ASSESSMENT OF GRANULOVIRUS, SPINOSAD, AND MATING DISRUPTION FOR REDUCING FRUIT DAMAGE FROM *CYDIA POMONELLA* L. (LEPIDOPERA: TORTRICIDAE) IN ORGANIC COASTAL CALIFORNIA APPLE ORCHARDS

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TITLE:	Assessment of Granulovirus, Spinosad, and Mating Disruption for Controlling *Cydia pomonella* L. (Lepidoptera: Tortricidae) in Organic Coastal California Apple Orchards
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ABSTRACT

Assessment of Granulovirus, Spinosad, and Mating Disruption for Controlling *Cydia pomonella* L. [Lepidoptera: Tortricidae] in Organic Coastal California Apple Orchards

Raven Marie Lukehart

Codling moth, *Cydia pomonella* [Lepidoptera: Tortricidae], is a major entomological pest of apples, pears, and walnuts cross the world (Pajac et al. 2016). Female codling moths lay eggs on the apple exocarp and larvae burrow within the fruit causing economic losses to fruit growers. Organic apple orchards in San Luis Obispo, CA currently have three codling moth, *Cydia pomonella,* control options commercially available including granulovirus (CpVG), spinosad, and mating disruption. In field tests on apple (*Malus*), we compare percent fruit injury between treatments of granulovirus (2.43 oz/ha Cyd-X® organically approved, Certis USA, Columbia MD), spinosad (4.05 oz/ha Entrust® Naturalyte® organically approved WP formation, Dow AgroSciences, Indianapolis IN), and a control. We also compared mating disruption in form of codling moth Codlemone® sex pheromone (257 ties/ha (506 mg)/acre Isomate®-OFM TT organically approved Pacific Biocontrol Corporation Vancouver, WA) against a control. Delta taps and 1 mg pheromone lures were used to trap males and track the degree day (DD) model for the two orchard's codling moth populations to determine application timing for each treatment. A preliminary DD model was used based on the University of California at Davis Agricultural Extension codling moth DD model.

During 2016 trials no detectible control was provided by spray treatments with an average fruit injury of 26% control, 23% granulovirus, 28% spinosad. During 2016 trials no detectible control was provided by mating disruption with an average fruit injury of 16% control and 16% pheromone. During 2017 trials there was detectible control provided by the treatments to the crop by both spray treatments and pheromone ties. 2017 average fruit injury for spray treatments was 51% control, 20% granulovirus, and 14% spinosad. 2017 average fruit injury for mating disruption was 29% control and 6% pheromone. Data suggest underlying relationship between location specific climate factors, cultivars, codling moth populations, and treatment efficacy.

Keywords: *Cydia pomonella*, codling moth, Lepidoptera, Tortricidae, granulovirus, pheromone disruption, malus, apples, organic, orchard, coastal California, and farm.

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CHAPTER 1

1. INTRODUCTION

1.1 Background

1.1.1 General Information

Consumer concern for health and safety of food and the environment is on the rise (Crowder and Regnold 2015, Klonsky 2012). Consumers could relieve their health and safety concerns by purchasing organic products (Crowder and Regnold 2015, Klonsky 2012). Organic growers have in recent years increased production to meet this growing consumer demand (Crowder and Regnold 2015). However, organic production is challenged by potential environmental threats such as pathogens, insect pests, plant and soil fertility, and weather, that can lead to failed or reduced crops (Kirchmann and Thorvaldsson 2000). Organic growers have limited choices of products to negate these potential environmental challenges and the products are often costlier and less effective (Klonsky 2012).

Cydia pomonella Linnaeus, codling moth, is a threatening entomological pest of apples, pears, and walnuts across the world (Arthurs, Lacey and Fritts 2005). Over wintering takes place in the pupal stage for codling moth, they emerge as adults in and around apple orchards during spring (Haseman and Johnson 1932). Mated females will lay eggs on the surface of fruit within just a few days of emergence (Haseman and Johnson 1932). Neonate larvae bore into the fruit calyx upon and develop internally leaving fruit damaged from feeding (Arthurs, Lacey and Miliczy 2007). Developed larvae exit the fruit, dropping to the orchard floor in search of debris to spin a cocoon, pupate, and finally reach adult stage (Haseman and Johnson 1932). Codling moth hibernaculum overwinter from November to April (Haseman and Johnson 1932). Weather

conditions at a location determine the rate/duration of these developmental processes (Lacey, et al. 2008). For example, the cooler Pacific Northwest states see two to three generations per year (Beers, et al. 1993), whereas the warmer Pacific Southwest states like California see three to four generations (Caprile and Vossen 2016). Conventional insecticides used to treat this pest have seen increased resistance and decreased efficacy due to their identical modes of action (Lacey, et al. 2008). New U.S. Environmental Protection Agency chemical use restrictions have been developed to control resistance and environmental toxicity (Arthurs, Lacey and Miliczy 2007, Brunner, et al. 2002, Lacey, et al. 2008). Resistance management requires new insecticide options with lower selective pressure along with diversification of modes of actions (Lacey, et al. 2008). Organic insecticides for codling moth are limited to a rotation of spinosad and codling moth granulovirus (CpGV), applied with horticultural oils (Wunderlich, et al. 2015, McGhee, Epstein and Gut 2009). Non-insecticidal control methods include codling moth mating disruption, resistant cultivars, cultural sanitation remedies, and area wide management plans (McGhee, Epstein and Gut 2009, Wunderlich, et al. 2015). Efficacy of the organic materials, in controlling codling moth, have been limited (Arthurs, Lacey and Miliczy 2007). Mating disruption, horticultural oils, resistant cultivars, cultural sanitation remedies, and area wide management plans are potential replacements for broad-spectrum insecticides with high levels of resistance, negative environmental impacts, and low applicator safety (Lacey, et al. 2008).

An integrated pest management (IPM) approach has been adopted by many growers for reducing inputs, involving a combination of several control measures as part of a long-term prevention plan (Fadamiro, Ciborowski and Hock 2003). Control measures include techniques such as biological control, habitat manipulation, modification of cultural practices, and use of resistant varieties (Fadamiro, Ciborowski and Hock 2003). Insecticides are used only after

monitoring indicates pest populations have met established guidelines such as action thresholds and targeted life stages based on insect development indicators such as degree days (DD) and pheromone trap captures (Lamichhane, et al. 2016, Coft and Riedl 1991, Gleason 2008).

1.1.2 Codling Moth Phonological Model

Glenn (1922) introduced the DD model for tracking codling moth development. Degree days are species specific developmental bench marks tracked by heat units calculated from daily maximum and minimum temperatures (Murray 2008). Developmental heat accumulation for codling moth is based on a lower threshold of 10 ºC and an upper threshold of 31.1ºC (Caprile and Vossen 2016). Temperature based DDs are widely used to determine the phenological model tracking codling moth developmental life stages, such as adult flights, egg eclosion, larval activity, and subsequent generations (Glenn 1922, Rock and Shaffer 1983).

Biofix is the date of the first sustained capture of three to five adult male moths, marking the start of the phonological model for the growing season (Glenn 1922). Treatment periods are based on estimations of important life stages based on trap captures and DD (Rock, Childers and Kirk 1978). Aligning spray intervals to specific developmental periods can significantly reduce the number of sprays needed (Rock, Childers and Kirk 1978). Emergence of the codling moth larvae from eggs is the most important threshold for determining spray application timing for this pest (Arthurs, Lacey and Miliczy 2007). Emergence occurs at 250 DD for the first generation and 1250 DD for the second generation; remaining generations typically occur after fruit is harvested (Caprile and Vossen 2016). An area wide pest management plan has been adopted by major apple growing regions involving using similar materials applied at specific developmental benchmarks, seasonally alternating modes of actions, and using IPM (Calkins and Senft 1995, Caprile and Vossen 2016).

1.1.3 Codling Moth Pest Management in Coastal California

No area wide IPM control program for Coastal California apple growers exists at this time. Management for codling moth in apples is usually achieved by area wide IPM control program in a long term plan against the codling moth population in the region with the objective of reducing the insect population to the economic threshold (Glenn 1922, Gleason 2008). Area wide IPM includes application of chemical or organic pesticides, use of biological controls, resistance management, as well as use of cultural practices on a calendar-based schedule or on a phonological based schedule (Glenn 1922, Gleason 2008). Developing an area wide plan for the Coastal California growers would assist in minimizing costs, since profit margins for fresh apples are narrow (Rosenberger, Engle and Meyer 1996).

1.1.4 Damage Potential by Codling Moth

Female codling moth adults lay their eggs on the fruit and upon eclosion the neonate larvae burrow into the fruit creating openings in the exocarp, rendering the fruit unmarketable (Calkins and Senft 1995, Lacey, et al. 2008). Codling moth has the potential to reduce marketable apples by 100 % of total production when left untreated (Iraqui and M'hamed 2016, Caprile and Vossen 2016). Apple and pear growers in the western United States spray nearly 907,185 kilograms (2 million pounds) of insecticide annually to treat for codling moth and other insect pests (Lacey, et al. 2008); there are no statistics available to determine what percentage of the total is solely for codling moth control.

Conventional growers suppress codling moth populations using broad-spectrum insecticides such organophosphates (Arthurs, Lacey and Miliczy 2007, Gleason 2008). Widespread use of organophosphates has reduced crop damage, while creating a variety of secondary problems such as negative environmental effects, insecticide resistance, outbreaks of

secondary pest outbreaks due to disruption of natural enemies, decreased safety of pesticide applicators, and increased food safety concerns (Arthurs, Lacey and Miliczy 2007, Tumbler 1998, Lacey, et al. 2008, Gleason 2008). Alternative pesticide options are needed to reduce these negative impacts.

Codling moth ganulovirus, spinosad, and codling moth mating disruption are three potential alternatives to organophosphates and are National Organic Program (NOP) allowable materials for organic growers (USDA 2017, Caprile and Vossen 2016). Granulovirus is a naturally occurring and species-specific virus formulated in microencapsulated protein occlusion bodies (OB) for application (Arthurs, Lacey and Fritts 2005). Granulovirus has little to no impact on beneficial organisms, while it has the potential to depress codling moth populations, reducing amounts of non-marketable fruit (Lacey, et al. 2008, Falcon and Hyber 1991). Spinosad is a naturally occurring soil bacterium with a broad host range and a moderate potential to harm beneficial insects, including honey bees and other pollinators (Rabea, Badawy and Nasr 2010). Spinosad has been shown to reduce codling moth populations and fruit damage caused by codling moth larvae in apples (Arthurs, Lacey and Miliczy 2007). Mating disruption in the form of female sex pheromone, Codlemone®, has been shown to reduce codling moth populations, is species specific, and has no effect on beneficial organisms (Sumedrea, et al. 2015). Efficacy of these organic materials in controlling codling moth varies from 2 to 90% reduction in fruit damage (Arthurs, Lacey and Miliczy 2007).

1.1.5 Granulovirus

Granulovirus [family *Baculoviridae*, genus *Granulovirus*] is a microbial pesticide for codling moth control (Arthurs, Lacey and Fritts 2005). Granulovirus has minimal impacts on the environment and beneficial insects, while also being safe for applicators and food crops (Arthurs,

Lacey and Fritts 2005). Granulovirus must be applied to coincide with eclosion of codling moth larvae from the egg (Arthurs, Lacey and Fritts 2005). Peak eclosion periods for treatment are determined using the codling moth phenological model in conjunction with monitoring pheromone traps (Arthurs, Lacey and Fritts 2005). Codling moth neonate larvae ingest CpGV OB (granules) applied during egg eclosion before initial entry into fruit (Arthurs, Lacey and Miliczy 2007). Granules dissolve in the alkaline midgut releasing virions (Arthurs, Lacey and Fritts 2005). Virions establish a transient infection breaking down the gut wall and allowing for virion movement into the tracheal matrix, epidermis, fat body, and other tissues (Lacey, et al. 2008). Over a span of 5-10 days infection causes cell lysis and eventually host death (Arthurs, Lacey and Fritts 2005).

Commercial preparations of CpGV 9 X 10^{11} occlusion bodies (OB)/oz (Cyd-X, certis USA, Columbia MD) (Arthurs, Lacey and Miliczy 2007). CpGV is applied in a full season program as multiple applications of 220 mL Cyd-x/ha (Arthurs, Lacey and Fritts 2005). Treatments are timed to coincide with codling moth egg eclosion for each generation (Arthurs et al., 2005, 2007). Generations are determined using a biofix and phenology model based on DD accumulation (Beers, et al. 1993). Two to four applications at 7-14 d intervals could be needed to provide residual control until roughly 95% eclosion (Arthurs, Lacey and Miliczy 2007). Application methods and rates are a major factor for how effective CpGV will be at depressing codling moth populations (Arthurs, Lacey and Fritts 2005, Arthurs, Lacey and Miliczy 2007).

Level of codling moth population control depends on dosage, frequency, timing of virus applications, and initial codling moth infestation level (Lacey, et al. 2008). Application rates of 4.6 x 10^{12} to 10^{13} Occlusion Bodies CpGV/ha have been shown to provide the best codling moth control when timed with the first codling moth generation egg eclosion (Lacey, et al. 2008).

Lacey et al. (2008) also found reduced rates of 10^7 Occlusion Bodies with increased frequency of application reduced fruit infestation to 0-42% per tree.

In one study, Granulovirus did not initially reduce fruit injury compared to the controls but did show potential to depress codling moth populations eventually reducing amounts of damaged fruit (Lacey, et al. 2008). Arthurs et al. (2007) found that CpGV treated trees sustained rates of fruit damage similar to untreated controls, but eradicated or injured a majority (67-71%) of neonate codling moth larvae. There was between 5-37% fruit injury in untreated plots, while CpGV treatments had 2-27% fruit injury. When measuring fruit injury there were some outliers, one factor explaining these outliers would be variation in application timing and rates (Arthurs et al. 2005; Arthurs et al. 2007). When compared with the codling moth insecticide spinosad (0-2% fruit injury), CpGV (2-27% fruit injury) offered limited protection. While fruit damage was not detectibly reduced by CpGV, high larval mortality with 81-98% larvae death within fruit, reducing mature larvae 54-98% was noted (Arthurs, Lacey and Fritts 2005, Granger, Brunner and Doerr 2003).

1.1.6 Spinosad

Spinosad is a highly selective neural toxin insecticide derived from the bacterial species *Saccharopolyspora spinose* sp. (*Actinomycetales*: *Pseudonocardiaceae*) (Hertlein, et al. 2011). Insects could ingest or come into contact with spinosad causing excitation of the nervous system leading to paralysis, impeding feeding, then mortality (Hertlein, et al. 2011). Low levels of cross resistance were found between spinosad and neonicotinoid and organophosphate pesticides in other species such as Colorado potato beetle, Leptinotarsa decemlineata (Say), and oriental fruit fly, Bactrocera dorsalis (Hendel), studies (Monta-Sanchez, et al. 2005, Hsu and Feng 2006). Commercial preperations of Spinosad (Entrust® Naturalyte® organically approved WP

formation, Dow AgroSciences, Indianapolis IN) is not known to cause codling moth cross resistance to any other known insecticides (Sparks, Crouse and Durst 2001). Codling moth populations have shown low levels of resistance to spinosad after repeated treatments during season long treatment studies leading to lower dosage label recommendations (Arthurs, Lacey and Fritts 2005). Spinosad is broad spectrum and can affect a variety of insects including Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, and Hymenoptera (Sparks, Dripps, et al. 2012). Disruption of biological predators can increase secondary pests (Arthurs, Lacey and Miliczy 2007). Van Steenwyke et al. (2005) documented elimination of the parasitoid *Trixoys pallidus* (Haliday) (Hymenoptera: Aphidiidae), an introduced parasitoid of walnut aphid, *Chromaphis juglandicola* (Kaltenbach) (Hemiptera: Sternorrhyncha), but no others. An increase in *Epitrimerus pyri* (Nalepa) (Acari: Eriophyidae) and *Cacopsylla pyricola* (Förster) (Homoptera: Psyllidae) was also reported following spinosad treatment (Van Steenwyk and Nomoto 2006).

Spinosad degrades quickly with little to no residual insecticidal activity 3-7 days after application (Williams, et al. 2004). Spinosad should be reapplied every 7-10 days, until =90% egg eclosion (Arthurs, Lacey and Miliczy 2007). Due to restrictions for resistance management, spinosad applications should be limited to 630 g/ha/season (Arthurs, Lacey and Miliczy 2007). Spinosad has been shown to effectively reduce fruit damage as low as 1.7% and increase larval mortality rates by 82%, by eliminating neonate larvae before entry into fruit (Arthurs, Lacey and Miliczy 2007).

1.1.7 Mating Disruption

Codling moth and other lepidopteran insects use sex pheromones for olfactory mating communication (Witzgall, et al. 2008) where females produce a blend of volatile organic

molecules detected by males when locating females. Mate finding can be obstructed by permeating the atmosphere with species-specific synthetically produced codling moth pheromone, Codlemone®, thereby reducing the need for companion insecticide applications (Witzgall, et al. 2008). Mating disruption has been widely used among apple and pear growers and has been shown to be successful in decreasing fruit damage (Sumedrea, et al. 2015). Pheromone disruption techniques only work to prevent mating, having no effect on females that have already successfully mated and are able to fly into treated areas unaffected by the pheromone (Sumedrea, et al. 2015).

Fruit injury was reduced by 93% with insecticide applications dropping by 65% in pounds of organophosphate during implementation of a four-year, 3,238 hectare, area wide IPM program in Michigan, involving mating disruption and reduced use of insecticides, trap monitoring, and sanitation (McGhee, Epstein and Gut 2009). Pheromone additions were more cost effective annually than conventional insecticides after two years (McGhee, Epstein and Gut 2009). Pheromone test plots only had 4 to 0.001% damaged fruit when combined with insecticides (Sumedrea, et al. 2015).

CHAPTER 2

2. MATERIALS AND METHODS

2.1 Field Sites

Apple orchards participating in this study were located in Avila Beach and See Canyon, two coastal California apple growing regions of San Luis Obispo, CA. Participating orchards were selected based on the following primary criteria: previously observed apple injury due to codling moth and little to no control materials (insecticides) applied for their control. The study included two orchards, San Luis Creek (Creek Side) and Gopher Glen. A pre-study assessing 45 kilograms of fruit was used to detect codling moth fruit injury occurring in participating orchards. Both farms tested positive for traceable levels of codling moth infestation during 2015 (Table 1).

Table 1. 2015 Codling moth fruit injury pre-treatment study results in two San Luis Obispo County apple orchards.

		San Luis Obispo County	*California*	
Farm	**Year**	**Sample (N)**	**Mean % Fruit Injury**	**Std Dev**
Gopher Glen	2015	12	24.71	± 0.10
San Luis Creek	2015	14	16.02	± 0.13

Average percent fruit injury of all harvested fruit was 24.71% for Gopher Glen orchard and 16.01 % for Creek Side orchard (Table 1), indicating suitability for the study. Other criteria included appropriate application equipment and materials on site, licensing, commercial apple production status, accessibility, and clearly defined borders around orchard plots.

Orchard studies were conducted in the 2016 and 2017 growing seasons. San Luis Creek and Gopher Glen orchards were originally planted in1971, with consistent replanting taking place until 2004. Both orchards utilize overhead sprinkler irrigation and were planted at 1,112 tree/ha spacing. Variation exists between plot sizes and apple cultivars (Table 2). Gopher Glen

totaled 5.67 hectares and Creek Side 10.52 hectares, with plots ranging from 0.81- 4.85 hectares (Table 1). Gopher Glen orchard was planted with high numbers of cultivars, thus, it was listed as 100+ cultivars in Table 1.

Table 2. Farm composition description of two San Luis Obispo County apple orchards used in study.

	San Luis Obispo County	*California*	
Farm	**Blocks**	**Cultivar (s)**	**Hectares**
Gopher Glen	Block 1-2	Mix 100+	4.05
	3 (control)	Golden Delicous	
		Arkansas Black	1.62
San Luis Creek	Freeway	Fuji	
		Jonagold	2.43
	Monte	Braeburn	2.43
	Gable (control)	Fuji	
		Empire	
		Granny Smith	
		Braeburn	4.85
	Heavensent (control)	Heavensent	.81

2.2 Detection Trapping

Pheromone baited Pherocon® (Trécé, Inc., Adair, OK USA) traps containing 1 mg Codlemone® longlife lures were hung in the upper 20% tree canopy each growing season to determine biofix (Figure 1) and to monitor the adult flights that indicate the start of a new generation. An average 5 males or more per week per tap marked the biofix (Caprile and Vossen 2016) (Figure 2). Lures were changed every 12 weeks and sticky trap liners were changed when liners became inundated with debris or lost adhesiveness. Traps were checked once a week in between flights and during major flight periods. Generations were tracked using the University of California Davis (UCD) Agricultural Extension phenology model, created by Caprile and Vossen (2016), that assumes lower and upper developmental thresholds of 10.0/31.1˚C , overwintering emergence at 250 Degree Days (DD), and generational flights at 1060 DD, 1100 DD, and 1200 DD. Generational flight determinations are based on historic flight and

temperature data (Caprile and Vossen 2016). Degree days were calculated using the UCD Agricultural Extension codling moth DD calculator and the temperature (ºF) readings from the San Luis Creek weather station, Pacific Gas and Electric, San Luis Obispo, CA. Spray periods occurred 250 DD after each generational flight (Arthurs, Lacey and Fritts 2005, Caprile and Vossen 2016). Treatments were reapplied one to four times to cover 90% egg eclosion periods from each flight. Reapplication was based on length of period for peak male trapping periods and label rates of treatment.

Figure 1. Trecé Pherocone® traps containing 1 mg Codlemone® lures and male codling moths trapped by the adhesive bottom.

Figure 2. Codling moth males captured in a monitoring trap showing the number trapped has reached the threshold for setting a biofix date.

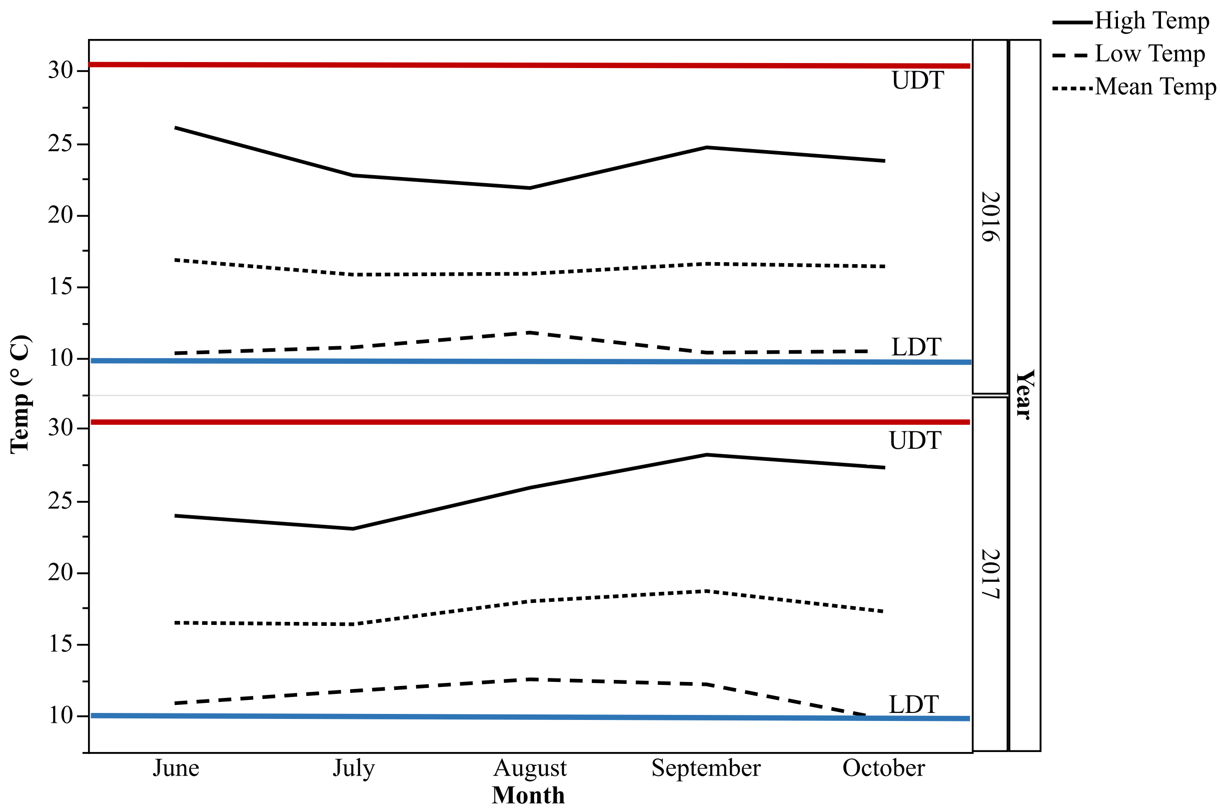

Figure 3. Average monthly high and low temperatures (C°) for 2016 – 2017 recorded at the San Luis Creek weather station, Pacific Gas and Electric Center, San Luis Obispo, CA. The red line indicates the estimated upper developmental threshold (UDT) of 31.1C° and the blue line indicates the lower developmental threshold (LDT) of 10C° for codling moth.

2.3 Codling Moth Trap Field Design

Pheromone traps in the Creek Side Freeway and Monte treatment plots were deployed in a grid format. The first two traps in the Freeway and Monte treatment plots were placed at the southern edge along the Gable control plot, with the last two traps placed on the northern border. San Luis Creek Freeway and Monte treatment plots had a total of six traps in a two by three grid. Grids were used to determine the level of male moth intrusion from Gable control plot into Freeway and Monte treatment plots. (Figure 4)

San Luis Creek Gable control plot had two pheromone traps placed within its boundary. Traps were set according to Trecé Pherocone® label recommendation of two traps per four hectares. The plot was divided into 2 ha sections with one pheromone trap placed in the center of each.

Gopher Glen orchard had one trap placed in the center of each treatment plot and two traps placed in the control plot. Traps within the control plot were set five rows and 120 meters apart. Gopher Glen orchard traps were placed at a higher than recommended rate, due to the variable topography of the orchard.

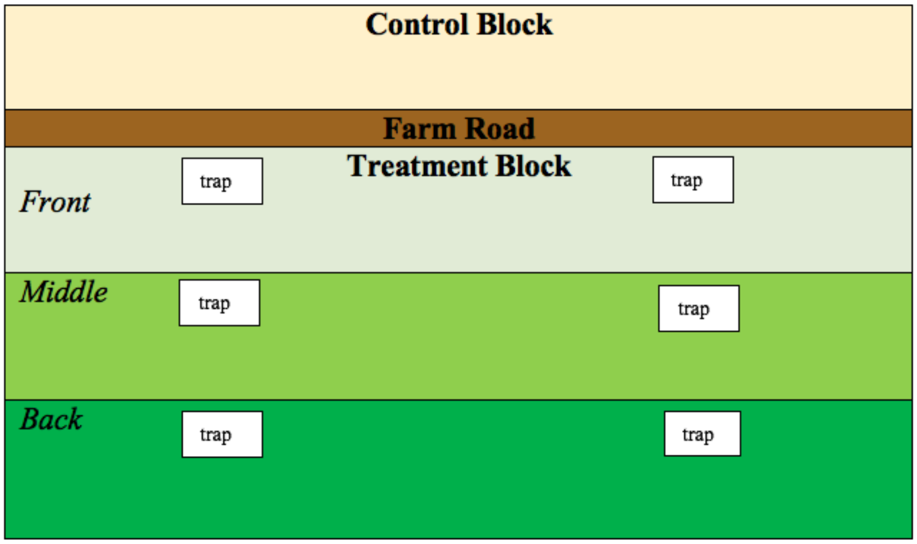

Figure 4. Trap location within the two treatment plots Monte and Freeway located at San Luis Creek Side bordering Gable control plot. Traps labeled front were placed the closest to the control on the south end of the plot, while traps labeled back were placed the farthest from the control while bordering the north end of the orchard.

2.4 Experimental Design

Commercial preparations of codling moth granulovirus containing 9 X 10^{11} occlusion bodies (OB)/oz, (Cyd-X® organically approved, Certis USA, Columbia MD) and spinosad (Entrust® Naturalyte® organically approved WP formulation, Dow AgroSciences, Indianapolis IN) were applied separately to the Creek Side Freeway and Monte plots in a full-season program for codling moth. Application rates were set at maximum label rates of 2.43 oz/ha (Cyd-X) or 4.05 oz/ha (Entrust).

Commercial pheromone ties (Isomate®-OFM TT organically approved Pacific Biocontrol Corporation Vancouver, WA) containing 506 mg codling moth female sex pheromone were applied to Gopher Glen Orchard treatment plots one and two at maximum label application rates of 247 ties/ha (Isomate).

2.5 Applications

2.5.1 Spray Applications

Granulovirus and spinosad treatments were timed to coincide with codling moth peak egg eclosion periods based upon the biofix date and a DD accumulation based phenology model (Caprile and Vossen 2016). The biofix date was identified using 1 mg pheromone traps placed in the tree canopy top 20% beginning in March and ending in November (Caprile and Vossen 2016). In 2016 and 2017, initial applications of granulovirus and spinosad were made at 250 DD post flight one and one to two further applications at 7 d intervals to provide residual control until 95% eclosion. Eclosion date was determined by summing 250 DD beginning on the first day of the flight. The second larval generation beginning at 1100 DD followed the same protocol, with two applications. The third generation at 1200 DD did not require an application, since flight captures were post fruit harvest (Caprile and Vossen 2016). Spray treatments were

applied late evening or early morning during calm wind conditions (< 0.5m/s) with a diesel powered 1000-l orchard blast sprayer (Jacto® Tualatin, OR). The sprayer was calibrated at 2,570 liters/ha at 0.89 mps to provide full coverage of leaves and fruit. All three plots were bordered by heavily wooded riparian areas or 30-m buffer zones, minimizing overspray or spray drift between treatments.

Monte plot received four commercial preparations of codling moth granulovirus containing 9×10^{11} OB/oz. Maximum label rates of 2.43 oz/ha CpGV was applied during each spray event. Applications of CpGV were applied 250 DD after each flight, in conjunction with egg eclosion events (Table 2). The first application was on 16 June 2016 and 30 June 2017 250 DD after the first flight, with a second application on 23 June 2016 and 7 July 2017, and a third application on 14 July 2017, seven days after the first application (Table 2). One to two applications were made after the second flight on 1 August 2016, 30 July 2017, and 7 August (Table 2).

Freeway plot received four commercial preparations of spinosad. Maximum label rates of 4.05 oz/ha spinosad were applied for each spray event. Applications of spinosad were applied 250 DD after each flight, in conjunction with egg eclosion events (Table 3). The first application occurred on 16 June 2016 and 30 June 2017, 250 DD after the first flight, with a second application on 23 June 2016 and 7 July 2017, and a third application on 14 July 2017, following a seven-day reapplication period (Table 3). The next round of applications occurred on 1 August 2016 and 30 July 2017, 250 DD after the second flight, with a final application on 7 August 2017 following the same 7 d reapplication interval (Table 3).

Table 3. Codling moth flight dates, spray application dates, and Degree Day (DD) accumulation during major events in 4 orchard plots located at San Luis Creek from 4 April 2016-4 September 2018.

	Date of biofix or Overwintering emergence	Date of 1st flight	DD accumulated since biofix	Spray application date for 1st flight	DD accumulated since biofix	Date of 2nd flight	DD accumulated since biofix	Spray application date for 2nd flight	DD accumulated since biofix	Date of 3rd flight	DD accumulated since biofix	Spray application date for 3rd flight
2016	4 Apr	22 May	500	16 June 23 June	750 840	11 July	1085	1 August	1265	27 July	1294	n/a
2017	4 Apr	5 June	645	30 June 7 July 14 July	895 1074 1137	10 July	1090	30 July 7 August	1335 1470	10 August	1515	n/a

2.5.2 Pheromone Tie Application

Commercial pheromone ties containing 506 mg codling moth female sex pheromone were applied to Gopher Glen Orchard plots one and two on 15 April 2016 and 2017. Ties were placed in the top 20% of tree canopy, on lateral branches. Application rates were 247 ties/ha (Isomate®). Treatments were timed to coincide with the first detection of male codling moth populations for the season, based on label recommendations. Reapplication occurred 90 days after the initial application to maintain a minimum of 58mg residual pheromone during the fruit bearing months of April-October. During both years, initial applications were made on April 15 (after first male capture of the season) and the second application was made on July 15. Plots one and two (4.05 hectares total), receiving Isomate® treatments, were located downwind from the un-treated control plot (1.62 hectares) minimizing drift of the pheromone.

The study was set up as a randomized complete plot design within each orchard. San Luis Creek Orchard contained one plot for CpGV (2.23 hectare/2,475 trees), one plot for spinosad (2.43 hectares/2,700 trees), and two plots untreated-control (0.81 hectares/900 trees). Gopher Glen Orchard had two plots mating disruption (4.05 hectares/4,500 trees) and one plot untreated-control (1.62 hectares/1,800 trees). There was some limitation in matching plot sizes due to natural buffers needed to delineate treatments.

2.6 Sampling and Analysis

2.6.1 Fruit Collection

One tree per every odd numbered row was randomly selected using a random number generator and marked for sampling during 2016. One tree per row was randomly selected using a random number generator and marked for sampling during 2017. Twenty-one to sixty-nine trees in total were selected for sampling from each plot. Marked trees were strip harvested during 2016 and all of the fruit was analyzed for codling moth damage. Twenty four apples were selected from the tree for sampling during 2017. Two apples were selected from a quadrant based on Cartesian coordinates and further divided into high and low canopy for a total of 24 apples per sample tree. Fruit samples were examined in the laboratory for codling moth larval entry and exit holes causing fruit injury (Figure 5). Mated female codling moths deposit eggs externally onto the apple exocarp. After egg eclosion, young larvae bored into the fruit creating punctures in the exocarp. Developed larvae exit the fruit after completing growth. Both entry and exit holes were deemed as fruit injury caused by codling moth. Fruit were dissected if visual inspections could not confidently determine codling moth-related fruit injury. Dissections revealed codling moth larval mortality or deep larval entries inside the fruit, if present (Figure 6).


Figure 5. Damage caused by codling moth larvae in apples.


Figure 6. Laboratory dissected apple revealing codling moth deep larval entry inside the fruit.

2.6.2 Data Collection

Data were collected for potential variables effecting codling moth fruit injury during sample collection and laboratory analysis. General information was collected such as orchard name, plot number, apple cultivar, date, treatment, weight, and tree location (row and number). From the 24 samples per tree, Additional information was collected from the 24 fruit samples per tree including number of fruit with codling moth fruit injury, number of fruit without codling moth injury, total kilograms of fruit harvested per sample, kilograms of marketable fruit per sample, kilograms of fruit injured by codling moth per sample, percent marketable fruit per sample, and percent codling moth fruit injury per sample. Percent data was calculated by dividing kilograms of marketable fruit or kilograms of fruit injured by codling moth by total kilograms of fruit harvested per sample for each tree.

2.6.3 Statistical Analysis

Data for experimental plots were analyzed using graphic comparisons and tabulated summary reports of ranges, maximum values, minimum values, means, and standard deviations for dependent variables. Analyses were run using JMP statistical software (SAS Institute, 2012). Statistical p and F values were not calculated due to the limitation of replication in this study. Participating growers applied one treatment to entire plots both years lacking replication within each orchard.

CHAPTER 3

3. RESULTS

3.1 Detection Trapping

2017 had detectibly more trap captures across all plots than 2016. Trap captures for 2017 averaged 2.0 ± 4.47 (n = 352; range 0 – 43), while 2016 trap captures averaged 1.23 ± 1.73 (n = 170; range 0 – 11).

There were three distinctive flights during this study, as indicated by male trap captures, for the each of the codling moth generations that occurred in 2016 and in 2017 (Figure 7). Average trap captures were 1.0 ± 1.73 (n = 170; range 0 – 11) capture per trap in 2016, averaging 0.4 ± 0.55 (n = 5; range 0 – 1) to 3.3 ± 2.58 (n = 14; range 0 – 11) captures during major flight events. 2017 had an average of 2.01 ± 4.47 (n = 352; range 0 – 43) captures per trap, averaging 4.8 ± 9.1 (n = 20; range 0 – 40) to 6.1 ± 5.46 (n =18; range 0 – 14) captures during peak flights. In 2016, average captures by week used to detect peak flights never reached the biofix threshold of an average of 5.0 captures per trap (Caprile and Vossen 2016), while 2017 average captures were above the biofix threshold during the first and second flights (Figure 7). The 2016 total captures for the season averaged 1.17 ± 1.73 (n =169; range 0 – 11) moths per trap, while 2017 averaged 2.0 moths ± 4.48 (n =350; range 0 – 43) per trap (Table 4). An initial biofix for flight one was not established due to the less than 5 moths/trap per week captures during 2016 peak flights. Spikes ranging 1-11 moth captures in the flight pattern were used to determine spray periods to account for the lower trap capture's in 2016. In 2016, individual trap captures over the span of the season varied between 0 and 11 while 2017 individual trap captures varied between 0 and 43 (Table 4). Variation between individual trap captures blocked by week were used to

detected peak flight periods for treatment. Individual trap captures peak at 0 to 43 captures during flights, followed by lag periods of 0 to 4 trap captures flights.

San Luis Creek Side

2016

Three generations were observed during the 2016 season (Figure 7). Overwintering moth emergence was observed on 4 April with 1.0 capture per trap (n = 1), the first generation was observed 22 May with 1.0 capture per trap (n = 1), the second generation was observed on 11 July with an average of 0.85 ± 1.75 (n = 14; range 0 – 6) captures per trap, and the third generation was observed 27 July with an average of 3.29 ± 2.58 (n = 14; range 0 – 11) captures per trap. Applications for flight one was not made since flight captures were below the biofix threshold of an average of 5.0 captures per trap at average (Caprile and Vossen 2016). Applications were applied to cover 90% egg eclosion from the second and third flights, even though flight captures were below the biofix threshold of an average of 5.0 captures per trap at (Caprile and Vossen 2016).

2017

Four separate flights or generations were observed during the 2017 season (Figure 7). Over wintering moth emergence was observed on 4 April with 5.0 captures per trap (n = 1), the first generation was observed on June 5 with an average of 7.0 ± 12.68 (n = 16; range 0 – 43) captures per trap, the second generation was observed on 10 July with an average of 5.43 ± 10.11 (n = 16; range 0 – 40) captures per trap, the third generation was observed on 10 August with an average of 2.85 ± 3.12 (n = 20; range 0 – 11) captures per trap, and the fourth generation was observed on 5 September with an average of 1.85 ± 1.98 (n = 20; range 0 – 6) captures per trap. Applications were applied to cover 90% egg eclosion from the first and second flights since

flight captures were above the biofix threshold of an average of 5.0 captures per trap (Caprile and Vossen 2016). Applications for flights three and four were not made since flights were below 5 moths/trap per week and flight four occurred post-harvest (Caprile and Vossen 2016).

Gopher Glen

2016-2017

The first captures of codling moth occurred between 1 April and 7 April for both years. Subsequent flights were not detected within the pheromone treatment plots, while peak flights in the control plot were observed on 22 May, 3 July, and 5 September. For the control, first flight peak averaged 12.0 ± 2.83 (n = 2; range 10 – 14) captures per trap, second flight peak averaged 13.5 ± 10.61 (n = 2; range 6 – 21) capture per trap, and third flight peak averaged 1.5 ± 0.71 (n = 2; range 1 – 2) captures per trap. Over all, control average trap captures were 3.21 ± 4.69 (n = 34; range 0 – 21) and treatment average trap captures were 0.35 ± 0.77 (n = 34; range 0 – 3). There were significant differences in trap catches between the control and the treatment plots ($t = -3.5$, $p = 0.001$) For both years mating disruption was applied twice, once in spring and once during summer to maintain a minimum of 58 mg ambient Codlemone®.

Table 4. A tabulation of trap captures of male codling moths caught weekly in pheromone traps in 4 San Luis Creek apple orchards between 27 March 2016 and 5 October 2017. 2017 trap captures averaged 36% more captures than 2016.

2016 *# Catches*				*2017* *# Catches*			
Mean	**Min**	**Max**	**Std Dev**	**Mean**	**Min**	**Max**	**Std Dev**
1.18	0	11	±1.73	2	0	43	±4.47

Number of Male Codling Moth Captures by Date: 2016-2017

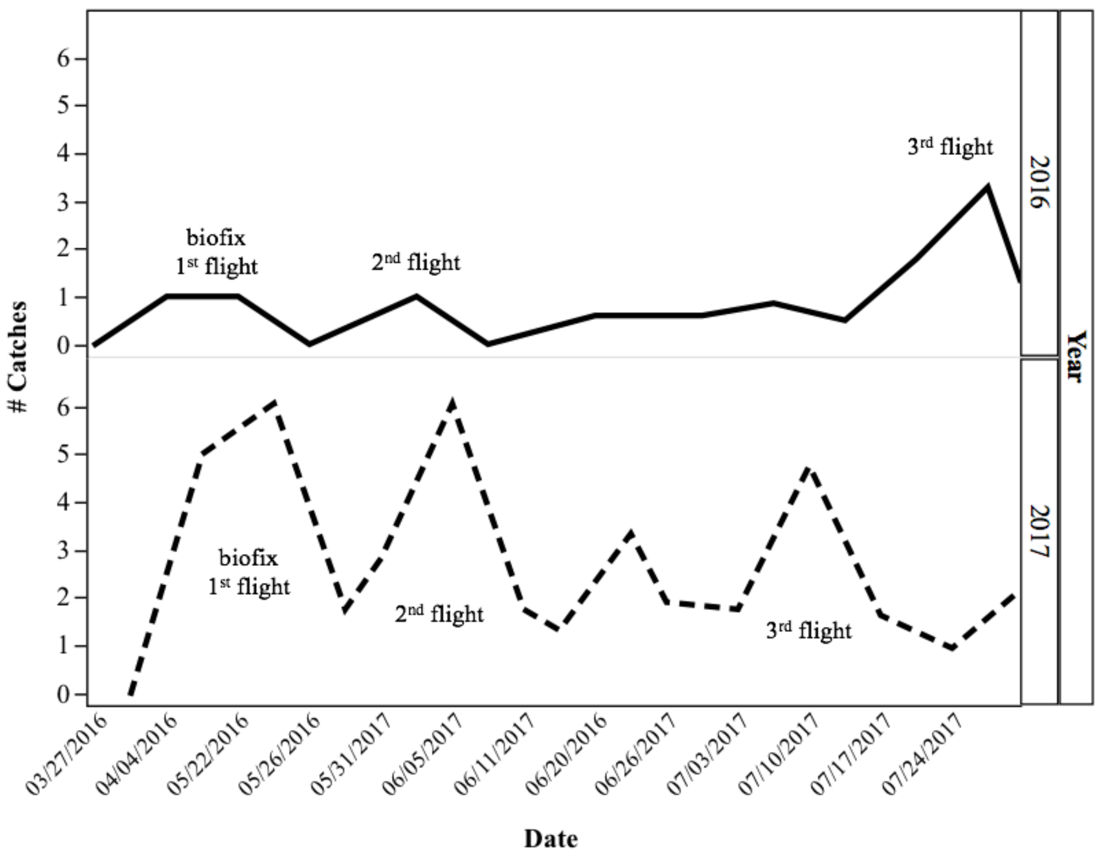

Figure 7. Mean number of trap captures of male codling moths caught weekly in pheromone traps in 5 apple orchards portraying moth flights between 27 March 2016 and July 24 2017.

3.2 Codling Moth Trap Field Design

San Luis Creek Side

2016

Average trap captures for each trap position within the treatment plots were 0.91 ± 1.39 (n = 53; range 0 – 6) for the front, 1.13 ± 1.73 (n = 46; range 0 – 9) for the middle, and 1.46 ± 2.2 (n = 46; range 0 – 11) for the back (Figure 4).

Average trap captures for each plot within the orchard were 1.29 ± 1.3 (n = 24; range 0 – 5) for control plots and 1.12 ± 1.8 (n = 146; range 0 – 11) for treatment plots.

2017

Average trap capture for each position within the treatment plots were 2.37 ± 4.74 (n = 89; range 0 – 33) for the front, 0.69 ± 1.32 (n = 72; range 0 – 6) for the middle, and 1.17 ± 1.88 (n = 89; range 0 – 10) for the back (Figure 4).

Average trap captures for the control and treatment plots were 3.44 (n = 66; range 0 – 43) for control plots and 1.63 (n = 216; range 0 – 33) for treatment plots.

Gopher Glen

2016-2017

By orientation within plot, average trap captures were 0.41 ± 0.94 (n = 17; range 0 – 3) for traps located the farthest from the control and 0.29 ± 0.59 (n = 17; range 0 – 2) for traps located nearest to the control.

By plot, control average trap captures were 3.21 ± 4.69 (n = 34; range 0 – 21) captures and treatment average trap captures were 0.35 ± 0.76 (n = 34; range 0 – 2).

3.3 Treatments

3.3.1 Spray Applications

Average percent fruit injury for each year including controls and treatments was 26.1 ± 0.19% (n = 37; range 9 – 100) for 2016 and 34.4 ± 0.28% (n = 135; range 0 – 100) for 2017.

Detectable differences between both years controls were measured, 2017 control averaged 44.95 ± 0.15% (n = 69; range 0 – 69.4), while the 2016 control averaged 26.7 ± 0.19% (n = 4; range 9 – 54).

2016

For 2016, percent fruit injury was determined by strip harvesting randomly selected trees and recording weight of fruit with codling moth fruit injury and fruit without. Average percent fruit injury for each treatment was 26.3 ± 0.19% (n = 4; range 9 – 54) control, 23.0 ± 0.13% (n= 14; range 9 – 52) granulovirus, and 28.3 ± 0.22% (n = 19; range 9 – 100) spinosad (Figure 8).

2017

During 2017, percent fruit injury was determined by harvesting samples from randomly selected trees and recording both count and weight of fruit with codling moth fruit injury and fruit without codling moth fruit injury. Average percent fruit injury by weight for each treatment was 50.99 ± 0.29% (n = 69; range 0 – 100) control, 20.04 ± 0.13% (n = 32; range 0 – 55.95) granulovirus, and 14.27 ± 0.12% (n = 34; range 0 – 55.42) spinosad (Figure 8). Average percent fruit injury by count for each treatment was 49.76 ± 0.29% (range 0 – 100) control, 19.79 ± 0.12% (range 0 – 54.17) granulovirus, and 13.85 ± 0.12% (range 0 – 54.17) spinosad.

Cultivar and climatic influence on fruit injury was reduced by comparing samples from within the same orchard and consisting of the same cultivar. Fuji samples taken at Creek Side within the control plot were compared to Fuji samples taken within the granulovirus plot. Fuji

samples within the granulovirus plot average percent fruit injury by weight was 80.7 ± 0.1% (n = 14; range 65 - 100) control and 26.2 ± 0.14% (n=15; range 11 - 56). Braeburn samples taken at Creek Side within the control plot were compared to Braeburn samples taken within the spinosad plot. Spinosad Braeburn average percent fruit injury by weight was 63.9 ± 0.18% (n = 29; range 22 - 90) control and 26.2 ± 0.14% (n=34; range 14.3 - 55).

Percent Fruit Injury by Treatment: 2016-2017

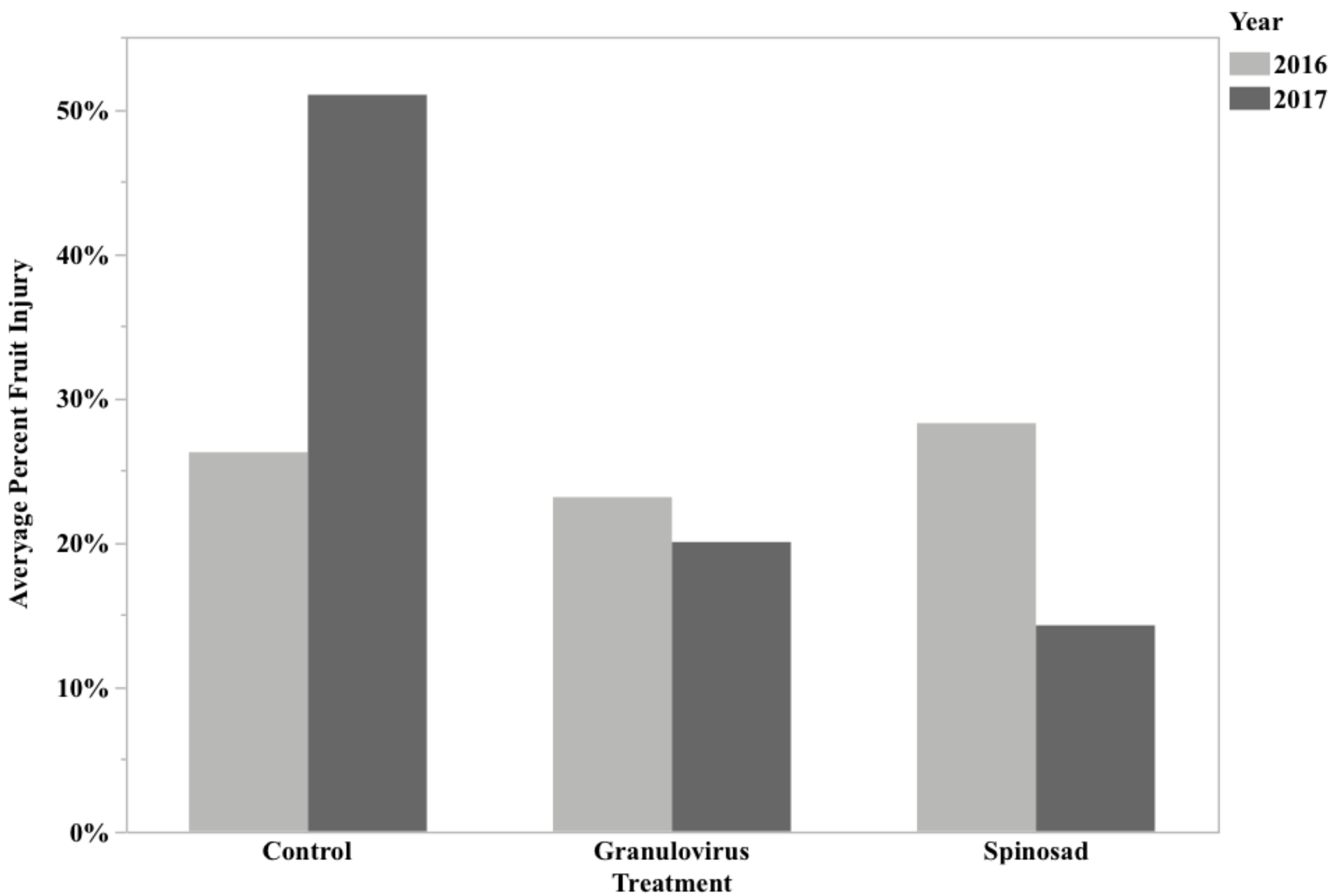

Figure 8. Mean percent of fruit damaged by codling moth larvae or stings per 24 apples at the four San Luis Creek orchard plots in San Luis Obispo, CA.

3.3.2 Mating Disruption

Fruit injury for 2017 averaged 13.02 ± 0.17% (n = 71; range 0 – 71.79), while 2016 trap captures averaged 15.56 ± 0.15% (n = 39; range 0 – 67.57). 2017 control averaged 29.81 ± 0.23% (n = 21; range 0 – 71.79), while the 2016 control averaged 15.77 ± 0.12% (n = 3; range 4.55 – 27.78).

2016

Average percent fruit injury was 15.8 ± 0.12% (n = 11; range 4.55 – 27.78) control and 15.5 ± 0.16% (n = 36; range 0 – 67.57) pheromone (Figure 9).

2017

Average percent fruit injury was 29.1 ± 0.05 % (n = 21; range 0 – 66.67) control and 5.58 ± 0.01% (n = 50; range 0 – 29.17) pheromone (Figure 9).

Cultivar and climatic influence on fruit injury was reduced by comparing samples from within the same orchard and consisting of the same cultivar. Gala samples taken at Gopher Glen within the control plot were compared to Gala samples taken within the mating disruption plot. Mating disruption Gala samples average percent fruit injury by weight was 31.8 ± 0.1% (n = 11; range 13 - 68) control and 10.9 ± 0.08% (n=11; range 0 - 29). Golden Delicious samples taken at Gopher Glen within the control plot were compared to Golden Delicious samples taken within the mating disruption plot. Mating disruption Golden Delicious samples average percent fruit injury by weight was 31.8 ± 0.23% (n = 21; range 0 - 72) control and 6 ± 0.07% (n=50; range 0 - 29).

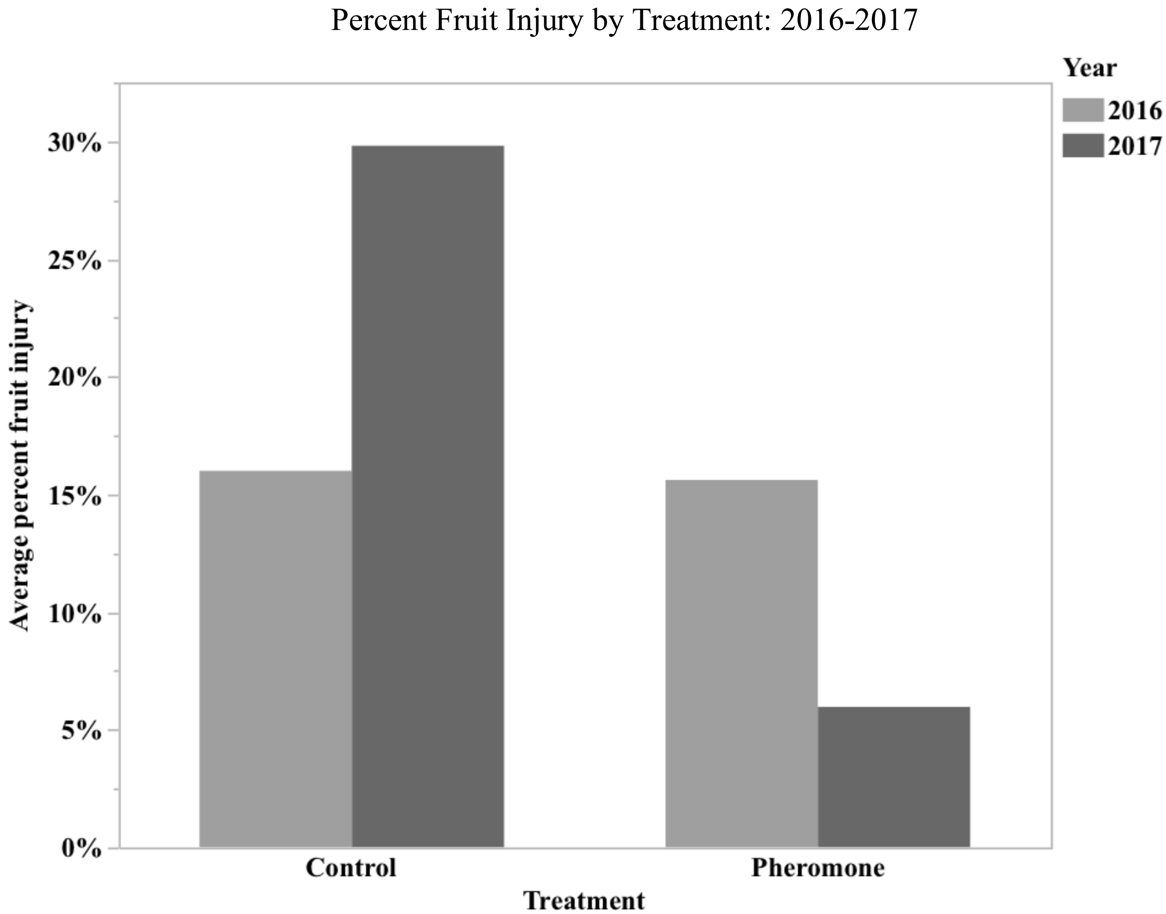

Figure 9. Mean percent weight of fruit damaged by codling moth larvae exit holes or stings per 24 apples at the two Gopher Glen orchard plots in San Luis Obispo, CA.

3.4 Apple Cultivar

During October 2017 fruit was sampled from San Luis Creek Side orchard within the granulovirus treatment plot to compare fruit damage between cultivars. Jonagold averaged 14.32 ± 0.02% (n = 16; range 0 – 29.2) fruit injury, while Fuji averaged 25.26 ± 0.03% (n = 16; range 12.5 – 54.2). San Luis Creek Side orchard within the spinosad treatment plot Braeburn averaged 63.95 ± 0.18% (n = 29; range 21.5 – 90.4) fruit injury, while Fuji averaged 90.71 ± 0.1% (n = 14; range 65.1 – 100) and Heavensent averaged 21.67 ± 0.12% (n = 15; range 3.8 – 51.9).

During July and August of 2017 fruit was sampled from Gopher Glen orchard within the mating disruption plot to compare fruit damage between cultivars. Gala had the highest rates of fruit injury averaging 11.36 ± 0.02% (n = 11; 0 – 29.17) of fruit sampled. Jonathan and Empire had the lowest rates of fruit injury respectively averaging 2.08 ± 0.01% (n = 8; range 0 – 4.17) and 0.83 ± 0.01% (n = 5; range 0 – 4.17%). Average percent fruit injury rates for the remaining four cultivars ranged between 3.13 and 9.17 ± 0.07% n = 17) with Burgundy 9.17 ± 0.02 (n = 5; range 4.2 – 12.5), Nittany 8.33 ± 0.03 (n = 5; range 0 – 16.67), Gravenstein 4.17 ± 0.01 (n = 8; range 0 – 4.17), and Mollies 3.17 ± 0.02 (n = 4; range 0 – 8.33).

Percent Fruit Injury by Cultivar within Mating Disruption Treatment at Gopher Glen: 2017

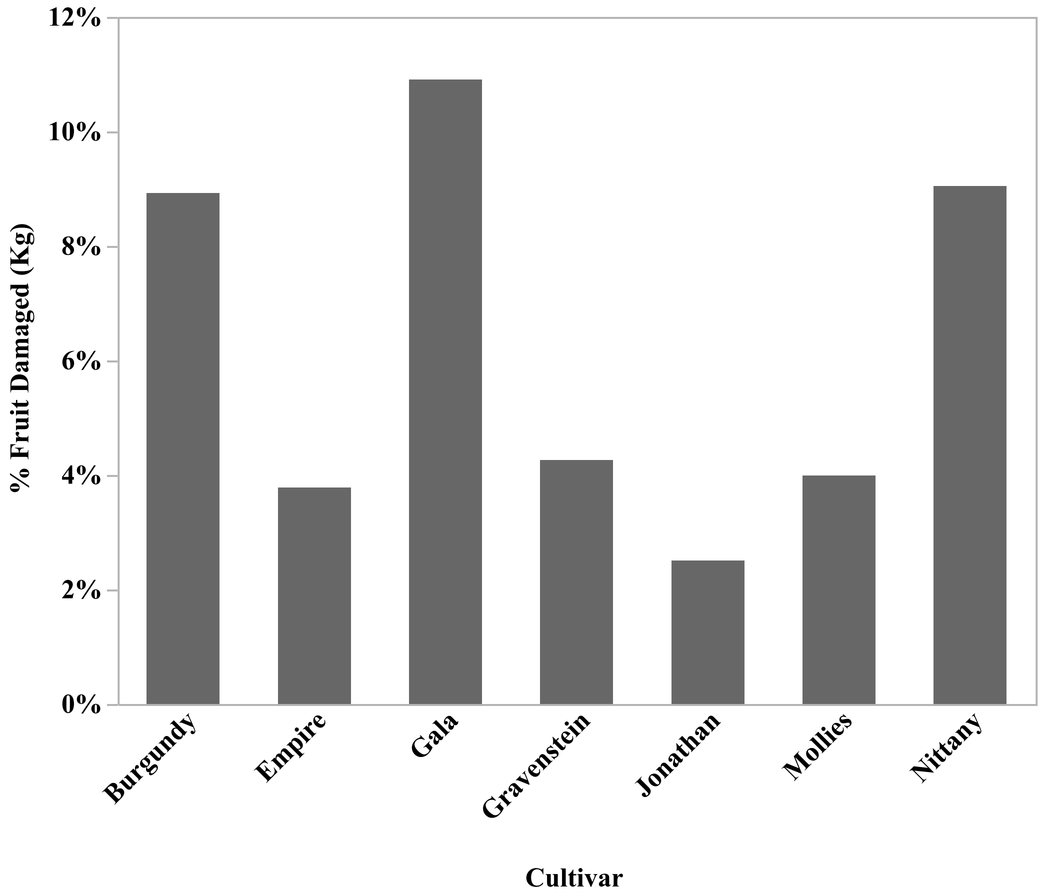

Figure 10. 2017 average percent fruit injury comparison between cultivars within the Gopher Glen orchard mating disruption treatment plot. Fruit injury averages were Gala 11.36 ± 0.02 (n = 11; range 0 – 29.17); Burgundy 9.17 ± 0.02 (n = 5; range 4.2 – 12.5); Nittany 8.33 ± 0.03 (n = 5; range 0 – 16.67); Gravenstein 4.17 ± 0.01 (n = 8; range 0 – 4.17); Mollies 3.17 ± 0.02 (n = 4; range 0 – 8.33); Jonathan 2.08 ± 0.01 (n = 8; range 0 – 4.17); and Empire 0.83 ± 0.01 (n = 5; range 0 – 4.17).

CHAPTER 4

4. DISCUSSION

Codling moth has the potential to measurably reduce percent marketable fruit by 16-51% in central coast apple orchards in San Luis Obispo, CA as documented in this study. Larvae damage the fruit exocarp and damage fruit flesh during larval feeding. Analyses showed that intervention can moderately reduce fruit injury caused by codling moth larval feeding by 24 to 36%. Results from this study demonstrated that granulovirus, spinosad, and mating disruption are effective on their own or combined in an areawide IPM plan for organic orchards in coastal California.

Percent fruit injury increased from 13 to 40% in control plots compared with plots receiving spinosad, granulovirus, or mating disruption where there was a 30% average decrease in fruit injury. The substantial reduction in fruit injury during the second year of the study indicated the materials were successful in increasing larval mortality and substantially reducing mating as evidenced by codling moth trap captures below 5 moths/trap per week. Treatments did not further reduce fruit injury when codling moth populations were below five moths/trap per week as observed from trap captures in 2016. An action threshold of 5 moths/trap per week when using mating disruption, granulovirus, or spinosad was developed as a result of this study. The same threshold also is used to determine the biofix date (Caprile and Vossen 2016). Orchards with trap captures below 5 moths/trap per week during the biofix and consecutive generational flights might not benefit from treatments with the materials used in this study.

Male moth flights were clearly documented by an increase of trap captures from 1 – 6 moths within 315 to 560 DD of the projected DD periods for the species (1060 DD, 1100 DD, and 1200 DD) during 2017. This suggested the phenological model used in this study accurately

tracked generational flights with enough precision to effectively target the eclosion periods, even when the resources that were available to the grower for tracking the model varied 315 to 560 DD from estimated generational flights. Some potential areas for inaccuracies were distance of weather station from plots, accuracy of the weather station, pheromone trap accuracy in detecting the initial biofix, and accuracy of the DD calculator provided by the UC Davis statewide IPM program website. Reduction of fruit injury achieved in 2017 by targeting egg eclosion periods based on DD calculating additionally supported the ability of the phenological model in tracking egg eclosion for timing of treatments even with the 315 to 560 DD difference.

The 2017 location-based trap capture numbers and percent fruit injury suggested infiltration of moths from external sources bordering the treatment plots. Moths have been known to travel over a mile in search of new habitat for oviposition sites or other resources (Hoover, Tepe and Foulk 2015). Habitat can include untreated commercial orchards, home garden trees, and ground litter (Hoover, Tepe and Foulk 2015). Traps located in the treatment plots adjacent to the control plots caught the highest number of moths of all traps in the study, suggesting infiltration from this untreated, adjacent habitat. The Gable plot was a contributor to the increased trap captures along treatment plot borders since Gable plot had an infestation indicated by an increase of average trap captures from 1.63 to 3.44 captures/trap per week and increased fruit damage from 20% to 50%. Gable plot consisted of a heavily planted orchard with excess wood habitat from minimal pruning and high stands of grasses and other orchard floor habitat. Gable plot has had no treatments for codling moth for 8 years, including 2016 and 2017, leading to infestation and contamination of bordering orchards. Other factors contributed to trap captures in the treatment plot outside of the intrusion from the Gable plot, as indicated by number of captures in traps located on the border farthest from the Gable plot. Traps in this

location had the second highest trap numbers with detectibly less captures than the Gable plot. Traps located in the center of the treatment plots had the fewest male moths due to the effectiveness of the materials and the isolation from external populations. High pressure areas such as neighboring orchard borders and orchard edges could require increased application rates or increased application intervals and areawide management to reduce codling moth damage.

Pheromone trap captures during 2016 and 2017 were not detectibly different between plots compared within Creek Side or Gopher Glen orchards. Pheromone traps were used to demonstrate the presence of male codling moths, not to determine population levels of codling moth. The pheromone traps used in this study are very sensitive, meaning they will attract male moths even at very low densities. None distinct difference between plot captures within each year, was due to trap sensitivity in capturing male moths. Codling moth populations were indicated using fruit injury, thus more fruit injury meant higher populations of codling moth in that orchard. In 2016, there was 52% less fruit injury in apple samples compared to the 2017 apple samples.

Percent fruit injury and trap count capture differences between 2016 and 2017 suggested initial insect population densities in treatment areas played a detectible role in the effectiveness of the insecticidal materials in reducing fruit injury. Trap captures indicated the population developmental stages and population thresholds for treatment decisions but were not an exact estimation of existing orchard populations; fruit damage can be used to detect a general increase or decrease in populations for comparison over time. Trap capture numbers of codling moth were below the action threshold of 5 moths/trap per week during 2016, while the following year flight peaks averaged 6 moths/trap per week indicating male moth levels were slightly lower in 2016 than 2017. During 2017, percent fruit injury in the control plots was double that of the previous

year. During 2017, percent fruit injury in treatments was detectibly lower than the control, while during 2016 the treatments and control plots had similar levels of fruit injury at 23 to 26%. Higher annual populations during 2017 resulted in increased fruit damage potential for the 2017 season, making the differences between treatment and control plots traceable with fruit sampling. Repeated annual applications can reduce codling moth populations over time (Caprile and Vossen 2016). Repeated annual applications cannot be responsible for increased effectiveness of treatments during 2017 since the percent fruit injury levels in control plots and all trap captures were 24 - 63% higher in 2017, indicating higher populations rather than reduced populations after the second year of applications.

Two different methods were used to quantify fruit damage between 2016 and 2017. During 2016, selected trees had 100% of fruit removed and visually categorized as damaged or not damaged, and then weighed. The following year 24 samples were taken in a quadrant based on Cartesian coordinates and further divided into high and low canopy from selected trees. Weight and count samples during 2017 were assessed to determine if weight and count methods of assessing fruit injury would significantly vary in their representation of the population. Lack of significant differences ($F = 0.03$, d.f. = 1, $p = 0.87$) between the two types of sampling suggested both sampling methods were accurate in representing the fruit injury caused by codling moth and thus can be statistically compared within this study.

Statistical analysis was limited to tabulated mean comparisons since replication limitations existed within this study preventing p and F value calculations. Due to the nature of the commercial orchards participating and the ambient physical characteristics of mating disruption, each treatment was limited to one plot. Treatment plots were comprised of one 2.4 to 4.85 ha plot. Participating orchards had the same geographical characteristics (location, size,

climate, and bordering habitat), identical infrastructure (overhead irrigation, planting densities, and age of trees), large sample sizes, and similar historical treatments minimizing outside influences. Analogous results from this study and historical studies of the topic support the minimization of influence from these factors. Results from 2017 supported past research that indicated spinosad is more effective in reducing fruit damage than granolvirus (Arthurs, Lacey and Fritts 2005, Caprile and Vossen 2016, Lacey, et al. 2008, Pajac, Pejic and Baric 2011, Granger, Brunner and Doerr 2003). Results from 2017 additionally supported past studies showing granulovirus, spinosad, and mating disruption reduce fruit injury compared to controls (Arthurs, Lacey and Fritts 2005, Brunner, et al. 2002, Granger, Brunner and Doerr 2003, Lacey, et al. 2008, McGhee, Epstein and Gut 2009, Pajac, Pejic and Baric 2011).

Differences between the average percent fruit injury in the Gopher Glen and Creek Side controls were attributed to cultivar resistance to codling moth damage, existing codling moth populations, and/or intrusion from neighboring orchards. Gopher Glen orchard had much lower detectable rates of fruit injury than Creek Side orchard, when comparing control plots between orchards. Gopher Glen orchard experienced 15% less fruit injury than Creek Side orchard, when averaging the two years. Compositional variation such as topography and weather, as described below, were potential contributors to increased fruit injury at San Luis Creek since these characteristics were different between the two orchards. Gopher Glen orchard is located at a higher elevation than Creek Side orchard, with very few orchards neighboring it. Gopher Glen orchard is split into smaller plots stair-stepping from the creek to the base of a mountain at 100 m above sea level. Gopher Glen orchard receives 58% more annual rainfall on average than Creek Side orchard, though July to September. Gopher Glen temperatures averaged 0.82 ºC higher than Creek Side with wind speeds averaging 0.32 mph slower. These differences can contribute to

measurable fruit injury differences between orchard controls, due to the differences in the rate of codling moth generational development. For both 2016 and 2017 the biofix for Gopher Glen was 1-2 weeks later than Creek Side due to the cooler winter and spring temperatures at Gopher Glen slowing down overwintering emergence. Rate of development differences during this study supported the importance of tracking individual populations for each orchard when targeting generational flights for treatment (Glenn 1922). Later emergence can push the second and third generations to late August and late September, by which time some cultivars have been harvested. Most of the fruit at Gopher Glen is harvested in September thru November, so this likely did not have an impact on fruit injury and does not explain the lower levels of percent fruit injury at Gopher Glen. Gopher Glen is bordered by a conventional orchard on its north-western edge. This orchard was treated with conventional sprays annually for the past eight years, decreasing the populations within the area and decreasing moth intrusion from these sites into the study control likely attributed to a lower percent fruit injury in the Gopher Glen control plot. Gable control at Creek Side was untreated for eight years creating a detectibly higher rate of intrusion noted by pheromone trap captures and increased fruit injury for the sites in this study. Increased initial codling moth population likely created the difference between Gopher Glen control and Creek Side control fruit injury levels. Initial codling moth population and climate-caused-developmental variation was accounted for by comparing treatments within orchards to controls within the same orchard rather than comparing treatments across all orchards. Mating disruption and control at Gopher Glen was not directly compared to granulovirus, spinosad, or controls at Creek Side.

Differences in resistance of cultivars to codling moth damage suggested cultivar played a role in fruit injury. A variety of cultivars at both San Luis Creek Side and Gopher Glen were

found to be more resistant to codling moth damage than others. Varieties Jonathan, Empire, and Jonagold had up to 11 % less fruit injury than other cultivars. Gopher Glen was comprised of over 100 apple cultivars, while San Luis Creek is comprised of six cultivars. Cultivar influence at Gopher Glen is likely reduced due to the intensity of cultivar variation, while San Luis Creek lacks this variation allowing for potential cultivar influence on fruit injury. Mating disruption and control at Gopher Glen was not directly compared to granulovirus, spinosad, or controls at Creek Side so the cultivar variation difference between the two orchards was avoided. Graph representations of the average fruit injury showed detectable difference in fruit injury between Fuji, Braeburn, and Heavensent and between Fuji and Jonagold compared within individual treatment plots of Creekside. Plots under different treatments at Creek Side were not cross compared for cultivar resistance since treatment had a detectable influence over fruit injury. Cultivar commonality existed within Creek Side plots between control and Freeway and between control and Monte reducing, but not eliminating, the influence of cultivar resistance to codling moth. Creek Side orchard included; control plots comprised of Fuji, Braeburn, and Heavensent; Monte plot comprised of Braeburn; and Freeway plot comprised of Fuji and Jonagold. Fuji fruit injury was 26%, compared to 14% Jonagold fruit injury in the granulovirus plot. Heavensent and Braeburn had low to moderate fruit injury with 22% and 64% respectively, compared to 81% Fuji fruit injury all within control plots. Fuji had the highest rates of fruit injury in both the control and granulovirus plots. Investigating apple cultivar resistance to codling moth should be pursed in future research as there were obvious differences detected during this study but were beyond the scope of the current objectives. Studies such as Joshi et al. (2015) have looked into general cultivar resistance, there are many cultivars and potential fruit characteristics yet to be studied. During habitat selection, mating, and egg depositing codling moths are likely stimulated

by fruit maturing period, fruit volatiles, and variations in the production and release of volatiles (Joshi, et al. 2015). Knowledge about cultivar characteristics that deter fruit injury can aid in the development of novel resistance mechanisms, thus reducing pesticide usage.

Other concerns outside of insecticidal efficacy and codling moth population levels included ease of application, safety, and potential side effects of the pesticides. Granulovirus and mating disruption proved to be the easiest to apply since both are species specific, require no resistance management, and have high safety ratings for both beneficial insects and applicators (Lacey, et al. 2008, Witzgall, et al. 2008). Spinosad was the most difficult since it is broad spectrum, potentially harmful to beneficial insects, and limitations exist for annual application quantities to manage for resistance (Sparks, Crouse and Durst 2001, Williams, et al. 2004, Lacey, et al. 2008). Most likely all three materials should be used together in a season-long IPM program.

Research investigating potential insecticide resistance due to long term use of granulovirus and spinosad products is necessary to avoid buildup of resistance. Granulovirus and spinosad are useful tools for control of populations resistant to other pesticides, but when used exclusively resistance development is a potential. Spinosad should be used in rotation with other materials to prevent or slow resistance. Studies in Germany and France reported resistance to spinosad after seven years (Fritsch, et al. 2005, Sauphanor, et al. 2006). Resistance to spinosad is especially a problem for certified organic apple growers limited to these two treatments options for codling moth and thus, avoiding resistance is key for this sector of agriculture. One potential solution is to develop more materials for use in organic situations, while also investigating commercial use of biological control agents such as entomopathogenic nematodes (EPNs) and insect predators and parasitoids.

Entomophathogenic nematode species in the families Steinernematidae and Heterorhabditidae have shown promise in controlling insect pests in several studies over the past 50 years and could also control codling moth (Koppenhöfer 2000, Grewal, Ehlers and Shapiro-Ilan 2005, Georgis, et al. 2006). *Steinernema carpocaspsae* (Rhabditida: Steirnernematidae) was one of the first EPN species to be commercialized after its discovery in codling moth cocooned larvae (Dutky and Hough 1955, Weiser 1955). The main obstacles for successful codling moth control with EPNs are EPN's sensitivity to temperature and moisture (Lacey, et al. 2008).

Bats are a predator of codling moth and potentially play a role in control (Hogan 2000). Bats can be attracted to orchards by installing bat shelters (Hogan 2000). Acari species groups *Anystis* (Trombidiformes: Anystidae) and *Balaustium* (Trombidiformes: Erythraeidae) are likely predators of codling moth eggs and larvae, but no significant quantitative studies have been conducted (MacLellan 1977). One species of specialized codling moth parasitoid exists, a koinobiont endoparasitoid *Ascogastar quadridentate* spp. (Hymenoptera: Braconidae) (Brown and Kainoh 1992). *Ascogastar quadridentate* attacks the eggs and emergences from the penultimate larval stage (Brown and Kainoh 1992). Parasitoid field studies across Europe have demonstrated low success rates of 5 to 20% (Mills 2005, Coutin 1974). There are many generalist species including *Tichomma enecator* and *Pristomerus vulerator* (Hymenoptera: Ichneumonidae), *Elodia tragica* (Diptera: Tachinidae), and 9 or more *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) endoparasitic polyphagous species that also attack codling moth eggs (Mills 2005, Pinto, et al. 2002). Success using *Trichogramma* has mostly had low efficacy in research trials, but one study in California by Mills (2003) suggested that indigenous and locally adapted *Trichogramma platneri* Nagarkattie reduced codling moth damage by 60% in walnuts and pears. *Trichogramma* are often habitat specialists, preferring either trees or

herbaceous plants and their behavior can be influenced by both plant structure and climate. Thus commercially available *Trichogramma* species has potential, but may not be equally suitable for all orchard types (Mills 2005). Inundative releases of *T. platneri* is a potential method worth further study for controlling codling moth in the orchards in this study since both the habit and climate is similar to that of the study performed by Mills (2003) and the species is currently offered for sale by commercial insectaries.

Granulovirus, spinosad, and mating disruption have proven to be effective options for controlling codling moth in coastal California orchards, though their short residual activity and unpredictable effectiveness can limit their adoption in conventional settings. Spinosad detectablity reduced fruit injury compared to granulovirus and controls supporting Arthur's (2005 and 2007) studies. Arthur's (2005 and 2007) studies showed spinosad to reduce fruit injury to 0-2 %, while granulovirus reduced fruit injury to 2-27 % from 5-37 % found in controls. Spinosad in this study reduced fruit injury to 14% showing the unpredictable efficacy of the product and potential influence of factors such as cultivar resistance to codling moth injury, imprecise tracking of site temperatures and DD calculations, intrusion from nearby harborages, expired or misused products, and malfunctioning equipment. Organic producers will likely use the three materials in conjunction, applying pheromone ties season long while alternating granulovirus and spinosad rather than using them as stand-alone methods. Spinosad can be used for the first generation to effectively reduce codling moth populations decreasing future risk of fruit injury but should not exceed 630g/ha/season to avoid resistance issues and should not be applied during blossom period to avoid bees (Arthurs, Lacey and Miliczy 2007, Caprile and Vossen 2016). Growers can use the less effective granulovirus for over wintering emerging larva and the second, third, and fourth generations or after they have reached the

threshold of 630g/ha/season of spinosad (Arthurs, Lacey and Miliczy 2007). Other materials and best management practices (BMP) can also enhance an IPM program such as horticultural oils for smothering codling moth eggs and larvae; wettable kaolin clay powder creating a physical barrier on the plants and physical irritant to the insect; mowing to remove harborage; pruning regularly; trunk banding to trap mature larvae in a cardboard band as they climb trunk seeking a site to pupate; use of parasitic or predatory nematodes or insects; attracting bats to the orchard; rotating materials with alternating modes of action for controlling resistance, and creating an areawide IPM with local grower participants working together for codling moth control (Caprile and Vossen 2016, Calkins and Senft 1995, Ingels, et al. 2001, Lacey, et al. 2008). An areawide IPM program is already being developed for San Luis Obispo coastal California orchards as a result of this study.

Coastal California orchards can follow BMPs to effectively reduce fruit injury caused by codling moth. The following BMPs were supported by the findings of this study. (1) Growers can start with general sanitation measures provided by the statewide IPM program developed by the University of California in 2009 (Caprile and Vossen 2016). Remove senesced host trees from nearby abandoned apple, pear, and walnut orchards to remove harboring sites of codling moth. Store empty picking bins, props, and cultivation tools in locations outside of orchard sites. (2) Regular pruning and hand thinning clusters to 1-2 fruit removing excess wood and fruit from orchard site (Ingels, et al. 2001, Caprile and Vossen 2016). Larvae move between fruits 1.3 to 1.9 cm and larger that are touching (Ingels, et al. 2001). (3) Removal of harvested fruit and fruit dropped by trees soon after picking before larvae leave fruit (Ingels, et al. 2001). (4) Establish an individual biofix date for each orchard by hanging pheromone traps with pheromone lures in top 20% of the canopy by mid-March, with one trap every 10 acres and at least two traps per orchard

(UC IPM Statewide Integrated Pest Management Program 2009). Use 1 mg lures if mating disruption has not been applied, use 10 mg supercharged lures in when pheromone dispensers are set out (UC IPM Statewide Integrated Pest Management Program 2009). (5) Apply mating disruption in the top 20% of the canopy either prior to moth emergence based on historical biofix dates or shortly after first moth emergence ca. March/April (Caprile and Vossen 2016). Early pheromone placement will disrupt mating of overwintering moths as they emerge, while a late application will require supplemental spray treatments (Caprile and Vossen 2016). Rate of application is based on the manufacturers guidelines and re-application is based on the fruit harvesting date. A minimum of 58 mg pheromone was maintained in the pheromone plot during this study. Pheromone disruption is not recommended for orchards less than 3-5 acres and can increase fruit damage if used in orchards of this size (UC IPM Statewide Integrated Pest Management Program 2009). (6) Orchards with a moderate to heavy infestation of codling moths will require spray treatments om addition to mating disruption, established by sampling a minimum of 200 fruit 900-1000DD from the biofix and determining fruit damage levels greater than 0.5 % or following the initial biofix of 5 moths/trap per week (UC IPM Statewide Integrated Pest Management Program 2009, Caprile and Vossen 2016). Spray applications can be applied to emerging overwintering populations as early as 250DD in orchards with a known infestation or orchards in their first year of mating disruption (Arthurs, Lacey and Fritts 2005, Calkins and Senft 1995, Lacey, et al. 2008). Materials that are documented to be harmful to pollenating hymenopteran species should not be applied until post petal fall, including spinosad (Rabea, Badawy and Nasr 2010). Granulovirus is specific to codling moth and can be applied in spring during blooming periods (Lacey, et al. 2008, Falcon and Hyber 1991). Consecutive applications should be made until 90% egg ecolsion for each generational flight (Calkins and Senft 1995).

As a result of this study four additional BMPs were developed for organic orchard management, with specifications for coastal California growers. (1) New orchards can utilize cultivar resistance to reduce fruit injury by planting varieties less susceptible to codling moth injury. Semi-highly to highly resistant varieties from this study include Mollies, Gravenstein, Braeburn, Heavensent, Jonagold, Jonthan, and Empire. (2) Fruit-baring orchards can utilize weekly or bi-weekly pheromone trapping beginning in mid-March and continue until harvest for treatment decision making. The biofix date is determined when trapping achieves 5 moths/trap per week. Orchards located closer to the coast will have a biofix around 1 April, while orchards further inland with less temperate climates will have a biofix around 14 April. Precision of daily high and low temperature measurements will determine how effective the phonological model will be in determining DD and predicting generational flights. Precise generational flight prediction is important in reduced spray programs focused on targeting egg ecolsion and larval stages. (3) An economic threshold of 5 moths/trap per week can be used to determine if treatments of granulovirus or spinosad will give growers detectible control. In orchards with a codling moth infestation, indicated by a fruit injury level of 30-100%, organic growers can benefit from spraying granulovirus and/or spinosad along with applying pheromone ties. Orchards below infestation fruit injury levels could keep control with application of pheromone ties exclusively. Spray periods for coastal California orchards will occur during early May, mid to late June, late July to early August, and mid-September. More than one application could be needed during each spray period. (4) Number of re-applications for each flight is determined by pheromone trap monitoring. Monitoring detects flights and lengths of flights. Re-application is recommended if peak flight periods are sustained for longer than one week. 250 Degree Days should be calculated from the first day or the flight as well as the last day of the flight to

determine 90% egg ecolsion. Pheromone ties can be applied 1 April coinciding with overwintering moth emergence. Mating disruption should be applied to neighboring orchards to reduce overall populations within the region and to reduce potential codling moth harborages that lead to intrusion into treatment orchards from non-treated orchards, this is why an areawide IPM approach must be developed.

An areawide IPM approach on the central California coast will be the most effective and economic way for organic apple growers to gain control over this pest, while reducing inputs. Codling moth is the biggest pest threat in apple production with a potential of 100% fruit injury (Caprile and Vossen 2016, Iraqui and M'hamed 2016). Actual damage from codling moth reached an average of 50% during this study with samples ranging as high as 100% fruit injury, thus coordinated grower action is needed. Central California coastal growers will initially need to relentlessly attack the codling moth populations with all three materials and a number of cultural practices before seeing a reduction in populations. Utilization of a customizable areawide IPM program is a promising solution to reduce and maintain codling moth populations in coastal California organic below the action threshold of 5 moths/trap per week. Notable economic savings can be generated as codling moth populations become depressed resulting in a decrease of labor during applications and decrease in total materials applied.

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