FACTORS INFLUENCING STERILE CODLING MOTH (C*ydia pomonella* L.) RECAPTURE, DISPERSION, AND EFFECTIVENESS AS A CONTROL TACTIC IN APPLE ORCHARD SYSTEMS

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ABSTRACT

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The sterile insect technique is a proven technology used in the control and eradication of a number of pest insects over large areas. However, using this technology on a farm scale is a new and unproven application that until now has not been explored. This dissertation examines the impact of integrating the sterile insect technique for codling moth *(Cydia pomonella)* into existing farm-scale commercial apple pest management programs, methods of release, the sterile insect's interactions with the orchard, how existing management schemes are compatible with releases of sterile *C. pomonella*, the role of sterile female moths and dispersive distances. The main objectives were to 1) determine the impact of release methods on moth dispersal; 2) measure moth dispersal in contemporary trellised or netted orchards, and in orchards planted on steep terrain; 3) determine male and female dispersal in orchards treated with pheromone mating disruption; 4) establish release densities and timings to manage *C. pomonella*; 5) integrate sterile codling moths into existing commercial apple pest management programs; 6) elucidate the role of sterile females in controlling *C. pomonella* males; 7) determine the probability of male and female codling moth catches from specified distances using traps baited with a pheromone/kairomone combination lure in a single-trap, multiple-release experimental design; and 8) apply this information for estimating trap plume reach, maximum moth dispersive distance and absolute pest density based on moth catch in traps. Comparison of releases by hand

at a single central location versus evenly released throughout the orchard showed higher overall recapture of sterile moths in all traps placed within the orchard when they were released at the center, suggesting that higher numbers of moths were retained in targeted areas with this method. For releases by hand or by unmanned aerial systems (UAS), recapture of sterile moths was higher when released by UAS. Orchard characteristics were found to impact sterile moth dispersal from single central locations; moths moved away from release points more in trellised orchards than in those with large old single trees. Male and female dispersal in orchards with mating disruption was similar, but shorter than in orchards without mating disruption. Sterile codling moths released on commercial farms controlled wild populations when released at densities ranging from 500 to 2000/ha and increasing numbers of sterile females were shown to increase the disruption of wild populations. Deploying sterile males and females at lower densities than the standard 2000/ha or targeting the peak flight of one or both generations showed promise as a means of making SIT a more cost-effective tactic for managing *C. pomonella* at a farm scale. In orchards with mating disruption, the plume reach of a single codling moth trap baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) was found to be very small, maximum dispersal distance was ca. 100-130m, corresponding to a trapping radius of ca 3-5ha. Pest density estimates, based on capture of a single moth in traps, were shown to correspond to 113-180 moths/ha.

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CHAPTER ONE

Introduction

INTRODUCTION

This introductory chapter provides background information that sets the context for understanding the five chapters detailing research experiments pertaining to the recapture, dispersion, and effectiveness of sterile codling moths as a control tactic in commercial apple production. The chapters that comprise the main body of this dissertation were written as manuscripts for publication.

APPLE PRODUCTION IN WASHINGTON STATE

The United States is ranked second worldwide in apple production. Washington state is ranked #1, producing 65.8% of all US fresh apples with a total of 3,750,000 tons of apples harvested from 165,000 acres. The state exports approximately 25% of all fresh apples (USDA NASS, 2019) and produces 24% of the US processed apples (Cho, 2004). In 2001 the cost to produce an acre of apples in Washington State was about $5800 to $6600, packing and marketing cost an additional $3,600 per acre, and the average break-even price for a box of apples was about $13.50 (Smith, 2001). Current production costs are about $5200 to $7400 and additional packing and marketing costs are $5,600 to $7,500 per average acre of production. Apple cultivars grown in the state are Red Delicious, Gala, Fuji, Granny Smith, Golden Delicious, Braeburn, Cripps Pink, Jonagold, Cameo, and several other minor and “club” varieties (DuPont, 2020). Costs of insecticides alone can be in excess of $800/ac annually in Washington Honeycrisp apple production (Gallardo and Galinato, 2020).

TAXONOMY AND BIOLOGY OF *CYDIA POMONELLA*

Cydia pomonella (Linneaus, 1758), the codling moth, is the key pest impacting apple production in Washington State and throughout apple growing regions of the world. The codling moth was first described as *Tinea pomonella* (Phalaena #270) in Linneaus (1758) and was subsequently changed and re-described many times. Brown (1979) finally clarified the confusing taxonomy of this species and established *Cydia pomonella* (L., 1758) as the most up to date synonym for this species. The taxonomy of the current genus, *Cydia*, has also gone through many changes since Linneaus first published genus and species descriptions in 1758; at least 19 different names have been used to describe species in this genus (Oboyski, 2011). *Cydia* belongs to the monophyletic subfamily Olethreutinae which are characterized by various structures as described in Horak (1999), and the tribe Grapholitini Guenée 1845 (Komai, 1999; Komai and Horak, 2006). Oboyski, 2011 provided a thorough review of the genus with re-drawn phylogenies from several papers. *Cydia pomonella* also infests pear, walnut, quince, crabapple, loquat, hawthorn and some stone fruits such as apricots, cherries, peaches, plums, and prunes (Newcomer and Whitcomb, 1925).

Life history

Cydia pomonella, like all Lepidoptera has four distinct life stages: egg, larva, pupa, and adult. There are five larval instars, all of which feed internally in the fruit of host plants, and the fifth instar is also found outside of the fruit seeking refuge to spin a cocoon and pupate. The adult moth, about ¾ inch long, is slightly greyish with a bronze band at the tip of the forewing. The eggs are typically deposited singly on leaves and fruit, and are round, flat, and semi-transparent and about the size of the head of a pin (Allman, 1928). The caterpillar, which is responsible for

fruit damage, is the most well-known stage of this pest and is commonly referred to as the "worm in the apple". The final larval instar pupates in sheltered places on or near the host tree. Wild-type females mate an average of 2.2 times during their lifetime (Hathaway, 1966). Throughout most of its range*, C. pomonella* has two to three adult emergences annually (Seigler and Plank, 1921; Hall, 1928; Geier, 1963). Typically, fifth instar larvae overwinter in cracks in the bark of host trees or other hidden areas in the orchard before breaking diapause and forming a pupa in the first warm days of early spring (Hall, 1928). Following pupation, a spring brood of moths emerge, mate, and lay the eggs of the first summer generation (Geier, 1963). Seigler and Plank (1921) report that some individuals of the first summer generation enter diapause and remain inactive until the following season, but most pupate, emerge as adults, mate, and lay the eggs of the second summer generation. Setyobudi (1989) found that in Oregon almost 40% of all first-generation individuals entered diapause. Most individuals, up to 77% (Setyobudi, 1989), of the second generation will enter diapause as fully fed, mature fifth instar larvae, but some will pupate and emerge as adults that mate and lay the eggs of the third generation (Geier, 1963). A small number of third generation larvae will survive when conditions are favorable and enter diapause to wait out the winter. Life history studies conducted by Seigler and Plank (1921), Isley and Ackerman (1923), and Brunner (1993), observed that that there were differences in the length of each stage depending on the time of year; stages occurring in mid-summer typically take less time to develop than those in early spring and fall.

Fruit injury caused by larval feeding

Codling moth causes damage directly to fruit by the action of larval feeding, rendering fruit unfit for sale as a fresh commodity. Due to regulations and market demands, there is a near zero tolerance for injury. Crop loss can be highly variable from location to location and year to year. When trees are unmanaged or poorly managed, losses can be up to 95% (Isley and Ackerman, 1923; Allman and Essig, 1929; Putman, 1963; Glass and Lienk, 1971; MacLellan, 1972; Westigard, 1973; Setyobudi, 1989; Wise and Gut, 2000, 2002). Losses in revenue *from C. pomonella* injury and costs of controlling this pest can be a significant burden for farmers. Farms with consistent management and crop loads have consistent year over year injury levels whereas those with higher variability in management practices and crop load experience more variation in the levels of damage (Isley and Ackerman, 1923). The variety of apple being grown is also important when considering susceptibility to codling moth damage; some are highly susceptible while others are more tolerant (Cutright and Morrison, 1935).

Movement of Cydia pomonella

Although there is general consensus that *C. pomonella* is a rather sedentary species (Geier, 1963), dispersion over longer distances has also been shown. Worthley (1932) captured most adults within 500 feet (~160 meters) of the release point. Using sweet baits, Steiner (1940) observed differences in dispersive distance based on whether moths were released inside or outside of orchard blocks; those released inside dispersed an average of 200 feet (~65 meters) and those released outside dispersed over 600 feet (~200 meters). Trematerra et al. (2004) found average dispersal distances of male *C. pomonella* to be up to ~130-200 meters. Similarly, Basoalto

et al. (2010) found dispersal distances of ~150-300 meters. In concurrence with previous studies, Adams et al. (2017) found the mean maximum dispersive distance of sterile male *C. pomonella* to be up to ca 260 meters in orchards not under mating disruption using mark recapture experiments. Mani and Weibolz (1977) found some marked individuals as far as 11 km from the point of release in the Rhine Valley, and Howell and Clift (1974) found a small number of individuals as far as 5.4 miles (~8.7 km) from release points. Keil et al. (2001) explored the genetic components of moth mobility and dispersion and demonstrated a correlation between movement and genotype. Using the sex attractant, codlemone, and pear ester as baits, Margaritopoulos et al. (2012) found that the majority of both sexes dispersed less than 80 meters, and a few traveled up to 200 meters. Following an accidental release of lab-reared fertile female codling moth into an infestation-free area of Washington State, 90% of the fruit injury was found within 1000 feet of the point of release and the rest was found within 2000 feet (White et al., 1973a). Neumann (1996) concluded that females disperse only 30-60 meters in the field, implying that females are relatively sessile compared to males. In flight mill studies Schumacher et al. (1997b), found males and females capable of similar flights. There is likely genetic variability in flight capacity with most of the population being sedentary and a small number highly dispersive (Gu et al., 2006; Schumacher et al., 1997a). It is clear that there is a high degree of variability in observed flight distances of individuals; most *C. pomonella* do not disperse over great distances, but a few are capable of it.

MANAGEMENT OF *CYDIA POMONELLA*

Monitoring and predicting Cydia pomonella activity

Accurate monitoring and prediction of phenological events is key to successful management of *C. pomonella*. The first phenology model for this pest was developed by Glenn (1922), but like most models in the era before the development of codling moth pheromone trapping tools it suffered from inaccurate methods of estimating in-field populations. Shelford (1927) further attempted to refine the Glenn (1922) phenology model, correcting for weather and climate. Early work by Geier (1963) found that fruit damage in the early spring began 7-10 days after temperatures above 60°f were sustained. Much work was fundamental to development of the PETE (Predictive Extension Timing Estimator) model (Reidl and Croft, 1974; Reidl et al., 1976; Welch et al 1978) which required extensive early season trapping to establish a biofix based on the first captures of moths. Jones et al. (2008) suggested it was not necessary to capture moths for biofix to predict wild moth emergence and flight. In Washington State, the degree day model ultimately developed by Jones et al. (2013) allowed prediction of emergence of moths without the need for extensive trapping. The Jones et al. (2013) model predicted that the first brood of moths would emerge after approximately 175 (°F) degree days from January 1 and begin to deposit eggs after 225-275 degree-days, and the second brood would emerge at 1175 degree-days. Knowing local conditions and degree day accumulation allows for accurate predictions, but the degree of accuracy needed varies by management strategies.

From historical times through today, *C. pomonella* populations have been monitored by assessing in-field damage of fruit, by wrapping trunks in bands of materials that simulate rough bark to assess overwintering densities (Allman, 1928; Yothers and Van Leeuwen, 1931), assessing

in-season adult activity by light trapping (Butt and Hathaway, 1966), and more recently attractant-based trapping. Proverbs (1965) observed that *C. pomonella* males were attracted to caged virgin females in British Columbia apple orchards. Armed with that knowledge, Butt and Hathaway (1966) studied the attraction of extracts made from female moths and ultimately found evidence for the presence of a female-produced sex pheromone. Subsequently McDonough et al. (1969) were able to extract pure pheromone from 3-day-old female moths and learn that the structure likely had an alcohol group and no carbonyl-containing groups. Eventually Roelofs et al. (1971) were able to determine that the sex pheromone is trans-8, trans-10-dodecadien-1-ol (commonly referred to as codlemone) and that the synthetic compound was highly attractive to males in the field. Since then, advances in synthesis and deployment have increased the efficacy of monitoring using sex pheromone-baited traps.

Several synergists have been developed to increase the attraction of monitoring traps to *C. pomonella* adults. The kairomone, ethyl (E, Z)-2,4-decadieonate (pear ester) isolated from pears was found to be attractive to both males and females (Light et al., 2001). This kairomone has been extensively studied (Knight and Light 2004a,b,c; Light and Knight, 2005; Knight and Light, 2005a,b; Knight et al, 2005; Schmera and Guerin, 2012), and is now commercially available in lure formulations. Trona et al. (2010) found that *C. pomonella* males are not attracted to pear ester in the absence of sex pheromone. In addition to pear ester, acetic acid has been found to be attractive to *C. pomonella* adults (Landolt et al. 2007). Multiple studies of attractiveness of acetic acid, or fermented sugar baits (acetic acid is a product of fermentation) have resulted in its inclusion in lure formulations (Yothers, 1930a,b; Landolt et al., 2007; Knight, 2010a,b; Judd, 2016).

A consequence of widespread adoption of *C. pomonella* mating disruption as the primary control for this pest is that monitoring traps are rendered ineffective when baited with the same pheromone compound as the mating disruption emitters. As a result, several options have been developed to override the inhibitory effect of mating disruption on attraction of moths to monitoring traps. Charmillot (1990) discovered that the potential to capture male *C. pomonella* in pheromone-treated orchards was greatly enhanced when traps were baited with 10 or 20 mg rather than the standard 1 mg of pheromone. The utility of a 10 mg load of pheromone for assessing *C. pomonella* activity in disrupted orchards was confirmed by researchers in North America (Barrett, 1995; Judd et al., 1996), leading to the high-load lure becoming the standard for monitoring in disrupted orchards More recently, various combinations of the sex pheromone and kairomones discussed above also have proved effective for monitoring *C. pomonella* in disrupted orchards (Knight 2010b; Knight and Light 2005ac, 2012; Gut et al., 2019).

Overview of Cydia pomonella management

C. pomonella has been a target of control efforts for centuries. From the 1870's through the mid-1900's, arsenical compounds (Paris Green, Lead Arsenate) were the primary insecticides used to control this pest in the United States (Peryea, 1998). Following the disuse of arsenicals, chlorinated pesticides such as DDT became the favorable option for *C. pomonella* control (Durkee et al., 2017). By the end of the 1960's the organophosphates were the primary insecticide used in apples, but their use has been declining for some time (Costa, 2018) due to the development of resistance (Varela et al., 1993) and regulatory pressures. Several technologies, such as insect growth regulators (Westigard, 1979; Westigard et al., 1986), *Cydia pomonella* Granulosis Virus (Westigard and Hoyt, 1988), the sterile insect technique (Proverbs et al., 1966; Dyck et al., 1992),

and pheromone-based mating disruption (Gut and Brunner, 1991, 1992, 1994, 1998) have been tested and variably implemented to replace organophosphates. There are currently several conventional and organic compounds available for use in apple against *C. pomonella*, but in Washington State it is now recommended to use insecticides as a supplement to a robust mating disruption program (DuPont, 2019).

Cydia pomonella granulosis virus

A highly virulent codling moth granulosis virus (CpGV) was first discovered in infected larvae near Valle de Allende, Chihuahua, Mexico in the 1960's (Tanada, 1964), and was found to be transmissible in frass among individuals (Tanada and Leutenegger (1968). Larval entry into apples was reduced by about 95% by field applications of experimental CpGV extracts every two weeks, and the LD50 for late instar larvae was found to be about 30 virus capsules/L_1 (Keller, 1973). Virus development and pest mortality is inversely proportional to dose (Sheppard and Stairs, 1977). Caterpillar death, followed by liquefaction usually occurs within five to ten days (Arthurs and Lacey, 2004). Commercially produced CpGV is extracted from mass-reared, infected *C. pomonella* larvae and contains homogenized larvae, glycerol, and water (Certis, 2009). CpGV is the most effective commercially produced biological agent used in the control of *C. pomonella* (Lacey et al., 2008). Lacey et al. (2008) provides a comprehensive history of CpGV along with formulation information, resistance development, and a discussion of use in IPM.

Pheromone-based mating disruption

In response to increased regulatory pressure and resistance development against insecticides, the first pheromone dispenser for codling moth mating disruption (CM MD) was

registered for commercial use in 1991 (Witzgall et al., 2008). Implementation of CM MD typically requires deploying large numbers of synthetic pheromone-loaded dispensers throughout the orchard to disrupt the normal mate finding behavior of codling moth (Płuciennik, 2013). Mating disruption using synthetic pheromone emitters for *C. pomonella* control is achieved via competition between the synthetic source of pheromone and wild-type females (Miller et al. 2010, McGhee et al 2014). On-farm use has increased steadily, and from 1995 to 2015 the acreage of Washington apples treated with disruption increased from 10% to nearly 90% (Willett and Curtiss, 2019). Similar increases in Argentina pears treated with CM MD from 1990 to 2011, have now seen least 30,000 ha treated, and damage was reduced from 5-6% to 0.26% (Cichon, 2011). New Zealand apple farms treated with mating disruption have experienced a 70% reduction in adult captures in traps, lower fruit damage, and reductions in insecticide applications by nearly half (Walker et al., 2013). In Poland, pheromone mating disruption treated orchards had a reduction in fruit damage up to 95% compared to untreated orchards, but when *C. pomonella* populations were high, damage was higher (Płuciennik, 2013). As of 2008, 80% of all acreage treated with mating disruption was still using the pheromone formulation registered in 1991 (Witzgall et al., 2008). In 1995 the USDA funded the codling moth areawide management project (CAMP) focused on CM MD in California, Oregon and Washington (Willett and Curtiss, 2019; Witzgall et al. 2008). Washington state had three sites, and CAMP was successful at all locations (Willett and Curtiss, 2019). Areawide CM MD was found to be highly successful in Michigan apple orchards where *C. pomonella* in traps and fruit damage declined to very low levels after implementation of the approach, as well as overall reductions in insecticide applications

within treated areas (McGhee et al., 2011). Similarly, Knight (1995) found significant cost savings from reduced pesticide sprays in disrupted orchards.

Sterile insect technique

The sterile inset technique (SIT) is a proven pest control tactic that has been successfully employed for management and eradication of several species (Dyck et al. 2021). Interest in SIT began with the exploration of sterilizing insects by Hunter (1912) with rice weevils and Morgan and Runner (1913) with cigarette beetles. Runner (1916) found high-dose X-rays sterilized all life stages of cigarette beetles and adult longevity was not different from untreated beetles. In the 1930's and 1940's Knipling (1955), Vanderplank (1944, 1947), and Serebrovskii (1940) theorized that sterilized insects could be used to control wild populations. Vanderplank (1944, 1947), determined that sterility could be induced through hybridization of two species of Tsetse flies in Tanganyika, and theorized that field releases of high numbers may be sufficient to inhibit natural mating. Serebrovskii (1940) developed the concept of using chromosomal translocations for pest population suppression and theorized that introductions of insects with sterility genes would persist in wild populations. Knipling (1955) reported that in 1937 he first theorized that irradiated sterile screw-worms, released into the wild populations could be used to suppress or eradicate these pests on an area-wide basis. Muller (1950 a,b) was the first to demonstrate that ionizing radiation could be used to sterilize *Drosophila* without compromising longevity or competitiveness. The sterile insect technique using ionizing radiation to sterilize large numbers of pest insects was put into practice beginning in the 1950's in the United States by Knipling, Bushland, Lindquist, Hopkins, Baumhover, and others at the USDA (Baumhover, 2002) for the control and eradication of the screw-worm in Curacao, Florida, and the Southeastern United

States. The screw-worm SIT program successfully achieved eradication in the US by the 1970's, in Mexico and Belize by the 1980's, and south throughout central America to Panama by the 1990's where a biological barrier was established to prevent re-infestation.

Codling moth SIT: Proverbs and others began working on codling moth SIT in British Columbia, Canada beginning in the 1960's and by 1992 a fully formed sterile insect release (SIR) program was initiated in the South Okanagan region of British Columbia, Canada (Thistlewood and Judd, 2019). Researchers in Washington State explored the use of SIT for codling moth management in the 1960's and 1970's, but ultimately abandoned the technique in favor of mating disruption.

Considerable work has been directed towards determining release rate and frequency needed to achieve eradication of codling moth (Proverbs, 1965; Proverbs and Newton 1962a,b,c; Proverbs et al., 1966; Proverbs et al., 1967; Proverbs et al., 1969; Proverbs et al., 1975; Proverbs et al., 1982). Hathaway (1966) demonstrated significant reductions in viable mating with increasing doses of gamma radiation in field and laboratory studies. White et al. (1969) released sterilized mixed-sex *C. pomonella* in Yakima, WA 6 days per week from May 16-Sept 14 in a small orchard plot and reduced fruit damage from almost 50% in 1965 without SIT to 1.57% in 1966 with SIT. Butt et al. (1970) found releases of SIT codling moths to be comparable to similar orchards treated with chemical insecticides, but never achieved the theorized eradication ratio of 40:1 determined by Proverbs et al. (1982). Butt et al. (1972) prepared a 32 square mile area for SIT with pesticide and sanitization treatments to first reduce codling moth populations, and then released mixed-sex sterilized adults from April to September 1971, and reduced native codling moth captures in traps and overwintering larvae by over 90% from 1970 to 1971 (Butt et

al., 1973). Following the accidental release of 336 fertile females, White et al. (1973a) successfully suppressed mating by following up with mass releases 24 and 48 hours later in addition to regularly scheduled daily releases. In a 20-acre Yakima, WA apple orchard, season-long releases of sterilized codling moths reduced infestation by 92% (White et al., 1973b). White et al. (1976a) ultimately experienced failure of the sterile insect technique for codling moth from 1971 to 1972 when infestation and fruit damage increased within the area that sterile adults were released and the sterile: wild ratio never exceeded 20:1, however they concluded that SIT combined with other control methods still suppressed wild populations. Modifying the technique from 1972 to 1973, White et al. (1976b) compared releases of mixed sexes, females only, and males only and found "69% and 27% less damaged fruit in the areas treated with releases of females only and mixed sexes, respectively, but a 100% increase in damaged fruit in the area treated with releases of males only", indicating that for codling moth, releases of mixed sexes may be necessary.

Commercial C. pomonella sterile insect release programs: Following several successful trials piloting control of *C. pomonella* with the sterile insect technique (Proverbs et al., 1982), plans for a full-fledged eradication program and rearing facility were put into effect in British Columbia, Canada in the early 1990's. The Okanagan-Kootenay Sterile Insect Release (OKSIR) rearing facility of BC, Canada cost $7.4 million to complete in 1993 and releases of sterile codling moth began in 1994. In 2004 the eradication program was expanded to include the Central and North Okanagan. Eventually the codling moth eradication program transitioned to a suppression program when it was clear that eradication could not be achieved. A suppression program required perpetual releases of sterilized adults, but there was concern that *C. pomonella* would not be adequately suppressed within the coverage area using only sterile

insects. Thus, many farms within the coverage area have supplemented SIT with other control tactics, including mating disruption (Judd and Gardiner, 2005; Thistlewood and Judd 2019). The total annual OKSIR program costs are currently over $3.7 million, of which $2.2 million goes to wages and benefits of permanent and seasonal staff. Costs are paid by general property taxes, an average of $6-12 per year paid by all property owners within the service area (revenue of ~$1.7 million in 2018), and by orchard owners at a rate of $139.26 per acre annually (revenue of ~$1.2 million in 2018). Approximately 2.2. million moths are produced each year for release from May through August. In addition to moth production and release, the OKSIR program provides many services including pest monitoring, public education and enforcement of laws concerning removal of infestations. More recently, the OKSIR facility has provided sterile codling moth to researchers in New Zealand (Horner et al., 2016; Horner et al., 2020), Washington State and Michigan. In South Africa, a codling moth rearing facility was established to produce 2 million moths weekly to treat up to 1000 hectares of apples and pears in combination with insecticides; significant reductions in wild codling moth captures, fruit injury, and the number of insecticide sprays were the result (Barnes et al. 2015). However, the program did not continue due to farmer reluctance to continue paying for control measures when the SIT program reduced codling moth damage to levels below the economic injury level (Barnes et al. 2015).

JUSTIFICATION AND OBJECTIVES OF THIS RESEARCH

The British Columbia *C. pomonella* SIT program does not consider economic viability if growers were to incur the full cost themselves; it is a Canadian government-subsidized program.

Although most sterile insect release programs have been conducted on an area-wide scale, SIT has the potential be used as an Integrated Pest Management (IPM) tool that will complement and add to existing codling moth management programs on individual farms. To accomplish this, development of cost-effective approaches to releasing sterilized moths and an understanding of the efficacy of SIT in various types of orchard plantings and settings is required.

Since the establishment of the British Columbia release program, and the subsequent transition from eradication to suppression, very little if any research has been conducted to determine if release densities and frequency can be reduced to obtain suppression; current release rates are still based on Proverbs et al. (1969) early work establishing a 40:1 sterile to wild ratio as a requirement for eradication. The cost of the current standard protocol of releasing 2000 sterile adults/ha weekly over the course of at least 20 weeks of *C. pomonella* activity is upwards of $2000/ha. Individual apple growers cannot bear this expense for controlling a single insect pest on their own. In addition, very little effort has focused on determining the potential of combining a modified SIT program with other management practices such as mating disruption and current chemical and biological control techniques to manage *C. pomonella*. Judd and Cossentine (1997) examined the combination of mating disruption and SIT and found reduced damage when employing the approach, with a 98% reduction in sterile/wild mating in disrupted orchards. The release of sterile codling moth to greatly limit mating of wild moths has the potential to provide farmers with a sustainable and environmentally sound control method that could be a game changer for managing this key pest of pome fruit.

This dissertation arose as a synthesis of several year's work in the laboratories of Dr.'s Miller and Gut at Michigan State University. Miller et al. (2015) set some of the fundamental

groundwork for the theories behind the movement and responses of insects dispersing throughout the landscape. The findings of McGhee (2014), and McGhee et al. (2014) were fundamental to understanding the role of and response to pheromone mating disruption by codling moths. Finally, Adams et al. (2017), and Kirkpatrick et al. (2018) demonstrated that the work of Miller et al. (2015) using mark-recapture studies to assess insect movement could be put into practice in the field to establish trap plume reach and maximum dispersal distances of mobile pest species.

The overall aim of the research was to determine the potential of the sterile insect technique as a pest management strategy that would complement and add to existing codling moth management and be used at the individual farm level. Due to the recent availability and relatively easy importation of high-quality sterile adults from the Canadian sterile codling moth facility in Osoyoos, BC one of the major barriers to investigating and implementing the use of sterile codling moth on US farms was addressed. Sterile codling moths are now being sold by the OKSIR facility as a commercial product for release onto farms in the US, though little research has been conducted on how to cost-effectively integrate this technology into current apple IPM programs. The project presented herein is a multi-faceted effort to establish the use of codling moth SIT in apple at a farm level. The overall aim was to understand how sterile adults disperse after release by various methods, how they interact with existing management practices, how effective they are as a control tactic in contemporary orchard systems and to elucidate approaches to using them that are cost-effective. The research builds on the work of previously discussed authors and knowing that farm operators in the US are beginning to use this technology without much guidance from research. In summary, the aim was to advance the use of SIT for

codling moth management at the farm level and to gain an understanding of how to implement the technique in an effective, and economical manner.

This research was conducted in Washington State rather than Michigan for several reasons. First, early attempts to ship sterile moths from Osoyoos, BC directly to Michigan proved difficult, and several shipments of moths arrived in poor condition or moribund. The proximity of Washington State to BC meant shorter transport times and much reduced likelihood of compromised shipments. Second, I was interested in the role and responses of sterile female moths, and the pheromone/kairomone lure used to attract them to traps is ineffective in Michigan, while in Washington State capture of over 50% females in traps is often achieved when they are baited with these lures. Third, the availability of high numbers of acceptable field plots in Washington, with growers and farm managers who were willing to modify existing practices if necessary, or otherwise tolerate release of sterile moths onto their farms meant there would be sufficient space to conduct these experiments with appropriate buffers between plots and within a reasonable time frame. Lastly, M3 Consulting Group was available as a key collaborator in Washington State that could facilitate importing moths, deliver them to field sites, and pilot Unmanned Aerial Systems to release moths at various research sites across the state.

Specifically, the objectives of the research were to 1) determine the impact of methods of release on codling moth dispersal; 2) determine the effectiveness of strategies for releasing sterile *C. pomonella* in contemporary trellised or netted orchards, and in orchards planted on steep terrain; 3) determine how *C. pomonella* males and females disperse in orchards treated with the two main technologies for disrupting *C. pomonella*, hand-applied or aerosol emitters releasing codlemone; 3) compare male and female moth movement in mating disrupted versus

non-disrupted orchards; 5) establish release strategies, densities and timings to cost-effectively manage wild *C. pomonella* on an individual farm basis; 6) determine the impact of integrating the sterile insect technique into an existing farm-scale commercial apple pest management programs at several release densities and timings coinciding with generational activities on wild moth populations and fruit damage; 7) elucidate the role of females in controlling *C. pomonella* by SIT; 8) determine the probability of male and female codling moth catches from specified distances using traps baited with a combination lure in a single-trap, multiple-release experimental design; and 9) to apply this information for estimating plume reach, maximum dispersive distance and absolute pest density using the quantitative tools developed by Miller et al. (2015). To accomplish these objectives, field experiments were conducted in Washington State apple orchards with releases of sterile *C. pomonella* from the OKSIR facility of Osoyoos, BC from the Spring of 2018 to the Fall of 2020.

CHAPTER TWO

Release Location, Altitude, and Time Influence Sterile Codling Moth (Lepidoptera: Tortricidae) Dispersion and Recapture

INTRODUCTION

The most important pest in apple production worldwide is the codling moth, *Cydia pomonella* (L.), with losses attributed to this insect ranging from 50-80% when controls are not applied (Westigard, 1973; Wise et al., 2015). Historically, management of this pest heavily relied on broad-spectrum insecticides, but over the past thirty years conventional management has become challenging due to the loss of compounds from restrictions or resistance (Varela et al., 1993; Knight et al., 1994), the high cost of new insecticides and fuel, concerns about worker and public safety, public interest in reducing pesticide use, increasing scrutiny of conventional spray practices, and grower motivation to adopt alternative tactics for *C. pomonella* control. Two alternative control technologies that have gained acceptance for managing *C. pomonella* are pheromone-based mating disruption (Gut et al., 2019), and the sterile insect technique (SIT) (Proverbs 1969 et al.; Dyck et al. 2005). However, both tactics are most effective against low populations, resulting in the technologies being integrated into pest management programs. Both technologies are often more expensive than insecticide-based programs (Williamson et al., 1996; Agnello et al., 2009; Thistlewood and Judd, 2019). Despite this limitation, *C. pomonella* mating disruption has been adopted as the primary control on an estimated 243,000 hectares of apples, pears and walnuts worldwide (Gut et al 2019), and the sterile insect technique has been broadly applied in British Columbia (Dyck et al. 2005) and South Africa (Barnes et al., 2015). The sterile insect technique, as successfully applied to several pests has typically been administered on an area-wide scale with cooperation between government agencies and industry stakeholders.

As implied by the name, the sterile insect technique requires sterilized laboratory reared insects that are released in high numbers into target areas to compete with their wild counterparts for mating partners, thereby greatly reducing or even eliminating fertile mating and offspring (Lance and McInnis 2005). Early development of the *C. pomonella* sterile insect technique occurred from the 1960's to 1980's in the western US and Canada. White et al. (1969) demonstrated fruit damage reductions from nearly 50% to less than 2% with releases of ca. 60,000 sterilized mixed-sex codling moths. In a 83km^2 area of the Wenas Valley, WA, Butt et al. (1972) deployed pesticides and sanitization to lower local populations prior to releasing 1.5 million mixed-sex sterilized codling moths. Subsequently, Butt et al., 1973 reported a reduction in wild adult captures and overwintering larval densities by over 90%. Proverbs et al., 1982 showed that damage could be kept at levels below thresholds, by first using insecticides and sanitation to decrease local *C. pomonella* populations, and then releasing of 1,000 sterile moths per hectare 2 to 3 times per week. In a 8.5ha apple orchard near Yakima, WA, White et al. (1973b) estimated a 92% reduction in fruit infestation following the release of over 500,000 mixed sex sterile codling moths.

Research scientists in Washington abandoned the sterile insect technique (SIT) for managing *C. pomonella*, opting instead to pursue pheromone-based mating disruption while researchers and industry in British Columbia investigated and implemented SIT in pome fruit production areas. Proverbs et al. (1966, 1967, 1969, 1975, 1982) conducted extensive research on the required overflooding ratio and release frequency needed to achieve codling moth control. This research culminated in an area-wide project aimed at eradicating *C. pomonella*. A clean-up program began in 1992 to reduce *C. pomonella* populations to ensure proper ratios of

sterile to wild moths (40 to 1) could be achieved. A multimillion-dollar facility was built to rear up to 16 million moths/week. Beginning in 1994, sterile moths were released. About 40% of the program costs were borne by fruit growers charged a per hectare fee, and the rest of the cost was covered by government expenditure of tax revenue (Bloem et al. 2007, Thistlewood and Judd 2019). The goal of the SIT program was to eradicate codling moths from pome fruit production areas. Although eradication was never achieved, *C. pomonella* densities and damage in the region were substantially reduced.

Maintaining the necessary over-flooding ratio of 40 sterile moths to 1 wild moth (Proverbs et al., 1982) proved difficult, as did the programs ability to eliminate *C. pomonella* from hosts on residential and other non-commercial properties (Thistlewood and Judd 2019). Beginning with the 2000-growing season, the objective of the program changed from eradication to area-wide suppression of *C. pomonella* using a combination of SIT, pheromone-based mating disruption and insecticides. This intensive suppression program has reduced wild *C. pomonella* populations by 94%, kept fruit injury to less than 0.2% on 91.5% of the acreage and reduced the amount of insecticides applied for *C. pomonella* control by 96% (https://www.oksir.org/). The success of the program in British Columbia has led other growing regions to pursue this approach including New Zealand (Horner et al., 2016), South Africa (Barnes et al., 2015), and the United States (McGhee et al., 2014; Adams et al., 2017).

Interest in pursuing SIT in Michigan developed following the publication by Adams et al. (2017) demonstrated that sterile codling moths move randomly and quickly across the orchard, disperse to a maximum of ca. 250m, and single traps were shown to have an effective trapping area of ca. 20ha. The use of sterile *C. pomonella* for population suppression or control was not of

interest in these studies, but wild-type male capture data in monitoring traps showed reductions in wild populations after the first season, and these findings suggested that *C. pomonella* SIT had the potential to be an effective management strategy even on a farm-scale. To transition the *C. pomonella* sterile insect technique from area-wide eradication to farm scale control, research on the release or distribution patterns (uniform coverage or single, central point), the method of release (i.e. hand, ATV, UAS), and the time of day that moths were deployed was first required to achieve levels of suppression that would support the integration of SIT into existing *C. pomonella* farm-scale IPM programs.

The overall aim with the research presented herein was to develop the information needed to improve the effectiveness of sterile *C. pomonella* release strategies in farm-scale management programs. Research assessed the impact on moth distribution and recaptures of: 1) deployment of moths from uniformly distributed multiple release sites or from a single central release site using hand and Unmanned Aerial Systems, 2) Release at four altitudes within or above the tree canopy, and 3) disbursement into orchards at different times of the day.

METHODS AND MATERIALS

Source and handling of codling moths

Mass-reared sterile codling moths were obtained from the Okanagan-Kootenay Sterile Insect Release (OKSIR) facility in Osoyoos, British Columbia, Canada. Permits allowed importation of sterile moths to the United States. At OKSIR, recently eclosed mixed-sex, internally-marked (calico red) codling moths were placed in petri dishes at an approximate 1:1 ratio with 800 total males and females, treated with 150gy of gamma radiation from a Cobalt-60 source, immediately

packed into battery-powered coolers (2.8 Cu. Ft. Portable Fridge/Freezer: Edgestar co. Austin, Texas) held at approximately 5°C, and shipped to Washington State for release.

Sterile *C. pomonella* arrived at field sites within 24 hours of when they were packed. Sexes could not be separated at field plots prior to release due to the manner in which they were packed and the tight schedule for release. Upon arrival at field sites, moths were placed in polystyrene cups (540-ml Fabri-Kal Corp. Kalamazoo, MI) in batches of up to 4,000/cup. Moths were externally marked using approximately 1.4gram of Dayglo florescent pigments (ECO11 Aurora Pink®, ECO15 Blaze Orange™, ECO18 Signal Green™, ECO19 Horizon Blue™) (DayGlo Color, Cleveland, OH)/petri dish to uniquely identify moths released into each orchard block. Moths were allowed to warm to ambient temperature, and then released at a density of 800 mixed sex moths per 0.4ha by the methods described below at pre-marked locations in the blocks.

Traps

Recapture of sterile codling moths released in these studies was quantified by trapping in orange Pherocon VI delta traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure. Traps were placed within the top 1/3 of pre-marked apple trees in a 20-trap grid pattern with spacing of approximately 30m (Figure 2.1A-D). Lures were placed and replaced every 6 weeks per label instructions. Trap liners were collected once weekly throughout the study period for examination in the laboratory and the number of sterile moths of each sex identified and recorded.

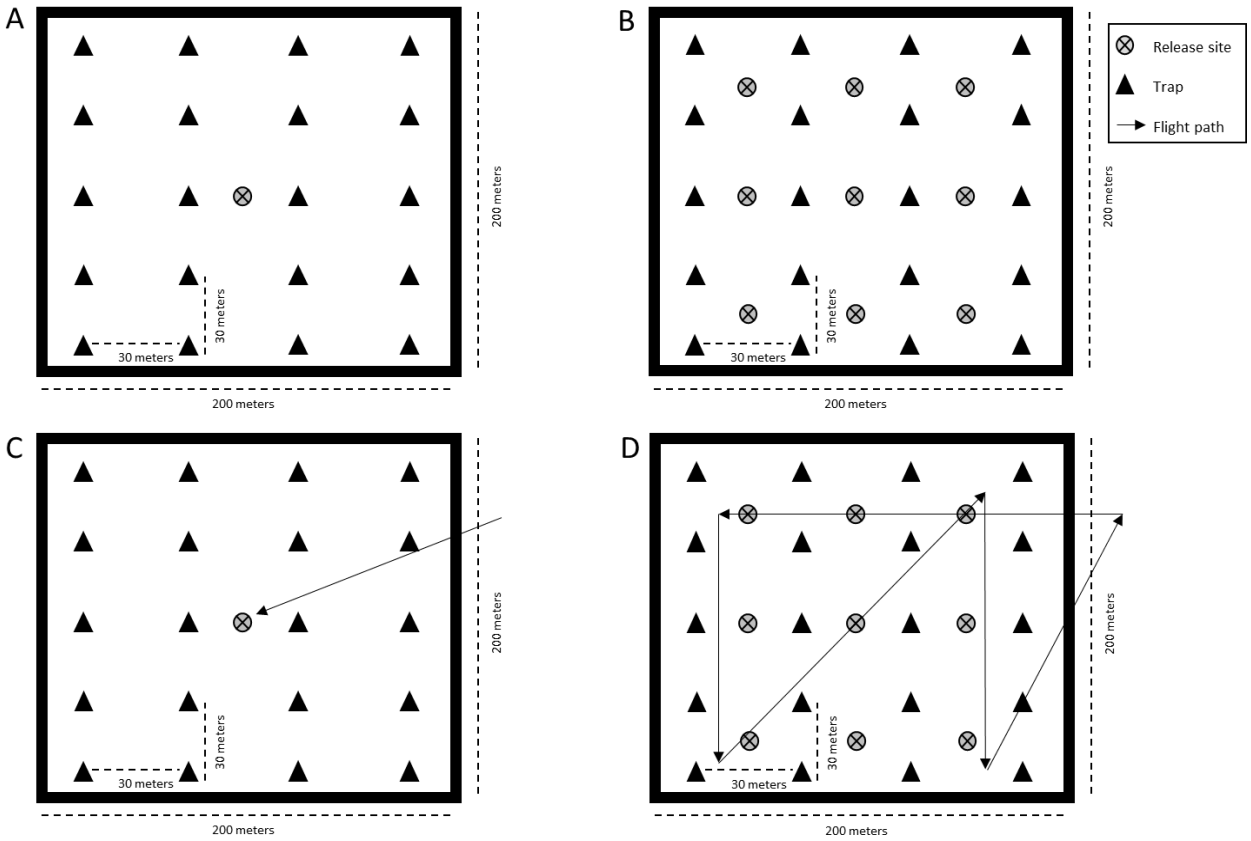

Figure 2.1. Trap and release site locations for *C. pomonella* released by A) hand release at the center of blocks, B) hand release at nine evenly spaced points, C) UAS release at the center of blocks, and D) UAS release distributed throughout blocks. UAS flight paths are approximate and for illustration purposes.

UAS

As described in Moses-Gonzales et al. (2021), two types of Unmanned Aerial Systems (UAS) were used throughout the course of the study: 1) octocopter airframes, referred to as the Hermes V.1 UAS (Spreading Wings S1000, SZ DJI Technology Co., Ltd., Shenzhen, China), and 2) Hermes V.2 UAS (Hermes V.2, M3 Consulting Group LLC., Dayton, United States) hexacopter airframes developed by M3 Consulting Group. Both UAS used the open-source Pixhawk 2.1 flight controllers (Pixhawk 2.1, Hex Technologies, Xiamen, Fujian, China). The Ground Control Station

(GCS) used to pilot the UAS was the open-source GCS Mission Planner (Mission Planner 1.3.58, ArduPilot Development Team and Community). A proprietary release device, described in Moses-Gonzales et al. (2021), using an internal paddlewheel to expel and meter sterile codling moths from the UAS, was used to deliver them to their release location (M3 Consulting Group LLC., Dayton, United States). All UAS missions were flown by M3 Consulting Group staff pilots under the supervision of the study authors.

MARK-RELEASE-RECAPTURE STUDIES

Experiment 1: Release Location by UAS vs. Hand

An independent-measures experiment to quantify the effect on sterile moth recapture and dispersal after release by hand vs UAS and releases at a single central point vs 9 evenly-spaced points was conducted from 2018-2020 in 16 4.05ha apple blocks within a larger 4850ha commercial orchard located near Brewster, Washington State. The site was comprised of a variety of apple cultivars, rootstocks, irrigation schemes, and tree training systems. Blocks were subject to existing management systems, including variable tree-training conditions and practices, various forms of insecticide treatments, and were treated with mating disruption using several technologies, including active emitters (i.e. ISOMATE® CM Mist Plus (Vancouver, WA)) at 2.5-5ha, or passive dispensers (i.e. ISOMATE® CM Flex, and Scentry NoMate® CM Spiral (Billings, MT)) at 300-400/ha throughout the experiment from 2018-2019.

To study their dispersal and assess movement from a single point of release compared to a more uniformly distributed release, marked sterile *C. pomonella* releases were replicated 14

times for each treatment during the 2018-2020 growing seasons. Treatments were: 1) release by hand at the center of blocks (Fig. 1A), 2) release by hand at nine uniformly spaced points throughout blocks (Fig. 1B), 3) release by UAS at 35m altitude in the center of blocks (Fig. 1C), and 4) release by UAS at 35m flying a pattern approximating the nine uniformly spaced points (Fig 1D). In 2018, eight 4.05ha blocks were rotated for a total of six replications of each treatment. In 2019, eight additional replications each of the two hand-released treatments were conducted in four 4.05ha apple orchards, and in 2020, eight replications each of the two UAS-released treatments were conducted in four 4.05ha apple orchards. The impact of treatments was assessed by dispersion and percent recapture.

Moths that were released by hand were taken directly to pre-marked release trees and released either at a single tree at the center of the block or at nine marked trees uniformly spaced within the block. Moths released by UAS were secured into the release device after being color marked, the device was mounted to the UAS, and flown to a point where the release device engaged, thus releasing moths in the appropriate manner, either in the center or in a uniformly distributed pattern. The release device was engaged remotely by the GCS control program upon reaching a specified point in space. Following release, moths were observed to fly in all directions.

Experiment 2: Release Altitude

The eight 4.05ha square apple orchard blocks used for this independent-measures experiment were located near Brewster, Washington State. The blocks were located within a larger 4850ha orchard with several planted varieties. The blocks were subject to variable

environmental conditions and commercial management practices. Codling moth mating disruption technology (ISOMATE® CM Mist Plus (Vancouver, WA)) was deployed at 2.5-5.0 emitters/ha. Sterile *C. pomonella* were released at a density of 800 mixed sex moths per 0.4ha in each block.

To assess the impact of release altitude above the ground on recapture and dispersal of released sterile codling moths, four height above ground level release treatments were compared: 30-35m, 20-25m, 10-15m, and 0-5m. Each treatment was replicated 8 times in experiments conducted in 2019. The two test plots used in this experiment were 16.2ha contiguous square orchards divided by narrow drive rows into four 4.05ha blocks (Fig. 2.2). For each replication, the four 4.05ha blocks simultaneously received one of the four altitude

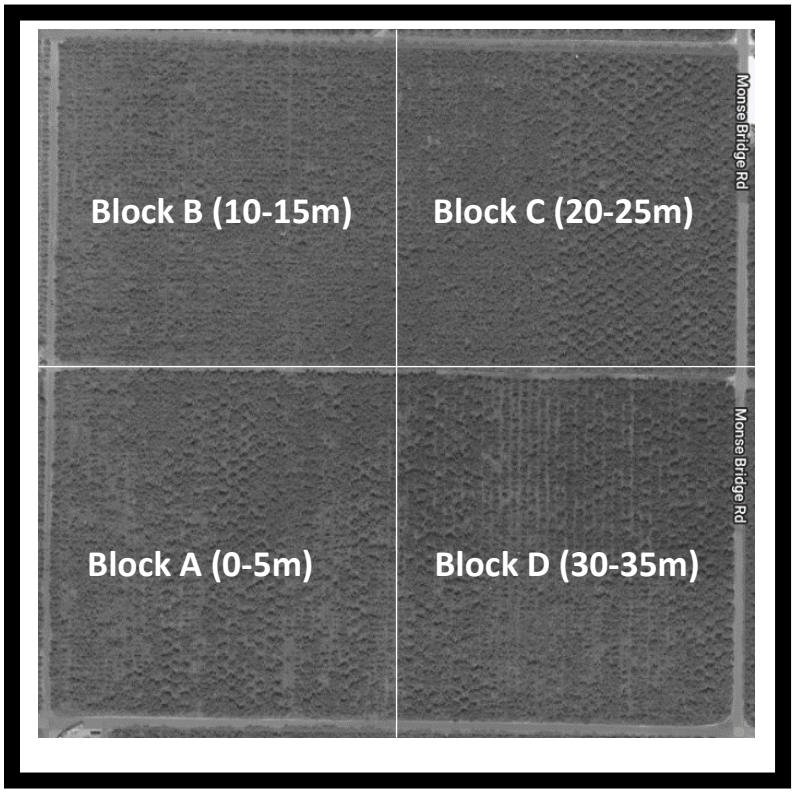

Figure 2.2. Example of orchard layout for experiment #2, release altitude. Blocks A, B, C, and D were each 4.05ha, combining to form a larger 16.2ha orchard. Two 16.2ha orchards were used in rotation for replications of releases at these altitudes above the ground in 2019.

treatments to ensure that moths were subject to similar environmental conditions at the time of release. The two 16.2ha orchard blocks were rotated every other week to prevent overlap of and interference between released moths. Each replicated release was conducted at or above a single point in the orchard block (Fig. 2.1A and Fig. 2.1C). At the 0-5m altitude moths were hand released at a pre-marked central location, while moths released at higher altitudes were deployed by UAS (as described above) at a pre-programed GPS location at the appropriate altitude above the center of the orchard. The lowest release altitude was performed by hand because it was an unsafe altitude for the UAS to fly. Due to wind and program imperfections release altitude and exact location was typically within a 3-dimensional five-meter range.

Experiment 3: Release Time

An independent-measures study quantified the impact of time of day on released sterile codling moths' dispersal patterns and percent recapture during the growing seasons of 2018 and 2019. Sterile codling moths were released by hand centrally into 4.05ha apple orchard blocks located within the same 4850ha orchard as the previous experiments near Brewster, Washington State (Figure 2.1A). Sterile codling moths were released as follows: 11:00 (8 replicates in 2019, 5 blocks), 12:00 (10 replicates in 2018, 4 blocks), 15:00 (4 replicates in 2018, 4 blocks; 8 replicates in 2019, 4 blocks), 18:00 (4 replicates in 2018, 4 blocks), 19:00 (8 replicates in 2019, 6 blocks), and 21:00 (10 replicates in 2018, 4 blocks). Orchard blocks were under existing commercial grower management systems. Blocks were subject to variable horticultural and pest management practices. All blocks were treated with mating disruption technologies including

active emitters (i.e. ISOMATE® CM Mist Plus (Vancouver, WA)) at 2.5-5.0/ha, and passive dispensers (i.e. ISOMATE® CM Flex, and Scentry NoMate® CM Spiral (Billings, MT)) at 300-40/ha. Moths were colored and hand released in the center of blocks at a density of 2000 mixed sex moths per 1ha.

DATA ANALYSIS

Recapture: For all three experiments, mean *C. pomonella* percent recapture and dispersion were used to measure the treatment impact of the release strategy. Significant treatment effects for percent recapture were determined by performing a log(x+1) transformation to normalize the proportional data, followed by ANOVA to determine global significance of treatments. The means were separated by multiple pairwise comparisons using Tukey's HSD (P=0.05) test.

Dispersion: The degree to which the released populations aggregate about the center of the orchard was determined by calculating Morisita's index of dispersion (Iδ) in two manners for each replicate. The index was calculated as $I\delta=n(\sum(xi^2)-\sum(xi))/(\sum(xi)^2-\sum(xi))$ where n=the number of traps and xi=the capture in individual traps (Morisita 1959, 1962). For the initial analysis, Iδ was calculated based on the absolute number of moths captured in each trap from each replicate. This was followed by calculating Iδ based on each traps' percentage of replicate total recapture. Iδ calculations return a number from 0 to *n*, where 0 represents an even distribution, 1 represents a random distribution and >1 represents an aggregated distribution; the degree of aggregation increases as *n* is approached. In the case of these studies, each trap was the unit of measure for

captured individuals, so *n*=20. An analysis of variance was conducted on both Morisita's indices calculated for each experiment to compare treatments and determine if there are significant treatment differences. If the results of the ANOVA indicated significant differences, post hoc Fisher's LSD tests (P=0.05) were used for mean separation. When recapture of sterile *C. pomonella* in traps was low (i.e. less than 5 total moths captured from all 20 traps) or all traps from a single replication individually recapture one and/or zero moths, the index is likely to be imprecise (Amaral et al., 2014). Also, if recapture of sterile *C. pomonella* is confined to 1-2 traps, high Iδ values are returned for that replicate, and the results may be skewed. In order to minimize inaccuracy in this measure for these experiments, absolute Iδ was calculated for replicates in which capture was >5 total moths and was not calculated when all traps individually recaptured 1 or 0 moths. For percent of total recapture, Iδ was not calculated when fewer than 4 total moths were recaptured in traps. For the release location experiment (expt. #1) aggregation analysis of the absolute number recaptured, one replication was eliminated from hand-central release, two replications from hand-even release, and one from each of the two UAS releases because they captured too few moths for accurate analysis. Also for experiment 1, the aggregation analysis based on the percent of total catch was calculated for all 14 hand-central release replicates, 12 of the hand-spread replicates, and 13 replicates for each of the UAS release types because the other replications recaptured four or fewer total moths. For the release altitude experiment (expt. 2), all of the 8 replications at each release altitude were used to calculate dispersion by Morisita's index as recaptures were consistently sufficient for analysis.

In contrast, many replications from the release time experiment (expt. 3) were eliminated from analysis because they did not meet the criteria for calculating an accurate Morisita's index

(release times of 11:00 [8 replications], 12:00 [10 replications], 15:00 [12 replications], 18:00 [4 replications], 19:00 [8 replications], 21:00 [10 replications] were tested) because they failed to recapture enough moths for analysis thresholds. At the 11:00 release time one replication did not recapture any moths, at the 15:00 release time two replications did not recapture any sterile moths, and at 19:00 two replications did not recapture any moths. Of the remaining seven replications from the 11:00 release, only four captured enough sterile moths for absolute Iδ analysis, and five for Iδ of % of total analysis. All 10 replications from the 12:00 release, and all four replications from the 18:00 release recaptured sufficient moths for analysis. For absolute Iδ of 15:00 releases, only five replications could be used, but for % of total Iδ analysis, nine replications were analyzed. From the release at 19:00, only three replications recaptured sufficient numbers of sterile *C. pomonella* for absolute Iδ analysis, and four could be used for % of total analysis. For absolute capture analysis with Iδ of releases at 21:00, nine of the replications recaptured enough sterile codling moths to be included in analysis, and all 10 replications were used for Iδ analysis of the % of total analysis.

Distance: Additionally, as traps were at fixed distances from the center of blocks in all three experiments, it was possible to determine if different numbers of moths from each treatment were recaptured in traps located at the various distances extending out from the center of blocks. Each trapping grid had six trap distances [15m (2 traps); 33.5m (4 traps); 45m (2 traps); 54m (4 traps); 62m (4 traps); 75m (4 traps)] from the center of the plot. The recapture per distance was Arcsine transformed and ANOVA used to determine if there were differences in recapture among and within treatments at each distance. Treatment and distance effects of the multiple pairwise comparisons were separated with post hoc Tukey's HSD test (P=0.05).

RESULTS

Experiment 1: Release Location by UAS vs. Hand

Recapture: There were significant differences in the recapture of moths released by the four methods (F=4.8407, df=3, P=0.0047) (Table 2.1). Specifically, a significantly lower recapture of 0.8%±0.3 was recorded in hand-nine uniform points release plots compared to the highest recapture of 3.6%±0.7 in the UAS evenly spread plots. There were no significant differences in recapture between the hand-center release (1.6%±0.7) and hand-nine uniform points release, the hand-center release and UAS Center release (2.5%±0.6) or the hand-center release and the UAS uniform release.

Dispersion: There were significant differences in dispersion based on Iδ indices calculated using the absolute number recaptured in traps for the four treatments (F=2.9316, df=3, P=0.0431) (Table 2.1). Fisher's LSD test revealed that differences in aggregation were between hand-center (mean Iδ=2.64±0.44) and hand-nine uniform points (mean Iδ= 1.68±0.22), and UAS-uniform (mean Iδ=1.43±0.14) releases, indicating that sterile moths released at the center of the orchard were significantly more aggregated around the central point of release than those released in a spread out pattern. Moths released by hand-at nine uniformly spaced points and UAS-center (mean Iδ= 1.87±0.27) were not significantly more or less aggregated from each other or the hand center release or UAS uniform release point treatments.

There were significant differences in dispersion based on Iδ indices calculated using the proportion of total recaptured sterile *C. pomonella* (F=4.021, df=3, P=0.012) (Table 2.1). Moths released by hand at the center of blocks were highly aggregated (average Iδ=3.25±0.56), those released by hand at nine uniform points were more dispersed (average Iδ=2.36±0.44), followed

by releases by UAS at the center (average Iδ=1.94±0.28), and the greatest dispersion was for moths released by UAS spread throughout the orchard approximating the hand release at nine uniformly spaced points (average Iδ=1.40±0.17). However, Fisher's LSD test revealed that only moths released by hand at the center were significantly more aggregated on average than those released by both UAS methods. There were no significant differences in dispersion using proportion recapture found between the two hand releases, nor between the two UAS releases.

	Release method	*ANOVA*	*Mean (± SEM)*
Percent Recapture	**Hand release – Center**	F=4.8407 df=3, 52 P=0.0047	1.6 ± 0.7 a,b
	Hand release – 9 Points		0.8 ± 0.3 b
	UAS release – Center		2.5 ± 0.6 a,b
	UAS release - ~9 Points		3.6 ± 0.7 a
Iδ – Absolute	**Hand release – Center**	F=2.9316 df=3, 47 P=0.0431	2.64 ± 0.44 a
	Hand release – 9 Points		1.74 ± 0.28 b
	UAS release – Center		1.87 ± 0.27 a,b
	UAS release - ~9 Points		1.43 ± 0.14 b
Iδ - % of Total Captured	**Hand release – Center**	F=4.0213 df=3, 48 P=0.0124	3.25 ± 0.56 a
	Hand release – 9 Points		2.36 ± 0.44 a,b
	UAS release – Center		1.94 ± 0.28 b
	UAS release - ~9 Points		1.40 ± 0.17 b

Table 2.1. Mean (±SEM) % recapture and Iδ for sterile *C. pomonella* released by four methods. Means with the same letters are not significantly different (Tukey's α = 0.05)

Distance: There were significant differences in recapture by distance from the center of the orchard (F=4.6925, df=5, P=0.0004) for moths released by hand at a single central location (Table 2.2). More moths released at the center of test orchards were recaptured at the closest traps (15m) than at traps 62m, and 75m away (Table 2.2). In addition, moths were significantly more likely to be captured in traps 33.5m from the release than those 75m from the center. The effect of distance from the center on recapture within treatments was variable. Although average recapture was low at all trap distances from the center (Table 2.2), there were no significant

differences in capture by distance (F=1.5887, df=5, P=0.1634) when moths were released at nine uniformly spaced locations. Sterile *C. pomonella* released by UAS at the center of the orchards were significantly more likely (F=3.8202, df=5, P=0.0023) to be captured in traps 15m from the center than those 54m and 75m away (Table 2.2). In addition, they were less likely to be

			Distance from center					
Release method		**ANOVA**	***15m***	***33.5m***	***45m***	***54m***	***62m***	***75m***
	Hand release-Center	F=4.6925 df=5, 274 P=0.0004	13.4 ± 3.5 a	7.3 ± 1.4 a	8.3 ± 3.6 a,b	6.5 ± 2.0 a,b	4.3 ± 1.0 b	3.4 ± 1.3 b
	Hand release-9 Points	F=1.5887 df=5, 274 P=0.1634	4.7 ± 1.2	3.4 ± 0.7	3.5 ± 1.2	3.0 ± 0.7	3.3 ± 0.8	2.2 ± 0.7
	UAS release-Center	F=3.8202 df=5, 274 P=0.0023	17.6 ± 3.7 a	14.3 ± 2.5 a,c	7.6 ± 2.0 a,b	7.4 ± 1.5 b,c	9.6 ± 2.1 a,b	5.7 ± 1.0 b
	UAS release-~9 Points	F=0.7507 df=5, 274 P=0.5862	15.5 ± 2.3	15.5 ± 1.7	13.5 ± 2.0	14.8 ± 1.9	13.3 ± 2.2	13.3 ± 1.9

Table 2.2. Comparison of mean (±SEM) number of sterile *C. pomonella* recaptured at distances from the center of orchard plot when released by four different methods. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

recaptured in traps 75m from the center than in traps 33.5m away from the point of release. There were no significant recapture differences by distance from the center of the block when moths were released by UAS approximating nine uniformly spaced points in the orchards (F=0.7507, df=5, P=0.5862); recapture was highest at all distances for this release method among the treatments (Table 2.2).

The effect of distance from the center on recapture between treatments also was variable. There were significantly different treatment impacts (Table 2.3) on moths recaptured 15m from the center of the orchard (F=5.2226, df=3, P=0.0021). Fewer moths were captured at

this distance when they were released by hand at nine uniform locations than by UAS at the center, and UAS approximating the nine locations (Table 2.3). There were significant treatment effects (Table 2.3) on recapture of moths at 33.5m from the center of the orchard (F=15.9328, df=3, P<<0.0001). Fewer sterile *C. pomonella* were recaptured when they were released by hand at nine uniformly spaced points than by UAS at the center, and by UAS approximating nine uniformly spaced points. There were also significantly fewer recaptured when they were released by hand at the center than UAS at the center, or UAS at nine uniformly spaced locations. There were no significant differences in recapture between the two hand released methods at this distance from the center. At a distance of 45m from the center of the orchard, there were significant differences in recapture of moths by treatment (F=6.0036, df=3, P=0.0008); fewer moths were recaptured when released by hand at a single central location and released by hand at nine uniformly spaced points than when they were released by UAS approximating nine uniformly spaced points (Table 2.3). There were significant differences (F=14.5591, df=3, P<<0.0001) in recapture of released moths among the four treatments at a distance of 54m from the center of the orchard (Table 2.3). Traps at this distance recaptured significantly more sterile codling moths when they were released by UAV in a pattern approximating nine uniformly spaced points than by hand at the center, by UAS at the center, and by hand at nine points. Also, significantly fewer moths released by hand at nine uniformly spaced locations were recaptured than those released by UAS at the center of the orchard, and by UAS approximating nine uniformly spaced points. Additionally, significantly fewer of the moths released by UAS at the center were recaptured than those by UAS approximating nine uniformly spaced points. There were significant differences (F=11.5004, df=3, P<<0.0001) in recapture of moths among release

strategies at a distance of 62m from the center of the orchard (Table 2.3). Fewer moths released by hand at the center of the orchard were recaptured than moths released by either UAS at the

Distance from release	Release method	# traps at distance	ANOVA	*Mean (± SEM) recapture by distance*
15m	**Hand release – Center**	2	F=5.2226 df=3, 108 P=0.0021	13.4 ± 3.5 a,b
	Hand release – 9 Points	2		4.7 ± 1.2 b
	UAS release – Center	2		17.6 ± 3.7 a
	UAS release - ~9 Points	2		15.5 ± 2.3 a
33.5m	**Hand release – Center**	4	F=15.9328 df=3, 220 P<<0.0001	7.3 ± 1.4 a
	Hand release – 9 Points	4		3.4 ± 0.7 a
	UAS release – Center	4		14.3 ± 2.5 b
	UAS release - ~9 Points	4		15.5 ± 1.7 b
45m	**Hand release – Center**	2	F=6.0036 df=3, 108 P=0.0008	8.3 ± 3.6 a
	Hand release – 9 Points	2		3.5 ± 1.2 a
	UAS release – Center	2		7.6 ± 2. 0 a,b
	UAS release - ~9 Points	2		13.5 ± 2.0 b
54m	**Hand release – Center**	4	F=14.5591 df=3,220 P<<0.0001	6.5 ± 2.0 a,b
	Hand release – 9 Points	4		3.0 ± 0.7 b
	UAS release – Center	4		7.4 ± 1.5 a
	UAS release - ~9 Points	4		14.8 ± 1.9 c
62m	**Hand release – Center**	4	F=11.5004 df=3, 220 P<<0.0001	4.3 ± 1.0 a
	Hand release – 9 Points	4		3.3 ± 0.8 a
	UAS release – Center	4		9.6 ± 2.1 b
	UAS release - ~9 Points	4		13.3 ± 2.2 b
75m	**Hand release – Center**	4	F=19.7768 df=3, 220 P<<0.0001	3.4 ± 1.3 a
	Hand release – 9 Points	4		2.2 ± 0.6 a
	UAS release – Center	4		5.7 ± 1.0 b
	UAS release - ~9 Points	4		13.3 ± 1.9 c

Table 2.3. Comparison of four treatments' mean (±SEM) number of sterile *C. pomonella* captured at distances from the center of the orchard plot when released by four methods. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

center or UAS approximating the nine uniformly spaced points. Likewise, Tukey's HSD test showed that significantly fewer moths released by hand at nine uniformly spaced points were recaptured than when moths were released both by UAS at the center and UAS approximating the nine uniformly spaced points. At a distance of 75m from the center of the orchard, there

were significant differences (F=19.7768, df=3, P<<0.0001) in recapture of moths among the treatments (Table 2.3). As indicated by Tukey's HSD test of recapture of sterile *C. pomonella* at this distance from the center of the orchard, more moths released by the UAS approximating nine uniformly spaced points were recaptured than those released by hand at the center, hand at nine uniformly spaced points, and UAS at the center of the orchard. Also, significantly fewer moths released by hand at the center and by hand at nine uniformly spaced locations were recaptured than those released by UAS at the center of the block.

Experiment 2: Release Altitude

Recapture: There were no significant differences in recapture when moths were released by UAS at 30-35m altitude, 20-25m altitude, 10-15m altitude, or by hand at 0-5m altitude (F=1.0562, df=3, P=0.3833). The mean proportion of moths recaptured did not exceed 2.5% in any treatment (Table 2.4).

Dispersion: There were no significant differences found among the four treatments based on absolute aggregation (F=0.5726, df=3, P=0.6377) or aggregation by percent of recapture (F=0.815, df=3, P=0.4964) Overall, all four release strategies for releasing sterile *C. pomonella* centrally resulted in 1.1-2.4% recapture and moderate aggregation around the point of release (Table 2.4).

	Release altitude	*# Reps*	*ANOVA*	*Mean (± SEM)*
Percent Recapture	0-5 m (hand)	8	F=1.0562 df=3, 28 P=0.3833	2.4 ± 0.62
	10-15 m (UAS)	8		2.1 ± 0.70
	20-25 m (UAS)	8		1.1 ± 0.39
	30-35 m (UAS)	8		1.5 ± 0.60
Iδ – Absolute	0-5 m (hand)	8	F=0.5726 df=3, 28 P=0.6377	2.27 ± 0.34
	10-15 m (UAS)	8		2.14 ± 0.24
	20-25 m (UAS)	8		2.90 ± 0.48
	30-35 m (UAS)	8		2.25 ± 0.65
Iδ - % of Total Captured	0-5 m (hand)	8	F=0.815 df=3, 28 P=0.4964	2.32 ± 0.36
	10-15 m (UAS)	8		2.18 ± 0.28
	20-25 m (UAS)	8		3.20 ± 0.58
	30-35 m (UAS)	8		2.66 ± 0.67

Table 2.4. Mean (±SEM) % recapture and Iδ for sterile *C. pomonella* released at four altitudes. Means with the same letters are not significantly different (Tukey's α = 0.05).

Distance: Three of the four release strategies resulted in significant differences in recapture by distance (Table 2.5). There were significant differences in recapture by distance

			Distance from center					
		ANOVA	***15m***	***33.5m***	***45m***	***54m***	***62m***	***75m***
Altitude of release	**0-5 m (hand)**	**F=9.9738 df=5, 154 P<<0.0001**	27.3±5.4 a	12.8±2.3 b	10.9±4.0 b,c	5.4±1.1 b,c	5.5±1.2 b,c	4.3±1.1 c
	10-15 m (UAS)	**F=4.5111 df=5, 154 P=0.0007**	19.0±3.8 a	8.3±1.8 b	9.39±3.6 a,b	8.7±1.9 b	6.0±1.6 b	4.0±0.8 b
	20-25 m (UAS)	**F=1.8878 df=5, 154 P=0.0995**	8.4±2.7	5.3±1.8	5.3±1.5	3.7±0.99	4.1±1.4	2.4±0.6
	30-35 m (UAS)	**F=4.5364 df=5, 154 P=0.0007**	13.9±4.3 a	9.0±2.2 a,b	2.8±1.1 b	3.8±1.1 b	5.2±1.7 b	3.0±0.6 b

Table 2.5. Comparison of mean (±SEM) number of sterile *C. pomonella* recaptured at distances from the central release point by release at each of four altitudes. Means with the same letters are not significantly different (Tukey's α = 0.05).

when sterile *C. pomonella* were centrally released by hand at 0-5m altitude (F=9.9738, df=5, P<<0.0001), by UAS at 10-15m altitude (F=4.5111, df=5, P=0.0007), and by UAS at 30-35m altitude (F=4.5364, df=5, P=0.0007). Hand release resulted in traps at 33.5m recapturing significantly more moths than those at 75m. Following release by UAS at 10-15m altitude, traps closest to the release point (15m) recaptured significantly more sterile *C. pomonella* than traps at 33.5m, 54m, 62m, and 75m. Following release by UAS at 30-35m altitude, more sterile codling moths were recaptured in traps 15m from the center than in traps 45m, 54m, 62m, and 75m away. Although the one-way analysis of variance did not indicate significant differences in recapture by distance following UAS release at 20-25m altitude (F=1.8878, df=5, P=0.0995), there were more moths recaptured in traps closest to the center of the block than in those furthest from the center.

Four of the six release distances resulted in significant differences in recapture by release altitude (Table 2.6). There were significant differences among the four altitude treatments in recapture in traps positioned 15m from the center of the orchard (F=3.7447, df=3, P=0.0156), 33.5m from the center of the orchard (F=3.7769, df=3, P=0.0124), 45m from the center of the orchard (F=2.7276, df=3, P=0.0518) and 54m from the center of the orchard (F=2.7276, df=3, P=0.0518). Recapture of moths in traps located 15m from the center of the orchard were higher following release by hand at 0-5m altitude compared to UAS at 20-25m altitude. More sterile *C. pomonella* were recaptured at a distance of 33.5m from the central release location when they were released at 0-5m altitude than when they were released at 20-25m altitude. More sterile codling moths were recaptured in traps placed 45m from the center of the orchard following hand-release from 0-5m than UAS at 30-35m altitude, and more moths were recaptured in traps

located 54m from the center of the orchard following release at 10-15m above the canopy compared to release 20-25m above the canopy. There were no treatment differences in recapture among the four release strategies in traps positioned 62m from the center of the orchard (F=0.8463, df=3, P=0.4711) or 75m from the center of the orchard (F=1.2028, df=3, P=0.3116).

Distance from release	Treatment (Altitude)	# traps at distance	ANOVA	*Mean (± SEM) recapture at distance*
15 meters	**0-5 m (hand)**	2	F=3.7447 df=3, 60 P=0.0156	27.3 ± 5.4 a
	10-15 m (UAS)	2		19.0 ± 3.8 a,b
	20-25 m (UAS)	2		8.4 ± 2.7 b
	30-35 m (UAS)	2		13.9 ± 4.3 a,b
33.5 meters	**0-5 m (hand)**	4	F=3.7769 df=3, 124 P=0.0124	12.8 ± 2.3 a
	10-15 m (UAS)	4		8.2 ± 1.8 a,b
	20-25 m (UAS)	4		5.3 ± 1.8 b
	30-35 m (UAS)	4		9.0 ± 2.2 a,b
45 meters	**0-5 m (hand)**	2	F=2.7276 df=3, 60 P=0.0518	10.9 ± 4.0 a
	10-15 m (UAS)	2		9.3 ± 3.6 a,b
	20-25 m (UAS)	2		5.3 ± 1.5 a,b
	30-35 m (UAS)	2		2.8 ± 1.1 b
54 meters	**0-5 m (hand)**	4	F=3.3041 df=3, 124 P=0.0226	5.4 ± 1.1 a,b
	10-15 m (UAS)	4		8.7 ± 1.9 a
	20-25 m (UAS)	4		3.7 ± 0.99 b
	30-35 m (UAS)	4		3.8 ± 1.0 a,b
62 meters	**0-5 m (hand)**	4	F=0.8463 df=3, 124 P=0.4711	5.5 ± 1.20
	10-15 m (UAS)	4		6.0 ± 1.60
	20-25 m (UAS)	4		4.1 ± 1.40
	30-35 m (UAS)	4		5.2 ± 1.70
75 meters	**0-5 m (hand)**	4	F=1.2028 df=3, 124 P=0.3116	4.3 ± 1.1
	10-15 m (UAS)	4		4.0 ± 0.8
	20-25 m (UAS)	4		2.4 ± 0.6
	30-35 m (UAS)	4		3.0 ± 0.6

Table 2.6. Comparison of four treatments' mean (±SEM) number of sterile *C. pomonella* captured at distances from the center of the orchard plot when released at four altitudes. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

Experiment 3: Release Time

Recapture: There were significant treatment differences found in the recapture of moths released at different times of the day (F=4.1328, df=5, P=0.0039). Releases conducted at 12:00 resulted in numerically higher recapture than all other treatments and Tukey's HSD test indicated that significantly more sterile *C. pomonella* were recaptured from releases conducted at 12:00 than releases at 11:00, 15:00, and 19:00 (Table 2.7).

	Treatment	*# Reps*	*ANOVA*	*Mean (± SEM)*
Percent Recapture	**1100 release**	7	F=4.1328 df=5, 41 P=0.0039	1.4 ± 0.9 a
	1200 release	10		3.7 ± 0.8 b
	1500 release	10		1.2 ± 0.6 a
	1800 release	4		1.2 ± 0.7 a,b
	1900 release	6		0.4 ± 0.2 a
	2100 release	10		1.7 ± 0.3 a,b
Iδ – Absolute	**1100 release**	4	F=4.1124 df=5, 29 P=0.0061	1.96 ± 0.38 a
	1200 release	10		1.90 ± 0.19 a
	1500 release	5		1.80 ± 0.21 a
	1800 release	4		2.91 ± 0.40 a
	1900 release	3		8.01 ± 3.56 b
	2100 release	9		3.13 ± 0.88 a
Iδ - % of Total Captured	**1100 release**	5	F=5.7990 df=5, 36 P=0.0005	2.60 ± 0.61 a
	1200 release	10		1.81 ± 0.19 a
	1500 release	9		2.65 ± 0.41 a
	1800 release	4		3.17 ± 0.47 a
	1900 release	4		8.33 ± 2.55 b
	2100 release	10		3.42 ± 0.80 a

Table 2.7. Mean (±SEM) % recapture and Iδ for sterile *C. pomonella* released at six different times of the day. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

Dispersion: Analysis of absolute recaptures revealed significant differences in Iδ indices among the treatments (F=4.1124, df=5, P=0.0061). The highest value and thus extent of aggregation was found for releases conducted at 19:00 – dispersion indices at 21:00, 18:00,

11:00, 12:00, and 15:00 were all significantly lower than at 19:00, but were not different from each other (Table 2.7).

Percent of Replication's Recapture: Similar to the results for absolute capture, time of release had a significant effect on dispersion (F=5.7990, df=5, P=0.0005) when Iδ indices were calculated based on the percent of a replicate's total capture (Table 2.7). The aggregation indices for moths released at 11:00 (Iδ=2.60), 12:00 (Iδ=1.81), 15:00 (Iδ=2.65), 18:00 (Iδ=3.17), and 21:00 (Iδ=3.24) were not significantly different from each other, but all were significantly less aggregated than releases conducted at 19:00 (Iδ=8.33).

Distance: Five of the six release times resulted in significant differences in recapture by distance (Table 2.8). When sterile codling moths were released at 12:00 they were more likely to be captured in traps close to the center of the orchard than traps farther from the center (F=16.8678, df=5, P<<0.0001). Tukey's HSD indicated that traps 15m from the center recaptured significantly more sterile *C. pomonella* than those 33.5m, 45m, 54m, 62m, and 75m away (Table 2.8). Traps 33.5m from the center of the orchard recaptured significantly more sterile *C. pomonella* than those 75m away from the center, and traps 45m from the orchard center recaptured more than those 62m, and 75m away when moths were released at 12:00. Recapture at all distances when moths were released 1500 day was low (Table 2.8), but there were significantly differences in the distance from the center where moths were recaptured (F=2.4175, df=5, P=0.0374). Traps 15m from the center of the orchard were significantly more likely to recapture sterile moths than traps 75m away from the point of release. Captures were low when sterile moths were released at 19:00 (Table 2.8), but there were significant effects globally on recapture based on distance of the trap from the center of the orchard (F=2.6104, df=5,

P=0.0283), and Tukey's HSD revealed that moths were more likely to be recaptured in traps 15m from the release point than moths 54m, 62m, and 75m away. Significantly more *C. pomonella* released at 21:00 were recaptured in traps close to the center of the block (F=12.7680, df=5, P<<0.0001) than in traps farther away (Table 2.8), and Tukey's HSD test showed that traps within 15m of the center of the orchard recaptured significantly more sterile moths than those 33.5m,

			Distance from center					
		ANOVA	**15m**	**33.5m**	**45m**	**54m**	**62m**	**75m**
Time of release	**1100 release**	F=0.9806 df=5, 94 P=0.4339	14.4 ± 6.6	10.4 ± 3.5	5.8 ± 3.7	7.3 ± 2.7	6.7 ± 2.3	4.0 ± 1.7
	1200 release	F=16.8678 df=5, 194 P<<0.0001	40.6 ± 6.7 a	16.15 ± 2.2 b	22.6 ± 4.3 b	11.9 ± 1.9 b,c,d	9.5 ± 1.8 c,d	5.7 ± 1.2 d
	1500 release	F=2.4175 df=5, 194 P=0.0374	9.3 ± 2.8 a	6.5 ± 2.4 a,b	6.3 ± 2.4 a,b	3.9 ± 1.2 a,b	4.3 ± 1.3 a,b	2.1 ± 0.5 b
	1800 release	F=2.5206 df=5, 74 P=0.0366*	9.5 ± 3.1	2.8 ± 0.9	11.4 ± 5.9	5.1 ± 2.8	2.8 ± 1.2	2.12 ± 1.3
	1900 release	F=2.6104 df=5, 114 P=0.0283	6.5 ± 4.0 a	2.0 ± 0.8 a,b	1.9 ± 1.5 a,b	0.5 ± 0.2 b	0.8 ± 0.5 b	1.0 ± 0.6 b
	2100 release	F=12.7680 df=5, 194 P<<0.0001	20.4 ± 4.4 a	9.1 ± 1.8 b	7.1 ± 1.6 b,c	3.7 ± 0.9 c	4.5 ± 0.9 b,c	2.5 ± 0.7 c

Table 2.8. Comparison of six treatments' mean (±SEM) number of sterile *C. pomonella* captured at distances from the center of the orchard plot when released at different times of the day. * indicates global significance but means not significantly different (Tukey's $\alpha = 0.05$).

45m, 54m, 62m, and 75m, and traps 33.5m from the center captured more than those 54m and 75m. Although ANOVA indicated that for the 18:00 time of release, the distance from the central release point moths are recaptured was significant globally (F=2.5206, df=5, P=0.0366), overall

captures were low and the Tukey's test did not reveal significant differences among the trap distances (Table 2.8). There was not a significant effect on recapture at the six trapping distances when sterile moths were released at 11:00 ($F=0.9806$, $df=5$, $P=0.4339$), but numerically there were more captured in traps close to the center.

All six trap distances resulted in significant differences in recapture by time of release (Table 2.9) and generated the following results: 15m from the center ($F=9.4846$, $df=5$, $P<<0.0001$), 33.5m from the center of the orchard ($F=10.4666$, $df=5$, $P<<0.0001$), 45m from the center of the orchard ($F=7.1408$, $df=5$, $P<<0.0001$), 54m from the center of the orchard ($F=11.6886$, $df=5$, $P<<0.0001$), 62m from the center of the orchard ($F=8.1937$, $df=5$, $P<<0.0001$) and 75m from the center of the orchard ($F=5.5968$, $df=5$, $P<<0.0001$). Traps placed 15m from the center recaptured more moths when they were released at 12:00 than at 11:00, 15:00, 18:00, 19:00, and 21:00 (Table 2.9). Also, significantly more moths were recaptured when they were released at 21:00 than 19:00. Traps placed 35m from the center recaptured more moths when they were released at 12:00 than at 15:00, 18:00, 19:00, and 21:00. In addition, moths released at 11:00 and 21:00 were recaptured significantly more than those released at 19:00. Traps placed 45m from the center recaptured more moths when they were released at 12:00 than at 11:00, 15:00, 19:00, and 21:00 (Table 2.9). Traps 54m from the central release point recaptured significantly different numbers of moths depending on the time of the day they were released, and more of the moths released at 12:00 were recaptured than moths that were released at 11:00, 15:00, 18:00, 19:00, and 21:00 (Table 2.9). Also, the 19:00 release had significantly lower recapture than the 1100 release and the 21:00 release. Traps placed 65m from the center recaptured more moths when they were released at 12:00 than at 15:00, 18:00, 19:00, and

Distance from release	Time of release	# traps at distance	ANOVA	*Mean (± SEM) recapture by distance*
15m	**1100**	2	F=9.4846 df=5, 81 P<<0.0001	14.4 ± 6.6 a,c
	1200	2		42.6 ± 6.8 b
	1500	2		9.3 ± 2.8 a,c
	1800	2		9.5 ± 3.0 a,c
	1900	2		6.5± 4.0 c
	2100	2		20.3 ± 4.4 a
33.5m	**1100**	4	F=10.4666 df=5, 174 P<<0.0001	10.4 ± 3.5 a,b
	1200	4		16.1 ± 2.2 b
	1500	4		6.5 ± 2.4 a,c
	1800	4		2.8 ± 0.9 a,c
	1900	4		2.0 ± 0.8 c
	2100	4		9.1 ± 1.8 a
45m	**1100**	2	F=7.1408 df=5, 84 P<<0.0001	5.8 ± 3.7 a
	1200	2		22.6 ± 4.3 b
	1500	2		6.3 ± 2.4 a
	1800	2		11.4 ± 5.9 a,b
	1900	2		1.9 ± 1.5 a
	2100	2		7.1 ± 1.6 a
54m	**1100**	4	F=11.6886 df=5, 174 P<<0.0001	7.3 ± 2.7 a
	1200	4		11.9 ± 1.9 b
	1500	4		3.9 ± 1.2 a,c
	1800	4		5.1 ± 2.8 a,c
	1900	4		0.5 ± 0.2 c
	2100	4		3.7 ± 0.9 a
62m	**1100**	4	F=8.1937 df=5, 174 P<<0.0001	6.7 ± 2.3 a,b,c
	1200	4		9.5 ± 1.8 b
	1500	4		4.3 ± 1.3 a,c
	1800	4		2.8 ± 1.2 a,c
	1900	4		0.8 ± 0.5 a
	2100	4		4.5 ± 0.9 c
75m	**1100**	4	F=5.5968 df=5, 174 P<<0.0001	4.0 ± 1.7 a,b
	1200	4		5.7 ± 1.2 b
	1500	4		2.1 ± 0.5 a
	1800	4		2.2 ± 1.3 a
	1900	4		1.0 ± 0.8 a
	2100	4		2.5 ± 0.7 a

Table 2.9. Comparison of mean (±SEM) number of sterile *C. pomonella* captured at distances from center of orchard plot after release at different times of the day. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

21:00 (Table 2.9). In addition, significantly fewer moths released at 19:00 were recaptured than those released at 21:00. Traps placed 75m from the center recaptured more moths when they were released at 12:00 than at 15:00, 18:00, 19:00, and 21:00 (Table 2.9).

DISCUSSION

There are practical and economic considerations when comparing the use of UAS versus manual releases of sterile *C. pomonella*. A practical advantage of the UAS method over hand release is that pesticide reentry intervals do not impact the ability to release moths in a timely manner. On the other hand, weather conditions and proximity to "no-fly" areas may impede the use of UAS. Under the variable climactic conditions in New Zealand, wind or rain occasionally delayed the application of sterile moths or even resulted in a reversion to ground release (Horner et al. 2020). Finally, because the aircraft used to release the moths are expensive, precautions to avoid a crash are necessary and may impact the cost of the program.

Many SIT programs utilize fixed-wing aircraft to distribute sterilized insects throughout target areas (Tan and Tan, 2013), but due to the high cost of this release method, sterile *C. pomonella* release programs have primarily relied on other methods such as hand release (White et al., 1969, 1973), modified all-terrain vehicles with a release device (Judd et al., 2011), helicopters and modified tractors with release devices (McMechan and Proverbs, 1972), and release from mountain bikes (Horner et al., 2016). Recently, moths have been delivered to target areas using unmanned aerial systems equipped with specially designed release devices (Seymour, 2018). Regardless of the various methods of release employed to deliver sterile insects, the goal is to quickly and uniformly distribute them throughout the target orchards

without compromising quality and survival. Knowing that sterile *C. pomonella* are capable of flights of up to 250m (Adams et al., 2017), at the outset of this study the hypothesis being tested was that on a farm-scale, uniform distribution of moths may not be necessary.

To determine the best means of farm-scale release of sterile *C. pomonella* recapture and dispersion of moths following their deployment by hand or by UAS and from a central location or multiple uniformly spaced sites was compared. The results show that *C. pomonella* adults released by hand at uniformly spaced locations in orchard blocks were recaptured significantly less than when released by UAS at 35m altitude on a flight path approximating the same locations. Although percent recapture was found to be different in releases at/above nine evenly spaced points, there was no difference in the degree of aggregation/dispersion found by either release method (Hand or UAS). However, when hand releases at the center of the orchard were compared to hand releases at nine uniformly spaced points and also UAS release approximating the same points, there was a higher degree of SIT moth aggregation about the center of the block when moths were released by hand at the center than when they were released at nine uniformly spaced points by hand or UAS, indicating that more sterile *C. pomonella* were retained in the targeted orchard block when they were released at the center. In addition, release altitude was not a significant factor in the dispersion or recapture of sterile codling moths.

In contrast, Bouyer et al. (2020) reported that sterile male *Aedes egypti* recapture was strongly impacted by release method and altitude. Deployment of sterile male *A. egypti* from a UAS in Brazil resulted in lower recapture rates of mosquitos compared to deployment by hand and as altitude of release increased so did dispersal from the point of release. The discrepancy with these results may be related to differences in the dispersal capabilities of *C. pomonella*

compared to mosquitoes. *Cydia pomonella* is a stronger flier than mosquitoes with greater maximum dispersive distances (Tremmaterra, 2004; Basoalto et al., 2010; Adams et al., 2017; Verdonschot and Besse-Lototskaya, 2014). The larger size of codling moths may also be beneficial with less impact from winds and forces associated with aerial release.

Moths released at different times of the day behaved generally like those in other trials – when released at the center of the orchard they dispersed throughout the 4.05ha area. Moths released at noon were recaptured at a higher rate than those released at other times of the day while also not having major differences in dispersion. Causes of this may be: 1) moths released late in the evening do not have sufficient time to acclimatize to field conditions before cessation of normal evening activities, thus are subject to 24 hours of potential mortality factors before conditions are appropriate for dispersing, 2) moths released in the middle afternoon are susceptible to shock caused by the rapid transition from chill-coma to >35°C causing either direct mortality or non-lethal damage resulting in low response rates, and 3) moths released at noon do not experience the previous two conditions at their release time and have sufficient time to acclimatize to temperatures and day lengths in the field. Typically, the warmest part of the day at the field sites where these experiments were conducted is from 13:00-17:00. Blomefield and Giliomee (2011) found evidence for why moths released during the heat of the day and late in the evening are not recaptured at high rates: successful mating occurs between 18:00 and 20:00 and longevity decreases with increasing temperatures. Moths released at or after typical evening mating periods do not experience the cues to elicit a mate-finding response. These data show that late afternoon and evening releases should be avoided, and moths should be released by noon the day they are received.

Application costs are a major consideration when implementing any SIT program and are acutely important for commercial applications of the technique by individual farms. Tan and Tan (2013) describe the merits of using UAS for release of SIT targets, but ultimately rejected their use at the time due to the high initial cost of aircraft in excess of US $2 million, but recently costs have decreased considerably, and the technique is now more likely to be used. For example, the costs for releasing *Aedes albopictus* was reduced from $20/ha by hand to $1/ha with UAS (Bouyer et al. 2020).

For the past few years, the New Zealand, British Columbia and Washington State, USA programs have explored UAS as a means of delivering sterile *C. pomonella* to target areas. A UAS flying at about 30 meters above the orchard can distribute 30,000 sterile moths over a 6ha orchard in less than 10 minutes. However, the cost remains high. Season-long release of moths is currently accomplished in Washington State by a commercial applicator for over $1100/ha (Courtney, 2021). Wider adoption of this method of release will require new approaches that focus on improved efficiencies. Releasing only during peak generation flight may provide a significant cost savings and still provide substantial *C. pomonella* population suppression. Similarly, releasing fewer than the currently accepted full release density of 2000 mixed sex sterile moths per hectare may be more cost-effective. SIT is density dependent and thus operates much like pheromone-based mating disruption. With mating disruption, the fraction of added control achieved by applying 1000 rather than only 750 dispensers per acre may not be worth the added cost. The same may be the case with SIT, adding more moths to achieve the theoretical overflooding ratio of 40:1 sterile to wild-type males for eradication may not be worth the additional cost when used in conjunction with other management techniques. Robinson and

Proverbs (1975), theorized that greater than 1000 sterile females/ha would be needed to sufficiently disrupt wild-type males from locating fertile females, though at the time, mating disruption had not yet been developed as a control tactic. Likely, deployment of fewer SIT females than theorized for eradication in combination with synthetic pheromone mating disruption has an additive impact on confusing wild-type males and increasing suppression and control.

These findings reveal that hand-applying sterile *C. pomonella* is a viable option. Hand and aerial release of moths provided similar recapture and dispersal of released moths. Labor costs for hand application versus the costs of paying for a UAS or service to release moths should be compared when deciding which approach is best for a given situation. In the approximately 4ha experimental blocks, an individual could walk from the truck to the pre-marked center of the orchard and back for a hand release in less than five minutes and walk to the nine uniformly spaced locations in 10-15 minutes. In contrast, UAS releases applied moths to 4 ha plots in five minutes from the time the flight began to when it returned for all methods tested.

In conclusion, users of SIT for on-farm control of *C. pomonella* should carefully plan where and when to release moths to maximize their effectiveness. Based on the findings reported herein, a single release at the center of 4 ha orchard either manually or by UAS at any altitude is sufficient to allow moths to disperse independently to the edges of the block while retaining the maximum number of moths within the targeted treatment area. Requiring only a single central release in each 4 ha orchard plot should reduce the cost of application. Additionally, moths should be released prior to noon. If moths are unavailable for release in the morning, applicators should consider holding them in cold storage until the next day.

CHAPTER THREE

Tree Architecture, Orchard Topography, and Use of Protective Netting Influence the Recapture and Dispersion of Hand-Released Sterile Codling Moths

INTRODUCTION

The codling moth, *Cydia pomonella* (L.), is a major pest of apples, pears and walnuts in growing regions throughout Europe, Asia, America, Africa, Australia, and New Zealand (Barnes 1991). Efforts to control *C. pomonella* using broad-spectrum insecticides are challenging due to the loss of effective compounds through restrictions or resistance (Varela et al 1993, Knight et al 1994, Mota-Sanchez et al. 2008). The availability of pheromone-based mating disruption since the early 1990's has provided growers with an effective and environmentally sound alternative for managing this pest (Gut et al. 2019). The technique entails dispersing synthetic sex pheromone into the crop to disrupt normal mate finding behavior, thereby controlling the pest by interfering with reproduction. It has been adopted as the primary control for codling moth on an estimated 243,000 hectares of apples, pears and walnuts worldwide (Gut et al 2019). Mating disruption is most effective when *C. pomonella* populations are low and thus, the approach has generally been integrated into a management program that includes other control tactics.

The sterile insect technique (SIT) is another alternative management strategy to insecticides that is effective at greatly reducing population densities of a number of targeted insect pests, i.e. fruit flies, disease vectoring flies, and moths (Dyck et al. 2005). The approach entails sterilization of large numbers of laboratory reared insects that are subsequently released to compete with and disrupt the normal mating behavior of wild-type pest populations (Knipling, 1955). The goal is to greatly reduce or even eliminate fertile matings and thus offspring (Lance and McInnis 2005). The potential for using sterile insects to suppress or eradicate *C. pomonella* populations was first explored in the 1960's and 1970's in the western US and Canada. Pilot

studies in Washington state demonstrated that mass releasing mixed-sex sterilized *C. pomonella* moths over the course of the growing season resulted in a reduction in overwintering larval densities by up to 92% (Butt et al. 1973, White et al. 1976). Concurrent with the Washington research, Canadian scientists were exploring the use of the sterile insect technique (SIT) to control *C. pomonella* throughout south central British Columbia; their efforts primarily focused on determining the overflooding ratio and release frequency required to achieve eradication (Proverbs et al. 1966, 1967, 1969, 1975, 1982). A pilot study demonstrated that *C. pomonella* population densities could be substantially lowered and maintained below damage thresholds by initially reducing population densities with insecticides and host removal, followed by the release of sterile insects 2-3 times weekly over the course of the season (Proverbs et al 1982). This study provided the impetus to embark on an area-wide project to eliminate *C. pomonella* throughout the pome fruit producing areas of British Columbia where a multimillion-dollar, 16 million sterile moths/week facility was built; about 40% of the ongoing costs of the program are paid by fruit growers charged a per hectare fee, and the remaining 60% of the cost is funded by a portion of property tax revenues collected by the provincial government (Bloem et al 2007, Thistlewood and Judd 2019). The first sterile moths were released on an area-wide basis in 1994 and the government-funded SIT program was carried out through the 1999 growing season with eradication as its ultimate goal. Beginning with the 2000-growing season, the objective of the program shifted to area-wide suppression of *C. pomonella* using a combination of SIT, pheromone-based mating disruption and judicious use of insecticides (Thistlewood and Judd 2019). This intensive SIT suppression program has successfully reduced wild *C. pomonella*

populations by 94%, kept fruit injury to less than 0.2% on 91.5% of the acreage and reduced the amount of insecticides applied for control of this key pest by 96% (https://www.oksir.org/).

Interest in SIT arose from several years of on-farm research using sterile moths from the Canadian facility to explore fundamental and applied questions about *C. pomonella* movement and response to pheromone-baited traps. This work determined that sterile *C. pomonella* males move randomly and quickly throughout an orchard landscape, dispersing in orchards without mating disruption to a maximum distance of ca. 250 m, producing a trapping area of ca. 20 ha around a single CML2-baited ((E,E)-8, 10-dodecadien-1-ol (codlemone)) trap (Adams et al., 2017). In SIT test plots, capture of wild-type males in monitoring traps was reduced or eliminated after the 1st season of releases, resulting in growers opting to withhold insecticides targeting *C. pomonella* within SIT release plots. This outcome indicated that *C. pomonella* SIT holds great potential as a management strategy for this key pest, even when applied to a single block or at the farm-scale. This transition from area-wide eradication to farm scale control, required revisiting the factors that could influence the efficacy of SIT, including the impact of orchard design (tree architecture, netting, etc.) on farm-scale dispersal of released moths.

As a means of improving fruit quality, obtaining high yields, and reducing variability in the crop, modern orchards have adopted substantial changes in orchard design, most notably high planting densities and modification of planting systems and canopy architectures (Wagenmakers, 1991). Vertical and inclined V-trellis systems are among the most common orchard systems currently used to produce fresh market apples. Trellised systems have compact canopies and improved light exposure compared to free standing systems (Stephan et al., 2008). Additionally, these high-density systems, have as many as 4500 trees/hectare. Trellised systems are often

referred to as a fruiting wall because trees are trained along a trellis wire in a vertical orientation to maximize light penetration (Wagenmakers, 1991). Vertical trellised blocks have trees growing in a single row in the same plane, and V-trellised blocks alternate every other tree growing at the opposite angle forming a V-shaped row. Standard planted blocks are characterized as having rows of single-planted, large, old (>30 years) trees, typically in densities as low as 364 trees/acre. The compact canopies associated with trellised systems may impact the movement of pest species, including potential candidates for SIT. For example, hedgerow barriers limited the movement of *Grapholita molesta*, with few moths able to navigate through or over the hedgerow (Garcia-Salazar et al. 2007).

Over the past few decades, the use of exclusion netting has increased worldwide as a means of preventing damage to apple and other horticultural crops from hail, wind, frost, sunburn, insects, birds, and frugivorous bats (Iglesias and Allegre 2006, Manja and Aoun 2019). Several studies have demonstrated the potential of netting to reduce damage from *C. pomonella* (Alaphilippe et al. 2016; Baiamonte et al. 2015; Sauphanor et al 2012; Siegwart et al. 2013). Anti-hail nets were found to reduce captures of males in pheromone or virgin-female baited traps and fruit damage at harvest (Tasin et al 2008). Net enclosures are becoming an important means for organic fruit growers to mitigate insect pests and other production issues (Granatstein et al. 2016). The most successful efforts to control *C. pomonella* using netting rely on Alt'carpo nets (www.alt-carpo.com) designed by the French extension service. Complete exclusion systems using this technology applied before bloom have provided substantial levels of *C. pomonella* control, however the costs of installing nets may be disadvantageous to many farmers because they can be as high as 25% of the total planting costs for the first three years and 7% of annual

costs thereafter (Chouinard et al. 2016). A network of 23 orchards relying almost exclusively on Alt'carpo netting for *C. pomonella* control sustained an average 0.2% fruit injury at harvest (Sauphanor et al. 2012). A similar complete exclusion system tested for pest control in southern Quebec from 2012-2016 provided significant control of *C. pomonella* compared to an unnetted control in 3 of 5 years (Chouinard et al. 2017). Interestingly, Sauphanor et al. (2012) found that under laboratory conditions a portion of moths of both sexes were able to pass through the Alt'carpo nets and females were able to lay fertile eggs through the nets when in close contact with a suitable surface to deposit eggs. Low recapture of male moths released in a netted orchard compared to an unnetted orchard led the authors to conjecture that the nets, in part, operate by impeding the reproduction of *C. pomonella* by interfering with male flight and thus their capacity to locate females.

The overall aim of the research presented herein was to develop the information needed to improve the effectiveness of strategies for releasing sterile *C. pomonella* in contemporary trellised or netted orchards, and in orchards planted on steep terrain. To achieve this goal, the distribution and proportion of sterile moths recaptured following release into orchards with different planting systems, slopes, or canopy closures was assessed. The first objective compared moth distributions and recaptures following hand-deployment in 8-foot (2.4 m) or 20-foot (6.1 m) height netted orchards versus open orchards. The second objective compared moth dispersion and recaptures in orchards without netting following hand-deployment of moths in vertical or V-trellised planting systems versus free traditional standing systems. The third objective compared moth dispersion and recaptures in apple orchards with steep slopes and flat slopes.

MATERIALS AND METHODS

Source of sterile moths

Mass-reared sterile *C. pomonella* adults were imported from the Okanagan-Kootenay Sterile Insect Release facility in Osoyoos, British Columbia, Canada for release in test orchards. Permits were obtained to allow the importer to hand-carry containers of sterile moths across the US/Canadian border in British Columbia. Recently eclosed mixed-sex, internally-marked (calico Red) *C. pomonella* were placed by the facility into petri dishes at an approximate 1:1 ratio with 400 males and 400 females, then sterilized with 33 krad of gamma radiation from a Cobalt-60 source, and immediately packed into battery-powered coolers (2.8 Cu. Ft. Portable Fridge/Freezer: Edgestar co. Austin, Texas) held at approximately 5°C for shipment to Washington State. Previous research has verified a range of 47-54% males or approximately a 1:1 ratio of M:F moths in petri dishes packed by the OKSIR facility (Adams et al. 2017). Studies examining the fitness of mass-reared sterilized *C. pomonella* after chilling and transportation have found no significant reduction in flight ability, mating ability, fecundity, fertility or longevity (Carpenter et al. 2013, Bloem et al. 2007, Blomefield et al. 2011).

Handling of sterile moths

Sterile moths arrived at field sites by noon the day they were packed or before noon the day after they were packed and were immediately released into field plots. Sexes could not be separated prior to release. Upon arrival at field sites, moths were dispensed into 540-ml polystyrene cups (Fabri-Kal Corp. Kalamazoo, MI) in batches of up to 4000/cup, colored using ca. 1.25ml/petri dish Dayglo florescent pigments (ECO11 Aurora Pink®, ECO15 Blaze Orange™, ECO18 Signal Green™, ECO19 Horizon Blue™) (DayGlo Color, Cleveland, OH) to uniquely identify

moths released in each orchard block, allowed to warm to ambient temperature, and then released by hand at a pre-marked central location in the blocks. Moths were gently tossed by hand from the containers of colored moths ca. 1-2 m into the canopy of pre-marked trees. Released moths primarily alighted on the leaves and stems of the surrounding trees in all directions, some fell to the ground, but McMechan and Proverbs (1972) found no difference in moth recovery when moths were deployed into trees or on the ground. Moths were released at a density of 1000/ha or 2000/ha.

Experiment 1: Netted versus unnetted orchards

This experiment was designed as an independent measures study and was conducted in 11 4ha apple orchard plots in George (2 sites) and Bridgeport (9 sites), Washington. All 11 blocks at both locations were managed without mating disruption, drip-irrigated, fruit bearing, and were under five years old, rows were spaced 3-4 meters apart with trees planted on trellis at ca. 1m intervals and trained to a tall-spindle system. Orchards in George, WA were grown under shade netting while the orchards in Bridgeport, WA had no netting. One orchard site at George, WA was enclosed with 2.4m high netting, while the other was planted under 6.1m high netting. The nine 4ha blocks in Bridgeport, WA all contained Scilate (Envy) apples on Geneva G.41 rootstock, the 4ha block under 6.1m net in George, WA had Granny Smith apples on Mark rootstock, and the 4ha block under 2.4m net in George, WA had Honeycrisp apples on Mark rootstock. In both netted blocks, fabric fully covered the area above the canopy, reached the ground on two sides parallel to tree rows, and was open on two sides perpendicular to tree rows to allow farm equipment entry to the blocks. The white polyethylene Extenday nets (Extenday

USA, Union Gap, WA) are designed to reduce light intensity by as much as 30% to mitigate the impacts of sunburn and overheating of fruit. Trees grown under both net heights and in blocks without netting were similar in height at approximately 2.4 meters. Thus, the open space above the treetops in the three tested net systems varied, with little to no space above trees in the 2.4 m net, 3-4 meters of space above trees under the 6.1 m net, and unimpeded space above trees in unnetted blocks. Rows in George, WA orchards were oriented approximately East/West while those in Bridgeport, WA were oriented approximately North/South.

Due to limited availability of appropriate orchards and moths for release, releases of marked *C. pomonella* were replicated over the course of the experiment as follows: netted orchards 2018 (2 times) 2019 (12 times), not netted orchards 2020 (14 times). Moths were released at a density of 800/acre in George, WA field sites and at 400/acre in Bridgeport, WA. The lower release density was used at the Bridgeport, WA blocks due to a consistently high recapture of moths in the unnetted blocks following initial releases of 800 moth/acre overloading the traps with moths to the extent of interfering with their retention.

Captures of male and female SIT marked moths were quantified using orange Pherocon VI delta traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure. Traps were placed in a 20-trap grid pattern in order to measure dispersion of moths released from the center of the block. Traps were separated by approximately 30 meters (Figure 3.1) and lures were replaced every 6 weeks. To maximize catch, traps were either placed within the top 1/3 of pre-marked trees (Yothers, 1927, Riedl et al 1979), or hung on the trellis wire at approximately the same height. Trap liners were collected once

weekly, numbers of wild-type and marked sterile moths captured were sexed and recorded in the laboratory.

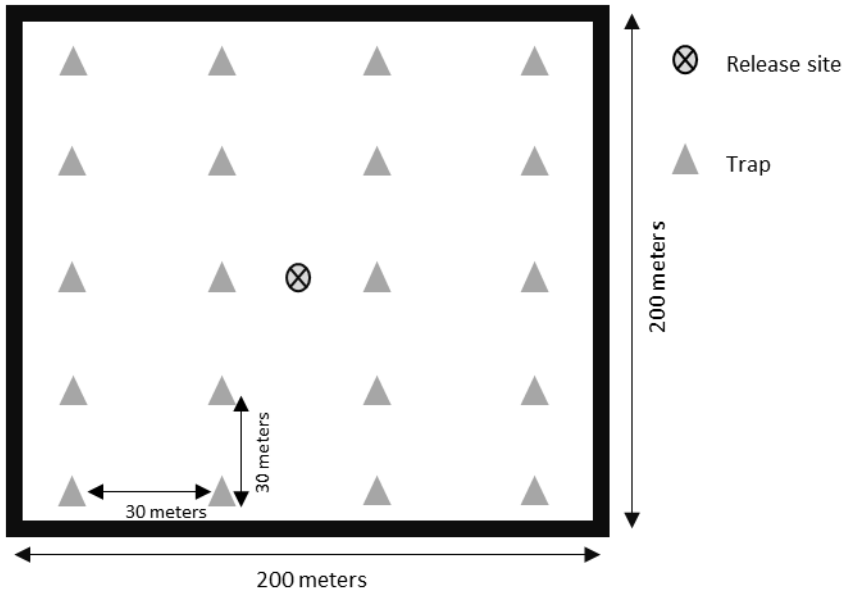

Figure 3.1. Layout of 20 traps in blocks with shade netting and without netting.

Experiment 2: Trellised versus free standing orchards under pheromone mating disruption

This experiment was an independent measures design to quantify the effect of three different orchard canopy structures on *C. pomonella* movement. It was conducted in 11 commercial apple orchard blocks located near Brewster, WA; each treatment orchard block was a 4ha section within a larger contiguous farm orchard. Three canopy structure treatments were analyzed: 1) standard planted free-standing single trees trained using the central leader system planted in 6.1m wide rows 3m apart, and 4-4.5m tall (control, 4 orchards); 2) "vertical trellis" with trees trained to tall spindle and planted in 3m rows 0.9m apart, and 2.4m tall (4 orchards); and 3) V-trellis with trees planted in 3.7m rows 0.6m apart, and 3.6m tall (3 orchards). Standard planted blocks are typically planted in densities as low as 364 trees/acre, whereas vertical

trellised systems can have tree densities as high as 1452 trees/acre, and V-trellised blocks can have densities as high as 1815 trees/acre (Willett, MJ, Pers. Comm.). Trellised systems are often referred to as a fruiting wall because trees are trained along a trellis wire in a planar orientation to maximize light penetration (Wagenmakers, 1991).

All experimental orchards were subject to existing commercial management systems and practices, including various forms of irrigation, pruning, and pest management treatments. Orchards were planted with several apple varieties (predominantly Granny Smith, Honeycrisp, and Gala) on various rootstocks, but all blocks had rows oriented approximately North/South. Additionally, all orchards were treated with pheromone mating disruption using several products, including active emitters (i.e. ISOMATE® CM Mist Plus (Vancouver, WA)) at 0.5-1/ac, or passive dispensers (i.e. ISOMATE® CM Flex, and Scentry NoMate® CM Spiral (Billings, MT)) at 300-400/ac throughout the experiment from 2018-2019.

Each 4ha treatment block received 2000 sterile codling moths/hectare/release. Releases were conducted 18 times for tall spindle, 18 times for V-trellis, and 19 times for control throughout 2018 and 2019 because there were a limited number of orchards available for releases of sterile moths. Moth releases were alternated among replicate blocks so that test plots received a new replicated release of moths dyed with a unique color at most every third week throughout the study period to prevent overlapping of released populations (traps were monitored for two weeks following release). Moths were externally marked using Dayglo pigmented powders as previously described and set free at central locations in apple orchards. Moth dispersal from the point of release was compared in the three training systems to

determine if *C. pomonella* have a directional preference up and down or across rows, as well as if there were differences in capture among the three treatments.

Thirty-two traps, baited as previously described, were placed in a concentric trapping pattern similar to that of Turchin and Theony (1993) to measure the dispersion and direction of moths released from the center of the block. Traps were placed in 8 transects radiating from the point of release; each transect corresponded to a cardinal direction (Fig. 3.2). Traps oriented to the North and South were in line with the orientation of the rows, while those to the East and West were across rows. Traps were placed approximately 10 meters apart within the top 1/3 of pre-marked apple trees or on a trellis wire approximating the top 1/3 of the tree to maximize catch (Yothers, 1927, Riedl et al 1979). Lures were replaced according to manufacturer specifications every 6 weeks and trap liners were collected once weekly for two weeks after releases. Trap liners were examined under UV light in the laboratory to separate colors of fluorescent marked moths and sexed sterile and wild moths were recorded.

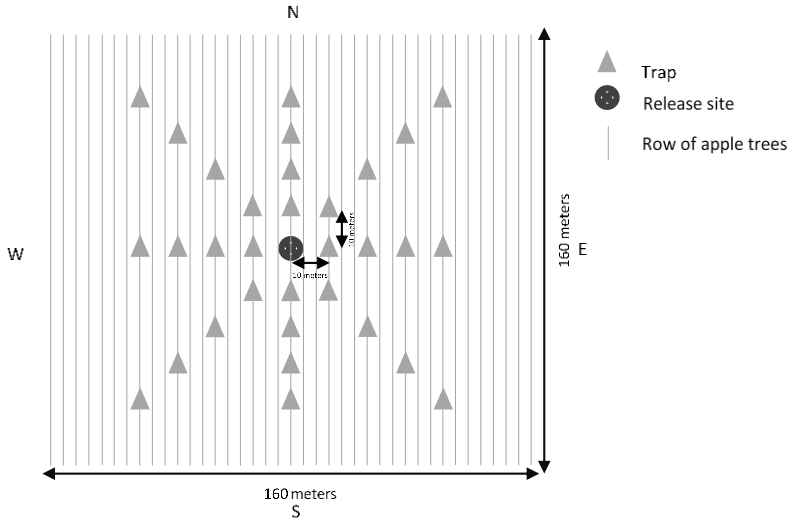

Figure 3.2. Pattern of single central release with a concentric trapping grid. Thirty-two Pherocon VI Delta traps baited with CM-DA COMBO™ Lure + AA Lure and replaceable sticky liner were checked weekly for two weeks after release. 2000 moths/hectare were released weekly.

Experiment 3: Steep slope vs flat planar orchards

This experiment was an independent measures design to quantity the effect of orchard slope on *C. pomonella* movement. It was conducted in 4 commercial apple orchard blocks located near Brewster, WA; each treatment orchard block was a 4ha section within a larger, ca. 4800ha, farm orchard. Two orchard slope treatments were analyzed: 1) orchards on flat ground with an average slope of less than 1° (2 blocks); and 2) orchards on a hill with an average of 14° slope (2 blocks). Blocks with steep slopes were trained to vertical trellis in a super spindle configuration, while those on flat ground were free standing in a central leader configuration. Both orchard types had rows oriented approximately North to South, but those on the hill were oriented up and down the hill.

As in experiment 2, these trials were conducted in commercial apple orchards with variable apple cultivars, rootstocks, pruning, irrigation and management. All orchards had mating disruption with active emitters (ISOMATE® CM Mist Plus (Vancouver, WA)) deployed at 0.5-1/ac. Releases of sterile codling moths at a density of 2000 sterile moths/hectare/release were conducted 18 times for each topographical treatment throughout the summer 2019 growing season. Similar to both previous experiments, releases were made at pre-marked central locations in the orchards (Fig. 3.1), and moth recapture and dispersal was compared to determine if moths' dispersal is different when released in blocks with hills than when released on flat ground. Moths were recaptured in the same trap and lure combination as described in both previous experiments. Traps were placed in the same 20-trap configuration as in experiment 1 (Fig. 3.1), and liners were replaced 7 and 14 days after release.

DATA ANALYSIS

Recapture: Sterile codling moth recaptures in baited traps were used to determine if released moths disperse differently in the presence or absence of exclusion netting, differential open space between the tree canopy and top net, in three different tree training systems, and steep slopes vs planar orchards. The mean percentage of sterile moths captured in each experiment was used to compare treatment effects. Percent of moths captured was normalized using Log(x+1) transformation to reduce heteroscedasticity and then subjected to ANOVA to determine if there were treatment differences. Post hoc Tukey's HSD tests ($p<0.05$) were performed to determine which treatment means were different.

Aggregation: Morisita's index of dispersion (Morisita, 1959 and 1962) was used to measure the degree to which moths aggregated or dispersed in the three experiments: 1) exclusion netting, 2) orchard training systems, and 3) orchard slopes. The Morisita formula is: $I\delta=n(\sum(xi2)-\sum(xi))/(\sum(xi)2-\sum(xi))$ where n = the number of traps and xi = the capture in individual traps. With this index, values of <1 indicates random dispersion, =1 is an even dispersion, and >1 suggests an aggregated dispersion; high Iδ values (maximum of n traps) indicate strongly aggregated populations. When captures in traps are low (i.e. less than 5 total moths captured in all traps) or all traps from a single replicate individually recapture one and/or zero moths, the index may be inaccurate (Amaral et al., 2014). In addition, if all captures from a single replication are concentrated in 1-2 traps, high Iδ values are returned, and this may skew the results. In order to minimize inaccuracy in this measure in experiments 1 and 3, Iδ was calculated for replicates in which capture was greater than five total moths and was not calculated when all traps individually captured 1 or 0 moths. Only one replication from blocks with 6.1 m high nets

(experiment 1) did not meet this threshold for analysis and was excluded. Six replicates conducted on a flat slope (experiment 3) violated minimum capture criteria for Iδ analysis, leaving 12 valid replications. To mitigate inaccuracy due to low catches in experiment 2 replicates, Iδ was calculated when at least 33 total moths were recaptured from the 32 traps; resulting in a total of 14 valid replications from standard planted treatments, and 17 each from the trellised treatments.

For experiments 1 and 3, Iδ indices were calculated two ways: 1) using absolute number of recaptured moths/trap/number released by replicate, and 2) using the percent recaptured/trap out of the total number of moths recaptured for each replicate and treatment. For experiment 2, Iδ was calculated using the absolute number of moths recaptured. Significant treatment effects on dispersion based on Iδ indices were determined using ANOVA to test the assumption that *C. pomonella* will not aggregate differently under nets, on slopes, or in each training system. To assess the degree to which *C. pomonella* aggregation was affected by slope, training system or net height, post hoc Fisher's LSD test (P=0.05) was used to separate treatment effects.

Distance: For experiments 1 and 2, differences in aggregation and dispersion were assessed by calculating the numbers of moths recaptured at selected distances from the central point of release. Trapping grids in experiment 1 consisted of 20 traps placed at six trap distances [15 meters (2 traps); 33.5 meters (4 traps); 45 meters (2 traps); 54 meters (4 traps); 62 meters (4 traps); 75 meters (4 traps)] from the central release point. Experiment 2 trapping grids had four trap distances (10 m, 20 m, 30 m, 40 m) each with eight traps. The mean percent sterile moth recapture was calculated for each distance and Arcsin(√(x)/100) transformed. An ANOVA was

conducted on Arcsin transformed recapture numbers to determine if there were differences among treatments.

Directionality: The direction of moth dispersion throughout the block was calculated for experiments 2 and 3 to assess whether *C. pomonella* disperse in specific directions in orchards planted in the different training system or with and without slopes. For experiment 2, the degree to which *C. pomonella* disperse along each transect in each orchard training system was quantified: traps were placed in 8 transects radiating out from the point of release, so dispersion in each of the 8 cardinal directions could be compared. Mean percent recaptures from each training system transect were Arcsin(√(x)/100) transformed and then analyzed using ANOVA to determine whether moths disperse more up and down or across rows, or exhibit a preference based on cardinal direction. Post hoc Tukey's tests (P=0.05) were used to separate means.

For experiment 3, whether moths were more likely to disburse uphill, downhill, or across the slope in either direction from the point of release was tested. Captures were also assessed in corresponding traps in blocks with flat slopes. Because there were four rows with five traps each (fig. 1), there were 4 traps at the extreme top and bottom of the slopes, and a total of 8 traps below and 8 above the release. Likewise, there were 5 traps at each extreme cross slope direction and 10 total traps on each side of the release point. Using these four general trap locations, it was determined using a t-test if either up or down slope or across the slope in either direction was significantly more likely to capture sterile moths in the two slope configurations. The direction of dispersal was not compared between the two slope treatments because captures in flat blocks were low overall.

RESULTS

Experiment 1: Netted versus unnetted orchards

Recapture: The number of moths recaptured were significantly different in the blocks with the two net heights and the blocks without nets, (F=14.401, df=2, P<<0.001). Results of Tukey's test showed that significantly more moths were recaptured in blocks without nets than in both blocks with nets, but there were no differences in recapture of moths under the two net heights. The mean percent recapture of moths in the block with the 2.4 m net was 5.8% ± 1.5, in the block with the 6.1 m net was 3.6% ± 1.1, and in blocks without nets was 11.5% ± 0.6 (Table 3.1).

	Net Height	***# Replicates***	***ANOVA***	***Mean (± SEM)***
Percent Recapture	2.4 meters	14	F=14.401	5.7% ± 1.5 *b*
	6.1 meters	14	df=2, 39	3.6% ± 1.1 *b*
	No net	14	P<<0.001	11.5% ± 0.6 *a*
iMor – Absolute	2.4 meters	14	F=7.211	2.80 ± 0.41 *a*
	6.1 meters	13	df=2, 38	1.73 ± 0.19 *b*
	No net	14	P=0.002	1.46 ± 0.03 *b*
iMor - % of Total Captured	2.4 meters	14	F=5.822	2.74 ± 0.43 *a*
	6.1 meters	13	df=2, 38	1.96 ± 0.29 *ab*
	No net	14	P=0.006	1.31 ± 0.04 *b*

Table 3.1. Mean (±SEM) % recapture and Iδ for sterile codling moths released in three apple orchard shade net systems.

Aggregation – Absolute Recapture: There were significantly different Iδ indices for the three treatments (F=7.211, df=2, P=0.002) based on absolute moth recapture in traps (Table 3.1). Fisher's LSD test showed that moths released in orchards without nets (Iδ=1.46 ± 0.03) were not significantly more aggregated than those released under 6.1 meter high nets (Iδ=1.73 ± 0.19),

but moths recaptured under 2.4 meter nets (Iδ=2.80 ± 0.41) were significantly more aggregated than those recaptured in blocks under 6.1 m nets and those without nets.

Aggregation – Percent of Replication's Recapture: When removing variability of overall recapture due to environmental conditions, moth quality and other cryptic factors by calculating Iδ based on the percent captured in each trap for each replication, significant differences were found in the extent of aggregation based on Morisita indices (F=5.822, df=2, P=0.006) (Table 3.1). Fisher's LSD test revealed that moths recaptured in blocks without nets (Iδ=1.31 ± 0.03) were significantly less aggregated than those under 2.4 m nets (Iδ=2.74 ± 0.43), but not significantly less aggregated than those recaptured in blocks with 6.1 m nets (Iδ=1.96 ± 0.29), and the degree of population aggregation of the released SIR moths in both netted blocks was not significantly different from each other.

Distance: In terms of recapture of moths at selected distances from the central release point in the blocks, significant differences were found among the treatments at most distances (Table 3.2). At 15 meters from the central release point there were differences in recapture among the treatments (F=21.206, df=2, P<<0.001): Tukey tests showed that significantly fewer moths were recaptured under 6.1 meter high nets (21.3 ± 5.2) than under 2.4 meter high nets (60.4 ± 7.9), or in orchards without nets (50.0 ± 2.8). At 33.5m distant from the release, significant differences were found among the treatments (F=10.149, df=2, P<<0.001): Tukey tests showed that significantly more moths were captured in blocks without nets (34.3 ± 2.1) than blocks with 6.1 meter nets (22.2 ± 3.6), and blocks treated with 2.4m nets (30.6 ± 4.8) were not different than either other treatment. At 45m from the release point, there were no significant differences in recapture among the treatments (F=2.781, df=2, P=0.068). Among the three treatments, traps at

54m from the center of the orchard captured significantly different numbers of sterile moths (F=5.225, df=2, P=0.006); significantly more moths were captured in blocks without nets (14.2 ± 1.2) than in blocks with 6.1m nets (10.4 ± 2.5), but the 2.4m net treatment (15.0 ± 3.5) was not different than either other treatment. At the fifth distance from the central release point, 62m, there were significant differences in recapture among the treatment (F=11.034, df=2, P<<0.001): Tukey tests showed that significantly more moths were recaptured in blocks without nets (22.7 ± 1.6) than in blocks with 2.4m high nets (17.7 ± 3.1) and 6.1m high nets (14.2 ± 2.8), but the two net treatments were not different. Traps at the farthest tested distance from the central release point recaptured significantly different numbers of sterile codling moths among the three net treatments (F=4.553, df=2, P=0.0119): the results of the Tukey's test showed that more moths

Treatment	**Distance from release**	**# traps**	**ANOVA**	***Mean (± SEM)***
No Net	15 meters	2	F=21.206	50.0 ± 2.8 *a*
2.4m net height		2	df=2, 81	60.4 ± 7.9 *a*
6.1m net height		2	P<<0.001	21.3 ± 5.2 *b*
No Net	33.5 meters	4	F=10.149	34.3 ± 2.1 *a*
2.4m net height		4	df=2, 165	30.6 ± 4.8 *ab*
6.1m net height		4	P<<0.001	22.2 ± 3.6 *b*
No Net	45 meters	2	F=2.781	13.4 ± 1.7
2.4m net height		2	df=2, 81	19.6 ± 4.5
6.1m net height		2	P=0.068	10.1 ± 2.9
No Net	54 meters	4	F=5.225	14.2 ± 1.2 *ab*
2.4m net height		4	df=2, 165	15.0 ± 3.5 *a*
6.1m net height		4	P=0.006	10.4 ± 2.5 *b*
No Net	62 meters	4	F=11.034	22.7 ± 1.6 *a*
2.4m net height		4	df=2, 165	17.7 ± 3.1 *b*
6.1m net height		4	P<<0.001	14.2 ± 2.8 *b*
No Net	75 meters	4	F=4.553	11.8 ± 1.0 *a*
2.4m net height		4	df=2, 165	11.6 ± 2.5 *ab*
6.1m net height		4	P=0.012	9.9 ± 2.0 *b*

Table 3.2. Mean (±SEM) number of sterile codling moths captured at distances from the point of release under different heights of shade netting in apple orchards.

were recaptured in traps from blocks without nets (11.8 ± 1.0) than blocks with 6.1m nets (9.9 ± 2.0), but traps under 2.4m nets (11.6 ± 2.5) did not recapture different numbers of moths than either other treatment.

Experiment 2: Trellised versus free standing orchards under pheromone mating disruption

Recapture: Significant differences in recapture were found between the three tree training systems (F=17.624, df=2, P<<.001). Tukey's HSD test showed that significantly more moths were recaptured in both trellised blocks than in standard planted blocks, but that recapture was not different among the trellised blocks (Table 3.3). The mean recapture of moths in standard planted blocks was 1.6% ± 0.3, in V-trellised blocks was 9.2% ± 1.5, and in Vertical-trellised blocks was 9.1% ± 1.5.

Aggregation: Significant differences in aggregation were found among the three tree training systems (F=6.871, df=2, P=.0025) (Table 3.3). Fisher's LSD test showed that moths released in standard planted orchards were aggregated around the point of release significantly more than those released in either trellised training system: the mean Iδ of moths released in standard planted blocks was 2.57 ± 0.44, in V-trellised blocks was 1.59 ± 0.09, and in Vertical trellised blocks was 1.39 ± 0.05. There was no significant difference in degree of aggregation between the trellised blocks.

	Training system	*Replicates*	*ANOVA*	*Mean ± SEM*
Percent Recapture	STD-32	19	F=17.624	1.6 ± 0.3 a
	V-Trellis	18	df=2,52	9.2 ± 1.5 b
	Vertical Trellis	18	P<<0.001	9.1 ± 1.5 b
Iδ – Absolute	STD-32	14	F=6.871	2.57 ± 0.44 a
	V-Trellis	17	df=2,45	1.59 ± 0.09 b
	Vertical Trellis	17	P=0.0025	1.39 ± 0.05 b

Table 3.3. Average % recapture and Iδ for sterile codling moths released in three tree training systems.

Distance: As previously determined, moths released in trellised orchards were captured significantly more than in standard planted single, stand-alone tree orchards. Distance from point of release analysis determined that for each distance, 10m (F= 18.115, df=2, P<<0.001), 20m (F= 17.176, df=2, P<<0.001), 30m (F=14.434, df=2, P<<0.001), and 40m (F=12.370, df=2, P<<0.001) from the point of release, there were significant differences among the three tree training systems (Table 3.4). Tukey's HSD test revealed that significantly more moths were captured in

Distance from release	*Training system*	*ANOVA*	*# traps*	*Mean ± SEM*
10 meters	Standard	F= 18.115	8	49.1 ± 9.3 a
	Vertical Trellis	df=2, 52	8	264.1 ± 40.1 b
	V-Trellis	P<<0.001	8	271.4 ± 41.5 b
20 meters	Standard	F= 17.176	8	31.1 ± 9.9 a
	Vertical Trellis	df=2, 52	8	200.3 ± 34.9 b
	V-Trellis	P<<0.001	8	216.6 ± 38.1 b
30 meters	Standard	F=14.434	8	26.4 ± 6.5 a
	Vertical Trellis	df=2, 52	8	141.0 ± 25.3 b
	V-Trellis	P<<0.001	8	143.3 ± 25.1 b
40 meters	Standard	F=12.370	8	20.5 ± 5.7 a
	Vertical Trellis	df=2, 52	8	121.6 ± 23.7 b
	V-Trellis	P<<0.001	8	100.6 ± 20.3 b

Table 3.4. Average number of moths recaptured at four distances in three orchard planting systems.

both trellised systems than in standard planted orchards, and moth recapture in the two trellised orchard types were not different at any of the distances. The estimated maximum population recapture distance of between 55-65 meters from the point of release was estimated as the point at which the three trend lines x-intercept on Figure 3.3.

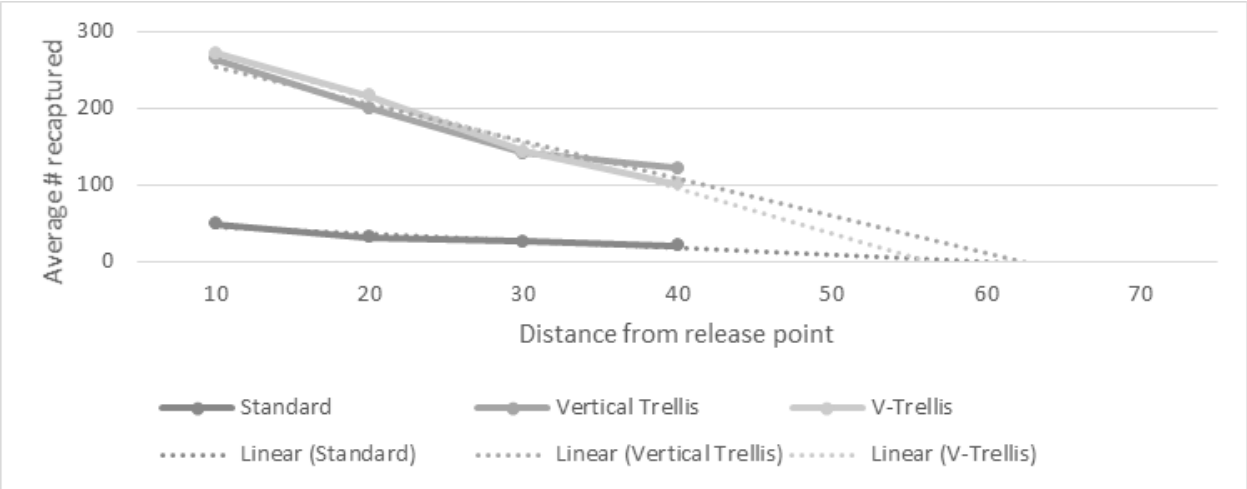

Figure 3.3. *The average number of codling moths captured in apple orchards with three tree architectures at four distances from release.*

Directionality: There were significant differences found in the direction of sterile moth dispersal in the standard planted orchards (F=2.570, df=7, P=0.0159), but not in the Vertical trellis planting system (F=0.404, df=7, P=0.8986), nor the V-trellised planting systems (F= 1.387, df=7, P=0.2157). Moths released in trellised orchards exhibited a slight, but not statistically significant directional preference for movement up and down rows, while those released in standard planted blocks had a minor but significant Westerly preference as revealed by Tukey's HSD (Table 3.5). However, the overall impact on dispersion is minimal as moths dispersed to the edges of the blocks in all directions in the three tested tree training systems (Fig. 3.4).

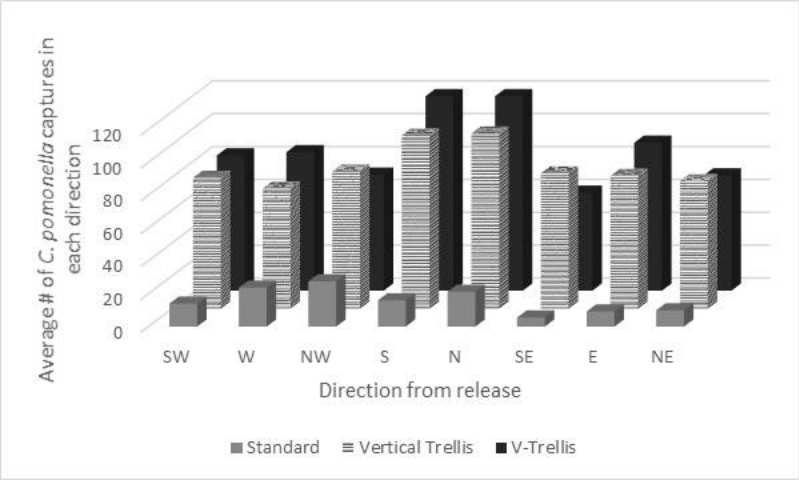

Figure 3.4. The average number of sterile *C. pomonella* captured at each direction in apple orchards with three different tree training systems. Traps to the North and South are up and down rows while those to the West and East are across rows.

Training system	*Transect Direction*	*ANOVA*	*# Traps*	*Mean ± SEM*
Standard Planting	Northwest	F=2.570 df=7, 144 P=0.0159	4	27.5 ± 7.7 a
	West		4	*23.7 ± 5.3* a
	Southwest		4	13.9 ± 3.0 a
	North		**4**	**21.2 ± 4.9 a**
	South		**4**	**16.0 ± 4.1 a**
	Northeast		4	9.9 ± 2.2 ab
	East		4	*9.2 ± 2.1* ab
	Southeast		4	5.5 ± 1.4 b
Vertical Trellis	Northwest	F=0.404 df=7, 136 P=0.8986	4	83.4 ± 15.2
	West		4	*73.0 ± 11.5*
	Southwest		4	79.6 ± 13.5
	North		**4**	**106.5 ± 19.7**
	South		**4**	**105.4 ± 21.0**
	Northeast		4	77.5 ± 16.5
	East		4	*80.8 ± 16.0*
	Southeast		4	82.4 ± 17.1
V-Trellis	Northwest	F= 1.387 df=7, 136 P=0.2157	4	70.6 ± 12.6
	West		4	*84.3 ± 14.9*
	Southwest		4	82.3 ± 16.5
	North		**4**	**118.3 ± 21.3**
	South		**4**	**118.3 ± 20.4**
	Northeast		4	70.1 ± 18.5
	East		4	*90.0 ± 22.0*
	Southeast		4	59.6 ± 10.8

Table 3.5. Average number of sterile codling moths captured at each direction of three tree training systems. Traps to the North and South (bold) are up and down rows while those to the East and West (italics) are across rows.

Experiment 3: Steep slope vs flat planar orchards

Recapture: Significantly more sterile moths (F=30.991, df=1, P<0.001) were recaptured in plots on a slope (4.8%±0.9) than without a slope (0.9%±0.3) (Table 3.6). However, the flat plots were not trellised, and as demonstrated in experiment 2, plots of this type typically have lower rates of recapture.

Aggregation: There was no significant difference in aggregation in the two treatments based on absolute recapture (F=3.128, df=1, P=0.0878). The Morisita index of absolute number of sterile moths recaptured on orchards with a hill was 1.53 ± 0.10, and for orchards with less than a 1° slope was 2.90 ± 0.90 (Table 3.6). There were significant differences in the mean trap percent of total capture between the two treatments (F=5.366, df=1, P=0.0281). The aggregation index for traps on the hill of 1.45 ± 0.13 was less than that for traps in a flat orchard of 3.29 ± 0.91, indicating that moths were less aggregated on the hill plots.

	Orchard Slope	*# Reps*	*ANOVA*	*Mean ± SEM*
Percent Recapture	14° steep hill	18	F=31.763	4.8±0.9 a
	1° Flat planar	18	df=1, 34 P<<0.001	0.8±0.3 b
Iδ - Absolute	14° steep hill	18	F=3.128	1.53±0.10
	1° Flat planar	12	df=1, 28 P=0.088	2.95±0.97
Iδ - % of total	14° steep hill	18	F=5.366	1.45±0.13 a
	1° Flat planar	12	df=1, 28 P=0.028	3.35±0.99 b

Table 3.6. Average % recapture and Iδ for sterile codling moths released in orchards with two slopes.

Direction uphill/downhill: In the two apple orchard blocks with a 14° slope, the four traps at the bottom of the slope recaptured a slightly lower but not significantly different number of

moths than the four traps at the top of the hill (F=3.305, df=1, P=0.078) (Table 3.7). In the flat blocks, the four traps corresponding to the hilltop traps and the hill bottom traps were also not significantly different from each other (F=0.652, df=1, P=0.425). These data indicate that sterile codling moths do not exhibit a significantly elevated uphill movement.

Orchard Slope	*Location of recapture*	*ANOVA*	*# Traps*	*Mean ± SEM*
14° Steep Hill	Extreme Uphill	F=3.305 df=1, 34 P=0.078	4	76.4±16.23
	Extreme Downhill		4	42.2±9.8
	All Uphill	F=1.261 df=1, 34 P=0.269	8	162.5±31.2
	All Downhill		8	118.8±26.0
	Extreme Left	F=0.208 df=1, 34 P=0.651	5	62.5±11.4
	Extreme Right		5	79.0±21.6
	All Left	F=0.015 df=1, 34 P=0.905	10	187.1±31.0
	All Right		10	193.1±45.4
<1° Flat Planar	Extreme Uphill	F=0.652 df=1, 34 P=0.425	4	5.7±2.1
	Extreme Downhill		4	11.9±4.8
	All Uphill	F=0.152 df=1, 34 P=0.700	8	18.7±5.8
	All Downhill		8	27.4±10.6
	Extreme Left	F=0.250 df=1, 34 P=0.620	5	10.3±4.5
	Extreme Right		5	14.9±5.4
	All Left	F=0.100 df=1, 34 P=0.754	10	29.0±10.0
	All Right		10	37.8±12.5

Table 3.7. Average number of sterile codling moths captured at each direction from the central release point in apple orchards with two slopes.

The eight traps down slope from the central release point did not capture significantly different numbers of moths than the eight traps up the slope in the apple orchards on the 14° slope (F=1.261, df=1, P=0.269). The orchards on the flat area also did not capture significantly

different numbers of moths in the eight traps corresponding to uphill and the eight traps corresponding to downhill (F=0.152, df=1, P=0.700). Further confirmation that *C. pomonella* do not tend to have a preference for dispersal uphill (Table 3.7).

Direction across slope: In the two orchard blocks with a 14° slope, the five traps at each extreme side of the block did not recapture different numbers of sterile codling moths (F=0.208, df=1, P=0.651) (Table 3.7). Likewise, the 10 traps on each side of the release point across the slope also did not recapture different numbers of moths (F=0.015, df=1, P=0.905).

In the orchard blocks with a flat planar slope, the traps that correspond to those on the extreme sides of hill blocks did not recapture different numbers of moths (F=0.250, df=1, P=0.620), nor did all traps on each side of the release point (F=0.100, df=1, P=0.754).

DISCUSSION

Results from these three experiments indicate that orchard structures (i.e. trellis and shade netting) and topography variably impact the dispersal of sterile codling moths, and they also have implications for understanding the behaviors of wild moths in these orchard systems. While many studies have focused on the maximum distance that a few *C. pomonella* will travel after release, few have explored the dispersion of a population of moths within the orchard close to the point of origin and none have compared this dispersion in different orchard training systems, under nets, or on slopes. Moths released at the center of blocks with trellis disperse more readily than those released into blocks with large, widely-spaced single planted trees. Additionally, it has been demonstrated that moths do not have a strong preference for dispersing up and down rows versus across in all tree training systems tested, indicating that the direction

of dispersion is not impacted by rows. Also, hills do not impact dispersion by causing more moths to move upslope than down.

Early studies exploring intra-orchard self-dispersion of released sterile codling moths demonstrated that when omnidirectional winds were common, moths released at a single point were capable of reaching the edges of the orchards (Howell and Clift, 1974), but these studies did not explore the impact of planting type on dispersal, nor were they able to ascertain more accurate information on movement within the orchard. Additional studies of on-farm self-dispersal of released sterile codling moths further refined the distance that some moths will disperse (Mani and Wildbolz, 1977), but they were not able to discern the impacts of topography, vegetation, or other orchard conditions on the whole population of released moths. The impact of tree training systems on released sterile codling moth recapture, aggregation, and dispersion was assessed, and greater dispersion and recapture of moths is found in orchards with trellis compared to standard planted orchards. As well, this is the first study to test the idea that rows may act as barriers to self-dispersal of released sterile codling moths – they do not. Rows are generally planted in a North/South orientation to take advantage of light and temperature patterns to increase yield and quality (Wagenmakers, 1991), and from these data it appears that moths do not orient or disperse based on magnetic directions or the presence of rows.

This is also the first study to directly measure the impact of shade netting on the dispersal of sterile codling moths. Netted orchards resulted in higher aggregation of sterile moths than did open orchards, and aggregation increased as net height decreased and the orchard canopy and net height overlapped. While nets have gained traction as a unique and effective strategy for managing codling moth (Tasin et al. 2008, Sauphanor et al. 2012, Baiamonte et al. 2015,

Alaphilippe et al. 2016, Chouinard et al. 2017). The current study has demonstrated that shade netting impacts the recapture of moths and impedes their movement. Sauphanor et al. (2012) conjectured that the nets, in part, suppress codling moth populations by interfering with male flight and thus their capacity to locate females. These results provide direct evidence that this mode of action is an important contributor to the effectiveness of nets for managing codling moth. The recapture and dispersion of sterile codling moths was substantially impeded when apple trees were grown under shade nets, especially when the top of the nets and orchard canopy were close. The results on the distance that moths are recaptured from the point of release clearly show that orchard blocks with nets close to the canopy arrest significantly more moths close to the release point and may impede longer distance movement of the released population. Specifically, because sterile moths were released weekly, they could not adapt to the presence of nets as observed by Siegwart et al. (2013). These data help explain why the presence of nets have been observed as a factor controlling wild populations.

Previous studies have also demonstrated that landscape level elevational differences are an important factor in the capture of wild *C. pomonella* (Vernon et al. 2006). However, the reported results refine the notion that elevation may impact the capture of moths by scaling down the test area to single plots and comparing on-farm recapture at different elevations on a hill. It was found that moths are not more likely to disperse uphill than downhill or in either direction across the slope, and in flat plots there is also no preference for movement in a directional manner.

It is clear from these three experiments that moths released into orchards with trellised trees are recaptured at higher rates than those released into orchards with large old stand-alone

trees. Hu and Whitty (2019) provide a basic explanation of the differences between 2D (trellised) and 3D (stand-alone) apple trees, and the same concepts can be applied to test orchards used in this study. One possible explanation of these results is the complexity of the systems, as well as the canopy density: trellised canopies are less dense and less three dimensionally complex. In trellised systems, not only is there more open space for moths to move, but there is also less canopy to interact with while they move from one point to the next. In addition, the odor plume emitted from lures in traps may be more apparent to moths and more defined in orchards with trellis due to the reduced complexity and three-dimensional structure of the canopy.

These results, in all three experiments, show that 1) sterile codling moths disperse from the point of release and some moths reached the traps in all directions furthest from the central release point, and 2) that aggregation around the point of release is typically moderate. These data suggest that although some moths may travel far and leave the block, the majority remain in the target area and are available to provide farm-scale control of existing wild populations when they are released at the center of the orchard. Self-dispersal of released sterile codling moths, regardless of orchard conditions, is vital to successfully integrating them into existing management systems. *C. pomonella*, although known to disperse for mate and host finding, is generally accepted as being a sedentary species (Geier, 1963). Variation in habitats may necessitate different degrees of dispersal (Gu et al., 2006), but the impact of orchard habitat variation on *C. pomonella* dispersal is poorly understood. Much work has gone into determining maximum dispersive distance of *C. pomonella* (Worthley, 1932; Steiner, 1940; Tremmaterra, 2004; Basoalto et al., 2010; Margaritopoulos et al., 2012; Adams et al., 2017), but no studies have explored the impact of physical structures such as nets or tree training/architecture on farm-

scale dispersal of released sterile codling moths, nor have they explored dispersal in orchards with mating disruption. The current study has demonstrated that over short distances the layout and architecture of the orchard impacts how *C. pomonella* disperse throughout the landscape when orchards are treated with pheromone mating disruption. Additionally, the estimated maximum dispersive distance of the population of released moths under mating disruption is less than that in the absence of mating disruption (Tremmaterra, 2004; Basoalto et al., 2010, Adams et al., 2017). Those studies found maximum dispersal distances of well over 200 meters in the absence of mating disruption, but these findings suggest that in the presence of mating disruption, dispersal of the majority of the population is less than 75 meters. The influence of mating disruption on *C. pomonella* dispersal and recapture needs further study.

From a practical standpoint, the movement of sterile codling moths into and out of orchards is of great importance to growers as they strive to make a SIT program economically viable. Farmers do not want to learn that the insects they released to control their pests have all flown away, nor do they want to suffer the disappointment of knowing that the insects did not fly to the areas of the farm where they were needed most. These results support employing different release tactics depending on orchard architecture type as well as under nets. Although in all three experiments moths dispersed to the edges of the plot in all directions, it is evident that moths do not disperse as readily in orchards with old stand-alone tree plantings. In these types of plantings, it may be prudent to not release moths at the center of the block for every release: either employ several distributed release points for each weekly release or rotate release points throughout the season based on a schedule or damage sampling. It is equally clear that more moths disperse to the edges of trellised blocks than in stand-alone tree blocks, and release

at the center is optimal to retain the maximum number of moths within the target block. It may be possible to utilize fewer sterile moths in orchards with trellis to affect the same level of control as in blocks with large stand-alone trees. As increasingly more orchards employ this tree training style (Wagenmakers, 1991), this knowledge will be vital to the success of this technology on individual farms. Likewise, in blocks with nets it may also be prudent to avoid releases at a single central location, but rather to select several evenly spaced locations within the farm to release sterile moths. Orchards with trees partly on steep hills, or wholly situated on steep slopes should have sterile moths released at the center of the slope because there is a slight but not significant preference for uphill dispersion.

In summary, it has long been known that some *C. pomonella* are capable of flying long distances beyond the edges of the orchard, but previous studies do not describe the within-farm movement of a released *C. pomonella* population on hills, up and down versus across rows, or under nets, nor has recapture been compared in different tree training systems, on hills or under shade nets. That evidence is reported here. *C. pomonella* move up and down rows just as readily as they move across them. Additionally, it has been demonstrated that in several planting systems this remains true. The presence of rows, either with or without trellis, does not act as a barrier for the movement of *C. pomonella* within the farm. Rather, released sterile codling moths disperse much more readily in trellised orchards, suggesting that this planting system is highly conducive to the sterile insect technique. Farmers with trellised orchards will find that a simple single point release at the center of a 10-acre bock is sufficient to treat the whole block with sterile codling moths. Orchardists that employ shade netting should be aware that sterile codling moths aggregate more around the point of release when nets are close to the top of the canopy.

And lastly, orchardists with apple blocks on hills need to understand the ways that sterile codling moths disperse in an orchard with uphill sections and modify releases accordingly.

CHAPTER FOUR

The Influence of Mating Disruption Technology and Monitoring Trap Lures on Dispersion and Recapture of Sterile *Cydia pomonella* (Lepidoptera: Tortricidae) Released into Apple Orchards

INTRODUCTION

The codling moth, *Cydia pomonella* (L), the most important economic pest in commercial apple production, was introduced into North America in shipments of infested fruit sometime in the 1750's (Essig, 1931). Crop loss is highly variable by location and year, and when orchards are unmanaged or poorly managed losses can be very high (Isley and Ackerman, 1923; Allman and Essig, 1929; Putman, 1963; Glass and Lienk, 1971; MacLellan, 1972; Westigard, 1973; Setyobudi, 1989; Wise and Gut, 2000, 2002). Upon the advent of chemical control techniques, in particular Paris green and lead arsenate from the 1870's through the mid 1900's, *C. pomonella* quickly became the target of various control tactics (Peryea, 1998). Following the disuse of arsenicals, chlorinated pesticides such as DDT became the favorable option for *C. pomonella* control (Durkee et al., 2017) until the end of the 1960's when the organophosphates replaced them as the primary insecticide used in apples. Reliance on organophosphates for *C. pomonella* control has declined (Costa, 2018), but several insecticidal compounds are currently available for use against this key pest (Van Steenwyk and Peters-Collaer, 2020). Efforts to control *C. pomonella* using broad-spectrum insecticides continue to be complicated by the loss of effective materials from restrictions or resistance (Varela et al., 1993; Knight et al., 1994; Mota-Sanchez et al., 2008).

In response to increased regulatory pressure and resistance development against insecticides, the first pheromone dispenser for *C. pomonella* mating disruption was registered for commercial use in 1991 (Witzgall et al., 2008). On-farm use has increased steadily, and from 1995 to 2015 the Washington State apple acreage treated with mating disruption increased from 10% to nearly 90% (Willett and Curtiss, 2019). Similarly, from 1990 to 2011 at least 30,000 ha of pears in Argentina were treated with *C. pomonella* mating disruption and damage declined from 5-6%

to 0.26% (Cichon, 2011), disrupted apple farms in New Zealand have had a 70% reduction in male captures in traps, lower fruit damage and insecticide applications reduced by nearly half (Walker et al., 2013), and in pheromone-treated orchards in Poland fruit damage was reduced by up to 95% compared to untreated orchards, but damage was higher when population densities were high (Płuciennik, 2013). *Cydia pomonella* mating disruption is most effective when pest populations are low, thus it is typically coupled with other control methods in an integrated pest management program (DuPont, 2019). Pheromone-based mating disruption has been adopted as the primary control technique for *C. pomonella* on ca. 243,000 hectares of apples, pears and walnuts because it is an effective and environmentally sound alternative for managing this key pest (Gut et al. 2019).

Mating disruption involves placing large numbers of synthetic sex pheromone dispensers into cropped areas to disrupt normal mating behavior and reproduction (Płuciennik, 2013). There are several dispenser technologies in use for deploying pheromone into target orchards, including, but not limited to the passively emitting reservoir dispensers NoMate CM Spirals (Scentry biologicals Inc.), Isomate-CM Flex and CTT (Pacific Biocontrol), Cidetrak CM Puzzle Piece (Trece Inc.), and Checkmate CM-XL 1000 (Suterra LLC.), and the actively emitting aerosol dispensers Isomate CM-Mist (Pacific Biocontrol), Semios CRS Plus (Semios Technologies Inc. Canada), NoMate® CM Smart Release (Scentry biologicals Inc.), and Checkmate Puffer CM (Suterra LLC.) (Benelli et al., 2019; Murray and Alston, 2020). Mating disruption of *C. pomonella*, using passive or active dispensers is achieved via competition between the synthetic source of pheromone and wild-type females (Miller et al. 2010, McGhee et al 2014). Passive dispensers are typically deployed at densities of 500-750 units/ha, whereas active dispensers may be deployed

at densities of 2–5 units/ha (Benelli et al., 2019), and the two approaches to distributing pheromone likely impact codling moth population dispersion differently (McGhee, 2014; McGhee et al., 2014). McGhee et al. (2014) found that the optimal ISOMATE CM MIST emitter rate is about 2.5 units/ha, and a single emitter disrupts trap finding 4.5 times more effectively than does a single monitoring trap baited with a codlemone lure. In addition to determining that active dispensers disrupt *C. pomonella* competitively, McGhee (2014) reported that active dispensers appear to cause moth captures in monitoring traps using long-lasting codlemone lures to be clustered in clean air upwind of active dispensers, suggesting that a density of emitters too low may result in farm areas with poor coverage, allowing for pockets of non-disrupted moths to reproduce.

Historically, *C. pomonella* populations were monitored by assessing in-field damage and wrapping trees in bands of materials that simulate rough bark (Allman, 1928; Yothers and Van Leeuwen, 1931), light trapping (Butt and Hathaway, 1966), the use of food baits (Yothers, 1927; Yothers, 1930a, 1930b)) or traps baited with caged virgin females. Once the female codling moth sex pheromone, *trans-8, trans-10-dodecadien-1-ol* (codlemone), was identified by Roelofs et al. (1971), it quickly became available for use in monitoring traps and was effective for assessing adult male activity. However, with the advent of pheromone mating disruption in the 1990's, it became problematic from a monitoring standpoint that mating disruption also interfered with the captures of moths in traps through competition with lures in the same way that pheromone dispensers compete with females. To overcome this inability to monitor *C. pomonella* activity in disrupted orchards, researchers identified attractants that could override the effect of the pheromone. A lure loaded with 10mg of codlemone was discovered to be an effective attractant

in disrupted blocks (Charmillot 1991), Light et al. (2001) found that adding pear ester (DA), *ethyl (E, Z)-2,4-decadieonate*, to the long-life codlemone lure (CM L2) increased the attraction of male and female moths to traps, and Landolt et al. (2007) found that the addition of acetic acid (AA) to the lure acts synergistically with pear ester. The advantages of adding pear ester to monitoring trap lures has been extensively studied (Knight and Light 2004a, 2004b, 2004c; Light and Knight, 2005; Knight and Light, 2005a, 2005b; Knight et al, 2005; Schmera and Guerin, 2012). Trona et al. (2010) found that male *C. pomonella* are not attracted to pear ester in the absence of sex pheromone. Multiple studies demonstrating the attractiveness of AA, or fermented sugar baits (AA is a product of fermentation) have resulted in its inclusion in lure formulations (Yothers, 1930a, 1930b; Landolt et al., 2007; Knight, 2010a 2010b; Judd, 2016). Judd (2016) found that in orchards receiving both mating disruption and sterile codling moths for control, females accounted for 81% of the sterile moth catch using the pear ester and acetic acid lure and that both AA-DA lures and CM-DA lures performed better than CM L2 lures. Currently, a lure containing codlemone, pear ester, and acetic acid is commercially available (PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.), providing a useful tool for assessing male and female *C. pomonella* moth dispersion in orchards with mating disruption.

The research report herein details the dispersal of sterile *C. pomonella* males and females following their release into mating disrupted and non-disrupted apple orchards by using the mark-release-recapture method. The first objective was to determine the impact of attractant-baited traps on the ability to assess moth dispersion. The second objective was to compare moth dispersal in orchards treated with the two main technologies for disrupting *C. pomonella*, passive or active dispersing emitters releasing codlemone, and pheromone-free orchards. These

objectives were accomplished by centrally releasing sterile male and female moths and recapturing at various distances away from the release point in traps baited with the CM-DA+AA lure in orchards under the different mating disruption schemes. The hypotheses being tested were that 1) dispersion of released sterile *C. pomonella* is impacted by CM-DA+AA lures in traps when they are present at the time of release compared to when moths disperse without interference from trap lures; 2) *C. pomonella* dispersion, as compared to pheromone-free control plots, is altered differently by in-field interaction with synthetic pheromone sources found in passive dispensers and active dispensers; and 3) female dispersion is not impacted by pheromone sources, but trap kairomone lures do impact dispersion. The hypotheses were tested by measuring moth recapture and dispersal 1) when CMDA+AA baited traps were present at the time moths were released versus when trap placement was delayed until 48 hours after the moths were released; and 2) when moths were released into orchards with passive, active, or no pheromone dispensers and recaptured in traps baited with the same lure.

METHODS AND MATERIALS

Source and handling of sterile moths

Mixed-sex and internally-marked with calico red dye *C. pomonella* adults, ca. 400 male and 400 females, were placed in petri dishes at the Okanagan-Kootenay Sterile Insect Release facility in Osoyoos, British Columbia, Canada and treated with 33 krad of gamma radiation from a Cobalt-60 source as described in Horner et al. (2020). Weekly importations of sterile *C. pomonella,* from this facility were transported by permit to field sites in Washington State in battery-powered coolers (2.8 Cu. Ft. Portable Fridge/ Freezer: Edgestar co. Austin, Texas) held at

approximately 5°C. Sterile moths arrived at field sites the day they were packed and sterilized, and were released the day they were received. At field sites, moths were placed in 540ml polystyrene cups (Fabri-Kal Corp. Kalamazoo, MI) in batches of up to 4000/cup, externally marked using ca. 1.25ml/800 moths Dayglo florescent pigments (ECO11 Aurora Pink®, ECO15 Blaze Orange™, ECO18 Signal Green™, ECO19 Horizon Blue™) (DayGlo Color, Cleveland, OH) to uniquely identify those released into test orchard blocks, and released by hand at pre-marked central locations in blocks. Moths were allowed to warm to ambient temperature before being gently tossed onto pre-marked trees. The release of marked sterile moths allowed for the measurement of *C. pomonella* population responses to pheromone sources in the field under a known population density and point of origin.

Traps and lures

Recapture of released sterile *C. pomonella* was quantified by the one of the two alternative trapping procedures described below using orange Pherocon VI delta traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure. Traps were placed within the top 1/3 (Reidl et al., 1979; McNally and Barnes, 1981) of pre-marked apple trees in a 16-trap grid pattern with spacing of approximately 40m (Figure 4.1). Lures were replaced at 6-week intervals per label instructions. Trap liners were collected once weekly throughout the study period for examination in the laboratory using UV illumination (400-405 nm, 12 UV LED bulb flashlight, Bioquip Products, Rancho Domingo, CA) to determine the color and sex of marked moths.

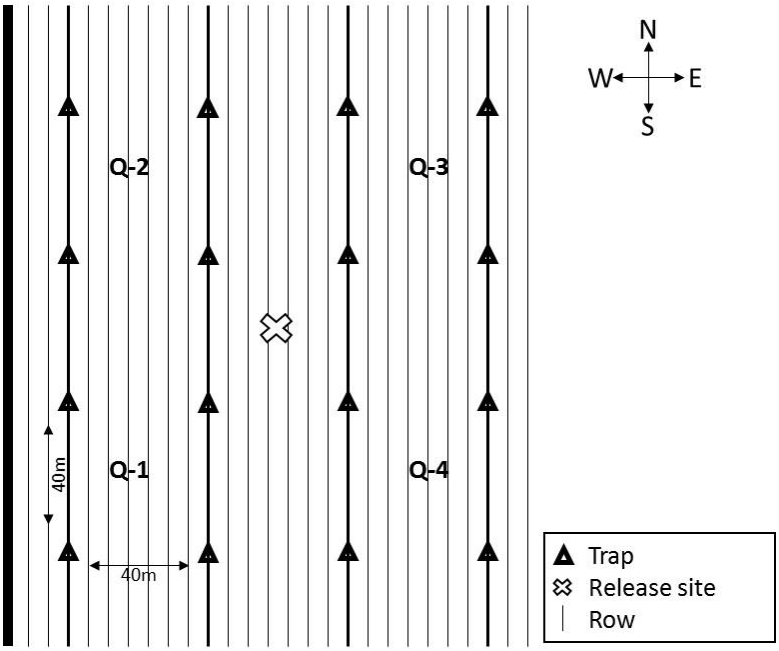

Figure 4.1. *Trap layout used in all treatments. Trap quadrants are denoted Q-1, Q-2, Q-3, and Q-4 for traps placed in the Southwest, Northwest, Northeast and Southeast portions of the orchard blocks, respectively. Four traps were placed in each quadrant.*

Experimental design

This mark-release-recapture experiment was conducted during July and August 2020 on large commercial apple farms located near Brewster, WA and Bridgeport, WA. All orchards were subject to existing commercial management systems and practices, i.e. several forms of irrigation, pruning, and pest management treatments. All blocks had rows oriented approximately North/South. Two factors were tested in a factorial design, mating disruption technology and the timing of trap deployment for a total of six treatments. The experiment was designed to test the hypothesis that centrally released sterile codling moths' dispersion and recapture are impacted by both the type of on-farm mating disruption technology and presence of traps at or after the time of release. Plots were treated with one of the following six mating disruption/trap deployment timing treatments: 1) control with no mating disruption/traps

present before moth release (‘*Control-Before’*); 2) control with no mating disruption/traps placed 48 hours after moth release (‘*Control-Delayed’*); 3) passively dispensed mating disruption (NoMate CM Spirals (Scentry biologicals Inc.) at 300-350 units/ha)/traps present before moth release (‘*Passive-Before’*); 4) passively dispensed mating disruption (NoMate CM Spirals (Scentry biologicals Inc.) at 300-350 units/ha)/traps placed 48 hours after time of moth release (‘*Passive-Delayed’*); 5) actively dispensed mating disruption (ISOMATE CM Mist Plus (Pacific Biocontrol) at 1-2 units/ha)/traps present before moth release (‘*Active-Before*’); and 6) actively dispensed mating disruption (ISOMATE CM Mist Plus (Pacific Biocontrol) at 1-2 units/ha)/traps placed 48 hours after time of moth release (‘*Active-Delayed*’). Releases for each treatment were replicated 18 times. The trapping period in all plots was 7 days from the time of release to allow for adequate numbers of moths to disperse and respond to the traps. In blocks receiving delayed trap deployment 48 hours after release, traps were removed from the plot before release, then, 48 hours after release, they were replaced in pre-marked trees.

Control plots: Moth dispersion was measured in non-disrupted plots that were located near Bridgeport, WA. There were four 4.05ha blocks assigned to the ‘Control-Before’ treatment and four 4.05 ha blocks assigned to ‘Control-Delayed’ treatment. These square blocks all had drip-irrigated Scilate (Envy) apples on Geneva G.41 rootstock in rows that were spaced 3-4 meters apart, and trees were planted on trellis at ca. 1m intervals and trained to a tall-spindle system. This orchard was isolated from other apple orchards by 1.6-3.2km across the Columbia River or 2.1-3.2km across open rangeland. Releases were alternated each week between plots to avoid interference between newly released moths and previously released moths. Moths were released at a density of 5 petri dishes of sterile moths/4.05ha block per replicate.

Mating disruption plots: Passive and active dispensers were placed by the orchard manager at densities and locations they determined to be adequate for codling moth control in their IPM programs, and the layout of traps and active dispensers in 'Active-Before' and 'Active-Delayed' blocks is shown in Figures 4.2 and 4.3 respectively. All orchard blocks receiving mating disruption treatments were located near Brewster, WA within a larger 4850ha orchard, planted with granny smith apples as standard planted free-standing 4-4.5m tall single trees trained using the central leader system planted in 6.1m wide rows 3m apart. Treatments were each assigned to one of four 16.2ha square orchards each with four 4.05ha blocks that were rotated weekly as above for replicates (ex. Fig. 4.2). Moths were released into mating disruption blocks at a density of 10 petri dishes of externally marked sterile codling moths/4.05ha block for each replicate.

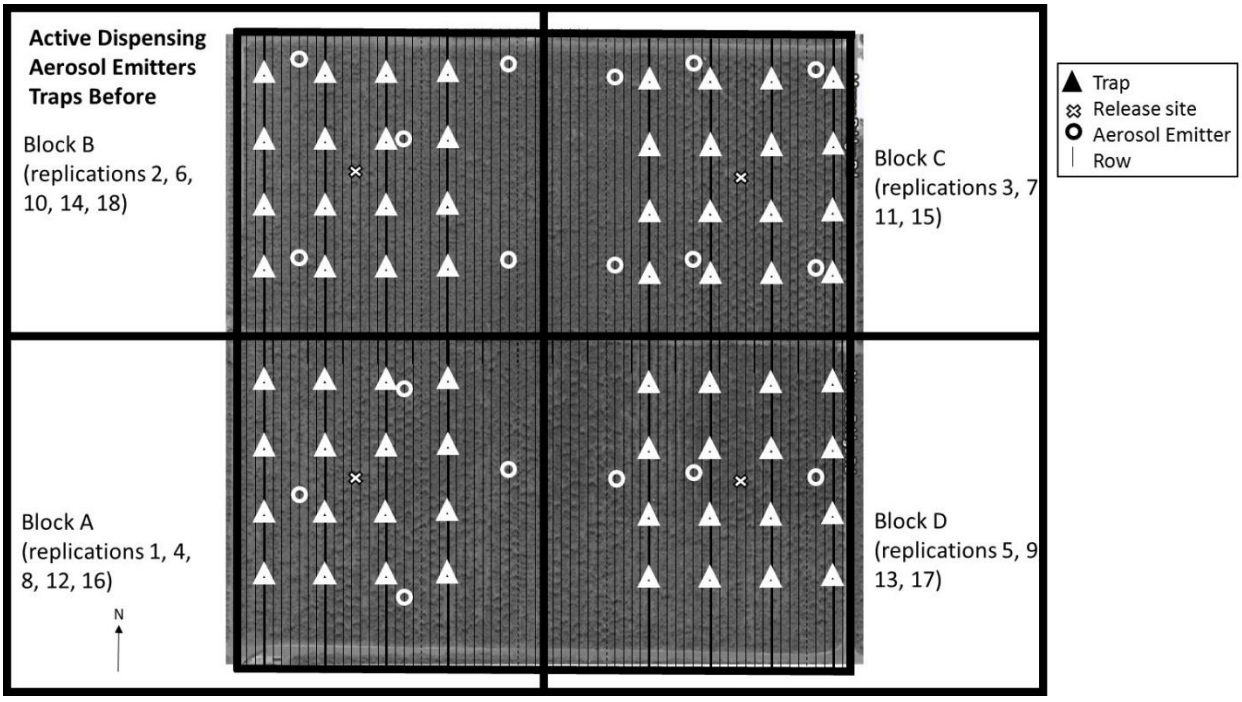

Figure 4.2. Layout of traps and active dispensers in adjacent blocks used for replications with trap placement before moth release ('Active-Before') treatments.

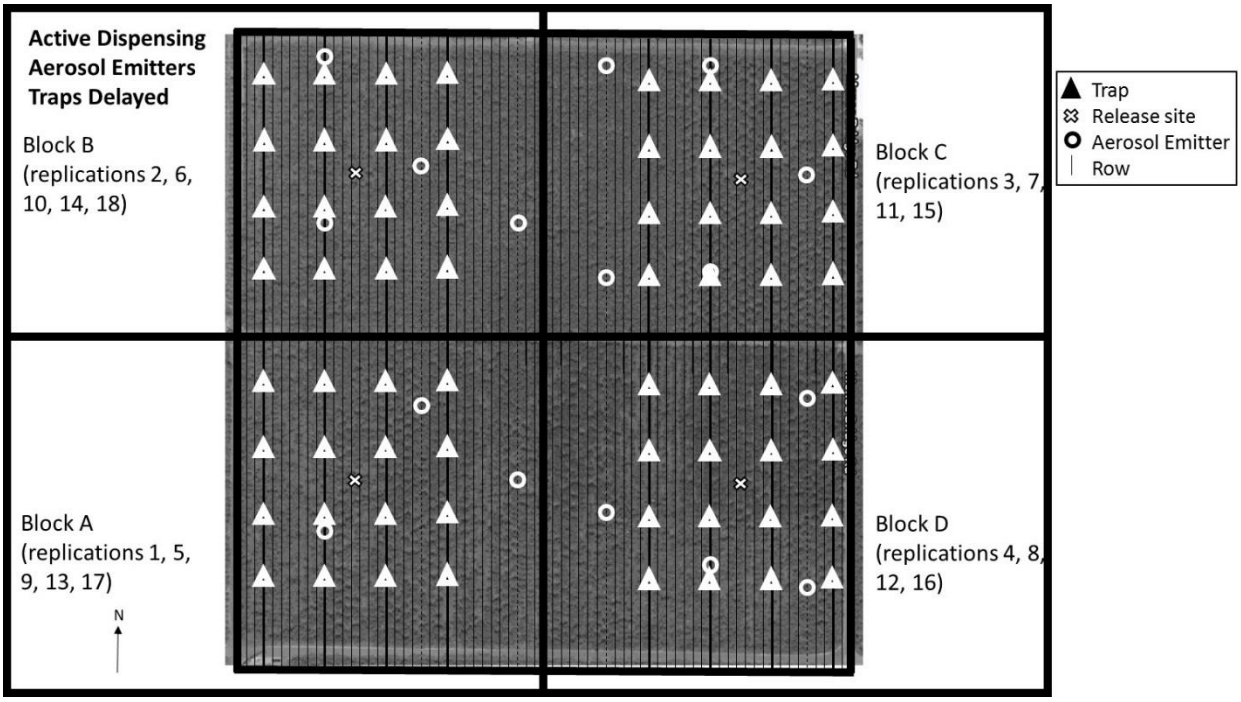

Figure 4.3. Layout of traps and active dispensers in adjacent blocks used for replications of delayed trap placement ('Active-Delayed') treatments.

DATA ANALYSIS

Recapture: Moth captures in lure baited traps were used to determine if released sterile codling moth recapture was different among the treatments. The mean proportion of released sterile moths recaptured in each experiment was used to compare treatment effects on both male and female codling moths. The percent of captured moths was normalized using log(x+1) transformation to reduce heteroscedasticity and then subjected to ANOVA to determine if there were treatment differences. Post hoc Tukey's HSD tests ($p<0.05$) were performed to determine which treatment means were different.

Aggregation: Morisita's index of dispersion (Morisita, 1959 and 1962) was used to measure male and female moth dispersion separately. The Morisita formula is: $I\delta=n(\sum(xi^2)-\sum(xi))/(\sum(xi)^2-\sum(xi))$: n = the number of traps and xi=capture in individual traps. When the index

returns values of <1 it indicates random dispersion, =1 is an even dispersion, and >1 suggests an aggregated dispersion; high Iδ values (maximum of n traps) indicate strongly aggregated populations. When captures from a replicate are low (i.e. less than 5 total moths recaptured from all traps) or all 16 traps from a replicate individually recapture one and/or zero moths, the index may be inaccurate (Amaral et al., 2014). In addition, if high numbers of moths from a single replication are concentrated in only 1-2 traps, high Iδ values are returned, and this may skew the results. For this experiment, Iδ indices were calculated for each replication, separately for male and female codling moth recaptures, two ways: 1) using xi=(number of recaptured moths in each trap/number released), and 2) using xi=(# moths recaptured in each trap/total number of moths recaptured in a replication)*100, or percent of total moths recaptured in each trap. In an effort to minimize inaccuracy described in Amaral et al. (2014) in using this index, Iδ was calculated when 1) catch from a replicate was >5 total moths, 2) at least one trap from a replicate captured >1 moth, and 3) when capture was not high in only 1-2 traps or distances. Due to this criteria, different numbers of replications were accepted for analysis of male and female capture. The % capture Iδ index calculation was used to demonstrate moth dispersion correcting for week to week recapture variations caused by cryptic environmental factors. These two measures together provided a more complete indication of aggregation for determining the impact of MD technology and trap timing on post-release dispersion. Significant treatment effects on dispersion, using Iδ indices calculated for each replication, were determined using ANOVA to test the assumption that CM aggregation will be similar among treatments. To assess the degree to which CM aggregation was affected by MD technology and trap deployment timing, post hoc Fisher's LSD test (P=0.05) was used to separate treatment effects.

Distance from release: Separately for male and females, dispersion was further assessed between treatments by using the percent of total catch at selected trap distances from the central release. Trapping grids consisted of 16 traps placed at three trap distances [28 meters (4 traps); 63m meters (8 traps); and 85 meters (4 traps)] from the central release. The percent of sterile moth recapture was calculated for each distance and log(x+1) transformed. An ANOVA was conducted on log transformed recapture numbers to determine if there were differences among treatments. Post-hoc Tukey's tests were performed to separate means if ANOVA results indicated significance. For this analysis replicates that recaptured >1 released codling moths were used, resulting in different numbers of accepted replications for males and females.

Evenness of Treatments: The 4.05ha square blocks were divided into trapping grids of four quadrants (Figure 1), each with 4 traps to measure evenness of male and female codling moth capture separately within and among treatments. The mean capture/quadrant was calculated for each replicate, log(x+1) transformed and subjected to analysis of variance to determine if there were differences in recapture among the four quadrants of a treatment (Fig. 1) and if treatments were different by capture in quadrants. If ANOVA results indicated significance, post hoc Tukey's tests were performed to determine which quadrants or treatments had higher capture. For this analysis replicates that recaptured >1 released codling moth were used, resulting in differing numbers of accepted replications for males and females.

Active Dispenser Blocks: Because the layout of active dispensers in test block quadrants was different (Fig. 4.2, 4.3), it was necessary to further analyze capture in these blocks in more detail to test quadrants for evenness of capture and demonstrate any interactions with dispensers. Replications with >1 moth recaptured were used as described previously. The mean

number of moths recaptured/quadrant/block was calculated, log(x+1) transformed and ANOVA performed to determine if there were differences in the number of moths captured by quadrant and by each of the two trap deployment timing treatments. Tukey's test was used for mean separation.

Finally, the number of moths recaptured based on trap proximity to active dispensers was determined. Three trap/emitter proximity criteria were established, a) near (<20m), b) intermediate (20-40m), and c) far (>40m) for analysis of recapture data. The mean number of CM recaptured in traps at these three distance criteria were log(x+1) transformed and compared using ANOVA to determine if there was an impact on recapture by proximity to emitters within treatments. Post-hoc Tukey's tests were used to separate means if ANOVA results indicated significance. Replications that had >1 moth recaptured were used for this analysis. Significant differences among the two active dispensing aerosol emitter treatments for each trap proximity measure were determined by t-test of the log(x+1) transformed mean numbers of sterile CM recaptured.

RESULTS

Male Recapture: Of the ca. 360,000 sterile male *C. pomonella* released, very few were recaptured in blocks treated with mating disruption. Significant differences in recapture of sterile male moths ($F_{5,102}$=83.4025, P<<0.0001) were observed between the six treatments (Table 4.1). Blocks without mating disruption had >15% recaptures, which were significantly higher than the <3% recaptures in blocks under mating disruption. Blocks with active dispensers and traps placed

48 hours after release had higher recapture than blocks with passive dispensers and traps placed 48 hours after release.

Female r*ecapture:* Although very few of the ca. 360,000 sterile female codling moths released in this experiment were recaptured, there were significant treatment effects ($F_{5,102}$=17.2323, P<<0.0001) on sterile female *C. pomonella* recapture (Table 4.1). Plots without mating disruption had >5.6 % recaptures which was significantly higher than the <1.9% recaptures recorded in plots with mating disruption. Additionally, among plots with traps placed 48 hours after release, those with active dispensers had higher recapture than plots with passive dispensers.

Male aggregation: There were significant treatment differences in aggregation based on the absolute value of sterile male codling moths recaptured ($F_{5,102}$=3.2235, P=0.0099). The least amount of aggregation was found in blocks without MD and when traps were placed 48 hours after release (Table 4.1).

Significant treatment differences for male aggregation based on the % of total moths recaptured ($F_{5,92}$=7.702, P<<0.0001) were observed. Aggregation was higher in blocks with mating disruption than in blocks without disruption, but not different among the mating disruption blocks (Table 4.1). The highest dispersion was found in blocks without mating disruption that received traps 48 hours after release

			♂		♀	
	Treatment	**# reps (♂/♀)**	**ANOVA**	**Mean ± SEM**	**ANOVA**	**Mean ± SEM**
Percent Recapture	**'Control-Before'**	18/18	F=83.4025 df=5, 102 P<<0.0001	25.6%±1.8 a	F=17.2323 df=5, 102 P<<0.0001	5.7%±0.6 a
	'Control-Delayed'			15.7%±1.4 a		7.0%±0.9 a
	'Passive-Before'			1.7%±0.5 b,c		1.8%±0.4 b,c
	'Passive-Delayed'			1.2%±0.6 c		1.1%±0.6 c
	'Active-Before'			1.6%±0.3 b,c		1.5%±0.4 b,c
	'Active-Delayed'			2.9%±0.5 b		3.5%±0.8 b
Iδ-Index Absolute Aggregation	**'Control-Before'**	18/18	F=3.2235 df=5, 92 P=0.0099	1.48±0.06 a,b	F=12.0602 df=5, 83 P<<0.0001	1.19±0.03 a
	'Control-Delayed'	18/18		1.29±0.05 b		1.24±0.04 a
	'Passive-Before'	15/14		1.80±0.18 a,c		2.00±0.11 b
	'Passive-Delayed'	12/10		1.78±0.22 a,c		1.77±0.21 b,c
	'Active-Before'	17/14		1.86±0.14 c		1.56±0.11 c
	'Active-Delayed'	18/15		1.73±0.10 a,c		1.51±0.06 c
Iδ-Index % of Total Aggregation	**'Control-Before'**	18/18	F=7.702 df=5, 92 P<<0.0001	1.36±0.06 a	F=7.3820 df=5, 84 P<<0.0001	1.19±0.04 a
	'Control-Delayed'	18/18		1.20±0.06 a		1.24±0.05 a,d
	'Passive-Before'	15/14		2.08±0.22 b		2.15±0.15 b,c
	'Passive-Delayed'	12/10		2.30±0.31 b		2.75±0.28 c
	'Active-Before'	17/14		2.25±0.23 b		1.77±0.13 b,d
	'Active-Delayed'	18/16		1.90±0.12 b		2.10±0.41 b

Table 4.1. Recapture and aggregation. Mean (±SEM) ♂ and ♀ recapture and aggregation (Iδ-Index measured by: 1) absolute number of moths captured/replicate, and 2) trap percent of total capture/replicate, of sterile *C. pomonella* released into 4.05ha apple orchard blocks with three mating disruption treatments: 1) control with no mating disruption, 2) passive mating disruption (Scentry NoMate® CM Spiral (Billings, MT)) at 300-350/ac, and 3) mating disruption with active dispensers (ISOMATE® CM Mist Plus (Vancouver, WA)) at ½-3ac).Trapping of sterile moths was performed by two methods (Before=traps present at the time of release, Delayed=traps placed in blocks at pre-marked trees 48 hours after release). Means with the same letters are not significantly different (Tukey's α = 0.05).

Female aggregation: There were significant treatment differences in aggregation based on the absolute value ($F_{5,83}$=12.0602, P<<0.0001) for females released in this experiment (Table 4.1). Blocks without mating disruption had the lowest aggregation, while those with passive dispensers had the highest.

There also were significant differences for female aggregation based on the % of total moths recaptured ($F_{5,84}$=7.3820, P<<0.0001) among the treatments (Table 4.1). Blocks without

mating disruption had the least aggregation, and those with passive dispensers had the most aggregation.

Male recapture by distance: There were significant between treatment differences in male recapture at two of the trap distances (Table 4.2), but Tukey's test only revealed minor differences between treatments in capture at the farthest distance from the central release location.

Female recapture by distance: There were significant differences in sterile female recapture by distance among the treatments (Table 4.2). Blocks without mating disruption had higher recapture at the farthest two distances than those with mating disruption.

Male recapture evenness within and between treatments: For each of the 'Control-Before', 'Control-Delayed', 'Passive-Before', 'Passive-Delayed', and 'Active-Before' treatments, there were no significant differences in male recapture by quadrant (Table 4.3), but in 'Active-Delayed' treated blocks there were significantly more moths captured in the Northwest (Q2) than in the Northeast quadrant (Q3) ($F_{3,68}$=4.6230, P=0.0053).

Between treatments, in the quadrant Southwest of the central release point (Q1) significantly more sterile male moths were recaptured in apple orchards that received the 'Control-Before' and 'Control-Delayed' treatments than all other treatments, and traps in 'Passive-Delayed' treated orchards recaptured significantly fewer moths than 'Active-Before' and 'Active-Delayed' treated orchards ($F_{5,100}$=29.8345, P<<0.0001) (Table 4.4).

Mean ± SEM recapture	♂							
		ANOVA	***Control-Before*** ***(n=18)***	***Control-Delayed*** ***(n=18)***	***Passive-Before*** ***(n=17)***	***Passive-Delayed*** ***(n=16)***	***Active-Before*** ***(n=18)***	***Active-Delayed*** ***(n=18)***
	28m	F=2.5421 df=5, 99 P=0.0330	45.14±2.09 a	37.72±1.68 a	45.92±4.50 a	51.64±5.40 a	47.51±3.15 a	37.13±3.03 a
	63m	F=1.3151 df=5, 99 P=0.2638	42.83±1.63	43.90±0.94	39.04±4.0	38.07±4.74	39.05±1.85	40.09±1.78
	85m	F=5.0707 df=5, 99 P=0.0004	12.02±0.81 ab	18.38±1.23 a	15.04±2.04 ab	10.29±2.28 b	12.44±2.06 ab	22.78±2.08 a
Mean ± SEM recapture	♀							
		ANOVA	***Control-Before*** ***(n=18)***	***Control-Delayed*** ***(n=18)***	***Passive-Before*** ***(n=16)***	***Passive-Delayed*** ***(n=11)***	***Active-Before*** ***(n=17)***	***Active-Delayed*** ***(n=16)***
	28m	F=2.2508 df=5, 99 P=0.0551	26.86±1.68	34.05±1.65	47.73±3.93	47.26±6.74	56.25±4.41	48.19±4.57
	63m	F=3.7113 df=5, 99 P=0.0040	52.68±1.19 a	46.15±1.00 a	40.22±2.68 ab	42.76±5.57 b	34.39±3.51 ab	39.56±3.45 ab
	85m	F=8.9693 df=5, 99 P<<0.0001	20.46±1.25 a	19.80±1.66 a	12.05±2.86 ab	9.98±3.93 b	9.36±1.58 b	12.26±1.50 ab

Table 4.2. Recapture distance. Comparison of treatment percent of total (±SEM) sterile male and female *C. pomonella* recaptured at three distances from point of release in 4.05ha apple orchards. Treatments were: 1) control with no mating disruption, 2) passive mating disruption (Scentry NoMate® CM Spiral (Billings, MT)) at 300-350/ac, and 3) mating disruption with active dispensers (ISOMATE® CM Mist Plus (Vancouver, WA)) at ½-3ac), with trapping performed by two methods for each (Before=traps present at release time, Delayed=traps placed 48 hours after release). Analysis includes only replications that captured ≥1 moth. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

In the Northwest quadrant (Q2), between treatments, traps in orchards treated with 'Control-Before' captured more moths than 'Passive-Before', 'Passive-Delayed', 'Active-Before', and 'Active-Delayed' treated orchards ($F_{5,100}$=18.3192, P<<0.0001). Additionally, traps in 'Control-Delayed' treated orchards had more male moths than 'Passive-Before', 'Passive-Delayed', and 'Active-Before' treated orchards, and 'Active-Delayed' treated orchards had significantly more male moths than 'Passive-Delayed' treated orchards (Table 4.4).

In the Northeast quadrant (Q3) significantly more male moths ($F_{5,100}$=36.2294, P<<0.0001) were recaptured in blocks that received 'Control-Before' and 'Control-Delayed' treatments than all other treatments, and the other treatments were not different from each other (Table 4.4).

Traps deployed in the quadrant Southeast (Q4) of the central moth release point captured significantly more male *C. pomonella* when orchards were treated with 'Control-Before' and 'Control-Delayed' than all other treatments ($F_{5,100}$=27.2393, P<<0.0001) (Table 4.4). Also, traps in 'Active-Delayed' treated orchards recaptured significantly more male moths than 'Passive-Delayed' treated orchards.

Female recapture evenness within and between treatments: For the 'Control-Delayed', 'Passive-Before', 'Passive-Delayed', 'Active-Before', and 'Active-Delayed' treatments, there were no significant differences in female recapture by quadrant (Table 4.3), but in 'Control-Before' treated blocks there was a slight but significantly higher female moth recapture in traps in the Southeast quadrant (Q4) than in the Southwest quadrant (Q1) ($F_{3,68}$=2.8017, P=0.0464).

			♂					♀				
	Treatment	**# reps (♂/♀)**	**ANOVA**	**Quadrant 1**	**Quadrant 2**	**Quadrant 3**	**Quadrant 4**	**ANOVA**	**Quadrant 1**	**Quadrant 2**	**Quadrant 3**	**Quadrant 4**
Mean ± SEM recapture	**Control-Before**	18/18	F=1.8611 df=3, 68 P=0.1444	107.8±10.6	135.4±12.5	124.5±12.4	145.1±12.0	F=2.8017 df=3, 68 P=0.0464	22.3±2.5 a	28.8±3.8 a,b	27.9±3.3 a,b	35.9±3.9 b
	Control-Delayed	18/18	F=0.8732 df=3, 68 P=0.4594	84.6±7.6	80.7±6.9	74.7±9.2	73.5±8.8	F=2.6842 df=3, 68 P=0.0535	34.6±3.5	45.7±6.7	23.6±3.6	35.6±5.0
	Passive-Before	18/16	F=1.6130 df=3, 68 P=0.1944	17.6±4.9	27.4±7.5	8.4±2.5	14.5±4.1	F=2.5190 df=3, 60 P=0.0665	22.8±6.1	31.3±7.0	9.6±2.8	19.0±4.5
	Passive-Delayed	16/11	F=0.4148 df=3, 60 P=0.7430	10.6±5.6	20.1±8.6	11.6±5.0	13.4±6.9	F=0.0816 df=3, 40 P=0.9697	11.1±6.3	27.4±15.8	12.3±5.7	21.6±13.9
	Active-Before	18/17	F=1.3690 df=3, 68 P=0.2597	15.7±2.9	21.0±4.9	9.2±2.1	16.3±4.0	F=0.6586 df=3, 64 P=0.5805	17.0±3.5	20.6±5.6	8.4±1.6	18.4±4.9
	Active-Delayed	18/16	F=4.6230 df=3, 68 P=0.0053	21.3±4.0 a,b	55.3±10.1 b	15.1±2.9 a	24.7±5.2 a,b	F=0.5599 df=3, 60 P=0.6436	30.3±7.6	53.1±11.7	25.8±5.3	46.7±11.2

Table 4.3. Recapture quadrant by treatment. Comparison of mean (±SEM) sterile male and female *C. pomonella* recapture in quadrants treated with: 1) control with no mating disruption, 2) passive mating disruption dispensers (Scentry NoMate® CM Spiral (Billings, MT)), and 3) mating disruption with active dispensers (ISOMATE® CM Mist Plus (Vancouver, WA)), and traps deployed 1) Before release, 2) Delayed 48 hours after release. Analysis includes only replications with non-zero recapture. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

Mean ± SEM recapture	♂							
		ANOVA	***'Control-Before' (n=18)***	***'Control-Delayed' (n=18)***	***'Passive-Before' (n=18)***	***'Passive-Delayed' (n=16)***	***'Active-Before' (n=18)***	***'Active-Delayed' (n=18)***
	Southwest quadrant (Q1)	F=29.8345 df=5, 100 P<<0.0001	107.8±10.6 a	84.6±7.6 a	17.6±4.9 b,c	10.6±5.6 c	15.7±2.9 b	21.3±4.0 b
	Northwest quadrant (Q2)	F=18.3192 df=5, 100 P<<0.0001	135.4±12.5 a	80.7±6.9 a,b	27.4±7.5 c,d	20.1±8.6 d	21.0±4.9 c,d	55.3±10.1 b
	Northeast quadrant (Q3)	F=36.2294 df=5, 100 P<<0.0001	124.5±12.4 a	74.7±9.2 a	8.4±2.5 b	11.6±5.0 b	9.2±2.1 b	15.1±2.9 b
	Southeast quadrant (Q4)	F=27.2393 df=5, 100 P<<0.0001	145.1±12.0 a	73.5±8.8 a	14.5±4.1 b,c	13.4±6.9 c	16.3±4.0 b,c	24.7±5.2 b
Mean ± SEM recapture	♀							
		ANOVA	***'Control-Before' (n=18)***	***'Control-Delayed' (n=18)***	***'Passive-Before' (n=16)***	***'Passive-Delayed' (n=11)***	***'Active-Before' (n=17)***	***'Active-Delayed' (n=16)***
	Southwest quadrant (Q1)	F=4.6784 df=5, 90 P=0.0008	22.3±2.5 a,c	34.6±3.5 a	22.8±6.1 a,b,c	11.1±6.3 b	17.0±3.5 c	30.3±7.6 a
	Northwest quadrant (Q2)	F=2.8846 df=5, 90 P=0.0031	28.8±3.8 a,b	45.7±6.7 b	31.3±7.0 a,b	27.4±15.8 a	20.6±5.6 a	53.1±11.7 a,b
	Northeast quadrant (Q3)	F=6.6609 df=5, 90 P<0.0001	27.9±3.3 a	23.6±3.6 a,c	9.6±2.8 b,c,d	12.3±5.7 c,d	8.4±1.6 d	25.8±5.3 a,d
	Southeast quadrant (Q4)	F=5.3818 df=5, 90 P=0.0002	35.9±3.9 a	35.6±5.0 a	19.0±4.5 a,b	21.6±13.9 b	18.4±4.9 a,b	46.7±11.2 a

Table 4.4. Recapture by quadrant among treatments. Comparison of quadrant mean (±SEM) sterile male and female *C. pomonella* recaptured by treatment in 4.05ha apple orchard blocks treated with one of three mating disruption (MD) treatments: 1) control with no mating disruption, 2) passive dispenser mating disruption (Scentry NoMate® CM Spiral (Billings, MT)), and 3) mating disruption with active dispensers (ISOMATE® CM Mist Plus (Vancouver, WA)) at ½-3ac). Trapping of sterile moths was performed by two methods for each mating disruption treatment (Before=traps present at the time of release, Delayed=traps placed 48 hours after release). Analysis includes only those replications with non-zero recapture. Means with the same letters are not significantly different (Tukey's $\alpha = 0.05$).

There were significant differences in female recapture among treatments within each of the four quadrants (Table 4.4). In the southwest quadrant (Q1), significantly fewer females were recaptured in orchards treated with 'Passive-Delayed' than in those treated with 'Control-Before' and 'Control-Delayed'. Additionally, significantly more female moths were recaptured in orchards treated with 'Control-Delayed' than in 'Passive-Delayed' and 'Active-Before' orchards.

In the Northwest quadrant (Q2), significantly more female moths were recaptured in traps in orchards treated with 'Control-Delayed' than those in traps in 'Passive-Delayed' and 'Active-Before' treated orchards (Table 4.4).

There were significant differences in recapture of moths in the Northeast quadrant (Q3) among all the treatments (Table 4.4). Higher female recaptures were recorded in traps in orchards treated with 'Control-Before' than in 'Passive-Before', 'Passive-Delayed', and 'Active-Before' treated orchards. There was also higher recapture in orchards treated with 'Control-Delayed' than 'Passive-Before' and 'Active-Before' treated orchards.

Traps deployed in the quadrant Southeast (Q4) of the central point of moth release captured fewer female moths in traps in 'Passive-Before', 'Passive-Delayed', and 'Active-Before' blocks than those placed in 'Control-Before', 'Control-Delayed', and 'Active-Delayed' blocks (Table 4.4).

Male recapture in active dispenser blocks: In the two 16.2ha orchards treated with active dispensers, 'Active-Before' and 'Active-Delayed', there was no difference in recapture of male *C. pomonella* by quadrant (Table 4.5). There were also no significant differences in recapture of male moths in traps whether they were positioned near, mid-distance or far away from the

emitter for either 'Active-Before' or 'Active-Delayed' treatments (Table 4.6). At the furthest distance (>40m) between trap and active dispenser, significantly more male moths were recaptured in 'Active-Delayed' treated blocks than 'Active-Before' blocks, but there were not differences between the two treatments at the near or mid distances (Table 4.7).

Female recapture in active dispenser blocks: There was no significant difference in recapture of sterile female *C. pomonella* by quadrant in the two 16.2ha orchards treated with active dispensers, 'Active-Before' and 'Active-Delayed' (Table 4.5). There also were not significant differences in female recapture in traps whether they were positioned near, mid-distance or far away from the emitter for either 'Active-Before' or 'Active-Delayed' treatments (Table 4.6). At the nearest distance between trap and active dispenser (<20m), there were no significant differences in recapture of female moths between the 'Active-Before' or 'Active-Delayed' treatments. However, at the intermediate trap/emitter proximity distance (20-40m) and the furthest distance (>40 trap), significantly more female moths were recaptured in 'Active-Delayed' treated blocks than 'Active-Before' treated blocks (Table 4.7).

♂				♀			
'Active-Before'		**'Active-Delayed'**		**'Active-Before'**		**'Active-Delayed'**	
F=0.4521 df=15, 56 P=0.9450		F=1.0167 df=15, 56 P=0.4524		F=0.3284 df=15, 56 P=0.9898		F=1.0330 df=15, 48 P=0.4401	
Orchard/ Block/ Quadrant #	**Mean±SEM**	**Orchard/ Block/ Quadrant #**	**Mean±SEM**	**Orchard/ Block/ Quadrant #**	**Mean±SEM**	**Orchard/ Block/ Quadrant #**	**Mean±SEM**
1/A/Quad. 1	16.0±5.3	*5/A/Quad. 1*	23.6±10.5	*1/A/Quad. 1*	17.4±7.9	*5/A/Quad. 1*	24.4±12.5
1/A/Quad. 2	15.2±7.4	*5/A/Quad. 2*	57.2±20.4	*1/A/Quad. 2*	15.2±8.8	*5/A/Quad. 2*	54.2±27.7
1/A/Quad. 3	11.4±6.7	*5/A/Quad. 3*	10.2±4.7	*1/A/Quad. 3*	8.6±4.0	*5/A/Quad. 3*	14.2±8.0
1/A/Quad. 4	12.6±6.8	*5/A/Quad. 4*	20.6±7.1	*1/A/Quad. 4*	20.8±12.7	*5/A/Quad. 4*	47.8±26.4
1/B/Quad. 1	22.4±8.0	*5/B/Quad. 1*	17.6±4.5	*1/B/Quad. 1*	18.4±7.9	*5/B/Quad. 1*	14.0±5.2
1/B/Quad. 2	23.4±9.8	*5/B/Quad. 2*	61.8±23.7	*1/B/Quad. 2*	29.0±15.0	*5/B/Quad. 2*	32.4±17.1
1/B/Quad. 3	8.6±2.9	*5/B/Quad. 3*	15.6±4.7	*1/B/Quad. 3*	6.8±2.7	*5/B/Quad. 3*	20.2±9.0
1/B/Quad. 4	25.0±11.2	*5/B/Quad. 4*	27.4±14.2	*1/B/Quad. 4*	23.6±10.8	*5/B/Quad. 4*	33.4±21.0
1/C/Quad. 1	12.3±3.0	*5/C/Quad. 1*	18.0±8.7	*1/C/Quad. 1*	8.5±4.7	*5/C/Quad. 1*	45.7±28.4
1/C/Quad. 2	12.5±4.3	*5/C/Quad. 2*	45.0±22.9	*1/C/Quad. 2*	10.5±7.0	*5/C/Quad. 2*	73.7±33.7
1/C/Quad. 3	9.3±3.8	*5/C/Quad. 3*	16.5±6.9	*1/C/Quad. 3*	6.3±2.5	*5/C/Quad. 3*	34.0±14.0
1/C/Quad. 4	7.8±3.0	*5/C/Quad. 4*	27.3±12.3	*1/C/Quad. 4*	6.5±4.6	*5/C/Quad. 4*	55.3±25.3
1/D/Quad. 1	10.5±4.7	*5/D/Quad. 1*	26.3±9.7	*1/D/Quad. 1*	19.0±7.1	*5/D/Quad. 1*	51.7±17.7
1/D/Quad. 2	33.8±15.7	*5/D/Quad. 2*	55.0±20.0	*1/D/Quad. 2*	22.0±11.4	*5/D/Quad. 2*	65.3±11.5
1/D/Quad. 3	7.0±2.6	*5/D/Quad. 3*	19.3±9.0	*1/D/Quad. 3*	10.3±3.4	*5/D/Quad. 3*	46.3±7.5
1/D/Quad. 4	18.8±7.4	*5/D/Quad. 4*	23.8±10.3	*1/D/Quad. 4*	16.3±5.4	*5/D/Quad. 4*	58.3±18.1

Table 4.5. Recapture in active dispenser treated blocks by quadrant. Comparison of sterile male and female *C. pomonella* quadrant mean (±SEM) recaptured by block in 4.05ha apple orchard blocks treated with mating disruption (ISOMATE® CM Mist Plus (Vancouver, WA)) at ½-3ac). Trapping of sterile moths was performed by two methods (Before=traps present at the time of release, Delayed=traps placed 48 hours after release). Analysis includes only those replications with non-zero recapture.

		♂				♀			
		ANOVA	***Near***	***Mid***	***Far***	**ANOVA**	***Near***	***Mid***	***Far***
Treatment	**'Active-Before'**	F=0.2433 df=2, 51 P=0.7850	4.6±1.0	4.2±0.8	3.4±0.7	F=0.3421 df=2, 51 P=0.7119	4.5±1.2	4.1±0.9	3.4±1.0
	'Active-Delayed'	F=0.9596 df=2, 51 P=0.3898	5.4±1.0	6.6±1.2	8.5±1.5	F=0.0884 df=2, 51 P=0.9155	9.8±2.4	11.8±2.6	9.1±2.0

Table 4.6. Recapture proximal to active dispensers by treatment. Comparison of mean (±SEM) sterile male and female *C. pomonella* recapture in 4.05ha orchard blocks treated with active dispenser mating disruption (ISOMATE® CM Mist Plus (Vancouver, WA)) at ½-3ac) by proximity of traps to active dispensers. Traps denoted "near" active dispensers were <20m away, those denoted "mid" distance were 20-40m distant, and those denoted "far" were >40m distant from active dispensers. Trapping of sterile moths was performed by two methods (Before=traps present at the time of release, Delayed=traps placed 48 hours after release).

		♂			♀		
		T-Test	**'Active-Before'**	**'Active-Delayed'**	**T-Test**	**'Active-Before'**	**'Active-Delayed'**
Proximity to dispenser	**Near**	*t*=0.6507 df=34 P=0.5196	4.6±1.0	5.4±1.0	*t*=1.7011 df=30 P=0.0993	4.5±1.2	9.8±2.4
	Mid	*t*=1.5922 df=34 P=0.1206	4.2±0.8	6.6±1.2	*t*=2.0983 df=27 P=0.0454	4.1±0.9	11.8±2.6*
	Far	*t*=2.6851 df=33 P=0.0113	3.4±0.7	8.5±1.5*	*t*=2.2566 df=29 P=0.0317	3.4±1.0	9.1±2.0*

Table 4.7. Treatment recapture in traps proximal to active dispensers. T-test comparison of treatment mean (±SEM) by trap method sterile male and female *C. pomonella* recapture in 4.05ha orchard blocks treated with active dispenser mating disruption (ISOMATE® CM Mist Plus (Vancouver, WA)) at ½-3ac) trap proximity of traps to active dispensers. Traps denoted "near" active dispensers were <20m away, those denoted "mid" distance were 20-40m distant, and those denoted "far" were >40m distant from active dispensers. Trapping of sterile moths was performed by two methods (Before=traps present at the time of release, Delayed=traps placed 48 hours after release). Significant differences are indicated with "*".

DISCUSSION

Mating disruption for *C. pomonella* has been shown to interrupt normal mate finding behavior by directly competing with female moths for the time and attention males expend on this activity (Tomaszewska et al., 2005; Miller and Gut, 2015; Gut et al. 2019). This is the case when pheromone is dispensed from passive reservoir dispensers (Miller et. al., 2010) or active dispensers (McGhee et al., 2014). Miller and Gut (2015) proposed the term "induced allopatry" to describe the possible mechanism of *C. pomonella* active dispensers' disruption where attraction to active dispensers releasing codlemone resulted in higher male catches near compared to far from dispensers. Additionally, McGhee (2014), Welter and Cave (2000), and Welter et al. (2000) reported that the specific location of single active dispensers in an orchard can influence capture in monitoring traps by causing moths to respond to traps in areas of the farm that had incomplete coverage of pheromone disruption. In the study reported herein, similar levels of recapture and patterns of dispersion were recorded in plots treated with either passive or active dispensers. In addition, similar levels of population disruption were recorded throughout the pheromone treated plots. Unlike the previous studies, *C. pomonella* traps were baited with lures containing codlemone plus two kairomones that are synergistically attractive to adults rather than lures releasing only codlemone. The combination of codlemone plus the two kairomones, pear ester and acetic acid, attract males and females to traps and overcomes the inhibitory effects of the pheromone disruption treatment on male captures (Landolt et al., 2007; Knight et al., 2005). The ability of moths to detect traps throughout the block despite dispersion within a mosaic of natural and synthetic pheromone plumes could explain why there was not a clustering of males upwind from dispensers as reported by McGhee (2014) and Welter

et al. (2000), as well as similar levels of male recapture in traps at near, mid and far distances from an active dispenser. These findings suggest that dispersion of the population of codling moths is random and effectively uniform even in the presence of devices emitting large quantities of pheromone, and that interference with trap finding is more likely the cause of previous findings. Clustering of males near active dispensers as was reported by McGhee (2014) and upwind as reported by Welter and Cave (2000) and Welter et al. (2000) may be a result of pheromone concentrations being very low directly upwind of active dispensers, allowing males to respond to codlemone-baited traps in an area of low pheromone coverage while realistically maintaining a uniform population distribution throughout the orchard. The findings of this study demonstrate that there is risk in utilizing lures in monitoring traps that are unevenly suppressed by mating disruption; damage is unlikely to follow the same pattern.

Although active dispensers are deployed at much lower densities per unit area than passive dispensers, the plume of pheromone emanating from these high-releasing devices is likely greater due to the aerosol nature and higher dose of active ingredient released from emitters. McGhee et al. (2014) found that a single MIST pheromone unit can suppress trap finding 4.5 times more than a single trap. As suggested by Baker and Hansson (2016) strong plume strands have the best chance of reaching males far downwind, resulting in moths continuously interacting with pheromone as they move upwind toward the source. Pheromone plumes from active dispensers have been estimated to travel up to 300m from the emitter and disruption has been demonstrated as far as 450m away (Welter and Cave, 2000; Welter et al., 2005). Taking advantage of the apparent large plume, active dispensers have been deployed at lower densities per orchard area than reservoir dispensers. The major risk in using a low-density of dispensers is

that pheromone distribution is dependent on wind flow and areas of little-or-no pheromone coverage can occur (McGhee et al., 2014; Gut et al., 2019). Wind, at the time of moth activity certainly plays a role in determining the direction that pheromone is carried throughout the orchard, and therefore the completeness of disruption. In the present study, there was no indication based on recapture of released moths that some areas of the orchard were devoid of pheromone. If winds were steady, or always from the same direction during periods of activity there likely would be areas of greater and lesser disruption due to the effect of air flow on plumes of pheromone. Not revealing a clear pattern where male captures were highest, may suggest that the wind is constantly shifting and pheromone concentrations in various regions of the orchard are in random and continuous flux. Also expected is that the use of the three-part lure allows trap detection equally in areas of low and high pheromone concentration following uniform and random moth dispersion throughout the orchard. Over a 7-day trapping period the impact of disruption downwind of emitters could not be discerned; there was no upwind or downwind pattern of movement, but rather moth dispersion appeared to be random and uniform throughout a constantly shifting chemical landscape.

The use of lures releasing both codlemone and two kairomones facilitated examining both male and female dispersal following release of sterile adults. Similar levels of recapture and patterns of aggregation throughout the orchard were found for males and females, suggesting a similar dispersal pattern of both sexes throughout the orchard following release. For male and female moths, recapture was highest and aggregation lowest in blocks without mating disruption. In pheromone treated plots, recapture of moths in traps baited with codlemone, pear ester and acetic acid was almost evenly split between the sexes. In contrast, Judd (2016) found

that the ratio of male to female recapture in mating disrupted blocks was highly influenced by the kinds of compounds emitted from the lure; traps baited with lures containing acetic acid and pear ester captured 1.7 times more females and 95% fewer males than traps baited with lures combining only codlemone and pear ester.

This research provides some insights into the plume reach of the CM-DA+AA lure, and how far the plume emanating from the pear ester and acetic acid lure attracts males and females. Plant volatile kairomones are generally considered to have short plume reaches (Braasch and Kaplan, 2012; Schlyter, 1992). Judd (2016) conjectured, based on male versus female Sterile:Wild ratios in traps baited with pear ester/acetic acid combination, that these kairomones likely are a short-range attractant for both males and females. The finding that more females were recaptured at all distances in pheromone-free plots when trap placement was delayed is consistent with the plume being short; disruption by the traps close to the release was observed. In blocks without mating disruption, higher numbers of both male and female moths were captured in traps close to the release point when traps are present at the time of release than when trap placement was delayed 48 hours after moth dispersal begins. Additionally, more moths were caught at distances farther from the release point when trap placement was delayed than when traps were not delayed. Judd (2018) also found that the CM DA combination yielded catches with male sterile to wild ratios that were significantly greater than female sterile to wild ratios and proposed that this likely meant that the codlemone component attracted males over a longer range. Similarly, the findings the current study are consistent with the codlemone component of the lure having a larger plume reach or active space over which to attract males than the kairomones attract both sexes.

A major hypothesis incorporated into the experimental design of the study reported herein was that the presence of lure baited traps in the plots at the time moths were released would impede the movement of moths by acting as disruptive influences. This likely effect on moth movement could be discerned by including a protocol in which moths had 48 hours to disperse before deploying attractant-baited traps. The concern with lure baited traps impeding movement was based on previous research showing that as the density of *C. pomonella* traps in an orchard increased, capture/trap decreased, suggesting competition of traps for the attention of males (McNally and Barnes, 1981). In the study reported herein, more male moths were recaptured in plots with active dispensers when traps were delayed 48 hours. In contrast, higher captures were also found in plots treated with no mating disruption and with passive dispensers when trap placement was not delayed. The results of the present study did not provide much support for the hypothesis that attractant-baited traps significantly interfere with moth dispersal. In the absence or presence of a mating disruption treatment, traps in the orchard at the time of moth release did not significantly impact recapture or aggregation of male moths at trap distances of 28m and 63m from the point of origin when compared to delaying trap placement 48 hours after release. In blocks with active dispensers there was a significant increase in capture at 85m from release when traps deployment was delayed, evidence for a slight degree of interference from the traps. However, the preponderance of evidence presented here, and described by Miller et al. (2015), supports random movement of sterile codling moths from the point of origin even in the presence of pheromone mating disruption rather than mating disruption technology or traps influencing where in the orchard moths disperse.

It is possible that with these data it was impossible to discern the effect of delaying trap placement on moth movement because sterile females were also released and were additionally competitive with traps for the attention of males. In this scenario, the presence of a large number of sterile females was having a major influence on male captures in traps and overriding the possible influence of the traps. Recent studies have suggested that sterile females may provide a major disruptive effect when released as part of a SIT program (Stringer et al., 2013; Horner et al., 2020). Unfortunately, due to the nature of sterile codling moth shipments being mixed sex, the disruptive impact of traps when releasing males only was not able to be studied. The level of interference caused by pheromone-producing released sterile *C. pomonella* females that self-disperse rapidly, randomly, and uniformly throughout the orchard in essentially the same pattern as males should be explored in the future.

An intermediate period of time was allowed for moths to respond to traps because previous work indicated sterile codling moth's field responses occur as late as 20 days after release, though most SIT moths locate traps within 7 days (R. Curtiss, unpublished data). One potential drawback of the 7-day trapping period used in this study was an inability to observe responses on a fine time scale. It is possible that dispersion of moths at 24- and 48-hours was different in all plots receiving traps at different times, but by day 7, due to continued random movement of the released CM population throughout the test area (Miller et al., 2015), differences decreased. Over time, differences in dispersion, recapture, and aggregation may be diluted until treatment differences are nullified. Also, the likelihood of moths interacting with traps increases over time if they disperse throughout the orchard randomly, and under this scenario differences among the treatments would also decrease as time increased. Of course,

there is an upper limit to the time allowed for moth response; eventually enough die to offset the increased likelihood of trap finding. Likewise, most mark-release-recapture studies of insects suffer from an inability to track the movements of individuals, and recovery of relatively small numbers of the released populations; the current study had the same shortcomings.

From the results of this study it appears that sterile *C. pomonella* dispersion and response to monitoring traps baited with the PHEROCON® CM-DA COMBO™ Lure + AA Lure is not impacted differently by passive and active disruption technologies over several-day time scales. This combination of pheromone and kairomone is an effective means of measuring male and female moth dispersal in the presence of pheromone. Male and female moth movement from the point of origin is over their lifespan, and response to traps in pheromone mating disrupted orchards occurs primarily when they are in close proximity to traps and not disrupted by the deployed synthetic pheromone. This random spread results in the nearly uniform capture of both males and females among the four quadrants where traps were placed surrounding moth release points. In orchards without mating disruption, dispersion of moths is more uniform than those under disruption, but not different among the pheromone-treated orchards as indicated by Morisita indices. Additionally, the presence of traps at the time of release and their use for measuring dispersal does not seem to impact the patterns of dispersal measured over the time scale of this study. These data demonstrate that both male and female adult *C. pomonella* disperse randomly throughout the orchard when released at a single central point, resulting in an effectively uniform distribution regardless of mating disruption technology employed, or presence of monitoring traps, highlighting the limitations of relying on monitoring traps for predicting population dispersal and movement. When monitoring traps baited with codlemone

are used in orchards with pheromone mating disruption, they may be more effective in locating areas with inadequate pheromone coverage than in locating concentrations of codling moths and corresponding damage. Monitoring traps baited with codlemone and the two kairomones, pear ester and acetic acid, are more accurate at predicting the locations of wild moth populations when used in orchards with pheromone mating disruption, and because males and females were found to disperse similarly, it may not be necessary to separate sexes in traps baited with this lure when making management decisions.

CHAPTER FIVE

Differential Sterile *Cydia pomonella* (L.) Release Densities and Generational Targeting for Codling Moth Control in Washington Commercial Organic Apple Orchards

INTRODUCTION

The codling moth, *Cydia pomonella* (L.), the key pest worldwide in apples also infests pear, walnut, quince, crabapple, loquat, hawthorn and some stone fruits (apricots, cherries, peaches, plums, prunes) (Newcomer and Whitcomb, 1925). It is a fruit quality pest because larval instars feed internally on the fruit of host plants rendering them unmarketable. Throughout most of its range, *C. pomonella* has two to three adult emergences annually (Seigler and Plank, 1921; Hall, 1928; Geier, 1963). Observations in Oregon suggest that in addition to favorable temperatures, there is a critical photoperiod of 13-13.5 hours needed to break early spring diapause of fifth instar larvae (Setyobudi, 1989). After diapause, larvae pupate, and then a brood of moths emerge, mate, and lay the eggs of the first summer generation (Geier, 1963). Seigler and Plank (1921) report that some individuals of the first summer generation enter diapause and remain inactive until the following season, but most pupate, emerge as adults, mate, and lay the eggs of the second summer generation. Setyobudi (1989) found that in Oregon almost 40% of all first-generation individuals entered diapause. Most individuals, up to 77% (Setyobudi, 1989), of the second generation will enter diapause as fully fed, mature fifth instar larvae, but some will pupate and emerge as adults that mate and lay the eggs of the third generation (Geier, 1963) when conditions are favorable. A small number of third generation larvae will survive when conditions are advantageous and enter diapause to wait out the winter.

Many of the *C. pomonella* phenology events are tied to temperature and environmental conditions and they can be predicted by tracking temperature. The first phenology model for this pest was developed by Glenn (1922), but it suffered from inaccurate methods of estimating in-field populations. Early work by Geier (1963) found that fruit damage in the early spring began 7-

10 days after temperatures above 60°f were sustained. Much work was fundamental to development of the PETE (Predictive Extension Timing Estimator) model (Reidl and Croft, 1974; Reidl et al., 1976; Welch et al 1978) which required extensive early season trapping to establish a biofix based on the first captures of moths. In Washington State, a degree day model developed by Jones et al. (2013) without the need for early season monitoring and biofix allowed for prediction of moth emergence. The Jones et al. (2013) model predicted that the first brood of moths emerges from pupae after ca. 175 (°F) degree days from January 1 with a base temperature of 50°F and begin to deposit eggs after 225-275 degree-days, and the second brood emerges at ca. 1200 degree-days. Knowing local conditions and degree day accumulation allows for accurate predictions, but the degree of accuracy needed varies by management decisions and methods employed for *C. pomonella* control.

As insecticide use continues to decline, many farmers are transitioning orchards to organic production. Unfortunately, there are limited effective control options available for organic farmers to manage *C. pomonella*. Pheromone mating disruption, using this pest's sex pheromone is an effective tactic currently applied on ca. 243,000ha of commercial apples, pears, and walnuts worldwide (Gut et al. 2019). The development of mating disruption was in response to increased regulatory pressure and resistance development against insecticides, and the first pheromone dispenser for *C. pomonella* mating disruption was registered for commercial use in 1991 (Witzgall et al., 2008). In addition to mating disruption, a highly virulent *C. pomonella* granulosis virus (CpGV) was discovered in infected codling moth larvae near Valle de Allende, Chihuahua, Mexico in the 1960's (Tanada, 1964), and was found to be transmissible in frass among individuals (Tanada and Leutenegger, 1968). Larval entry into apples was reduced by

about 95% by field applications of experimental CpGV extracts every two weeks, and the LD50 for late instar larvae was found to be about 30 virus capsules/L_1 (Keller, 1973). Virus development and pest mortality is inversely proportional to dose (Sheppard and Stairs, 1977). Caterpillar death, followed by liquefaction usually occurs within five to ten days (Arthurs and Lacey, 2004). Commercially produced CpGV is extracted from mass-reared, infected codling moth larvae and contains homogenized larvae, glycerol, and water (Certis, 2009). CpGV is the most effective commercially produced biological agent commonly used in the control of codling moth (Lacey et al., 2008). Lacey et al. (2008) provides a comprehensive history of CpGV along with formulation information, resistance development, and a discussion of use in Integrated Pest Management programs.

In addition to chemical and organic control, *C. pomonella* has also been a target of the sterile insect technique (SIT). The tactic of using ionizing radiation to sterilize large numbers of pest insects was put into practice beginning in the 1950's in the United States by Knipling, Bushland, Lindquist, Hopkins, Baumhover, and others at the USDA (Baumhover, 2002) for the control and eradication of the screw-worm in Curacao, Florida, and the Southeastern United States. The screwworm SIT program achieved eradication by the 1970's in the US. By the 1980's eradication was achieved in Mexico and Belize, and by the 1990's the pest was eradicated south throughout Central America to Panama where a biological barrier was established to prevent re-infestation (Baumhover, 2002). Area-wide eradication of insect pests became the modus operandi of sterile insect release programs worldwide, and there have been many successes, including pink bollworm, for which eradication was declared on 19 October 2018 after 18 years

of a three-pronged approach of pheromone mating disruption, SIT, and use of transgenic cotton (Purdue, 2018).

Proverbs and others began working on *C. pomonella* sterile releases in British Columbia, Canada beginning in the 1960's and by 1992 a fully formed area-wide sterile insect release (SIR) program was initiated in the South Okanagan region of British Columbia, Canada (Thistlewood and Judd, 2019). Much of Proverbs work was used to determine release rate and frequency with the goal to achieve eradication (Proverbs, 1965; Proverbs and Newton 1962a, 1962b, 1962c; Proverbs et al., 1966; Proverbs et al., 1967; Proverbs et al., 1969; Proverbs et al., 1975). They estimated a target of 40:1 sterile to wild-type male moth release densities timed weekly throughout the growing season would achieve eradication.

Researchers in Washington State explored the use of sterile *C. pomonella* in the 1960's and 1970's, but ultimately abandoned the technique in favor of mating disruption. Hathaway (1966) effected significant reductions in viable mating with increasing doses of gamma radiation in field and laboratory studies without sacrificing vigor. Hathaway et al. (1966) and Hathaway et al. (1968) used aerosol chemical sterilizing agents to sterilize large numbers of *C. pomonella* for release near Yakima, WA. White et al. (1969) released sterilized mixed-sex *C. pomonella* in Yakima, WA six days per week from May 16-Sept 14 in a small orchard plot and reduced fruit damage from almost 50% in 1965 without SIR to 1.57% in 1966 with SIR. Butt et al. (1970) found control following releases of sterile *C. pomonella* to be comparable to treatments with chemical insecticides, but never achieved the theorized eradication ratio of 40:1. Butt et al. (1972) prepared a 32 square mile area for SIR by treating with pesticides and sanitization to reduce *C. pomonella* populations, and then released mixed-sex sterile moths from April to September

1971, and reduced native *C. pomonella* capture and overwintering larvae by over 90% from 1970 to 1971 (Butt et al., 1973). Following the accidental release of 336 fertile females, White et al. (1973a) successfully suppressed mating by following up with mass releases 24 and 48 hours later in addition to regularly scheduled daily releases. In a 20-acre Yakima, WA apple orchard, season-long releases of sterile *C. pomonella* reduced infestation by 92% (White et al., 1973b). White et al. (1976) ultimately experienced failure of the sterile insect technique for *C. pomonella* from 1971 to 1972 when infestation and fruit damage increased within the area that sterile moths were released and the sterile: wild ratio never exceeded 20:1, however they concluded that SIR with other control methods still suppressed wild populations.

The Okanagan-Kootenay Sterile Insect Release (OKSIR) rearing facility of BC, Canada, completed in 1993 at a cost of $7.4 million, began sterile *C. pomonella* releases in 1994. In 2004 the area-wide program was expanded to include the Central and North Okanagan. What began as an eradication program eventually transitioned to a suppression program when it was clear that eradication could not be achieved due to constant immigration of moths from surrounding untreated areas. A suppression program required perpetual releases of adults, but it was not clear if *C. pomonella* would continue to be suppressed within the coverage area using only sterile insects. Many farms within the coverage area used other control tactics such as mating disruption and insecticides in addition to SIR (Judd and Gardiner, 2005; Thistlewood and Judd 2019). According to the OKSIR program website (www.oksir.org), the total annual program costs were over $3.7 million, of which $2.2 million was wages and benefits of permanent and seasonal staff. Costs were paid by general property taxes, an average of $6-12 per year paid by all property owners within the service area (revenue of ~$1.7 million in 2018), and by orchard owners at a

rate of $139.26 per acre annually (revenue of ~$1.2 million in 2018) (Bloem et al. 2007; Thistlewood and Judd, 2019). Approximately 2.2. million moths were produced each year for release from May through August at densities of 2000 sterile adults/ha weekly. In addition to moth production and release, the OKSIR program provided many services including pest monitoring, public education and enforcement of laws concerning removal of infestations.

Very little effort has focused on modifying release rates and frequency to implement a cost-effective *C. pomonella* SIT program that is no longer pursuing eradication on an area-wide basis but has transitioned to suppression or management at a farm-scale. Sterile codling moths have become available as a commercial product for release on individual farms in the US, but recommended release rates are based on the early work of Proverbs et al. (1969) establishing a 40:1 sterile to wild ratio to achieve area-wide eradication independent of additional control tactics such as mating disruption and chemical insecticides. Judd and Cossentine (1997) examined the combination of mating disruption and SIT and found reduced damage from *C. pomonella* in all treatments, and a 98% reduction in sterile/wild mating.

The current study seeks to explore the impact of integrating the *C. pomonella* sterile insect technique into existing farm-scale commercial apple pest management programs at several release densities and timings coinciding with generational activities of wild moth populations. The benefits of successful *C. pomonella* SIT program modifications from the current recommended release density of 2000 sterile moths/ha weekly for the entire season to the use of fewer moths/ha/week or fewer moths/year would be advantageous for farmers opting to use this technology at their own cost on individual commercial apple farms. Also, integrating SIT into farm IPM programs that include mating disruption will be an additional tool in the *C. pomonella*

control toolbox. The specific objectives were to determine the efficacy of four alternative release strategies that could potentially reduce the cost of *C. pomonella* ST: 1) treating the peak of the first generation flight only, 2) treating the peak of the second generation flight only 3) treating the peaks of both the first and second generation flights only, or 4) releasing for the full season at reduced densities of 500 or 1000 sterile moths/ha/week rather than the standard weekly density of 2000/ha.

METHODS AND MATERIALS

Sterile moths released in these trials were purchased from the Okanagan-Kootenay Sterile Insect Release facility in Osoyoos, British Columbia, Canada, and imported weekly by permit into the United States by M3 Consulting Group. Sterile moths were internally marked with calico red dye incorporated into the larval diet to discern sterile from wild moths captured in traps in field plots. Approximately 800 moths/container, 1:1 mixed-sex and recently eclosed, were sterilized as described in Horner et al. (2020). Chilled moths were transported by the importer and study authors to field sites in battery-powered coolers (2.8 Cu. Ft. Portable Fridge/ Freezer: Edgestar co. Austin, Texas) held at approximately 5°C. Weekly at field sites, appropriate numbers of moths by treatment were gently released directly from shipping containers by tossing them by hand into trees at pre-marked central locations in test blocks upon warming up to ambient temperatures.

Commercial apple orchard blocks used in this experiment were located between Soap Lake and Ephrata, WA, and were transitioned from conventional to organic management from

2015-2017. The ca. 120ha apple, cherry, and pear orchard was divided into 27 roughly square-shaped 3.25ha fruit-bearing apple blocks interspersed with non-bearing apple, and cherry and pear blocks. A total of 21 apple blocks were randomly selected to receive the various treatments using a random number generator in Microsoft excel (Table 5.1). Each treatment was replicated in three orchard blocks. All blocks, throughout the 2019 and 2020 seasons received mating disruption with Isomate®-CM Flex twin-tubes (Pacific Biocontrol Corp.) at ca. 700 dispensers/ha and twice weekly sprays of Codling Moth Granulosis Virus (CYD-X®, Certis USA L.L.C.) at 0.11L/ha. To further mitigate *C. pomonella* fruit damage, the farmer also employed thinning of infested fruit, tree banding in traditional *C. pomonella* hotspots in the fall of every year and destroyed bands by the end of winter.

The timing of moth releases was based on daily accumulated degree days (ADD) with an annual start date of January 1, tracked from April 9, 2019/20 to October 31, 2019/20 and compared with the 2009 to 2018 ten-year average on the Washington State University AgWeatherNet Growing Degree Days (GDD) model with a base temperature of 50°C at the weather station located in Ephrata, Grant County, Washington. This was the closest weather station to test orchards. GDD models were used to estimate the generational flights of wild *C. pomonella* for targeted releases and ADD were charted to compare with actual wild moth capture in traps post facto to confirm that capture peaks corresponded with estimated flights.

Treatment	Apple Variety(s) in blocks	Grower Block #
0/ha	Honeycrisp	3
0/ha	Honeycrisp	14
0/ha	Gala	23
500/ha	Gala	12
500/ha	Honeycrisp/Golden Delicious/Granny Smith	18
500/ha	Granny Smith	26
1000/ha	Granny Smith	8
1000/ha	Honeycrisp	11
1000/ha	Braeburn	27
2000/ha	Honeycrisp	1
2000/ha	Fuji	6
2000/ha	Gala	21
Generation 1	Gala	15
Generation 1	Gala	20
Generation 1	Fuji	25
Generation 2	Honeycrisp	4
Generation 2	Honeycrisp/Fuji	17
Generation 2	Gala	24
Generation 1,2	Honeycrisp	13
Generation 1,2	Honeycrisp/Fuji	16
Generation 1,2	Gala	22

Table 5.1. Treatments, apple varieties, and grower's orchard block number used in 2019 and 2020. Treatment release densities are the approximate number of mixed-sex *C. pomonella* released/ha into blocks weekly, while the generationally targeted releases were for a six-week period encompassing the designated generation.

Moths were released into test orchards from 29 April 2019 to 16 September 2019, and 28 April 2020 to 15 September 2020. Moths were hand-released in the center of each plot. Monitoring traps were present in blocks two weeks prior to first release, and two weeks after last release. There were two main modified release strategies, 1) different weekly release densities, and 2) reduced number of treatment weeks corresponding with generational flights. The following three density treatments were applied weekly for consecutive weeks in 2019 and 2020: 1) 2000/ha which is the current standard in British Columbia and Washington State and

served as the positive control, 2) 1000 moths/ha or half of the standard density and 3) 500 moths/ha or a quarter of the standard density. There also were three generational treatments, all at densities of 2000 moths/ha: 1) releases during the six weeks comprising the predicted peak of first generation flight (week 3 to 8, 2019, and week 5 to 10, 2020), 2) releases during the six weeks comprising the predicted peak of second generation flight (week 16 to 21, 2019 and week 15 to 20, 2020), and 3) releases during the six weeks comprising the predicted peaks of first and second generation flights. Additionally, there was a negative control in which no moths were released over the course of the season.

Capture of released sterile and wild *C. pomonella* was quantified using orange Pherocon VI delta monitoring traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.), and, to maximize captures, placed in the top 1/3 of the canopy (Reidl et al., 1979; McNally and Barnes, 1981), of pre-marked apple trees in an 8-trap grid pattern with spacing of approximately 50m between traps (Fig. 5.1). The monitoring trap and lure used in this study attracts male and female *C. pomonella*. Traps were deployed from 15 April (week 1) to 16 September 2019 (week 23), and 21 April (week 1) to 22 September 2020 (week 24). Lures were replaced at six week intervals following label instructions, and trap sticky liners were collected once weekly after week 5 of 2019 throughout the study period for examination in the laboratory and sexed sterile and wild moths captured in traps were recorded. Upon discovering high numbers of codling moths in traps in week 5 of 2019, trap collection was modified from once monthly to once weekly to prevent reduced captures due to overflooding of trap sticky bottoms with adults. Sterile were discerned from wild-type (WT) *C. pomonella* in the laboratory by

crushing every specimen to observe for evidence of calico red internal dye after separation of the sexes.

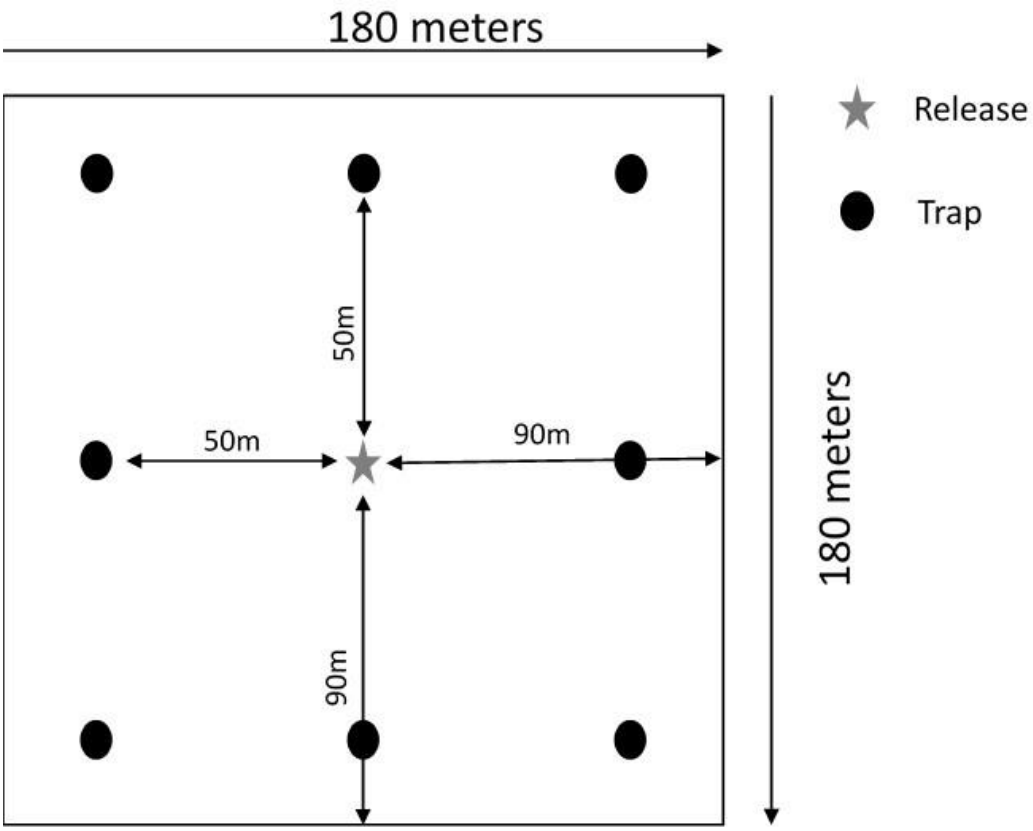

Figure 5.1. Generalized trap layout for all 21 apple orchard blocks used in 2019 and 2020. Blocks each had a single central sterile *C. pomonella* release point with traps at fixed distances from the center. Traps were orange Pherocon VI delta traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure. Lures were changed per label instructions, and trap sticky bottoms replaced as needed weekly if there were moths captured.

In-tree *C. pomonella* damage to fruit was assessed mid-season (mid- to late-July) and end-of-season prior to harvest (late-August to early-September) in test blocks annually by visual inspection. In each block a total of 600 randomly selected fruit were examined for *C. pomonella* infestation at each damage assessment as follows: 1) 10 fruit from mid-canopy height on each of

15 randomly selected trees on the edges of the orchard, 2) 10 fruit from the upper canopy in each of 15 randomly selected trees on the edges of the orchard, 3) 10 fruit from mid-canopy height on each of 15 randomly selected trees in the center of the orchard, and 4) 10 fruit from the upper canopy in each of 15 randomly selected trees in the center of the orchard. Fruit at the tops of trees were accessed either by ladder or removal using pole pruners.

DATA ANALYSIS

Accumulated degree days for 9 April to 31 October 2019, 2020, and the 10-year 2009-2018 average were plotted weekly and compared with the aggregated capture of sexed wild *C. pomonella* captured from all test plots.

Within each treatment, comparison of wild-type male and sterile male catch was used to assess differences in capture both weekly and annually to demonstrate the impact of releases of sterile male *C. pomonella* at different release densities and generational timings on wild-type male populations. To compare weekly catch for each treatment, a t-test was conducted for each week's arcsin(√x/100) transformed data for mean wild-type male and SIT male catch. In addition, for each treatment the annual capture of SIT males was compared to wild-type males, and t-tests (α=0.05) were performed on arcsin(√x/100) transformed proportion of total capture.

Mean and proportion capture of sterile males, sterile females, wild males, and wild females, individually, were calculated to assess differences in weekly and annual capture of moths across treatments. Data were arcsin(√x/100) transformed, and ANOVA performed to compare treatments. If ANOVA results indicated significance, Fisher's test was used to separate

means. Additionally, 2019 to 2020 changes in mean and proportion capture of sterile males, sterile females, wild males, and wild females in each treatment plot were compared among the treatments. Year 1 (2019) to year 2 (2020) changes in mean capture were assessed for each treatment by t-test of arcsin(√x/100) transformed annual mean moth catch/trap. The percent change in capture from year 1 to year 2 was calculated using the formula (((2020 capture – 2019 capture)/(2019 capture))*100). The percent change was negative in some of the plots, thus the % change data was transformed (arcsin (√(x+(abs(lowest Neg. value)+1)/100))) prior to analysis. ANOVA was used to compare year over year percent change in catch among the treatments. If differences were indicated Fisher's LSD was employed to separate treatment means.

To assess the impact of releasing varying numbers of sterile female *C. pomonella* and the resulting potential disruption of wild male catch, the sterile female:wild male ratio of catch was compared across treatments. For each week, by treatment, the weekly mean percent capture of sterile females and wild males was calculated, and data were arcsin(√x/100) transformed before performing ANOVA and Fisher's LSD to separate treatment effects. The annual mean capture of sterile females to wild males was also compared across treatments by calculating the mean treatment percent of combined capture that was SIT female, and ANOVA and Fisher's LSD on (x^2) transformed data was used to separate treatment means. Additionally, year 1 to year 2 change in SIT female:wild male catch was calculated among and between treatments. The two year change in the ratio between treatments was subjected to ANOVA on (arcsin(√(x+(abs(lowest Neg. value)+1)/100))) transformed percent change in capture from 2019 to 2020 (((2020 capture – 2019 capture)/(2019 capture))*100). The within treatment changes in year 1 to year 2 capture were determined by t-test on arcsin(√x/100) transformed annual average moth capture/trap.

Finally, for each treatment, the proportion of sterile male, sterile female, wild-type male, and wild-type female *C. pomonella* captured were directly compared. Weekly, by treatment, the proportion capture of SIT male, SIT female, wild male, and wild female *C. pomonella* was calculated from the total treatment moth capture. Differences in capture were compared by arcsin(√x/100) transforming the SIT male, SIT female, wild male, and wild female *C. pomonella* proportion captured for each treatment, performing ANOVA, then Fisher's test for means separation.

Fruit damage was compared among the treatments twice annually. For each damage assessment, the % damaged fruit observed in each block was arcsin(√x/100) transformed, ANOVA conducted to compare treatments, and if necessary, Fisher's LSD was performed to separate means. Treatment effects on the within-season percent change in damage from mid-season to pre-harvest were determined by calculating the % change in damage by (((mid-season damage – pre-harvest damage)/(mid-season damage))*100) and subjecting the arcsin(√x/100) transformed values to ANOVA followed by Fisher's LSD test for mean separation. In addition, the year 1 to year 2 changes in damage within treatments were compared by t-test of arcsin(√x/100) transformed annual average/block % damage, and year 1 to year 2 change in % damaged fruit between treatments by ANOVA on arcsin(√x/100) transformed % change in damage from 2019 to 2020 (((2020 damage – 2019 damage)/(2019 damage))*100), and year over year changes in damage were determined for each treatment by t-test of arcsin(√x/100) transformed annual average/block % damage.

RESULTS

Predicted and actual degree day accumulation

A plot of estimated 10-year average and actual observed accumulated degree days in real time showed that generation one flight was estimated to begin in week 3 of 2019 and week 5 of 2020, and generation two was estimated to have begun in week 15 in both years. Recorded capture of wild-type moths in traps corresponded with this predicted timing (Fig. 5.2). Capture of wild moths in traps may have lagged behind flight by up to one week due to the once weekly recording of data. Wild-type male and female moth capture generally aligned each week with nearly equal numbers caught in traps. Generation one peak for wild male and female catch was between week 4 and 7 both years and Generation two peaked between weeks 15 and 19 both years.

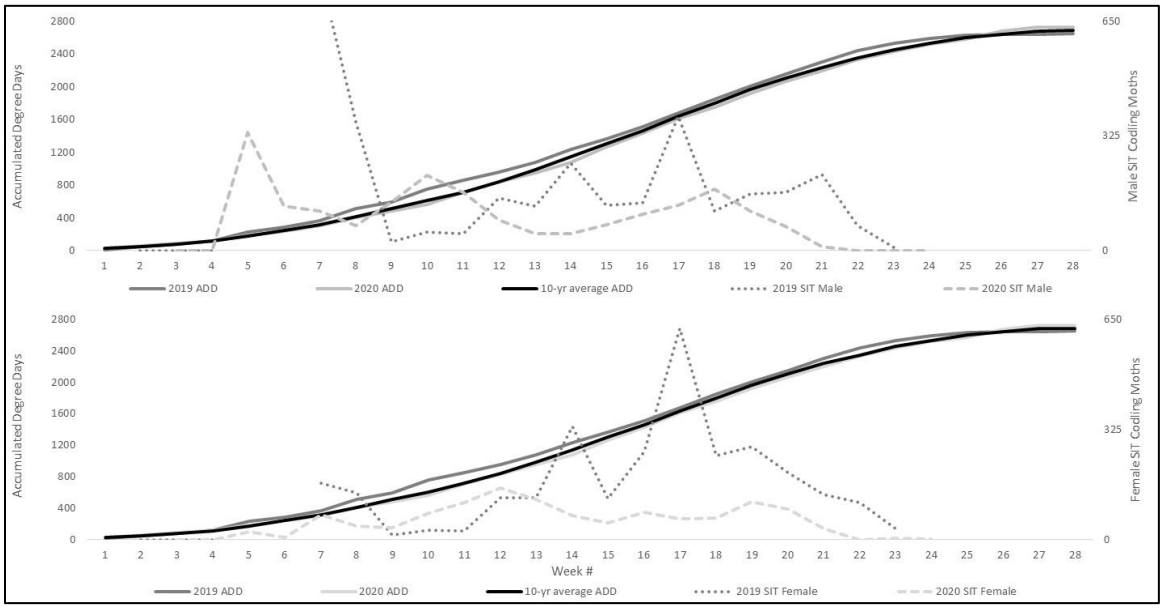

Figure 5.2. Accumulated degree days in 2019, 2020, the 2010-1020 10-year average (left y-axis), and 2019 and 2020 total sterile male (top) and female (bottom) *C. pomonella* capture by week (right y-axis). Based on degree day accumulation and the 10-year average, generation one is estimated to have begun in week 3 of 2019 and week 5 of 2020, and generation two is estimated to have begun in week 15 in both years. Generation one releases began in week 3 of 2019 and week 5 of 2020 due to differences in ADD, and Generation two releases began in week 16 of 2019 and week 15 of 2020.

Comparative capture of sterile and wild males within each treatment

Weekly mean captures of sterile (SIT) and wild-type (WT) males for the six treatments and the negative control are presented in Figures 5.3-5.9. Wild captures were low, never exceeding a mean of 5 males/trap, throughout 2019 and 2020 in all of the treatments, including the non-SIT control (Fig. 5.6). Season-long weekly releases at the standard density of 2000 moths/ha resulted in high captures of sterile moths and significantly greater weekly capture of sterile compared to wild males for most weeks and overall in 2019 and 2020 (Fig. 5.3). Recaptures of sterile males released at a density of 2000 moths/ha were lower in 2019 compared to 2020 and mean catch in 2019 peaked at just under 20 males per trap, while in 2020 catch peaked at nearly 50 males/trap. Season long weekly releases at the reduced density of 1000 moths/ha resulted in high captures of sterile moths and significantly greater weekly capture of sterile compared to wild males for most weeks and overall in 2019 and 2020 (Fig. 5.4). Recaptures of sterile males at this density were lower in 2019 than in 2020 and mean catch in 2019 peaked at just under 20 males per trap, while 2020 catch peaked at nearly 30 males/trap. Recaptures of sterile males following season long weekly releases at the lowest density of 500 moths/ha were extremely low in 2019 and only significantly greater than captures of wild moths during three weeks at the end of the season (Fig. 5.5). High captures of sterile moths and significantly greater weekly capture of sterile compared to wild males were recorded for most weeks in 2020, and overall in 2020 but not in 2019 (Fig. 5.5). At a release density of 500 moths/ha, mean 2019 catch peaked at fewer than 5 males per trap, while 2020 catch peaked at about 15 males/trap. When targeting the generational emergence of wild moths with releases at the standard density of 2000 moths/ha during six weeks of the first and six weeks of the second generation, high captures of sterile

moths and significantly greater weekly capture of sterile compared to wild males for most release weeks and overall in 2019 and 2020 were observed (Fig. 5.7). Recaptures of sterile males were lower in 2019 compared to 2020. Mean catch in 2019 peaked at ca 16 males per trap, while in 2020 catch peaked at ca. 35 males/trap. Six weekly releases at the standard density of 2000 moths/ha during the first generations resulted in a significantly higher catch of sterile compared to wild males in 2019 and 2020 (Fig. 5.8). Recaptures of sterile males were lower in 2019 than in 2020. Mean catch in 2019 peaked at just over 10 males per trap, while 2020 catch peaked at greater than 30 males/trap. Six weekly releases at the standard density of 2000 moths/ha during the second generation resulted in high captures of sterile moths and significantly greater weekly capture of sterile compared to wild males for several weeks in 2019 and 2020 and overall in both years (Fig. 5.9). Recaptures of sterile males were lower in 2019 than in 2020. Mean catch in 2019 peaked at about 20 males per trap, while 2020 catch peaked about 35 males/trap.

Sterile males were routinely captured in low numbers in the negative control block where sterile moths were not deployed and in the blocks in which sterile moths were not released during generation targeting. As many as 6 sterile moths/trap were recaptured in the control block and during two weeks of 2020 there were significantly more sterile than wild males captured (Fig. 5.6). Similarly, up to 5 sterile moths/trap were captured during the second generation in 2019 and 2020 in plots in which SIT moths were only released over a six-week period during the first generation (Fig. 5.8). Very few sterile males were recaptured during the first-generation period in both years in plots in which SIT moths were not released (Fig. 5.9).

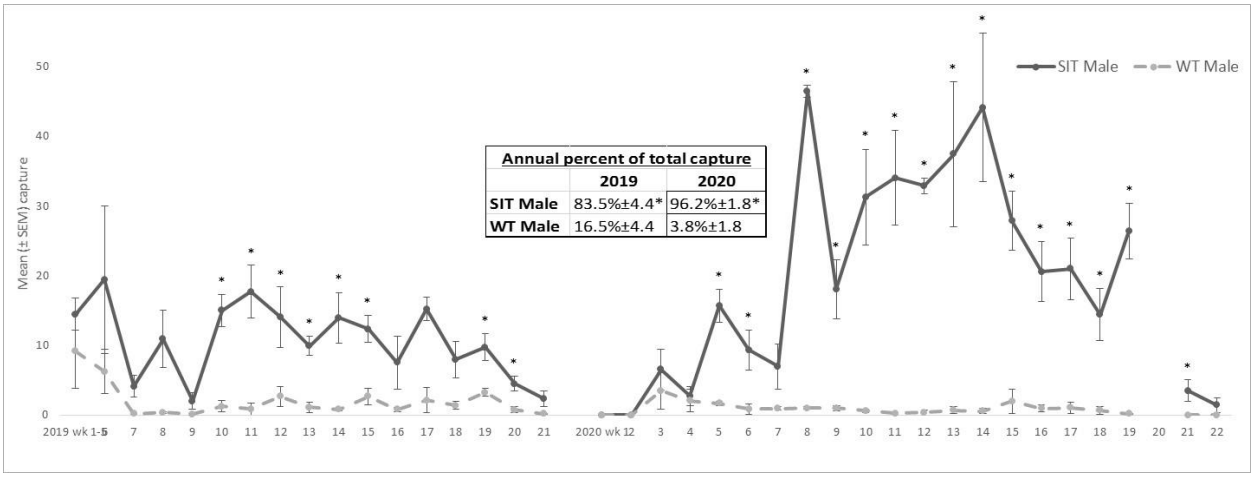

Figure 5.3. *Release of 2000/ha.* Comparative mean (± SEM) catch per trap (n = 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 2000/ha over the entire season. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

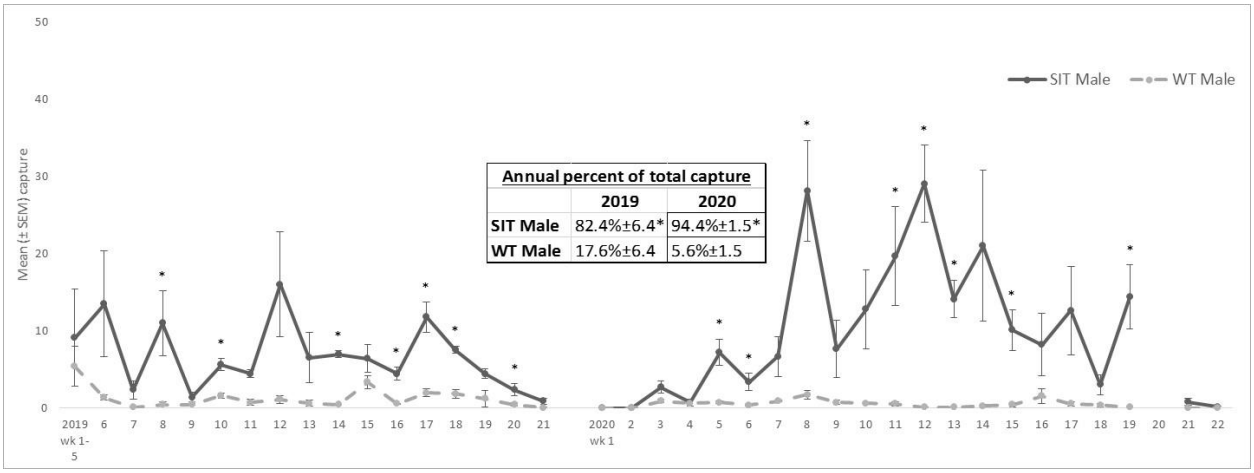

Figure 5.4. *Release of 1000/ha.* Comparative mean (± SEM) catch per trap (n = 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 1000/ha over the entire season. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 is missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

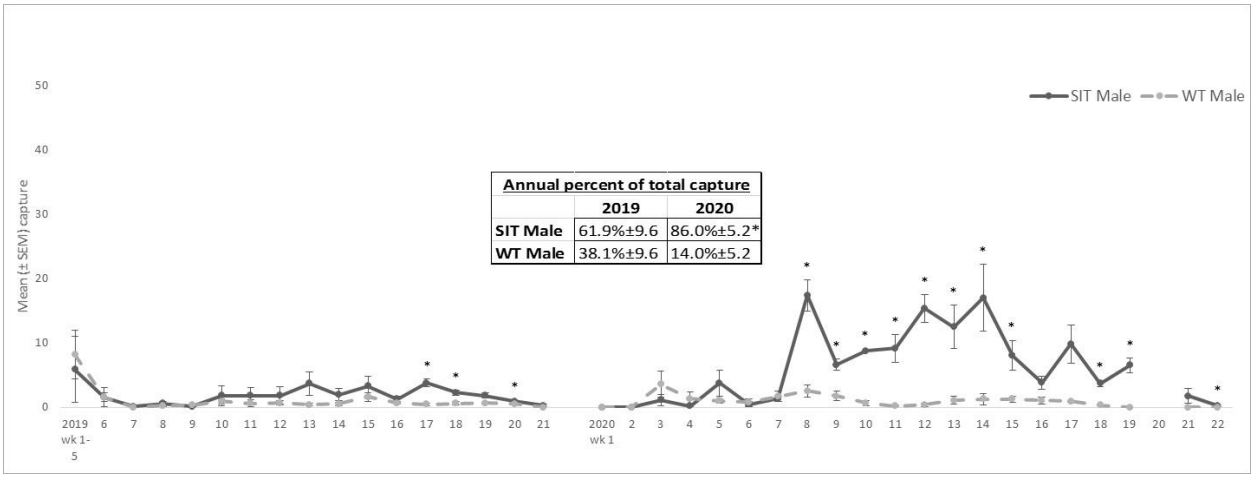

Figure 5.5. *Release of 500/ha.* Comparative mean (± SEM) catch per trap (n = 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 500/ha over the entire season. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 is missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

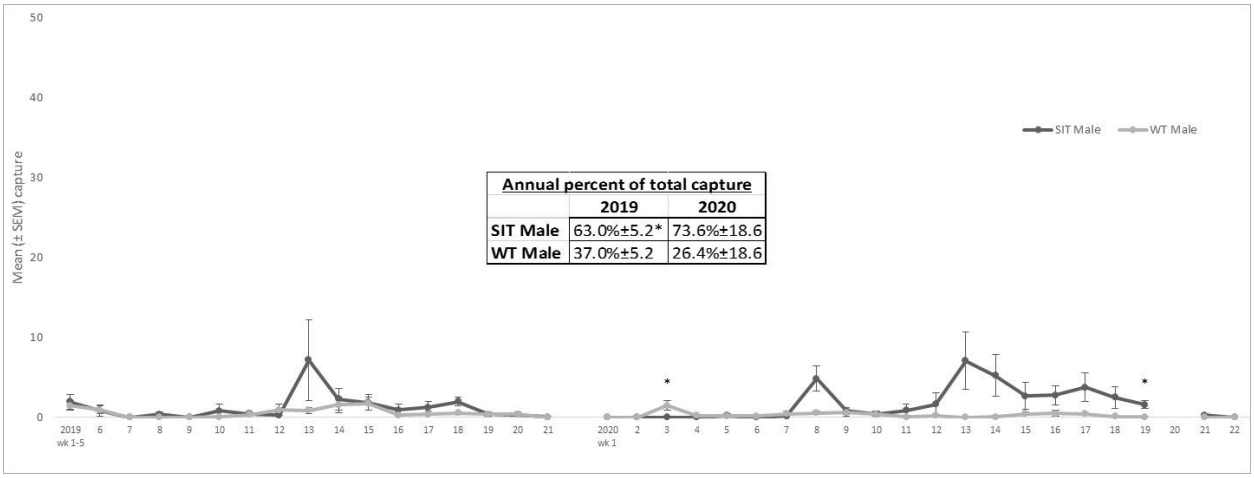

Figure 5.6. *Release of 0/ha.* Comparative mean (± SEM) catch per trap (n = 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 0/ha over the entire season. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 is missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

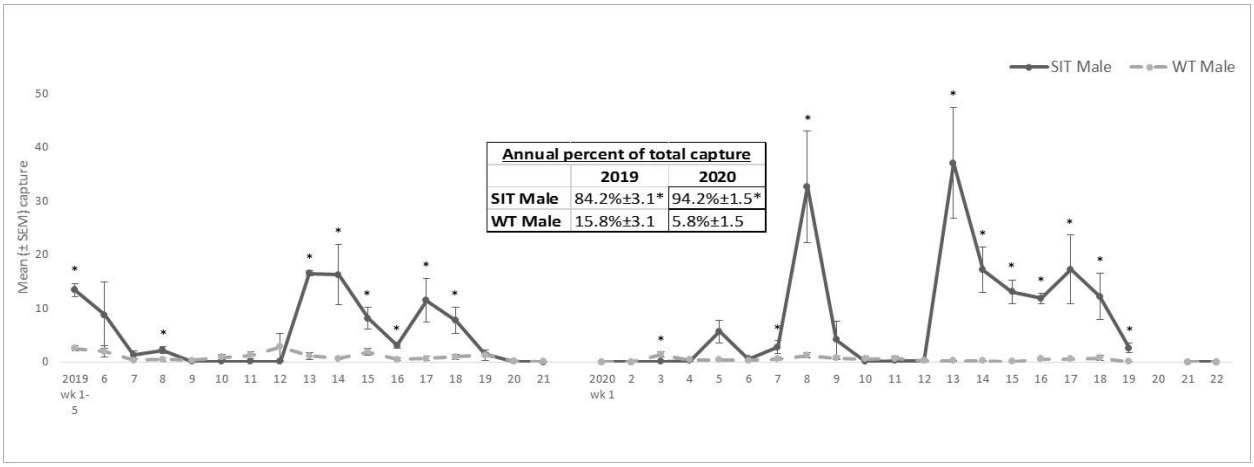

Figure 5.7. *Release during first and second generation.* Comparative mean (± SEM) catch per trap (n = 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 2000/ha during six weeks of the first- and second-generation flights. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

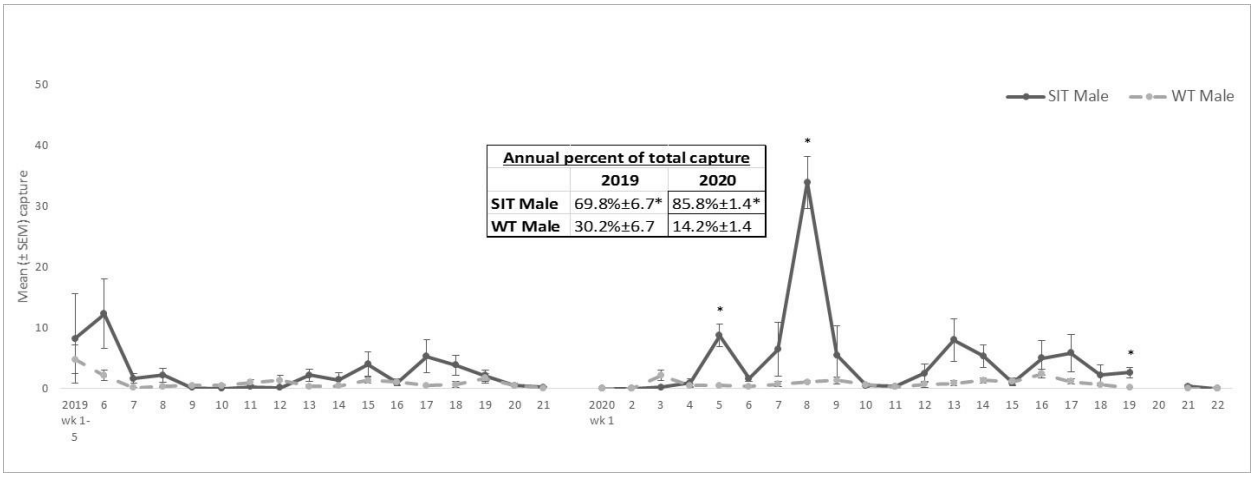

Figure 5.8. *Release during first generation.* Comparative mean (± SEM) catch per trap (n = 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 2000/ha during six weeks of the first-generation flight only. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

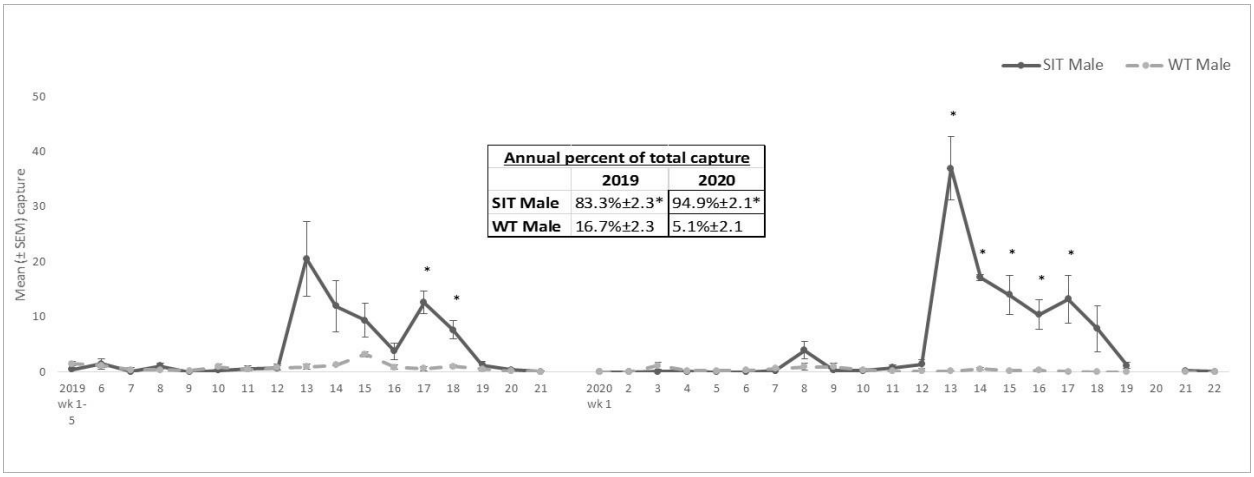

Figure 5.9. *Release during second generation.* Comparative mean (± SEM) catch per trap (n= 3) of wild (WT) and sterile (SIT) male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a density of 2000/ha during six weeks of the second-generation flight only. Annual percent of combined capture of wild and sterile males in 2019 and 2020 is shown in the inserted table. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences in mean and percent capture of SIT and WT males of $p < 0.05$ are indicated on figures and in the inserted table with "*".

Comparative capture of sterile males at varying SIT release densities

Comparative weekly mean capture of sterile males following the release of moths at densities of 0, 500, 1000 or 2000/ha are presented in Figures 5.10-5.11. Significantly higher weekly recaptures were recorded in plots treated with 2000 moths/ha compared to 0/ha throughout 2019 and 2020 (Fig. 5.10, upper). Recaptures of released SIT moths also were significantly higher in plots treated with 2000 moths/ha compared to 500/ha on the vast majority of sampling dates in 2019 and 2020 (Fig. 5.10, upper). Captures were generally 5 to 15-fold higher following the release of 2000 compared to 500 sterile moths/ha. Recaptures of released SIT males were significantly higher in plots treated with 1000 moths/ha compared to 0/ha on the vast majority of sampling dates in 2019 and 2020 (Fig. 5.10, lower). However, significantly fewer moths were recaptured in plots treated with 1000 moths/ha compared to 2000/ha on 12

sampling dates in 2020 and 3 sampling dates in 2019 (Fig. 5.10, lower). Captures were generally 2- to 3-fold higher following the release of 2000 compared to 1000 sterile moths/ha. Significantly higher weekly recaptures were recorded in plots treated with 1000 moths/ha compared to 500/ha on 6 sampling dates in 2019 and 3 sampling dates in 2020 (Fig. 5.11). Captures were generally 2- to 4-fold higher following the release of 1000 compared to 500 sterile moths/ha. Overall, mean annual recaptures of sterile males were significantly higher following the release of 2000/ha or 1000/ha compared to 500/ha, and higher in 2020 that in 2019 (Table 5.2).

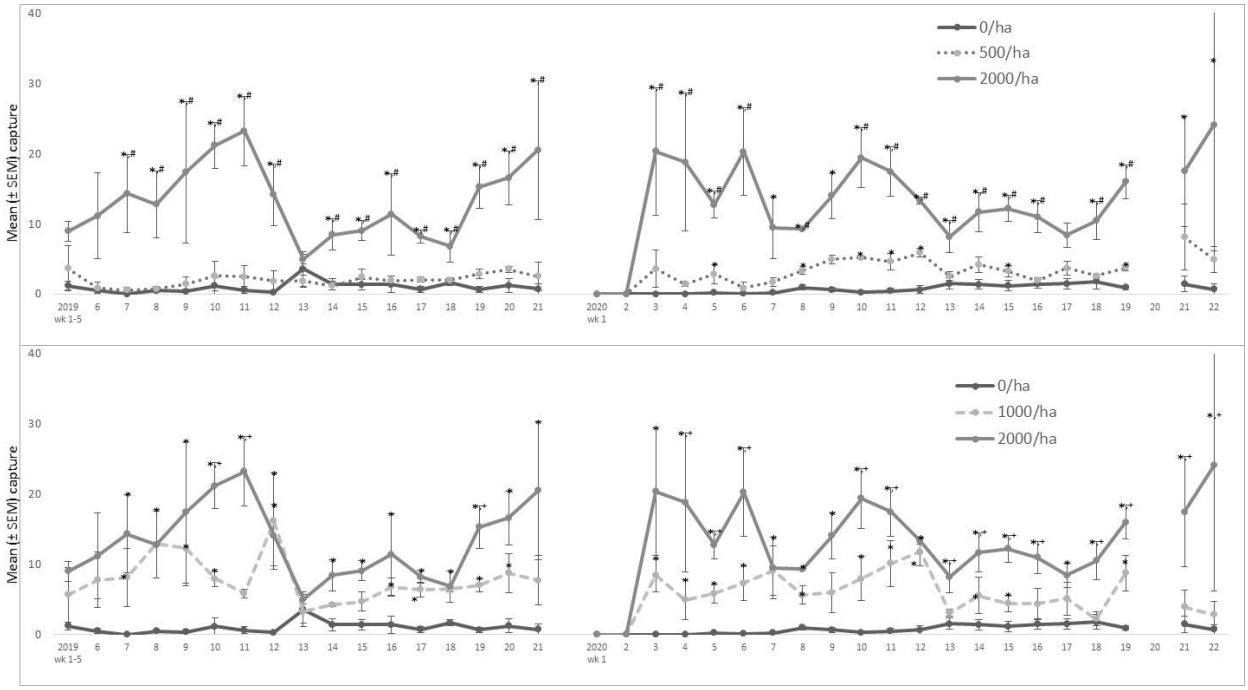

Figure 5.10. Comparative mean (± SEM) proportion catch (n = 3) of sterile male *C. pomonella* weekly throughout the 2019-2020 season following SIIT release at densities of 0/ha, 500/ha, 1000/ha, or 2000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "#" for more than 500/ha, "+" for more than 1000/ha, "/" for more than 2000/ha.

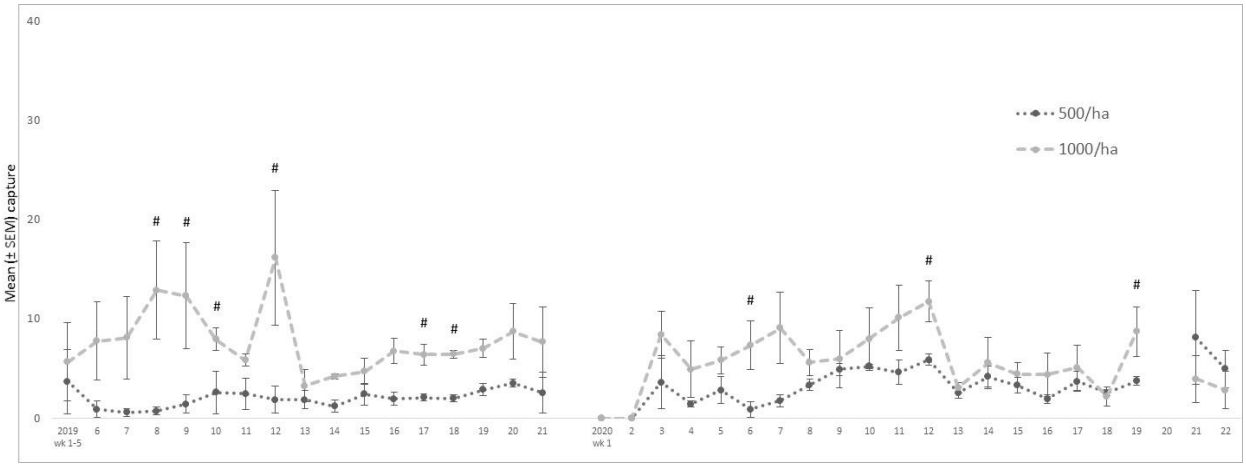

Figure 5.11. Comparative mean (± SEM) proportion catch (n = 3) of sterile male *C. pomonella* weekly throughout the 2019-2020 season following SIIT release at densities of 500/ha or 1000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "#" for more than 500/ha, "+" for more than 1000/ha, "/" for more than 2000/ha.

Comparative capture of sterile males at varying SIT release timings

Comparative weekly mean captures of sterile males following weekly release of moths for the entire season, six weeks during generations one and two, six weeks during generation one, or six weeks during generation two are presented in Figure 5.12. Higher captures were consistently recorded in the season long timing compared to the other three timings. In 2019, recaptures of moths during six weeks of the first-generation *C. pomonella* flight resulted in very low captures of sterile males, and significantly fewer recaptures compared to the season-long program during nearly all sample dates (Fig. 5.12, upper and lower). In 2020, catches of sterile males in the plot receiving six generation one releases were higher than in 2019, however fewer sterile males were captured in these plots compared to plots treated season-long during

		ANOVA	0/h	500/ha	1000/ha	2000/ha	Generation 1	Generation 2	Generation 1,2
Sterile Male	2019 Mean total capture	*F=9.9613 df=6,14 P=0.0002*	21.8±10.5 e	34.4±8.4 c	114.9±28.3 ab	181.0±25.3 a	51.4±19.8 de	72.0±10.0 bcd	90.6±5.7 bd
	2020 Mean total capture	*F=10.2847 df=6,14 P=0.0002*	35.1±14.7 d	128.3±19.1 bc	202.7±54.1 b	400.5±51.1 a	90.7±25.5 cd	107.8±19.4 bc	157.7±39.1 bc
	Mean Year Over Year % Change	*F=1.1260 df=6,14 P=0.3967*	72.6%±77.2	361.9%±191.6	105.8%±68.8	139.1%±67.5	95.1%±54.5	59.6%±42.3	70.7%±32.2
	2019 to 2020 capture difference		*t=0.5812 df=4 P=0.5923*	*t=4.9409 df=4 P=0.0078 Δ*	*t=1.4997 df=4 P=0.2081*	*t=4.0879 df=4 P=0.0150 Δ*	*t=1.1959 df=4 P=0.2978*	*t=1.7230 df=3 P=0.1834*	*t=1.8283 df=2 P=0.2090*
Sterile Female	2019 Mean total capture	*F=36.7963 df=6,14 P<<0.0001*	2.3±1.3 e	9.3±1.7 d	26.0±3.1 b	59.8±3.9 a	8.9±3.1 d	13.4±2.3 cd	19.9±0.4 bc
	2020 Mean total capture	*F=8.9126 df=6,14 P=0.0004*	3.5±1.9 d	36.0±13.7 b	39.2±13.8 b	98.9±21.4 a	13.2±5.7 cd	26.9±3.1 bc	29.4±4.4 bc
	Mean Year Over Year % Change	*F=0.6766 df=6,14 P=0.6710*	64.3%±94.0	258.9%±79.0	67.9%±79.9	69.4%±44.0	120.7%±154.7	105.4%±34.4	48.6%±24.7
	2019 to 2020 capture difference		*t=0.2554 df=3 P=0.8149*	*t=2.3276 df=2 P=0.1454*	*t=0.9128 df=2 P=0.4577*	*t=1.9854 df=2 P=0.1855*	*t=0.4548 df=3 P=0.6802*	*t=3.4508 df=4 P=0.0260 Δ*	*t=2.1847 df=2 P=0.1605*
Wild Male	2019 Mean total capture	*F=2.8653 df=6,14 P=0.0490*	10.7±2.9 b	19.7±3.2 ab	21.7±5.3 ab	33.7±5.7 a	17.0±4.4 b	14.0±1.3 b	17.6±4.7 b
	2020 Mean total capture	*F=1.2512 df=6,14 P=0.3395*	6.3±2.2	20.7±8.5	10.6±2.2	18.0±10.7	15.3±5.3	5.8±2.4	8.7±1.8
	Mean Year Over Year % Change	*F=0.8465 df=6,14 P=0.5553*	-18.3%±45.6	16.3%±55.0	-50.2%±3.2	-52.9%±21.1	-11.4%±16.4	-59.2%±14.7	-43.5%±14.9
	2019 to 2020 capture difference		*t=1.2400 df=4 P=0.2827*	*t=0.0361 df=3 P-0.9734*	*t=2.1378 df=3 P=0.1221*	*t=1.4523 df=3 P=0.2424*	*t=0.2709 df=4 P=0.7999*	*t=2.5448 df=2 P=0.1259*	*t=1.8225 df=3 P=0.1659*
Wild Female	2019 Mean total capture	*F=2.8847 df=6,14 P=0.0480*	6.8±2.7 c	12.8±2.0 bc	18.3±5.9 ab	28.5±7.3 a	15.0±3.3 abc	22.2±6.3 ab	20.2±1.7 ab
	2020 Mean total capture	*F=1.5724 df=6,14 P=0.2270*	2.9±1.1	10.6±4.7	4.4±1.5	9.9±5.0	11.9±4.0	4.2±1.4	6.6±1.5
	Mean Year Over Year % Change	*F=1.1077 df=6,14 P=0.4057*	4.1%±80.2	-16.6%±34.4	-73.6%±11.0	-69.1%±7.9	-23.4%±12.4	-75.3%±10.4	-66.0%±10.3
	2019 to 2020 capture difference		*t=1.2232 df=3 P=0.3086*	*t=0.6136 df=3 P=0.5829*	*t=2.7918 df=3 P=0.0683*	*t=2.2891 df=4 P=0.0839*	*t=0.6243 df=4 P=0.5663*	*t=3.4390 df=3 P=0.0384 Δ*	*t=5.5775 df=4 P=0.0045 Δ*

Table 5.2. Mean (± SEM) annual per trap catch of wild and sterile male and female *C. pomonella* from the 2019-2020 season by treatment. Collection of Pherocon VI delta monitoring traps baited with PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure was from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Week #20 of 2020 had no trap collection due to a 121,200ha forest fire preventing access to test orchards. ANOVA was used to compare capture means and year over year change in capture by treatment and significant differences of $p < 0.05$ are indicated different letters. Negative values in year over year percent change in capture indicate a reduction in capture while positive values indicate an increase in capture. T-tests ($P<0.05$) were used to determine if treatment blocks had a year over year change in capture, and significant differences from 2019 to 2020 are indicated with "Δ" next to the P-value.

four of the six weeks (Fig. 5.12, upper). Mean captures of sterile males were not statistically different in plots receiving moths weekly for six weeks during the second generation or season-long on most sample dates during that release period (Fig. 5.12, middle). The pattern of recapture in plots treated with sterile moths for six weeks during each of the two generations were similar to those recorded in plots receiving moths for one or the other generation as described previously. Recaptures of moths in the dual six-week program were very low and

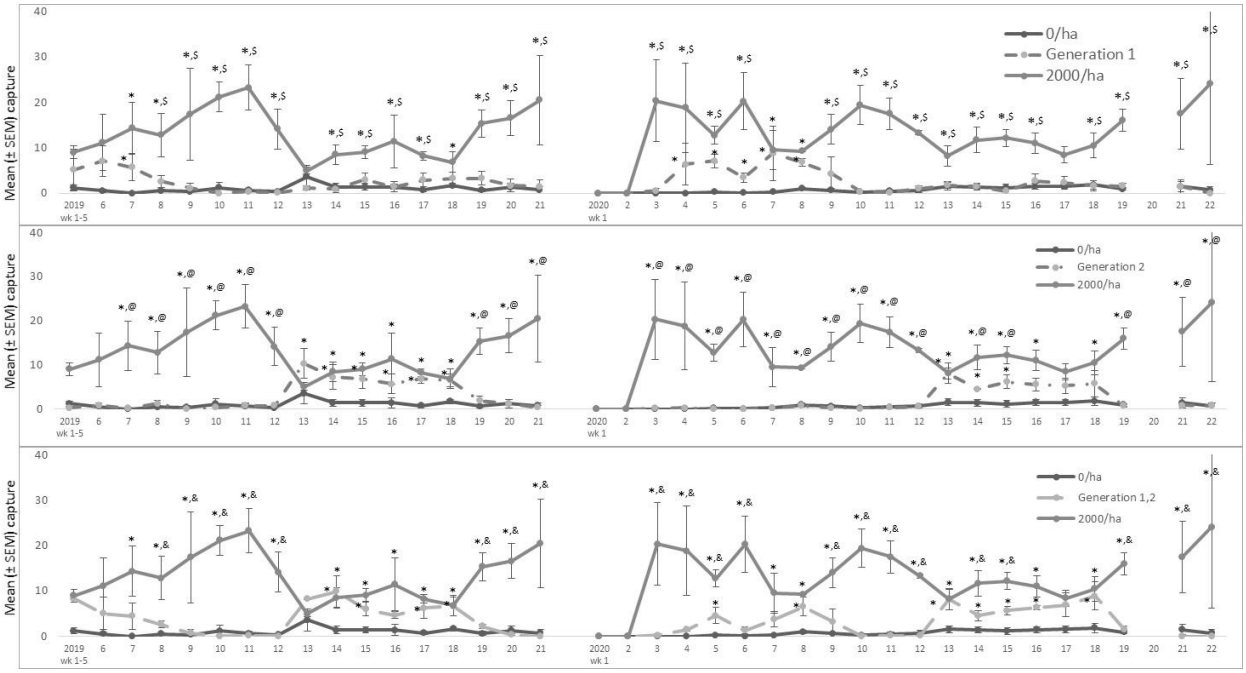

Figure 5.12. Comparative mean (± SEM) proportion catch (n = 3) of sterile male *C. pomonella* weekly throughout the 2019-2020 season following the release of 2000 SIT moths/ha weekly for 21 weeks (2000 ha), six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "/" for more than 2000/ha, "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

significantly fewer recaptures compared to the season-long program were recorded on many sample dates (Fig. 5.12, lower). Mean captures of sterile males during the six weeks of second-generation releases in the dual six-week program were not statistically different from the season-long program on most sample dates (Fig. 5.12, lower). Overall, mean annual recapture of sterile males were significantly higher following six weekly releases during both generations or second generation only compared to six weekly releases during first generation (Table 5.2).

Comparative capture of sterile females at varying SIT release densities

Comparative weekly mean proportion capture of sterile females following the release of moths at densities of 0, 500, 1000 or 2000/ha are presented in Figures 5.13-5.14. Significantly higher weekly proportional recaptures were recorded in plots treated with 2000 moths/ha compared to 0/ha throughout 2019 and 2020 (Fig. 5.13). Weekly proportional recaptures of released SIT females also were significantly higher in plots treated with 2000 moths/ha compared to 500/ha on the majority of sampling dates in 2019 and 2020 (Fig. 5.13, upper). Proportional recaptures of released SIT moths at 500/ha were extremely low in 2019 and not significantly different from the control throughout the first generation and for most of the second generation (Fig. 5.13, upper). Proportional recaptures of released SIT moths at 500/ha in 2020 were significantly higher than the control on many sampling dates, especially following the second-generation releases (Fig. 5.13, upper). Weekly proportional recaptures of females were significantly higher in plots treated with 1000 moths/ha compared to 0/ha on the majority of sampling dates in 2019 and 2020 (Fig. 5.13, lower). However, significantly fewer moths were

recaptured in plots treated with 1000 moths/ha compared to 2000/ha on 8 sampling dates in 2020 and 6 sampling dates in 2019 (Fig. 5.13, lower). Captures were generally 2 to 3-fold higher following the release of 2000 compared to 1000 sterile moths/ha. The patterns of sterile female proportional recaptures in plots treated with 1000 or 500 sterile moths/ha were similar in both years, except during four 2019 and one 2020 early-season releases when captures were significantly higher following the release of 1000/ha (Fig. 5.14). Overall, mean annual recapture of sterile females were significantly higher following the release of 2000 or 1000/ha compared to 500/ha, and higher in 2020 than in 2019 (Table 5.2).

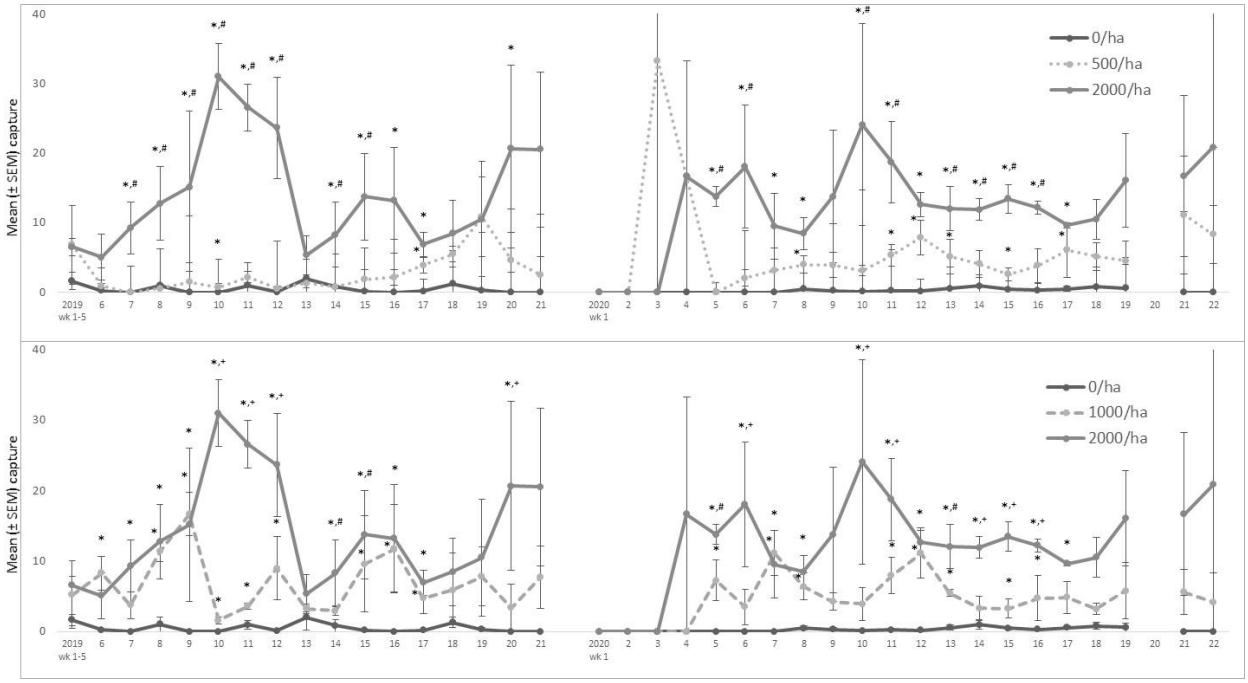

Figure 5.13. Comparative mean (± SEM) proportion catch (n = 3) of sterile female *C. pomonella* weekly throughout the 2019-2020 seasons following sterile moth releases at densities of 0/ha, 500/ha, 1000/ha, or 2000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "#" for more than 500/ha, "+" for more than 1000/ha, "/" for more than 2000/ha.

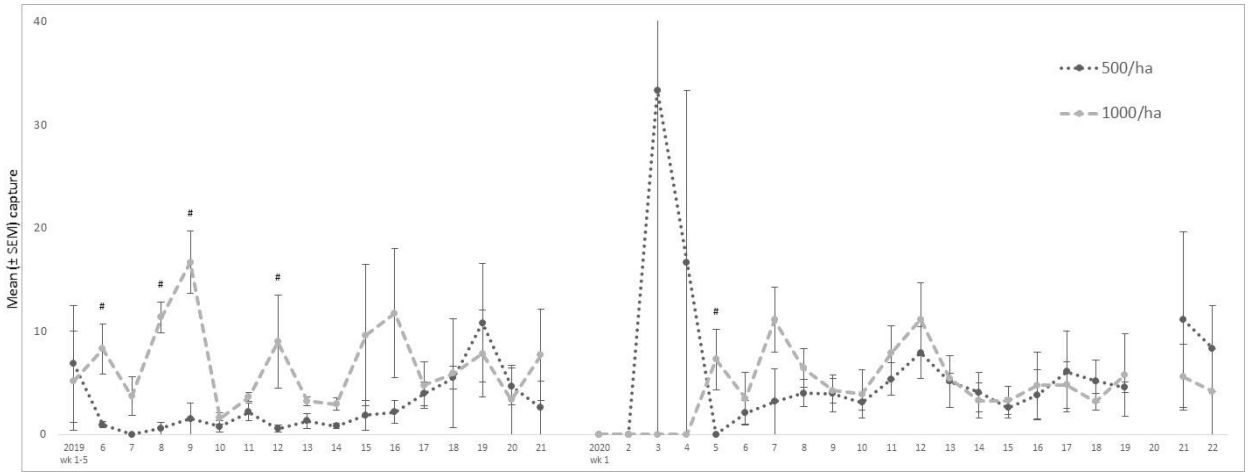

Figure 5.14. Comparative mean (± SEM) proportion catch (n = 3) of sterile female *C. pomonella* weekly throughout the 2019-2020 seasons following sterile moth releases densities of 500/ha or 1000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "#" for more than 500/ha, "+" for more than 1000/ha.

Comparative capture of sterile females at varying SIT release timings

Comparative weekly mean proportional capture of sterile females following weekly release of moths for the entire season, six weeks during generations one and two, six weeks during generation one, or six weeks during generation two are presented in Figure 5.15. Higher captures were consistently recorded in the season long timing compared to the other three timings. The pattern of proportional recapture in plots treated with sterile moths for six weeks during each of the two generations followed this trend, though during several release weeks capture was similar to season-long release timings (Fig. 5.15). In 2019, releases of moths during six weeks of the first-generation *C. pomonella* flight resulted in very low recaptures of sterile females, and significantly fewer recaptures compared to the season-long program during nearly all sample dates that did not receive moths (Fig. 5.15, upper). In 2020, catches of sterile females

in the plot receiving six first generation only releases were higher than in 2019, and captures of sterile females during release weeks were not significantly different than captures in the season-long program (Fig. 5.15, upper). Mean proportional captures of sterile females were not statistically different during release weeks in plots receiving moths weekly for six weeks during the second generation or season-long on most sample dates (Fig. 5.15, middle). Overall, mean annual recapture of sterile females were higher in the season-long program, but not significantly

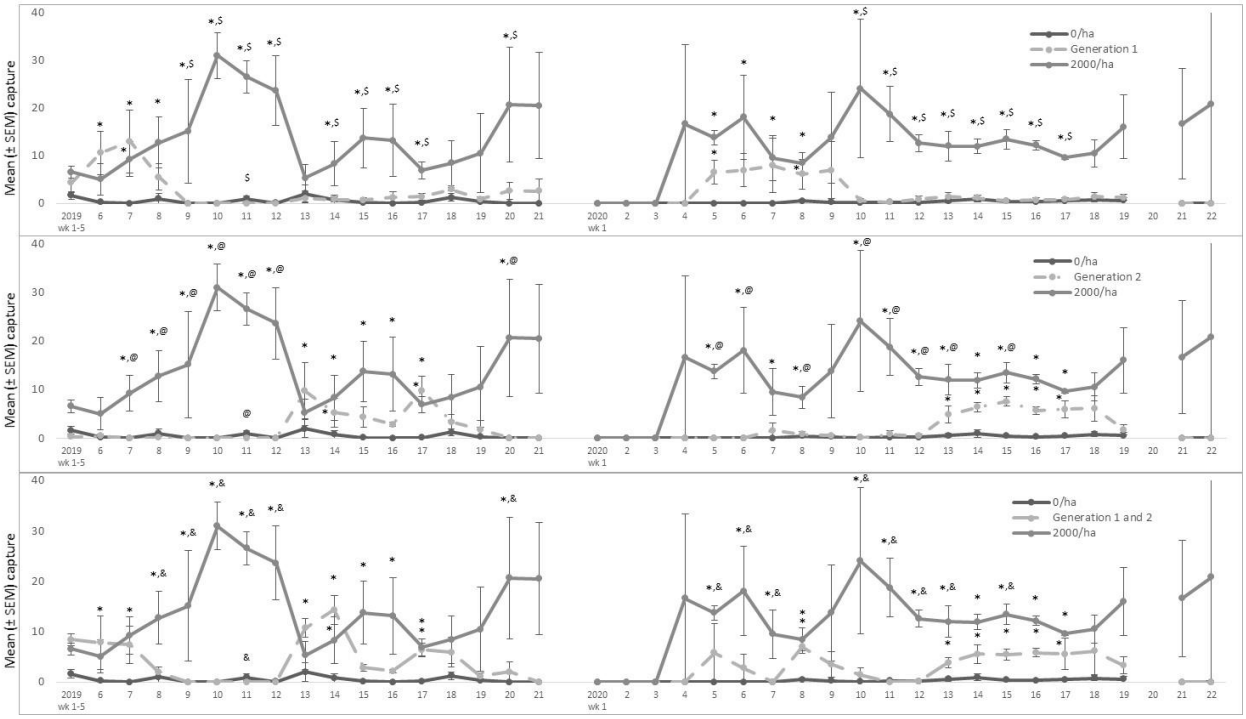

Figure 5.15. Comparative mean (± SEM) proportion catch data (n = 3) of sterile female *C. pomonella* weekly throughout the 2019-2020 season following the release of 2000 SIT moths/ha weekly for 21 weeks (2000 ha), six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "/" for more than 2000/ha, "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

different following six weekly releases during generation one, generation two or both generations (Table 5.2). The lone exception was in 2020 when significantly fewer sterile females were captured in the generation one only treatment compared to all other timings.

Comparative capture of wild males at varying SIT release densities

Wild type male catch was generally low across all of the release densities, with average total captures per trap per plot for the entire season ranging from 10-34 in 2019 and 6-18 in 2020 (Table 5.2). Weekly proportion captures of wild males are presented in Figures 5.16-5.17.

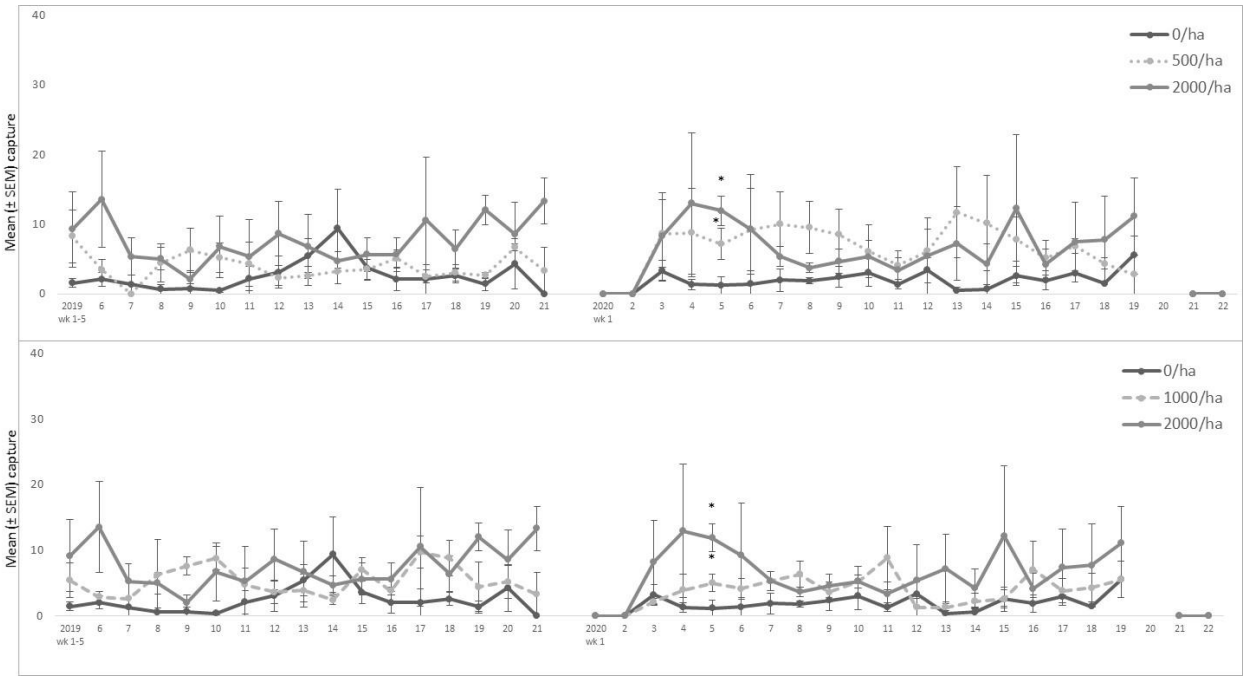

Figure 5.16. Comparative mean (± SEM) proportion catch (n = 3) of wild male *C. pomonella* weekly throughout the 2019-2020 following sterile releases at 0/ha, 500/ha, 1000/ha, or 2000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "#" for more than 500/ha, "+" for more than 1000/ha, "/" for more than 2000/ha.

In 2019 and 2020 the proportions of wild males captured weekly were not significantly different in all but a single week, following releases of sterile moths at 2000/ha, 1000, ha, 500/ha, or 0/ha (Fig. 5.16-5.17).

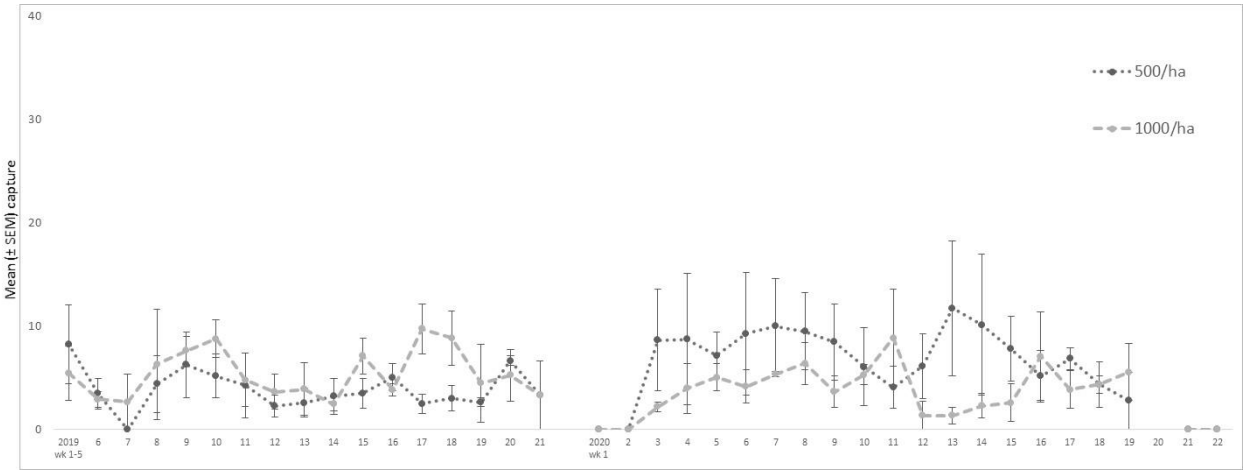

Figure 5.17. Comparative mean (± SEM) proportion catch (n = 3) of wild male *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at densities of 500/ha and 1000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "#" for more than 500/ha, "+" for more than 1000/ha.

Comparative capture of wild males at varying SIT release timings

Wild male catch was generally low across all of the release timings, with average proportion of captures per plot for the entire season ranging from 9-20 in 2019 and 13-30 in 2020 (Table 5.2). Weekly proportion captures of wild males are presented in Figure 5.18. In 2019 and 2020 the proportions of wild males captured weekly were not significantly different in all but one week, following releases season-long, for six weeks during the first generation, six weeks during the second generation or six weeks during both generations (Fig. 5.18).

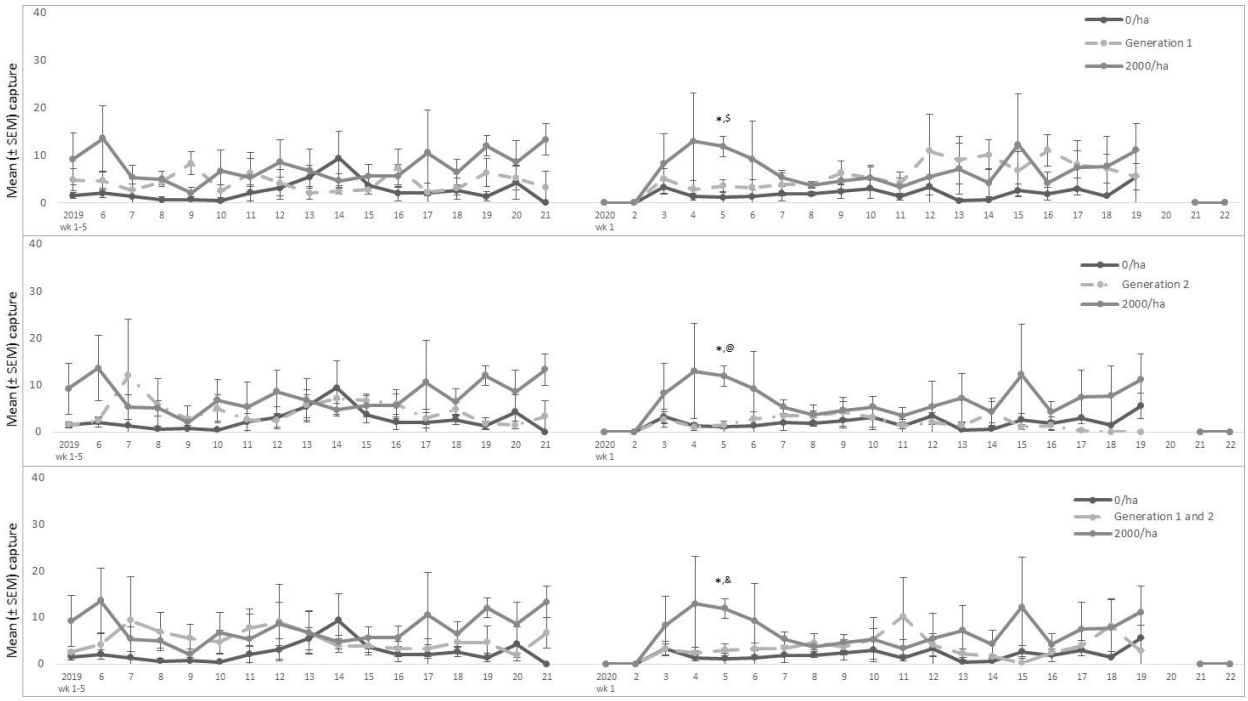

Figure 5.18. Comparative mean (± SEM) proportion catch (n = 3) of wild male *C. pomonella* weekly throughout the 2019-2020 season following the release of 2000 SIT moths/ha weekly for 21 weeks (2000 ha), six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "/" for more than 2000/ha, "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

Comparative capture of wild females at varying SIT release densities

Wild female catch was generally low across all of the release densities, with average total captures per plot for the entire season ranging from 7-28 in 2019 and 3-11 in 2020 (Table 5.2). Weekly proportion captures of wild females in plots treated with varying release densities are presented in Figures 5.19-5.20. In 2019 and 2020 the proportions of wild females captured weekly were not significantly different in all but two weeks, following releases at 2000/ha, 1000, ha, 500/ha, or 0/ha (Fig. 5.19-5.20).

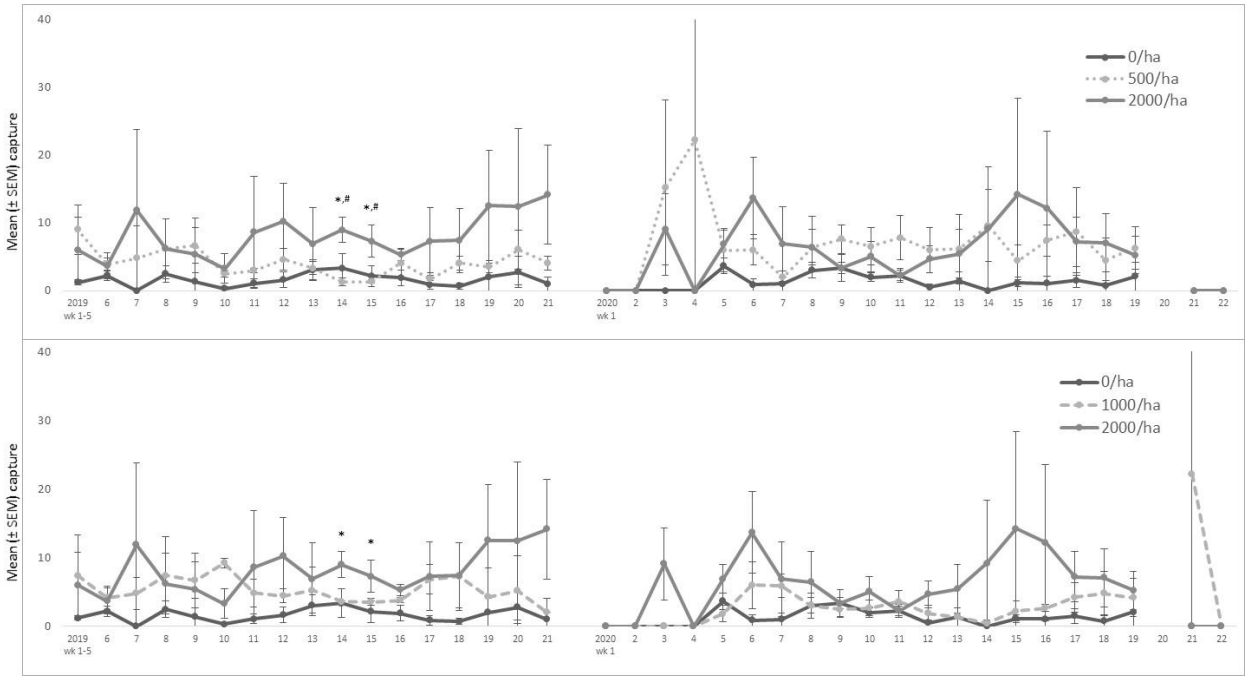

Figure 5.19. Comparative mean (± SEM) proportion catch (n = 3) of wild female *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at densities of 0/ha, 500/ha, 1000/ha or 2000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "#" for more than 500/ha, "+" for more than 1000/ha, "/" for more than 2000/ha.

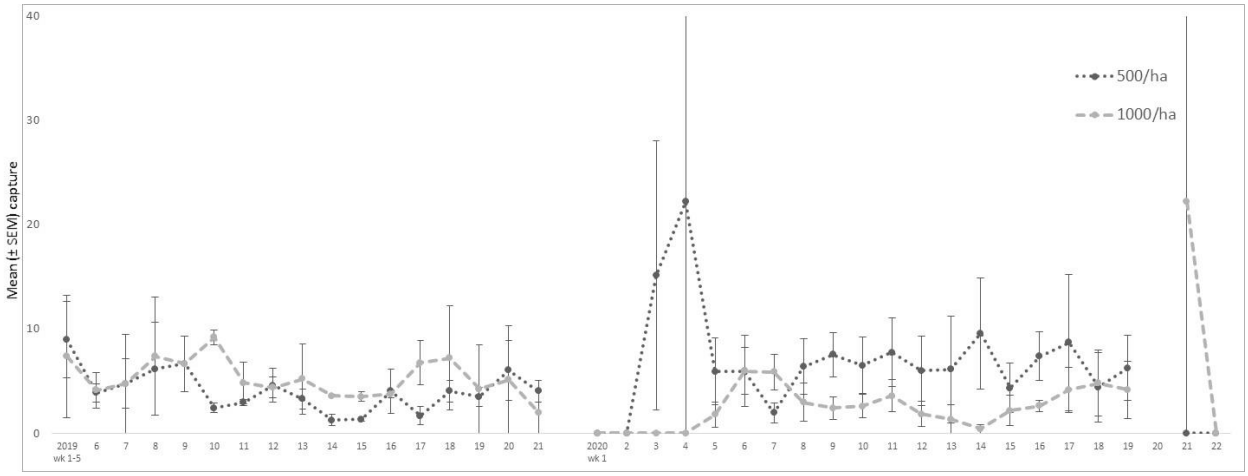

Figure 5.20. Comparative mean (± SEM) proportion catch (n = 3) of wild female *C. pomonella* weekly throughout the 2019-2020 season following sterile releases at a densities of 500/ha or1000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "#" for more than 500/ha, "+" for more than 1000/ha.

Comparative capture of wild females at varying SIT release timings

Wild female catch was generally low across all of the release timings, with average proportion of captures per plot for the entire season ranging from 15-22 in 2019 and 4-12 in 2020 (Table 5.2). Weekly proportion captures of wild females in plots treated at varying release timings are presented in Figures 5.21-5.22. In 2019 and 2020 the proportions of wild females captured weekly were not significantly different in all but two weeks, following releases season- long, for

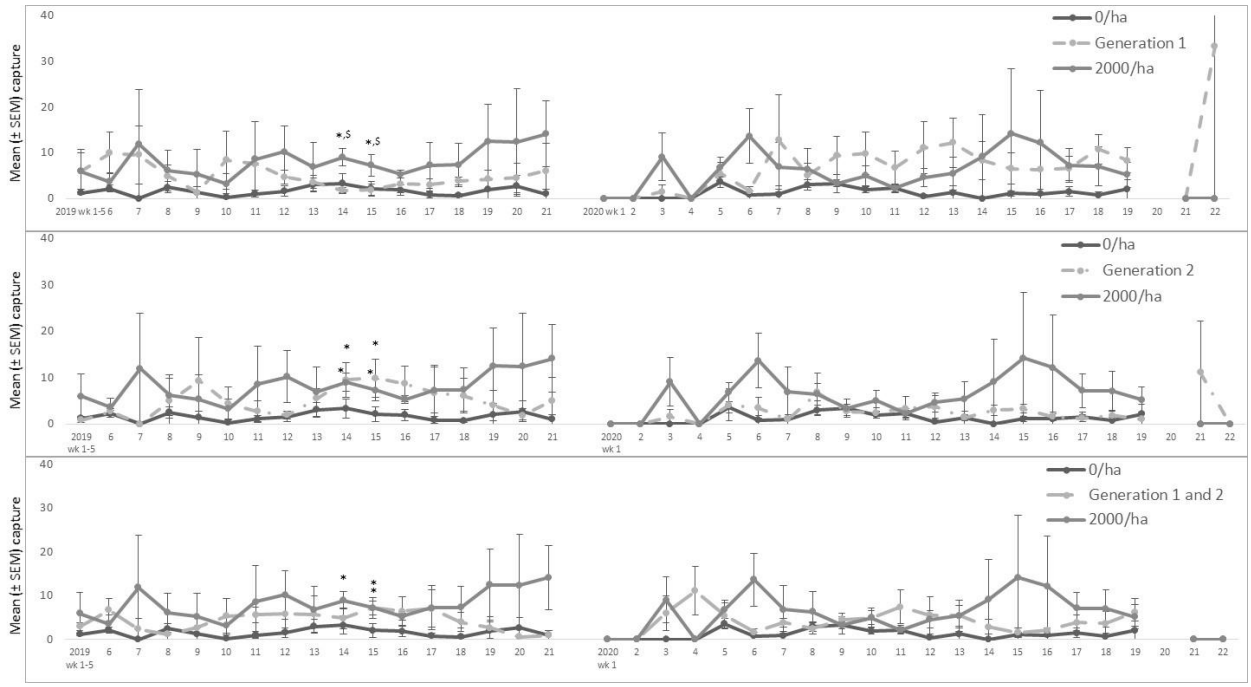

Figure 5.21. Comparative mean (± SEM) proportion catch (n = 3) of wild female *C. pomonella* weekly throughout the 2019-2020 season following the release of 2000 SIT moths/ha weekly for 21 weeks (2000 ha), six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "/" for more than 2000/ha, "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

six weeks during the first generation, six weeks during the second generation or six weeks during both generations (Fig. 5.22). Overall, fewer females were caught in 2020 than in 2019 in plots treated for six weeks of both generations or second generation only, but catch was the same in both years in plots treated first generation only for six weeks (Table 5.2).

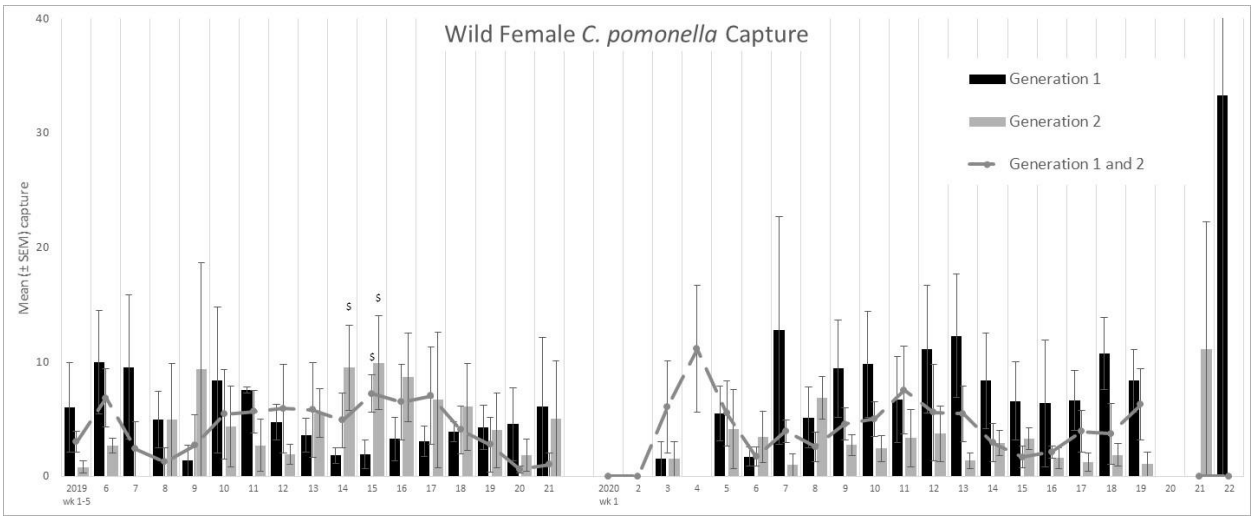

Figure 5.22. Comparative mean (± SEM) proportion catch (n = 3) of wild female *C. pomonella* weekly throughout the 2019-2020 season following the release of 2000 SIT moths/ha weekly for six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

Proportion capture of sterile females to wild males when varying SIT release densities

The proportional catch of sterile females to wild males was assessed as an indirect measure of the potential impact of releasing sterile females on disrupting the male ability to locate wild females. The weekly proportion of sterile females to wild males was significantly higher in blocks treated with 2000/ha, 1000/ha, and 500/ha mixed sex sterile codling moths compared to blocks not treated with SIT (0/ha) (Fig. 5.23-5.24). Releasing 2000 or 1000 moths/ha resulted in similar proportions of sterile females to wild male captures throughout 2019 and 2020

(Fig. 5.23, lower). In contrast, the weekly proportion of sterile females to wild males was significantly higher in blocks treated with 2000/ha compared to 500/ha mixed-sex sterile codling

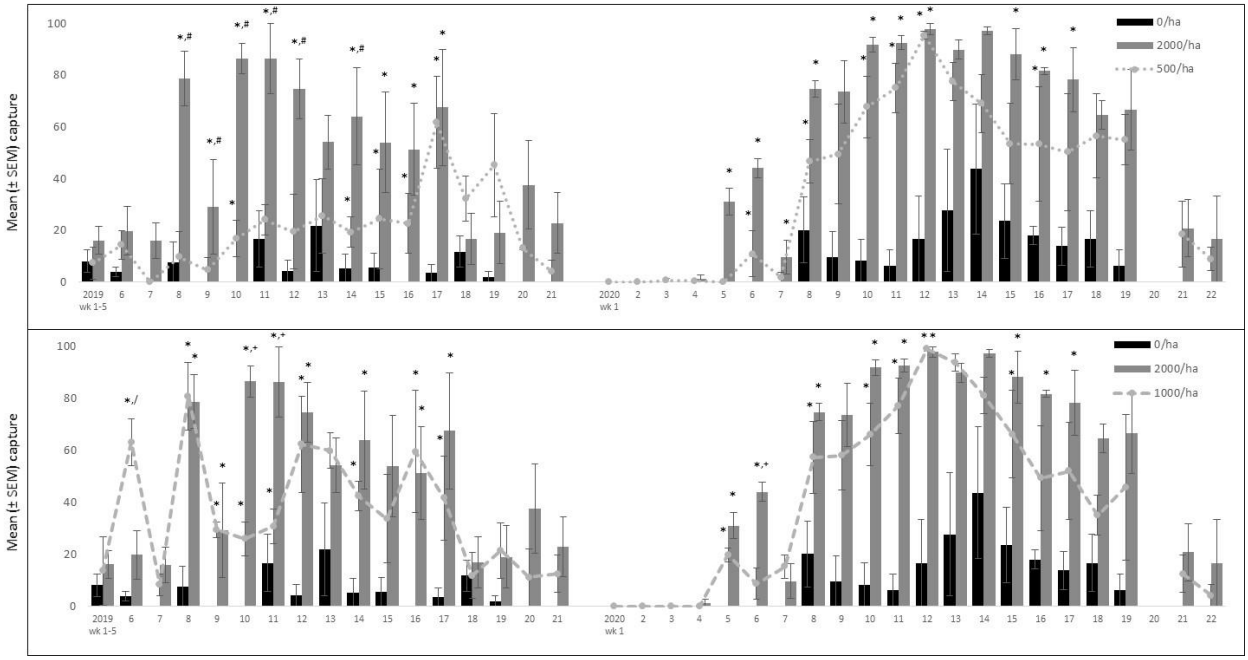

Figure 5.23. Comparative mean (± SEM) proportional catch (n = 3) of sterile *C. pomonella* females to wild male weekly throughout the 2019-2020 season following releases at densities of 0/ha, 500/ha, 1000/ha, and 2000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "#" for more than 500/ha, "+" for more than 1000/ha, "/" for more than 2000/ha.

moths on six of the sampling dates in 2019 (Fig. 5.23, upper). The weekly proportion of sterile females to wild males was similar in blocks treated with 1000/ha or 500/ha mixed sex sterile codling moths, with the exception of a few weeks early in the season both years when the proportional catch was higher in the plots receiving 1000 moths/ha (Fig. 5.24). Overall, the annual sterile female to wild male proportional catch was significantly higher when moths were released at densities of 2000 or 1000 moths/ha compared to 500 moths/ha in 2019 and 2000/ha compared to 500/ha in 2020 (Table 5.3). Similar annual sterile female to wild male proportional

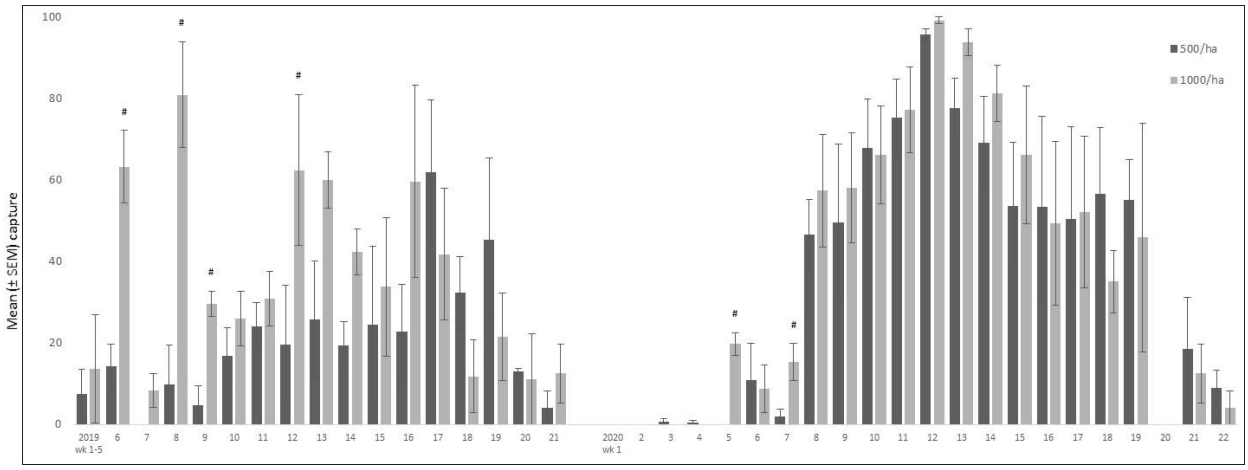

Figure 5.24. Comparative mean (± SEM) proportional catch (n = 3) of sterile *C. pomonella* females to wild male weekly throughout the 2019-2020 season following releases densities of 500/ha and 1000/ha. Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "#" for more than 500/ha, "+" for more than 1000/ha.

		ANOVA	0/ha	500/ha	1000/ha	2000/ha	Generation 1	Generation 2	Generation 1 and 2
Sterile Female to Wild Male	2019 Mean % SIT Female	*F=7.4492 df=6,14 P=0.0010*	15.1%±4.4 d	31.3%±1.5 d	54.5%±4.0 ab	63.7%±4.8 a	33.3%±9.3 cd	46.4%±4.5 bc	53.7%±7.0 ab
	2020 Mean % SIT Female	*F=3.5850 df=6,14 P=0.0230*	35.2%±20.4 c	60.7%±12.3 bc	74.4%±7.0 ab	86.7%±4.0 a	41.0%±10.1 c	80.1%±8.0 ab	75.6%±3.4 ab
	Mean Year Over Year % Change	*F=0.0859 df=6,14 P=0.9968*	130.3%±108.6	95.8%±41.3	37.9%±16.6	36.8%±6.1	82.8%±107.5	72.6%±1.8	46.5%±22.9
	2019 to 2020 capture difference		*t=0.6487 df=2 P=0.5831*	*t=2.7069 df=2 P=0.1137*	*t=2.5685 df=3 P=0.0826*	*t=3.6107 df=4 P=0.0225* Δ	*t=0.5530 df=4 P=0.6097*	*t=3.8233 df=4 P=0.0187* Δ	*t=2.7558 df=3 P=0.0704*

Table 5.3. Mean (± SEM) annual per trap percent catch of sterile female to wild male *C. pomonella* from the 2019-2020 season by treatment. Collection of Pherocon VI delta monitoring traps baited with PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) bisexual lure was from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Week #20 of 2020 had no trap collection due to a 121,200ha forest fire preventing access to test orchards. ANOVA was used to compare capture means and year over year change in capture by treatment and significant differences of $p < 0.05$ are indicated with different letters. Negative values in year over year percent change in capture indicate a reduction in capture while positive values indicate an increase in the proportion of females captured. T-tests ($P<0.05$) were used to determine if treatment blocks had a year over year change in capture, and significant differences from 2019 to 2020 are indicated with "Δ" next to the P-value.

catches were recorded in plots receiving 2000 or 1000 moths/ha (Table 5.3). For all release densities, there was not a significant change in the mean annual proportion of sterile females to wild males from 2019 to 2020 (Table 5.3).

Proportion capture of sterile females to wild males when varying SIT release timings

The weekly proportion of sterile females to wild males was significantly higher in blocks treated with mixed sex sterile codling moths for six weeks of the first generation, six weeks of the second generation or six weeks during each generation compared to blocks not treated with SIT (0/ha) (Fig. 5.25). Releasing 2000 moths/ha season-long or second generation only resulted

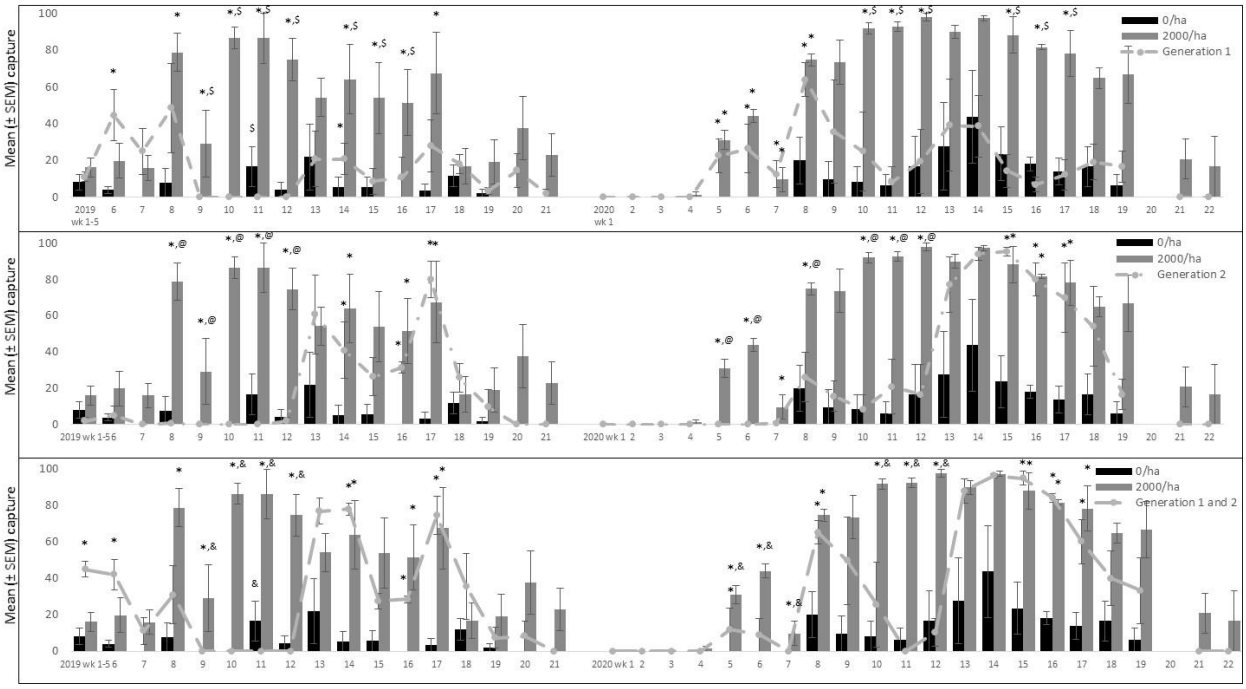

Figure 5.25. Comparative mean (± SEM) proportional catch (n = 3) of sterile *C. pomonella* females to wild male weekly throughout the 2019-2020 season following the release of 2000 SIT moths/ha weekly for 21 weeks (2000 ha), six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "*" for more than 0/ha, "/" for more than 2000/ha, "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

in similar proportions of sterile females to wild male captures during the period in which moths were deployed (Fig. 5.25, middle, lower). In contrast, the weekly proportion of sterile females to wild males was significantly higher in blocks treated season-long compared to first generation only treatment during the six weeks that moths were deployed in the latter (Fig. 5.25, upper, lower). The weekly proportion of sterile females to wild males was similar in blocks treated with 2000/ha mixed sex sterile codling moths for six weeks during both generations, first generation only, and second generation only during the periods when plots received moths (Fig. 5.26). Overall, the annual sterile female to wild male proportional catch was significantly lower when moths were only released during six weeks of the first generation compared to season-long or second generation releases (Table 5.3). For generation one and generation one and two release timings, there was not a significant change in the mean annual proportion of sterile females to wild males from 2019 to 2020 (Table 5.3).

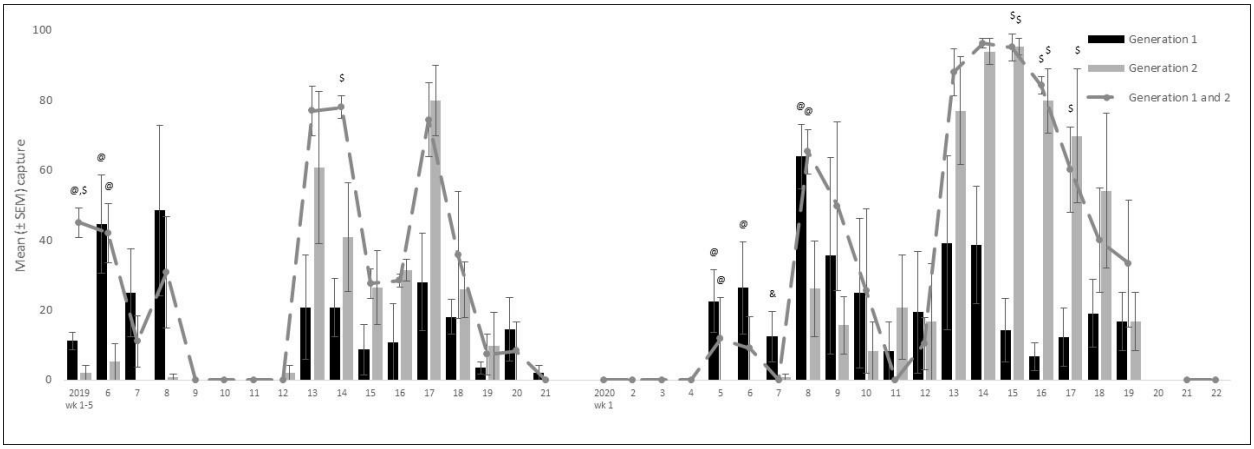

Figure 5.26. Comparative mean (± SEM) proportional catch (n = 3) of sterile *C. pomonella* females to wild male weekly throughout the 2019-2020 season following the release of 2000 SIT moths for six weeks during first and second generation (generation 1,2), six weeks during first generation only (generation1), or six weeks during generation two only (second generation). Data were collected from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Data for week #20 of 2020 a missing due to a forest fire preventing access to test orchards. Significant differences of $p < 0.05$ are indicated on figures with "$" for more than generation 1, "@" for more than generation 2, "&" for more than generations 1,2.

Annual mean proportional catch of sterile and wild males and females

Comparison of the annual mean proportional catch of sterile males, sterile females, wild males, and wild females is presented in Table 5.4. Across all treatments, significantly greater proportions of sterile males or females were captured compared to wild males or females. Additionally, with a few exceptions, a greater proportion of the catch were sterile males compared to sterile females, wild males or wild females. Across all treatments, a lower proportion of wild females were captured in 2020 compared to 2019 (Table 5.4).

Fruit injury

Assessments taken at mid- and end-of-season revealed low levels of *C. pomonella* fruit injury across all treatments (Table 5.5). Damage did not exceed 1.0% in any of the treatments or sample dates. Damage in the control plot not receiving sterile moths never exceed 0.56%, resulting in no instances of a significant reduction in damage in SIT treated plots. The percent change in damage from mid- to end-of-season in both years and from 2019 to 2020 did not differ among the treatments. Generation one only releases was the only treatment that had a significant change in damage from 2019 to 2020, with a reduction in mean damage from 0.5% to 0.06%.

Treatment	Year	ANOVA	SIT ♂	SIT ♀	W.T. ♂	W.T. ♀
0/ha	2019	*F=18.3591 df-3,64 P<<0.001*	46.2%±5.3 a	5.2%±2.1 c	29.4%±5.5 b	19.1%±3.1 b
	2020	*F=14.8273 df=3,80 P<<0.001*	55.7%±7.9 a	4.2%±1.1 d	22.2%±6.8 bc	8.4%±2.8 cd
500/ha	2019	*F=25.5243 df=3,64 P<<0.001*	47.7%±3.2 a	11.3%±1.9 c	20.8%±3.3 b	20.3%±2.5 b
	2020	*F=18.7504 df=3,80 P<<0.001*	60.2%±4.9 a	14.6%±2.1 b	18.9%±5.6 b	6.4%±1.0 b
1000/ha	2019	F=113.1811 df=3,64 P<<0.0001	65.1%±2.7 a	13.0%±1.6 b	11.4%±1.9 b	10.4%±1.2 b
	2020	F-54.1210 df=3,80 P<<0.0001	77.0%±2.0 a	12.6%±1.8 b	7.8%±2.6 b,c	2.6%±0.7 c
2000/ha	2019	F=115.6353 df=3,64 P<<0.0001	66.5%±2.3 a	12.9%±1.4 b	10.1%±2.1 b	10.6%±1.6 b
	2020	F=55.9006 df=3,80 P<<0.0001	76.7%±2.2 a	14.4%±2.2 b	6.8%±2.6 c	2.0%±0.4 c
Generation 1	2019	F=8.4996 df=3,64 P<<0.0001	42.4%±6.2 a	8.6%±2.4 c	23.1%±4.5 b	25.9%±5.2 ab
	2020	F=10.5329 df=3,80 P<<0.0001	55.7%±6.7 a	8.1%±1.7 b	17.4%±4.7 b	18.8%±6.0 b
Generation 2	2019	F=13.5259 df=3,64 P<<0.0001	41.4%±5.7 a	5.1%±1.6 b	27.4%±5.8 a	26.2%±4.8 a
	2020	F=5.9108 df=3,80 P=0.0011	51.4%±7.9 a	11.0%±2.3 b	23.7%±7.2 b	13.9%±4.3 b
Generation 1 and 2	2019	F=6.2757 df=3,64 P=0.0008	43.6%±7.2 a	9.6%±2.2 c	27.3%±5.6 ab	19.6%±3.9 bc
	2020	F=7.7532 df=3,80 P=0.0001	57.8%±7.5 a	12.9%±2.7 b	17.2%±5.5 b	12.1%±4.1 b

Table 5.4. Weekly mean percent capture of sterile male, sterile female, wild male, and wild female *C. pomonella* in organic apple orchards in Washington State. Twenty-one 3.25ha organic apple blocks were randomly assigned one of three replicated treatments of weekly releases of sterile codling moth at 0/ha, 500/ha, 1000/ha, 2000/ha , or six weeks of 2000/ha for Generation 1, six weeks of 2000/ha for Generation 2, or six weeks of 2000/ha for both Generation 1 and six weeks of 2000/ha for Generation 2. Generation 1 releases began in week 3 of 2019 and week 5 of 2020 due to differences in Accumulated Degree Days, and Generation 2 releases began in week 16 of 2019 and week 15 of 2020. Traps were deployed in week 1, and first collected in week 2. In 2019, between weeks 4 and 7, traps were not collected due to early low capture and initial planning for once monthly trap collection, subsequently through the rest of 2019 and 2020 this was changed to weekly trap collection upon discovering high numbers of moths in traps. Collection of traps was from 27 May 2019-17 September 2019 and 4 May 2020-28 September 2020. Week #20 of 2020 has no trap collection data due to a 121,200ha forest fire preventing access to test orchards.

ANOVA	0/ha	500/ha	1000/ha	2000/ha	Generation 1	Generation 2	Generation 1 and 2
2019 Mid-Season Mean Percent Damage							
F=3.9307 *df=6,14* *P=0.0163*	0.06%±0.06 b	0 c	0 c	0.28%±0.15 a b	0.06%±0.06 b	0.11%±0.06 b	0.44%±0.06 a
2019 End-of-Season Mean Percent Damage							
F=1.0594 *df=6,14* *P=0.4305*	0.28%±0.15	0.22%±0.06	0.22%0.06	0.11%±0.06	0.50%±0.10	0.17%±0.17	0.83%±0.07
2019 Mid- to End-of-Season Mean Percent Change in Damage							
F=1.7458 *df=6.14* *P=0.1828*	66.7%±33.3	100.0%±0	100%±0	-44.4%±29.4	91.7%±8.3	25.0%±25	46.5%±17.5
2020 Mid-Season Mean Percent Damage							
F=2.4973 *df=6,14* *P=0.0742*	0.56%±0.40	0	0	0.06%±0.06	0	0.22%±0.15	0.33%±0.17
2020 End-of-Season Mean Percent Damage							
F=0.7044 *df=6,14* *P=0.6513*	0.17%±0.17	0.44%±0.24	0.17%±0.10	0.22%±0.22	0.06%±0.06	0	0.22%±0.15
2020 Mid- to End-of-Season Mean Percent Change in Damage							
F=0.8213 *df=6,14* *P=0.5717*	-9.09%±9.09	100%±100	100%±100	26.67%±26.67	100%±100	-100%±100	-31.7%±22.42
2019-2020 Year over Year Mean Percent Change in Damage							
F=0.6892 *df=6,14* *P=0.6621*	-66.67%±66.67	116.67%±116.67	-33.33%±88.19	100%±152.75	-266.67%±88.19	-100%±100	-177.78%±131.00
T-test of 2019 to 2020 Change	t=0.6003 df=4 P=0.5807	t=0.2672 df=2 P=0.8143	t=0.7543 df=2 P=0.5294	t<0.0001 df=3 P=0.9999	t=3.6968 df=3 P=0.0344 Δ	t=1.000 df=3 P=0.4227	t=0.9548 df=3 P=0.4101

Table 5.5. 2019 and 2020 mean±SEM percent damage at mid- and end-of-season. A total of 600 apples, 300 fruit from block edges and 300 fruit from the interior of the block, were visually observed per treatment block for *C. pomonella* infestation at each damage assessment. Analysis of variance (P<0.05) was used to compare treatment means. Within-season and year-over-year changes in damage can be used to demonstrate treatment effectiveness. Twenty-one 3.25ha organic apple blocks were randomly assigned one of three replicated treatments of weekly releases of sterile codling moth at 0/ha, 500/ha, 1000/ha, 2000/ha , or six weeks of 2000/ha for Generation 1, six weeks of 2000/ha for Generation 2, or six weeks of 2000/ha for both Generation 1 and six weeks of 2000/ha for Generation 2. Generation 1 releases began in week 3 of 2019 and week 5 of 2020 due to differences in Accumulated Degree Days, and Generation 2 releases began in week 16 of 2019 and week 15 of 2020. T-tests (P<0.05) were used to determine if treatment blocks had a year over year change in damage, and significant differences from 2019 to 2020 are indicated with "Δ" next to the P-value.

DISCUSSION

The overall aim of this research was to develop farm scale SIT programs that could cost-effectively manage *C. pomonella* populations. The approach used in the British Columbia SIT program, and since employed in commercial apple orchards in Washington State, entails weekly sterile moth releases of 2000/ha over the 16-20 week period of *C. pomonella* activity in these production regions. Implementing this program currently costs Washington apple growers $1161/ha (Courtney, 2021). Two general approaches to reducing costs were tested 1) to deploy sterile moths at reduced densities of 500/ha or 1000/ha rather than the current standard of 2000/ha, or 2) to deploy sterile moths during only a portion of the time when wild moths are active. The British Columbia sterile insect facility currently charges $16 for the 2000 mixed-sex sterile moths needed every week per hectare. Thus, reducing the deployment density to 1000 or 500 would lower the cost of moths to $8 or $4/ha/week, respectively. If deployed for 20 weeks this would amount to a savings of up to $240/ha for season-long control. Limiting releases to only 6 weeks during either generation would result in savings for both the costs of moths and the costs of deployment. The savings in the costs of moths alone generated by releasing the standard density of 2000/ha for 6 rather than 20 weeks would be $264/ha. Either program would provide up to a 30% reduction in the cost of using SIT. Additional savings could be attained if moths did not have to be released uniformly across the block using specialized equipment, such as the modified four-wheeler used in the Canadian program or unmanned aerial systems that are currently being used in Washington State. The results of this project demonstrate that hand release of moths from a central location is an effective strategy for deploying sterile codling moths in individual apple blocks for the purpose of managing this pest. Following weekly hand

release, moths were consistently captured in traps placed throughout the 3.25 ha apple blocks. Recapture of sterile moths increased with increasing release density. Unmanned aerial systems are a convenient and effective means of deploying sterile moths, however growers are currently paying about $900/ha for the importation/release service to obtain sterile moths from Canada and release them by air on Washington Farms.

The sterile insect technique has been shown to be an effective tactic for eradication of insect pests when used on an area-wide scale (Knipling, 1955, 1957, 1959, 1960; Baumhover, 2002; Klassen and Curtis, 2005; Purdue, 2018). It has also been used successfully for area-wide suppression of pest populations (Klassen and Curtis, 2005; Bloem et al., 2007). The codling moth eradication/suppression program of British Columbia has been administered as an area-wide program since the early 1990's (Thistlewood and Judd 2019), and as with any effective IPM program, complimentary technologies are integrated when necessary (Judd and Cossentine, 1997; Judd and Gardiner, 2005). Area-wide integrated pest management is defined as long-term coordinated management of pest populations within a geographic area that is delimited by the extent of dispersal into and out of the area (Dickerson et al., 1999; Lindquist, 2000; Klassen, 2005).

The use of SIT on a farm-block scale is novel and has generally not been considered as a viable means of using the technique due to the potential immigration of *C. pomonella* adults from surrounding areas. Further challenging the utility of SIT on a farm-scale is the potential movement of sterile moths out of treated orchards, thus reducing the number of sterile moths available to successfully compete with wild moths. The release of moth into 3.25 ha apple blocks revealed that some sterile moths will move out of the blocks they were released in, as sterile

individuals were captured in blocks not under SIT. However, these data demonstrate that despite the loss of some sterile moths due to emigration, the release of sterile moths at a farm scale contributes to a reduction in wild population densities over time. Similarly, Horner et al. (2020) demonstrated that following the deployment of SIT in New Zealand apple orchards at a targeted deployment density of 40:1 sterile:wild moths, capture of wild male *C. pomonella* decreased over several years. Releasing sterile moths into the individual plots used in the current study in subsequent years should continue to reduce *C. pomonella* densities. Previous field trails with SIT in Washington State also demonstrated decreases in wild populations and damage over time in individual apple orchards (White et al., 1969; Butt et al., 1973; White et al., 1973b; White et al., 1976). As revealed in the New Zealand program (Horner et al. 2020), this approach to using SIT will provide good suppression of *C. pomonella*, but eradication of the population is not likely to be achieved without expanding the effort to an area-wide program.

Fruit damage provides the most direct measure of efficacy and means of comparing the performance of different management programs. The release of 500, 1000 or 2000 sterile codling moths provided similarly low levels of fruit injury at harvest. Deploying sterile codling moths season-long, first generation only or second-generation only also provided the same degree of fruit protection. In the year prior to starting this project, the grower had incurred upwards of 20% *C. pomonella* infestation. As indicated by the relatively low wild adult captures in traps and low fruit injury at harvest in the control plots not receiving sterile moths (0/ha), the intensive management program combined with sterile moth releases successfully controlled *C. pomonella*. Under this intensive management regime, there was no measurable contribution of SIT to the

high level of control achieved. Additional years of sterile moth releases may have may have resulted in clearer treatment separation.

Despite not being able to show differences in fruit damage, captures in pheromone-baited traps provided insights into which reduced-cost strategies might prove most useful for growers. Recapture of sterile moths was consistently lower following first compared to second generation releases. In addition, the greatest 2019 to 2020 decline in average capture of wild-type males were in blocks in which sterile moths were released during the second generation. Previous studies have also found that sterile males are less active in the spring than the summer, resulting in low ratios of sterile to wild males based on captures in pheromone traps (Thistlewood and Judd 2019). Possible reasons for the low activity in spring include higher mortality of released moths, reduced response to the pheromone or lower dispersal due to the cooler spring temperatures (Judd et al. 2004, Thistlewood and Judd 2019). The relatively low activity of sterile moths released during the spring suggests that this is not the most efficient use of this expensive technology in Washington State or that higher release densities may be required in the spring.

Lower activity of sterile males in the spring also points to the importance of having a reliable means of knowing when wild males are emerging and active so that releases are well-timed. The *C. pomonella* model developed by Jones et al. (2013) was used in this study to predict emergence and flight, and to time the deployment of sterile moths to coincide with wild moth activity. However, in 2019 and 2020 wild *C. pomonella* flight was not observed until two weeks after the model predicted emergence, resulting in early releases of sterile moths when no wild moths were present. In addition, there were several weeks when captures of both sterile and wild moths were very low. While using moth captures and traps to assist in determining when

flight begins may not be necessary for modeling *C. pomonella* activity as proposed by Jones et al. (2008, 2013), it is likely very useful in determining when to release sterile moths in particular orchard blocks. Factors that are block specific and environmental conditions such as temperature, wind and precipitation can have a major impact on *C. pomonella* activity. Worthley (1932) found that flight, and response to bait pails, increased with increasing temperature, decreasing atmospheric humidity, increasing barometric pressure, increasing length of moonlight and clear skies, and was stopped by light rain and winds. Likewise, Pitcairn et al. (1990) found consistent flight and response to pheromone-baited traps occurred when evening temperatures exceeded 15.8°C, windspeed was <3.62m/s, and rain was minimal. Precipitation, albeit artificial, has been demonstrated to control *C. pomonella* in apple (Knight, 1998). Releasing sterile moths during periods of unfavorable conditions for flight are probably unproductive. Monitoring individual orchards to assess *C. pomonella* activity is the best means of ensuring that sterile moths are being released at the times when males are active.

Sterile male recapture in attractant-baited traps also revealed that increases in release density resulted in consistent increases in sterile male activity. Releasing 2000 moths/ha generally resulted in at least a two-fold greater catch compared to releasing 1000/ha and similarly, releasing 1000/ha generally resulted in at least a two-fold greater catch compared to releasing 500/ha. This suggests that fine-tuning the release density to respond to lower or higher wild population densities may be a viable approach to cost-effectively managing *C. pomonella* using SIT. One approach would be to release higher densities of sterile moths during the peak flight period and lower densities at the start or end of a flight period.

In this study, attention was paid to both the recapture of males and females. This was accomplished by baiting traps with a lure that attracts both sexes. The results provide insight into the potential importance of releasing both sexes when targeting *C. pomonella* with SIT. Judd (2016) found that in orchards receiving equal numbers of sterile females and males, females accounted for 81% of the sterile moth catch in traps baited with pear ester and acetic acid lures. In the current study, traps were baited with lures containing the kairomones plus the sex attractant, codlemone, and sterile male captures were consistently higher than female captures. However sterile females comprised up to 15% of the catch and the seasonal patterns of sterile male and female captures were similar. The proportion of sterile females to wild males captured in traps increased as release densities increased, and all treatments had reductions in wild male capture concurrent with increases in sterile female capture. In addition, there were dramatic reductions from 2019 to 2020 in the numbers of wild females captured and modest decreases in the numbers of wild males in most treatments. These findings suggest that sterile females may be impeding male captures in traps and by inference inhibiting wild males from locating wild females. The potential importance of females in achieving control by SIT is consistent with the findings of White et al. (1976); when they released sterile females only, sterile males only and mixed sexes, 69% and 27% damage reductions were recorded where females only or mixed sexes were released, respectively, while damaged increased by 100% when only males were released. The hypothesis that sterile females provide control by serving as mobile pheromone emitters, interfering with mating between wild males and females in a competitive manner similar to commercial mating disruption technologies should be further explored.

Use of SIT has been proven to be an effective tactic for managing *C. pomonella* in areawide programs carried out in British Columbia, Canada (Thistlewood and Judd, 2019), South Africa (Barnes, 2015) and New Zealand (Horner et al., 2020). However, the programs cost upwards of $1120/ha and in all cases a large portion of the expense has been taxpayer funded. In the current study, strategies have been proposed and tested for using SIT at a farm-scale and in a cost-effective manner whereby the growers can afford the technology on their own. Cost-effective strategies include treating for only a portion of a generation or at a reduced deployment density. Additional strategies not yet tested include only releasing moths every other week or releasing more or fewer sterile moths as the density of wild populations change. The current study was unable to demonstrate the effectiveness of the modified programs based on fruit injury at harvest, as the grower's standard program provided a high level of *C. pomonella* control. However, all of the lower-cost SIT approaches tested in this study resulted in a decrease in the level of damage from 2019 to 2020 and there was a concurrent decrease in the number of wild males and females captured in pheromone-baited traps. Further field trials in orchards with higher wild populations and fruit damage at harvest are needed to confirm the efficacy of the modified programs.

Currently, the ability of growers to fine-tune their SIT program to make it more economical is greatly hampered by the presence of only a single source for obtaining sterile *C. pomonella*, and by the facility being located in Canada. The permit and shipping process are quite difficult and currently the moths can only be imported by M3 Consulting Group. The facility increases production beginning in the winter to provide moths to North American growers in the summer and will only supply enough moths to treat about 1200ha in the United States. The same

number of moths must be imported to the US weekly, making it extremely difficult to modify the density of moths released or the number of weeks that moths are released. Farms that choose to purchase and release sterile moths must decide early in the year how many moths they need for the growing season, and the facility delivers the same number of moths every week for the entire season. A SIT facility in the US that is able to supply adequate numbers of moths for more than 1200ha is needed to provide growers with the flexibility desired to modify release strategies and implement a cost-effective program.

CHAPTER SIX

Estimating plume reach and trapping radius for male and female *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) captured in pheromone/kairomone baited traps in apple orchards under mating disruption

INTRODUCTION

Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), is the key pest of apples worldwide and also infests pear, walnut, quince, crabapple, loquat, hawthorn and some stone fruits (apricots, cherries, peaches, plums, prunes) (Newcomer and Whitcomb, 1925). It has been the target of control efforts in North America since first being detected in the 1750's (Essig, 1931). *C. pomonella*, causes damage directly to fruit by the action of larval feeding, rendering fruit unfit for sale as a fresh commodity. When apple orchards are unmanaged or poorly managed, losses can be substantial (Isley and Ackerman, 1923; Allman and Essig, 1929; Putman, 1963; Glass and Lienk, 1971; MacLellan, 1972; Westigard, 1973; Setyobudi, 1989; Wise and Gut, 2000, 2002).

Control of *C. pomonella* with broad-spectrum insecticides has been complicated by the loss of effective compounds through resistance or restrictions (Varela et al., 1993; Knight et al., 1994; Mota-Sanchez et al., 2008). In response to resistance leading to control failures and a general need for alternative control tactics, the use of pheromone as a direct control strategy for *C. pomonella* was explored beginning with field trials in the 1970's (Cardé et al., 1977; Vickers and Rothschild, 1991). For the past thirty years pheromone-based mating disruption has provided growers with a commercially viable means of managing codling moth (Gut et al., 2019). Mating disruption interferes with *C. pomonella* reproduction by dispersing synthetic sex pheromone into the crop to disrupt normal mate finding. The technique is used on an estimated 243,000 hectares of commercial apples, pears and walnuts worldwide as the primary control for codling moth (Gut et al 2019).

A consequence of widespread use of *C. pomonella* mating disruption is that monitoring traps are rendered ineffective when baited with pheromone dispensed at a lower release rate

than the mating disruption dispensers that emit the same pheromone. As a result, several synergists have been developed to increase the attraction of monitoring traps in disrupted orchards. The kairomone, ethyl (E, Z)-2,4-decadieonate (pear ester), isolated from pears, was found to be attractive to both male and female *C. pomonella* (Light et al., 2001), and has been extensively studied (Knight and Light 2004a; Knight and Light 2004b; Knight and Light 2004c; Light and Knight, 2005; Knight and Light, 2005a; Knight and Light, 2005b; Knight et al, 2005; Schmera and Guerin, 2012). The kairomone is now commercially available in three lure formulations in combination with *C. pomonella* pheromone. In addition to pear ester, acetic acid has been found to be attractive to both sexes of *C. pomonella* (Landolt et al. 2007). Multiple studies of attractiveness of acetic acid, or fermented sugar baits (acetic acid is a product of fermentation) have resulted in this compounds inclusion in commercial lure formulations (Yothers, 1930a, 1930b; Landolt et al., 2007; Knight, 2010a; Knight 2010b; Judd, 2016).

The addition of kairomones has allowed *C. pomonella* pheromone-baited monitoring traps to capture adults in orchards with mating disruption, but there are still aspects of monitoring program results using these baits that are unknown. Extrapolating capture in traps to absolute pest density and determining the minimum effective number of traps baited with these lures needed per area under mating disruption needs to be determined. Recent studies by Miller et al. (2015) and Adams et al. (2017 a, b) demonstrated that the number of moths caught in traps baited only with *C. pomonella* pheromone can be translated into estimates of pest density and trapping area. Their studies were conducted in orchards not under mating disruption, and the use of their experimental and quantitative methods should now be applied as a means of estimating these parameters using the kairomone synergists currently in widespread use for

monitoring *C. pomonella* in orchards under mating disruption. A greater understanding of catch in traps baited with kairomone-based lures in mating disrupted orchards can now be attained using the methods detailed in Miller et al. (2015) and Adams et al. (2017 a,b), to estimate the maximum dispersive distance, plume reach, effective trapping area, and expected pest density within the trapping area under mating disruption when using monitoring traps baited with a lure containing *C. pomonella* pheromone (CM), pear ester (DA), and acetic acid (AA).

The overall aim of this work was to provide a better understanding of wild capture in monitoring traps using pheromone and kairomone lures in fruit production systems employing *C. pomonella* mating disruption. This information can be used to improve *C. pomonella* management. The primary objective was to determine the probability of male and female *C. pomonella* catches from specified distances using traps baited with PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) in a single central trap, multiple-release experimental design, and to apply this information for estimating maximum dispersive distance, plume reach, and absolute pest density using the quantitative tools developed by Miller et al. (2015). The hypothesis being tested is that male *C. pomonella* will have a smaller dispersive distance in pheromone-controlled orchards than in orchards without mating disruption, and that females' dispersive distances will be similar to males. This research represents the first efforts to determine the plume reach, dispersive distance, trapping area, and estimates of pest density for female *C. pomonella* through the use of kairomone-baited traps.

METHODS AND MATERIALS

Source of sterile adults

Sterile, mixed-sex *C. pomonella* adults were obtained from the Okanagan-Kootenay Sterile Insect Release (OKSIR) facility in Osoyoos, British Columbia, Canada. Upon eclosion, moths at the OKSIR facility were immediately placed in petri dishes at an approximate ratio of 1:1 males:females (ca. 800 moths/petri dish) and treated in a Co^{60} irradiator as described in Horner et al. (2020). The dishes of irradiated moths were then packed into battery-powered coolers (2.8 Cu. Ft. Portable Fridge/Freezer: Edgestar co. Austin, Texas) held at approximately 2-5°C and shipped to Washington State. Moths always arrived before noon the same day they were packed allowing for immediate release into field plots. Because moths were transported as mixed-sex batches in chill coma directly from the shipper to field sites for immediate release, the sexes could not be separated prior to release.

Handling of sterile adults

Immediately upon arrival at field sites, moths were dispensed into 540-ml polystyrene cups (Fabri-Kal Corp. Kalamazoo, MI) in batches corresponding to the number being released at each distance, but never more than 4000/cup. Moths for each release distance were uniquely colored using ca. 1.25ml/800 moths of Dayglo florescent pigments (ECO11 Aurora Pink®, ECO15 Blaze Orange™, ECO18 Signal Green™, ECO19 Horizon Blue™) (DayGlo Color, Cleveland, OH), allowed to warm to ambient temperature, and then released at pre-marked locations at distances of 20m, 40m, 60m, and 80m from the central pheromone-baited trap location. Moths were gently tossed by hand from the containers of colored moths ca. 1-2 m into the canopy of

pre-marked trees. Released moths primarily alighted on the leaves and stems of the surrounding trees in all directions, and some fell to the ground, but McMechan and Proverbs (1972) found no difference in moth recovery when moths were deployed into trees or on the ground.

Estimating dispersive distance, plume reach, and trapping area, for male and female codling moths

A mark-release-recapture study to estimate dispersive distance, plume reach, and trapping area for male and female codling moths was conducted in several commercial apple orchards in North-Central Washington State during the summer 2018-2020 field seasons. Orchards in which the experiment was conducted had a variety of apple cultivars, rootstocks, irrigation schemes, and tree training systems. The experiment was conducted in two 40-acre blocks in Brewster, WA in 2018, one 40 acre block in Loomis, WA, one 24-acre block in Ellisforde, WA, and two 40-acre blocks in Brewster, WA in 2019, and five 40-acre blocks in Brewster, WA in 2020. All orchards were treated with pheromone mating disruption for *C. pomonella* control using either actively dispensing aerosol emitters (i.e. ISOMATE® CM Mist Plus (Vancouver, WA)) at 0.5-1/ac, or passively dispensing reservoir dispensers (i.e. ISOMATE® CM Flex, and Scentry NoMate® CM Spiral (Billings, MT)) at 700-800/ha. Conventional chemical controls were applied as needed for pests other than *C. pomonella*. All orchards had been under mating disruption for several years, thus only granulosis virus was used as a supplemental control for *C. pomonella*.

The experiment employed a cardinal direction mark-release-recapture design with a single central trap following modified protocols developed by Adams et al. (2017a) (Fig. 6.1A). Release locations were marked with flagging tape in the four cardinal directions from the single

trap at distances of 20, 40, 60, and 80 meters. In each replicate, approximately equal numbers of females and males were released, and the number of moths was increased with increasing distance. Each of the four 20 m release points received ~400 sterile males/~400 sterile females, the four 40m release points each received ~800 sterile males/~800 sterile females, the four 60m release sites each received ~1600 sterile males/~1600 sterile females, and each of the four 80m release sites received ~3200 sterile males/~3200 sterile females.

Moths at each release distance were marked with a unique color at the time of release and recaptured at the central trap location. Captures of male and female SIT marked moths were quantified using Orange Pherocon VI delta traps (Trece Inc., Adair, OK) baited with a PHEROCON® CM-DA COMBO™ Lure + AA Lure (Trece, Inc.) designed to attract both male and female codling moths (Knight and Light, 2012). The 2-part lure was held above the replaceable sticky liner with a pin through the top of the trap. To maximize catch, traps were placed within the top 1/3 of pre-marked trees (Yothers, 1930a, Riedl et al 1979). Lures were changed every six weeks. Traps were monitored for 14 days following release. Trap sticky liners were removed and replaced if moths were present when traps were checked weekly and subsequently examined in the laboratory using UV illumination (400-405 nm, 12 UV LED bulb flashlight, Bioquip Products, Rancho Domingo, CA) to determine the color and sex of marked moths.

DATA ANALYSIS

Due to high variability in recapture of codling moths from block to block, year to year, and month to month, criteria were established for minimum recapture to qualify for analysis. For both male and female captures, several replications across all plots from May through September

each year had low or no capture, and it was not restricted to a single plot, month, or mating disruption technology. To minimize stochasticity due to low or no capture, replications were not included in the data analysis if fewer than two moths were captured from each distance released, or fewer than 20 moths were captured in total. Males and females were analyzed separately, so different numbers of acceptable replications were used based on these criteria. Captures from 18 replicates were used for analysis of male data, and 12 for analysis of female data.

Terminology for data analysis and data were plotted following the quantitative methods of Miller et al. (2015) to yield 1) an untransformed graph of the released moths over distance from trap, 2) plot of 1/proportion of released moths recaptured over distance of release from central trap (MAG plot), and 3) (annulus area)*(proportion of *C. pomonella* recaptured)/distance of release from central trap (Miller plot). The untransformed plot confirms that release distances were selected appropriately when a concave line with an asymptotic approach to zero catch is observed. The slope of the MAG plot, linear over close release distances, can be used to determine plume reach of monitoring traps using the standard curve of Miller et al. (2015), Fig. 4.12. The maximum dispersive distance for 95% of the responding population is estimated by a second-order polynomial fitted to the Miller plot data with the point at which the line crosses the x-axis estimating the maximum distance 95% of the population can disperse (Adams et al., 2017a). The average proportion caught out of all insects in the full trapping area (Tfer) for these experiments was calculated by dividing the mean of the proportion caught at a specific distance (spTfer) × annulus area by the mean annulus area [mean (spTfer × annulus area)/mean annulus area] (Eq. 5.2, Miller et al., 2015), and was used to estimate population density per trapping area. Areas of trapping annuli were calculated as per Miller et al. (2015).

RESULTS

Captures of males in the replicates having sufficient recaptures to allow for analysis (n=18) ranged from 23-113 with an average recapture of 53 total moths. The range of capture of females in the replications having sufficient recaptures to allow for analysis (n=12) was 22-152 with an average of 65 total females caught.

Female Recaptures: Of the 288,000 females released from all distances combined, 0.27% were recaptured through the course of the experiment. At each of the four release distances, 20m, 40m, 60m, and 80m, the mean proportions of females captured were 0.125±0.017 (mean±SEM), 0.085±0.019, 0.054±0.011, and 0.081±0.013, respectively.

Female movement: As predicted for insects that move randomly (Miller et al., 2015), catch decreased as distance released from the central trap increased (Fig. 6.1B). The Miller plot estimated the maximum dispersive distance for 95% of the released population of females (where the curve intersects the x-axis) to be approximately 128 meters (Fig. 6.1C). The MAG plot (Fig. 6.1D) produced a straight line over the four data points with a slope of 0.077 and y-intercept of -0.510, and using the standard curves of Miller et al. (2015), a negative y-intercept corresponds to a very small plume reach, likely much less than 5 m. Using these data, the trapping radius of ~128 meters corresponded to a trapping area of 5.15ha for females using a PHEROCON® CM-DA COMBO™ Lure + AA Lure in an Orange Pherocon VI delta trap in apple orchards under mating disruption. The mean T_{fer} was 0.002 (n=12).

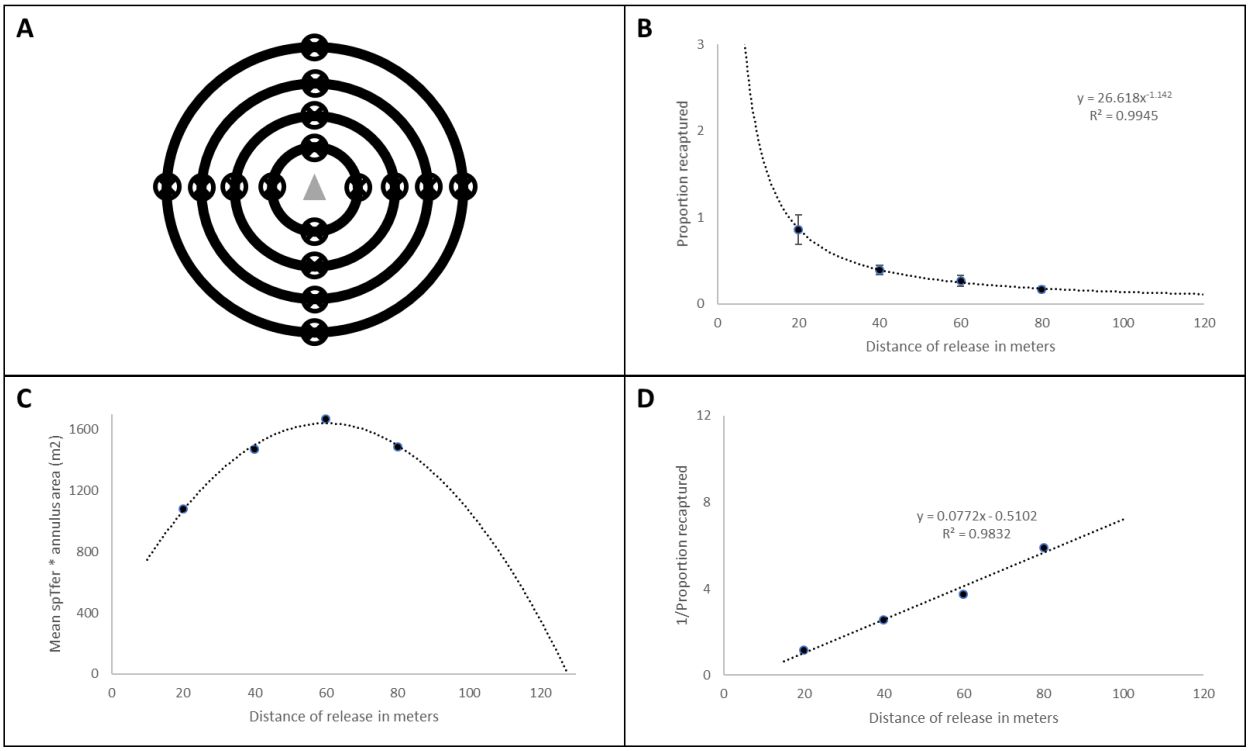

Figure 6.1. Female *C. pomonella* mark-release-recapture experimental design and data plots following the protocols of Miller et al. 2015. A) Cardinal direction release pattern (circles in line) with central trap location (triangle). B) Mean probability of catch at a specific distance (spT_{fer})/distance; mean of 12 replicates. C) Miller plot transformation (inverse of proportion caught by distance). D) Inverse of proportion caught by distance (MAG plot).

Male recaptures: Of the 432,000 males released from all distances combined, 0.23% were recaptured through the course of the experiment. At each of the four release distances, 20m, 40m, 60m, and 80m, the mean proportions of males captured were 0.161±0.031 (mean±SEM), 0.063±0.011, 0.035±0.006, and 0.016±0.003, respectively.

Male movement: As with female moths, catch decreased as distance released from the central trap increased (Fig. 6.2B). The Miller plot estimated the maximum dispersive distance for 95% of the released population to be approximately 100 meters (Fig. 6.2C). The MAG plot (Fig. 6.2D) produced a straight line over the four data points with a slope of 0.1451 and negative y-intercept (-0.7322); using the standard curves of Miller et al. (2015), a negative y-intercept

corresponds to a very small plume reach, likely much less than 5m. Using these data, the trapping radius of ~100 meters corresponded to a trapping area of 3.14ha for males in apple orchards under mating disruption, using a PHEROCON® CM-DA COMBO™ Lure + AA Lure in an orange Pherocon VI delta trap. The mean T_{fer} was 0.002 (n=18).

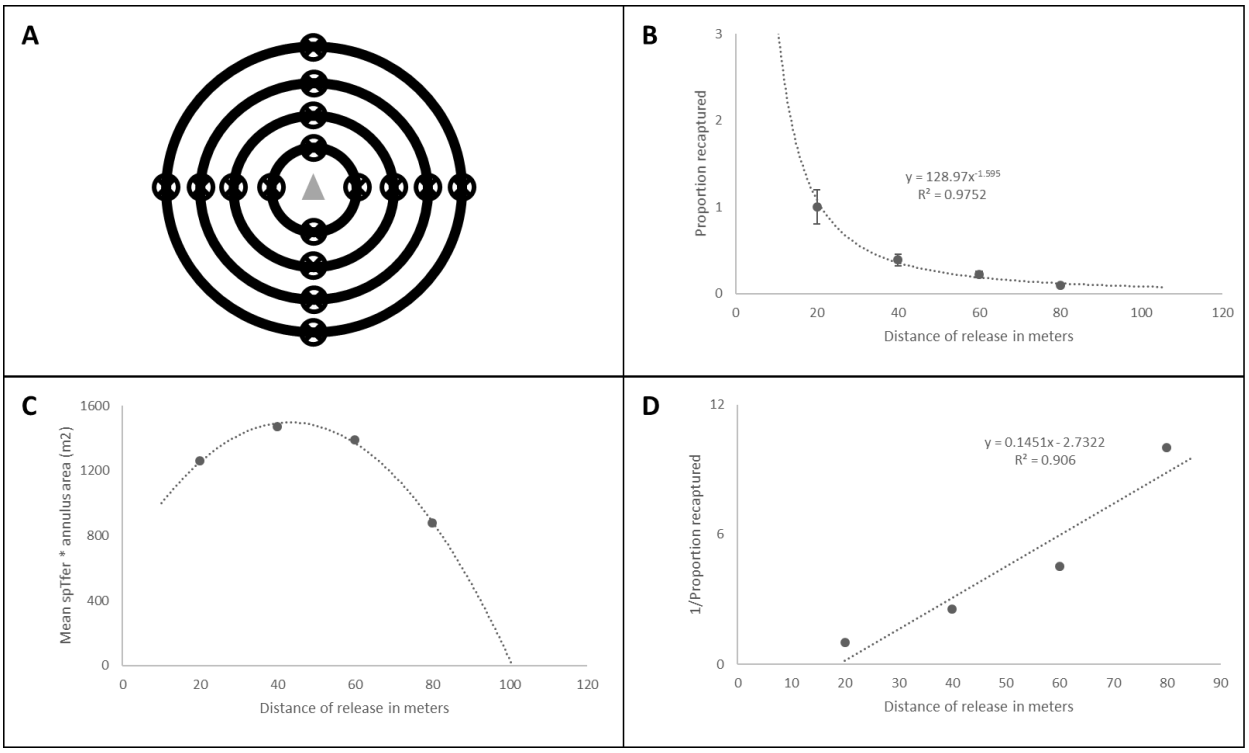

Figure 6.2. Male *C. pomonella* mark-release-recapture experimental design and data plots following the protocols of Miller et al. 2015. A) Cardinal direction release pattern (circles in line) with central trap location (triangle). B) Mean probability of catch at a specific distance (spT_{fer})/distance; mean of 18 replicates. C) Miller plot transformation (inverse of proportion caught by distance). D) Inverse of proportion caught by distance (MAG plot).

DISCUSSION

This study was conducted from spring through the peak of the growing season, and into harvest. In addition, replicates were conducted in multiple locations over a period of three years. Thus, released moths were exposed to a variety of environmental conditions such as varying temperature, humidity, sunlight, and seasonality, all of which can impact *C. pomonella* adult flight

(Vickers and Rothschild 1991). Due to the fluctuating conditions over the course of the study that can impact adult flight, recapture of released sterile moths was variable, and in some instances, no moths or low numbers of moths were recaptured. For pheromone plus kairomone-baited traps placed in commercial apple orchards under mating disruption, the average probability of capture of *C. pomonella* adults in the trapping area (T_{fer}) was 0.002 for males and 0.002 for females. According to Miller et al. (2015) T_{fer} values measured over an entire trapping area are always small. Despite the varying conditions under which the experiment was conducted and the seemingly low captures, results of the mark-release-recapture experiment and quantification of the data using the protocols developed by Miller et al (2015) provided consistent and useful insights into the dispersal patterns of male and female *C. pomonella*. They also facilitated the first account of dispersive capabilities, plume reach, and trapping radius for both female and male *C. pomonella* in apple orchards under mating disruption.

Plume reach, maximum dispersive distance, trapping area and proportion catch

The estimated plume reach for a monitoring trap baited with a combination pheromone plus kairomone lure deployed in apple orchards under mating disruption was very small (likely <1m) for both male and female *C. pomonella* adults. A somewhat larger, but still small plume reach of <5m was estimated for *C. pomonella* males responding to a trap baited with pheromone only and deployed in apple orchards not under the influence of a pheromone mating disruption treatment (Adams et al. 2017). A short plume reach for male and female *C. pomonella* responding to attractant-baited traps is consistent with findings reported in other trapping studies (Knight and Light 2005d, Judd 2016). Recording differential recaptures of marked *C. pomonella* adults

released at varying distances from traps led Knight and Light (2005b) to conclude that the active space of a pear ester lure for both sexes was much shorter than that of codlemone only lures for male moths. Based on sterile and wild adult ratios of male to female captures in traps baited with a pear ester/acetic acid combination lure, Judd (2016) surmised that the plume reach for both males and females was small. Additionally, he reported that traps baited with a pheromone plus pear ester lure yielded catches with male sterile to wild ratios that were significantly greater than female sterile to wild ratios and proposed that this likely meant that the kairomone component was attractive to females over a very short distance and the codlemone component attracted males over a slightly greater range. A single trap multiple release test of *Drosophila suzukii* response in cherry orchards to a sticky red panel trap baited with a commercial lure containing plant volatile-based attractants generated a similar tiny plume reach of <3m (Kirkpatrick et al. 2018). A short plume reach for kairomone-baited traps appears to be the consensus among researchers testing the response of insects to these more generally attractive compounds (Braasch and Kaplan, 2012; Schlyter, 1992). These data confirm and provide support for these previous findings.

Maximum dispersive distances for 95% of the released sterile *C. pomonella* adults were estimated to be 100m for males and a slightly larger 128m for female over the course of two weeks of catch (Figures 6.1, 6.2). Combining this value with the short plume reach of <1m generates a trapping radius only slightly greater than the maximum dispersal distance and calculated trapping areas of 3.14 ha and 5.15 ha, respectively. The maximum dispersive distance for 95% of captured adult males and the resulting calculated trapping area for *C. pomonella* responding to a pheromone-baited trap in non-disrupted apple orchards were substantially

greater - 260m and 21 ha, respectively (Adams et al. 2017). The high dispersal of *C. pomonella* in conventionally treated apples is consistent with early studies of their movement. Steiner (1940) observed that moths would move ~610 meters from the point of emergence, Worthley (1932) captured most adults within ~160 meters of the release point. Trematerra et al. (2004) recorded average dispersal distances for male *C. pomonella* to be up to ~130-200 meters, and similarly Basoalto et al. (2010) reported dispersal distances of ~150-300 meters.

The substantially smaller dispersive distance and trapping area for pheromone plus kairomone-baited traps deployed in mating disrupted orchards compared to pheromone-baited traps in non-disrupted orchards reveals that male *C. pomonella* movement is greatly impacted by the pheromone treatment. The reduced area traveled by males is a result of high-releasing synthetic sources of pheromone drawing the attention of males as they search for mates (Miller and Gut 2016). *C. pomonella* males appear to make multiple visits to dispensers over the course of an evening (Miller et al., 2006), further reducing dispersal over the course of their lifetime. The trapping area of lure-baited traps attractive to female *C. pomonella* in either pheromone-treated or orchards using insecticides to manage this pest has not previously been examined. In the current study, the maximum dispersive distance and the resulting calculated trapping area for *C. pomonella* females responding to a pheromone plus kairomone baited trap in apple orchards under mating disruption was 4 fold smaller than the maximum dispersive distance and trapping area for males detected in pheromone-baited traps in non-disrupted orchards. It is possible that the more limited movement of females is due to them searching for oviposition sites within which to deposit eggs and being more sedentary than males if host fruit are abundant. Studies of female *C. pomonella* movement in non-disrupted apple orchards are needed to assess the potential

impact of the mating disruption treatment on female movement and whether the trapping area would be similar or different under the two management regimes.

The greatly reduced trapping area for pheromone plus kairomone-baited traps deployed in mating disrupted orchards compared to pheromone-baited traps in non-disrupted orchards has important ramifications for those relying on moth captures in traps to make management decisions. The recommended monitoring trap density in disrupted and conventional orchards is one codling moth trap for every hectare (Gut and Wise, 2016, Knight and Light 2005c). While this trapping density may be sufficient in orchards not under disruption where the trapping area covered by a pheromone-baited trap is an estimated 21ha, it may be inadequate in orchards under disruption where the trapping area covered by a pheromone plus kairomone-baited trap may only be 3.1 ha. Additionally, although results of this study indicate that male and female moths will disperse at least 100m in orchards under mating disruption, the likelihood of moths being captured in monitoring traps 100m away from where they emerge is very low. Deploying an inadequate number of monitoring traps in disrupted apple orchards may provide misleadingly low estimates of moth population densities.

Estimating pest density from moth captures in monitoring traps

Pest density can be readily calculated by dividing capture in CM-DA COMBO™ Lure + AA Lure-baited monitoring traps in mating disrupted orchards by T_{fer}, (Miller et al. 2015, Adams et al. 2017a, Kirkpatrick et al. 2018). Using the T_{fer} values generated in this study, 0.002 for males or 0.002 for females, captures of 1, 5, 10, 50, and 100 males equates to ca. 180, 898, 1795, 8976, and 17925 male *C. pomonella* per hectare. Captures of 1, 5, 10, 50, and 100 females corresponds

to population densities of ca. 113, 567, 1135, 5673, and 11346 females per hectare. Although the ratio of males to females is approximately 1:1 for wild or released sterile moths, they do not respond in exactly the same manner to CM-DA COMBO™ Lure + AA Lure-baited monitoring traps in mating disrupted orchards, likely due to cues eliciting differential responses to the attractant and differences in their propensity to move. Knight and Light (2005) found that it was less risky to base action thresholds using pear ester-baited traps on combined male and female captures, rather than one sex or the other. The results of the current study also indicate that it may be more useful to estimate field population density by taking into account captures of males and females. In this case, capture of a single moth, regardless of gender, equates to 100-200 moths/ha and capture of 10 moths would predict a density of 1100-1800 moths/ha.

Extrapolating female capture in traps to on-farm damage

Codling moth females, which mate an average of 2.2 times (Hathaway, 1966), have been found to deposit an average of 55 eggs (Huang et al., 2008) to as many as 160 eggs/female (Blomefield and Giliomee, 2012) over their lifetime. Estimates of the density of moths/ha calculated above reveal that a single female moth captured in a trap corresponds to 113 female moths/ha. If each of those females is capable of laying 55-160 eggs over her lifetime, a single female captured in a monitoring trap corresponds to between 6215-18080 eggs laid/ha in a single generation. If each of those eggs is laid singly and hatch to attack and infest apples, it becomes problematic as huge crop losses can occur within a short period of time. The results demonstrate that even if zero or very few moths are captured in monitoring traps, high numbers of females may be present within the trapping area and that significant crop losses may occur. The likelihood

of sustaining damage even when catches are very low, led Knight and Light (2005c) to propose that practitioners relying on traps baited with pear ester lures to make management decisions in mating disrupted orchards should use an action threshold of a cumulative ≥ 2-3 total moths/trap or ≥ 1-2 females per trap to trigger a treatment.

Conclusion

The Miller et al. (2015) methods for quantifying insect pest movement were first field validated for male *C. pomonella* responding to a sex pheromone-baited trap by Adams et al. (2017a) and further applied for monitoring *D. suzukii* using a kairomone-baited trap (Kirkpatrick et al. 2018) and for *Halyomorpha halys* (Stål) using an aggregation-pheromone baited trap (Kirkpatrick et al. 2019). The approach has now been field validated in the current study for *C. pomonella* females and males using PHEROCON CM-DA COMBO™ Lure + AA Lure-baited monitoring traps deployed in apple orchards under mating disruption in Washington State. The results demonstrate that by the time a single male or female moth is captured in a monitoring trap in a pheromone-treated orchard, there are already many individuals present in an orchard, and immediate management actions to supplement the disruption treatment may be needed to keep the population in check.

These experiments are the first to estimate dispersal distance, plume reach, trapping radius, and trapping area for female *C. pomonella* released into commercial apple orchards. This study is also the first to estimate these metrics for male *C. pomonella* using a trap baited with a pheromone plus kairomone lure and deployed in mating disrupted apple orchards. The plume reach for both male and female captures was very small. The trapping areas were 4- to 7-fold

smaller for traps baited with a pheromone plus kairomone lure and deployed in mating disrupted apple orchards compared to traps baited with pheromone only in apple orchards not under pheromone mating disruption. The implications for monitoring of field populations include the need to have a sufficient number of traps to accurately assess population density, use of a treatment threshold of only a few adults captured per trap, and caution in interpreting catch as reduced dispersal of adults can mean that population densities are higher than anticipated.

CHAPTER SEVEN

Summary and Conclusions

The sterile insect technique and pheromone mating disruption for codling moth management are sister technologies that diverged in the 1980's. Both technologies successes and limitations have been documented by a number of researchers. The two management strategies can be successful at managing codling moth (*Cydia pomonella)* populations, but typically are deployed over large areas and require additional control measures to prevent increases in pest numbers. However, there have been few instances where these two control technologies have been intentionally combined on a farm-scale to enhance control of *C. pomonella*. The overall aim of this research was to 1) study how sterile *C. pomonella* farm-scale dispersion is impacted by conditions at release and different orchard systems under mating disruption, 2) understand how sterile moths disperse in orchards with mating disruption, 3) study and develop on-farm management schemes for integrating the two technologies, and 4) develop information needed for management decisions based on moth catch in monitoring traps deployed in orchards under mating disruption.

My initial studies investigated the dispersal and recapture of sterile *C. pomonella* released under several different conditions in orchards with mating disruption. I compared releases at a single central location with releases spread evenly throughout the orchard and found that aggregation and recapture was lower when moths were spread evenly. More single point-released moths were likely retained in target blocks and they quickly distributed themselves throughout the 4.05ha orchards used in these studies. Current commercial sterile *C. pomonella* release programs in Washington State and British Columbia release moths using expensive specialized equipment such as drones and modified all-terrain vehicles fitted with complex release devices. Recapture of *C. pomonella* adults released from an unmanned aerial vehicle

(UAV) at any altitude up to 35m above the ground, spread uniformly or from a single point, was higher than when moths were released by hand. However, hand release was shown to be an adequate low-tech alternative that has many advantages. A UAV requires specialized licenses to operate, is expensive, prone to crashes, and unable to fly in a variety of conditions, and release by hand does not suffer from any of these limitations. These studies demonstrate that a single central release by hand in a 4.05ha apple orchard is sufficient to allow sterile codling moths to self-disperse throughout the orchard. These findings formed the foundation of the use of this release method for all subsequent studies described in this dissertation.

The results of experiments comparing the influence of orchard architectures, topography, and types of shade netting on sterile moth dispersal and recapture showed that the characteristics of the orchard are an important factor in moth dispersal. Orchard shade net was shown to reduce recapture and increase aggregation of released sterile *C. pomonella* compared to similar blocks without netting. Tree trellising was also found to influence the dispersion and recapture of sterile moths. Blocks with trellis were shown to be highly conducive to single point central release, with low aggregation and high recapture compared to blocks with standard planted free-standing single trees trained using the central leader system. An experiment was also conducted to address anecdotal in-field observations indicating that hills and orchard slopes play a role in monitoring trap capture and pest distribution - it is believed that *C. pomonella* aggregate in uphill orchard locations. To address the possibility that in orchards with hills a central release would be disadvantageous, dispersion, recapture, and direction of catch was compared between orchards with steep slopes and flat orchards. It was found that sterile moths disperse uniformly in all directions from the central release point and do not exhibit uphill or

downhill preference, further strengthening the use of this method across orchard types. Taken together, these findings provide further support for the observation that sterile moths quickly and uniformly self-disperse throughout farm-scale release areas.

An experiment directly assessing the impacts of synthetic pheromone on sterile moth recapture and dispersion was conducted in orchards employing passive emitting and active emitting pheromone dispensers compared with pheromone-free orchards. These are the two most common types of pheromone mating disruption found in Washington apple orchards. Also, the influence of monitoring traps on sterile codling moth dispersal in these three scenarios was explored. Monitoring traps employed a pheromone/kairomone combination lure that allowed sterile males and females to perceive traps even when otherwise disrupted by orchard pheromone treatments. This monitoring trap lure facilitated quantification of dispersal and recapture in these test plots. The results of this study demonstrated that sterile male recapture was lower in blocks with both types of mating disruption than in blocks without pheromone, and that aggregation was slightly higher when traps were deployed at the time of release compared to when they were delayed 48 hours. Sterile female moths performed similarly to males, but at lower recapture rates across all treatments. It was also found that for both males and females in no-pheromone and active emitting dispenser plots, when trap placement was delayed, higher numbers of moths were captured in traps at the farthest distance than in plots with passive emitting pheromone dispensers. This study further demonstrated the rapid and effectively uniform dispersal of released sterile *C. pomonella* - capture was similar across orchard quadrants by treatment for both sexes. Contrary to previous studies of moth dispersal in orchards with active-emitting pheromone dispensers by McGhee (2014), male moths did not cluster near

pheromone sources when they were allowed to disperse and respond to traps for seven days. The extended trapping period used in this study compared to McGhee (2014) demonstrated that although sterile moths may individually interact with synthetic pheromone sources in the orchard, the overall population dispersion follows predictable patterns. The population randomly disperses from the point of release in all directions resulting in an effectively uniform population distribution. The findings of all the experiments detailed in this dissertation corroborate these observations, but further information regarding capture in monitoring traps was needed to understand the sterile moths' interactions with the pheromone/kairomone lure employed throughout these studies.

The sterile *C. pomonella* release density currently employed by the government-run sterile release program of British Columbia and adopted by the commercial release program in Washington State is 2000 sterile moths per hectare for the full 16-22 week growing season. Using the simple central hand-release method, release densities of 0/ha, 500/ha, and 1000/ha, were compared with 2000/ha in small on-farm plots to measure impacts of varying release densities on fruit damage and native population captures. All farm plots were under pheromone mating disruption and organic management and had experienced several years of high *C. pomonella* damage and capture in monitoring traps previous to experimental releases of sterile moths. In addition to varying release density, moths were released at 2000/ha for the six weeks coinciding with predicted emergences of first, second, or first and second generations and compared with season-long releases of 2000/ha. Across all plots fruit damage was low and differences in crop loss could not be discerned. However, increasing release densities resulted in increased recapture of sterile *C. pomonella*, suggesting that it is possible to flood areas of high pest pressure

with large numbers of sterile moths to effect control. Capture of wild moths in monitoring traps decreased over time in all release plots with the greatest declines found in those receiving 1000/ha and 2000/ha over the entire season. In addition, it was shown that sterile females play an important role in reducing wild populations, likely due to competition with wild females for the attention of wild males. These findings demonstrate that coupling mating disruption with sterile *C. pomonella* release is possible and increases the overall load of disruption in the orchard, resulting in greater control than when they are not combined.

Finally, an experiment to estimate male and female codling moth's dispersive distances, plume reaches of the pheromone/kairomone lure, and trapping areas, was conducted to estimate population densities of wild moth capture in monitoring traps per trapping area in orchards under mating disruption. Dispersive distances for 95% of the released males and females were found to be about 100m and 130m respectively in orchards with mating disruption, less than half of the dispersive distance found for males in orchards without mating disruption from previous studies by Adams et al. (2017). The plume reach of the pheromone/kairomone lure was found to be very small and resulted in trapping areas of ca. 5ha for females and ca. 3ha for males, less than ¼ the trapping area found in orchards without mating disruption by Adams et al. (2017). These findings indicate that in orchards with mating disruption, *C. pomonella* movement is greatly impacted by pheromone treatment, and that monitoring traps may need to be placed at higher densities to accurately measure populations. The impact of mating disruption is clear, and as designed, it interferes with males' normal mate-finding activities. Population density estimates calculated based on moth capture in pheromone/kairomone-baited monitoring traps in orchards with mating disruption, showed that captures of small numbers of

males or females correspond with large numbers of moths within the trapping area, and could result in substantial crop losses.

The above detailed investigations show that the sister technologies of pheromone mating disruption and sterile insect release are highly compatible for use in Washington State apple orchards on a single farm scale. Releases of sterile *C. pomonella* at several densities in orchards with mating disruption resulted in reductions in wild moth captures over time, indicating that even small numbers of sterile moths, combined with mating disruption, may have positive impacts on codling moth management. It has also been demonstrated that the use of specialized equipment that distribute moths uniformly throughout the orchard is not necessary; they dispersed themselves throughout the 4ha release areas despite variability in orchard structures or pheromone mating disruption. The studies presented in this dissertation provide a solid framework for integrating the sterile insect technique into integrated pest management programs that also employ pheromone mating disruption, and provides the information needed to make informed management decisions based on monitoring trap data from orchards under mating disruption.

It is my hope that the findings presented here will be adopted and practiced by farmers and farm managers. These studies should aid in administration of IPM programs that combine mating disruption and the sterile insect technique and help inform decisions made from monitoring trap results. Hopefully these studies result in better management procedures that result in greater control of *C. pomonella*, reduced need for pesticides and more sustainable farming practices.

APPENDICES

APPENDIX A

Photographs

PHOTOGRAPHS

Figure A.1. Photograph of UAS in flight

Figure A.2. Photograph of side by side netted orchards near George, WA used in experiment 1 of Chapter 3. Net on right is 6.1m high and net on left is 2.4m high.

Figure A.3. Photograph of the inside of 2.4m high net near George, WA.

Figure A.4. Photograph of the inside of 6.1m high net near George, WA.

Figure A.5. Photograph of approximate height traps were hung in trees and typical orchard with standard planted large stand-alone trees used in experiment 2 of Chapter 3.

Figure A.6. Photograph of typical "V-trellised" orchard in Brewster, WA used in experiment 2 of Chapter 3.

Figure A.7. Photograph of typical "Vertical Trellised" orchard used in experiment 2 of Chapter 3. The section of orchard pictured had a slope of 14° and was used in experiment 3 of Chapter 3.

APPENDIX B

Record of Deposition of Voucher Specimens

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: 2021-02

Author and Title of thesis:

Robert T Curtiss III

Factors Influencing Sterile Codling Moth (*Cydia pomonella* L.) Recapture, Dispersion, and Effectiveness as a Control Tactic in Apple Orchard Systems

Museum(s) where deposited:

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

Table A.1. Record of voucher specimens

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Tortricidae	*Cydia pomonella*	adult	5 Male	pinned
Tortricidae	*Cydia pomonella*	adult	5 Female	pinned

LITERATURE CITED

LITERATURE CITED

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