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DEVELOPMENT AND DELIVERY OF BIOPESTICIDE-BASED PEST MANAGEMENT FOR MICHIGAN APPLE PRODUCERS

By

David Lawrence Epstein

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ABSTRACT

DEVELOPMENT AND DELIVERY OF BIOPESTICIDE-BASED PEST MANAGEMENT FOR MICHIGAN APPLE PRODUCERS

By

David Lawrence Epstein

Field investigations were conducted to develop effective and economical biopesticide-based management programs for control of two tortricid moth species, codling moth (CM) (*Cydia pomonella* L) and Oriental fruit moth (OFM) (*Grapholita molesta* Busck). Experiments were conducted: i) to determine mechanisms by which pheromone-mediated mating disruption (MD) operates for control of CM and OFM, ii) to evaluate pheromone dispenser deployment strategies, iii) to evaluate effects of moth behavior on dispenser deployment strategies, and iv) to evaluate use of CM *Granulosis* virus for CM population suppression. I found that: i) MD of both moth species is improved by increasing density and distribution of pheromone release sites, ii) CM adults are distributed throughout the tree canopy during evening activity periods, iii) MD dispensers placed in the top and mid tree canopy resulted in lower percentages of mated females than distribution at one height, only, iv) adult CM leave the tree canopy to inhabit drive-row grass and herbicide strip vegetation during daytime inactivity periods, v) frequent applications of a low rate of virus yielded excellent, economical control of CM, and vi) an areawide approach to deploying CM MD reduced male moth capture and incidence of larval injury to fruit with less

insecticide use than MD in small individual orchard blocks or insecticide only programs.

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INTRODUCTION

Apple Agriculture in Michigan

With over 107,000 acres of orchards, Michigan is the leading producer of tree fruits in the Midwest (Michigan Department of Agriculture, 2008), and the third largest apple producing state in the U.S.A, behind Washington and New York (Michigan Agricultural Statistics, 2009). Michigan had 36,500 bearing acres of apple in 2008 worth a farm level value of $117.4 million (Michigan Agricultural Statistics, 2009).

Codling Moth and Oriental Fruit Moth

Management of over two-dozen insect pests in apple in the upper Midwest is a difficult task (Howitt 1993, Epstein and Gut 2000, Epstein et al. 2002), and IPM programs for these crops are complex. The most challenging species to manage are the direct pests whose larvae feed internally within fruit (internal feeders) rendering it unmarketable. Two lepidopterans from the family, Tortricidae, codling moth, *Cydia pomonella* (L.) and Oriental fruit moth, *Grapholita molesta* (Busck), are introduced species that have become the principal direct pests of North American apples. The larvae of both species overwinter as mature larvae in litter on the ground or under bark on the tree (Borror *et al.*, 1989). Three full generations of Oriental fruit moth occur in Michigan; the first generation emerges as adults around the last week of April (Epstein and Gut, 2000). Codling moth typically emerge as adults in Michigan the second to third week of May. There are generally two full generations of codling moth per year in Michigan (Epstein and Gut, 2000). Larvae of both species tunnel into the fruit shortly upon eclosion, and are morphologically distinguishable only by the presence (Oriental fruit moth) or absence (codling moth) of an anal comb located ventrally on the terminal abdominal segment (Epstein and Gut, 2000). Oriental fruit moth has become an increasingly important pest of apples east of the Mississippi river within the past ten years. Many Michigan apple growers now treat codling moth and Oriental fruit moth with the same high degree of concern. In the absence of control measures, greater than 75% of the apple crop in Michigan orchards can be damaged by the two species (Wise and Gut 2000, 2002).

Biopesticides

Pest control for Michigan apple growers is currently a major challenge, and is likely to become more difficult in the foreseeable future due to loss of compounds through U.S. Environmental Protection Agency (EPA) regulatory restrictions to protect citizens from the harmful effects of pesticides in food, air, and water (Food Quality Protection Act, 1996, H.R. 1627), the development of pest resistance to currently used pesticides, and a shortage of economic and effective alternatives. A pheromone-trap bioassay (Riedl et al., 1985) was conducted to assess the susceptibility of codling moth to azinphosmethyl, the primary organophosphorous (OP) insecticide used for codling moth control in Michigan since the middle of the 20th century (Gut et al., 2001; Gut and Wise, 2004). Resistance testing revealed that high levels of codling moth resistance were present on farms around Michigan (Mota-Sanchez et al., 2008). Based on the MI resistance bioassays, it appears likely that many codling moth control failures are related to a reduced effectiveness of OP's.

New control tactics, with novel modes of action are needed to sustain an economically viable Michigan apple industry. Among the most promising candidates is a group of tactics collectively referred to as biopesticides. Biopesticides are biological pesticides derived from natural sources that the EPA categorizes into three major classes. The first two, microbial pesticides (a microorganism is the active ingredient), and plant-incorporated-protectants (genetic manipulation of plant to self-produce toxins) are toxic control agents. The third class, biochemical pesticides, manages pests through non-toxic means (U.S. EPA, 2006). Biochemical pesticides include insect sex pheromones and plant volatiles.

Mating Disruption

a. Background / Current Status

Pheromones are chemicals secreted by an animal that influence the behavior or development of others of the same species (Wilson and Bossert, 1963). Mating (sex) pheromone is used by individuals of a species to attract mates for reproduction. In the case of codling moth and Oriental fruit moth, the female calls the male by emitting sex pheromone, sensed by males from distances of up to 40m or more (Rodriguez-Saona and Stelinski, 2009) on wind borne plumes consisting of a series of filaments of pheromone interspersed with pockets of zero to low concentrations due to natural turbulence in the air (Sanders 1997). Responsive male moths follow the pheromone plume to the female; engage in courtship behaviors and copulate. Codling moth female sex pheromone was first identified by Roelofs *et al.* (1971), and Oriental fruit moth female sex pheromone was discerned by Cardé *et al.* (1979).

The deployment of synthetic sex pheromone to interfere with mate finding orientation and subsequent mating behavior is called mating disruption. Mating disruption, as it is commercially practiced today, is largely achieved through the manual application of reservoir-type release devices (Cardé and Minks, 1995). Approximately 100-fold quantities of synthetic pheromone are released from these dispensers as compared to the pheromone release rate of a calling female (Cardé and Minks, 1995).

Pheromone mating disruption is a feasible tactic for control of codling moth and Oriental fruit moth in fruit orchards. In the U.S.A., disruption products for codling moth are currently deployed on more than 130,000 acres of apple and pear, and over 45,000 acres of apple and peach are being treated with Oriental fruit moth disruption products (Gut *et al.*, 2004, Witzgal et al., 2008). As it has been practiced for the past two decades, mating disruption for codling moth and Oriental fruit moth has not generally been a stand-alone tactic capable of achieving adequate protection of apple fruit from larval entries without additional application of insecticides targeting these two pests. Efforts to improve application of this technology are necessary to limit agricultural use of pesticides, reduce control program costs and to facilitate widespread adoption of mating disruption by the grower community.

b. Mechanisms of MD

Although mating disruption of codling moth and Oriental fruit moth with synthetic pheromone has been commercially deployed in apple orchards internationally since the early 1990's, there is still no consensus among the scientific community as to the mechanism(s) by which disruption acts to interfere with moth orientation and mating behavior. There are four principal mechanisms that are thought to underlie mating disruption: (1) desensitization – including (a) *adaptation* of males' peripheral sensors (antennae) due to exposure to constant, high levels of the pheromone stimulus that prevents males from being able to detect pheromone. Recovery can occur shortly upon removal of the stimulus source so that the male can detect pheromone upon stimulus reintroduction; and (b) *habituation* of the males' central nervous system resulting from long-term exposure to high pheromone concentrations that inhibits the male from responding, even upon withdrawal of the high concentration stimulus source. Habituated males become less responsive to the pheromone stimulus for long periods after exposure to high concentrations, (2) Camouflage, or masking of the pheromone plumes emitted from females by a high background concentration of pheromone. The male's sensory apparatus is functioning properly in this model, but is inefficient for locating females in a uniform and high background level of pheromone in the orchard; (3) False-plume following (competition) preventing the male from locating the female; the males can still sense and respond to the pheromone stimulus but are inhibited from finding mates due to time and energy spent pursuing and/or becoming arrested on false trails from synthetic dispensers placed throughout the orchard and (4) Sensory imbalance resulting from a compromise in the chemical composition of the female pheromone, such that the active ingredient released from dispensers distorts the normal perception of the signal (Bartell, 1982; Cardé, 1990; Cardé and Minks, 1995; Gut, *et al.*, 2004; Gut *et al.* 2008, Sanders, 1997).

Miller *et al.* (2006 a, b) divided the four proposed mating disruption behavioral mechanisms into two categories, competitive (false-trail following or competitive attraction) that is predicated on males being attracted to dispensers, and non-competitive (desensitization, camouflage and sensory imbalance) that operates by interfering with the male's ability to sense and respond normally to pheromone. Competitive attraction is distinguished from the other mechanisms by requiring attraction to pheromone dispensers as the first step in response to a mating disruptant. Three main lines of evidence support competitive attraction as the primary mechanism operating in the field using current mating disruption technologies (Miller et al., 2006b, Gut et al., 2008). In practice, mating disruption appears to be highly density dependent. If mating disruption operated in a non-competitive means, all males within the adequately treated area, regardless of density, should be unresponsive to females. Males of four tortricid moth species, Oriental fruit moth (*Grapholita molesta*), obliquebanded leafroller (*Choristoneura rosaceana*), redbanded leafroller (*Argyrotaenia velutinana*) and codling moth (*Cydia pomonella*) have all been observed readily approaching their respective polyethylene tube pheromone dispensers (Stelinski *et al.* 2005b; Judd *et al.* 2004). The most persuasive evidence is provided by Miller *et al.*'s (2006b) examination of dosage response profiles generated from published work with sufficient data for analysis. Disruption profiles for 11 of 13 data sets adhered to the predictions of a competitive attraction model.

Cardé *et al.* (1997, 1998) point out that two or more of these mechanisms may act together to successfully prevent moth mating in a synergistic or additive fashion, such as false-plume following resulting in short-term adaptation to pheromone upon male moth contact with a dispenser. Additionally, varying physiological response to pheromone among moth species (Stelinski *et al.* 2005b) and species variation in chemical characteristics of pheromones (Gut *et al.*, 2004) provide evidence that some moth species are inherently more difficult to disrupt than other species.

c. Pheromone Dispenser Point Source Distribution

Nine codling moth and seven Oriental fruit moth disruption products commercially are available in the U.S.A. Five hand-applied reservoir dispensers, Isomate® C+ and CTT (Shin-Etsu Chemical Co., Tokyo, Japan), Scentry NoMate® codling moth Spirals (Scentry Biologicals, Billings, Montana), Hercon Disrupt codling moth (Hercon Environmental, Emigsville, PA), and CheckMate® codling moth XL 1000 (Suterra, Bend, OR) are available in the U.S. market for codling moth. Isomate® M 100, Isomate® M Rosso, Scentry NoMate® Oriental fruit moth Spirals, and CheckMate® SF are the reservoir dispensers available for Oriental fruit moth. Label rates for Oriental fruit moth reservoir dispensers vary from 250 to 500 per hectare.

Label rates for reservoir dispensers for codling moth disruption vary from 300 to 1000 per hectare, and growers typically select a rate at the lower end due to economic and labor concerns. The hand application of codling moth dispensers is a labor intensive process (approximately 5 hours / ha at the full label rate), adding labor costs as well as competition for labor at times of the growing season when many Michigan growers are busy working in other crops, and has been identified as a major impediment to widespread adoption of mating disruption (Epstein *et al.*, 2002b).

The predominant formulation currently used in the U.S.A. for codling moth is the Isomate-C Plus polyethylene tube dispenser (Shin-Etsu Chemical Co., Tokyo, Japan) with a label rate of 1000 per ha (Thomson et al. 2001, Witzgal *et al.*, 2008). However, there are several commercially available disruption products for codling moth that claim that disruption can be achieved with reduced numbers of dispensers per ha. For example, Isomate-CTT (Shin-Etsu Chemical Co., Tokyo, Japan) dispensers contain twice as much pheromone as the Isomate-C Plus dispenser and are designed for application at a rate of 500 / ha (Alway 2002). Some researchers (Shorey and Gerber 1996, Knight 2004, Welter *et al.* 2005b) suggest that mating disruption efficacy can be maintained while reducing the density of release sites, if overall release rate of pheromone per ha is maintained or increased. A saving in labor is postulated for a given number of Isomate dispensers assembled into clusters rather than distributed individually (Knight 2004), or with the use of battery-powered aerosol emitters, deployed at a rate of 5 per ha, dispensing daily rates of (*E,E*)-8-10-dodecadienol, codling moth sex pheromone, equivalent to a full label rate of Isomate-C Plus (Welter 2005). However, Suckling and Angerilli (1996) falsified this idea for lightbrown apple moth, *Epiphyas postvittana* (Walker). Their study revealed that if the total number of dispensers was held constant, plots with higher densities of release sites best-disrupted moth catches in pheromone traps. Most recently, Stelinski et al., (2007) reported very low levels of 24-26% disruption of codling moth male orientation to pheromone traps in plots treated with aerosol dispensers deployed at the recommended rate of 2.5 units per ha.

Miller *et al.* (2006 a, b) argue that competitive attraction is the predominant mechanism of disruption, and that practical implications for improving application of mating disruption are that 1) attractiveness should be competitive with females, 2) dispenser density should be high, and 3) distribution of pheromone dispensers should be uniform rather than clumped. Understanding how distribution of pheromone release devices in an orchard affects orientational disruption of male moths is necessary to optimize efficacy of mating disruption for codling moth and Oriental fruit moth.

d. Canopy Distribution of Reservoir Dispensers

Should competitive attraction be the predominant mechanism in mating disruption, a factor to consider in point source distribution of reservoir pheromone dispensers is location of dispensers within the tree canopy to compete with calling females. However, the distribution of calling codling moth females within the tree canopy has yet to be directly studied. Research with pheromone baited traps showing male flight behavior to be concentrated in the top third of the tree canopy is primarily responsible for the standard recommendation that reservoir dispensers be applied within the top meter of the tree canopy (Barret 1995, Knight 2000, McNally and Barnes 1980).

Behavioral and biological studies of codling moth also support moth activity being centered in the tops of tree canopies. Geier (1963) stated that moths spend the majority of their time in tree crowns when not in flight (though providing no data to support this statement), Witzgall *et al.* (1998) visually observed male moths flying and searching branches in the upper half of tree crowns, and Stoeckli *et al.* (2008) found highest mean values of larval infestation of fruit in the top and middle tree canopy heights, providing further support for deployment of pheromone dispensers high in the canopy. The location of both adult males and females during daylight hours also has not been well documented, and it is largely assumed that adult moths confine themselves within the tree canopy during periods of biological inactivity.

Research data on direct sampling of calling female moths in tree canopies is not reported in the literature. In discussing pheromone trap placement for capture of male moths, McNeil (1991) points out that trap placement within the tree canopy can impact the structure and trajectory of a pheromone plume and therefore the probability and distance over which males will be able to locate the source. Kührt *et al.* (2005) found that height within the tree canopy significantly affected air temperature within the canopy, a factor possibly affecting microhabitat selection by calling females. Improved knowledge of codling moth bionomics, including the location of calling females, diel behavior patterns and the affects of factors such as age and prevailing climatic conditions on female behavior, could be of critical importance in optimizing dispenser placement within the tree canopy and in ultimately improving the performance of mating disruption of tortricid moth pests.

e. High-Density Mating Disruption Formulations

Miller *et al.* (2006a and b) have recently presented a strong case that pheromone dispenser point source density is strongly correlated to pheromone treatment efficacy, and that high-density formulations are optimal to take advantage of false trail following found in the competition model of mating disruption. Machine-applied formulations that disperse pheromone via numerous small releasers are being evaluated for disruption of Oriental fruit moth and codling moth. Hercon® flakes (Hercon, Emigsville, PA) and Scentry® fibers (Scentry, Billings, MT) are formulations targeting codling moth control that are applied either by ground or air, allowing large areas to be treated in a much shorter time than with hand-applied reservoir dispensers. A sticker is added so that fiber and flake particles will adhere to foliage and wood. These types of dispensers are formulated to be applied at much higher densities (about 37,000 / ha vs. 1,000 hand-applied dispensers / ha), compared with Isomate dispensers, and release pheromone at rates similar to those emitted by female moths (Swenson and Weatherston 1989, Welter and Cave 2005). Fibers and flake have the potential to provide adequate levels of codling moth disruption, but have limited commercial viability due to insufficient product adherence and lack of rainfastness (Stelinski et al., 2008)

Paraffin-based emulsified wax is another dispensing formulation being tested for use in mating disruption (Atterholt 1998, de Lame 2003, Miller 2006a and b). Stelinski *et al.* (2006) developed a tractor-mounted mechanical dispensing system for high-density application of wax dispensers for mating disruption of Oriental fruit moth. The wax applicator delivered *about* 160 wax drops per tree at a speed of 22.5 minutes / hectare (Stelinski et al., 2006). A single application of wax drops provided 97% orientation disruption of pheromone-baited traps for 55d and completely prevented mating of tethered virgin females throughout the season (Stelinski et al., 2007c). A version of this wax formulation is currently marketed by ISCA Technologies (Riverside, CA, USA) under the trade name, SPLAT.

Pheromone also has been formulated into microcapsules or beads that are delivered through conventional spray equipment at densities in excess of one hundred thousand per hectare. Each capsule releases pheromone at a rate below that of a calling female resulting in an approach that appears to disrupt mate finding by camouflaging the female's signal (Stelinski et al., 2005b). Sprayable pheromone has been quite effective for Oriental fruit moth (Il"Ichev et al., 2006), while codling moth control using the approach has proved more difficult (Stelinski et al., 2007b). The highest level of disruption is achieved when the sprayable product is applied using very low gallonages of water (Knight and Larsen, 2004). However, high or low gallonage applications require multiple sprays through the season and reapplication after a rain event to maintain disruption (Stelinski et al. 2007b, Waldstein and Gut 2005).

f. Areawide Mating Disruption

A major challenge to the expanded adoption of tortricid moth mating disruption as it is currently practiced with reservoir-type release devices is the cost associated with deploying a pheromone program that also requires the application of numerous insecticide sprays per growing season to prevent unacceptable levels of larval feeding injury to fruit. An areawide or whole-farm deployment strategy can help to achieve attainment of high performance disruption that provides acceptable control with reduced insecticide use.

Areawide management promotes a cooperative effort by neighboring growers with contiguous acreage to deploy mating disruption on all of the acreage on their farms to directly address key concerns regarding orchard perimeters in smaller, individualized mating disruption programs; the immigration of mated females into a disrupted orchard, and lower pheromone concentrations along orchard perimeters (Gut and Brunner, 1998; Knight, 1995). Areawide disruption programs of 1,100 contiguous ha of apples and pears in Australia, and 1,200 ha of mixed stone fruits in the Tulbagh valley in South Africa resulted in excellent control of Oriental fruit moth (Il'ichev *et al.,* 2002; Barnes and Blomefield, 1997). Successful areawide mating disruption programs targeting codling moth were conducted in pome fruit in the western U.S.A. during the years 1994 to 1998 (Calkins *et al.*, 2000; Brunner *et al.*, 2001) and in Michigan during the years 2004-2009 (McGhee *et al.*, 2009). None of these programs relied solely on the use of mating disruption to achieve successful control, but incorporated judicious and timely applications of insecticides. Direct comparisons with conventional programs outside the project areas revealed reductions in the number of insecticides applied for codling moth and Oriental fruit moth control. Areawide disruption projects have also successfully controlled pink bollworm in large, contiguous areas of cotton in the southwestern U.S.A. (Staten *et al.*, 1987) and Egypt (Jones and Casagrande, 2000).

Cydia pomonella Granulosis Virus

Since it was first collected in Mexico over 40 years ago (Tanada, 1964) and underwent its initial orchard trials in the 1960's, codling moth *granulosis* virus (CpGV) has been researched as a codling moth control (Falcon *et al.*, 1968). The *Granulosis* virus is a highly lethal baculovirus, protected by a protein occlusion body made from granulin. The occlusion body is ingested by neonate larvae and dissolved in the alkaline midgut. Virus particles are released, initiating a primary infection of midgut epithelium cells, which, in turn produces budded viruses that spread secondary infection to other tissues (i.e. neuronal cells and fat bodies) (Lacey and Shapiro-Ilan 2003; Jehle *et al.* 2006, *Weeden et al.* 2007). Mortality occurs within 5 to 10 days and cadavers release billions of viral occlusion bodies into the environment (Jehle *et al.*, 2006). There are no known negative effects on plants, mammals, birds, fish, or non-target insects (Weeden *et al.* 2007).

Although laboratory bioassays confirmed CpGV to be highly active against neonate larvae (Jaques *et al.* 1987), effectiveness in the field was inconsistent

(Charmillot *et al.* 1993, Huber and Dickler 1977; Jaques et al. 1987; Laing and Jaques 1980; Rashid *et al.* 2001; Vail *et al.*, 1991). Recent efforts to reformulate *Granulosis* virus products in Europe, Canada and New Zealand have resulted in reports of consistently good codling moth control and CpGV products are now applied on more than 100,000 ha of sustainable codling moth control programs in combination with mating disruption in Europe (Charmillot *et al.* 1998; Lacey and Shapiro-Ilan 2003; Eberle and Jehle 2006).

Three commercial formulations are available in Michigan, Carpovirusine (Arysta LifeScience, Cary, NC), Cyd-X (Certis USA, Columbia, MD), and Virosoft^{CP4} (BioTepp, Canada). Carpovirusine contains 10^{13} viral particles / liter, and is applied at a label rate of 0.5-1 liter virus/ 1000 liters water / hectare. Cyd-X contains 3 x 10^{13} viral particles / liter, and is applied at a label rate of 74 – 266 ml / hectare. Virosoft^{CP4} contains 4 x 10^{13} viral particles / liter and is applied at a label rate of 250 ml / hectare. The virus requires frequent reapplication using standard farm spray equipment due to very short residual activity in the field resulting from viral inactivation from exposure to ultraviolet radiation and high temperatures (Lacey and Shapiro-Ilan, 2003). Researchers are currently investigating CpGV use patterns comparing the efficacy of frequent applications (5-7 day re-application intervals) of virus at low rates vs. higher rates at 14-21 day application intervals, efficacy against different codling moth generations, and vertical transmission of CpGV between generations (Epstein *et al.*, 2006; Hull and Krawczyk 2005; Quénin and Laur 2006).

Multi-year programs combining CpGV and mating disruption in Europe, New Zealand and the United States have noted a pattern where initially high numbers of codling moth adults and high percentages of partial larval entries into fruit declined over 2-4 year period resulting in reduced pest densities and reduced injury to fruit (Charmillot *et al.* 1998; Wearing *et al.* 1994).

Control failures have also been reported. Reduced susceptibility of codling moth to CpGV of up to 1000-fold less, based on LC_{50} values, was first reported in organic apple orchards in 2005 in Germany and France, where CpGV and pheromone mating disruption constitute the backbone of organic management of codling moth (Fristch *et al.* 2005, Jehle *et al.* 2006, Eberle 2006). Asser-Kaiser *et al.* (2007) described codling moth resistance to CpGV as the interaction of three factors: sex-linkage (located on the Z-chromosome of the moth, favoring rapid resistance development under continued selection pressure), concentration dependent dominance (resistance is higher in heterozygous males at low virus concentrations), and uniformity of the selective agent (Mexican isolate, CpGV-M).

Newly identified CpGV isolates of varying virulence have been identified (CpGV-I12 from Iran; Madex Plus, Andermatt Biocontrol, Switzerland; and 3 unregistered isolates from Jehle lab in Germany) and tested in Europe (Eberle *et al.* 2008; Jehle 2008). All new isolates show good efficacy against resistant populations and offer a broad genetic basis for development of resistance management strategies along with the development of resistance monitoring techniques (Jehle 2008).

Dissertation research

The overall aim of this thesis research was to develop and deliver effective and economical biopesticide-based management programs for codling moth (CM) and Oriental fruit moth (OFM) control that rely on mating disruption, granulosis virus, and the judicious use of reduced-risk or conventional insecticides for control of these key pests. To accomplish these goals experiments were conducted to better understand mechanisms by which pheromone mediated mating disruption operates for control of CM and OFM, evaluations of pheromone dispenser types and deployment strategies, how moth behavior may affect dispenser deployment strategies, and use of codling moth *Granulosis* virus with pheromone disruption for CM population suppression. Specific objectives were to determine: 1.) the effect of dispenser point source distribution on CM and OFM orientation disruption, 2.) within-canopy adult distribution of CM in disrupted and non-disrupted apple orchards, 3.) diel distribution of adult CM in disrupted and non-disrupted apple orchards, 4.) efficacy and optimum patterns of use of CM Granulosis virus formulations, and 5.) the effectiveness of a whole-farm or areawide program for codling moth control that relies on mating disruption, granulosis virus, and the judicious use of reduced-risk or conventional insecticides for control of this key pest.

CHAPTER ONE

Higher densities of distributed pheromone sources provide disruption of codling moth (Lepidoptera: Tortricidae) superior to that of lower densities of clumped sources

Also published as:

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ABSTRACT

Field experiments quantified the effect of synthetic pheromone release-site density and distribution on: 1) orientational disruption of male codling moths, *Cydia pomonella* L., to pheromone-baited traps, and 2) fruit injury. A clustering test varied pheromone release-site density from 0 - 1000 Isomate-C Plus dispensers / ha while maintaining the total number of dispensers at 1000. Percent orientational disruption of pheromone-baited traps increased significantly as a function of increasing density of release sites. Fruit injury decreased as the density of release sites increased and was lowest in plots treated with Isomate-C Plus dispensers distributed as 1,000 point sources / ha. We also manipulated point source density of 0.1 ml paraffin-wax drops containing 5 % codlemone ((*E*,*E*)-8,10-dodecadien-1-ol), and thus, total amount of pheromone deployed per ha. Percent disruption of traps baited with either 1.0 or 0.1 mg codlemone lures increased with increasing density of wax drops deployed. Both trapping and field observations confirmed that wax drops were attractive to male codling moths, suggesting that disruption was mediated by competitive attraction. Development of dispensers that can be mechanically applied at high densities has potential to improve the efficacy and economics of codling moth disruption at high population densities.

INTRODUCTION

Mating disruption of codling moth (CM), *Cydia pomonella* L., in the U.S.A. is implemented on over 54,000 ha of pome fruits and walnuts, with varying degrees of success (Gut et al. 2004). The predominant formulation currently used in the U.S.A. is the Isomate-C Plus polyethylene tube dispenser (Shin-Etsu Chemical Co., Tokyo, Japan, Thomson et al. 2001) applied at densities of 500 to 1000 per ha, which equates with ca. 1 to 2 per fruit tree. Some researchers (Shorey and Gerber 1996, Knight 2004) suggest that efficacy can be maintained while reducing the density of release sites, if overall release rate per ha is maintained or increased. A saving in labor is postulated for a given number of Isomate dispensers assembled into clusters rather than distributed individually (Knight 2004). However, Suckling and Angerilli (1996) falsified this idea for lightbrown apple moth, *Epiphyas postvittana* (Walker). Their study revealed that if the total number of dispensers was held constant, plots with higher densities of release sites best-disrupted moth catches in pheromone traps.

There are several commercially available disruption products for CM in addition to Isomate-C dispensers. For example, Isomate-CTT (Shin-Etsu Chemical Co., Tokyo, Japan) dispensers contain twice as much pheromone as the Isomate-C Plus dispenser and are designed for application at a rate of 500 / ha (Alway 2002). Other products, such as Hercon Disrupt CM flakes (Hercon, Emigsville, PA) and Scentry NoMate CM Fibers (Scentry, Billings, MT) are applied at higher densities (ca. 37,000 / ha), compared with Isomate dispensers, and release pheromone at rates similar to those emitted by female moths (Swenson and Weatherston 1989, Welter and Cave 2005). Understanding how distribution of pheromone release devices in an orchard affects orientational disruption of male moths is necessary to optimize efficacy of mating disruption for CM.

The overall goal of this investigation was to determine whether CM disruption is improved by increasing density and distribution of pheromone release sites. Studies were also conducted to determine whether male moths approach dispensers in order to provide insight into the mechanism(s) by which disruption may be operating. Our specific objectives were to determine whether: 1) a given number of Isomate-C Plus dispensers were equally efficacious whether clumped or distributed, and 2) CM disruption could be improved over that by Isomate dispensers if multiple paraffin-wax drops were deployed per tree as Stelinski et al. (2005a) demonstrated was effective for Oriental fruit moth, *Grapholita molesta* (Busck). A trapping study and direct field observations were conducted to determine whether wax drops attract male CM. Pheromone release rate from wax drops was also quantified by gas chromatography.

MATERIALS AND METHODS

Experiment 1: Reducing release-site density while maintaining total pheromone applied per ha constant. This study was conducted 10 May to 30 September, 2003. The objective was to evaluate the effect of clustering pheromone release sites on disruption efficacy while maintaining a total of 1000 Isomate-C Plus dispensers / ha. These dispensers contained 205 mg of 53.0 %

codlemone ((*E,E*)-8,10-dodecadien-1-ol), 29.7 % 12OH, 6.0 % 14OH, and 11.3 % inert ingredients. Densities of release sites per ha were: 0, 10, 25, 100, 500, and 1,000. Dispensers were applied individually in the top m of trees for the 500 and 1000 / ha treatments. Two dispensers were spaced 2.5 cm apart in the 500 release site / ha treatment. Multiple dispensers in the 10, 25 and 100 release site / ha treatments were attached to horizontally mounted, (1 intersection / cm^2) galvanized-metal hardware cloth (TWP Wire Mesh, Berkeley, CA, U.S.A.), each dispenser spaced 2.5 cm apart as per Suckling and Angerilli (1996). The 100 release sites / ha treatment consisted of 10 dispensers per cluster; the 25 release sites / ha treatment consisted of 40 dispensers per cluster, and the 10 release sites / ha treatment consisted of 100 dispensers per cluster. Pheromone release sites were spaced equidistantly throughout each 0.4 ha plot (Fig. 1). Experimental design was a randomized complete block with two replicates (blocks) located at the Trevor Nichols Research Complex (TNRC) of Michigan State University in Fennville, MI and one each in two commercial orchards in South Lyon and Flushing, MI. The two blocks at TNRC were separated by at least 300 m and treatment plots at all commercial orchards were separated by at least 45 m. Experimental sites and tree composition are described in Table 1. Disruption of male moth orientation to sex pheromone was assessed using 3 pheromone traps (LPD Scenturian Guardpost, Suterra, Bend, OR) / 0.4 ha plot baited with 1 mg codlemone rubber septum lures and placed in the upper third of tree canopies on a diagonal transect across each plot (Fig. 1). Lures were red rubber septa loaded with codlemone (> 98 % isomeric and chemical purity, Suterra, Bend, OR). New pheromone lures were deployed every two weeks during each moth generation. Moths captured in traps were counted and removed once weekly.

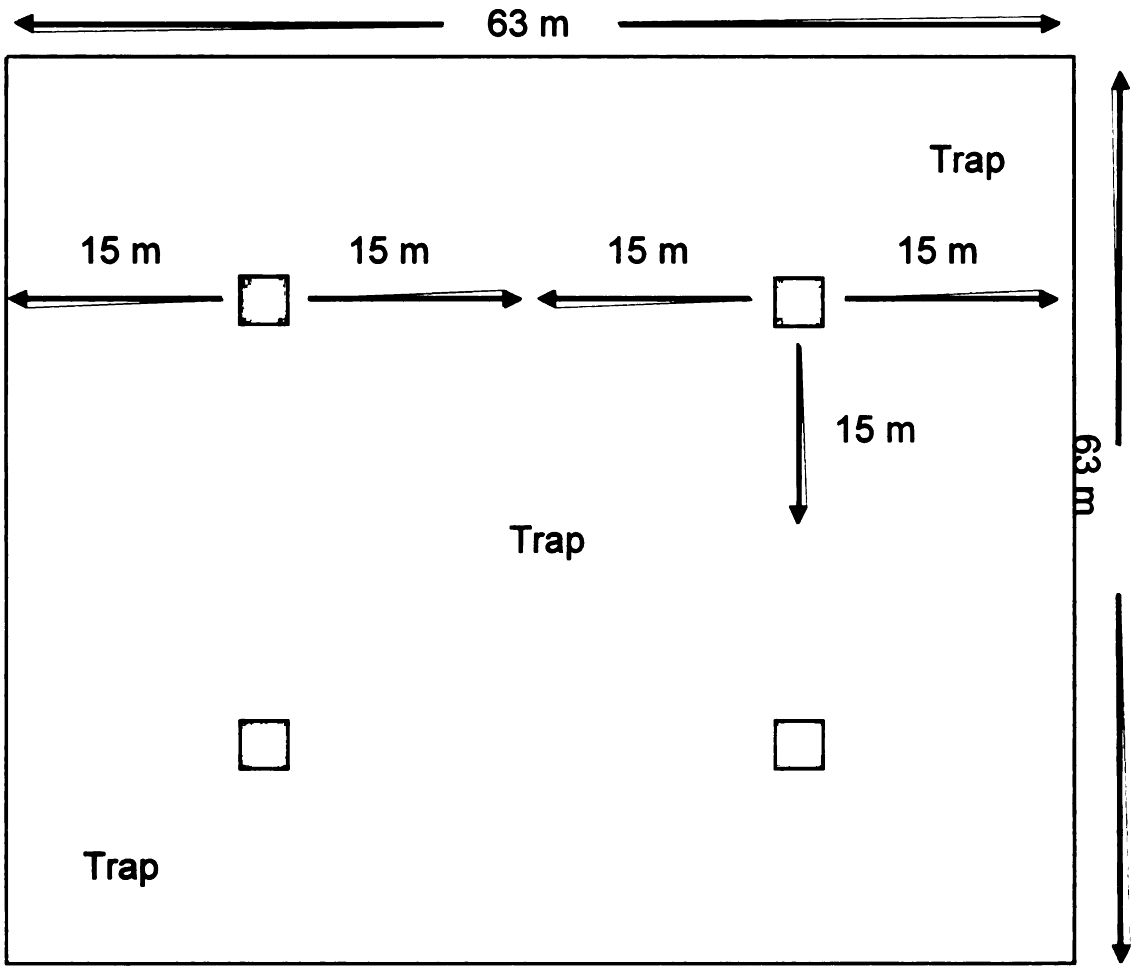

Figure 1. Diagram of a 0.4 ha orchard plot containing 4 release sites of clustered Isomate-C Plus dispensers. Shaded boxes (not to scale) represent 100 clustered Isomate-C Plus dispensers. Other release-site densities were likewise regularly distributed.

Table 1. Description of orchards used in Experiment 1 in which density of release sites (Isomate-C Plus dispensers) per ha was varied without varying total amount of pheromone deployed per ha.

Orchard No.	Cultivar(s)	Tree Age	Canopy Height	Tree Spacing
1	Red Delicious	16 yrs	3.5 – 4.5 m	3 x 6 m
2	Golden Delicious	10 yrs	3.5 – 4.5 m	1.8 x 4.6 m
	Red Delicious	10 yrs	3.5 – 4.5 m	3 x 5.5 m
	Red Delicious	20 yrs	4 – 5 m	3 x 5.5 m
	Rome	8 yrs	3 – 4 m	2.4 x 5.5 m
3	Braeburn	7 yrs	2.4 – 3.1 m	2.7 x 4.9 m
	Macintosh	7 yrs	2.4 – 3.1 m	2.7 x 4.3 m
	Fuji	7 yrs	2.4 – 3.1 m	2.4 x 4.6 m
	Jonagold	7 yrs	2.4 – 3.1 m	2.4 x 4.6 m
	Northern Spy	7 yrs	2.4 – 3.1 m	2.4 x 5.5 m
4	Gala	20 yrs	2.4 – 3.1 m	2.4 x 4.6 m
	Gala	20 yrs	3 – 4 m	2.7 x 4.9 m

CM fruit injury was evaluated in all plots following first generation moth flight and immediately prior to harvest. Thirty apples per tree, fifteen high in the canopy and 15 low in the canopy, were examined from 20 trees per plot (600 fruit / plot total).

Experiment 2: Increasing both point-source density and total pheromone applied per ha from paraffin-wax drops. Paraffin-wax dispensers (Stelinski et al. 2005a) were used because a larger range of densities could be compared than with Isomate-C Plus dispensers, while applying less total pheromone / ha than could be achieved with Isomate-C Plus dispensers. This study was conducted 13

May to 3 June of 2004 at TNRC. This experiment ran for only 2 wk because disruption efficacy was rapidly lost thereafter. Experimental site, tree composition, and maintenance protocol of orchards are described in Stelinski et al. (2005a). Orientational disruption trials were conducted comparing five application densities of 0.1 ml drops of paraffin wax containing 5% pheromone. Paraffin-wax dispensers were formulated as described in Stelinski et al. (2005a). Briefly, paraffin-wax formulation consisted of: 30 % paraffin wax (Gulf wax, Royal Oak Sales, Inc., Roswell, GA), 4 % soy oil (Spectrum Naturals, Inc., Petaluma, CA), 2 % Span 60 (Sorbitan monostearate, Sigma-Aldrich Co., St. Louis, MO), 1 % vitamin E ([±]-α-tocopherol, Sigma Chemical Co., St. Louis, MO), 5 % codlemone (Bedeukian Co., Danbury, CT, > 98% purity confirmed by gas chromatography), and 58 % (by total weight) de-ionized water. Wax drops were hand-applied to branches of trees using 5 ml plastic syringes. Densities of wax drops compared were: 0 per tree, 3 per tree (820 / ha, 3.3 g A. I. / ha); 10 per tree (2,700 / ha, 11 g A. I. / ha); 30 per tree (8,200 / ha, 33 g A. I. / ha); and 100 per tree (27,300 / ha, 109 g A. I. / ha). Experimental design was a randomized complete block with treatments applied to 0.07 (16 tree) ha plots replicated 5 times. Replicate orchards (blocks) were separated by at least 35 m and treatment plots by at least 15 m. Orientational disruption of male catches was assessed using two pheromone traps, baited with either 1.0 or 0.1 mg of codlemone, placed in two of four central trees of each plot. Traps were hung ca. 2 - 3 m above ground level in the upper third of the tree canopy. Wax dispensers were never placed closer than 50 cm from monitoring traps. Moths captured in traps were counted and removed twice weekly.

Experiment 3: Evaluating attractiveness of paraffin-wax dispensers to male codling moths. We compared captures of male CM in traps baited with 0.1 ml paraffin-wax drops formulated as above with that of rubber septum lures loaded with 1 mg of codlemone (described above) known to be highly attractive. These tests were conducted 6 July through 19 September, 2004 in plots not being used for mating disruption studies. Paraffin-wax dispensers containing pheromone were pipetted onto 2 x 5 cm strips of aluminum foil and allowed 24 h to dry before strips were deployed in traps. Fresh lures were installed every two weeks into plastic delta traps deployed in unsprayed 0.4 ha plots of apples, as above. Unbaited traps were used as a negative control. Paraffin-wax treatments were replaced every four weeks. The experiment was arranged in a randomized complete block with 6 replicates. Traps were spaced ca. 26 m apart and rotated weekly. Traps were hung ca. 1.5 - 2 m above ground level in the upper third of the tree canopy. Moths captured in traps were counted and removed twice weekly.

Experiment 4: Direct observation of codling moth males in plots treated with various densities of wax drops. Direct observations of male CM visits to wax drops in trees were conducted as detailed in Stelinski et al. (2005a). Observations were conducted on 17 nights during first CM generation. One wax drop containing pheromone was observed in control plots that were otherwise untreated.

Experiment 5: Measuring pheromone release rate from 0.1 ml wax drops. Release rate of codlemone from paraffin-wax dispensers was determined using protocol reported by Stelinski et al. (2005a). Briefly, paraffin-wax drops (0.1 ml) were applied to 1 x 5.5 cm pieces of flat, wooden Craft Sticks (Diamond Brands, Cloquet, MN) using an Eppendorf repeat pipetter. Resulting drops were ca. 7 mm in diameter and 2 mm thick. Samples were attached with plastic ties to branches of five 5 - 7 m tall apple trees at a height of 2 - 3 m within the tree canopy. Samples were maintained in the field and in a laboratory fume hood from 3 August to 8 October, 2004. Five samples were collected immediately at application to determine pheromone concentration in wax drops at test onset. Thereafter, three samples were collected approximately every 24 h for 4 d and then every other d for 80 d. Pheromone extracted from samples was quantified using a gas chromatograph (HP-6890, Hewlett-Packard Co.) fitted with a DBWAXETR polar column (model # 122-7332, J&W Scientific, Folsom, CA) of length 30 m and internal diam. 250 μm. Initial oven temperature was 130 °C for 2 min and then it was ramped at a rate of 2.5°C / min to 160°C, where it was held for 2 min. The program then ran at 40°C / min to a final temperature of 230 °C. The carrier gas, He, entered the column at 20 psi.

Statistical analysis. For orientational disruption and trapping studies, data were transformed to ln (x + 1) (which normalized distributions and homogenized variance) and then subjected to analysis of variance (ANOVA). Fruit injury data were arcsine transformed prior to ANOVA. Differences in pairs of means were separated using the LSD test (SAS Institute 2000). Fruit injury data were also subjected to regression analysis to describe the overall relationship between fruit injury and $\log_{10}$ of pheromone release-site density. Observational data of moth visits to paraffin wax drops in the field were subjected to χ^2 analyses. Percent orientational disruption was calculated as 1 - (mean moth catch per trap in the pheromone-treated plot / mean moth catch per trap in the control plot) x 100.

RESULTS

Experiment 1: Reducing release-site density while maintaining total pheromone applied per ha constant. Overall, percent disruption of pheromone-baited traps increased significantly as a function of increasing density of pheromone release sites (Fig. 2 A). Traps in plot centers treated with 1,000 or 500 release sites / ha captured significantly ($F = 14.2$, df = 5, 18, $P < 0.01$) fewer males than those in plots treated with 0, 10, or 25 release sites / ha (Fig. 2 A). Also, significantly ($P < 0.01$) fewer males were captured in plots with 1000 release sites / ha than in plots with 100 release sites / ha (Fig. 2 A). There was no significant ($P > 0.05$) difference in male catch in central traps among plots treated with 10 and 25 release sites / ha (Fig 2 A).

Traps placed on plot borders treated with 500 and 1,000 point sources / ha captured significantly ($F = 8.3$, df = 5, 18, $P < 0.01$) fewer males than those placed on plot borders with 0, 10, 25, or 100 release sites / ha (Fig. 2 B). There was no significant difference among male captures in border traps of plots treated with 10 or 100 release sites / ha (Fig. 2 B). As with central traps, orientational disruption of border traps was highest for the two highest release-site density treatments (Fig. 2 B).

Percentage of infested fruit decreased as density of release sites increased. Fruit injury in both plot centers and borders was significantly (*F's* = 4.4, 5.0, respectively, df = 5, 18, $P < 0.01$) reduced relative to control in plots treated with 1,000 release sites / ha (Fig. 3 A, B). Among pairs of means, there was no significant ($P > 0.05$) difference between fruit injury in plots with 100 through 1000 release sites / ha. Overall relationships between decreasing fruit injury as a function of increasing $\log_{10}$ of release-site density were described by $y = -0.23x + 3.8$ ($R^2 = 0.84$) and $y = -0.25x + 4.2$ ($R^2 = 0.8$) for plot centers and borders, respectively. Slopes for these relationships were significantly (*F's* = 20.5, 13.1, df = 1, 5, $P < 0.05$) negative.

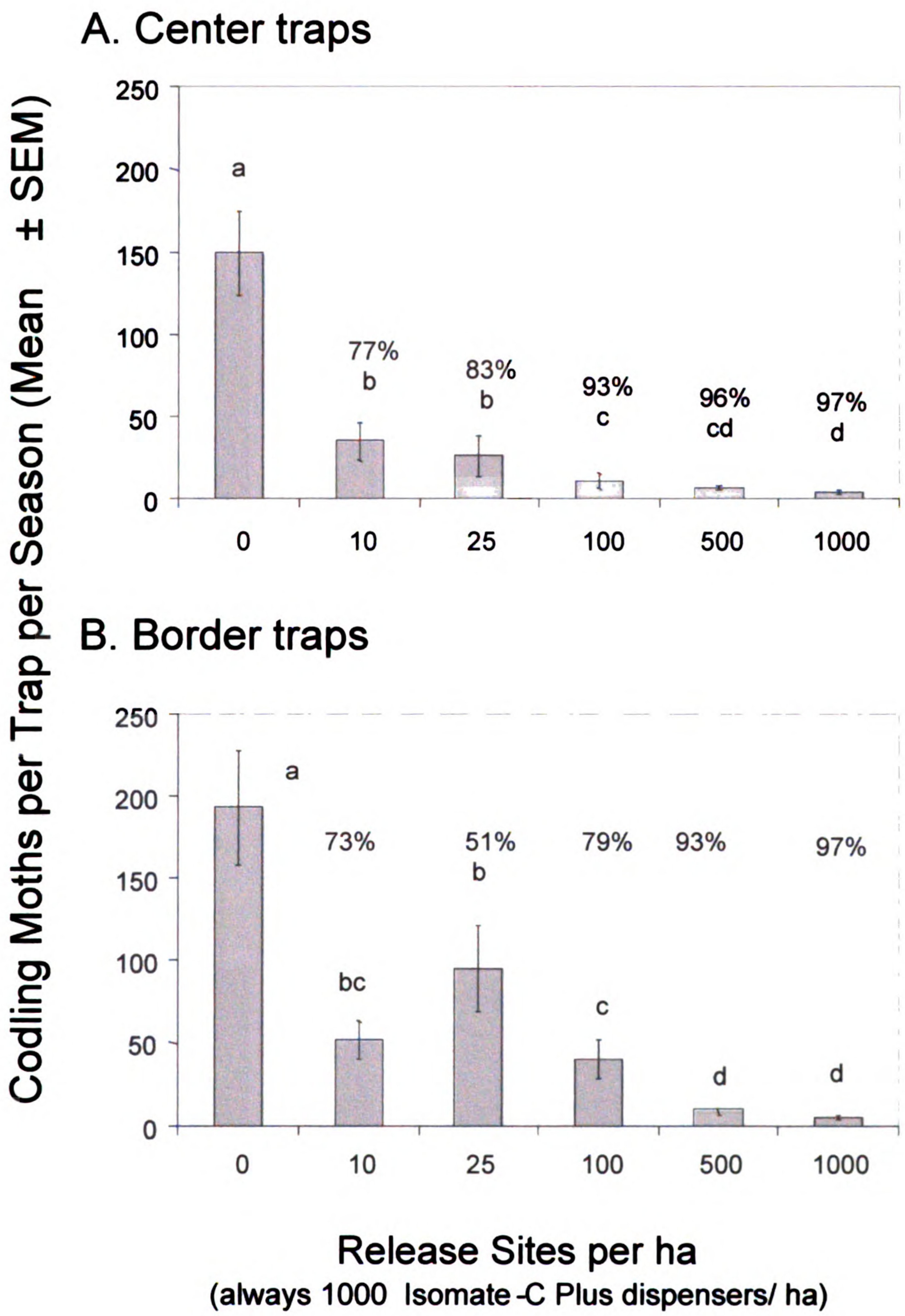

Figure 2. Mean captures of codling moth males in lure-baited delta traps in A) centers and B) borders of plots containing various release-site densities of Isomate-C Plus dispensers while maintaining the total number of dispensers at 1000 / ha. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

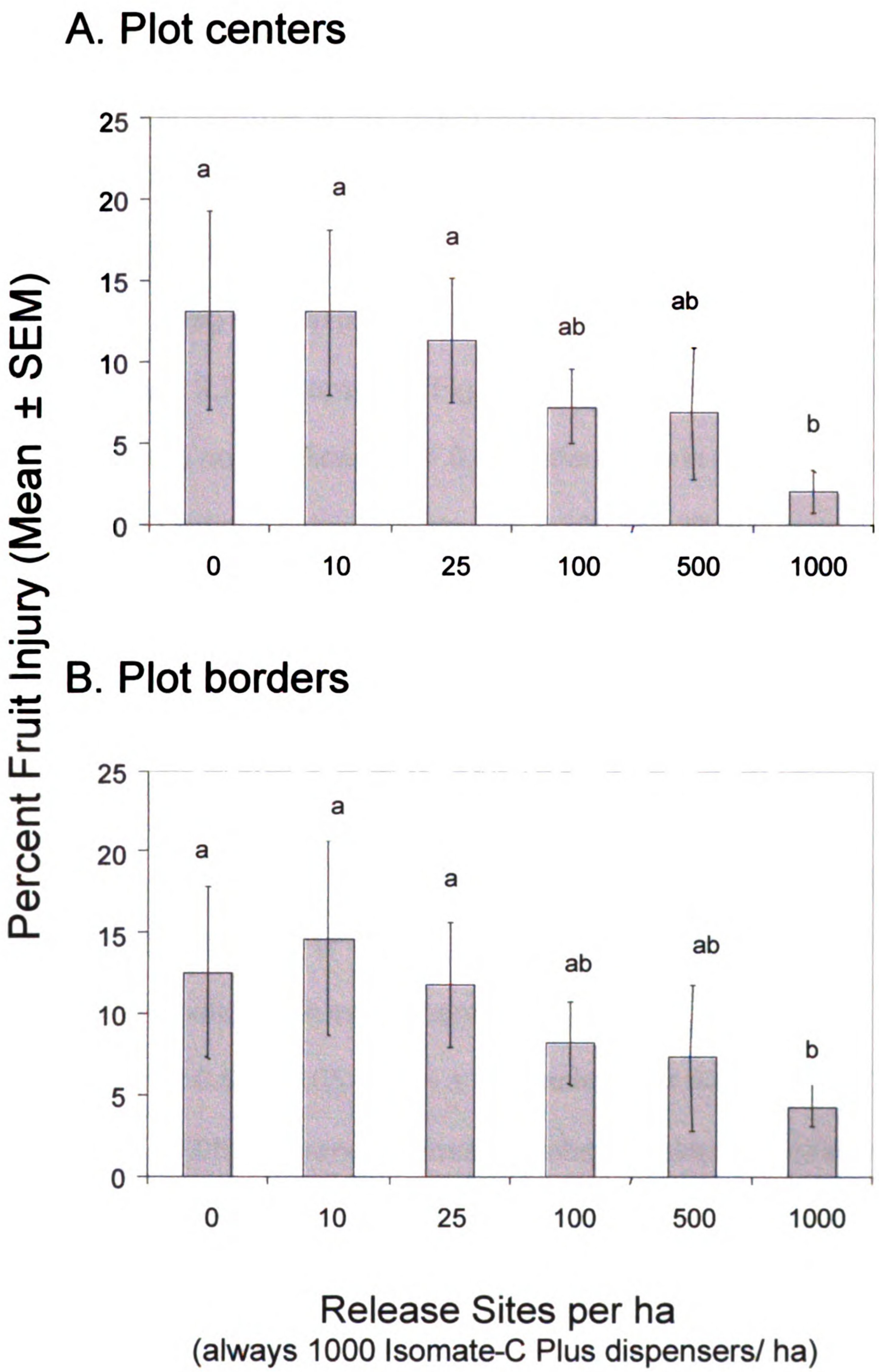

Figure 3. Mean percent fruit injury due to codling moth larval infestation in A) centers and B) borders of plots containing various release-site densities of Isomate-C Plus dispensers while maintaining the total number of dispensers at 1000 / ha. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

Experiment 2: Increasing both point-source density and total pheromone applied per ha from paraffin-wax drops. Mean numbers of moths captured in traps decreased as a function of increasing wax-drop density. Significantly (*F's* = 11.4,12.2, respectively, df = 4, 20, $P < 0.01$) fewer males were captured in traps baited with 1.0 and 0.1 mg lures in plots treated with 27,300 wax drops / ha than in plots with 0, 820, and 2,700 drops / ha (Fig. 4 A, B). However, this analysis of pairs of means revealed no significant ($P > 0.05$) differences in numbers of males caught with both lure loadings between plots with 8,200 and 27,300 point sources (Fig. 4 A, B). Overall, percent disruption of traps baited with either 1.0 or 0.1 mg lures increased with increasing density of point sources deployed (Fig. 4 A, B). Ninety-four and 99 % orientational disruption was achieved with 27,300 point sources / ha for 1.0 and 0.1 mg lures, respectively.

Experiment 3: Evaluating attractiveness of paraffin-wax dispensers to male codling moths. Significantly ($F = 16.2$, df = 3, 20, $P < 0.01$) more male CM were captured in traps baited with each pheromone treatment than in blank controls which captured 0.06 ± 0.05 (mean ± SE) males over the season. There was no statistical (P > 0.05) difference in mean number of males captured in traps baited with red septa (65.5 ± 13.4), 0.1 ml wax drops with 98% pure codlemone (81.8 ± 18.5), or 0.1 ml wax drops with codlemone extracted from Isomate-C Plus dispensers (74.3 ± 9.2).

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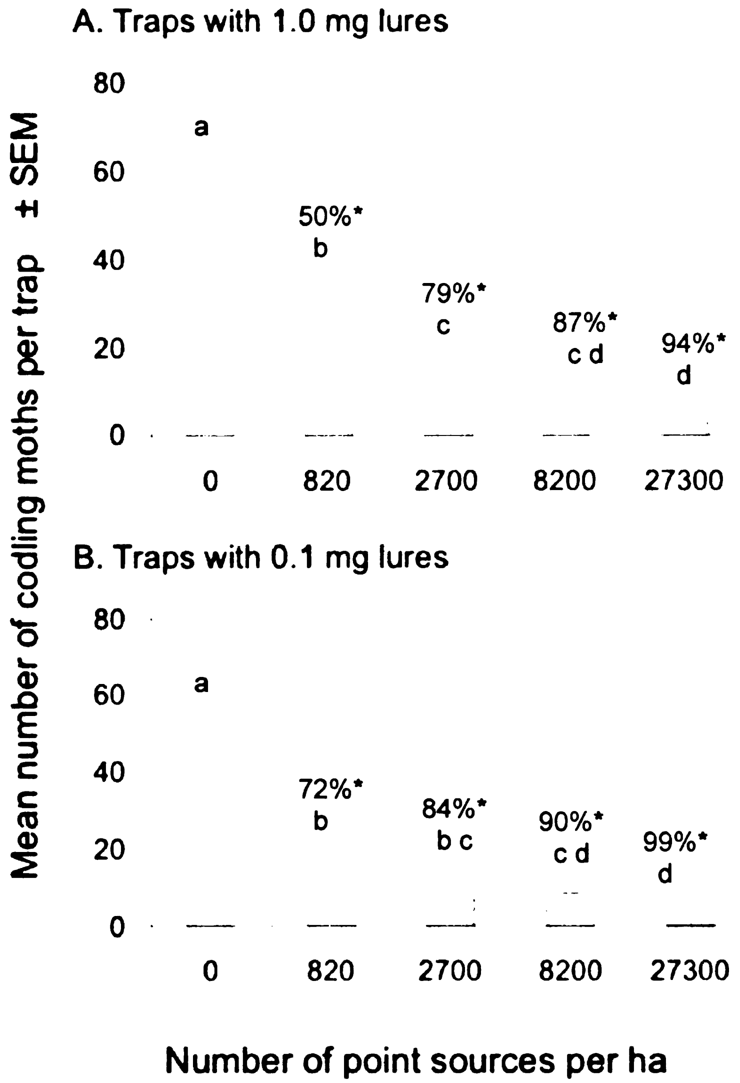

Figure 4. Mean captures of codling moth males in lure-baited delta traps in plots containing various densities of 0.1 ml paraffin-wax drops containing 5 % codlemone. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

Experiment 4: Direct observation of codling moth males in plots treated with various densities of wax drops. CM males approached wax drops in treated plots at all dispenser densities (Fig. 5). Significantly ($\chi^2 = 7$, df = 1, $P < 0.01$) more males approached a single drop under observation in otherwise untreated plots than in plots with 820 drops / ha and likewise significantly ($\chi^2 = 20$, df = 1, P

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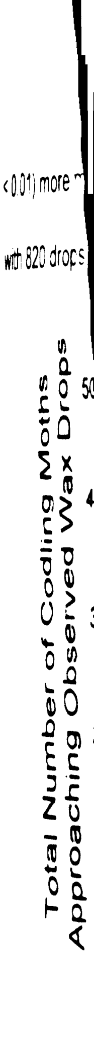

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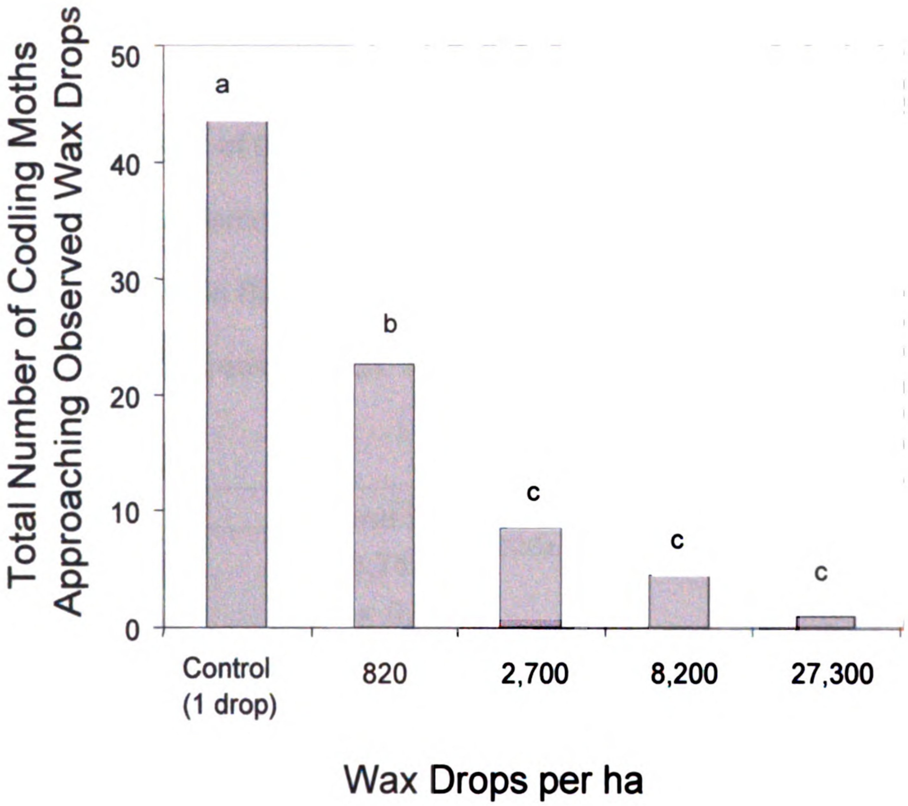

Figure 5. Numbers of codling moth males observed approaching paraffin-wax drop dispensers of pheromone in plots receiving various densities of dispensers. All moths observed approached within 130 cm of dispensers. Observations were conducted on 17 evenings between 17 May and 12 June. Means followed by the same letter are not significantly different at $\alpha < 0.05$.

males observed made direct contact with drops, 88% of observed males approached within 20 cm of wax drops, and all observed moths came within 130 cm. Following landing on foliage near wax drops, males fanned their wings and walked rapidly. None of the visits lasted longer than 2 min, and 90 % flew away from dispensers within 60 s.

Experiment 5: Measuring pheromone release rate from 0.1 ml wax drops.

Release profiles of codlemone from wax drops in a laboratory fume hood and in the field are shown in Fig. 6, fitted with exponential decay curves. Laboratory samples yielded an R^2 of 0.98 and a decay constant value of – 0.028, while field samples gave an R^2 of 0.94 and a higher decay constant value of – 0.051. Release rate of codlemone from a wax drop was ca. 2.1 and 3.3 µg / h over the first 14 d of release in the laboratory and field, respectively. Between d 14 and 35, release rate decreased to ca. 1.3 to 0.5 µg / h.

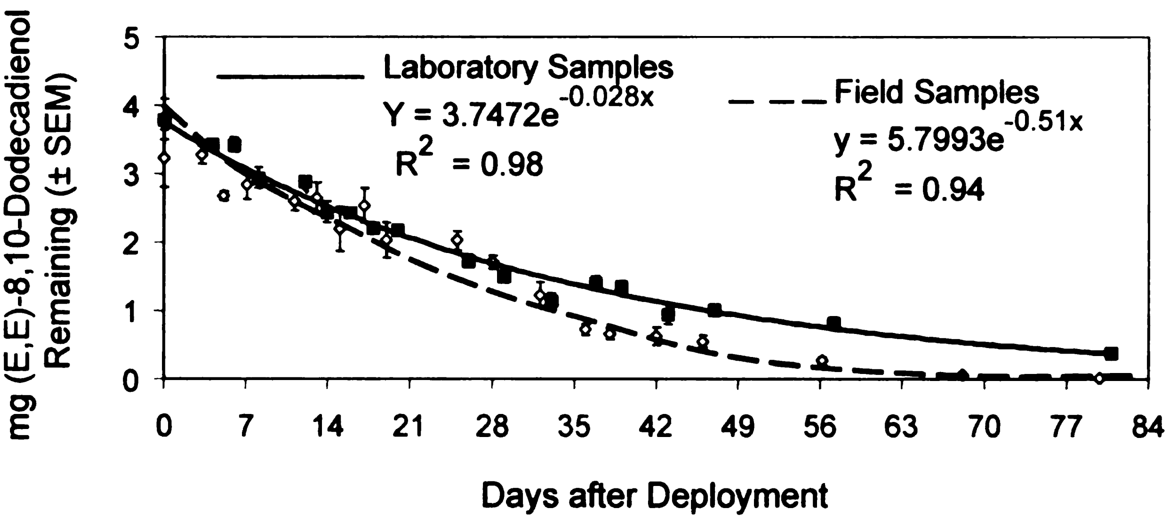

Figure 6. Release profiles of 0.1 ml paraffin-wax drops containing 5 % codlemone fitted with exponential decay curves.

DISCUSSION

The highest density of pheromone-dispenser release sites yielded the best orientational disruption of male CM to traps and lowest fruit injury. Increased orientational disruption as a function of increasing release-site density occurred both when the total amount of pheromone per ha was increased, and when it was held constant. These results corroborate findings of previous studies of release-site density on disruption for other moth species (Charlton and Cardé 1981, Palaniswamy et al. 1982, Suckling et al. 1994, de Lame 2003). In the current study, 96-97 % orientational disruption was achieved with 1000 Isomate C Plus ropes / ha distributed as 1000 point sources / ha, and 94-99 % disruption was achieved with 27,300 paraffin-wax drops per ha. Given that Isomate-C Plus dispensers release pheromone at ca. 20 µg / h (Knight 1995b), and paraffin-wax drops released ca. 2 µg / h during the first 2 weeks in the field, the amount of pheromone released / ha / h for 1000 Isomate dispensers and 27,300 wax drops was ca. 20 and 55 mg, respectively.

Alford and Silk (1983) and Suckling and Angerilli (1996) also varied release-site density while maintaining total number of pheromone point sources per ha constant. The former study was conducted with the spruce budworm, *Choristoneura fumiferana* (Clemens), and Hercon flakes (Hercon, Emigsville, PA) as the dispenser of synthetic pheromone. Intermediate densities of 33 and 100 points / m^2 of 60 and 20 flakes per release site yielded higher disruption than a uniform distribution of single flakes or 9 release sites of 222 flakes / site. These results are consistent with the current study in that the treatment with fewest release sites disrupted the least. That intermediate densities of flakes proved slightly better than the completely uniform distribution of one flake per release site in the Alford and Silk study is likely because clumps of 20 and 33 flakes / site competed with females to a higher degree than single flakes / site, given the low pheromone release rate from these dispensers. However, even with intermediate clumping, treatments with 20 and 33 flakes / release site were applied at high densities of 2000 and 660 total release sites / ha. Suckling and Angerilli (1996) studied the lightbrown apple moth and used polyethylene, reservoir-type dispensers similar to those used in our study. Our results were consistent with the above studies in that clumping of dispensers decreased disruption.

Current results are inconsistent with those of Knight (2004), who found that highly-spaced clusters of 100 Isomate-C Plus dispensers (4 / ha) disrupted CM males comparably to 500 dispensers / ha distributed individually. However, Knight's cluster treatment was supplemented by a 10-20 m-wide border application of Isomate-C Plus dispensers at a density equivalent to 1000 / ha, which was not applied to the highly-distributed Isomate-dispenser treatment. In addition, CM density in the current study was high (> 300 moths / trap for the season in control plots based on 3 traps per 0.4 ha) but was low in the Knight study (< 8 moths / trap for the season in control plots based on 2 traps / ha). Thus, contradicting results between our study and Knight's are likely due to differences in experimental design and CM population density.

On both plot borders and in plot centers, fruit injury was lowest when Isomate-C Plus dispensers were most highly distributed. Fruit injury in the 1,000 point source / ha treatment was almost two-fold less than in the 500 point source / ha treatment (Fig. 3 A, B). In addition, there was a significant overall relationship between increased release-site density and decreased fruit injury. It is important to note that seemingly small differences in percent disruption as measured by traps (eg. 93 – 96 % vs. 97 - 99 %) may be associated with widely differing crop protection outcomes.

CM males have been observed orienting to and landing within close proximity (mean 50 cm) of Isomate-C Plus dispensers placed directly within trees in pheromone-treated orchards (Stelinski et al. 2005b). In our recent flight-tunnel studies with CM males, pre-exposure of moths to Isomate-C Plus dispensers decreased male responsiveness to otherwise highly attractive lures (Stelinski et al. in review). This suggests that desensitization following pre-exposure to Isomate dispensers may have contributed to disruption of CM. More research will be required to determine relative importance of desensitization following pre-exposure to high-release, reservoir dispensers versus pure competitive attraction to false plumes.

Both trapping experiments and field observations confirmed that wax drops were highly attractive to male CM, suggesting that orientational disruption by wax drops functioned primarily by competitive attraction (false-plume following). Deployment of paraffin-wax dispensers at 8,200 and 27,300 drops / ha has recently been shown to provide better mating disruption of Oriental fruit moth, *Grapholita molesta* (Busck), than standard applications of Isomate-M Rosso dispensers in small plot trials (Stelinski et al. 2005a). However, this wax formulation still needs improvement for CM under high population densities in MI. Although 99 % orientational disruption was achieved in the current study with 27,300 drops / ha, this result lasted only ca. two weeks, after which disruption was not better than 50 % relative to control plots. This may have been due to the drop in codlemone release rate after d 14 (Fig. 6) or codlemone degradation (Millar 1995) after this interval. That wax drops deployed within delta traps were attractive to CM for up to four wk in our trapping study, while disruption failed after 2 wk, suggests photo-degradation of codlemone was a problem. A recently improved formulation incorporating antioxidants, as well as UV and visible light blockers deployed at 27,300 drops / ha proved equally effective to Isomate C-Plus dispensers at 1000 / ha for an entire moth generation (Stelinski et al. unpublished data) and holds promise as a cost-effective alternative mating disruption formulation for CM that can be machine-applied. The addition of insecticides to this formulation might enhance efficacy through removal of attracted males (Krupke et al. 2002).

ACKNOWLEDGEMENTS

We thank Krista Buehrer, Racheal Mallinger, Alison Gould, and Kevin Vogel for assistance with field application of pheromone dispensers and collection of trapping data. This research was funded in part by Michigan State University Project GREEEN.

CHAPTER TWO

Higher densities of uniformly distributed pheromone sources provide disruption of Oriental fruit moth (Lepidoptera: Tortricidae) superior to that of lower densities of clumped sources

Portions of this chapter are in press as:

de Lame' F.M., D.L. Epstein, L.J. Gut, H. Goldfarb, and J.R. Miller. 2010. Impact of Varying Density of Pheromone Point Sources on the Success of Mating Disruption of *Grapholita molesta* (Lepidoptera: Tortricidae), Econ. Entomol.

ABSTRACT

Field experiments quantified the effect of synthetic pheromone release-site density and distribution on: 1) orientational disruption of male Oriental fruit moths, *Grapholita molesta* (Busck) to pheromone-baited traps, and 2) fruit injury. A clustering test varied pheromone release-site density from 0 - 500 Isomate-M Rosso dispensers per ha while maintaining the total number of dispensers at 500. Percent orientational disruption of pheromone-baited traps increased significantly as a function of increasing density of release sites. Fruit injury decreased as the density of release sites increased and was lowest in plots treated with Isomate-M Rosso dispensers distributed as 500 point sources / ha.

INTRODUCTION

Mating disruption of Oriental fruit moths, *Grapholita molesta* (Busck), in the U.S.A. is implemented on over 45,000 acres of apple and peach (Gut *et al.*, 2004, Witzgal et al., 2008) and has a long history of success worldwide (Rothschild 1975, 1979, Vickers et al. 1985, Pfeiffer and Killian 1988, Audemard et al. 1989, Sexton and Il'ichev 2000, de Lame et al. 2007). Isomate® M 100, Isomate® M Rosso, Scentry NoMate® Oriental fruit moth Spirals, and CheckMate® SF are the reservoir dispensers available for Oriental fruit moth (OFM), with label rates varying from 250 to 500 per hectare.

Because the input costs of mating disruption products are often relatively expensive, many growers are apprehensive about using these products. Additionally, the application of hand applied dispensers is a labor intensive process (approximately 2 h per acre), adding labor costs as well as competition for that labor at times of the growing season when many growers are busy working in other crops.

Some researchers (Shorey and Gerber 1996, Knight 2004) suggest that mating disruption efficacy can be maintained while reducing the density of release sites, if overall release rate per ha is maintained or increased. A saving in labor is postulated for a given number of reservoir dispensers assembled into clusters rather than distributed individually (Knight 2004). However, Rothschild (1975) and Stelinski et al. (2005) showed that orientation disruption of male *G. molesta* to traps increased with increasing numbers of pheromone point sources.

Stelinski et al. (2005) showed decreased mating of tethered virgin females with increased numbers of pheromone dispensers. Only Rothschild (1975) kept the amount of pheromone applied per area constant. We report on studies investigating the effect of reducing point source densities while maintaining overall equivalent amounts of pheromone per unit area on orientation disruption of *G. molesta* using Isomate OFM-Rosso (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan). Understanding how distribution of pheromone release devices in an orchard affects orientational disruption of male moths is necessary to optimize efficacy of mating disruption for OFM.

MATERIALS AND METHODS

Experiments directly compared effects of varying numbers of pheromone point sources per unit area on *G. molesta* orientation disruption. Total pheromone per unit area was kept constant, while varying the number of evenly distributed point sources. Experiments were set up as randomized complete block designs with individual orchards as blocks.

This experiment was replicated in two apple orchards at TNRC, and in two commercial orchards in South Lyon and Flushing, MI, 4 May to 23 September, 2003 (Table 2). The two blocks at the TNRC were separated by at least 300 m and treatment plots at all commercial orchards were separated by at least 45 m. Four-hundred and ninety-four (123g active ingredient) Isomate-M Rosso polyethylene tube dispensers (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) were applied per ha in the upper third of tree canopies. The densities of release sites per ha were: 0 (0 point sources / plot, or 0 PTS), 10 PTS, 25 PTS, 90 PTS, 250 PTS, and 500 PTS. For the 500 PTS treatment, the dispensers were applied individually. For the 250 PTS treatment, two dispensers were spaced 2 - 3 cm apart at each release site. The 90 PTS treatment consisted of 5 dispensers per cluster; the 25 PTS treatment consisted of 20 dispensers per cluster, the 10 PTS treatment consisted of 50 dispensers per cluster. Pheromone release sites were spaced equidistantly throughout each 0.4 ha plot (Figure 7).

Table 2. Description of orchards used in which density of release sites (Isomate-C Plus dispensers) per ha was varied without varying total amount of pheromone deployed per ha.

Orchard No.	Cultivar(s)	Tree Age	Canopy Height	Tree Spacing
1	Red Delicious	16 yrs	3.5 – 4.5 m	3 x 6 m
2	Golden Delicious	10 yrs	3.5 – 4.5 m	1.8 x 4.6 m
	Red Delicious	10 yrs	3.5 – 4.5 m	3 x 5.5 m
	Red Delicious	20 yrs	4 – 5 m	3 x 5.5 m
	Rome	8 yrs	3 – 4 m	2.4 x 5.5 m
3	Braeburn	7 yrs	2.4 – 3.1 m	2.7 x 4.9 m
	Macintosh	7 yrs	2.4 – 3.1 m	2.7 x 4.3 m
	Fuji	7 yrs	2.4 – 3.1 m	2.4 x 4.6 m
	Jonagold	7 yrs	2.4 – 3.1 m	2.4 x 4.6 m
	Northern Spy	7 yrs	2.4 – 3.1 m	2.4 x 5.5 m
4	Gala	20 yrs	2.4 – 3.1 m	2.4 x 4.6 m
	Gala	20 yrs	3 – 4 m	2.7 x 4.9 m

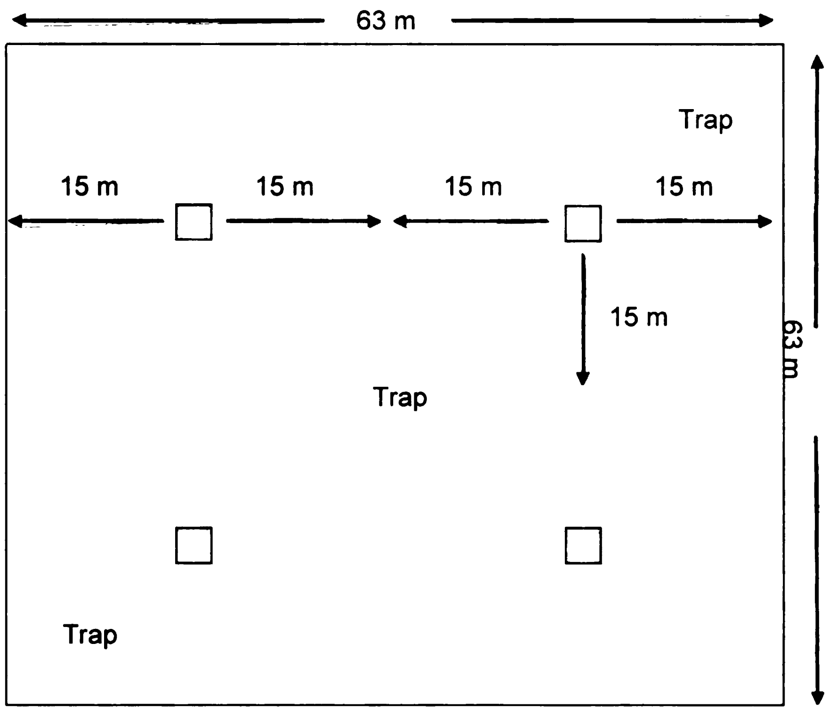

Figure 7. Diagram of a 0.4 ha orchard plot containing 4 release sites of clustered Isomate-M Rosso dispensers. Shaded boxes (not to scale) represent 50 clustered Isomate-M Rosso dispensers. Other release-site densities were likewise regularly distributed.

Multiple dispensers in the 10 PTS, 25 PTS, and 90 PTS treatments were attached to horizontally-mounted, (1 intersection/cm^2) galvanized-metal hardware cloth (TWP Wire Mesh, Berkeley, CA); dispensers were spaced 2.5 cm apart, as per Suckling and Angerilli (1996). Disruption of male moth orientation to sex pheromone was assessed using three pheromone-baited traps (LPD Scenturian Guardpost, Suterra, Bend, OR) per 0.4 ha plot, placed on a diagonal transect across each plot in the upper third of tree canopies. Two traps were located in plot outer corners, taking into account edge effects, while one was located in the center of each plot. The lures were red rubber septa loaded with 0.1 mg *G. molesta* sex pheromone. New pheromone lures were deployed every four weeks during each moth generation. Moths captured in traps were counted and removed once weekly. OFM fruit injury was evaluated in all plots in July and immediately prior to harvest. Thirty apples per tree, fifteen high in the canopy and 15 low in the canopy, were examined from 20 trees per plot (600 fruit / plot total).

The level of significance for all statistical tests was $\alpha = 0.05$. For orientational disruption and trapping studies, data were transformed to $\ln (x + 1)$ (which normalized distributions and homogenized variance) and then subjected to analysis of variance (ANOVA) with orchard as a blocking factor. Means were separated with Tukey's HSD test (StatSoft, Inc. 1984-2008). The Kruskal-Wallis test was also used to analyze orientation disruption data. If significant differences were identified using the Kruskal-Wallis test, means were separated with multiple comparisons of the mean ranks. The Wilcoxon Signed Rank test was used to analyze the effect of trap location (center or edge) on percent orientation disruption of *G. molesta* in plots treated with Isomate-M Rosso. Fruit injury data were arcsine transformed prior to ANOVA. Differences in pairs of means were separated using the LSD test (SAS Institute 2000). Fruit injury data were also subjected to regression analysis to describe the overall relationship between fruit injury and $\log_{10}$ of pheromone release-site density.

RESULTS

Overall, percent disruption of pheromone-baited traps increased significantly as a function of increasing density of pheromone release sites ($F=12.71$, $df = 5$, $P < 0.000$) (Figure 8). Only at the highest densities of point sources per ha tested (250/ha & 500/ha), did dispensers perform well, with most plots achieving at least 95% orientation disruption.

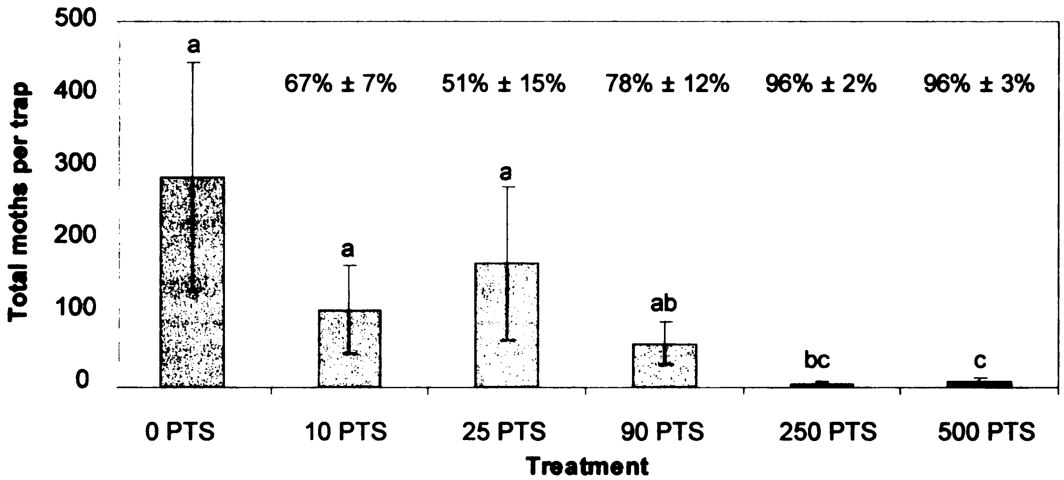

Figure 8. Numbers of male *G. molesta* captured in lure-baited traps in plots treated with Isomate-M Rosso during *G. molesta* generations one, two, and three (mean ± S.E.). Bars labeled with the same letter are not significantly different (Tukey, $P \geq 0.05$). Percent orientation disruption values (mean ± S.E.) are written above treatment bars.

Orientation disruption was 40% lower in traps placed in plot edges than traps placed in plot middles in the 10 PTS plots, 57% lower in 25 PTS plot, and 43% lower in the 100PTS plot (Table 3). It was only when point source density increased to 250 and 500 PTS that orientation disruption was more uniform across the entire plot. Orientation disruption at 250 PTS and 500 PTS was 6%

and 7% lower in edge traps respectively. There was no significant effect of generation ($S = 2.56$, df = 2, $P = 0.28$).

Table 3: Percent orientation disruption to traps placed in the center versus the edge of plots treated with Isomate-M Rosso (mean ± S.E.). Values in a row followed by the same letter are not significantly different (Wilcoxon Signed-Rank Test $P \geq 0.05$).

	Trap Location			
Treatment	Center	Edge	Z	P
10 PTS	77.7 ± 9.4a	58.7 ± 10.6a	1.41	0.16
25 PTS	86.5 ± 4.5a	29.8 ± 15.5b	2.85	0.004
90 PTS	99.8 ± 0.1a	64.1 ± 12.7b	2.67	0.008
250 PTS	99.1 ± 0.4a	95.9 ± 1.6b	2.67	0.008
500 PTS	100.0 ± 0.0a	94.3 ± 4.2b	2.02	0.04

Percentage of infested fruit decreased as density of release sites increased. Fruit injury in both plot centers and borders was significantly reduced relative to control in plots treated with 500 release sites / ha (F's = 4.4, 5.0, respectively, df = 5, 18, $P < 0.01$). Among pairs of means, there was no significant ($P > 0.05$) difference between fruit injury in plots with 100 through 500 release sites / ha. Overall relationships between decreasing fruit injury as a function of increasing $\log_{10}$ of release-site density were described by $y = -0.23x + 3.8$ ($R^2 = 0.84$) and $y = -0.25x + 4.2$ ($R^2 = 0.8$) for plot centers and borders, respectively. Slopes for these relationships were significantly (F's = 20.5, 13.1, df = 1, 5, $P < 0.05$) negative.

DISCUSSION

These studies show that decreasing point source density, even while keeping the amount of pheromone applied per ha constant, is not a viable option for reducing the cost of deploying *G. molesta* mating disruption with hand-applied dispensers. Only at the highest densities of point sources per ha tested did dispensers perform well, achieving at least 95% orientation disruption. Also, orientation disruption was significantly higher in center traps versus edge traps in all treatments except for the 4 PTS treatment, and variability in percent orientation disruption was consistently higher in edge traps versus center traps, although the difference was smaller for the 4 PTS treatment. These results agree with prior studies of tortricid mating disruption that identify orchard borders as being more susceptible to higher levels of fruit injury, reinforcing the need for area-wide mating disruption programs to make the best use of the mating disruption technique (Thompson et al. 1999, Il'ichev et al. 2002, Gut et al. 2004).

These results are at odds with Rothschild (1975), who found no significant difference in the numbers of *G. molesta* captured in pheromone-baited traps when the dispensers were applied at a range of 25 to 200 point sources per hectare. Stelinski et al. (2007) achieved 94% and 97% orientation disruption of *G. molesta* with a perimeter application of 2.5 Puffers/ha releasing 600 mg/ha/d of pheromone. Average total moth captures per trap in Stelinski et al.'s (2007) control plots were 26.4 during the first generation, and 70.1 during generations two and three combined. Moth captures in the control plots in the current experiments were up to eight times higher (Fig. 2). Low moth populations may explain the higher than expected level of *G. molesta* orientation disruption with Puffers in Stelinski et al.'s (2007) experiments. Stelinski et al. (2007) states that puffers may result in mating disruption by different mechanism(s) than hand-applied dispensers. Most hand-applied dispensers cause mating-disruption by competitive attraction (Miller et al. 2006b). Perhaps Puffers function by other mechanisms, such as camouflage, desensitization, or sensory imbalance (Miller et al. 2006a). Alternatively, perhaps aerosolizing pheromone leads to better distribution of the compounds throughout an area, versus diffusion of pheromone through a matrix. Further studies should be conducted to investigate the effect of varying point source densities of Puffers versus hand-applied dispensers while keeping the rate of pheromone released per ha constant.

CHAPTER THREE

Within-canopy adult distribution of codling moth (Lepidoptera: Tortricidae) in mating disrupted and non-disrupted apple orchards

ABSTRACT

A study aimed at determining the location of searching codling moth (*Cydia pomonella* [L.]) (Lepidoptera, Tortricidae) males and calling females in mating disrupted and non-disrupted plots was conducted in Michigan, USA in 2005. A leaf blower, converted into a vacuum for sampling codling moth adults on branches and in the tree canopy, had a 20-24% success in recovering released moths in orchard conditions. A series of four collections was made during the hours of 09:00-18:00 from May 25 through June 15, and a second series of four collections was completed during the hours of 18:00-22:00 from July 20 to August 22. Only eight codling moth adults were collected during the four daylight samples; one female and two male moths were sampled from the top third of the tree canopy, four males were sampled from the middle third of the tree canopy, and one male was sampled from the lower third of the tree canopy. Distributions of adults were also assessed during daytime hours (09:00-18:00) by fogging trees with various pyrethroid insecticides. No codling moth adults were collected in any of these samples. In contrast to the low number of moths collected in the daytime samples, 94 moths were collected during four twilight samples, with equal numbers sampled in disrupted and non-disrupted plots. In mating disruption plots, 42% of females were found in the top third of the tree canopy, 46% were found in the middle third, and 12% were recovered in the lower third. The 22 females sampled from non-disrupted plots were more evenly distributed, with 36.4% in the top third, 36.4% in the middle third, and 27.2% in the lower third of the tree canopy.

INTRODUCTION

Mating disruption of codling moth (*Cydia pomonella* [L].) using various hand-applied dispensers has become an accepted practice, with approximately 77,000 ha of apple in North America treated in 2008 (Witzgal *et al.* 2008). Research with pheromone baited traps showing male activity to be greater in the upper than the lower portion of the tree canopy has resulted in the standard recommendation that dispensers be applied within the top meter of the tree canopy (Barret 1995, Knight 2000, McNally and Barnes 1980). Witzgall *et al.* (1998) visually observed male moths flying and searching branches in the upper half of tree crowns, providing further support for deployment high in the canopy. However, the distribution of codling moth females within the tree canopy has yet to be determined.

The studies reported here summarize an investigation of the location of codling moth males and females in apple orchards during daylight and twilight hours. To directly measure moth spatial distribution within the tree canopy, a vacuum sampling technique was developed. The long-term aim of this work is to improve the effectiveness of codling moth mating disruption based on a solid understanding of adult distribution within the canopy.

METHODS AND MATERIALS

Vacuum: A 10 horsepower (Briggs & Stratton Intek engine) leaf blower (MacKissic Inc., PA, USA) with a 34cm diameter impeller and 322KPH air velocity, was converted into a vacuum powerful enough to remove codling moth adults from sampled surfaces. A 2m length of 15cm diameter reinforced rubber air hose (Cathey Co, Lansing, MI) was attached to the leaf blower air intake opening. The open end of the rubber hose was attached to a 2m long wand constructed of 15cm diameter heat duct sheet metal. A handle constructed of 1m long, 2.5cm diameter metal was attached to the wand with hose clamps. Four 3.5cm long bolts were attached at 90-degree spacing 2.5 cm from the end of the sheet metal wand. Moths were collected from trees into a 19-liter nylon mesh paint strainer bag (Master Craft, El Monte, CA, USA) inserted into the wand, folded back over the bolts, and secured with rubber bands to prevent the bag from being suctioned into the impeller.

Recovery Efficacy of Vacuum: Mark, release and recapture trials were used to test the efficiency of the vacuum. An initial 2005 test of whether the vacuum was capable of collecting moths from apple trees was done using 2m tall potted Red Delicious trees in a glass greenhouse at Michigan State University. The procedure entailed three individual releases of twenty laboratory-reared moths onto four trees and immediately vacuuming the trees to recover moths.

Two release/recapture trials were performed in the field in 2005 with the 10 hp leaf blower vacuum. Moths were marked by placing them in a 19L plastic pail and spraying 0.5g dye in 75ml acetone through cheesecloth covering the top opening of the pail. Forty laboratory-reared moths were marked and released on two occasions onto individual 16-year old trees, 3 X 6m Red Delicious, 4m-5m in height, at the Trevor Nichols Research Complex.

Orchard Vacuum Trials: All orchard vacuum collections were done in six 0.4 ha apple plots at the Trevor Nichols Research Center, Fennville, MI

(42°35'38.10"N, 86°06'05.92"W). Four plots were 16 year old Red Delicious (3 x 6m planting), 4m-5m in height, and two plots were 23 year old Macspur, (5.5m x 6m), 5m in height. Two of the Red Delicious plots and one Macspur plot were treated with Isomate C+ (Shin-Etsu Chemical Co., Tokyo, Japan) at the full label rate of 1000 dispensers/ha. The remaining three plots were not treated with pheromone. Daytime samples were collected between the hours of 9:00 to 16:00, twilight collections occurred between 18:00-21:30 hours. Twelve trees from each of the six plots were sampled on four dates for both daytime and twilight samples in 2005. Six of the 12 sample trees were located on plot perimeters and six trees were located in plot middles. Thirty-second samples were collected from the top third, middle third, and lower third of each tree. The lower third of the tree sample included the trunk to the soil surface, and weeds growing within 0.5m of the tree trunk. Sample contents were transferred from the nylon mesh collection bag located in the vacuum wand into 4L freezer bags, and identified in the laboratory. Only adult codling moths were counted in these collections, even though the vacuum was powerful enough to remove cocooned larvae (5 individuals) and pupae (7 individuals) from the trunks and scaffold branches of the trees in 2005 field trials. One trap (LPD Scenturian Guardpost, Suterra, Bend, OR) baited with a 1 mg codlemone rubber septum lure was placed in the upper third of the tree canopy in each non-mating disrupted plot to monitor codling moth flight.

Tree Fogging: Twelve trees were fogged with pyrethroid insecticides (tetramethrin and sumithrin) on two separate dates in 2005 to test this method for sampling codling moth adults in the tree canopy. Aerosol foggers were attached to the trunks of each tree. Trees were covered with 4ml plastic, and the foggers were activated until empty of contents. After a 10-minute interval, the plastic covers were removed, and trees shaken to dislodge codling moth adults, as well as other insects, onto tarps placed on the ground under each tree. All specimens were collected off of the tarps and taken to the laboratory for later identification.

Statistical Analysis: Statistical analyses were done using the chi-square goodness of fit test at alpha = 0.05.

RESULTS

Recovery Efficacy of Vacuum: The leaf blower vacuum recovered 70%-80% of moths released onto potted trees, and 24% of moths released in trees at the Trevor Nichols Research Center orchards in 2005. Recovery of released moths (14 releases) from covered trees within screened tents in 2006 was 20.6%.

Orchard Vacuum Trials: A total of 8 moths were collected during 576 individual daylight samples in 2005, 6 males and 2 females. The 2 females were captured from the top third of tree canopies (Table 4). Of total males captured, one was located in the top third, four in the middle third, and one in the bottom third of the tree canopies (Table 4). Twilight samples (576 individual vacuum samples) resulted in the capture of 94 moths distributed throughout the tree canopies; 15 males and 18 females in the top third of tree canopies, 23 males and 19 females from the middle third, and 10 males and 9 females from the bottom third of the tree canopies (Table 4). Moth captures were significantly higher in the evening hours for all tree canopy heights sampled (Table 5).

Table 4. Total moths collected with vacuum in 2005 orchard samples during daylight (09:00-16:00) and evening twilight (18:00-21:30) hours. (Day. Cap. = daytime captures, Eve. Cap. = evening captures).

	Top 1/3 Canopy		Middle 1/3 Canopy		Low 1/3 Canopy		Total	
	Day Cap.	Eve. Cap.	Day Cap.	Eve. Cap.	Day Cap.	Eve. Cap.	Day	Eve.
Male	1	15	4	23	1	10	6	48
Female	2	18	0	19	0	9	2	46

Table 5. 2005 moth captures were significantly higher in the evening hours for all tree canopy heights sampled (576 samples collected) (Numbers within row with same letter not significant at $p < 0.05$).

2005 Day vs. Night Total Moths (w/in Height)			
Height	Daytime Captures	Evening Captures	Chi Square Statistics
All	8b	94a	$X^2 = 72.5$, df = 1, $p < 0.0000$
High	3b	33a	$X^2 = 25$, df = 1, $p < 0.0000$
Middle	4b	42a	$X^2 = 31.4$, df = 1, $p < 0.0000$
Low	1b	19a	$X^2 = 16.2$, df = 1, $p < 0.0001$

Evening moth samples were not significantly higher between the top 1/3 of the tree canopy and the middle third, but were significantly higher in the top 1/3 of the canopy when comparing high and low, significantly higher in the middle 1/3 canopy compared to the lower 1/3, and significantly higher in the combined lower

2/3 of the tree canopy as compared to the top 1/3 (Table 6). No differences were seen either by gender or time of evening sampled.

Table 6. Statistical comparisons of moths collected with vacuum in 2005 orchard samples during twilight (18:00-21:30) hours (Numbers within row with same letter not significant at $p < 0.05$).

2005 Evening (w/in Height)			
Height	Total Captures	Total Captures	Chi Square Statistics
High vs. Mid	High: 33a	Mid: 42a	$X^2 = 1.1$. df = 1, $p < 0.2987$
High vs. Low	High: 33b	Low: 19a	$X^2 = 3.8$, df = 1, $p < 0.0522$
Middle vs. Low	Mid: 42b	Low: 19a	$X^2 = 8.7$, df = 1, $p < 0.0032$
High vs. lower 2/3	High: 33b	Mid + Low: 61a	$X^2 = 8.3$, df = 1 $p < 0.0039$

Moths were distributed evenly between plot perimeters and plot centers in both pheromone mating disrupted and non-disrupted plots in the 2005 vacuum samples (Figure 9). In pheromone mating disruption plots there were significantly more males in the combined lower 2/3 of tree canopy vs. top 1/3 of canopy (Table 7). No significant differences in male capture were seen in comparisons of high vs. middle, high vs. low or middle vs. low. In disrupted plots, 46% of female moths and 43% of male moths were collected from the middle third of the tree canopies, while 42% of females and 22% of males were in the top third of the tree canopies (Tables 7, 8 and 9). An equal percentage of females in non-disrupted plots were collected from the top (36%) and middle (36%) thirds of the tree canopies. Male distribution in plots not treated with pheromone was skewed toward the top two thirds of the tree canopies, with 52% collected from the middle and 40% from the top (Tables 7, 8 and 9). Thirty-five percent of males were collected from the bottom third of the tree canopies in disrupted plots, while only 8% were vacuum collected in the lower canopy in non-disrupted plots.

Tree fogging with pyrethroid insecticides applied on 2 occasions to 12 covered trees during daylight hours did not result in the capture of any codling moth in 2005.

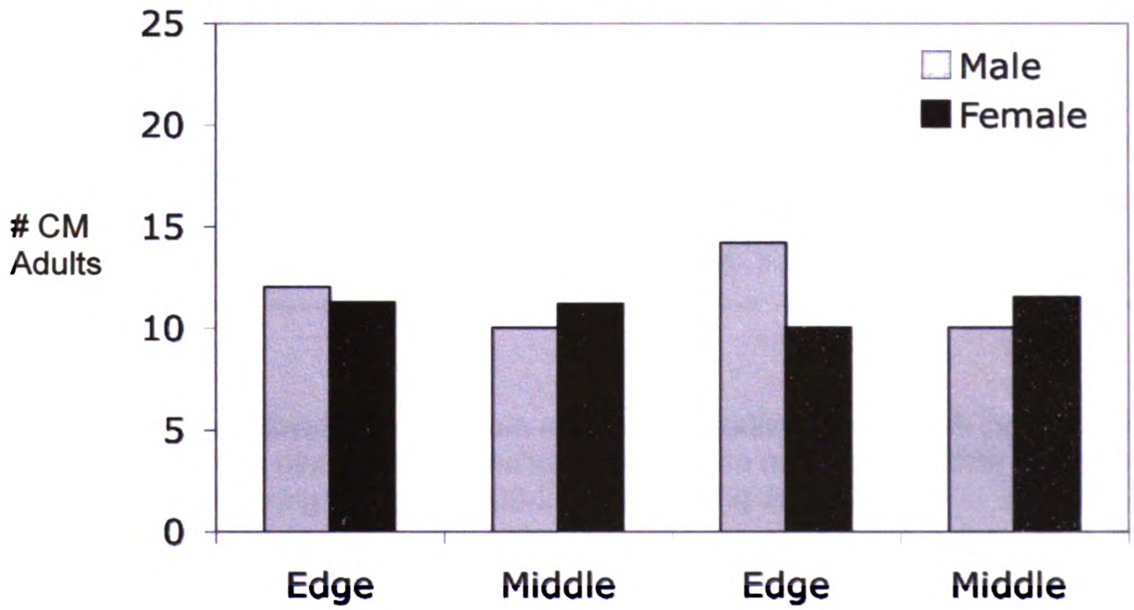

Figure 9. Vacuum Samples showed that males and females were present in both orchard edges and middles.

Table 7. Statistical comparisons of moths collected with leaf blower vacuum in plots with and without pheromone mating disruption. Samples were collected during twilight (18:00-21:30) hours in 2005 (Numbers within row with same letter not significant at $p < 0.05$).

2005 Male Moth Captures in MD Plots vs. Non-MD Plots			
MATING DISRUPTED PLOTS - MALES			
Height	# Moths	# Moths	Chi Square Statistics
High vs. Middle	High: 5a	Mid: 10a	$X^2 = 1.7$, df = 1, $p < 0.1966$
High vs. Low	High: 5a	Low: 8a	$X^2 = .692$, df = 1, $p < 0.4054$
Middle vs. Low	Mid: 10a	Low: 8a	$X^2 = .692$, df = 1, $p < 0.6375$
High vs. low 2/3	High: 5a	Mid + Low: 18b	$X^2 = 7.35$, df = 1, $p < 0.0067$
NON-MATING DISRUPTED PLOTS - MALES			
Height	# Moths	# Moths	Chi Square Statistics
High vs. Middle	High: 10a	Mid: 13a	$X^2 = .391$, df = 1, $p < 0.5316$
High vs. Low	High: 10a	Low: 2b	$X^2 = 5.33$, df = 1, $p < 0.0209$
Middle vs. Low	Mid: 13a	Low: 2b	$X^2 = 8.07$, df = 1, $p < 0.0045$
High vs. low 2/3	High:10a	Mid + Low: 15a	$X^2 = 1$, df = 1, $p < 0.3137$

Table 8. Percent distribution of male and female codling moths collected with leaf blower vacuum in plots with and without pheromone mating disruption. Samples were collected during twilight (18:00-21:30) hours in 2005.

	MD		No MD	
	Male	Female	Male	Female
High	21.7%	41.6%	40.0%	36.4%
Middle	43.5%	45.8%	52.0%	36.4%
Low	34.7%	12.5%	8.0%	27.3%

Table 9. Statistical comparisons of female moths collected with leaf blower vacuum in plots with and without pheromone mating disruption. Samples were collected during twilight (18:00-21:30) hours in 2005 (Numbers within row with same letter not significant at $p < 0.05$).

2005 Female Moth Captures in MD Plots vs. Non-MD Plots			
MATING DISRUPTED PLOTS - FEMALES			
Height	# Moths	# Moths	Chi Square Statistics
High vs. Middle	High: 10a	Mid: 11a	$X^2 = .048$, df = 1, $p < 0.8267$
High vs. Low	High: 10a	Low: 3b	$X^2 = 3.77$, df = 1, $p < 0.0522$
Middle vs. Low	Mid: 11a	Low: 3b	$X^2 = 4.57$, df = 1, $p < 0.0325$
High vs. low 2/3	High: 10a	Mid + Low: 14a	$X^2 = .667$, df = 1, $p < 0.4141$
NON-MATING DISRUPTED PLOTS - FEMALES			
Height	# Moths	# Moths	Chi Square Statistics
High vs. Middle	High: 8a	Mid: 8a	$X^2 = 0$, df = 1, $p < 1$
High vs. Low	High: 8a	Low: 6a	$X^2 = 0.29$, df = 1, $p < 0.5928$
Middle vs. Low	Mid: 8a	Low: 6a	$X^2 = 0.29$, df = 1, $p < 0.5928$
High vs. low 2/3	High: 8a	Mid + Low: 14a	$X^2 = 1.6$, df = 1, $p < 0.2009$

DISCUSSION

Orchard vacuum samples show codling moth adults to be distributed throughout all parts of the tree canopies during evening periods when female calling and male searching activity is occurring. Higher numbers of moths were captured at mid-tree canopy than in either the top 1/3 canopy or low 1/3 canopy, with significantly more moths captured in the combined lower 2/3 than in the top 1/3 of the tree canopy (Table 6).

The pheromone treatment did have a fairly pronounced effect on the distribution of codling moth adults. In plots under pheromone mating disruption, female moths were captured in approximately equal numbers from the top 1/3 and mid 1/3 of tree canopies, with few captured in the lower 1/3 of tree canopies (Tables 8 and 9). In non-mating disrupted plots, female moths were captured in relatively equal amounts from all three strata (Tables 8 and 9). The effect of the pheromone treatment was more evident with respect to male distribution. Male moths were collected in greater numbers in the lower 2/3 of trees in mating disrupted plots, and the upper 2/3 of trees in non-mating disrupted plots, suggesting that the presence of dispensers had an inhibitory effect on male behavior. The largest number of males was collected from the middle tree canopy in both disrupted and non-disrupted plots (Tables 7 and 8).

Current protocols for the deployment of hand applied mating disruption dispensers call for dispensers to be placed exclusively in the top meter of tree canopies, based on research showing male activity to be concentrated in the top third of the tree canopy (Barret 1995, Knight 2000, McNally and Barnes 1980, Witzgall *et al.* 1998). Our research with the vacuum sampling of male and female moths disputes these findings for males, and is unique in determining the locations of calling female moths. The results of this study indicate the need to conduct additional research addressing whether placement of dispensers at a range of heights within the tree may improve the overall performance of mating disruption targeting codling moth.

The low number of moths captured with the vacuum during daylight collections, paired with zero capture of moths with the pyrethroid treatments raises the question of whether moths move to other habitats during periods of behavioral inactivity. Tree fogging with pyrethroid insecticides applied on 2 occasions to 12 covered trees during daylight hours did not result in the capture of any codling moth in 2005. It is possible that the failure to collect moths using these techniques resulted from a lack of moths in the tree at the time of sampling, inadequacy of the techniques for sampling inactive adults, or a combination of both.

ACKNOWLEDGEMENTS

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CHAPTER FOUR

Effects of varying height in the canopy of hand-applied reservoir dispensers on efficacy of pheromone-mediated mating disruption of codling moth (Lepidoptera: Tortricidae) in apple

ABSTRACT

Trials investigating the effects of varying height in the tree canopy of codling moth (*Cydia polmonella* L.) mating disruption reservoir dispensers were conducted in 2007. Two Isomate-C Plus dispensers / tree were placed at heights of 2m, 4m and/or a combination of one dispenser at each height. Pheromone-baited traps and tethered virgin female moths were similarly placed in plots at 2m and 4m to assess treatment effects. Traps placed high in tree canopies captured greater numbers of male moths than traps placed low, regardless of dispenser positioning. The fewest moths were captured where both traps and dispensers were placed high in the tree. Mating of virgin female moths was highest when moths were tethered at the 2m height in plots with dispensers placed at 4m, and when moths were tethered at the 4m height in plots with dispensers placed at 2m. Overall, mating of females was lowest where dispensers were equally distributed at 2m and 4m in the tree canopy.

INTRODUCTION

Mating disruption of codling moth (*Cydia pomonella* [L].) using various hand-applied dispensers has become an accepted practice, with approximately 77,000 ha of apple in North America treated in 2008 (Witzgal *et al.* 2008). Research with pheromone baited traps showing male flight behavior to be concentrated in the top third of the tree canopy has resulted in the standard recommendation that dispensers be applied within the top meter of the tree canopy (Barret 1995, Knight 2000, McNally and Barnes 1980). Witzgall *et al.* (1998) visually observed male moths flying and searching branches in the upper half of tree crowns, providing further support for deployment high in the canopy.

Epstein *et al.* (unpublished, chapter 3 of this dissertation) developed a vacuum sampling technique to directly measure male and female moth spatial distribution within the tree canopy in orchards with and without codling moth mating disruption. Results from this study showed male moths were collected in greater numbers in the lower 2/3 of tree canopies in plots under pheromone mating disruption, while female moths were captured in approximately equal numbers from the top 1/3 and mid 1/3 of tree canopies in disrupted plots. In plots not treated with pheromone, male moths were collected in greater numbers in the upper 2/3 of trees, and female moths were captured in relatively equal amounts from all parts of the tree. More males were found in the middle tree canopy in both disrupted and non-disrupted plots (Epstein *et al.*, unpublished).

Recent work by Miller *et al.* (2006a and 2006b) provides strong evidence of competitive attraction as a primary mechanism by which pheromone mating disruption of codling moth operates in the field. Optimum disruption is likely to be achieved when calling females and competing dispensers are in close proximity. Thus, if calling female moths are distributed in equal amounts in both upper and middle tree canopies (Epstein, unpublished), dispenser placement should not be confined solely to the top 1/3 tree canopy as is currently practiced, but placed in the middle tree canopy as well.

The studies reported here summarize an investigation of the effects of distributing pheromone mating disruption dispensers at varying heights within the tree canopy on orientational disruption of male moths to pheromone baited traps, and mating of tethered virgin female moths. The long-term aim of this work is to improve the effectiveness of codling moth mating disruption based on improved understanding of adult distribution within the canopy and the impact of dispenser placement on moth behavior.

METHODS AND MATERIALS

Four 1.2ha replications of 4 dispenser height treatments were randomly assigned to orchard plots at the Trevor Nichols Research Complex, Fennville, MI (42°35'38.10"N, 86°06'05.92"W) in a 16 year old Red Delicious planting, 3 x 6m tree spacing, 4m-4.5m tree height. All pheromone mating disruption plots were treated with Isomate C+ (Shin-Etsu Chemical Co., Tokyo, Japan) at the full label rate of 1000 dispensers/ha. The four dispenser height treatments were: 1) two Isomate C+ dispensers at 4m height in the tree canopy (High), 2) two dispensers at 2m height in the tree canopy (Low), 3) one dispenser at 4m and one dispenser at 2m in the tree canopy (Mixed), and 4) no dispensers deployed.

Inhibition of moth catch in pheromone traps was the principal means of assessing treatment effects. Four traps (LPD Scenturian Guardpost, Suterra, Bend, OR) baited with 1 mg codlemone rubber septum lures (Suterra, Bend, OR) were placed in each treatment (64 total), two at a height of 4m, two at a height of 2m. In all disrupted plots, traps were positioned at least 1 m away from an Isomate dispenser. Lures were replaced every two weeks. Traps were checked twice a week beginning at the start of first generation CM flight activity, from May 11 to August 24, 2007. At each collection date, the number of moths captured was recorded for each trap, trap liners cleaned or replaced, and traps at 2m were rotated to a 4m height, and traps at 4m were rotated to a 2m height within the same trees. Branches were flagged with colored plastic tape at the 2 and 4m heights to ensure that the traps returned to the same high and low positions upon rotations.

In addition to orientational disruption to traps, mating disruption was assessed based on mating of tethered, virgin females. Female *C. pomonella* used for tethering were reared from pupae purchased from Benzon Research Inc., Carlisle PA. Females were sorted in the pupal stage and placed into 50-ml plastic cages containing 5% sucrose in plastic cups with cotton dental wick protruding from their lids. Female *C. pomonella* were deployed within two days of pupal emergence for 16h (17:00 – 09:00hrs) in all treatment plots on five dates coinciding with peak moth presence during the first and second generations (13 June, 15 June, 26 July, 1 August and 2 August). Four female moths were tethered per treatment replicate (64 total), two at 4m and two at 2m in the tree canopy, during each deployment period. Female moths were secured to branches of trees with polyester thread (Jo-Ann Stores, Inc., Hudson, OH) tied to the base of the left wing. Moths were given at least 60 cm of thread and were observed for at least 30 s after deployment to ensure they remained tethered and mobile upon the branch. Approximately 68% of tethered females were recovered over the five deployments. Female moths were placed into alcohol in 8-dram glass vials upon collection, and dissected in the laboratory within 24h to determine mating status. Mating status was based on the presence or absence of a spermataphore in the bursa copulatrix.

Generalized linear models were evaluated to assess the characteristics of male moth capture in pheromone-baited traps and of mating of female moths. Moth capture and mating data were log transformed to address concerns with normality of error distribution. Generalized linear model analyses were performed in R version 2.10.0 (R Development Core Team, 2009, R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org); the mating data as a binomial distribution. The ANOVA command in R was used to test parameter significance of each model using analysis of deviance, and the Tukey multiple comparisons test was used for means separation.

RESULTS

Traps placed high in the tree canopy captured greater numbers of male moths at all dispenser height treatments (df=3, F=2.994, P< 0.0297), with the fewest moths were captured when both traps and dispensers were placed high in the tree (figure 10). Significantly fewer moths were captured in all pheromone treatments as compared to the no pheromone control (Table 10, Figure 11).

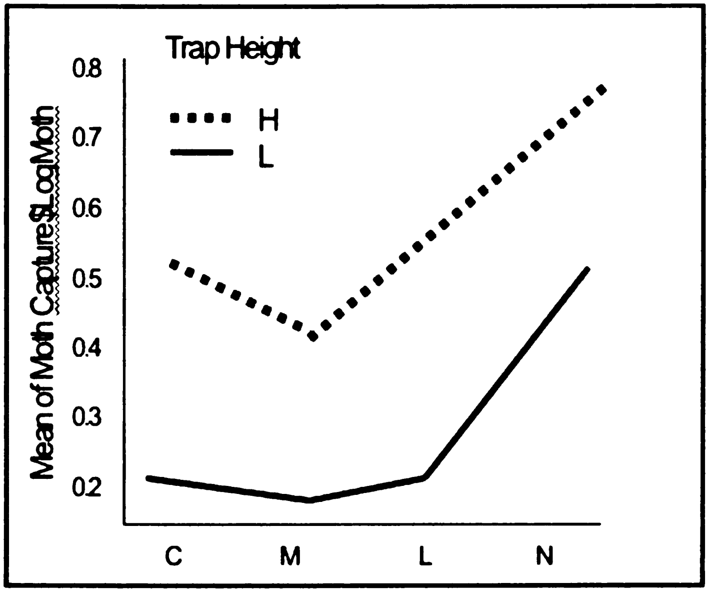

Figure 10. Interaction plot of effects of treatment and trap height on male codling moth capture (Mixed = dispensers high and low, H = high dispenser placement, L = low dispenser placemen and N = no pheromone dispensers).

Table 10. Tukey multiple comparison of the means of male moth capture in varying dispenser height treatments (M = Mixed, 1 dispenser each @2m and 4m; H = high, 2 dispensers @ 4m; L = low, 2 dispensers @2m; and N = no pheromone dispensers) * indicates significant p value @ $\alpha < 0.05$.

Comparison	Difference	Lower	Upper	P Adj
M-N	-0.2843	- 0.3512	-0.2173	0.0000*
H-N	-0.3521	0.4190	0.2852	0.0000*
L-N	-0.2426	-0.3099	-0.1753	0.0000*
H-M	-0.0679	-0.1347	-0.0010	0.0449*
L-M	0.0417	-0.0255	0.1089	0.3815
L-H	0.1096	0.0424	0.1767	0.0002*

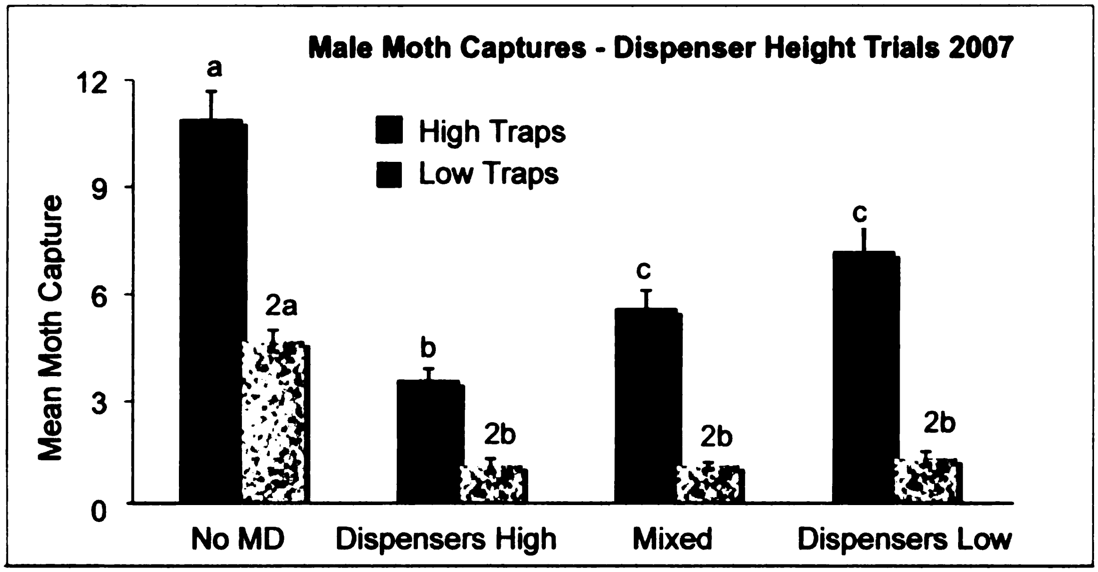

Figure 11. Captures of male moths in plots where pheromone dispenser and trap height was varied (High = 2@ m, Low = 2 @ 2m, Mixed = 1 @ m and 1 @ 2m). Means followed by the same letter are not significantly different at α < 0.05.

Evaluations of moth mating, though, reveal that a distribution of dispensers at a combination of heights, 4m and 2m, resulted in the lowest overall mating in these trials ($df = 222$, $z = -3.7$, $p < 0.00025$). Mean percent mated female moths was 15% in the mixed plots, followed by 19% mating in plots with 2 dispensers @ 4m, 21% mating in plots with 2 dispensers @ 2m and 46% in plots where no dispensers were deployed. Mating of virgin female moths was highest where moths were tethered at a 2m height in plots with dispensers placed at 4m and where moths were tethered at the 4m height in plots with dispensers placed at (Tables 11-13, Figure 12).

Table 11. Mating of tethered female moths in pheromone dispenser trials was lowest where dispensers were arranged both at 2m and 4m heights in the tree canopy.

Treatment	df	z value	Pr(>|z|)
No Dispensers	222	-0.267	0.78931
Mixed (1 ea. @ 2m & 4m)	222	-3.662	0.00025 *
High (2 dispensers @ 4m)	222	-3.525	0.00042 *
Low (2 dispensers @ 2m)	222	-2.855	0.00431 *

Table 12. Percent of tethered virgin female codling moths mated when deployed on five dates at 2m and 4m heights in tree canopies in plots with varying dispenser height treatments (Mixed = dispensers @ 2m and 4m, High = dispensers at 4m, Low = dispensers @ 2m)

Pheromone Treatment	Female Height	13-Jun	15-Jun	26-Jul	1-Aug	9-Aug	Means
No MD	4m	1.00	0.86	0.25	0.40	0.00	0.50
No MD	2m	0.67	0.75	0.33	0.20	0.17	0.42
Mixed	4m	0.00	0.50	0.20	0.00	0.00	0.14
Mixed	2m	0.17	0.50	0.17	0.00	0.00	0.17
High	4m	0.67	0.40	0.00	0.00	0.13	0.24
High	2m	0.14	0.20	0.17	0.00	0.17	0.14
Low	4m	0.43	0.20	0.20	0.20	0.33	0.27
Low	2m	0.14	0.20	0.00	0.00	0.40	0.15

Table 13. Tukey multiple comparison of the means of mating in varying dispenser height treatments (M = mixed, 1 dispenser each @2m and 4m; H = high, 2 dispensers @ 4m; L = low, 2 dispensers @2m; and N = no pheromone dispensers) * indicates significant p value @ $\alpha < 0.05$.

Treatments	Diff	lwr	upr	p adj
M-N	-0.3571	-0.5685	-0.1458	0.0001*
H-N	-0.3103	-0.5069	-0.1137	0.0004*
L-N	-0.2640	-0.4679	-0.0600	0.0052*
H-M	0.0469	-0.1583	0.2520	0.9346
L-M	0.0932	-0.1190	0.3054	0.6673
L-H	0.0463	-0.1513	0.2439	0.9298

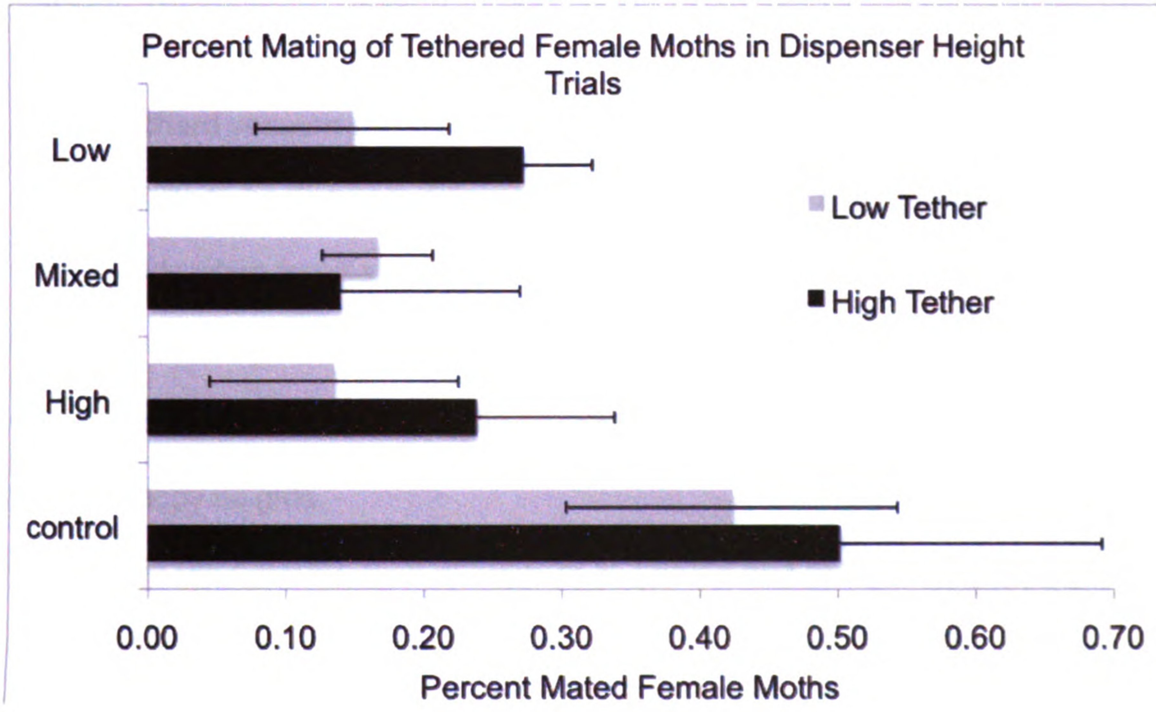

Figure 12. Mating of tethered females at 2m and 4m in plots where dispenser height was varied (High = 2@ 4m, low = 2@ 2m, Mixed = 1@ 2m, 1@4m).

DISCUSSION

Many researchers have concluded that codling moth distribution and behavioral activity is greatest in the top 1m of tree canopies, both in pheromone mating disrupted orchards and in non-disrupted orchards (Barret 1995, Knight 2000, McNally and Barnes 1980, Riedl *et al.* 1979). Weissling and Knight (1995) found that males responded in greatest numbers to tethered females high in tree canopies in mating disrupted orchards where dispensers were all placed at either 2m or 4m, but that there was no statistical difference in the number of mated females tethered at 2m or 4m in either treatment. These findings have resulted in the recommendation that pheromone dispensers be placed high in the tree canopy.

Orchard vacuum samples in 2005 showed both codling moth male and female adults to be distributed throughout all parts of the tree canopy during the time periods when female calling and male searching activity is occurring (Epstein, unpublished). Weissling and Knight (1995) also found that high captures of male and female moths on unbaited sticky traps occurred at mid and upper-canopy heights.

With the determination of Miller *et al.* (2006a and b) that competitive attraction is a primary mechanism in pheromone-mediated disruption of codling moth, it follows that a distribution of female moths throughout the tree canopy calls for a similar distribution of dispensers in the upper and mid canopy levels to reduce codling moth mating. The findings of this study shows that a distribution of pheromone dispensers in the upper and mid canopies of trees did, indeed, result in reduced overall mating of tethered female moths, and suggests that recommendations for placement of pheromone ties should be revisited based on recent findings of moth distribution and mechanism of codling moth pheromone disruption.

ACKNOWLEDGEMENTS

We thank the Michigan Apple Research Committee for funds supporting this research.

CHAPTER FIVE

Codling Moth, *Cydia pomonella* (L.), adult diel activity in apple orchards

ABSTRACT

Release / recapture studies of marked adult codling moths were conducted in 2006 and 2007 in screened tents to discern within orchard habitats for adult moths during the daylight hours, 09:00 – 18:00. A leaf blower, converted into a vacuum for sampling codling moth adults in the tree canopy and from ground surfaces, had a 20-24% success in recovering released moths in orchard trees and a 22% recovery rate from the orchard herbicide strip and drive-row grass. Of moths recaptured, 9% of females and 9% of males were recaptured from the ground (herbicide strip and drive-row grass) 16 hours post release, 40% of females and 8% of males from the ground 40 hours post release, and 52% of females and 24% of males from the ground 64 hours post release. Weather data loggers monitored over three months during release / recapture trials showed little variance in temperature between the three habitats measured, tree 22.1°C, H-strip 22.6°C, and grass 22.1°C, but relative humidity was more than 12% higher in the grass than other habitats; tree 78.7%, H-strip 79.5%, and grass 92%.

INTRODUCTION

The studies reported here summarize a two-year investigation of the location of codling moth males and females in apple orchards during daylight hours, 09:00 – 18:00. It is widely understood that codling moth is behaviorally active during evening twilight hours, approximately the hours of 19:00 – 22:00 (Castrovillo and Cardé 1979, Geier 1963, Witzgall *et al.* 1998). Very little is reported in the literature regarding codling moth behavior and habitat selection during periods of biological inactivity, and it is largely assumed that adult moths confine themselves within the tree canopy during inactive periods (Geier 1963).

However, direct sampling of tree canopies during the hours of 09:00 – 18:00 by Epstein *et al.* (2005, unpublished, chapter 3 of this dissertation) located only eight moths in 576 samples of tree bark and foliage over four dates in 2005. By comparison, evening twilight samples located 94 moths on tree bark and foliage in 576 samples over four dates (Epstein unpublished, chapter 3 of this dissertation). Additionally, direct visual observations of codling moths emerging from orchard drive-row grass during evening twilight hours, 19:00 - 20:00 (Epstein, unpublished), raised questions as to whether codling moth adults leave the tree canopy after periods of twilight biological activity.

Improved knowledge of codling moth bionomics, including diel behavior patterns and the affects of factors such as age and prevailing climatic conditions on female behavior, could lead to improved control of this key tortricid pest. In particular, the long-term aim of this work is to improve the effectiveness of codling moth mating disruption based on a greater understanding of diel patterns of moth activity.

MATERIALS AND METHODS

Vacuum: A 10 horsepower (Briggs & Stratton Intek engine) leaf blower (MacKissic Inc., PA, USA) with a 34cm diameter impeller and 322KPH air velocity, was converted into a vacuum powerful enough to remove codling moth adults from sampled surfaces. A 2m length of 15cm diameter reinforced rubber air hose (Cathey Co, Lansing, MI) was attached to the leaf blower air intake opening. The open end of the rubber hose was attached to a 2m long wand constructed of 15cm diameter heat duct sheet metal. A handle constructed of 1m long, 2.5cm diameter metal was attached to the wand with hose clamps. Four 3.5cm long bolts were attached at 90-degree spacing 2.5 cm from the end of the sheet metal wand. Moths were collected from trees into a 19-liter nylon mesh paint strainer bag (Master Craft, El Monte, CA, USA) inserted into the wand, folded back over the bolts, and secured with rubber bands to prevent the bag from being suctioned into the impeller. A 90-degree sheet metal register box was attached to the wand end for vacuuming ground surfaces. Collection bags for ground collections were made from fiberglass window screening tapered to 10cm at the closed end to avoid clogging the airflow through the wand.

Screen Tents: Four tents measuring 3m high, 5.5m wide, and 6.5m long were constructed at the Trevor Nichols Research Complex, Fennville, MI (42°35'38.10"N, 86°06'05.92"W). The tent structure was covered with mosquito netting walls and ceiling, sewn together to slip over the tent structure as one piece (Quality Awning Shops, Inc., Lansing, MI). Mosquito netting walls were staked into the ground with tent stakes to minimize open space where the tents met the ground. The tents were erected over single apple trees, encompassing drive row grass. Trees were 2.75m high Red Delicious, spaced 3X6m. Data loggers (Hobo Pro series, Onset Computer Corporation, Bourne, MA, USA) were placed in the tree and in the herbicide strip and grass at soil level to record daily temperature and relative humidity levels from 9 June to 29 August.

Release / recaptures. Release / recaptures of marked moths were conducted in 2006 and 2007 in screened tents to identify daytime (09:00-18:00) habitats for adult moths within the orchard. Moths were recaptured with the vacuum 16 hours post release in 2006 (15 releases), and 40 hours (13 releases, 6 dates) and 64 hours (17 releases, 7 dates) post release in 2007. Adult *C. pomonella* were reared from pupae purchased from Benzon Research Inc., Carlisle PA. Females and males were sorted in the pupal stage and placed into 50-ml plastic cages containing 5% sucrose in plastic cups with cotton dental wick protruding from the lids. Releases with wild type moths, collected as cocooned larvae from the first generation and reared to adulthood on corrugated cardboard in 50-ml plastic cages in the laboratory, were also conducted in 2006 (1 release, 30 ea, male and female) and 2007 (2 releases, 40 females and 20 males on 8 August, and 40 females and 25 males on 9 August).

Moths were marked by placing 20 male and or female individuals at a time into 13cm Petri dishes coated with differing colors of 0.4g luminescent powder (Bioquip Inc., CA, USA) for easy differentiation of gender upon recapture. The moths accumulated powder after several minutes of movement in the dish. Moths were released into tents within two days of pupal emergence between 18:00-19:00 hours and recaptured at 16 hours, 40 hours, and 64 hours post release, between the hours 10:00 to 14:00.

Release cages consisted of two 50-ml plastic containers with two 5cm X 10cm windows cut from each, so that the two containers could be oriented with windows non-aligned for transport, and aligned for moth release (Figure 13).

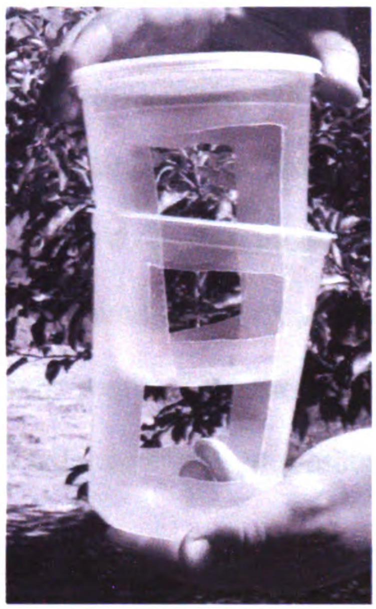

Figure 13. Release cages used in moth release / recapture trials.

Moths were released by placing 2 open release containers, one with 20 males and one with 20 females, within the tree canopy. Male and female moths were released in equal numbers and were segregated into separate release containers prior to all releases. All tree, herbicide strip and grass habitats were systematically vacuumed without time limit to recapture moths. Contents of mesh vacuum collection bags were transferred into 4L freezer bags for transport to the laboratory for identification.

Statistics. All statistical analyses were performed using version 2.10.0 of the R computing language (R Development Core Team, 2009, R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org). Spearman's rank correlation tests were performed to assess the level of correlation between percent moth recapture, after non-recovered moths were removed, in each possible habitat category (tree, drive-row grass, herbicide strip) by either Julian date, hours between release and recapture, and by average daily temperature, respectively. Furthermore, a 4 + 4 ANOVA modeling proportion recapture, after non-recovered moths were removed, by block and habitat was performed with post-hoc multiple comparisons made using the Tukey Honest Significant Difference method. For the latter analysis, proportion recapture was normalized using the arcsin transformation.

RESULTS

Fifteen release / recapture trials in screened tents in 2006 resulted in 47.7% of 596 released moths being recovered from one of five habitats after 16 h. Of recovered moths, 29.2% were found in the tree, 5.3% in the grass, 8.8% in the herbicide strip, 32.4% on the tent screen surface and 24.3% did not leave the release cage (Table 14). In 2007, 25.3% of released moths were recovered after 40 h (13 releases) and 45.7% were recovered after 64 hours (17 releases) (Table 14). Significantly more moths were recovered from the tree than either the grass or the herbicide strip (Table 15). No statistically significant correlation was found between percent moth recapture in varying habitats and Julian date, hours between release and recapture or ambient temperature.

Although not statistically significant, higher percentages of female moths were consistently collected from drive-row grass and weeds under the trees than male moths, 14.6% of females and 13.5 % of males after 16 hours, 17.4% of females and 3.4% of males after 40 hours, and 16.9% of females and 7.8% of males after 64 hours (Table 14). When analyzed temporally, no male moths were found in the grass or herbicide strip in June, 3.7% of males were recovered from the ground in July and 11.7% from the ground in August (Table 16). Seven percent of female moths were recovered from ground surfaces in June, 11% in July and 22.6% in August (Table 16).

Table 14. Percent male and female codling moth adults recovered in five habitats in release / recapture trials in 2006-2007 (* = release cage).

Moth Recovery 16, 40 and 64 Hrs Post Release in 2006 - 2007 Trials						
16 Hrs	Male	Female	Total	Mean % Recovered	% Males Recovered	% Females Recovered
Tree	31	52	83	29.2	24.6	32.9
Grass	6	9	15	5.3	4.8	5.7
H-strip	11	14	25	8.8	8.7	8.9
Screen	49	43	92	32.4	38.9	27.2
Rel. cage*	29	40	69	24.3	23.0	25.3
Recovered	126	158	284			
# Released	297	299	596			
40 Hrs						
Tree	34	26	60	32.1	38.2	26.5
Grass	2	12	14	7.5	2.3	12.3
H-strip	1	5	6	3.2	1.1	5.1
Screen	31	19	50	26.7	34.8	19.4
Rel. Cage*	21	36	57	30.5	23.6	36.7
Recovered	89	98	187			
# Released	370	370	740			
64 Hrs						
Tree	26	27	53	19.3	25.5	15.7
Grass	6	18	24	8.8	5.9	10.5
H-strip	2	11	13	4.7	1.9	6.4
Screen	17	15	32	11.7	16.7	8.7
Rel. Cage*	51	101	152	55.5	50.0	58.7
Recovered	102	172	274			
# Released	300	300	600			

Table 15. Tukey multiple comparison of the means of percent moth recapture in varying habitats (H = Herbicide Strip, G = Grass; T = Tree; * indicates significant p value @ alpha = 0.05).

Comparisons	Difference	Lower	Upper	p adj
H-G	0.1697	-0.7557	1.0951	0. 5718
T-G	2.1459	1.2205	3.0712	0.0110*
T-H	1.9762	1.0508	2.9015	0.0003*

Table 16. Percent moths recovered from grass plus herbicide strip habitats over three months of release / recapture trials in 2006-2007.

	June		July		August	
	Male	Female	Male	Female	Male	Female
16 Hrs	0.0	0.0	0.0	0.0	12.5	14.7
40 Hrs	0.0	6.3	0.0	3.7	8.8	35.9
64 Hrs	0.0	7.5	11.1	29.3	13.9	17.3
Mean	0.0	6.9	3.7	11.0	11.7	22.6

Data loggers (Hobo Pro series, Onset Computer Corporation, Bourne, MA, USA) placed in the tree and in the grass at soil level showed that the mean temperature in the 2 microclimates did not vary over a 3-month period (tree 22.1, grass 22.1), but that mean relative humidity varied by 13.3% between the tree (78.7%) and the grass (92.0%).

Release / recapture of wild type moths was conducted one time in 2006 (Recovery 16 hrs post release) and on two dates in 2007 (Recovery 64 hrs post release). Twenty-four percent of wild type moths released were recovered 16 hrs post release, and 16% of moths were recovered 64 hrs post release. All male moths that left the release cage in 2006 were recovered 16 hrs post release from the tr

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the tree (43%), while the lone female moth recovered was found in the grass (Table 17). Twenty-five percent of female wild type moths recovered after 64 hrs were recovered from the drive-row grass, 18.8% from the tree and 6.3% from the herbicide strip (Table 17).

Table 17. Recovery of wild type moths in five habitats 16 and 64 hours post release in release / recapture trials in 2006-2007 (* = release cage).

16 Hrs	Male	Female	Total	Mean % Recovered	% Males Recovered	% Females Recovered
Tree	6	0	6	20.7	42.86	0.00
Grass	0	1	1	3.4	0.00	6.67
H-strip	0	0	0	0.0	0.00	0.00
Screen	0	2	2	6.9	0.00	13.33
Rel. cage*	8	12	20	69.0	57.14	80.00
Recovered	14	15	29			
# Released	60	60	120			
64 Hrs						
Tree	2	3	5	25.0	50.00	18.75
Grass	0	4	4	20.0	0.00	25.00
H-strip	1	1	2	10.0	25.00	6.25
Screen	1	5	6	30.0	25.00	31.25
Rel. cage*	0	3	3	15.0	0.00	18.75
Recovered	4	16	20			
# Released	45	80	125			

DISCUSSION

This study provides evidence that adult codling moths do leave the tree canopy in apple orchards to inhabit drive-row grass and herbicide strip vegetation during daytime periods of inactivity. Significantly more moths were recovered from the tree than either the grass or the herbicide strip (Table 15), but both male and female lab-reared moths were recovered from the grass and herbicide strip at all recovery time periods, 16, 40 and 64 hrs post release (Table 14). Wild type females (6.7%) were recovered from the grass after 16 hrs, and wild type males (25%) and females (31.3%) were recovered from ground habitats (combined grass and herbicide) after 64 hrs (Table 17).

Although not statistically significant, a numerical trend was seen with recovery of 6.9% of female moths from ground habitats in June, 11% in July and 22.6% in August (Table 16). Males were also recovered in higher percentages from ground habitats as the summer months progressed, 0% in June, 3.7% in July and 11.7% in August (Table 16).

Data loggers maintained over the duration of the study showed that mean relative humidity varied by 13.3% between the tree (78.7%) and the grass (92.0%). The higher availability of moisture found in the grass could provide an explanation for why moths may seek shelter in this habitat during hot, dry summer days.

Adult codling moth behavior during the hours of 09:00 and 18:00 has received little study and is not well understood. Researchers investigating behavioral management tactics should consider the impact of moth behavior during periods of inactivity to better understand any potential impacts on control. For example, adult moth behavior that physically removes the adult from the tree canopy could affect efforts to manage codling moth with pheromone mediated mating disruption. Should mating disruption operate, at any level, by desensitization from long-term exposure to high pheromone concentrations, moth displacement to habitats away from dispensers placed in the tree canopy might influence di

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ACKNOWLEDGEMENTS

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CHAPTER SIX

Efficacy and optimum use patterns of CM Granulosis virus [family *Baculoviridae*, genus *Granulovirus*] formulations for control of Codling Moth, *Cydia pomonella* (L.), in Michigan apple

ABSTRACT

Commercial on-farm trials performed as part of this project show that codling moth (CM) *granulosis* virus (CpGv) is an effective, biologically based control option for CM in Michigan. Three commercially available formulations of CpGV, Carpovirusine, Cyd-X and Virosoft^{CP4}, were evaluated over the 2005 and 2006 growing seasons in small plot trials to evaluate application rates and frequency, and in larger on-farm trials to evaluate efficacy of all three formulations as supplements for codling moth control. Frequent applications of a low rate of all three CpGv formulations targeting first and / or second-generation larval populations consistently yielded excellent and economical control of CM. The average number of second-generation larvae recovered in cardboard bands and fruit injury at harvest was substantially reduced in all virus-treated plots compared to plots not treated with virus. There was no statistically significant difference in efficacy between the three formulations tested.

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INTRODUCTION

Since it was first collected in Mexico over 40 years ago (Tanada, 1964) and underwent its initial orchard trials in the 1960's, codling moth *granulosis* virus (CpGV) has been investigated as a codling moth control (Falcon *et al.*, 1968). CpGV is highly specific, infecting CM and a few closely related species, and is noninfectious toward beneficial insects, fish, wildlife, livestock, and humans (Gröner 1986). Although laboratory bioassays confirmed CpGV to be highly active against neonate larvae (Jaques et al. 1987), effectiveness in the field was inconsistent (Huber and Dickler, 1977; Laing and Jaques, 1980; Jaques et al., 1987; Vail et al., 1991; Charmillot, 1993; Rashid et al., 2001), resulting in hesitancy by fruit growers to incorporate *CpGV* products into their IPM programs (Arthurs *et al.*, 2005).

Two factors limiting field performance are the rapid breakdown of the virus by UV radiation, and the lack of consistent quality of virus products (Lacey and Shapiro-Ilan, 2003). Recent efforts to reformulate *Granulosis* virus products in Europe, Canada and New Zealand have resulted in reports of consistently good codling moth control, and CpGV products are now applied on more than 100,000 ha of sustainable codling moth control programs in combination with mating disruption in Europe (Charmillot *et al.* 1998; Lacey and Shapiro-Ilan 2003; Eberle and Jehle 2006). The entrance of these products into the market provides a renewed opportunity to develop effective and economically viable use patterns for this promising biopesticide.

Three commercial CpGv formulations are available in Michigan, Carpovirusine (Arysta LifeScience, Cary, NC), Cyd-X (Certis USA, Columbia, MD), and Virosoft^{CP4} (BioTepp, Canada). All formulations of the virus have short residual activity in the field, resulting from viral inactivation from exposure to ultraviolet radiation and high temperatures (Lacey and Shapiro-Ilan, 2003). Researchers are currently investigating CpGV use patterns comparing the efficacy of frequent applications (5-7 day re-application intervals) of virus at low rates vs. higher rates at 14-21 day application intervals, efficacy against different codling moth generations, and vertical transmission of CpGV between generations (Epstein *et al.* 2006; Hull and Krawczyk 2005; Quénin and Laur 2006).

Here we report on field trials at an MSU research station and on commercial farms conducted 2005 and 2006, to evaluate application rates, frequency of application and efficacy of the three commercially available formulations.

MATERIALS AND METHODS

Small Plot Trials to Evaluate Application Rates and Frequency: Three Carpovirusine spray programs for CM control were evaluated in an experimental apple block in Southwest Michigan located at the MSU Trevor Nichols Research and Extension Center (42°35'38.10"N, 86°06'05.92"W). The experimental design was a randomized block (blocked from North to South) with 4 replicates for each treatment. A 1.4 ha block of Smoothee apples was subdivided into 12-tree plots each measuring 0.03 ha. One or two tree buffers were established between

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plots. Each block was randomly assigned one of the following four treatments: 1) application of 325 mL / ha of Carpovirusine (10^{13} viral particles / liter) weekly for a total of 6 sprays per generation 2) application of 650 mL / ha every 14 days for a total of 3 sprays per generation, 3) application of 975 mL / ha twice per generation with a 21 day interval between sprays, and 4) an untreated control. The first application in the virus plots was timed for the start of egg hatch, 100 degree-days after biofix, base 11°C. All virus treatments were applied with an airblast sprayer at a rate of 950 L/ha.

Fruit injury and larval survival were used to evaluate the effectiveness of treatments in controlling CM. Fruit damage assessments on 100 fruit per plot were conducted at the end of the first and second CM generations (July 21 and September 7). Banding of trees with corrugated cardboard strips (Uline Inc., Waukegan IL) and counting the number of healthy and infected larvae that enter the bands to pupate was used to estimate larval densities and mortality from viral infection. Bands (6-7 cm wide) were placed on each of the 12 trees per plot prior to larvae exiting the fruit. Bands were removed after infestation and examined in the lab for the number and health of larvae present. Banding was only done at the end of the first generation. Fruit injury data was analyzed using ANOVA and mean separation with Fishers LSD for multiple comparisons.

Small-plot experiment to compare Carpovirusine and Virosoft: A small plot experiment to compare two codling moth granulovirus (CpGV) materials was conducted in apple blocks at the Trevor Nichols Research Complex in southwest Michigan. Two Carpovirusine spray programs were compared to one Virosoft spray program and an untreated control. The experimental design was a randomized block with 4 replicates for each treatment. Each plot consisted of twelve trees, primarily of the Red Delicious variety, and was randomly assigned one of the following four treatments: 1) application of Virosoft (4×10^{13} viral particles / liter) at the rate of 150 mL / ha weekly for a total of 5 sprays per generation 2) application of Carpovirusine (10^{13} viral particles / liter) at 630 mL / ha weekly for a total of 5 sprays per generation, 3) application of Carpovirusine at 975 mL / ha every 12 days for a total of 3 sprays per generation, and 4) an untreated control. The first application in the virus plots was timed for the start of egg hatch, 100 degree-days base 11ºC after biofix. All virus treatments were applied with an airblast sprayer at a rate of 950 L / ha.

Fruit injury and larval survival were the measures of effectiveness of treatments in controlling CM. Fruit damage assessments on 150 fruit per plot were conducted at the end of the first and second CM generations, July 10 and September 14, respectively. For each entry hole it was determined whether it was a shallow "sting", just breaking through the skin of the apple, or deep entry hole penetrating into the flesh. Apples were cut open to follow tunnels and larvae were removed and identified. Banding of trees with corrugated cardboard strips and counting the number of healthy and infected larvae that enter the bands to pupate was used to estimate larval densities and mortality from viral infection. Two bands (6-7 cm wide) were placed on each of four trees per plot prior to larvae exiting the fruit. Each banded tree had the trunk and a major scaffold limb banded. Bands were removed after infestation and examined in the lab for the number and health of larvae present. Fruit injury data were analyzed using ANOVA and means separated using the Tukey-Kramer HSD method for multiple comparisons.

Large On-Farm Plot Trials to Evaluate Efficacy: Trials were conducted in 2005 and again in 2006 in Kent and Ottawa counties, Michigan. The overall experimental design in 2005 was a direct comparison of the effectiveness of CM pest management programs that included multiple applications of CM granulosis virus with a grower standard program that did not include virus. In most cases the standard plot at each site was treated with organophosphorous (OP) insecticides such as Guthion (Azinphosmethyl) and Imidan (Phosmet), synthetic pyrethroids (SP) such as Asana (Esfenvalerate) and Pounce (Permethrin), or Provado (Imidacloprid).

Virus formulations tested were Carpovirusine (Arysta LifeScience, Cary, NC, 10^{13} viral particles / liter), Cyd-X (Certis USA, Columbia, MD, 3×10^{13} viral particles / liter), and Virosoft^{CP4} (BioTepp, Canada, 4×10^{13} viral particles / liter). Each formulation was tested on three to four farms. Granulosis virus products were applied three times per generation at the following low label rates: 450 mL / ha for Carpovirusine, 150 mL / ha for Cyd-X, or 130 mL / ha for ViroSoft^{CP4}.

Supplemental insecticides were applied at the discretion of the grower and may have included one or more of the following insecticides: Calypso (Thiacloprid), Rimon (Novaluron), Assail (Acetamiprid) and Intrepid (Acetamiprid). Levels of fruit injury and larval survival were used to evaluate the effectiveness of treatments in controlling CM. Fruit damage assessments on 600 fruit per plot, 30 from each of 20 trees, were conducted at the end of the first and second CM generations. Banding of trees with corrugated cardboard strips and counting the number of healthy and infected larvae that enter the bands to pupate was used to estimate larval densities and mortality from viral infection. Corrugated cardboard bands (6-7 cm wide) were placed around the trunk or major scaffold limbs of 20 trees in each treatment at mid-season and prior to harvest. Bands were removed after infestation and examined in the lab for the number and health of larvae present.

In 2006, Cyd-X and Carpovirusine were directly compared at four sites; ViroSoft was included at three sites. In all cases, virus was applied on an alternating basis with a Calypso (Thiacloprid) or Rimon (Novaluron) treatment. Two virus applications and 1-2 applications of Calypso or Rimon were made against each of the two CM generations at the following rates: 450 mL / ha for Carpovirusine, 150 mL / ha for Cyd-X, or 130 mL / ha for ViroSoft^{CP4}. Fruit damage assessments on 600 fruit per plot, 30 from each of 20 trees, were conducted at the end of the first and second CM generations. Banding of trees with corrugated cardboard strips and counting the number of healthy and infected larvae that enter the bands to pupate was used to estimate larval densities and mortality from viral infection. Corrugated cardboard bands (6-7 cm wide) were placed around the trunk or major scaffold limbs of 20 trees in each treatment at mid-season and prior to harvest. Bands were removed after infestation and examined in the lab for the number and health of larvae present.

RESULTS

Small Plot Trials to Evaluate Application Rates and Frequency: First Generation Results: Densities of internal feeders were high in the plots, as indicated by the nearly 50% injury recorded in the untreated check (Table 18). Additionally, an average of *ca.* 14 larvae were recovered in tree bands placed in the untreated plots (Table 19). Under this level of pest pressure, the three virus treatments significantly reduced the occurrence of deep entries (Table 18). They also reduced by ca. 80% the number of larvae completing development and spinning up in bands placed around the tree trunks (Table 19). Adults emerging from most of the injured fruit were OFM. Only 15% of the individuals completing development in the untreated check were CM. The only virus treatment in which CM were recovered was the 975 mL rate applied only twice.

Table 18. Mid season codling moth (CM) or Oriental fruit moth (OFM) fruit injury in small plots treated with Carpovirusine (Virus) at the indicated rate or untreated (Means followed by the same letter do not differ significantly, p = 0.05).

		Mean entries (%)		Larvae in apples (%)	
Treatment	Rate (mL/ha)	Shallow	Deep	Codling Moth	Oriental Fruit Moth
Untreated	na	2.0	45.6a	15	85
Virus	325	2.0	13.0b	0	100
Virus	650	3.0	16.0b	0	100
Virus	975	3.0	12.6b	20	80

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Table 19. Density and health status of larvae recovered in cardboard bands placed at base of trees in small plots treated with Carpovirusine (Virus) at the indicated rate or untreated (Means followed by the same letter do not differ significantly, p = 0.05).

		Mean larvae recovered per 12 trees	
Treatment	Rate (mL/ha)	Healthy	Unhealthy
Untreated	na	14.3a	0
Virus	325	1.5b	0.3
Virus	650	2.0b	0
Virus	975	3.8b	0

Second generation results. Second generation fruit injury exceeded 57% in the untreated check (Table 20). All of the virus treatments again significantly reduced the occurrence of deep entries (Table 20). However, significantly more damage occurred in the plot treated twice at a high rate of 13.2 oz/acre than in the plot treated frequently at the low rate of 4.4 oz/acre. The high rate / low frequency virus application also allowed for a relatively high survival of CM, as compared to frequent applications of a low rate of virus (Table 20). The majority of adults emerging from infested fruit in plots managed under the more effective, higher application frequency, virus programs were OFM.

Table 20. Pre-harvest codling moth (CM) or Oriental fruit moth (OFM) in small plot trials treated with Carpovirusine (Virus) at the indicated rate or untreated (Means followed by the same letter do not differ significantly, p = 0.05).

		Mean entries (%)		Larvae in apples (%)	
Treatment	Rate (mL/ha)	Shallow	Deep	Codling Moth	Oriental Fruit Moth
Untreated	na	4.0	53.6a	25	75
Virus	325	8.0	8.6b	0	100
Virus	650	8.0	13.0bc	10	90
Virus	975	10.0	20.6c	40	60

Small-plot experiment to compare Carpovirusine and Virosoft: First generation results: The density of internal feeders was high, as indicated by the mean percentage of infested fruit in the untreated plots (Table 21). Shallow entries, or stings, were numerically higher in the virus treated plots but not statistically different than in the untreated plots. All of the virus treatments significantly reduced the number of deep entries into the fruit. There was no statistically significant difference in the number of deep entries between the products or application intervals. Although not statistically significant, the majority of larvae found in the injured fruit were codling moth in all cases except in the Carpovirusine treatment at 8.5 ounces applied weekly where the proportion of oriental fruit moth larvae was higher. The number of healthy and unhealthy

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Table 21. Evaluation of 1st generation fruit injury in virus treated and untreated plots. Means followed by the same letter do not differ significantly, Tukey's HSD, p = 0.05. (Carpovirusine (Carpo-V); codling moth (CM); Oriental fruit moth (OFM).

			Mean Entries (%)		Larvae in Apple (%)	
Treatment	Rate (mL/ha)	Frequency (days)	Shallow	Deep	CM	OFM
Untreated	n/a	n/a	8.8	33.5a	93.6	6.4
Virosoft	150	7	11.2	11.2b	64.2	35.8
Carpo-V	630	7	15.5	12.0b	37.5	62.5
Carpo-V	975	12	10.0	10.5b	58.9	41.1

Table 22. The health status of 1st generation CM larvae recovered from cardboard bands in virus treated and untreated plots (Means followed by the same letter do not differ significantly, Tukey's HSD, p = 0.05)

			Mean larvae recovered from bands	
Treatment	Rate (oz/A)	Frequency (days)	Healthy	Unhealthy
Untreated	na	na	8.8 a	2.3 a
Virosoft	150	7	0.5 b	0.3 b
Carpovirusine	630	7	0.0 b	0.0 b
Carpovirusine	975	12	0.0 b	0.0 b

codling moth larvae recovered from bands in untreated plots was significantly higher than in the virus treated plots (Table 22). The number of unhealthy larvae in bands did not differ statistically among virus treatments.

Small-plot experiment to compare Carpovirusine and Virosoft: Second generation results: The infestation level for the second generation of codling moth was high as shown by the percentage of injured fruit in the untreated plots (Table 23). Like the data for the first generation injury ratings, all virus treatments significantly reduced the number of deep entries. The 630 mL / ha Carpovirusine treatment applied every seven days had the fewest deep entries but not significantly fewer than the number of entries in the other two virus treatments. The percentage of shallow entries (stings) on the fruit was lowest in the untreated plots and highest in plots treated with CM virus. Significantly more stings were found on fruit in the treatment consisting of Carpovirusine at the 630 mL / ha rate applied on a 7-day interval compared to the others. The majority of the larvae recovered from injured fruit were identified as oriental fruit moths in the virus treated plots while the opposite was true in the untreated plots. Only the 630 mL / ha Carpovirusine treatment had a significantly lower proportion of codling moth larvae in the fruit. The number of healthy and unhealthy codling moth larvae recovered from bands in untreated plots was significantly higher than in the virus treated plots (Table 24).

Table 23. Evaluation of 2nd generation fruit injury in virus treated and untreated plots (Means followed by the same letter do not differ significantly, Tukey's HSD, p = 0.05).

			Mean entries (%)		Larvae in apples (%)	
Treatment	Rate (oz/A)	Frequency (days)	Shallow	Deep	Codling Moth	Oriental Fruit Moth
Untreated	na	na	30.0b	50.2a	61.1a	38.9b
Virosoft	150	7	39.0ab	11.7b	26.1ab	73.9ab
Carpovirusine	630	7	47.5a	5.7b	3.6b	96.4a
Carpovirusine	975	12	42.0ab	10.8b	25.0ab	75.0ab

Table 24. The health status of 2nd generation CM larvae recovered from cardboard bands in virus treated and untreated plots (Means followed by the same letter do not differ significantly, Tukey's HSD, p = 0.05).

			Mean CM recovered from bands	
Treatment	Rate (oz./A)	Frequency (days)	Healthy	Unhealthy
Untreated	na	na	11.3 a	0.8
Virosoft	2	7	1.3 b	0
Carpovirusine	8.5	7	1.0 b	0
Carpovirusine	13	12	1.5 b	0

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Large On-Farm Plot Trials to Evaluate Efficacy: Results: The extent to which the virus program contributed to CM control varied by site in 2005 (Tables 24-26). No first generation fruit damage was recorded in the Carpovirusine plot, and second-generation fruit protection was equivalent in the virus and no virus plots (Table 25). The best summer fruit protection was recorded at the site with the highest CM densities (Tables 25-27, site 1). Fruit injury at harvest was 0.3 % and 39.3% in the Carpovirusine and no-virus treated orchards, respectively, 0.3 % and 49.7% in the ViroSoft and no-virus treated orchards, respectively, and 7.2 % and 57.7% in the Cyd-X and no-virus treated orchards, respectively. At sites 2 and 4, little or no fruit damage was recorded prior to harvest in the ViroSoft treated plots (Table 26). Similar levels of fruit injury were recorded in the ViroSoft and no virus plots at site 3. Fruit damage prior to harvest was higher in the Cyd-X plots at sites 2 and 3, compared to the no virus plots (Table 27). The number of CM larvae recovered in tree bands was substantially lower in all three of the virus treatments than the no virus plots. Although fruit were damaged, larval survival was low.

Table 25. Codling moth (CM) fruit injury and larval densities in plots treated with Carpovirusine (Carpo-V) or not treated with virus (standard) at four sites.

		Percent Injury		Total Larvae in Bands	
Site	Treatment	1st gen.	2nd gen.	1st gen.	2nd gen.
1	Carpo-V		0.3		
	Standard		39.3		
2	Carpo-V	0.7	6.5	2	1
	Standard	1.9	8.0	18	59
3	Carpo-V	0.4	12	5	1
	Standard	0.2	0	18	1
4	Carpo-V	0	0.5	1	0
	Standard	0.7	0.5	0	0

Table 26. Codling moth (CM) fruit injury and larval densities in plots treated with ViroSoft or not treated with virus (standard) at four sites.

		Percent Injury		Total Larvae in Bands	
Site	Treatment	1st gen.	2nd gen.	1st gen.	2nd gen.
1	ViroSoft	1.2	0.3	36	8
	Standard	1.5	49.7	17	503
2	ViroSoft	0	0	5	1
	Standard	0.7	0.5	18	1
3	ViroSoft	0.4	10		
	Standard	0.2	0.8		
4	ViroSoft	0.2	0	3	0
	Standard	0.2	0.8	2	0

Table 27. Codling moth (CM) fruit injury and larval densities in plots treated with Cyd-X or not treated with virus (standard) at three sites.

		Percent Injury		Total Larvae in Bands	
Site	Treatment	1st gen.	2nd gen.	1st gen.	2nd gen.
1	Cyd-X	2.0	7.2	57	17
	Standard	1.3	57.7	57	92
2	Cyd-X	0.7	1.7	3	1
	Standard	0.3	0	0	0
3	Cyd-X		0.4		
	Standard		0		

Table 28. Codling moth (CM) larval densities in plots treated with one of three granulosis virus formulations compared to plots not treated with virus (standard) based on tree banding.

	Mean CM larvae recovered		
Treatment	1st gen.	2nd gen.	Season
Carpovirusine	2.7	0.7	1.7
Standard	12.0	20.0	16.0
Cyd-X	30.0	9.0	19.5
Standard	28.5	46.0	37.3
ViroSoft^{CP4}	14.7	3.0	8.8
Standard	12.3	167.7	90.0

In on-farm trials in 2006, the average number of second-generation larvae recovered in cardboard bands was substantially reduced in all virus-treated plots compared to plots not treated with virus (Table 28). The three management programs that included virus also substantially reduced fruit injury at harvest (Figure 14). Fruit injury at harvest was 9 % and 1.5% in the virus vs. no-virus treated orchards. To a large extent, however, this difference in damage could be attributed to a single comparison for each virus formulation. Specifically, at three sites, fruit injury was extremely high in the no virus, standard block. Differences in fruit injury in the no virus compared to virus plots at the three sites were as follows: 39.3% vs. 0.3% using Carpovirusine, 57.7% vs. 7.2% using Cyd-X, and 39.7% vs. 0.3% using ViroSoftCP4 (Figure13).

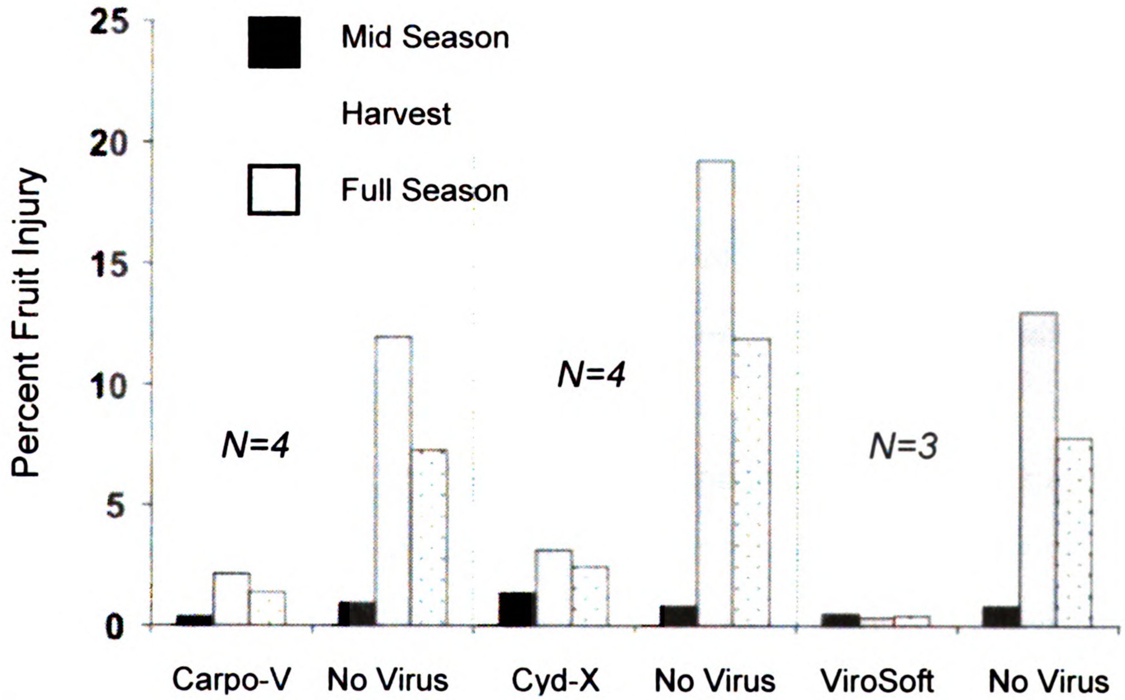

Figure 14. On-farm comparison of CM control in plots treated or not treated with one of three CpGv virus formulations: Carpovirusine (Carpo-V), Cyd-X, or ViroSoft^{CP4} (ViroSoft). N = Number of farms trials conducted on. represented by

DISCUSSION

Small plot trials conducted to determine optimum application rates and frequency of application of CpGv formulations show that a tactic of frequent application of low rates of all three commercially available formulations yielded the best results, as measured by levels of codling moth injury to fruit and numbers of larvae recovered from bands in treated and untreated plots (Tables 18-20). There were fewer codling moth deep entries into the fruit and fewer healthy larvae recovered in the low rate frequent application plots for both first and second generation larvae as compared to both the untreated plot and plots receiving less frequent applications of a higher virus rate.

Frequent application addresses concerns of viral inactivation of CpGv from exposure to ultraviolet radiation and high temperatures (Lacey and Shapiro-Ilan, 2003). Lacey and Shapiro-Illan (2008) state that CpGv has a relatively rapid speed of kill, and that the LD50 has been estimated as low as 1.2–5 granules per larva, indicating that a strategy that maintains a low rate of material through frequent applications to prevent product breakdown can be effective.

Frequent application of low rates of virus should also prove to be the most economical program for Michigan apple producers. Three applications of products, such as Carpovirusine, are equal in the price of product used to one application of the high rate. In temperate climates, growers are weekly applying fungicides and or insecticides during codling moth first generation, so the addition of CpGv formulations to the spray tank does not incur additional expenses in time and tractor use. All CpGv formulations tested using the low rate frequent application strategy in our on-farm trials substantially reduced fruit injury at harvest (Table 27, Figure 14). These data are supported by evidence from multi-year programs combining CpGV and mating disruption in Europe, New Zealand and the United States where a noted pattern of initially high numbers of codling moth adults and high percentages of partial larval entries into fruit declined over 2-4 year period resulting in reduced pest densities and reduced injury to fruit (Charmillot *et al.* 1998; Wearing *et al.* 1994).

CHAPTER SEVEN

Quantifying the Benefits of Areawide pheromone mating disruption programs for codling moth (Lepidoptera: Tortricidae)

Portions of this chapter are being submitted for peer review to the American Entomologist as:

McGhee, P.S., L.J. Gut, and D.L. Epstein. Quantifying the Benefits of Area-wide pheromone mating disruption programs for codling moth (Tortricidae)

ABSTRACT

A four-year research and implementation project in Michigan apple production demonstrated that deploying pheromone-mediated mating disruption for codling moth in an areawide (AW-CMMD) program where mating disruption is established on all apple producing acreage on individual and adjacent farms, significantly improved control of codling moth compared to mating disruption applied to individual apple blocks (CMMD) on farms with other blocks not under disruption, or apple blocks not treated with mating disruption (No-CMMD). Pheromone-baited traps were deployed at 1 per ha over 850 ha of AW-CMMD, 20 ha CMMD, and 20 ha of No-MD. Average captures of CM in AW-CMMD declined each season while they remained steady or increased, respectively, in the CMMD and No-MD programs. Additionally, injury to fruit and the overall number of insecticides targeting CM decreased each year in the AW-CMMD program while injury levels remained similar with slight insecticide reductions from year to year in CMMD orchards and injury increased significantly while insecticide use varied little from year to year in the No-MD orchards. Growers implementing AW-CMMD realized a mean savings of $55 to $65 /ha as compared to orchards utilizing insecticide only programs.

INTRODUCTION

Codling moth (CM) (*Cydia pomonella* L.) is a primary internal feeder of apples, and damage from this pest makes fruit unmarketable. Without effective control, losses can range from 50 to 90% of the crop (Wise and Gut 2000, 2002). Michigan apple orchards have a history of high CM pressure, and growers have experienced increasing difficulty in controlling this key internal-feeding pest (Howitt 1993, Epstein and Gut 2000, Epstein *et al.* 2002). As of 2002, infestation levels in excess of 10% had occurred on MI farms, and reduced pack-outs and load rejections due to the detection of infested fruit had become common. Controlling this pest with one or more broad-spectrum compounds became difficult, with failures reported throughout the State of Michigan. CM resistance to the organophosphorous insecticide, azinphos-methyl was reported at levels of greater than 10 fold, compared to susceptible populations throughout major apple growing regions in MI (Mota-Sanchez *et al.* 2008).

Areawide pest management aims to suppress the population levels of mobile pest species over a large geographic range. This strategy relies less on reactive population controls such as the application of insecticides after pest populations reach treatment thresholds, and more on proactive measures (i.e. pheromone-mediated mating disruption) designed to suppress pest populations from ever reaching treatment thresholds. Pheromones are semiochemicals that elicit intraspecific behavioral responses between members of a species (Gut *et al.*, 2004). The deployment of synthetic sex pheromone to interfere with mate finding orientation and subsequent mating behavior is called mating disruption.

Areawide (AW) pheromone management promotes a cooperative effort by neighboring growers with contiguous acreage to deploy mating disruption on all of the acreage on their farms to directly address key concerns regarding the immigration of mated females and lower pheromone concentrations in the borders of smaller, individualized disruption programs (Gut and Brunner, 1998; Knight, 1995). Deploying sex pheromones for AW pest management has been utilized for various pests in diverse cropping systems around the world including: South Africa, Italy, the Western U.S.A., Australia, and Argentina, (Barnes and Blomefield, 1997; Staten *et al.*, 1997; Jones and Casagrande, 2000; Calkins *et al.*, 2000; Il'ichev *et al.*, 2002).

The objective of this four-year research project was to determine whether the deployment of a pheromone based AW mating disruption (AW-CMMD) program would be an effective management approach under Michigan apple production conditions, specifically smaller farms (≥40 ha) often separated from neighboring orchards by fields and woodlots. The project also was designed to directly assess, for the first time, the added benefit of using pheromone on an areawide basis versus on an individual block basis. Thus, we compared AW-CMMD with mating disruption applied to smaller (<4 ha) individual orchards (CMMD) and orchards not treated with MD (No-CMMD) in the same region. Numbers of moths captured, levels of CM injury to apples, insecticide usage targeting CM and control program economics were used to evaluate management approaches.

METHODS AND MATERIALS

Experimental Layout. The MI apple production region commonly referred to as The Ridge is located 16 km northwest of Grand Rapids Michigan (43"07'51.97"N; 85"46'46.65"W; 278m elevation). The Ridge was chosen for this study due to recurring codling moth (CM) outbreaks and increased levels of fruit injury, >5%, at harvest. Eight adjacent commercial apple orchards comprising an area of 320 fruit bearing hectares were selected to implement an areawide CM mating disruption (AW-CMMD) strategy in 2004. Four farms located closely to, but distinctly outside of the immediate AW-CMMD region, deployed CM pheromones to a single, 2-4 ha, apple block (CMMD). Each of these four farms had a paired 2-4 ha apple block that was left untreated with pheromones (NO-CMMD) as a negative control. Supplemental insecticides were used when necessary throughout all farms and treatments to control codling moth populations that exceeded economic treatment thresholds. Ten additional farms joined the AW-CMMD project in 2005-2007 bringing the total area treated to 850 ha.

Pheromone Disruption. Growers participating in the AW-CMMD and CM-MD orchards chose which hand-applied disruption product to deploy in their orchards. All pheromone dispensers were placed in orchards prior to the start of first generation CM flight, approximately May 10 – May 15, at label rates. Products used included Isomate® C+ and CTT (Shin-Etsu Chemical Co., Tokyo, Japan), Scentry NoMate® codling moth Spirals (Scentry Biologicals, Billings, Montana), and CheckMate® codling moth XL 1000 (Suterra, Bend, OR).

Monitoring. Large Delta style traps (LPD Scenturian Guardpost, Suterra, Bend, OR) baited with long life CM L2 lures (Trécé Inc., Adair, OK) and Biolure CM 10x (Suterra Inc.) were used to monitor male adult activity throughout each growing season. Traps were deployed at 1 trap per ha in each treatment. CM L2 and Biolure 10x baited traps were deployed so that they were arranged in an alternating pattern with 1 each per 2 ha. The L2 lure, releases pheromone at a rate similar to a female CM and was used to measure the effectiveness of the pheromone MD treatments. The 10x lure releases pheromone at a much higher rate, and captures in these traps measured relative CM density. Moth captures were recorded each week throughout the growing season. Sticky liners were replaced after cumulative captures exceeded 30 moths per trap or at the beginning of each flight. Pheromone lures were replaced at the onset of adult emergence for each generation.

The location of each trap was recorded using a Garmin etrex Vista GPS device (Garmin, USA, Olathe, KS). Trap locations were plotted on USGS aerial imagery using Terrabrowser V1.5b3 (http://www.Chimosoft.com). Trap locations were used to plot moth captures in each orchard in order to assist in the assessment of CM populations and to facilitate management decisions. Mean moth captures were calculated weekly for each 4 ha apple block and a corresponding color coordinated map overlay was generated according to assigned pest pressure: no moths, low (1-3 moths), medium (4-10 moths), or high (11+ moths) catch using Omnigraffle Pro (Omni Development, Inc., Seattle, WA). Each week participating apple growers were presented with a summary report detailing the numbers and corresponding locations of moths captured on their farms. A public information kiosk was established at the Fruit Ridge Orchard Supply Store, Sparta, MI, located centrally within the AW-CMMD project region. This kiosk provided a summary of moth captures for all farms and treatments, while the color coordinated map indicating relative moth captures illustrated the weekly trends in moth populations.

Evaluation of Control Programs: Fruit Injury. Fruit injury assessments consisted of evaluating 600 fruit per block; 30 fruit from each of 20 trees (15 fruit from high and 15 from low in the canopy) in each orchard. Each fruit was inspected by hand on the tree for CM feeding. Fruit injury assessments were conducted after 1st CM flight (ca. July 7) and just prior to harvest beginning in the fall (Aug 21-Sept 14) of each year. Equal numbers of fruits were sampled from the orchard perimeters and interiors. All damaged fruit was collected, cut open, and any larvae found were identified as either oriental fruit moth (OFM) or codling moth by presence (OFM) or absence (CM) of an anal comb. The total number of injured fruits and larval species was recorded.

Evaluation of Control Programs: Chemical Control Programs. Records of all pesticide applications were obtained from each grower. The number and kinds of insecticides applied for CM control were summarized for each season. The mean number of insecticide applications was directly compared for each treatment.

Evaluation of Control Programs: Economics. A general economic analysis was used to evaluate the economic impact of using AW-CMMD verses the grower standard, NO-CMMD. The economic analysis assessed net cost or savings of implementing MD relative to the grower's "standard comparison" approach, based upon a ranging analyses (Connor and Epstein, unpublished). This analysis used a five-year (1995-1999) average on prices received in Michigan for fresh and processed apples. Costs considered were: 1) damage cost - the revenue lost when damaged fruit cannot be sold or receives a lower price, and 2) cost of arthropod pest control inputs including MD plus cost of MD installation for orchards where MD programs were implemented. Costs for fuel and labor to apply insecticides were not included as some control applications regularly occur with tank mixes to control other pests and diseases present in the orchard. The value of apples in each plot was estimated and presented within a range depending on crop market destination, either for processing or the fresh market. The Connor / Epstein model (Unpublished) estimates that a 1% reduction in injury from pests feeding internally on apples is equivalent to a range of $17.50 per ha for orchards where 100% of fruit is destined for a processing market to $52.50 per ha where 100% of fruit will be sold fresh.

Data analyses. We calculated the average and standard error of the number of male CM captured in 1 mg pheromone-baited traps for each generation in all treatments over the course of 4 years. Data were square root transformed to meet the requirements of normality according to skewness and kurtosis and then subjected to an analysis of variance (ANOVA). Means were separated by Fischer's protected least significant difference (PLSD) ($P = 0.05$)

(StatView 5.0.1, SAS Institute). The untransformed means and standard errors are presented in table 1.

Percentage data from fruit injury analysis were arcsine transformed to stabilize variances and then subjected to analyses of variance (ANOVA). Means were separated by Fisher's protected least significant difference (PLSD) ($P = 0.05$) (Systat 13, Systat Software, Inc., Chicago, Ill, www.systat.com). The untransformed means and standard errors are presented in figure 1.

The mean number of total insecticide applications as well as the number of organophosphorous insecticide applications targeting codling moth were directly compared for all management programs and standard errors of the means reported.

Product cost for pheromone ($108/ac), organophosphate insecticides ($15.37/ac/ application), and non-OP insecticides ($33.40/ac/applicaiton) were calculated based on local insecticide prices obtained from a chemical distributer. The average price for pheromones, and insecticides targeting CM was determined for CMMD and No-CMMD blocks.

RESULTS

Moth Capture. Catch of male codling moth in 1x baited traps decreased from 42 ± 12.5 to 1.5 ± 0.8 in AW-CMMD orchards over four years. Reduced moth captures were realized by the second generation in year one, with only an average of 7.8 ± 2.8 in moths captured. Captures of moths also were greatly reduced from 28.7 ± 11.8 to 7.7 ± 3.5 in the Block-CMMD orchards by the 4th year. Statistically the two mating disruption treatments only differed in the first generation of year 2 when 6x more moths were captured in the Block-CMMD treatment compared to the AW-CMMD treatment (Tables 29-31). Overall moth captures were significantly lower in both AW-CMMD and Block-CMMD vs. No-CMMD orchards. Moth catch was sustained at high levels over all four years in the No-CMMD treatment with 59, 37, 99, and 25 males per trap during first generation for 2004-2007, respectively (Table 29). The same pattern of high captures was observed during second generation each year. Weekly moth captures in all pheromone treatments remained at consistently low levels. Orchards employing AW-CMMD rarely saw captures over 2 moths per trap while populations in Block-CMMD peaked near 5 moths per trap. In contrast, a pattern of higher and more variable moth captures was observed over the course of each season in the No-CMMD orchards with maximum weekly captures averaging as high as 28 individuals per trap.

Table 29. Captures of male moths was significantly lower in AW-CMMD vs. No-CMMD for every generation in all 4 years, except for the 1st generation, 2004.

	DF	SS	Mean Sq	F-Value	P-Value
1st gen. 2004	2	13.1	6.6	0.948	0.4126
2nd gen. 2004	2	89.9	44.9	7.793	0.0060
1st gen. 2005	2	53.5	26.7	8.745	0.0045
2nd gen. 2005	2	33.1	16.6	8.042	0.0061
1st gen. 2006	2	107.8	53.9	16.961	0.0003
2nd gen. 2006	2	119.5	59.8	21.858	<0.0001
1st gen. 2007	2	35.1	17.5	10.862	0.0025
2nd gen. 2007	2	52.6	26.3	11.542	0.0020

Table 30. Mean captures of 1st and 2nd generation male codling moth in AW-CMMD, Block-CMMD, and No-CMMD apple orchards 2004-2007.

Year	Treatment	1st gen ± SEM	2nd gen ± SEM
2004	AW-CMMD	42.0 ± 12.5a	7.8 ± 2.8a
	Block-CMMD	28.7 ± 11.8a	7.9 ± 1.6a
	No-CMMD	58.9 ± 13.1a	78.3 ± 42.0b
2005	AW-CMMD	4.3 ± 2.6a	7.2 ± 3.3a
	Block-CMMD	26.7 ± 10.5b	12.5 ± 3.3a
	No-CMMD	36.8 ± 21.0b	37.8 ± 24.0b
2006	AW-CMMD	16.7 ± 4.1a	9.0 ± 2.4a
	Block-CMMD	15.8 ± 8.9a	10.3 ± 2.1a
	No-CMMD	99.3 ± 21.4b	92.6 ± 29.6b
2007	AW-CMMD	1.5 ± 0.8a	2.2 ± 1.2a
	Block-CMMD	7.7 ± 3.5a	9.9 ± 4.7a
	No-CMMD	24.5 ± 7.0b	36.7 ± 10.9b

Table 31. Fishers PLSD table for effect of treatment on male moth capture in pheromone-baited traps. Significantly different P values, alpha = 0.05, are bolded and marked with the symbol, * (AW = AW-CMMD, NoMD = no disruption, Block = Block-CMMD).

Comparison	Year	Gen.	Mean Diff.	Crit. Diff.	P-Value
AW – NoMD	2004	1st	-1.708	3.479	0.3082
AW – Block	2004	1st	0.764	3.479	0.6429
NoMD - Block	2004	1st	2.472	4.017	0.2065
AW – NoMD	2004	2nd	-5.579	3.176	**0.0022***
AW – Block	2004	2nd	-0.347	3.176	0.8174
NoMD - Block	2004	2nd	5.232	3.668	**0.0087***
AW – NoMD	2005	1st	-4.197	2.388	**0.0024***
AW – Block	2005	1st	-3.240	2.388	**0.0120***
NoMD - Block	2005	1st	0.957	2.694	0.4536
AW – NoMD	2005	2nd	-3.602	1.960	**0.0018***
AW – Block	2005	2nd	-1.105	1.960	0.2431
NoMD - Block	2005	2nd	2.497	2.212	**0.0300***
AW – NO-MD	2006	1st	-5.959	2.435	**0.0002***
AW – Block	2006	1st	0.273	2.435	0.8115
NoMD - Block	2006	1st	6.231	2.747	**0.0003***
AW – NoMD	2006	2nd	-6.501	2.258	**<0.0001***
AW – Block	2006	2nd	-0.347	2.258	0.7434
NoMD - Block	2006	2nd	6.154	2.547	**0.0002***
AW – NoMD	2007	1st	-3.720	1.768	**0.0007***
AW – Block	2007	1st	-1.455	1.876	0.1158
NoMD - Block	2007	1st	2.265	1.876	**0.0223***
AW – NoMD	2007	2nd	-4.541	2.100	**0.0006***
AW – Block	2007	2nd	-1.675	2.228	0.1262
NoMD - Block	2007	2nd	2.866	2.228	**0.0163***

Fruit Injury. Immediate reductions in CM injured fruit were observed in orchards deploying AW-CMMD compared to Block-CMMD and No-CMMD orchards. In 2004, CM injury to fruit in AW-CMMD was 0.7% and 1.0%, significantly less than either the Block or No-CMMD programs (Figure 15). The AW-CMMD program had significantly less injury due to CM feeding than No-CMMD orchard blocks for every CM generation in all four years (Figure 15 and Table 32). Block-CMMD had significantly less injury due to CM feeding than No-CMMD orchard blocks for both CM generations in 2005 and 2007, and for first CM generation in 2006 (Figure 15 and Table 33). The AW-CMMD program had significantly less injury due to CM feeding than Block-CMMD both CM generations in 2004, and for all four years at harvest (Figure 15 and Table 33).

Injury in orchards treated with pheromone gradually decreased over 4 years of pheromone usage while there was a steady increase in injury levels in the NO-CMMD orchards, with final harvest injury above 3% in 2007. In the fourth season, CM injury to fruit in the AW-CMMD was 0.6% and 3.1% less than Block and No-CMMD orchards, respectively. Fruit protection was also realized in the Block-CMMD orchards with a total reduction of about 0.5% over 4 years. On average, CM injury on AW-CMMD farms was virtually undetectable by year 4.

Table 32. Fruit injury from CM was significantly lower in AW-CMMD vs. No-CMMD for every generation in all 4 years.

	DF	SS	Mean Sq	F-Value	P-Value
1st gen. 2004	2	0.02	0.008	21.959	<0.0001
2nd gen. 2004	2	.015	.008	6.824	.0078
1st gen. 2005	2	.032	.016	14.123	<0.0001
2nd gen. 2005	2	.085	.043	18.600	<0.0001
1st gen. 2006	2	.041	.021	7.868	.0014
2nd gen. 2006	2	.041	.020	12.275	<0.0001
1st gen. 2007	2	.051	.026	12.250	.0003
2nd gen. 2007	2	.091	.046	29.634	<0.0001

Table 33. Fishers PLSD table for effect of treatment on injury to fruit from CM feeding. Significantly different P values, alpha = 0.05, are marked with the symbol, * (AW = AW-CMMD, NoMD = no disruption, Block = Block-CMMD).

Comparison	Year	Gen.	Mean Diff.	Crit. Diff.	P-Value
AW – NoMD	2004	1st	-0.052	0.023	0.0003*
AW – Block	2004	1st	-0.067	0.023	<0.0001*
NoMD - Block	2004	1st	0.015	0.026	0.2237
AW – NoMD	2004	2nd	-0.064	0.041	0.0043*
AW – Block	2004	2nd	-0.052	0.041	0.0161*
NoMD - Block	2004	2nd	0.012	0.045	0.5664
AW – NoMD	2005	1st	-0.073	0.028	<0.0001*
AW – Block	2005	1st	-0.010	0.032	0.5104
NoMD - Block	2005	1st	0.063	0.037	0.0015*
AW – NoMD	2005	2nd	-0.119	0.040	<0.0001*
AW – Block	2005	2nd	-0.053	0.045	0.0214*
NoMD - Block	2005	2nd	0.066	0.053	0.0161*
AW – NO-MD	2006	1st	-0.069	0.037	0.0005*
AW – Block	2006	1st	0.004	0.056	0.8740
NoMD - Block	2006	1st	0.074	0.060	0.0171*
AW – NoMD	2006	2nd	-0.069	0.029	<0.0001*
AW – Block	2006	2nd	-0.048	0.045	0.0349*
NoMD - Block	2006	2nd	0.021	0.048	0.3739
AW – NoMD	2007	1st	-0.114	0.049	<0.0001*
AW – Block	2007	1st	-0.049	0.053	0.0692
NoMD - Block	2007	1st	0.065	0.064	0.0446*
AW – NoMD	2007	2nd	-0.152	0.042	<0.0001*
AW – Block	2007	2nd	-0.066	0.045	0.0067*
NoMD - Block	2007	2nd	0.087	0.055	0.0033*

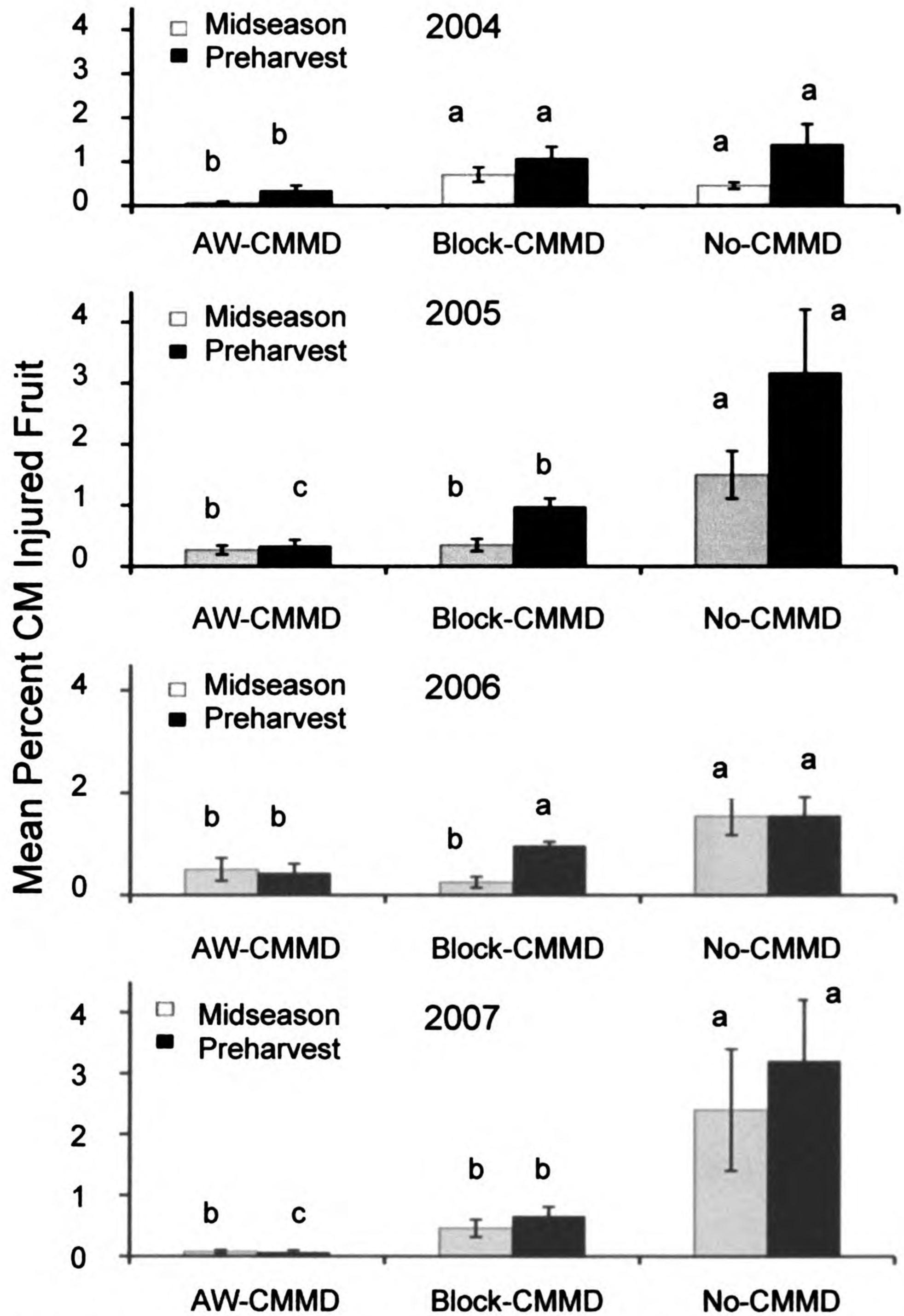

Figure 15. AW-CMMD was significantly better at protecting fruit from injury by CM larvae than both Block-CMMD and No-CMMD in all four years, 2004-2007.

Chemical control programs. Insecticide management regimes were similar across all programs, beginning in 2004, and relied primarily on organophosphorous compounds averaging 5 of the 6 seasonal sprays applied. Both the AW-CMMD and Block-CMMD programs saw decreased insecticide usage in the 2nd – 4th years, while the overall numbers of applications remained the same in the No-CMMD program orchards (Figure 15). AW-CMMD insecticide applications were reduced by at least 1 spray from each of the previous years over the duration of the project. There were only 3 targeted sprays in the AW-CMMD in 2007, of which only 1 spray was an OP. A similar

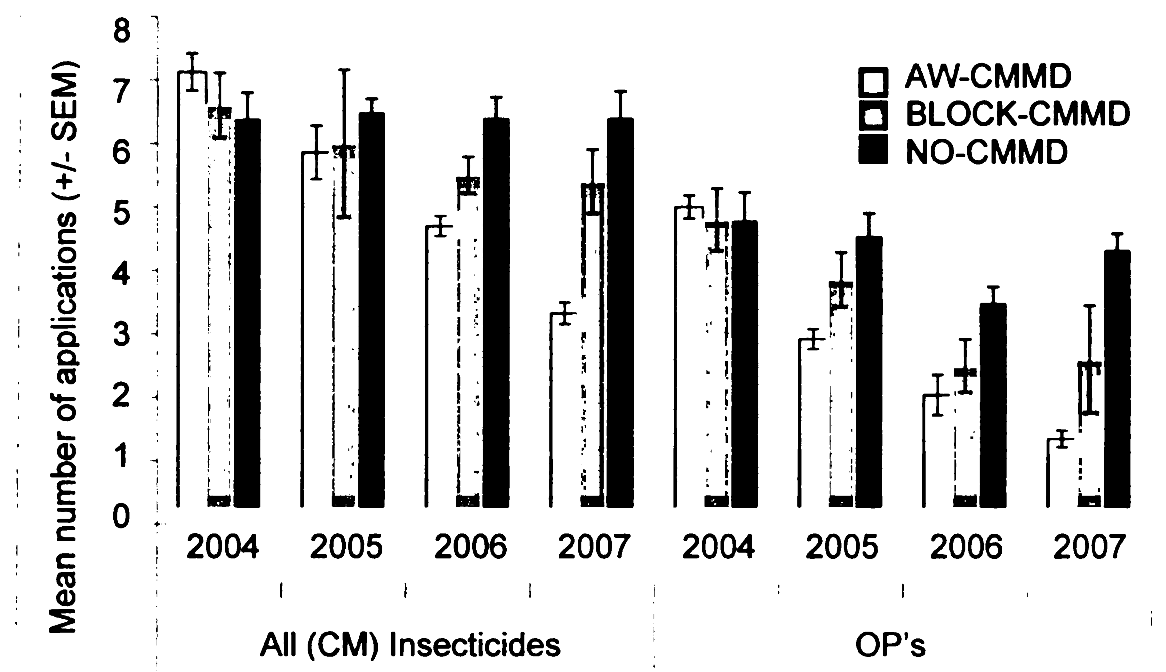

Figure 16. Number of insecticide applications for farms under areawide disruption (AW-CMMD), disruption in individual orchard blocks (Block-CMMD) and no disruption (No-CMMD) over the years 2004-2007.

insecticide applications was realized in the Block-CMMD programs, but with fewer reductions in total seasonal and OP sprays, 5 and 3 respectively.

Economics. Costs: Dispenser input costs ranged between \$200 and \$275 / ha depending on product chosen and rate of dispensers deployed per ha. Labor costs for applying dispensers ranged from \$37.50 and \$50.00 / ha. Labor costs for monitoring traps ranged from \$75 to \$100 / ha.

Economics: Returns: Insecticides: Average cost for application of an insecticide spray targeting CM control was \$87.50 / ha. AW growers realized a mean reduction of 50% in CM insecticides over 4 years (7 applications in 2004 to 3.5 applications in 2007). The reduction from 7 to 3.5 CM insecticide applications represented a savings of \$306.25 / ha.

Fruit Injury: After 4 years of AW-CMMD injury to fruit from CM feeding was a mean of 3.1% less than in insecticide only programs. Based on a 1% reduction in internal injury to apples being valued at a range of \$17.50 per ha for orchards where 100% of fruit is destined for a processing market to \$52.50 per ha where 100% of fruit will be sold fresh, AW-CMMD growers received an average range of \$61.25 / ha to \$183.75 / ha additional income compared to growers using insecticide only.

The cost of purchasing pheromone dispensers, labor for applying dispensers and for monitoring pheromone-baited traps ranged between \$312.50 / ha and \$425 / ha. Mean savings realized from implementing AW-CMMD by year four of the project ranged between \$367.50 / ha to \$490 / ha, meaning that growers implementing AW-CMMD realized a mean savings of \$55 to \$65 /ha.

DISCUSSION

Although CM mating disruption has been practiced in apple orchards around the world for nearly 2 decades, many practical and economical concerns with the approach have slowed its widespread adoption. In apple growing regions like Michigan, challenges of incorporating pheromone disruption into pest management programs include costs for managing a multitude of insect and disease pests, availability of labor for hanging pheromone dispensers, and a farmland topography in which orchards are interspersed with other crop or non-crop habitats. Indeed, MI orchards are often surrounded by woodlots and neglected blocks that support populations of pest insects. Our results show that mating disruption is both effective and economical under Michigan conditions, especially when applied on a whole-farm basis.

It is well documented that great successes with mating disruption can be achieved where large, contiguous areas are treated with pheromone. (Il'ichev *et al.,* 2002; Barnes and Blomefield, 1997; Calkins *et al.*, 2000; Brunner *et al.*, 2001). Growers in the Michigan areawide project were rewarded with a similar level of success. Overall the results have been impressive, with pest densities, as measured by moth captures in pheromone traps and fruit injury at harvest, declining to very low levels following implementation of an area-wide approach. In addition, direct comparisons with conventional programs outside the project area have revealed dramatic reductions in the number of insecticides applied for control of the targeted pest. However, each of the areawide projects cited above has fallen short of demonstrating superior efficacy of mating disruption applied on an areawide versus an individual block basis.

This Michigan areawide project marks the first time that the efficacy of mating disruption deployed in an areawide fashion (AW-CMMD) was measured against mating disruption deployed in individual farm plots (Block-CMMD). Results show that an areawide approach further improved upon the benefits of deploying mating disruption in individual blocks within a larger orchard in terms of both fruit protection from CM injury and reductions in insecticide applications for CM control (Table 33 and Figures 15 and 16).

Researchers have established that the success of mating disruption programs is strongly influenced by moth density and the ability to detect moth immigration into disrupted orchards (Carde´ and Minks 1995, Knight and Light 2005, Miller *et al.* 2006a). A key contributor to the success or failure of mating disruption is the program for monitoring pest populations, and a portion of the success of areawide mating disruption projects has often been attributed to the more intensive monitoring programs that are implemented (Calkins *et al.*, 2000; Brunner *et al.*, 2001). Inadequate monitoring can result in undetected CM populations exceeding damage thresholds and ultimately crop loss at harvest due to infested fruit. Growers in the Michigan areawide project relied on traps baited with standard or high-load pheromone lures and were encouraged to place traps at a density of one trap per hectare. Damage was kept to a minimum and decisions to not treat based on moth captures allowed growers to reduce the number of insecticide applications.

Still, monitoring of CM in disrupted orchards is limited using codlemone-baited traps. The codlemone lure is attractive to males, only, leaving half the moth population unmonitored. Another limiting factor is the ability of the monitoring trap, baited with the same sex pheromone as is found in dispensers, to outcompete both dispensers and calling females.

The recent identification of ethyl (*E, Z*)-2,4-decadienoate (pear ester) as a CM lure provides a new tool to improve CM monitoring and the timing of insecticide applications in mating disrupted orchards (Light *et al.* 2001). Current research is underway investigating the use of pear ester alone and in combination with codlemone to better measure moth activity. The possible advantages of the pear ester-based lures over the sex pheromone lure are 1) they attract females and thus moth catch is more directly linked to egg laying and the potential for worms in the fruit, and 2) attraction does not appear to be impeded or suppressed by pheromone-based mating disruption. As orchard production systems move toward greater reliance on pheromones and narrow-spectrum and relatively expensive insecticides, more precise trapping systems are required to monitor CM activity and aid in making cost-effective management decisions.

The cost of purchasing pheromone dispensers, labor for applying dispensers and for monitoring pheromone-baited traps ranged between $312.50 / ha and $425 / ha. Mean savings realized from implementing AW-CMMD by year four of the project ranged between $367.50 / ha to $490 / ha, meaning that growers implementing AW-CMMD realized a mean savings of $55 to $65 /ha, demonstrating that AW-CMMD is a cost efficient management approach.

Following the success of the AW-CMMD program established in the Fruit Ridge, additional AW-CMMD programs were established on apple farms in four additional MI counties (11 farms on approximately 400 ha). After two years, these AW programs were yielding similar results to those seen on the Fruit Ridge (Epstein, unpublished). These areawide programs are continuing in 5 counties after completion of the MSU organized research project, with growers hiring private concerns to maintain the intensive monitoring program. The MI areawide project has had a major impact in advancing the adoption of mating disruption by apple producers in the state of Michigan. Combining both pheromones and a few well-timed sprays using these compounds is effective and economical and has proven to maintain CM populations at low levels year after year.

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SUMMARY AND CONCLUSIONS

Pest control for Michigan apple growers is currently a major challenge, and is likely to become more difficult in the foreseeable future due to loss of compounds through U.S. Environmental Protection Agency (EPA) regulatory restrictions to protect citizens from the harmful effects of pesticides in food, air, and water (Food Quality Protection Act, 1996, H.R. 1627), the development of pest resistance to currently used pesticides, and a shortage of economic and effective alternatives. The overall aim of this thesis research was to develop and deliver effective and economical biopesticide-based management programs for codling moth (CM) and Oriental fruit moth (OFM) control that rely on management tactics generally considered to be sustainable into the foreseeable future: mating disruption, granulosis virus, and the judicious use of reduced-risk or conventional insecticides.

My initial studies (chapters 1 and 2 of this dissertation) addressed deployment strategies for hand-applied reservoir dispensers for mating disruption of CM and OFM. Some researchers (Shorey and Gerber 1996, Knight 2004) reported that efficacy of disruption performance was maintained while reducing the density of release sites, if overall release rate of pheromone per ha was maintained or increased, thereby providing the grower savings in labor for pheromone deployment (Knight 2004). My results contradicted these findings, instead showing that the highest density of pheromone-dispenser release sites yielded the best orientational disruption of male CM to traps and lowest fruit injury. These results are in agreement with Miller *et al.* (2006 a and b) who argues that competitive attraction is the predominant mechanism of disruption, and identifies practical implications for improving application of mating disruption as 1) dispenser attractiveness should be competitive with females, 2) dispenser density should be high, and 3) distribution of pheromone dispensers should be uniform rather than clumped.

Should competitive attraction be the predominant mechanism in mating disruption, a factor to consider in point source distribution of reservoir pheromone dispensers is location of dispensers within the tree canopy to compete with calling females. Previous research with pheromone baited traps showing male activity to be greater in the upper than lower portion of the tree canopy resulted in the standard recommendation that dispensers be applied within the top meter of the tree (Barret 1995, Knight 2000, McNally and Barnes 1980). However, there was little in the literature directly investigating male distribution without use of behavioral stimuli inducing flight, and the distribution of CM females within the tree canopy was unknown. I developed a vacuum system for sampling male and female moths directly from the tree canopy that revealed adults to be distributed throughout all parts of the tree during evening periods when female calling and male searching activity is occurring. In fact, higher numbers of moths were captured at mid-tree canopy than in either the top 1/3 canopy or low 1/3 canopy, with significantly more moths captured in the combined lower 2/3 than in the top 1/3 of the tree canopy (Chapter 3, table 3). In plots under pheromone mating disruption, female moths were captured in approximately equal numbers from the top 1/3 and mid 1/3 of tree canopies (Chapter 3, tables 5 and 6).

Discovering that the presence of female moths was equally distributed between the top and middle portions of the tree canopy led me to investigate the effects of pheromone dispenser placement at different heights within the tree canopy. If competitive attraction is a primary mechanism in pheromone-mediated disruption of codling moth, it follows that a distribution of female moths throughout the tree canopy calls for a similar distribution of dispensers in the upper and mid canopy levels to reduce CM mating. The results showed that a distribution of pheromone dispensers in the upper and mid canopies of trees did, indeed, result in reduced overall mating of tethered female moths, and suggests that recommendations for placement of pheromone ties should be revisited based on recent findings of moth distribution and mechanism of codling moth pheromone disruption (Chapter 4).

Studies investigating the canopy distribution of adult moths provided me with another question regarding moth behavior. Vacuum samples during daytime hours, 09:00 to 18:00, when adults are typically inactive, yielded a few moths. Coupled with visual observations of moths emerging from orchard drive-row grass at twilight, these findings initiated adult moth release / recapture studies to investigate the possibility that moths were leaving the tree canopy after twilight activity periods to rest on the orchard floor. This potentially removes adults from the influence of pheromone sources in the tree for up to 12 hours per day. The release / recapture investigation showed that significantly more moths were recaptured from the tree canopy, but also provided evidence that adult codling moths do leave the tree canopy in apple orchards to inhabit drive-row grass and herbicide strip vegetation during daytime periods of inactivity (Chapter 5). The importance of this behavior, if any, to optimizing mating disruption efficacy is yet to be determined.

Positive reports of multi-year programs combining reformulated *Granulosis* virus products and mating disruption in Europe and New Zealand encouraged me to investigate efficacy and use patterns for this biopesticide under Michigan conditions beginning in 2005 (Charmillot *et al.* 1998; Wearing *et al.* 1994). Small plot trials at research stations and larger on-farm experiments established that frequent applications of a low rate of all three CpGv formulations available in Michigan targeting first and / or second-generation larval populations consistently yielded excellent and economical control of CM. The low rate frequent application strategy of CpGv use should prove to be the most economical program for Michigan apple producers, since three applications of CpGv products are equal in price of product used to one application of the high rate. In temperate climates, growers are weekly applying fungicides and or insecticides during codling moth first generation, so the addition of CpGv formulations to the spray tank does not incur additional expenses in time and tractor use. Virus used in combination with mating disruption has been shown to be effective at reducing high CM populations and resulting larval entries into fruit over a 2-4 year period (Charmillot *et al.* 1998; Wearing *et al.* 1994). The combined use of these two biopesticides allows growers to control two different CM life stages, mating disruption targeting adults and CpGv targeting neonate larvae.

Codling moth *Granulosis* virus products were incorporated into control programs on several farms in Michigan as part of a four-year areawide (AW) CM mating disruption project from 2004 to 2007. Participating growers deployed a uniform distribution of high densities of pheromone-dispensers across 800 ha of contiguous apple plantings in Sparta, MI, and used CpGv and other insecticides to gain control of CM populations that had been increasingly difficult to control in years leading up to the start of the AW project. Results showed that an AW approach further improved upon the benefits of deploying mating disruption in individual blocks within a larger orchard in terms of both fruit protection from CM injury and reductions in insecticide applications for CM control (Chapter 7, Table 5 and Figures 15 and 16).

The above investigations show that biopesticide-based management programs for CM and OFM control in Michigan apple production are both effective and economical. As products and delivery strategies have been developed and their efficay demonstrated, growers have embraced their use. Commercial use of codling moth pheromone-mediated mating disruption products for CM, alone, has grown in Michigan from 50 to 100 ha in the year 2000 to over 3200 ha in 2010 (D. Thomson, Pacific BioControl Corp., Seattle, WA, personal communication). Researchers will continue to further develop and refine current use protocols for biopesticide-based products as additional investigations reveal relationships between insect behavior / physiology and product development and use.

LITERATURE CITED

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

Alway, T. 2002. Codling Moth Mating Disruption Revisited in 2002, Orchard IPM Update newsletter, WA State Univ. Tree Fruit Research and Extension Center. 4: 1-5.

Asser-Kaiser, S., E. Fritsch, K. Undorf-Spahn, J. Kienzle, K. E. Eberle, N. A. Gund, A. Reineke, C. P. W. Zebitz, D. G. Heckel, J. Huber, J. A. Jehle. 2007. Rapid Emergence of Baculovirus Resistance in Codling Moth Due to Dominant, Sex-Linked Inheritance, Science: 317: 1916-1918, September.

Atterholt, C.A., M.J. Delwiche, R. E. Rice, and J.M. Krochta. 1998. Study of biopolymers and paraffin as potential controlled-release carriers for insect pheromones, J. Agric. Food Chem. 46: 4429-4434.

Audemard, H., C. Leblon, U. Neumann, and G. Marboutie. 1989. Bilan de sept années d'essais de lutte contre la Tordeuse orientale du pêcher *Cydia molesta* Busck (Lep., Tortricidae) par confusion sexuelle des mâles. J. Appl. Ent. 108:191-207.

Arthurs, S. P., L. A. Lacey and R. Fritts, Jr. 2005. Optimizing Use of Codling Moth Granulovirus: Effects of Application Rate and Spraying Frequency on Control of Codling Moth Larvae in Pacific Northwest Apple Orchards, J. Econ. Entomol. 98(5): 1459-1468

Barnes, B.N. and T.L. Blomefield. 1997. Goading growers towards mating disruption: the South African experience with Grapholita molesta and Cydia pomonella (Lepidoptera: Tortricidae). IOBC WPRS Bulletin 20: 45-56.

Barret, B.A. 1995. Effect of synthetic pheromone permeation on captures of male codling moth (Lepidoptera: Tortricidae) in pheromone and virgin female moth-baited traps at different tree heights in small orchard blocks. Environ. Entomol. 24(5): 1201-1206.

Bartell, R.J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. Physiological Entomology 7: 353-364.

Borror, J.B., C.A. Triplehorn and N.F. Johnson, 1989. An introduction to the study of insects. Sixth Edition, Saunders College Publishing, Fort Worth, TX, 875 pp.

Brunner, J., S., Welter, C., Calkins, R., Hilton, E., Beers, J., Dunley, T., Unruh, A., Knight, R. VanSteenwyk and P., Van Buskirk. 2001. Mating disruption of codling moth: a perspective from the Western United States. IOBC WPRS Bulletin 25: 207-215.

Calkins, C.O., A.L., Knight, G. Richardson, and K.A. Bloem. 2000. Area-wide population suppression of codling moth. In: Tan, K-H. (ed) Area-wide Control of Fruit Flies and Other Insect Pests, Peneritg Universiti Sains Malaysia, Pulau Pinang, pp. 215-219.

Cardé, A.M., T.C. Baker, and R.T. Cardé . 1979. Identification of a four-component sex pheromone of the female Oriental fruit moth, Grapholitha molesta (Lepidoptera: Tortricidae), Journal of Chemical Ecology: 5(3)

Cardé, R.T. 1990. Principles of mating disruption. In Ridgeway, R.L. and R.M. Silverstein (eds) Behavior-modifying chemicals for pest management: applications of pheromones and other attractants, pp. 47-71, New York, Marcel Dekker.

Cardé, R.T. and A.K. Minks. 1995. Control of moth pests by mating disruption: successes and contraints. Annual Review of Entomology 40: 559-585.

Cardé, R.T., A. Mafra-Neto, R.T. Staten and L.P.S. Kuenen. 1997. Understanding mating disruption in the pink bollworm moth. IOBC/WPRS Bulletin 20: 191-201.

Cardé, R.T., R.T. Staten and A. Mafra-Neto. 1998. Behavior of pink bollworm moths near high-dose, point sources of pheromone in field wind tunnels: insights into mechanisms of mating disruption. Entomol. Exp. et Appl. 89: 35-46.

Castrovillo, P.J. and R.T. Cardé. 1979. Environmental regulation of female calling and male pheromone response periodicities in the codling moth (*Laspeyresia pomonella*), Journal of Insect Physiology, 25(8): 659-667.

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

Charmillot P J, D . Pasquier, N.J. Alepaiz and M. Meiwald. 1993. A biological preparation for control of the codling moth Cydia pomonella L.: the granulosis virus. Revue Suisse Vitic Arboric Hortic 25: 225-231.

Charmillot, P.J., Pasquier, D., Scalco, A., and Hofer, D. 1998. Control of codling moth Cydia pomonella L. by an attract and kill technique. Rev. Suisse Vitic. Arboric. 29: 111-117.

de Lame, F.M. 2003. Improving mating disruption programs for the oriental fruit moth, Grapholita molesta (Brusck): efficacy of new wax-based formulations and effects of dispenser application height and density. M.S. thesis, Michigan State University, East Lansing.

de Lame, F. M., J. R. Miller, C. A. Atterholt, and L. J. Gut. 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.

Devotto, L. and M. Gerding. 2005. Control of the codling moth (Cydia pomonella) using the area-wide approach in Chile. In Book of Abstracts, International Conference on Area-Wide Control of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques. Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA), 9-13 de Mayo de 2005, Vienna, Austria.

Eberle, K. E. and J.A. Jehle. 2006. Field resistance of codling moth against *Cydia pomonella* granulovirus (CpGV) is autosomal and incompletely dominant inherited, J. of Invertebrate Pathology, 93: 201-206.

Eberle, K.E., S. Asser-Kaiser, S.M. Sayed, H.T. Nguyen, and J.A. Jehle. 2008. Overcoming the resistance of codling moth against conventional Cydia pomonella granulovirus (CpGV-M) by a new isolate CpGV-I12, Journal of Invertebrate Pathology 98: 293–298.

Epstein, D. & L. Gut. 2000. A Pocket Guide for IPM Scouting in Michigan Apples. Michigan State University Extension Bulletin E-2720, 72pp.

Epstein, D., L.J. Gut, A.L. Jones and K. Maxson-Stein. 2002. A Pocket Guide for IPM Scouting in Stone Fruits. Extension Bulletin E-2840, East Lansing, MI, Michigan State Univ., 83 p.

Epstein, D.L., D. Waldstein, and C. Edson. 2002b. Michigan Apple Integrated Pest Management Implementation Project Final Narrative Report to the Pew Charitable Trusts, http://www.ipm.msu.edu/pdf/MAIPMIPFinalReport.pdf

Epstein, D.L., L.J. Gut, and J. Wise. 2006. Early season codling moth management decision-making, MI State University IPM Program Fruit Crop Advisory Team Newsletter 21(6).

Falcon, L.A., W.R. Kane, and R.S. Bethell. 1968. Preliminary evaluation of a granulosis virus for control of codling moth. Journal of Economic Entomology 61: 1208-1213

Food Quality Protection Act. 1996. H.R. 1627 http://www.epa.gov/pesticides/regulating/laws/fqpa/

Fritsch, E., Undorf-Spahn, K., Kienzle, J., Zebitz, C. P. W., Huber, J. 2005. Codling moth granulovirus: Variations in the susceptibility of local codling moth populations, Bulletin of the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section.

Fruit and Tree Nuts Situation and Outlook Yearbook. Market and Trade economics Division, Economic Research Service, U.S. Department of Agriculture, October 2008, FTS-2008.

Geier, P.W. 1963. The life history of codling moth, (*Cydia pomonella* (L.) Lepidoptera, Tortricidae) in the Australian capital territory, Aust. J. Zool., 11, pp. 323-67.

Groner, A. 1986. Specificity and safety of baculoviruses, CRC Press, Publisher Place, Boca Raton, Fla.

Gut, L. and J. Brunner. 1998. Pheromone-based management of codling moth (Lepidoptera: Tortricidae) in Washington apple orchards. Journal of Agricultural Entomology 15: 387-405.

Gut, L. J, J. Wise, J. Miller, R. Isaacs & P. McGhee. 2001. New insect controls and pest management strategies. Proc. Mich. State Hort. Soc. 129: 66-75.

Gut, L.J., L.L. Stelinski, D.R. Thomson, and J.R. Miller. 2004. Behavior-Modifying Chemicals: Prospects and Constraints in IPM, p. 73-121, In: Integrated pest management: potential, constraints and challenges, Koul, O., G.S. Dhaliwal and G.W. Cuperus, (eds), CAB International, Oxfordshire, UK: 73-121.

Gut, L. & J. Wise. 2004. Increasing concern about codling moth resistance to insecticides. Fruit CAT Alert 19(8): 1-2.

Gut, L.J., J.R. Miller, L. L. Stelinski, and D. L. Epstein. 2007. Competitive Attraction as a Primary Mechanism of Moth Mating Disruption in Tree Fruit Crops, IOBC/wprs Bulletin, Vol. 30 (4), ISBN 92-9067-199-6 [xii + 279 pp.].

Howitt, A.H. 1993. Common Tree Fruit Pests. Michigan State University. Michigan State University Extension Bulletin, NCR 63.

Huber, J. and E. Dickler. 1977. Codling moth granulosis virus: Its efficiency in the field in comparison with organophosphorus insecticides. J. Econ. Entomol. 70: 557-561

Hull, L. and G. Krawczyk. 2005. An Evaluation of Cyd-X(r) in Pennsylvania Orchards for Codling Moth Control, Proceedings of the 76th Annual Western Orchard Pest & Disease Management Conference, Portland, OR.

Il'ichev, A.L., L.J. Gut, D.G. Williams, M.S. Hossain, P.H. Jerie. (2002). Area-wide approach for improved control of oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera:Tortricidae) by mating disruption Gen. Appl. Ent. 31, pp. 7-16.

Il'ichev, A.L., L.L. Stelinski, D.G. Williams and L.J. Gut. 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.

Jaques, R. P., J. E. Laing, D. R. Laing, and D. S. K. Yu. 1987. Effectiveness and persistence of the granulosis virus of the codling moth, Cydia pomonella (L.) (Lepidoptera: Olethreutidae), on apple. The Canadian Entomologist 119(12): 1063-1067.

Jehle, J.A., S. Sayed and B. Wahl-Ermel. 2006. What do we (need to) know about low-susceptibility of codling moth against Cydia pomonella granulovirus (CpGv)! Proceedings of the Ecofruit 12th International Conference on Organic Fruit Growing, Weinsberg, Germany.

Jehle, J. A. 2008. The Future of Cydia pomonella Granulovirus in Biological Control of Codling Moth. Ecofruit, *In*: Boos, Markus (Ed.) Ecofruit - 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference at Weinsberg/Germany, pp. 265-270.

Jones, O.T. and E.D. Casagrande. 2000. The use of semichemical-based devices and formulations in area-wide programmes: A commercial perspective. In: Tan, K-H. (ed) Area-wide Control of Fruit Flies and Other Insect Pests, Peneritg Universiti Sains Malaysia, Pulau Pinang, pp. 285-293.

Judd, G.J.R., M.G.T. Gardiner , N.C. DeLury and G. Karg. 2004. Reduced antennal sensitivity, behavioural response, and attraction of male codling moths, Cydia pomonella, to their pheromone (E,E)-8,10-dodecadien-1-ol following various pre-exposure regimes, Entomologia Experimentalis et Applicata: 114(1): 65-78.

Knight, A.L. 1995. The impact of codling moth (Lepidoptera: Tortricidae) mating disruption on apple pest management in Yakima Valley, Washington. Journal of the Entomological Society of British Columbia 92: 29-38.

Knight, A. L.1995b. Evaluating pheromone emission rate and blend in disrupting sexual communication of codling moth (Lepidoptera: Tortricidae). Environ. Entomol. 24: 1396-1403.

Knight, A. 2000. Monitoring codling moth (Lepidoptera: Tortricidae) with passive interception traps in sex pheromone-treated apple orchards. J. Econ. Entomol. 93(6): 1744-1751.

Knight, A. L., and T. E. Larsen. 2004. Improved deposition and performance of a microencapsulated sex pheromone formulation for codling moth (Lepidoptera: Tortricidae) with a low volume application. J. Entomol. Soc. B. C. 101: 79-86.

Knight, A. L. 2004. Managing codling moth (Lepidoptera: Tortricidae) with an internal grid of either aerosol or dispenser clusters plus border applications of individual dispensers. J. Entomol. Soc. Brit. Columbia. 101: 69-77.

Knight, A. L. and D. M. Light. 2005. Seasonal Flight Patterns of Codling Moth (Lepidoptera: Tortricidae) Monitored with Pear Ester and Codlemone-baited Traps in Sex Pheromone-Treated Apple Orchards, Environ. Entomol. 34(5): 1028-1035.

Krupke, C. H., B. D. Roitberg, and G. J. R. Judd. 2002. Field and laboratory responses of male codling moth (Lepidoptera: Tortricidae) to a pheromone-based attract-and-kill strategy. J. Econ. Entomol. 31: 189-197.

Kührt, U., J. Samietz and S. Dorn. 2005. Effect of plant architecture and hail nets on temperature of codling moth habitats in apple orchards, Entomologia Experimentalis et Applicata, 118(3): 245-259.

Lacey, L.A. and D.I. Shapiro-Ilan. 2003. The potential role for microbial control of orchard insect pests in sustainable agriculture, Food, Agriculture & Environment 1(2): 326-331.

Lacey, L.A. and D.I. Shapiro-Ilan. 2008. Microbial Control of Insect Pests in Temperate Orchard Systems: Potential for Incorporation into IPM, Annu. Rev. Entomol. 53: 121–44.

Laing, D.R., Jaques, R.P., 1980. Codling moth: techniques for rearing larvae and bioassaying granulosis virus. J. Econ. Entomol. 73, 851–853

Light, D. M., A. L. Knight, C. A. Henrick, D. Rajapaska, B. Lingren, J. C. Dickens, K. M. Reynolds, R. G. Buttery, G. Merrill, J. Roitman, and B. C. Campbell. 2001. A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). Naturwissenschaften 88:

333-338.

McGhee, P., D.L. Epstein and L.J. Gut. 2009. Michigan Growers Successfully Managing Codling Moth With Areawide Mating Disruption, Fruit Growers News, March.

McNally, P.S. and Barnes, M.M. 1980. Inherent characteristics of codling moth pheromone traps. Environ. Entomol. 9: 538-541.

McNeil, J.N. 1991. Behavioral Ecology of Pheromone-Mediated Communication in Moths and its Importance in the Use of Pheromone Traps, Annu. Rev. Entomol. 1991. 36:407-30.

Michigan Agricultural Statistics. 2009. url: http://www.nass.usda.gov/Statistics_by_State/Michigan/Publications/Annual_Statistical_Bulletin/stats09/fruit.pdf

Millar, J. G. 1995. Degradation and stabilization of (*E*,*E*)-8,10-dodecadien-1-ol, the major component of the sex pheromone of the codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 88: 1425-1432.

Miller, J. R. L. J. Gut, F. M. de Lame and L. L. Stelinski. 2006a. 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. L. J. Gut, F. M. de Lame and L. L. Stelinski. 2006b. Differentiation of Competitive vs. Non-competitive Mechanisms Mediating Disruption of Moth Sexual Communication by Point Sources of Sex Pheromone (Part II): Case Studies, J Chem Ecol, 32: 2115–2143

Mota-Sanchez, D., J. C. Wise, R. Vander Poppen, L. Gut and R.M. Hollingsworth. 2008. Resistance of codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae) larvae in Michigan to different modes of action causes reduced residual activity. Pest Manag. Sci.: 64: 881-90.

Palaniswamy, P., R. J. Robs, W. D. Seebrook, G. C. Lonergan, C. J. Weisner, S. H. Tan, and P. J. Silk. 1982. Mating suppression of caged spruce budworm (Lepidoptera: Tortricidae) moths in different pheromone atmospheres and high population densities. J. Econ. Entomol. 75: 989-993.

Pfeiffer, D. G. and J. C. Killian. 1988. Disruption of olfactory communication in oriental fruit moth and lesser appleworm in a Virginia peach orchard. J. Agric. Entomol. 5(4): 235-239.

Quénin, H. and P. Laur. 2006. Carpovirusine granulosis virus formulation: control of resistant strain of codling moth and study of the vertical transmission of the virus, Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference, Portland, OR.

Rashid, T., D. T. Johnson, D. C. Steinkraus and C. R. Rom. 2001. Effects of microbial, botanical, and synthetic insecticides on Red Delicious apple arthropods in Arkansas. HortTechnology 11: 615-621

Riedl, H. A., S. A. Hoying, W.W. Barnett and J. E. Detar. 1979. Relationship of Within-Tree Placement of the Pheromone Trap to Codling Moth Catches, Environ. Entomol., 8(4): 765-769.

Riedl, H. A., A. Seaman & F. Henrie. 1985. Monitoring susceptibility to azinphosmethyl in field populations of the codling moth (Lepidoptera: Tortricidae) with pheromone traps. J. Econ. Entomol. 78: 692-699.

Rodriguez-Saona, C.R. and L.L. Stelinski. 2009. Behavior-Modifying Strategies in IPM: Theory and Practice, In: Integrated Pest Management: Innovation-Development, R. Peshin, A.K. Dhawan (eds.), Springer Netherlands, pp. 263-215.

Roelofs, W., A. Comeau, A. Hill, and G. Milicevic. 1971. Sex Attractant of the Codling Moth: Characterization with Electroantennogram Technique, Science, 15 October: 174 (4006), pp. 297-299

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

Rothschild, G. H. L. 1979. A comparison of methods of dispensing synthetic sex pheromone for the control of oriental fruit moth, Cydia molesta (Busck) (Lepidoptera: Tortricidae), in Australia. Bull. Ent. Res. 69: 115-127.

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

SAS Institute. 2000. SAS/STAT User's Guide, version 6, 4th ed., vol. 1. SAS Institute, Cary, NC.

Sexton, S. B. and A. L. Il'ichev. 2000. Pheromone mating disruption with reference to oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) literature review. Gen. Appl. Ent. 29: 63-68.

Shapiro-Ilan, D. I., R.F. Mizell III, T.E. Cottrell, and D. L. Horton. 2003. Measuring field efficacy of Steinernema feltiae and Steinernema riobrave for suppression of plum curculio, Conotrachelus nenuphar, larvae. Biological Control 30 (2), June, 496-503.

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

Staten, R. T.; H. M. Flint, R. C. Weddle, E. Quintero, R. E. Zarate, C. M. Finnell, M. Hernandes, A. Yamamoto. 1987. Pink Bollworm (Lepidoptera: Gelechiidae): Large-Scale Field Trials with a High Rate Gossyplure Formulation, J. Econ. Entomol., 80(6): 267-1271.

StatSoft. StatSoft, Inc., Tulsa, Oklahoma, USA, http://www.statsoft.com/contact-us/

Stelinski, L. L., L. J. Gut, R. E. Mallinger, D.L. Epstein, T. P. Reed, J. R. Miller. 2005a. 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., L. J. Gut, D. Epstein and J. R. Miller. 2005b. Attraction of four tortricid moth species to high dosage pheromone rope dispensers: Observations implicating false plume following as an important factor in mating disruption IOBC/wprs Bulletin 28(7): 313-317.

Stelinski L.L., L.J. Gut, K. C. Ketner and J. R. Miller. 2005. Orientational disruption of codling moth, *Cydia pomonella* (L.) (Lep., Tortricidae), by concentrated formulations of microencapsulated pheromone in flight tunnel assays. J. Appl. Entomol. 129: 481-488.

Stelinski, L. L., J.R. Miller, R. Ledebuhr, and L.J. Gut. 2006. Mechanized applicator for large-scale field deployment of paraffin-wax dispensers of pheromone for mating disruption in tree fruit, J. Econ. Entomol. 99(5): 1705-1710.

Stelinski, L.L., L.J. Gut, D. Epstein, P. McGhee and M. Haas. 2007. 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., P. McGhee, M. Haas, A. L. Il'ichev and L.J. Gut. 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., J.R. Miller, R. Ledebuhr, P. Siegert and L.J. Gut. 2007c. Season-long disruption of *Grapholita molesta* (Lepidoptera:Tortricidae) by one machine application of pheromone in wax drops (SPLAT-OFM). J. Pest Sci. 80: 109-117.

Stelinski, L.L., P.McGhee, M. Grieshop, J. Brunner and L.J. Gut. 2008. Efficacy and mode of action of female equivalent dispensers of pheromone for mating disruption of codling moth, Cydia pomonella (L.). Agric. Forest Entomol. 10: 389-97.

Stoeckli, S., K. Mody and S. Dorn. 2008. Influence of Canopy Aspect and Height on Codling Moth (Lepidoptera: Tortricidae) Larval Infestation in Apple, and Relationship between Infestation and Fruit Size, J. Econ. Entomol. 101(1): 81

Suckling, D. M., G. Karg, S. J. Bradley, and C. R. Howard. 1994. Field electro-antennogram and behavioral responses of *Epiphyas postvittana* under low pheromone and inhibitor concentration. J. Econ Entomol. 87: 1477-1487.

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

Swenson, D. W. and I. Weatherston. 1989. Hollow-fiber controlled-release systems. In: A. R. Jutsum and R. F. S. Gordon (eds.), Insect pheromones in plant protection. Whiley, Chichester, UK, pp. 173-197.

Tanada, Y. (1964). A granulovirus of the codling moth, Carpocapsa pomonella L. J Insect Pathol 6, 378–380.

Thomson, D. R., L. J. Gut, and J. W. Jenkins. 1999. Pheromones for insect control: strategies and successes, pp. 385-412. *In*: F. R. Hall and J. J. Menn (eds.), Methods in Biotechnology, vol. 5: biopesticides: use and delivery. Humana Press Inc., Totowa, N.J.

Thomson, D., J. Brunner, L.J. Gut, G. Judd, and A. Knight. 2001. Ten years implementing codling moth mating disruption in the orchards of Washington and British Columbia: starting right and managing for success! Pheromones for insect control in orchards and vineyards. IOBC/WPRS Bull. 24: 23-30.

Unites States Environmental Protection Agency. 2006. What Are Biopesticides. url: http://www.epa.gov/pesticides/biopesticides/whatarebiopesticides.htm

Vail, P.V., J.S. Tebbets, D.C. Cowan, and K.E. Jenner. 1991. Efficacy and persistence of Granulosis virus against investations of Plodia interpunctella (Hubner) (Lepidoptera: Pyralidae) on raisins, J. Stored Prod. Res. 27: 103-107.

Vickers, R.A., Rothschild, G.H.L. and E.L. Jones, 1985. Control of Oriental Fruit moth at a district level by mating disruption with synthetic female pheromone. *Bull.Entomol.Res.* 75: 625- 634.

Waldstein, D. W. and L. J. Gut. 2005. Effects of rain and sunlight on oriental fruit moth (Lepidoptera: Tortricidae) microcapsules applied to apple foliage. J. Agric. and Urban Entomol 21: 117-128.

Wearing, C.H., Walker, J.T.S., Thomas, W.P., Clearwater, J.R., Suckling, D.M., Charles, J.G., Shaw, P.W., White, V. and Burnip, G.M. 1994. Pest control for organic apple production in New Zealand, Proc. 43rd New Zealand Weed and Pest Control Conference, The Horticulture and Food Research Institute of New Zealand Ltd., Palmerston North, New Zealand.

Weeden, Shelton, Li, and Hoffman. 2007. Biological control: a guide to natural enemies in North America, Cornell University, url: http://www.nysaes.cornell.edu/ent/biocontrol/pathogens/baculoviruses.html

Weissling, T. J. and A. L. Knight. 1995. Vertical distribution of codling moth adults in pheromone-treated and untreated plots, Entomologia Experimentalis et Applicata, 77(3): 271-275.

Welter, S., and F. Cave. 2005. Rapid Assessment of New Pheromone Mating Disruption Devices Using an EAG. Proceedings of the Western Orchard Pest and Disease Management Conference, 5-7 January 2005, Portland, OR.

Welter, S.C., C. Pickel, J. Millar, F. Cave, R.A. Van Steenwyk, and J. Dunley. 2005. Pheromone mating disruption offers selective management options for key pests, California Agriculture: 59 (1), p. 16.

Wilson, E. O. and W. H. Bossert. 1963. Chemical communication among animals. Recent Progress in Hormone Research, 19, 673-716.

Wise, J. and L. Gut. 2000. Control of codling moth and Oriental fruit moth with new insecticide chemistries, 1999: Arthropod Management Tests 25: 40.

Wise, J. and L. Gut. 2002. Apple: season long control of lepidopteran pests with Intrepid, 2001: Arthropod Management Tests 27: A55.

Witzgall, P., Backman, A., Svensson, M., Koch, U. Rama, F., El-Sayed, A., Brauchli, J., Arn, H., Bengtsson, M., and Lofqvist, J. 1998. Behavioral observations of codling moth, Cydia pomonella, in orchards permeated with synthetic pheromone. BioControl 44:2 11-237.

Witzgall, P., L. Stelinski, L. Gut and D. Thomson. 2008. Codling moth management and chemical ecology. Ann. Rev. Entomol. 53: 503-22.

Zingg, D. 2008. Madex Plus and Madex I12 overcome virus resistance of codling moth. In: FOEKO (Ed.), Ecofruit—Proceedings of the 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, FOEKO, Weinsberg (Germany), pp. 256–260.