IMPACT OF HIGH RELEASING MATING DISRUPTION FORMULATIONS ON (MALE) CODLING MOTH, *Cydia pomonella* L., BEHAVIOR

By

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ABSTRACT

IMPACT OF HIGH RELEASING MATING DISRUPTION FORMULATIONS ON (MALE) CODLING MOTH, *Cydia pomonella* L., BEHAVIOR

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New high-releasing pheromone mating disruption technologies applied at low point-source densities were compared to that of industry standard dispensing systems that emit lower concentrations of pheromone and are deployed at higher point source densities for control of codling moth in Michigan apple. Meso and aerosol dispensers show the most promise as cost-effective alternatives to high-density dispensers for mating disruption of CM. Males exposed to pheromone released from aerosol emitters show that they become sensitized rather than desensitized to pheromone emitted from lure baited traps. Dosage response experiments reveal aerosol emitters disrupt codling moth by the behavioral mechanism, competitive attraction, and that the optimal density is 5-7 units per ha. Pheromone conservation of 50% or more can be achieved by reducing the overall concentration, rate of emission, and period of release without a loss in percent disruption making increased dispenser density economically viable.

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INTRODUCTION

Apple Agriculture in Michigan

Michigan grows over 15,800 hectares of apples (Michigan Department of Agriculture -MDA, 2011), making it the third leading producer of apple behind the states of Washington and New York. There are *ca.* 850 family-operated apple farms in Michigan; the average operation is 40ha, while 35% exceed 80ha. Michigan's 5-year average farm level production of 345 metric tons is valued at $104.1 million (MDA, 2011) and contributes $700-900 million annually to Michigan's economy. About 40% of Michigan apples go to fresh markets ready to eat, while the remainder are processed into other products, including fresh-cut slices, cider, applesauce, and pie slices. Newer orchards are trending to high-density plantings (about 1200 - 2000 trees ha^{-1}) that come into production earlier than traditional central leader orchard plantings (245-500 trees ha^{-1}) and bring desirable varieties to market quickly.

Codling Moth Impact and Biology

Codling moth (CM) (*Cydia pomonella* L.) is the primary internal feeding pest of apples; the damage it causes renders 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 controlling this pest with one or more broad-spectrum compounds has become difficult. Failures have been reported throughout North America (Howitt 1993).

By 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. Not surprisingly, CM resistance to organophosphorous, pyrethroid, and carbamate insecticides, including azinphosmethyl, has been detected at levels of greater than 10 fold compared to susceptible populations throughout the major MI apple growing regions (Mota-Sanchez et al. 2008).

Codling moth (CM) is an introduced species that is the principal direct pest of North American apple. Two full CM generations and occasionally a partial 3rd generation occur in MI and in most other primary apple production regions in North America. Mature larvae overwinter under bark on the tree or in litter on the ground (Howitt 1993). In Michigan, first generation adults emerge around the second to third week of May (Howitt 1993, Mota-Sanchez et al. 2008). Soon after emerging, female moths release a sex pheromone to attract males, mate, and begin depositing eggs on developing fruit and leaves. The majority of adult flight and mating occurs over a 3-4 h period beginning at dusk and when temperatures exceed 15C with calm wind (Batiste et al. 1973). Mated females produce between 90-150 eggs and deposit them singly on leaves or fruit (Putman 1962). Eggs hatch in 8-14 d (beginning around 125dd base 10C after biofix), and bore into fruit within a few h. Feeding larvae tunnel to the endocarp and consume the protein rich seeds. The larvae can be confused with another internal-feeding tortricid, oriental fruit moth, *Grapholita molesta*, when both occur in the same orchard. The two species are distinguishable by the absence of the anal comb on the terminal abdominal segment of CM and it's characteristic tunneling to the fruit endocarp and subsequent feeding on the seeds.

When fully grown, larvae exit the fruit and spin a cocoon on or near the host tree where they pupate. Depending on day length and temperature they either enter diapause or emerge as the next generation of adults in 2-3 weeks. Second generation emergence typically begins in late July in MI (Howitt 1993).

Codling moth Management

Control of codling moth in apple is a major challenge for growers, and is likely to become more difficult in the foreseeable future due to: loss of effective insecticides through regulatory restrictions, development of resistance to available materials, and increased cost of newer registered compounds or other control tactics. Broad-spectrum insecticides have been effective for many years and have served as the backbone of economical CM management programs as they target more than one life stage, an array of pests, and have fairly good residual efficacy. Over the past few years however, the use of broad-spectrum materials such as methyl parathion, azinphos-methyl and chlorpyrifos has been curtailed due to safety concerns for workers, consumers, and the environment.

Several alternatives to broad-spectrum insecticides have been registered over the past 10 yrs. Included are insecticides with novel modes of action, including neonicotinoids such as acetamiprid, spinosyns such as spinetoram, and diamides such as Rynaxypyr®. Although effective for controlling codling moth and other important pests, these new chemistries have some characteristics that can limit their usefulness: 1) critical timing of application due to shorter residual activity and life stage specificity, 2) they often must be consumed in order to be effective. 3) prolonged onset of poisoning before the pest stops feeding resulting in crop injury, 4) rapid development of insecticide resistance due to cross-resistance with other compounds rotation, and 5) non-target effects that can disrupt biological control and cause secondary pest problems.

Codling moth sex pheromone

Pheromones are semiochemicals used for communication between members of a species that elicit intra-specific behavioral responses (Gut et al. 2004). Codling moth pheromone was first identified as (E,E)-8,10-dodecadien-1-ol (Roelofs et al. 1971) and further characterized by the subtle behavioral effects of minor components including but not limited to (E,Z)-8,10-dodecadien-1-ol, and 1-tetradecanol (Bartell and Bellas 1981, Arn et al. 1985, El-Sayed et al. 1999, Witzgall, Bengtsson, et al. 1999). Codling moth pheromone has been synthesized and placed in lures for the purpose of population monitoring or in dispensers for control by mating disruption.

Attractants and traps for monitoring

Effective pest management relies on the early detection of insect pests prior to crop injury. Monitoring adult codling moth facilitates early detection and estimates of population density. Growers have relied on multiple methods including food-baited, light, and sex pheromone traps to achieve those goals and to establish management thresholds. Pheromone baited traps are advantageous in that they are species-specific, lures last several weeks, require no power source, and are generally easy to maintain. Moth catch in pheromone traps is influenced by many variables including: trap location within the tree canopy, distribution and density in the crop, lure loading rate, and weather. Wing and delta style traps, such as Pherocon ICP and VI, baited with a 1 mg codlemone lure are often used for monitoring codling moth populations. Efficient lures should release pheromone that is similarly attractive compared to a calling female, but last for months before becoming depleted. The rational being that not catching males should correspond with a failure of males to locate actual females. Unfortunately, low load lures (with ca. 1mg codlemone) sometimes fail to catch moths in orchards under mating disruption. Such falsely negative catches can result in unacceptable fruit injury. Lures containing more codlemone (Charmillot 1991, Gut and Brunner 1998), or pear ester, or a combination of codlemone and pear ester have proven more effective for monitoring CM activity in disrupted orchards (Knight et al. 2005). Pheromone traps baited with these alternatives to standard lures are likely more apparent to moths in disrupted orchards and are not completely suppressed, providing growers with an effective means of assessing the population density within the orchard and of timing insecticide sprays.

Pheromone-based Mating Disruption (MD)

Mating disruption has proved to be a feasible control tactic for some key pests of fruit crops (Gut et al. 2004, McGhee et al. 2011). This approach to pest control entails dispensing synthetic sex attractants into a crop so as to interfere with mate finding, thereby controlling the pest by curtailing reproduction. In practice, the success of mating disruption depends on the cost-effective delivery of an appropriate blend, amount, and spatial distribution of pheromone for an extended period (Suckling 2000, Gut et al. 2004). Mating disruption, as it is commercially practiced today, is largely achieved through the manual application of reservoir-type release devices (Witzgall et al. 2008) throughout the cropping system.

The major mechanisms typically offered as explanations for MD of moths include: 1) competitive attraction (Miller, Gut, de Lame, and Stelinski 2006a), 2) camouflage (masking), and 3) desensitization (Witzgall et al. 2008). Over the past several years, Michigan State University researchers have concluded that a combination of mechanisms operating in sequence explains the success of MD for CM. Specifically, competitive attraction appears to be required to bring males close to a dispenser (Stelinski, Gut, Vogel, et al. 2004, Stelinski et al. 2006) releasing high rates of pheromone at which time males become desensitized and rendered temporarily unresponsive to pheromone at the levels emitted by females (Miller et al. 2010). Behavioral observations of pink bollworm implicated the combination of false-plume following and habituation as important contributing mechanisms of disruption almost a decade ago (Cardé et al. 1998) and current evidence with CM is consistent with those conclusions (Miller, Gut, de Lame, and Stelinski 2006b).

Mating disruption has been widely adopted for control of CM in fruit orchards since the 1990's. In the U.S., disruption products for CM are currently deployed on more than 77,000 ha of apple and pear (Witzgall et al. 2008). Approximately 90% of the apple acreage in WA employs CM disruption. However, less than 20% of Michigan apples use this technique and an even smaller percentage of the acreage is treated in other eastern apple production regions (Gut et al. 2004).

Hand applied reservoir formulations constitute the majority of commercialized CM products used in apple (Thomson et al. 2001). Isomate-C Plus, Isomate CM FLEX polyethylene tube dispensers (Shin-Etsu Chemical Co., Tokyo, Japan), CheckMate CM (Suterra LLC, Bend, OR) and NoMate CM (Scentry Biological, Billings, MT) are applied at densities of 300 to 1000 ha^{-1} and must be placed high in the canopy for effective control. Isomate products (ShinEtsu are loaded with a 3-component blend, while all other CM disruption products use codlemone only. The efficacy of hand-applied dispensers is correlated with pheromone point-source densities; higher pheromone point-sources provide better and more consistent suppression of male moth captures in traps and reduction in injury to fruit (Epstein et al. 2006).

Application of dispensers is typically accomplished with the use of ladders, extension poles, or mechanical pruning towers. It is labor intensive and takes up to 1.5 man h per ac. at a time when labor demand for many growers is greatest due to other important orchard activities, including weed and disease management and final pruning cleanup. Deploying dispensers at the recommended label rate costs growers up to \$272 ha^{-1} for the product and an additional \$37-\$62 ha^{-1} for labor. Growers must apply the pheromone treatment prior to moth emergence and therefore before fruit set to achieve control. Spring frosts at bloom time, when CM emerges, can reduce or destroy the season’s crop. Growers are faced with purchasing and treating all of their acreage before they can determine if there will be a crop worth protecting.

Hand-applied high point-source density pheromone dispensers, like Isomate Flex and Scentry NoMate, are widely used for management of CM in apple. These dispensers are deployed at 500-1000 ha^{-1} to achieve acceptable fruit protection. They remain the dominant mating disruption formulation used in North America for CM control. The behavioral mechanism of disruption for these dispensers was unknown for many years. Miller et al. (2010) discovered that dispensers such as these operate by competitive attraction, whereby the frequency with which male insects find calling females or monitoring traps (surrogates for calling females) is reduced because males are diverted from orienting to these sources of pheromone due to preoccupation with more numerous nearby dispensers that first attract responders and then arrest and possibly deactivate them for a period of time.

New types of pheromone delivery systems are needed to allow more apple growers to adopt this novel pest management practice. Several companies have invested in strategies that aim to reduce labor cost while maintaining or improving efficacy including: 1) Micro-encapsulated sprayable formulations, 2) paraffin wax emulsions, 4) laminate flakes and hollow fibers, 5) meso dispensers, and 6) aerosol emitters.

Microencapsulated formulations of pheromones can be delivered with standard air-blast sprayers. Although such formulations are highly desirable from a practical standpoint, their efficacy has been inconsistent and short-lived in the field (Knight et al. 2004, Stelinski, McGhee, et al. 2007). A recently developed low volume application method has improved efficacy of sprayable formulations for codling moth (Knight and Larsen 2004). However, the need for many applications to maintain efficacy remains problematic. Wins-Purdy et al. (2007) reported evidence supporting habituation, as the likely non-competitive behavioral mechanism for obliquebanded leafroller, *Choristonura rosaceana* disruption through flight tunnel and electroantennogram assays whereas flight tunnel assays on high and low concentration CM MEC formulations suggest that an initial but short-lived disruption mechanism like camouflage is followed by a longer period of false-plume following to clumps of microcapsules (Stelinski, Gut, Ketner, et al. 2005).

Female equivalent formulations, such as flakes and fibers, represent another option for automated delivery of pheromones (Gut et al. 2004, Stelinski et al. 2008, Witzgall et al. 2008, Stelinski et al. 2009). The presumable advantages of these technologies are the rapid machine application or many thousands of point-sources ha^{-1}. Theoretically these formulations use thousands of point-sources to compete with calling females with the advantage of improving efficacy relative to hand-applied devices that are deployed at lower densities. These dispensers appear to disrupt CM by competitive attraction (Miller, Gut, de Lame, and Stelinski 2006a). Fibers and flakes have been evaluated for their potential at managing CM in orchards, but have provided levels of control inferior to the currently used hand-applied dispenser technology and have proved difficult to apply (Stelinski et al. 2009).

Meso dispensers describe controlled deliver devices that fall between aerosol emitters or ‘Mega-dispensers’ that release huge amounts of pheromone and are applied at only a few per hectare and standard hand-applied dispensers that release much less pheromone and are applied at several hundred per ha. A meso dispenser releases substantially more pheromone than the standard, hand-applied dispenser. The idea is that the higher release can allow for application of meso dispensers at much lower rates, 25-100 per ha and thus greatly reduce the labor required for hand application. However, the impact a meso dispenser has male behavior would likely need to be greater than that of current commercial formulations (Isomate CM FLEX, Scentry NoMate CM) considering they would be applied at substantially lower densities if they operate by the same mechanism of disruption. Such dispensers would need to attract males from a larger area than current commercial hand-applied formulations. Alternatively, meso-dispensers could operate by desensitization so as to elevate male response threshold. The approach, in part, has been validated by work where some success with CM control was achieved using clusters of 10-100 Isomate C-Plus standard dispensers applied at reduced densities (Epstein et al. 2006).

An intriguing pheromone-based approach entails the formulation and release of insect sex attractants via aerosol-emitting devices, such as “puffers” (Shorey and Gerber 1996; Stelinski, Gut, et al. 2007), “misters” (Mafra-Neto and Baker 1996), or “microsprayers” (Isaacs et al. 1999). Aerosol emitting devices are deployed at only 2-5 ha^{-1}, but each unit releases a substantial quantity of pheromone every 15-30 min. The reach of the plume emanating from aerosol dispensers is likely much larger than that from standard dispensers. Additionally, pheromone at such high dosages might survive on plant surfaces at behaviorally meaningful dosages (Gut et al. 2004) over areas much larger than would be expected per dispenser emitting lower rates of pheromone. Experiments in California pear orchards registered suppression of male CM catch in pheromone baited traps up to 450m downwind of a single Puffer (Welter and Cave 2000a). In addition to being attracted to pheromone emitters, males under treatment by aerosol dispensers may receive a pheromone dose sufficient to elevate their response threshold. Aerosol emitters deployed at 2.5 ha^{-1}, but delivering vastly greater amounts of pheromone (14 mg/hr), were suspected to camouflage the low concentration pheromone plumes emitted by calling female moths (ca. 7 ng/hr) (Bäckman et al. 1997). In CA, OR and WA, high levels of orientational disruption have been achieved with deployment of 2.5 puffers ha^{-1} (Shorey and Gerber 1996, Knight 2005, Hansen 2008). These trials suggest that a high release, low point-source pheromone dispensing strategy could be effective. However, the aerosol treatment is often supplemented with a border application of hand-applied pheromone dispensers and/or companion insecticides. Aerosols have not performed as well in Michigan field trials; less than 75 % disruption of male CM orientation to pheromone-baited traps typically occurs in treated plots compared with untreated controls (Stelinski, Gut, et al. 2007).

Aims of Research

The overall aim of this dissertation research was to understand how newly developed high-releasing pheromone mating disruption technologies applied at low point-source densities provide CM disruption and to compare their efficacy to that of industry standard dispensing systems that emit lower concentrations of pheromone and are deployed at higher point source densities. Meso and aerosol dispensers show the most promise as cost-effective alternatives to high-density dispensers for mating disruption of CM. Recent research (McGhee et al. 2012) with hand applied dispensers revealed that lowering the release rate of ai by 80%, 50%, and 25% of the normal rate did not significantly reduce efficacy. Optimizing the use of meso and aerosol dispensers will require an understanding of how their distribution and release rate parameters impact orientational disruption of male moths. Thus, the objectives of my research were to: 1) measure the effectiveness of meso pheromone dispensers on orientational disruption of CM, 2) determine the optimal point-source density and potency of meso dispensers that provides the highest level of moth disruption, 3) evaluate the effectiveness of aerosol emitters on orientational disruption of CM. 4) determine the optimal parameters of aerosol emitters that provide effective CM disruption including: point-source density, dispenser potency, frequency of pheromone emission/distribution, and functional operation period, and 5) elucidate the behavioral response of CM and behavioral mechanism of mating disruption to sex pheromone released from high-releasing aerosol emitters. To accomplish these objectives, field experiments were conducted in apple using two meso pheromone dispensing technologies, Isomate CM RING and Trécé Cidetrak CM meso, and two aerosol emitter technologies, Suterra CM Puffer, and Isomate CM MIST.

CHAPTER ONE

Meso dispensers provide a viable option for pheromone-based mating disruption of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae)

ABSTRACT

Experiments were conducted in commercial apple orchards to determine if codling moth, *Cydia pomonella* L., mating disruption can be realistically achieved using “meso” dispensers that contain ca. 10x more (ca. 750-1150mg) pheromone than current “standard” commercial formulations (75-100mg) when applied at - 10-80 ha^{-1}. Dispenser efficacy was measured by the reduction of male captures in pheromone-baited traps, and mating status of female moths (measured by recovered spermatophores) captured in kairomone-baited traps. A significant suppression of catch in pheromone traps was achieved in plots treated with Cidetrak meso dispensers (73-87%) and the effect was similar to that achieved in plots treated with Cidetrak standard dispensers applied at 10x greater densities. Cidetrak CM/PE formulated with a combination of codlemone and pear ester kairomone, , had slightly less mated females, 13%, compared to orchards with Cidetrak CM dispensers or no pheromone. Isomate CM RING dispensers deployed at 10-100 dispensers ha^{-1} compared to Isomate CM FLEX applied at 1000 dispensers ha^{-1} reduced moth captures only minimally. None of the meso treatments provided the high level (>90%) of mating disruption typical of low-emitting dispensers applied at high densities (ca. 1000 ha^{-1}). Meso dispensers offer a labor-saving alternative to high-density dispensers but protection only when CM populations are low or where companion insecticides for other pests is common.

INTRODUCTION

Codling moth, *Cydia pomonella* L., is a cosmopolitan pest of apple and walnut (McDonough et al. 1972, Barnes 1991). Pheromone-based mating disruption for codling moth has been commercially available for over two decades (Cardé and Minks 1995, Thomson et al. 1998) and is the principal management tool for this pest on more than 170,000 hectares worldwide (Witzgall et al. 2008). While this novel option has been widely adopted, and has decreased the amount of pesticides applied for codling moth (Gut and Brunner 1998, McGhee et al. 2011), further expansion of its use will likely require a reduction in cost and improvements in efficacy.

Reservoir formulations constitute the majority of commercialized codling moth disruption products used in pome fruits (Witzgall et al. 2008). The various products include: Isomate-C Plus or Isomate CM FLEX polyethylene reservoir dispensers (Shin-Etsu Chemical Co., Tokyo, Japan), CheckMate CM (Suterra LLC, Bend, OR) and NoMate CM (Scentry Biological, Billings, MT). All reservoir dispensers are hand-applied at densities of 300 to 1000 per ha and placement high in the tree canopy is recommended for best effect. The efficacy of hand-applied dispensers is positively correlated with pheromone point-source densities; higher pheromone point-sources provide better and more consistent suppression of male moth captures in traps and reduction in injury to fruit (Epstein et al. 2006). Application of dispensers is typically accomplished with the use of ladders, extension poles, or mechanical pruning towers. Such procedures are labor intensive, taking at least 4 person-hours ha^{-1} at a time when demand for labor is especially high in many growing regions due to other important orchard activities, including weed and disease management and final pruning cleanup. Deploying dispensers at the recommended label rate costs growers up to \$275 USD ha^{-1} for the product and an additional \$45-\$75 ha^{-1} for labor. Growers must apply the pheromone treatment prior to moth emergence, and consequently before fruit set to achieve optimal control. Spring frosts at bloom time, just as CM begins to emerge, can reduce or destroy the season's crop. Growers in this situation often must purchase and deploy pheromone prior to knowing if there will be a crop to protect.

New pheromone delivery systems that require less labor and time to apply are needed to entice more apple growers to adopt this environmentally sound pest management practice. Welter (Welter and Cave 2004) coined the term meso dispenser to describe controlled delivery devices that fall between aerosol emitters (Mega-dispensers) releasing 336 mg pheromone/d and are applied at a density of only a few ha^{-1} and standard hand-applied dispensers that releasing ca. 100ug pheromone/d and are applied at a 1000 ha^{-1}. Meso dispensers aim to release enough pheromone from each unit to achieve effective disruption while using only 50-100 devices ha^{-1}. Mega dispensers like aerosol emitters build on the success of hand-applied reservoir dispensers, but lower the costs of application. They may be especially useful in high canopy crops, such as walnuts, where applying dispensers high in the canopy is especially difficult. The approach, in part, has been validated by work where some success with CM control was achieved using clusters of 10-100 standard dispensers applied at reduced point source-densities (McDonough et al. 1972, Barnes 1991, Knight 2004, Epstein et al. 2006).

Several companies have engineered meso-dispensers based on their current hand-applied reservoir technology. Shin-Etsu Chemical Co. developed a larger Isomate polyethylene tube, Isomate CM RING, 10x larger and releases more active ingredient than Isomate CM TT dispensers. Trécé Inc. (Adair, Oklahoma, USA) developed a solid PVC matrix dispenser that can be manufactured in various sizes, thus allowing latitude in the amount of pheromone released per dispenser. The concept behind each of these technologies is to provide a sufficiently high pheromone loading and release rate such that fewer dispensers are required per ha.

Trécé has loaded their PVC dispenser with codlemone and pear ester with the aim of improving disruption (Knight and Light 2005a, Knight et al. 2012). Pear ester (PE), ethyl (E,Z)-2,4-decadienoate (Et-2E,4Z-DD), is a plant kairomone that is attractive to codling moth larvae (Knight and Light 2001. Witzgall et al. 2008) and adults (Light et al. 2001, Coracini et al. 2004). More male and female moths are captured in traps baited with a combination of pear ester and codlemone in apple and walnut orchards (Light et al. 2001, Coracini et al. 2004, Witzgall et al. 2008). Combining codlemone and pear ester might enhance competition of artificial pheromone sources with females leading to a more effective disruption than dispensers releasing only codlemone.

Here we report results of experiments conducted between 2010-2013 to determine the effectiveness of meso dispensers for disruption of codling moth. Experiment 1 directly compared the efficacy of the Shin-Etsu CM RING meso dispenser to that of the standard Isomate CM FLEX dispenser. Experiment 2 directly compared the efficacy of the Trécé Cidetrak® CM (647) and Cidetrak® (645 & 646) meso pheromone dispensers containing either codlemone alone or a combination of codlemone and pear ester. The hypotheses tested in these comparisons were; 1) meso dispensers deployed at low densities provide a similar level of disruption to standard dispensers deployed at high densities and, 2) addition of pear ester improves disruption over commercial formulations containing only codlemone. Experiment 3 determined the optimum loading rate and application density of PVC meso dispensers. It directly compared the efficacy of various loading rates and deployment densities of Trécé Cidetrak® meso dispensers containing codlemone and pear ester for codling moth (CM) mating disruption in apple using large-plot field trials. The hypothesis tested was that disruption efficacy increases with increasing point-source potency and application density.

MATERIALS AND METHODS

Experiment 1 - Efficacy of the Isomate CM RING meso dispenser

Experiments were conducted during the summer in 2009 and 2010 to determine the efficacy of a novel mega dispenser, Isomate CM RING, for disruption of codling moth. The dispenser is essentially a continuous loop of 10 standard Isomate Flex dispensers. The experimental design was randomized complete block, consisting of 5 treatments replicated on 7 apple farms located in the Ridge and West Central fruit-growing regions of MI. Orchards ranging in size from 50 ha were subdivided and treatments randomly assigned to 2 ha plots. Pheromone treatments consisted of: (i) Isomate CM RING at 100 ha^{-1}, (ii) Isomate CM RING at 100 ha^{-1} but deployed in 10 discrete point-sources ha^{-1} with each point-source consisting of 10 RINGS/tree, (iii) Isomate CM FLEX at 1000 dispensers ha^{-1} as a positive control, (iv) CM FLEX at 100 ha^{-1} and (v) no pheromone (negative control). All pheromone dispensers were deployed in the upper 1/3 of the tree canopy.

Treatment effects were assessed by comparing adult male captures in pheromone traps and fruit injury. Capture of males in traps was monitored weekly using 6 plastic Trécé Pherocon VI traps baited with Trécé L2 CM lures placed in a grid in the plot center; 1 trap/0.2ha. Traps were elevated in the tree canopies on 2.5m bamboo poles. Fruit injury assessments were conducted twice, once after completion of 1st generation CM and again prior to harvest by visually inspecting 1,200 half fruits per treatment. All injured fruit were collected and any larvae found were identified to species; oriental fruit moth (OFM) or codling moth (CM). If supplemental insecticides and disease management applications were warranted, they were applied to all treatments at a given site.

Moth catch data from 2009 and 2010 were pooled for statistical analysis. Catch data + 1 was log transformed to correct for skewness and kurtosis and subjected to a linear mixed model analyses (Systat Ver. 13, Systat Software Inc.

2009). Years and farms were blocked; the main fixed effect was pheromone treatments. Treatment effects were compared using Tukeys HSD test, $p<0.05$.

Experiment 2 - Efficacy of Cidetrak® CM meso dispensers

Trials testing the efficacy of Cidetrak CM meso dispensers were conducted on four Michigan Farms; two in the Hartford / Lawrence region of Southwest Michigan and two Mid-Michigan Farms located near Sparta. The Cidetrak meso dispenser is essentially a continuous, uncut, strip of standard Cidetrak dispensers. Experiments were designed to directly compare the following four treatments: (i) Trécé Cidetrak® CM “standard” (647) containing 75/55mg codlemone per dispenser, (ii) a “meso” treatment (Trécé 645) containing 750mgs of codlemone, (iii) a meso treatment (Trécé 646) loaded with 750/550mgs codlemone/pear ester and (iv) a no-mating disruption (negative control). Orchards ranging from 18 to 30 ha were subdivided into 2.0-2.4 ha plots and randomly assigned a treatment. All Cidetrak® formulations were applied in early May to the top third of the tree canopy at 790- 845 ha^{-1} for Cidetrak® standard dispensers (Trécé 645) and 79-84 ha^{-1} for (Trécé 645 and 646) meso dispensers. The three disruption treatments (Trécé 647, 645 or 646) were deployed such that each ha received 59.3/43.5, 59.3/0, and 59.3/43.5g codlemone/pear ester, respectively. Traps were deployed as previously described to monitor adult response; 3 traps baited with Trécé CM L2 codlemone lures used for males and 3 traps baited with Trécé CM/DA +AA lures for females.

Captured females were collected, returned to the lab, and dissected to determine mating status.

Native CM populations were supplemented with releases of sterile codling moths obtained from the Okanagan-Kootenay Sterile Insect Release (SIR) program in Oosoyos, Canada. The addition of SIR moths provided an evaluation using a known and equal pest density across treatments. Releases of SIR moths were conducted in all orchards. One-day-old moths produced by the rearing facility were shipped overnight via FedEx in Styrofoam coolers with cold packs. Upon receipt, groups of SIR CM were color marked using ca. 0.1g of DayGlo pigment powders (green, blue, pink, orange) per 133 moths. Moths were held in 296 ml Solo™ containers and transported immediately to the field for release. Each DayGlo color group was assigned to a treatment.

Three replicated releases of sterile codling moths were conducted for each experiment. Each release consisted of 1600 total moths per treatment block, ca. 800 male and 800 female codling moths. Moths were released in late afternoon by tossing them into the air between two trees at 4 different locations in each treatment block. Moths immediately flew into tree canopies upon release. A quality control release was conducted on each release day. Six cups, each containing 133 moths, were tossed separately over a blue polypropylene tarp (3.7m x 6m) in an orchard not used for experimental treatment purposes. The numbers of moths not capable of flying were counted on the tarp. The percentage of total moths flying ranged between 75-98%. The actual number of flight capable moths released into each treatment was thus estimated.

Experiment 3 - Optimizing CM meso dispensers.

Two sizes of Trécé Cidetrak CM/PE dispensers, 20cm and 51cm long, were deployed at two point-source densities in commercial apple orchards to determine the optimal potency and density required to effectively disrupt codling moth. The evaluations focused on the reduction in wild male CM captures, mating status of wild females, and captures of released sterile CM. The experiment was conducted on three Michigan Farms - one Southwest Michigan farm, and two Mid-Michigan Farms located near Sparta. The orchards ranged in from 20-40 ha with mature bearing trees trained to a vertical axis 3-4m tall. The orchards were managed according to commercial pruning, irrigation, herbicide, fungicide, and insecticide practices.

Disruption trials were conducted comparing four treatments: (i) no pheromone control; (ii) 20cm Cidetrak CM/PE, 1 dispenser per point-source; (iii) 51cm Cidetrak CM/PE, 2 dispensers per point-source; (iv) 51cm Cidetrak CM/PE, 4 dispensers per point-source, Figure 1. The experiment was arranged as a randomized complete block design with three replicates, each replicate consisted of 1 farm location, with treatments deployed in 2 ha plots. The experiment was divided into two 8-wk intervals to evaluate dispenser point-source densities comprising (i) May-June and (ii) July-August. Pheromone treatments were evaluated at 10 units ha^{-1} May - June. Treatments were applied to the top third of the tree canopy prior to the start of CM flight. Similar numbers of units for each treatment were field-aged off-site in separate commercial apple orchards. The original pheromone plots were now supplemented with 10 of the aged dispensers placed in the top third of the canopy in the first wk of July so as to increase the total point-sources from 10 to 20 units ha^{-1} total. Pheromone treatments were formulated accordingly: 20cm straps - 600mg codlemone, (*E, E*)-8,10-dodecadien-1-ol, (CM) + 440mg pear ester, ethyl (2E,4Z)-decadienoate, (PE); 51cm straps - 1500mg CM + 1100mg PE. Disruption was assessed by monitoring wild and SIR CM adults in traps as described in Experiment 2. Fruit injury evaluations were omitted because the experimental treatments were altered midway through the growing season; thus, injury could not be attributed to a particular dispenser density. Due to miscommunication, moths from 2 sites were not identified to sex in CM/DA +AA traps. Statistical analyses on female catch were not performed due to lack of replication.

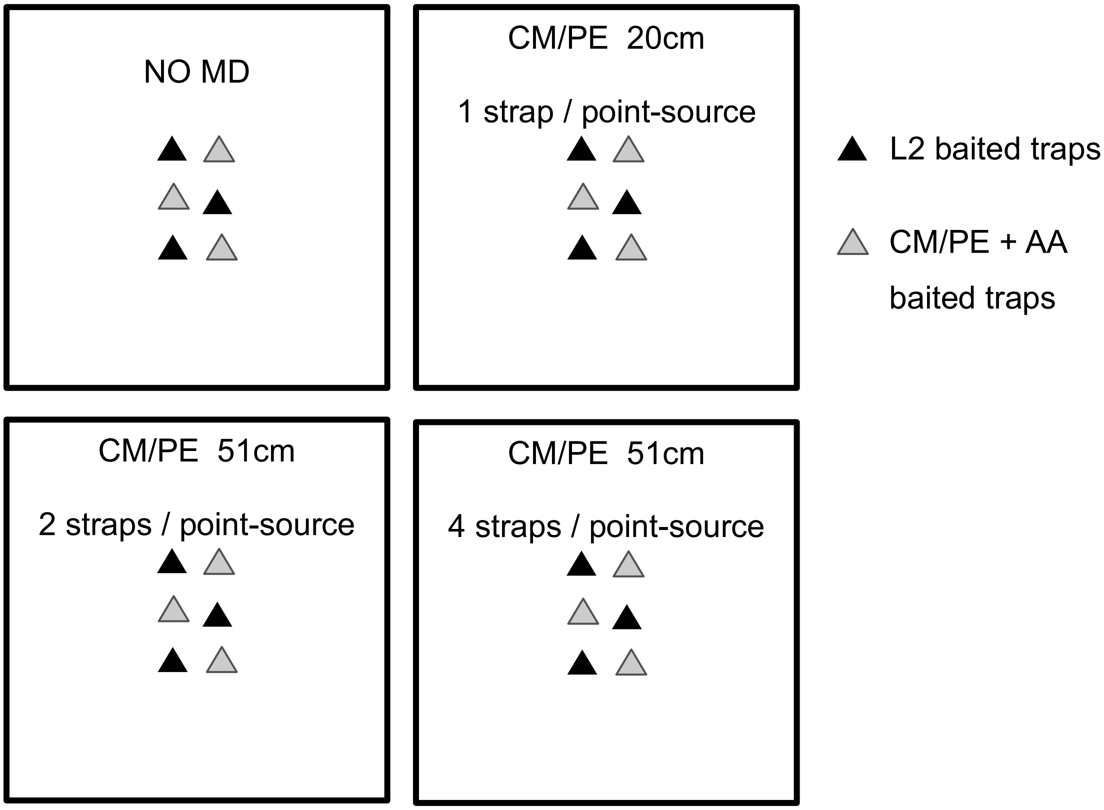

Figure 1. Generalized layout of Cidetrak® CM meso plots, MI, 2013. Dispensers deployed at a density of 10 point-sources ha^{-1} until July 1st and then increased to 20 point-sources ha^{-1}.

RESULTS

Experiment 1. Efficacy of the Isomate CM RING meso dispenser

Codling moth captures were low to moderate at 6 sites and extremely high at the 7th site, (from the teens to hundreds of individuals per trap), respectively. The CM FLEX deployed at 1000 ha^{-1} significantly suppressed moth captures for both generations compared to the other pheromone treatments and the No MD control (Figure 2). The RING treatment deployed at 100 point-sources ha^{-1} was the only other treatment with statistically lower moth captures than the No MD control for both first and second generations, and it was not different from FLEX at 1000 point-sources ha^{-1}. Moth catch in CM FLEX and RING treatments deployed as 100 point-sources ha^{-1} were similar. The two CM RING treatments also were similar, with only a slight numerical difference in moth catch suppression during the first generation. Isomate CM FLEX at 1000 point-sources ha^{-1} reduced captures by 84% and 78% for 1st and 2nd generations respectively. CM FLEX and CM RING dispensers applied at 100 point-sources ha^{-1} provided ca. 64% and 25% disruption for the two generations, respectively.

Fruit injury was highest in the negative control for both mid-season (2%) and pre-harvest (2.5%) samples. Pheromone treatments generally had about 50% less fruit injury than the NO MD treatment (Figure 3). The major exceptions were the high level of reduction in injury for the Flex 1000 at midseason and the lack of reduction of fruit injury for the Ring 100 treatment prior to harvest.

However, there were no statistical differences in fruit injury among any of the treatments.

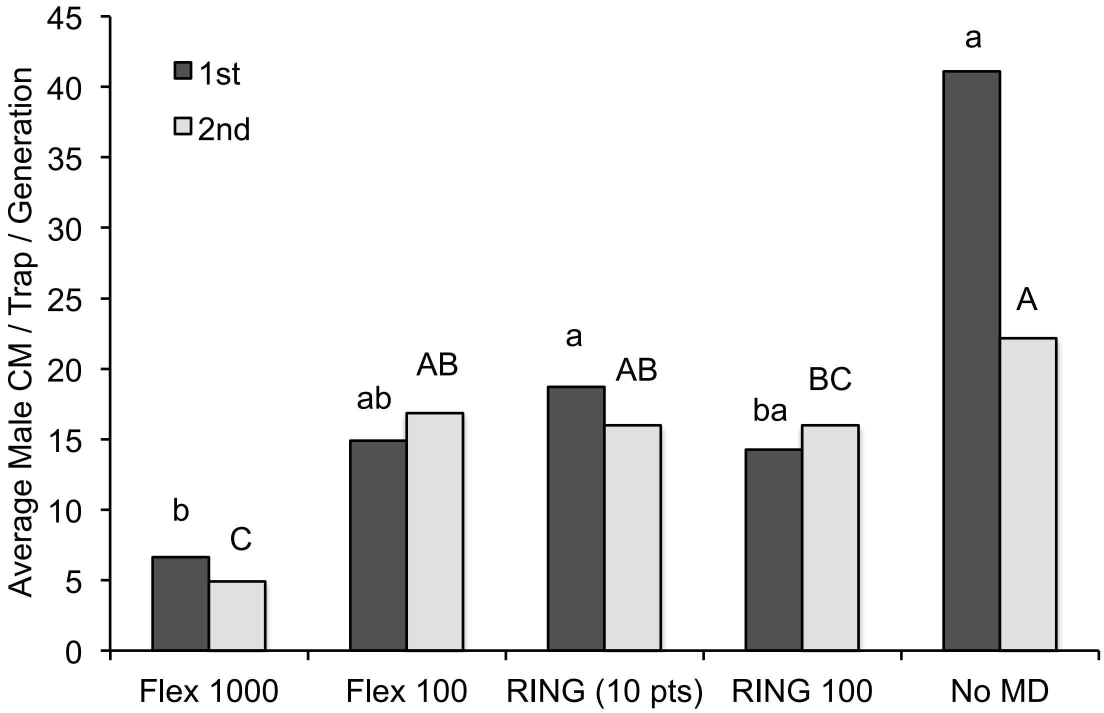

Figure 2. The average number of wild male codling moths captured in L2 baited traps 1st and 2nd generation in plots treated with Isomate CM FLEX dispensers deployed at 1000 or 100 dispenser point-sources ha^{-1} and Isomate CM RINGs deployed at 100 or 10 point-sources ha^{-1} (10 RINGS on each of 10 trees ha^{-1}), MI, 2009-2010.

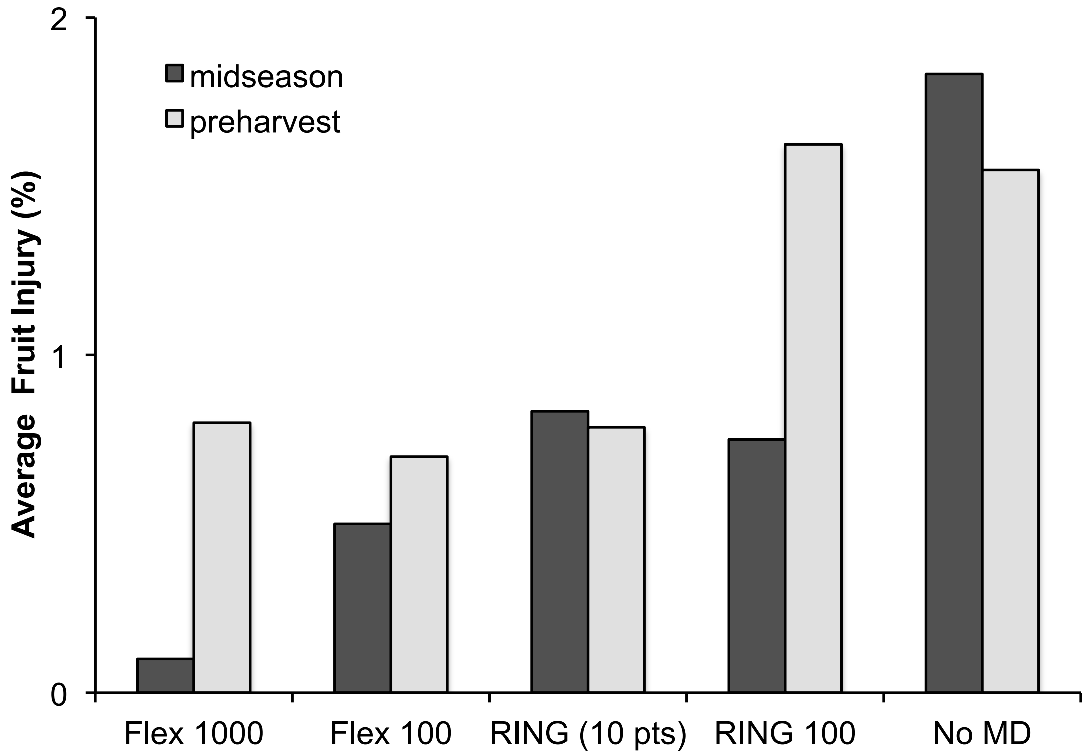

Figure 3. The average codling moth injured fruit (%) at midseason and pre-harvest in plots treated with Isomate CM FLEX dispensers deployed at 1000 or 100 dispenser point-sources ha^{-1} and Isomate CM RINGs deployed at 100 or 10 point-sources ha^{-1} (10 RINGS on each of 10 trees ha^{-1}), MI, 2009-2010.

Experiment 2. Efficacy of Cidetrak CM meso dispensers

Seasonal captures of male codling moth were moderate; the mean catch in the no pheromone control plots was 90 males. The loss of the apple crop due to a severe crop freeze in 2012 contributed to a lower than normal CM population, especially for the summer or 2nd generation. However, wild male codling moths were captured at every site throughout the season. All Cidetrak®

treatments reduced wild male CM captures in pheromone-baited traps compared to the No MD control for both 1st and 2nd generations (Figure 4). There were no statistically significant differences between Cidetrak® “standard” and “meso” treatments for captures of wild male moths. Cidetrak “standard” and meso pheromone treatments provided 78% and 65-59% reductions in male captures compared to the negative control during 1st and 2nd flights. The pattern of disruption for SIR moths was similar to that for wild moths. All Cidetrak® treatments significantly reduced male moth captures below those in the negative control (Figure 5).

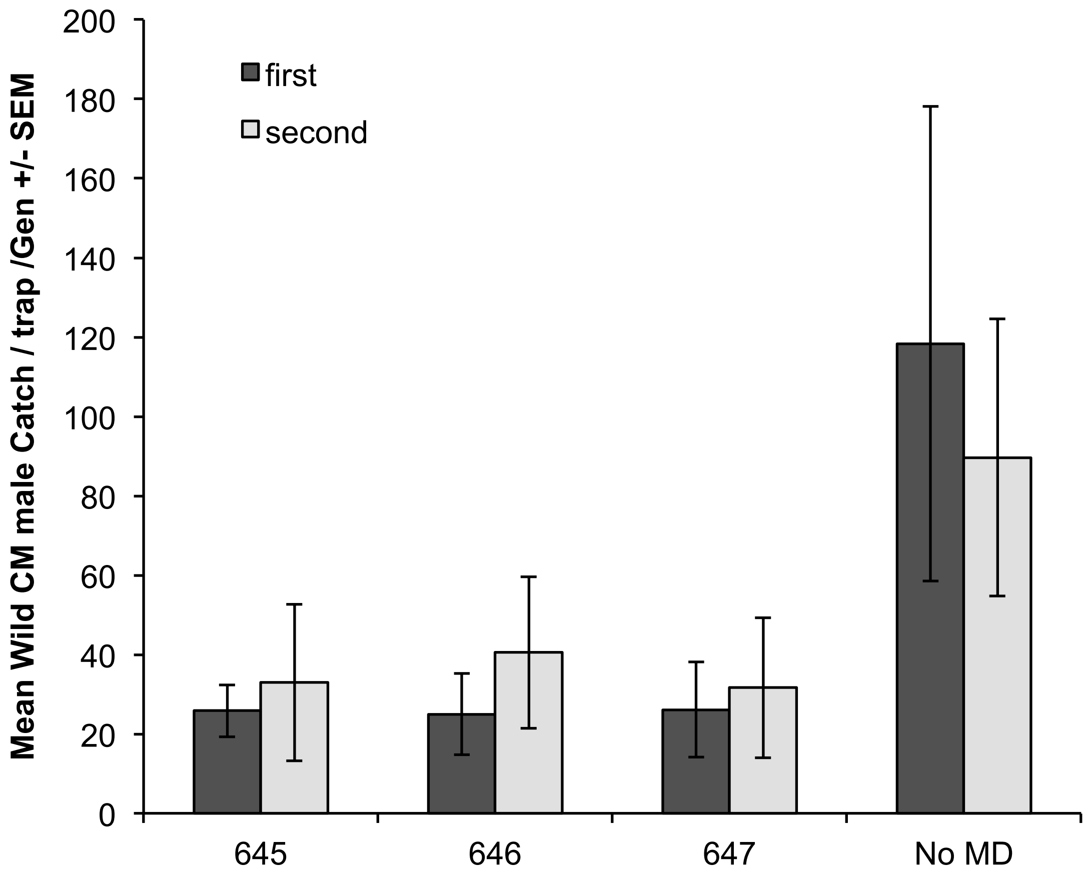

Figure 4. The average number of wild male codling moths captured in L2 baited traps 1st and 2nd generation in plots treated with 3 types of Trécé Cidetrak® CM dispensers (645, 646, and 647), MI, 2012.

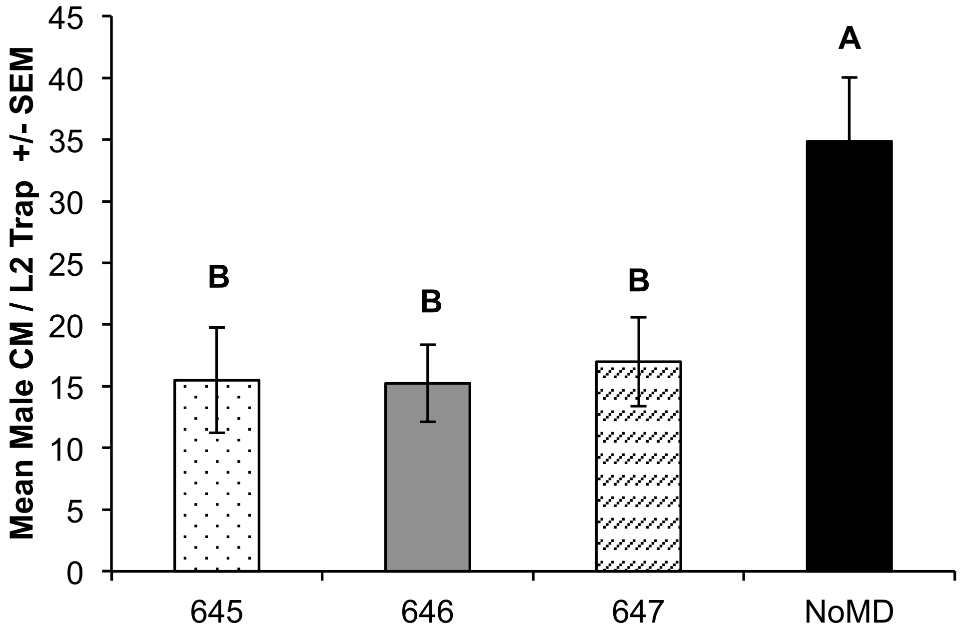

Figure 5. The average number of SIR CM captured in pheromone traps during 14 releases in plots treated with 3 types of Trécé Cidetrak® CM dispensers (645, 646, and 647), MI, 2012. Fisher's Protected LSD, (Df = 3, f= 5.13 p = 0.004).

Female densities also were atypically low due to lack of fruit the previous season, but moths were captured at each site. Overall, more than twice as many female moths were captured in the no pheromone control compared to Cidetrak® treatments (Figure 6). Cidetrak® 645 and 646 treatments captured slightly more female CM overall than the 647 treatment. Although there were no statistically significant differences by treatment in the number of mated females recovered or the number of spermatophores per female. There were fewer mated females in orchards receiving meso dispensers (ca. 37%) compared to either the 647 or no MD orchards (ca. 50% each). Regardless of treatment the vast majority of mated females recovered contained only 1 spermatophore (96% +/- 1.5% SEM).

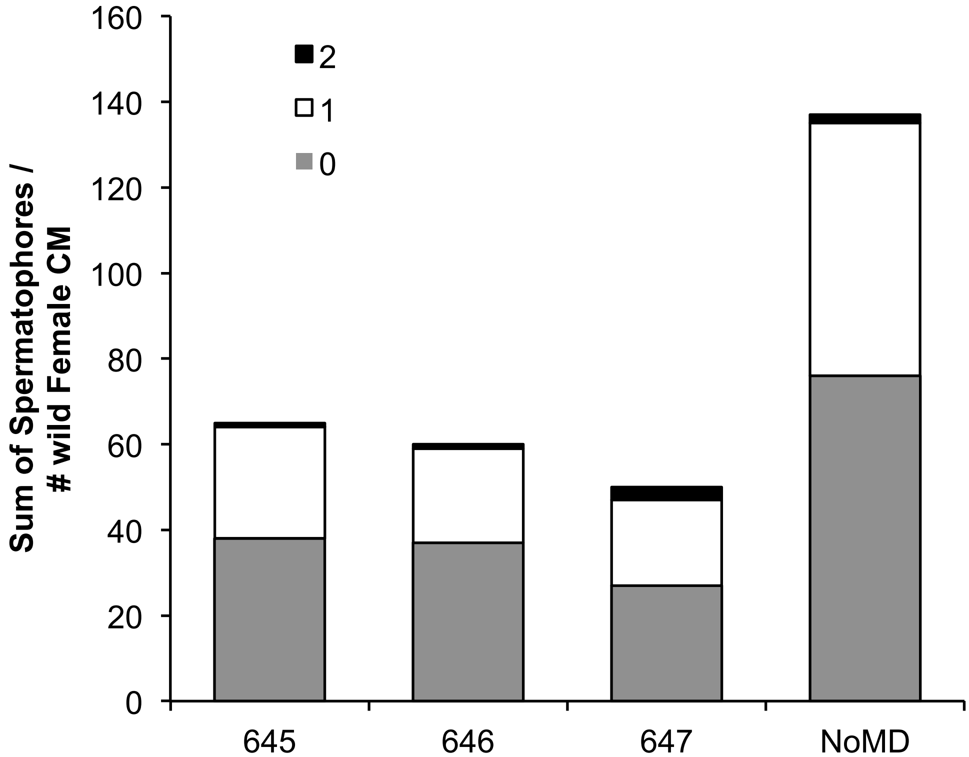

Figure 6. Female CM mating and numbers of spermatophores per female in plots treated with 3 types of Trécé Cidetrak® CM dispensers (645, 646, and 647), MI 2012.

Experiment 3. Optimizing CM meso dispensers

Seasonal patterns of codling moth captures in traps were low and varied among locations in 2013, most likely due to the freeze of 2012. In many orchards, there was less than 10% of a crop left in 2012 to sustain fruit-infesting codling moth populations. Wild codling moth adults were present in the no pheromone treatments at peak levels of only 3-5 moths/trap/week during 1st generation and dropped to less than one moth/trap/week in the 2nd generation.

Under these pest densities, 1st generation male captures were similar across treatments, including the no pheromone control (Figure 7).

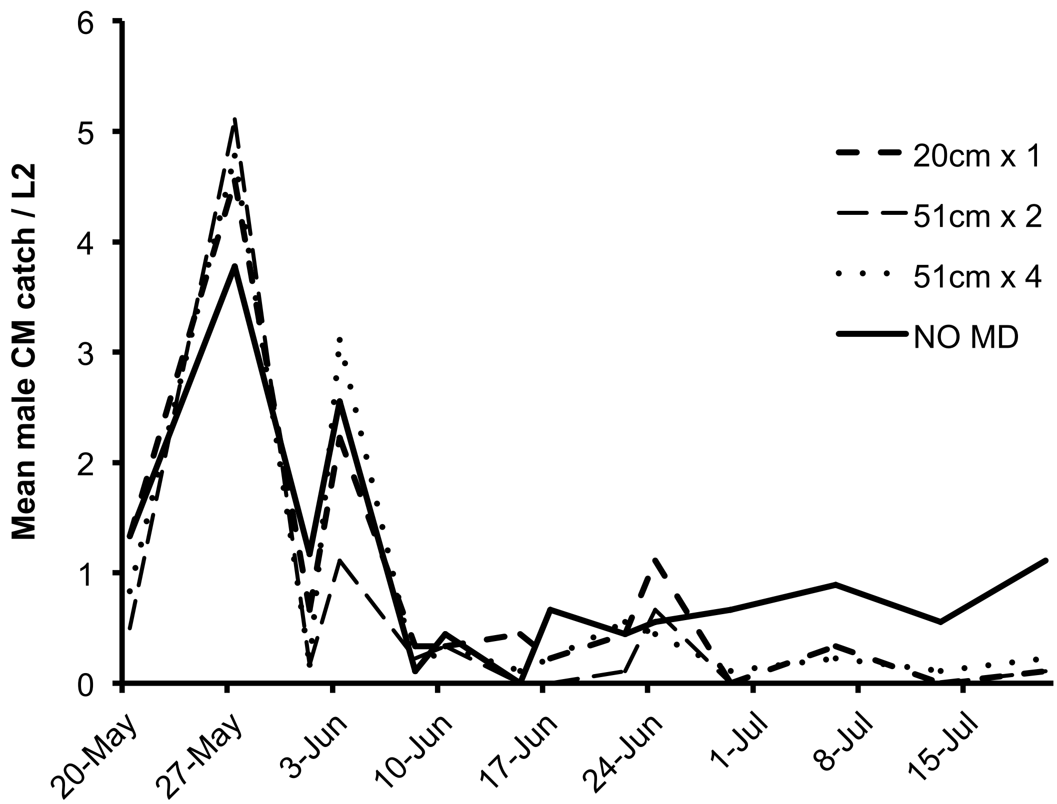

Figure 7. Seasonal captures of wild male codling moths in L2 baited traps in plots treated with Trécé Cidetrak® CM meso dispensers or not treated with pheromone (No MD), MI 2013.

Due to the low native populations, captures of released SIR moths was the principal means of assessing treatment effects. All Cidetrak® meso treatments reduced SIR male recaptures in L2 baited traps compared to the negative control when deployed at either 10 and 20 point-sources ha^{-1} (Figures 8 & 9) respectively; 10 point-sources ha^{-1} [F=6.132 (3, 32) p = 0.002], and 20 point-sources ha^{-1} [F=12.64 (3,32) p< 0.001].

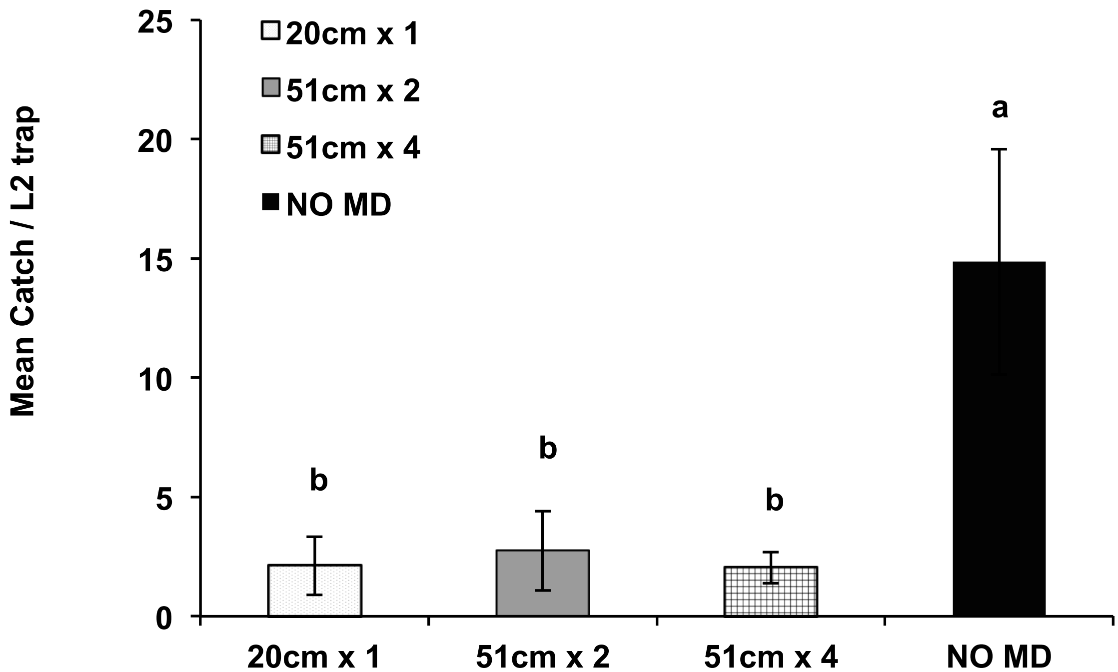

Figure 8. The average number of SIR CM captured in plots treated with Trécé Cidetrak® CM meso dispensers deployed at 10 point-sources per ha^{-1} or not treated with pheromone (No MD), MI 2013. ANOVA, pairwise comparisons Tukey's HSD test, (F=6.132 (3, 32) p = 0.002).

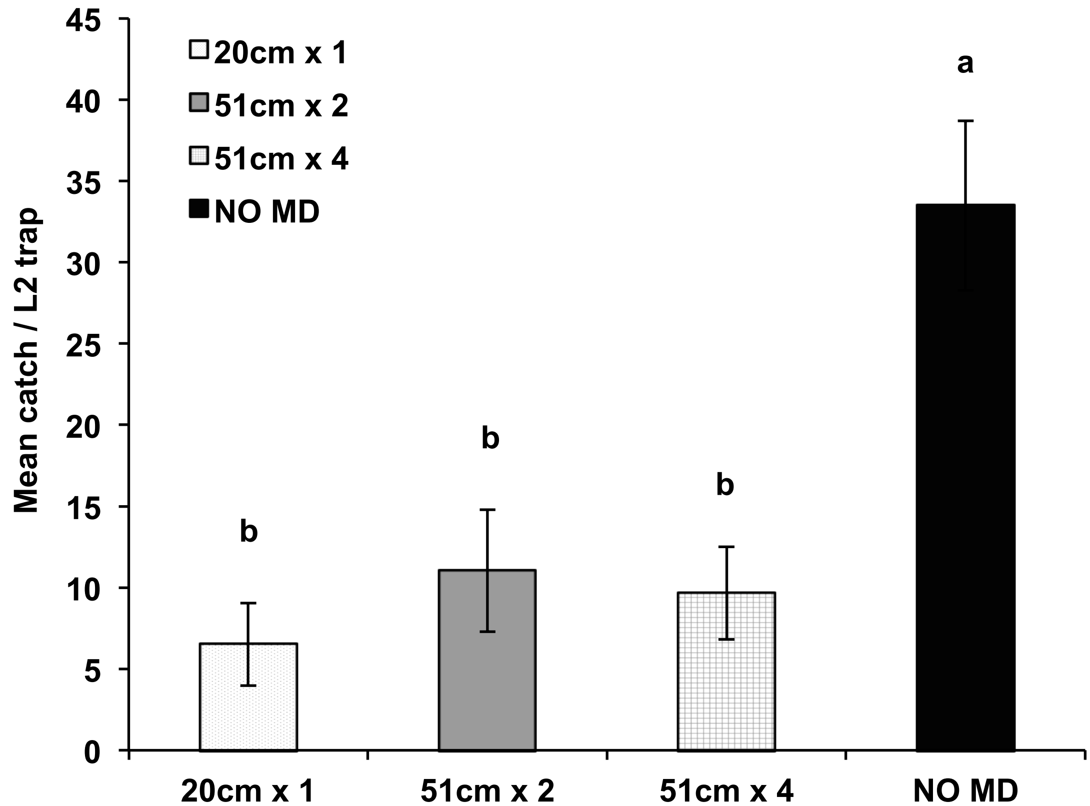

Figure 9. The average number of SIR CM captured in plots treated with Trécé Cidetrak® CM meso dispensers deployed at 20 point-sources per ha^{-1} or not treated with pheromone (No MD), MI 2013. ANOVA, pairwise comparisons Tukey's HSD test, $F = 12.64$ (3, 32), $p < 0.001$.

Traps baited with CM/DA +AA caught equal numbers of unmated female CM in all pheromone treatments and the no pheromone control (Figures 10 & 11). In the 10 point-source ha^{-1} trial, there were more mated females captured in the single 20cm meso treatments compared to either 51cm pheromone treatment and the untreated control. The majority of females mated once with multiple-matings obtained in only the single 20cm dispenser and double 51cm dispenser treatments. More females were captured during the experiment with 20 point-sources ha^{-1} vs 10 point-sources ha^{-1} treatments. This was also the case for male captures in pheromone baited traps and most likely reflects increased activity due to warmer evening temperatures in summer. The ratio of mated to unmated females was reduced when dispensers were deployed at 20 point-sources ha^{-1}, Figure 10.

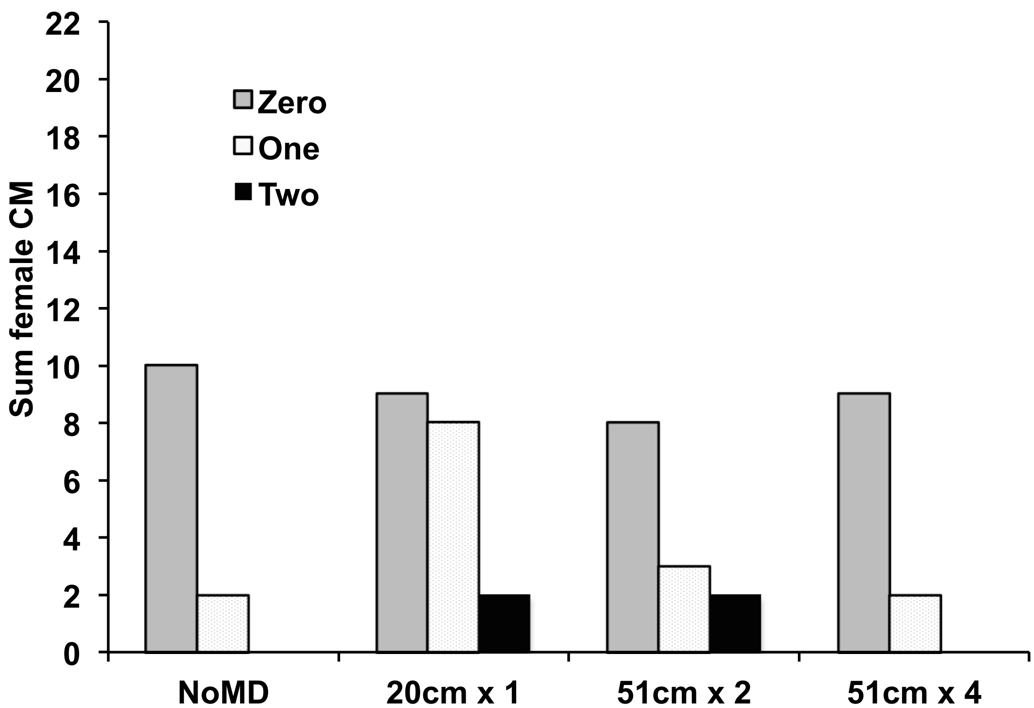

Figure 10. Sum of wild female CM with 0, 1, or 2 spermatophores, captured in plots treated with Trécé Cidetrak® CM meso dispensers deployed at 10 point-sources ha^{-1} or not treated with pheromone (No MD), MI. 2013 (1 site).

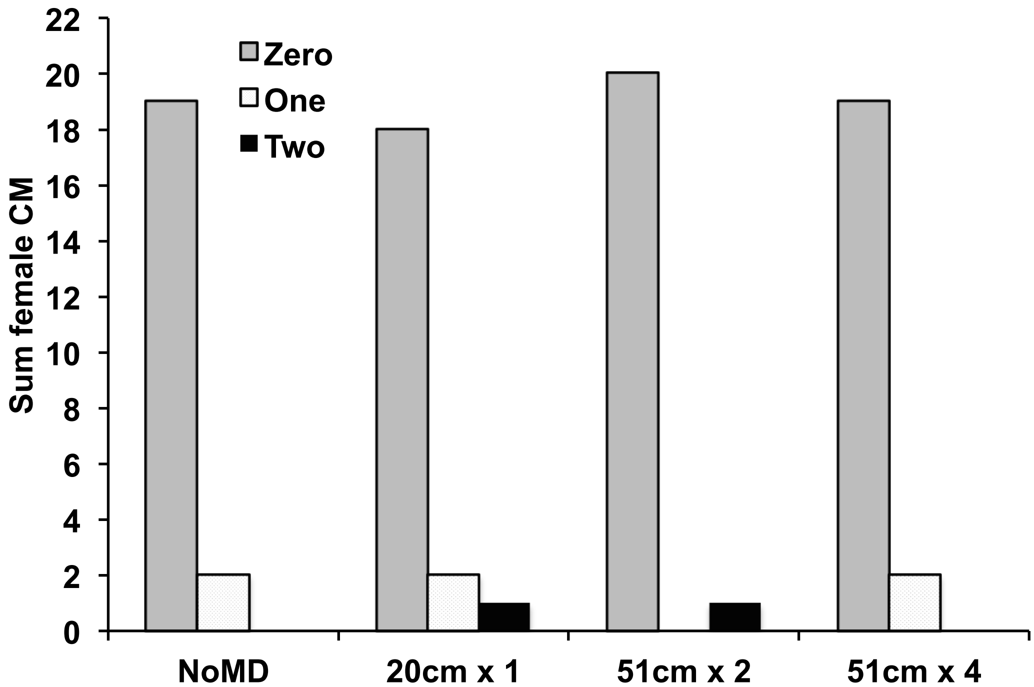

Figure 11. Sum of wild female CM with 0, 1, or 2 spermatophores, captured in plots treated with Trécé Cidetrak® CM meso dispensers deployed at 20 point-sources ha^{-1} or not treated with pheromone (No MD), MI. 2013 (1 site).

DISCUSSION

The Isomate RING and Cidetrak meso technologies were designed with the common aim of reducing deployment density and thus application time and cost while maintaining efficacy similar to standard hand-applied dispensing systems. Our studies focused on determining field efficacy rather than application time. Isomate CM RING dispensers deployed at reduced densities compared to Isomate CM FLEX applied at 1000 dispensers ha^{-1} reduced moth captures only slightly. The level of inhibition provided by the CM RING dispenser deployed at reduced densities compared to the FLEX dispenser was much lower (avg. 45%) than what is considered necessary to achieve robust mating disruption using reservoir dispensers (Miller et al. 2010, Knight et al. 2012). Higher levels of catch suppression were achieved in plots treated with Cidetrak meso dispensers applied at low densities. Inhibition of catches of wild and released moths in 2012 averaged 78 and 56%, respectively. In 2013, meso treatments reduced catch on average by 85% when deployed at 10 units ha^{-1} and 73%, at 20 units ha^{-1}.

The addition of pear ester as an active ingredient did not improve the efficacy of the Cidetrak meso dispenser. In the 2012 and 2013 experiments, the 20cm meso dispenser containing both PE and CM pheromone provided a similar level of disruption for the SIR moths; ca 75%. Similarly, Knight et al. (2012) found few differences in the disruption of traps or calling females for standard Cidetrak dispensers loaded with codlemone alone or with the addition of pear ester. Although pear ester is known to attract codling moth males, any improvement in disruption may be difficult to demonstrate in large field studies and fairly low codling moth densities.

It is perplexing that the proportion of mated females captured in CM/DA +AA was similar across treatments, including the no pheromone control. The lack of an effect of the pheromone treatments on mating may indicate that high levels of mating occur when only about 80% disruption of male catches in traps is achieved. However, it also may reflect the preferential capture of mated codling moth females in CM/DA bated traps (Knight and Light 2005b). This combination lure may be most useful as a monitoring tool, rather than a means of evaluating the impact of the pheromone treatment.

Increasing the size, and presumably the potency, of either mega dispensing system did not enhance disruption. The 20 cm and 51 cm Cidetrak dispensers deployed at a common density provided 70-80% inhibition of male catches compared to the no pheromone control. The RING dispenser is 10x larger and loaded with 10x more pheromone than the FLEX dispenser, and thus might be expected to provide superior disruption on a per dispenser basis. However, the Isomate CM RING applied at 100 ha^{-1} did not out-perform the Isomate CM FLEX dispenser when the two dispensers were deployed at the same density. We suspect that the Isomate meso and standard dispensers tested produced pheromone plumes of similar size and potency.

Perhaps, most surprisingly, there was no added effect of clustering 10 RING dispensers as a single point source (in one tree) at a density of 10 point-sources ha^{-1}. I postulated that the clustered RING pheromone treatment would mimic the very high release of a codling moth aerosol emitter. The intent was to make a passive dispenser that could be deployed at similar low densities of 5-6 units ha^{-1} recommended for aerosol emitters (Welter and Cave 2004). Codling moth catch suppression of >95% has been reported using aerosol emitters at low densities in apple and walnuts (Shorey and Gerber 1996, Welter and Cave 2004, Knight 2005). The less than satisfactory performance of the RING 10x mega dispenser at low densities may, in part, be due to the differences in how this technology dispenses pheromone compared to the aerosol emitter. Isomate CM RING dispensers contain pheromone in a hollow polyethylene reservoir and passively release it by diffusion through the membrane wall. Aerosol emitters retain pheromone in a pressurize reservoir that protects it from degradation and the pheromone is actively released in large quantities (ca. 3.5 mg/emission) as an aerosol. Isomate CM RING dispensers emit pheromone passively in small quantities all along the surface of the dispenser, while aerosol emitters actively disperse pheromone from a single point directly into the orchard atmosphere. The success of aerosols may be due to the large pheromone plume size and concentration, while Isomate CM FLEX dispensers rely on manually distributing the pheromone by deploying 1000 point-sources ha^{-1}. The CM RING mega dispenser apparently does not release pheromone in a manner that fosters its movement over a large expanse, nor is it deployed at a high enough density to manually achieve the efficacious distribution of pheromone.

The moderate levels of disruption achieved using meso dispensers agree with results from other studies documenting 70- 80% reduction in male catch is easily attainable at low point-source densities. Miller (Miller et al. 2010) generated dosage response profiles for Isomate-C Plus dispensers releasing ca. 5 µg codlemone/hr and demonstrated that two dispensers per cage (ca. 50 ha^{-1}) provided 43% reduction in moth capture. Increasing the density to 8 dispensers/cage (ca. 200 ha^{-1}) resulted in 69% reduction in catch. (Epstein et al. 2006) clustered individual Isomate–C Plus dispensers at different densities while maintaining the overall pheromone concentration ha^{-1}, and showed good suppression of moth catch in pheromone traps at reduced point-source densities. Plots treated with as few a 10 units ha^{-1} reduced catch by 75%. Attaining a level of disruption above 90% is a much more difficult task. To date, achieving this high level of codling moth disruption requires either a high density of point-sources (Miller et al. 2006, 2010,) or a high release of pheromone from an aerosol emitter (Shorey and Gerber 1996, Knight 2005).

Dosage-response studies for both low-releasing reservoir-dispensers and very high-releasing aerosol emitters have demonstrated that codling moth disruption using these devices is achieved via competition (Miller et al. 2010, McGhee et al. 2014) The graphical profile for disruption outcomes occurring by competition reveals that the first few dispensers deployed provide the biggest impact (Miller, Gut, de Lame, and Stelinski 2006b). Cidetrak meso dispensers applied at low densities of 10, 20 or 80 units/ ha^{-1} provided similar levels of moderate disruption. This would suggest that meso dispensers also operate via competition between dispensers and female moths. However, delineating the mechanism of disruption for meso dispensers awaits a dosage response study in which dispensers of uniform size and pheromone release are applied at a range of densities. Graphical analyses of the results would also assist in determining the optimal deployment density for these moderately releasing devices.

Based primarily on the results for the Cidetrak dispenser, meso dispensers applied at low densities appears to be a viable option for pheromone-based management of codling moth. A significant level of disruption of male captures in pheromone traps can be achieved by deploying as few as 10 dispensers ha^{-1}. The approach falls short of the disruption level achieved using high-density reservoir dispensers. Except where codling moth population densities are low, the meso approach will likely require supplemental insecticide sprays. However, many growers are applying insecticides to control other pests and welcome the option of using a dispensing system that requires little time and labor to apply. The meso approach should be especially appealing to producers of tree crops with very tall canopies where positioning the dispensers high in the canopy is difficult.

CHAPTER TWO

High-dosage codling moth aerosol pheromone emitters enhance rather than disrupt attraction

ABSTRACT

Field studies over 3 summers (2010-2012) in Michigan commercial apple orchards documented the effects of codlemone, (*E, E*)-8,10-dodecadien-1-ol, released from aerosol pheromone emitters (Suterra CM Puffer and Isomate CM MIST) on subsequent male codling moth behavior. Pheromone plumes originating from a single aerosol emitter reduced male captures in baited traps up to 180m away. Surprisingly, traps within 5m of the emitter captured many males. Catch of released, marked, and sterilized moths demonstrated that males moved preferentially from areas of no or low pheromone concentration towards aerosol emitters, where they were captured in nearby traps. Additionally, moths bypassed baited traps placed between the release and recapture locations. Dispersal of released moths in aerosol-treated orchards (1 unit ha^{-1}) was twice that of moths in orchards treated with high point-source density pheromone dispensers (Isomate CM FLEX, 1000 ha^{-1}) or those left untreated. Moths caged for 24 h on aerosol-treated foliage and then released into apple orchards had an elevated response to traps than were males exposed to 2 Isomate FLEX dispensers or caged on untreated foliage. Overall, these findings suggest that aerosol emitters operative competitively, by causing males to displace away from females. We propose the term *induced allopatry* for the phenomenon of mating disruption by spatial segregation of the sexes where movement towards and aggregation near the high pheromone sources diverts males away from females that are releasing very low quantities of pheromone.

INTRODUCTION

Although more pheromone is used for codling moth, *Cydia pomonella* L., mating disruption than any other agricultural insect pest (Witzgall et al. 2008), the actual mechanism whereby sexual communication is inhibited is only recently becoming clearer. Competitive attraction was proposed and validated as the primary mechanism for codling moth disruption using passively emitting, hand-applied pheromone dispensers (Miller et al., 2010, McGhee et al., 2014). Here, the ability of the males to find females is suppressed because males waste time following false plumes from the dispensers releasing moderate (5 μg/hr) dosages (Tomaszewska et al., 2005) of pheromone. Non-competitive mechanisms include desensitization (habituation and adaptation), camouflage, or sensory imbalance where the male moths' ability to perceive and respond normally to pheromone is impaired (Miller, Gut, de Lame, & Stelinski, 2006a; 2006b). Habituation affecting the processing of and normal responsiveness to olfactory information already in the central nervous system was the disruptive mechanism suggested for codling moth (Shorey and Gerber, 1996) but later dismissed (Judd et al. 2005; Stelinski, Gut, & Miller, 2005) because airborne concentrations of pheromone under field settings are much lower than those known to induce this phenomenon in a laboratory flight tunnel.

Little is known about the events happening once CM males have encountered a high dose of pheromone in the field. Miller (Miller et al., 2010) deduced from dosage-response curves recorded in large field cages that codling moths were deactivated for the remainder of one diel activity period after approaching an Isomate C-Plus rope dispenser as directly observed by Stelinski (Stelinski et al. 2006). However, the behavioral impacts of exposure to the much higher dose (up to 28 mg of pheromone per hour) released from aerosol emitters, such as CM Puffers (Suterra, Bend, OR) and Isomate CM MIST (PBC, Vancouver, WA) has not been explored.

The reach of the plume emanating from aerosol dispensers is likely much larger than that from standard dispensers. Additionally, pheromone at such high dosages might survive on plant surfaces at behaviorally meaningful dosages (Gut et al. 2004) over areas much larger than would be expected per dispenser emitting lower rates of pheromone. Experiments in California pear orchards registered suppression of male CM catch in pheromone baited traps up to 450m downwind of a single Puffer (Welter, 1999). In addition to being attracted to pheromone emitters, males under treatment by aerosol dispensers may receive a pheromone dose sufficient to elevate their response threshold. Aerosol emitters deployed at low densities (2.5 ha^{-1}), but delivering vastly greater amounts of pheromone (14 mg/hr), were suspected to camouflage the low concentration pheromone plumes emitted by calling female moths (ca. 7 ng/hr) (Bäckman, Bengtsson, & Witzgall, 1997), however, a recent dosage-response study falsified that hypothesis (McGhee et al., 2014) and supported competitive attraction.

The reliability of aerosol devices has improved, but mechanical failure of individual units under field conditions could leave wide gaps between pheromone plumes where mating could occur, thus leaving fruit unprotected. Understanding the behavioral changes elicited in CM by high-releasing devices could help optimize aerosol technology.

Therefore, we conducted field studies in large Michigan apple orchards during the 2010-2012 field seasons to document the effects of CM male exposure to codlemone (E, E 8-10 Dodecadien-ol) released from a standard reservoir-dispenser (Isomate CM FLEX (2-4 μg/hr)), a meso reservoir dispenser (Isomate CM RING (20 μg /hr)), or an aerosol emitter (Suterra CM Puffer (28mg/hr)). The objectives were to determine whether: (i) exposure to pheromone suppressed or enhanced male catch in pheromone-baited monitoring traps, and (ii) the effect varied depending on the concentration of pheromone emitted from the release devices.

METHODS

Experimental plots

All experiments were conducted on large commercial apple farms in Michigan's central apple-growing region near Sparta, (43.114729°, -85.715304°; 43.332007°, -85.846757°; 43.327993°, -85.836386°). Each farm had a minimum of 40 ha of bearing fruit trees. The 4 ha treatment blocks were always separated by at least 18m of untreated orchard. The apple plantings varied only slightly from a 3m x 5.5m and 5.5m x 6m tree x row spacing oriented in north-south and with a maximum canopy height of 5m. All farms had orchards of mixed cultivars including, Paula red, Ida red, gala, empire, and golden delicious.

Pheromone release devices

Four pheromone dispensers were evaluated. Isomate CM Flex (ShinEtsu Corporation, Tokyo, Japan) releasing a few µg of pheromone/h represented the standard reservoir dispenser. Isomate CM RING (ShinEtsu Corporation, Tokyo, Japan) is a meso dispenser manufactured longer (1m) than a single Flex dispenser (20cm). Either the CM Puffer (Suterra LLC, Bend, OR) or Isomate CM MIST (Pacific Biocontrol, Vancouver, WA) represented aerosol emitters releasing mgs of pheromone/h. These aerosol emitters were loaded with 71g of codlemone, and released ca. 7 mg of pheromone every 15 min over a 12 hr cycle (0500-1500hr).

Experiment 1 - Efficacy of Aerosol Emitters

A randomized complete block design was used to test the hypotheses that i) aerosol pheromone emitters disrupt male captures in traps similarly to Isomate CM FLEX, ii) the patterns and extent of male codling moth movement differs in pheromone- and non-pheromone-treated apple orchards, and iii) that pattern varies depending on the intensity of the pheromone treatment. One replicate of each of the following treatments was deployed in 2.5 ha plots at each of three sites: 1) Isomate CM MIST at 0.4 dispensers ha^{-1} and emitting ca. 12 mg/h, and 2) Isomate CM FLEX deployed at 1000 ha^{-1} but releasing only ca. 2-4 µg/h, and 3) a no-pheromone negative control. Male CM captures were then monitored with 15 Trécé 0.1mg codlemone-baited Pherocon VI (Trécé, Adair, OK) traps/plot (Figure 12). Traps were secured to a 2.54 cm x 2.4 m bamboo pole and hung over a limb in the upper 1/3 of the tree canopy. Foliage was trimmed from around the trap openings so as not to impede pheromone plume dispersion and moth captures. Lures were changed in all traps at mid-season between codling moth generations. Nine traps were randomly placed in trees adjacent to those with a MIST unit, and 6 traps were placed equidistantly between two MIST-emitter trees to test the hypothesis that moths are drawn towards MIST emitters (Figure 12). Traps were separated by a minimum of 34m. The same spatial distribution of traps was replicated in CM FLEX and No MD treated plots so as to permit direct comparison to the CM MIST-treated orchards. Traps were monitored weekly and pheromone lures changed every other wk.

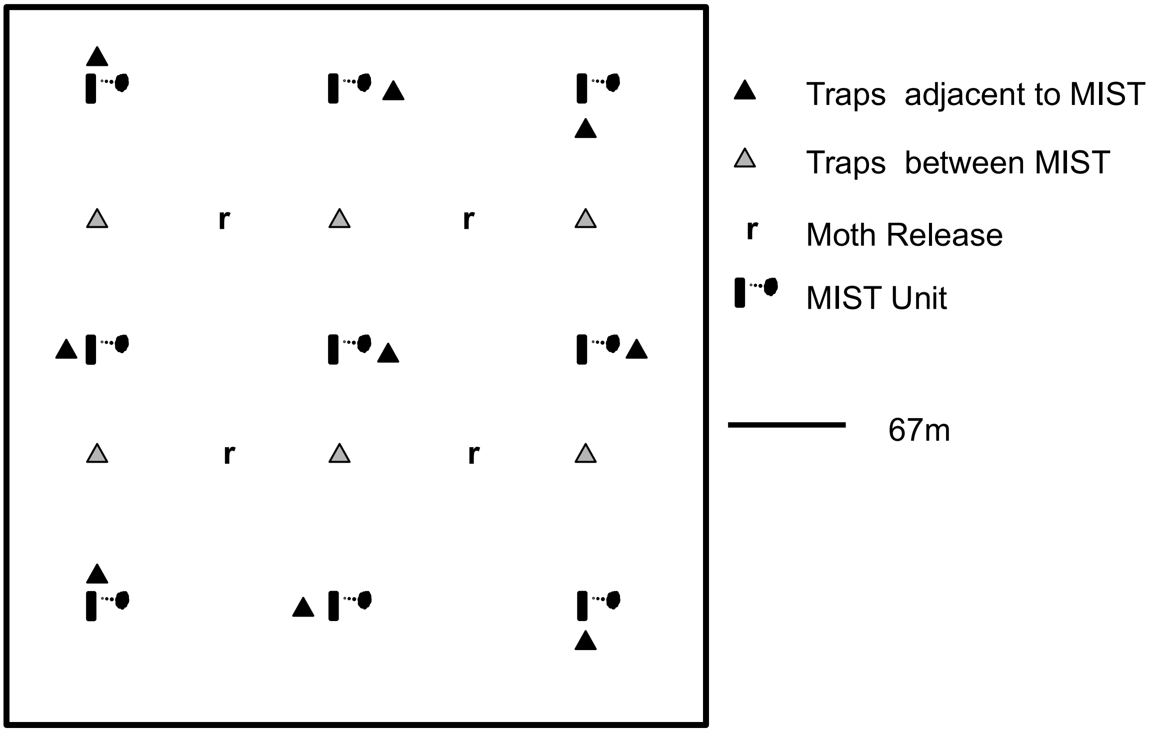

Figure 12. Generalized plot layout of Isomate CM MIST emitters, monitoring traps, and sterilized CM release sites in Isomate MIST treated orchards with similar trap and moth release locations in Isomate CM FLEX, and NO MD plots, 2011.

Codling moths from the Okanagan- Kootenay Sterile Insect Release (SIR) program in Osoyoos, British Columbia, Canada were released into orchards to ensure equal moth densities across treatments. Male and female moths from this facility were sterilized by exposure to 250 Gy of γ radiation from a Cobalt60 source (Gammacell 220; Nordion, Canada; dose rate of 125 Gy/min) (Bloem et al. 1999). SIR moths were marked internally by Calco-Red dye in the larval diet, enabling easy differentiation from wild moths. Moths received a light dusting

(0.1mg per 800 moths) with a Dayglo® powder (DayGlo Color Corp., Cleveland, OH): according to treatment regime. Moths were transported to field sites in an open and chilled chest so moths were exposed to daylight and remained accustomed to the normal MI diel cycle.

All plots received three successive releases of moths; each comprising of ca. 1600 male and 1600 female codling moths. The 3600 moths were released as groups of 800 moths at 4 locations near the center of each treatment plot quadrat. Cups with moths were gently warmed in the palms of the experimenters' hands prior to release and were gently tossed into the air. Moths immediately dispersed into the closest tree canopies.

The proportion of released moths captured was calculated. Arcsine transformation was required prior to analyses with an independent-samples t-test to compare moth catch in traps adjacent to and between emitter locations for each treatment. Variance to mean ratios of moth catch was calculated to assess if populations were more or less clustered under the influence of treatments.

Experiment 2 – CM capture pattern across a plot containing one high-releasing pheromone dispenser

The design of this experiment was randomized complete block. Three farms, each with two pheromone treatments and a negative control, were used to determine the effect of pheromone released from a single high-emitting pheromone point-source on moths within a 4 ha orchard. One CM Puffer or one cluster of 32 Isomate RING dispensers (RING Mega) was deployed per 4ha plot. Dispensers were placed in a tree near the up-wind border as per Figure 13.

Individual Isomate CM RINGs were loosely bound together and placed as a single point-source emitting a high overall dosage of pheromone. The intent was to produce a dispenser that might function similarly to a Puffer, but release pheromone passively. All dispensers for this and all following studies were deployed in the top 1/3 of the canopy top.

The impact of released pheromone on male movement was inferred by capture patterns of wild and released CM across a grid of Pherocon VI delta traps baited with CM L2 (3mg) lures in each orchard (Figure 13). Twenty traps were deployed in each treatment block; traps were uniformly spaced every 46m so that 1 trap was deployed per 0.2 ha. The trap grid was arranged so that an entire row containing 4 delta traps was on the prevailing upwind side of the Puffer (Figure 13). Traps were placed in the canopy and maintained as in Experiment 1.

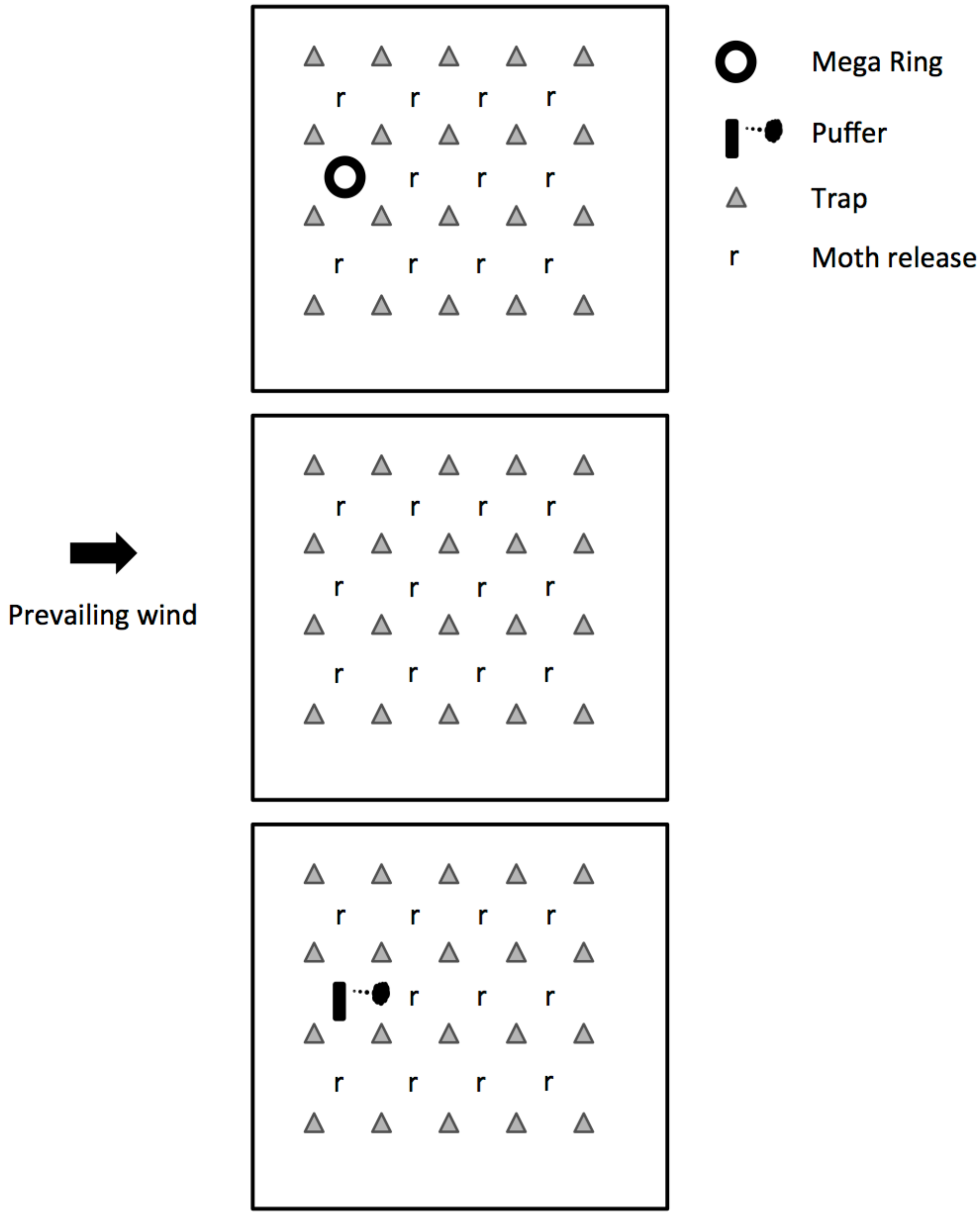

Figure 13. Representative plot layout for Experiment 1 testing male CM captures in a trapping grid in presence of a single Isomate RING Mega, or Suterra CM Puffer, or no pheromone.

Fifty SIR moths (1:1 sex ratio) were released at 12 locations equidistant from the 4 traps in each treated plot (Figure 13) to ensure the population would quickly become uniformly distributed within the orchard relative to the grid of traps. No moths were released along orchard perimeters. The experiment was replicated spatially at 3 farms and temporally with releases on July 26, Aug. 2, Aug. 9, and Aug. 16. Traps were monitored weekly and the numbers and colors of marked moths captured recorded.

Statistical Analyses

The mean and variance of moth captures was determined for each type of plot treatment and variance to mean ratios (VMR) were calculated to characterize codling moth redistribution from release sites. The degree of moth dispersion is indicated by: values <1 imply a random distribution male population; values >1 imply populations that are under-disbursed or clumped, and values of 0 indicate populations are uniformly distributed. The further the value is from 0, the greater the dispersion. Treatments were compared by subjecting variance to mean ratios of moth recaptures for three releases to pairwise t- test.

Experiment 3 - Pheromone Pre-Exposure

The experimental design was a randomized complete block consisting of five treatments detailed below (Isomate CM MIST Direct, Isomate CM MIST Indirect, Isomate CM FLEX Direct, Isomate CM FLEX Indirect, and No MD) replicated at 3 locations to test the hypothesis that CM adults pre-exposed to high dosages of sex pheromone disperse more in orchards receiving identical mating disruption regimes.

On designated trees in each plot type, SIR codling moth adults were caged on shoot terminals in a 19 L nylon-mesh paint-strainer bag, #11513, (Trimaco - Morrisville, NC 27560) (Figure 14). Moths were dusted (0.1mg per 800 moths) with either: red, pink, green, or blue powder, or marked only internally with calico red, for each respective pre-exposure treatment.

Figure 14. Nylon cage for exposing marked codling moth to Isomate CM MIST, CM FLEX, and no pheromone treatments prior to release. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

Caged moths were exposed to pheromone in MIST, FLEX, or untreated orchards. Treatment pre-exposures are defined accordingly: i) (MIST Direct) moths caged on foliage receiving 5 emissions of pheromone from a CM MIST unit held 0.3 m away and given 5 min to dry, ii) (MIST Indirect) moths caged on untreated foliage and exposed to pheromone indirectly from MIST emitters deployed uniformly in the plot at 2.5/ha, iii) (FLEX Direct) moths caged on foliage treated with 2 CM FLEX dispensers, iv) (FLEX Indirect) moths caged on untreated foliage and exposed to pheromone from FLEX dispensers deployed uniformly in orchard at 1000/ha or, v) (No Exposure) moths caged on untreated foliage in an orchard not treated with pheromones. Three replicated cohorts of each pre-exposure treatment were generated at each of 3 farms. Eight hundred moths (1:1 MF) were placed into each mesh cage between 1800-1900h. Following 24h of exposure to a treatment, moths were collected by removing the shoot terminal and cage from the tree. One cage of moths from each pre-exposure treatment was transported immediately to the area equidistant between 4 traps in the NW corner of each treatment orchard (Figure 15). Mesh bags were removed and moths were gently shaken into a tree canopy.

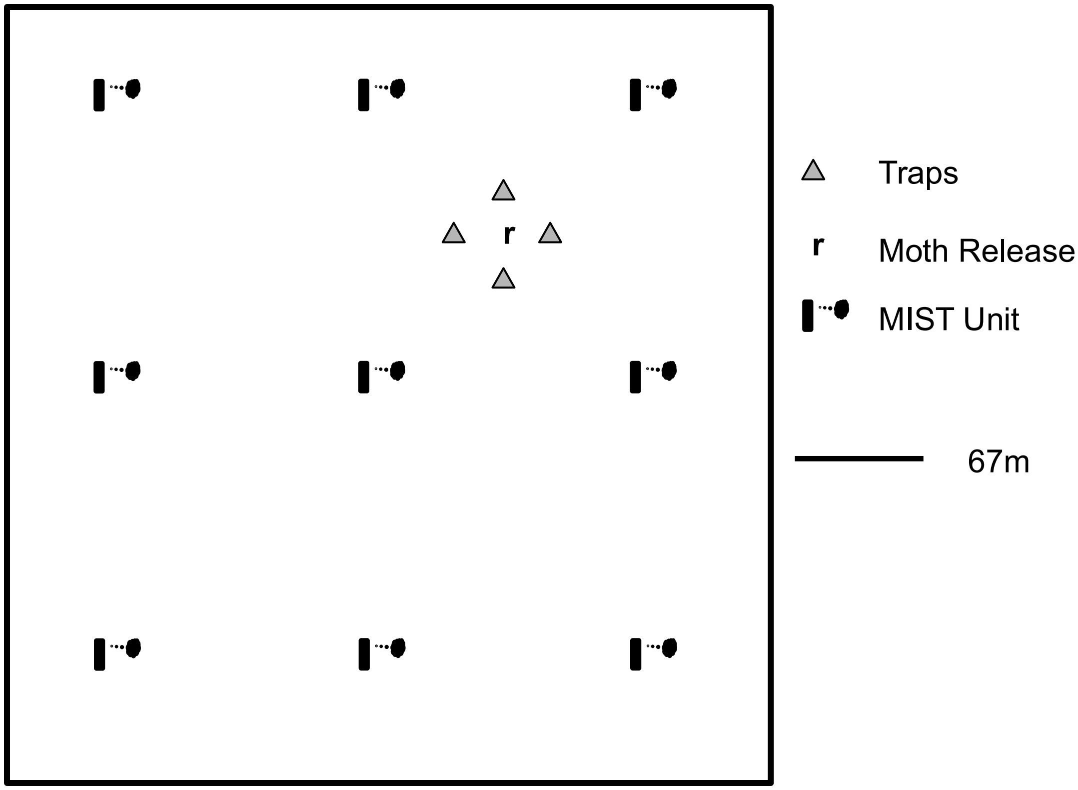

Figure 15. Generalized plot layout of Isomate CM MIST emitters (in emitter treated plots), monitoring traps, and sterilized moth release sites used to determine the effect of pheromone pre-exposure on male moth orientation to traps in apple orchards, 2012.

Three replicate samples of 15 leaves from each pre-exposure treatment (24h exposure) were collected in PAKVF4 mylar bags (Sorbentsystems, Los Angeles, CA), chilled, and transported to Michigan State University within 2 h for quantification of release rate using a volatile capture system (VCS) described by Tomaszewska et al. (2005)

Male movement was inferred by patterns in capture of male moths in 4 Trécé CM L2-baited Pherocon® IV traps deployed in each cardinal direction ca. 18m from the release location in each type of disrupted orchard. The number of moths captured in traps was recorded 12h following release.

Statistical Analyses

The sum of moths captured after 12h was recorded for each pheromone pre-exposure in each orchard treatment. Moth captures (x+ 0.5) were square root transformed to normalize the distributions and homogenize variance, then subjected to analyses of variance (ANOVA, GLM) using pheromone pre-exposure (MIST direct, MIST indirect, FLEX direct, FLEX indirect, None) and orchard treatment (MIST, FLEX, Untreated) as the main effects. Pairs of means for pheromone pre-exposure and orchard treatment were separated using Fisher's LSD test p=0.05 (Systat Ver.13, San Jose, CA).

RESULTS AND DISCUSSION

Experiment 1 - Efficacy of Aerosol Emitters

Both Isomate MIST and FLEX treatments reduced wild and sterile CM male captures in 0.1mg-baited monitoring traps compared to the No MD control

(Figure 16). Captures of male moths in Isomate MIST and FLEX-treated blocks was statistically lower than that for the No MD treatment $F(2,21)=30.58$, $p<0.0001$; but these means did not differ from one another (Figure 5). Interestingly, in MIST-treated orchards, a statistically ($t(10) = 3.27$, $p<0.008$) greater proportion of catch occurred in traps adjacent to vs. between emitter locations, (Figure 17). In MIST-treated orchards, traps adjacent to the emitters caught 2.7x more sterile moths than those located between emitters. This was surprising especially since traps were baited with very low load lures, 0.1mg, which should have been easily disrupted. This pattern of catch was not observed for traps in the same spatial arrangement in FLEX and No MD orchards (Figure 17).

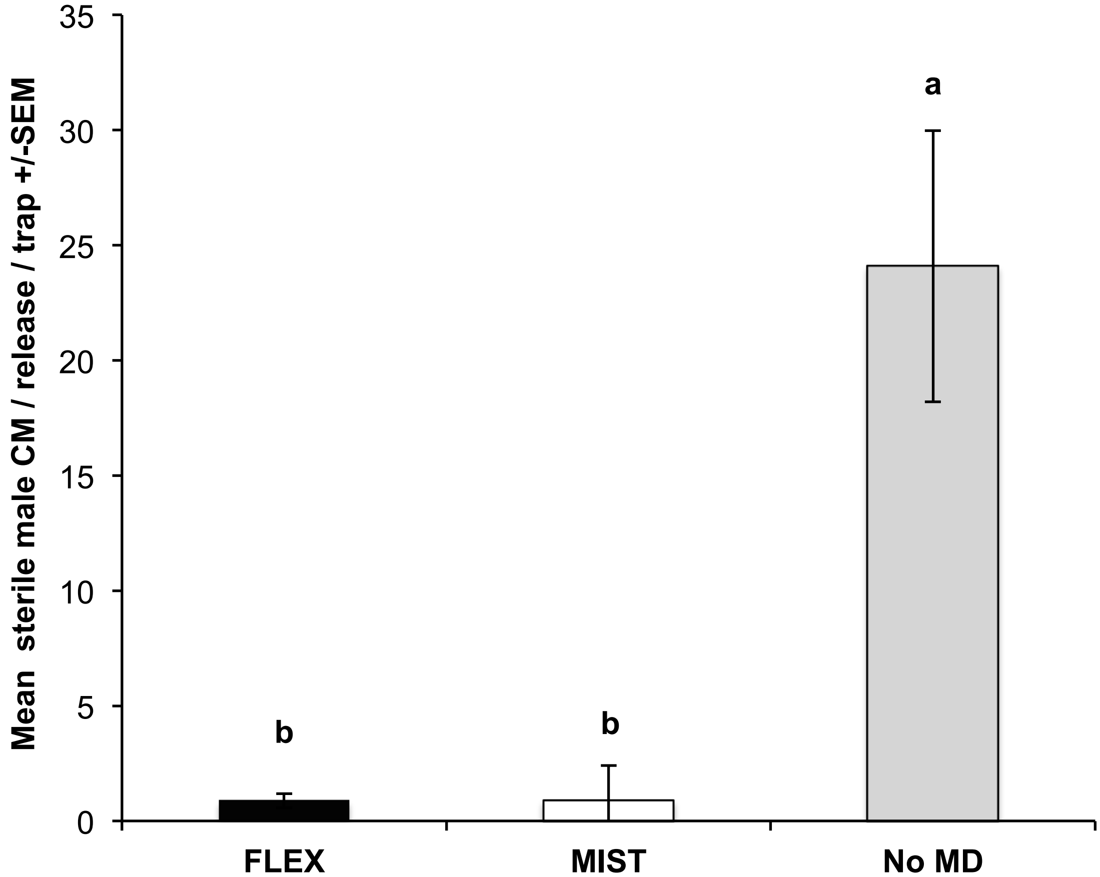

Figure 16. Captures of released, sterilized, male codling moth +/- SEM, in plots treated with Isomate CM FLEX, Isomate CM MIST, or no pheromone, 2011. General Linear Model Analyses (2,15) F=20.158 p<0.0001, pairwise comparisons of treatments Tukeys HSD test.

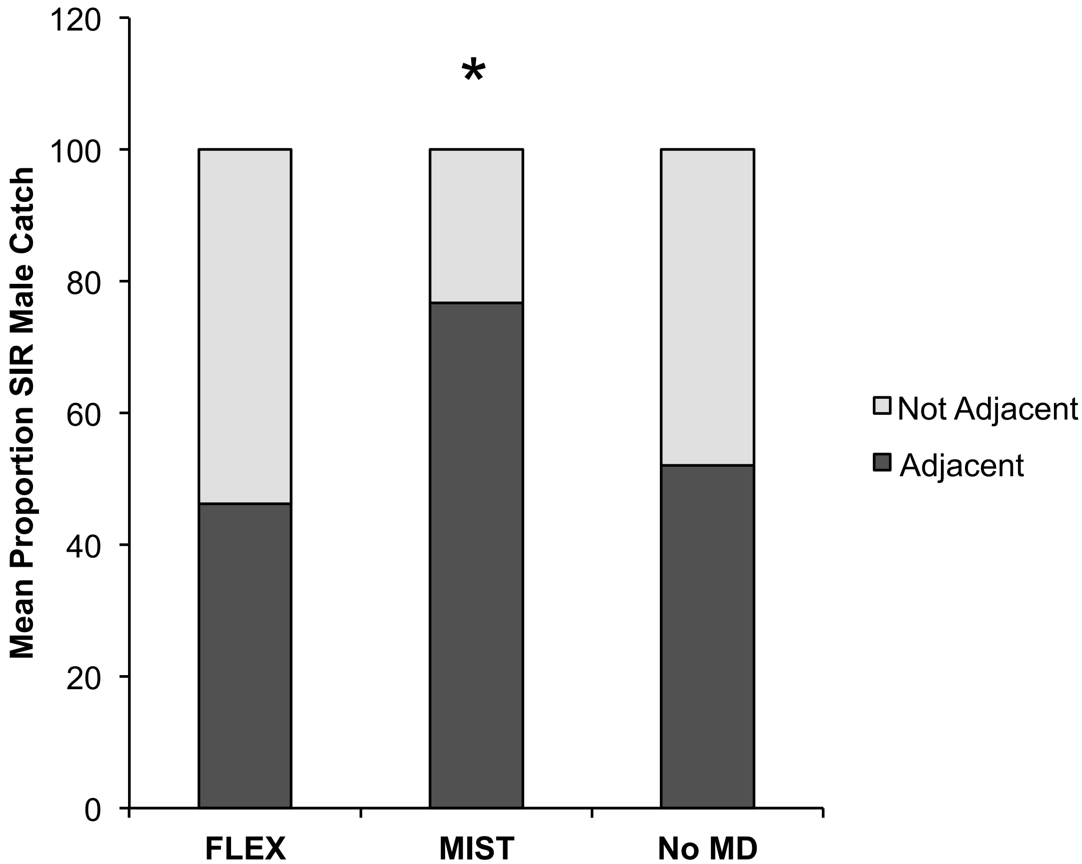

Figure 17. Location of sterilized male codling moth captures in traps located adjacent to or not adjacent to Isomate CM MIST emitters deployed in apple orchards and in traps similarly located in Isomate CM FLEX and no pheromone treated orchards, 2011. Students paired T test, $t(10) = 3.27$, $p<0.008$, *indicates significant differences.

We surmise that aerosol emitters actively dispensing large quantities of pheromone are attractive to male moths. The disparity in moth catch in traps located adjacent to or distant from MIST emitters refutes the hypothesis that aerosol devices operate by camouflaging females or traps. Pheromone concentration should be greatest, and therefore more effective at reducing catch in traps located nearest the emitters under the camouflage scenario, but the opposite was true. In contrast, moths responded equally to adjacent and distant traps in FLEX treated and untreated orchards. Since trap densities and placement were equivalent among the three pheromone treatments, the difference in moth captures is explained best by the influence MIST emitters have on moth behavior. Greater captures of moths nearest to MIST units can be explained by moths moving towards higher concentrations of pheromone and possibly congregating in larger numbers. Additionally, males in these areas may fail to disperse similarly to males in areas of less pheromone. Alarmingly, this suggests that males may move into aerosol treated orchards from otherwise untreated areas, thus, the best protection of aerosol emitters may be happening at some distance from the emitter and not adjacent to it.

Given the differences in pheromone release from aerosol emitters, Isomate FLEX, monitoring traps, and females, plume reaches can be expected to vary accordingly. Pheromone plumes generated by aerosol emitters have been estimated up to 300m from the point-source (Welter and Cave, 2000b) and as such may have the ability to draw moths from further distances than does

Isomate FLEX. MIST emitters were the most competitive at attracting males but did not suppress catch in adjacent traps.

Experiment 2 – Area of influence of high-concentration pheromone dispensers

Wild CM males were captured at all three sites during first generation flight. A total of 2435 males (41/trap) was captured in the RING treatment, 1996 (33/tap) under the Puffer treatment, but only 314 (5/trap) in the untreated blocks. Moth captures were greatest under the RING Mega treatment on two farms and in the Puffer treatment on the third farm, suggesting that males were not inhibited from responding to low-emitting pheromone traps.

We had anticipated captures would be highest in untreated plots where the only competing pheromone sources for males' attention were native females or baited traps but, the highest captures of wild CM males occurred in aerosol emitter plots. The most plausible explanation for the remarkably low catch in the untreated plots is that plumes from the high-releasing emitters overlapped the control plots and attracted males towards them, as in Experiment 1. Lower-emitting sources (females and traps) were apparently bypassed in favor of more concentrated pheromone plumes from the high-emitting sources. Wild CM males were apparently drawn from great distances to the pheromone-treated plots.

On the other hand, SIR moths were released only inside each plot. Overall captures of marked SIR moths was similar across treatments unlike the pattern observed for wild moths. Total captures for RING Mega, Puffer, and the negative control were 402, 430 and 360 males, respectively. However, the spatial pattern of SIR captures inside plots resembled those of the wild moths and was clumped for all treatments. The average variance to mean (VM) ratios of catch was 14.5, 2.7, and 20.3, for wild and 6.2, 3.4, and 3.1 for SIR moths respectively for Puffer, NO MD, and RING Mega treatments. The higher VM ratio indicates that moths in the pheromone treated plots were more clumped than in the No MD plots. The distribution of SIR catch in the Puffer treatment was significantly more clumped, ca. 2x greater, than that for moths in the No MD $t(6)=5.6$ $p<0.001$ and RING Mega $t(6)=3.1$ $p<0.05$ treatments. Moths were not distributed differently between the No MD and RING treatments (Figure 18). Moth captures was highest at all three farms for traps nearest to and directly upwind of the Puffer (Figure 19a-c), providing further evidence that aerosol emitters are attractive to CM males. Traps positioned directly upwind of Puffers captured the most moths (Figure 18). Captures of moths in traps upwind of the Puffer and near the orchard perimeter corroborates previous research (Knight 2005) suggesting that CM control along borders in orchards treated with Puffers may be poor.

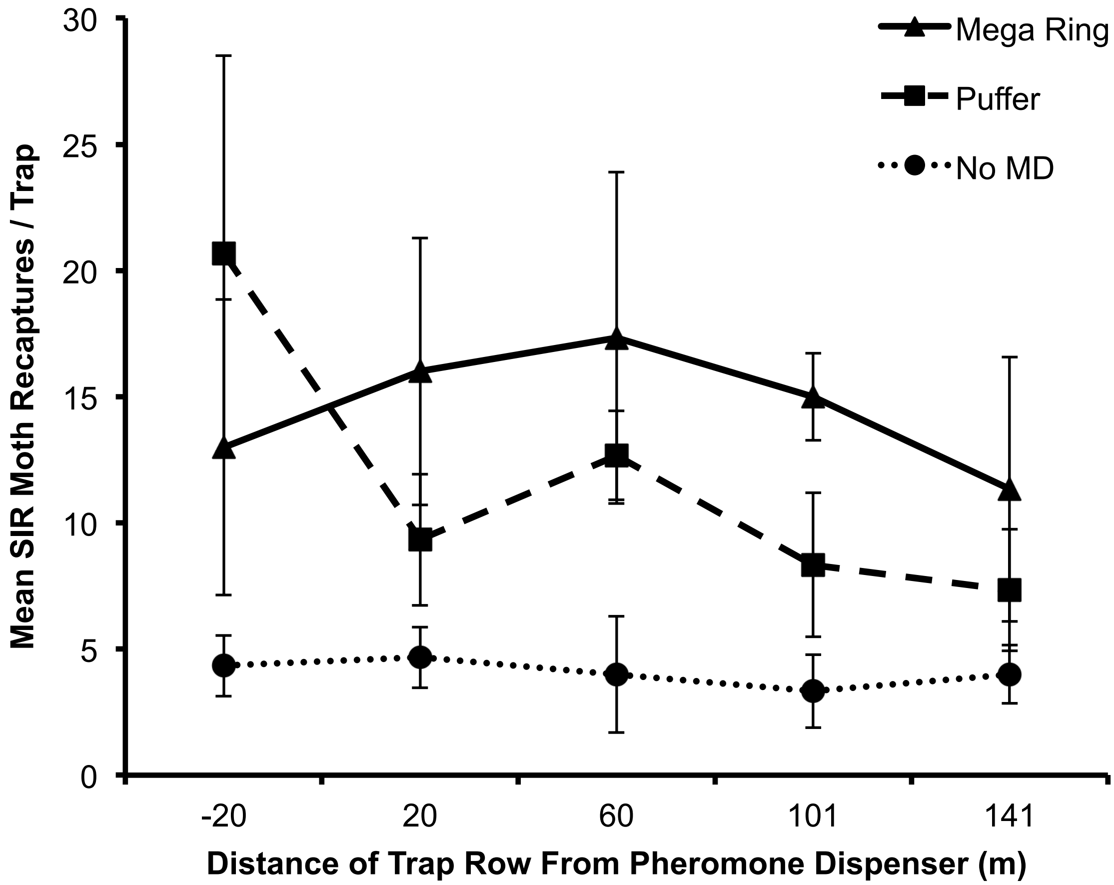

Figure 18. Captures of sterilized male CM in L2-baited monitoring traps, according to distance of row containing traps from the Suterra CM Puffer, Isomate CM RING Mega dispenser, or likewise in No MD orchards.

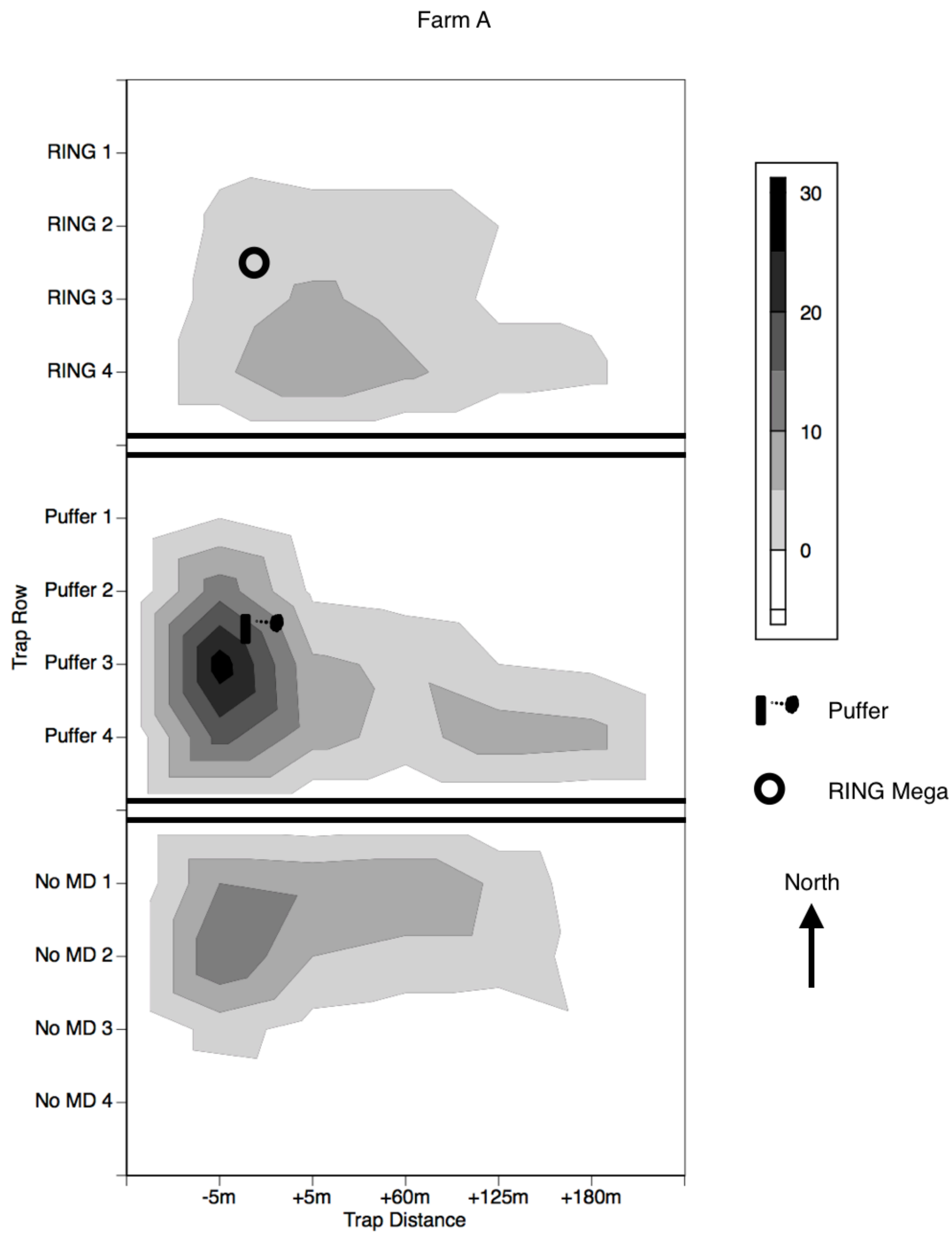

Figure 19a. Spatial representation of SIR moth captures in plots containing one Suterra CM Puffer, or one Isomate CM RING Mega, or no pheromone, 2010.

Farm B

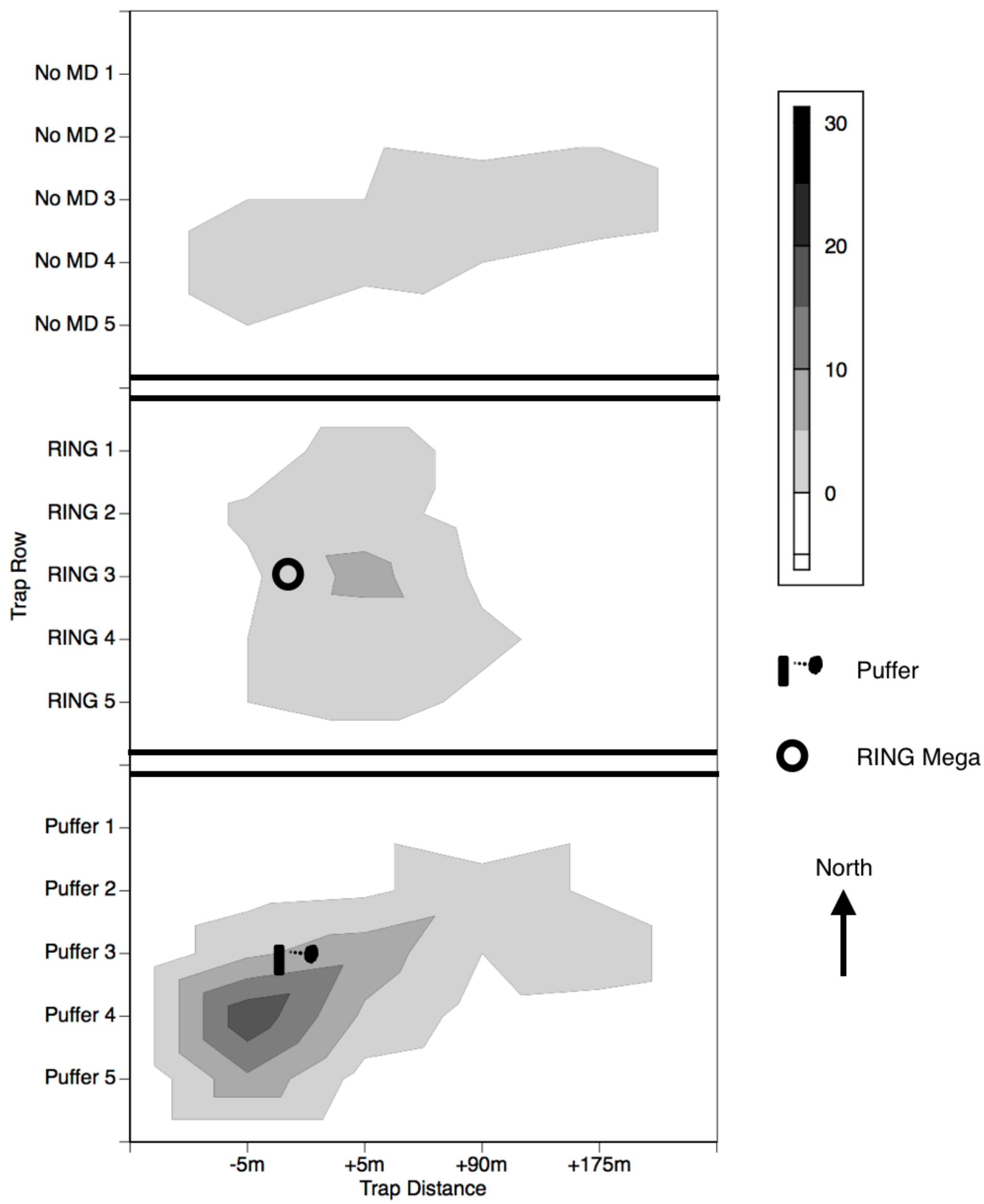

Figure 19b. Spatial representation of SIR moth captures in plots containing one Suterra CM Puffer, or one Isomate CM RING Mega, or no pheromone, 2010.

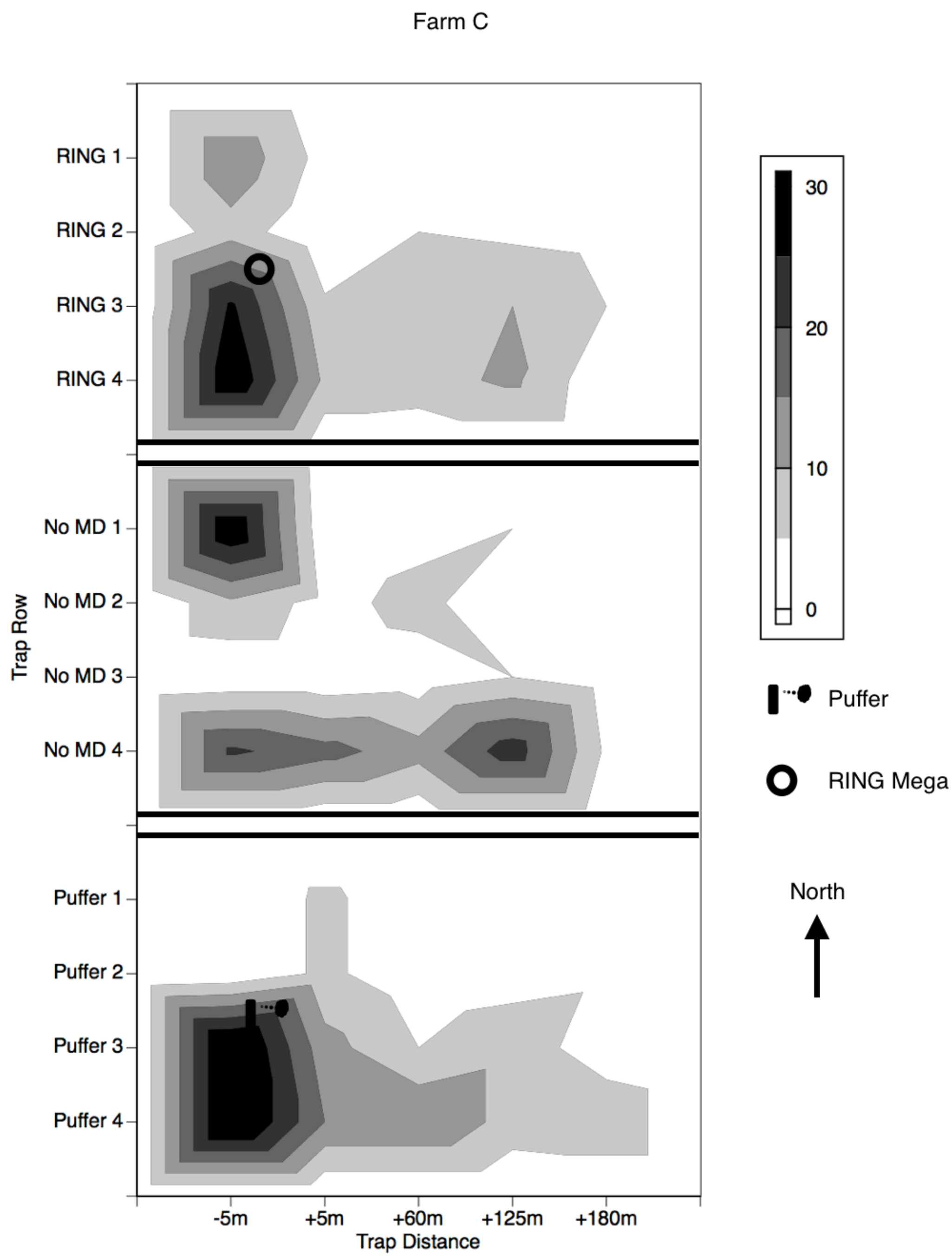

Figure 19c. Spatial representation of SIR moth captures in plots containing one Suterra CM Puffer, or one Isomate CM RING Mega, or no pheromone, 2010.

Captures of differentially marked SIR moths in apple plots treated with and without pheromone revealed a directional pattern of moth movement in response to high concentrations of pheromone (Figure 20). Moths released in the control plots were captured in all three treatment plots. Overall, 35% of recovered moths released in the control plots were captured in Puffer plots; the highest capture rates occurred in traps nearest the Puffers. Conversely, few moths released in the Puffer plots were captured in other plots. Traps upwind of the Puffer captured 2.5x more moths on average than those downwind. These data are strikingly similar to the pattern of moth catch in Experiment 1 and provide further evidence that moths are attracted to aerosol emitters. Moths released in the RING Mega plots were primarily captured within those plots, and thus did not follow the pattern of dispersion seen for moths released in the untreated orchards. The difference in moth captures between the aerosol and RING emitters is likely related to active vs. passive emission, respectively, the former yielding the highest instantaneous releases, and likely the largest plumes.

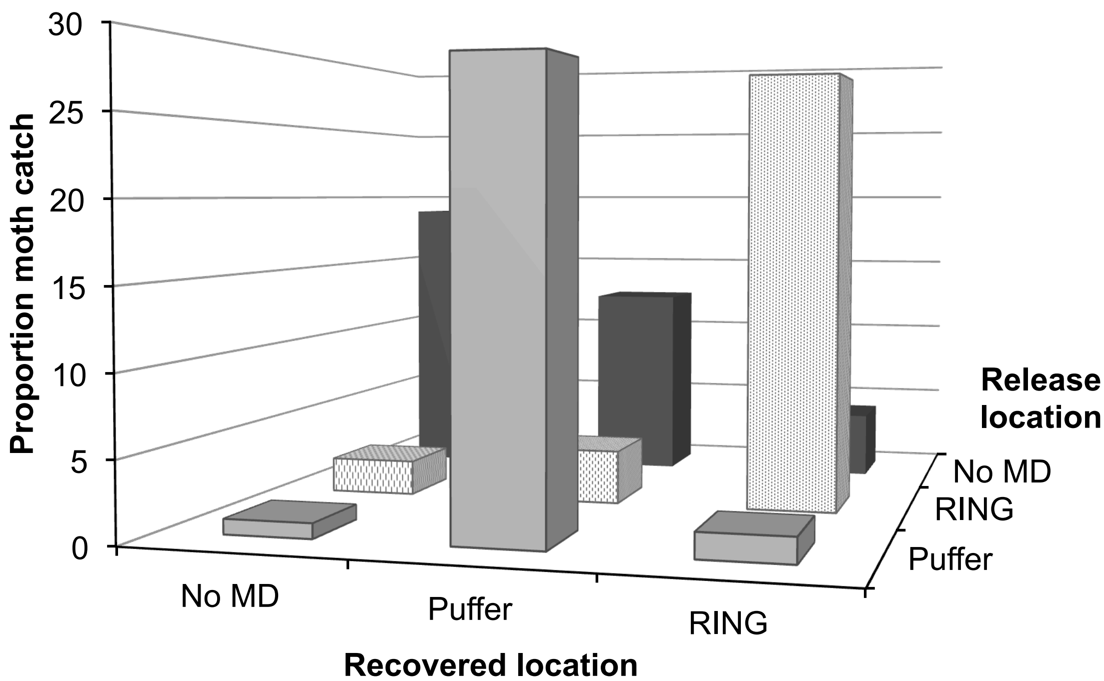

Figure 20. Location of SIR CM male captures in traps relative to their original release and first exposure to pheromone.

Higher CM catch nearest to the Puffer suggests that males were similarly drawn toward the source of the pheromone plume produced by these very high-releasing devices. These observations reiterate those of Huang et al. (2013) where codling moth males favor more vs. less concentrated pheromone plumes. Moths entering Puffer-treated orchards from otherwise untreated areas were captured in greater numbers in traps nearest the Puffer. Captures immediately upwind of the Puffer would suggest that males sensitivity to low emitting pheromone sources is not diminished after exposures to high concentration plumes from Puffers or recovers shortly thereafter.

The long-range impact of plumes generated from Puffers may have unintended impacts on pest management practices in adjacent orchards not treated with pheromone. In California, consultants working in walnut orchards not under but adjacent to those under CM Puffer mating disruption have reported reduced or no moth captures in traps deployed in the untreated orchards. Indeed, the California label for Suterra CM Puffers contains a disclaimer, declaring the potential of reduced moth captures in neighboring orchards due to the drift of pheromone plumes preventing moths from finding lure-baited traps. Our current findings suggest an alternative explanation for the inhibited catches in orchards adjacent to Puffer-treated orchards. Males may be moving out of orchards not treated with pheromone and into Puffer-treated orchards, reiterating, the best protection of aerosol emitters may be happening at some distance from the emitter and not adjacent to it.

Experiment 3 - Pheromone Pre-Exposure

Captures of SIR CM males were good overall; the percent capture was highest (12%) in the untreated orchards for moths exposed to MIST direct, and lowest in FLEX treated orchards for moths exposed to 2 FLEX dispensers for 24h. Both pheromone pre-exposures ($F=5.51$, $df=4, 83$; $p=0.001$) and plot treatment ($F=12.97$, $df=2,83$; $p<0.0005$) significantly enhanced the numbers of moths recaptured, however, there was no interaction between the two variables. Significantly fewer released moths were captured in orchards treated with Isomate FLEX than those treated with Isomate MIST or left untreated; the latter treatments were statistically equivalent (Figure 21).

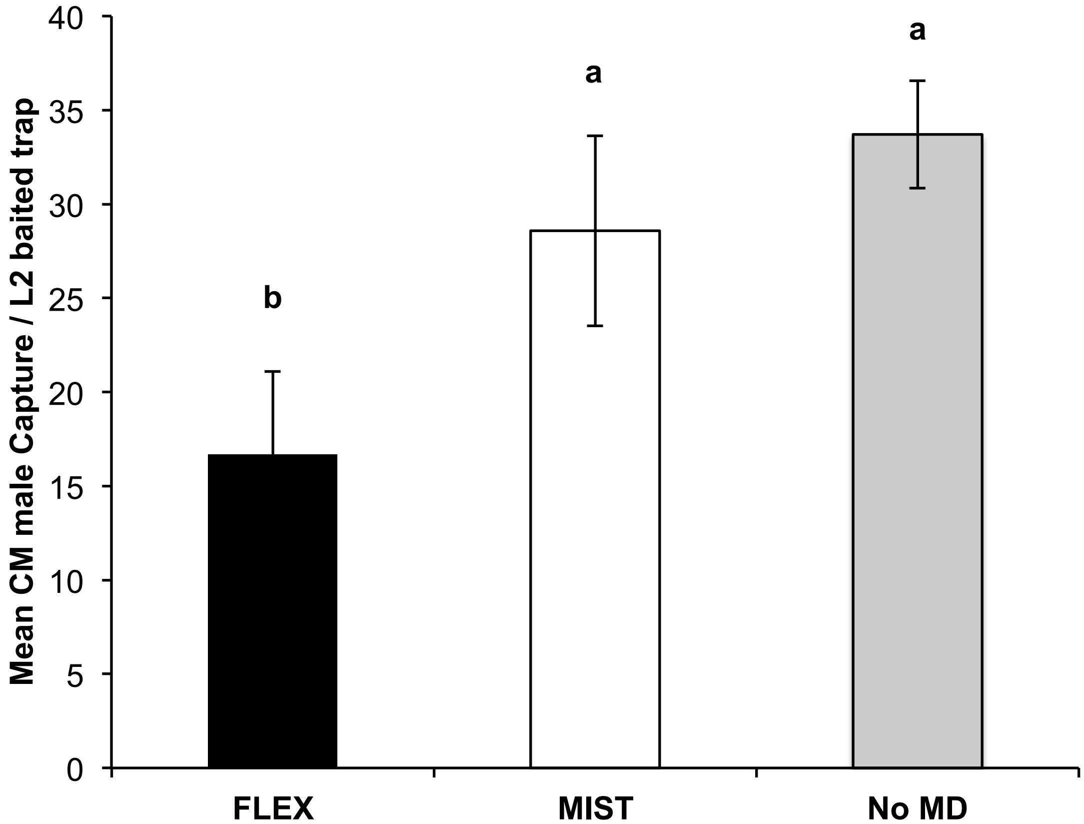

Figure 21. Mean captures of sterilized male CM subjected to 24h pheromone pre-exposure treatments, 1 night following release into apple orchards (+/- SEM) in L2 baited traps, 2012. ANOVA (2,15) F= 4.288 p<0.04, pairwise comparisons of treatments Tukeys HSD test.

The response of CM pre-exposed to pheromone varied according to the means of exposures. Surprisingly, more males pre-exposed to MIST directly or indirectly were captured in traps than naïve moths not exposed to pheromone, or moths exposed indirectly to pheromone released from FLEX dispensers (Figure 23). Moths directly exposed to two CM FLEX dispensers for 24h or caged on untreated foliage in MIST orchards responded similarly to pheromone-baited traps. Catches of moths from both FLEX treatments were not statistically different from those of naïve moths (Figure 22).

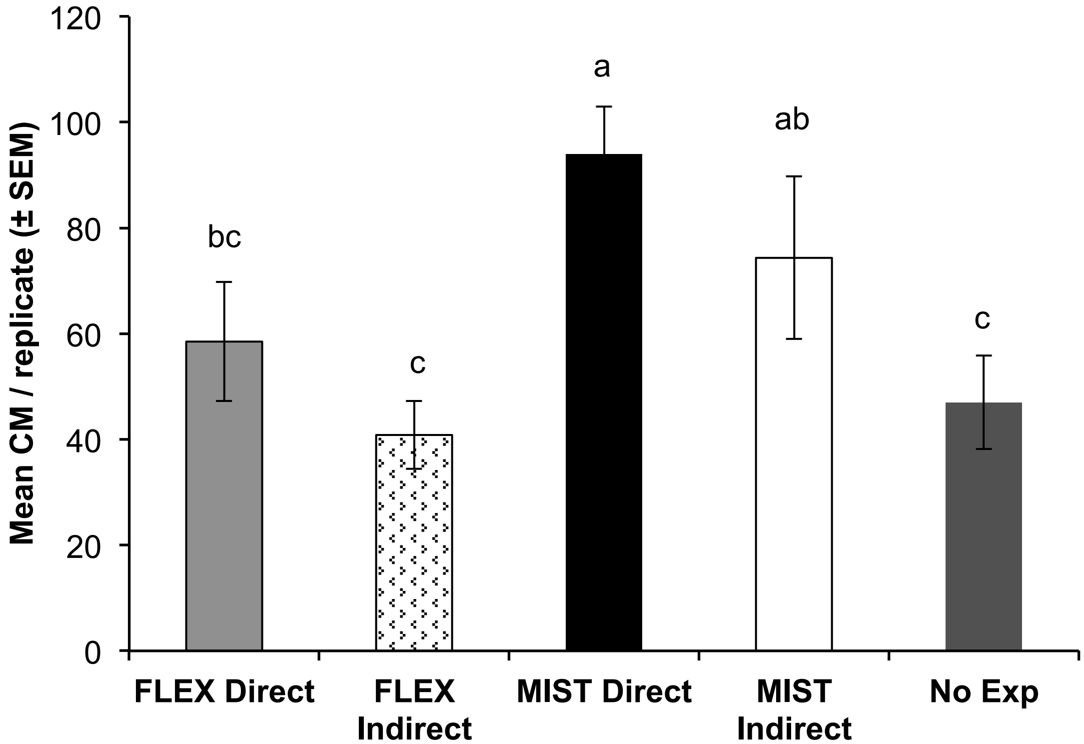

Figure 22. Mean captures of sterilized male CM subjected to 24h pheromone pre-exposure treatments, 1 night following release into apple orchards (+/- SEM) in L2 baited traps, 2012. General Linear Model Analyses (8,83) F=5.51 p<0.001, pairwise comparisons of treatments Tukeys HSD test.

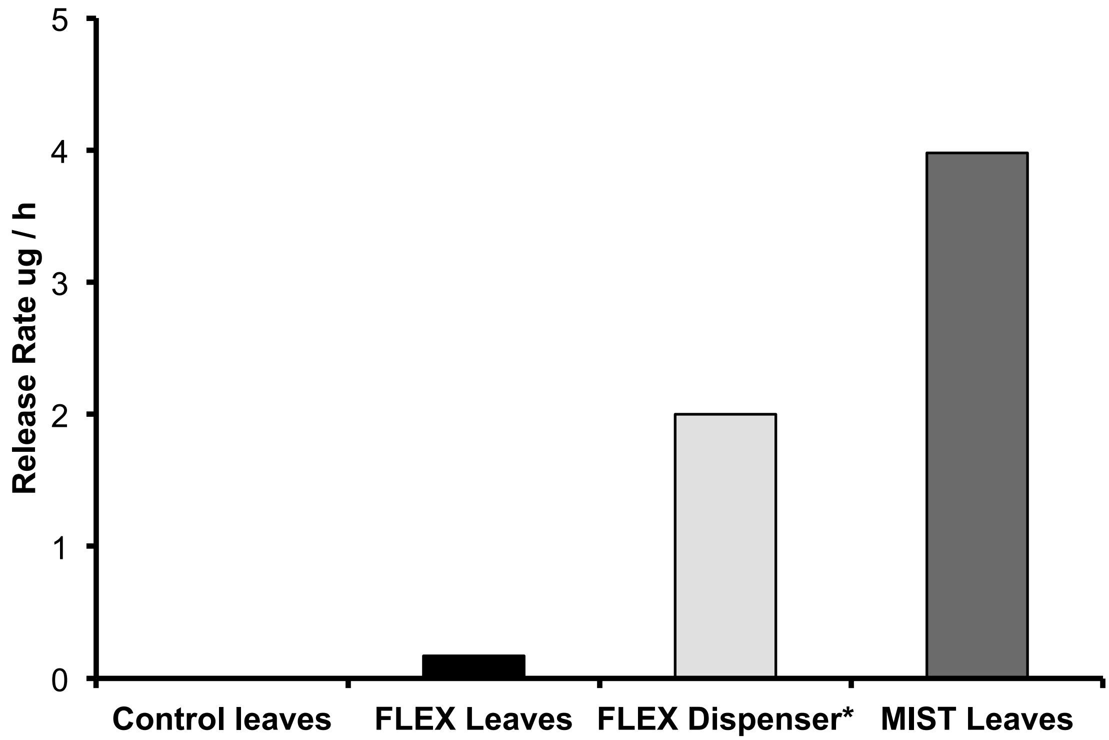

Figure 23. Concentration of codlemone, 8,10-dodecadien-1-ol, collected by volatile capture from 20 leaves, 24 hours post-pheromone treatment; and from 1 FLEX dispenser*.

Codlemone was collected in substantial quantities from leaves treated directly with CM MIST and at a much lower concentration from leaves in cages with two FLEX dispensers (Figure 23). Minor quantities of the isomers (Z, E) and (E, Z)-8, 10-12OH were collected from MIST-treated leaves (Figure 24). The other alcohol components, 12-OH and 14-OH, contained in the commercial Isomate CM FLEX dispenser, were collected from leaves associated with two FLEX dispensers in a sleeve cage. Leaves treated with MIST had ca. 20x more codlemone than leaves confined with two CM FLEX dispensers (4 µg/h and 0.2µg/h captured, respectively).

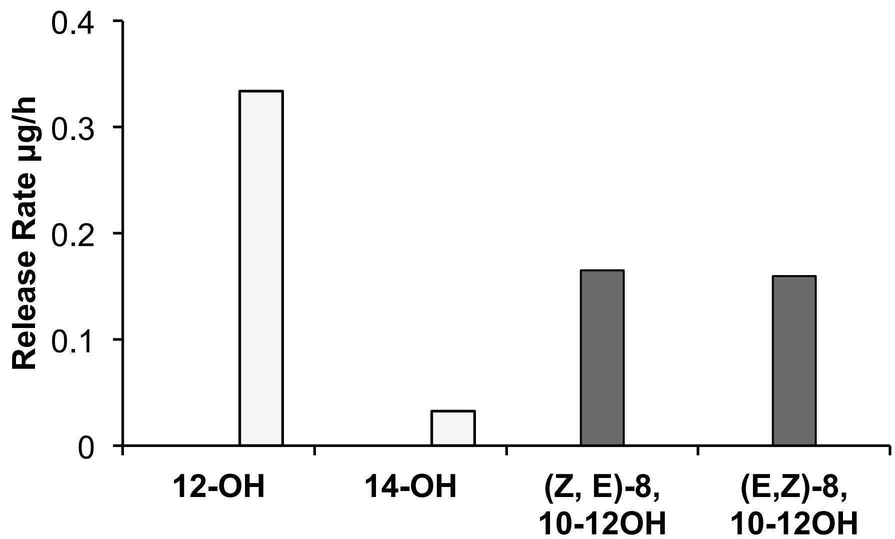

Figure 24. Concentration of minor CM pheromone components from 20 MIST Direct and 20 FLEX Direct treated leaves, collected by volatile capture, 24 hours post-pheromone treatment.

Capture of codling moths following pre-exposure to pheromone provides further insight into the underlying behavioral mechanism of orientational disruption via aerosol (CM MIST) vs. hand applied (CM FLEX) dispensers. Moths held in FLEX-treated plots, but not directly exposed to dispensers in cages, responded similarly to naïve moths. These results suggest that pheromone plumes emanating from dispensers in FLEX treated orchards either did not reach caged moths, or that the airborne concentration was lower than that required to induce increased search. In contrast, moths pre-exposed directly or indirectly to pheromone released from a MIST device were captured in greater numbers than naïve moths not exposed to pheromone. Male pre-exposures to codlemone can cause long-lasting adaptation (LLA) in male CM under laboratory settings. (Judd et al. 2005, Stelinski, Gut, and Miller 2005). It was asserted, however, that the dosage and duration of exposure required to achieve LLA is unlikely to occur under field conditions. Our findings following forced exposure to high levels of codlemone under field conditions confirm that such exposures did not cause LLA in the field setting, as the male's ability to find a pheromone source increased rather than decreased. Furthermore, males exposed to pheromone plumes emanating from aerosol dispensers or coming in close contact with exposed foliage are quite capable of finding traps and they continue to actively respond to pheromone plumes emanating from lure-baited traps. These results suggest that exposures to high concentrations of pheromone increases/stimulates rather than decreases search behavior. Alternatively, released moths might have quickly recovered from LLA (ca. 1-2h post release), initiated search, and were captured in traps in high numbers. However, fewer unexposed moths were captured in all plots vs. greater catch of moths pre-exposed to pheromone, reiterating pre-exposure to pheromone increases catch.

Moth exposures to pheromone in Experiment 3 may actually be greater than those recorded from 20 leaf VCS samples. Scaling the release rate of codlemone for total number of leaves per cage suggests rates could have been as high as 7.5 μg/h for MIST treated cages. A single Isomate CM FLEX dispenser releases 1.7 μg/h after 21d of field aging and remains fairly constant until day 120 (Dr. Juan Huang – Michigan State University, personal communication). Thus, moths in cages with two CM FLEX dispensers may have been exposed to rates as high as 3.4 µg/h. These rates are both much higher than what has been reported to induce long lasting adaptation (Judd et al. 2005, Stelinski, Gut, Miller 2005). However, field observations of codling moth males approaching ropes show that visits are brief and very few contact the source (Stelinski et al. 2004).

Minor quantities of codlemone isomers were collected from MIST treated leaves and from leaves in cages with 2 CM FLEX dispensers. While (E, Z)-8, 10-12OH is known to be antagonistic to male CM behavior (El-Sayed et al. 1998), the quantities recovered in samples (Figure 11) were well below that reported (10pg/min) to cause abnormal behavior (El-Sayed, 2004). However, SIR moths may have been exposed to higher dosages of these minor pheromone components when confined in close proximity to CM FLEX dispensers in the mesh cages. The increased response of moths pre-exposed to leaves treated with CM FLEX seems to refute a possible antagonistic effect of these compounds on male search behavior in our experiment.

Codling moth males have previously been reported to move towards pheromone treated orchards (Witzgall, Bäckman, et al. 1999) and to elevate their search in pheromone treated vs. untreated orchards. Similarly, Stelinski et al. (2005) found CM exposure to high dosages of pheromone stimulated responsiveness but also elevated their response thresholds in flight tunnel assays. Further evidence supporting enhanced CM response to higher pheromone sources includes the common practice of using higher dosage lures (10mg) in pheromone traps to monitor CM in pheromone treated orchards. Captures in pheromone-treated orchards are much higher in traps baited with high load lures than with standard lures.

Codling moth males moved toward aerosol emitters and may move a considerable distance in apparent response to this high-releasing pheromone source. A small portion of released moths in Experiment 1 moved up to 457m, or one quarter of a mile. In addition, it appears that as they moved toward the high-emitting pheromone source, males bypassed other lower-releasing pheromone sources, such as lure-baited traps.

Overall, these findings suggest that aerosol emitters operate competitively, but through displacement of males away from females. We propose the term *induced allopatry* to describe the phenomenon of mating disruption by spatial segregation of the sexes where movement towards and aggregation near the high pheromone sources diverts males attention away from females that are releasing very low quantities of pheromone.

CHAPTER 3

Aerosol emitters disrupt codling moth, *Cydia pomonella*, competitively

Also published as:

McGhee, P.S., L.J.Gut., and J.R.Miller 2014. Aerosol Emitters Disrupt Codling Moth, *Cydia pomonella*, Competitively Pest Management Science.

ABSTRACT

Isomate® CM MIST aerosol emitters (Pacific BioControl Corp, Vancouver, WA) containing 36 grams of codlemone, (*E, E*)-8,10-dodecadien-1-ol, were deployed at various densities in a commercial apple orchard to generate dosage-response profiles in order to elucidate the behavioral mechanism of disruption. Moth captures decreased asymptotically as Isomate® CM MIST densities increased. Data fit to Miller-Gut and Miller-de Lame plots yielded straight lines, with positive and negative slopes respectively. Catch of male moths decreased from 28 / trap in the control to 0.9 / trap at the highest emitter density. Disruption of > 90% was realized at emitter densities greater than 5 units per ha. The resulting set of profiles explicitly matched the predictions for competitive rather than non-competitive disruption. Thus, these devices likely disrupt by inducing false-plume following rather than by camouflaging traps and females. Five MIST units per ha would be necessary to achieve the same level of CM control provided by a standard pheromone treatment with passive reservoir dispensers. The need for only a few aerosol emitters, 2.5 - 5 units per ha, mitigates the cost of labor required to hand apply hundreds of passive reservoir dispensers; however, a potential weakness in using this technology is that the low deployment density may leave areas of little or no pheromone coverage where mate finding may occur. This technology is likely to benefit substantially from treatment of large contiguous blocks of crop.

INTRODUCTION

Pheromone-based mating disruption has contributed significantly to the management of insect pests in agriculture for over 30 years (Cardé and Minks 1995, Gut et al. 2004, Cardé 2007). Mechanisms of mating disruption fall into two main categories: competitive (e.g., false-plume following) (Miller, Gut, de Lame, and Stelinski 2006b, Byers 2007) and non-competitive disruption (e.g., camouflage, desensitization, and sensory imbalance) (Bartell 1982, Miller, Gut, de Lame, and Stelinski 2006b). Passive reservoir, hand-applied dispensers (Isomate® CM, Scentry NoMate® CM, and Suterra CheckMate® CM) dominate disruption formulations for codling moth, *Cydia pomonella* L. (CM). Of the estimated 162,000 ha of fruit crops treated with pheromone for CM management in 2008, about 80% were treated with passive reservoir, hand-applied formulations deployed at densities of 500-1000 / ha^{-1}. Individual dispensers are loaded with 50-120 mg of codlemone and release upwards of 5 µg of the active ingredient h^{-1} (Tomaszewska et al. 2005). These hand-applied dispensers disrupt CM by first attracting them to the dispensers and then deactivating them for several hours, (Miller et al. 2010) probably by sensory adaptation or habituation occurring when the males closely approach the dispensers (Stelinski, Gut, Pierzchala, et al. 2004). Although such passive reservoir dispensers have performed well, their deployment requires significant labor and time (Agnello and Reissig 2009) at a period when growers in many regions are performing other important horticultural practices, including pruning and treating for plant diseases.

Broadcasting larger quantities of pheromone using much lower dispenser densities (2-5 / ha) *e.g.*, via aerosol emitters (Suterra Puffer® and Isomate® MIST) could reduce deployment demands Shorey and Gerber 1996, Knight 2005, Hansen 2008). However, a challenge of aerosol emitters is the greater variability in catch inhibition; reported levels of disruption vary from 98% (Shorey and Gerber 1996, Knight 2005) to less than 70% (Stelinski, Gut, et al. 2007). Factors that might contribute to efficacy variability include: pest density,(Stelinski, Gut, et al. 2007) canopy size(Suckling et al. 2007), plume size (Welter and Cave 2000a) pheromone concentration (Casado et al. 2011), and the positioning of aerosol units within an orchard (Knight 2005). Two critical components to any mating disruption program have largely been unexplored for aerosol emitters: optimal dispenser density and the behavioral mechanism of disruption. It is possible that the high release rates of aerosol emitters might be sufficient to shift disruption from competitive to non-competitive mechanisms, as has been demonstrated for Oriental fruit moth, *Grapholita molesta* (Reinke et al. 2013) and citrus leafminer, *Phyllocnistis citrella* (Stelinski et al. 2008).

In the current study, we used dosage-response curves and the quantitative tools of Miller *et al.* (Miller, Gut, de Lame, and Stelinski 2006b, Miller et al. 2010) to demonstrate for aerosol emitters that: 1) CM disruption is achieved through competition; it does not shift to a non-competitive mechanism even at very high release rates, and 2) the optimal number of emitters is *ca*. 5 units per hectare.

METHODS

Isomate® CM MIST aerosol emitters (Pacific BioControl Corp, Vancouver, Washington) containing 36 grams of codlemone, (*E, E*)-8,10-dodecadien-1-ol, were deployed at various densities (Figure 2) in a commercial apple orchard to generate a dosage-response profile. The emitters were programmed to deliver 1 dose of codlemone (*ca.* 3.5 mg) every 15 min from 1700 – 0500 hours. The experimental was conducted on one 180 ha commercial apple farm located in Grant, MI and releases of known moth densities were replicated three times. Five, 4 ha plots were randomly assigned one of the following treatment densities: Isomate® CM MIST at, 0.6 units / ha, 1.25 units / ha, 7.5 units / ha, 15 units / ha, and a no-pheromone control. Treatments were separated by at least 180m. The control was placed on the prevailing upwind side of the farm to reduce the chance of pheromone drift/contamination.

This orchard was comprised of ca. 30-year-old red delicious and Ida Red cultivars on a 4.4 m x 6 m tree x row spacing with a 5m maximum canopy height. Emitters were uniformly spaced in a grid pattern throughout treatment plots (Figure 25). Treatment effects were assessed as CM catch suppression in 8 Trécé CM L2-baited Pherocon® IV traps spaced uniformly throughout each plot and in the top 1/3 of the canopy on bamboo poles. Sterilized codling moths obtained from the Okanagan-Kootenay Sterile Insect Release (SIR) Program in Osoyoos, British Columbia, Canada were released into the plots so that even density populations were guaranteed. SIR moths were lightly dusted with different color Dayglo™ pigment powders, ca. 0.1 mg per 800 moths to distinguish treatments and ensure there was no interaction between plots. Moths were transported to field sites in chilled containers. Warmed moths were released at locations no closer than 10 m from traps or emitters by tossing them into the tree canopies. Approximately 335 male and 335 female moths were released at each location for a total of 2,680 males and 2,680 females per treatment. The number of SIR males captured per treatment was recorded 5 days after each release. Releases occurred on: Aug. 21, Aug. 28, and Sept. 4, 2013. Dosage response profiles were generated using the mean number of moths captured / trap.

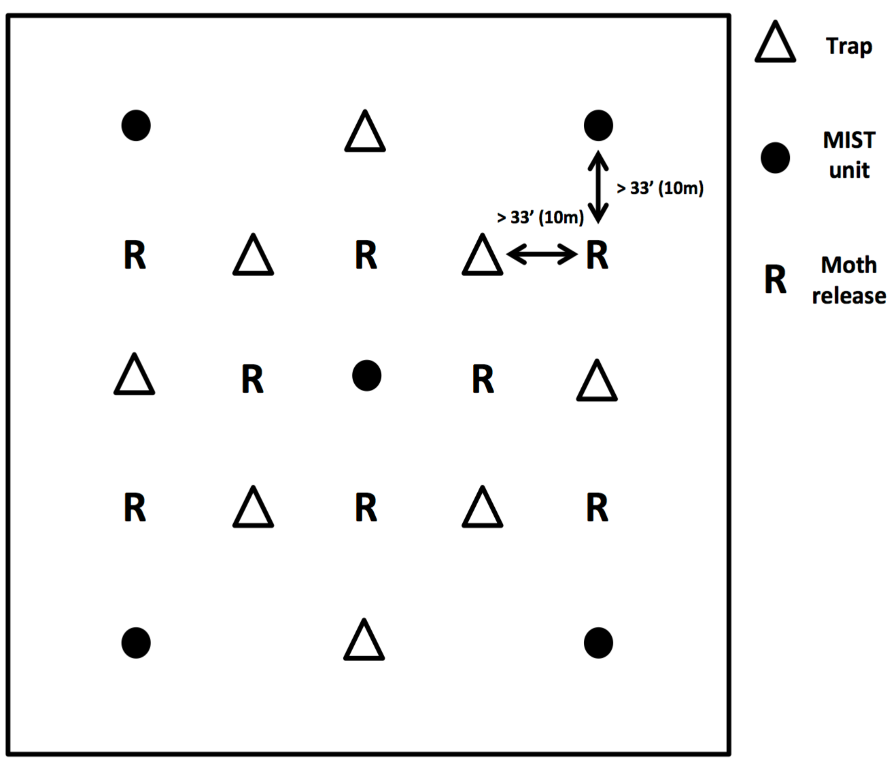

Figure 25. Example of plot layout indicating relationship of Isomate® CM MIST units, L2 baited monitoring traps, and locations of moth release.

I calculated the mean and standard error of the total number of male moths captured in plots treated with the same number of MIST point sources for 3 releases. Percent disruption was calculated as (1 - (mean catch in treatment ⁄ mean catch in control)) x 100. Prior to analysis, data were square root transformed, followed by checks for normality by examining skewness and kurtosis, and subject to ANOVA and Tukey's HSD (Systat Ver.13, San Jose, CA) to determine treatment effects. Graphical plots of catch *vs.* point source density, 1 ⁄ catch *vs.* point source density (Miller-Gut plot), and catch *vs*. point source density x catch (Miller- de Lame plot) were generated to determine the type of disruption mechanism underlying the pattern of moth captures (Miller, Gut, de Lame, and Stelinski 2006b). A competitive disruption profile is concave on an untransformed plot, gives a straight line with positive slope on a Miller-Gut plot, and a straight line with negative slope on a Miller-de Lame plot. By contrast, a non-competitive profile gives a straight line with negative slope on an untransformed plot, an up-curving line on a Miller-Gut plot, and a re-curving line on a Miller-de Lame plot. The slope of a Miller-de Lame plot of competitive disruption reveals the disruption potency of each dispenser (D_a) relative to the trap used to measure the competitive disruption, which gives a value of 1.0.

RESULTS

A large proportion of SIR moths was captured; mean recapture was 8.5% in the control treatment. Significantly fewer moths were recovered in traps in the MIST treatments at dispenser densities of 7.5 and 15 / ha compared to the control treatment ($F=4.37$; $df=4,14$; $p<0.05$). Catch of male moths decreased from 28 / trap in the control to 0.9 / trap at the highest emitter density. Disruption of > 90% was realized at emitter densities greater than 7.5 units per ha (Table 1).

Table I. Male moth catch (mean ± SEM) and % disruption, measured with L2 baited Pherocon® IV monitoring traps, in plots treated with varying densities of Isomate® CM MIST.

Dispensers / ha	Mean Catch ± SEM	Percent Disruption
0	27.9 ± 11.2 a	0
0.6	14.9 ± 9.5 ab	47
1.25	6.3 ± 2.5 ab	77
2.5	4.8 *	83
5	2.6 *	91
7.5	2.0 ± 0.9 bc	93
15	0.9 ± 0.3 bc	97

*Calculated values from Miller-Gut plot.

One way ANOVA, means separation Tukey's HSD ($F=4.37$; $df=4,14$; $p<0.05$)

Dosage response profiles for Isomate® CM MIST explicitly matched the predictions for competitive rather than non-competitive disruption (Figures 26a-c). Moth captures decreased asymptotically as Isomate® CM MIST densities increased (Figure 26a). Data fit to Miller-Gut and Miller-de Lame plots yields straight lines, with positive and negative slopes respectively (Figures 26b & c). This set of results conclusively demonstrates that Isomate® CM MIST dispensers compete with traps for male responses.

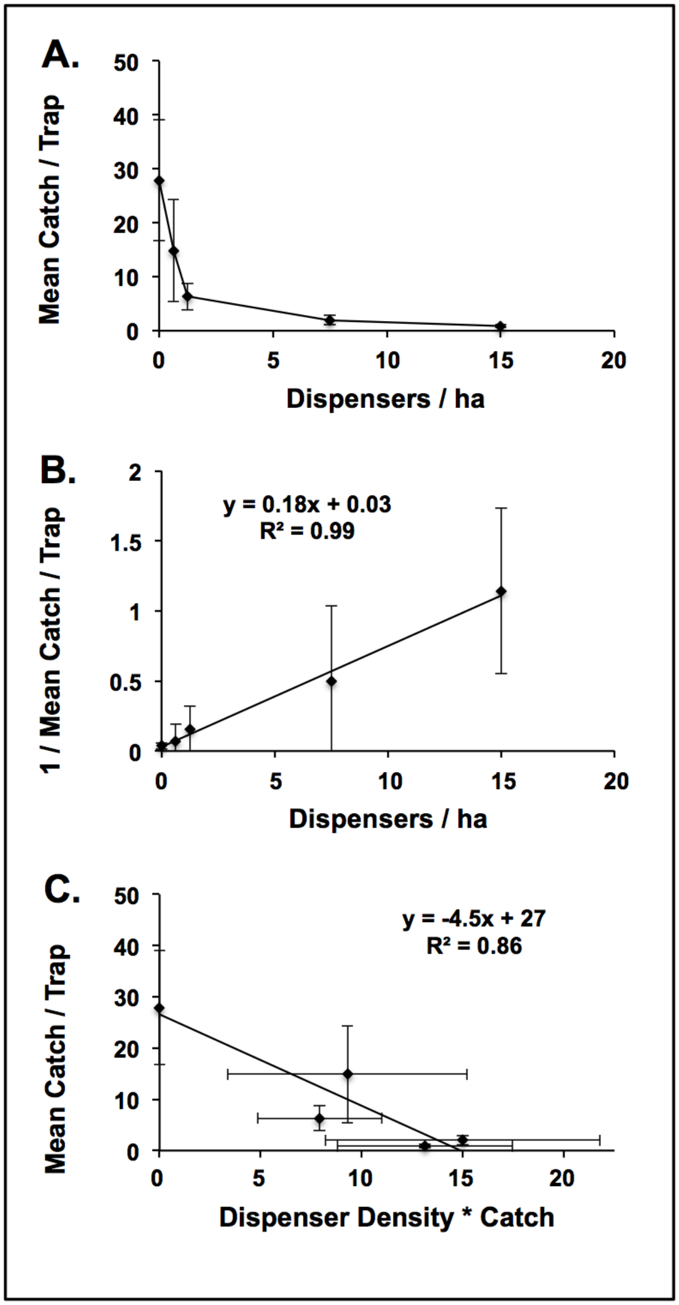

Figure 26. Plots of (A) male moth catch vs. point source density, (B) 1 ⁄ catch vs. point source density, and (C) catch vs. point source density*catch in Isomate® CM MIST mating disruption.

DISCUSSION

This is the first report to show competitive attraction as the likely behavioral mechanism of pheromone disruption for CM using aerosol emitters. Under this type of disruption, the first few dispensers deployed in the crop provide the greatest reduction on moth catch per dispenser. Additional dispensers are helpful, but the per unit impact declines dramatically and overall disruption approaches 100% only asymptotically. The lack of a switch to non-competitive disruption, even under very high pheromone release rates, means that the limitations of the asymptotic effect cannot be overcome even by aerosol emitters.

A Miller-de Lame slope of - 4.5 (Figure 26c) reveals, for the first time, a D_a value for a pheromone dispenser that is greater than 1, indicating that a single MIST unit suppresses trap finding 4.5 times more effectively than does deploying one trap. Da is a measure of the potency of each pheromone dispenser in reducing catch in a baited monitoring trap relative to the suppression effect of that trap (Miller et al. 2010). It is attained as the slope of the Miller-de Lame plot. In a study using large field-cages with known densities of codling moth, Isomate CM dispensers were determined to have a D_a value of 0.2, (Miller et al. 2010) indicating *ca*. 5 dispensers are necessary to match the disruptive activity of a single monitoring trap. We can calculate, based on a label rate of 1000 Isomate dispensers / ha, that 200 monitoring traps would provide similar CM suppression as does the standard Isomate CM treatment. However, traps would need to be monitored constantly in order to maintain the sticky liners efficiency at 100%, as moths and non-target species accumulate over time and reduce catch efficiency. Comparatively, D_a values reveal that Isomate CM MIST is 22.5 times more effective at suppressing moth captures than is each passive reservoir dispenser. We can also deduce from the slope of the Miller-de Lame plot, that 4 - 5 additional monitoring traps with sticky liners would provide a similar level of moth disruption as a single CM MIST unit, and, therefore 20-25 traps / ha would disrupt CM similarly to 5 MIST units / ha.

Mean catches of 4.8 and 2.6 moths per trap for Isomate® CM MIST at densities of 2.5 and 5 units per ha were calculated from Miller-Gut plots and reflect trap inhibition of 83% and 91% respectively. Passive reservoir, hand-applied codling moth pheromone dispensers deployed at rates of 500-1000/ha provide similar levels of trap suppression where pest densities are low to moderate and the impact is generally sufficient to suppress economic injury to fruit at harvest (Charmillot 1991, Thomson et al. 1998). Based on these results five MIST units per hectare would be necessary to achieve the same level of CM control provided by a standard treatment with passive reservoir dispensers.

Practitioners have two viable options for codling moth mating disruption that present quite different options for dispensing pheromone; yet operationally they share some common features. Passive reservoir, hand-applied dispensers and aerosol emitters operate competitively and thus, disruption efficacy is density dependent. This means that control using either dispensing system will be harder to achieve for higher density populations and that it will likely require the use of companion insecticides to reduce pest numbers to levels that are more amenable to disruption. In addition, the asymptotic nature of the disruption profiles for both passive reservoir dispensers and aerosol emitters dictates that economics play a major role in choosing the application rate. The gain in efficacy must justify the cost of additional dispensers. Users must also weigh the relative benefits of increasing dispenser density versus applying a companion insecticide.

The principal difference in the two approaches is the density of units deployed and consequently the means of distributing pheromone. Passive reservoir formulations distribute pheromone via the application process itself, while aerosol emitters largely rely on the wind to distribute pheromone. The former is intended to distribute pheromone uniformly in the canopy with closely spaced dispensers, while the latter relies on natural aerial redistribution of pheromone to avoid gaps due to the widely-spaced deployment of aerosol emitters. A major limitation of passive reservoir, hand-applied dispensers is the labor and cost of deploying hundreds of devices/ha. The need for only a few aerosol emitters mitigates this shortcoming, however a potential weakness of using this technology is that the low deployment density may leave areas of little or no pheromone coverage where mate finding might occur and failure of a unit would leave large areas unprotected relative to passive reservoir dispensers. Indeed, edges are known to be problematic in aerosol-treated crops and supplemental treatment of borders with reservoir dispensers is recommended (Knight 2005). The aerosol tactic is likely to benefit substantially from treatment of large contiguous blocks of crop and is less desirable for small and irregular fields.

CHAPTER FOUR

Tactics for maintaining efficacy while reducing the amount of pheromone released by aerosol emitters disrupting codling moth, *Cydia pomonella* L.

ABSTRACT

Experiments were conducted in commercial apple orchards to determine if improved efficiencies in pheromone delivery may be realized using aerosol pheromone emitters for codling moth mating, *Cydia pomonella* L., disruption. Specifically, we tested how reducing: pheromone concentration, diel period of emitter operation, and frequency of pheromone emission from aerosol emitters affected orientational disruption of male CM to pheromone-baited monitoring traps. Isomate CM MIST formulated with 50% less codlemone (3.5 mg/ emission) provided disruption equal to the commercial formulation (7 mg / emission). Decreased periods of emitter operation (3 and 6 h) and frequency of pheromone emission (30 and 60 min) provided a similar level of orientational disruption than the current standard of releasing over a 12 h period or on a 15 min cycle, respectively. These three modifications provide a means of substantially reducing the amount of pheromone necessary for CM disruption. The savings accompanying pheromone conservation could lead to increased adoption of CM mating disruption and moreover, provide an opportunity for achieving higher levels of disruption by increasing dispenser densities.

INTRODUCTION

Aerosol pheromone emitters aim to improve the cost-effectiveness of codling moth (CM), *Cydia pomonella* L mating disruption (MD) by reducing application labor. They are deployed at densities of 2.5 - 5 ha^{-1}, and each unit releases ca. 7 mg of pheromone every 15-30 min. In Washington, high levels of CM orientational disruption were achieved with Suterra Checkmate CM Puffers deployed in a grid pattern at 2.5 puffers ha^{-1} and releasing 7 mg codlemone (E, E 8-10 Dodecadien-ol) every 15 min during the flight period (Knight 2005). These trials suggested a high-release, low point-source pheromone-dispenser might be effective for disruption of CM. However, the Puffer treatment is usually supplemented with a border-application of hand-applied pheromone dispensers, such as Isomate CM FLEX, and/or companion insecticides. Aerosol emitters (MSU Microsprayer, Suterra Checkmate CM-O Puffer) did not perform as well in Michigan field trials when deployed as a stand-alone tactic (Isaacs et al. 1999, Stelinski, Gut, et al. 2007).

Little is known about the optimal parameters of operation (pheromone release rate, dispenser density, operation period and duration) for aerosol emitters to achieve highly efficacious CM disruption. Experiments examining the response of male CM to lure-baited traps in aerosol-emitter-treated orchards will help optimize these devices. Deploying more than 2.5 ha^{-1} is cost prohibitive and supplementing with high-density formulations increases the cost of both labor and pheromone. Do we need the extremely high amounts of codlemone released by current technologies to achieve disruption? Aerosol emitters dispense pheromone even when conditions (wind, rain, temperature) are unfavorable for moth flight. McGhee et al. (2012) found polyethylene reservoir-dispensers formulated with substantially less codlemone (25-75%) than Isomate C+ provided similar high-level disruption (95%) of CM males. Given these positive results we suspect that aerosol emitters releasing pheromone at lower rates (10-50% less) could be equally effective. Improved CM disruption using aerosol emitters may be realized without significantly increasing cost by deploying higher densities of emitters (5-10 ha^{-1}) than currently recommended (2.5 ha^{-1}) but releasing lower pheromone concentration. This would also likely help mitigate the problems encountered on orchard perimeters when using the low-density approach. Understanding what parameters are optimal for aerosol emitters could lead to increased adoption of this strategy through improved efficacy without increasing cost.

Here we address three questions important to aerosol emitter optimization: 1) Do 25 or 50% reduced pheromone release rates (17.75-35.5g ai ha^{-1} / y) disrupt CM equal to current high release formulations (71g ai ha^{-1} / y), 2) Do reduced diel periods of pheromone release (0, 3, 6 h) disrupt CM equal to the standard 12 h diel operation period, and 3) Do reduced frequencies of pheromone release (0, 30, 60 min) disrupt CM equal to standard frequency of release every 15 min. The success of any of these options in maintaining efficacious CM disruption while substantially reducing the pheromone required per ha^{-1} ultimately provide opportunities for reducing the cost of CM aerosol emitter technologies.

METHODS

All experiment were conducting using Isomate® CM MIST aerosol emitters (Pacific BioControl Corp, Vancouver, Washington) loaded with 18-72 grams of codlemone, (*E, E*)-8,10-dodecadien-1-ol, depending on the experiment. The units are programmable, allowing the operator to change the frequency of emission and period over which pheromone is released. Isomate® CM MIST loaded with 17.75, 35.5 or 71 g of codlemone released 1.75, 3.0 or 7.0 g of pheromone per emission, respectively.

Experiment 1 – reduced pheromone concentration (0%, 25%, 50%, and 100%).

The design of this experiment was randomized complete block (RCB). It was conducted in 2011 on two commercial apple farms near Sparta, MI, and two farms near Watervliet, MI. Each farm consisted of a minimum of 40ha of apple. Six 2ha plots of mature apple trees (3-5m) were randomly assigned one of the following treatments on each farm: Isomate CM MIST 100% (7 mg puff), 50% (3.5 mg puff), 25% (1.75 mg puff), 25% x 2 emitters/ac, and a negative control (No MD). Isomate CM MIST units were deployed at 1 per 0.4 ha for all but the 25% x2 treatment (2 units per 0.4 ha) with emitters uniformly spaced ca. 63 m apart. Emitters were programmed to deliver 1 dose of pheromone every 15 min from 1700 – 0500 h.

Treatment effects were judged by moth captures in 5 Trécé CM/PE +AA (codlemone/pear ester + acetic acid) and 7 Trécé CM L2 baited Delta style traps spaced uniformly throughout each plot and elevated on bamboo poles. Traps were monitored weekly and sticky trap liners were changed as necessary to keep the trapping surface clean. The number and sex of wild moths was recorded each wk. Females captured in CM/PE +AA-baited traps were dissected to determine mating status by counting spermatophores.

Sterile codling moths were obtained from the Okanagan-Kootenay Sterile Insect Release (SIR) program in British Columbia, Canada. The addition of SIR moths provided an evaluation using a known and equal pest density across treatments. Releases of SIR moths were conducted in all orchards. One day old moths were shipped overnight via FedEx in Styrofoam coolers. Upon receipt groups of SIR CM were dusted with ca. 0.1g of DayGlo® pigment powders (green, blue, pink, orange) per 800 moths. Moths were immediately transported in 473 ml solo containers to the field for release. Each DayGlo® color group was assigned to a treatment.

Approximately 1600 moths (1:1, M:F) were released in each plot per replicate. Moths were released in late afternoon by tossing them into the air between two trees at 4 different locations spaced uniformly in the plot. Released moths flew directly into the canopy of the nearest trees.

Moth catch data were transformed to sqrt (x + 0.5) (which normalized the distributions and homogenized variance) and then subjected to a general linear model (GLM) (Systat 13, 2009) blocked by farms. Differences in pairs of means were separated using Tukey's highly significant differences (HSD) test.

Experiment 2 – reduced diel period of operation (0, 3, 6, 12 h)

The experimental design was RCB. It was conducted in 2012 on four farms located in regions described in Experiment 1 to test the hypothesis that reducing the period of pheromone emission from 12 h to 6 or 3 h does not significantly reduce male CM orientational disruption to traps. Isomate CM MIST 50% ai aerosol emitters (35.5g ai) were uniformly distributed at 2.5 ha^{-1} as the basal treatment. Four 4 ha plots of mature apple trees were randomly assigned one of the following daily emission protocols on each farm: 12 h (48 emissions), 6 h (24 emissions), 3 h (6 emissions), and a No MD treated orchard as a negative control. MIST units began operating at 1800 h and ceased operation after 12, 6, and 3 h respectively, delivering 1 emission of codlemone (3.5mg) every 15 min over the course of the release period.

Treatment effects were determined by releasing SIR moths into orchards as described above and measuring capture of male moths in pheromone baited traps. Sixteen hundred moths (1:1, M:F) were released into each treatment for a total of 3 releases. Moth catch data were analyzed as in Experiment 1.

Experiment 3 – Reduced frequency of pheromone release (0, 15, 30, 60 m)

The Experiment 2 design, orchards, and analyses were employed to test the hypothesis that reducing the frequency of pheromone emission below 1x every 15 min does not diminish orientational disruption of CM. The following treatments were randomly assigned on each farm using Isomate CM MIST 50% ai aerosol emitters (35.5g ai) at intervals: 15 min (4x/h), 30 min (2x/h), 60 min (1x/h), or 0 min (No MD) as a negative control. Emitters were programmed in July immediately following the completion of Experiment 2; pheromone was released between 1700 and 0500 h. SIR moths were released at three times and catch data was analyzed as per Experiment 1.

RESULTS

Experiment 1 – reduced pheromone concentration (0%, 25%, 50%, and 100%).

Isomate CM MIST reduced moth captures equally in plots treated with 25% (5 units/ha^{-1}), 50% and 100% (2.5 units/ha^{-1}) loading rates compared to the No MD control, Figure 27. Male moth catch was suppressed 61%, 53%, and 62% respectively in the three treatments. There was no statistical difference in SIR moth captures in L2 baited traps among Isomate CM MIST 25% (5 units/ha^{-1}), 50% (2.5 units/ha^{-1}), and 100% (2.5 units/ha^{-1}) treatments. Isomate CM MIST at 25% (2.5 units/ha^{-1}) had the highest moth catch of the pheromone treatments and was not statistically lower than the control indicating that this density and concentration failed to provide orientational disruption.

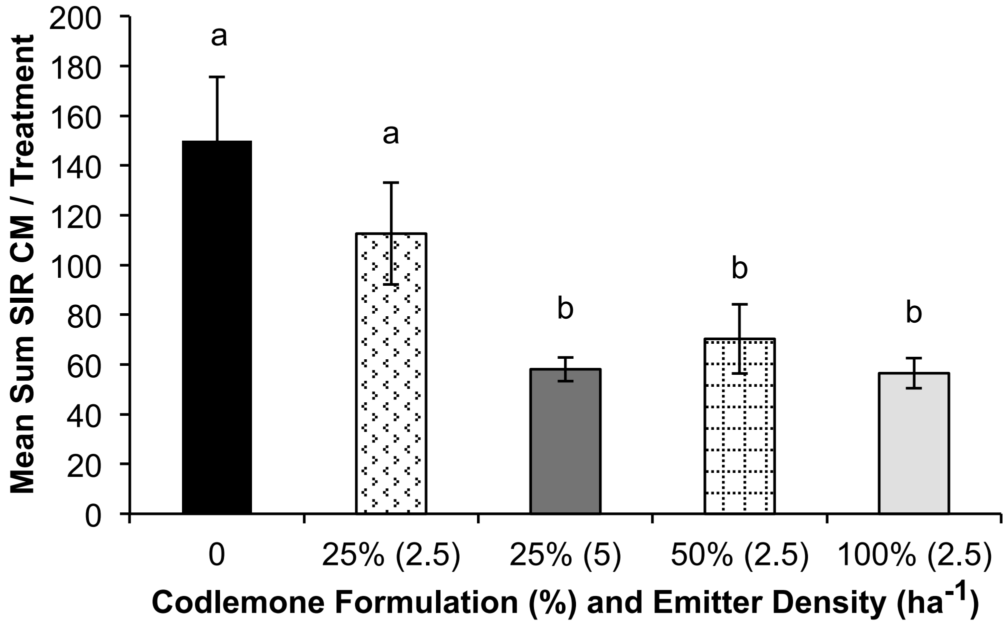

Figure 27. Captures of male CM in apple plots treated with Isomate CM MIST formulated with different concentrations of codlemone (25%, 50%, or 100% at 2.5 units/ha^{-1}, and 25% at 5 units/ha^{-1}), General Linear Model Analyses (4,42) $F=2.74$ $p<0.05$, pairwise comparisons of treatments Tukeys HSD test $p<0.05$

Experiment 2 – reduced diel period of operation (0, 3, 6, 12 h)

Few released and wild moths were caught. Captures of SIR moths was significantly lower in all pheromone treated blocks than in the untreated control, Figure 28. Male moth catch was suppressed 56%, 47%, and 74% respectively in the 3, 6, and 12 h treatments. The pattern of captures was similar for wild moths. Moth catch in plots with MIST units operating for 3, 6 and 12 h did not differ statistically, only the 3 and 12 h treatments had significantly lower catch than the control, Figure 29.

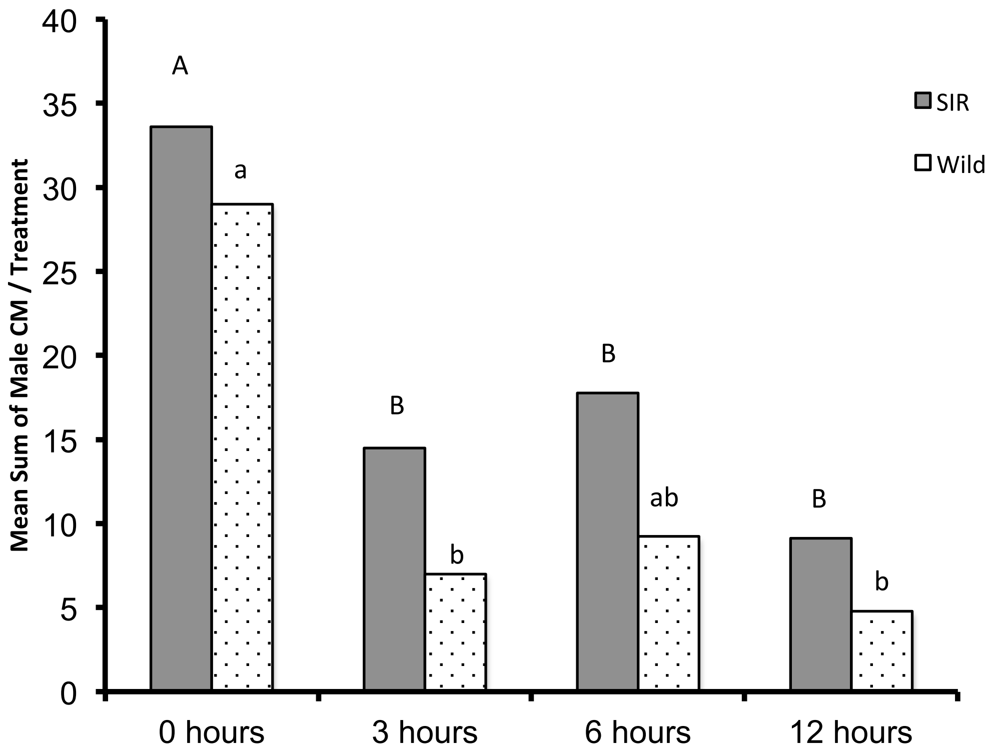

Figure 28. Captures of male CM in Isomate CM MIST treated apple plots dispensing codlemone with increasing duration (0, 3, 6, 12h), General Linear Model Analyses (3,53) $F=4.05$ $p<0.01$, pairwise comparisons of treatments Tukeys HSD test $p<0.05$, SIR moth catch, (3,57) $F=11.204$ $p<0.001$, pairwise comparisons of treatments Tukeys HSD test $p<0.05$, wild moth catch

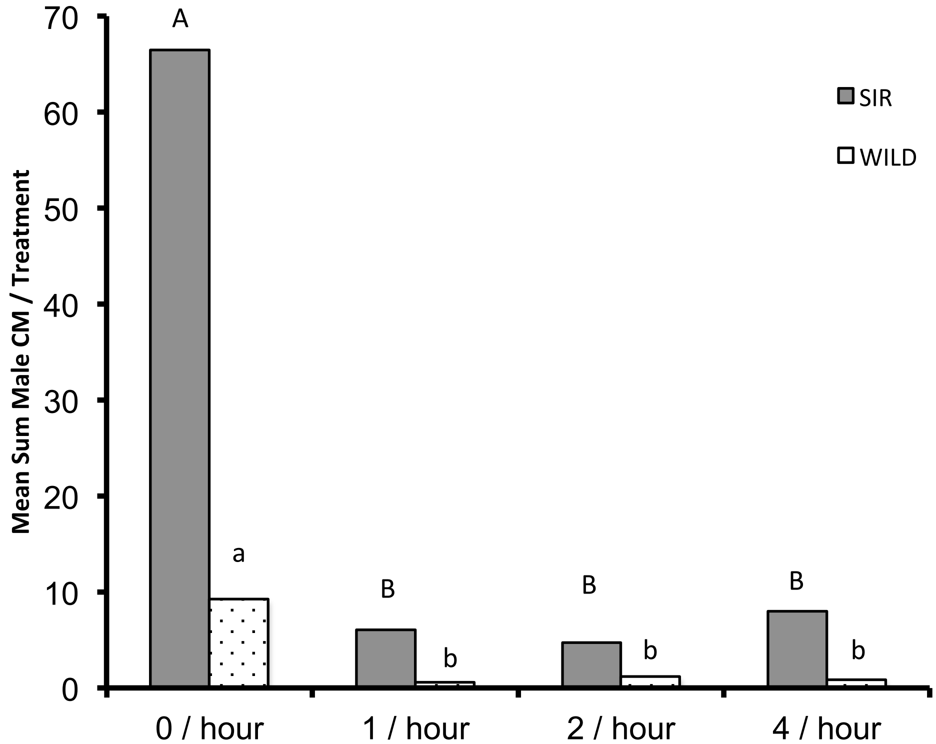

Figure 29. Captures of male CM in Isomate CM MIST treated apple plots dispensing codlemone at different intervals (15, 30, 60 min) between 1700-0500 h, General Linear Model Analyses (3,54) $F=16.58$ $p<0.01$, pairwise comparisons of treatments Tukeys HSD test $p<0.05$, SIR moth catch, (3,38) $F=5.38$ $p<0.01$, pairwise comparisons of treatments Tukeys HSD test $p<0.05$, wild moth catch

Experiment 3 – Reduced frequency of pheromone release (0, 15, 30, 60 m)

More moths were captured during Experiment 3 than Experiment 2. Captures of marked and wild moths was statistically lower in all pheromone-treated plots than in the control plots, Figure 29. Moth catch in plots with MIST units releasing pheromone once, twice or four times per hour did not differ statistically. Suppression of SIR catch was ca. 90% overall for the pheromone treatments compared to the control.

DISCUSSION

Our results indicate, indeed, that reducing pheromone concentration by 50% while maintaining the quantity (7 mg) of the formulation released with each emission provided disruption similar to that of the full concentration. The overall catch in MIST-treated plots examining reduced pheromone concentration was not as dramatically lower than the negative control as previously observed (Experiment 1, Chapter 2). Smaller plot size likely resulted in higher catch. Treatment plots in Experiment 1 were ½ the size (2 ha) of those in Chapter 2 (4 ha) and likely contributed to higher moth catch. Early in the development of aerosol emitters, researchers recognized that the geometry associated with deploying the devices at low densities meant that larger plots would be easier to cover with the pheromone plume than smaller plots (Baker et al. 1997). The Suterra Checkmate Puffer CM-O label warns that use in orchards less than 16 ha will lead to less than optimal results and recommends 5 units ha^{-1}. Regardless of plot size, CM catch was the same in 100% and 50% pheromone plots using equal emitter densities. We postulate CM disruption using 50% would likely improve with plot size similarly to when the 100% pheromone concentration was tested in 4 ha plots (Chapter 2). Reducing pheromone concentration 50% would correspondingly decrease the formulation expense.

Pheromone conservation can also by achieved by reducing the diel period and frequency of emissions. Altering the diel period of operation from 12 to 6 or

3h gave similar orientational disruption. Interestingly, catch of SIR and wild male CM was inconsistent for the 6h treatment. Male SIR captures under 6h operation was similar to both pheromone treatments (3 and 12h) and the negative control, whereas wild moth catch was similar only to the pheromone treatments but not the control.

Likewise, disruption of CM catch was very high across all reduced frequencies of pheromone emissions (15, 30, and 60 min intervals). Altering the emission frequency from 15 min to 30 or 60 min saves 50-75% pheromone without a loss in efficacy. Dispensing pheromone more frequently is not warranted and does not provide a higher level of disruption.

Here we present three tactics using aerosol emitters that reduce the amount of pheromone necessary for CM disruption in apple. Adopting any of these strategies could result in alternative deployment strategies, such as increased dispenser densities, where the savings provided by pheromone conservation can offset the additional cost of more emitters. This strategy might negate the recommendation (Knight, 2005, Suterra Checkmate CM-O Puffer label) of applying additional high point-source density dispensers around orchard perimeters. Future research combining any or all three of these techniques could result in even greater conservation of pheromone.

SUMMARY AND CONCLUSIONS

Pheromone mating disruption continues to play a vital role in codling moth pest management in apple. Mating disruption, as it is commercially practiced today, is still largely achieved through the manual application of reservoir-type release devices (1000 ha^{-1}) throughout the cropping system. New pheromone delivery systems including meso and aerosol emitters were developed to reduce cost and labor and might entice more apple growers to adopt this novel pest management practice, but not much was known about how to deploy these devices efficiently or what behavioral mechanisms are at play in disruption. The overall aim of this dissertation research was to understand how high-releasing pheromone technologies deployed at low point-source densities perform compared to that of industry standard disruption technology that emits lower concentrations of pheromone and are deployed at higher point-source densities and determine the behavioral mechanism(s) of disruption.

My initial studies investigated the possibility of deploying more potent pheromone dispensers (meso) at reduced densities (10-100 ha^{-1}) compared to traditional hand-applied dispensers (Isomate CM FLEX, Scentry No Mate CM, Checkmate CM) at relatively high densities (1000 ha^{-1}). Isomate CM RING meso dispensers failed at reducing male CM catch in traps compared to Isomate CM FLEX applied at 1000 dispensers ha^{-1}. The level of inhibition provided by the CM RING dispenser applied at reduced densities was much lower (45%) compared to the FLEX dispenser (>90%). The RING dispenser, while physically larger than a FLEX dispenser, may not be more attractive to CM males or have a larger active space. I found no evidence based on captures of male CM that RING dispensers were a viable option for disrupting this pest. Failure of the CM RING deployed at low densities to disrupt male catch in traps might result from low pheromone emission from the dispenser or deficiencies in the plume structure; the increase in dispenser size may not correlate to increased pheromone emission rates or plume size. Conducting a volatile capture experiment on CM RING would reveal the rate and concentration of pheromone emission and could then be compared to commercially successful dispensers such as CM FLEX

In contrast, higher levels of male catch suppression were achieved in plots treated with Cidetrak CM meso dispensers applied at low densities. Inhibition of male catch reached levels as high as 85% using ten 20 cm dispensers ha^{-1}. Increasing the overall size of Cidetrak dispensers from 20 cm to 51 cm did not increase the level of disruption. Differences between the two dispensers, size and therefore quantity of pheromone, may not be great enough to alter the response of CM males or possibly the degree of attraction is already maximized with the smaller dispenser. Volatile capture experiments performed on both Cidetrak CM meso dispensers would likely provide insights into why they perform similarly.

Meso dispensers formulated with codlemone and pear ester sought to increase disruption above that of dispensers containing only codlemone. The results of experiments show these dispensers perform similarly at reducing male catch. While I did not find the addition of pear ester enhanced male disruption, these formulations use less codlemone and therefore may be less expensive to manufacture while providing a similar level of disruption. Further studies investigating the effect pear ester released from meso dispensers on female behavior might prove to be beneficial; possibly targeting female mating or oviposition successes such as finding and depositing eggs on choice larval developmental locations.

Cidetrak CM meso applied at low densities, 10, 20 or 80 units/ha^{-1}, provided similar levels of moderate disruption. The pattern of male catch suggests that meso dispensers also operate via competition between dispensers and female moths. However, confirming the behavioral mechanism of disruption for Cidetrak CM meso awaits a dosage response study in which dispensers of uniform size and pheromone release are applied at a range of densities. Graphical analyses of the results would also assist in determining the optimal deployment density to achieve 95% or greater male disruption in traps that is necessary for high performance mating disruption.

Relatively few scientific studies have been published on the performance of aerosol emitters compared to other mating disruption technologies. These devices are deployed at very low densities, but each unit releases very large quantities of pheromone. My initial interest was to determine if aerosol emitters could provide levels of mating disruption equivalent or better than that achieved using standard dispensers. The first experiments comparing Isomate MIST to Isomate FLEX revealed a similar reduction in the numbers of CM males responding to traps (Figure 16). Deploying fewer aerosol dispensers will greatly reduce the time and labor needed for growers using mating disruption for CM control.

The pattern of catch in my initial aerosol emitter studies showed that more moths were captured in traps nearest to the emitters, indicating male CM are either congregating in larger numbers near the pheromone source or that males already in these areas fail to disperse in a manner similar to those in areas of less concentrated pheromone. The movement of the released sterile males from untreated areas (Chapter 2, experiment 1) into aerosol treated orchards indicates this was due to moths moving towards high concentrations of pheromone. This explanation follows that moths evolved to follow a concentration gradient to the source and conforms to competitive attraction as the mechanism of mating disruption reported for codling moth (Miller, Gut, de Lame, and Stelinski 2006a) where moths bypass lower concentration sources, such as females and traps, in favor of more concentrated pheromone plumes from dispensers. Thus, the best protection of aerosol emitters may be happening at some distance from the emitter and not adjacent to it.

It appeared to me that CM was capable of responding to high concentrations of pheromone in orchard environments. If males fly up to aerosol emitters, it follows that they might not become incapacitated (long lasting adaptation) after exposures to high dosages of pheromone as previously suggested (Judd et al. 2005; Stelinski, Gut, and Miller 2005). Captures of codling moths following pre-exposure to pheromone provides further insight into the underlying behavioral mechanism of orientational disruption via aerosol (CM MIST) vs. hand applied (CM FLEX) dispensers. Moths held in FLEX treated orchards, but not directly exposed to dispensers in cages, responded similarly to naïve moths suggesting that pheromone plumes emanating from dispensers in FLEX treated orchards either did not reach caged moths, or that the airborne concentration was lower than that required to induce increased search. In contrast, moths pre-exposed directly or indirectly to the large quantity of pheromone released from a MIST device were captured in greater numbers than naïve moths not exposed to pheromone. These data provide evidence that such exposures to high concentrations of pheromone do not cause long lasting adaptation in the field setting, as the male’s ability to find a pheromone source increased rather than decreased. Additionally, males exposed to pheromone plumes emanating from aerosol dispensers or coming in close contact with exposed foliage are quite capable of finding traps and they continue to actively respond to pheromone plumes emanating from lure-baited traps indicating that exposures to high concentrations of pheromone increases/stimulates rather than decreases search behavior. Males become sensitized rather than desensitized.

These findings suggested that aerosol emitters operate competitively and the resultant dosage response experiment confirmed this to be true (Chapter 3). Moth captures decreased asymptotically as emitter densities increased and the Miller-Gut and Miller-de Lame plots yielded straight lines, with positive and negative slopes respectively. I propose that aerosol emitters operative competitively, by induced allopatry, where spatial segregation of the sexes occurs when males move towards and aggregate near high concentration pheromone sources thus diverting attention away from females that are releasing very low quantities of pheromone. Additionally, the dosage response profile shows that 5-7 units ha^{-1} are necessary to provide a high level (>95%) of male orientation to traps; slightly more than the current label recommendation of 2-4 units ha^{-1}. A potential weakness of using aerosol emitters is that the low deployment density may leave areas of little or no pheromone coverage, where mate finding might occur, and mechanical failure of a unit would leave large areas unprotected relative to passive reservoir dispensers. The aerosol tactic is likely to benefit substantially from treatment of large contiguous blocks of crop and is less desirable for small and irregular fields.

Increasing the density of aerosol units, as described above, to achieve highly effective MD is cost prohibitive. This revelation provided me with the question regarding optimization of aerosol emitters. Determining the minimal thresholds for rate, period, and concentration of pheromone necessary to disrupt male CM could result in cost savings of the expensive active ingredient making increased unit density an affordable possibility. These studies show that indeed reducing either the rate (4 to 2 emissions/hr), period of operation (12 to 6 hr), or concentration of pheromone (150 g to 71 g) in the canister by 50% provided >90% disruption of male moths. Future research combining any or all three of these techniques could provide a greater reduction in pheromone requirements. The savings accompanying pheromone conservation could lead to increased adoption of CM mating disruption and moreover, provide an opportunity for achieving higher levels of disruption by increasing dispenser densities.

The above investigations show aerosol pheromone emitters suppress male CM catch in the range provided by commercially available dispensers (Isomate CM FLEX) in Michigan apple orchards (Figure 16). This technology provides growers with an effective labor savings option for CM management. Studies presented in this dissertation, especially dosage response and optimization experiments, provide a solid framework for determining the behavioral mechanism of disruption, dispenser density, and quantity of pheromone required to achieve a high level of disruption using low density devices.

APPENDIX

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2014 – 03

Author and Title of thesis:

Peter Scott McGhee

Impact Of High Releasing Mating Disruption Formulations On (Male) Codling Moth, *Cydia pomonella* L., Behavior

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Table 2. Record of voucher specimens

Family	Genus-Species	Life Stage	Quantity	Preservation
Tortricidae	*Cydia pomonella* (L)	adult	10 male	pinned
Tortricidae	*Cydia pomonella* (L)	adult	10 female	pinned

LITERATURE CITED

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Agnello, A. M., and H. Reissig. 2009. Mechanically Applied Pheromone Products for Mating Disruption of Codling Moth and Oriental Fruit Moth in Apples. New York Fruit Quarterly. 17.

Arn, H., P. M. Guerin, H. R. Buser, S. Rauscher, and E. Mani. 1985. Sex-Pheromone Blend of the Codling Moth, *Cydia pomonella* - Evidence for a Behavioral Role of Dodecan-1-Ol. Experentia. 41: 1482–1484.

Baker, T. C., T. Dittl, and A. Mafra-Neto. 1997. Disruption of sex pheromone communication in the blackheaded fireworm in Wisconsin cranberry marshes by using MSTRS(TM) devices. Journal of Agricultural Entomology. 14: 449–457.

Barnes, M. 1991. Tortricids in Pome and Stone Fruits, p. 808. In vanDerGeest, S., Evenhuis, H.H. (eds.), Tortricid Pests, Their Biology, Natural Enemies, and Control. Elsevier Science.

Bartell, R. J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. Physiol Entomol. 7.

Bartell, R. J., and T. E. Bellas. 1981. Evidence for naturally occurring, secondary compounds of the codling moth female sex pheromone. Australian Journal of Entomology. 20: 197–199.

Batiste, W., W. H. Olson, and A. Berlowitz. 1973. Codling Moth: Diel Periodicity of Catch in Synthetic Sex Attractant vs. Female-Baited Traps. Environmental Entomology. 2: 673–676.

Bäckman, A.-C., M. Bengtsson, and P. Witzgall. 1997. Pheromone release by individual females of codling moth, *Cydia pomonella*. J Chem Ecol. 23: 807–815.

Byers, J. A. 2007. Simulation of mating disruption and mass trapping with competitive attraction and camouflage. Environmental Entomology. 36: 1328–1338.

Cardé, R. T. 2007. Using pheromones to disrupt mating of moth pests. Perspectives in ecological theory and integrated pest management. Cambridge University Press, Cambridge. 122–169.

Cardé, R. T., and A. K. Minks. 1995. Control of moth pests by mating disruption: successes and constraints. Annu. Rev. Entomol. 40: 559–585.

Cardé, R. T., R. T. Staten, and A. Mafra-Neto. 1998. Behaviour of pink bollworm males near high-dose, point sources of pheromone in field wind tunnels: insights into mechanisms of mating disruption. Entomologia Experimentalis et Applicata. 89: 35–46.

Casado, D., F. Cave, and S. Welter. 2011. Effect of Pheromone Dose Reduction on the Plume of Aerosol Puffers. Proceedings of the 85th Orchard Pest and Disease Management Conference. 85: 1–56.

Charmillot, P. J. 1991. Mating disruption technique to control codling moth in western Switzerland, pp. 165–182. In Ridgeway, R.L., Silverstein, R.M., Inscoe, M.N. (eds.), Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants. Dekker, New York.

Coracini, M. D. A., M. Bengtsson, I. Liblikas, and P. Witzgall. 2004. Attraction of codling moth males to apple volatiles. Entomologia Experimentalis et Applicata. 110: 1–10.

El-Sayed, A. M. 2004. Behavioral Effect of (E)-8,(Z)-10-Dodecadien-1-ol and (E)-8,(E)-10-dodecadienyl acetate on the upwind orientation of male codling moth, *Cydia pomonella* to pheromone source. Behaviour. 141: 313–325.

El-Sayed, A. M., M. Bengtsson, S. Rauscher, J. Löfqvist, and P. Witzgall. 1999. Multicomponent sex pheromone in codling moth (Lepidoptera: Tortricidae). Environmental Entomology. 28: 775–779.

El-Sayed, A. M., R. C. Unelius, I. Liblikas, J. Löfqvist, M. Bengtsson, and P. Witzgall. 1998. Effect of codlemone isomers on codling moth (Lepidoptera: Tortricidae) male attraction. Environmental Entomology. 27: 1250–1254.

Epstein, D. L., L. L. Stelinski, T. P. Reed, J. R. Miller, and L. J. Gut. 2006. Higher densities of distributed pheromone sources provide disruption of codling moth (Lepidoptera : Tortricidae) superior to that of lower densities of clumped sources. Journal of Economic entomology. 99: 1327–1333.

Gut, L. J., and J. F. Brunner. 1998. Pheromone-based management of codling moth (Lepidoptera : Tortricidae) in Washington apple orchards. Journal of Agricultural Entomology. 15: 387–406.

Gut, L. J., L. L. Stelinski, D. Thomson, J. R. Miller. 2004. Behaviour-modifying chemicals: prospects and constraints in IPM, pp. 73–121. In Koul, O., dhaliwal, G.S., cuperus, G.W. (eds.), Integrated Pest Management: Potential, Constraints and Challenges. CABI.

Hansen, M. 2008. Puffers Save Labor. Good Fruit Grower.

Howitt, A. J. 1993. Common tree fruit pests. Michigan State University Extension.

Huang, J., L. J. Gut, and J. R. Miller. 2013. Codling Moth, *Cydia pomonella*, Captures in Monitoring Traps as Influenced by Proximately to Competing Female-Like- vs. High-Releasing Pheromone Point Sources. J Insect Behav. 26: 660–666.

Isaacs, R., M. J. Ulczynski, B. Wright, L. J. Gut, and J. R. Miller. 1999. Performance of the microsprayer with application for pheromone-mediated control of insect pests. Journal of Economic entomology. 92: 1157–1164.

Judd, G. J., M. G. T. Gardiner, N. C. DeLury, and G. Karg. 2005. Reduced antennal sensitivity, behavioral 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: 65–78.

Knight, A. L. 2004. How Can We Make Codling Moth mating Disruption Work? Proceedings of the 78th Western Orchard Pest and Disease Management Conference, Portland, OR.

Knight, A. L. 2005. Managing codling moth (Lepidoptera: Tortricidae) with an internal grid of either aerosol Puffers or dispenser clusters plus border applications of individual dispensers. Journal of the Entomological Society of British Columbia. 101: 69–78.

Knight, A. L., and D. M. Light. 2001. Attractants from Bartlett pear for codling moth, Cydia pomonella (L.), larvae. Naturwissenschaften. 88: 339–342.

Knight, A. L., and D. M. Light. 2005a. Developing action thresholds for codling moth (Lepidoptera : Tortricidae) with pear ester- and codlemone-baited traps in apple orchards treated with sex pheromone mating disruption. Canadian Entomologist. 137: 739–747.

Knight, A. L., and D. M. Light. 2005b. Seasonal flight patterns of codling moth (Lepidoptera : Tortricidae) monitored with pear ester and codlemone-baited traps in sex pheromone-treated apple orchards. Environmental Entomology. 34: 1028–1035.

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. Journal of the Entomological Society of British Columbia. 101: 79–86.

Knight, A. L., D. Light, and V. Chebny. 2012. Evaluating Dispensers Loaded With Codlemone and Pear Ester for Disruption of Codling Moth (Lepidoptera: Tortricidae). Environmental Entomology. 41: 399–406.

Knight, A. L., R. J. Hilton, and D. M. Light. 2005. Monitoring codling moth (Lepidoptera: Tortricidae) in apple with blends of ethyl (E, Z)-2,4-decadienoate and codlemone. Environmental Entomology. 34: 598–603.

Knight, A. L., T. E. Larsen, and K. C. Ketner. 2004. Rainfastness of a microencapsulated sex pheromone formulation for codling moth (Lepidoptera: Tortricidae). Journal of Economic Entomology. 97: 1987–1992.

Light, D., A. L. Knight, C. Henrick, D. Rajapaska, B. Lingren, J. C. Dickens, K. Reynolds, R. G. Buttery, G. B. Merrill, J. Roitman, and B. Campbell. 2001. A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). Naturwissenschaften. 88: 333–338.

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

McDonough, L. M., D. A. George, B. A. Butt, J. M. Ruth, and K. R. Hill. 1972. Sex pheromone of the codling moth: structure and synthesis. Science. 177: 177–178.

McGhee, P. S., D. L. Epstein, and L. J. Gut. 2011. Quantifying the benefits of areawide pheromone mating disruption programs that target codling moth (Lepidoptera: Tortricidae). American Entomologist. 57: 94–100.

McGhee, P. S., L. J. Gut, and J. R. Miller. 2014. Aerosol Emitters Disrupt Codling Moth, *Cydia pomonella*, Competitively. Pest. Manag. Sci.

McGhee, P. S., L. J. Gut, M. J. Haas, J. R. Miller, and J. Huang. 2012. Field Evaluations of Isomate FLEX CM and Cidetrak CM Meso hand Applied Mating Disruption. Proceedings of the 86th Annual Orchard Pest and Disease Management Conference.

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 2): Case studies. J Chem Ecol. 32: 2115–2143.

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 I): Theory1. J Chem Ecol. 32: 2089–2114.

Miller, J. R., P. S. McGhee, P. Y. Siegert, C. G. Adams, J. Huang, M. J. Grieshop, and L. J. Gut. 2010. General principles of attraction and competitive attraction as revealed by large-cage studies of moths responding to sex pheromone. Proceedings of the National Academy of Sciences. 107: 22–27.

Mota-Sanchez, D., J. C. Wise, R. V. Poppen, L. J. Gut, and R. M. Hollingworth. 2008. Resistance of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), larvae in Michigan to insecticides with different modes of action and the impact on field residual activity. Pest. Manag. Sci. 64: 881–890.

Putman, W. L. 1962. The Codling Moth, *Carpocapsa pomonella* (L.) (Lepidoptera: Tortricidae): a review with special reference to Ontario. Proceeding of the Entomological Society of Ontario. 93: 22–59.

Reinke, M. D., P. Y. Siegert, P. S. McGhee, L. J. Gut, and J. R. Miller. 2013. Pheromone release rate determines whether sexual communication of Oriental fruit moth is disrupted competitively vs. non-competitively. Entomologia Experimentalis et Applicata. 150: 1–6.

Roelofs, W. L., A. Comeau, A. Hill, and G. Milicevic. 1971. Sex attractant of the codling moth: characterization with electroantennogram technique. Science. 174: 297–299.

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

Stelinski, L. L., J. R. Miller, and M. E. Rogers. 2008. Mating Disruption of Citrus Leafminer Mediated by a Noncompetitive Mechanism at a Remarkably Low Pheromone Release Rate. J Chem Ecol. 34: 1107–1113.

Stelinski, L. L., L. J. Gut, A. V. Pierzchala, and J. R. Miller. 2004. Field observations quantifying attraction of four tortricid moths to high-dosage pheromone dispensers in untreated and pheromone-treated orchards. Entomologia Experimentalis et Applicata. 113: 187–196.

Stelinski, L. L., L. J. Gut, and J. R. Miller. 2005. Occurrence and duration of long-lasting peripheral adaptation among males of three species of economically important tortricid moths. ann. entom. soc. amer. 98: 580–586.

Stelinski, L. L., L. J. Gut, and J. R. Miller. 2006. Orientational Behaviors and EAG Responses of Male Codling Moth After Exposure to Synthetic Sex Pheromone from Various Dispensers. J Chem Ecol. 32: 1527–1538.

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. Journal of Applied Entomology. 129: 481–488.

Stelinski, L. L., L. J. Gut, K. J. Vogel, and J. R. Miller. 2004. Behaviors of naive vs. pheromone-exposed leafroller moths in plumes from high-dosage pheromone dispensers in a sustained-flight wind tunnel: Implications for mating disruption of these species. J Insect Behav. 17: 533–554.

Stelinski, L. L., L. J. Gut, M. Haas, P. S. McGhee, and D. L. Epstein. 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. S. McGhee, M. Haas, A. L. Il'Ichev, and L. J. Gut. 2007. Sprayable Microencapsulated Sex Pheromone Formulations for Mating Disruption of Four Tortricid Species: Effects of Application Height, Rate, Frequency, and Sticker Adjuvant. Journal of Economic entomology. 100: 1360–1369.

Stelinski, L. L., P. S. McGhee, M. J. Grieshop, J. F. Brunner, and L. J. Gut. 2009. Efficacy and release rate of reservoir pheromone dispensers for simultaneous mating disruption of codling moth and oriental fruit moth (Lepidoptera: Tortricidae). Agricultural and Forest Entomology. 102: 389–397.

Suckling, D. M. 2000. Issues affecting the use of pheromones and other semiochemicals in orchards. Crop Protection. 19: 677–683.

Suckling, D. M., J. M. Daly, X. Chen, and G. Karg. 2007. Field electroantennogram and trap assessments of aerosol pheromone dispensers for disrupting mating in *Epiphyas postvittana*. Pest. Manag. Sci. 63: 202–209.

Thomson, D., J. F. Brunner, L. J. Gut, G. J. Judd, and A. L. Knight. 2001. Ten years implementing codling moth mating disruption in the orchards of Washington and British Columbia: starting right and managing for success! IOBC wprs Bulletin. 24: 23–30.

Thomson, D., L. J. Gut, and J. W. Jenkins. 1998. Pheromones for insect control, pp. 385–411. In Hall, F.R., Menn, J.J. (eds.), Biopesticides. Biopesticides.

Tomaszewska, E., V. R. Hebert, J. F. Brunner, V. P. Jones, M. D. Doerr, and R. J. Hilton. 2005. Evaluation of Pheromone Release from Commercial Mating Disruption Dispensers. J. Agric. Food Chem. 53: 2399–2405.

Welter, S. 1999. Defining the active area of pheromone plumes from aerosol based emitter, Paramount Puffer.

Welter, S. C., and F. Cave. 2004. Evaluation of Alternative Pheromone Dispensing Technologies.

Welter, S., and F. Cave. 2000a. Definition of the Active Space of Pheromone Plumes from Aerosol Emitters, "Paramount Puffers." Proceedings of the 74th Annual Western Orchard Pest and Disease Management Conference, Portland, OR.

Welter, S., and F. Cave. 2000b. Defining the Active Area of Pheromone Plumes from Aerosol Based Emitter, The Paramount Puffer. 1–19.

Wins-Purdy, A. H., G. J. Judd, and M. L. Evenden. 2007. Disruption of pheromone communication of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) using microencapsulated sex pheromones formulated with horticultural oil. Environmental Entomology. 36: 1189–1198.

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

Wise, J. C., and L. J. Gut. 2002. Season long control of lepidopteran pests with Intrepid, 2001. Arthropod Management Tests.

Witzgall, P., A.-C. Bäckman, M. Svensson, U. T. Koch, F. Rama, A. M. El-Sayed, J. Brauchli, H. Arn, M. Bengtsson, and J. Löfqvist. 1999. Behavioral observations of codling moth, *Cydia pomonella*, in orchards permeated with synthetic pheromone. Biocontrol. 44: 211–237.

Witzgall, P., L. L. Stelinski, L. J. Gut, and D. Thomson. 2008. Codling Moth Management and Chemical Ecology. Annu. Rev. Entomol. 53: 503–522.

Witzgall, P., M. Bengtsson, A. El Sayed, A.-C. Bäckman, S. Rauscher, A.-K. K. Borg-Karlson, R. Unelius, and J. Löfqvist. 1999. Chemical communication in codling moth: towards environmentally safe control methods. IOBC wprs Bulletin. 22.