TOWARDS OPTIMIZATION OF SEX-ATTRACTANT PHEROMONE USE FOR DISRUPTION OF TORTRICID MOTH PESTS IN TREE FRUIT

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Entomology

2012

ABSTRACT

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As applied to insects, mating disruption is the practice of deploying synthetic sex attractant pheromones into an environment so as to interfere with normal mate finding, thereby reducing pest populations through diminished reproductive success. Recent investigations into the mechanisms of mating disruption have revealed that competitive attraction is the primary mechanism by which mating disruption operates for moth pests. In the competitive mechanism, attraction to a pheromone is the required first step toward disruption. Research presented here used the competition framework to inform development and testing of new mating disruption formulations and deployment tactics with the aim of maximizing efficacy while minimizing costs. A novel release matrix consisting of paraffin wax and ethylene vinyl acetate (hot glue) was developed for inexpensive production of an easy-to-apply and modifiable pheromone dispenser. In the laboratory, this matrix regulated the release of the pheromones of several tortricid moth pests within a desired range over several months. In the field, this matrix proved to be a good lure for possible use in monitoring programs. When applied at high densities (up to 10800 ha^{-1}), matrix dispensers on string suppressed sexual communication of Oriental fruit moth, obliquebanded leafroller, and codling moth populations, although sometimes not as well as high-releasing commercial dispensers. In large field cages, disruption of

Oriental fruit moth operated competitively when dispensers released pheromone at *ca*. 0.04 µg hr^{-1}. But disruption switched to a non-competitive mechanism when pheromone was released at *ca.* 60 µg hr^{-1}. These studies also demonstrated that an attract-and-remove scenario would enhance Oriental fruit moth control. Trapping after attraction improved suppression of sexual communication 10-fold over competitive disruption achieved by releasing pheromone at *ca.* levels released by female moths. A patent-pending microtrap was developed primarily for use in an attract-and-remove control program. Laboratory and field investigations reported here justify the trap design and function for codling moth. An attract-and-remove study also demonstrated proof-of-concept that obliquebanded leafroller could also be controlled under an attract-and-remove strategy. Collectively, this research demonstrates several ways costs may be reduced while maintaining or improving efficacy when using sex pheromones for pest management. These developments should encourage broader adoption of this environmentally friendly method of pest control.

ACKNOWLEDGEMENTS

I first want to thank my major professors, Drs. Larry Gut and Jim Miller. Their unique, complementary personalities and skill sets have provided all those around them, including me, with a vision of what true teamwork and partnership can accomplish. Larry's ability to narrow in on the essence of a problem and the focus of thought to understand how to solve those problems gave me insight into the archetypal requirements of an applied researcher. Jim's infective, sometimes exhaustive energy and unrelenting curiosity have shown me that success and supreme enjoyment in this field can be one and the same. I came to Michigan State University as a budding entomologist and, thanks to Jim and Larry, am leaving a true scientist.

I also wish to thank my committee members: Drs. Rufus Isaacs and Dan Guyer. Their critiques and input have greatly improved this dissertation and, therefore, the publications that will forever be a written record of my time here. They have also provided great insight and advice on my doctoral work, making me a broader and more well-educated scientist.

A big thanks goes out to those who have been instrumental in the work in Larry's and Jim's labs over the years. Peter McGhee has been the glue holding the tree fruit research program together. His knowledge, insight and connections have contributed greatly to the field portions of the dissertation. Juan Huang's expertise in gas chromatography and pheromone analysis and ability to be a sounding board for novel ideas and concerns have helped streamline projects and solve problems before they became problems. Piera Siegert's constancy, perseverance, and ability to keep her "eyes on the prize" were essential to create and maintain the large field cages at the

level required for the years of great results they generated. I cannot forget the multitude of undergraduate students that have passed through the labs and their daily commitment to the advancement of science, especially Krista Buehrer, Douglas Aho, Tyler Joseph, Matthew Julian, and Liz Jagenow.

Finally, I must recognize my loving wife, Amy LaPorte. Her patience and understanding through the 16-hour workdays, sleepless nights, and 7-day workweeks has been incredible. Her natural entomological curiosity even made her a valued and enjoyable temporary international technician. For all you have done, and not done, for the sake of my sanity, I love you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: INTRODUCTION TO PHEROMONE USE IN PEST MANAGEMENT

PHEROMONES FOR MONITORING

For over 80 years, intraspecific insect chemical communication has been a topic of research in the entomological community (eg. Valentine 1931). Within 15 years of the definition of the term “pheromone” (Schneider 1962) these chemical signals were being identified for insect pests including tortricid moths such as codling moth, C*ydia pomonella* L., (Roelofs et al. 1971), obliquebanded leafroller, *Choristoneura rosaceana* Harris, (Roelofs and Tette 1970), and Oriental fruit moth, *Grapholita molesta* (Busck), (Roelofs et al. 1969). Initially, synthesized pheromones were used to trap insects either for monitoring phenology and population levels of a pest species in various cropping systems, or for control by mass trapping (Roelofs et al. 1970, El-Sayed et al. 2006).

Early pheromone dispensing systems for use as lures consisted of sand-filled vials (Wolf et al. 1967), polyethylene plastic bags (Toba et al. 1969), polyethylene plastic caps (Glass et al. 1970, Roelofs et al. 1970), polyvinyl chloride pellets (Fitzgerald et al. 1973), or plastic film laminates (Kydonieus et al. 1976). The discovery of natural rubber as a carrier, either in rubber band form (Madsen and Vakenti, 1972) or as sleeve-type rubber stoppers (Roelofs et al. 1972), provided researchers with an easy-to-handle, simple, long-lasting lure capable of emitting pheromone at desired levels for numerous pest species over several months. The rubber stopper, also called the rubber septum, is still the most widely used lure type on the market for most agricultural pests (Trécé, Adair, OK). Sulfur, present in the natural rubber septum, is an isomerization catalyst for some pheromones containing conjugated diene structures in at least one of their pheromone components (Fujiwara et al. 1976, Shani and Klug 1980). Research on synthetic rubber septa designed to protect these pheromones from isomerization (Brown and McDonough 1986) has led to the current employment of a gray halo-butyl elastomer for these sensitive compounds (Knight 2002).

PHEROMONES FOR MATING DISRUPTION

Researchers quickly proposed that pheromone could also be used as a mating disruptant, confusing target pests with overwhelming levels of their own pheromone (Shorey et al. 1972, Gaston et al. 1977). Many dispensing technologies have been developed over the years, but there remains little consensus as to whether it is better to dispense pheromone from regularly spaced point sources or to broadcast pheromone throughout the crop (Gut et al. 2004). Hollow fibers (Cardé et al. 1977, Stelinski et al. 2008), plastic flakes (Witzgall et al. 1999), emulsifiable wax (Atterholt et al. 1999, deLame et al. 2007), and microencapsulated sprayables (Knight and Larsen 2004, Stelinski et al. 2007b) are all mechanically-applied disruption systems designed to dispense high densities of dispensers, each releasing pheromone at rates near that of a female moth. Hand-applied reservoir dispensers (Taschenberg et al. 1974, Witzgall et al. 2008), the most common dispenser type currently used, release pheromone at approximately 250x the rate of a female and are applied at moderate densities (250-1000 ha^{-1}). Aerosol-emitters (Shorey et al. 1996, Knight 2004, Stelinski et al. 2007a) reduce labor requirements as they are applied at rates of only one or a few dispensers per hectare. These low-density devices inundate a given area with extremely high rates of pheromone more than 1000x that of a calling female. Most of these systems are still employed commercially today (Gut et al. 2004, Witzgall et al. 2008).

The most direct measure of mating disruption efficacy is the calculation of relative crop damage and marketable volume. However, other methods have been widely employed to determine the real-time effects of disruption during a growing season (Gut et al 2004). One prominent method uses the monitoring tactics described above using capture counts of male moths in pheromone-baited traps as a measure of control. Capture of zero or very few moths is considered an indicator of a highly efficacious disruption treatment. The rationale behind this measure is that the ability of males to locate a lure releasing synthetic pheromone is indicative of their ability to locate calling female moths. Tethering virgin females to branches in the field and assessing their mating status is a similar, but more direct, measure of mating disruption efficacy. However, the approach is used infrequently as tethering entails the time-consuming and complicated system of tying thread to many colony-raised females, transporting them to the field, attaching them to tree branches, protecting them from predation, then finally retrieving them and dissecting them to determine mating status (Stelinski et al. 2005). Moreover, studies using both pheromone-baited traps and tethered females have revealed similar results using both techniques (eg. Stelinski et al. 2005, 2008). Pheromone-baited traps appear to be a less-labor intensive, simpler and effective measure of disruption.

MECHANISMS OF MATING DISRUPTION

Mating disruption interferes with the complex process of attraction and mating in insects. The behaviors involved can differ among even closely related species. The introduction of artificial blends and/or supernormal amounts of pheromone into a crop for control of a pest can impact attraction and mating behavior in various ways (Bartell 1982, Cardé and Minks 1995) (Figure 1). Different release technologies and active ingredients may be necessary depending on the mechanisms(s) of disruption to which a particular pest species is vulnerable (Gut et al. 2004).

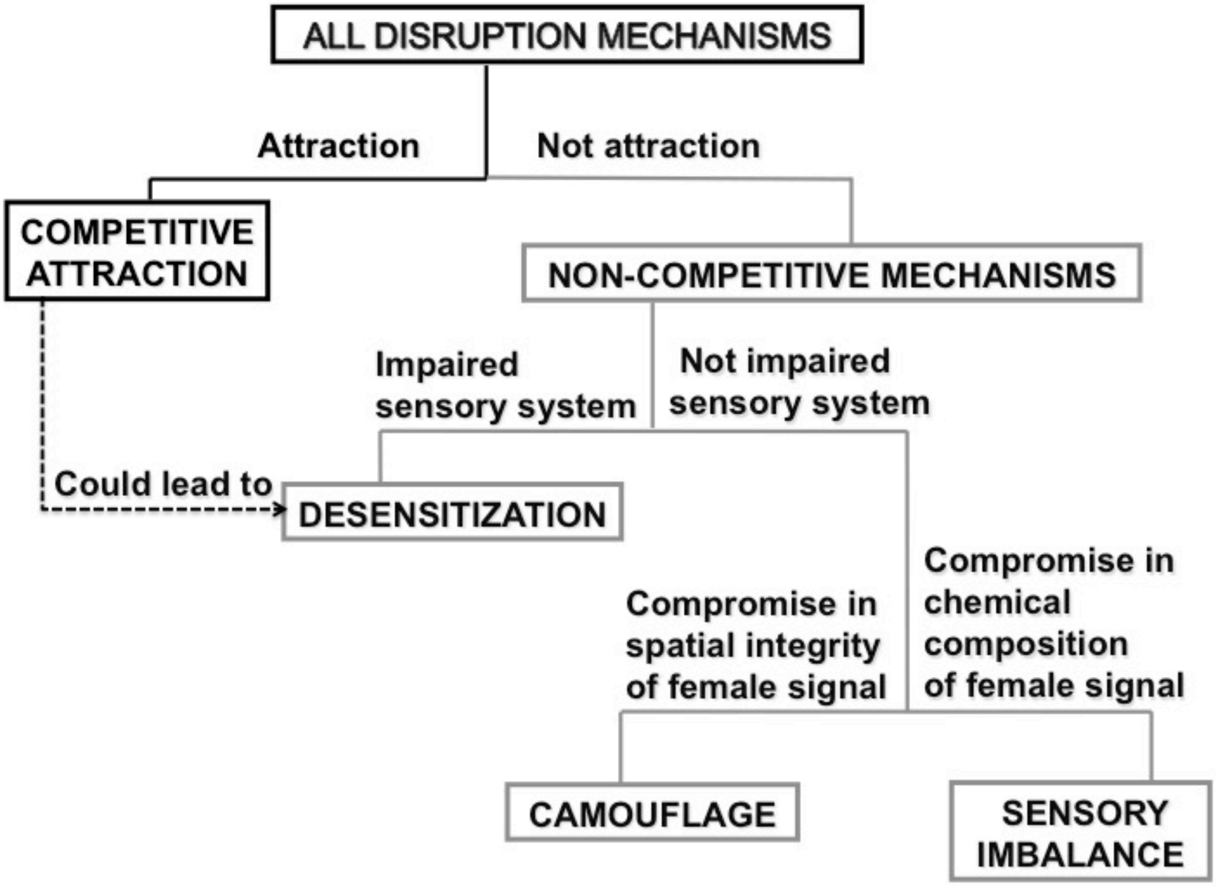

Figure 1. Mechanisms of mating disruption.

Non-competitive mechanisms

Several mechanisms for disruption by pheromone have been proposed. Most can be considered non-competitive, as they do not require competition for initial orientation towards a discrete plume for them to occur. The three most commonly discussed non-competitive mechanisms are sensory imbalance, camouflage, and desensitization (Bartell 1982, Cardé and Minks 1995).

Sensory imbalance is the release of an off-ratio blend of pheromone that creates an imbalance of sensory input. This impairs the responder from correctly deciphering the natural pheromone ratio that would be emitted by the female.

Camouflaging is the act of masking individual pheromone plumes by inundating the area with a background of similar compounds. The margins of a natural plume would dissolve into the background, rendering the female sensorially invisible to the male.

Desensitization operates on the physiology of the insect; either by reducing the responsiveness of the olfactory receptors at the periphery (adaptation) or rendering the central nervous system incapable of response to a pheromone signal (habituation). Mating disruption is achieved by preventing the male from behaviorally responding to a pheromone signal.

These mechanisms can be separated and defined. Unfortunately, because they necessarily do not require orientation, they are currently indistinguishable in field experiments. Indeed, before Miller et al. (2006a) differentiated these mechanisms from competitive attraction, interpretation of mechanisms was anecdotal and speculative. Miller et al. (2006a) extended knowledge of enzyme kinetics to develop mathematical models capable of separating competitive from non-competitive mechanisms using captures of insects in monitoring traps.

Competitive Attraction

Competitive attraction operates by presenting artificial plumes to males so as to out-compete those emanating from female moths. Recently Miller et al. (2006a,b) developed a mathematical framework for differentiating between competitive and non-competitive mechanisms of disruption. The fundamental difference between the two is the rate at which randomly distributed individuals are behaviorally removed from the mating pool. Under non-competitive disruption, individuals are subtracted from the mating pool at a particular rate as dispensers are added to the habitat (Figure 2). This relationship holds because each pheromone dispenser disrupts a discrete area of crop, inside which orientations to female plumes are impossible. If such plumes from dispensers were to cover the entire crop, complete shutdown of orientation females and monitoring traps would be expected. Under competitive disruption, individuals are removed from the population in a manner predicted by the following equation (Miller et al. 2006a) for the simplest case where males are certain to find traps, traps are 100% efficient, and dispensers operate with the same efficacy as traps:

Catch in a monitoring trap used to assess disruption = (number of traps * number of males)/(1 + (number of traps + number of dispensers))

Under this relationship the first dispensers added have the largest numerical impact on catch suppression. Each additional dispenser reduces catch, but the magnitude of the reduction for each given dispenser progressively diminishes in a manner where zero catch is approached asymptotically (Figure 2). If one transforms these profiles, the

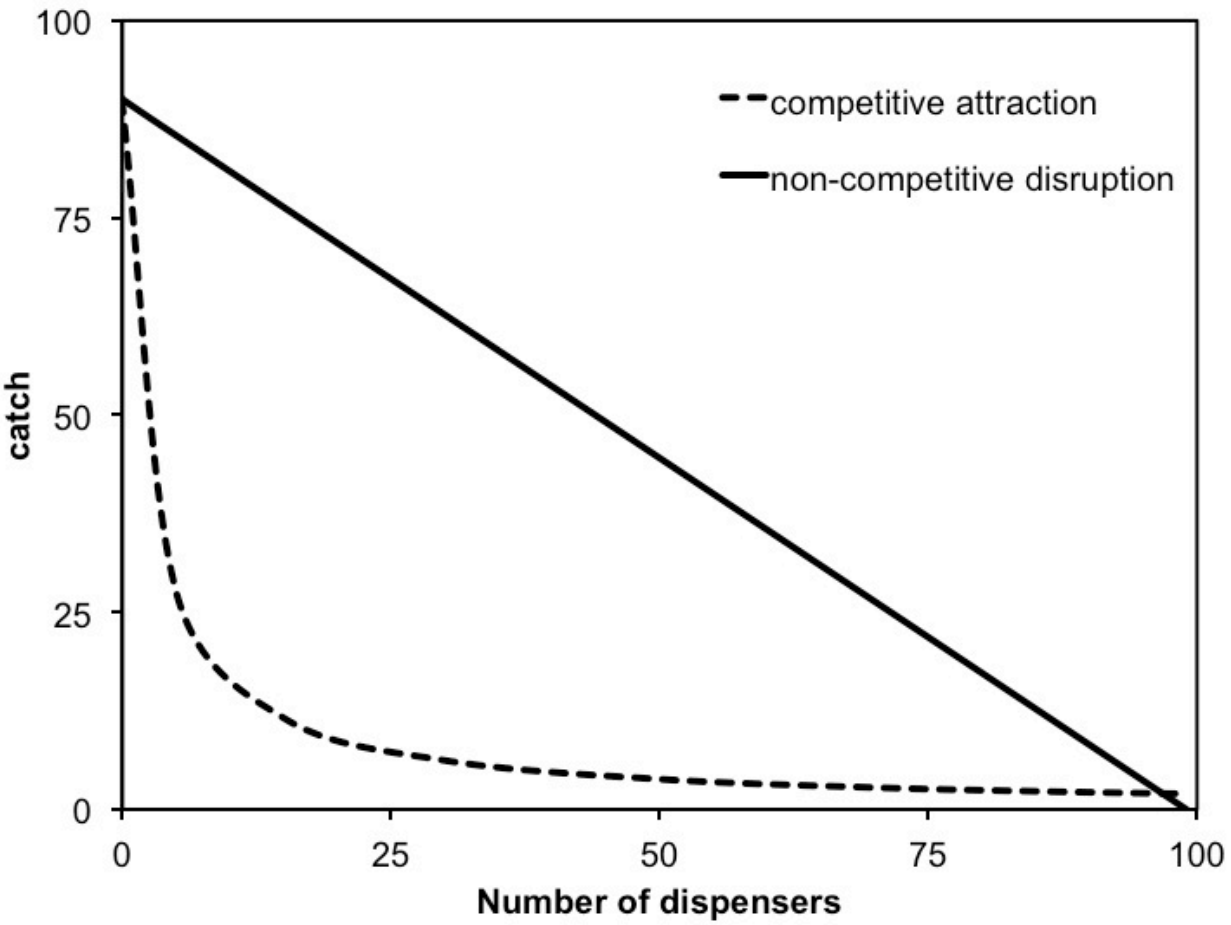

Figure 2. Plot of theoretical competitive attraction and non-competitive disruption curves as catch of pest vs. disruption dispenser density.

competitive attraction profile becomes more unique. Plotting the dispenser density against 1/catch (Miller-Gut plot), the competitive attraction profile becomes a straight line with a positive slope (Figure 3). A non-competitive profile becomes concave. Plotting catch against dispenser density x catch (Miller-de Lame plot), the competitive attraction profile remains straight but with a negative slope (Figure 4). The non-competitive profile is distinctly re-curved with its apex approximately halfway along the y-axis. These profiles highlight distinct differences in the behavior of individuals in a population under competitive vs. non-competitive disruption.

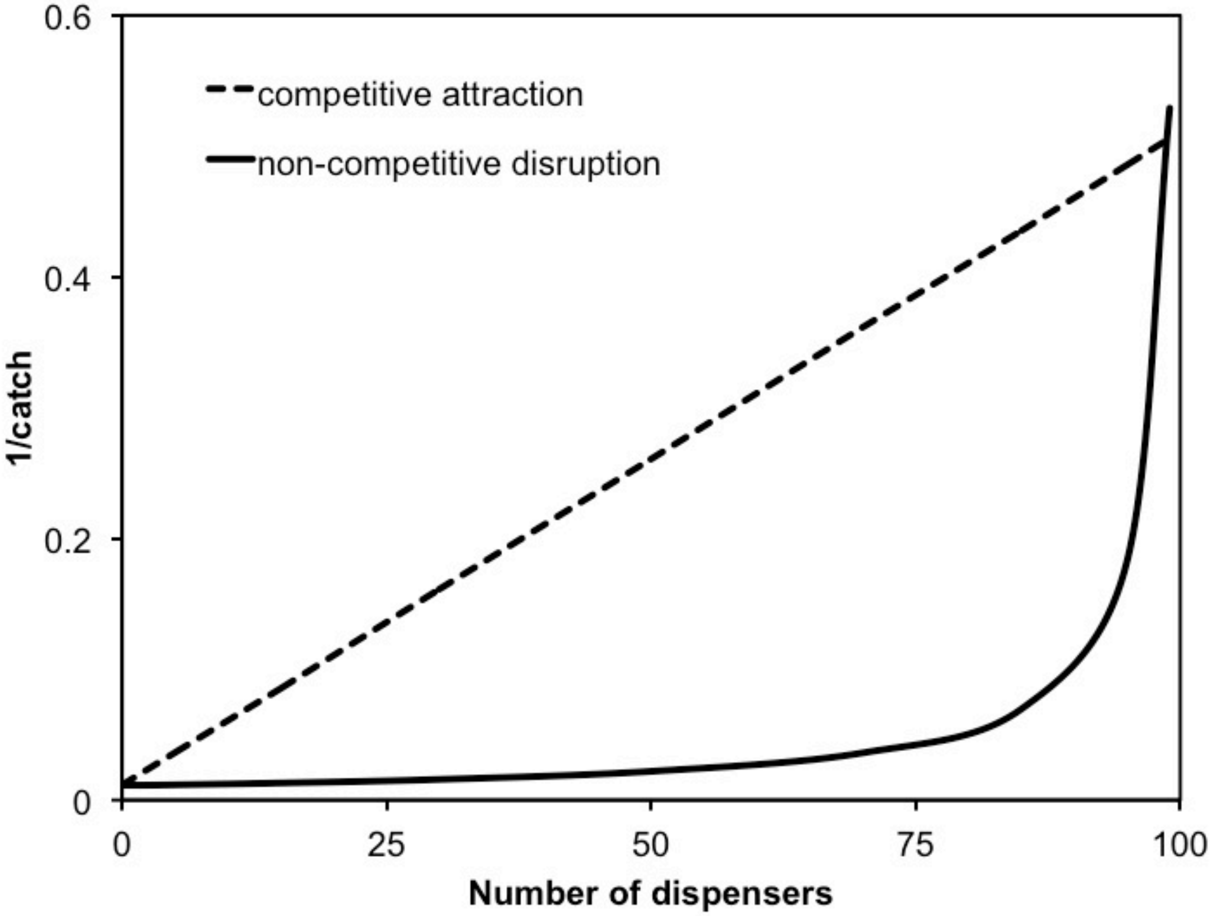

Figure 3. Miller-Gut transformation of theoretical competitive attraction and non-competitive disruption curves. Plot is 1/catch of pest vs. disruption dispenser density.

Eleven of 13 previous studies analyzed using the above calculations followed the competitive attraction profiles (Miller et al. 2006b). The conclusion from these analyses is that competitive attraction is the primary method of pheromone-based mating disruption currently being used by available products.

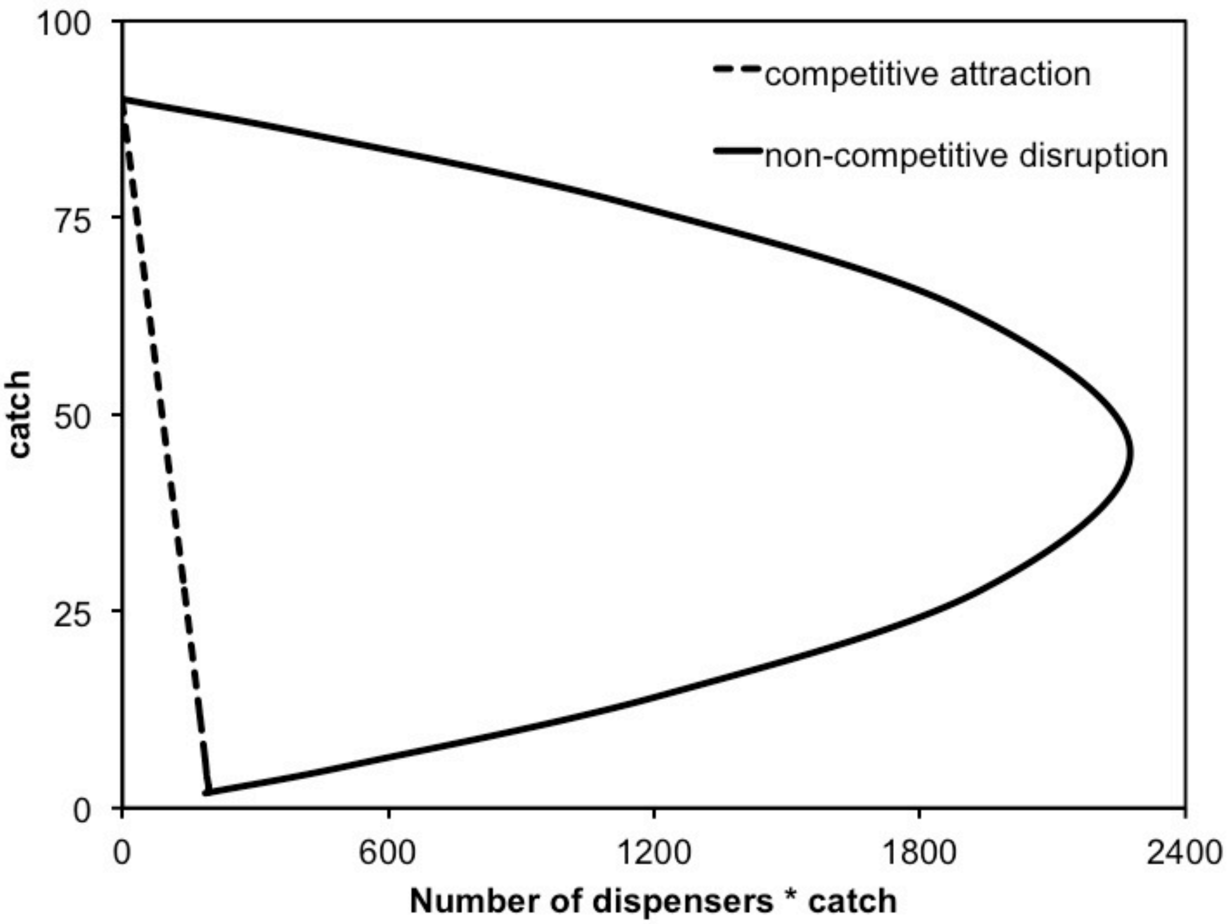

Figure 4. Miller–de Lame transformation of theoretical competitive attraction and non-competitive disruption curves. Plot is catch of pest vs. dispenser density*catch.

ATTRACT AND REMOVE

Mating disruption for pest control has improved in the last 10-15 years. Many new products have been developed, some of which have been very successful at controlling the targeted pest while reducing the quantity of supplemental insecticide sprays (Staten et al. 1996, Il'ichev et al. 2002). There appears, however, to be a limit to how well a pheromone treatment alone can disrupt the mate-finding ability of an insect species under the competitive attraction mechanism. By coupling competitive attraction with the supplementary effect of removing the pest individual from the population either by killing it or permanently deactivating (trapping) it, superior control can possibly be achieved.

Attract and Kill

Attract-and-kill, or lure-and-kill as it is sometimes called, is the act of attracting an insect to a device that then kills them with a lethal dose of insecticide. This tactic has been implemented for control of several insect pest species, including: fruit flies, sap beetles, and shoot borers (Daterman et al. 2001, Hossain et al. 2008, Pinero et al. 2009). The reliability with which tortricid moth pests can be lured within close proximity to a pheromone source provides promise that they should be particularly vulnerable to attract-and-kill.

Insecticides typically used in agricultural systems today are designed to break down rapidly so as not be a prolonged environmental threat. Contrastingly, attract-and-kill treatments strive to be effective as long as possible. The chemical class of choice for attract-and-kill treatments has been pyrethroids (Losel et al. 2000, Poullot et al. 2001, Sukovata et al. 2004, Evenden and McLaughlin 2005) due to their high efficacy in low doses and residual action. Most attract-and-kill formulations combine the killing agent with pheromone in a viscous paste that can readily be applied to the crop, typically as a drop or dollop.

Many tortricid moths have been targets of attract-and-kill research such as: Oriental fruit moth (Evenden and McLaughlin 2004), codling moth (Krupke et al. 2002, Lösel et al. 2002, Knight 2003), obliquebanded leafroller (Curkovic and Brunner 2006), and light brown apple moth, *Epiphyas postvittana* (Walker), (Suckling and Brockerhoff

1999). While these studies generally have shown promise in the laboratory, few have demonstrated a supplementary effect of the insecticide in the field. In most cases the attract-and-kill formulation provides control principally via mating disruption. It is likely the majority of the formulations tested did not have pheromone release rates optimized for sustained source contact. Pheromone must release at rates high enough to attract males from afar while not releasing at rates that deter the insect from contacting the source. If pests do contact the source, adequate amounts of insecticide might not be transferred due to inadequate transfer of toxicant from the formulation.

Mass Trapping

Before pheromones were combined with insecticides for attract-and-kill, pheromone was added to adhesive traps to physically remove pests from the population. This method is called “ mass trapping” (e.g., Glass et al. 1970, Roelofs et al. 1970, and Taschenberg et al. 1974). Early experiments used high trap densities in an attempt to control pest populations, with some good results. Glass et al. (1970) showed delayed egg laying of redbanded leafroller, *Argyrotaenia velutiana* (Walker), when they applied 1700 cup-style sticky traps in 20 ha of apple trees (210 traps ha^{-1}). Taschenberg et al. (1974) provided moderate control of redbanded leafroller and grape berry moth, *Paralobesia viteana* Clemens, in smaller 1.1 ha grape vineyards using 175-334 traps. Roelofs et al. (1970) performed two mass trapping experiments for control of redbanded leafroller that varied dramatically in their results. Under high pest pressure, application of 2400 traps in 8 ha of apples (300 traps ha^{-1}), reduced mating less than 50% and allowed 40% crop damage. Under lower pest pressure, 1100 traps in 6 ha of apples (183 traps ha^{-1}), reduced mating up to 99%. Only one apple was damaged in the second study.

High lure and trap costs and trap maintenance necessitated a reduction in trap densities for application in a commercial system. Pink bollworm, *Pectinophora gossypiella* (Saunders), could be adequately controlled using trap densities as low as 11-20 traps ha^{-1} (Huber et al. 1979, Mafra-Neto and Habib 1996). Unfortunately, most species were not as susceptible to low trap densities. Codling moth control was demonstrated using a similarly low trap density of 10 traps ha^{-1} (Madsen et al. 1976, Willson and Trammel 1980) under low moth populations. Moderate populations required 30-40 traps ha^{-1} to noticeably reduce crop damage (Willson and Trammel 1980). Attempts to control low populations of several other species of tortricid moths: Oriental fruit moth, lesser apple worm, obliquebanded leafroller, redbanded leafroller, and threelined leafroller were unsuccessful using the same 30-40 traps ha^{-1}(Willson and Trammel 1980). Recently, control of dogwood borer was not possible with traps placed at 5 and 20 traps ha^{-1}(Leskey et al. 2009).

Renewed international research using higher trap densities has met with greater success. In China, the rates of mating of caged virgin females of the Chinese tortrix, *Cydia trasias* (Meyrick), was reduced by 65-70% in urban plantings of Chinese scholar trees, *Sophora japonica* L., using traps in transects spaced 9 m apart (Zhang et al. 2002). Damage of leaf petioles and seedpods by subsequent generations was reduced by as much as 81% and 96%, respectively. Population densities of overwintering generations were also significantly reduced. In India, the overuse of insecticides for the control of brinjal shoot and fruit borer in eggplant has led to reduced crop yields and increased production costs. Incorporation of mass trapping in conjunction with an integrated pest management program has yielded positive results (Cork et al. 2005). One hundred traps ha^{-1} and a rigorous practice of removing infested shoots greatly decreased fruit infestation in later harvests. Two years into the study, plots under the IPM regime produced four times the amount of healthy fruit. One supporting factor for the IPM plots was the reemergence of several parasitoid species virtually eliminated by the standard insecticide treatments.

Recent work into modeling of mass trapping systems produced mixed support for mass trapping. Byers (2007) concluded that mass trapping is more effective than mating disruption for reducing mating occurrences. He used a computer-simulated population of male and female insects as well as a number of traps or dispensers randomly placed in a virtual block of trees one hectare in size for mass trapping or mating disruption, respectively. Model assumptions included: 1) a female is only capable of mating once in a lifetime, 2) once a male enters the effective attraction radius (EAR) of a trap he is necessarily caught, 3) when a male enters the EAR of a female or a dispenser he is occupied for a discrete time, 4) when a female EAR is overlapped by that of a dispenser or trap the artificial pheromone source gets priority, and 5) the ratio of females to artificial pheromone sources is between 1:10 and 5:1. The model indicated: 1) the larger the EAR of a dispenser or trap, i.e. the greater the attraction ratio compared to a female, the lower the mating frequency, 2) the longer the moths remain orienting to a source, the lower the mating frequency, 3) the more dispensers or traps, the lower the mating frequency, and 4) the fewer the females, the lower the mating frequency.

Using mathematical modeling, Yamanaka (2007) concluded that the majority of the effects from mass trapping are actually mating disruption from the pheromone source in the trap, not the capture itself. Some assumptions were different from those of Byers (2007): 1) when pheromone plumes overlap, probability of orientation to either plume is 50%, 2) probability of capture in a trap once the insect is orienting to a plume is 50%, 3) the ratio of females to traps is 312:1, and 4) the EAR of a lure is 15 times that of a female. Under these assumptions the additional benefit of removing males with traps was negligible. Many females can be found inside the EAR of one trap. The chances are low of capturing a male once it orients to a trap. Yamanaka (2007) concluded that long living pests should be easier to control when at endemic levels. Also, each individual male is more important due to the potentially larger number of matings an individual can have. The model showed that overlapping of trap EAR's did not increase control, creating an upper limit, beyond which the addition of traps will show little improvement in control. Under these assumptions, increasing lure efficiency is more important than trap efficiency to improve control.

Most recently, Miller et al. (2010) used large field cages to compare the effects of near-female-equivalent dispensers and high-releasing dispensers on disruption of codling moth male catch in monitoring traps. The former dispensers consisted of monitoring traps with lures, but no sticky liner. The authors also included treatments with liners to compare the effects of attract-and-remove to disruption. Both dispenser types followed the competition equation of Miller et al. (2006a,b, 2010). The addition of the sticky liner to the competing traps improved control significantly. They reduced male orientations to point sources 4-fold over the high-releasing dispensers and 14-fold over the near-female-equivalent dispensers.

DISSERTATION RESEARCH.

The goals of this project were three-fold: 1) to test the effects of near-female-equivalent dispensers placed at extremely high densities to take advantage of the competitive attraction mechanism, 2) to determine the mechanisms involved in Oriental fruit moth control using large field cages, and 3) to develop an effective and inexpensive attract-and-remove system for enhanced pest disruption.

Chapters 2 and 3 feature the development and application of a novel pheromone release matrix for monitoring and control of codling moth, Oriental fruit moth, and obliquebanded leafroller. The novel matrix was developed to create a dispenser that was easy to produce, inexpensive, long lasting, and easy to apply in extremely high densities. As mating disruption of most species operates by competitive attraction, the plan was to develop an application method to greatly overwhelm the pest population with artificial dispensers, effectively eliminating the chances of males finding actual females.

Disagreement and confusion exists as to which mechanism(s) are involved in mating disruption, especially among tortricid moth species. While competitive attraction appears to be the primary mechanism used in pheromone-based mating disruption, a rich body of evidence exists that supports other, non-competitive mechanisms, especially for Oriental fruit moth. Chapter 4 examines the mechanisms by which

Oriental fruit moth disruption occurs in response to the rate of release of pheromone point sources. A goal was to shed light upon why Oriental fruit moth is easier to control than codling moth using a pheromone-based approach.

Further development of trapping technology and the understandings of pheromone action since the early mass trapping experiments supports renewed study of this tactic. Chapters 5 and 6 detail the construction of a novel trap and its use in an attract-and-remove scenario. Several trap types are described, with explanations for the decision to use the cube-shaped design for use in trapping of codling moth.

CHAPTER TWO: CONTROLLED RELEASE OF CODLEMONE FROM ETHYLENE-VINYL ACETATE AND PARAFFIN WAX FORMULATIONS: IMPLICATIONS FOR ALTERNATIVE INSECT LURES

ABSTRACT

Paraffin wax is used in entomological research for controlled release of various pheromone compounds, while ethylene-vinyl acetate (EVA) is used for controlled-release of some medical drugs. Here we explored use of wax and EVA alone and in combination as matrices for controlled release of the sex pheromone of codling moth, *Cydia pomonella*, codlemone ((E,E)-8,10-dodecadien-1-ol). Codlemone release increased in direct proportion to the percentage of EVA in wax. Addition of powdered carbon as a photo-degradation protectant generally lowered the release rate of codlemone. In most cases, the pheromone release profile followed the Higuchi square root of time model, suggesting that diffusion through the matrix was the mechanism limiting release. Wax and EVA blends were evaluated as matrices for monitoring trap lures in orchards. Some of these custom lures equaled the performance of commercial rubber septum lures.

INTRODUCTION

Since its identification and synthesis some four decades ago (Roelofs et al. 1971), the sex pheromone of coding moth, *Cydia pomonella*, (E,E)-8,10-dodecadien-1-ol (codlemone), has been widely deployed in diverse formulations for mating disruption, attract-and-kill, and population monitoring (Witzgall et al. 2008). Compared to disruption programs, release of pheromones for monitoring is especially demanding. For codling moth, as an example, it requires the attraction of a maximal number of insects into sufficiently close proximity for ensnarement throughout as much of the growing season as possible; ideally 150-180 days. This means the pheromone, or pheromone components in the case of a blend, must: (1) be released in consistent rates similar to those of the female, (2) not contain antagonists inhibitory to close-range attraction, and (3) be protected from isomerization or other types of degradation.

Paraffin wax can be an effective pheromone release matrix for mating disruption formulations (Atterholt et al. 1999, Stelinski et al. 2006, Behle et al. 2008). While best known for its adhesive properties, ethylene-vinyl acetate (EVA) has become a controlled release matrix for various medicines (e.g. Cho et al. 2005, Kim et al. 2006). Both EVA and paraffin waxes have melting points at or below the boiling point of the majority of pheromones used for monitoring moth populations. These materials are desirable as potential lure matrices because they are inexpensive, easy and flexible to manufacture, and non-toxic.

Here we report laboratory and field experiments on the performance of EVA as a potential release matrix for codlemone. We also explored combining EVA with paraffin wax so as to generate a flexible set of solids with diverse release characteristics. The performance of these matrices was determined through comparisons to various release rate models and diffusional characteristics (Higuchi 1963, Korsmeyer et al. 1983, Shoaib et al. 2006). Models tested include zero order, first order, and Higuchi square root of time. The Korsmeyer-Peppas model refined understanding of the mechanism of release (Korsmeyer et al. 1983). This model helped determine whether diffusion through the matrices tested occurs by traditional Fickian methods or through anomalous (non-Fickian) diffusion, typically involving both Fickian diffusion and polymeric relaxation or disentanglement (Qui and Zhang 2000, Shoaib et al. 2006).

Codlemone is sensitive to degradation from exposure to ultraviolet light, requiring the addition of antioxidants or UV stabilizers for long-term protection (Millar 1995). As such, some of the formulations received activated carbon powder, a known photo-degradation inhibitor (Funt et al. 1993) and possibly a release-rate modifier.

MATERIALS AND METHODS

Pheromone and Matrix Materials

(E,E)-8,10-dodecadien-1-ol (codlemone), was obtained from ShinEtsu Ltd., Tokyo, Japan. Paraffin canning wax (Gulfwax) was obtained from Royal Oak Sales, Inc., Roswell, GA. Ethylene vinyl acetate (EVA) hot melt adhesive (GIA 1051) was obtained from Glue Machinery Inc. Melting points for the wax and EVA formulations were 53 and 75°C, respectively. Low-sulfur activated carbon powder produced from coconut shells (GX203) was obtained from PICA USA, Inc., Columbus, Ohio.

Matrix Assembly

Measured amounts of wax and/or EVA were heated in a glass beaker on a hot plate to just above melting. Carbon was then added to the molten matrix with vigorous stirring. Finally, the liquid pheromone was added and the formulation was immediately poured either onto pieces of waxed paper or into conical plaster-of-Paris molds, depending on the study.

In laboratory studies, the formulation was dripped onto wax paper to produce *ca.* 6mm diameter hemispherical individual lumps. The wax paper was then cut into 10mm x 40 mm pieces, each containing a single drop. A piece of aluminum foil (30 mm x 100 mm) was folded lengthwise into thirds then placed under the wax paper for structural support. The ends of the foil plate were folded over, enclosing the ends of the wax paper. Drop plus plate combinations weighed 1.0 ± 0.02 (S.E.M.) g.

For the field studies, formulations were poured into conical depressions (24mm high x 15.5 mm dia.) in plaster blocks. To serve as a handle and hanger for each formulated piece, a bent 0.5 cm length of *ca*. 8 cm long steel wire was submerged into each molten lump. Pre-soaking the molds in water greatly aided cooling and release of the lure from a mold without damage.

Release Quantification

Drops were weighed weekly on a Cahn C-35 Ultra-Microbalance (Thermo Electron Corporation, Beverly, MA). The ±0.1µg microbalance accuracy was sufficient to record the weekly weight loss of pheromone, typically in the range of 0.2-1.0mg. Drops were hung at room temperature (22-25°C) in a laboratory fume hood with wind velocity of ca. 0.4 m/sec for aging between weighings. For each treatment, drops containing all ingredients but without pheromone served as controls. The weight of the corresponding control treatment was subtracted from its pheromone-containing counterpart.

Experiment 1. Matrix Composition

The formulations tested were: (1) paraffin wax only, (2) 1:1 blend of paraffin wax plus EVA, and (3) EVA alone, each loaded with: no pheromone (control), codlemone at 10% by weight, or codlemone plus powdered carbon also loaded at 10% by weight. Five replicates of all treatments were tested simultaneously between 17 March 2008 and 19 October 2008.

Experiment 2. Codlemone Loading Rate

To evaluate the effects of active ingredient concentration on release rate, codlemone was loaded into a 1:1 blend of paraffin wax and EVA at: 0 (control), 1, 3, 10, or 20% by weight. All concentrations were tested both with and without carbon added at 10% by total weight. Five replicates of all five treatments were hood-aged simultaneously between 14 September 2008 and 29 January 2009.

Experiment 3. Lure Study 1

This field-trapping study was conducted in apple orchards at the Michigan State University Trevor Nichols Research Center near Fennville, MI. The experimental design was randomized complete block with 5 replicates. The matrix formulation with carbon from Experiment 2 was loaded with codlemone at 0.05, 0.5, or 0.2% by total weight. Codling moth captures in Trécé delta VI monitoring traps baited with each of these lures were compared with those in traps baited with a Trécé CML2 rubber septum. Traps were hung in the apple trees at a height of 2.5 m and distributed in linear transects with

15m between traps. Traps were rotated to new positions within each transect twice weekly beginning 15 May 2007 and ending 14 September 2007.

Experiment 3. Lure Study 2

This field-trapping study was performed in commercial apple orchards near Tatura, Victoria, Australia. The lure matrix was that of Experiment 2, but included carbon at 5% by total weight and codlemone at 0.1, 0.3, 0.5 or 0.7% by total weight. The treatments were slightly different from Lure Study 1 in an effort to further improve the pheromone loading to maximize moth capture. These formulations were compared to a Trécé CML2 septum. Traps were hung in the apple trees at a height of 2.5 m and distributed in linear transects with 24m between traps. The placement of traps within each of four replicate transects was rotated weekly beginning 5 November 2008 and ending 11 February 2009.

Analyses of Release Profiles

Release profiles, as measured by percent pheromone remaining, were tested for fit to the models commonly useful (Higuchi 1963, Shoaib et al. 2006) in differentiating among the factors most influential in shaping release kinetics (Table 1). The Korsmeyer-Peppas model characterizes drug release from a polymeric system (Korsmeyer et al. 1983). The first 60% of the drug released is fitted to the Korsmeyer-Peppas equation in Table 1. Values of $n \leq 0.5$ indicate release controlled by Fickian diffusion. Values of $n \geq 1.0$ indicate case II or super case II transport (Korsmeyer et al. 1983). Values of $0.5<n<1.0$ indicate anomalous (non-Fickian) diffusion.

For laboratory release rate studies, elapsed days of release was square root transformed then subjected to analysis of variance (ANOVA) to differentiate among formulation treatments. For lure efficacy studies, moth catch data were transformed to ln (x + 0.5) (which normalized the distributions of residuals and homogenized variances) and were then subjected to ANOVA. Differences between pairs of means were separated using the least significant difference test (SAS Institute, 2000). In all cases, the critical significance level was $\alpha<0.05$.

Table 1. Release rate models. Formula variables are: C = pheromone concentration, C_o = initial pheromone concentration, k = rate constant, t = time, M_t / M_∞ = the fraction released at time t, n = release exponent (Korsmeyer et al. 1983, Shoaib et al. 2006).

	Controlling variable	**Formula**	**Linear plot**
Zero order	Evaporation	$C = kt$	% remaining *vs.* time
First order	Concentration	$\log C = \log C_o - kt/2.303$	Log of % remaining vs. time
Higuchi	Diffusion	$C = kt^{1/2}$	% remaining *vs.* square root of time
Korsmeyer -Peppas		$M_t / M_\infty = kt^n$	Log of % remaining *vs.* log of time

RESULTS AND DISCUSSION

Experiment 1. Matrix Composition Study

The percentage of codlemone remaining in paraffin wax, EVA, and their combination is shown in Figure 5 as a function of the square root of time. Release from all three matrix types was significantly different between formulations in pairwise comparisons ($P<0.001$). Emission was slower from paraffin wax than EVA. The wax/EVA matrix released pheromone at a rate intermediate to the respective single-component matrices. For paraffin wax and the wax/EVA blend, carbon slowed the release of codlemone ($P<0.001$ and $P<0.001$, respectively). Addition of carbon to EVA reduced the emission rate of codlemone slightly, but this difference was not statistically significant (P=0.82).

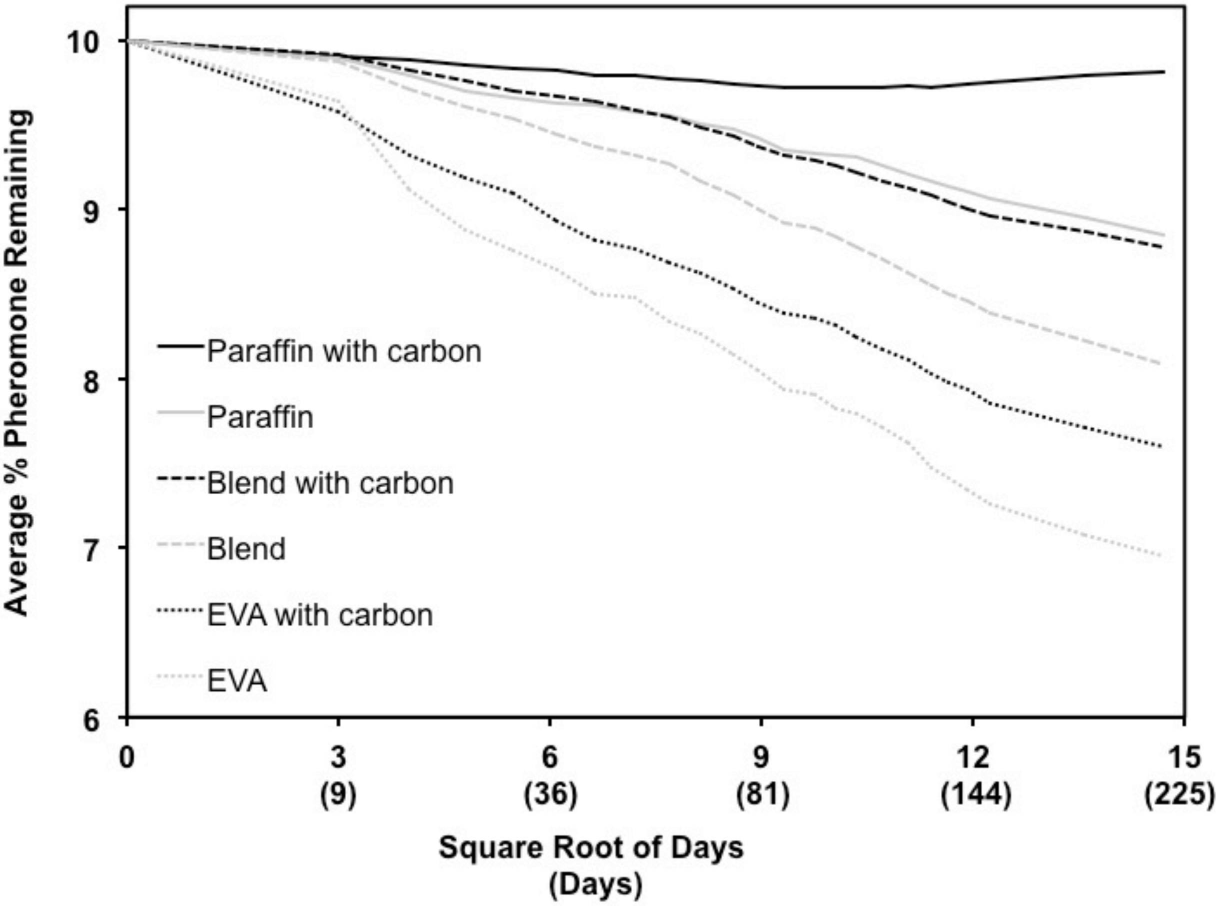

Figure 5. Percent codlemone remaining by weight over time in various matrices developed to control release of codlemone. Standard errors are not included to prevent cluttering.

The quasi-crystalline paraffin wax may have diminished codlemone movement through that matrix relative to the non-crystalline EVA. Also, the alcohol moiety of codlemone imparts notable polarity (Wohlfarth 2011). EVA is also polar in proportion to its percentage of vinyl acetate. Wax is, however, highly nonpolar. We suggest that these differences in polarity between the matrices and codlemone result in the pheromone being more soluble in EVA than in the wax, enabling the pheromone to move more readily to the surface of the matrix.

The addition of carbon slowed the release of codlemone when the matrix contained wax (Figure 5). Powdered carbon is highly porous and adsorptive, which makes it likely that the carbon adsorbed a portion of the pheromone and may have released it at a slower rate than the diffusive rate through the matrix. Alternatively, the carbon might have permanently adsorbed some of the codlemone so as to slow release by effectively reducing the available codlemone concentration in the matrix. The carbon, however, had no significant effect on codlemone release when the matrix consisted solely of EVA. Here, the carbon apparently did not permanently adsorb pheromone. Perhaps the carbon differentially favored adsorption of EVA over codlemone. It is also possible that the amorphous EVA solid was more adept at sequestering the carbon powder than was the crystalline wax. Additional testing is required to fully determine carbon’s role in the modification of codlemone release.

Release profiles for formulations of Experiment 1 containing wax fit the first order model best (Table 2) indicating pheromone release was likely concentration limited. The formulations containing EVA only released codlemone according to the Higuchi model, indicating diffusion-controlled release. Further scrutiny via the Korsmeyer-Peppas equation revealed release from the EVA matrix operated by Fickian diffusion, while release from the matrices containing wax generally operated under anomalous (non-Fickian) diffusion. This suggests that pheromone release from the wax-containing matrices operated under a combination of Fickian diffusion and polymeric relaxation.

This relaxation typically occurs for matrices near transition temperatures due to creation of glassy polymers (Crank 1979). A glassy polymer stretches and contracts slower than a non-glassy one; thus, molecular pores frequently open and close within the matrix. The melting point of the EVA was likely high enough that this matrix never reached its glass transition temperature during this laboratory study. It is possible, however, that the wax did reach the point of polymeric relaxation. The paraffin wax with carbon was not included in the analysis, as it essentially stopped releasing pheromone early in the study. Model fit could not be performed.

Table 2. R^2 fit of release rate transformations from codlemone release rate experiments. Fits better than competing models by 0.01+ are in bold. Korsmeyer-Peppas diffusional exponent determines diffusional mechanism.

Treatment	Zero order	First order	Higuchi	Diffusional exponent
Paraffin wax	0.972	0.977	0.976	0.64
Paraffin wax with carbon	0.917	0.920	**0.995**	0.50
Blend	0.971	0.979	0.974	0.75
Blend with carbon	0.973	0.978	0.969	0.78
EVA	0.897	0.928	**0.989**	0.48
EVA with carbon	0.907	0.929	**0.996**	0.50
1%	0.978	**0.980**	0.924	0.996
1% with carbon	0.908	0.921	**0.959**	0.71
3%	0.931	0.965	**0.975**	0.69
3% with carbon	0.780	0.812	**0.945**	0.44
10%	0.877	0.898	**0.969**	0.54
10% with carbon	0.709	0.719	**0.883**	0.40
20%	0.842	0.852	**0.930**	0.65
20% with carbon	0.794	0.807	**0.912**	0.60

Experiment 2. Codlemone Loading Study

The higher the initial codlemone concentration in the wax/EVA mixture, the greater the release rate throughout the study (Figure 6). Among the formulations without carbon, all pairwise comparisons of release rates from loading rate treatments were highly significant (P<0.002). The same was true for formulations containing carbon (P<0.02).

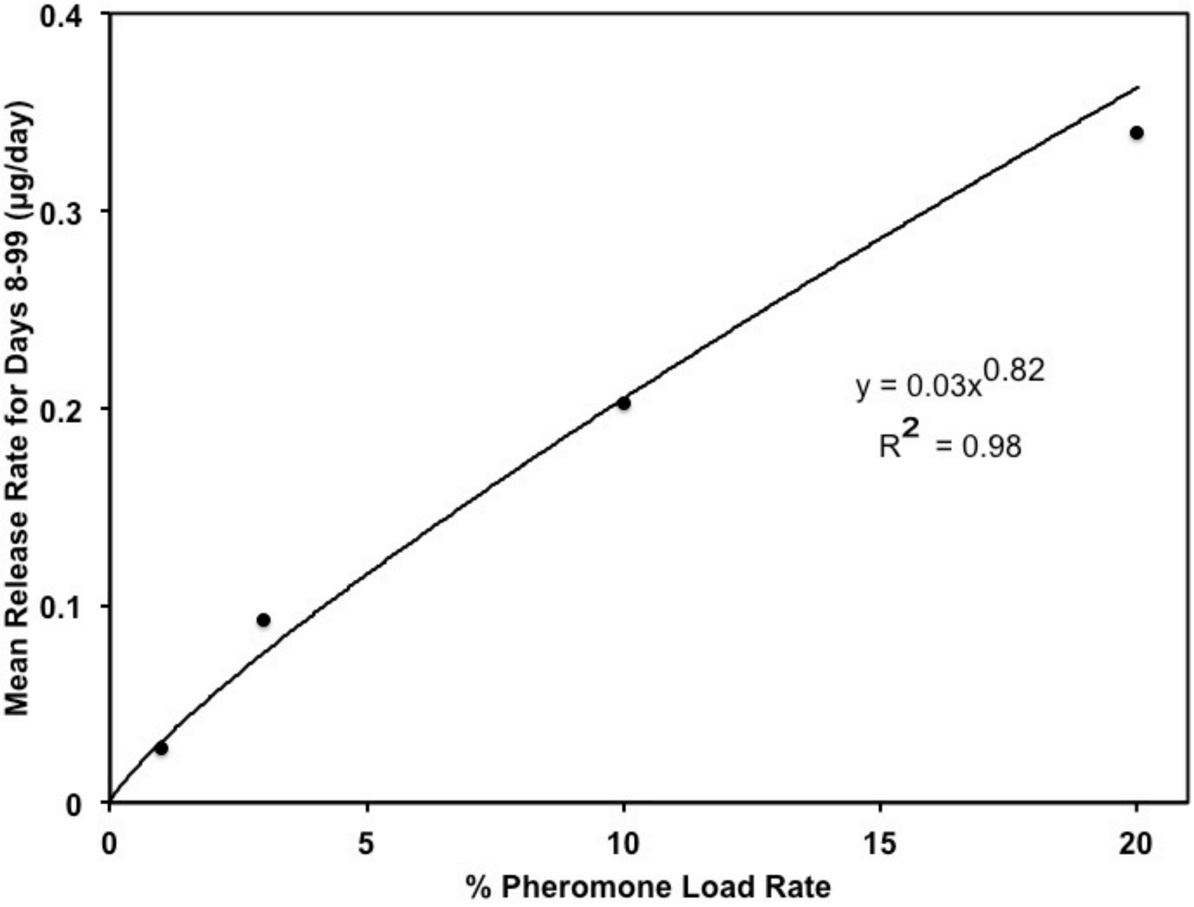

Figure 6. Release rate of codlemone as a function of loading rate in EVA/paraffin wax matrix.

The effect of carbon on codlemone release rate was variable in this test. At 1% and 3% loads of codlemone, carbon had no significant effect (P=0.50 and P=0.39, respectively) (Figure 7A). At a pheromone load of 10%, carbon reduced the release rate significantly (P<0.001) (Figure 7B). At 20% codlemone, the reduction of release rate when carbon was added was just below significance (P=0.067) (Figure 7B). It is possible that the low loading rates dispersed the pheromone in the matrix to such a degree that concentrated areas of neat codlemone did not form, whereas I speculate that the 20% codlemone load overwhelmed the carbon's binding capacity.

The best release profile fit for most formulations was the Higuchi model (Table 2), indicating pheromone release was primarily diffusion-controlled. Again, application of the Korsmeyer-Peppas equation suggested release from the EVA/paraffin wax matrix blend operated under anomalous (non-Fickian) diffusion. The loading rate of the pheromone did not change the diffusion mechanism.

Experiment 3. Lure Studies

All lure formulations yielded high catches of male codling moths in traps (P<0.001) (Table 3). The 0.05% and 0.2% lures generated lower catches than did the rubber septum (P<0.001 and P=0.025, respectively). The difference in codling moth captures in traps baited with the 0.5% and rubber septum treatments was not significant (P=0.669) (Table 3).

Table 3. Captures of codling moth in a Michigan lure study to compare novel wax/EVA matrix lures loaded with different percentages of codlemone. The mean catch is from two codling moth generations. Mean catch values followed by the same letter are not significantly different (P<0.05).

Lure	**Mean Catch ± S.E.M**
0.05%	132.4 ± 60.0 b
0.20%	68.6 ± 40.5 c
0.50%	172.8 ± 57.5 ab
septum	165.8 ± 40.5 a
check	6 ± 2.5 d

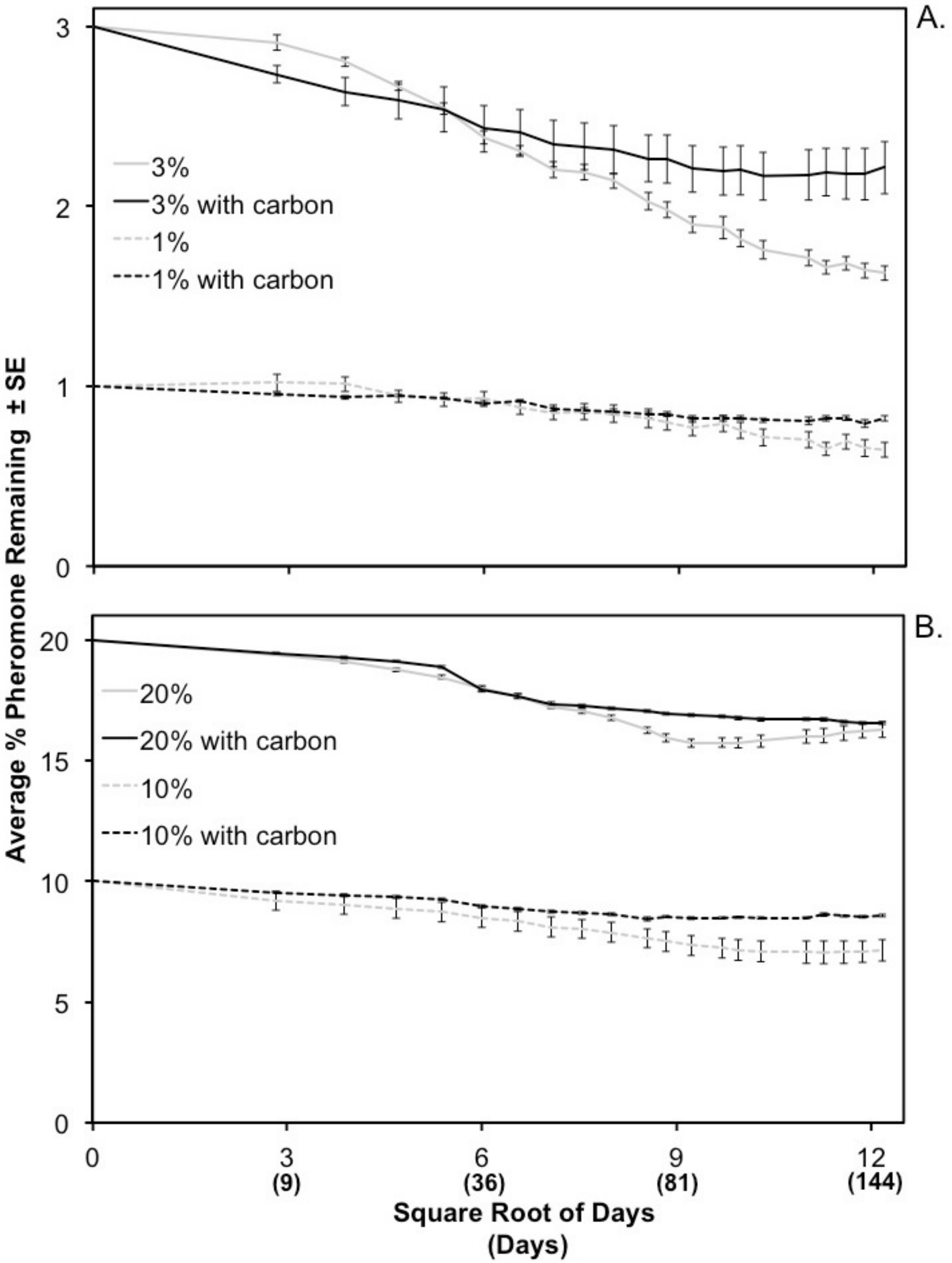

Figure 7. Percent codlemone remaining by weight over time in an EVA/paraffin wax matrix for lures loaded with different amounts of codlemone: (A) 1% and 3% codlemone, by weight. (B) 10% and 20% codlemone, by weight.

Codling moth captures for all lure treatments were statistically equal (P≥0.085) in the lure study performed in Australia (Table 4). The matrix lures described here successfully released codlemone at levels very attractive to codling moths. It released pheromone at attractive levels over 3-4 months of the season, showing it has the capability to maintain viability as a lure for multiple moth generations within a season. No commercial lures currently marketed make this claim.

Table 4. Captures of codling moth in an Australian lure study to compare novel wax/EVA matrix lures loaded with different percentages of codlemone. The mean catch is from the first codling moth generation. No significant differences were found among treatments.

Lure	**Mean Catch ± S.E.M**
0.1% load	90.5 ± 44.0
0.3% load	57.0 ± 18.2
0.5% load	53.0 ± 18.0
0.7% load	62.3 ± 18.7
CML2 septum	81.5 ± 12.8

Implications for Alternative Insect Lures

This research establishes that wax/EVA blends have the potential for use as extended-life lures for monitoring of codling moth and perhaps other insects. Wax and EVA can easily be combined at different levels in conjunction with different loading rates and other additives such as carbon to tailor the release of the compound to the desired rate. The range in which the rates can be varied includes optimal attraction rates for monitoring traps of codling moth. Future research will determine the abilities of these matrices to desirably release the pheromones of other insect species.

APPENDIX A

ALTERNATIVE MATRIX ADDITIVES FOR ULTRAVIOLET PROTECTION OF PHEROMONES WITH CONJUGATED DIENES

As a potential ultraviolet protection additive, several compounds were tested as potential additives for their potential to occlude the transmission of light into the solid matrix. Cellulose and cornstarch were tested for their effects on codlemone release from the novel matrix. These compounds were chosen for their ability to remain suspended within the molten matrix and their ability to render the matrix opaque. Materials and Methods were as above. The matrix was a blend of paraffin wax and EVA at a 1:1 ratio. Both additives were combined with the matrix at 25% by weight. Figure 8 reports the release from these components and shows a comparison to no-additive and carbon added treatments reported above. Zero order, first order, and Higuchi models were tested for best fit. Results from matrices with cellulose (R^2=0.925, 0.963, and 0.987, respectively) and starch (R^2=0.899, 0.922, and 0.955, respectively) indicate pheromone release was concentration limited with the addition of either material. The Korsmeyer-Peppas diffusional exponents for cellulose and starch were 0.62 and 0.74, respectively, indicating pheromone release occurred by non-Fickian diffusion.

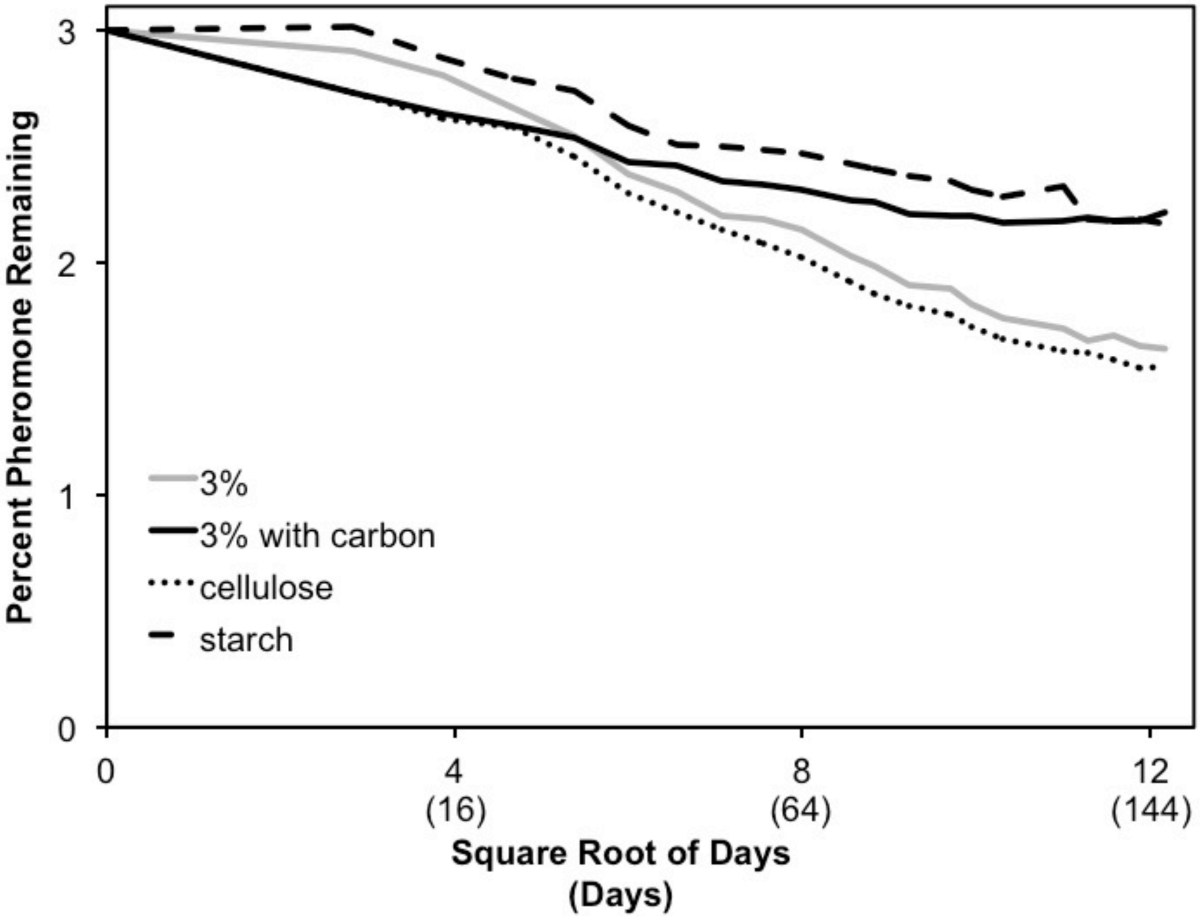

Figure 8. Percent codlemone remaining by weight over time in an EVA/paraffin wax matrix for lures loaded with different additives. Standard errors are not included to prevent cluttering.

APPENDIX B

DEVELOPMENT OF MATRIX AS A LURE FOR MONITORING OF ORIENTAL FRUIT MOTH

The novel matrix was also tested for its potential use as a trap lure for Oriental fruit moth. Materials and methods were as Experiment 3 Lure studies 1 and 2 except the following. The lures were contained a three-part Oriental fruit moth pheromone blend containing (Z)-8-dodecenyl acetate, (E)-8-dodecenyl acetate, and (Z)-8-dodecenol at a ratio of 95:4:1. For Oriental fruit moth lure study 1, the matrix lure loads were 0.001, 0.003, 0.005 or 0.007% pheromone by total weight. For lure study 2, the matrix lure loads were 0.007, 0.01, 0.03,or 0.05% pheromone by total weight. No lure and commercial rubber septum treatments were included for negative and positive checks, respectively. For lure study 1, all pheromone lures were statistically equal in their capture of Oriental fruit moth (Table 5). The highest novel matrix capture, however, was 64% that of the rubber septum. High variability between replicates contributed to the large standard error. For lure study 2, Captures in all treatments were statistically similar to the rubber septum save the lures loaded with Oriental fruit moth pheromone at 0.01% (Table 6). These studies indicate that the novel matrix is capable of use as a trap lure for Oriental fruit moth, but further refinement is necessary to improve its performance to beyond that of the rubber septum.

Table 5. Capture of Oriental fruit moth in a Michigan lure study to compare novel wax/EVA matrix lures loaded with different percentages of Oriental fruit moth pheromone. The mean catch is from the first moth generation. Moth catch values followed by the same letter are not significantly different ($P<0.05$).

Lure	Mean Catch ± SE
0.001%	54.0 ± 30.4ab
0.003%	70.6 ± 21.8a
0.005%	81.0 ± 35.0a
0.007%	96.2 ± 59.3a
Septum	150.6 ± 48.8a
check	10.8 ± 3.6b

Table 6. Capture of Oriental fruit moth in an Australian lure study to compare novel wax/EVA matrix lures loaded with different percentages of Oriental fruit moth pheromone. The mean catch is from the first moth generation. Moth catch values followed by the same letter are not significantly different (P<0.05).

Lure	**Mean Catch ± SE**
Septum	35.0 ± 9.8a
0.007%	11.5 ± 4.3ab
0.01%	11.0 ± 4.5b
0.03%	25.8 ± 7.1ab
0.05%	17.5 ± 6.6ab

CHAPTER THREE: HIGH-DENSITY MATING DISRUPTION OF VARIOUS TORTRICID PESTS USING LOW-RELEASING PHEROMONE DISPENSERS IS NOT SUPERIOR TO COMMERCIAL HIGH-RELEASING PHEROMONE DISPENSERS

ABSTRACT

Many mating disruption systems operate by competitive attraction, where dispensers compete with females for male visits. If there are no additional mechanisms upon reaching the source, competitive disruption should improve in accordance with the ratio of artificial pheromone dispensers to available females. Here we compared codling moth, Oriental fruit moth, and obliquebanded leafroller disruption using high densities of low-releasing paraffin wax/EVA dispensers on continuous strings deployed in rows of fruit trees with conventional deployments of Isomate hand-applied dispensers. In the laboratory, all of these pheromones could be loaded into the paraffin wax/EVA matrix so as to release at near-female rates for extended periods. In all cases, release followed a Higuchi square root of time profile. In field tests using plots up to 0.2 ha per treatment, the string-deployed dispensers disrupted competitively without any evidence of prolonged post-visit deactivation. Wax dollops at up to 10800 ha^{-1} provided disruption similar but not superior to commercial hand-applied dispensers at 1000 ha^{-1}. Thus, for codling moth, a high density of low-releasing string-deployed dispensers is not recommended over conventional disruptive dispensers that invoke long-lasting deactivation after attraction.

INTRODUCTION

Hand-applied pheromone dispensers such as the Isomate products are currently the dominant choice when using mating disruption to manage moth pests in orchards. Such dispensers are deployed at rates of 100-1000 ha^{-1}, depending on the target pest species (Witzgall et al. 2008). Each dispenser typically releases pheromone on the order of 500-1000 times that emitted by a female of the target species (Lacey and Sanders 1992, Backman et al. 1997, Cardé et al. 1998, Il'ichev and Williams 2006). These application and release rates were determined mainly by trial and error. In recent years, more emphasis has been placed on understanding the mechanisms under which mating disruption operates in an attempt to optimize efficacy of mating disruption while keeping costs to a minimum.

Recent publications suggest that moth pests are disrupted primarily through competitive attraction (Miller et al. 2006a, b, 2010), where the first step in the disruption process is attraction to individual point sources of pheromone. If such attraction does not lead to additional disruptive events like desensitization in close proximity to dispensers, disruption by competitive attraction is a matter of direct competition between authentic and false females. To suppress moth populations through competition alone, dispensers should be highly attractive to males of the target species and must considerably outnumber the females. If disruption occurred exclusively by competition, release of pheromone at rates above that required for attraction would be wasteful.

Here we compared season-long disruption of codling moth (*Cydia pomonella*), Oriental fruit moth (*Grapholita molesta*), and obliquebanded leafroller (*Choristoneura* *rosaceana*) under conventional densities of Isomate dispensers with that under high densities of a novel pheromone dispenser releasing at female-like rates (Chapter 2). In order to aid in high-density application, an system, where each point source is attached along a continuous string that is then applied evenly throughout the orchard rows, is described .

MATERIALS AND METHODS

Pheromone and Matrix Materials

Commercial pheromone dispensers acquired from ShinEtsu Ltd. (Tokyo, Japan) included: Isomate C+ (182 g a.i. ha^{-1}), Isomate CM/OFM (423 g a.i. ha^{-1}), Isomate M-Rosso (250 g a.i. ha^{-1}), and Isomate OBLR/PLR+ (227 g a.i. ha^{-1}) (Table 7). Technical pheromone, (E,E)-8,10-dodecadien-1-ol (codlemone), (Z)-11-tetradecenyl acetate, (E)-11-tetradecenyl acetate, and (Z)-11-tetradecenol, were also obtained from ShinEtsu Ltd. Obliquebanded leafroller pheromone blend contained (Z)-11-tetradecenyl acetate, (E)-11-tetradecenyl acetate, and (Z)-11-tetradecenol at a ratio of 93.4:4.2:2.3. (Z)-8-dodecenyl acetate, (E)-8-dodecenyl acetate, and (Z)-8-dodecenol pheromones obtained from Bedoukian Research Inc., Danbury, CT were blended at a ratio of 95:4:1 for the Oriental fruit moth pheromone blend.

Paraffin canning wax (Gulfwax) was obtained from Royal Oak Sales, Inc., Roswell, GA. Ethylene vinyl acetate (EVA) hot melt adhesive (GIA 1051) was obtained from Glue Machinery Inc. Low-sulfur activated carbon powder produced from coconut shells (GX203) was obtained from PICA USA, Inc., Columbus, Ohio.

Matrix Assembly – Laboratory Experiment

The release matrix was produced by combining measured amounts of wax and EVA in a beaker, and heating them on a hot plate to just above their melting points. Carbon was then added with vigorous stirring, followed by liquid pheromone. Experimental formulations were immediately dripped onto wax paper to produce *ca.* 6mm hemispherical individual lumps. The wax paper was then cut into 10mm x 40mm pieces, each containing a single drop. A piece of aluminum foil (30mm x 100mm) was folded lengthwise into thirds then placed under the wax paper for structural support. The ends of the foil plate were folded over, enclosing the ends of the wax paper. Each drop and plate combination weighed 1.0 ± 0.02g

Matrix Assembly – Field Studies

Matrix formulations were produced as above. Matrix drops were molded around lengths of cotton string at 60cm intervals. A mold block was designed for rapid production of large numbers of drops on a continuous string while maintaining consistent drop spacing. A custom-made continuous plaster mold block measuring 244 x 4 x 3 cm (l x w x h) was anchored to the center of a 244 x 60 cm table. Concave 2 x 1 cm depressions were formed at 2.5cm intervals along the mold block, resulting in 96 molds per block (Figure 9). Nails at 5cm intervals were driven into the table top along its long edges. Cotton string was then woven across the table around each nail and over each depression in the mold. Once the string was in place atop the recently water-soaked mold, the molten matrix formulation was dispensed into each depression by pneumatic glue gun (Champ 10s, Glue Machinery Inc., Baltimore, MD). Water was then fogged onto the drops for rapid cooling. Once solidified, the drops were pulled from the

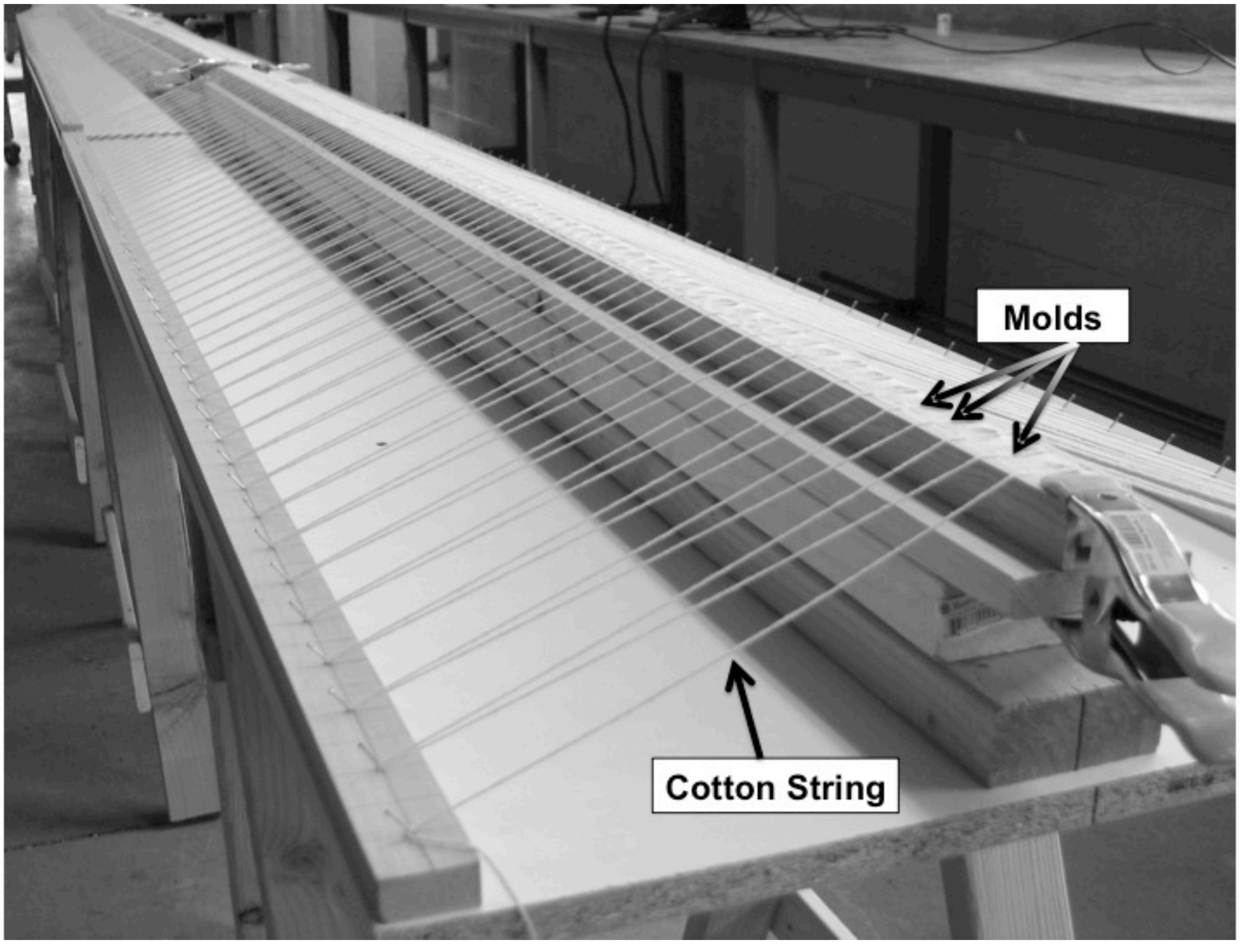

Figure 9. Continuous plaster mold block for production of Matrix drops on string. Cotton string is woven through individual molds.

mold and the string was wound onto spools of various sizes depending on study. Spools for a given moth species were placed into large plastic bags, and stored at -10^{o}C until deployed in the field. Average drop mass was 1.0g. As effects of pheromone cross-contamination between codling moth and Oriental fruit moth blends have been documented (Arn et al. 1974, Evenden and McClaughlin 2005), care was taken to not cause cross-contamination of pheromones by creating all dispensers necessary for a given year's studies for one species before progressing to another species in the order:

codling moth, obliquebanded leafroller, Oriental fruit moth, codling moth/Oriental fruit moth combination.

A custom-made string applicator consisted of an external backpack frame modified to carry a freely rotating spool. String from the spool was fed through a tube to the front of the human applicator at belt height, then through an extendable, hand-held, hollow painter’s pole. The pole could be extended to reach the top of the canopy of each tree type. The applicator started at one end of a row of trees, tied the string to a branch, then walked down the row laying the string into the crop canopies for the length of the row (Figure 10). This application method allowed application of approximately 10-fold more dispensers in the same amount of time as an application of the Isomate standard.

Experiment 1. Species Release Rate Quantification

The four treatment formulations were: (1) codling moth pheromone, (2) Oriental fruit moth pheromone, (3) obliquebanded leafroller pheromone, and (4) no pheromone control. All formulations were tested both with and without carbon added at 10% by total weight. In all cases, pheromone was loaded at 3%, by weight. Five replicates of all ten treatments were performed simultaneously between 14 September 2008 and 29 January 2009. Drops were hung at room temperature (22-25°C) in a laboratory fume hood for aging between weighings.

Drops were weighed weekly using a Cahn C-35 Ultra-Microbalance (Thermo Electron Corporation, Beverly, MA). Microbalance sensitivity (0.1μg) was sufficient to record the weekly weight loss of pheromone, typically between 0.2 and 1.0mg. The weight of the no pheromone control treatments were always subtracted from the pheromone-containing treatments.

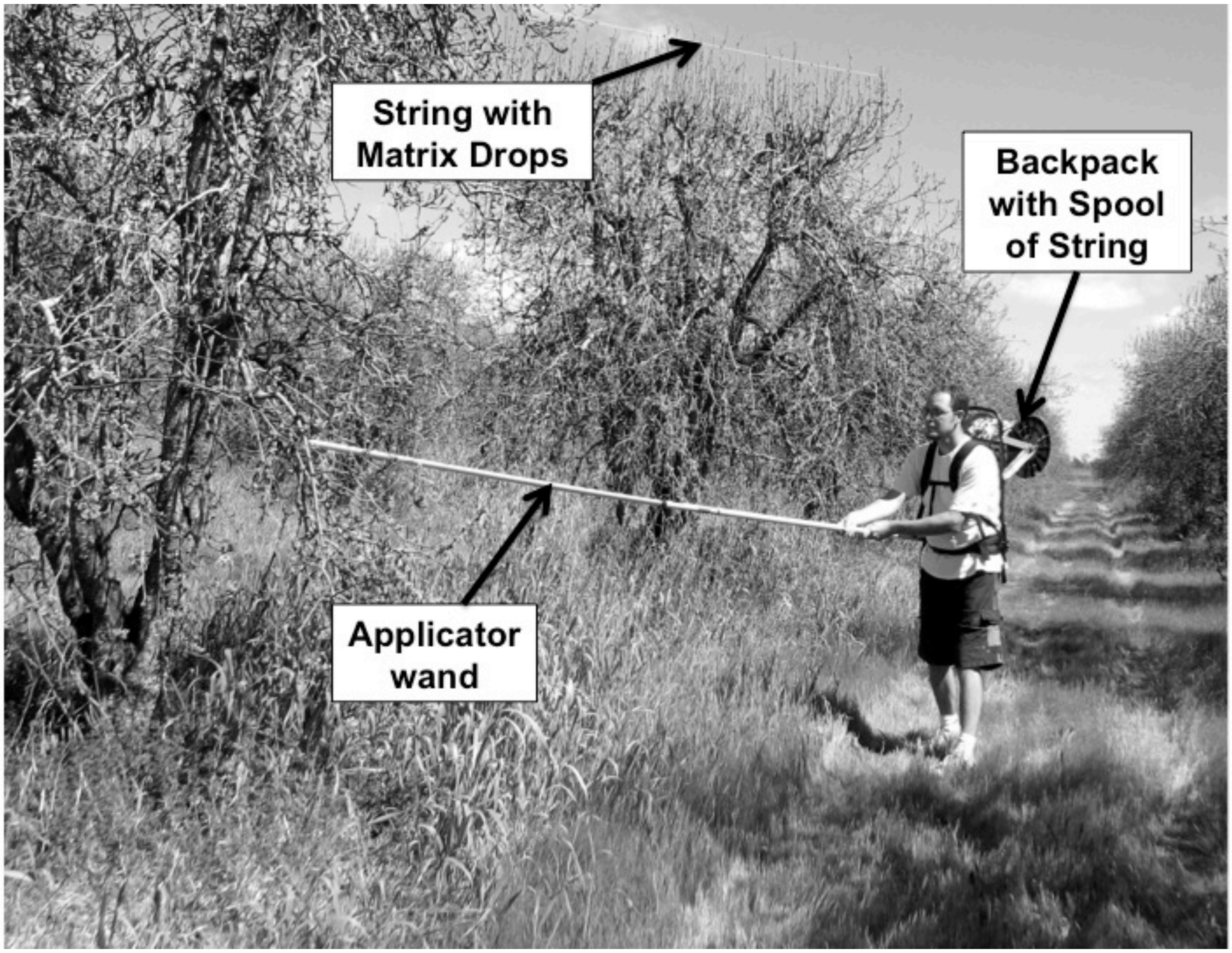

Figure 10. Backpack style Matrix applicator. Spool on backpack contains cotton string with Matrix drops. String goes through wand applicator and is directed to the crop.

Release profiles were tested for fit to the models commonly useful (Shoaib et al. 2006) in differentiating among the factors most influential in shaping release kinetics. The models tested were zero-order, first-order, and Higuchi square root of time. Procedures for the release profile comparisons are described in Chapter 2.

Experiment 2 – Effects of Dispenser Spacing on Disruption

Plots 0.03 ha in size (16 trees on a 4x4 grid) were laid out in research orchards located at the Trevor Nichols Research Center (TNRC), near Fennville, Michigan. Apple orchard blocks were divided into plots with a minimum of 15 m between treatments. All plots contained free-standing apple trees approximately 3m in height, planted at 350-500 trees ha^{-1}. All plots were maintained with regular horticultural practices, but with no insecticide sprays.

Five replicates of five treatments were organized by randomized complete block design. The distance between dispensers in designated treatments was 10 m, 6 m, 3 m, 1 m, or 0.5 m. Dispensers consisted of individual dollops of matrix loaded at 1% by weight with codlemone. Each bead was connected by 1 cm of cotton string to a 25 x 40 mm plastic clip (Kwiklok, Yakima, WA) that could be inserted onto a branch. Three dispenser spacing treatments (10 m, 6 m, and 3 m) contained a maximum of one dispenser per tree. These dispensers were clipped to branches in the top 1/3 of the tree canopy. To apply dispensers for the remaining two treatments (1 m and 0.5 m), the first dispenser was clipped at the tree trunk at the base of the lowest branch. Each subsequent dispenser was clipped to a branch as close to the treatment distance as possible, beginning along the tree trunk and extending to the perimeter of the canopy. Resulting dispenser densities (per hectare) were: 155, 410, 1090, ≈6000, and ≈25000, respectively. Following application, two dispensers were removed and replaced with monitoring traps baited with a monitoring lure loaded with 0.1 mg codlemone. Traps were checked twice a week for the entire season. Lures were replaced each generation and liners were replaced if contaminated or saturated with insects. Treatments were applied on 2 June 2008 and were maintained until after the end of the second codling moth generation on 11 August 2008.

Experiment 3 – Multiple Species Disruption Studies

Plots 0.06-0.08 ha in size were as in Experiment 3. Five replicates of eight treatments were organized by randomized complete block design. Three string dispenser treatments included: Oriental fruit moth pheromone only, codling moth only plus Oriental fruit moth only in separate dispensers, and codling moth and Oriental fruit moth combined in the same dispenser. Each matrix dispenser containing one pheromone was loaded at 1% by weight. Combination dispensers were loaded with codling moth pheromone at 1% and Oriental fruit moth pheromone at 0.5%. Four commercial hand-applied mating disruption treatments included: Isomate C+ only, Isomate M-Rosso only, Isomate C+ plus Isomate M-Rosso, and Isomate CM/OFM. An untreated control was also included. Application rates for all treatments are listed in Table 7. Two monitoring traps were placed in the corners (southwest: northeast and southeast: northwest for codling moth and Oriental fruit moth, respectively) of each plot at least 5 m from the perimeter. Codling moth traps were baited with either a CM 0.1 mg or Trécé CM L2 monitoring lure. Oriental fruit moth lures were baited with Trécé OFM lures. Traps were checked twice a week for the entire season. Lures were replaced each generation and liners were replaced if contaminated or saturated with insects. Treatments were applied on 8 May 2008 and were maintained until after the end of the second codling moth generation on 25 August 2008.

The multiple species study was replicated on a larger scale on commercial orchards near Tatura, Australia. Plots of 0.2 ha were located 6 km east of Tatura in

Victoria, Australia. It was a single, commercial pear block with a history of codling moth infestation. The block consisted of 4-5 m high pear trees maintained with no insecticides. The block was divided into adjacent 0.2 ha plots. Plot separation was not possible due to the high number of treatments.

Table 7: Mating disruption dispenser application rates for all field studies. Matrix application rates are approximate due to application method. CM = codling moth. OFM = Oriental fruit moth. OBLR = obliquebanded leafroller.

	Disruption study application rates (dispensers ha^{-1})		
	CM/OFM combination		**OBLR**
Treatment	**USA 2008**	**Australia 2008-2009**	**2007, 2008**
Matrix CM		10800	
Matrix CM/OFM	10800	10800	
Matrix CM: Matrix OFM	10800: 2700	10800: 2700	
Matrix OFM	2700	2700	
Matrix OBLR			10800
Isomate C+	1000	1000	
Isomate CM/OFM	500	500	
Isomate C+: Isomate M-Rosso	1000: 500	1000: 500	
Isomate M-Rosso	250	250	
Isomate OBLR/PLR+			500

Four replicates of nine treatments were organized by randomized complete block design. Four high-density custom dispenser treatments included: codling moth only, Oriental fruit moth pheromone only, codling moth only plus Oriental fruit moth only, and codling moth and Oriental fruit moth combination. Each matrix dispenser containing one pheromone was loaded at 1% by weight. Combination dispensers were loaded with codling moth pheromone at 1% and Oriental fruit moth pheromone at 0.5%. Four commercial hand-applied mating disruption treatments included: Isomate C+ only, Isomate M-Rosso only, Isomate C+ plus Isomate M-Rosso, and Isomate CM/OFM. An untreated control was also included. Application rates for all treatments are listed in Table 7. Two monitoring traps were placed at the corners (southwest: northeast and southeast: northwest for codling moth and Oriental fruit moth, respectively) of each plot at least 5 m from the perimeter. Codling moth traps were baited with either a CM 0.1 mg or Trécé CM L2 monitoring lure. Oriental fruit moth lures were baited with Trécé OFM lures. Traps were checked twice a week for the entire season. Lures were replaced each generation and liners were replaced if contaminated or saturated with insects. Treatments were applied on 7 October 2008 and were maintained until 15 December 2008.

To quantify the release of pheromone from the matrix dispensers from the second part of Experiment 4, samples of four dispensers of each type were removed from the field weekly for two months and sealed in foil bags. Dispensers were stored at -10^{O}C until analysis via volatile collection. Volatile collection was performed as in Tomaszewska et al. (2005). All dispensers were allowed to equilibrate to room temperature for 24 h prior to volatile collection. The volatile collection apparatus consisted of compressed air flowing through Teflon tubing connected to a Teflon collection jar. A glass tube containing an adsorbent polyurethane foam cartridge (Supelco, Bellefonte, PA) was attached to the jar outlet. Individual dispensers were suspended within the collection jar. Volatile collections were made for 2 h at 20^{O}C with a flow rate of 10 L per min. Pheromone was extracted from the foam cartridges by three 150ml acetone rinses. Extracts were then appropriately diluted for analysis using an Agilent 6890N Gas Chromatograph and an Agilent 7683 auto sampler. Operating procedures and GC conditions are given in Tomaszewska et al. (2005). The quantification of residues in the extract was performed by electronic peak area measurement and comparison to an internal standard of methyl myristate. Release profiles of the major component for each species were tested for fit to the models as above.

Experiment 4 – Obliquebanded Leafroller Disruption Studies

Plots 0.06-0.08 ha in size were as in Experiment 3. Five replicates of three treatments were organized by randomized complete block design. The three treatments were: high-density custom dispensers, Isomate OBLR/PLR+, and an untreated control. Each matrix dollop was loaded with the obliquebanded leafroller blend at 1% by weight. Application rates can be found in Table 7. Two monitoring traps were placed in the southeast and northwest corners of each plot at least 5 m from the perimeter. Traps were baited with Trécé OBLR monitoring lures. Traps were checked twice a week for the entire season. Lures were replaced each generation and liners were replaced if contaminated or saturated with insects. Treatments were applied on 12 June 2007 and were maintained until after the end of the second obliquebanded leafroller generation on 24 August 2007. Treatments were re-applied on 2 June 2008 and were again maintained until after the end of the second obliquebanded leafroller generation on 25 August 2008.

Statistical Analysis

For laboratory release rate studies, time was transformed by square root then release rates were subjected to analysis of variance (ANOVA). For disruption efficacy studies, data were transformed to ln (x + 0.5) (which normalized the distributions of residuals and homogenized variance) then subjected to ANOVA. Differences between pairs of means were separated using Fisher's protected least significant difference (SAS Institute, 2000). In all cases, the significance level was $\alpha<0.05$.

RESULTS AND DISCUSSION

Experiment 1. Species Release Rate Quantification

The percentages of pheromones remaining in the EVA/paraffin wax matrix are shown in Figure 11 as a function of the square root of time. Pairwise comparisons of the pheromones from all species were significantly different when carbon was present ($p<0.001$). Without carbon in the matrices, codling moth pheromone released from the matrix significantly slower than either of the other species' pheromones ($p<0.001$), but no difference was found between Oriental fruit moth and obliquebanded leafroller pheromone ($p=0.857$). Oriental fruit moth and obliquebanded leafroller laden matrix treatments without carbon released pheromone significantly faster than their respective treatments with carbon ($p\leq0.001$). No difference was detected between the codling moth treatments with and without carbon ($p=0.087$).

The release of pheromone from the matrix varied with respect to species. This is likely due to the molecular structures of the various pheromone components. The alcohol group on a pheromone molecule imparts higher polarity than an acetate group (Wohlfarth 2011). The matrix blend used here is largely nonpolar. We suggest the major components (acetates) of Oriental fruit moth and obliquebanded leafroller pheromones were more soluble in the matrix than codlemone and therefore moved more readily

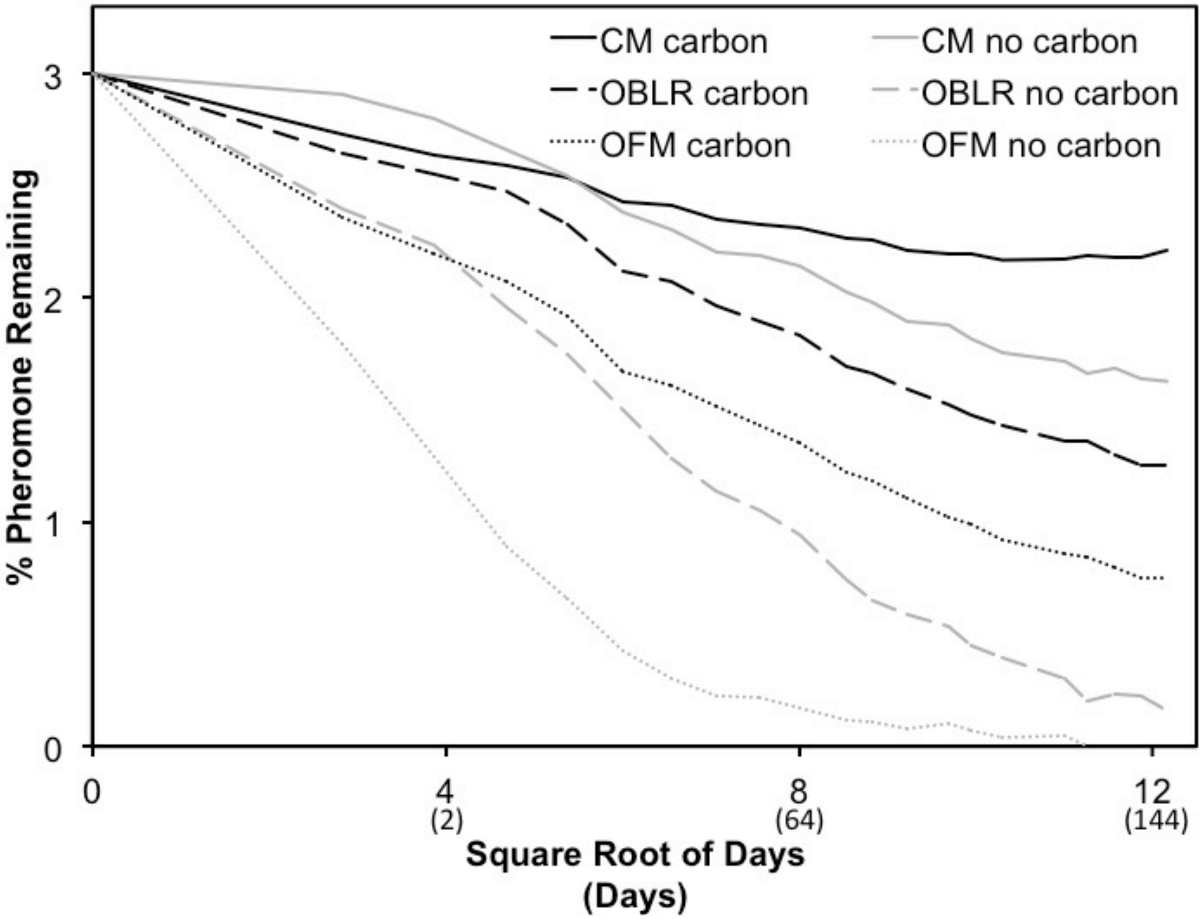

Figure 11. Percent pheromone remaining by weight over time in the EVA/wax matrix for various tortricid moths. CM = codling moth. OFM = Oriental fruit moth. OBLR = obliquebanded leafroller. Standard errors are not included to prevent cluttering.

through it. Oriental fruit moth pheromone components are 12 carbon molecules. Obliquebanded leafroller pheromone is a similar blend of acetate and alcohol components, but each is 14 carbons long. The added length reduces the evaporative rate (Gut et al. 2004). For acetates in this study the longer chain slowed the rate of diffusion. When carbon was present, the release rates of pheromones for these two species were significantly different. While the difference was not significant when carbon is absent, the obliquebanded leafroller pheromone consistently released from the matrix at a slower rate than the Oriental fruit moth pheromone.

Powdered carbon is highly porous and adsorptive. The addition of carbon to the matrix slowed the release of pheromones, significantly in the cases of Oriental fruit moth and obliquebanded leafroller. The carbon potentially adsorbed a portion of the pheromone, releasing more slowly than the natural rate of diffusion through the matrix. The carbon also might have permanently adsorbed some of the pheromone, reducing the release rate by effectively reducing pheromone concentration within the matrix, as indicated by the codling moth treatment where release ceased just over three months into the study. At that time, only one third of the pheromone had released.

Release profiles for most formulations fit the Higuchi model best (Table 8) indicating pheromone release was diffusion-controlled. The codling moth treatment with carbon appeared to stop releasing pheromone by day 100. As such, fit analysis did not include data beyond day 99. Due to the high rate of release from the Oriental fruit moth matrix without carbon, the treatment was exhausted by day 99. Additionally, the mechanism of release appeared to shift once the pheromone concentration reached very low levels. As such, the 'OFM no carbon' matrix release profile was halved, each analyzed separately. During the first half (to day 50) of the study, where the majority of release occurred, the first order release model was a slightly better fit than the Higuchi model (R^2=0.998 and R^2=0.989, respectively). During the second half (days 50-99) of the study the Higuchi release model was a better fit than the first-order model (R^2=0.858 and R^2=0.770, respectively).

Table 8. R^2 fit of release rate transformations from pheromone release rate experiments. Fits better than competing models by 0.01+ are in bold. CM = codling moth. OFM = Oriental fruit moth. OBLR = obliquebanded leafroller. Matrix formulations are samples from Australia species combination field study. *CM with carbon and OFM no carbon analyses included only up to day 99.

Treatment	Zero order	First order	Higuchi
CM no carbon	0.875	**0.965**	0.948
CM with carbon*	0.581	0.901	**0.984**
OFM no carbon*	0.662	**0.968**	0.884
OFM with carbon	0.522	0.974	**0.984**
OBLR no carbon	0.653	0.973	0.982
OBLR with carbon	0.774	0.974	**0.985**
Matrix CM	0.91	0.954	0.959
Matrix OFM	0.179	0.641	**0.861**
CM in Matrix CM/OFM	0.587	0.666	**0.851**
OFM in Matrix CM/OFM	0.585	0.561	**0.836**

Release profile best fits of most of the treatments were consistent with other laboratory release studies (Chapter 2), indicating pheromone release is diffusion controlled. The Higuchi (diffusion-controlled) model (Higuchi 1963) fits best where release from an insoluble matrix is dependent upon the diffusive capability of the pheromone through the matrix. As with the first order release model, the concentration in the matrix affects the release rate from the matrix. Unlike first order release, however, the gradient itself is not as important as the capability of the molecules to traverse through the matrix. In the case of the EVA/paraffin wax matrix, the molecular structure construction was either tightly bound enough, of low enough polarity, or both to restrict the diffusion of the straight carbon chain pheromones through the matrix.

Experiment 2 – Effects of Dispenser Spacing on Disruption

Treatments with dispenser spacing of 3 m or more (no more than one dispenser per tree) yielded statistically equal codling moth catch (p>0.054) (Figure 12A). Reducing

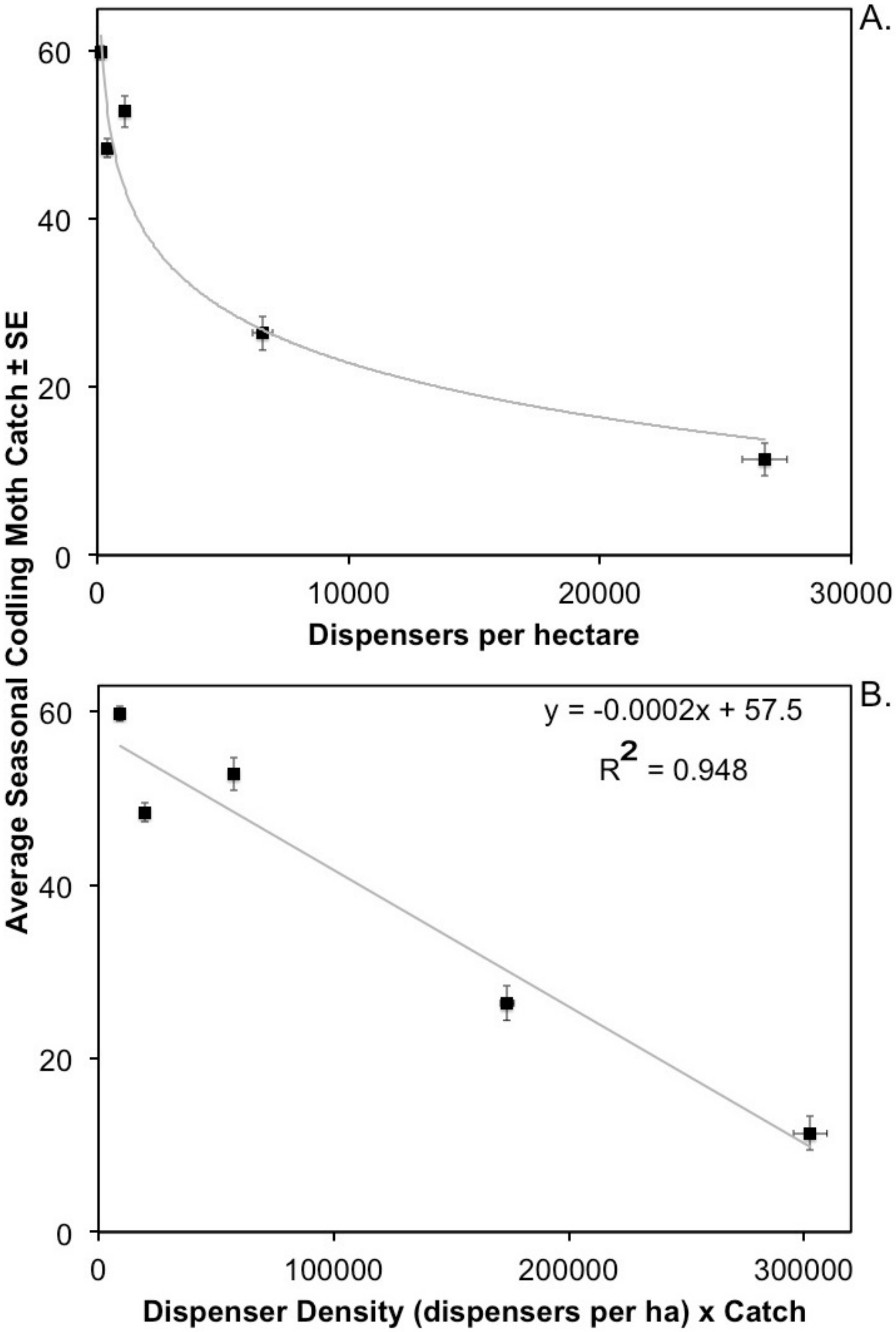

Figure 12. Effects of varying the density of EVA/wax dispensers on codling moth catch in monitoring traps. (A) Average number of male codling moths captured in monitoring traps *vs.* dispenser density. (B) Miller-de Lame plot of codling moth catch *vs.* dispenser density x catch. Dispenser spacings tested were 10, 6, 3, 1, and 0.5 m.

the dispenser spacing to 1 m (approximately 12 dispensers per tree) significantly (p<0.001) reduced capture by almost 50%. Halving the dispenser spacing to 0.5 m (approximately 49 dispensers per tree) again significantly (p=0.001) reduced capture by more than 50%.

Miller et al. (2006a,b and 2010) suggest that the primary mating disruption mechanism for many tortricid pests is competitive attraction. Under pure competitive attraction, the higher the dispenser density, the better the control of the target pest. In the current research, the highest dispenser densities were more effective at reducing capture of codling moths in the monitoring traps (Figure 12A). The profile followed an inverse relationship. A Miller-de Lame analysis (Figure 12B) as described in Miller et al. (2006a) documented a linear relationship between catch and dispenser density x catch. Both of these profiles are strongly indicative of competitive attraction being the primary disruption mechanism. Under a competitive attraction mechanism, attraction of individuals in a population is divided among competing dispensers. This necessarily indicates that capture in a monitoring trap is always theoretically possible, regardless of the number of dispensers. Even 25000 dispensers per hectare were unable to completely shut down codling moth catch in monitoring traps (Figure 12A).

Experiment 3 – Species Combination Disruption Studies

Codling moth pheromone treatments reduced capture of male codling moths by 77-94% compared to the control treatment (Figure 13). The commercial dispenser treatments were more effective at reducing codling moth capture than the matrix treatments (p<0.001). Separating codling moth pheromone with Oriental fruit moth pheromone into individual dispensers *vs.* combining them in the same dispenser had no effect for either dispenser type (p>0.305). Oriental fruit moth pheromone treatments reduced capture of male Oriental fruit moths (53-95%, compared to the control). No difference was found between an Isomate treatment and its corresponding matrix treatment (p>0.201). Treatments combining the pheromones of both species in the same dispenser were more effective than the separate pheromone treatments; they reduced Oriental fruit moth capture a further 70-82% (p<0.032).

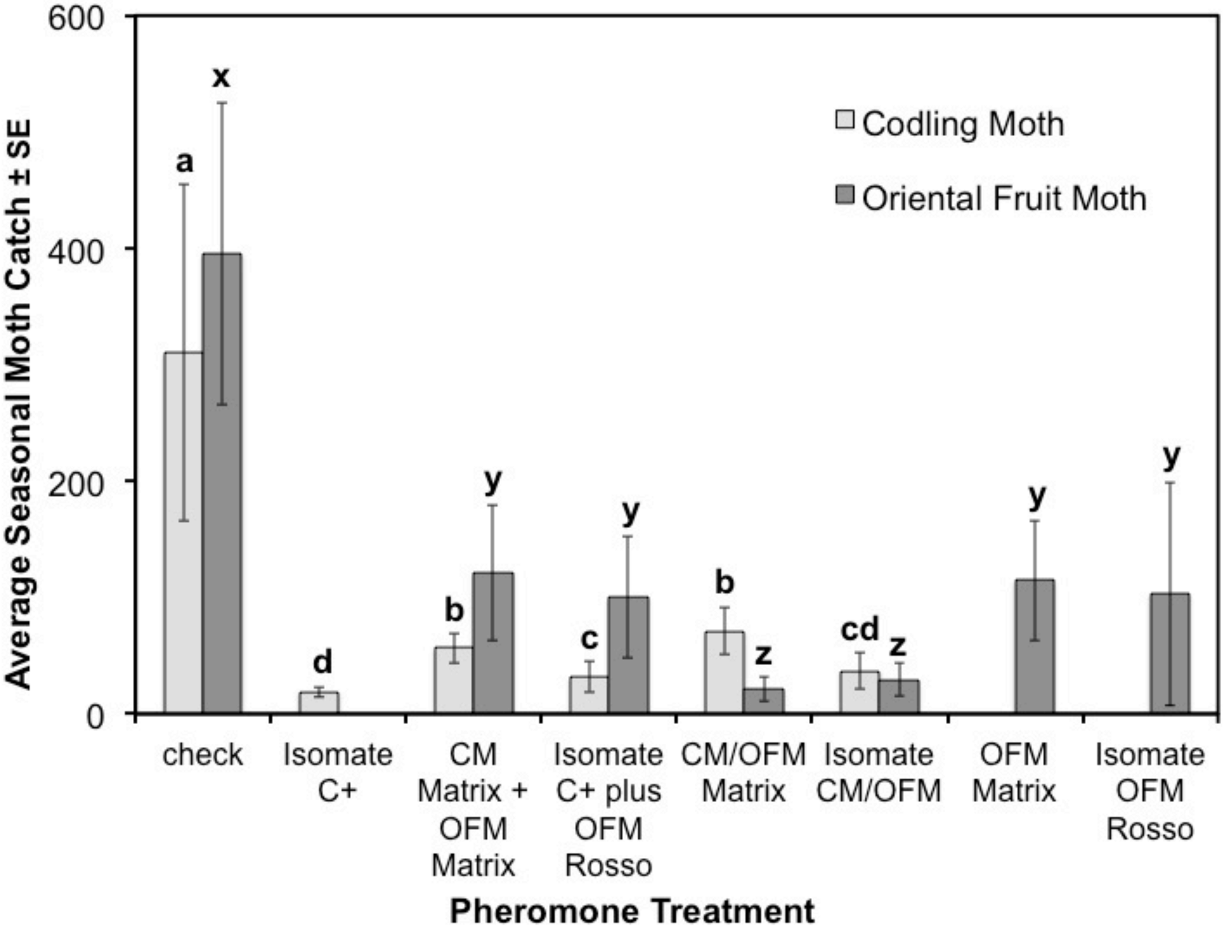

Figure 13. Average number of male codling moths and Oriental fruit moths captured in monitoring traps in USA high-density combination dispenser disruption study. Treatments with the same letter are not significantly different (P<0.05).

In Australia the string treatments reduced capture of codling moth in monitoring traps by 45-87%. But, the Isomate dispensers were more effective at reducing codling moth capture than the matrix treatments ($p<0.020$) (Figure 14). Again, combining pheromones did not significantly reduce the effect on capture for either the matrix or commercial dispensers ($p=0.054$ and $p=0.998$, respectively). Oriental fruit moth populations were extremely low. All Oriental fruit moth pheromone treatments reduced capture ($p<0.001$), but statistical comparisons of pheromone treatments were not possible due to the low catches.

On day 0 the Oriental fruit moth pheromone from Matrix OFM drops was over 1.2 µg/h (Figure 15). All other pheromone release on day 0 was 400-800 ng/h. By day 14, most pheromone release was below 400 ng h^{-1}. By day 56, all dispensers were still releasing pheromone at more than 10 and 20 times that of a female codling moth or Oriental fruit moth, respectively (Backman et al. 1997, Lacey and Sanders 1992). Analysis of release profiles of all three dispensers indicated that the Higuchi square root of time release model is the best fit to the data (Table 8). The release profile results are consistent with those in Experiment 1 and of results in Chapter 2.

The release rates in the laboratory study and the species combination disruption study performed in Australia (Figures 4-6,15) indicate the matrix dispensers used here released pheromone at levels that would elicit attraction. The results for all field studies should then show a significant reduction in catch of the target species using the matrix dispensers on string. For Oriental fruit moth, no dispenser type proved superior, suggesting both dispenser types were eliciting the same disruption mechanism. In the case of codling moth in both species combination studies, however, the Isomate

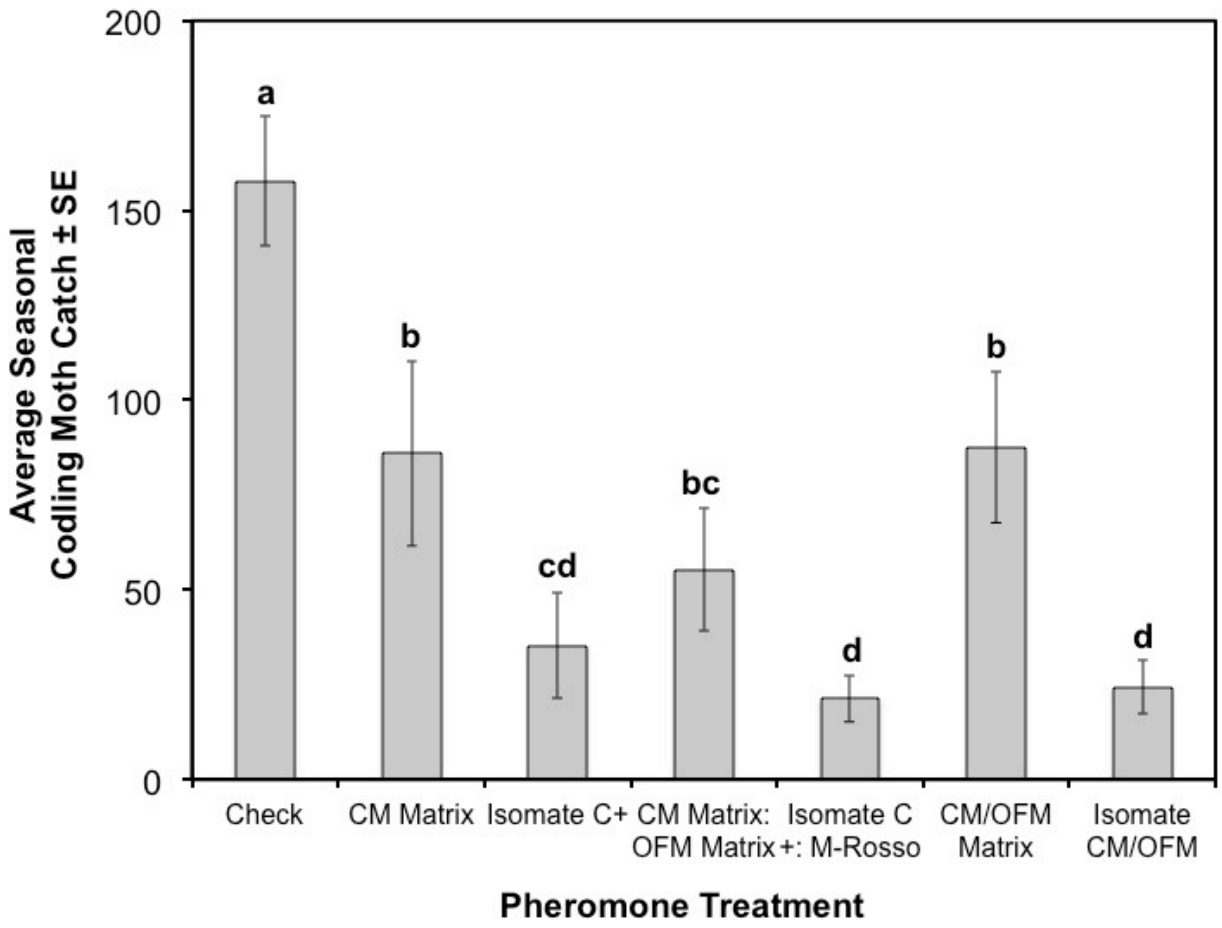

Figure 14. Average number of male codling moths captured in monitoring traps in Australia high-density combination dispenser disruption study. Treatments with the same letter are not significantly different (P<0.05).

treatment reduced male catch better than the corresponding matrix bead treatment (Figures 13-14), sometimes significantly. In the release rate quantification study, codlemone release from the matrix dispensers effectively ceased by day 100. In both of the species combination studies, male codling moth catches in all matrix treatments were similar to their corresponding Isomate treatments early in the test, but increased towards the end of the studies, indicating the attractiveness of the matrix beads waned

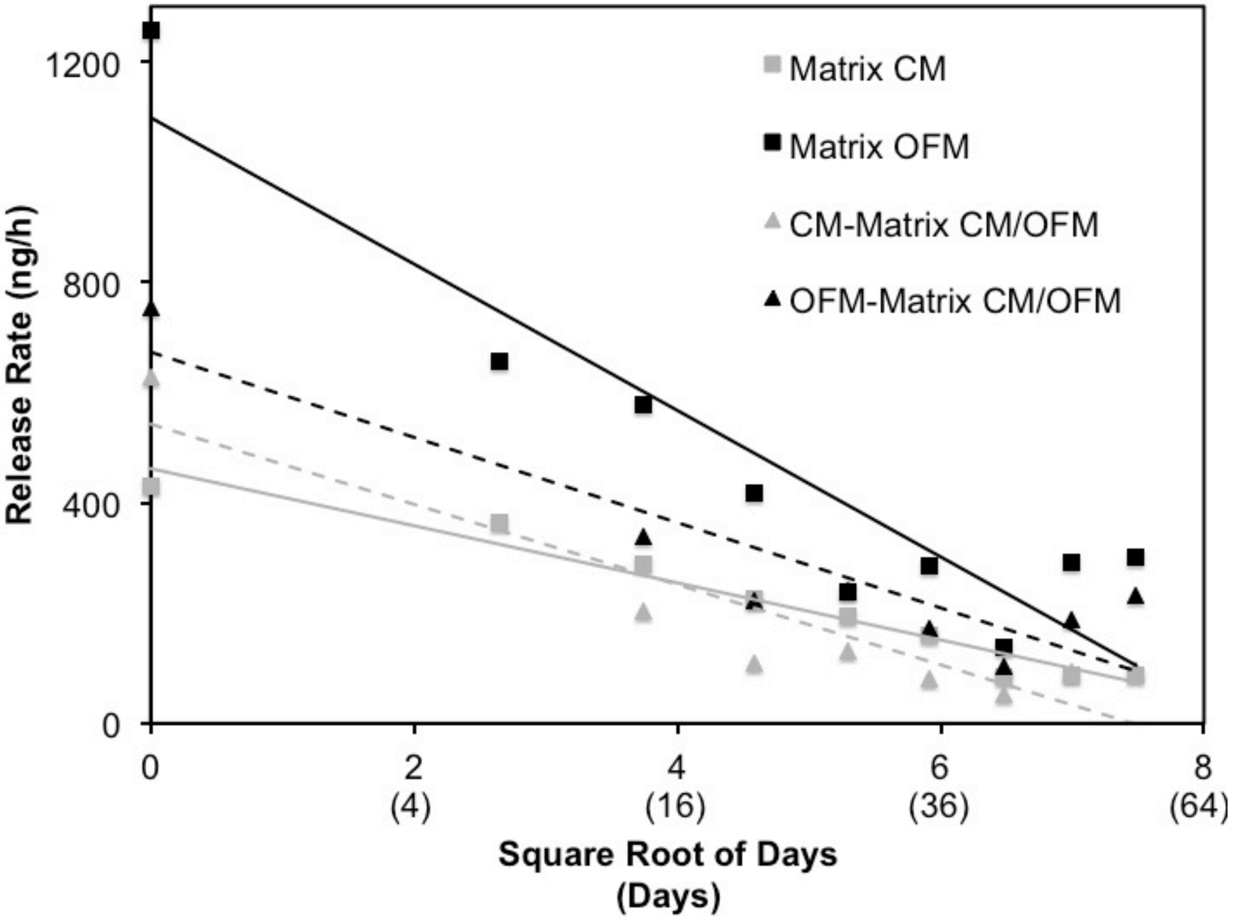

Figure 15. Average release rate of the pheromone (main component) from matrix drops in the Australia species-combination disruption study. CM = codling moth. OFM = Oriental fruit moth. Standard errors are not included to prevent cluttering.

towards the end of the study. This could indicate disruption occurred primarily through competitive attraction, but the higher-releasing dispensers could be more attractive, drawing proportionally more moths per dispenser than the matrix beads.

The high release rate of the commercial dispensers could also have invoked a secondary noncompetitive mechanism. For codling moth, Miller et al. (2010) reported that in large field cage studies codling moths were likely incapacitated once they completed an attraction event towards an Isomate dispenser. This incapacitation could have been some form of desensitization whereby the codling moths were unable or unwilling to orient to the low-releasing lures in the monitoring traps in each field cage. For Oriental fruit moths, Chapter 4 documents a shift in mechanism from competitive attraction to a noncompetitive mechanism when using near-female-equivalent dispensers and Isomate OFMTT dispensers, respectively, in large field cages. Here the same was not true when the moths were exposed to the matrix dispensers. Pheromone release rate for all matrix dispensers was significantly higher than female codling moths and Oriental fruit moths (Lacey and Sanders 1992, Backman et al. 1997) and nearly twice the rate of the dispensers used in Chapter 4 (40 and 90+ ng h^{-1}, respectively), but significantly lower than the Isomate dispensers (Cardé et al. 1998, Il'ichev and Williams 2006) (Figure 15). These results suggest codlemone release rate must be increased, but Oriental fruit moth release rate is potentially sufficient to elicit a non-competitive disruption mechanism.

Combining pheromone into the same dispenser had variable effects, depending on the target insect. While not significant, the addition of Oriental fruit moth pheromone to codling moth dispensers consistently increased the number of codling moth males captured in the monitoring traps (Figure 15). Supplementing Oriental fruit moth dispensers with codlemone had the contrasting effect of decreasing Oriental fruit moth capture. Addition of various Oriental fruit moth pheromone components inhibits close-range attraction of codling moth males (Arn et al. 1974, Evenden and McClaughlin 2005). Conversely, Evenden and McClaughlin (2005) reported an increase in Oriental fruit moth attraction when codlemone was combined with Oriental fruit moth pheromone.

In the studies reported here, the combination of pheromones in the dispensers could have reduced their attractiveness to codling moths relative to the monitoring trap lures, drawing relatively more moths to the more attractive traps. Conversely, the combination of pheromones could have increased the relative attractiveness of the dispensers to Oriental fruit moth males, reducing capture in the monitoring traps.

Experiment 4 – Obliquebanded Leafroller Disruption Studies

Results from both years were similar. Therefore, years were combined. Both dispenser types significantly reduced moth capture ($p<0.001$) (Figure 16). No difference was found between dispenser types ($p=0.116$). Division of pheromone into many more dispensers did not improve disruption over a dispenser releasing at a high rate. It merely divided the attraction. Stelinski et al. (2004a) observed disproportionately more obliquebanded leafroller males orienting towards Isomate OBLR/PLR+ dispensers than captured in monitoring traps, indicating higher relative attraction to the high-releasing dispensers. In the current study, it is possible that the matrix dispensers did not release pheromone at a rate to elicit this higher relative attraction. It is also possible the matrix dispensers released pheromone at levels much too low to effect non-competitive disruption.

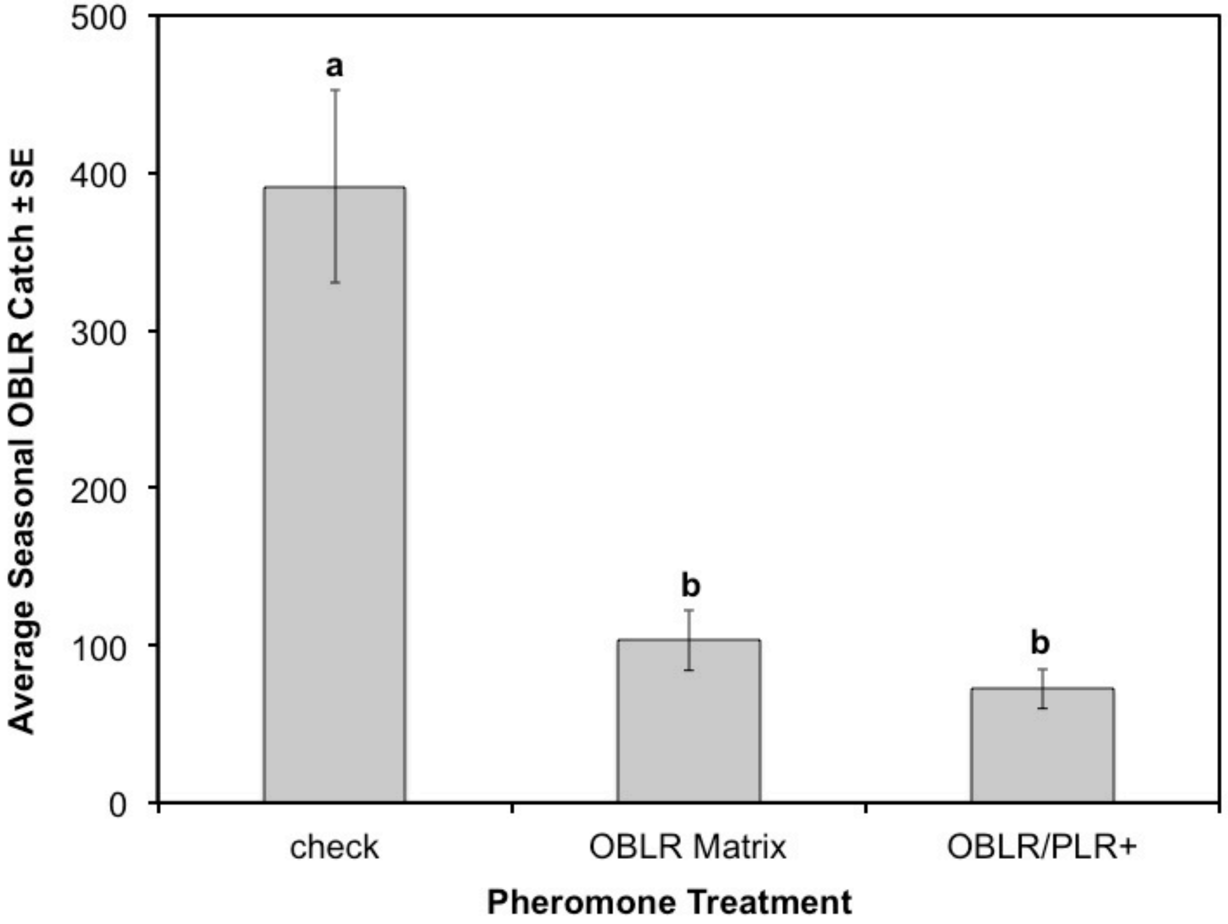

Figure 16. Average number of male obliquebanded leafrollers captured in monitoring traps in a high-density dispenser disruption study comparing a novel pheromone release matrix to Isomate OBLR/PLR+. Treatments with the same letter are not significantly different (P<0.05).

APPENDIX

GRAPE BERRY MOTH PHEROMONE RELEASE PROFILE FROM NOVEL MATRIX

Materials and Methods were as above for Experiment 1. The addition of carbon to the matrix significantly ($p<0.001$) reduced release rate of grape berry moth pheromone components (Z9-dodecenyl acetate and E9-dodecenyl acetate) (Figure 17). Release profiles for grape berry moth were extremely similar to those for Oriental fruit moth pheromone. This is likely due to very similar pheromone molecules between the two species. Zero order, first order, and Higuchi models were tested for best fit. Results from matrices with (R^2=0.888, 0.965, and 0.982, respectively) and without carbon (R^2=0.569, 0.745, and 0.816, respectively) indicate pheromone release was concentration limited.

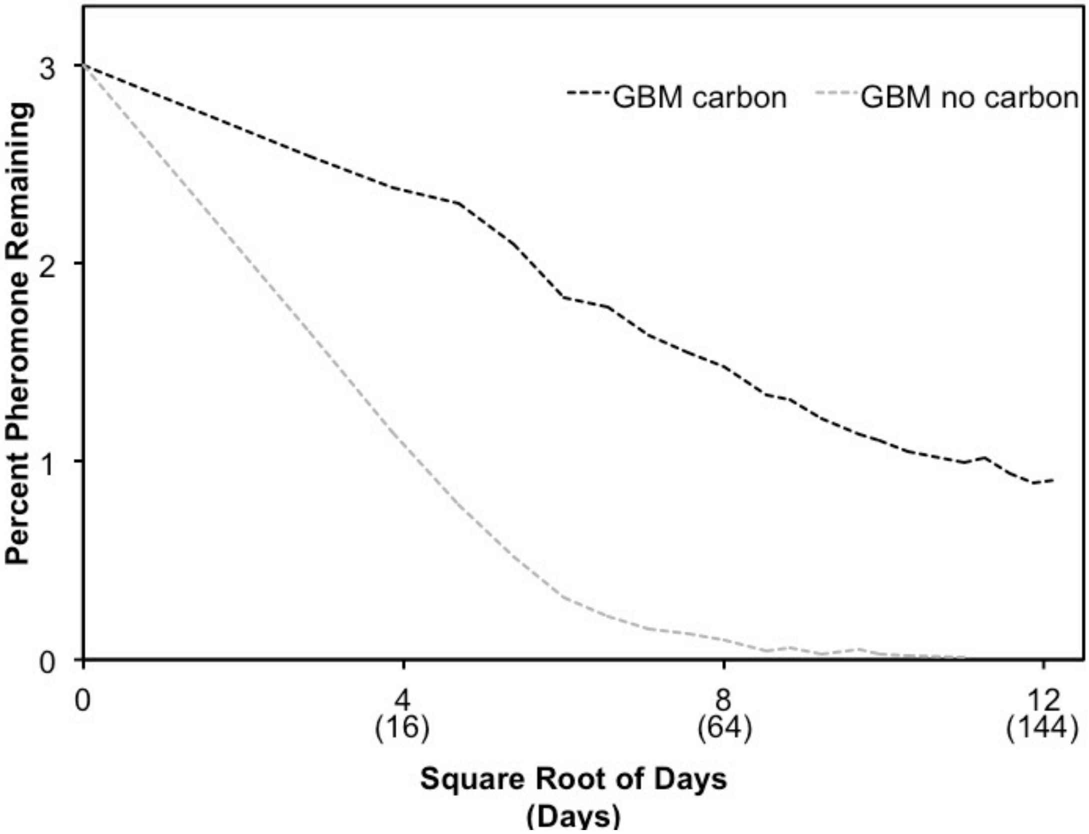

Figure 17. Percent pheromone remaining by weight over time in the EVA/wax matrix for grape berry moth. See Figure 5 for more species.

CHAPTER FOUR: PHEROMONE RELEASE RATE DETERMINES WHETHER SEXUAL COMMUNICATION OF ORIENTAL FRUIT MOTH IS DISRUPTED BY COMPETITIVE OR NON-COMPETITIVE MECHANISMS

ABSTRACT

Large cages were used to determine communicational disruption mechanisms for Oriental fruit moth. Near-female-equivalent pheromone dispensers (0.04 µg h^{-1}) operated by competitive attraction. In contrast to codling moth, Oriental fruit moth disruption shifted to a non-competitive mechanism for high-releasing dispensers (60 µg h^{-1}). The near-female-equivalent pheromone dispensers were also used to quantify the additive effect of an attract-and-remove control strategy compared to competitive mating disruption. A 5-fold reduction of Oriental fruit moth captures was found under attract-and-remove compared to mating disruption using near-female-equivalent dispensers. Surprisingly, the presence of females in equal numbers to released males had no significant impact on the effectiveness of any dispenser type in these disruption experiments lasting seven days.

INTRODUCTION

Despite decades of research, dozens of successful commercial products, and a large body of literature on pheromone-based control of insects (Cardé and Minks 1995, Miller et al. 2006a,b), the details of how mating disruption operates to control insects are still largely speculative. While it is clear that mating disruption diminishes mate finding and mating, the behavioral mechanisms involved can vary among even closely related species and can include: competitive attraction, desensitization, camouflage, or sensory imbalance (Cardé and Minks 1995).

Miller et al. (2006a,b) offered mathematical tools for differentiating between competitive attraction (false plume following) and non-competitive mechanisms (desensitization, camouflage, and sensory imbalance). They analyzed cases from the literature for fit to the two classes of mechanisms. Under competitive disruption, catch drops dramatically initially but non-linearly with density of pheromone dispensers. The impact per dispenser continually diminishes as pheromone dispenser density increases (Miller et al. 2006a,b). Contrastingly, the impact per dispenser is expected to be constant for non-competitive disruption so as to generate a straight line when dispenser density is plotted *vs.* catch suppression (Miller et al. 2006a,b). Eleven of the 13 cases analyzed supported competitive attraction, judged to be the primary mechanism for current mating disruption products for Lepidoptera.

Codling moth (*Cydia pomonella* L.*)*, a major insect pest of apples and pears, is an exemplar for competitive disruption. Using 20 large field-cages, Miller et al. (2010) validated a competitive-attraction equation under unique conditions where dosage-response profiles for differing disruption treatments under known, manipulatable moth densities could be generated simultaneously. Regardless of release rate from the pheromone dispensers tested, codling moth sexual communication was always inhibited competitively, i.e., attraction to the dispensers was always the first step in the behavioral sequence yielding disruption as measured by diminished catch in optimized monitoring traps.

Oriental fruit moth (*Grapholita molesta*), a closely related tortricid, is also an economically important tree-fruit pest in many parts of the world. Unlike codling moth, it may be susceptible to non-competitive mechanisms. Flight tunnel and field studies documented desensitization to synthetic pheromone (Rumbo and Vickers 1997, Figueredo and Baker 1992). Miller et al. (2006b) reported a field study using 12 ml dollops of emulsifiable wax releasing Oriental fruit moth pheromone blend at 150 to 40 µg h^{-1} that followed the predictions of a noncompetitive model including a linear plot of the data and a quadratic curve in the Miller-de Lame plot. Oriental fruit moth is also easier to control using lower densities of dispensers (Kovanci et al. 2005, Stelinski et al. 2007a) or fewer sprayable pheromone applications (Il'ichev et al. 2006) compared to codling moth.

Gut et al. (2004) proposed that Oriental fruit moth is more susceptible to disruption because they are sensitive to very low concentrations of pheromone. Alternatively they could be disrupted non-competitively. The specific objectives of the current research using Oriental fruit moth and the large field-cage system of Miller et al. (2010) were to: 1) corroborate that disruption of this tortricid operates competitively for point sources releasing at female-like rates, 2) and if so, determine whether the data validate the competition equation of Miller et al. (2010), so far validated only for codling moth, 3) quantify whether, and to what extent, an attract-and-remove tactic improves disruption over attraction alone, 4) determine if disruption can shift to a non-competitive mechanism at supernormal dispenser release rates, and 5) determine whether and to what extent the presence of female Oriental fruit moths influences disruption outcomes.

MATERIALS AND METHODS

Insect Rearing

Oriental fruit moths were obtained from a 9-yr-old laboratory colony originally collected as larvae from apple orchards in Southwestern Michigan. Moths were reared at 24^{O}C on a standard pinto bean diet under a 16:8h (L:D) photoperiod. Pupae were segregated by sex, and placed in 0.03 m^{3} cubical mesh cages for emergence. Sexes were kept in separate laboratory rooms to prevent males from being exposed to pheromone before the start of experiments. Adults of both sexes were provided a 10% sucrose solution and held under laboratory conditions on the natural Michigan photoperiod until they were released in the field cages, usually within 2-4 d after emergence.

Field Cages and Disruption Assessment

Each of 20 mesh-covered field cages covered 12 standard apple trees (details in Miller et al. 2010). Suppression of catch (disruption) was quantified using one Pherocon VI delta trap (Trécé, Adair, OK) hung in the upper half of a central tree of each cage and baited with a red rubber septum Oriental fruit moth monitoring lure (Trécé) pinned beneath the trap roof. Lures were replaced weekly. Capture of moths was recorded for 6 d.

Release Protocol

On the day of release, 1- to 4-day-old male and female Oriental fruit moths were captured in small glass vials (1–3 individuals of one sex per vial) and lightly dusted with fluorescent powder (DAYGLO Color Corp., Cleveland, OH). Males and females received contrasting colors. Colors were changed from week-to-week to enable detection of any trap visitation from wild individuals or those from previous studies. Such events were rare. Vials containing moths were protected from intense sunlight during transport to the field and vials were randomized before moths were released evenly throughout each cage. The vast preponderance of moths flew directly into the dense canopy of the closest tree at the time of release, permitting moths to be initially distributed uniformly throughout the cages. Moths incapable of horizontal or upward flight (<2%) were destroyed whenever possible and replaced by able individuals. Releases occurred in the early afternoon.

Experimental Design

The design of all experiments was a randomized complete block design, i.e., one of each treatment combination (randomly assigned to a cage) was present every day of each run lasting one week (a block). Three replicates were accumulated through the 2010 and 2011 growing seasons. The experiments were alternated so that no given experiment experienced conditions unique to one time of the season.

Experiment 1. Varying Densities of Low-Releasing Sticky and Non-Sticky Dispensers

To evaluate the effect of varying point source density of low releasing dispensers, commercial Oriental fruit moth monitoring lures (Trécé) were used to disrupt sexual communication. Dispenser densities per cage were: 0, 1, 2, 4, 8, and 17, always uniformly distributed within a cage. Near-female dispensers used were lures releasing 0.04 µg h^{-1}, near the rate for maximal attraction into a trap (Baker et al. 1980). Each lure used as a dispenser was placed inside a Pherocon VI delta trap body as described above for the monitoring trap. The above densities of dispensers within trap bodies were of two types: without and with sticky liners. This design permitted assessment of the added effect of attract-and-remove over attract only. All cages received 72 male Oriental fruit moths. Another factor in this experiment was females absent *vs.* females present (see above). Treatments included females present and females absent. Treatments with females received 72 females dispensed in the cages using the same methods as males.

Experiment 2. Varying Densities of High-Releasing Dispensers

Uniformly distributed Isomate OFMTT (ShinEtsu, Tokyo, Japan) "rope" dispensers releasing Oriental fruit moth pheromone blend at 60ug h^{-1} for the major component were deployed at: 0, 4, 6, 10, 15, 20, and 30 per cage. All dispensers were attached to 25 x 40 mm plastic clips (Kwiklok, Yakima, WA) and clipped onto branches in the upper half of the tree canopy. All cages received 48 male Oriental fruit moths. Treatments included females present and females absent. Treatments with females received 48 females dispensed in the cages using the same methods as males.

Data Collection and Statistics

Oriental fruit moth captures in monitoring traps and sticky dispensers (identical to monitoring traps) were counted daily for the first three days of each experiment. A final count was performed just before beginning the next experimental run, three or more days later. Treatment means were transformed into dispenser density*catch (Miller-de

Lame plot, Miller et al. 2006a). To develop best fit lines for transformed data in Experiment 2, the plotting procedure was modified slightly from those outlined in the original publication to facilitate trend line fits to a quadratic equation using Excel. Dispenser density x catch was placed on the y-axis (x-axis for standard Miller-de Lame plot) and catch alone was placed on the x-axis (y-axis for standard Miller-de Lame plot). R-square values of lines were compared for fit to theoretical curves generated using the competitive attraction and non-competitive mechanism equations (Miller et al. 2006a). D_a values were compared using a 2-tailed t test. Data were normalized by log (x+1) transformation then subjected to multi-way analysis of variance (ANOVA). Differences in pairs of means were separated using the least significant difference test (Fisher's protected LSD) (SAS Institute 2000). The significance level was always $\alpha < 0.05$.

RESULTS

Experiment 1. Varying Densities of Low-Releasing Dispensers

No difference in male captures was found when females were added to the study (p=0.938). For subsequent analyses, female present and female absent treatment combinations were pooled. The addition of sticky liners to the competing low-releasing dispensers significantly reduced capture of male Oriental fruit moths in the central monitoring trap, compared to the non-sticky dispensers (p=0.0003) (Figure 18). Only two competing sticky traps were necessary to significantly reduce capture in the monitoring trap, while eight were required to do so for competing non-sticky dispensers.

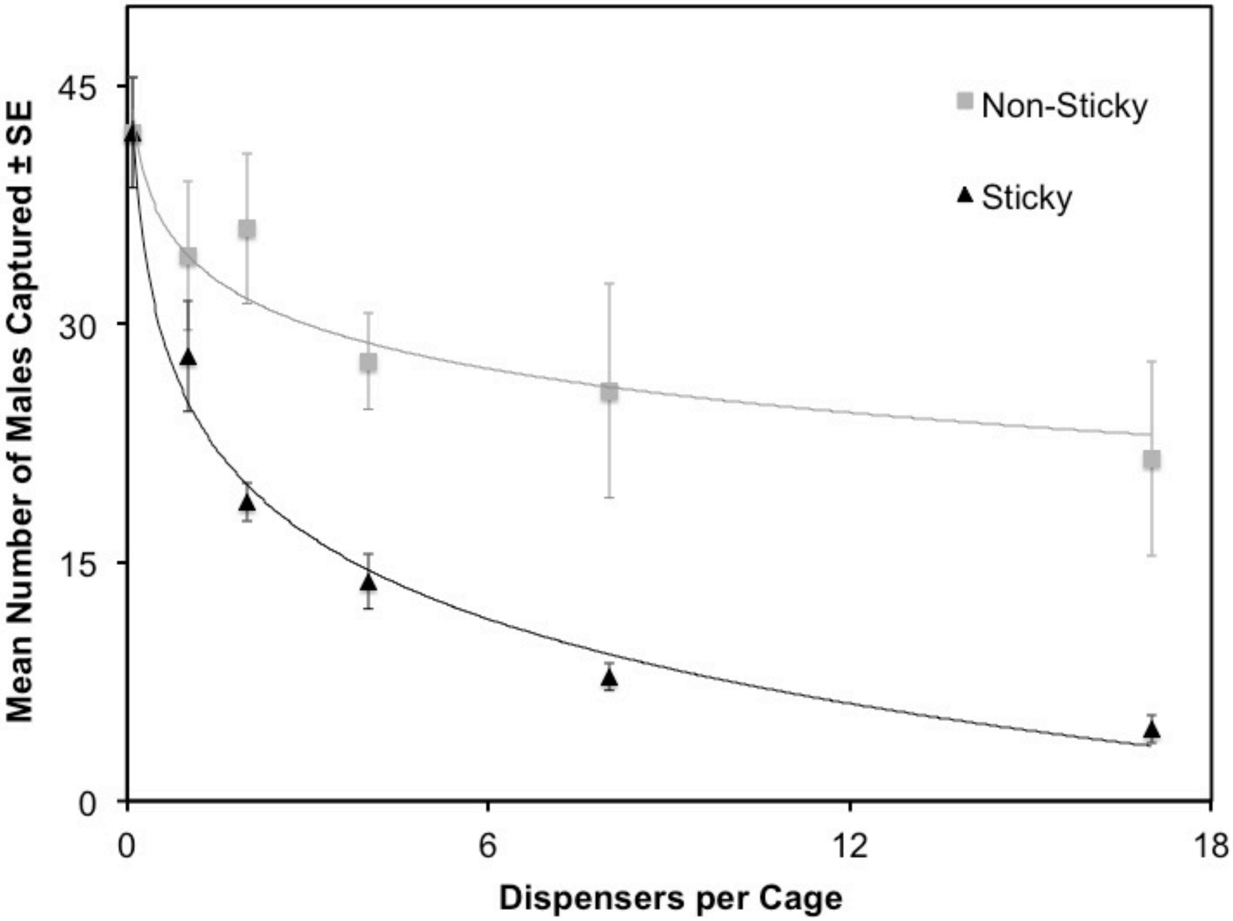

Figure 18. Untransformed plots of the mean number of male Oriental fruit moths captured in the central monitoring trap using low-releasing dispensers deployed at varying densities. Sticky = lure-baited delta trap with liner. Non-sticky = lure-baited delta trap without a liner.

Whether the disruption profile fit a competitive *vs.* non-competitive model was initially assessed by whether the data followed a linear or inverse function when plotted as dispenser density *vs.* catch. The inverse function was the best fit for both the sticky and non-sticky treatment combinations (Figure 18). The Miller-de Lame plot is a more stringent test of whether the experimental results follow a competitive or non-competitive mechanism (Figure 19). The linear model was a better fit than the quadratic

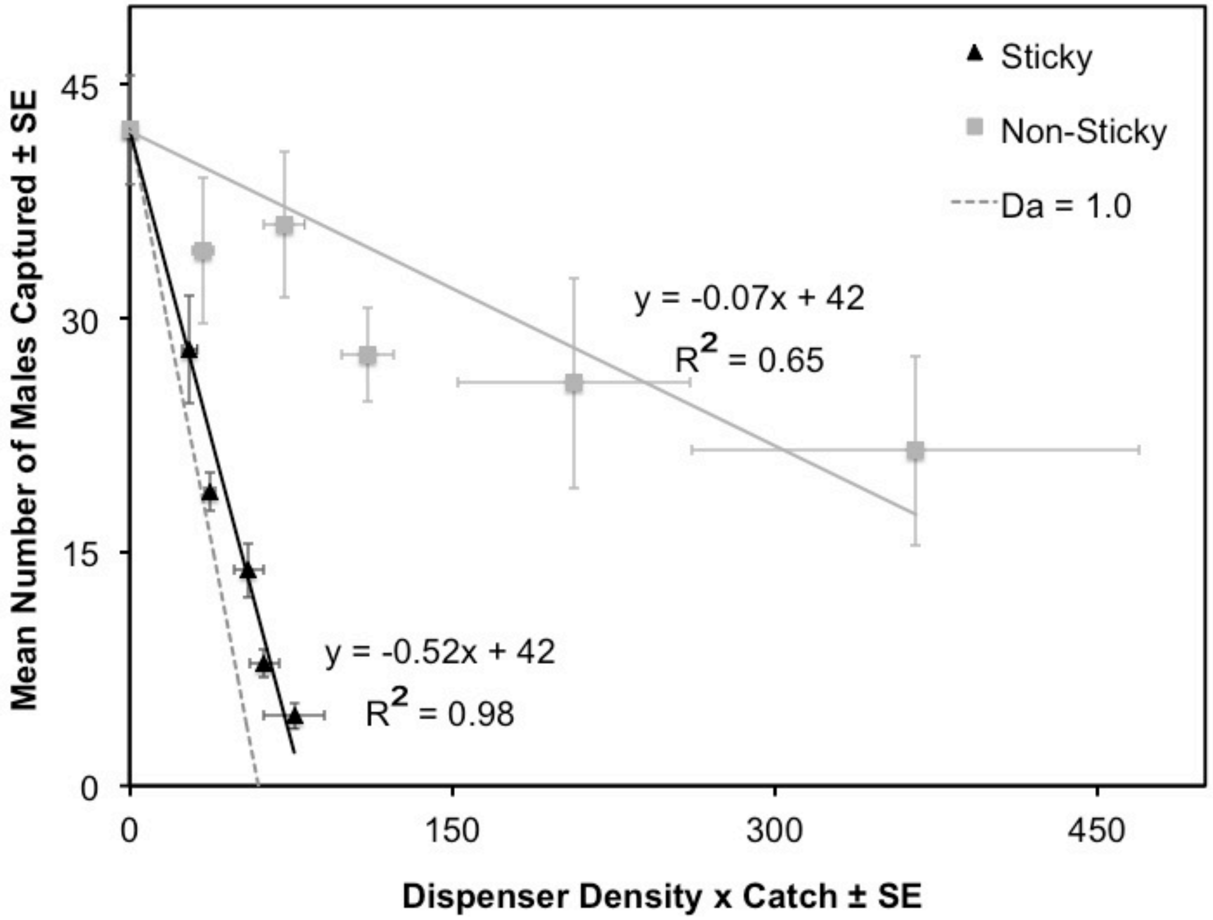

Figure 19. Miller-de Lame plots of mean male Oriental fruit moth captures in central monitoring trap using low-releasing dispensers. The best-fit linear model is shown for both treatment blocks. Treatment blocks indicate if the dispenser did or did not include a sticky liner. A line indicating a dispenser activity (D_a) of 1.0 is also included for comparison.

model for both the sticky (R^2=0.98 and R^2=0.20, respectively) and non-sticky (R^2=0.86 and R^2=0.77, respectively) treatment combinations, indicating the near-female-equivalent dispensers operated via competitive attraction.

Experiment 2. Varying Densities of High-Releasing Dispensers

No difference was found between the female present/absent treatment combinations in the untransformed or Miller-de Lame transformed data (p=0.157 and p=0.605, respectively). For subsequent analyses, data from female present and female absent treatments were pooled. Disruption increased linearly with increasing dispenser density. (R^2=0.89) (Figure 20). But as assessed by contrasting pairs of data, fifteen or more dispensers were required for a significant reduction in male Oriental fruit moth

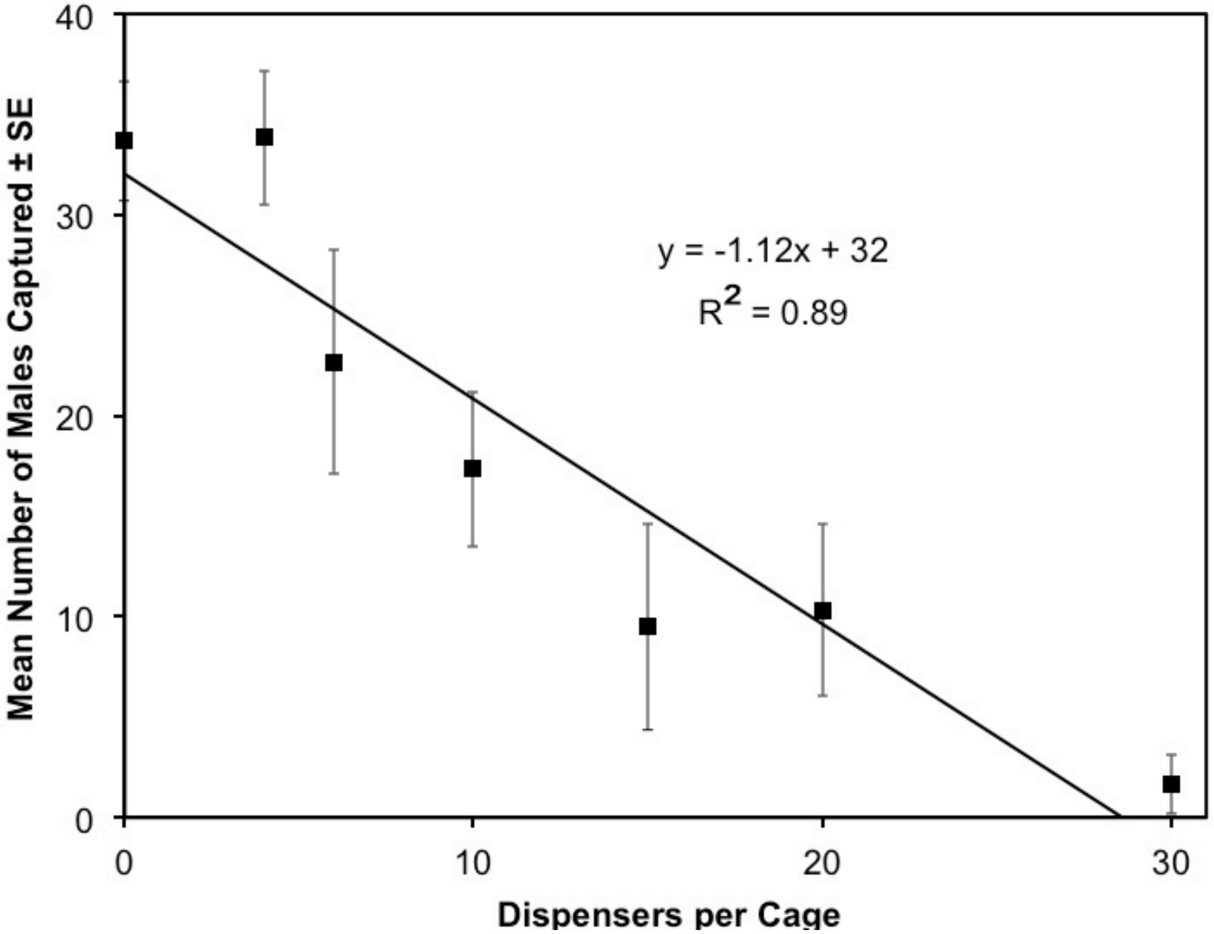

Figure 20. Untransformed plot of the mean number of male Oriental fruit moths captured in the central monitoring trap using high-releasing dispensers deployed at varying densities.

captures in the central monitoring trap ($p<0.001$). The Miller-de Lame plot corroborated the untransformed observation (Figure 21). Here, the quadratic model was a better fit than the linear model ($R^2=0.56$ and $R^2=0.06$, respectively), indicating disruption occurred non-competitively.

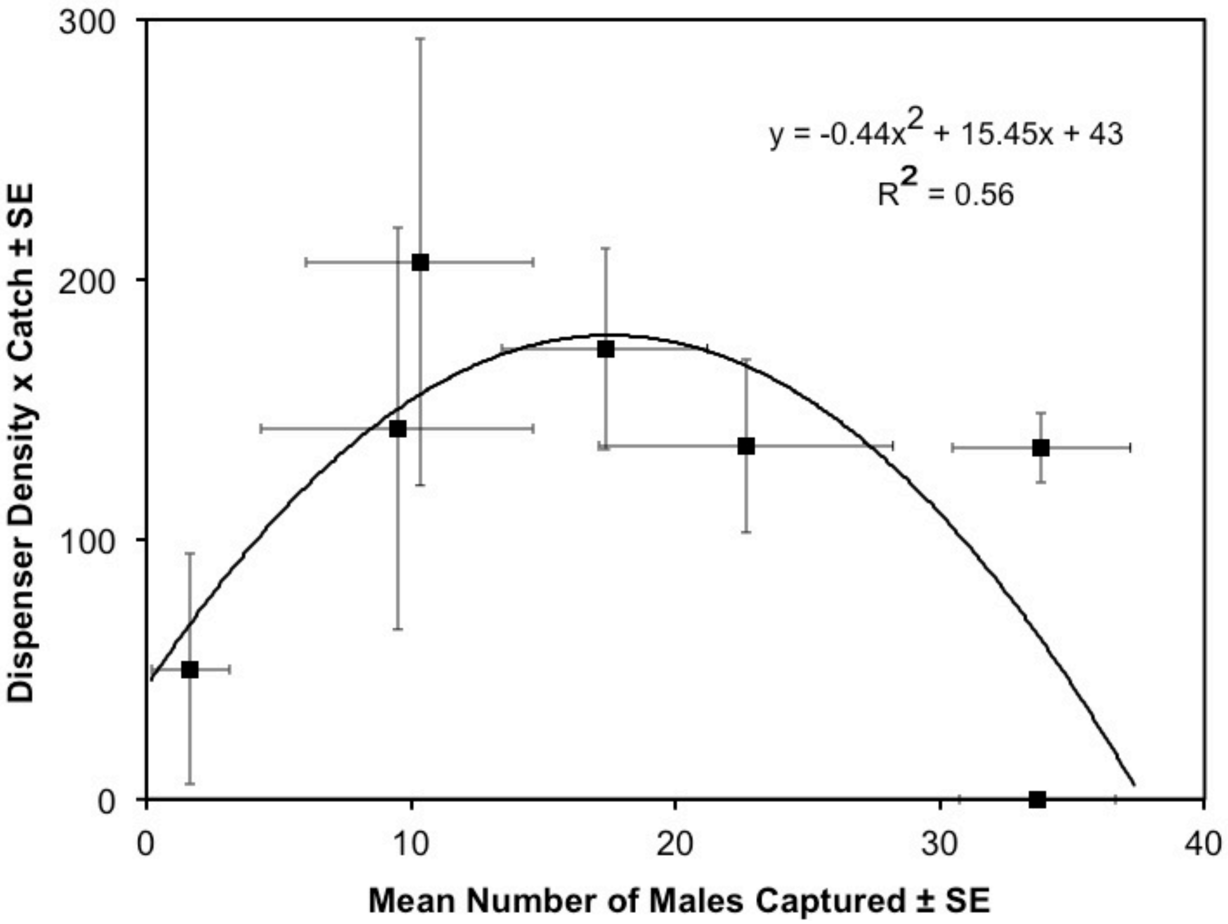

Figure 21 Miller-de Lame plot of mean male Oriental fruit moth captures in central monitoring trap using high-releasing dispensers. The best-fit quadratic model is shown.

DISCUSSION

The current results confirm the conclusion of Stelinski et. al. (2005) that Oriental fruit moth is disrupted competitively at near-female release rates of pheromone. The key supportive evidence from the current test using Oriental fruit moth lures is dosage-response plots that are: 1) concave on non-transformed data (Figure 18) and 2) linear with negative slope on a Miller-de Lame plot (Figure 19) and an x-intercept that nearly matches the mean number of Oriental fruit moth males released per cage (87 ± 9.5 *vs.* 60 ± 5.2, respectively). Dispenser activity values (D_a) can be calculated by dividing slopes on a Miller-de Lame plot by trap findability x efficiency (Miller et al. 2010). D_a values for traps with and without liners were 0.70 ± 0.05 *vs.* 0.12 ± 0.05, respectively. Because identical traps with liners were competing with the central monitoring trap, their D_a value should be nearly 1.0 under disruption by competitive attraction if all traps caught equally (Miller et al. 2010). The perimeter traps, however, captured fewer moths than central traps, resulting in a Da level significantly lower than 1.0 (p=0.002). This was likely due the artificially restricted trapping area of the perimeter traps caused by the cage walls. Using the reasoning of Miller et al. (2010), the x-intercept in Figure 19 for traps without liners was 828, indicating that Oriental fruit moth males got ten times more visits to non-sticky *vs.* sticky traps over their lifetimes in the large cages. The greater disruption provided by sticky *vs.* non-sticky traps (Figure 18) provides further support for the case that a mass-trapping regime would be more effective than an equal number of mating disruption dispensers operating only by competitive attraction. Collectively, the data from Experiment 1 corroborate the competitive attraction equation of Miller et al. (2010) and its use in quantifying competitive disruption.

Miller et al. (2006b) tentatively concluded that 12 ml dollops of emulsified wax formulation of Oriental fruit moth pheromone releasing at >40 µg h^{-1} disrupted Oriental fruit moth non-competitively. A weakness of that study was insufficient pheromone dispenser densities to convincingly populate the Miller-de Lame plot. The current more rigorous experiments prove this shift in disruption mechanisms for Oriental fruit moth and that it is caused by increased release rate of pheromone. This is the first time such a shift in disruption mechanism has been demonstrated against a background of otherwise identical experimental conditions in the field. Moreover, this is the first case where a commercial Isomate rope formulation has been shown to operate non-competitively.

Non-competitive disruption of monitoring traps indicates that attraction of Oriental fruit moth males was not a necessary first step in the disruption of traps (Figures 20-21). Males must have been rendered unable to respond to the traps before a first visit to the ropes. However, this does not mean that Oriental fruit moth males were never attracted to the ropes. During this experiment, males sometimes were observed approaching ropes and commonly were found sitting on foliage nearby the Isomate dispensers. Such attraction to rope dispensers but not traps suggests threshold elevation above the lure release rate but not the higher Isomate release rate of pheromone. We tentatively conclude that desensitization not first requiring attraction was the more likely behavioral explanation for the non-competitive disruption of Oriental fruit moth.

The data of Figure 20 can be used to estimate the area over which each Isomate dispenser non-competitively disrupted the Oriental fruit moth traps. Because it took *ca.* 30 dispensers to reduce catch from *ca.* 30 males per trap in the negative control to near zero, the slope of the Figure 20 regression line was necessarily nearly -1. Thus, each Isomate dispenser disrupted *ca.* 1/30th of the large-cage total area of 360m^2, or 12m^2 per dispenser. This value is a remarkably good match to the 13m^2 that Miller et al. (2006b) reported for non-competitive disruption of Oriental fruit moth traps by each 12 ml dollop of an emulsified wax formulation of Oriental fruit moth sex pheromone. Given that the area disrupted per high-releasing dispenser is very close to the two-dimensional area occupied by our average apple tree, it appears that at least one dispenser would be required per tree for excellent non-competitive disruption of Oriental fruit moth. This dispenser density is similar to that recommended for Isomate C+ (Witzgall et al. 2008) disrupting codling moths only competitively (Miller et al. 2010). However, under disruption by competitive attraction, codling moth males may happen to orient to a female or trap (proxy for a female) before their first visit to an Isomate dispenser that disrupts them only for the remainder of that given night (Miller et al. 2010). When Oriental fruit moth is disrupted non-competitively, such chance orientations to females would be unexpected. We suspect that this is the key reason why Oriental fruit moth is more easily disrupted than codling moth (Gut et al. 2004). Indeed, it is possible that other insects thought to be “easily disrupted” are so because it is possible to disrupt them non-competitively. Further examination of other “easily disrupted” species is required (Gut et al. 2004).

We postulated that the females in the low-releasing dispenser study would act as dispensers, reducing the recapture of males in the monitoring traps. This, however, was not the case. At no point was there any significant difference between dispenser density treatments with vs. without females. Additionally, no discernable difference was found in the high-releasing dispenser study between the female present and absent treatments. Anecdotal evidence exists to suggest females were calling. In the low-releasing dispenser experiment, evidence of mating was found, as a small proportion of the male Oriental fruit moths captured in traps had trace amounts of the contrasting dye color applied to females on their claspers. It is possible the presence of females emitting low levels of pheromone induced male flights and orientations. The postulated reduced capture in traps when females were present was potentially offset by the higher male activity in the presence of many female sources. These results suggest that the simpler model including males only is adequate for discerning the effects of varying dispenser densities on the capture of males in monitoring traps.

In the large-cage (Miller et al. 2010), competitive attraction was the primary mechanism reducing male codling moth catch, regardless of pheromone release rate. Here, we show disruption of Oriental fruit moth can occur via competitive or non-competitive mechanisms, depending on the pheromone release rate from each dispenser. The two release rates tested were quite disparate. As such, we could not determine the release rate at which control switches from a competitive to a non-competitive mechanism. Further work would refine that critical release rate.

CHAPTER FIVE: DEVELOPMENT OF A NOVEL MICROTRAP FOR CAPTURE OF CODLING MOTH, *CYDIA POMONELLA*

ABSTRACT

Various designs have been introduced over the years for capture of codling moths in monitoring traps. Recent research on high-density trapping for management of tortricid pests (Reinke et al. 2012) led to the development of a 4 cm cube as a novel microtrap. The present report details the development of the cube and several other microtrap designs. Initial flight tunnel studies compared various trap designs including several cylindrical shapes, vanes, cubes, and a pyramid. Moth captures in cylindrical traps were extremely sensitive to trap orientation; few moths were caught when the entrances to the traps were perpendicular to wind flow. However, cube- and pyramid-shaped traps captured up to 60% of released moths. A 4 cm cube with 1.3 cm holes on all sides captured as many codling moths as the Pherocon VI delta trap (Trécé Inc., Adair, OK) in both choice and no-choice tests in large field-cages. Open-field studies using sterile codling moths revealed various box shapes and a pyramid design were equally effective at capturing moths, but round openings in the traps (15 moths trap^{-1}) were more effective than long slits (6 moths trap^{-1}), and larger cube- shaped traps captured more moths than smaller cube traps (6.6, 3.1, and 2.6 moths trap^{-1} for 8 cm, 4 cm, and 2.5 cm cubes, respectively).

INTRODUCTION

Codling moth, *Cydia pomonella* (L.), is a major pest of apples and pears in most pome fruit growing regions of the world. Management of this widespread pest has advanced since the discovery of its main pheromone component, E,E-8,10 dodecanol (codlemone) (Roelofs et al. 1971). Codling moth pheromone has been successfully utilized for monitoring (Madsen and Vakenti 1973) and pest management (El-Sayed et al. 2006, Witzgall et al. 2008).

Codling moth monitoring systems require the combination of a strong attractant and effective capture and retention mechanisms. Over the years many trap designs have been evaluated for codling moth monitoring, including wing-style traps such as the Pherocon 1C originally produced by Zoecon Corp. and later by Trécé (Madsen and Vakenti 1973, Ahmad and Algharbawi 1983, Knodel and Angelo 1990, Vincent et al. 1990, Kehat et al. 1994, Knight et al. 2002, Fadamiro 2004), delta-style traps (Ahmad and Algharbawi 1983, Knodel and Angelo 1990, Knight et al. 2002, Fadamiro 2004, Knight and Fischer 2006), non-sticky funnel-type traps (Howell 1984, Knodel and Angelo 1990, Vincent et al. 1990, Kehat et al. 1994), passive intercept traps (Weissling and Knight 1994, Knight 2000), and blacklight traps (Weissling and Knight 1994). Wing-style and delta-style traps are currently the most widely used commercially.

While pheromone-baited traps have been used primarily for monitoring codling moth phenology and assessing relative pest population size, they have also been employed as a means of pest control through mass trapping. Early attempts to control codling moth through the deployment of 10-40 traps ha^{-1} reduced codling moth damage, but not at levels that would be commercially acceptable (Madsen and Carty

1979, Willson and Trammel 1980). In their review of mass trapping for control of various lepidopteran pests, El-Sayed et al. (2006) concluded that improvement of the technique would require a very efficient and inexpensive trap design that could be deployed at a high density. Recent studies examining the mechanisms of mating disruption support the potential of high-density trapping for codling moth control (Miller et al. 2010). Large field cage studies revealed a supplementary and substantial benefit of permanent removal of codling moth males using high densities of pheromone lure-baited sticky traps over mating disruption using high-releasing dispensers. Miller et al. (2010) concluded that high rates of pheromone released from the dispensers eliminated further orientations by attracted males for one evening, while the trapping system resulted in permanent removal of males from the mating pool.

Recently, Reinke et al. (2012) reported that high densities (250-1000 ha^{-1}) of a small, cube-shaped trap provided 92% inhibition of male captures in central monitoring traps, while mating disruption dispensers only provided 71% inhibition.

Herein, we report on wind tunnel and field studies comparing the efficiency of various trap designs for capturing codling moth. The aims were: 1) to develop highly effective microtraps that could be deployed as attract-and-kill devices for codling moth control, and 2) to evaluate the utility of the best trap designs for potential use in codling moth monitoring programs.

MATERIALS AND METHODS

Insect Colonies

For flight tunnel and cage experiments, codling moth males were removed from a 5-yr-old laboratory colony established with wild moths from untreated apple orchards located in Michigan, USA. Moths were reared at 24°C and 60% RH on pinto bean-based diet (Shorey and Hale, 1965) under a 16:8 (L/D) photoperiod. Pupae were sorted by sex, and adults emerged in $0.03 m^3$ plastic cages that included small cups of 5% sucrose solution with cotton dental wicks protruding from their lids. For field studies using sterile moths, adult codling moths were obtained from the Okanagan–Kootenay Sterile Insect Rearing Facility (Osoyoos, B.C., Canada). The moths were picked up at the Canadian border and shipped overnight to Michigan State University via FedEx in Styrofoam coolers with cold packs. Upon receipt in the morning, groups of 150 moths (equal numbers of males and females) were sorted into 10 oz. solo containers, dyed using DayGlo pigment powder (green, blue, pink or orange) (DAYGLO Color Corp., Cleveland, OH), and transported immediately to the field for release. Colors were rotated between experiments to discern released moths from wild individuals or from sterile moths released in earlier studies.

Trap Assembly

All cubical, pyramidal, vertical vane, and funnel traps were constructed of 17.5 x 17.5 cm delta trap sticky inserts (Alpha Scents Inc., Portland, OR). Cubical and pyramid traps (Figure 22F and G) consisted of inserts cut into equal sized panels folded and cemented together in the desired shape using hot-melt adhesive. A single orifice of equal size and shape was cut into each side of the trap. Traps were constructed such

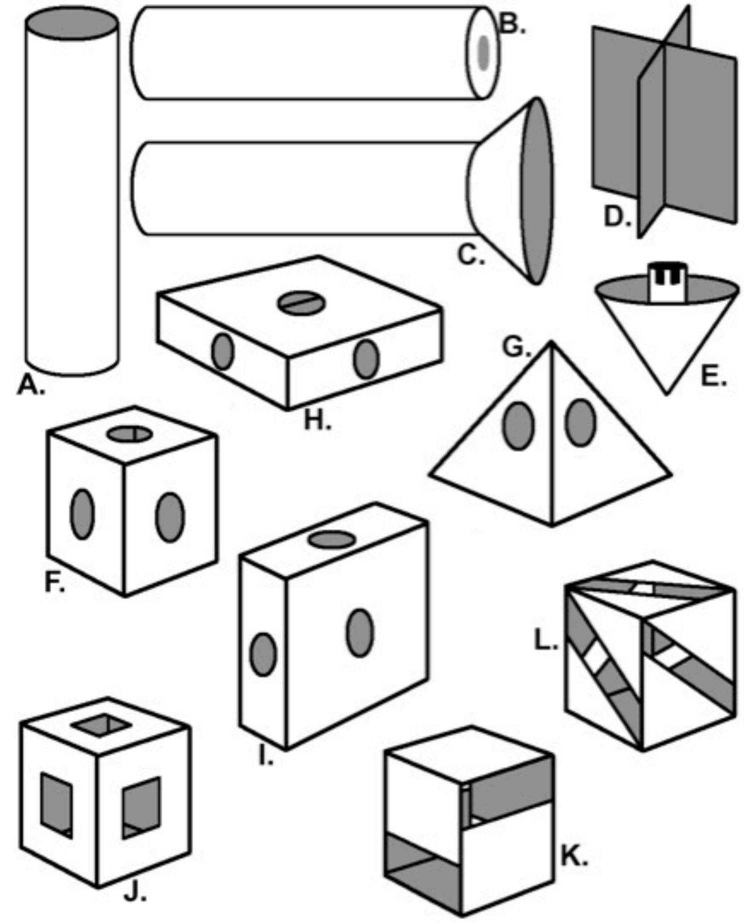

Figure 22. Codling moth microtrap designs evaluated in flight tunnel and field studies. A. tube, B. tube with small openings, C. tube with cone, D. vertical vanes, E. funnel, F. cube with round holes, G. pyramid, H. flat box, I. upright box, J. cube with square holes, K. cube with straight slit, L. cube with angled slit. Gray areas in figure indicate sticky surfaces. See Table 9 for trap sizes.

that adhesive surfaces always were on the inside of the trap. One codling moth lure was placed on the bottom interior surface of each trap. The funnel trap (Figure 22E) consisted of a conical piece 4 cm tall with a 4 cm opening on the top and closed at the bottom with the adhesive side facing inside. A cup 1.0 cm tall and 1.0 cm in diameter containing the codling moth lure was suspended over the funnel to promote a consistent airflow over the lure. Each vane of the vertical vane traps (Figure 22D) measured 2.5 cm wide by 8 cm tall and included adhesive on both sides. One codling moth lure was placed equidistant from the top and bottom at the nexus of two vanes on the downwind side of the trap.

Tube traps (Figure 22A-C) were constructed of 15 cm lengths of white poly-vinyl chloride (PVC) plastic tubing with an internal diameter of 3.2 cm. Tanglefoot adhesive (Contech Enterprises Inc., Victoria B.C., Canada) was applied to the internal surface of each tube. A single codling moth lure was placed inside each tube, equidistant from the two ends. Additions to the tube traps in the treatments with small or cone openings were created using delta trap sticky inserts as above. In the tube treatment with small openings (Figure 22B), ends were created to reduce airflow and moth access to a 1.3 cm orifice at either end of the tube. The sticky side of the trap insert piece was oriented towards the trap interior. In the tube treatment with a cone end (Figure 22C), a conical piece 3 cm deep with a 7 cm diameter entry was applied to the downwind opening of the tube with the sticky surface on the interior of the cone.

Sizes and dimensions for all trap designs varied depending on the study, and are provided below for each experiment. Lures used in all traps for all studies were a rubber septum loaded with 0.1mg of codling moth pheromone produced by Trécé Inc. (Adair, OK).

Flight Tunnel Study

Initial microtrap design efficacy comparisons were performed in a Plexiglas sustained-flight wind tunnel designed after that of Miller and Roelofs (1978). The rectangular flight tunnel was 2.4 m long and measured 1.3 x 0.8 m in cross-section. It was maintained at 15°C and 50-70% RH. Light was maintained at 1800-2000 lux inside the flight tunnel. Stelinski et al. (2004b) describe other particulars of the flight tunnel.

Male codling moths, 1-4 days old, were removed from the laboratory colony 1 h prior to the end of photophase and placed into cylindrical (17-cm-long × 8-cm-dia) wire-mesh release cages. Each cage contained 1-2 moths. The cages were placed in the wind tunnel for 1 h of acclimation prior to the beginning of the study. Experiments ran for a maximum of 1 h each day.

A lure, aged between 2 and 7 days, was placed into each trap tested on a given day. The trap was hung from a ring stand such that the center of the trap was 15 cm from the upwind end of the tunnel and 25 cm above the tunnel floor. A wire mesh cage containing male codling moths was placed at the downwind end of the flight tunnel, also 25 cm above the tunnel floor. Each male was allowed 3 min to respond, and then removed from the flight tunnel. The behaviors recorded were anemotactic flight followed by landing on the trap and capture within the trap. A replicate was terminated early only if the moth remained captured in the trap for a minimum of 30 sec. Wire mesh cages and ring stands were washed with acetone daily, after use.

One or two traps were tested daily. All replicates for an individual trap were performed over one day. Traps tested are listed in Table 9, with the first design tested at the top of the list, and subsequent designs in order from top to bottom. Ten, one moth replicates were performed for each trap design tested. Flight tunnel experiments were performed from 15 June 2009 to 6 August 2009.

Large Cage Study

Trap comparisons were conducted in 16 mesh-covered field cages enclosing a dozen 2.4-3.0 m tall Jonathon apple trees (details in Miller et al. 2010). Individual trap treatment cages received one trap hung in the upper canopy (2 m) of a central tree. Treatment cages in choice experiments received one trap of each type hung at the same height in the canopies of adjacent central trees. All cages received 36 colony-reared male codling moths. Vials containing moths were transported to the field in insulated coolers and randomized before moths were evenly released throughout each cage. Moths incapable of horizontal or upward flight (<2%) were destroyed whenever possible and replaced by able individuals. Releases occurred in the early afternoon. Treatments included a single 4 cm cube microtrap per cage, a single Pherocon VI delta trap (Trécé Inc., Adair, OK) per cage, or one cube and one delta trap placed in adjacent trees in a single cage (choice experiment). Each treatment was replicated 3 times. All treatments and replications were randomized and performed simultaneously between 15 and 21 September 2009.

Field Studies

Plots were in a commercial apple orchard near Alpine, Michigan. The eight plots contained free-standing apple trees approximately 3m in height and planted at 400 trees ha^{-1}. Each plot consisted of a central “release tree” and four trees at cardinal directions 15 m from the central tree. For each experiment, four different trap designs were hung in the upper third of the canopies of the four perimeter trees.

All plots received approximately 150 codling moths at a ratio of 1:1 (male:female). Cups containing dyed moths were transported to the field in coolers to minimize moth injury and mortality during transport to the field. Cups were randomized before moths were released into each central release tree. Moths were released by tossing them into the canopy.

Three experiments were performed between 21 July 2011 and 25 August 2011. Experiment 1 compared 4 trap designs (l x w x h): cube (4 x 4 x 4cm), pyramid (4.5 x 4.5 x 4cm), flat box (5 x 5 x 2.5cm), and upright box (5 x 2.5 x 5cm) (Figure 22F-I). External dimensions were chosen to maintain similar internal volumes (60-64 cm^3). Experiment 2 compared trap entry orifice shapes: round, square, straight slit, angle slit (Figure 22F, J-L). Cube traps (4 cm) were used for all experiments. Minimum orifice dimension was 1 cm to allow easy moth entry. Experiment 3 compared a Pherocon VI delta trap to cubes of various sizes: 2.5 cm, 4 cm, and 8 cm. All experiments were conducted as a complete block design with eight replicates performed simultaneously for each experiment. To eliminate the effect of direction compared to the central release tree each trap treatment was placed in each cardinal direction for two replicates.

Statistical Analysis

For the flight tunnel study, the behavioral data were compared among treatments and to zero catch using a generalized linear model. For large cage and field studies, data were square root transformed (to normalize the distributions of residuals and homogenize variance) then subjected to ANOVA. Differences in pairs of means were separated using Fisher's protected least significant difference (SAS Institute, 2000). Significance level was $\alpha<0.05$.

RESULTS

Flight Tunnel Study

Trap designs varied significantly in their capacity to attract and capture moths in the flight tunnel (Table 9). The vertical vane, cube, and pyramid traps were very effective at attracting and capturing codling moths Each provided greater than 60% attraction and 50% capture of released moths. A 15 cm tube with Tanglefoot adhesive on the internal surface, attracted 90% of the codling moths released, but captured only 20% when placed horizontally such that the openings were parallel with wind flow. A similar trap with restricted openings on both ends (1.3 cm) attracted significantly fewer moths and catch remained low (Table 9). The horizontal tube trap, oriented vertically such that the openings were perpendicular to wind flow, attracted no moths. Inserting a cone on the downwind end of the horizontal tube significantly increased trap performance. This trap design attracted and captured 70% of the released moths. The funnel trap attracted very few moths and none were captured.

Table 9. Codling moth capture in microtraps of different design tested in a wind tunnelValues within a column followed by the same letter are not significantly different ($p<0.05$).

	% Number of Codling Moths:	
Trap design	**On trap**	**Capture**
15cm tube-3.2cm dia. Horizontal	90ab	20yz
15cm tube-3.2cm dia. Vertical	0e	0z
15cm tube-3.2cm dia. With cone	70abc	70x
15cm tube-3.2cm dia., 1.3cm openings	40cd	20yz
Funnel (4cm x 4cm)	10de	0z
Vertical vanes (2.5cm x 8cm)	70abc	50xy
2.5cm cube (0.8cm holes)	100a	50xy
4cm cube (1.3cm holes)	60bc	60x
4cm pyramid (1.3cm holes)	90ab	60x

Large Cage Study

Under no-choice conditions, individual 4 cm cubes captured an average of 19.4 codling moth males per cage (Figure 23). Individual delta traps captured an average of 17.4 moths. No difference was found between the no-choice treatments (p=0.53). In the choice study, the cube and delta traps captured an average of 11.3 and 9.8 codling moths, respectively. Again, captures in these traps were not significantly different (p=0.50).

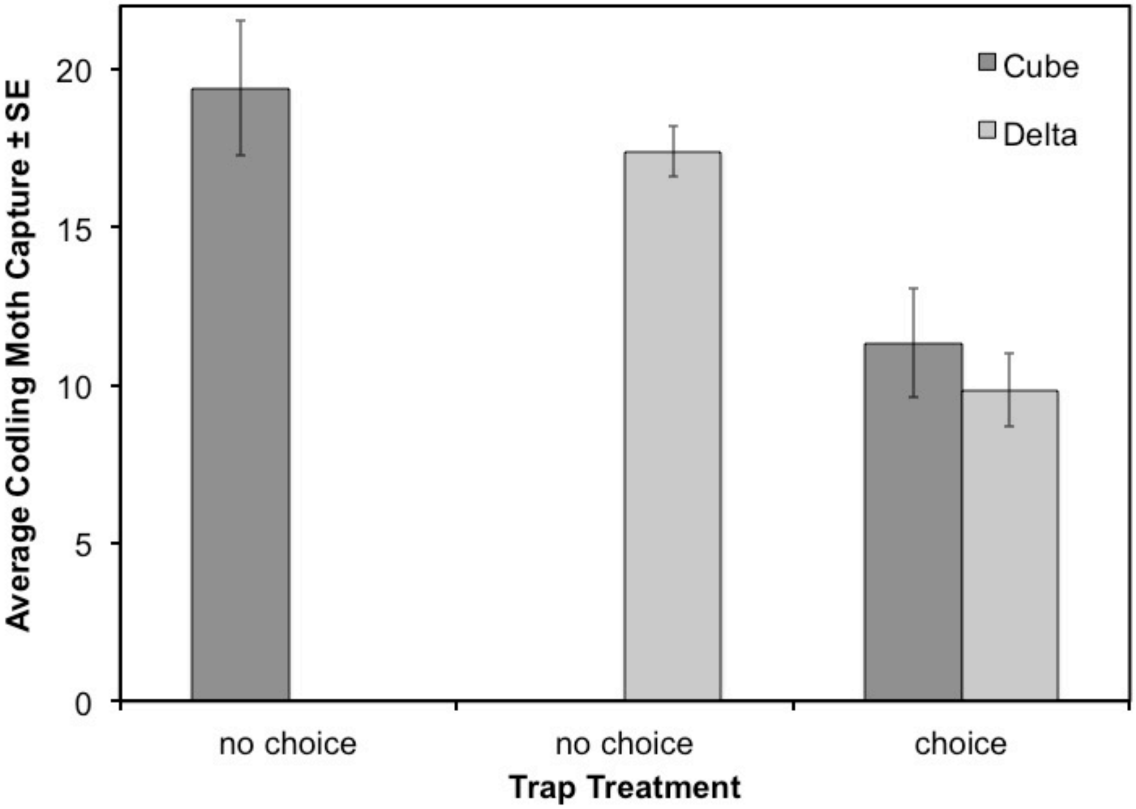

Figure 23. Large field- cage study comparing 4 cm cube microtrap to Pherocon VI delta trap for capture of codling moth males. Thirty-six male codling moths released per treatment.

Field Studies

In the experiment comparing trap shape, the pyramid captured the highest number of codling moths, averaging 7.6 per trap (Figure 24). The cube captured the least, averaging 4.6 codling moths per trap. No significance difference was found between treatments (p>0.582).

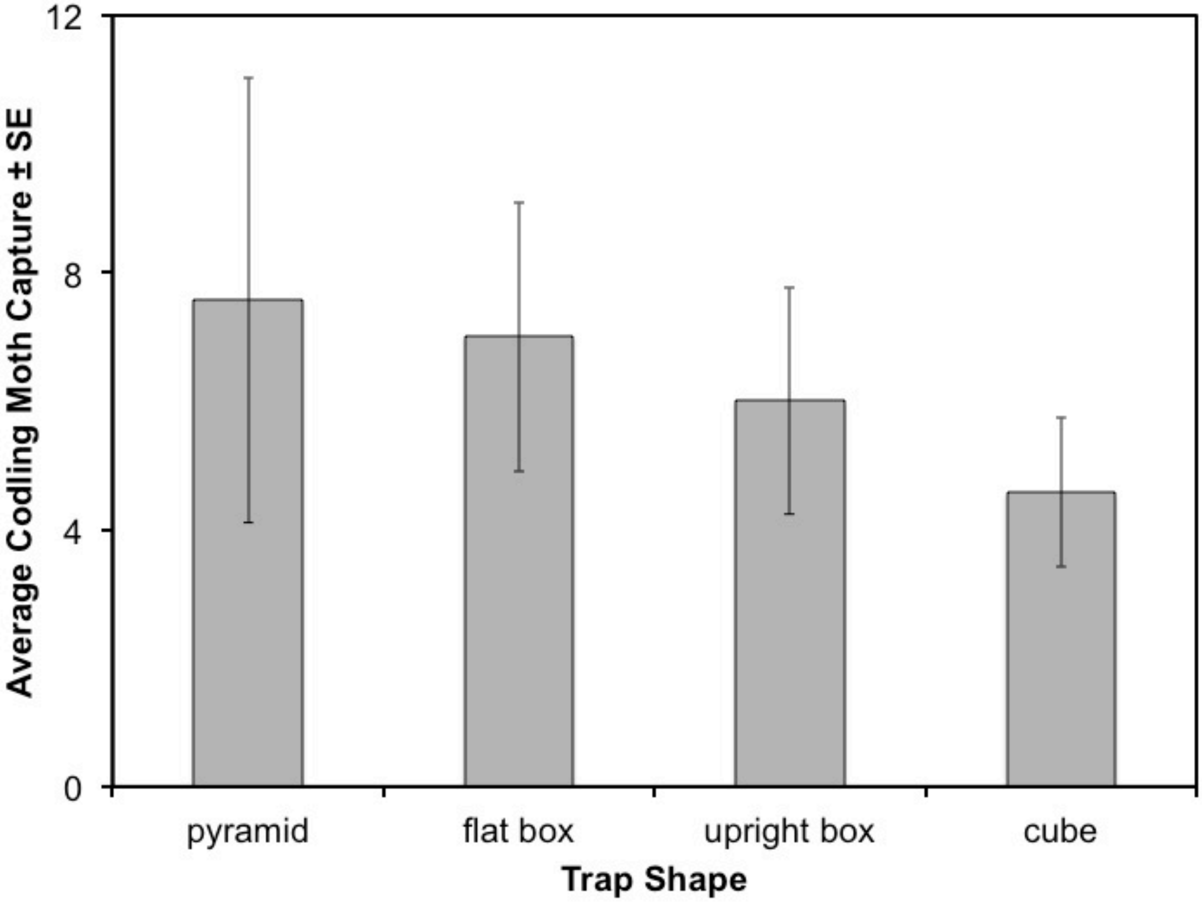

Figure 24. Field study comparing impact of microtrap shape on capture of codling moth males.

In the study comparing orifice entry shapes, the cube with the round orifice captured the most codling moths at 15.0 moths per trap (Figure 25). The cube with the square orifice captured statistically equal numbers of moths compared to the round orifice (p=0.186). Both straight-slit and angled-slit treatments captured statistically fewer moths than the round orifice treatment (p=0.004), but not the square cubes (p=0.077 and p=0.067, respectively).

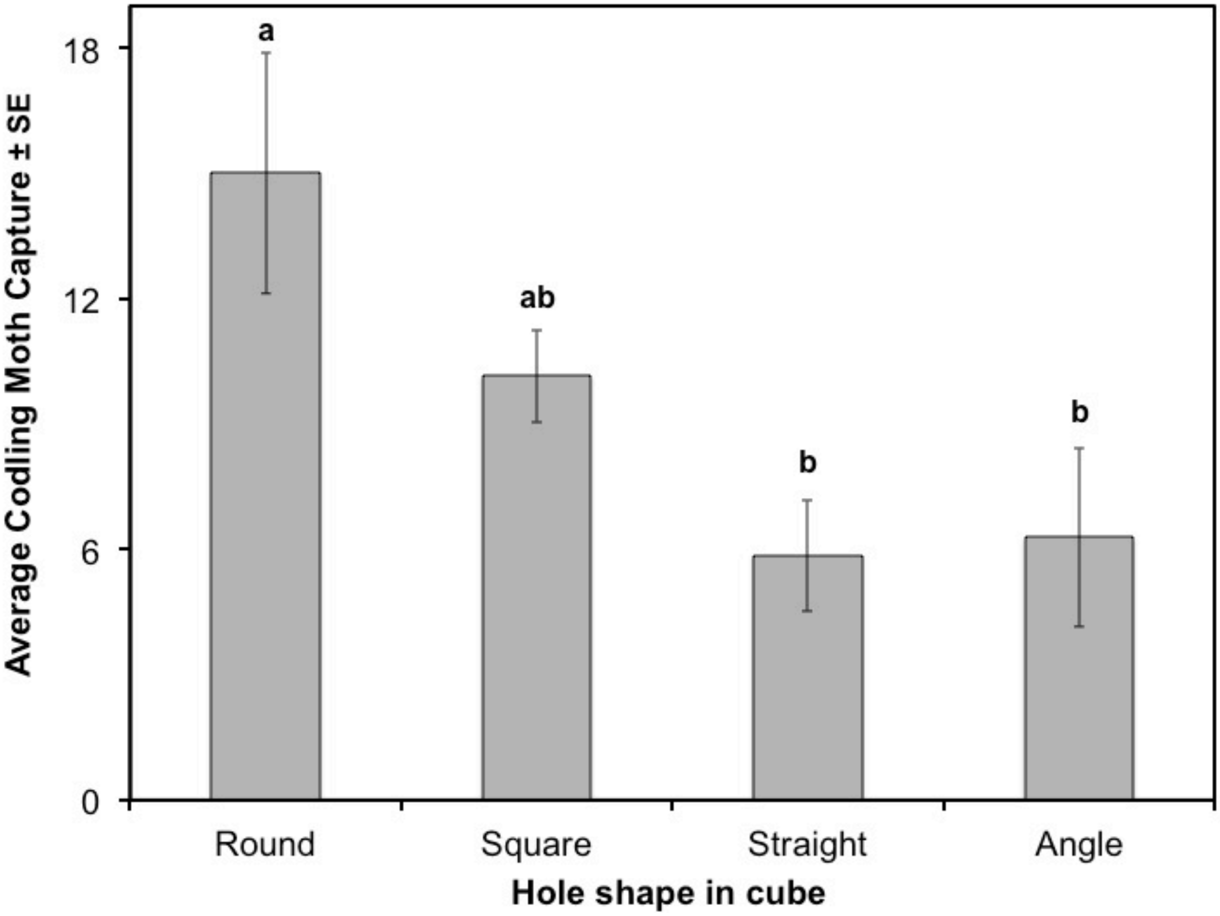

Figure 25. Field study comparing impact of microtrap orifice shape on capture of codling moth males. Treatments with the same letter are not significantly different (P<0.05).

The largest cube (8 cm) was the most effective cube treatment, capturing 6.6 codling moth males per trap (Figure 26). Moth captures in the 8 cm and the 4 cm cubes were not significantly different from the Pherocon VI delta trap's capture of 7.7 moths (p=0.485 and p=0.052, respectively). The 2.5 cm cube captured an average of 2.6

moths per trap, significantly fewer than the delta (p=0.025), but not different than either the 4 cm or 8 cm cubes (p=0.715 and p=0.110), respectively).

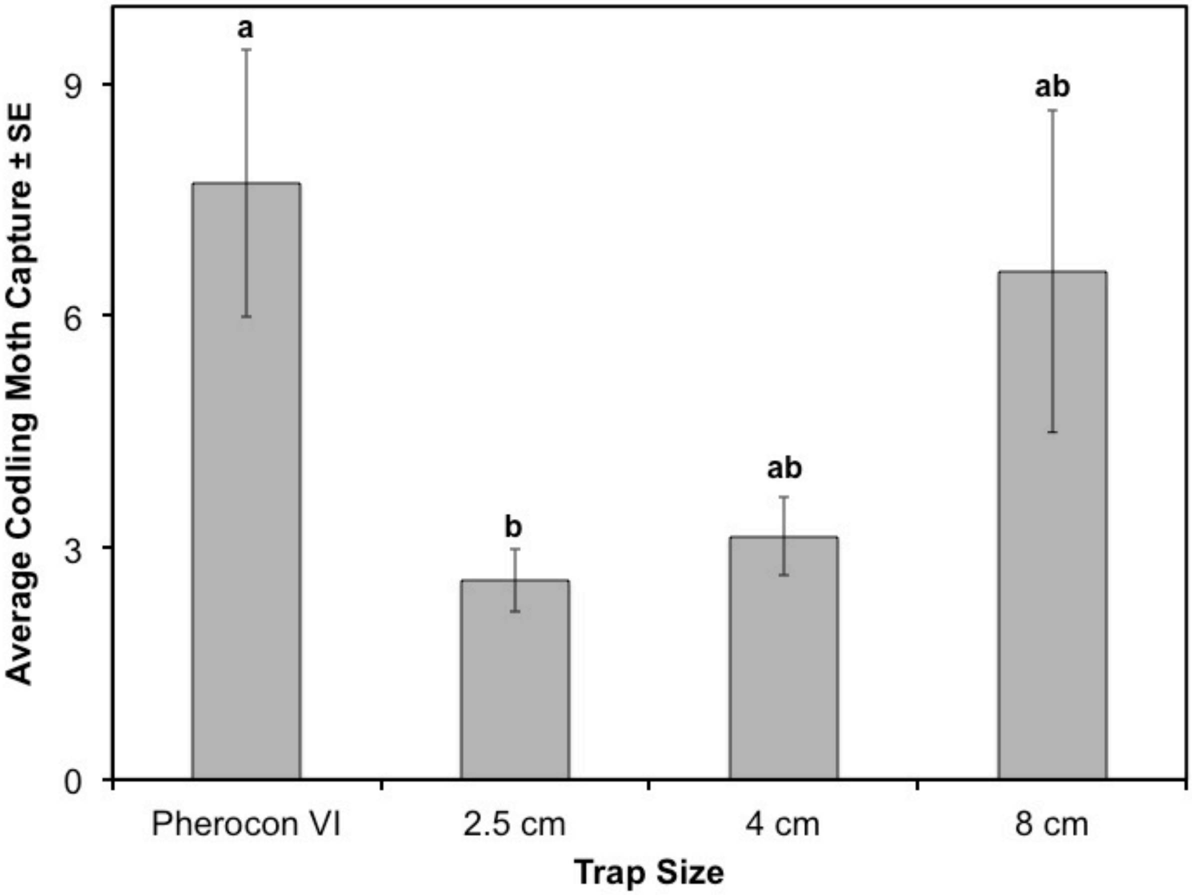

Figure 26. Field study comparing impact of microtrap size on capture of codling moth males. Treatments with the same letter are not significantly different (P<0.05).

DISCUSSION

The impetus for development of a new pheromone-baited trap for capture of codling moths was to explore the potential of high-density trapping using microtraps for codling moth control. Flight tunnel studies were conducted to rapidly screen a number of trap designs. A simple trap constructed of lengths of PVC plastic tube with a pheromone lure and adhesive on the interior to attract and capture the moths (Figure 22A) had some ability to capture codling moths, but was extremely sensitive to its orientation in respect to airflow (Table 9). The tube trap when oriented such that the openings were perpendicular to wind flow, failed to attract or catch moths. The simple tube trap was subsequently modified in an attempt to improve moth retention once they were attracted to and contacted the trap. Introducing an adhesive-laden landing area at the opening of the trap in the shape of a cone (Figure 22C), greatly improved moth retention. A tube trap with small (1.3 cm) openings on each end (Figure 22B) was designed to improve catch and limit entry by larger non-target insects or other contaminants. Unfortunately, the trap was less attractive than the original tube with the larger openings. Reducing the airflow through the trap by restricting the size of the openings may have negatively impacted the plume size or shape such that many fewer moths found the source. The major drawback of all tube trap designs was their sensitivity to orientation with respect to wind direction. Orientations that limited wind flow through the trap always resulted in little or no attraction. Due to constantly changing wind directions in field settings, maintaining an optimal orientation on a trap so sensitive to directionality is impossible.

The funnel and vertical vane traps (Figure 22D-E) were designed principally to mitigate the orientational limitations of the tube trap. The funnel trap proved ineffective and was dropped from consideration for field-testing. The vane trap was among the most efficient trap designs tested in the flight tunnel. It attracted and captured a majority of the released codling moths (Table 9). Although highly promising, the vane trap’s easy access raised concerns of unacceptable amounts of non-targets and debris, as well as predation of trapped moths, prompting development of the cube and pyramid traps.

The cube and pyramid traps were designed to allow wind flow and moth entry from all directions, thus alleviating the need to maintain a certain orientation with respect to wind direction (Figure 22F-G). The cube and pyramid traps proved highly effective, as both designs consistently captured and retained a majority of the moths released in the flight tunnel assays (Table 9). Additionally, the small openings reduced the probability that larger non-target insects would coincidentally enter the trap and saturate the adhesive. Observations of moths approaching and entering the traps revealed a propensity to first land on the surface and then crawl to the entrance. Having flat surfaces for codling moths to alight upon may have contributed to the enhanced performance. The use of titanium tetrachloride smoke plumes revealed consistent airflow through the cube from all directions. Small orifices on flat surfaces appeared to induce airflow that created steady plumes significantly larger than the trap itself.

Flight tunnel tests revealed that several microtrap designs were viable candidates for field-testing. The tube with a cone placed at one end, vane, pyramid, and cube traps were highly attractive and provided at least 50% capture. A combination of factors favored the 4 cm cube as the best design for initial field studies to determine its potential as a microtrap for use in a high-density trapping program for codling moth control. The 4 cm cube was effective in capturing moths, less vulnerable to non-target animals, relatively easy to construct, and provided one of the highest surface areas treated with adhesive. Initial testing in large field cages revealed that under choice and no choice conditions, moth capture in the microtrap was equivalent to that in the commercially used delta trap (Figure 23). The surface area coated with adhesive for the delta and 4 cm cube traps were 271 cm^2 and 86 cm^2, respectively. The smaller area available for catching moths in the cube trap did not impede performance under the conditions of the field cage experiments. In open-field trap comparisons, a larger trapping surface generally increases catch (Knight and Light, 2005) presumably due to reduced likelihood of saturation. In the field cages, a known number of moths were released and there was no emigration or immigration. Under these conditions the delta trap and small 4cm cube trap each captured an average of *ca.* 20 moths, suggesting that at this density the adhesive-treated surfaces of both trap types had not saturated. These results suggest that the cube trap could be used in a season-long attract and remove program based on deployment of high densities of traps. The expected catch when placed in a commercial orchard at densities of 500 or more traps per hectare should be well below 20 moths per trap.

The cube trap has been used successfully in an attract-and-remove program for control of codling moth (Reinke et al. 2012). In studies conducted in 0.2 ha orchard plots, cubes deployed at 500 traps ha^{-1} reduced codling moth capture by 92%. Moreover, they provided superior control of moth populations compared to that achieved using commercial hand-applied dispensers applied at the same rate.

To refine the design with the aim of further improving microtrap performance, field studies were conducted in 2011 focusing on the impact of trap shape and size or orifice design on moth catch. Reinke et al. (2012) reported that codling moths were caught on the sides and bottom interior surfaces, but not on the upper interior of the cube microtrap. The uneven box traps (Figure 22H-I) were designed to increase the relative surface area on the bottom, and perhaps also foster catch on the upper surface, or to minimize the amount of upper surface area. These design changes, however, did not significantly increase catch compared to the cube or pyramid trap (Figure 24). Few moths were captured on the upper surface of either box trap. As in Reinke et al. (2012), all codling moths were captured on the bottom or side sticky surfaces for all trap types. The pyramid and box traps did numerically capture more moths, suggesting that additional research on the designs is warranted.

Alternative orifice sizes and shapes were developed with the aim of providing easier access into the trap by searching moths. The round shape of the orifice in the cube trap proved to be the best option, with higher catches recorded compared to all other orifice treatments (Figure 25). Concerns over the small size of the round entry point impeding some walking and wing-fanning moths to enter led to designs with wider gaps (Figure 22K-L). The large slits however, may have allowed moths to leave from the bottom of the trap compared to when the opening was a smaller square or round orifice.

Cube size had the most significant impact on moth catch. The smallest cube captured the fewest moths. Trap saturation was likely not the reason for the lower catch, as mean captures in the experiment were well below the 20 moths found in the field cage experiments to not impede catch in the 4 cm cube. The smaller size may have impacted the size or shape of the pheromone plume, thus reducing trap findability (Miller et al. 2010). The larger 8 cm cube captured more moths than the smaller cubes and performed as well as the delta trap (Figure 26). Although not statistically significant, the 4 cm cube trapped fewer moths than the delta trap. This is inconsistent with the nearly equal catches recorded in the large field cages. Differences in experimental set up could have contributed to the conflicting results. In the open field study, four traps were in close proximity and competing for males, while in the cage studies there was no competition in the no-choice experiment and only two traps competing in the choice study. Two of the traps in the open field study had large trapping surfaces. It is possible the smaller cube traps did not perform as well in this more competitive environment. In addition, differences in trapping space between trap designs were largely negated in the field cages comparison, as only moths released within the confines of the cage were available for capture. In the open field comparisons, moths could arrive from any distance.

Studies reported herein focused on developing a new trap design that when baited with a pheromone lure would effectively capture codling moth males. Several designs proved effective, but a simple 4 cm cube appeared to have the most utility for further development. A logical next step would be to test the efficiency of the cube design for capturing other moth pest species. Preliminary testing has indicated that the cube microtrap has potential as a novel trap design for many other insect species, including Oriental fruit moth (*Grapholita molesta*), obliquebanded leafroller (*Choristoneura rosaceana*), grape berry moth (*Paralobesia viteana*), cranberry fruitworm (*Acrobasis vaccinii*), and cherry fruitworm (*Grapholita packardi*).

CHAPTER SIX: POTENTIAL OF HIGH-DENSITY PHEROMONE-BAITED MICROTRAPS FOR CONTROL OF CODLING MOTH, *CYDIA POMONELLA,* AND OBLIQUEBANDED LEAFROLLER, *CHORISTONEURA ROSACEANA*

Also published as:

REINKE, M. D., MILLER, J. R., and GUT, L. J. 2012. Potential of high-density pheromone-releasing micro-baited microtraps for control of codling moth, *Cydia pomonella*, and obliquebanded leafroller, *Choristoneura rosaceana*. *Physiol. Entomol.* 37: 53-59.

ABSTRACT

Recent large-cage studies with codling moth, *Cydia pomonella* (L.), reveal that removal of moths from an apple orchard using traps is more effective at reducing capture in a central monitoring trap than is a mating disruption protocol without kill/capture. Here studies using open orchard 0.2-ha plots comparing a high-density trapping scenario to mating disruption confirm those results. Two tortricid moth pests of tree fruit are studied, codling moth and obliquebanded leafroller, *Choristoneura rosaceana* (Harris). Codling moth treatments included Isomate CM FLEX, non-sticky traps baited with Trécé CM lures, and sticky traps baited with Trécé CM lures, all at equal application rates of 500 dispensers ha^{-1}, and a no pheromone control. The traps are of a novel design& small, easy to apply, and potentially inexpensive to produce. Mating disruption using Isomate CM FLEX and non-sticky traps reduce codling moth capture in standard monitoring traps by 58% and 71%, respectively. The attract-and-remove treatment with sticky traps reduces capture by 92%. Obliquebanded leafroller treatments include Isomate OBLR/PLR Plus, Pherocon IIB traps baited with Trécé OBLR lures, both applied at 500 dispensers ha^{-1}, and a no pheromone control. Mating disruption reduces capture in monitoring traps by 69%. The attract-and-remove treatment reduces capture by 85%. Both studies suggest that an attract-and-remove approach has the potential to provide superior control of moth populations compared to that achieved by mating disruption operating by competitive attraction alone.

INTRODUCTION

Semiochemically-mediated mass trapping as a control tactic has long been of interest for various lepidopteran species in agricultural systems. Roelofs et al. (1970) report a reduction of fruit damage by moderate populations of redbanded leafroller, *Argyrotaenia velutinana*, is possible using 180-300 monitoring traps ha^{-1} in apple orchards. Likewise, redbanded leafroller and grape berry moth, *Paralobesia viteana*, damage is reduced to moderate levels in grape vineyards using 160-300 monitoring traps ha^{-1} (Taschenberg et al. 1974).

Concerns over substantial costs of mass trapping systems (Huber et al. 1979, El-Sayed et al. 2006) and reduced individual trap effectiveness due to overlapping pheromone plumes (Madsen et al. 1976) prompted tests with reduced trap densities. Willson and Trammel (1980) used 10-40 traps ha^{-1} in an unsuccessful attempt to control various tortricid species in apple orchards. That study did show damage from codling moth in particular can be reduced compared to the control, but it is still above acceptable levels for commercial practice. Madsen and Carty (1979) also demonstrated the potential for codling moth control using traps at 10 and 36 ha^{-1}, but the lack of replication and control treatments precludes firm conclusions. Leskey et al. (2009) document low efficacy at trap densities of 5 and 20 traps ha^{-1} against dogwood borer, *Synanthedon scitula*, in apple orchards.

Other recent studies with higher trap densities have fared better. Zhang et al. (2002) provide control of the Chinese tortrix, *Cydia trasias*, in urban street plantings of Chinese scholar-trees when traps are deployed at densities equivalent to 110 ha^{-1}.

Cork et al. (2005) show that, in combination with other integrated pest management tactics, control of a pyralid, *Leucinodes orbonali*, can be achieved using 100 traps ha^{-1}. Eggplant production increases significantly in comparison to the standard, intense chemical program.

The consensus from these studies (El-Sayed et al. 2006) is that mass trapping has several hurdles to overcome before it can become a viable economic control tactic: the pheromone lure must be highly attractive and capable of drawing targets from long distances yet able to bring them very close to the source, the trap must be a design that is efficient at luring in and trapping the target species, the trap must be capable of capturing many individuals, and the traps must be inexpensive to allow many to be deployed per ha.

Miller et al. (2006a, b) document that competitive attraction, or false plume following, is the dominant mechanism of semiochemical control among Lepidoptera. Under this mechanism, pheromone point sources directly compete with calling females. Over successive evenings, males of the targeted species will continue to orient towards individual pheromone plumes. To achieve control through competitive attraction, traps and dispensers must considerably outnumber females. This precludes the use of low numbers of dispensers or traps for controlling all but the lowest of population levels.

In the present study, a high-density trapping regime is compared to mating disruption of codling moth, *Cydia pomonella* (L.), and obliquebanded leafroller, *Choristoneura rosaceana* (Harris). While the first of the four limitations to an effective attract-and-remove strategy listed above can be overcome by the use of commercially available lures, the others need to be addressed. In this study, trap densities equivalent to standard hand-applied mating disruption dispensers are employed to test the hypothesis that attraction combined with male moth removal is superior to mating disruption. A patent pending, novel trap design is deployed against codling moth. It is suggested that this microtrap has the capacity to accomplish the attract-and-remove scenario without the compromises inherent with large, complex, and high-maintenance traps typically used for monitoring.

MATERIALS AND METHODS

Orchards

Research was performed at two Michigan State University research stations, Clarksville Horticultural Experiment Station and Trevor Nichols Research Center located in Ionia and Allegan counties, respectively. Two replicates were performed at Clarksville where treatments were placed in isolated 0.2-ha apple orchard plots. Each plot measured 44.5 x 44.5 m and was surrounded by open grass or a border row of mature poplar trees. Each plot received a single treatment. Treatments were deployed in a randomized complete block design. All orchard plots were trellis-planted at 1750 trees ha^{-1} and maintained as organic orchards, but with no insect sprays. The two remaining replicates were placed in two 1.8-ha apple orchard blocks at Trevor Nichols Research Center. Orchards were divided into 0.2-ha plots measuring 40 x 50 m set up diagonally from each other with a minimum of 15 m between treatments at the corners. Each 0.2-ha plot received one treatment. Treatments were deployed in a complete block design with the following randomization restriction. To prevent pheromone spread between treatment plots, treatments here were deployed on a west-east orientation from lowest total pheromone concentration to highest as an adjustment for the westerly daytime prevailing winds. All plots contained free-standing apple trees planted at 500 trees ha^{-1}. All plots were maintained with regular horticultural practices, but with no insecticide sprays.

Codling moth trapping

Treatments included; (1) Isomate CM Flex (ShinEtsu Ltd., Tokyo, Japan) applied evenly at 500 ha^{-1} (100 per plot), (2) microtraps of a novel design described below without a sticky internal surface, each baited with one Trécé codling moth rubber septum (CM L2) lure, and applied evenly at 500 ha^{-1} (100 per plot), (3) microtraps with a sticky internal surface, each baited with a rubber septum lure, and applied evenly at 500 ha^{-1} (100 per plot), and (4) an untreated control plot. The non-sticky microtrap treatment was included to quantify their possible disrupting effect. Doing so permitted assessment of the supplementary effect of removing moths using the sticky traps. Each plot received two monitoring traps (orange Trécé Pherocon VI delta traps, with a Trécé CM L2 monitoring lure) placed in the southwestern and northeastern corners of the plot at least 15 m from the block perimeter. Treatments were applied on 7 May 2010 and were maintained until after the end of the second codling moth generation on 8 September 2010. The lures in the microtraps of treatment (3) were changed between codling moth generations on 29 July 2010. For first generation moths, Trécé CM L2 monitoring lures were used. Low captures in the microtraps in first flight led to the suggestion that L2 lures release at an above-optimal rate to lure codling moth males into the microtraps. To test this hypothesis, all microtrap lures were changed to gray septum 0.1-mg lures produced by Trécé for the second codling moth generation. All traps were checked weekly. Moths in monitoring traps were counted and removed. Moths captured in the microtraps were counted, but were not removed, and were designated as being caught on the bottom, side, or top surfaces. Each microtrap was given a unique designation permitting mapping of moth population differences within each plot. All damaged or saturated traps and/or liners were replaced.

Microtrap design

The microtrap is a 4-cm cube. The four sides and top have one 12 mm diameter hole in the center of each surface. The bottom has two 5 mm diameter holes placed equidistant from each other and the sides of the microtrap. The purpose of the smaller holes on the bottom is to reduce the likelihood of the lure and/or the captured moths from falling out of the trap. This microtrap design captures codling moths effectively in a flight tunnel (Chapter 5). Traps were constructed from modified Alpha Scents (Portland, Oregon) plastic delta sticky inserts. A clip made of bent steel wire was glued to one side of the cube for attaching microtraps to trees.

Obliquebanded leafroller trapping

Treatments included; (1) Isomate OBLR/PLR Plus (ShinEtsu Ltd., Tokyo, Japan) applied evenly at 500 ha^{-1} (100 per plot), (2) Trécé Pherocon IIB traps each baited with one Trécé OBLR monitoring lure and applied evenly at 500 ha^{-1} (100 per plot), and (3) an untreated control. The Trécé Pherocon IIB traps were the smallest commercially available traps for attract-and-remove of obliquebanded leafroller due to the yet unproven efficacy of the microtrap at capturing this species. Two orange Trécé Pherocon VI delta traps, with a Trécé OBLR rubber septum monitoring lure, were placed in the southeastern and northwestern corners of the plot at least 15 m from the block perimeter. Treatments were applied on 1 June 2010 and were maintained until after the end of the second obliquebanded leafroller flight on 8 September 2010. All traps were checked weekly. Moths in monitoring traps were counted and removed. Moths captured in all other traps were counted, but were not removed. Each Trécé Pherocon IIB trap was given a unique designation and individually maintained to facilitate mapping of moth population differences within each plot. All damaged or saturated traps and/or liners were replaced. Shoot damage assessment was performed 21-23 July 2010. Ten shoots were checked on each of 30 trees per plot. Each shoot tip was inspected for the presence of larvae and/or feeding damage.

Data analysis

For orientation disruption and trapping studies, the weekly moth capture in the two traps in each plot were summed. Weekly data were log transformed [ln (x + 1)] (which normalized the distributions and homogenized variance) then subjected to analysis of variance (ANOVA). Trapping treatment trap captures were summed by generation for individual traps, then log transformed [ln (x + 1)] and subjected to ANOVA. Shoot injury data were log transformed [ln (x)] prior to ANOVA. Differences in pairs of means were separated using the least significant difference test (SAS Institute, 2000). In all cases, the significance level was $\alpha < 0.05$.

RESULTS

Codling moth trapping

For first generation, total moth captures for the Isomate, non-sticky microtrap, and sticky microtrap treatments were significantly lower than for the untreated control (*P*<0.001, P<0.001, and P<0.001, respectively) (Figure 27). No significant difference was observed between the Isomate and non-sticky trap treatments (*P*=0.089). Capture in monitoring traps was significantly lower in the sticky microtrap plots than both the

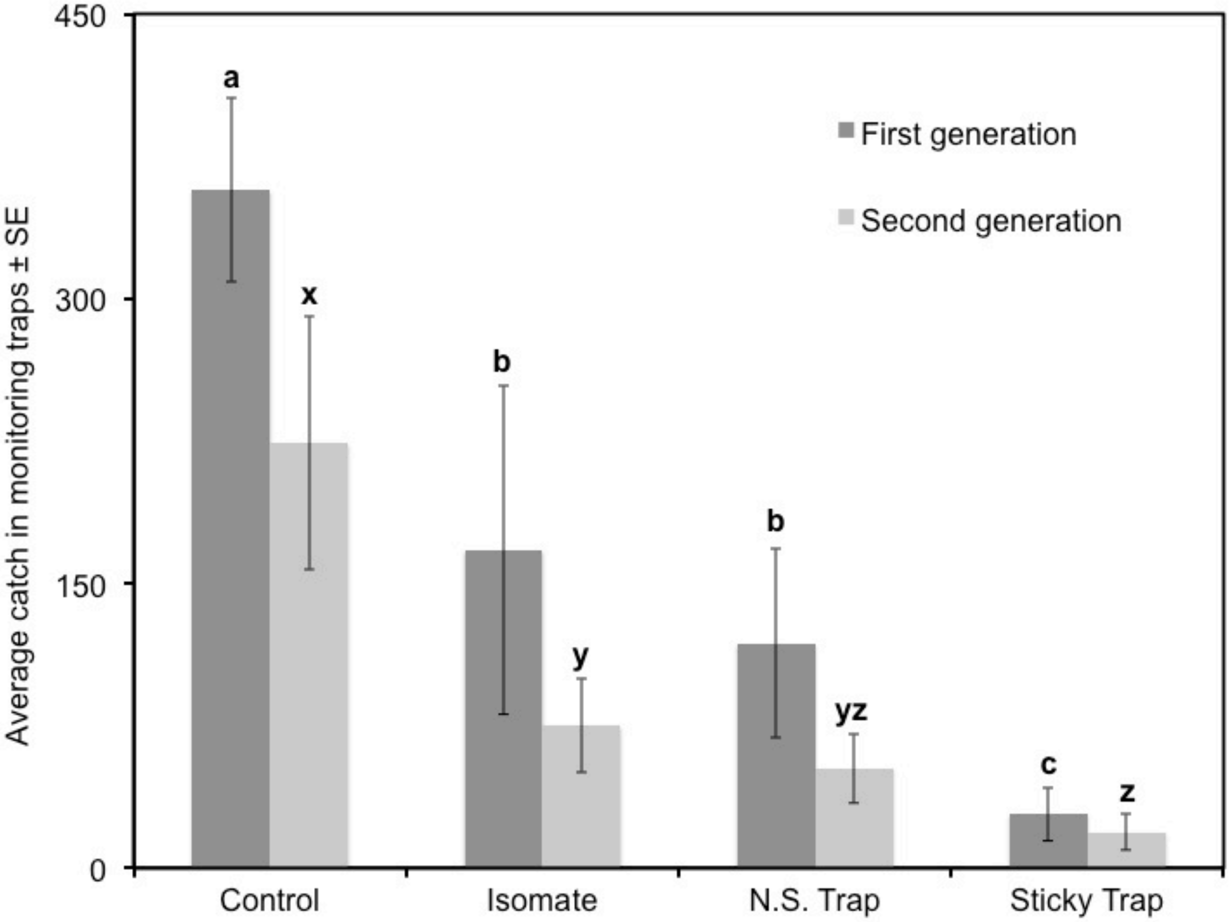

Figure 27. Capture of codling moth, *Cydia pomonella*, males in monitoring traps. Average catch per plot is used with catches summed for both monitoring traps in each plot for each generation. Treatments with the same letter are not significantly different (P<0.05).

Isomate and non-sticky microtrap treatments (*P*<0.001 and *P*<0.001, respectively) (Figure 27). Weekly captures in the trapping treatment were summed for each trap in the first codling moth generation. These counts were mapped using a surface map (Figure 28).

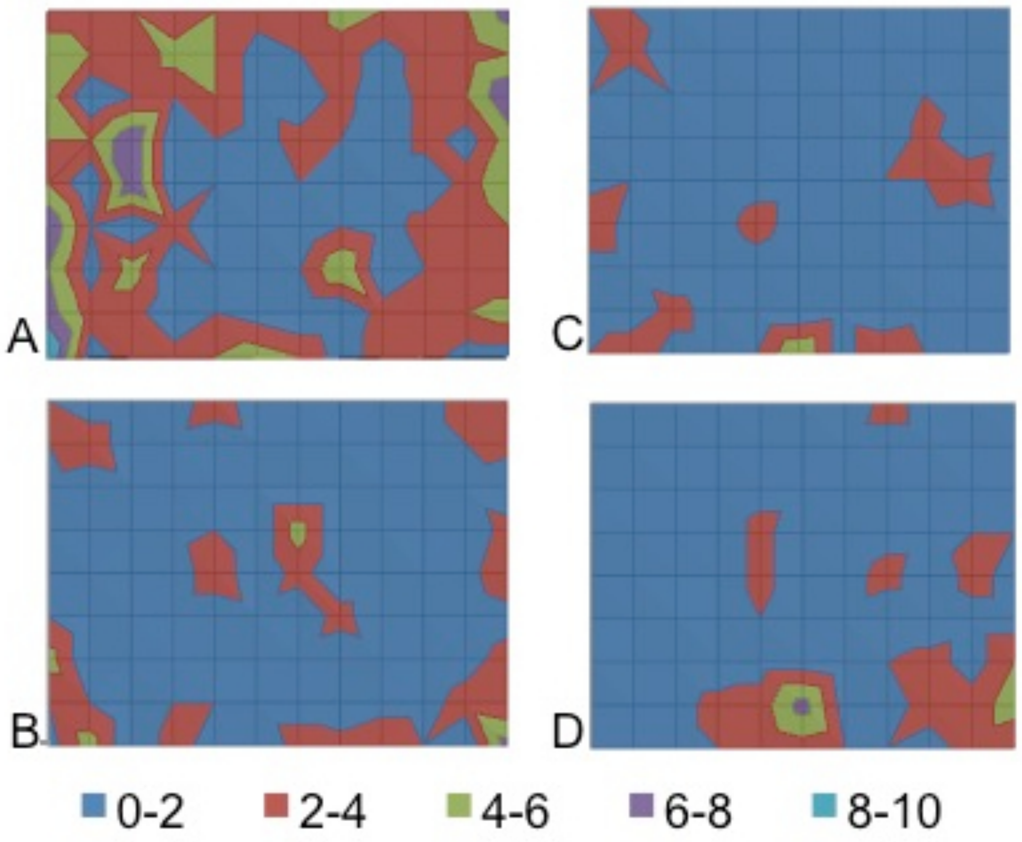

Figure 28. Surface map showing cumulative number of codling moth, *Cydia pomonella,* males captured in each microtrap in the trapping treatment during first generation. Colors designate cumulative moth counts in each trap. Large surfaces with the same color are indicative of multiple traps with identical counts. Letters A-D designate individual replicates found in Figures 34 & 35. For interpretation of the references to color in all figures, the reader is referred to the electronic version of this dissertation.

For the second generation, total moth captures in monitoring traps for the Isomate, non-sticky microtrap, and sticky microtrap treatments were significantly lower than for the untreated control (*P*<0.001, *P*<0.001, and *P*<0.001, respectively) (Figure 27). No significant difference was observed between the Isomate and non-sticky trap treatments (*P*=0.488). Capture in monitoring traps was, however, significantly lower in the sticky microtrap plots than the Isomate plots (*P*=0.023), but not the non-sticky microtrap plots (*P*=0.108) (Figure 27). Weekly captures in the trapping treatment were summed for each trap in the second codling moth generation. These counts were mapped using a surface map (Figure 29).

Microtrap design

All interior surfaces of the microtrap were sticky. However, only the sides and bottom effectively captured high numbers of codling moths. Of the 644 males captured in the microtraps during first generation, 60% were captured on the bottom interior surface (Table 10). The remaining 40% were captured on the four sides. During second generation, total capture increased, significantly (P<0.001), to 1308; the bottom captured 53%. The remaining 46% and 1% were captured on the sides and top, respectively.

Table 10. Total number of codling moth, *Cydia pomonella*, males*,* captured in microtraps, including breakdown of catches on sections of traps.

	Capture (percentage of grand total)			
Generation	**Bottom**	**Sides**	**Top**	**Grand Total**
One	386 (60%)	258 (40%)	0	644
Two	689 (53%)	601 (46%)	18 (1%)	1308
Total	1075 (55%)	859 (44%)	18 (1%)	1952

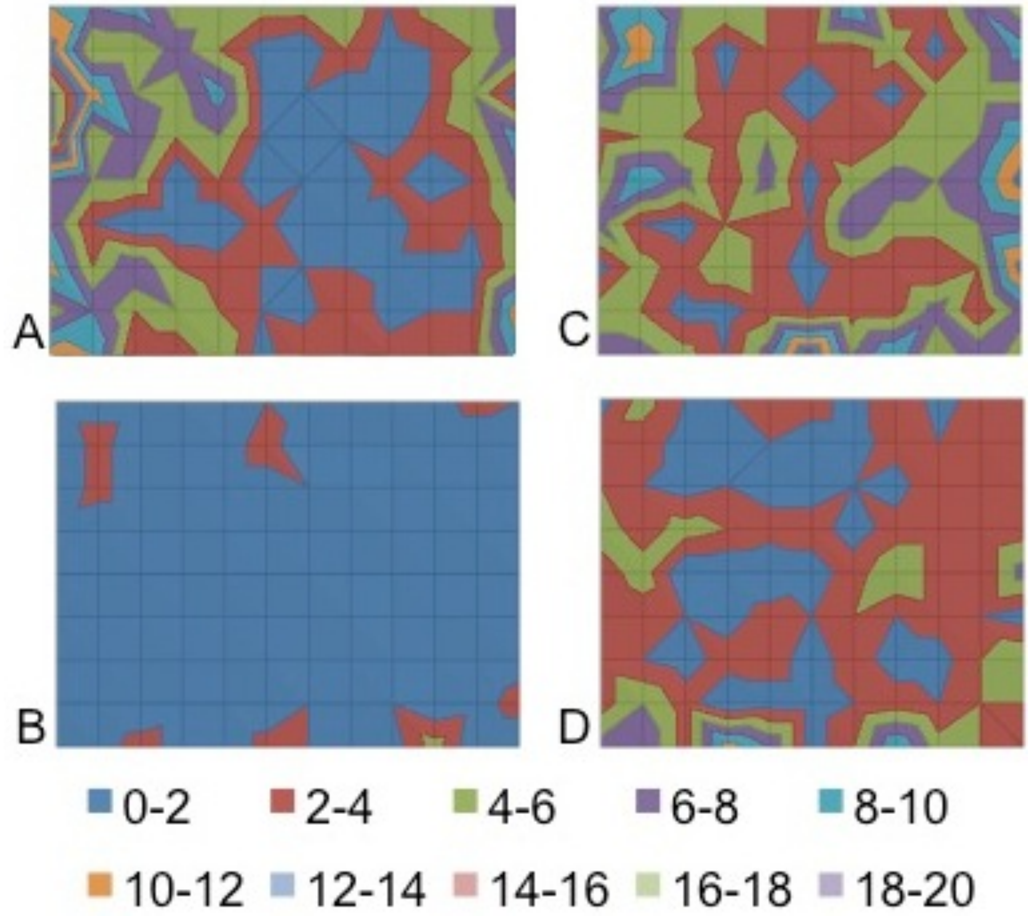

Figure 29. Surface map showing cumulative number of codling moth, *Cydia pomonella,* males captured in each microtrap in the trapping treatment during second generation. Colors designate cumulative moth counts in each trap. Large surfaces with the same color are indicative of multiple traps with identical counts. Letters A-D designate individual replicates found in Figures 34 & 35.

Obliquebanded leafroller trapping

For first generation, moth captures in the Isomate and trapping treatments were reduced by 57% and 76%, respectively, compared to the untreated control (Figure 30). These reductions were both significant (*P*<0.001 and *P*<0.001, respectively). Captures were significantly reduced in the trapped plots more than the Isomate plots (*P*=0.049). Weekly captures in the trapping treatment were summed for each trap in the first obliquebanded leafroller generation. These counts were mapped using a surface map (Figure 31).

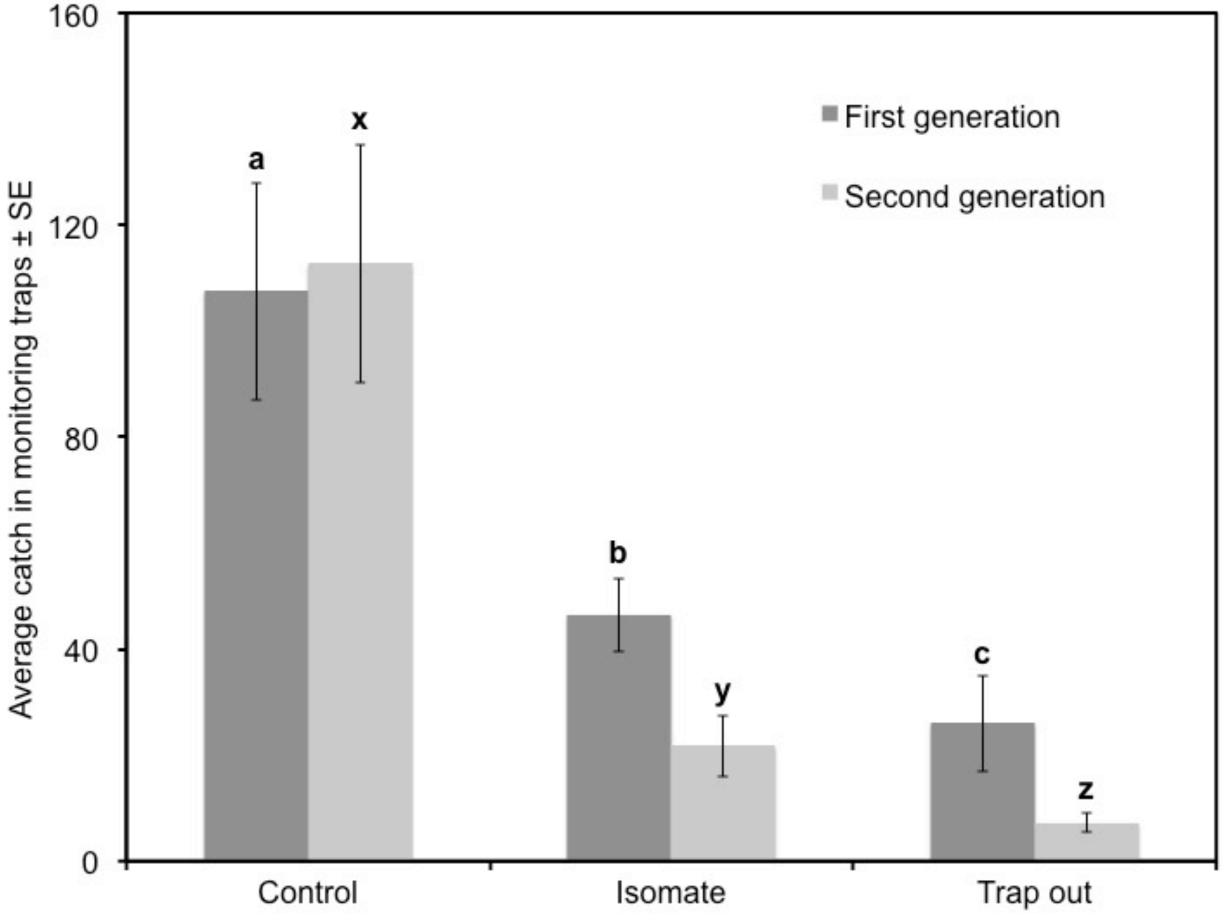

Figure 30. Capture of obliquebanded leafroller, *Choristoneura rosaceana,* males in monitoring traps. Average catch per plot is used with catches summed for both monitoring traps in each plot for each generation. Treatments with the same letter are not significantly different (P<0.05).

For the second generation, total moth captures in the monitoring traps for the Isomate and trapping treatments were reduced by 81% and 94%, respectively, compared to the untreated control (Figure 30). These reductions were both significant (*P*<0.001 and *P*<0.001, respectively). Captures in monitoring traps were significantly reduced in the trapped plots more than the Isomate plots (P=0.007). Weekly captures in the trapping treatment were summed for each trap in the second obliquebanded leafroller generation. These counts were mapped using a surface map (Figure 32).

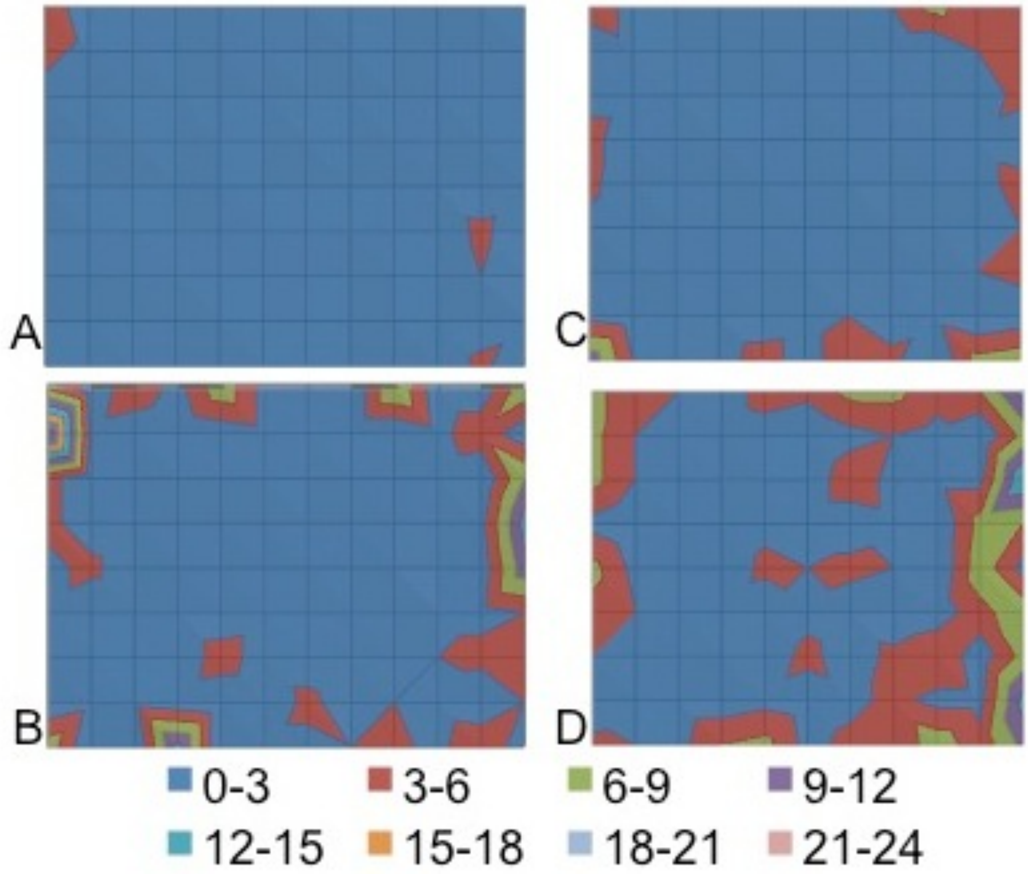

Figure 31. Surface map showing cumulative number of obliquebanded leafroller, *Choristoneura rosaceana,* males captured in each trap in the trapping treatment during first generation. Colors designate cumulative moth counts in each trap. Large surfaces with the same color are indicative of multiple traps with identical counts. Letters A-D designate individual replicates found in Figures 34 & 35.

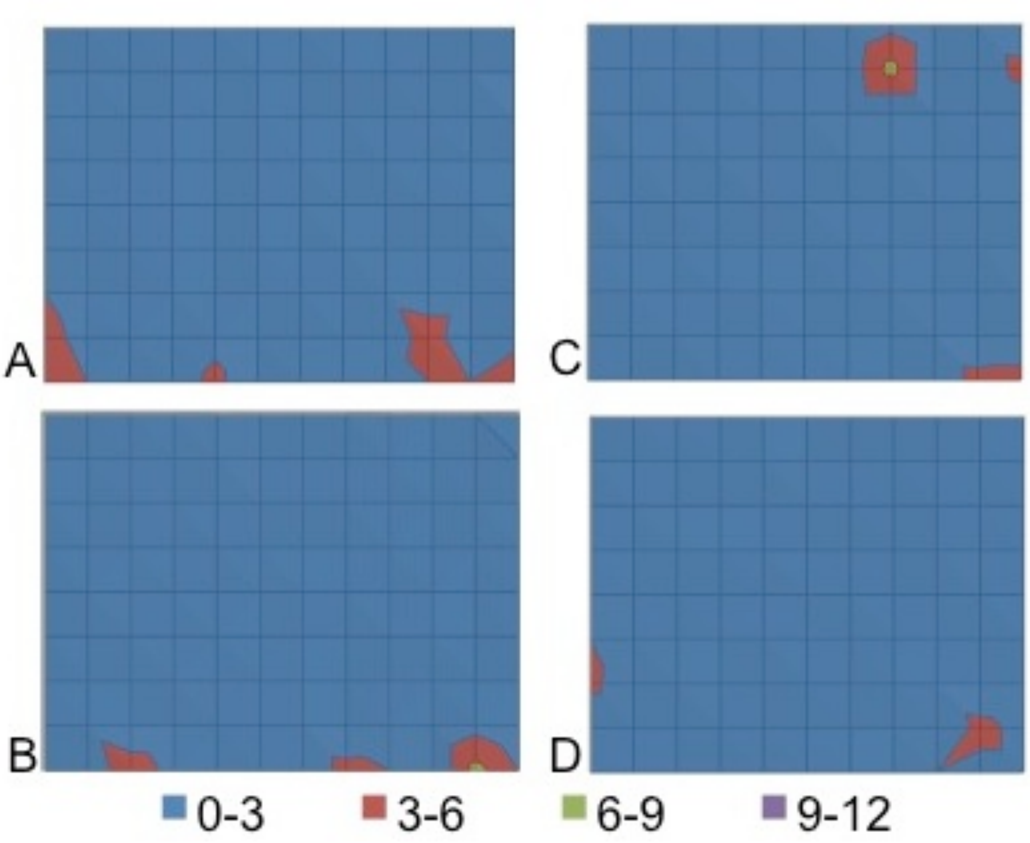

Figure 32. Surface map showing cumulative number of obliquebanded leafroller, *Choristoneura rosaceana,* males captured in each trap in the trapping treatment during second generation. Colors designate cumulative moth counts in each trap. Large surfaces with the same color are indicative of multiple traps with identical counts. Letters A-D designate individual replicates found in Figures 34 & 35.

Mid-season shoot damage was significantly reduced in the Isomate mating disruption and high-density trapping treatments compared to the check (*P*<0.024 and *P*<0.003, respectively) (Figure 33). No significant difference was found between these pheromone treatments (*P*=0.099).

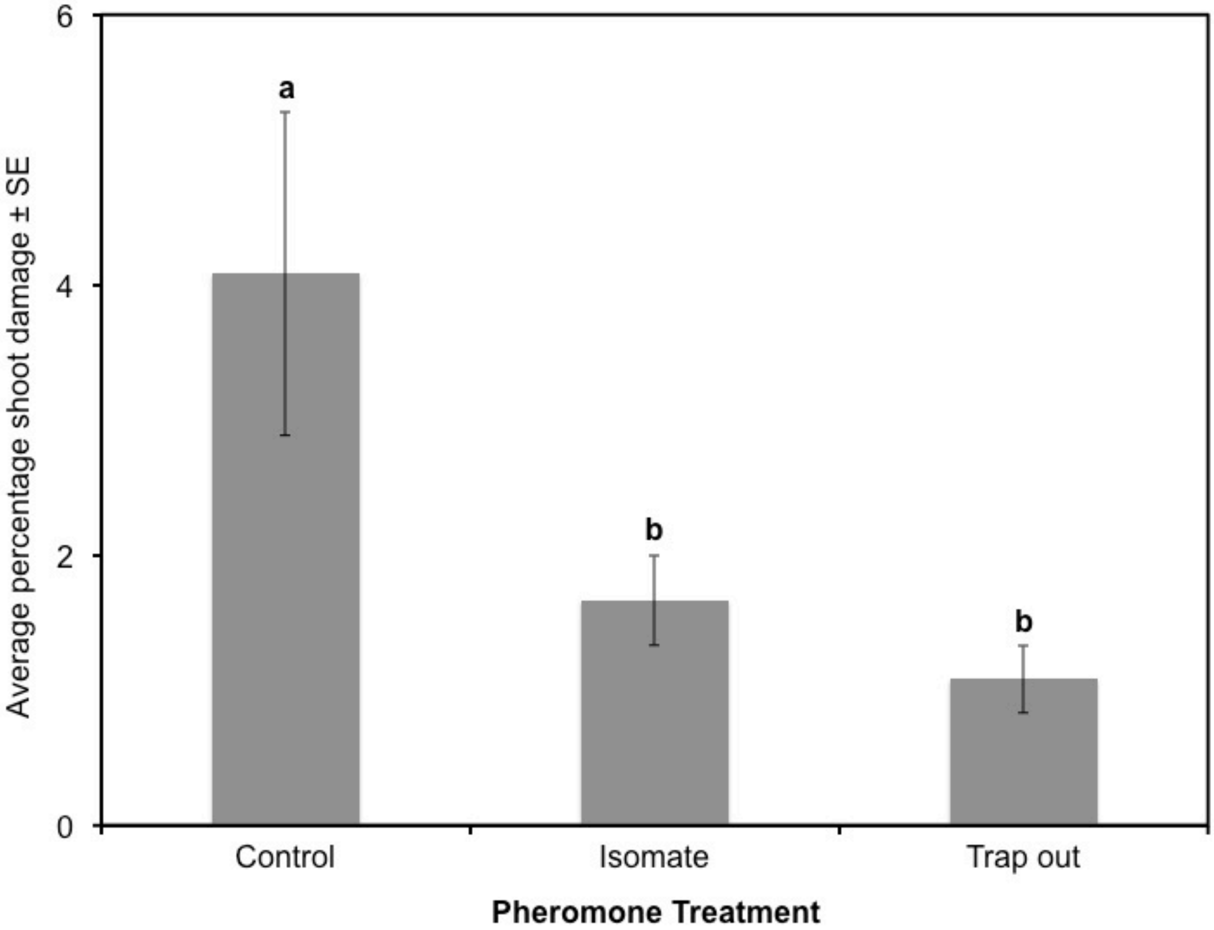

Figure 33. Percentage of mid-season shoots damaged by obliquebanded leafroller larvae, *Choristoneura rosaceana*. Treatments with the same letter are not significantly different (P<0.05).

DISCUSSION

El-Sayed et al. (2006) suggest that traps at high density would operate more as mating disruption dispensers than traps. They imply that mass trapping cannot out-perform mating disruption. However, computer simulations comparing mating disruption to mass trapping (Byers, 2007) and research on mating disruption mechanisms conducted in large field cages (Miller et al. 2010) do not support this proposition. In the latter study, known numbers of codling moths were released into large field cages with known dispenser and trap densities ranging from 25-500 ha^{-1}. A conclusion for codling moth is that standard hand-applied mating disruption is more effective than lures placed in linerless “disrupting” traps at reducing capture of males in monitoring traps. Without liners, the lure/trap combination is operating as a low-releasing dispenser. Once liners are added to the traps, however, there is a supplementary decrease in the capture of males compared to the standard disruption. At the highest density of sticky traps, capture in the central monitoring trap is reduced 4-fold (75%) compared to the hand-applied dispensers and 9-fold (89%) compared to the non-sticky low-releasing dispensers (Miller et al. 2010).

Miller et al. (2010) deduce that high rates of pheromone released from the hand-applied dispensers deactivate attracted males, while low-releasing dispensers do not. This temporary deactivation eliminates further orientation by individuals on a given evening, but allows orientations during the next flight period. The addition of a capturing medium results in permanent deactivation.

The current codling moth open-field study confirms and extends the large-cage work. Captures in the Isomate and non-sticky microtrap plots are reduced by 58% and 71% compared to the untreated plot, respectively (Figure 27). Because non-sticky microtraps depressed catch in the monitoring traps to levels equal to Isomate dispensers, it is tentatively concluded that males are retained for most of each activity period. Laboratory flight-tunnel studies indicate this trap design is capable of retaining male codling moths longer than a large plastic delta trap (Chapter 5). This effect may be due to the design in which the male has a flat surface to alight upon at the source of the pheromone plume, in the case of the microtrap, but a small orifice to enter and exit the trap. Once the moth crawls into the non-sticky trap, the small orifices make exit difficult. Retaining the male codling moths inside the non-sticky traps for an extended time would equate to nightly deactivation. This condition would, however, be temporary, just as it is for the hand-applied Isomate dispensers.

As expected, the addition of a capturing medium to the inside of the trap enhanced reduction of capture in the monitoring traps compared to all mating disruption treatments. Captures of codling moth males in the sticky microtrap plots are reduced by 92% compared to the control and 80% and 72% to the hand-applied and non-sticky microtrap treatments, respectively. Capture of obliquebanded leafroller in the trapping treatment is reduced by 85% compared to the control and by 51% compared to the mating disruption treatment. Both studies prove that the attract-and-remove tactic can provide superior control of codling moth and obliquebanded leafroller populations in apple orchards.

The microtrap is effective at capturing codling moth males. The higher captures during second generation can be attributed to the lower release rate of the 0.1 mg lure (Table 10, Figs 28, 29). The CM L2 lure used during first generation probably releases at a rate not conducive to optimal male entry into the trap. If the 0.1 mg lure had been used over the full season, even more male codling moth would have been captured during first generation.

The vertical sides and horizontal bottom of the microtrap are equally important in capturing the high numbers of moths (Table 10). The top interior sticky surface is ineffective at capturing moths, but could cause them to fall into the trap. Future work is necessary to determine if a sticky top contributes to overall trap efficiency.

Midseason shoot damage assessment confirms the monitoring trap counts that indicate both mating disruption and high-density trapping are effective at diminishing obliquebanded leafroller populations. Codling moth fruit damage could not be assessed due to infestations of apple flea weevil that resulted in negligible fruit set. Fruit counts in all plots at Clarksville Horticultural Experiment Station were reduced to levels too low for differentiation between mating disruption vs. trap-out. Further investigation into codling moth control using high trap densities and an attract-and-remove strategy is needed.

Deploying traps at high densities has the added benefit of reducing the burden of each individual trap from capturing all nearby moths. At a density of 500 traps ha $^{-1}$, the largest distance between traps is 5 m. As such, plume overlap likely occurs (Murlis et al. 2000, Byers, 2007, Yamanaka, 2007). This suggests that, under a competitive attraction scenario, a male moth has the potential to encounter the plumes of multiple traps. At high moth densities, where seasonal capture would be around 2000 moths ha^{-1} as indicated in this and other publications (Roelofs et al. 1970, Madsen and Carty, 1979), mass trapping with low trap densities would likely result in trap saturation. With trap densities reported here, those catches would be shared by a larger number of traps. If one trap begins to saturate, surrounding traps would be capable of capturing those moths escaping the saturating microtrap. Trap saturation at these trap densities, however, is doubtful when capturing codling moth or obliquebanded leafroller males. In the unlikely event a trap did saturate, it would still have a substantial effect as a mating disruption dispenser, as indicated by the activity of the non-sticky microtrap treatment.

Figs. 28-29 and 31-32 provide a visual record of population density on a fine scale. Hotspots are easily discernible by islands of contrasting color against the blue background. These population centers envelope 2-4 traps. It appears the hotspots are confined to no more than 2-4 trees in low-density apple plantings, and 5-6 trees in 1-2 rows in a trellis designed orchard. By placing traps at high densities it is possible to have at least one trap in each of these high population centers, facilitating earlier capture and removal from the population.

In addition to revealing hotspots, the surface maps assist visualization of population dynamics on a near-individual-tree scale. Codling moth males are captured throughout the blocks, but there is a marked edge effect in all four replicates (Figs 28, 29). In three of the four replicates there is a marked increase in moth capture during second generation. This is due to multiple factors. Firstly, the lures used in the microtraps were changed between generations to one with a release rate probably more conducive to moth entry into the trap, thus increasing capture of a larger proportion of responding males. This is supported by comparing generation specific captures in the monitoring traps. While capture in the microtraps increases significantly during second generation, capture in the monitoring traps decreases (Figure 27). Secondly, both research stations historically have high codling moth populations during second generation in unmanaged blocks. Replicate A at Clarksville (Figure 34) and both replicates at Trevor Nichols Research Station (Figure 35) are in close proximity to apple orchard blocks. In all three cases, the largest moth captures are along edges adjacent to these other apple blocks. These conditions facilitate immigration pressure. Even at the high trap densities tested, the pressure is too high to prevent immigration into the interior of the blocks. Alternatively, replicate B at Clarksville is isolated from other apple orchard blocks, as indicated in Figure 34. The nearest untreated apple block is 300 m to the southwest, on the other side of a stand of mature hardwood trees. This reduces immigration pressure. Captures in the microtraps in replicate B decrease in the second generation, even with the higher trap efficiency due to new lures.

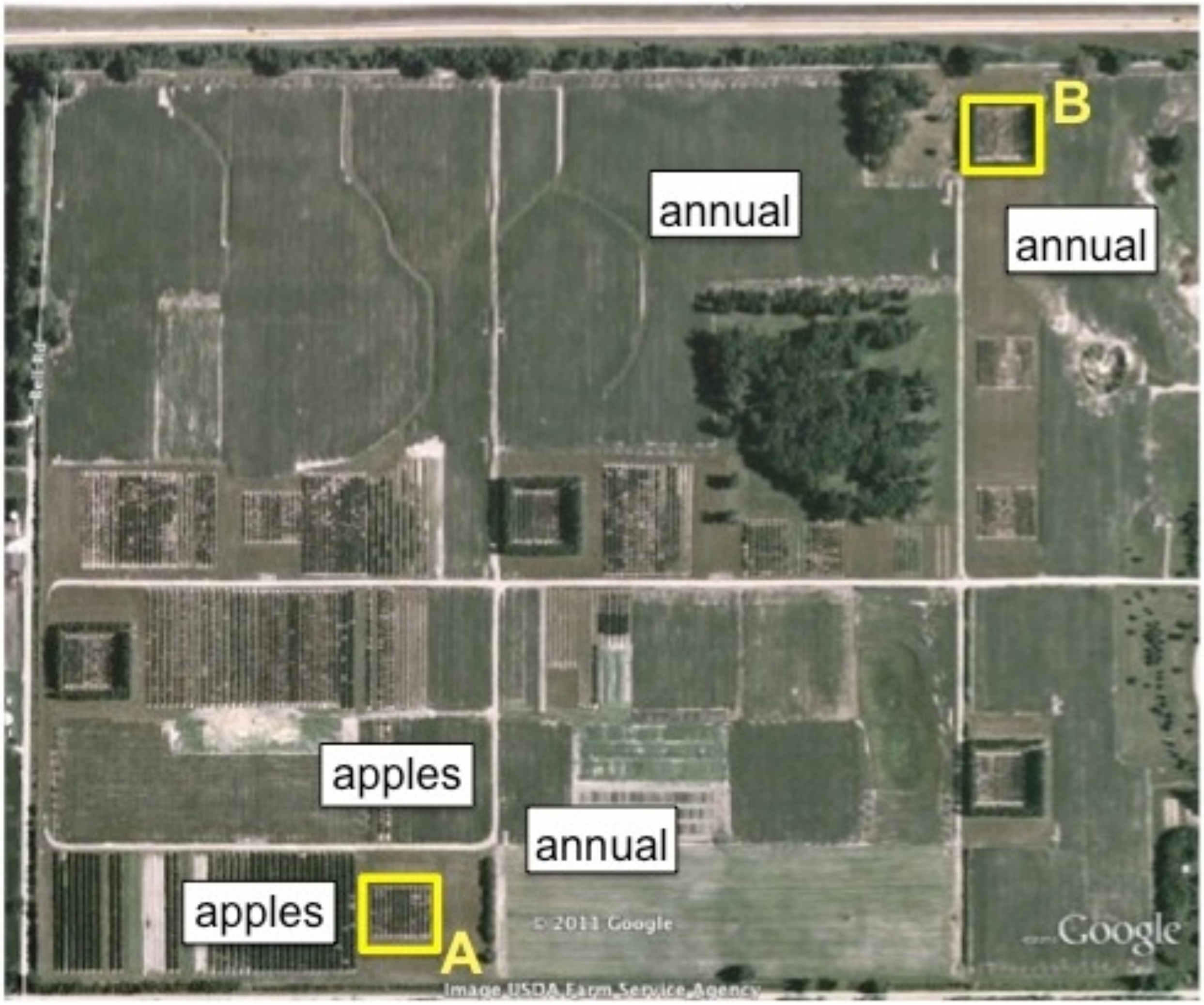

Figure 34. Google Earth map showing placement of trapping treatment replicates for both codling moth, *Cydia pomonella,* and obliquebanded leafroller, *Choristoneura rosaceana,* at Clarksville Horticulture Experiment Station, Clarksville, Michigan, U.S.A. Letters A & B designate individual replicates.

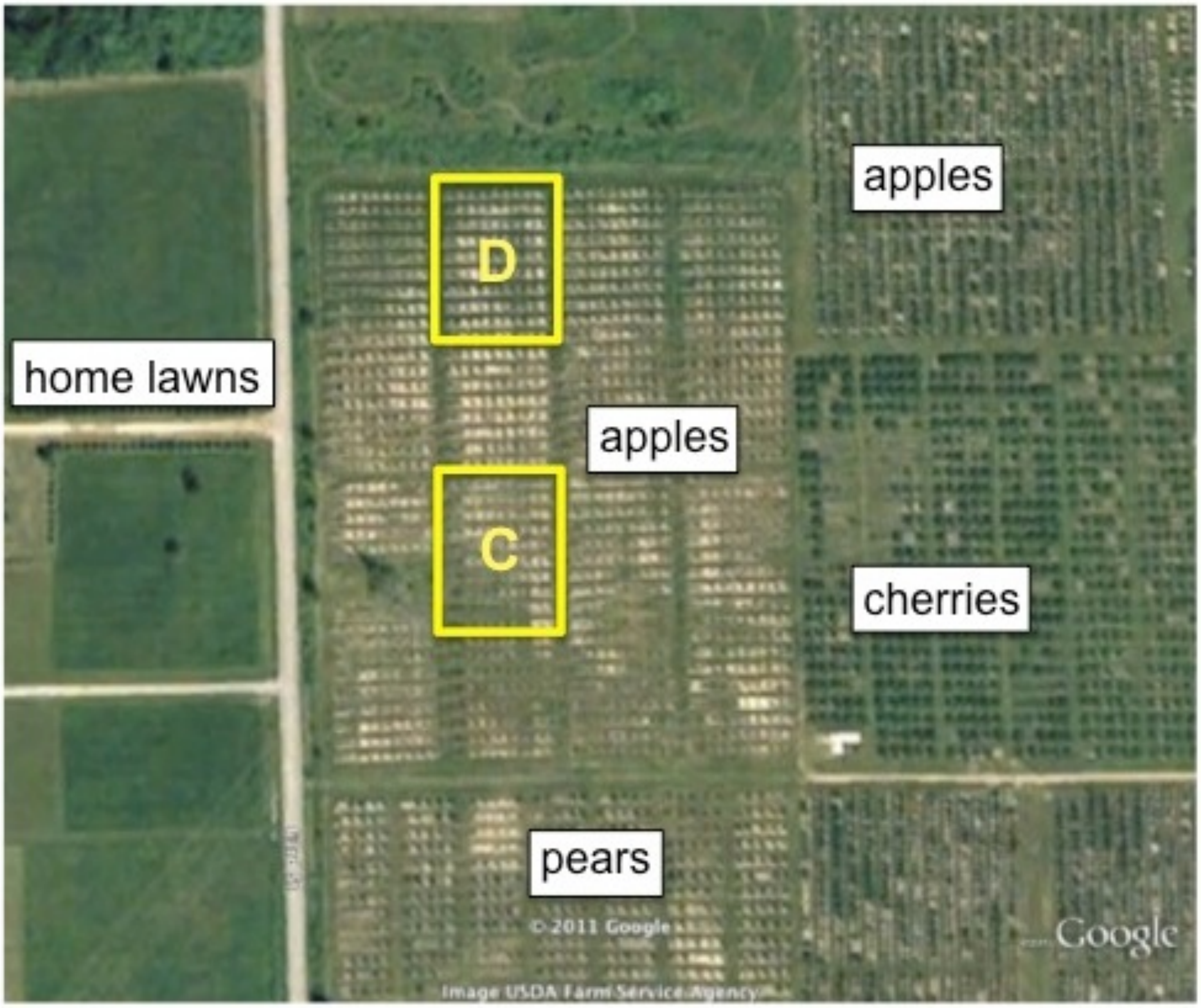

Figure 35. Google Earth map showing placement of trapping treatment replicates for both codling moth, *Cydia pomonella,* and obliquebanded leafroller, *Choristoneura rosaceana,* at Trevor Nichols Research Center, Fennville, Michigan, U.S.A. Letters C & D designate individual replicates.

Spatial maps of obliquebanded leafroller captures suggest a similar population structure (Figs. 31, 32). Use of the same lure type for both generations allows captures from both generations to be directly compared. The majority of the catches during first generation are at the perimeter of each plot. Male capture in Replicate A increases slightly during the second generation. Captures in the remaining three replicates markedly decrease during second generation. In all replicates, all traps with cumulative captures of 3+ moths per trap during second generation are within 10 m of the block perimeter. These maps indicate obliquebanded leafroller populations are successfully controlled using an attract-and-remove program. Obliquebanded leafroller captures appear to be primarily due to immigration into the experimental plot.

While the novel microtrap shows promise for use in an attract-and-remove regime for codling moth, more development is required. The high capture rates in the present study indicate the microtrap has the potential to efficiently capture codling moth males. Future work will test its efficacy against other lepidopteran pest species in various agricultural systems. The microtrap is small and constructed of inexpensive materials, but development of a cost-effective assembly process is required before microtraps can be considered economically viable.

CHAPTER SEVEN: SUMMARY AND CONCLUSIONS

Synthetic pheromones of insects found immediate application in monitoring of pests. Using sex pheromones as potent and species-specific lures has been instrumental to IPM programs through definitive determination of what pests are present and, when linked to phenology models, when control measures such as insecticide applications can be most effective. Early investigations into whether synthetic pheromones could effect direct pest control by mass trapping concluded that this tactic was impractical because of prohibitive costs of the many traps necessary and the labor to install and service them. Broadcasting synthetic pheromone to disrupt mating proved to be a more successful tactic for using pheromones for direct suppression of pest populations. However, the path to commercial products was one of trial-and-error. Various formulations, dosages, and deployment patterns were tried. Then, the better-performing treatments were adopted until superior products came along.

Deep understanding of mating disruption mechanisms has lagged behind its practical use. It has long been stated that knowledge of the mechanism(s) involved, as well as the biology, ecology, and behaviors of a pest, can help in improving a pheromone-based control system beyond what can be achieved by trial-and-error (Cardé et al. 1995, Gut et al. 2004). Recently, Miller et al. (2006a,b, 2010) achieved a breakthrough in understanding of mating disruption mechanisms by drawing parallels with enzyme kinetics. Unique, large-cage experiments wherein the densities of moths, traps, and pheromone dispensers could be set and manipulated led to the discovery of a fundamental competition equation. This approach led to mathematical procedures for definitively differentiating between major classes of disruption mechanisms and offered methodologies for determining how disruption efficacy might be improved through specific manipulations of dispenser types, release rates, and deployment spacings, Moreover, the new conceptual framework and analytical tools provided the theoretical justification for an insight that mass-trapping should be superior to mating disruption operating only competitively. This suggested that the tactic of mass trapping should be revisited with fresh thinking that included the idea that traps need not be as large and costly as are traditional monitoring traps.

This dissertation research builds upon the competition-equation framework and takes early steps to exploit its insights for pheromone-based management of tortricid pests of tree fruit. The over-arching question toward which this research contributes was: specifically how might a pheromone-based pest management system be designed that maximizes efficacy while minimizing material (particularly, expensive pheromone compounds) and labor costs.

The sub-questions I addressed were: 1) is it possible to produce a novel dispenser matrix that is less expensive to manufacture than current commercial formulations and one that is more flexible in achieving improved release rates, dispenser spacings, and application methodologies? Answering affirmatively through the development of a flexible and inexpensive paraffin wax/ethylene vinyl acetate (EVA) dispenser (Chapter 2) led to question 2), when applied in the field on continuous string at densities considerably higher than conventional hand-applied dispensers, does the new matrix releasing at female-like rates yield superior disruption? and 3) does such a dispenser disrupt competitively or non-competitively? The answer to question 3 was competitive disruption (Chapter 3), while that for question 2 was no – disruption was no better for the new matrix at high densities than for higher-releasing dispensers at lower densities (Chapter 3). For codling moth, I conclude that it is possible to reduce the release rate per dispenser below what is optimal, and that an increase in dispenser density does not always compensate for a reduction in release rate. The current commercial hand-applied (rope) dispensers for codling moth realize an advantage by apparently raising the threshold for male response upon close visit to a dispenser so that males are removed from the mating pool for a longer period than occurs for dispensers releasing at female-like rates. However, the Chapter 3 experiments established that it is possible to combine the pheromones of codling moth and Oriental fruit moth into a common dispenser, a development that could increase overall disruption efficacy for reduced cost.

Using the large-cage system of Miller et al. (2010), I answered, for the first time, question 4) by what disruption mechanism do the commercial rope dispensers for Oriental fruit moth operate, and 5) how is the disruption mechanism related to dispenser release rate? Disruption operated competitively at a female-like release rate (0.04 μg h^{-1}) (Chapter 4), but shifted to non-competitive disruption at the rope release rate (60 μg h^{-1}). This is the first time such a shift in mechanism has been linked specifically with a defined shift in dispenser release rate. Such a shift was not achieved for codling moth. These findings raise the prospect that being able to achieve a sufficiently high release rate for non-competitive disruption may be linked to ease of disrupting mating of various species. As a general principle deserving further experimentation, I postulate that easy disruption may reflect achievement of non-competitive disruption, while species that have been difficult to disrupt experience only competitive disruption.

The field-cage system was used to answer question 6) -- does removal of Oriental fruit moth males attracted to a pheromone point source give benefit over temporary attraction alone? The answer was yes, and the level of benefit was 10-fold when pheromone was released at a female-like rate. This positive result and similar results for codling moth (Miller et al. 2010) led to question 7) – is it possible to design and produce a microtrap that is nearly as effective as the standard monitoring trap for tortricid moths? Chapter 5 answers affirmatively for codling moth and presents the rationale for a microtrap design deemed sufficiently unique and useful that Michigan State University is pursuing a patent on and around this device.

Finally, I answered (Chapter 6) the obvious next question, 8) – does this microtrap, deployed in the field at densities similar to that for commercial mating disruption dispensers, suppress sexual communication at a level superior to that for the conventional mating disruption dispenser. Satisfyingly, the answer for codling moth was yes. I provide evidence that such a microtrap has potential to control other tortricid moths.

Collectively, the results from this set of experiments provide solid leads that should encourage and guide additional research toward the overall goal of making pheromone-based insect control more effective while reducing costs. Potential research directions might include: 1) What is the optimal release rate and dispenser spacing to achieve non-competitive disruption for Oriental fruit moth? Large field cage studies varying dispenser release rates while keeping dispenser spacing equal, or vice versa, would reveal the critical pheromone release rate whereby disruption changes from competitive to a non-competitive mechanism or the dispenser density necessary to effectively control an entire area non-competitively. 2) Field cage studies similar to those presented in Chapter 4 or proposed here on other tortricid moth pests known to be easy or difficult to control would be needed to validate the argument that easy-to-control pests are those that can be controlled non-competitively. 3) Does attract-and-remove using high trap densities provide superior control of pests that can be disrupted non-competitively using higher-releasing dispensers, such as Oriental fruit moth? Experiments similar to those performed here on codling moth and obliquebanded leafroller would be necessary to compare the two strategies. 4) Is the patent-pending microtrap design effective at capturing pest species beyond codling moth? As mentioned at the end of Chapter 5, exploratory studies have been conducted on other pest species in Michigan, but more extensive testing, such as direct comparisons to other trap designs, is necessary. Developments, such as these, should lead to broader adoption of this environmentally friendly pest control tactic.

LITERATURE CITED

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AHMAD, T. R., and ALGHARBAWI, Z. A. 1986. Effects of pheromone trap design and placement on catches of codling moth males. *J. Appl. Entomol.* 102: 52-57.

ARN, H., SCHWARZ, C., LIMACHER, H., and MANI, E. 1974. Sex attractant inhibitors of codling moth *Laspeyresia-pomonella* L. *Experientia* 30: 1142-1144.

ATTERHOLT, C. A., DELWICHE, M. J., RICE, R. E., and KROCHTA, J. M. 1999. Controlled release of insect sex pheromones from paraffin wax and emulsions. *J. Controlled Release* 57: 233-247.

BACKMAN, A. C., BENGTSSON, M., and WITZGALL, P. 1997. Pheromone release by individual females of codling moth, *Cydia pomonella*. *J. Chem. Ecol.* 23: 807-815.

BAKER, T. C., CARDÉ, R. T., and MILLER, J. R. 1980. Oriental fruit moth (Lepidoptera, Tortricidae) pheromone component emission rates measured after collection by glass-surface adsorption. *J. Chem. Ecol.* 6: 749-758.

BARTELL, R. J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera - a review. *Physiol. Entomol.* 7: 353-364.

BEHLE, R. W., COSS, A. A., DUNLAP, C., FISHER, J., and KOPPENHOFER, A. M. 2008. Developing wax-based granule formulations for mating disruption of Oriental beetles (coleoptera: Scarabaeidae) in turfgrass. *J. Econ. Entomol.* 101: 1856-1863.

BROWN, D. F., and MCDONOUGH, L. M. 1986. Insect sex pheromone formulation to increase the stability of conjugated dienes. *J. Econ. Entomol.* 79: 922-927.

BYERS, J. A. 2007. Simulation of mating disruption and mass trapping with competitive attraction and camouflage. *Environ. Entomol.* 36: 1328-1338.

CARDÉ, R. T., BAKER, T. C., and CASTROVILLO, P. J. 1977. Disruption of sexual communication in *Laspeyresia pomonella* (codling moth), *Grapholitha molesta* (Oriental fruit moth) and *Grapholitha prunivora* (lesser appleworm) with hollow fiber attractant sources. *Entomol. Exp. Appl.* 22: 280-288.

CARDÉ, R. T., and MINKS, A. K. 1995. Control of moth pests by mating disruption - successes and constraints. *Annu. Rev. Entomol.* 40: 559-585.

CARDÉ, R. T., STATEN, R. T., and MAFRA-NETO, A. 1998. Behaviour of pink bollworm males near high-dose, point sources of pheromone in field wind tunnels: Insights into mechanisms of mating disruption. *Entomol. Exp. Appl.* 89: 35-46.

CHO, C.-W., CHOI, J.-S., and SHIN, S.-C. 2005. Controlled release of furosemide from the ethylene-vinyl acetate matrix. *Int. J. Pharm.* 299: 127-133.

CORK, A., ALAM, S. N., ROUF, F. M. A., and TALEKAR, N. S. 2005. Development of mass trapping technique for control of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Lepidoptera : Pyralidae). *Bull. Entomol. Res.* 95: 589-596.

CRANK, J. 1979. *The mathematics of diffusion*. Clarendon Press, Oxford.

CURKOVIC, T., and BRUNNER, J. F. 2006. Evaluation of permethrin for attracticide development against *Choristoneura rosaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae) males. *Crop Protect.* 25: 973-976.

DATERMAN, G., EGLITIS, A., CZOKAJLO, D., SACK, C., and KIRSCH, P. 2001. Attract and kill technology for management of European pine shoot moth (*Rhyacionia buoliana*) and western pine shoot borer (*Eucosma sonomana*). *Journal of Forest Science (Prague)* 47: 66-69.

DE LAME, F. M., MILLER, J. R., ATTERHOLT, C. A., and GUT, L. J. 2007. Development and evaluation of an emulsified paraffin wax dispenser for season-long mating disruption of *Grapholita molesta* in commercial peach orchards. *J. Econ. Entomol.* 100: 1316-1327.

EL-SAYED, A. M., SUCKLING, D. M., WEARING, C. H., and BYERS, J. A. 2006. Potential of mass trapping for long-term pest management and eradication of invasive species. *J. Econ. Entomol.* 99: 1550-1564.

EVENDEN, M. L., and MCLAUGHLIN, J. R. 2004. Initial development of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae). *Environ. Entomol.* 33: 213-220.

EVENDEN, M. L., and MCCLAUGHLIN, J. R. 2005. Male Oriental fruit moth response to a combined pheromone-based attracticide formulation targeting both Oriental fruit moth and codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* 98: 317-325.

FADAMIRO, H. Y. 2004. Monitoring the seasonal flight activity of *Cydia pomonella* and *Argyrotaenia velutinana* (Lepidoptera: Tortricidae) in apple orchards by using pheromone baited traps. *Environ. Entomol.* 33: 1711-1717.

FIGUEREDO, A. J., and BAKER, T. C. 1992. Reduction of the response to sex-pheromone in the Oriental fruit moth, *Grapholita-molesta* (Lepidoptera, tortricidae) following successive pheromonal exposures. *J. Insect Behav.* 5: 347-

363.

FITZGERALD, T. D., CLAIR, A. D. S., DATERMAN, G. E., and SMITH, R. G. 1973. Slow release plastic formulation of the cabbage looper pheromone cis-7-dodecenyl acetate: Release rate and biological activity. *Environ. Entomol.* 2: 607-610.

FUJIWARA, H., SATO, Y., and NISHI, K. 1976. Physicochemical studies on the sex pheromone of insects part 1 behavior of the sex pheromone of *Spodoptera litura* in rubber cap. *Journal of Takeda Research Laboratories* 35: 52-59.

FUNT, J., SIFLEET, W., and TOMME, M. 1993. Carbon black in plastics, pp. 389-408. *In* DONNET, J., BANSAL, R. and WANG, M. [eds.], Carbon black: Science and technology, second edition, revised and expanded ed. Marcel Dekker, Inc., New York.

GASTON, L. K., KAAE, R. S., SHOREY, H. H., and SELLERS, D. 1977. Controlling the pink bollworm by disrupting sex pheromone communication between adult moths. *Science (Washington D C)* 196: 904-905.

HIGUCHI, T. 1963. Mechanism of sustained action medication: Theoretical analysis of rate of release of solid. *J. Pharm. Sci.* 52: 1145-1149.

HOSSAIN, M. S., BARTELT, R. J., HOSSAIN, M. A. B. M., WILLIAMS, D. G., and CHANDRA, S. 2008. Longevity of pheromone and co-attractant lures used in attract-and-kill stations for control of carpophilus spp. *Entomol. Exp. Appl.* 129: 148-156.

HOWELL, J. F. 1984. New pheromone trap for monitoring codling moth (Lepidoptera, Olethreutidae) populations. *J. Econ. Entomol.* 77: 1612-1614.

HUBER, R. T., MOORE, L., and HOFFMANN, M. P. 1979. Feasibility study of area-wide pheromone trapping of male pink bollworm moths in a cotton insect pest management program. *J. Econ. Entomol.* 72: 222-227.

GLASS, E. H., ROELOFS, W. L., ARN, H., and COMEAU, A. 1970. Sex pheromone trapping red-banded leaf roller moths and development of a long-lasting poly ethylene wick. *J. Econ. Entomol.* 63: 370-373.

GUT, L., STELINSKI, L. L., THOMSON, D. R., and MILLER, J. R. 2004. Behaviour-modifying chemicals: Prospects and constraints in IPM pp. 73-122. *In* KOUL, O., DHALIWAL, G. S. and CUPERUS, G. W. [eds.], Integrated pest management: Potential, constraints and challenges. CABI Publishing, Cambridge, MA.

IL'ICHEV, A. L., GUT, L. J., WILLIAMS, D. G., HOSSIAN, M. S., and JERIE, P. H. 2002. Area-wide approach for improved control of Oriental fruit moth *Grapholita* *molesta* (busck) (Lepidoptera: Tortricidae) by mating disruption *Gen. Appl. Ent.* 31: 7-16.

IL'ICHEV, A. L., STELINSKI, L. L., WILLIAMS, D. G., and GUT, L. J. 2006. Sprayable microencapsulated sex pheromone formulation for mating disruption of Oriental fruit moth (Lepidoptera: Tortricidae) in Australian peach and pear orchards. *J. Econ. Entomol.* 99: 2048-2054.

IL'ICHEV, A. L., and WILLIAMS, D. G. 2006. Combined control of codling moth *Cydia pomonella* l. And Oriental fruit moth *Grapholita molesta* Busck (Lepidoptera: Tortricidae) by mating disruption on pears in Australia. *General and Applied Entomology* 35: 29-37.

KEHAT, M., ANSHELEVICH, L., DUNKELBLUM, E., FRAISHTAT, P., and GREENBERG, S. 1994. Sex-pheromone traps for monitoring the codling moth - effect of dispenser type, field aging of dispenser, pheromone dose and type of trap on male captures. *Entomol. Exp. Appl.* 70: 55-62.

KIM, J., KIM, W.-J., KIM, S.-J., CHO, C.-W., and SHIN, S.-C. 2006. Release characteristics of quinupramine from the ethylene-vinyl acetate matrix. *Int. J. Pharm.* 315: 134-139.

KNIGHT, A. L. 2000. Monitoring codling moth (Lepidoptera : Tortricidae) with passive interception traps in sex pheromone-treated apple orchards. *J. Econ. Entomol.* 93: 1744-1751.

KNIGHT, A. L. 2002. A comparison of gray halo-butyl elastomer and red rubber septa to monitor codling moth (lepidoptera: Tortricidae) in sex pheromone-treated orchards. *Journal Entomological Society of British Columbia* 99: 123-132.

KNIGHT, A. L., LARSON, D., and CHRISTIANSON, B. 2002. Flight tunnel and field evaluations of sticky traps for monitoring codling moth (Lepidoptera: Tortricidae) in sex pheromone-treated orchards. *J. Ent. Soc. B.C.* 99: 107-116.

KNIGHT, A. L. 2003. Testing an attracticide hollow fibre formulation for control of codling moth, *Cydia pomonella* (lepidoptera: Tortricidae) *Journal Entomological Society of British Columbia* 100: 71-78.

KNIGHT, A. L. 2004. Managing codling moth (Lepidoptera: Tortricidae) with an internal grid of either aerosol puffers or dispenser clusters plus border applications of individual dispensers. *Journal Entomological Society of British Columbia* 101: 69-78.

KNIGHT, A. L., and LARSEN, T. E. 2004. Improved deposition and performance of a microencapsulated sex pheromone formulation for codling moth (lepidoptera:

Tortricidae) with a low volume application *Journal Entomological Society of British Columbia* 101: 79-86.

KNIGHT, A. L., and LIGHT, D. M. 2005. Factors affecting the differential capture of male and female codling moth (Lepidoptera : Tortricidae) in traps baited with ethyl (e, z)-2,4-decadienoate. *Environ. Entomol.* 34: 1161-1169.

KNIGHT, A. L., and FISHER, J. 2006. Increased catch of codling moth (Lepidoptera: Tortricidae) in semio-chemical baited orange plastic delta-shaped traps. *Environ. Entomol.* 35: 1597-1602.

KNODEL, J. J., and AGNELLO, A. M. 1990. Field comparison of nonsticky and sticky pheromone traps for monitoring fruit pests in western new-york. *J. Econ. Entomol.* 83: 197-204.

KORSMEYER, R., GURNY, R., DOELKER, E., BURI, P., and PEPPAS, N. 1983. Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.* 15: 25-35.

KOVANCI, O. B., SCHAL, C., WALGENBACH, J. F., and KENNEDY, G. G. 2005. Comparison of mating disruption with pesticides for management of Oriental fruit moth (Lepidoptera: Tortricidae) in North Carolina apple orchards. *J. Econ. Entomol.* 98: 1248-1258.

KRUPKE, C. H., ROITBERG, B. D., and JUDD, G. J. R. 2002. Field and laboratory responses of male codling moth (Lepidoptera: Tortricidae) to a pheromone-based attract-and-kill strategy. *Environ. Entomol.* 31: 189-197.

KYDONIEUS, A. F., QUISUMBING, A. F., and HYMAN, S. 1976. Application of a new controlled release concept in household products, pp. 295. *In* PAUL, D. R. and HARRIS, F. W. [eds.], Controlled release polymeric formulations. ACS Symposium Series 33.

LACEY, M. J., and SANDERS, C. J. 1992. Chemical-composition of sex-pheromone of Oriental fruit moth and rates of release by individual female moths. *J. Chem. Ecol.* 18: 1421-1435.

LESKEY, T. C., BERGH, J. C., WALGENBACH, J. F., and ZHANG, A. 2009. Evaluation of pheromone-based management strategies for dogwood borer (Lepidoptera: Sesiidae) in commercial apple orchards. *J. Econ. Entomol.* 102: 1085-1093.

LOSEL, P. M., PENNERS, G., POTTING, R. P. J., EBBINGHAUS, D., ELBERT, A., and SCHERKENBECK, J. 2000. Laboratory and field experiments towards the development of an attract and kill strategy for the control of the codling moth, *Cydia pomonella*. *Entomol. Exp. Appl.* 95: 39-46.

LÖSEL, P. M., POTTING, R. P. J., EBBINGHAUS, D., and SCHERKENBECK, J. 2002. Factors affecting the field performance of an attracticide against the codling moth *Cydia pomonella*. *Pest Manage. Sci.* 58: 1029-1037.

MADSEN, H. F., and VAKENTI, J. M. 1972. Codling moths: Female-baited and synthetic pheromone traps as population indicators. *Environ. Entomol.* 1: 554-557.

MADSEN, H. F., and VAKENTI, J. M. 1973. Codling moths: Use of codlemone-baited traps and visual detection of entries to determine need of sprays. *Environ. Entomol.* 2: 677-679.

MADSEN, H. F., and CARTY, B. E. 1979. Codling moth suppression by male removal with sex pheromone traps in 3 British-Columbia Canada orchards. *Can. Entomol.* 111: 627-630.

MADSEN, H. F., VAKENTI, J. M., and PETERS, F. E. 1976. Codling moth: suppression by male removal with sex-pheromone traps in an isolated apple orchard. *J. Econ. Entomol.* 69: 597-599.

MAFRA-NETO, A., and HABIB, M. 1996. Evidence that mass trapping suppresses pink bollworm populations in cotton fields. *Entomol. Exp. Appl.* 81: 315-323.

MILLAR, J. G. 1995. Degradation and stabilization of E8,E10-dodecadienol, the major component of the sex-pheromone of the codling moth (Lepidoptera, Tortricidae). *J. Econ. Entomol.* 88: 1425-1432.

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

MILLER, J. R., GUT, L. J., DE LAME, F. M., and STELINSKI, L. L. 2006. Differentiation of competitive vs. Non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (part i): Theory. *J. Chem. Ecol.* 32: 2089-2114.

MILLER, J. R., GUT, L. J., DE LAME, F. M., and STELINSKI, L. L. 2006. Differentiation of competitive vs. Non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (part 2): Case studies. *J. Chem. Ecol.* 32: 2115-2143.

MILLER, J. R., MCGHEE, P. S., SIEGERT, P. Y., ADAMS, C. G., HUANG, J., GRIESHOP, M. J., and GUT, L. J. 2010. General principles of attraction and competitive attraction as revealed by large-cage studies of moths responding to sex pheromone. *Proc. of the Nat. Acad. Sci. U.S.A.* 107: 22-27.

MURLIS, J., WILLIS, M. A., and CARDÉ, R. T. 2000. Spatial and temporal structures of pheromone plumes in fields and forests. *Physiol. Entomol.* 25: 211-222.

PINERO, J. C., MAU, R. F. L., MCQUATE, G. T., and VARGAS, R. I. 2009. Novel bait stations for attract-and-kill of pestiferous fruit flies. *Entomol. Exp. Appl.* 133: 208-216.

POULLOT, D., BESLAY, D., BOUVIER, J.-C., and SAUPHANOR, B. 2001. Is attract-and-kill technology potent against insecticide-resistant lepidoptera? *Pest Manage. Sci.* 57: 729-736.

QUI, Y., and ZHANG, G. 2000. Research and development aspects of oral controlled-release dosage forms, pp. 465-504. *In* WISE, D. L. [ed.], Handbook of pharmaceutical controlled release technology. Marcel Dekker, New York.

REINKE, M. D., MILLER, J. R., and GUT, L. J. 2012. Potential of high-density pheromone-releasing micro-baited microtraps for control of codling moth, *Cydia pomonella*, and obliquebanded leafroller, *Choristoneura rosaceana*. *Physiol. Entomol.* 37: 53-59.

ROELOFS, W. L., COMEAU, A., and SELLE, R. 1969. Sex pheromone of the Oriental fruit moth. *Nature* 224: 723-723.

ROELOFS, W. L., and TETTE, J. P. 1970. Sex pheromone of oblique-banded leaf roller moth. *Nature* 226: 1172-&.

ROELOFS, W. L., GLASS, E. H., TETTE, J., and COMEAU, A. 1970. Sex pheromone trapping for red-banded leaf roller control theoretical and actual. *J. Econ. Entomol.* 63: 1162-1167.

ROELOFS, W., COMEAU, A., HILL, A., and MILICEVIC, G. 1971. Sex attractant of the codling moth - characterization with electroantennogram technique. *Science (Washington D C)* 174: 297-299.

ROELOFS, W. L., BARTELL, R. J., HILL, A. S., CARDÉ, R. T., and WATERS, L. H. 1972. Codling moth sex attractant - field trials with geometrical isomers. *J. Econ. Entomol.* 65: 1276-1277.

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

SAS-INSTITUTE. 2000. Sas/stat user's guide: Volume 6. SAS Institute, Cary, NC.

SCHNEIDER, D. 1962. Electrophysiological investigation on the olfactory specificity of sexual attracting substances in different species of moths. *J. Insect Physiol.* 8:

15-30.

SHANI, A., and KLUG, J. T. 1980. Sex pheromone on egyptian cotton leafworm (*Spodoptera littoralis*) its chemical transformations under field conditions. *J. Chem. Ecol.* 6: 875-882.

SHOAIB, M., TAZEEN, J., MERCHANT, H., and YOUSUF, R. 2006. Evaluation of drug release kinetics from ibuprofen matrix tablets using hpmc. *Pakistan Journal of Pharmaceutical Science* 19: 119-124.

SHOREY, H. H., and HALE, R. L. 1965. Mass-rearing of larvae of 9 noctuid species on a simple artificial medium. *J. Econ. Entomol.* 58: 522-524.

SHOREY, H. H., KAAE, R. S., GASTON, L. K., and MC LAUGHLIN, J. R. 1972. Sex pheromones of Lepidoptera part 30 disruption of sex pheromone communication in *Trichoplusia ni* as a possible means of mating control. *Environ. Entomol.* 1: 641-645.

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

STATEN, R. T., EL-LISSY, O., and ANTILLA, L. 1996. Successful area-wide program to control pink bollworm by mating disruption.

STELINSKI, L. L., GUT, L. J., PIERZCHALA, A. V., and MILLER, J. R. 2004a. Field observations quantifying attraction of four tortricid moths to high-dosage pheromone dispensers in untreated and pheromone-treated orchards. *Entomol. Exp. Appl.* 113: 187-196.

STELINSKI, L. L., GUT, L. J., VOGEL, K. J., and MILLER, J. R. 2004b. Behaviors of naive vs. Pheromone-exposed leafroller moths in plumes from high-dosage pheromone dispensers in a sustained-flight wind tunnel: Implications for mating disruption of these species. *J. Insect Behav.* 17: 533-554.

STELINSKI, L. L., GUT, L. J., MALLINGER, R. E., EPSTEIN, D., REED, T. P., and MILLER, J. R. 2005. Small plot trials documenting effective mating disruption of Oriental fruit moth by using high densities of wax-drop pheromone dispensers. *J. Econ. Entomol.* 98: 1267-1274.

STELINSKI, L. L., MILLER, J. R., LEDEBUHR, R., and GUT, L. J. 2006. Mechanized applicator for large-scale field deployment of paraffin-wax dispensers of pheromone for mating disruption in tree fruit. *J. Econ. Entomol.* 99: 1705-1710.

STELINSKI, L. L., GUT, L. J., HAAS, M., MCGHEE, P., and EPSTEIN, D. 2007a. Evaluation of aerosol devices for simultaneous disruption of sex pheromone communication in *Cydia pomonella* and *Grapholita molesta* (Lepidoptera: Tortricidae). *J. Pest Sci.* 80: 225-233.

STELINSKI, L. L., MCGHEE, P., HAAS, M., IL'ICHEV, A. L., and GUT, L. J. 2007b. Sprayable microencapsulated sex pheromone formulations for mating disruption of four tortricid species: Effects of application height, rate, frequency, and sticker adjuvant. *J. Econ. Entomol.* 100: 1360-1369.

STELINSKI, L. L., MCGHEE, P., GRIESHOP, M., BRUNNER, J., and GUT, L. J. 2008. Efficacy and mode of action of female-equivalent dispensers of pheromone for mating disruption of codling moth. *Agric. For. Entomol.* 10: 389-397.

SUCKLING, D. M., and BROCKERHOFF, E. G. 1999. Control of light brown apple moth (Lepidoptera: Tortricidae) using an attracticide. *J. Econ. Entomol.* 92: 367-372.

SUKOVATA, L., KOLK, A., and CIEŚLAK, M. 2004. Effect of attract and kill formulations and application rates on trap catches of European pine shoot moth (Lepidoptera: Tortricidae) and shoot damage in scots pine saplings. *J. Econ. Entomol.* 97: 1619-1623.

TASCHENBERG, E. F., CARDÉ, R. T., and ROELOFS, W. L. 1974. Sex pheromone mass trapping and mating disruption for control of red-banded leaf-roller and grape berry moths in vineyards. *Environ. Entomol.* 3: 239-242.

TOBA, H. H., KISHABA, A. N., and WOLF, W. W. 1969. Polyethylene bags for dispensing synthetic sex pheromone of the cabbage looper. *J. Econ. Entomol.* 62: 517-518.

TOMASZEWSKA, E., HEBERT, V. R., BRUNNER, J. F., JONES, V. P., DOERR, M., and HILTON, R. 2005. Evaluation of pheromone release from commercial mating disruption dispensers. *J. Agric. Food Chem.* 53: 2399-2405.

VALENTINE, J. M. 1931. The olfactory sense of the adult mealworm beetle *Tenebrio molitor* (Linn.). *Journal of Exp. Zool.* 58: 165-228.

VINCENT, C., MAILLOUX, M., HAGLEY, E. A. C., REISSIG, W. H., COLI, W. M., and HOSMER, T. A. 1990. Monitoring the codling moth (Lepidoptera, Olethreutidae) and the obliquebanded leafroller (Lepidoptera, Tortricidae) with sticky and nonsticky traps. *J. Econ. Entomol.* 83: 434-440.

YAMANAKA, T. 2007. Mating disruption or mass trapping? Numerical simulation analysis of a control strategy for lepidopteran pests. *Popul. Ecol.* 49: 75-86.

WEISSLING, T. J., and KNIGHT, A. L. 1994. Passive trap for monitoring codling moth (Lepidoptera, Tortricidae) flight activity. *J. Econ. Entomol.* 87: 103-107.

WILLSON, H. R., and TRAMMEL, K. 1980. Sex-pheromone trapping for control of codling moth (Lepidoptera, Olethreutidae), Oriental fruit moth (Lepidoptera, olethreutidae), lesser appleworm (Lepidoptera, Olethreutidae), and 3 tortricid leafrollers in a new-york apple orchard. *J. Econ. Entomol.* 73: 291-295.

WITZGALL, P., BACKMAN, A. C., SVENSSON, M., KOCH, U., RAMA, F., EL-SAYED, A., BRAUCHLI, J., ARN, H., BENGTSSON, M., and LOFQVIST, J. 1999. Behavioral observations of codling moth, *Cydia pomonella*, in orchards permeated with synthetic pheromone. *BioControl* 44: 211-237.

WITZGALL, P., STELINSKI, L., GUT, L., and THOMSON, D. 2008. Codling moth management and chemical ecology. *Annu. Rev. Entomol.* 53: 503-522.

WOHLFARTH, C. 2011. Permittivity (dielectric constant) of liquids, pp. 6-187 to 6-208. *In* HAYNES, W. M. [ed.], Crc handbook of chemistry and physics: A ready-reference book of chemical and physical data, 92nd ed. CRC Press, Boca Raton, FL.

WOLF, W. W., KISHABA, A. N., HOWLAND, A. F., and HENNEBERRY, T. J. 1967. Sand as a carrier for synthetic sex pheromone of cabbage loopers used to bait blacklight and carton traps. *J. Econ. Entomol.* 60: 1182-1184.

ZHANG, G. F., MENG, X. Z., HAN, Y., and SHENG, C. F. 2002. Chinese tortrix *Cydia trasias* (Lepidoptera: Olethreutidae): Suppression on street-planting trees by mass trapping with sex pheromone traps. *Environ. Entomol.* 31: 602-607.