newman-Keuls' multiple comparison test following significant ANOVA ($P < 0.05$).

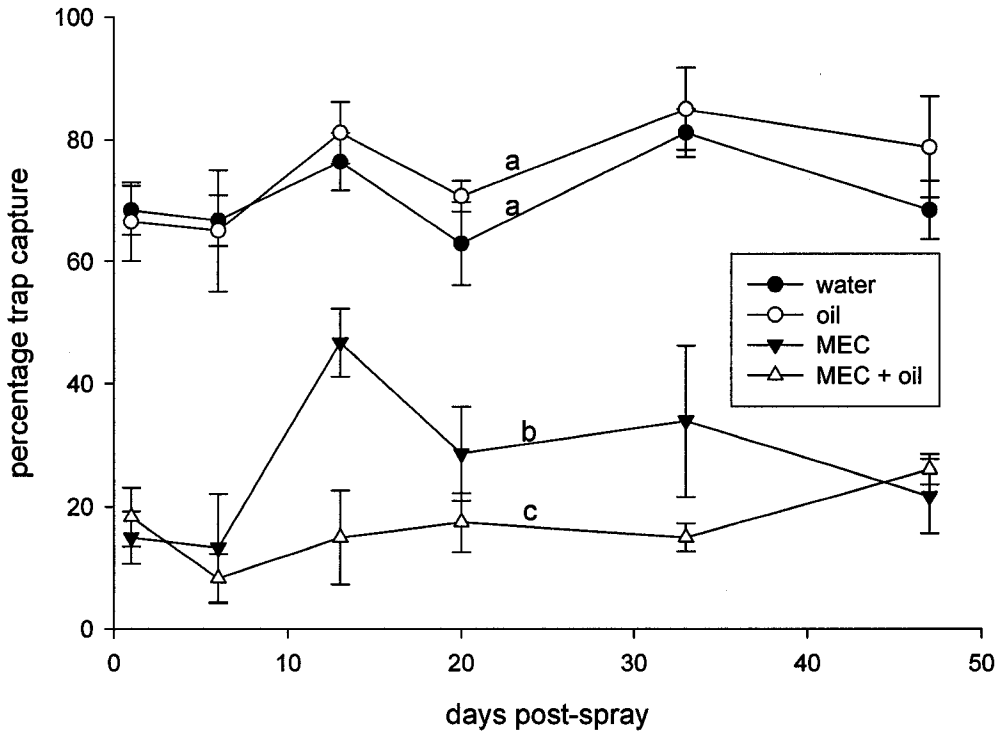

Figure 2.3 Mean (± SE) percentage of male *C. rosaceana* caught in female-baited traps in Exp. 2. Males were released 75 sec after a 1 hr treatment exposure on a metal disc sprayed with either water, 2% Purespray Green® (oil), MEC-LR® applied at 1 μg a.i.$^{-1}$ cm^{-2} (MEC), or MEC-LR® at 1 μg a.i.$^{-1}$ cm^{-2} + 2% Purespray Green® (MEC + oil). Formulations were aged on discs for 1, 6, 13, 20, 33, or 47 days (N = 6-7 groups of 10 males/treatment). Treatments with different letters are significantly different by Student-Newman-Keuls' multiple comparison test following significant ANOVA ($P < 0.05$).

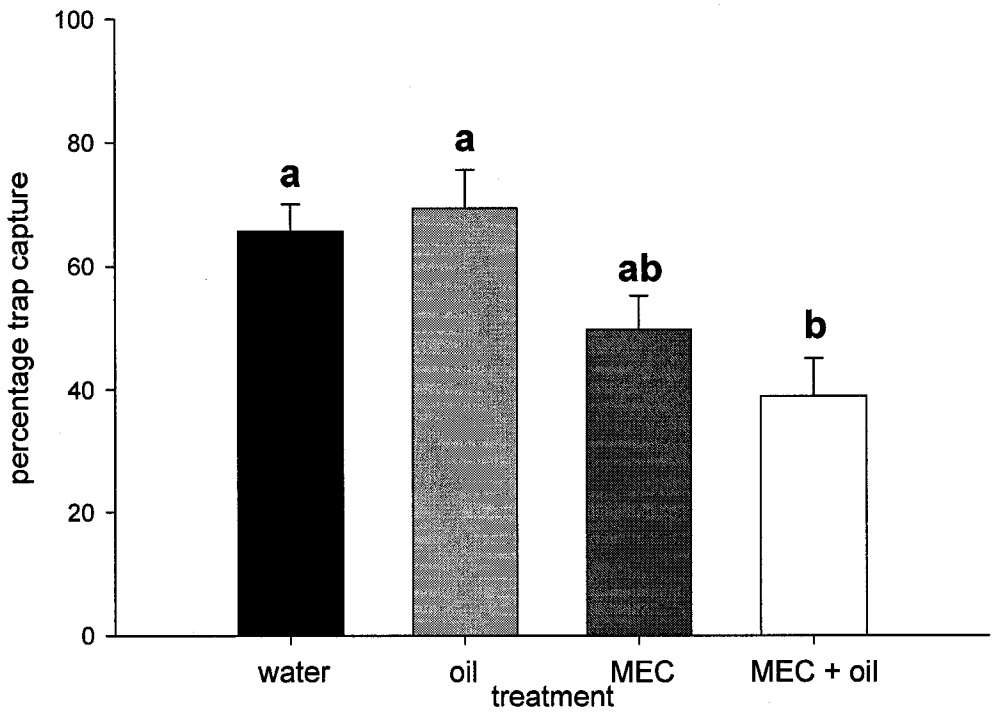

Figure 2.4 Mean (+ SE) percentage of male *C. rosaceana* caught in female-baited traps in Exp. 3 tested 1 hr after a 1 hr exposure on a metal disc sprayed with either water, 2% Purespray Green® (oil), MEC-LR® applied at 1 μg a.i.$^{-1}$ cm^{-2} (MEC), or MEC-LR® at 1 μg a.i.$^{-1}$ cm^{-2} + 2% Purespray Green® (MEC + oil). Formulations were aged on discs for 6 days (N = 7 groups of 10 males/treatment). Means with a different letter are significantly different by Student-Newman-Keuls' multiple comparison test following significant ANOVA ($P < 0.05$).

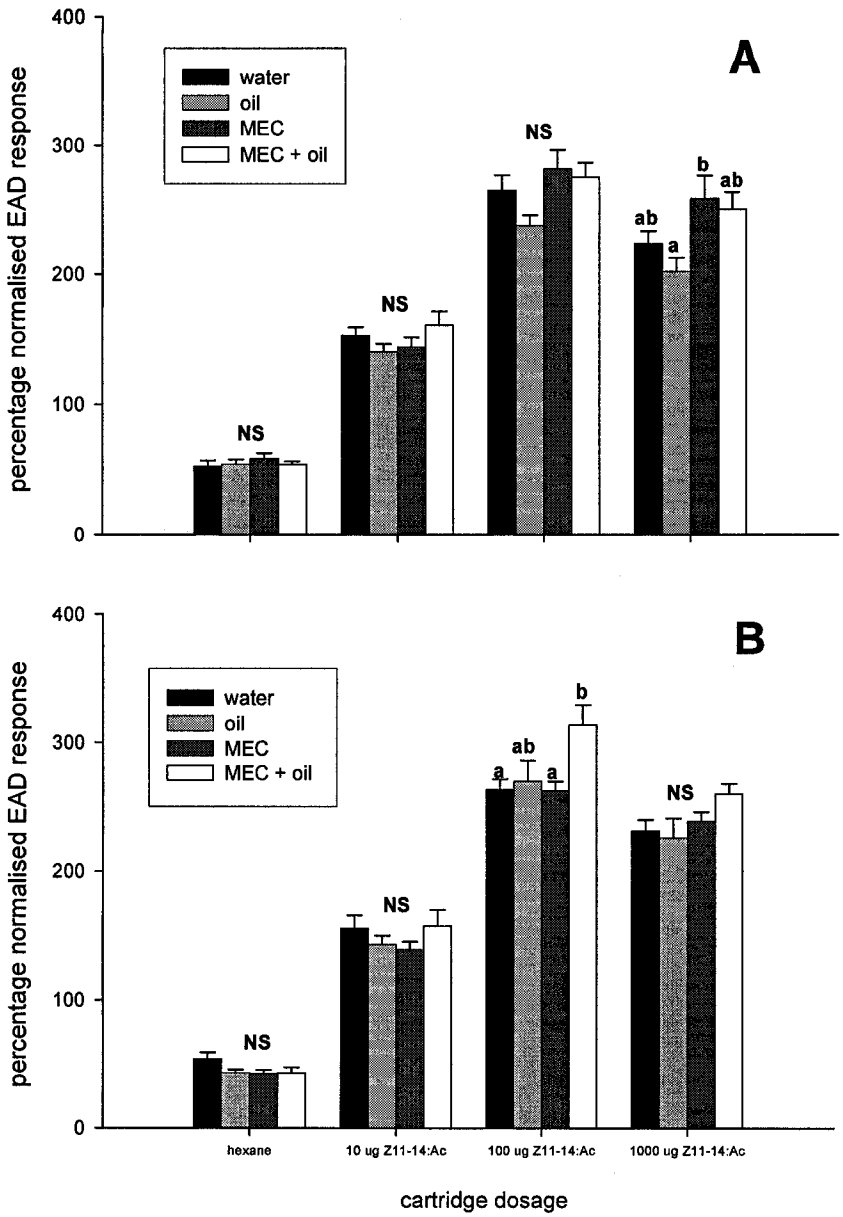

Figure 2.5 Mean (+ SE) percentage normalised EAD responses generated from excised antennae of male *C. rosaceana* assayed 75 sec after a (A) 1 hr or (B) 24 hr exposure on a metal disc sprayed with either water, 2% Purespray Green® (oil), MEC-LR® applied at 1 μg a.i.$^{-1}$ cm^{-2} (MEC), or MEC-LR® at 1 μg a.i.$^{-1}$ cm^{-2} + 2% Purespray Green® (MEC + oil) treatments. Formulations were aged on discs for 6 days before male exposure treatments, N = 9-10 antennae/treatment. Means within each cartridge dose loading with a different letter are significantly different by Student-Newman-Keuls' multiple comparison test following significant ANOVA (P < 0.05).

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CHAPTER 3 . DISRUPTION OF PHEROMONE COMMUNICATION OF *C. ROSACEANA* USING MICROENCAPSULATED SEX PHEROMONES FORMULATED WITH HORTICULTURAL OIL

A version of this chapter was accepted June 29, 2007 for publication in Environmental Entomology

3.1 Introduction

Historically, *Choristoneura rosaceana* (Harris) has been considered a minor pest of apple *Malus domestica* (Borkh), but in recent years its importance has increased to the point that it is now considered a major secondary pest of apples throughout the temperate fruit growing regions of North America (Lawson et al. 1996, Waldstein et al. 2001, Trimble and Appleby 2004). Broad-spectrum insecticides have traditionally been used to control *C. rosaceana*, but with increasing concerns about environmental impact and development of resistance to insecticides, the need for effective, environmentally sound control methods compatible with integrated pest management (IPM) programmes has increased.

Pheromone-based mating disruption has been successfully employed as a specific and effective management tool for many economically important Lepidoptera (Cardé and Minks 1995, Stelinski et al. 2003, Judd and Gardiner 2004). Although hand-applied dispensers are currently the most widely used technology method, microencapsulated (MEC) pheromone formulations represent a promising alternative (Polavarapu et al. 2001, Albajes et al. 2002, Judd et al. 2005, Il'ichev et al. 2006). MEC pheromone formulations have several distinct advantages over hand-applied dispensing systems: (1) easy application reduces labour costs associated with hanging dispensers (Waldstein and Gut 2004, Kovanci et al. 2005a), (2) tank mixing with compatible pesticides, fertilizers, or horticultural oils reduces frequency of spraying (Waldstein and Gut 2004), and (3) easily adjustable application rates make them

suitable as late-season supplements to enhance existing, hand-applied mating disruption (Kovanci et al. 2004).

Despite the advantages of MEC formulations, they are consistently outperformed by hand-applied dispensers with comparable initial pheromone loads. Sprayable formulations targeting the oriental fruit moth *Grapholita molesta* (Busck) were effective for 4 weeks but were significantly less effective than hand-applied dispensers for reducing trap catch 4 to 8 weeks after treatment (Kovanci et al. 2005b). Similarly, Trimble et al. (2004) concluded that lower pheromone release rates from *G. molesta* sprayable formulations over the course of the growing season were likely responsible for reduced efficacy compared to hand-applied dispensers. Mating disruption of *C. rosaceana* in orchard blocks treated with MEC formulations ranged from 50-80% trap catch reduction, whereas disruption in blocks treated with hand-applied dispensers ranged from 84-98% trap catch reduction (Trimble and Appleby 2004). Although Trimble and Appleby (2004) did not specifically examine the longevity of the sprayable formulation against *C. rosaceana*, the authors concluded that enhanced field longevity would significantly increase the efficacy of MEC pheromones.

The release-rate properties of MEC formulations often limit their effectiveness and longevity in the field. MEC pheromone formulations are generally characterized by a high initial release rate that quickly drops within several days and flattens out at low levels (Hall and Marrs 1989, Polavarapu et al. 2001, Albajes et al. 2002). Rapid loss of pheromone under field conditions is likely compounded by photodegradation of both pheromone and the microcapsule polymer wall upon exposure to ultraviolet light (Waldstein and Gut 2004). Reduced longevity of these MEC formulations means they often require multiple applications, unlike hand-applied dispensers, which

release pheromone at a stable and elevated rate for up to 120 days (Trimble et al. 2004, Il'ichev et al. 2006). A second factor affecting the efficacy and longevity of MEC pheromones is the poor retention of microcapsules on crop foliage, especially if overhead irrigation is employed (Knight et al. 2004). If male proximity to the sprayed surface does play a significant role in mating disruption (Chapter 2), then good retention of microcapsules on foliage could be important to improving the efficacy of sprayable formulations. However, exposure to rainfall has been shown to significantly reduce microcapsule density on apple foliage. Waldstein and Gut (2004) found that rainfall greater than 10 mm significantly reduced microcapsules containing *G. molesta* pheromone on apple leaves in the field by up to 76%. Similarly, Knight et al. (2004) found that increasing both the intensity and duration of simulated rainfall under lab conditions increased the removal of microcapsules from apple leaves. Although adjuvants such as spray stickers are commercially available, they only moderately improve the rainfastness of pheromone microcapsules on leaves (Knight et al. 2004).

One possibility for improving both the release-rate properties and rainfastness of MEC pheromones might be the use of light-grade horticultural oil as a spray adjuvant. Horticultural oils are used extensively as adjuvants for many pesticides and fungicides (Lasota and Dybas 1991, Steurbaut 1993, Taverner et al. 2001, Rae 2002, Zabkiewicz 2002), but to the best of my knowledge have not been tested with MEC pheromones. The use of horticultural oil in pest management has a long history (Agnello 2002), and is increasingly finding a place in current apple IPM programmes (Fernandez et al. 2005). Given the increasing use of horticultural oil in tree fruit pest management it would be useful to know whether it is compatible with MEC pheromone, and whether disruption of pheromone communication and mating by

application of MEC pheromone might be improved by combining it with horticultural oil. Judd et al. (2006a) found that formulations of MEC pheromone and horticultural oil release pheromone at a more constant rate than do water-only formulations, and in laboratory trials release more pheromone at time intervals greater than six days after application. I tested whether this elevated and sustained release-rate profile of oil-formulated MEC pheromones in the laboratory translated into improved field efficacy for disrupting pheromone communication in *C. rosaceana.*

The objective of this chapter was to examine and compare the relative level and longevity of disruption caused by MEC pheromones formulated with and without horticultural oil after their application to plots in apple orchards. Using both field trapping and laboratory flight-tunnel bioassays, I tested the hypothesis that a sprayable formulation consisting of horticultural oil and MEC pheromone would disrupt mate-finding behaviour of male *C. rosaceana* more effectively, and for a longer period of time, than a formulation consisting of MEC pheromone alone.

3.2 Materials and Methods

3.2.1 Insect Cultures

All experiments were conducted using a laboratory colony of *C. rosaceana* originally collected in the Okanagan and Similkameen Valleys of British Columbia (BC), and maintained since 1995. The colony was infused prior to this study with approximately 200 wild insects collected as larvae from apple orchards in the Similkameen and Okanagan Valleys. Insects were reared on a modified pinto bean-based diet (Shorey and Hale 1965) at 23°C under a 16:8 hr L:D photoregime in a controlled-environment chamber. Pupae were separated by sex and placed in separate 10 litre plastic buckets. For flight-tunnel experiments, emerged adults were collected daily, placed in different 10 litre plastic buckets, and held under rearing

conditions until needed. Males were separated from females to ensure no pheromone exposure prior to experiments. For field experiments, emerged adults were collected daily, put in 10 litre buckets, provisioned with a cotton wick containing water, and placed in a shaded area near one of the test orchards to acclimatise to field conditions.

3.2.2 Pest Management Products.

3M MEC-LR®, a formulation of microencapsulated (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac) was provided by 3M Canada (London, ON) and stored at 4°C until use. 3M MEC-LR® constitutes 20% *Z*11-14:Ac by weight, and was prepared for field spraying by tank-mixing 35 g of commercial product in 100 litres of water. Purespray Green® horticultural oil (Batch # 655-0602, Petro-Canada, Mississauga, ON) is a highly purified, *n*C23 horticultural mineral oil prepared with an emulsifier that allows mixing in water (emulsifier used is proprietary information of Petro-Canada). This oil was chosen because of its organic certification (Organic Materials Review Institute, Eugene, OR) and high purity (>99.9% paraffin content), which reduces the risk of phytotoxicity and makes it suitable for summer use when applied at concentrations of 2% or less. Purespray Green® was diluted to a concentration of 2% in water (v:v) by tank-mixing 2 litres of the commercial oil in 98 litres of water.

3.2.3 Field Sprays

Three experimental, high-density apple orchards at the Pacific Agri-Food Research Centre (Agriculture and Agri-Food Canada, Summerland, BC) were used in this study. These orchards received no synthetic insecticide sprays in 2006. All three orchards were irrigated solely by means of drip irrigation throughout the experimental period (28 June – 23 August, 2006). Orchard 1 (48 m × 78 m, 15 rows of trees) contained Spartan apples, orchard 2 (33 m × 110 m, 12 rows of trees) contained McIntosh apples, and orchard 3 (65 m × 110 m, 22 rows of trees) contained Gala

apples. Within each orchard, three 20 m × 25 m plots (0.05 ha total area) were established as far apart as possible, with at least a 5 m buffer between plots and orchard edge. Following a randomised complete block design, each plot was assigned one of the following three treatments: (1) water control, (2) MEC Z11-14:Ac mixed in water and applied at 100 g a.i./ha, and (3) MEC Z11-14:Ac applied at 100 g a.i./ha in a 2% (v:v) Purespray Green® plus water mixture. Treatment (3) was prepared by first adding the MEC Z11-14:Ac to water and agitating for 5 min before adding oil. All sprays were applied at a rate equivalent to 1021 litres water/ha that approximated the label-recommended commercial application rate of 1000 litres/ha for Purespray Green®. All sprays were applied early in the morning with a tractor-drawn airblast sprayer and plots in each orchard were randomly chosen to receive sprays in the following order: 1) water, 2) MEC, 3) MEC + oil. Orchards 1, 2, and 3 were sprayed on 5, 6, and 7 July 2006, respectively. Due to the relative proximity of control and treatment plots (20 to 30 m), plastic sheeting (3 m high) was erected vertically around each control plot during treatment spraying to minimise drift, although no drift between plots was observed during any application.

3.2.4 Flight-tunnel Assessment of Disruption

Male *C. rosaceana* were exposed to leaves harvested from treated plots to test the disruption effect and longevity of the pheromone formulations in a laboratory flight tunnel. All flights were done in the pushing-type flight tunnel described in detail in chapter 1. The mean light intensity in the centre of the tunnel was 1 lux. For all experiments, the wind speed in the centre of the tunnel was held constant at 0.3-0.4 m/sec, and the tunnel temperature was 22-24°C.

Moths were assayed in flight-tunnel tests at 7, 15, 21, 28, and 42 days after pheromone application in the field. Assays using leaves from each of the 3 orchards

were conducted on three consecutive days to ensure equal treatment ageing for each replicate orchard. On each flight day, 14 leaves were picked from each of the 3 experimental plots within a given orchard. Only 1 leaf was picked per tree, and only trees towards the centre of each plot were used. Leaf picking alternated between the bottom and top halves of adjacent trees, and only relatively flat leaves facing the inside of the row were picked. Leaves from each plot were sealed in separate plastic bags, brought back to the lab, and a 3.5 cm diameter leaf disc was cut from each leaf. To minimise variation associated with sampling a section of the leaf, all discs were cut to border both the mid-vein and leaf edge. Leaf discs were placed in the bottom of cylindrical wire-mesh release devices (3.5 cm diameter × 1.5 cm high) with the adaxial leaf surface facing upward.

Six female moths (4- to 48-hr-old) were briefly chilled at 2°C, placed in individual wire-mesh cages measuring 3 cm^3, and positioned at the downwind end of the flight tunnel 60 min before the onset of scotophase. Thirty min before lights-off, male moths (24- to 96-hr-old) were chilled at 2°C. At the onset of scotophase, 6 males (2 per foliar treatment) were placed on leaf discs in individual release devices. A removable metal lid was placed on each release device, and all 6 release devices were immediately brought into the tunnel room. Release devices containing males on MEC- and MEC + oil-treated leaves were placed in a small fume hood adjacent to the tunnel, and control males on water-treated leaves were placed directly beside the fume hood. This process was repeated every 12 min until all 42 male moths were in the tunnel room. Forty-five min after lights-off, 1 cage containing a calling female was placed on a small metal platform positioned 45 cm above the tunnel floor and 20 cm from the upwind end. Individual male moth flights to the calling female commenced exactly 1 hr after lights-off. One release device containing a male moth still sitting on

the treated leaf disc from the first randomly-selected treatment was placed on a sliding cart at the side of the tunnel, and pushed into the centre of the pheromone plume by a lever operated from outside the tunnel. Males were given 5 sec in the plume, and then released when the lid was lifted by means of an attached line operated from outside the tunnel. Male responses were scored as positive (+) or negative (–) for wing fanning, take-off from the release device, lock-on to pheromone plume, oriented upwind flight, and contact with the cage housing the female (source contact). Time to source contact was recorded as the time from the lid being lifted to the male contacting either the female cage or the platform. Males were given a maximum of 1 min to respond and leave the release device, and were quickly aspirated out of the tunnel after each flight. Flights were initiated every 2 min so that each male spent 60-70 min on the treated leaf disc under scotophase conditions before the bioassay. In total, $N = 11$-14 individual male flights per treatment were done for each replicate orchard, at each time period after treatment application. After each flight day, the tunnel was wiped down with ethanol, and the wire-mesh release devices, female cages, and the female resting platform were rinsed with acetone and baked for ≥ 12 hr at 200°C.

I conducted a preliminary flight-tunnel experiment to determine whether foliar treatment with a 2% oil-water mix (v:v) had any effect on the mate-finding behaviour of male moths. Flights were conducted as described above, but untreated leaves were picked from the orchard, randomly divided into two groups, and hand-sprayed with either a 2% oil-water mix or water only at a rate equivalent to 1000 litres water/ha using a Preval® hand sprayer (Precision Valve Corporation, Yonkers, NY) positioned 25 cm above leaf. Leaf discs were cut from air-dried leaves as above. Over 3 days,

52 water-exposed and 53 oil-exposed males were alternately flown to calling females in the flight tunnel.

3.2.5 Field Assessment of Disruption

Concurrent with flight-tunnel bioassays of disruption, I conducted a field trial to measure how effectively catches of male moths in female-baited traps were disrupted by the various treatments in the three orchards. Marked, laboratory-reared male moths were released and recaptured 7 days before any spray application to test whether there was any pre-existing bias in catches among plots within each orchard. Subsequent releases were made 1, 5, 12, 19, 26, 35, 42, and 49 days after pheromone spray. Males were released into orchards 1, 2, and 3 on three consecutive days to ensure that treatment applications in each replicate orchard were equally aged when the assays were conducted. Within each orchard, each plot had 9 male release points uniformly spaced throughout with 5 - 7 males released from each point. No release point was closer than 5 m from the plot edge. Each plot also had 4 female-baited Delta traps (PheroTech Inc., Delta, BC) hung 1.5 m above the ground (Fig. 3.1), spaced equidistantly from each other, and located 5.5 m from plot centre. For each release, male moths (24- to 96-hr-old) were collected from their outdoor holding area in the early afternoon, chilled at 2°C, and randomly divided into three equal groups of 40 - 62 moths to be released into each of the three plots within an orchard. Each group of males destined for a different plot was placed in a separate Petri dish and lightly dusted with uniquely-coloured Day-Glo® Daylight Fluorescent Powders (Switzer Brothers Inc., Cleveland, OH). For each plot, 5 - 7 males were loaded into each of 9 small plastic release devices (Judd et al. 2006b). Forty-eight female moths (24- to 48-hr-old) were collected at the same time as the male moths, chilled at 2°C, and placed individually in small fibreglass mesh bags (8 cm × 5 cm). All moths were

immediately transported to test orchards in a cooler. Within each plot, 2 female-containing mesh bags were fixed to the inside roof of each of the 4 Delta traps, and each of the 9 male release devices was hung approximately 1 m above the ground in the canopy and immediately opened to allow the males to escape. After 2 nights in the orchard, sticky trap inserts were collected, returned to the lab, and examined under UV light to identify marked moths. The number of males of each colour caught in each trap was recorded.

3.2.6 Scanning Electron Microscopy

To examine the physical interaction of oil and microcapsules, scanning electron micrographs were conducted of apple leaf surfaces sprayed with both the MEC formulated in water, and the MEC + 2% Purespray Green® formulated in water. The MEC in water solution was prepared by adding 0.5 g of MEC Z11-14:Ac to one litre of water and mixing thoroughly for 20 min using a stir bar. The MEC + Oil solution was prepared by adding 0.5 g of MEC Z11-14:Ac to 980 ml of water and mixing thoroughly for 10 min with a stir bar, then adding 20 ml of Purespray Green® and agitating for a further 10 min. One hundred ml of each solution was transferred to separate Preval® hand sprayers, and treatments sprayed onto the upper surface of freshly-picked apple leaves at a distance of 25 cm and a spray rate equivalent to the field application rate. Once dried, leaf surfaces were coated with gold/palladium and SEM pictures were carried out with a Philips FEI LaB6 Environmental Scanning Electron Microscope operated at 7 kV accelerating voltage.

3.2.7 Statistical Analyses

For the flight-tunnel bioassays, analysis of variance (ANOVA) was performed on the percentage of males exhibiting a particular behaviour at each post-spray assay time (SAS Institute 1998). Percentage data were arcsine square root transformed

prior to analysis to improve normality, and Fisher's LSD test was used to identify significantly different treatment means at each post-spray assay time. To test for differences between the MEC and MEC + oil treatments over time, the percentage of male moths that contacted the source from each treatment was first converted to percent disrupted by using the formula: percentage disrupted = (C-T)/C, where C = percentage of males from control group making source contact, and T = percentage of males from treatment group making source contact. Percentages were then arcsine square-root transformed, and a one-way repeated measures (RM) ANOVA was conducted (PROC GLM, SAS Institute 1998), with treatment as the main factor and each post-spray assay time as the repeated measure. At each post-spray assay time, differences in time to source contact for males from different treatment groups exhibited significant heteroscedasticity, and were analysed using a Kruskal-Wallis test on ranks followed by Dunn's multiple comparison procedure using Sigmastat® 3.0.1 software (SYSTAT Software Inc., San Jose, CA). Time to source contact on day 7 was excluded from the analysis due to the low number of MEC + oil-exposed males that contacted the source. In the preliminary flight-tunnel experiment, the percentage of water-exposed and oil-exposed males that made source contact were compared using a χ^2 test, and time to source contact for the two groups was compared using a Mann-Whitney U test (Sigmastat® 3.0.1).

For the field experiment, the percentages of released males recaptured in each treatment group 7 days before spraying, and each subsequent post-spray assay time, were analysed using a two-factor randomised complete block ANOVA with orchard as the blocking factor. As with flight-tunnel data, field recapture data were converted to percentage of males disrupted, arcsine square-root transformed, and analysed with a two-factor randomised complete block RM ANOVA with treatment as the main

factor, orchard as a blocking factor, and each time replicate as the repeated measure (SAS Institute 1998). For all statistical tests, significance levels were set at $\alpha = 0.05$.

3.3 Results

3.3.1 Flight-tunnel Assessment of Disruption

At each post-spray assay time, greater than 90% of all males exposed to water-treated control leaves exhibited all flight behaviours in response to a calling female (Table 3.1). The majority of MEC- and MEC + oil-exposed moths exhibited both wing-fanning and take-off behaviours in response to the female-produced plume, at all times post-spray. However, at 7 and 14 days after leaf treatment there was a reduced proportion of MEC- and MEC + oil-exposed males that locked onto the plume and conducted oriented upwind flight to the calling female compared to water-exposed males (Table 3.1). On day 7 the percentage of moths engaging in each behaviour while exposed to the MEC + oil treatment was numerically lower than those exposed to the MEC treatment but none of these differences were statistically significant. In all treatments, most males that successfully locked on to the pheromone plume also flew upwind and made source contact.

The percentage of males making source contact among treatments was significantly different at day 7 ($F_{2,4} = 12.84$, $P = 0.018$), day 14 ($F_{2,4} = 15.60$, $P = 0.013$), and day 21 ($F_{2,4} = 11.37$, $P = 0.018$) post-spray (Fig. 3.2a). At day 28 ($F_{2,4} = 1.07$, $P = 0.42$) and day 42 ($F_{2,4} = 5.86$, $P = 0.065$) post-spray the percentages of males that made source contact between treatments were no longer significantly different (Fig. 3.2A). When these data were converted to the percentage of males disrupted, RM ANOVA showed no significant difference in level of disruption between MEC-exposed moths (mean = 32.7% disruption) and MEC + oil (mean = 42.0% disruption) ($F_{1,4} = 1.97$, $P = 0.23$) over the course of the experiment (Fig.

3.2B). The effect of time was highly significant ($F_{4,8} = 17.98$, $P = 0.001$), however the time × treatment interaction was not significant ($F_{4,8} = 1.52$, $P = 0.28$).

Control males contacted the calling female significantly faster than males exposed to treatment leaves at day 15 (H = 10.79, df = 2, $P = 0.005$) and at day 21 (H = 25.84, df = 2, $P < 0.001$) post-spray (Table 3.2). Although MEC + oil-exposed moths took slightly longer to locate the female than MEC-exposed moths on both day 15 and 21, these differences were not statistically significant. At day 28 (H = 5.5, df = 2, $P = 0.063$) and day 42 (H = 5.4, df = 2, $P = 0.068$) post-spray, the flight times to source contact were not significantly different among treatments.

I found no difference in mate-finding behaviour of males resting on water-sprayed leaves for 1 hr compared with males resting on oil-treated leaves for 1 hr. Forty-three out of 52 control moths compared with 43 out of 53 oil-exposed moths made source contact ($\chi^2 = 0.01$, df = 1, $P = 0.92$), and the time to source contact by control moths (13.9 ± 1.3 sec) was not significantly different (T = 1720, df = 1, $P = 0.45$) than that of oil-exposed moths (13.7 ± 1.65 sec).

3.3.2 Field Assessment of Disruption

Seven days before any spray application there was no difference in the percentage of released males recaptured in female-baited traps among orchards or plots used for each treatment ($F_{2,4} = 0.457$, $P = 0.662$) (Fig. 3.3A). After applying foliar treatments, the percentage of released males recaptured among treatments was significantly different on day 1 ($F_{2,4} = 25.1$, $P = 0.005$), day 5 ($F_{2,4} = 33.4$, $P = 0.003$), day 12 ($F_{2,4} = 48.5$, $P = 0.002$), day 19 ($F_{2,4} = 12.9$, $P = 0.018$), day 26 ($F_{2,4} = 35.3$, $P = 0.003$), day 35 ($F_{2,4} = 37.2$, $P = 0.003$), and day 42 ($F_{2,4} = 10.5$, $P = 0.025$) post-spray (Fig. 3.3A). On day 49, trap catches increased markedly, and catches in all treatments were statistically equal ($F_{2,4} = 5.65$, $P = 0.068$). The two-factor RM

ANOVA on percent males disrupted showed no significant difference in the level of disruption between MEC-exposed moths (mean = 80.1% disruption) and MEC + Oil-exposed moths (mean = 84.3% disruption) ($F_{1,2} = 0.44$, $P = 0.57$) over the course of the experiment (Fig. 3.3B). The effect of time on the percent disruption was significant ($F_{7,14} = 5.84$, $P = 0.003$), but the time × treatment interaction was not ($F_{7,14} = 1.63$, $P = 0.21$) (Fig. 3.3B).

3.3.3 Scanning electron microscopy

SEM images showed that the spherical microcapsules sit up on the substrate when sprayed with water (Fig. 3.4). However, with the addition of 2% oil, some of the microcapsules appear to sit in small pools of oil that collect in depressions on the leaf surface (Fig. 3.5).

3.4 Discussion

My results indicate that MEC Z11-14:Ac formulated both with and without horticultural oil significantly disrupted mate-finding behaviour of male *C. rosaceana* for at least 21 days in flight-tunnel assays and 42 days in field assays, compared to water-sprayed controls. The flight-tunnel experiment showed that for at least 21 days after applying foliar treatments, one hour of contact with treated leaves significantly reduced male mate-finding behaviour. Since these flight-tunnel experiments were conducted with females calling in clean air, the only source of synthetic pheromone acting as a disruptant arose from the leaf surfaces on which males rested. These results illustrate an important difference in the mechanism(s) by which MEC pheromones likely cause disruption compared with point-source pheromone dispensing systems (Judd et al. 2005). The possibility that males were disrupted by false-trail following (*sensu* Bartell 1982), a mechanism of communication disruption invoked by attractive, point-source dispensing systems (Miller et al. 2006), is

eliminated in these flight-tunnel assays. Therefore, it seems likely that only non-competitive mechanisms of communication disruption are invoked by this unattractive MEC pheromone treatment. Possible disruptive mechanisms that caused the observed behavioural effect in the flight-tunnel tests include masking of the female plume by synthetic pheromone arising from the leaf surface, transient antennal adaptation while males were sitting on treated foliage, a longer-lasting form of antennal adaptation induced by the one hour pre-exposure (*sensu* Stelinski et al. 2003), or some form of central nervous system habituation. Given my previous results in chapter 2, it seems likely that habituation may be invoked when male *C. rosaceana* rest on MEC pheromone-treated leaves.

Field assays showed significant treatment efficacy for 42 days, while flight-tunnel assays using treated foliage from the same plots demonstrated treatment efficacy for only 21 days. This difference may be a result of several factors, including the males' direct placement in the female-produced plume and the small size (3.5 cm diameter) of the treated leaf disc on which males sat in flight-tunnel assays. Under field conditions, the effects of atmospheric permeation with pheromone from a multitude of pheromone-treated leaves may expose the male to much higher levels of pheromone, combined with a longer time period for pheromone exposure that may induce neurological effects. Flight-tunnel assays allow a detailed analysis of the entire sequence of male pheromone-mediated behaviours under controlled, artificial conditions. In turn, my field trials allow examination of male response to females under more natural conditions, with equal initial densities of released males among treatments. Although comparing results for formulation longevity between the two assays is difficult, the use of both assays simultaneously makes the test for differences between the MEC and MEC + oil treatments more robust.

This study tested the hypothesis that adding horticultural oil to a MEC pheromone formulation would enhance the effectiveness of the formulation over time. I hypothesized that under field conditions the oil might stabilize pheromone release rate over time, either by absorbing and re-releasing pheromone, or by physically blocking its release from the microcapsule. Scanning electron micrographs taken of a newly-sprayed MEC + oil-sprayed leaf surface show microcapsules embedded in oil to varying degrees (Fig. 3.5). I have shown that MEC pheromone formulations are compatible with horticultural oils, as disruption was equivalent with and without oil. In both flight-tunnel and field assays, the significant effect of time indicates that both treatments lost efficacy as the formulations aged in the field. However, the oil did not significantly increase the efficacy of MEC pheromones over time, as no significant treatment × time interaction was found in either assay. In the flight-tunnel assays, assessment of the individual, pheromone-mediated behaviours showed no increased effect of the addition of oil to MEC (Table 3.1). Similarly, there was no effect of the addition of oil to MEC on the time it took successful males to make source contact (Table 3.2). In the field assays, percent source contact by males exposed to MEC or MEC + oil treatments rose in tandem as the formulations aged. Although the field results showed a trend toward the MEC in water treatment losing efficacy more rapidly than the MEC + oil treatment, there was no clear indication that the addition of the oil helped to suppress trap catch over time. An initial reduction in the control trap catch on the first day post-spray was probably the result of unseasonably cool and wet weather, though it is possible that a small amount of drift from pheromone to control plots that contributed to this.

There may be several explanations as to why I saw no increase in the longevity of the MEC + oil formulation in these assays. It is possible that oil is

absorbed relatively quickly by the waxy cuticle of leaves, and that microcapsules initially sitting in pools of oil eventually end up exposed in a similar fashion to those sprayed without oil (Fig. 3.4). Horticultural mineral oils are generally known for their short residual activity (Ebbon 2002), penetrating plant tissues either through the cuticle or stomata (Zabkiewicz 2002). After several weeks in the field there may simply not be enough oil persisting on plant surfaces to have a significant effect on either pheromone release rate or microcapsule rainfastness. Although a host of studies have demonstrated a positive effect of petroleum-derived spray oils on the physical rainfastness of various herbicides and insecticides on plant tissues (Taylor and Matthews 1986, Wilson 1989, Kudsk et al. 1991, Kudsk 1992), these effects are usually tested within hours or days of the spray being applied, not over several weeks, as in this study. However, as I was unable to develop a reliable method for directly counting the microcapsules on foliage, the long-term effects of oil on microcapsule rainfastness remain to be tested directly.

Although the addition of oil did not significantly improve the longevity of the formulation, in both flight-tunnel and field assays it did seem to have a small, though statistically non-significant, benefit when disruption over the course of the 49-day experiment is considered. This difference is likely not explained by the presence of oil alone, as the results of the preliminary test showed that exposure to oil has no effect on male mate-finding behaviour. As the results from chapter 2 indicate that the oil increased the initial deposition of microcapsules on metal surfaces, it is plausible that the oil may have similarly increased the initial deposition of microcapsules on leaf surfaces. This could be achieved either by increasing the deposition of spray droplets on leaves (McWhorter 1993), or by increasing the adherence of the actual microcapsules onto leaves once the spray droplet made contact with the leaf surface.

A solution of the horticultural oil Actipron in water (12.5 g/L) had an equilibrium surface tension of 32.3 mN/m compared to 72.8 mN/m for a water spray, with the oil acting as a surfactant to reduce both the dynamic and equilibrium surface tension properties of the spray (Anderson et al. 1987). When sprayed on plant tissues, the presence of this oil increased spray retention and droplet spreading, both characteristics that could help microcapsules adhere to apple leaf surfaces. Furthermore, surfactants generally increase spray retention to a greater degree on 'non-wettable' species than easily wetted species (Anderson et al. 1987). As the top surface of an apple leaf is relatively non-wettable due to the waxy cuticle and the presence of small groups of trichomes (Knight et al. 2004), an oil adjuvant added to MEC pheromone formulations could significantly increase the initial deposition of microcapsules on apple leaves.

The major mechanisms of communication disruption postulated involve the effects of synthetic sex pheromone treatment on male mate-finding behaviour (Bartell 1982, Cardé 1990). However, recent evidence for *C. rosaceana* suggests that exposure to synthetic sex pheromones may negatively affect female sexual behaviour as well. Gökçe et al. (2007) tested *C. rosaceana* females under laboratory conditions and found that a constant exposure to an attractive, 3-component pheromone blend significantly reduced the proportion of calling females and shortened the average female calling period. This suggests a secondary mechanism of communication disruption in this species, and could possibly have contributed to the prolonged period of formulation longevity (≥ 42 days) in field disruption assays compared to flight-tunnel assays (≥ 21 days) in this chapter. In flight-tunnel assays, all female *C. rosaceana* were calling in clean air at the upwind end of the tunnel, and were checked for appropriate calling behaviour prior to conducting the assay. In field assays,

females in MEC- and MEC + oil-treated plots were constantly exposed to pheromone-permeated air, which may have reduced female calling and contributed to a reduced trap catch compared to controls. The results of Gökçe et al. (2007) corroborate those of Evenden (1998) who found that *C. rosaceana* females placed in pheromone-treated orchard plots marginally delayed the onset of calling compared to females in control plots. These results suggest that this possible mechanism needs to be explored further under field conditions to assess what role it may play in disrupting communication in *C. rosaceana.* Interestingly, the majority of studies reporting that MEC formulations lack efficacy and longevity in the field have used synthetic lure-baited traps instead of female-baited traps to assess communication disruption (Albajes et al. 2002, Trimble et al. 2004, Kovanci et al. 2005a,b, Walton et al. 2006). Lure-baited traps require far less time and effort to maintain than female-baited traps over a full insect flight period. However, lure-baited traps may underestimate the efficacy of mating disruption if the female moth is negatively affected as well. Any future field communication disruption trials with *C. rosaceana* should employ female-baited traps or tethered, virgin females instead of lure-baited traps to optimally assess communication disruption.

My study showed no significant improvement of the MEC formulation through the addition of horticultural oil, however it clearly demonstrated that the addition of oil does not reduce the effectiveness of the MEC pheromone treatment. One possibility for maximizing any potential synergistic effects of oil and pheromone might be to apply repeated applications of oil alone after the initial MEC + oil spray. This would repeatedly coat the microcapsules with oil, replacing oil absorbed into the leaf, and potentially stabilize pheromone release rates over a longer period of time. Although the risk of chronic phytotoxic effects can increase with repeated

applications of horticultural oil (Hodgkinson et al. 2002), eight field applications of a 2 % solution of the *n*C23 summer oil Orchex 796® did not cause any significant phytotoxicity on apples in the western USA (Brunner et al. 1996). Furthermore, using this spraying scheme against *C. rosaceana* could provide additional control through the more conventional insecticidal action of horticultural oil. Orchex 796® caused over 90% mortality when applied topically to eggs of *C. rosaceana* (Brunner et al. 1996), and my results in chapter 3 and those of Whitehouse (2006) indicate that Purespray Green® is an effective ovicide when applied topically against both newly-laid and fully-developed egg masses. The combination of mating disruption to target adult stages along with control of other life stages by horticultural oil application could be an effective and relatively straightforward control method.

The concept of using horticultural oil as an adjuvant for MEC pheromones is also compatible with more frequent, low-rate applications of pheromone. In this study I examined a single, high-rate application of MEC pheromone, however more frequent, low-rate applications of MEC pheromones are increasingly being recommended to maintain more consistent levels of pheromone throughout the adult flight period (Polavarapu et al. 2001, Kovanci et al. 2005b, Il'ichev et al. 2006). The low phytotoxicity of the oil would be compatible with multiple sprays during the growing season, with the added benefits of the oil on controlling the egg stage of the insect. Furthermore, several studies on mating disruption in *C. rosaceana* have shown that disruption is not more effective at higher pheromone application rates (Agnello et al. 1996, Lawson et al. 1996, Evenden et al. 1999). In the only other study to examine mating disruption of *C. rosaceana* with MEC pheromones, Trimble and Appleby (2004) concluded that increasing the rate of pheromone per spray would not improve the level of disruption. The concept of using more frequent, low-rate

applications of pheromone and oil needs to be tested against *C. rosaceana*, because at lower application rates the benefits of oil on the release-rate properties of MEC pheromone may be realized.

At the initiation of this work, the Controlled Delivery Products Division of 3M in London, ON, was interested in improving MEC-LR® formulations for use against leafrollers. Although this product is no longer commercially available in Canada, the compatibility of MEC formulations and horticultural oils demonstrated in this study could apply to a wide range of currently-available MEC products. As growers seek safer alternatives for control of insecticide-resistant lepidopteran pests, both horticultural oils and pheromone-mediated mating disruption will become more prominent tools. If IPM strategies for different pests are to be optimised, it is important to demonstrate which control strategies are compatible with each other, and if it is possible to combine them in a positive, synergistic fashion. This chapter represents a first step in field-testing the mating disruption effect of a sprayable pheromone in combination with a horticultural oil, with formulations remaining active for six weeks. This novel approach may provide a viable IPM tool for organic and conventional growers alike by targeting different life stages of the pest insect simultaneously.

Table 3.1 Mean (± SE) percentage of male *C. rosaceana* displaying various behaviours in a flight tunnel in response to a calling female 1 hr following placement on leaf discs from orchards sprayed with either water, MEC *Z*11-14:Ac, or MEC *Z*11-14:Ac + 2% Purespray Green® treatments, at increasing post-spray assay times. $N = 3$ replicates of 11 – 14 males flown at each post-spray assay time per treatment.

		Mean ± SE percentage males displaying behaviours				
		Days post-spray[a]				
Behaviour	Treatment	Day 7	Day 14	Day 21	Day 28	Day 42
Wing fanning	Water	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	MEC	70.9 ± 8.8a	70.0 ± 4.5b	79.4 ± 6.4b	86.5 ± 9.9a	97.2 ± 2.8a
	MEC+oil	61.1 ± 5.5a	80.2 ± 5.5b	95.2 ± 4.7a	89.7 ± 6.8a	91.9 ± 4.8a
Take off flight	Water	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	MEC	65.7 ± 19.1b	70.0 ± 4.5b	79.4 ± 6.3b	86.5 ± 9.9a	92.5 ± 4.1ab
	MEC+oil	55.5 ± 5.5b	80.2 ± 5.5b	92.8 ± 7.1ab	89.7 ± 6.8a	89.3 ± 3.0b
Lock on to plume	Water	100 ± 0.0a	92.5 ± 4.1a	92.5 ± 4.4a	97.6 ± 2.4a	100 ± 0.0a
	MEC	39.6 ± 16.5b	59.9 ± 3.1b	56.7 ± 8.6a	84.1 ± 8.8a	87.7 ± 8.5a
	MEC+oil	24.6 ± 10.0b	57.7 ± 6.2b	73.8 ± 9.8a	66.7 ± 7.8a	81.2 ± 7.6a
Upwind flight	Water	97.4 ± 2.4a	92.5 ± 4.1a	92.5 ± 4.4a	95.2 ± 2.4a	100 ± 0.0a
	MEC	39.6 ± 16.5b	57.3 ± 5.1b	56.7 ± 8.6a	84.1 ± 8.8a	87.7 ± 8.5a
	MEC+oil	19.0 ± 7.6b	53.7 ± 6.2b	69.1 ± 9.6a	66.7 ± 7.8a	78.6 ± 7.4a

[a]Means within a column for each behavioural category followed by the same letter are not significantly different (Fisher's LSD, $P > 0.05$)

Table 3.2 Mean (± SE) flight time (sec) from moth release to source contact for male moths flown in the flight tunnel that successfully made source contact after 1 hr pre-exposure on leaves treated with either water, MEC *Z*11-14:Ac, or MEC *Z*11-14:Ac + 2% Purespray Green® treatments at various post-spray assay times.

Treatment	Day 15	Day 21	Day 28	Day 42
Water	12.0 ± 1.1a	9.5 ± 0.9a	13.2 ± 2.1a	12.2 ± 0.9a
MEC	17.4 ± 2.4b	18.7 ± 2.6b	16.4 ± 2.3a	15.6 ± 1.8a
MEC + Oil	19.4 ± 2.5b	20.1 ± 2.2b	16.7 ± 2.1a	17.8 ± 2.1a

Means within columns followed by the same letter are not significantly different by Dunn's multiple comparison test on ranks ($P > 0.05$).

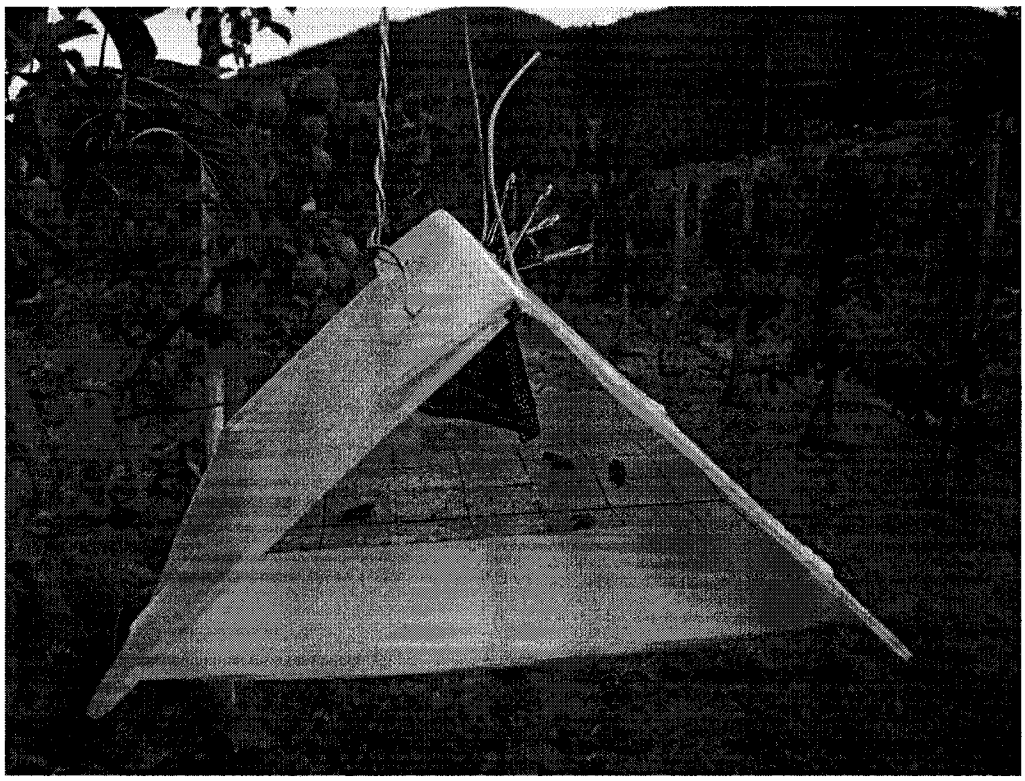

Figure 3.1 Female-baited Delta trap hung at a height of 1.5 m in an experimental apple orchard at the Pacific Agri-Food Research Centre, Summerland, BC.

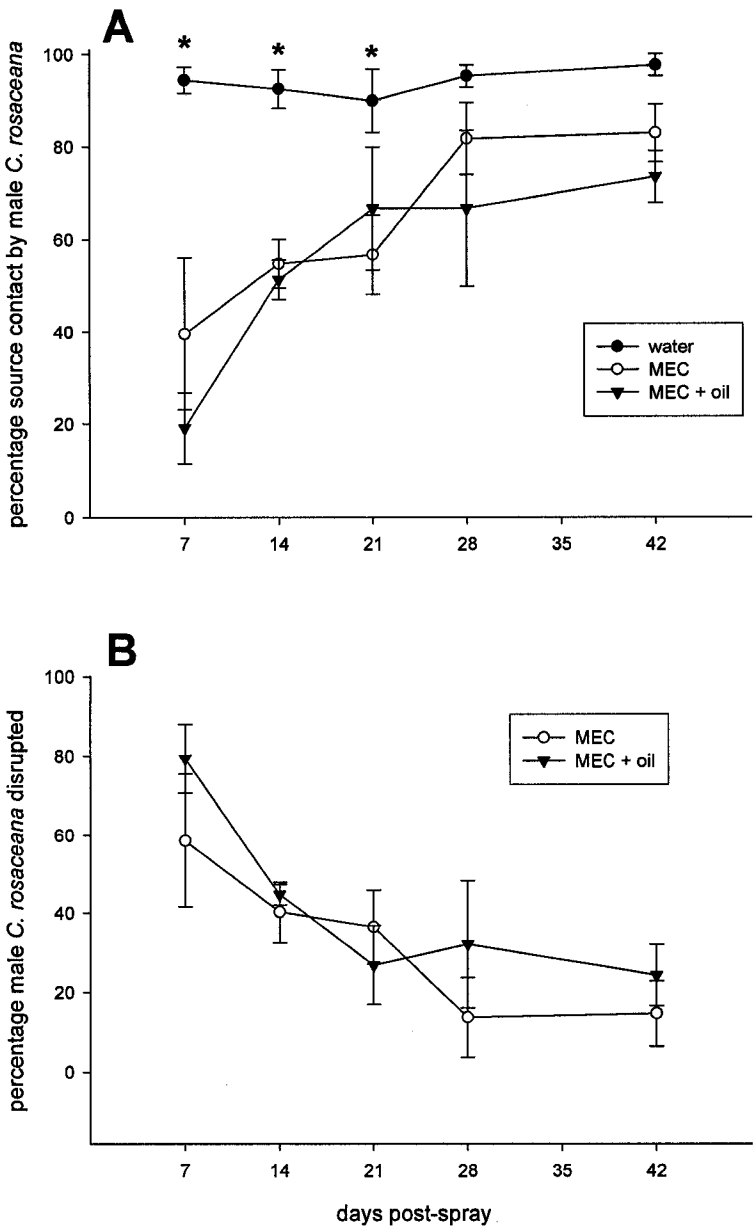

Figure 3.2 (**A**) Mean (± SE) percentage of male *C. rosaceana* that made contact with cage containing calling female when flown in flight tunnel after 1 hr exposure on leaf discs sprayed with either water, MEC Z11-14:Ac, or MEC Z11-14:Ac + 2% Purespray Green® treatments, at day 7, 14, 21, 28, and 42 after spray application. * indicates a significant treatment difference by ANOVA at $P < 0.05$. (**B**) Mean (± SE) percentage male *C. rosaceana* disrupted, converted from percentage source contact data from (A) at day 7, 14, 21, 28, and 42 after spray application.

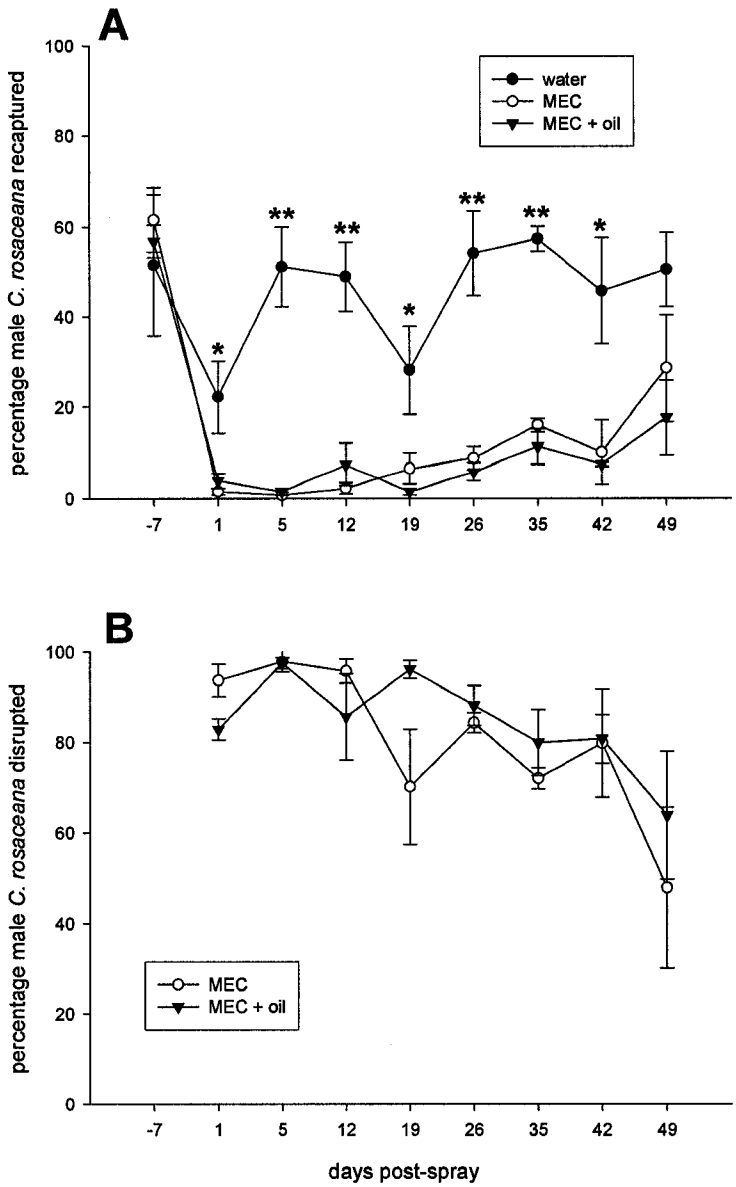

Figure 3.3 (**A**) Mean (± SE) percentage recapture of male *C. rosaceana* released into orchard plots sprayed with either water, MEC Z11-14:Ac, or MEC Z11-14:Ac + 2% Purespray Green® horticultural oil at 7 days before spray application, and at 1, 5, 12, 19, 26, 35, 42, and 49 days after spray application between June 28th and August 23rd, 2006. * and ** indicates a significant treatment difference by ANOVA at $P < 0.05$ and $P < 0.01$, respectively. (**B**) Mean (± SE) percentage male *C. rosaceana* disrupted, converted from percentage recapture data from (A) at 1, 5, 12, 19, 26, 35, 42, and 49 days after spray application.

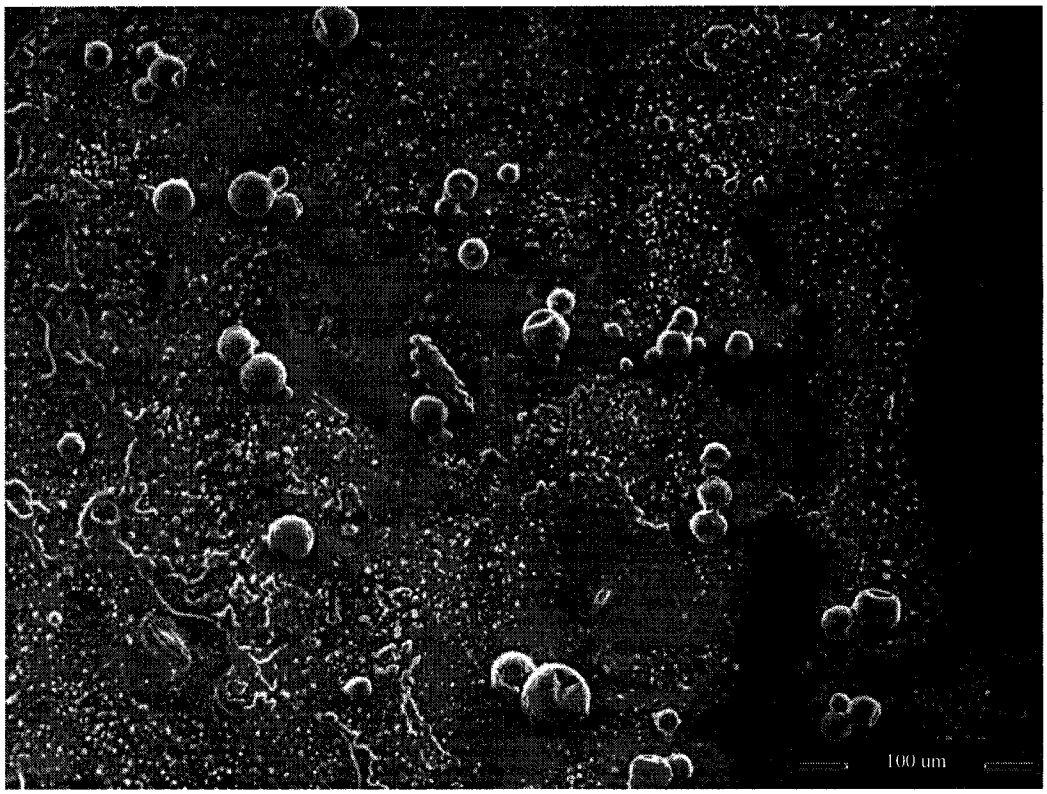

Figure 3.4 Scanning electron micrograph of the adaxial surface of a mature apple leaf sprayed with 3M MEC-LR formulated in water and applied at 100 g a.i. ha^{-1}. Microcapsules of various sizes are visible resting on the leaf surface.

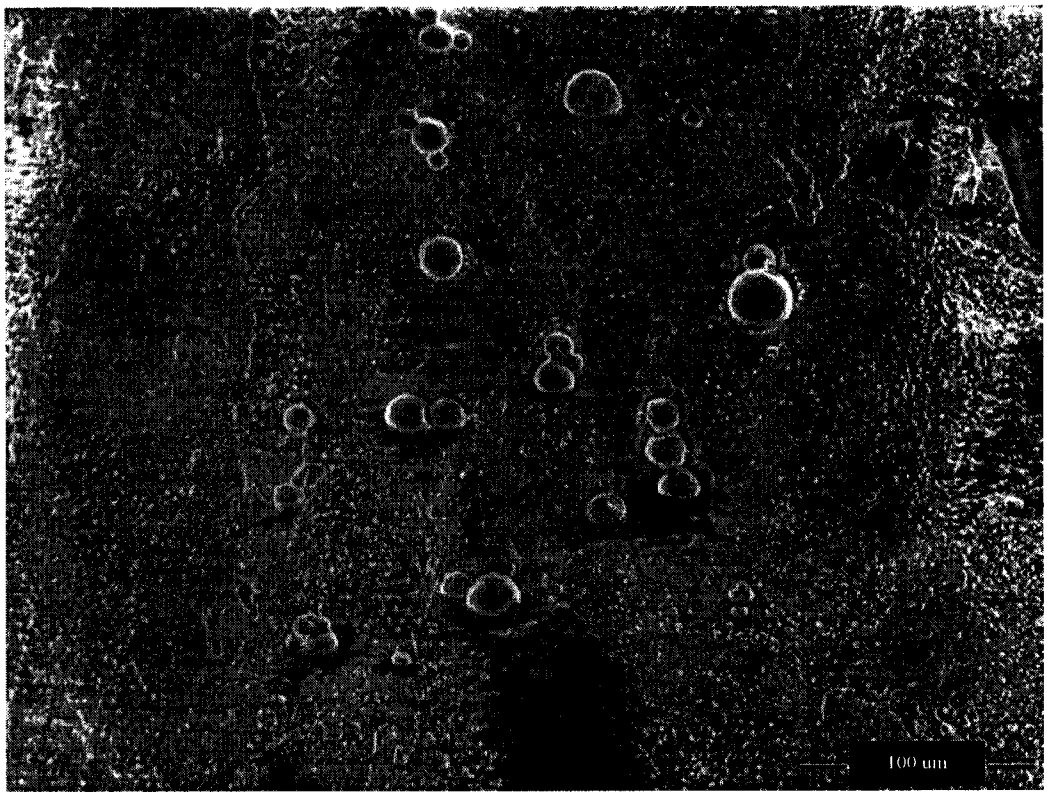

Figure 3.5 Scanning electron micrograph of the adaxial surface of a mature apple leaf sprayed with 3M MEC-LR (100 g a.i. ha^{-1}) formulated in 2% (v:v) Purespray Green® horticultural oil. Microcapsules of various sizes are visible sitting embedded in pools of oil that have collected in depressions in the leaf surface.

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CHAPTER 4. EFFECTS OF HORTICULTURAL OIL ON OVIPOSITION BEHAVIOUR AND EGG SURVIVAL IN *C. rosaceana*

4.1 Introduction

The obliquebanded leafroller *Choristoneura rosaceana* (Harris) is a polyphagous pest of pome fruit across North America. Over 30 years of control with organophosphate insecticides in eastern Canada and the USA has led to widespread insecticide resistance, a reduction in natural enemies, and a significant increase in economic damage from this pernicious pest (Reissig et al. 1986, Lawson et al. 1996, Smirle et al. 1998, Pree et al. 2001, 2002). Concerns over insecticide resistance, worker safety, environmental damage, and future restrictions on the use of azinphos-methyl (DiFonzo 1997) have provided the impetus for the development of less harmful control strategies that are compatible with integrated pest management (IPM) programs. Petroleum-derived spray oils have been employed for over a century to control arthropod pests in agricultural systems, yet traditional usage has been restricted to dormant applications because of impurities in oil formulations that cause significant phytotoxicity (Agnello 2002, Fernandez et al. 2005). However, recent advances in the purification and synthesis of horticultural oils have led to a wide range of highly-refined products that are suitable for multiple foliar applications during the growing period (Agnello 2002). This has made possible the use of horticultural oils to target insects like *C. rosaceana* throughout their lifecycle.

Horticultural oils are generally thought to kill immature and adult arthropods by entering spiracles, penetrating a short distance into the trachea, and impeding gas exchange (Taverner 2002). The ovicidal effects of oils are often ascribed to the oil coating the egg and suffocating it (Pearce and Chapman 1952, Fiori et al. 1963). Long carbon chain oils (i.e. > *n*C23) are thought to be effective at smothering eggs because they spread evenly over the egg surface and have a low volatility (Pearce and

Chapman 1952, Fiori et al. 1963). However, recent evidence suggests that oil sprayed directly onto foliage (i.e. a residual application) may also impact insect eggs through contact toxicity (Larew and Locke 1990, Riedl et al. 1995), and may deter oviposition in several insect orders (Larew and Locke 1990, Mensah et al. 1995, Sun 2002, Liu et al. 2006, Nguyen et al. 2007).

In this chapter, I examined the effects of both a residual and topical application of horticultural oil on egg mortality in *C. rosaceana*, as well as the effect of a residual oil treatment on female oviposition behaviour. I tested the hypothesis that a residual 2% oil treatment would reduce the total reproductive output of mated *C. rosaceana* females by both deterring oviposition and reducing the percentage survival of eggs laid. I also tested the hypothesis that a topical application of oil to *C. rosaceana* eggs would cause significant egg mortality.

4.2 Materials and Methods

4.2.1 Insects and Oviposition Substrate Materials

C. rosaceana used in this study came from a 10-year-old colony originally collected in the Okanagan and Similkameen valleys of British Columbia (BC) and periodically restocked with wild insects. Larvae were reared individually in 25 ml Solo® cups on a modified pinto bean-based diet (Shorey and Hale 1965) at 23°C and 50-60% RH under a LD 16:8 hr photoregime. Pupae were collected weekly, separated by sex, and placed individually in clean 25 ml Solo® cups until adult eclosion. Adults were collected each day, provided with water through a dental wick, and held under rearing conditions until use in bioassays. In all bioassays, adult males were 24-96-hr-old and females were 24-48-hr-old. Biossays used both wax paper (McNairn Packaging Inc., Whitby, ON) and apple leaf substrates from an unsprayed,

experimental McIntosh apple orchard at the Pacific Agri-Food Research Centre (PARC) in Summerland, BC for oviposition.

4.2.2 Oil Formulation and Application

Purespray Green® horticultural oil (batch # 655-0602, Petro-Canada, Mississauga, ON) is a highly purified, *n*C23 horticultural mineral oil, with a molecular weight of 325, a paraffin content >99.9 % (by wt), an aromatic content <0.01% (by wt), and an average boiling point (ASTM D 1160) of 223.9°C (Petro-Canada, technical data sheet). Purespray Green® is combined with a proprietary emulsifier and approved for spraying by organic producers in the USA (Organic Materials Review Institute, Eugene, OR). For all bioassays, control preparations consisted of distilled water sprays. Treatment applications of 2% oil (v:v) consisted of 2 ml Purespray Green® mixed in 98 ml of distilled water. Purespray Green® was stored at room temperature before use. Spraying was done with a 160 ml hand-held, disposable aerosol spray gun (Preval® Sprayer, Precision Valve Corporation, Yonkers, NY). Sprays were applied at a rate of 1 ml/100 cm^2, equivalent to the label-recommended application rate of 1000 litres ha^{-1} for Purespray Green®. All sprays were applied from a distance of 25 cm to either wax paper or apple leaves taped to a plastic board inclined at a 20° angle. For apple leaves, only the adaxial surface of the leaf was sprayed and leaves oriented downward during spraying.

4.2.3 Egg Mass Surface Area to Egg Number Relationship

As individual *C. rosaceana* eggs are difficult to visually distinguish within a newly-laid egg mass, an initial experiment was conducted to determine the relationship between egg mass surface area and number of eggs laid. Surface area measurements were made of 35 egg masses laid on clean, untreated wax paper using a digital image of each egg mass and Image Pro Plus v4.5.1 software (Media

Cybernetics Inc., Bethesda, MD). The eggs were counted at the *blackheaded* stage (Hammer 1912), when each egg is clearly visible. This relationship was used to estimate the initial number of eggs laid per egg mass for all choice and no-choice oviposition assays.

4.2.4 No-choice Oviposition Assays

Three no-choice oviposition experiments used either wax paper or apple leaf substrates to test the hypothesis that a 2% residual oil treatment would have both a toxic effect on eggs and act as an oviposition deterrent to female *C. rosaceana.* All assays were conducted in an environmental chamber at 23°C and 50-60% RH under a LD 16:8 hr photoregime. For all experiments, single pair matings were conducted in cylindrical wire mesh arenas (7 cm diameter × 5 cm high), and each mated female was weighed to the nearest 0.1 mg with a Mettler Toledo XS105 digital balance.

In experiment 1, wax paper discs (7 cm diameter) were sprayed with either water or 2% oil and inserted onto the floor of each wire mesh arena (7 cm diameter × 5 cm high). One mated female was introduced into each arena ($N = 23$) 1 hr before the onset of scotophase and given 48 hr to oviposit. A second experiment used apple leaves as the oviposition substrate. Individual leaves were picked from the McIntosh experimental apple orchard at PARC, rinsed with distilled water, and allowed to dry. Leaves were taped to a plastic board and sprayed individually with either water or 2% oil. Treatment applications were allowed to dry and one leaf was inserted onto the floor of a wire mesh arena. To prevent leaf desiccation, oviposition devices were placed on moist paper towels inside a gardening-type clear plastic humidity dome (50 cm × 25 cm × 15 cm high). One mated female was introduced into each arena and given 48 hr to oviposit. The third experiment used treated apple leaves that had aged in the field for 3 days after treatment application as oviposition substrates. Individual

apple leaves (one per tree) were marked with flagging tape and sprayed with either water or 2% oil. Leaves were left on the tree for 72 hr before they were picked, brought to the lab, and the oviposition assay conducted as in experiment 2. In all 3 no-choice experiments, I recorded the number of egg masses laid per female and the surface area of each egg mass. Egg masses were held in an environmental chamber to develop until the blackheaded developmental stage, when the number of eggs could be accurately counted using a digital image of each egg mass. After all viable larvae had emerged, the remaining dead blackheaded eggs were counted to determine the total number of larvae that hatched per egg mass.

4.2.5 Choice Oviposition Assays

Choice oviposition assays were conducted using both wax paper and apple leaf substrates to test the hypothesis that a 2% (v:v) residual oil treatment would act as an oviposition deterrent to female *C. rosaceana*. Wax paper assays were conducted in a Conviron® environmental chamber at 23°C and 50-60% RH under a LD 16:8 hr photoregime. Pairs of male and female moths were set up in individual cylindrical wire mesh arenas (7 cm diameter × 5 cm high), provisioned with wet cotton wicks, and given 24 hr to mate. Five mated females were then transferred to a cylindrical wire mesh oviposition arena (30 cm diameter ×10 cm high) and provided with a wet cotton wick (N=21). The oviposition substrate consisted of a circle of brown waxed paper (30 cm diameter) positioned on the bottom of the chamber. Prior to insertion into the chamber, one-half of each waxed paper circle was sprayed with 2% (v:v) Purespray Green®, while the other half was sprayed with distilled water. After a 48-hr oviposition period, the number of egg masses on treatment and control halves was recorded, and the surface area of each egg mass was measured as above. Egg masses touching both treated sides were excluded from analyses.

Apple leaf choice oviposition assays were conducted outside in the unsprayed, experimental McIntosh apple orchard at PARC between 24 July and 4 August, 2006. Newly-eclosed males and females were held outside for 24 hr in 10 litre buckets to acclimate to outdoor conditions before assays. Ten male and 10 female moths were introduced into an oviposition cage (45 cm × 45 cm × 45 cm) constructed from wood and plastic mesh, and provisioned with a wet cotton wick. Oviposition cages containing moths (N=12) were placed between apple trees on the orchard floor in the late afternoon, and moths were given 24 hr to mate. Apple shoots (45 cm long) with 10 mature apple leaves were clipped from trees, rinsed with distilled water, and allowed to dry. Shoots and leaves were taped flat to a plastic board, and the leaves of one entire shoot were sprayed with either distilled water or 2% Purespray Green®. After drying, two shoots of each treatment were inserted into floral picks and positioned vertically inside each oviposition cage in the late afternoon. Females were given 1 full scotophase to oviposit and shoots were collected the next morning. The number of egg masses laid on treatment and control leaves was recorded, and the surface area of each egg mass measured.

4.2.6 Topical Application of Oil to Eggs

Eggs laid on wax paper were treated topically with Purespray Green® at various concentrations to test the topical ovicidal activity of the oil. Egg masses laid on wax paper by individual mated females were placed singly in petri dishes (3.5 cm diameter) and held at 23°C and 50-60% RH until development to the blackheaded stage was complete, when the number of eggs per mass was counted. Blackheaded egg masses were randomly assigned to treatment groups (N=10 per treatment) and sprayed with a 0.1, 0.5, 1.0, 2.0, or 3.0% emulsion of Purespray Green® or a distilled water control. Treatments were allowed to dry and egg masses were placed

individually in petri dishes and held under the same conditions until larvae hatched and percent hatch was recorded.

4.2.7 Statistical Analyses

In no-choice assays, treatment differences in total number of eggs laid, percentage egg survival, and total number of emerged larvae were separated using two-sample *t*-tests, except in cases of significant heteroscedasticity where the Mann-Whitney U non-parametric test was used. Fecundity is positively correlated with female weight in *C. rosaceana* (Carrière 1992). However, my no-choice data revealed a significant treatment × female weight interaction, therefore female weight before oviposition was not used as a covariate in any of the analyses. In choice oviposition assays, treatment differences in number of egg masses laid, number of eggs per egg mass, and total number of eggs laid were analysed using paired *t*-tests. Percentage mortality of eggs treated topically were analysed with a one-way Kruskal-Wallis test followed by Dunn's test for multiple treatment comparisons against a control. All analyses set significance at $\alpha = 0.05$, and all tests were done using Sigmastat® 3.0.1 software (SYSTAT Software Inc., San Jose, CA).

4.3 Results

4.3.1 Egg Mass Surface Area to Egg Number Relationship

The egg mass surface area was a strong predictor of the number of eggs laid per mass (Fig. 4.1).

4.3.2 No-Choice Oviposition Assays

No-choice assays indicated that a residual 2% oil treatment tested immediately after spraying reduced both the number of eggs laid and the percentage egg survival. On both the freshly-sprayed wax paper and apple leaf oviposition substrates, females laid significantly fewer eggs on 2% oil treatments compared to controls (Table 4.1).

Percentage egg survival on the oil-treated surfaces was significantly less than controls (Table 4.1). Reductions in both the number of eggs laid and the percentage egg survival contributed to a significant reduction in the total number of emerged larvae on oil-treated surfaces. The freshly sprayed 2% oil treatment caused a 50% reduction ($t = 6.9$, df = 56, $P < 0.001$) in the total number of emerged larvae on the wax paper, and a 58% reduction ($t = 4.1$, df = 41, $P < 0.001$) in total number of emerged larvae on apple leaves (Table 4.1). When the sprayed apple leaves were allowed to age under field conditions for 3 days before oviposition assays, the 2% oil treatment caused only a non-significant reduction in both the number of eggs laid and the percentage egg mortality (Table 4.1). These contributed to a 25% reduction ($t = 2.1$, df = 42, $P = 0.04$) in the total number of larvae that emerged on oil-treated leaves (Table 4.1).

4.3.3 Choice Oviposition Assays

A 2% oil treatment acted as an oviposition deterrent when applied to wax paper, as females laid significantly fewer egg masses on the oil-treated side of the wax paper disc than on the water-treated side (Table 4.2). There was no effect of treatment on the mean number of eggs per egg mass, nor on the total number of eggs laid (Table 4.2). When given real apple leaves under more natural conditions, females laid marginally fewer egg masses on oil-treated leaves than on control leaves ($P = 0.07$), but a significantly lower mean total number of eggs on oil-treated leaves compared to control leaves (Table 4.2).

4.3.4 Topical Application of Oil to Eggs

A topical application of oil to blackheaded egg masses caused a significant increase in egg mortality ($H = 49.3$, df = 5, $P < 0.001$) (Fig. 4.2). Emulsions of 1.0, 2.0, and 3.0% oil caused significant egg mortality compared to a water-sprayed

control, with the 2.0 and 3.0% oil treatments causing >99% egg mortality. Emulsions of 0.1 and 0.5% oil did not cause significant egg mortality compared to control sprays (Fig. 4.2).

4.4 Discussion

The results of this study indicated that a 2% emulsion of Purespray Green® oil can deter oviposition and exert ovicidal effects in *C. rosaceana.* Both no-choice and choice oviposition assays showed a significant reduction in the number of eggs laid by mated female *C. rosaceana* presented with a newly-sprayed, residual 2% oil treatment. Similar reductions in eggs laid as a result of horticultural oil treatment have been documented in several key lepidopteran pests, including *Helicoverpa punctigera* (Wallengren) (Mensah et al. 1995), *Cydia pomonella* (L.) (Reidl et al. 1995), *Phyllonorycter ringoniella* (Matsumura) (Sun 2002), *Helicoverpa armigera* (Hübner) (Mensah et al. 2005), *Ostrinia nubilalis* (Hübner) (Mensah et al. 2005), and *Phyllocnistis citrella* (Stainton) (Liu et al. 2006). These results suggest that gravid females are able to assess and reject oil-sprayed surfaces either through tactile or chemosensory means, or some combination of the two. Ovipositing female *C. rosaceana* can detect and avoid conspecific egg masses under laboratory conditions, a behaviour most likely mediated by an oviposition-deterring pheromone (Poirier and Borden 1991). This suggests that female *C. rosaceana* are capable of using chemosensory means to reject an otherwise-suitable surface for oviposition. Conversely, Ramaswamy et al. (1987) inactivated various chemoreceptors of *H. virescens* females and suggested that mechanosensilla located on the tarsi and the ovipositor allowed females to assess physical properties of the leaf surface during oviposition. As *C. rosaceana* strongly prefer to oviposit on the glabrous, adaxial

surface of apple leaves (pers. observ.), females may also use mechanoreceptors to detect the oily residue on the leaf surface and reject the treated leaf for oviposition.

Close-range olfactory discrimination could also play a role in female rejection of oil-treated surfaces. In the Lepidoptera, chemosensory receptors involved in oviposition are present on the antennae, proboscis (Rivet and Albert 1990), tarsi (Ma and Schoonhoven 1973, Renwick and Radke 1982, Klijnstra and Roessingh 1986), and ovipositor of females (Klijnstra and Roessingh 1986, Fenemore 1988). Given the low volatility of Purespray Green®, it seems likely that contact chemoreceptors (Renwick and Chew 1994) in the tarsi and ovipositor of the female could play some role in detecting the presence of the oil. However, while the oviposition deterrent properties of horticultural oils have been documented in several pest species across a broad range of taxa, I am unaware of any study that has specifically examined whether the underlying mechanisms behind the deterrence are primarily tactile or chemosensory. Further research into these mechanisms is necessary to elucidate how horticultural oils deter gravid females from laying eggs.

Choice oviposition tests revealed a difference in the effects of the oil between oviposition substrates: 2% oil sprayed on apple leaves caused a greater reduction in the number of eggs laid than 2% oil sprayed on wax paper surfaces (Table 4.2). This difference may be due in part to the oil masking or suppressing host-plant volatiles used by the female to locate and accept suitable oviposition sites (Mensah et al. 2005). Mensah et al. (2005) found that the similar oil Caltex Canopy® applied at 2% (v:v) significantly reduced the magnitude of plant volatiles emitted from cotton leaves, although this effect disappeared four days after spraying. The authors hypothesised that the newly sprayed oil formed a physical barrier that was blocking the release of important volatiles from the leaves. Alternately, the choice oviposition tests on apple

leaves may have shown a greater treatment effect because females performed more of the behavioural sequence leading to oviposition than in the wax paper assays. The restrictive size of the wax paper oviposition arena (30 cm diameter ×10 cm high) eliminates the searching, orientation, encounter, and landing behaviours that a gravid female would normally complete before the contact evaluation of an oviposition surface (Singer 1986, Renwick and Chew 1994). The larger oviposition cage employed in the choice apple leaf tests (45 cm × 45 cm × 45 cm) and choice of 2 sprayed shoots per treatment requires the female to carry out at least the orientation, encounter, and landing behaviours in the sequence. Any olfactory effect exerted by the oil during this stage, either through female detection of inhibitory compounds present in the oil or by a physical suppression of positive plant volatile cues used by the female, would decrease the probability of a female alighting and ovipositing on an oil-sprayed leaf.

No-choice assays indicated that Purespray Green® had a toxic effect on *C. rosaceana* eggs, as a newly-sprayed residual 2% oil treatment significantly reduced larval hatch. The reasons for this remain unclear, since a residual treatment should not physically interfere with normal gas exchange across egg membranes, the generally-accepted explanation for the ovicidal effect of oil on arthropod eggs (Smith and Pearce 1948, Pearce and Chapman 1952, Fiori et al. 1963, Riedl et al. 1995, Taverner 2002). Taverner et al. (2001) demonstrated that oils absorb into insect membranes and displace protective lipids. Smith and Pearce (1948) proposed that oils could penetrate the chorion of eggs, potentially interfering with cellular processes or increasing egg desiccation, which could explain the toxic effect of the oil in these assays. The proprietary emulsifier added to the oil may also contribute to the toxic

effect, as emulsifiers are known to have wide-ranging effects on many types of cell tissues (Taverner 2002).

When applied topically to egg masses, Purespray Green® caused significant mortality of blackheaded eggs at concentrations of 1.0, 2.0, and 3.0% compared to a water-sprayed control. Using an identical spraying protocol, Whitehouse (2006) found that a topical application of Purespray Green® to newly-laid *C. rosaceana* egg masses caused significant mortality compared to controls at concentrations of 0.25% (v:v) and higher. The higher oil concentration necessary to cause significant mortality in blackheaded eggs compared to newly laid eggs suggests a greater resistance to the oil in the latter stages of egg development. This is consistent with Smith and Pearce (1948), who found that *Grapholita molesta* (Busck) eggs were less susceptible to oil treatments in the final third of the incubation period, when egg respiration rates increased sharply. These authors concluded that a continuous, >24 hr suppression of respiration by the oil was the primary cause of egg mortality. Oils with a long carbon chain (> *n*C23) like Purespray Green® are generally considered to have the best ovicidal activity because of a good spreading coefficient and a low volatility (Pearce and Chapman 1952, Fiori et al. 1963). This creates a physical barrier over the egg surface for an extended period of time, potentially killing the egg through a combination of anoxic conditions, a build up of toxic metabolites, and a disruption of membrane function (Smith and Pearce 1948). Collectively, my results and those of Whitehouse et al. (2006) suggest that a topical application of 2% Purespray Green® can effectively control *C. rosaceana* eggs at any developmental stage.

Although a newly sprayed residual oil treatment reduced both the number of eggs laid and the proportion of hatched larvae of *C. rosaceana*, these effects were not significant when oil-sprayed leaves were allowed to age under field conditions for

three days (Table 4.1). This is consistent with the results of Mensah et al. (2005), who found that a 2% oil treatment lost efficacy three days after treatment. This could be a result of oil degradation (Cornish et al. 1993) or absorption into the waxy cuticle of the leaf (Hodgkinson et al. 2002), and suggests that multiple oil sprays over the course of the oviposition period would be necessary to maintain a consistent and effective level of oil residue on foliage.

An intriguing potential impact of a residual oil treatment on egg development is the reduced retention of egg masses on oil-treated surfaces. This is rarely proposed as important in the literature, possibly because the majority of moth species examined prefer to oviposit on hairy leaf surfaces (Ramaswamy 1988), where eggs adhere to dense trichomes that prevent an even distribution of the oil. For *C. rosaceana*, egg masses laid on 2% oil-treated wax paper and apple leaf surfaces appeared to lose adherence around the margins of the egg mass after several days. Egg masses on water-sprayed surfaces did not appear to lose adhesion to either type of substrate. This effect could be more pronounced in the field, where apple leaves are constantly subjected to physical movement from wind, rain, etc. Assuming that adhesion to the substrate is important for optimal egg development, a reduced physical retention of egg masses on glabrous leaf surfaces may play a role in reducing egg survival in *C. rosaceana.*

As growers seek alternatives to organophosphate insecticides for controlling difficult insect pests like *C. rosaceana*, horticultural oils should play an increasingly more prominent role as they show little potential for insect resistance, low mammalian toxicity, and little impact on beneficial insects compared to broad-spectrum insecticides (Agnello 2002, Fernandez et al. 2005). This study documents the effects of horticultural oils on oviposition behaviour and egg hatch in *C.*

rosaceana, and suggests that larger-scale field trials are necessary to further assess the efficacy of Purespray Green®. A major obstacle to the broader acceptance and use of horticultural oils is growers' perception of a high risk of chronic phytotoxicity, which could negatively affect tree growth and fruit quality over time (Hodgkinson et al. 2002, Fernandez et al. 2005). I saw no evidence of acute phytotoxicity (*sensu* Hodgkinson et al. 2002) on apple foliage sprayed with 2% Purespray Green®, as the high paraffin content (>99.9%) and extremely low aromatic content (<0.01%) of this oil probably minimise these effects. However, more work is necessary to assess the chronic impacts of multiple oil sprays over multiple years on pome fruit production. This type of work is necessary to demonstrate the long-term safety of these oils and encourage a more widespread adoption of their use.

Table 4.1 Mean ± SE number of eggs laid, percentage egg survival, and total number of larvae emerged from egg masses laid by *C. rosaceana* in no-choice oviposition assays on wax paper and apple leaf substrates treated with either distilled water or 2% Purespray Green® in distilled water. (DAT = days after treatment sprays, W = water, O = 2% oil).

Oviposition substrate	DAT	*n*		Mean (± SE) total number eggs laid per female[a]				Mean (± SE) percentage egg survival[a]				Mean (± SE) total number larvae emerged[a]			
		W	O	Water	2% oil	*t*	*P*	Water	2% oil	*t*	*P*	Water	2% oil	*t*	*P*
wax paper	0	29	29	593.7 ± 34.9	440.9 ± 34.1	3.1	0.003	79.9 ± 0.02	52.5 ± 0.03	6.3	<0.001	460.3 ± 23.0	231.9 ± 23.4	6.9	<0.001
apple leaves	0	20	23	512.7 ± 36.5	347.7 ± 76.8	N/A*	0.004	76.5 ± .04	39.2 ± 0.06	5.3	<0.001	403.1 ± 38.3	168.4 ± 43.2	4.1	<0.001
apple leaves	3	21	23	507.9 ± 35.2	445.3 ± 48.4	1.1	0.30	86.3 ± 0.04	76.7 ± 0.05	1.5	0.154	436.8 ± 34.6	326.8 ± 38.9	2.1	0.040

[a]Treatment differences evaluated by two-sample *t*-test, $\alpha = 0.05$. *treatment differences evaluated by Mann-Whitney U test, $\alpha = 0.05$.

Table 4.2 Mean ± SE number of egg masses, number of eggs laid per egg mass, and total number of eggs laid by *C. rosaceana* in choice oviposition assays on wax paper and apple leaf substrates treated with either distilled water or 2% Purespray Green® in distilled water. (DAT = days after treatment sprays).

Oviposition substrate	DAT	*n*	Mean (± SE) number of egg masses[a]				Mean (± SE) number of eggs per egg mass[a]				Mean (± SE) total number eggs laid[a]			
			Water	2% oil	*t*	*P*	Water	2% oil	*t*	*P*	Water	2% oil	*t*	*P*
wax paper	0	21	4.0 ± 0.3	2.9 ± 0.4	2.6	0.017	160.4 ± 17.8	151.2 ± 19.0	0.3	0.772	635.7 ± 67.4	457.5 ± 75.8	1.7	0.104
apple leaves	0	12	6.2 ± 0.6	3.6 ± 0.8	2.0	0.072	318.7 ± 18.6	269.7 ± 45.2	1.2	0.271	1957.1 ± 195.7	986.8 ± 202.9	2.9	0.014

[a]Treatment differences evaluated by paired *t*-test, $\alpha = 0.05$.

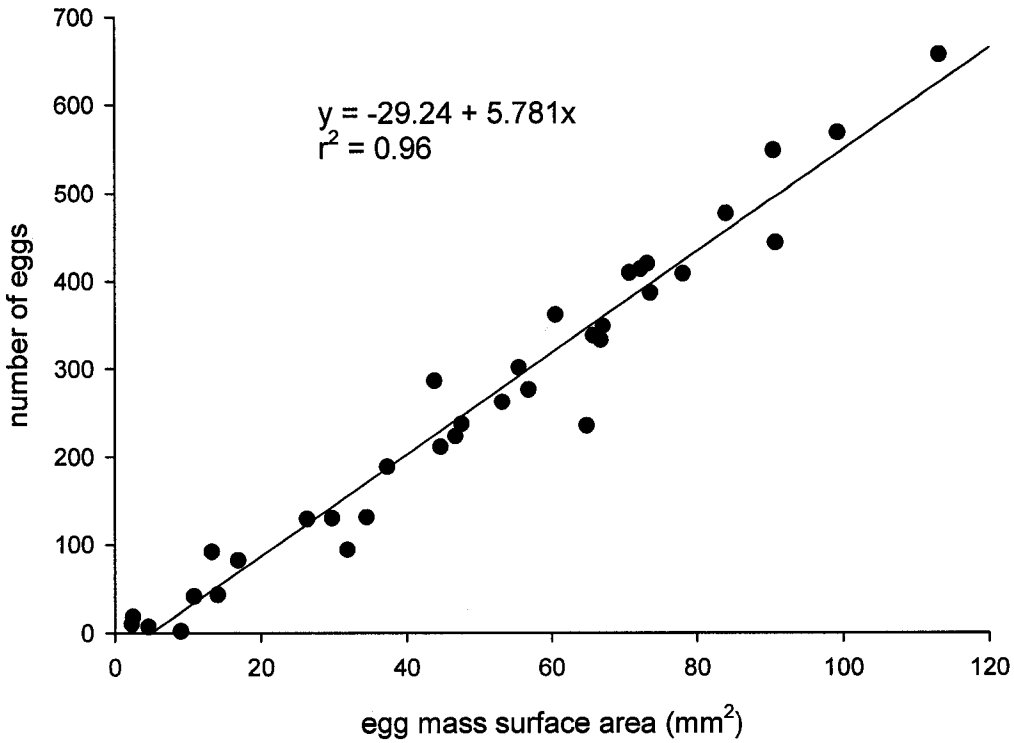

Figure 4.1 The relationship between egg mass surface area (mm^2) and the number of eggs in each egg mass for *C. rosaceana* eggs laid on clean wax paper, $N = 35$.

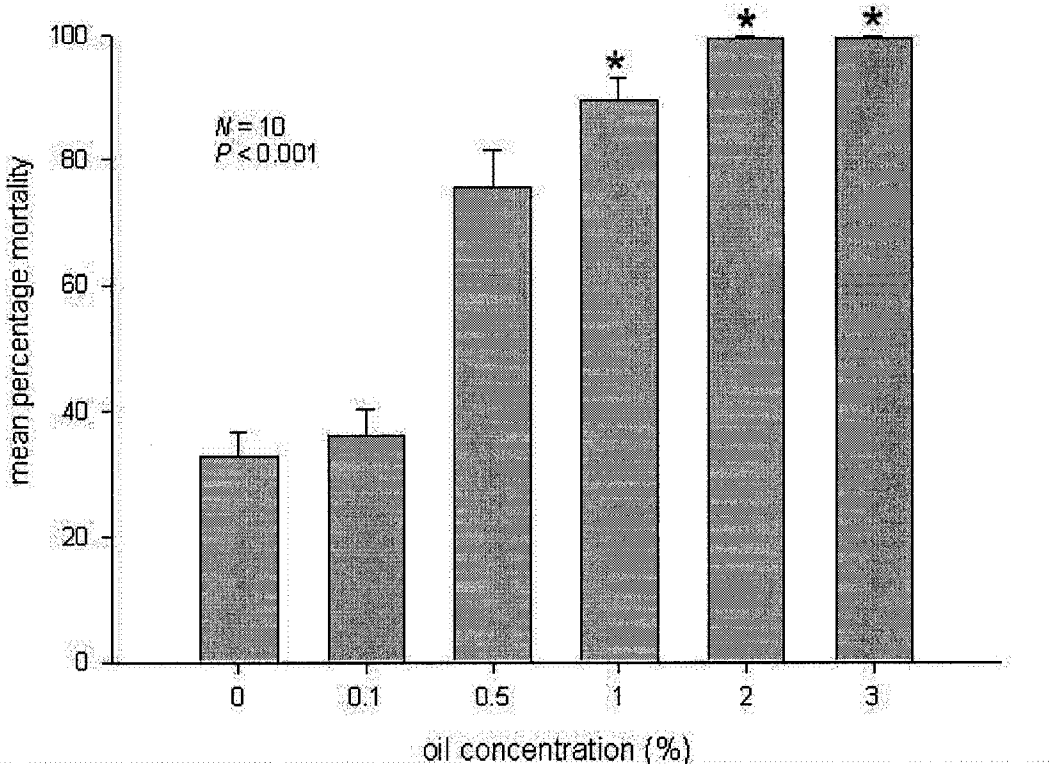

Figure 4.2 Mean (+ SE) percentage egg mortality for *C. rosaceana* eggs at the *blackheaded* stage treated topically with emulsions of 0.1, 0.5, 1, 2, and 3% Purespray Green® horticultural oil, and a distilled water control. * indicates a significant difference from the control group as assessed by Dunn's test for multiple treatment comparisons against a control following a significant Kruskall-Wallis non-parametric test.

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CHAPTER 5 . CONCLUDING DISCUSSION

5.1 Synopsis of Findings

This thesis examined the potential for synergy between microencapsulated (MEC) pheromones and horticultural oils, with a view to developing an integrated pest management (IPM) strategy that achieves better results than either tactic employed independently against *Choristoneura rosaceana* (Harris). In chapter 2, I found that mate-finding behaviour of male *C. rosaceana* was significantly disrupted for up to an hour after a one-hour exposure to a MEC- or MEC +oil-treated surface. This strong treatment effect in behavioural assays occurred although there was no antennal adaptation when tested 75 seconds after a one-hour exposure to a MEC- or MEC +oil-treated surface. This suggests that nervous system habituation may play an important role in communication disruption in this species. Flight-tunnel assays demonstrated that the addition of oil to the MEC pheromone marginally enhanced disruption of mate-finding behaviour over MEC alone during the 47-day course of the experiment. This finding may be due to the oil increasing the initial deposition of microcapsules on disc surfaces, as demonstrated here, or stabilizing the release rate of pheromone from microcapsules (Judd et al. 2006). Interestingly, both MEC and MEC + oil formulations lost little efficacy over the 47-day-long experiment. This finding points to the importance of close insect contact to MEC-treated surfaces for achieving and maintaining significant disruption of communication. It is also possible that tests of different rates of MEC pheromone or oil might distinguish a difference between the MEC and MEC + oil treatments. A lower concentration of pheromone in these treatments may have caused the disruptive effect to dissipate more rapidly over time, and consequently any differences between the oil and water MEC formulations would be magnified.

In chapter 3, I examined whether adding an oil adjuvant would increase the efficacy over time of MEC pheromones applied in the field. Although both flight-tunnel assays with treated leaves and small-plot field assays demonstrated a non-significant increase in the level of communication disruption caused by the MEC+oil treatment, there was no statistical evidence that the addition of oil increased the efficacy of MEC formulations over time. This discrepancy between the results of chapters 2 and 3 may be due in part to the oil being absorbed or removed from leaf surfaces under field conditions. My results from chapter 3 indicated that the 2% oil treatments lost efficacy after three days in the field, similar to the results of Mensah et al. (2005) and consistent with the idea that horticultural oils generally show little residual activity (Agnello 2002, Jacques and Kuhlmann 2002, Fernandez et al. 2005). Horticultural oils with higher *n*C*y* values generally persist longer on plant surfaces and could be considered for use instead of an oil like Purespray Green® in tree fruit IPM. However, oils higher than *n*C23 have a much greater chance of inducing chronic phytotoxicity (Jacques and Kuhlmann 2002). As an alternative, an application of MEC + Purespray Green® followed by multiple applications of Purespray Green® in water throughout the adult flight period (Brunner et al. 1996, Hilton et al. 2002, Fernandez et al. 2005) could help to maintain a consistent residual level of oil on foliage. If the oil does stabilize pheromone release rate by absorbing pheromone or physically blocking its release from microcapsules (Judd et al. 2006), this application protocol may help maintain a consistent pheromone release rate over time by constantly renewing the oil layer surrounding microcapsules. This may also serve to increase the rainfastness of microcapsules on foliage throughout the adult flight period. My results from chapter 4 suggest that repeated oil applications throughout the adult flight season would also provide effective control of the egg

stages of *C. rosaceana*. The fact that oil control of egg stages should be a density-independent control strategy (Fernandez et al. 2005) may also help to overcome an inherent weakness of mating-disruption regimes, namely that mating disruption tends to break down at high insect population densities (Cardé 1990, Gut et al. 2004). The combination of density-dependent and -independent control methods may provide more robust control of pests at higher population densities.

5.2 Future Research Directions

This thesis used flight-tunnel behavioural assays and small-plot mark-recapture experiments to carry out a first evaluation of an MEC pheromone formulated with a 2% horticultural oil. To the best of my knowledge, this thesis represents the first published study to examine the concept of using a horticultural oil adjuvant in a MEC formulation against any pest species. The moderate increase in communication disruption gained from this technique, along with the added ovicidal and oviposition-deterring properties of the oil, suggest that large-scale field trials assessing both disruption as well as fruit damage should be carried out using the information and techniques from this study. Although the MEC formulation used here is no longer commercially available, the principles behind formulating MEC with horticultural oils should apply to other MEC formulations. *C. rosaceana* is considered a difficult pest to control by mating disruption alone (Agnello et al. 1996, Knight et al. 1998), however commercially-available MEC formulations have been tested for both *Grapholita molesta* (Busck) (Trimble et al. 2004, Kovanci et al. 2005, Il'ichev et al. 2006) and *Cydia pomonella* (L.) (Knight et al. 2004). These primary pests of apple orchards across North America are both considered easier to disrupt than *C. rosaceana* (Stelinski et al. 2005), and the addition of horticultural oil to MEC

formulations against these species could enhance disruption more than in *C. rosaceana.*

As the tree fruit industry seeks alternatives to organophosphate insecticides to deal with established and emerging pest problems, both MEC pheromones and horticultural oils are destined to play an important role in many IPM programmes. Although each of these technologies has been deemed inadequate as a stand-alone method of control against *C. rosaceana* (Trimble and Appleby 2004, Fernandez et al. 2005), this thesis demonstrated the potential for combining these control methods in a synergistic fashion, and that the potential exists for attaining effective control using a combined strategy where each method alone might fail.

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