EXPLORING STERILE INSECT TECHNIQUE FOR MANAGEMENT OF CODLING MOTH IN MICHIGAN

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ABSTRACT

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The use of sterile insect technique (SIT) for codling moth (*Cydia pomonella* L.) management has been proven successful at suppressing wild populations, allowing for the reduction of insecticide applications. Most of this research has occurred in British Columbia, Canada and Washington, United States. In this thesis, I demonstrate how this tool might be utilized in Michigan apple orchards. I first looked at how male and female *C. pomonella* respond to traps baited with different chemical cues in Michigan compared to Washington in a 2-year study. I found a difference in the sex ratios of recaptured codling moths between the two states, demonstrating that lure performance is different based upon regional differences. In addition to monitoring traps, I investigated how to apply sterile insect technique in Michigan where a farm-scale approach is necessary due to the relatively small size of orchards in this region. I compared the timing of releases during the season (1st generation, 2nd generation, or season long) in addition to the rate of moths released (half or full rate). The full rate released for first generation or season long had consistently low wild male moth captures throughout the season. Finally, we tested the method of the release (self-released, released in the canopy, or released on the ground) and found that there was no significant difference between the release methods tested. These results can inform future development of codling moth management programs for Michigan apple orchards that include the sterile insect release.

This thesis is dedicated to my partner, Karan Dhillon, who has loved, supported, and motivated me not only through this program but throughout our relationship. I would not have been able to achieve this without your support.

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CHAPTER 1: Introduction and Literature Review

Overview of Codling moth

Codling moth (*Cydia pomonella* L., Lepidoptera: Tortricidae) is one of the most important pests in apple production and can cause losses ranging from 50%-90% of the crop (Wise et al. 2022). The United States is ranked third worldwide in apple production and the apple harvest in Michigan is valued at almost 300 million dollars annually in 2020 (Crop Value Summary USDA 2021). Codling moths are also a concern in pear, walnut, quince, crabapple, loquat, and hawthorn plants as well as stone fruits such as apricots, cherries, peaches, plums, and prunes (Newcomer and Whitcomb 1924).

The number of codling moth generations per year is dependent upon temperature and climate. In Michigan apple orchards, we see two full generations per year (Wise et al. 2022). Recently a partial third generation has been observed (Wise et al. 2022). Female moths can lay 30 - 70 eggs at one time and deposit them on developing fruit and leaves of fruit trees. After the eggs hatch, they bore into the fruit making a path through the flesh, and eventually to the seeds, which they typically consume; they feed until they reach full growth at 4 weeks (Pajac 2011). There are two types of direct damage that can occur: a deep entry into the apple from a larva successfully eating in the apple to feed on the seeds and a shallow entry where the larva has done some feeding but is not able to successfully enter the fruit (Wise et al. 2022). In Michigan the first generation typically emerges in mid-May (Howitt 1993, Wise et al. 2022). The second generation emerges in late July (Howitt 1993, Wise et al. 2022). Most of the adult flight occurs during dusk when temperatures are above 15 ^{o}C (Batiste 1973).

Management

Codling moth has been a difficult pest to manage since its introduction to North America in the mid-1700s (Slingerland 1898). Various management strategies have been developed and include insecticide sprays, virus biopesticide sprays, and pheromone-mediated mating disruption. Effective management techniques are important as there is a low damage threshold in commercial production, with conventional growers targeting no more than 1-2% of the crop damaged to remain profitable (Balasko et al. 2020).

Insecticides

Due to codling moth's multi-generational life cycle, applications of insecticide are typically needed several times per season (Lacey et al. 2008). In fact, 70% of insecticides applied to apple orchards are to help control codling moths (Franck et al. 2007). A total number of moths trapped of 5-7 during the first generation or 3-5 during the second generation in a single trap since the beginning of the season indicates that a control method for the population needs to be put into place (Wise et al. 2022). Codling moth pesticide sprays primarily target the eggs and larvae. The earliest spray can be applied at 100 DD° base 10°C (100 DD° base 50°F) after biofix when trying to target eggs, using ovicide sprays such as acetamiprid (Assail) and methoxyfenozide (Intrepid) which belong to the insect growth regulator benzoylyreas and ecdysone agonists chemical classes, respectively. If trying to target the larvae, sprays should be applied at 250 DD° after the biofix (Wise et al. 2022). Larvicides targeting neonate codling moth come from the chemical classes diamide, neonicotinoid, pyrethroid, organophosphates, spinosyn and include acetamiprid (Assail), chlorantraniliprole (Altacor, Voliam flexi), lamda-cyhalothrin (Warrior), phosmet (Imidan), and spinetoram (Delegate) (Wise et al. 2022).

The first case of recorded pesticide resistance in codling moth was to arsenates in 1928 (Hough 1928). Resistance has since been documented to DDT, nicotinoids, spinosyns, organophosphates, pyrethroids, and carbamate insecticides (Beers et al. 2003, Balasko et al. 2020). This significant increase in resistance has resulted in previously reliable control methods becoming less effective at protecting crops. In addition to the moths developing resistance to key insecticides, stricter chemical regulations have been introduced by EPA and some of the most effective tools have been lost such as azinphos-methyl (Guthion) and chlorpyrifos (Lorsban). Resistance development can be minimized by rotating different chemical sprays (IRAC 2020). The Insecticide Resistance Action Committee (IRAC) has identified 8 modes of action for improved pesticide effectiveness for codling moth control, by making sure a diverse array of these modes of action are being used, resistance build up can be slowed (IRAC 2020).

An alternative to chemical sprays are baculoviruses, a biopesticide. Baculovirus sprays target the young larvae, killing them before they burrow into the fruit. The first *C. pomonella* granulovirus (CpGV) (*Baculoviridae*) was found in Mexico in 1964 (Tanada 1964). It is species specific, making it safe to non-target organisms (Lacey et al. 2005a). There are four formulations of CpGV, the original one from Mexico (CpGv-M), one from Russia (CpGV-R), one from England (CpGV-E) (Berling et al. 2009), and the newest MADEX HP (Certis, Columbia, MD), which can target both codling moth and oriental fruit moth (*Grapholita molesta* Busck Lepidoptera: Tortricidae). Resistance against CpGV-M was reported in Germany in 2007 and then seen in France shortly after (Asser-Kaiser et al. 2007, Schmitt et al. 2013, Zichová et al. 2013). MADEX HP was released in 2013 (certisbio.com). The development of new viruses for the populations that developed resistance has been proven to be successful at overcoming resistance (Berling et al. 2008). Viruses will become more important as pesticide resistance continues to grow. A disadvantage to CpGV is that it is sensitive to solar radiation (Arthurs and Lacey 2004, Lacey et al. 2005b, Lacey et al. 2004) and therefore needs frequent reapplication.

Mating Disruption

Pheromone-mediated mating disruption is an Integrated Pest Management (IPM) method used in over 242,896 ha of apple and pear orchards worldwide (Witzgall et al. 2008). This is a beneficial tool in suppressing pest populations (Witzgall et al. 2010), that uses a female-produced sex pheromone to distract and confuse the active mating wild adults to ultimately suppress the population (Sarfraz 2006). The dispensed pheromone competes with the wild female insects for the attention of the males. By releasing pheromones at high volumes the targeted pest is confused (Shorey et al. 1972, Gaston et al. 1977). This environmentally friendly process is safe for non-target species and is highly compatible with other integrated pest management tactics (Witzgall et al. 2010).

A combination of codlemone ((E-E)-8, 10-dodecadien-1-ol) and other aliphatic alcohols was the first commercially available pheromone dispenser (Isomate-C®) for codling moths and this became available in the US in 1991 (Beers et al. 2003). One method to control codling moth damage is to hand apply sex pheromone dispensers throughout the orchard, and these dispensers will then compete with the female moths for the male moths' attention, leading to a reduction in mating. This is the most commonly used form of disruption in Michigan (Wise et al. 2022), though an alternative form of mating disruption is the use of aerosol emitters, which differ from dispensers as they are deployed at a low density of 1 to 2 per acre. For mating disruption to be successful the orchards need to have a low population of codling moths as well as a reliable monitoring system (Fernández et al. 2010).

Sterile Insect Technique

Sterile Insect Technique (SIT) is the environmentally-friendly process of sterilizing mass reared insects, which are then released into an infested area and compete with the existing wild population for reproduction. This competition results in the wild population being reduced and potentially eradicated. This idea has often been credited to Edward F. Knipling who saw the potential of SIT for many global pests (Knipling 1955). The system works similarly to a mating disruption system in which the released insects are competing for the attention of the wild insects, and mating events do not produce viable eggs, leading to a reduction in the pest population (Lance et al. 2000). Sterilization can occur in three ways: ionizing radiation, genetic modification, or exposure to chemicals (Horner et al. 2016).

SIT was first developed in the US in the 1930's but was not applied on a significant scale until the 1950's on the New World screwworm (*Cochliomyia hominivorax*, Coquerel 1858, Diptera: Calliphoridae) (Knipling 1955, Knipling 1960), a program that is still in operation. The New World screwworm lays eggs in open wounds of warm-blooded animals. In livestock they pupate in the skin, causing myiasis and this eventually can lead to death of the animal if left untreated (Knipling 1960). Initial calculations demonstrated that eradication through SIT could occur in five generations when a 9 to 1 ratio of sterile to wild flies was achieved (Knipling 1960). The initial experiment occurred on Sanibel Island, Florida where the wild population would be less impacted by migration effects. Sterile flies were released at a rate of 100 per square mile per week for three months, within 2 months 80% of the egg masses found on the island's livestock were sterile (Knipling 1960). This experiment was then repeated on the island of Curacao and total eradication of the fly was seen by the fourth generation. The success of this program led to the US making plans to produce sterile flies for a program on mainland Florida (Knipling 1960). Since then, there has been eradication of this species in regions of the USA, Mexico, and parts of Central America (Vargas-Terán et al. 2005). Populations still exist south of Panama in Columbia (Vargas-Terán et al. 2005). This initial success story led to many other effective SIT programs.

Another success story of SIT is the cotton bollworm (*Helicoverpa armigera;* Hübner, Lepidoptera: Noctuidae). It is considered one of the most damaging pests globally, with a current estimated loss of over 3 million dollars a year (Haile et al. 2021, Sharma 2005). As the pest is a global concern there have been many pest management strategies developed, one being SIT. Several attempts at eradication of the cotton bollworm have been made using SIT in the US Virgin Islands (Snow 1971, Laster et al. 1996). Both the first and second trial runs of irradiation were unsuccessful, but they did lead to a much better understanding of how to rear and release sterilized insects. After this, an experiment in North Carolina, USA was able to reduce the population of cotton bollworm by 73.5% (Carpentar and Gross 1993).

Lepidopteran sterilization is environmentally friendly and is an effective suppression tactic that works in many environments as well as alongside on-going pest management strategies (Bloem et al. 2001). Sterile Insect Technique has been developed for codling moths using gamma radiation. Codling moth SIT was originally conceived by Proverbs in 1965 (Thistlewood and Judd 2019) and after several cost-benefit analyses it has been shown that an SIT program would have a positive impact in the British Columbia apple industry (Thistlewood and Judd 2019, Holm 1985, 1986, Jeck and Hansen 1987). However, it wasn't until The Okanagan Valley Sterile Insect Release (SIR) Program in Osoyoos, Canada was launched in 1992 (Dyck et al. 1993) that the method really took off. This program was initially paid for by the Canadian federal and British Columbia governments, equating to 5.33 million USD of funding (Bloem et al. 2005). After 5 years of the program, 91% of the orchards in the treated areas had no detectable level of damage at harvest (Bloem et al. 2005). In addition, there was an 82% decrease in the amount of insecticides used.

With the success of the Canadian program, codling moth SIT has begun to be used outside of Canada with moths being imported to places such as the United States, Brazil, New Zealand, South Africa and Italy (Kovaleski and Mumford 2007, Horner et al. 2016, Bloem et al. 2010, Preti et al. 2021). Previous work has shown no reduction in codling moth flight ability or mating ability after 72 hours in shipment (Bloem et al. 1999, Carpenter et al. 2013). Male moths shipped from Canada to South Africa were equally attracted to the female moths from Canada and the ones found in South Africa, showing that the moths from Canada were compatible to a brand-new environment (Bloem 2009). This is important to understand since the successful eradication of codling moths from Brazil had an economic benefit value of 100 million dollars (Kovaleski and Mumford 2007).

Release Methods

In order to have a successful SIT program it is important to understand how to release the moths. The two most important things to understand is what quantity of moths to release as well as the mechanism in which you are releasing them.

Rates. The quantity of moths released is important to understand before setting up a new SIT program, because releasing too many or too few moths will lead to either management failure or by exceeding the cost-benefit point. There are two main ideas behind calculating the rate of moths to release, first is an overflooding ratio and the second is a simple per hectare rate.

An overflooding ratio is the ratio of sterilized insects to wild insects in a population and it is important to understand these ratios in order to calculate at what ratio the population will be eradicated. Overflooding ratios are calculated based on field experience and computer modeling of population growth and decay. There are many factors that influence this overflooding ratio including but not limited to: migration, fecundity, fertility, weather, and the desired timeline for eradication (Bloem and Bloem 1996). For codling moths an overflooding ratio of 40:1 sterile:wild moths has been adopted in most SIT programs (Dyck et al. 1993). This 40:1 ratio was determined in experiments evaluating the sterile and wild codling moth catch from sex pheromone traps (Dyck et al. 1993).

The idea of overflooding ratios was first explored using mathematical models (Knipling 1970). Later it was expanded on by Proverbs et al. (1982) who calculated that based on average apple and pear yields, a minimum 20% mortality of overwintering larvae, and an initial 5% damage at harvest, that a release of 1,000,000 moths would achieve the targeted overflooding ratio of 40:1 in the 2600 hectares of land the program was covering.

Another release method utilizes 2000 moths (1:1 male:female) per hectare released once a week (Nelson 2021) or ca. 46 grams of moths (~1600 individuals) released per hectare (Bloem et al. 2004, Bloem et al. 2001). This method of release does not require any mathematical equations to assume the wild population and the release rate is kept consistent throughout a season and then supported with other pest management strategies such as sprays.

Method of Release. The most common methods used to release SIT codling moths are hand applications, unmanned aerial vehicles, self-releasing, and all-terrain vehicles. Giving adequate consideration to the method of moth release is important because it can affect the moth's competitiveness with the wild population. Additionally, the release method has a significant impact on the scalability of SIT for the growers operating at different spatial scales. Smaller growers may be less able to afford mechanized releases that reduce labor but increase the evenness of distribution of the moths throughout an orchard while larger growers are able to benefit from it as the costs become more scalable. It is important to understand how each method is performed and how it affects moth distribution through an orchard.

Hand-applied moths have been the most common release method used. Hand releasing is done by gently tossing the moths into the top 1/3rd of the tree after a 10 or so minute period that allows the moths to warm. This warming period is often rushed by placing the moths between warm hands or on the warmed hood of a car. It is labor intensive when looking for an even dispersion of the moths throughout the orchard; however, it does not require any special equipment and is therefore one of the cheapest options for releases.

Unmanned aerial vehicles (UAVs) allow for moth releases to be done quickly without the operator even entering the orchard. A fixed rotary-winged aircraft modified with a custom designed release mechanism that stores and disperses the moths has been used since 2016 in Washington (Esch et al. 2021). The use of a UAV also enables more even distribution of the moths through the orchard as it is able to fly across the entire area and release in even quantities. However, UAV require training and licensing to operate and therefore can be quite expensive. This could potentially make the use of SIT codling moths inaccessible for many smaller growers, although a crop consulting company could provide this service.

Self-releasing the moths involves placing the moths in a container such as a paper bag or delta trap that will allow them to acclimate to their environment before self-dispersing through the orchard. It is unknown as to how evenly the moths can distribute after this release method. Allowing the moths to self-release could be the least labor-intensive option of release method as release sites are more limited, however in larger orchards the labor could become equivalent to the other methods. This is another affordable option for growers, although there are concerns that this method would increase the rate of predation on the released codling moths.

The final method that is often used for moth releases is distribution from All Terrain Vehicles such as 4 wheelers, motorcycles with sidecars, and sometimes trucks. In British Columbia, 4 wheelers are used to distribute moths through orchards by blowing them across the orchard floor under the tree canopies with the use of a fan powered automated system (Proverbs et al. 1982, Bloem et al. 2004). Motorcycles with sidecars have also been utilized for ground releases as they were the most cost-effective vehicles (Jeck and Hansen 1987). This system is swift and allows for even dispersal throughout the orchard. However, concerns are raised about increased moth mortality rates due to them being open to predators on the floor.

Michigan has 657 apple farms of 775 farms that are below 100 acres, with 357 of those being 9 acres or less (NASS USDA 2017). In these small orchards, hand applied or self release methods are more likely to be used compared to an areawide method that can utilize ATVs or UAVs at a cheaper cost per acre. It is currently unknown whether hand applied or self released is better than these other methods.

Monitoring Strategies

In IPM programs, one of the most important strategies to use is a monitoring system to allow for detection of the pest population. There are many ways in which codling moths can be monitored to help understand population dynamics. These include sex pheromone traps, kairomone traps, food-baited and light baited traps. Traps are typically attached to a pole (plastic or bamboo) that is able to reach into the mid-canopy (Wise et al. 2022). At the top of the pole there is a Triangle, Wing or Diamond trap that has a sticky liner placed inside to catch the moths. These sticky liners should be checked weekly and replaced once the liner is either dirty or every 6 weeks (Wise et al. 2022). Two inch sewing pins are often used to keep the chemical lures in place inside the trap. There should be at least 1 trap for every 5 to 8 acres (Wise et al. 2022).

Lures

Lures are a critical component in codling moth trapping systems. Using an effective and reliable lure enables accurate monitoring of codling moth populations. The most prominent codling moth lures contain the codling moth sex pheromone, codlemone ((E-E)-8, 10-dodecadien-1-ol). Codlemone is commonly used in mating disruption, attract-and-kill, as well as population monitoring (Roelofs et al. 1971, Vakenti and Madsen 1973, El-Sayed 2006, Witzgall et al. 2008). A commonly used lure containing this sex pheromone is the long-life lure (L2) (Trécé, Adair, Oklahoma), and it specifically targets the male codling moth population. Over the last two decades, consideration has been given to whether catching the female proportion of the population would be more beneficial to estimating population numbers.

This consideration resulted in the development of "combo" lures consisting of a blend of codlemone with other semiochemicals. One of the most common semiochemicals used is pear ester, ethyl (E,Z)-2,4-decadienoate, which a primary odorant found in ripe pears which attracts both male and female moths (Thwaite et al. 2004, Light et al. 2001). The Codling Moth Dual Action lure (CMDA) (Trécé, Adair, Oklahoma) is made of equal parts of pear ester and codlemone, which has been seen to increase catches (Knight et al. 2005). Despite the increased catch the CMDA lure still attracts <10% of females in pome fruits, a relatively small amount when the sex ratio of a codling moth population is 50:50 male: female (Hawkins and Hilton 2008).

To make this lure more attractive to female moths acetic acid was added, and this addition of 10 ml of glacial acetic acid made the lure 200% more attractive (Landolt et al. 2007). Acetic acid is a microbial fermentation product from sugar baits used for monitoring and attract-and-kill traps for many types of insects (Utrio and Eriksson 1977). The addition of acetic acid to a pear ester amplifies the response to its odors and can lead to an increase in both male and female moth catch (Landolt et al. 2007). However acetic acid alone has been found to be no more attractive than an unbaited trap (Landolt et al. 2007).

An additional combo lure for codling moth is a 4-component (4K) lure that contains pear ester, dimethyl nonatriene (DMDT, (E)-4,8,dimethyl-1,3,7-nonatriene), acetic acid, and pyranoid linalool oxide (6-ethenyl-2,2,6-trimethyloxan-3-ol) (Trécé, Adair, Oklahoma). Pear ester, DMDT, and pyranoid linalool oxide (pyrLOX) are all plant kairomones. This kairomone based lure has been reported to be highly attractive to female moths and females can make up more than 60% of captures (Knight et al. 2019). Geographical disparities between lure effectiveness have been observed between Washington and Italy. In particular, the 4K lure caught significantly more females in Washington when compared to other lures; however in Italy all lures caught females at an equal proportion (Preti et al. 2021a). Even though these lures are designed to attract female moths, apple growers in Michigan have reported low female catch (Larry Gut, personal communication).

Thesis objectives

The aim of my research is to understand how Sterile Insect Releases fit into Michigan codling moth IPM programs where growers would use this tool at the farm-scale, rather than as an area-wide program. My first objective was to determine the effectiveness of different lures to monitor male and female SIR codling moth in Michigan and compare this to lure performance in Washington apple orchards. My second objective was to determine the optimal release strategies for SIR codling moths for multiple scales of Michigan apple orchards, focused on comparing release timings and release methods.

CHAPTER 2: Evaluation of regional and lure effects on recapture of codling moth

Introduction

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is the most economically detrimental pest of apples in the US. Washington accounts for 65.8% and Michigan grows about 7% of all US apple production (USDA NASS Census 2017). Codling moth is also a direct pest of pear, walnut, quince, loquat, and hawthorn. Codling moths are native to Southeastern Europe but this species is now found across the globe (Crosby and Slingerland 1914). The earliest record of codling moth in North America was in 1750 in New England (Crosby and Slingerland 1914). From this introduction, codling moth quickly spread across North American and is now found everywhere apples are grown. Broad spectrum insecticides were historically required to prevent fruit loss to this pest (Gut and Brunner 1998). Beginning in the early 1990s, management of codling moth has been focused on integrated pest management, with catches in pheromone-baited monitoring traps used as the foundation for management decisions (Thomson 2001).

To effectively trap moths, lures were developed that contain the correct blend of pheromone to attract the male moths. The identification (Roelofs et al. 1971) and later synthesis of codlemone (E8, E10-dodecadienol) opened the door to using pheromone baited sticky straps as a monitoring tool (Roelofs et al. 1971, Vakenti and Madsen 1973, El-Sayed 2006, Witzgall et al. 2008). Over the years lures have been improved and refined by changing the pheromone blend, the volume per dispenser, and by the addition of plant volatiles and other attractive volatiles (Fernádez et al. 2010, Preti et al. 2021b, Knight et al. 2005).

Synthetic pheromone is also used to disrupt mate finding. In 1991, ISOMATE®-C (Pacific Biocontrol Crop., Ridgefield, WA) became the first commercial product registered for the control of codling moths through the use of mating disruption. Later pheromones became available in aerosol emitters and a sprayable formulation that releases large amounts of pheromone (Gut and Brunner 1998. In orchards with mating disruption, lures must be able to attract moths against this background of competitive sources in order to get an accurate representation of the population.

Currently there are more than 10 different lures available for attracting codling moths. One of the first, and still most commonly used lures is the L2 (Long-life) (Trécé, Adair, Oklahoma). Loaded into gray rubber septa, the L2 lure is a codlemone ((E-E)-8, 10-dodecadien-1-ol) only lure, used mainly for monitoring codling moth in orchards without mating disruption. As these lures contain only the female produced codlemone, they catch only males. The ability to catch females could potentially improve pest management decisions, while also removing females from the system before they lay eggs.

More recently, a variety of combo lures have been developed by blending codlemone with a variety of additional semiochemicals. Pear ester (ethyl (E,Z)-2,4-decadienoate), the primary odorant found in ripe pears, has been shown to attract both male and female codling moths in Washington (Light et al. 2001), and Australia (Thwaite et al. 2004) however it showed a slight male bias. Codlemone when combined with pear ester and loaded into a gray septa to create a combination lure, named the Codling Moth Dual Action (CMDA) lure (Trécé, Adair, Oklahoma), is designed to catch under mating disruption. Knight later suggested that female catch in traps, using this new lure, was better correlated with the first egg hatch than was the male catch alone (Knight and Light 2005a, b). The CMDA lure attracts <10% of females in pome fruits (Hawkins and Hilton 2008). When acetic acid (AA) is added to this lure, it becomes 200% more attractive to female moths, than a pear ester lure alone in field experiments (Landolt et al. 2007).

Acetic acid is a microbial fermentation product from sugar baits used for monitoring and attract-and-kill traps for many types of insects (Utrio and Eriksson 1977). The odors from ripening fruits such as pear ester are amplified by acetic acid and adding it to lures can lead to an increase in both male and female moth catch (Landolt et al. 2007). Acetic acid is necessary as a co-lure and is typically formulated into a plastic cup with a membrane (Trécé, Adair, Oklahoma) designed to be hung next to the septa lure.

A recent lure that has been released for codling moth monitoring is a 4-component (4K) lure that contains pear ester, dimethyl nonatriene (DMDT), (E)-4,8,dimethyl-1,3,7-nonatriene), acetic acid, pyranoid linalool oxide (pyrLOX) (6-ethenyl-2,2,6-trimethyloxan-3-ol) (Trécé, Adair, Oklahoma). Pear ester, DMDT, and pyrLOX are all plant kairomones. DMDT is costly to synthesize and when compared to pear ester, it has a short longevity in the field (Knight et al. 2014). These 4K lures are recommended to be used in partnership with an acetic acid lure and are a small black PVC rectangle that, when compared to the gray septa, is said to be longer lasting (Trécé, Adair, Oklahoma, USA).

Geographically, disparities between lure effectiveness have been observed between Washington and Italy. In particular, the 4K lure caught significantly more females in Washington when compared to other lures, however in Italy all lures caught females at an equal proportion (Preti et al. 2021a). In contrast, in Washington USA, over 60% of captures with the 4K lure were female moths (Knight et al. 2019). From a review of currently available lures, there is not one lure that stands out as the best lure for trapping codling moths, thus a comparison of lures' performance in Michigan orchards is needed.

In British Columbia there has been a government supported area-wide sterile insect release program for codling moths since 1992 (Dyck et al. 1993). This multimillion dollar facility rears

16 million moths per week for release across all the three apple producing provinces of BC. Monitoring is key to the success of the program and effective lures are key to that success (Bloem et al. 2007). These sterilized moths are not only a control tactic for population suppression but also a powerful tool to allow for experimental testing of monitoring strategies without relying on an unknown wild population.

The goal of this study was to determine the relative effectiveness of several commercially available pheromone and pheromone/semiochemical based lures to capture male and female codling moths in mating disrupted and non-mating disrupted apple orchards in both Michigan and Washington. With the availability of sterile codling moths, a comparison of performance of these lures in orchards that had populations of sterile moths and the background non-sterile population was also tested.

Materials and Methods

A mark, release, recapture study was conducted in commercial apple orchards in MI and WA in 2020 and 2021 to compare the recapture efficiency of six lures. This study was conducted over two years in Michigan and a single year in Washington. In 2020, the experiment was conducted at three sites near Grand Rapids, Michigan (48.117, -119.69), and seven sites near Brewster, Washington (43.043, -85.709). Between May and August. In 2021, the experiment was repeated in Michigan, at four sites between May and July (Table 2.1).

Lures. The following lures (treatments) were evaluated in this experiment: 1) CMDA, 2) CMDA + AA, 3) CMDA + five AA, 4) CML2, 5) 4K, and 6) 4K plus AA (Trécé, Adair, Oklahoma). Six Pherocon® delta traps (Trécé, Adair, Oklahoma) were placed 30 m away from a central release point in six equidistant points within each plot (Figure 2.1). Traps were numbered and then hung in the mid-canopy using a 1.8 m long bamboo pole. The rubber septa lures were hung near the top center of the delta trap with a 2.5 cm sewing pin (Michaels, Irving, Texas), while the 4K and 4K + AA lures were suspended from a manufacturer supplied hook-on double-sided tape in the same general location as the other lures. Lures were replaced every six weeks and lures were randomly rotated among trapping locations each week of the study. Traps were checked weekly and sticky bottoms changed at each collection.

Orchards. In 2020, the experiment was replicated across 1 site with 3 repeated blocks over 9 weeks in Michigan and 1 site with 7 blocks over 4 weeks in Washington respectively (Table 2.1). In 2021 the experiment was replicated in Michigan across 1 site with 4 blocks over 5 weeks (Table 2.1). In both years and in both states experimental plots were 0.4 Hectare located in the center of the orchard with at least 150 m buffer to orchard edges. Michigan based orchards for both years were operated with conventional management as well as no mating disruption programs. Washington based orchards were operated with conventional management but had a 0.5x rate aerosol emitter mating disruption program running.

Codling moth sourcing and handling. Sterilized codling moths were purchased from the Okanagan-Kootenay Sterile Release Insect Release Program in Osooyos, British Columbia, Canada. Moths (50:50 Male:Female) were shipped in 60mm Petri dishes (800 moths per Petri dish) placed into an insulated cooler with ice packs. A box of 100 Petri dishes was shipped weekly to both Michigan State University, East Lansing, Michigan and Brewster, Washington. Once the moths arrived, they were transported to the release sites in the same insulated shipping cooler. If the moths could not be released on the same day, they were stored in a refrigerator at 3.8 °C overnight for release the following day. Moths were marked using Fire Orange™, Aurora Pink®, Horizon Blue™, or Signal Green™ powder Eco DayGlo pigment (DayGlo, Cleveland, OH, USA) for different release sites to minimize potential crossover among release sites. Moths were dyed in groups of 4000 by placing them in a 500 ml cup with 21 g of the DayGlo powder and gently rotating them within the cup for 30-60 seconds.

Treatments were evaluated using the release of the marked sterile moths described previously (4000 per week). Moths were released in the center of the plot, and the release point consisted of a 21 cm x 15.25 cm x 34.93 cm paper bag (Gordon Food Services, Wyoming, MI, USA) that was stapled to the tree trunk at the height of 1.5 m.

Data Collection. Once a week, sticky liners were transported back to the lab to be evaluated with the use of a 12 LED 395 nm UV flashlight (Morpilot, CA, USA). The number of sterile males, sterile females, wild males, and wild females were recorded. In cases where marked moths from different experiments arrived in traps (indicated by DayGlo powder), termed bycatch, that catch data was noted but not analyzed. Released sterile moths could be distinguished from wild moths because larvae are fed a diet dyed with calico red that turns an adult's abdomen red.

Statistical Analysis. All data analysis was conducted in R-studio (Version 1.2.5001). Data was analyzed using an GLM model followed by an ANOVA and a Tukey's means comparison. Data was removed if the average capture across all replications was less than 1 moth or if the average temperature from 8 to 10 pm was less than 15.5 C on 50% or more of the dates. Codling moths have been shown not to fly when temperatures drop below 15.5 C (Rothwell and Gut 2013).

Results

Michigan. The number of sterile males released and the lure type in Michigan in 2020 did not affect the number of moths caught in traps (F = 0.73, *df* = 5, 145, *p* = 0.60 Figure 2.2). The number of both sterile and wild females in 2020 was similar across all lures (F = 1.310, *df* = 5, 141, *p* = 0.26 and F = 1.062, *df* = 5, 139, *p* = 0.38, respectively). Wild male moth catch was also similar across all lures (F = 1.462, *df* = 5, 141, *p* = 0.20).

By lure type, sterile male, sterile female, and wild male capture in Michigan in 2021 were all similar for type of moth captured (sterile v. wild) and lure type (Sterile male: F = 1.26, *df* = 5, 114, *p* = 0.28; Sterile female: F = 0.995, *df* = 5, 114, *p* = 0.424; Wild male: F = 0.62, *df* = 5, 114, *p* = 0.68; Figure 2.5). Wild female catch is not shown due to the low number of moths captured.

Washington. In Washington in 2020, significant difference was detected in the sterile male moth catch among lure treatments (F = 2.675, df = 5, 60, p = 0.03). Despite this, there was no significant difference between the direct comparison of lures when running the linear hypothesis. The number of sterile females per trap in Washington was significantly higher than Michigan female catch (F = 2.7766, df = 5, 60, p = 0.02). The 4K + AA lure caught 40% more moths than the L2 lure (z = -3.062, p = 0.02; Figure 2.5). Wild catch was not sexed in Washington due to limited catch. There was no significant difference in wild moth capture among lures (F = 1.08, df = 5, 60, p = 0.38).

Tables and Figures

Table 2.1. Site specific information of Year 1 and 2 including apple varieties, tree spacing and density, and mating disruption. All sites were under conventional management. High density plots consisted of trees being planted less than 5 feet apart from each other while low tree density had trees planted above 5 feet away from each other.

Year	**Site Location**	**Weeks of Data Collection**	**Plot Name**	**Apple Varieties**	**Tree Density**	**Mating Disruption**
Year 1 (2020)	Grand Rapids, Michigan	June - August	1A	Ida Red, Jonathan	Low	Not Present
			1B	Rome, Ida Red	Low	Not Present
			1C	Fuji, Jonagold	High	Not Present
Year 2 (2021)	Grand Rapids, Michigan	Mid-May to Mid-June	3A	Jonagold, Honey Crisp, Gala	High	Not Present
			3B	Jonagold, Honey Crisp, Gala	High	Not Present
			3C	Jonagold, Honey Crisp, Gala	High	Not Present
			3D	Jonagold, Honey Crisp, Gala	High	Not Present

Figure 2.1. Diagram of a single plot for codling moth sterile release; black lines represent rows of apple trees, the R represents the release point (where 4000 moths were released), and the numbered circles represent the pheromone traps. Moths were placed in a paper bag and attached to tree trunks for release.

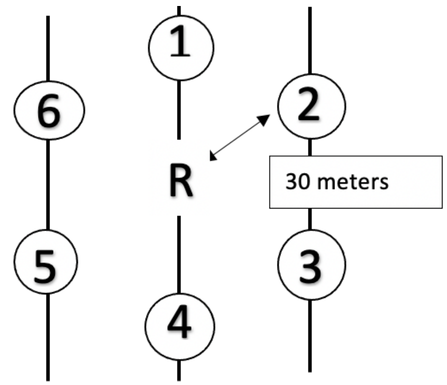


Figure 2.2. Mean (±SEM) weekly catch of sterile male moths, sterile female moths, wild male moths, and wild female moths per lure (L2, CMDA, CMDA + AA, CMDA + 5AA, 4K, 4K + AA) in Michigan between June and August 2020. Traps were placed in apple orchards with conventional management and no mating disruption.

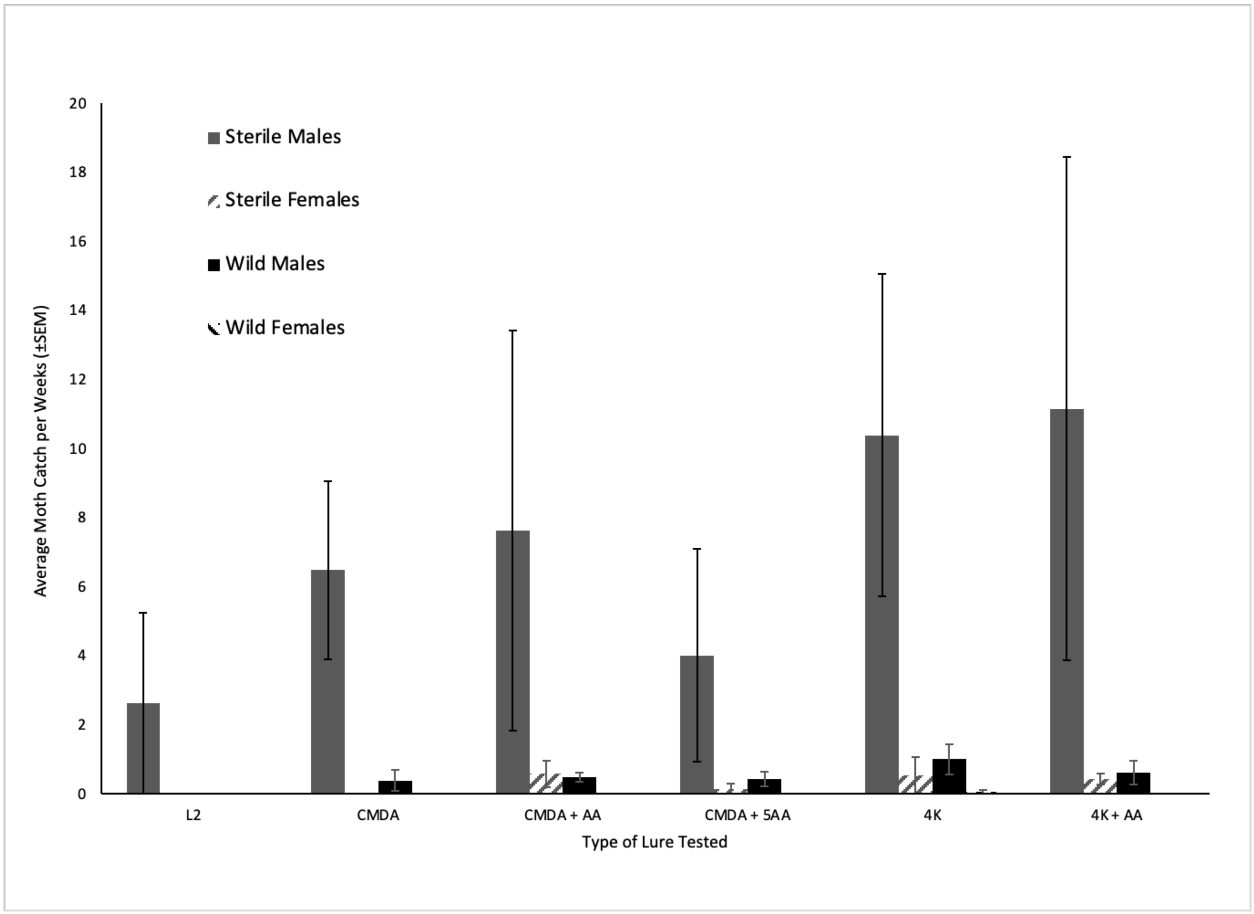


Figure 2.3. Average weekly catch of sterile male moth capture across time in Michigan in 2020 per lure (L2, CMDA, CMDA + AA, CMDA + 5AA, 4K, 4K + AA) with ±SEM error bars. Traps were placed in apple orchards with conventional management and no mating disruption.

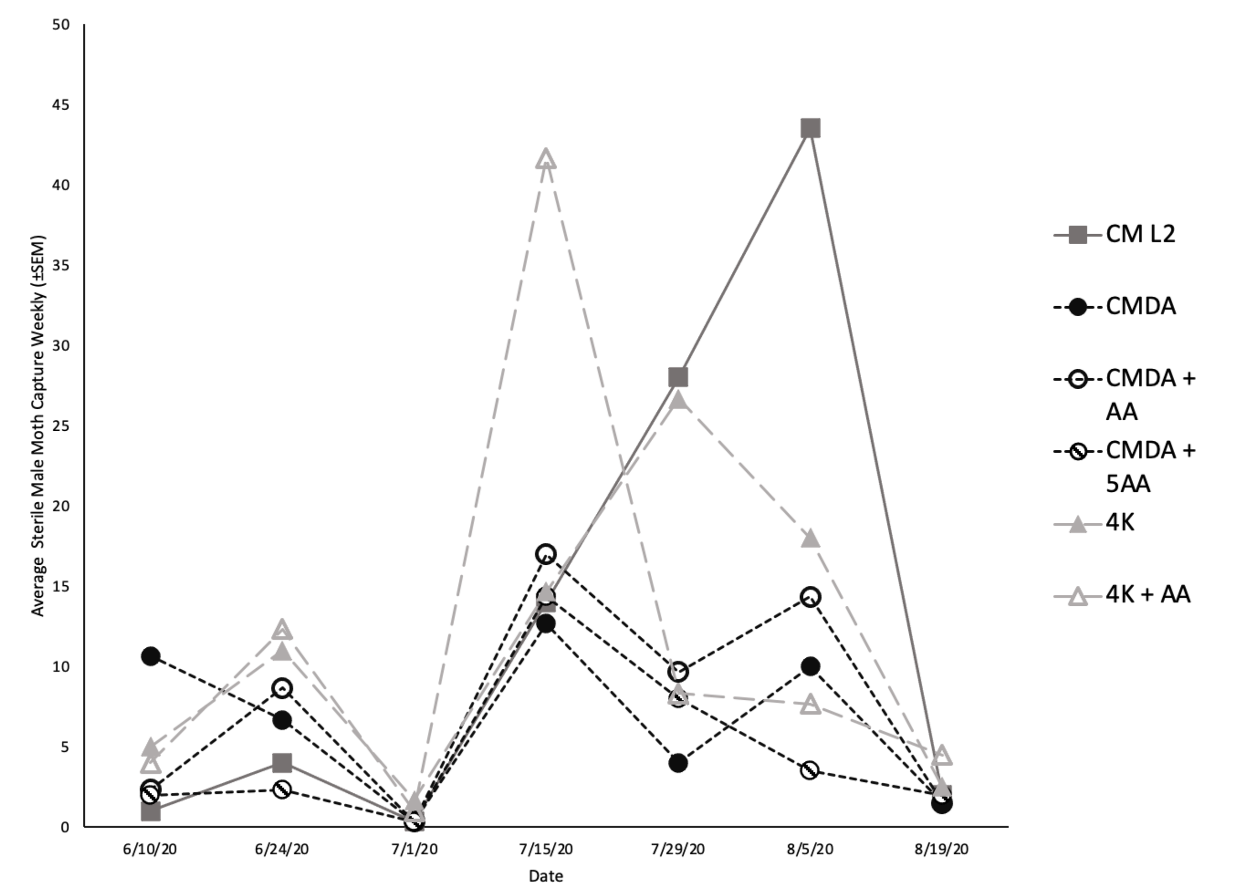


Figure 2.4. Average weekly catch of sterile male moths, sterile female moths, wild male moths, and wild female moths per lure (L2, CMDA, CMDA + AA, CMDA + 5AA, 4K, 4K + AA) in Michigan in May through June 2021 with ±SEM Error Bars. Traps were in orchards with conventional management and no mating disruption.

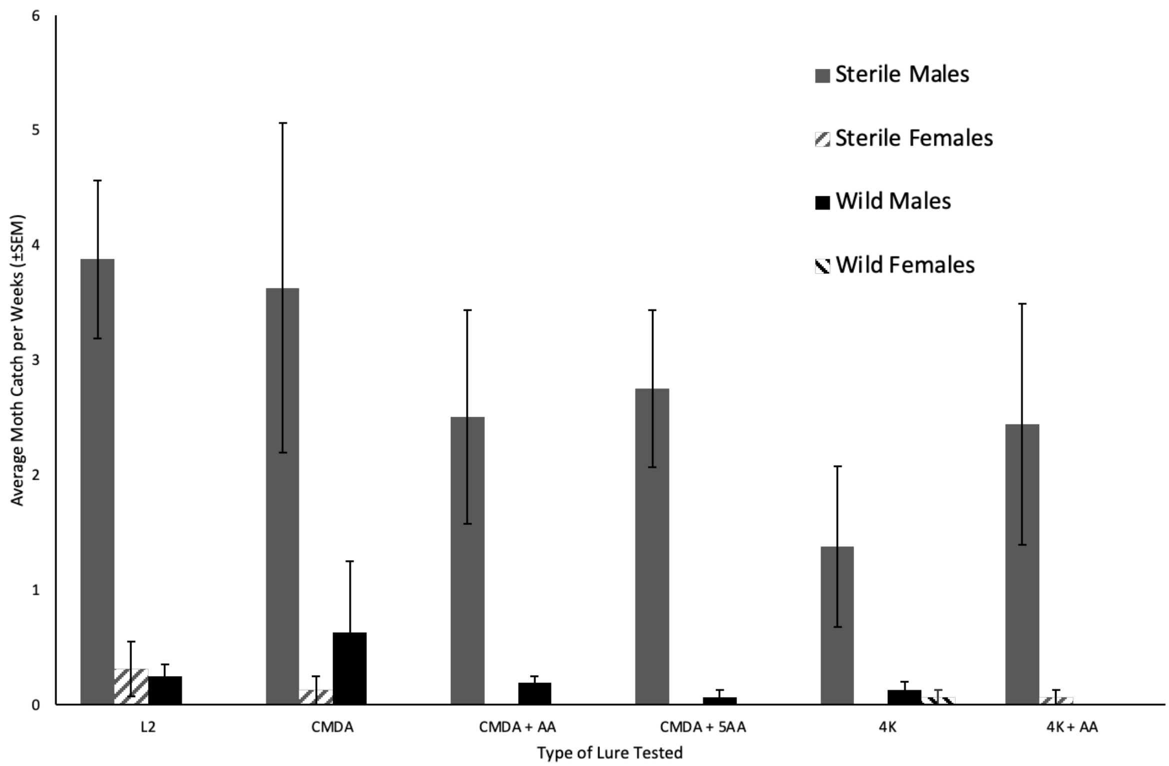
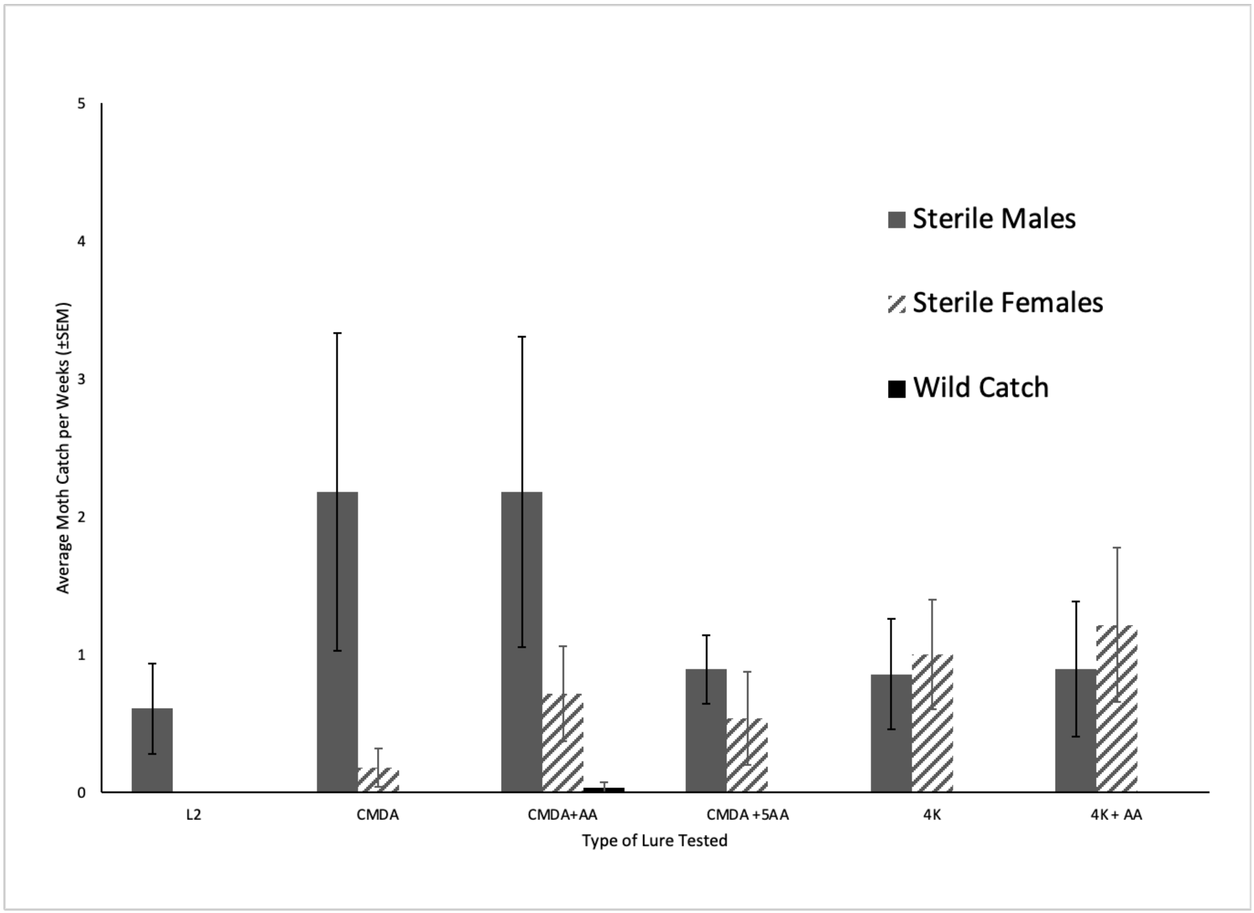


Figure 2.5. Average weekly catch of sterile male moths, sterile female moths, wild male moths, and wild female moths per lure (L2, CMDA, CMDA + AA, CMDA + 5AA, 4K, 4K + AA) in Washington in June through August 2020 with ±SEM Error Bars. Traps were in apple orchards with conventional management and 0.5x rate aerosol emitter mating disruption**.**



Discussion

This study evaluated the effectiveness of various lures for monitoring male and female sterile codling moths released in apple orchards in Michigan and Washington. By using moths of a common phenotype and a known weekly density, this experiment tested whether regional environmental factors affected lure efficacy for male and female codling moth rather than local phenotype or population density. These results suggest that regional environmental factors influence the sex ratio of recaptured moths, as well as total catch, when moth phenotype and density were controlled for.

The ability to catch female moths could potentially improve pest management decisions as this is the sex laying eggs, while the traps would also be removing females from the system. I wanted to compare catch data between WA and MI, looking at various lures, because there have been anecdotal reports of lures designed to catch female moths not catching females in MI and other Eastern US states. In this study I found that indeed, the ratio of male to female catch data was different by region. Results demonstrate that the six lures tested caught similar numbers of female codling moths in Michigan and Washington across both 2020 and 2021, however the sex ratio of males to females was different between the two states.

In previous studies done in Washington the 4K + AA lure has been seen to catch over 60% females (Knight et al. 2019). In Michigan, in both 2020 and 2021, male:female capture with the 4K+AA lure was 22:1 and 11:1 respectively, with virtually no female moths caught for the remaining lures (Figure 2.2 and 2.4). In contrast, in Washington in 2020, while overall captures were low, the 4K + AA lure provided a male:female ratio of 1:1 (Figure 2.5). As both the moth phenotype and lure lot numbers were constant across regions, these data strongly suggest that environment factors, rather than phenotype, drove this discrepancy.

The CMDA lure has been shown to catch less than 10% females of total capture, in Washington (Hawkins and Hilton 2008). These experiments I found similar results, with the CMDA lure catching at a 10:1 male:female ratio in Washington. The addition of acetic acid, the CMDA lure has been shown to improve the female capture by 200% (Landolt et al. 2007). In this research I saw that the ratio of male to female increased to 10:4 with the addition of acetic acid, which is a 400% increase in female capture over CMDA without acetic acid. In addition, Knight and Light (2005a) reported that CMDA lures caught significantly more male codling moths than the standard L2 lure in both mating disrupted and non-mating disrupted orchards in Washington. Our results support these previous findings but indicate that codling moth chemical ecology is regionally variable.

It has been reported that traps baited with acetic acid and either pear ester or DMDT catch significantly more males than traps baited with acetic acid alone (Light et al. 2001). My data revealed that CMDA and CMDA +AA lures catch more male codling moth in Washington, where CMDA and CMDA + AA lures have the highest male capture across the season. In Michigan, in 2020, I saw a trend in catch by lure type, with both the 4K and the 4K + AA catching numerically the highest, followed by the CMDA and CMDA+AA, with the L2 catching the least (Figure 2.2). This trend was not repeated in 2021 (Figure 2.4) The difference between years may have been a result of missing the first part of the season in 2020.

In addition to understanding the sex ratio of these lures I wanted to understand how the different lures perform throughout the season. The seasonal variability in performance has been noted by others as likely due to the limited flight windows available in early spring (Judd and Gardiner 2004). Seasonal variability of lures would influence growers understanding of their wild populations if they are dependent on a single lure. In this study I measured a seasonal influence on catch data in Michigan in 2020. This study has demonstrated that, in Michigan, there is not one lure that is the most effective at catching male moths season long to help make pest management decisions. None of the lures were statistically different from one another, in Michigan in either 2020 or 2021.

In these studies, there was no difference in recapture by lure type in the early part of the season, however after July 1st, where a spray was applied, there was a significant difference between the lures (Figure 2.3). All lures had increased in catch in the second half of the season, with the 4K+AA and the L2 outperforming the rest. The 4K+AA lure immediately caught more starting in July but trailed off going into August. Over time captures in the CM L2 steadily increased until it caught as much as the highest catch in 4K+AA. The L2 lure has been the standard lure for monitoring for male moths, however the catch with L2 are much higher in the later half of the season.

It is important to note the seasonality of lures as the action threshold would have to be changed and adapted throughout the season. Releasing a consistent population of moths for the entire duration of the season, allowing for us to know the population being recapturing, from this it’s seen that catch was highly influenced by something other than population or phenology (Figure 2.3). More experiments need to be done to truly know how these lures affect our understanding of the wild populations where factors such as temperature, humidity, wind, and precipitation are controlled.

This data illustrates the expected low proportion recapture rate (Adams et al. 2016), especially in the early part of the season when first generation control decisions are being made. In the second half of the season, the flight window (period of time when ambient temperatures are above lower flight threshold) is longer and provides more time for male moths to respond to traps.

Figure 3 also shows a spray event of Thiamethoxam around July 1st which gives us a dip in recapture.

Although the Washington experiment was only performed for a few weeks, our intent was to look at lures in different states but under similar conditions, i.e., without mating disruption. The ratio of male to female recapture is consistent with previous studies conducted in this region. The marked differences in male:female recapture ratios in MI provide evidence that there are environmental differences affecting female capture between the two regions. I conclude this because both the phenotype and density of moths were held constant between Washington and Michigan sites.

These data suggest that different lures should be used at different times in the season to gain optimal knowledge of the codling moth population status. This is useful information for allowing growers to make more informed decisions about control tactics and will be important to investigate further in an expanded study. Each lure has its own benefits, and I suggest it is best to use multiple types of lures and train trap checkers on what results from each lure means.

These results falsify the genetic difference hypothesis as I used moths from the same genetic strain, from the BC facility, in both regions. and by region as I was controlling for phenology. Hypotheses that still need to be tested are climatic differences such as temperature, humidity, and wind speed. While regional temperatures looked similar, humidity in Michigan and Washington are considerably different, with there being a 26 point difference in average relative air humidity from April to September between Brewster, Washington and Grand Rapids, Michigan (calculated from, tititudorancea.com).

These data suggest that environmental rather than phenological differences between Michigan and Washington affect the performance of codling moth lures. Results from Washington may not be applicable in less arid growing regions such as Michigan. Given that over 25% of US apple production occurs in the humid Great Lakes region (USDA NASS 2017), research results tested only under arid climates should be interpreted with caution. Beyond climatological differences, there are additional factors that may impact lure efficacy such as background plant volatiles, orchard layout, local geography, and deployment strategies. This research now needs to be repeated in Michigan in orchards that have pheromone mating disruption as well as in other states.

Optimizing lure usage in each region and orchard may be required to accurately monitor codling moth abundance and is therefore an important part of making informed IPM decisions. Researchers and crop consultants need to be aware that different lures catch better at different times of the year and in different regions of the country. I did not find one lure that was superior to the rest, over the entire season. The use of a single lure for the duration of an entire season would not be advisable. I recommend orchard managers rely on multiple lure types when monitoring this important pest.

CHAPTER 3: Evaluation of timing, rate, and release method on sterile codling moth performance

Introduction

Codling moth, *Cydia pomonella*, (L.) (Lepidoptera: Tortricidae) is the most important pest for apple growers across the US. Codling moth also impacts pears, walnuts, and several other crops since it arrived in the US in the 1750s (Crosby and Slingerland 1914; Newcomer and Whitcomb 1924) and growers continue to struggle to control this insect. As the second largest apple producer in the United States, Michigan's 300 million dollar apple industry (NASS USDA 2020) is critically important to the state economy, and finding sustainable pest management solutions has been identified as an industry priority.

To date, conventional broad spectrum synthetic insecticides have been the key control tool for codling moth. Concerns about pesticide resistance in codling moths requires researchers to constantly investigate new methods to help control this key pest (Balasko 2020). Sterile Insect Technique (SIT) is an environmentally friendly control tactic that uses mass reared and sterilized insect pests, to release into an infested area, where they compete with wild populations for mating opportunities. Mating with these released insects result in infertile eggs and ultimately helps reduce the population (Knipling 1955). This technique has been successfully used against cotton bollworm (*Helicoverpa armigera,* (Hübner), Lepidoptera: Noctuidae) as well as New World screwworm (*Cochliomyia hominivorax,* (Coquerel), Diptera: Calliphoridae) and more recently has been applied to codling moths in Canada. In 1992 a government sponsored area-wide SIT program against codling moth was started in Osoyoos, British Columbia, Canada (Dyck et al. 1993). Moths were released throughout the fruit growing regions of the Okanagan valley and after 5 years 91% of the orchards participating had no detectable level of damage at harvest (Bloem et al. 2005).

The success of this program in British Columbia inspired interest in adopting the tool in Argentina (Botto and Glaz 2010), South Africa, (Bloem et al. 2010), New Zealand (Horner et al. 2016), and Washington State U.S.A. (Bloem et al. 2001). This study investigates the use of this control tool in Michigan's apple orchards.

In order for SIT to be beneficial to Michigan growers we need to optimize release rates and strategies to minimize economic costs. The Canadian SIT program benefits from a government support structure and taxes collected from growers in a mandatory participation system comprising the entire industry, which allows them to release moths at ‘full rate’ (2000 moths /ha) over every orchard. In addition, most of the Canadian orchards are contiguous and contained within valleys along the Okanagan and Similkameen rivers. In contrast, Michigan has 775 apple orchards spread across the state, with 657 of these orchards less than 40 ha, and more than half (357) of those being 3.6 ha or less (USDA NASS 2017). It is within this diffuse and complex Michigan landscape, with only voluntary farm-scale participation, that SIT must work in order to be adopted by Michigan growers.

Release Rate. The British Columbia program initially started releasing moths at a targeted rate of 40 sterile moths to 1 wild moth, this overflooding ratio was modeled by Knipling and Proverbs to be enough to eradicate a population of codling moths within a year and half, provided this 40:1 ratio was achieved during the first flight of releases (Knipling 1970, Proverbs et al. 1982).

While eradication was not achieved, populations were quickly reduced to below damage thresholds using this target number. Maintaining this 40:1 ratio proved difficult as the wild population was hard to measure and fluctuated from year to year. Over the years the program has tried releasing high numbers, and other researchers have released as many as 4000 moths per hectare (1:1 male:female) (Nelson 2021, Bloem et al.2004, Bloem et al. 2001). However, these increases in release number still did not achieve eradication. This situation stimulated research to determine whether using this tool as part of an IPM strategy, could reduce moth populations to manageable levels, rather than for eradication. Currently, there is limited information about how many moths are needed to produce good control in managed orchards. In this research, I wanted to see if it would be possible to reduce the rate of moths typically released and still achieve control of damage from wild moths.

Timing of Releases. In addition to the number of moths released it is also important to understand the optimal time to release moths. When the Canadian SIT program began, moths were released all season long, twice per week (Bloem et al. 2007). With their early success and the need to expand the program to more provinces, there was the need to find efficiencies in how sterile moths were allocated. Program managers tried a number of different reduced release strategies including; reduction in the rate released season longs, releases reduced to once per week for the duration of the season, releases only one part of the season, or no releases when there had not been wild catch in monitoring traps for 4 or more years. Program managers were able to make these adjustments and maintain damage below allowed economic thresholds. Over a 6 year period of adjusting the release rates, there was a slight drop in the percent of orchards with no detectable damage (from 88% to 81%) (Bloem et al. 2007). This suggested that efficiencies could be found in how these insects were distributed. Because the Canadian program is supported by grower taxes, reducing rates can be politically challenging. However, releases done in the U.S. at the farm scale will need to be done as cost effectively as possible. With 46% of Michigan's orchards being less than 3.6 ha, it is important to understand potential cost saving measures for smaller operation growers who cannot afford a full scale SIT program (NASS

USDA 2017). With limited research being done on these reduced SIT programs, it is important to see how these programs would affect wild populations of moths in Michigan.

Current Release Strategies. The release process for SIT codling moths can be labor intensive and expensive, accounting for 40% of operational budget (Tan and Tan, 2013). In British Columbia, sterile moths are distributed through the orchard with the use of all-terrain vehicles (ATVs) equipped with custom made automated dispensers that release the moths by blowing them across the orchard floor (Proverbs et al. 1982). Recently, unmanned aerial vehicles (UAVs) have been shown to be the fastest way to release the moths (Moses-Gonzales et al. 2021). However, the cost of the technology is high and requires specially trained pilots, which could make adoption of this tool inaccessible for many smaller growers.

Others have tried to find efficiencies in release strategies. In New Zealand an experiment was done that compared releases on mountain bikes to motor vehicles, unmanned airplanes and unmanned hexacopters. While there were differences in recapture and distribution of moths, they found that bike releases produced better recapture results than ATV releases. In addition to recapture, the paper looked at the spatial distribution of moths, and found that, when released by unmanned aerial systems (UAS), the distribution was uneven from week to week, likely due to changes in wind speed and direction (Lo et al. 2021). While these ATVs and UAVs may provide for a more even distribution of moths throughout an orchard, they are expensive and require trained professionals to operate. Investing in this kind of equipment is not an option for most smaller scale growers. In the case of smaller operations, hand applied releases, while time consuming, is likely the most cost effective method.

Hand releasing moths involves taking chilled moths to the field in coolers and throwing the moths into the trees at mid-canopy, after allowing the moths to warm up. This method is slower than mechanized releases but requires no special equipment or training. In our previous experience with this release method, many moths often end up on the ground if not sufficiently warmed. Another method would be to place moths in paper bags secured to the trees, allowing moths to self release and disperse in their own time. The self release method could speed up the release process, as chilled moths could just be deposited into designated release stations, eliminating the warming up period for workers. Finally, I wanted to test the idea of just releasing moths directly onto the orchard floor. I expected that this method would be the least effective, as chilled moths would be vulnerable to predators such as birds and ants, and considered this a negative control, or worst case scenario for hand releases.

Objectives. The goal of this study was to optimize the release strategy for sterile codling moth release in commercially run apple orchards in Michigan. I compared three key aspects of release 1) different release rates, 2) different timings of release, and 3) different release methods. Release rate was either a) 2000 moths per hectare (full rate) or b) 1000 moths per hectare (half rate). Timing of release was a) all season long, b) first generation only, or c) the second generation only. Release methods were a) allowing moths to self release, b) tossing moths into the trees, or c) tossing moths on the ground. These various rates, timings, and methods were chosen to determine potential opportunities for savings in the application of sterile insect release for farm-scale application.

Materials and Methods

Codling moth sourcing and handling. Gamma radiation sterilized codling moths were purchased from the Okanagan-Kootenay Sterile Insect Release Program in Osoyoos, British Columbia, Canada. Moths were shipped in insulated coolers with ice packs in sets of 100, 60mm petri dishes containing ca. 800 moths per Petri dish (50:50 male:female). The moths were shipped weekly to both Michigan State University, East Lansing, Michigan and M3 agricultural technologies in Mount Pleasant, Michigan. Once the moths arrived, they were transported to the release sites in the same insulated shipping cooler, usually on the same day. If the moths could not be released on the same day, they were stored in a refrigerator at 3.8 °C overnight for release the following day.

Marking moths. For the release methods experiment and 3 plots of the timing experiment, moths were marked using Aurora Pink®, Horizon Blue™, or Signal Green™ powder Eco DayGlo pigment (DayGlo, Cleveland, Ohio) to distinguish between release methods. Moths were dyed in groups of approximately 1333 by placing them in a 500 ml cup with 7 g of the DayGlo powder and gently rotating them within the cup for 30-60 seconds.

Release Rate and Timing Experiment. Releases of sterile moths occurred over 16 weeks at locations near Bear Lake (44.448, -86.229) and Ludington (43.880, -86.378) and for 15 weeks in Potterville (42.632, -84.789) and Flushing (43.025, -83.911) in 2021. All 6 treatments and the control blocks were repeated 3 times at those locations. These treatments were as follows: 1) full rate season long, 2) half rate season long, 3) full rate first generation only, 4) half rate first generation only, 5) full rate second generation, 6) half rate second generation and 7) conventionally managed control blocks. The full rate consisted of 2000 moths (50:50 male:female) per ha per week, while the half rate consisted of 1000 moths (50:50 male:female) per ha per week. First generation releases occurred from May 25th to July 11th of 2021 and second-generation releases occurred from July 12th to August 30th of 2021. Trapping data was collected weekly in all plots for the entire season.

Moths were released with the use of UAV by M3 Agriculture Technologies in Bear Lake and Ludington but by hand in Potterville and Flushing. Hand releases were made by walking down the central row of the plots and releasing moths by tossing them into the tree canopies at approximately 1.8 meters at 4 to 6 points along the orchard row. Orchard plots ranged from 4-8 ha and monitored with 1.5 Pherocon® delta traps/ha (Trécé, Adair, Oklahoma), 50% baited with L2 (Long-life) (Trécé, Adair, Oklahoma, USA) and 50% baited with Codling Moth Dual Action (CMDA) lures (Trécé, Adair, Oklahoma, USA). Traps were placed in the upper third of the canopy on the outside of the tree and lures changed every six weeks to optimize capture (Wise et al. 2022). Moths were dyed using DayGlo powder allowing for us to distinguish between the plots. All but 3 plots were roughly 4 ha squares, the first being 8 ha square plot, the second one a 6 ha square plot and the third being a rectangular 4 ha plot. Quantity of traps and SIT moth release density were adjusted to maintain the treatment ratios in the different sized plots. For the 4 ha rectangular plot I placed one additional L2 and CMDA baited traps to ensure adequate trap coverage. Plot specifics are detailed in Table 3.1.

Release Methods Experiment. I conducted a release method experiment during 2020 and 2021 consisting of three treatments using a mark, release, recapture approach. I evaluated 3 different release methods: 1) releasing by throwing moths into the tree, 2) by scattering them on the ground surrounding the tree, and 3) by placing the moths inside a paper bag allowing them to self-disperse. At each experimental plot 1333 moths were released for each release treatment (a total of 4000 moths were released). Moths were released at a release point in the center of the plot, this point was consistent throughout the entire season. The first release method consisted of using a swift upwards throwing motion of the Petri dish into the canopy of a tree. These moths were dyed green. Moths released on the ground were gently scattered from the petri dish around the base of the tree such that they were delivered in a single layer. The second release method is the simplest to execute as the moths are scattered across the floor around the tree. The scatter is done slowly so that the minimal amounts of moths are laying on top of one and another. These moths were dyed blue. Self released moths were allowed to emerge naturally from a 21 cm x 15.25 cm x 34.93 cm open paper bag (Gordon Food Services, Wyoming, Michigan) stapled to the trunk of the tree at the height of 1.5 m. The moths were then placed inside of the bag., this method is designed to give the moths time to acclimate to their environment before flying. A new bag was stapled to the tree each week to make sure that the bags had not disintegrated due to the natural elements. These moths were dyed pink.

Each plot was 4 ha (40468 m^2) and had a grid of 16 equally distanced traps across it with the release site being in the center. Pherocon VI orange delta traps (Trécé, Adair, Oklahoma) were numbered and then hung in the upper-canopy using a 1.8 m long bamboo pole. Traps were baited with an L2 lure (Trécé, Adair, Oklahoma, USA) that was pinned inside a delta trap with a 2.5 cm sewing pin. The lure was replaced every six weeks. Traps were checked weekly and the sticky liner changed at each collection.

The experiment was replicated at two sites near Grand Rapids, Michigan (48.117, -119.69) and one site near Flushing, Michigan (43.025, -83.911). Sites in Grand Rapids were trellis planted, trees are supported with a framework that is used to train the trees to grow in a more compact manner, while sites in Flushing were free standing, trees are spaced around 3 meters apart from each other and are allowed to grow in a more natural tree shape. Due to the large difference between the planting styles these plots were not analyzed together. In 2020 the experiment was conducted 9 times in a free standing and 14 times in trellised orchards during June and August and in 2021 it was conducted 9 times in a free standing and 7 times in trellised orchard times between May to August. Orchards were under commercial management that did not include mating disruption.

Data Collection. Sticky liners were collected weekly and transported back to the lab to be evaluated with the use of a UV flashlight (Morpilot, CA, USA) which aided in identifying the fluorescent markings. I recorded six categories of moths from traps: sterile males, sterile females, bycatch sterile males, bycatch sterile females, wild males, and wild females. Bycatch is considered sterile moths that came from nearby experiments and were distinguishable as they had not been dyed by one of the three pigments. Sterile moths could be distinguished from wild moths by the red stained internal organs, a result of larvae being fed a diet containing Nile Red dye (Prifti et al. 2014).

Statistical Analysis. All data analysis was conducted in R-studio (Version 1.2.5001). For the timing and rate experiments I ran an ANOVA on the total recaptured moths and treatment across traps in capture for 16 weeks for 3 repetitions of each treatment. For the release methods experiment, orchards in Grand Rapids and Flushing were analyzed separately. I first modeled the data using a generalized linear modeling (R function: *glm*). I conducted hypothesis testing on GLM models using an ANOVA, with a Gaussian model and identity link function. In addition, I analyzed moth recapture over distance. Distances from release points to recapture traps were not always equally distanced in each plot. Traps that were within 5 m from each other were combined into a single data point.

Results

Timing and Release Rate Experiment. In an ANOVA comparing sterile male capture to treatment, I found no significant difference between the treatments for the average capture repeated over 16 weeks (F = 0.634, *df* = 6, 14, *p* = 0.702, Figure 3.1)**.** Figure 3.1 shows the sterile male moth recapture throughout the season for every treatment when looking at recapture across trap and site for all replicates. The graph demonstrates when I was releasing each generational treatment. It is an indicator as to when potential uncontrollable events may have occurred that would have also affected the wild moth capture.

In an ANOVA comparing wild male capture among the treatments, I found no significant difference between the timing and release rate treatments (F = 0.655, *df* = 6, 14, *p* = 0.687, Figure 2). Figure 3.2 shows the wild male moth capture across every trap and site for the entire season. It should be noted that the control recapture of wild male moths is low for the entire duration of the season. Orchard plots with full rate for 1st generation (May 17th through July 5th in this experiment) and full rate season long releases had consistently low catch throughout the season. In the half rate season long treatment, I found that the wild population increased to 8 moths per trap per site on June 28th 2021 however they then reduced to 1 moth per trap per site by August 9th 2021. Applications with the half rate for 1st generation had a population build up to 10 moths per trap per site by June 7th 2021 however it then decreased to 4 moths per trap per site on June 14th 2021, the population then build back up with 7 moths per trap per site being caught on August 2nd 2021. Plots with the full rate for 2nd generation started with an average moth catch of 6 moths per trap per site on May 17th 2021 which then decreased to 1 moth per site per trap on May 31st 2021, this then build back up to 5 moths per trap per site on July 5th 2021 and decreased again to 1 moth per trap per site on August 23rd 2021. Finally, in the half rate for

2nd generation releases started with 6 moths per trap per site on May 31st 2021 which then decreased to 4 moths per trap per site on June 28th 2021 which further decreased to 2 moths per trap per site on August 23rd 2021.

Release Method Experiment. Comparison of releases in free standing orchards that were self, on the ground, or in trees found that they were not significantly affected by distance in an ANOVA test. In the trellised plot I found a significant effect of distance on all the release styles (Self release: $F = 10.64$, $df = 1$, $p = 0.0172$; On ground: $F = 10.76$, $df = 1$, $p = 0.0168$; In tree: $F = 14.06$, $df = 1$, $p = 0.0095$). In 2021 I found no significant relationship between distance and release method in both trellis and free standing orchards in the ANOVA test for sterilized male moth recapture (Figure 3.3).

Figure 3.4 shows the results for the recapture of moths by distance for 2020 and 2021. In both years I saw that in free standing orchards there was a decrease in capture as distance increased with a slight increase in capture after 100 m. In the trellis planted orchards in 2020 there was an increase in capture before and decrease that is consistent for all methods apart from the self released moths which increased to ca. 80 at100 m. In 2021 trellis planted orchards I observed a similar curved slope with a peak being visible in all release methods (Figure 3.4).

Tables and Figures

Table 3.1. Site specific information of all replicates for the timing and release rate experiment including plot location, plot size, number of traps, planting style, the release mechanism used, and mating disruption system.

Timing	Rate	Location	Plot Size	Number of Traps	Planting Style	Release Mechanism	Mating Disruption System
Season Long	Full	Ludington	4 ha	6	Free Standing	UAV	Full Rate NoMate® Spirals
	Half	Ludington	4 ha	6	Free Standing	UAV	Full Rate NoMate® Spirals
1st generation	Full	Ludington	6 ha	6	Free Standing	UAV	Full Rate NoMate® Spirals
	Half	Ludington	4 ha	6	Free Standing	UAV	Full Rate NoMate® Spirals
2nd generation	Full	Ludington	4 ha	6	Free Standing	UAV	Full Rate NoMate® Spirals
	Half	Ludington	4 ha	8	Free Standing	UAV	Full Rate NoMate® Spirals
Control	Control	Ludington	4 ha	6	Free Standing	UAV	Full Rate NoMate® Spirals

Table 3.1. (Cont.)

Season Long	Full	Bear Lake	4 ha	6	Free Standing	UAV	No Program
	Half	Bear Lake	8 ha	12	Free Standing	UAV	No Program
1st generation	Full	Ludington	4 ha	6	Free Standing	UAV	No Program
	Half	Ludington	4 ha	6	Free Standing	UAV	No Program
2nd generation	Full	Ludington	4 ha	6	Free Standing	UAV	No Program
	Half	Bear Lake	4 ha	6	Trellised	UAV	No Program
Control	Control	Bear Lake	4 ha	6	Trellised	UAV	No Program
Season Long	Full	Flushing	4 ha	6	Trellised	Hand	Half Rate Isomate Twin Tube Combo
	Half	Potterville	4 ha	6	Free Standing	Hand	Isomate® Twin Tube CM
1st generation	Full	Flushing	4 ha	6	Trellised	Hand	Half Rate Isomate Twin Tube Combo
	Half	Flushing	4 ha	6	Free Standing	Hand	Half Rate Isomate Twin Tube Combo

Table 3.1. (Cont.)

2nd generation	Full	Flushing	4 ha	6	Trellised	Hand	Half Rate Isomate Twin Tube Combo
	Half	Flushing	4 ha	6	Free Standing	Hand	Half Rate Isomate Twin Tube Combo
Control	Control	Flushing	4 ha	6	Trellised	Hand	Half Rate Isomate Twin Tube Combo

Figure 3.1. Mean catch of sterile male codling moths per trap in plots where sterile moths were released at the following rates and timings between May and August of 2021: full rate season long, half rate season long, full rate 1st generation, half rate 1st generation, full rate 2nd generation, half rate 2nd generation, and no sterile moths released (control).

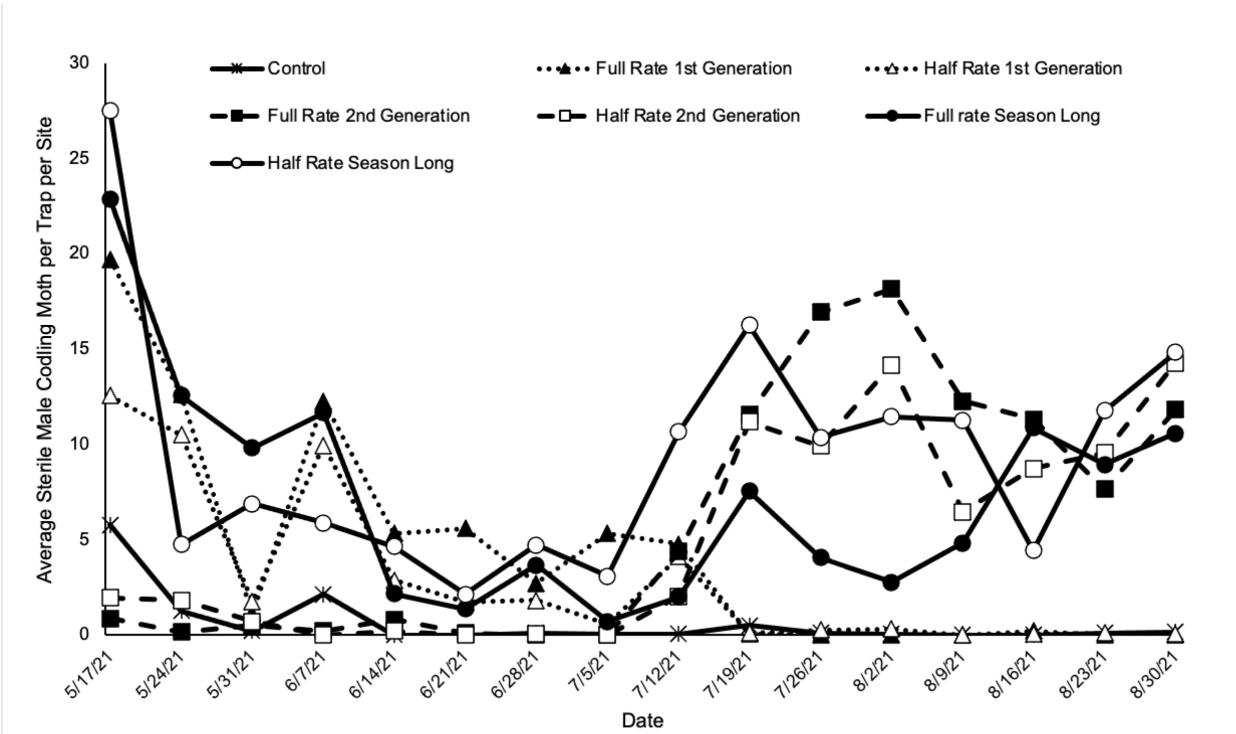


Figure 3.2. Mean catch of wild male codling moths per trap in plots where sterile moths were released at the following rates and timings between May and August of 2021: full rate season long, half rate season long, full rate 1st generation, half rate 1st generation, full rate 2nd generation, half rate 2nd generation, and no sterile moths released (control).

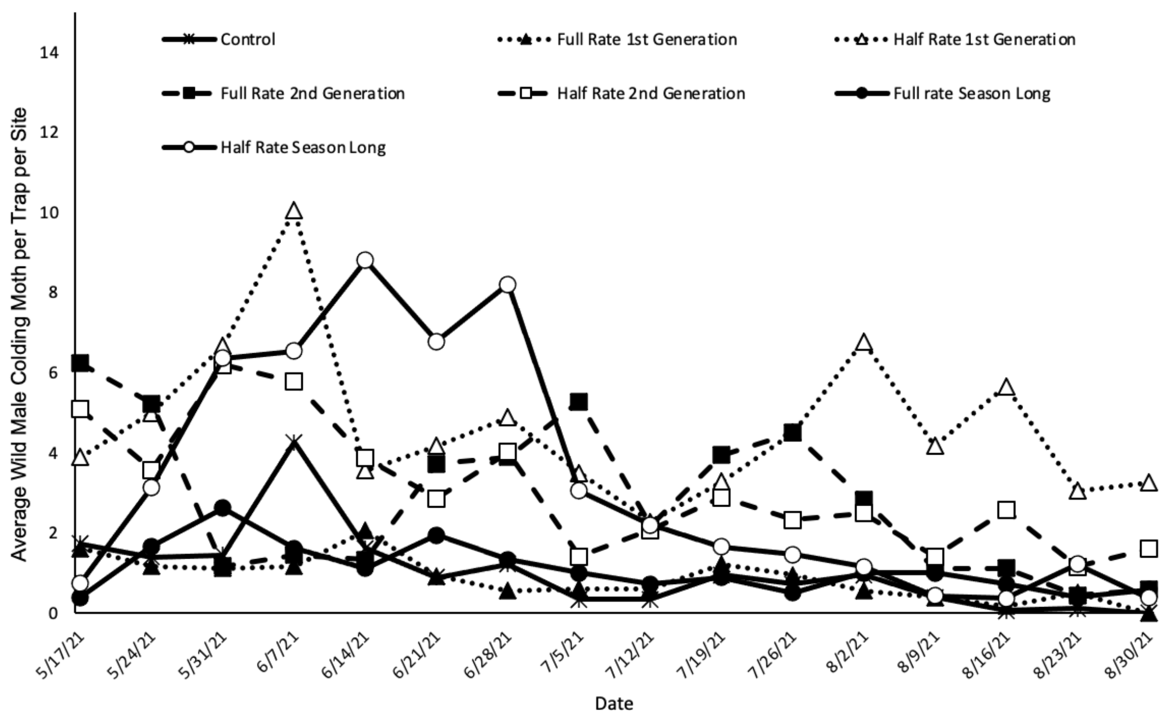


Figure 3.3. Comparison of sterile codling moth release methods in Flushing, Michigan versus Grand Rapids, Michigan orchards in 2020 and 2021. Mean (±SEM) number of sterile moths caught per trap each week when released in trees, on the ground, or from a bag.

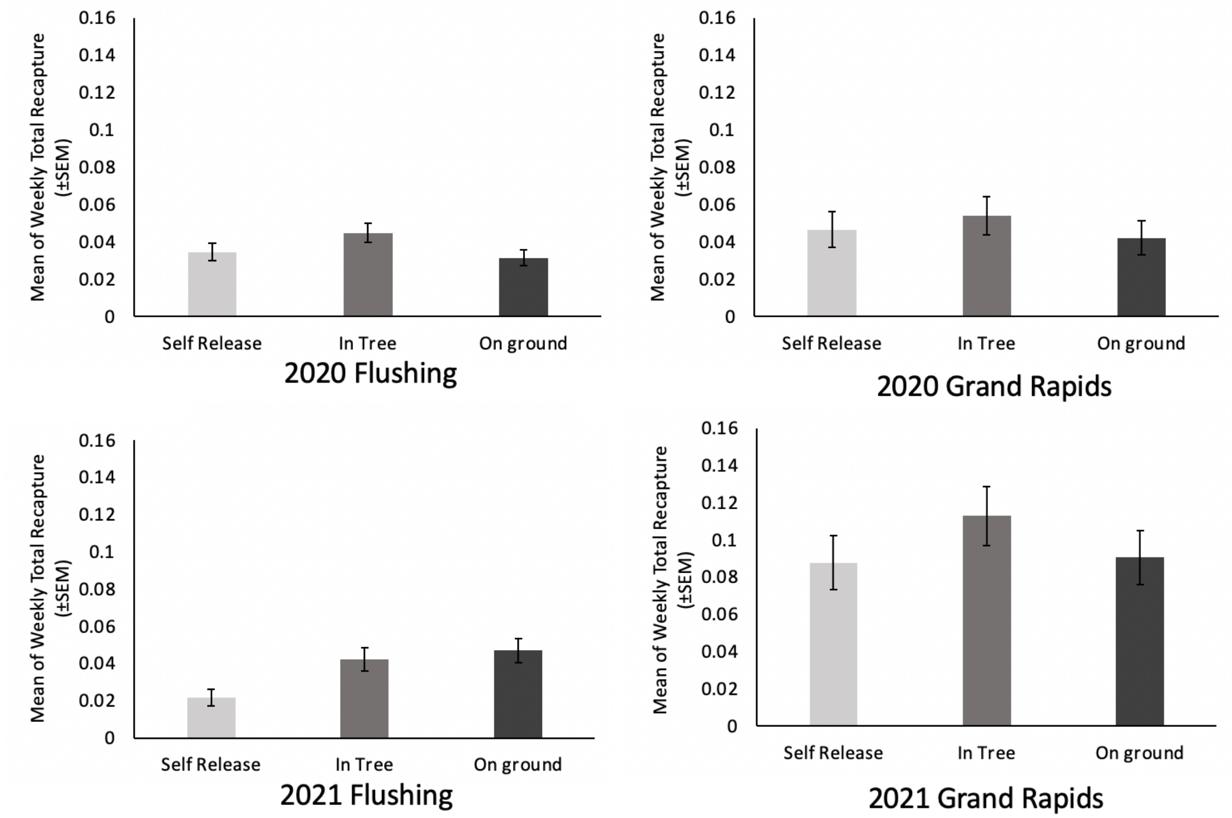
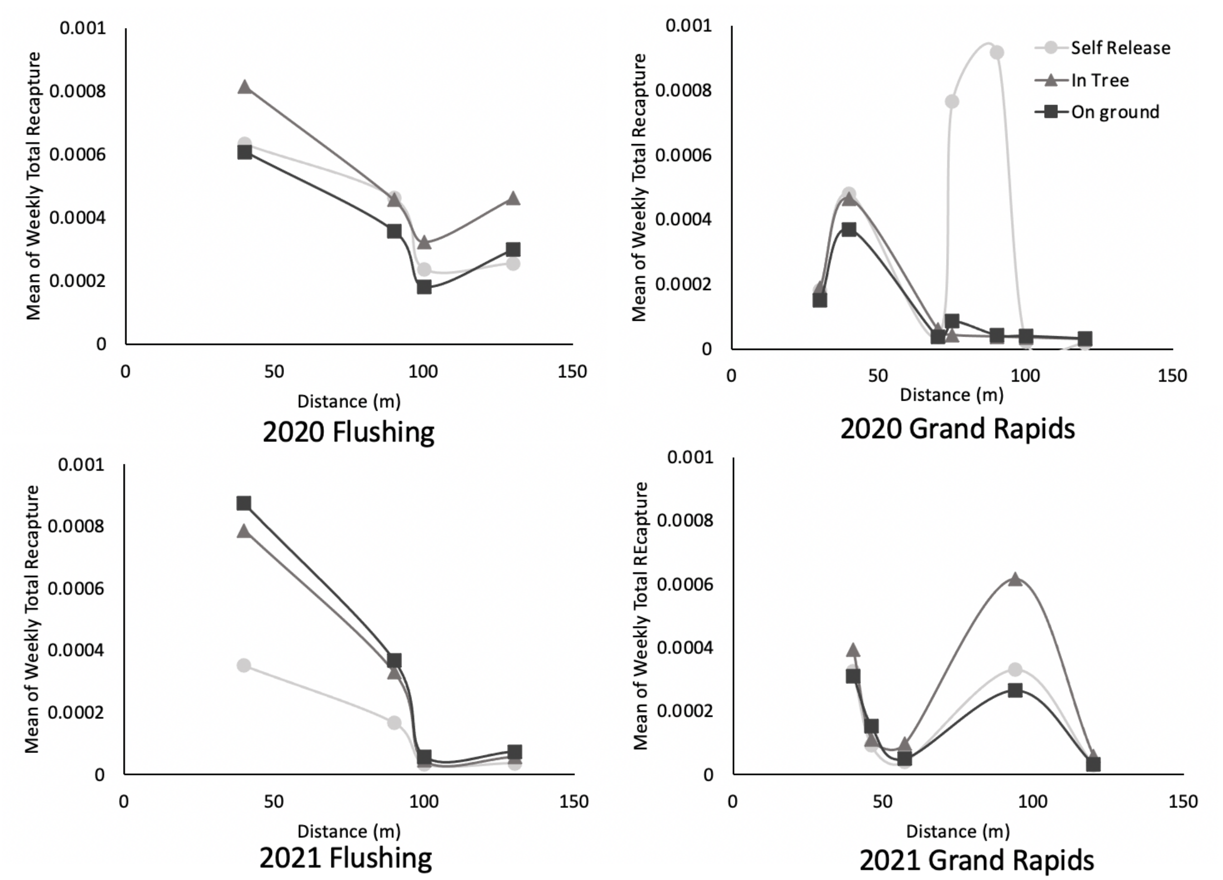


Figure 3.4. Comparison of distance recaptured of sterile codling moth release methods in Flushing and Grand Rapids, Michigan in 2020 and 2021. Mean recaptured at each trap distance for the duration of the experiment.



Discussion

The experiments performed in this chapter were intended to help understand how SIT could be used in Michigan apple orchards on a farm scale. By testing multiple rates and timing of the SIT moth releases we looked specifically at potential savings for Michigan growers. I also looked at how different release methods could impact moth dispersion throughout the orchard as well as recapture rates of the different release methods.

Release Rate and Timing Experiment. In the release rate and timing experiment, there was no difference seen between the treatments, this is due to the control receiving the lowest wild moth capture compared to all treatments. I intended to compare each release rate and timing to the control. However, as the control was so low, I did not analyze between the different timings. Figure 3.2 shows trends in wild catch data for 2021 by various release rate and timing. I am unable to know the effect that the second-generation releases had as we were not able to successfully perform this experiment over multiple years. Due to this, I cannot state whether the 2nd generation releases, and season long release would influence recapture.

I did not analyze, or report female captures for this experiment as female captures often averaged to below 0 by week. I currently theorize that traps in Michigan orchards are unable to capture female moths at the same ratio as the Washington and British Columbia region because of climate differences (Andrews 2022). More research would need to be done about this in the future.

This experiment needs to be performed over multiple years in order to truly understand the effect that the 2nd generation releases have on the following 1st generation moth populations. This is similar to the mating disruption system where it is impossible to truly know how beneficial the system is with only a year of work.

Across all treatments there may have been a potential effect from pesticides sprays. Unfortunately, we do not have access to spray records, but each grower has his own program and these differences in programs could have contributed to low captures in the control blocks as well as the high variability between sites. This could be a big factor into why our control orchards did not show a larger wild population, as some growers may have been spraying more heavily. In addition to these pesticide sprays it is also important to note that mating disruption was applied in some of the orchards in this study (Table 3.1). This could have had a large impact on the study as mating disruption and SIT both function by reducing mating and therefore they likely interact with each other.

Release Method Experiment. In addition to the release rate and release timing experiment we looked at the release method of SIT codling moths and how that could impact recapture and distribution of the moths through the orchard. In this experiment I found that the release method does not significantly affect the recapture of the moths in the orchard. This is beneficial to know that the method of release does not impact the recapture rates as it allows growers to have more flexibility with the release program that they use.

I observed that the two different orchard locations had different distributions of the moths when released at a central point (Figure 3.4). I would hypothesize that it is due to wind, micro-climates, or elevation; further research with larger replication and diversity in sites would have to be done on this to explore an explanation. In both years of sampling at the Flushing orchard across all release methods, I observed a population drop at 100 m, this may indicate that moth releases should occur every 200 m to allow even dispersal of the moths throughout the orchard. In contrast to this I saw a different pattern in the trellised orchards, this pattern also differed from year to year. This experiment had a small sample size which may be why there was difference between data sets. In 2020 trellis plots I saw that the same general curve of recapture slightly decreased over time however the self release method had a large unexplained peak at 80-100 m after the initial drop off.

In the future this experiment should be performed with more replications in a variety of locations. We were able to control for mating disruption in this experiment but were unable to control for insecticide sprays. These sprays would have had an impact on population recapture and should be looked to be control in the future. It would also be interesting to look at the impact of hail netting on SIT success with these different release methods being utilized.

Future research should collect damage data for the release rate and timing experiment, this type of data would have been extremely beneficial in terms of showing the effectiveness of the different treatments. I experienced crop failures across the experiments due to frost damage which meant we were unable to compare treatments for their impact on apple infestation levels.

The experiments performed during this research showed that the translation of SIT programs from one state to another should be thoroughly researched to enable the best results. Growers and stakeholders in the Michigan apple industry should be aware that while cost reduction may be possible with the use of reduced rates or timing of releases, more research needs to be done. In the future researchers should look at doing season-long SIT experiments where sites can be controlled and there is a large number of replicates over several years.

CHAPTER 4: Conclusions and future directions

This thesis explores release rates, timing and release technique of SIT codling moths and the response of SIT and wild moths to pheromone/kairomone based lures. This system has been extensively studied in British Columbia, Canada and in Washington State in the United States with limited research on how the program might work in Michigan. Michigan orchards are quite different from those in western North America due to climate differences and orchard size, with 46% of Michigan apple orchards being less than 9 acres in size (NASS USDA 2017). The research done in this thesis has begun to shed light on how SIT codling moth programs could be implemented in Michigan orchards to work within the integrated pest management strategies that are already in place.

In Chapter 2, I compared the effectiveness of pheromone, pheromone/semiochemical, and kairomone based lures for capture of male and female codling moths. The experiment was performed in both mating disrupted and non-mating disrupted apple orchards in both Michigan and Washington in 2020 and 2021. While data from Washington was limited, I include it here to test whether there are differences in male:female catch ratios between Michigan and Washington. I found that I caught a 1:1 ratio of male to female moths when using a 4K + AA lure in Washington, which agrees with Knight et al. (2019) who reported a 60% of catch being female in Washington. However, in Michigan I caught 22:1 and 11:1 ratio in 2020 and 2021, respectively.

Additionally, I found that the CMDA lure caught a 10:1 male:female ratio in Washington, and a 10:4 ratio with the addition of acetic acid. This increase in female capture is closer to the sterile moth population that we are releasing (1:1 male:female), which is potentially beneficial for crop consultants trying to gauge the population in an orchard. Landolt et al. (2007) saw that the addition of acetic acid to a CMDA lure could increase capture by 200%, whereas I found a 400% increase in capture in Washington when I added acetic acid to this lure. In Michigan I found that the CMDA lure caught 0 average female moths in 2020 and in 2021. This confirms the reported inconsistency between Washington female moth capture and the catches in Michigan. In 2020 in Michigan, season long releases of the same weekly populations illustrate variable recapture rate of a known population, for each of the lures. These data are important because they illustrate how the different lures catch differently throughout the season.

An important finding from this research is validating the disparity in female catch ratios between Michigan and Washington. While the cause of this difference is still unknown, conducting this experiment with lures from the same lot and moths from the same rearing facility falsifies these two variables as possible causes. Future research should explore environmental factors such as humidity or background plant volatiles.

Additionally, I showed the seasonal variability in catch between multiple lures. The lack of a clear, season-long winner suggests that multiple lures are needed to reliably monitor codling moth populations. For this reason, I would advise against depending on any single lure for an entire season, as farmers would be unable to accurately predict the wild population or action thresholds. More research is needed to better understand how and when to use all of these different lures.

In Chapter 3 I studied the seasonal timing and rate of moth releases in addition to the release method. The first experiment evaluated the release rates and the timing of releases, using half rate and full rate of moths, released either season long, for the first generation only, or for the second generation only. The objective of this experiment was to find efficiencies that would lead to cost savings for growers. Purchasing sterile moths at full rate for only the 1st generation would help reduce the cost of the SIT program for growers, but it is difficult to draw conclusions from these results as pesticide sprays were applied by different growers at slightly different rates and timings. This complication was the result of having to work with multiple grower cooperators in small blocks of trees, so future research might benefit from working in multiple orchards managed by the same farmer. Sterile insect technique works best when applied to larger continuous acres, and so a persistent challenge for future researchers in the eastern US is conducting this research on small irregular blocks. In Michigan 84% of growers have less than 100 acres of orchard, and this is usually not continuous. This suggests that a combination of SIT and other approaches may be needed to fit the more diverse cropping systems of this region.

The second experiment compared 3 different release methods; self release from paper bags, tossing the moths into the tree, or tossing the moths onto the ground. The goal of this experiment was to find potential savings in the application of the sterilized insect releases for smaller scale operations, and look for potential points of failure, i.e. workers dumping moths on the ground. I found that between the three methods of release that were tested, there was no difference in recapture rates. I observed that orchard location had an impact on distribution of the moths through the orchard, which could be due to a variety of environmental reasons. Across all three release methods a drop off in moth recapture occurred at approximately 100 meters from the release point, suggesting that moth releases should occur every 200 meters. This finding aligns with a 2012 study showing that most male and female moths dispersed within 80m, with some flying up to 200m away from the release point (Margaritopoulos et al. 2012).

One of the potential challenges of SIT research that others should keep in mind, with codling moths, is the flight capacity of the moths requires us to have experimental blocks that are bigger than most Michigan growers plant. Previous researchers have shown (Adams et al. 2017) that moths can disperse over 40 acres in just a few nights. As stated above most Michigan orchards are 9 acres or less (NASS USDA 2017), and therefore too small to prevent immigration of wild moths from outside the block. In addition, there is nothing to keep released moths within the borders of each experimental block. Finally, the current cost of sterile moths, plus overnight shipping, is an additional limiting factor for designing experiments and release programs.

In order for a SIT codling moth program to be successful implemented in Michigan there is a lot of research that still needs to be done to make sure that the program is as efficient and economical as possible. This thesis is just the tip of the iceberg in terms of questions about the translation of the established program in British Columbia to the different climate, orchard structure, and pest populations in Michigan orchards.

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: 2022-01

Author: Megan Abigail Andrews

Title of thesis: Exploring sterile insect technique for management of codling moth in Michigan

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Tortricidae	*Cydia Pomonella*	adult	20	pinned

LITERATURE CITED

LITERATURE CITED

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