SAN JOSE SCALE MATING DISRUPTION IN APPLES

By

Jessika Maas

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ABSTRACT

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Recently San Jose scale (*Quadraspidiotus perniciosus* Comstock) has reemerged as a critical pest within Michigan apple orchards. San Jose scale is an excellent candidate for the development of pheromone-mediated mating disruption as an alternative strategy to insecticides, as it is a weak flyer, and the sex-pheromone is known and has been in use for decades as a monitoring tool. The response of male San Jose scale to increasing densities of ISOMATE® dispensers primed with San Jose scale sex pheromone was utilized to determine the mechanism of mating disruption for this pest in a two-year study. Dispensers were deployed in naturally infested apple orchard plots at six different rates from 0 to 926 per ha. The dispenser density experiment revealed that San Jose scale exhibits competitive mating disruption, as evidenced by the curvilinear decrease in the number of males caught in monitoring traps with increasing dispenser densities. In a separate efficacy experiment, two rates of the ISOMATE® dispenser were compared to one rate of CIDETRAK® dispensers and an untreated control. There was no statistical difference among dispenser rates or types, but all performed significantly better than the control. However, an assessment of their pheromone release rate over time revealed significant differences in their output levels throughout the season. This study also revealed that the first flight of male San Jose scale in southwestern Michigan occurred after petal fall in both 2020 and 2021. Further research is needed to determine how best to incorporate mating disruption into current apple pest management programs, but this work demonstrates the viability of pheromone-mediated mating disruption as a pest management tool for San Jose scale. It is anticipated that this research will enable manufacturers of mating disruption tools to start the process of registration for commercial use.

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CHAPTER 1: Literature Review of *Quadraspidiotus perniciosus*

San Jose scale (*Quadraspidiotus perniciosus* Comstock, 1831, (Hemiptera: Diaspididae)) is an important pest within the United States apple, peach, and plum industries. *Quadraspidiotus perniciosus* has been a pest of concern for at least 150 years, since it arrived in California from China in the late 1870s and spread to the east coast through nursery trade (Marlatt 1906). *Q. perniciosus* poses a unique risk due to its small size that allows infestations to go unnoticed until this scale insect becomes a major pest (Gentile and Summers 1958).

Throughout the United States *Q. perniciosus* has become a common pest within many tree fruit cropping systems, however with in the northern United States it has become a common pest within apples. *Q. perniciosus* can affect apple trees by their feeding behaviors on surfaces of the tree. The most noticeable signs of an infestation can appear when *Q. perniciosus* feeds on the fruit of an apple tree. The identifying characteristics of *Q. perniciosus* apple damage is a halo-like red discoloration around a settled crawler, this halo can increase in size as the crawlers continue to grow and can merge with nearby crawler damage (Gentile and Summers 1958). Damage on the apple typically occurs around the calyx and the stem, however if populations become highly abundant scales can be found on the sides of the apple (Richards 1962). Damage on mature apple trees typically occur on the woody tissue. The damage can be noticed in the cortex with patches of reddish tissue filled with sap, this can result in bark damage, or die-back of the limb or tree (Gentile and Summers 1958). With damaged fruit becoming unmarketable and tree die-back this pest can feasibly cause large amounts of economic damage.

An important and unique aspect of *Q. perniciosus* pest management is related to the fundamental biology of scale insects. *Q. perniciosus* is an armored scale, covered with a waxy cuticle for much of its life cycle (Marlatt 1906). Due to its waxy cuticle the insect is protected from external chemical interactions, making chemical control difficult during this stage. Over-wintering immature scale have a black waxy cuticle, known as the black cap phase, which protects them from harsh conditions while they await warmer temperatures to finish developing (Mague and Reissig 1983). Once they emerge in late April to mid-May, the 0.6 mm winged males can be identified by their yellowish coloring and characteristic black band stretching horizontally across their thorax (Marlatt 1906, Gentile and Summers 1958). Mature females are 1 mm and apterous, remaining sedentary during the mating process (Marlatt 1906). Males are weakened fliers and tend to walk instead of flying to find a viable female (Gentile and Summers 1958). Once they have discovered a female, they climb on to the female and insert their stylus under the female's pygidial region (Gentile and Summers 1958). Once the females have mated, they can produce around four hundred live nymphs, known as crawlers, over the span of 6 weeks (Marlatt 1906). The crawlers can be identified by their characteristic oval bodies with yellow-orange coloring. Previous research indicates that crawlers are the stage most likely to disperse and cause new infestations, as they can be transported between trees and orchards by birds, humans, wind, and insects (Gentile and Summers 1958). About 26 hours after they emerge, crawlers settle to feed on the cortex layer of the woody tissue of the tree or on ripening fruit. Once the scale has had a chance to establish, it will begin to secrete a white waxy coating that will become its protective scale (Marlatt 1906, Gentile and Summers 1958). After eighteen days, male and female scale can be differentiated by an elongated protective covering (Marlatt 1906). During this stage males grow legs, antennae, and wings, while females continue to grow a molt (Marlatt 1906). Twenty-six days after birth the adult males emerge from the protective scale and are ready to fly to a mate (Marlatt 1906).

In addition to its cuticular "armor," *Q. perniciosus* has overlapping generations and highly clumped distribution which add to the challenge of chemical control for this pest. In the late 1800s, lime-sulfur applications were used as an effective control method for *Q. perniciosus* in apple orchards (Marlatt 1906). However, *Q. perniciosus* was the first insect to have documented resistance to lime-sulfur in 1908 (Melander 1914). Although insecticide resistance is a serious obstacle to effective chemical control of *Q. perniciosus*, aspects of its own biology compound this effect. *Q. perniciosus* often grow on branches in extremely high density, with younger scale settling under the caps of dead/older scale. This layering effect can decrease the potency of insecticide applications and complicates the pest management of many scale insects (Downing and Logan 1977). In the 1940s the use of DDT was able to provide ample control for *Q. perniciosus* until it was banned in the 1970s (Spector 1975). Later, a combination of horticultural oils and organophosphate insecticides became the common pest management strategy.

In 1985 it was recommended that growers with a low infestation could apply oils when the apple blossoms were still closed (Reissig et al. 1985). However, growers with high infestations were recommended to use pre-bloom oils sprays as well as a post-bloom insecticide treatment to target the crawler stage (Madsen and Morgan 1970). For heavily infested orchards it was advised there be two post-bloom sprays: when crawlers were first detected and then again two weeks later (Madsen and Morgan 1970). The use of a phenological model helped time sprays for pre and post bloom applications in the western U.S. (Jorgensen 1981). This model was later validated for western New York apple orchards by Mague and Reissig (1983) and used to recommend timing of sprays against crawlers (Reissig et al. 1985).

Precise timing of a spring oil application has been critical to the success of this strategy since spraying too close to bloom could cause the flowers to drop their petals, causing a reduction in fruit (DeOng 1926). The organophosphate chlorpyrifos was typically mixed with the horticultural oils to increase control of *Q. perniciosus*. Chlorpyrifos is an acetyl-cholinesterase (AChE) inhibitor that has been used to control pests in many crops for over fifty years (Buzzetti et al. 2016). In 1994, field caught *Q. perniciosus* were starting to exhibit resistance to chlorpyrifos (Rice and Jones 1996). By 2015 the apple industry in Chile was seeing control failures for *Q. perniciosus*, and researchers found that field populations of *Q. perniciosus* were showing upwards of 31-fold resistance (Buzzetti et al. 2016). This discovery prompted researchers to assess different management methods for *Q. perniciosus*. In the meantime, the U.S. Environmental Protection Agency has banned the use of chlorpyrifos starting in February 2022 (https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos).

With increased resistance and restrictions on insecticides, researchers have turned to non-insecticidal strategies to control *Q. perniciosus* including mating disruption. Mating disruption has been successful in controlling other key tree fruit pests including *Cydia pomonella, Grapholita molesta, Aonidiella aurantia*, etc. (Vacas et al. 2009, Miller et al. 2010, Witzgall et al. 2010). Mating disruption involves the manipulation of the chemical ecology of insect pests which utilize pheromone to locate mates; synthetic sex pheromone is deployed into a cropping area to disrupt the ability of male pests to successfully locate female mating partners (Gut et al. 2019).

Mating disruption is a mathematically complex pest management tool, which functions according to two primary mechanisms: competitive and non-competitive disruption (Miller and Gut 2015). Competitive disruption occurs when the male's ability to find a female is inhibited, while not being completely repressed. There are three main modes of competitive disruption: 1) competitive attraction where dispensers compete with females calling for males (Gut et al. 2019), 2) induced allopatry, when the dispenser draws a male away from a female (Miller and Gut 2015), and 3) when males have induced arrestment (Miller et al. 2006). Non-competitive mating disruption happens when the dispensers put out enough pheromone to incapacitate the males and keep them from finding a female (Gut et al. 2019). There are multiple ways that non-competitive mating disruption may interfere with the male's ability to find a mate. The most prevalent way is induced allochrony, where the deployment of pheromone dispensers exhausts males compelled to continuously search for a female (Miller and Gut 2015). The other way is by desensitization, where there is so much pheromone in the area that it maxes out the male receptors to a female's call (Miller and Gut 2015).

However, the use of mating disruption has its challenges. Mating disruption can only become a viable option for growers to use if it is economically comparable to other control methods (Gut et al. 2019). Making mating disruption economically competitive with other forms of control can be difficult because the cost of the pheromone is high. Synthetic pheromones are manufactured in small batches which then can create a low supply when the demand is high (Gut et al. 2019). When implementing a mating disruption program behavioral and environmental factors need to be considered (Gut et al. 2019). For mating disruption to be effective, crucial biological traits would need to be known about the pest with the most important being the male response to the pheromone (Gut et al. 2019). Environmental factors can also play a significant role in the success of a mating disruption program. The environmental conditions within a mating disruption plot could impact pheromone stability, release rate, and movement (Gut et al. 2019). One of the major environmental factors to consider is the amount of UV light that a dispenser can endure (Weatherston 1990). Heat and UV light can break down the chemicals within a dispenser faster than dispensers not exposed to those factors (Weatherston 1990).

It is economically important to investigate the effects that mating disruption has on *Q. perniciosus* due to its economic importance as a pest of apple and the cost of current management tools. Since males fly to females emitting pheromones, Q. perniciosus is the perfect candidate for mating disruption. The sex pheromone for Q. perniciosus was isolated in the 1970s and identified as 7-methyl-3-methylene-7-octen-1-yl propanoate, (Z)-3,7-dimethyl-2,7-octadien-1-yl propanoate (Anderson et al. 1979, Gieselmann et al. 1979). In recent years, the San Jose sex pheromone has been put into dispensers to evaluate the possibility of its use as a control method. Vacas et al. (2009) demonstrated that pheromone mating disruption could be applied for the successful control of *Aonidiella aurantia*, another scale insect pest of tree fruit, with comparable control to conventional insecticides. This discovery was intrinsic in the planning of this thesis, that mating disruption was possible for scale insects.

This thesis assesses mating disruption for *Q. perniciosus* as a pest management tool within Michigan apple orchards. The project first examined the dose response of the pest by conducting an experiment to determine the optimum deployment density and the mechanism of disruption. This experiment was conducted by setting up a randomized complete block design while deploying six levels of dispensers loaded with the San Jose sex pheromone within a 0.08 ha block in four different orchards at one site located in Allegan County over two years. The level of disruption was assessed by the number of adult males caught in two pheromone baited v-traps centrally placed within each plot. The mating disruption mechanism was determined by comparing the mean of males caught within the traps from each orchard at each level of dispenser density. This was compared to the untransformed graph in the Miller 2015 paper, which exemplified the curves that would show competitive vs. non-competitive disruption.

This project also investigated the difference between two dispenser types. The twin-tube ISOMATE® (ShinEtsu, Inc., Tokyo, Japan) dispensers were compared to CIDETRAK® (Trécé, Inc., Adair, OK, USA) meso dispensers within six, 0.2 ha blocks located in three different sites. The sites were in the Leelanau, Eaton, and Allegan counties. The number of adult *Q. perniciosus* males was recorded using four v-traps placed within the blocks. The control plots were used to compare the efficacy of each dispenser for suppressing male flight of *Q. perniciosus*. The pheromone release rates of the ISOMATE® and CIDETRAK® dispensers were compared over time after being deployed in an experimental orchard in Ingham County. Eight dispensers were brought in at 0, 7, 14, 21, 28, 42, 60, 90, and 120 days past deployment. The dispensers were returned to the lab to be run through a volatile capture, then the recovered volatiles were inserted into a gas chromatography machine to assess the levels of pheromone that remained. These levels were then plotted, and the release rate of both dispensers were compared to each other.

Finally, we report on the observed phenology of *Q. perniciosus* male flight as it relates to the degree-day model proposed by Jorgenson et al. (1981) and later validated by Mague and Reissig (1983) in western New York. We also report on the phenology of *Q. perniciosus* male flight as it relates to apple bloom and petal fall in southwestern Michigan.

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CHAPTER 2: San Jose scale mating disruption in apples

Introduction

San Jose scale (*Quadraspidiotus perniciosus* Comstock, 1831, (Hemiptera: Diaspididae)) feeds on a variety of fruit and nut crops and woody ornamental plants and was first discovered in the United States in the late 1800s (Gentile and Summers 1958). This pest is now commonly found in orchards across North America and is notorious for being the first insect pest to be recorded developing resistance to an insecticide (Melander 1914). The economic impact of San Jose scale in tree fruit crops has ebbed and flowed with changes in the availability and efficacy of different insecticides (Reissig et al. 1985, Howitt 1993). A degree-day based model was developed to aide in timing control strategies based on work by Jorgensen et al. (1981) who established minimum and maximum developmental thresholds of 10.5 ˚C and 32.2 ˚C respectively, but there is evidently a wide range of accumulated degree-day associated with the observed first appearance of different life stages in the field which can make it tricky to use degree-day to time management strategies (Mague and Reissig 1983, Reissig et. al 1985).

Early season pest management of San Jose scale in apple orchards has primarily been tied to crop phenology. Petroleum oil alone or in combination with various insecticides were once widely recommended for controlling overwintered scale when applied at the ½-in green stage (Stafford and Summers 1963, Rice 1974, Dinabandoo and Bhalla 1975, Reissig et al. 1985) however, it has become difficult to time an oil-based spray so as not to cause crop injury. The first male flight is another key timing for control with an insecticide application, but this timing commonly overlaps with apple bloom (Mague and Reissig 1983) when pollinators are present. The third key timing is about 4-6 weeks later to target crawlers using either direct scouting for the pest

(Madsen and Morgan 1970) or timed according to the degree-day based forecast model (Reissig et al. 1985).

In the northeastern United States, San Jose scale can complete two generations (Mague and Reissig 1983). They overwinter as immature scales that resume development with sap flow in spring once temperatures reach 10 ˚C (Gentile and Summers 1958). Males emerge and start flying short distances (up to 22 m upwind) to find immobile females for mating during apple bloom (Mague and Reissig 1983, Howitt 1993). Females can produce around 400 live young during their 6-week lifespan (Gentile and Summers 1958, Khan et al. 2018). Dispersal of the pest is primarily through the crawler stage via wind or when they hitchhike on vertebrates interacting with infested trees (Gentile and Summers 1958). As the crawlers settle and develop into the scale form, they use their piercing-sucking mouthparts to penetrate the plant tissue and feed on sap (Khan et al. 2018, Howitt 1993). Large populations can increase tree susceptibility to early decline (Gentile and Summers 1958). The second flight begins in late summer. This generation of crawlers settle and develop protective scales for overwintering. A single application of insecticide targeting second generation crawlers is often insufficient for their control since they are active for around six weeks and emerge at different intervals (Downing and Logan 1977). When populations are high, to control this pest, growers may need to make multiple insecticide applications that can be costly and harmful to the environment. Thus, more sustainable management options are needed for the control of this insect as a pest in orchards.

For insect pests that rely on pheromones to attract and find mates, pheromone-mediated mating disruption has been used successfully to control key crop pests by deploying their known sex pheromone thereby disrupts a male's ability to find a female (as reviewed by Miller and Gut 2015). The San Jose scale sex pheromone (7-methyl-3-methylene-7-octen-1-yl propanoate, (Z)-

3,7-dimethyl-2,7-octadien-1-yl propanoate) has been identified (Anderson et al. 1979, Gieselmann et al. 1979) and used for years to monitor male San Jose scale population levels (Rice 1974, Rice and Hoyt 1980, Mague and Reissig 1983) and for setting a biofix for the forecasting model (Reissig et al. 1985). Since they are weak fliers and thus slow dispersers, pheromone-mediated mating disruption could be a viable alternative to applying a foliar insecticide for managing this pest in orchards. To develop this strategy, the pheromone needs to be deployed in a biologically meaningful way at the correct rate and density.

In this study, we evaluated the viability of pheromone-mediated mating disruption as a control strategy for San Jose scale in apple orchards. Here we describe the results of a dose-response experiment to determine the mechanism of San Jose scale mating disruption. We also report on the results of an efficacy study comparing three different mating disruption density treatments considered for commercial use in apple production. We also measured the release rates of the experimental dispensers used in this study and report on the phenology of male San Jose scale as it relates to accumulated degree-day base 10 ˚C from 1 January and apple bloom in Michigan.

Materials and Methods

Study sites. The dispenser rate study was conducted in established (20+ year-old), semi-dwarf, central leader style, experimental apple orchards with a history of San Jose scale infestation at the Michigan State University Trevor Nichols Research Center (TNRC) (42.5946, -86.1554) in 2020 and 2021 (Figure. 2.1). This study was replicated 4 times each year in Red Delicious and Jonamac plots; individual plots were 0.08 ha (Figure. 2.2A).

In 2021, the efficacy of different types of pheromone dispensers and deployment rates were tested in six established (20+ year old), semi-dwarf, central leader style, apple orchard plots (0.2 ha). Two plots were at TNRC (Red Chief or Smoothie), three at a commercial farm in Eaton County (42.6347, -84.7891; Gala, Golden Delicious, Honeycrisp, Ida Red, McIntosh, Paula Red, Zestar), and one at a commercial farm in Leelanau County (45.026336, -85.697641; Gala, Ginger Gold, Honeycrisp). These sites were selected based on their history of San Jose scale infestation (Figure. 2.1). Plots within each rep at the Eaton County site were oriented so that they contained the same cultivars. All plots were between 1 and 10 m apart (Figure. 2.2B). All orchards were

Figure 2.1. Map of Sites. A map of Michigan with apple orchard field sites indicated by black dots. The dispenser density experiments were conducted at site 2 in 2020-2021 and the dispenser type study was conducted at sites 1-3 in 2021.

managed using a grower standard pest management program but insecticides with known efficacy against San Jose scale were excluded.

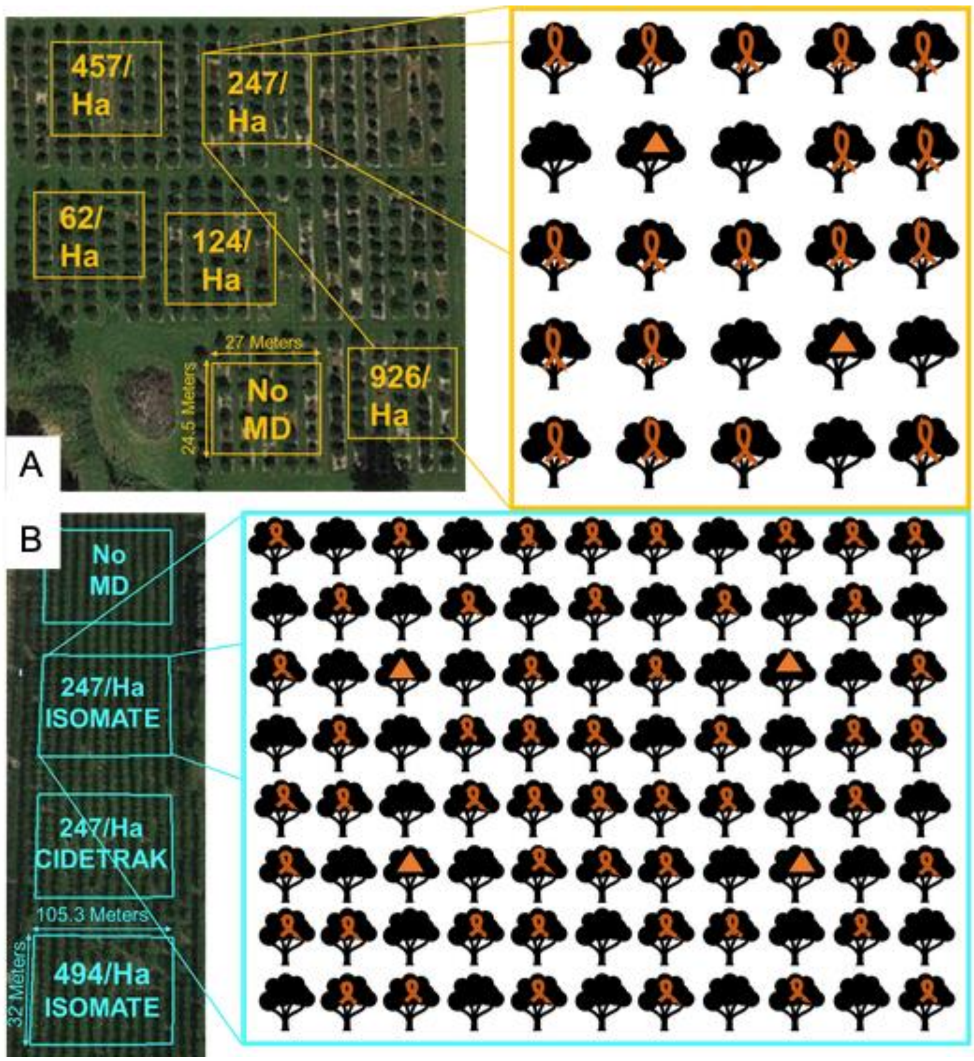

Figure 2.2. Experimental Plot Diagram. Diagram of experimental plots in apple orchards to assess different pheromone dispenser densities (A) and types (B) on San Jose scale mating disruption. Plots were 1-10 m apart with about 30 (A) and 140 (B) trees per plot. Each plot was assigned a different dispenser treatment, blocks were replicated 4-times (A) and 6-times (B). Red twists indicate dispensers and orange triangles indicated traps.

Dispenser density study. To develop a dose response curve, we compared the number of San Jose scale males caught in traps deployed in plots with increasing point sources of polyethylene ISOMATE® twin-tube reservoir dispensers containing 60 mg of San Jose scale pheromone (7-methyl-3-methylene-7-octen-1-yl propanoate; ShinEtsu, Inc., Tokyo, Japan), which according to the manufacturer were expected to emit pheromone for up to 200 days. Dispensers were attached to tree branches in the upper canopy at rates of 0, 62, 124, 247, 457, and 926 per ha and deployed on 18 May in 2020 and on 28 April in 2021. Plots with different treatments were at least 1-10 m apart and plots without dispensers (no mating disruption control) were placed upwind from all other treatments. Plots with the two highest dispenser rates were shifted within each block in 2021 so that they did not overlap with plots used in the previous season. We used data collected in the no MD plots to relate the phenology of male San Jose scale to degree-day accumulations in Michigan using the MSU Enviroweather data on demand tool (https://mawn.geo.msu.edu/).

Dispenser type study. Three different potential commercial mating disruption treatments were compared to a no mating disruption control for their relative ability to reduce the number of males caught in traps. Plots were assigned the following dispenser density and dispenser type treatments with the no mating disruption treatment placed upwind from the others: untreated control (no mating disruption), 247 ISOMATE® dispensers, 494 ISOMATE® dispensers, or 247 CIDETRAK® MESO dispensers containing 252 mg of pheromone (Trécé, Inc., Adair, OK, USA). CIDETRAK® dispensers were hung in trees with a clip and expected to emit pheromone for up to 180 days. Since the Leelanau site was not large enough to fit all 4 treatments, the CIDETRAK® lure was not tested at this location.

To compare the relative ability of males to locate females in plots under different mating disruption treatments, PHEROCON® V traps baited with a PHEROCON® (Trécé, Inc.) San Jose Scale lure was used to monitor male activity. Traps were replaced every 1-2 weeks, lures were replaced every 6 weeks, beginning in May 2020 and April 2021. Two traps were deployed in each

0.08 ha plot; four traps were deployed in each 0.2 ha plot (Figure 2.2A, B). Trap inserts were examined under a microscope and the total number of San Jose scale males per trap was recorded.

Dispenser aging study. To evaluate the pheromone release rate of the ISOMATE® twin-tube and CIDETRAK® meso dispensers, we deployed 64 dispensers of each type in an apple orchard at the Michigan State University Entomology research farm in Lansing, MI on 5 May 2021. Dispensers were hung on each apple tree down 5 rows to simulate the weathering process in a commercial orchard. Eight dispensers were placed in the freezer (-20°C) 0, 7, 14, 21, 28, 42, 60, 90, and 120 days post deployment.

Volatiles from dispensers were collected in sealed 1 L Teflon chambers (Jensen, Coral Springs, Florida) with two 0.64 cm diameter ports in their lids. Air was purified by passing it through a charcoal filter before entering the collection chambers at a rate of 958 ml/min through one port via Teflon tubing. Air exited at the other port through Super Q adsorbent trap (25 mg, Alltech Assoc., Deerfield, IL). Volatiles were collected from 8 samples simultaneously. Containers were cleaned between samples with acetone three times and heated at 120 ˚C for 2 hr.

For each field-aged timepoint, 8 replications of both types of dispensers were analyzed. Dispensers were removed from the freezer and allowed to equilibrate at room temperature in a fume hood for 1 hr. Individual pheromone dispensers were suspended on a metal wire inside a Teflon container and its pheromone emission was collected for 2 hr. Trapped pheromone was eluted from Super Q trap with 150 µl hexane under a gentle nitrogen stream. Methyl myristate (5 µg in hexane) was added as an internal standard into each sample, of which 1 µl was analyzed by capillary gas chromatography (Hewlett-Packard HP6890 equipped with a Hewlett Packard 7863 autosampler and an HP-Innowax polyethylene glycol column (30 m × 250 µm i.d., 0.25 µm film thickness)) with a split-less injector at 250 ˚C and flame ionization detector at 300 ˚C). Following injection, column temperature was held at 50 ˚C for 5 min, increased at 25 ˚C/min to 155 ˚C and held for 5 min; then increased at 0.5 ˚C /min to 165 ˚C and held for 3 min; finally increased at 30 ˚C/min to 225 ˚C and held for 2 min. Helium was used as a carrier gas at a flow rate of 1.1 ml/min. The pheromone released by each dispenser type was identified based on its retention time compared to that of the synthetic reference compound (7-Methyl-3-methylene-7-octen-1-yl-propanoate; Shi-Etsu Chemical Co., Japan) and quantified by comparing its peak area with that of the internal standard.

Statistical analysis. To compare the seasonal abundance using growing degree days base 10.5 °C of San Jose scale males, we calculated the average number of insects per trap in the plots without mating disruption in 2020 and 2021. To determine the efficacy of different rates of dispensers on mating disruption we fit our data with generalized linear mixed models with negative binomial link function (‘glmmTMB,’ R Core Team, 2021). The mean number of males caught in traps was used as the dependent variable and dispenser rates were used as the independent variable, plot and date were random effects. Each year of this two-year study was analyzed separately due to the differing start dates as well as the differences in scale population pressure. A post hoc Tukey’s test was used to determine differences among treatments (‘emmeans’).

To determine the efficacy of different dispenser types, the mean number of scale males caught in traps was analyzed with a generalized linear mixed model with negative binomial link function, site, and date as random effects (‘glmmTMB’). A post hoc Tukey’s test was used to determine differences among treatments (‘emmeans’).

Pheromone release rates over time were analyzed with a generalized linear mixed model (‘glmmTMB’) using maximum likelihood estimation. Dispenser type and the number of days in the field were fixed effects and the amount of pheromone collected during the volatile capture process was the dependent variable. A post hoc Tukey's test was used to compare the effect of dispenser type on the amount of pheromone released over time.

Results

San Jose scale seasonal abundance. From data collected in plots without mating disruption, seasonal activity of San Jose scale males was lower in 2020 than in 2021 with 39% more males caught in traps in 2021 (Figure 2.3). We detected two temporally discrete peaks in activity in 2021, whereas in 2020, there were few scales first flight, and the second flight was much more drawn out over time (Figure 2.3). Peak timings for male flight, however, aligned between the two years when plotted against accumulated degree-days, with the first flight occurring between 140 - 195 degree-days (base 10.5 ˚C from 1 January) and the second flight occurring between 594-1030 degree-days. Phenology was delayed in 2020 relative to 2021 resulting in later

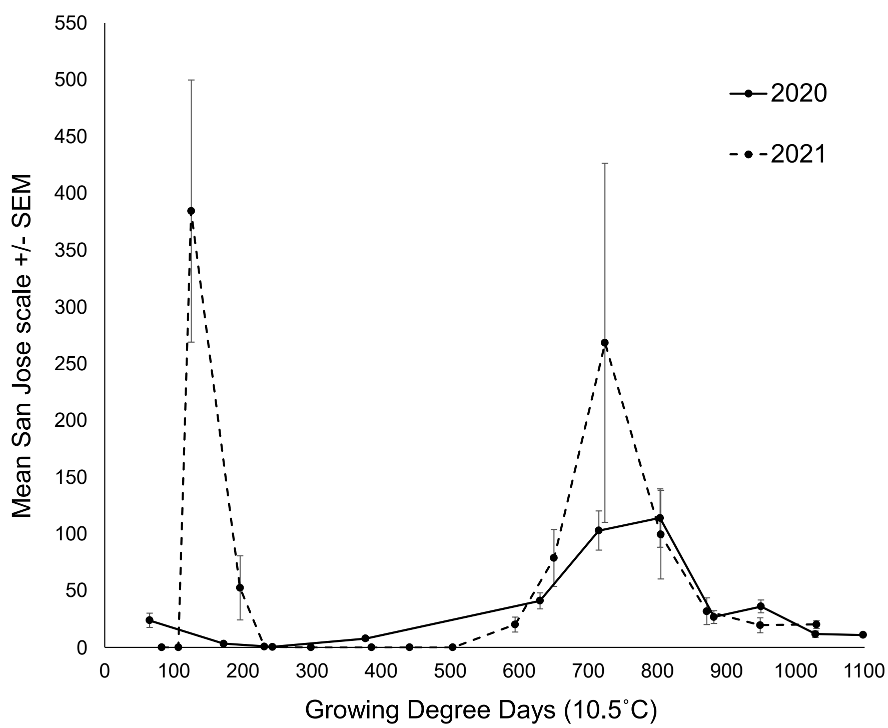

Figure 2.3. Seasonal Abundance of San Jose scale. The mean seasonal abundance of San Jose scale males in traps in plots without mating disruption treatments in an experimental apple orchard (Site 2) in 2020 (black line) and 2021 (dashed line).

bloom and petal fall dates. Full bloom in Red Delicious blocks was recorded on 18 May 2020 and 3 May 2021. Petal fall was recorded on 26 May 2020 and 17 May 2021. This means that the observed first flight of male San Jose scale occurred sometime between 27 May – 1 June in 2020, and between 19 May and 24 May in 2021, a time frame immediately following petal fall each year.

Dispenser density study. In 2020, there was a significant difference in the number of males in traps among the different dispenser densities ($\chi^2 = 430.47$; df = 9, 5; $p < 0.01$; Figure 2.4A). The mean number of San Jose scale was significantly different across all treatments ($t > 2.63$; df = 519; $p<0.01$; Figure 2.4A). There were 83% fewer males in plots with 247 dispensers/ha compared to the plots without mating disruption but there was only a 14% reduction in males between 247 dispensers per hectare and 926 dispensers per hectare. In 2021, there was a significant difference in male catch across all treatments ($\chi^2 = 375.90$; df = 9, 5; $p < 0.01$) and there was a significant difference across all treatments ($t > 1.99$; df = 759; $p < 0.01$; Figure. 4B). There was an 85% reduction in the mean number of males per trap in the 247 dispensers per hectare treatment

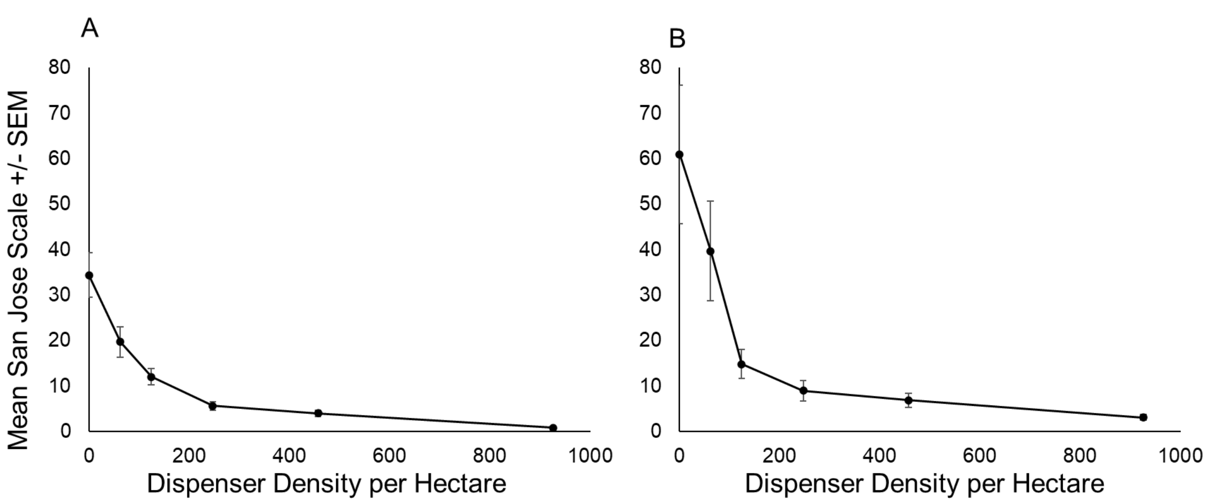

Figure 2.4. **Comparison of Dispenser Densities in 2020 and 2021.** Mean San Jose scale males per trap (+/- SEM) in 2020 (A) and 2021 (B) at increasing dispenser density starting at 0 to 926 dispensers per hectare. Different letters above dots indicate statistically different means ($p < 0.05$).

and a 95% reduction in the 926 dispensers per hectare compared to the no mating disruption plots.

Dispenser type study. There was a significant difference in the numbers of males per trap across the treatments with different dispensers (χ^2 = 31.78; df= 3; $p < 0.01$; Figure. 2.5). There was a > 80% reduction in the average number of males per trap in plots with all three of the dispenser treatments compared to plots without mating disruption (t > 4.34; df = 1531; $p < 0.01$). There was no significant difference between the mating disruption dispenser types or densities (t = 0.47; df = 1531; p = 0.77). There was an 82% difference between no mating disruption and CIDETRAK® (t = 0.45; df = 1534; p <0.01).

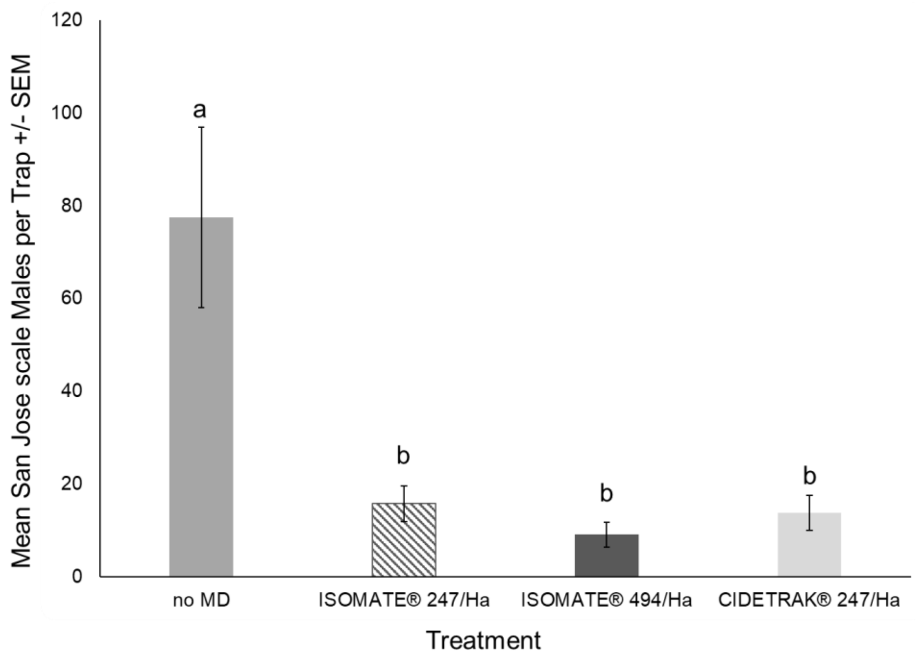

Figure 2.5. Comparison of High and Low Densities and Types of Dispensers. The effect of pheromone dispenser types and rates on San Jose scale males per trap (+/- SEM) in Michigan apple orchards in 2021 (MD = mating disruption). Different letters above bars indicate statistically different means ($p < 0.05$).

Dispenser aging study. When comparing the amount of pheromone released over time, there was a significant difference between the ISOMATE® and CIDETRAK® dispensers (χ^2 = 8.75; df= 1; $p < 0.01$; Figure 2.6). After 120 days, there was a 76% decrease in the amount of pheromone released for the ISOMATE® dispenser in contrast with a 98% decrease in the amount of pheromone released by the CIDETRAK® dispenser. At day 0, pheromone release from CIDETRAK® started nearly 100% higher than for the ISOMATE®. After 14 days in the field, the amount of pheromone released dropped by 78% in the CIDETRAK® dispensers compared with a drop of 48% in the

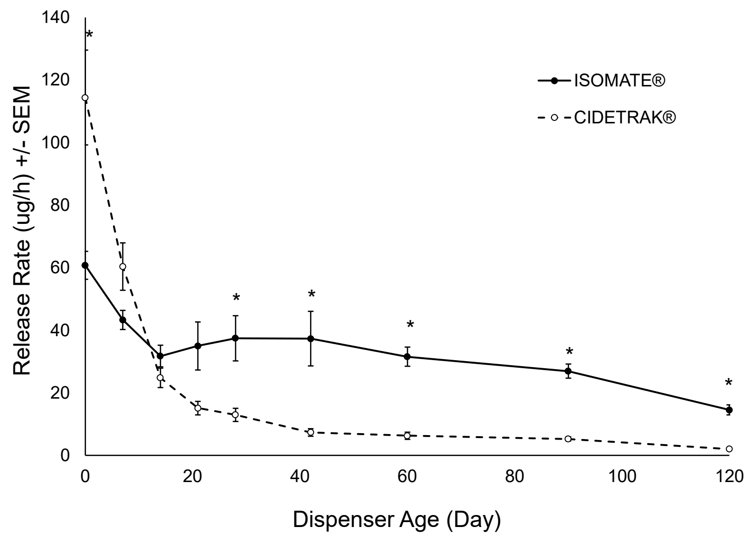

Figure 2.6. Pheromone Release Rate of Two Dispenser Types. Comparison of release rates for two pheromone dispensers collected over time from an experimental apple orchard in Michigan in 2021. A "*" indicate statistically different means with a $p < 0.001$.

ISOMATE® dispensers (Figure 2.6).

Discussion

Mating disruption is a viable strategy for managing San Jose scale in apple orchards. More specifically, per hectare dispenser density of 247 provides effective mating disruption. Increasing dispenser density did not increase control. We also found that the ISOMATE® dispensers sustain higher release rates for longer compared to the CIDETRAK® dispensers.

After two growing seasons, the dispenser density experiment revealed that San Jose scale exhibits a competitive mating disruption response, as evidenced by the curvilinear decrease in the number of males caught in monitoring traps with increasing dispenser densities (Miller and Gut

2015). These conclusions are promising when adopting this as a pest management strategy, especially given that other studies utilizing mating disruption for a similar species, California red scale (*Aonidiella aurantia*), demonstrated comparable results (Vacas et al. 2010, Grafton-Cardwell et al. 2021).

Deploying pheromone dispensers can be time intensive and expensive. Different lure types and numbers of point sources that could reduce installment time would be beneficial to apple growers for reducing the cost and potential barriers to implementation (Farrar et al. 2018). We were able to compare experimental dispensers from two different manufacturers for their ability to suppress male flight of San Jose scale. We found that both dispenser types easily disrupted San Jose scale at the population levels in our study. There was a difference, however, in dispenser handling time. With the same number of units deployed per area, the CIDETRAK® dispenser required more manual labor due to the larger size of the dispenser and the handling time of the plastic hook for placing it on a tree branch. The commercial grower we worked with also asked us to remove them at the end of the season to reduce plastic debris in the orchard. Conversely, the ISOMATE® twin-tube dispenser would be difficult to retrieve at the end of the season because it is small and difficult to relocate. These practical implications should be the focus of future studies to better align the use of this technology with the circumstances of apple production.

To further understand how both dispensers will interact within the environment (Gut et al. 2019), the pheromone release rates of the ISOMATE® twin-tube and CIDETRAK® meso dispensers used in this study were compared. We found that the CIDETRAK® dispensers released more pheromone at the beginning of the season before quickly declining, whereas the ISOMATE® twin-tube dispenser release rate decreased more gradually over the season. Our results indicated that San Jose scale is sensitive to these dispensers, and given the cost of producing synthetic pheromones, it might be possible to load dispensers with less pheromone and still achieve effective mating disruption for this pest.

The Predictive Extension Timing Estimator (PETE) model (Jorgensen 1981) for predicting San Jose scale phenology was developed in the western US and predicts first male flight starting at ca. 112 degree-day (base 10.5 ˚C from 1 March), but Mague and Reissig (1983) reported a range in first flight ca. 94-140 degree-days in New York apple orchards. These timings overlapped with bloom in the cultivars that they were studying. Our observations placed peak 1st flight emergence between 140-195 degree-days, which is later than those of Mague and Reissig (1983) and resulted in the peak flight occurring immediately after petal fall. Whether this is a shift in the phenological development of this pest in the Great Lakes Region over time, or if the population in Michigan could be characterized as a separate ecotype is not clear. Predicting the first male flight is important for both timing the deployment of pheromone mating disruption, and for timing strategies against other life stages of this pest. Given the increasing variability with respect to weather in spring due to climate change, it will be important to collect new phenological records, not just in apple, but in other important fruit crops impacted by this pest will facilitate reevaluation and refinement of forecasting models (Kistner et al. 2018).

In conclusion, we determined that pheromone-mediated mating disruption of San Jose scale is a viable management strategy. We will need to examine in future studies the economic viability of San Jose scale mating disruption compared to conventional control methods (Gut et al. 2019). Since San Jose scale does not have an action threshold (Reissig et al. 1985), it is difficult to compare its cost to other management methods and to develop recommendations for commercial apple production. However, our results provide a first step in transitioning research to application. We expect that as restrictions on effective pesticides continue, there will be greater incentive for using biorational alternatives and this will be reflected in the development and registration of these types of products for commercial sale. There is already an estimated 1 million hectares of crops that utilize mating disruption as a viable control alternative (Gut et al. 2019). While many pests controlled by mating disruption are lepidopteran species, Vacas et al. (2010) has demonstrated that mating disruption worked for California red scale. Future research should explore different options for deployment technologies that could result in significant cost savings. Along with further refinements to the dispensers, establishing an economic threshold for San Jose scale in apples will be necessary to compare the efficacy of mating disruption to current insecticide spray programs. It would also be ecologically interesting to explore how using mating disruption for a sedentary insect like San Jose scale might affect its parasitoid community (Shapira et al. 2018) and if females are able to overcome mating disruption by adopting new calling strategies (Kuhns et al. 2012). We will also need to assess if this strategy is effective in other apple growing areas where this pest is a problem outside of Michigan, as well as other tree fruit systems

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

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

Voucher Number: 2022-03

Author: Jessika Maas

Title of thesis: San Jose scale mating disruption in apples

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

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Diaspidiae	*Quadraspidiotus perniciosus*	adult males	10	alcohol
Diaspidiae	*Quadraspidiotus perniciosus*	larvae	10	alcohol
Diaspidiae	*Quadraspidiotus perniciosus*	cap	1 twig	pinned

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