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NOVALURON SUBLETHAL ACTIVITY IN CODLING MOTH, *CYDIA POMONELLA* (LINNAEUS), VIA TRANSOVARIAL TRANSMISSION AND EFFECTS ON NATURAL ENEMIES

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NOVALURON SUBLETHAL ACTIVITY IN CODLING MOTH, *CYDIA POMONELLA* (LINNAEUS), VIA TRANSOVARIAL TRANSMISSION AND EFFECTS ON NATURAL ENEMIES

By

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ABSTRACT

NOVALURON SUBLETHAL ACTIVITY IN CODLING MOTH, *CYDIA POMONELLA* (LINNAEUS), VIA TRANSOVARIAL TRANSMISSION AND EFFECTS ON NATURAL ENEMIES

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Control of codling moth, *Cydia pomonella* (Linnaeus), and obliquebanded leafroller, *Choristoneura rosaceana* (Harris), have developed new tactics with the passage of the Food Quality Protection Act (FQPA 1996) and the imminent cancelation of key pesticides, organophosphates (OP). These new chemistries are called reduced-risk or OP alternative compounds. Novaluron, an insect growth regulator is one such compound. Although novaluron has no direct adult toxicity, sublethal effects in the form of reduced egg viability have been seen. Field based bioassays performed with this compound on codling moth have shown that exposure to treated leaves or fruit elicits this reduced egg viability for up to 21 d post spray in fruits. Residue analysis of fruits and leaves showed a significant correlation between the residue levels and reduced egg hatch as well. Obliquebanded leafroller was used to determine the transovarial nature of this compound, and bioassays resulted in detectable levels of novaluron present in egg samples. Experiments with *Trichogramma platneri* Nagarkatti, has shown similar reduced viability after adult exposure. However, exposure of adults to eggs laid from treated codling moth and from topically treated eggs have both shown 0% adult emergence. Novaluron can be utilized as an organophosphate replacement, but further research into timings and proper use should be investigated.

Dedicated to Jae Deuk Kim (1929-2004), I hope I made you proud

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CHAPTER 1

LITERATURE REVIEW

I. *Cydia pomenella*

A. Geographic Distribution and Host

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a pest distributed worldwide. It originated in Asia and made its way to the United States through colonial introductions (Howitt 1993). Codling moth is prevalent throughout the United States from the Atlantic to the Pacific coast. This pest usually has two generations per year in the eastern regions of the US and 2-4 generations along the west coast. Codling moth has a wide range of hosts including pome fruits, stone fruits, and walnuts. Tree fruits infested by this pest in Michigan include apple (*Malus* spp.), pear (*Pyrus* spp.), peach (*Prunus* spp.), and plum (*Prunus* spp.).

B. Life History

The average length of codling moth adults across their expanded wings is 19 mm. The wings are grey-brown in color with grey and white bands weaved in and the tips of the wings containing bronzed areas (Howitt 1993).

Codling moth is usually a bivoltine pest in Michigan with the overwintering larvae constructing silken cocoons under lose bark, tree limbs, in wooden storage bins and crates, or in litter beneath the tree (Sanderson 1908, Chandler 1928, Howitt 1993). These larvae remain inactive during the winter months and remodel the cocoon with an exit tube in the early spring months (Sanderson 1908, Quayle 1921). Larvae then undergoes pupation and emerge as adults in 15-20 days (Sanderson 1908, Howitt 1993). First adult emergence, also called "first flight," in an apple orchard typically occurs

around bloom of 'Delicious'. Peak adult emergence occurrs 17-21 days later depending on temperature. Adult females begin to lay eggs 2-3 days after emergence if night temperatures are above 17 °C. First generation codling moth typically lay their eggs on the surface or underside of leaves, and move to fruit deposition as fruit increase in size. Eggs usually take 6-14 days to hatch, and once emerged, the larvae seek out fruit to penetrate into. Larvae tend to seek out rough areas like the calyx or scab spot, which allow for easier access (Howitt 1993). Larvae penetrate into the fruit and feed internally for 14-20 days, until the mature larvae drops from the apple and seek out pupation areas. Once adults have emerged, the "second flight" occurs between 1200 – 1800 DD_{50}, and the cycle begins again. During warmer summers, a third generation can take place late in the season.

C. Pest Status

Codling moth is one of the major pests in the United States and throughout the world where apples are produced (Hoyt et al. 1983, Barnes 1991). Moths are internal feeders of the fruit, and the majority of the economic damage is caused by late season infestation. Early season damage leads to premature apple drops, which eventually contribute to decreased yields at harvest. However, late season damage results in harvested fruit that are unmarketable for fresh or processed market. If this pest is not monitored and controlled, resulting damage can lead to 90% crop loss for the year (Caprile and Vossen 2005).

II. Obliquebanded leafroller

A. Geographic distribution and host

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris), is a significant pest that hinders tree fruit production in North America (Chapman et al. 1968). This moth is a polyphagous insect with two generation per year that feed on more than 50 different species (Sanderson and Jackson 1909, Raizenne 1952, Howitt 1993). Within the past 20 years, this leafroller has become an increasingly important pest in the tree fruit arena.

B. Life History

Obliquebanded leafroller is a bivoltine pest in Michigan with the overwintering generation remaining in the larval stage until the next spring when they emerge and continue development (Chapman et al. 1968, Howitt 1993). Overwintered larvae continue to feed and mature until early June when they begin to pupate (Onstad et al. 1985). The pupal stage lasts from 14-20 days. Upon emergence adults quickly mate and females begin depositing eggs. The eggs are green to yellow in color and are laid in masses on the foliage of the tree. Eggs take approximately 14 days to hatch. Newly emerged larvae seek out foliage or fruit to feed upon. Larvae feed for approximately 14-20 days to before pupating. On average, the pupal stage can last 7-14 days depending on temperature. Adults emerge, mate, and deposit eggs onto the foliage of the tree, and the cycle repeats itself. Overwintering larvae seek loose bark, wooden crates, or tree limbs for shelter during the winter months (Chapman and Lienk 1971).

C. Pest Status

OBLR larvae are external feeders of flowering buds, leaves, and fruit surfaces (Chapman et al. 1968, Reissig 1978, Howitt 1993). Early season damage from the overwintering larvae can cause fruit drop or deformed fruit. However, late season damage

causes shallow or deep holes on the surface of the fruit making fruit unmarketable for fresh produce (Stelinski et al. 2007a). Damage from this pest can range from 3-20% crop loss (Angello et al. 1996, Ho 1996, Lawson et al. 1996). The larvae that feed on the foliage elicit the typical leafroller behavior where the leaf is rolled upon itself and the larvae find shelter inside. Larvae may also bind leaves to fruit on the tree and feed on the surface of the fruit and the leaves.

III. Control Methods

A. Organophosphates

Control of these pests has generally relied upon broad-spectrum insecticides like organophosphates (OP) (Reissig 1978, Howitt 1993, Wise et al. 2008). OP's are labeled as Group 1 under IRAC, and their mode of action is inhibition of acetylcholinesterase. Inhibition of this enzyme prevents hydrolysis of acetylcholine in the nervous system, ending nerve impulse transmission. Organophosphates have historically provided control over codling moth and obliquebanded leafroller (Gratwich et al. 1965, Oatman and Libby 1965, Madsen and Morgan 1970), but insect resistance has become widespread in recent decades. However, the negative effects of OP's in the environment and toxicity towards mammals has lead to regulatory changes in the use of these compounds. With the impending phase-out of these compounds by the Food Quality Protection Act (Anonymous 1996) slated for 2012 (Anonymous 2006), industry has moved toward use of softer and more selective insecticides. The evidence of resistance development (Reissig et al. 1986, Varela et al. 1993, Knight et al. 1994, Carriére et al. 1996, Lawson et al. 1997b, Dunley and Welter 2000, Ahmad et al. 2002, Smirle et al. 2002, Reuveny and

Cohen 2004, Mota-Sanchez et al. 2007, Whalon et al. 2008) has also expedited exploration into newer chemistry classes.

B. Pheromones

Of the new techniques being used to control codling moth and obliquebanded leafroller, the use of pheromones for mating disruption is on the rise using sex pheromones for monitoring and disrupting mating (Moffitt 1978, Vickers and Rothchild 1991, Lawson et al. 1996, Judd et al. 1997, Calkins 1998, Gut and Brunner 1998, Knight et al. 1998, Evenden et al. 1999, Thomson et al. 1999, Calkins and Faust 2003, Stelinski et al. 2004, Stelinski et al. 2005, Stelinski et al. 2007b, Stelinski et al. 2008, Witzgall et al. 2008). Pheromone use has steadily risen due to increased concerns about negative environmental impacts brought on by insecticides. Pheromones are species specific and nontoxic towards beneficial organisms.

This method of control has some limitations. Use of pheromones to control codling moth has been to suppress low density populations or to apply the technique in conjunction with insecticides (Barnes et al. 1992, Howell et al. 1992, Carde´ and Minks 1995, Witzgall et al. 2008). The potential migration of mated females into treated orchards is a concern in areas using this technique. In areas with high codling moth populations (>1000 overwintering larvae per hectare), supplementing mating disruption with applications of insecticides is needed to maintain population suppression (Brunner et al. 2002, Witzgall et al. 2008). Use of mating disruption against codling moth has led to a potential increases in secondary pest outbreaks by the leafroller species (Madsen and Morgan 1970, Walker and Welter 2001). Issues with resistance in pheromone usage have yet to be described and increasing the effectiveness of this technology for long-term use

and significant reductions in populations without the need for supplemental insecticides remain as challenges (Witzgall et al. 2008).

C. Biological Control

With the impending phase out of the OP's, biological control methods have been studied to target these two pests. Potential biological control agents include nematodes, granulovirus, and parasitoids. All methods have been extensively studied and offer various control measures, but the range of control varies with the population densities of the pests targeted.

The use of entomopathogenic nematodes predominantly targets the overwintering larvae of codling moth or obliquebanded leafroller. Studies conducted using *Steinernema* spp. have demonstrated that under ideal conditions populations of the two tortricidae species can be controlled (Dutky 1959, Kaya et al. 1984, Lacey and Unruh 1998, Bélair et al. 1999, Unruh and Lacey 2001, Lacey et al. 2005, Lacey et al. 2006, Lacey and Shapiro-Ilan 2008). Moisture is a crucial factor leading to increased efficacy of entomopathogenic nematodes in a field setting.

The *Cydiia pomonella* granulovirus (CpGV) is an effective control agent for codling moth and is harmless towards humans and beneficial insects (Falcon et al. 1968, Huber 1986, Gröner 1990, Lacey et al. 2002, Arthurs and Lacey 2004, Arthurs et al. 2005, Lacey et al. 2008, Eberle et al. 2009). Although granulovirus provides effective control over neonate larvae, the larvae often survive long enough to create shallow entry stings on the surface of the fruit decreasing the value from fresh market to processed (Felt 1917, Glen and Clark 1985, Arthurs et al. 2005, Lacey and Shapiro-Ilan 2008). There have also

been cases of field populations of codling moth developing resistance to continued use of granulovirus (Eberle and Jehle 2006, Eberle et al. 2008).

Another well studied area of biological control of these two Tortricid pests is the use of parasitoids to manage populations. There are numerous parasitoids associated with various stages of codling moth; the Families Ichneumonidae (Bezemer and Mills 2001, Hougardy et al. 2005), Braconidae (MacLellan 1972, Wearing 1989, Suckling et al. 2002), Eulophidae (Mattiacci et al. 1999, Zaviezo and Mills 1999, Tscudi-Rein and Dorn 2001, Hausmann et al. 2005, Häckermann et al. 2007, Hein and Dorn 2008) and Trichogrammatidae (List and Davis 1932, Webb and Alden 1940, Dolphin et al. 1972, Karadzhov 1974, Yu et al. 1984, Hassan et al. 1988, Falcon and Huber 1991, Li 1994, Zhang and Cossentine 1995, Cossentine et al. 1996, Smith 1996, Bloem et al. 1998, Cossentine and Jensen 2000, Mills et al. 2000, Mansfield and Mills 2002, Pinto et al. 2002, Mansfield and Mills 2004, Mills and Kuhlmann 2004) have been explored as potential classical biological control agents (Mills 2005).

Parasitoids targeting obliquebanded leafroller that have been tested in field and laboratory settings (Pogue 1985, Li et al. 1999, Vakenti et al. 2001, Wilkinson et al. 2004, Sarvay et al. 2007) include the Families Ichneumonidae (Cossentine et al. 2004b, 2007,Cossentine 2008), Braconidae (Cossentine et al. 2004a, 2005, Cossentine 2008), Tachinidae (Hagley and Barber 1991, Biddinger et al. 1994), Eulophidae (Brunner 1993, 1996, Brunner et al. 2001), andTrichogrammatidae (Lawson et al. 1997a, McGregor et al. 1998).

These biological control methods should not be a stand-alone control tactic in high pest pressure areas, but rather be supplemented with selective insecticide treatments

and/or mating disruption. With the environmental limitations of nematodes and granulovirus, the parasitoid complex appears to provide a more stable control tactic.

D. Insect Growth Regulators

Among the newer control tactics being implemented, insect growth regulators (IGR) can be easily implemented into Integrated Pest Management (IPM) programs due to reduced potential for environmental and ecological impacts, and have played a substantial role in IPM of US pome fruits over the las decade. These compounds are a class of chemistries that alters the growth and development of the targeted pest. There are three classes of IGR's on the market; juvenile hormone mimics, ecdysone inhibitors, and chitin synthesis inhibitors. Juvenile hormone mimics (IRAC Group 7) is a compound that causes premature molting, deformation of wings and reproductive parts, examples within this group are pyriproxyfen and fenoxycarb. Ecdysone inhibitors (IRAC Group 18) is a compound that blocks the ecdysone needed to signal and insect to molt, examples within this group are tebufenozide and methoxyfenozide. Chitin synthesis inhibitors (IRAC Group 15,16) is a compound that prevent the insect from undergoing proper chitin formation, examples with this group are novaluron and diflubenzuron.

Insect growth regulators have been primarily used to target the larval and egg stages of codling moth and leafroller species based on the toxic effects to these stages seen in other Lepidoptera pests (Ishaaya et al. 1996, 1998, 2003). With strong toxic effects on eggs and larvae, insect growth regulators have been noted to demonstrate sub-lethal effects as well as acute toxicity on a variety of different pests, including Lepidoptera, Coleoptera, and Diptera (Broadbent and Pree 1984, Elek 1998, Pons 1999, Sun and Barrett 1999, Casa-Giner et al. 1999, Knight 2000, Charmillot et al. 2001, Cutler

et al. 2005, Pineda et al. 2006, Kostyukosky and Trostanetsky 2006, Wise et al. 2007, Gökçe et al. 2009).

IV. Chitin Synthesis Inhibitors

Within the insect growth regulator class are the chitin synthesis inhibitors (CSI), which are benzoylphenyl ureas that prevent the insect from undergoing proper chitin synthesis after the molting process (Ishaaya and Casida 1974, Post et al. 1974) or cause abnormal endocuticle deposition (Mulder and Gijswijt 1973), but the exact step where inhibition occurs is still unclear. However, inhibition of chitin may take place at the polymerization stage of chitin biosynthesis (Hajjar and Casisa 1978) or in chitin precursor transport (Nakagawa and Matsumura 1994). Benzoylphenyl ureas are selective CSI compounds that mainly act by ingestion, but do exhibit ovicidal and contact toxicity (Ascher and Nemny 1974, Wright and Harris 1976, Horowitz et al. 1992). Chitin synthesis inhibitors have been shown to have minimal effects on parasitoids and other natural enemies (Ishaaya 1990, Ishaaya et al. 2001). Studies conducted on parasite larvae inside treated hosts have demonstrated effects of the compounds on the larvae (Granett and Weseloh 1975, Broadbent and Pree 1984), but not on adult predators or mites feeding on treated hosts (Elliott and Anderson 1982, Jones et al. 1983, Broadbent and Pree 1984).

Diflubenzuron (Dimilin™), one of the first marketed CSI and the most studied, was generally used to control insects in Diptera, Lepidoptera, and Coleoptera orders (Grosscurt and Jongsma 1987). Diflubenzuron was mainly used for its ovicidal and larvicidal properties, which resulted in mortality of larvae after a molt or prevention of neonate larvae from hatching. However, diflubenzuron has demonstrated sub-lethal activity on codling moth, which results in reduced viability of the subsequent eggs laid

(Hoying and Riedl 1980, Elliot and Anderson 1982). Moore et al. (1978) demonstrated diflubenzuron was transferred between sexes in boll weevils (*Anthonomus grandis* Boheman) and resulted in reduced viability of subsequent eggs laid after treated males were allowed to mate with untreated females.

The IGR, novaluron, is a benzoylurea insecticide (IRAC Group 15), a chitin synthesis inhibitor. Novaluron, has recently been registered for use in tree fruit production and has similar sub-lethal effects when adults were exposed (Cutler et al. 2005, Kostyukovsky and Trostanetsky 2006, Wise et al. 2007a, Gökçe et al. 2009). Novaluron is a strong control agent against Lepidoptera by both ingestion and contact (Ishaaya et al. 2007). It has strong toxic effects on the eggs and larvae of obliquebanded leafroller (Wise et al. 2007b), however sub-lethal effects from adult exposure and presence of novaluron in egg masses have yet to be reported. Experiments by Gökçe et al. (2009), show sublethal effects on codling moth, reducing egg viability after adult exposure.

V. Thesis Research

The goal of the research herein is to demonstrate the mechanisms of the sublethal effects on codling moth, oblqiuebanded leafroller, and *Trichogramma platerni* after adult exposure to novaluron. Comparisons between transovarial and horizontal transmission of this insecticide will also be explored.

The future steps of this research are to: 1) determine physiological changes that occur in the adults after exposure, 2) determine what stage in the embryo development that mortality occurs.

CHAPTER 2

REDUCED EGG VIABILITY IN CODLING MOTH *CYDIA POMONELLA* (L.) (LEPIDOPTERA: TORTRICIDAE) FOLLOWING ADULT EXPOSURE TO NOVALURON, EXPOSURE TO DIFFERENT SEXES, AND EFFECTS OF TOPICAL AND HORIZONTAL TRANSFER TO EGGS

1. INTRODUCTION

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is an important direct pest of pome fruits in the United States (US) and around the world (Hoyt et al. 1983, Barnes 1991). There is a wide array of insecticides used to control codling moth in orchards, ranging from organophosphates (OPs) to neonicotinoids and insect growth regulators (IGRs) (Wise et al. 2008). However, since the US passed the Food Quality Protection Act in 1996 (FQPA), insecticides belonging to organophosphate, carbamate, and synthetic pyrethroid classes are under increased scrutiny regarding their continued use within the country. Because of the US Environmental Protection Agency (EPA) phase-out of azinphosmethyl (US EPA 2006) and increasing evidence of resistance (Varela et al. 1993, Knight et al. 1994, Dunley and Welter 2000, Reuveny and Cohen 2004, Mota-Sanchez et al. 2008, Whalon et al. 2008), new control strategies are being explored and implemented to control codling moth.

Insect growth regulators have played an important role within US pome and stone fruit Integrated Pest Management (IPM) programs over the last several decades. IGRs are generally considered to have reduced toxic effects on arthropod predators and parasitoids when compared to many of their competitors in the marketplace (Croft 1990, Suh et al. 2000, Cloyd and Dickinson 2006). IGRs have thus been primarily used as selective insecticides targeting Lepidoptera pests, such as codling moth and leafrollers, based on their direct toxicity to egg and larval stages (Hoying and Riedl 1980, Elliott and

Anderson 1982, Matolín and Kuldová 1982, Soltani and Solatni-Mazouni 1992, Pons et al. 1999, Knight 2000, Charmillot et al. 2001, Brunner et al. 2005). Several IGRs registered for use in US pome fruits, such as diflubenzuron, tebufenozide, methoxyfenozide, pyriproxifen, and novaluron, have in some cases shown sublethal effects in Lepidoptera, Coleoptera, and Diptera pests (Elek 1998, Casa-Giner et al. 1999, Sun and Barrett 1999, Cutler et al. 2005, Pineda et al. 2006, Kostyukosky and Trostanetsky 2006).

In the case of the chitin synthesis inhibitor (CSI) insecticide, diflubenzuron, treatment of codling moth pupae results in lower amounts of protein in female ovaries and fewer oocytes, and adult exposure reduces subsequent egg viability (Elliot and Anderson 1982, Soltani and Soltani-Mazouni 1992). The extent and duration of the sub-lethal effects from diflubenzuron vary depending on the dose and mode of exposure (Elliot and Anderson 1982, Casa-Giner et al. 1999). The IGR, novaluron, is a benzoylurea insecticide (IRAC Group 15), a chitin synthesis inhibitor. It interferes with cuticle formation (Post et al. 1974), but the exact step where inhibition occurs is still unclear. However, several studies have demonstrated that inhibition of chitin may take place at the polymerization stage of chitin biosynthesis (Hajjar and Casida 1978) or in chitin precursor transport (Nakagawa and Matsumura 1994). Novaluron has been reported to have similar sublethal effects for several key pests of US crops (Wise et al. 2007a, Alokhin et al. 2008). While novaluron is known to have direct lethal activity on codling moth eggs (Wise et al. 2008), the extent of its sublethal effects on adult fecundity and egg viability and the effect of different modes of exposure have yet to be described. Yet, there is another possible explanation for novaluron's activity: putatively from

horizontal transfer when previously exposed adults contact unexposed eggs through contaminated tarsi or scales which has been documented with another IGR (pyriproxyfen) against mosquitoes (Chism and Apperson 2003).

The objectives of this research were (a) to determine the sublethal effects of novaluron on the fecundity and egg viability of codling moth following exposure through contact, ingestion, or topical spray, (b) to determine the duration of novaluron's sub-lethal effects following the three exposure regimes, (c) to determine the contribution of male and female exposure in the decrease in percent egg hatch, and (d) to determine if adult mediated horizontal transfer contributed to egg mortality

2. MATERIALS AND METHODS

2.1 Insect material

Codling moth pupae were obtained from the Yakima Agricultural Research Laboratory (Wapato, Washington), reared on an artificial diet, and conditioned in constant light for 24 – 48 h at 21 °C and 60% RH. Male and female pupae were separated according to their abdominal structure into 1-liter plastic containers (Peterson 1965). They were incubated at 21 °C, 60% RH and 16:8 h (L:D) photoperiod until adult eclosion. After adult emergence, adult moths were transferred into 1-liter containers and incubated according to the type of experiment.

2.2 Chemical material

There were two treatments. The IGR novaluron (Rimon 0.83EC by Chemtura U.S.A. Corporation, Middlebury, CT) was prepared to 0.155 g l^{-1} AI equivalent to labeled field rate of 145 g AI ha^{-1}. Distilled water served as an untreated control. Due to the inability of novaluron to fully homogenize in water, Latron B-1956 (a spreader and

sticker by Dow Agro Sciences LLC, Indianapolis, IN) was added at 0.038:1 liter of solution to the novaluron and control treatments.

2.3 Novaluron effects on fecundity and egg viability of codling moth following three modes of exposure

2.3.1 Ingestion exposure

Newly emerged moths (five each male and female) were placed in 1-liter plastic cages. Each cage was provisioned with a 30 ml plastic cup (SOLO cup company, Urbana, IL) containing 30 ml of treatment solution and a protruding cotton dental wick (TIDI® Products, Neenah, WI). The insects were incubated for 3 d. After this period, the insects were transferred into new containers that were internally lined with wax paper (33.5 cm × 13 cm) and the total number of eggs deposited on wax paper was recorded for 7 d. The wax paper was replaced every day, and eggs were incubated at 21 °C, 60% RH and 16:8 h (L:D) photoperiod for 14 d, after which the number of eggs hatched and unhatched were recorded. Treatments were replicated ten times in a randomized block design, with one replicate set-up per day over ten days.

2.3.2 Contact exposure

Prior to the start of this experiment, pieces of wax paper as previously described were sprayed until run-off with the novaluron solution or distilled water. The papers were left to dry in a fume hood for 2 h. Five male and five female newly emerged moths were placed into 1-liter plastic assay chambers that were internally lined with novaluron or water treated wax paper. Adult moths were exposed to the treated wax paper for 3 d. After 3 d, adults were transferred to clean chambers lined with clean wax paper and incubated for 7 d. The insects were incubated under the same conditions as the ingestion

bioassay using a 30 ml cup with a 20% sugar water mixture (w/w) as a food source. Each day the papers were replaced with fresh ones. The collected wax papers were incubated for 14 d, and the number of hatched and unhatched eggs was recorded. Treatments were replicated ten times as described above.

2.3.3 Topical spray exposure

Two-d old adult codling moths were used in this experiment. Five females and five males were transferred into wire mesh cages (5.4 cm in diameter by 9.3 cm long). The moths were then sprayed with 2 ml of novaluron or control solutions per cage using an airbrush sprayer (Model 200NH, Badger Co.). After each application, the moths were left to dry at room temperature for 24 h. The insects were transferred into 1-liter plastic assay chambers lined with wax paper. The moths were incubated under the same conditions as mentioned above with a 20% sugar water source for 7 d. The wax papers were changed daily and the collected papers were incubated 14 d, and the number of eggs hatched and unhatched was recorded. Treatments were replicated ten times as described above.

2.4 Duration of sublethal effects following three modes of exposure

In this experiment, the duration of novaluron's sublethal effect was evaluated following each exposure method. Each of the above mentioned exposure methods were repeated for the 12 d incubation period in this duration experiment. Four consecutive 3-d oviposition time periods were set (0-3 DAT, 4-6 DAT, 7-9 DAT and 10-12 DAT), and after each interval adults were transferred into new assay chambers with clean wax paper surrounding the sides. The eggs laid on the removed wax paper were then allowed to incubate for a total of 14 d, and the number of eggs hatched and unhatched was recorded.

2.6 Sex Treatments

2.6.1 Female only treatment

Newly emerged codling moth adults (1-3 d old) were used for this experiment. The female only experiment consisted of exposing five females per rep to novaluron by both contact and ingestion for 3d. The experiment consisted of 6 replicates. After 3 d, female moths were placed into clean 1-liter containers lined with wax paper with five males. Moths were allowed to mate and lay eggs for 7 d after which moths were removed and wax papers were collected. The collected eggs were incubated at 21 °C, 60% RH and 16:8 h (L:D) photoperiod for 14 d after which the number of eggs unhatched and hatched were counted.

2.6.2 Male only treatment

The age of the moths used and exposure method of novaluron was similar to the methods stated above. For the male treated experiment, only males were exposed and after the 3 d exposure period, moths were placed into the oviposition chambers with five females. The moths were allowed to mate and deposit eggs. Moths were incubated in the chambers for 7 d and then were removed for incubation of the eggs. The collected eggs were incubated at 21 °C, 60% RH, and 16:8 h (L: D) photoperiod for 14 d after which the number of eggs unhatched and hatched were counted. This experiment was replicated 6 times.

2.6.3 Male and female treatment

The third experiment consisted of treating both females and males for 3 d, and after the exposure period, moths were handled as previously described methods. The collected eggs were incubated at 21 °C, 60% RH, and 16:8 h (L: D) photoperiod for 14 d

after which the number of eggs unhatched and hatched were counted. This experiment was replicated six times.

2.7 7 Day Topical Exposure

2.7.1 Exposure of Eggs to Treated Adult Females

Five male and five female newly emerged (1-3 d old) codling moth adults were placed into 1-liter containers lined with wax paper for 7 d to allow for mating and subsequent egg deposition. After the 7 d oviposition period, the eggs were marked and adults were removed. Then five females that were exposed for 3 d to novaluron by contact and ingestion were placed into the container with the marked eggs. Egg exposure to the treated females was continued for an additional 6 d after which moths were removed and the control and treatment eggs on wax papers were incubated for an additional 14 days to allow 99% egg hatch. The experiment was replicated six times and the numbers of hatched and unhatched eggs were counted. Containers holding the treated moths were visually inspected every 12 h to verify that adults were contacting previously laid eggs. Any new eggs laid by novaluron exposed females were removed.

2.7.2 Exposure of Novaluron to Eggs after Seven Day Egg Deposition

For this experiment, five males and five females were placed into 1-liter containers lined with wax paper for 7 d to allow for egg deposition. After the 7 d period, adults were removed and the wax paper deposited with eggs was sprayed with novaluron until runoff (same concentration as previously stated). Novaluron residues/eggs were allowed to dry and eggs incubated for 14 d. The experiment was replicated six times and the number of hatched and unhatched eggs was counted.

2.8 Statistical analysis

All percentage data were arcsine square-root percent transformed prior to analysis to normalize the data. The effects of novaluron on fecundity of codling moths were tested with a paired t-test for each exposure method to detect differences in egg viability between treated and untreated moths.

Percent of egg hatch data for the duration of sub-lethal effects experiments were subjected to two-way ANOVA (treatment and time period as the main effects) for each exposure method. When significant interaction between time and treatment was detected, the data were then subjected to paired t-test for each time period. All statistical analyses were carried out using the SAS statistical programme (SAS 2002).

Effects of different exposure methods for both sexes on egg hatch were submitted to one way analysis of variance and the Tukey's multiple comparison test (SAS 2002). Differences in percent egg hatch between treated and untreated were analyzed after arcsine transformation (acrsine x percent hatch) of the data. Novaluron effects on fecundity were analyzed using one-way analysis of variance followed by Tukey's multiple comparison test for the sex treatments. All statistical analyses were carried out using the SAS statistical programs (SAS 2002).

3. RESULTS

3.1 Novaluron effects on fecundity and egg viability of codling moth following three modes of exposure

The fecundity of codling moth was not significantly affected by novaluron for any of the exposure methods ($P > 0.1$) (Table 2.1). The egg viability resulting from novaluron treated adults was significantly lower than in the control for all exposure methods (Figure 2.1). Difference in egg viability was observed in the topical spray exposure method

where the mean hatch rate was 70 percent lower in the treatment than in the control ($t = 17.03$, $df = 18$, $P < 0.00$). The results from ingestion and contact exposure methods exhibited mean hatch rates that were 55 percent lower in the treatments than in the controls (ingestion: $t = 7.85$ $df = 18$, $P < 0.00$; contact: $t = 13.42$, $df = 18$, $P < 0.00$).

Treatment	Contact Exposure	Ingestion Exposure	Topical Spray Exposure
	# Eggs / ♀	# Eggs / ♀	# Eggs / ♀
Novaluron	19.17 ± 3.65 a	16.75 ± 5.49 a	19.25 ± 2.51 a
Control	25.07 ± 5.17 a	19.46 ± 3.51 a	21.95 ± 2.18 a

Table 2.1. Fecundity of *Cydia pomonella* adults (Mean ± SEM) and percent (%) egg hatch (Mean ± SEM) following the treatment of adults with novaluron using three different exposure methods, n=10. Means in column followed by a different letter are significantly different (paired t-test, P<0.05).

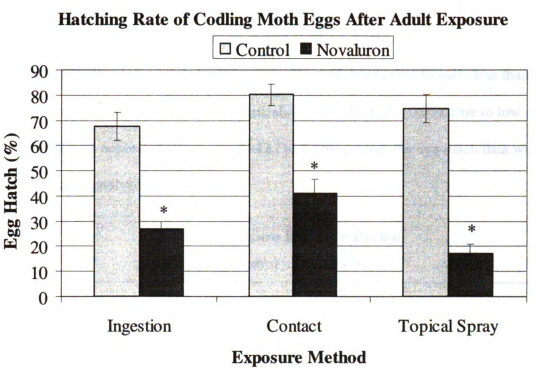

Figure 2.1. Percent hatch (mean ± SEM) following the treatment of adults with novaluron using three different exposure methods, n=10. Columns with * are significantly different from the control (paired t-test, $P < 0.05$)

3.2 Duration of sublethal effects following three modes of exposure

The duration of novaluron sublethal effects varied depending on the exposure method and the ovipositon time period (Fig 2.2-2.4). While egg deposition patterns were uniform between treated and untreated cohorts throughout the study, there was a gradual decline in the total numbers of eggs laid across the four time periods (combined average eggs deposited per replicate were: 92 in the 0-3 DAT period, 50 in the 4-6 DAT, 10 in the 7-9 DAT, and 4 in the 10-12 DAT). The codling moths subjected to contact exposure of novaluron (Fig 2.2) laid significantly lower numbers of viable eggs ($F= 81.52$, $df= 1, 42$, $P < 0.00$), and sublethal effects persisted for 9 d. There was a significant interaction between treatments and time period ($F= 3.65$, $df= 2, 42$, $P < 0.05$). Significant differences

between the novaluron treatment and the control were recorded at the 0-3 DAT period (t= 6.17, df=18, P < 0.00) and the 4-6 DAT period (t= 6.14, df=16, P < 0.00). The hatch rate of eggs laid by novaluron treated moths was 12%, which was significantly less than that achieved in the control at the 7-9 DAT period (t= 5.88, df=8, P < 0.01). Due to low numbers of eggs deposited during the 10-12 DAT time period, the egg hatch data were not statistically analyzed.

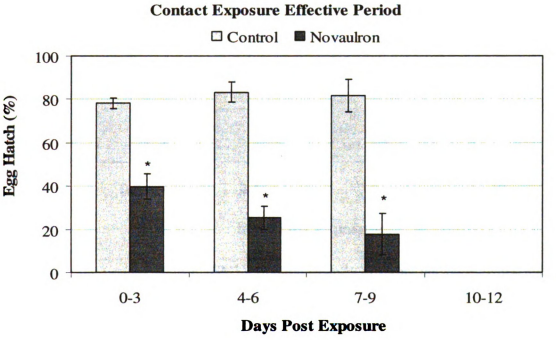

Figure 2.2. Hatch rates (mean ± SEM) were significantly different between treated and control (paired t-test, P<0.05) when marked with *. Statistical analysis was not conducted for data during 10-12 d time period (no data collected).

Ingestion exposure of codling moth to novaluron caused a significant reduction in egg viability, and this sublethal effects persisted for 9 d (F= 216.23, df= 1, 49, P < 0.00) (Fig 2.2). The interaction between the treatments and the time period was also significant (F= 5.15, df= 2, 49, P < 0.05). The differences were significant at each time period: 0-3 DAT (t= -13.50, df=8, P < 0.00), 4-6 DAT (t= 11.24, df=18, P < 0.00), and 7-

9 DAT (t= 5.84, df=13, P < 0.00). Due to low numbers of eggs deposited during the 10-12 DAT time period, the egg hatch data were not statistically analyzed.

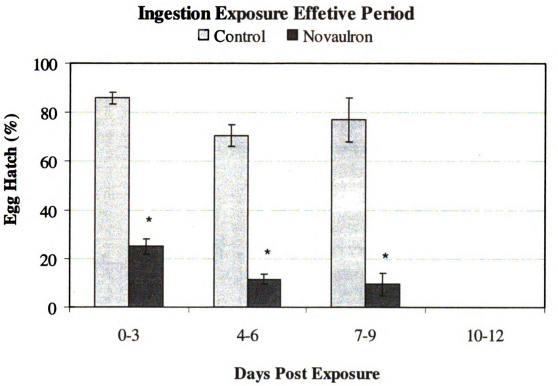

Figure 2.3. Hatch rates (mean ± SEM) were significantly different between treated and control (paired t-test, P<0.05) when marked with *. Statistical analysis was not conducted for data during 10-12 d time period (no data collected).

Topical spray exposure of codling moths to novaluron resulted in significant reduction in egg viability throughout all time periods (F= 622.99, df= 1, 46, P < 0.00), and the interaction between treatments and time period was significant (F= 4.76, df= 2, 46, P < 0.05). Significant differences between the treatment and the control were recorded at 0-3 DAT (t= 14.56, df=18, P < 0.00), 4-6 DAT (t= 15.84, df=18, P < 0.00), 7-9 DAT (t= 18.03, df=10, P < 0.00) and 10-12 DAT (t= 7.31, df=7, P < 0.00) (There was a replicate average of 11 eggs laid for the 10-12 DAT cohort, thus analysis was conducted.) Even though egg viability was significantly lower than the control throughout the study

periods, the intensity of the effect diminished after 6 d (Fig 2.3). The lowest percentage egg hatch was 8.1 percent at period 4-6 DAT and increased to 12.2 percent and 19.1 percent at 7-9 and 10-12 DAT respectively. It is not absolutely clear whether or not the lower egg deposition in these later time periods may also have contributed to the appearance of recovery from novaluron effects.

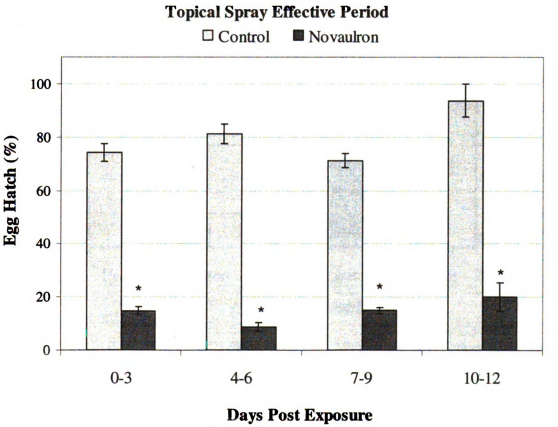

Figure 2.4. Hatch rates (mean ± SEM) were significantly different between treated and control (paired t-test, $P<0.05$) when marked with *.

3.3 Sex treatments

Results obtained from these replicated experiments where reduced fertility associated with transfer of novaluron via the female or male adult moths is presented in Figure 2.4. Eggs laid by treated females only, demonstrated that average egg mortality

was 90 ± 0.24% which was highly significant (t = 19.19, df = 10, p < 0.0001). Eggs laid after treated males were mated with untreated females was also significant (t = 2.6, df = 10, p = 0.0264), but exhibited an average of only 76 ± 1% egg mortality. When females and males were exposed to novaluron, the results were also highly significant (t = 21.45, df = 10, p < 0.0001) and similar to the previous female-only treatments.

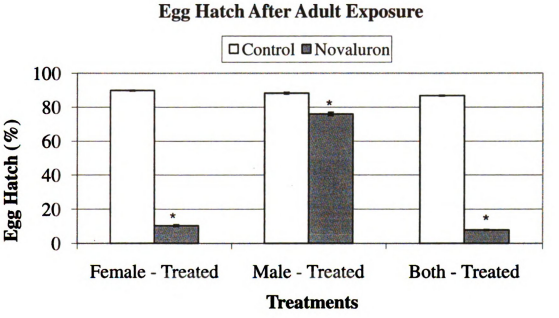

Figure 2.5. Mean percent hatch of codling moth eggs after different sex treatments. The hatch rate (mean ± SEM) with * is significantly different from the control (paired t-test, p < 0.05). Egg hatch rates are means of six replicates

These differences were not due to differential fecundity as compared to female fecundity (Table 2.2). Thus fecundity of female moths through different novaluron exposure mechanisms (both male & female treated, female treated alone, or male treated alone before copulation) revealed no significant difference (t = 1.00, df = 10, p = 0.3427; t = 0.83, df = 10, p = 0.4264; t = 1.74, df = 10, p = 0.1117).

Treatment	Female Treated	Male Treated	Both Treated
	# Eggs	**# Eggs**	**# Eggs**
Novaluron	221.7±38.7a	239.0±33.4a	199.3±39.2a
Control	183.3±25.4a	156.0±33.9a	156.5±17.7a

Table 2.2. Mean number (±SEM) of eggs laid by codling moth adults. Means in columns followed by different letters indicates significance (paired t-test, $p < 0.05$).

3.4 7 Day Topical Exposure and Horizontal Transfer

Eggs exposed to Novaluron treated females showed no significant difference in hatch (t = -0.33, df = 10, p = 0.7451). In contrast, topical application of Novaluron to already deposited eggs resulted in a significant reduction in hatch in the 7 day topical application treatments (Figure 2.5). Hatch of untreated eggs deposited on wax paper,that were held for 7 d and subsequently sprayed with novaluron was significantly recuded compared to control (t = 4.5, df = 10, p = 0.011).

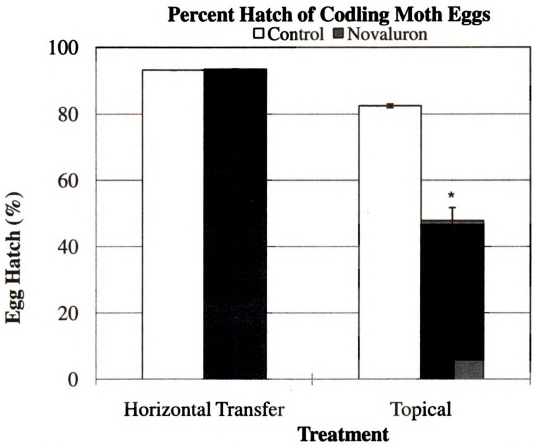

Figure 2.6. Mean percent hatch of codling moth eggs after different exposure methods. The hatch rate (mean ± SEM) with * is significantly different from the control (paired t-test, $p < 0.05$). Egg hatch rates are means of six replicates.

4. DISCUSSION

Novaluron treatment of codling moth adults using different exposure techniques did not affect the fecundity of this pest. Similar results were reported by Alyokhin et al. (2008), who did not observe any reduction in fecundity of *Leptinotarsa decemlineata* (Say) after feeding on novaluron treated foliage. Kostyukovsky and Trostanetsky (2006) also reported that novaluron treatment did not affect egg laying of *Tribolium castaneum*.

Novaluron treatment of codling moth adults, however, did result in significantly reduced egg viability following contact, ingestion and topical exposure. Similar

reductions in egg hatch of *Conotrachelus nenuphar* (Herbst) (Wise et al. 2007) and *Leptinotarsa decemlineata* (Say) (Alokhin et al. 2008) resulting from adult exposure to novaluron were reported by other researchers. The results of this study do not elucidate the mechanism(s) responsible for this effect of novaluron, but other studies of benzoylurea insecticides suggest contributions from physiological effects on female ovaries and basal oocytes, and transovarial movement into larvae and eggs (Soltani and Soltani-Mazouni 1992, Kostyukosky and Trostanetsky 2006).

The sublethal effects of exposure to novaluron persisted throughout most or all of the measured time periods, but the inherent viability of adult test subjects was a limiting factor in testing the duration of these effects. Following the novaluron topical spray exposure, relative increases in codling moth egg hatch were observed after 6 d, indicative of a diminishing effect, but egg viability remained significantly lower compared to the controls. Kostyukovsky and Trostanetsky (2006) reported novaluron's effects on the fertility of *Tribolium castaneum* as time dependent, and the compound prevented total egg hatch up to 4 weeks at 100 mgL^{-1} dose. This suggests that for species like the codling moth, with relatively short-lived adult stages, the compound may remain active long enough to prevent recovery. Similar persistence of other IGRs in hemolymph of codling moth was reported by other researchers (Retnakaran et al. 1995, Sun et al. 2003a, Sun et al. 2003b).

The horizontal transfer experiment results suggest that the mechanism for the reduced viability of eggs is not horizontal transfer between adult and egg. However, results from the different sex exposures have suggested that not only is there an effect through female exposure, but male exposure results in a significant decrease in egg

viability, demonstrating that this could be a physiological and/or transovarial transmission affect of novaluron. The exact physiological or morphological path that the compound takes in the adult moth is yet to be determined. More research is needed to understand the mechanism for how male and female exposed moths contribute to the eventual toxicity of the eggs.

Lethal and sublethal effects of IGRs on adult Lepidopteran pests are generally tested using a contact exposure method (Soltani and Soltani-Mazouni 1992, Waldstein and Reissig 2001). In addition to the contact bioassay method, however, ingestion and topical spray exposure methods should be investigated in order to capture the most complete understanding of environmental interaction of the pest and the insecticide. Contact and topical spray activity of novaluron against glasshouse whitefly was reported by Ishaaya et al. (1998) who explored increased potency by adding other actives. The three treatment methods used in our study help to provide a more complete reflection of the cumulative exposure for codling moth in a natural system.

To date, novaluron's direct lethal activity on codling moth eggs has been the primary basis for establishing the optimal spray timing in apple IPM programs (Wise et al 2008). Results from some field efficacy trials, however, suggest that there is very little difference in fruit protection when sprays target the beginning of egg laying (codling moth biofix plus 50 DD_{50}) compared to a later timing (codling moth biofix plus 150 DD_{50}) (Wise et al. 2004). The early spray timing targets the start of codling moth oviposition with eggs being laid on top of novaluron residues, thereby maximizing the compound's ovicidal activity. With the later spray timing, there would be exposure of a greater number of gravid females to fresh residues, as well as more larvae exposed as

they hatch from eggs covered with residues. Thus, the fruit protection observed from the later treatment timing is a combined result of novaluron's direct ovicidal and larvacidal activity and sublethal effects on exposed adults. Even though novaluron's ovicidal activity will likely remain the primary factor for optimizing spray timing, the contribution of this compound's sub-lethal mode of activity should also be considered.

With the phase-out of azinphosmethyl in the US following the FQPA and the incidence of codling moth resistance to OPs (Varela et al. 1993, Knight et al. 1994, Dunley and Welter 2000, Reuveny and Cohen 2004, Mota-Sanchez et al. 2008, Whalon et al. 2008), insecticides with novel modes of action appear to be a good alternative for IPM programs. Successful incorporation of new compounds, like novaluron, into pest management programs will require effective education efforts with fruit growers and careful consideration of optimal program partnering to cover the spectrum of insect pests. Further research is needed to fully understand the mechanisms responsible for novaluron's sublethal effects seen in this study. In addition, field studies are needed to understand the extent of these effects under field conditions.

CHAPTER 3

REDUCED VIABILITY OF CODLING MOTH EGGS AFTER ADULT EXPOSURE TO FIELD AGED RESIDUES

1. INTRODUCTION

A range of tactics are available for codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) control, including organophosphates, insect growth regulators (IGR), and pheromone based mating disruption. However, the use of organophosphates will be phased out by 2012 (US EPA, 2006) as a result of the promulgation of the Food Quality Protection Act (FQPA, 1996) in the US, which placed organophosphates, carbamates, and pyrethroids under intense scrutiny. In addition to the curtailing and phase out of these pesticides, codling moth control has been hindered by the development of resistance to key insecticides, including azinphosmethyl (Varela et al. 1993, Knight et al. 1994, Dunley and Welter 2000, Reuveny and Cohen 2004, Mota-Sanchez et al. 2007, Whalon et al. 2008). Both the FQPA and resistance have driven the search for new alternatives to control this major pest of pome fruits in the US and throughout the world.

The use of insect growth regulators (IGR) in IPM systems in pome and stone fruit production in the Midwest and Eastern portions of the US has been identified in the USDA Pest Management Strategic Plans for pome fruit as an important option for the sustained production of these crops (Baniecki and Dabann 2003, Alston et al. 2006). There have been several studies showing the lethal and sublethal effects of different IGR's on a variety of crops and insect species. Examples of these IGR's include tebufenozide (Pons et al. 1999, Sun et al. 1999), methoxyfenozide (Sun et al. 1999,

Pineda et al. 2006), pyriproxifen (Eliahu et al. 2007), and novaluron (Cutler et al. 2005, Kostyukosky and Trostanetsky 2006, Wise et al. 2007a).

Among the numerous IGR's marketed as "reduced risk" (US EPA 1997), benzoylureas have a unique mode of action that is commonly referred to as chitin synthesis inhibition (Post et al. 1974). The exact step where chitin formation inhibition is unclear, but studies by Hajjar and Casida (1978) demonstrated that inhibition may take place at the polymerization stage. Studies by Nakagawa and Matsumura (1994), have also demonstrated that inhibition may occur in the chitin precursor transport stage. The majority of studies with benzoylureas on codling moth have been with diflubenzuron (Elliot and Anderson 1982, Soltani and Soltani-Mazouni 1992, Casa-Giner et al. 1999). Novaluron (IRAC Group 15) is one of the newer benzoylureas marketed for tree fruit production. Like other compounds in the group, novaluron has been targeted for codling moth control based on its larvicidal and ovicidal properties. However, according to previous laboratory morbidity experiments where codling moth adults, significant reduced egg viability was observed after exposure to novaluron (Gökçe et al. 2009). These laboratory results suggested that novaluron could be used in field settings where females are exposed without mortality, but where high mortalities are incurred via the sublethal effects.

The objectives of this study of novaluron effects on codling moth were to determine (a) if reduced egg viability observed in previous laboratory experiments will be observed following exposure to field aged residues and (b) determine the contribution of leaf and fruit residues to sublethal effects over time.

2. MATERIALS AND METHODS

2.1 Insect material

Codling moth pupae were obtained from the Yakima Agricultural Research Laboratory (Wapato, Washington), reared on artificial diet, and conditioned in constant light for 24 – 48 h at 21 °C and 60% RH. Male and female pupae were separated according to their abdominal structure into 1-liter plastic containers (Fabri-Kal, Kalamazoo, MI) (Peterson 1965). They were incubated at 21°C, 60% RH and 16:8 h (L: D) photoperiod until adult eclosion.

2.2 Chemical material

The IGR novaluron (Rimon 0.83EC Chemtura U.S.A. Corporation, Middlebury, CT) was prepared to 0.155 g l^{-1} AI equivalent to labeled field rate of 145 g AI ha^{-1}, and distilled water to serve as an untreated control. Due to the inability of novaluron to fully homogenize in water, Latron B-1956 (a spreader and sticker by Dow Agro Sciences LLC, Indianapolis, IN) was added at 0.038:1 liter volume to the novaluron and control solutions.

2.3 Exposure material

Exposure containers were constructed using 1-liter plastic containers with 3.5cm of water saturated flora foam (Smithers-Oasis Company, Kent, OH) along the bottom of the container. One disposable Pasteur pipette (VWR Scientific Products) was placed inverted 1-2 cm into the flora foam, and 1cm of paraffin wax was pour onto of the flora foam. Once the wax cooled and hardened, the pipette was removed. Lids for the 1-liter

exposure containers were modified with 5 cm x 5 cm hole with screen mesh taped to the lid. Once all containers were made they were set aside until use and before usage the flora foam was rewetted using a funnel and water to account for any moisture that had evaporated.

2.4 Field based bioassay

Apple terminals (Red Delicious, 9 yr old, picked at random) containing fruit or leaves were collected after application from trees located at the Trevor Nichols Research Station in Fennville, Mi. Six trees were marked as controls and another six were treated with novaluron on May 18, 2007 with a FMC 1029 airblast sprayer calibrated to deliver 935.3 liters of water per ha (100 gal / acre).

Excised terminals were separated into those comprised of leaves or fruit only, immediately placed into the exposure containers and placed in coolers at 8°C, for transport back to the laboratory (approximately 90 minutes). Adult codling moths, 1-3 d old (5 female and 5 males), were placed into the containers and incubated at 21°C, 60% RH and 16:8 h (L:D) photoperiod for three days before all the moths were transferred to oviposition containers; oviposition containers were made out of a 1-liter plastic containers lined with wax paper (33.5 x 13 x 56.75 cm), fitted with a 1oz soufflé cup with a 20% sugar water solution, and sealed with a fitted lid (SOLO cup company, Urbana, IL) with a dental wick (TIDI® Products, Neenah, WI) protruding from the top. Females were allowed to lay eggs for 6 d, after which all adults were removed and wax papers were incubated (16:8 photoperiod at 22 °C) for an additional 12 d until egg hatch. Egg eclosion was assessed by examination using a Nikon SMZ1000 (Mager Scientific,

Inc., Dexter, MI) stereomicroscope and segregated into the number of hatched and unhatched eggs. Each trial was replicated 6 times per collection date.

2.5 Residue profiles

A series of fruit and foliage samples were taken from field plots at each of the four post-application timings (0 d, 7 d, 14 d, and 21 d). Samples of approximately 25 leaves and 25 fruit (minimum 10 g each) were placed into a 4 oz glass bottle (The Glass Group Inc., Park Hills, Missouri), placed in a cooler with ice packs, and transported to the Michigan State University Pesticide Analytical Laboratory in East Lansing, MI. A pesticide extraction procedure as described in Wise et al. (2006) was used to separate dislodgeable residues on the surface of the fruit and leaf samples from the subsurface residues (in the plant cuticle and internal tissues). Surface and subsurface residues over time were quantified by GC analysis of the extracted tissues.

To determine the amount of residue on the fruit and leaf surfaces, replicate samples of apple fruit and leaves were placed in 150 ml of acetonitrile and sonicated for 10-15 s. The acetonitrile was decanted through 5 g of anhydrous sodium sulfate to remove all water. The sample was dried via rotary evaporation and brought up in acetonitrile for high-performance liquid chromatography (HPLC) or gas chromatography / mass spectrometry (GC/MS) analysis.

To determine the residue from the fruit and leaf sub-surfaces, the remaining solid fruit and leaf samples were ground with 200 ml of dichloromethane. The extracts were then vacuum-filtered, and the filtrate was passed through 5 g of anhydrous sodium sulfate.

The samples were dried via rotary evaporation and brought up in acetonitrile. Any remaining particulates were removed by passing the sample through a 0.45 μm filter.

Novaluron samples were analyzed using GC/MSD (Agilent 6890 gas chromatograph with a 5973N MSD) equipped with a Zebron ZB-5ms 30 m, 0.25 mm I.D. column and a 0.25 μm film thickness. The GC/MSD settings for analysis were as follows: the oven was held at 115 °C for 5 min with a ramp of 9 °C per min to 280 °C, followed by 30 °C per min to 310 °C. The inlet was held at 200 °C in a pulsed splitless mode with 78324 Pa and a pulse pressure of 103421 Pa, with a purge flow of 50.0mL per min of helium gas. The MSD transfer line was held at 285 °C. The MSD was set to scan 28–535 Da. The injector was rinsed 3 times with acetone and 3 times with dichloromethane before each injection to eliminate contamination between injections. All compounds were quantitated against a standard curve, and recovery data were recorded. Level of detection (LOD) and level of quantitation (LOQ) recoveries ranged from 50 to 150 percent.

2.6 Statistical analysis

Egg hatching percentages recorded in the field bioassay were normalized using an arcsine square-root transformation and then subjected to ANOVA with means separations (PROC MIXED, SAS Institute 2002). If the significant interactions between time and treatment were detected, the data was subjected to paired t–tests for each time period (PROCTTEST), to check for statistical segregation of treatments.

To determine the relationship between sublethal effects on codling moth and novaluron residues, correlation analysis was performed on moth egg hatch data from the four post-application residual activity bioassays and the novaluron residue data for each

sample date (PROC CORR). Regression analysis (PROC REG) was used to determine the relationship between the amount of surface and subsurface residues of leaves and fruit and codling moth egg hatch.

3. RESULTS

3.1 Field bioassay

Exposure of adult codling moth to terminals collected from apple trees showed varying significance in the effect of novaluron on egg hatch (Figure 3.1, 3.2). There was a decrease in the percentage hatch of codling moth eggs laid by adults exposed to terminals treated with novaluron in both fruit ($p = 0.0005$, df = 10, t = 4.99) and leaves ($p = 0.0001$, df = 10, t = 6.07) at 0 d. At 7 d, only fruit terminals showed a significant difference compared to the control ($p = 0.0073$, df = 10, t = 3.36). At day 14, both fruit terminals and leaf terminals showed no significance (there were reduced reps in this test). Results from day 21 showed no significance for leaf terminals, but significance was seen for moths exposed to fruit terminals ($p = 0.0106$, df = 10, t = 3.14). Exposure to surface residues of novaluron yielded results similar to those achieved in laboratory trials conducted only on day 0 for both fruit and leaves and on day 7 for fruit.

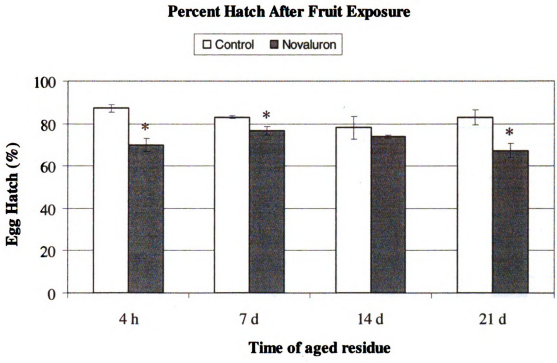

Figure 3.1. Mean percent hatch of codling moth eggs after exposure to field aged residues on fruit only surfaces. Hatch percent (mean ± SEM) with * is significantly different from the control (paired t-test, $p < 0.05$). Egg hatch rates are means of six replicates.

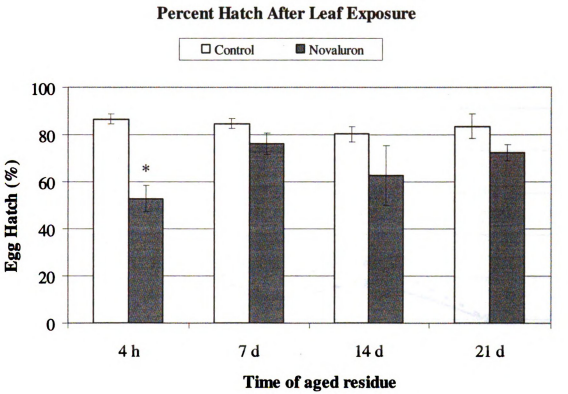

Figure 3.2. Mean percent hatch of codling moth eggs after exposure to field aged residues on leaf only surfaces. Hatch percent (mean ± SEM) with * is significantly different from the control (paired t-test, $p < 0.05$). Egg hatch rates are means of six replicates.

3.2 Residue sampling

Residue profile analysis was conducted on both apple leaves and fruit, and samples were taken at the same time terminals were collected for exposure experiments. The surface residues collected for both leaves and fruit showed high levels of novaluron at 0 d with a gradual decrease from 0 d to 14 d, then a more dramatic decline at 21 d

(Figure 3.3).

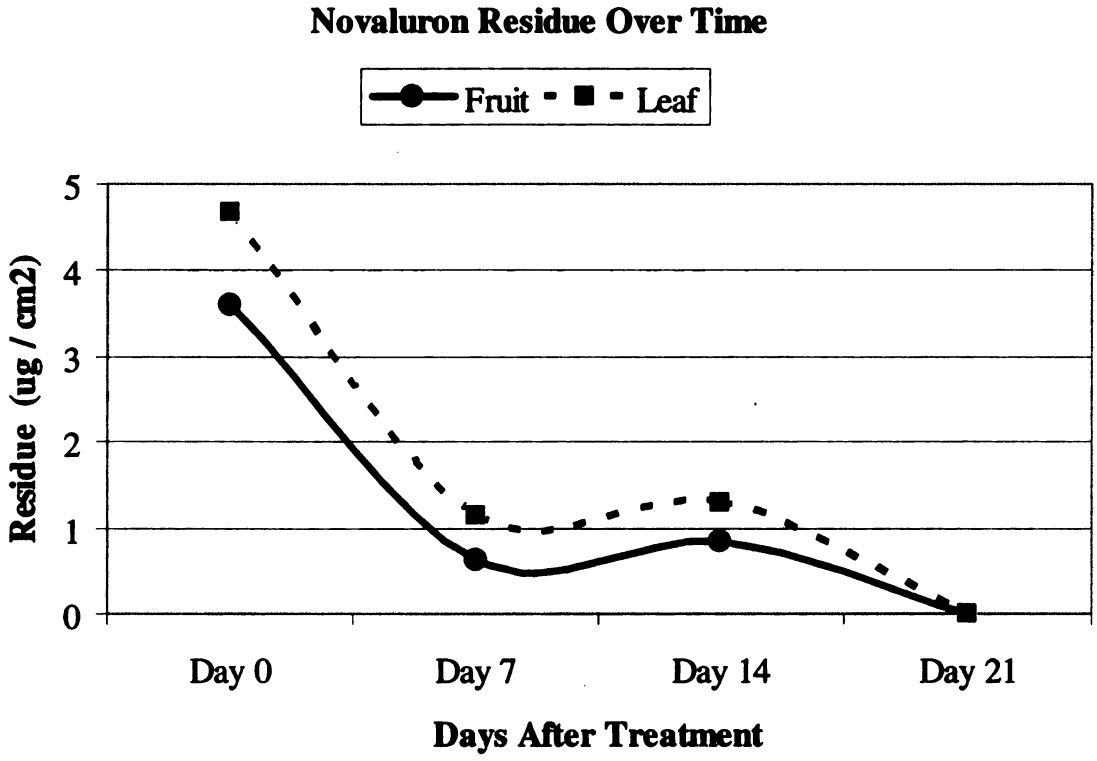

Figure 3.3 Residue profile analysis of leaf and fruit surface residues at different time periods after application.

Regression correlation (r^2) of fruit and leaf surface residue with codling moth fertility showed strong relationships. For the surface residues of leaves, there was a significant negative correlation between overall surface residues and percent hatch ($r^2 = 0.33$, $p = < 0.0001$). When surface residues were analyzed with percent hatch at each time interval, significance was seen only at 0 d ($r^2 = 0.60$), this could result in the low r^2 value that was observed when overall data was analyzed. Surface residues of fruit demonstrated a significant negative correlation between residues and percent hatch ($r^2 = 0.16$, $p =$

0.0104) as well. Surface residues analyzed with percent hatch for each time interval for fruit showed similar results as those seen from leaves. Significance was only seen at 0 d and 7 d (r^2 = 0.65 and 0.51 respectively). This suggests that both fruit and leaf residue exposures are important in inducing sublethal effects from novaluron

4. DISCUSSION

The objective of this research was to determine the effects of novaluron on codling moth egg viability after adult exposure to field aged residues. The main method of exposure for adults in this experiment was contact with leaf and fruit surfaces. Exposure of codling moth to leaf-only substrates showed significance at only 0 d, while exposure to fruit-only substrates showed significance at 0 d, 7 d, and 21 d. Exposure of colding moth to fruit at 14 d did not show significance. This can likely be attributed to a decrease in the number of repetitions used to conduct the bioassays. Insufficient newly emerged codling moths were available to complete the full 6 repetitions. Results from field bioassays conducted have shown similar results to laboratory results obtained by Gökçe et al. (2009). Similar results of reduced egg viability were seen in *Conotrachelus nenuphar* (Herbst) (Wise et al. 2007), *Leptinotarsa decemlineata* (Say) (Alokhin et al. 2008), and *Tribolium castaneum* (Herbst) (Kostyukovsky and Trostanetsky 2006).

Results from the residue profile analysis demonstrated that novaluron is present on both leaf and fruit surfaces up to 14 d and then decreases dramatically at 21 d. There were minimal amounts of precipitation during the time intervals when terminals were collected: 7.36 mm (0 d – 7 d), 13.46 mm (7 d – 14 d), and 33.52 mm (14 d – 21 d) (Michigan Automated Weather Network). Thus, the reduction in novaluron residues was due to degradation of the compound rather than wash off. Leaf surface provided more

residue ($\mu g/cm^2$) than fruit surfaces, but only exposure to fruit surfaces caused a significant decrease in egg viability beyond 0 d. Possible factors that explain why fruit exposure causes more sublethal effects can be: a) actual moth contact time on fruit vs. leaves may have been different, b) behavior of moths on fruit vs. leaves may be different, or c) dislodgeable residues pick-up by adults may be different on fruit vs. leaves, even if residue $\mu g/cm^2$ were similar. Studies conducted on attractiveness of codling moth to leaves vs. fruit have demonstrated a higher attractiveness towards fruit (Hern and Dorn 1999). The α-farnesene found in fruit has been the key chemical leading to the attractiveness (Yan et al. 2003, Witzgall et al. 2005). This compound has been shown to increase oviposition behavior of female codling moths after exposure. This attractiveness towards fruit is another possible explanation towards the lower egg viability after fruit-only exposure.

Novaluron has generally been used as a larvicide and ovicide for codling moth. However, results obtained from this study and laboratory studies by Gökçe et al. (2009) have demonstrated adult exposure as an alternative method for codling moth control. Although reductions in egg viability were not as dramatic as laboratory trials, the occurrence of sublethal effects is a promising result for the later spray timings for this compound. Novaluron application is recommended at 50 – 100 DD_{50} after codling moth biofix. This timing is used to allow for novaluron presence on the surface of fruit and leaves before egg laying begins. However, later spray timings can provide exposure to greater number of adults along with exposure of novaluron to newly hatched larvae. The later timing will increase residue concentration on fruit by minimizing effects of growth

dilution, thus maximizing fruit surface effects. The later spray timing can encompass both the sublethal effects as well as the larvicidal activity.

With no known direct toxicity to adult natural enemies, novaluron is a highly suitable organophosphate replacement/alternative in an IPM program (Suh et al. 2000, Cloyd and Dickinson 2006). However, additional research into the behavior of codling moth when exposed to treated leaf- or fruit-only surfaces is needed to provide further evidence for discrepancies seen between the two exposure methods. Research into spray timings that allow for effective control of codling moth, along with coverage for other pests present in the orchard at similar times should be investigated as well. Also research into the exact location to which novaluron is transferred once exposed to adult females should be investigated.

CHAPTER 4

Reduced Fertility and Transovarial Transmission by Obliquebanded Leafroller, *Choristoneura rosaceana* (Harris), after Exposure to Novaluron

1. INTRODUCTION

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris), has become a significant pest of tree fruit production in North America within the past 20 years. This moth is a polyphagous insect, usually with two generations per year that feeds on more than 50 different species (Sanderson and Jackson 1909, Howitt 1993). Larvae are external feeders of flowering buds, leaves, and fruit surfaces (Howitt 1993). Early season damage from overwintering larvae can cause fruit drop or deformed fruit. Late season damage causes shallow or deep holes on the surface of the fruit (Stelinski et al. 1996). Control of obliquebanded leafroller has generally relied upon broad-spectrum insecticides like organophosphates (Reissig 1978, Howitt 1993). However, with the impending phase-out of organophosphates by the Food Quality Protection Act (Anonymous 1996) and evidence of resistance development (Reissig et al. 1986, Carriére et al. 1996, Lawson et al. 1997, Ahmad et al. 2002, Smirle et al. 2002), newer chemistries have been developed to combat this pest.

Among the newer control tactics being implemented, insect growth regulators (IGR) fit well into Integrated Pest Management (IPM) programs due to reduced potential for environmental and ecological impacts. These compounds are a class of chemistries that alter the growth and development of the targeted pest. Insect growth regulators have been primarily used to target the larval and egg stages of codling moth (*Cydia pomonella* (L.)) and leafroller species (Tortricidae) based on the direct toxic effects to these stages

seen in other Lepidopteran pests (Ishaaya et al. 1996, 1998, 2003). Along with strong toxic effects on eggs or larvae, insect growth regulators demonstrate sublethal effects on a variety of different pests, including Lepidoptera, Coleoptera, and Diptera (Casa-Giner et al. 1999, Knight 2000, Charmillot et al. 2001, Cutler et al. 2005, Pineda et al. 2006, Kostyukosky and Trostanetsky 2006, Wise et al. 2007, Gökçe et al. 2009).

Within the insect growth regulator class are the chitin synthesis inhibitors that prevent the insect from undergoing proper chitin synthesis after the molting process (Post et al. 1974); however, the exact step where inhibition occurs is still unclear. Inhibition of chitin may take place at the polymerization stage of chitin biosynthesis (Hajjar and Casisa 1978) or in chitin precursor transport (Nakagawa and Matsumura 1994). The chitin synthesis inhibitors have minimal effects on parasitoids and other natural enemies (Ishaaya 1990, Ishaaya et al. 2001). Diflubenzuron is a chitin synthesis inhibitor that has sub-lethal activity on codling moth resulting in reduced viability of the eggs laid by treated adults (Hoying and Riedl 1980, Elliot and Anderson 1982).

Novaluron, a recently registered insect growth regulator, has similar sublethal effects when adults are exposed (Cutler et al. 2005, Kostyukovsky and Trostanetsky 2006, Wise et al. 2007a, Gökçe et al. 2009). Novaluron is a strong control agent towards Lepidoptera by both ingestion and contact (Ishaaya et al. 2007). It has strong toxic effects on the eggs and larvae of obliquebanded leafroller (Wise et al. 2007b); however, sublethal effects from adult exposure have yet to be reported. Delivery of novaluron that results in lowered egg viability can be explained by three mechanisms; a) physiological, b) transovarial transfer, or c) horizontal transfer. Although studies with novaluron on physiological mechanisms have yet to be conducted, studies with other benzoylurea

insecticides suggest effects on female ovaries and basal oocytes as possible explanations for the reduced egg viability (Soltani and Soltani-Mazouni 1992). Experiments by Gökçe et al. (2009) suggest that the sublethal effects seen in codling moth may be due to transovarial transmission. However, another possible explanation for how novaluron is delivered to obliquebanded leafroller eggs is from horizontal transfer, when previously exposed adults transfer the toxin by tarsal contact with clean eggs, which was seen with another insect growth regulator (pyriproxyfen) in mosquitoes (Chism and Apperson 2003).

The objectives of this study were (a) to determine the sublethal effects of novaluron on the egg viability of obliquebanded leafroller following adult exposure, and (b) to detect the presence of novaluron in egg masses laid by treated females as evidence for transovarial transmission.

2. MATERIALS AND METHODS

2.1. Chemicals

Novaluron (Rimon 0.83 EC, Chemtura U.S.A. Corporation, Middlebury, CT) was prepared according to the recommended field rate (0.24 g liter^{-1} active ingredient equivalent to a field rate of 224 g active ingredient ha^{-1}). Latron B-1956 (a spreader and sticker from Dow Agro Sciences LLC, Indianapolis, IN) was added at 0.038:1 vol:vol to novaluron to fully homogenize the novaluron and to control solutions.

2.2. Insects and Bioassay Materials

Obliquebanded leafroller from a laboratory colony, originally collected from unsprayed apple orchards in southwestern Michigan in 2000, was used for the following experiments. The colony was reared on a modified pinto bean diet (Shorey and Hale 1965) at 23 ± 1 °C, 60% RH, and 16:8 h (L:D) photoperiod. Pupae were collected, sexed, and placed into 1-liter plastic containers until eclosion. Adults were provided with a 10% sucrose solution in a 30 ml soufflé cup with a dental wick protruding from the top.

Adults (1-3 d old) were used. Experiments were replicated six times for the bioassays and four times for the residue studies. Each replicate consisted of five females and five males for the bioassays and 30 males and 30 females for the residue studies. Wax paper was spreayed with either the novaluron or control solution until drip using a handheld sprayer and allowed to fully dry before lining a clean 1-liter plastic container. Exposure containers also contained a 30 ml soufflé cup with a dental wick protruding from the lid filled with novaluron solution or the control solution. Adult moths were incubated in exposure containers for 3 d and then transferred to untreated containers for egg deposition. Egg masses collected for the bioassays were placed into petri dishes sealed with parafilm and incubated at 23 ± 1 °C, 60% RH, and 16:8 (L:D) h photoperiod for an additional 14 d.

2.3. Effects of Adult Exposure to Novaluron on Egg Survival

Exposure of adult moths was carried out as mentioned above, and then adults were transferred to clean 1-liter containers lined with clean wax paper for egg deposition. Moths were allowed to lay eggs for 7 d. After the 7 d egg laying period, egg masses were photographed, to estimate size, and an area measurement was taken using NIS Elements

software (Nikon). The numbers of eggs per egg mass was estimated using: -86.483 + 699.524x, where x = egg mass area (Sun et al. 2000). Egg masses were incubated as previously stated, and petri dishes were checked daily to count the number of larvae that had hatched.

2.4. Effects of Novaluron by Adult Horizontal Transfer

Untreated moths were allowed to lay eggs for 1-2 d before the adults were removed, and the egg masses were counted. Egg masses were photographed and the area was calculated as above. Then five unmated females that had been exposed to novaluron as above were introduced into the containers with the clean eggs. The unmated moths were incubated in the container for 7 d and were checked every 12 h to ensure moths were in contact with the egg masses. After the 7 d, the moths were removed, and the egg masses were incubated another 7 d to allow for hatch. The egg mass areas were analyzed in the same fashion as above after the incubation period was over. Controls for this experiment were carried out in a similar fashion, with Latron plus water used as the control for the novaluron solution.

2.5. Statistical Analysis

The total number of larvae emerged from egg masses was compared to the total number of eggs. The data collected from both bioassays was used as percent hatch and was normalized using an arcsine square root transformation before being subjected to a paired t-test comparing novaluron to the control (SAS 2002).

2.6. Residue Profile Analysis of Eggs Laid by Treated Adults

Adults were exposed to novaluron or a control solution as above. After the 3 d exposure period they were transferred to a clean 1-liter plastic container lined with clean wax paper for egg deposition. The moths were allowed to lay eggs for 7 d and then removed from the egg laying container. Eggs were collected from the wax paper (minimum 0.25 g/rep x 4 reps) and placed into a 25 ml glass vial. Acetonitrile (10 ml) was added and the vials were sent to the Michigan State University Pesticide Analytical Laboratory to test for the presence of novaluron using Gas Chromatography/Mass Selective Detector (Wise et al. 2007a). Controls for this experiment were of eggs laid by untreated adults.

2.7. Residue Profile Analysis of Eggs Exposed to Adult Horizontal Transfer

Untreated moths were allowed to lay eggs for 1-2 d before removal. Once the moths were removed, five untreated females exposed to novaluron by the methods described above were introduced into each container with the clean eggs. Moths were incubated in the container for 7 d and were checked every 12 h to ensure that moths were in contact with the egg masses. After 7 d, the moths were removed and egg masses were collected (minimum 0.25g/rep x 4 reps) and placed into a 25 ml glass vial. The vials were prepared and sent to the Pesticide Analytical Laboratory for detection of novaluron as previously stated. Controls for this experiment consisted of eggs laid from untreated adults.

3. RESULTS

3.1. Effects of Adult Exposure to Novaluron on Egg Survival

Results obtained from replicated experiments of exposure of adult obliquebanded leafrollers to novaluron demonstrated reduced fertility of eggs laid (Figure 4.1). Eggs laid by treated adults demonstrated that average egg hatch was 3 ± 2% which was significantly lower than in the control 20 ± 7% (t = 2.55, df = 10, p = 0.029).

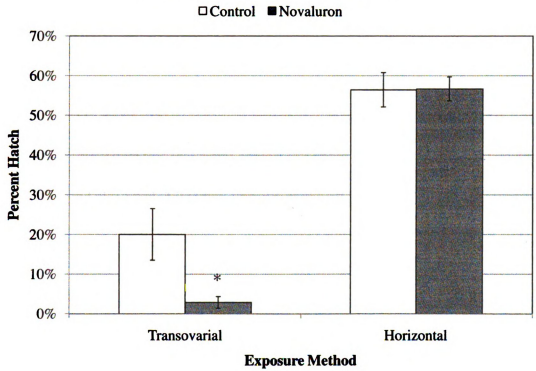

Figure 4.1. Mean percent hatch of obliquebanded leafroller eggs from different exposure methods. The hatch percent (mean ± SEM) with * is significantly different from the control (paired t-test, $p < 0.05$). Egg hatch rates are means of six replicates.

3.2. Effects of Novaluron by Adult Horizontal Transfer

Data obtained from the horizontal transmission experiment showed no reduction in egg fertility (Figure 4.1). The average hatch from the treatment eggs was 57 ± 3% which had no significant difference than the control average of 56 ± 4% (t= -0.03, df= 10, p = 0.973).

3.3. Residue Profile Analysis of Egg Masses

Analysis of egg masses laid by treated adults using a Gas Chromatography/Mass Selective Detector produced detectable levels of novaluron present in the eggs (0.034 ng/g ± 0.012). Eggs collected from the horizontal exposure and control experiments both yielded no detectable levels of novaluron.

4. DISCUSSION

The presented study has demonstrated that reduced egg viability occurs on obliquebanded leafroller after exposure to novaluron. Results from the horizontal transfer experiment demonstrate that incidental contact of female moths on previously laid eggs does not transfer sufficient compound to cause sublethal effects resulting in decreased viability. The results from the transovarial bioassays along with results obtained from novaluron residue detection in egg masses demonstrates a high likelihood that the reduced fertility occurs due to a transovarial passage of novaluron from adult to the eggs. A difference between egg viability of the control from the two different exposure methods was unexpected. A possible explanation between these differences can be attributed to exposure of the adults to Latron in the controls. The effects Latron has after ingestion and/or contact with obliquebanded leafroller have yet to be studied and can be a cause for lower egg viability seen in this experiment.

Reduced fertility after adult exposure to novaluron was seen not only in codling moth and obliquebanded leafroller, but similar results have been reported for Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Alyokin et al. 2008), the red flour beetle, *Tribolium castaneum* (Herbst) (Kostyukovsky and Trostanetsky 2006), and plum curculio,

Conotrachelus nenuphar (Herbst) (Wise et al. 2007). However, studies with other benzoylurea insecticide have demonstrated different physiological effects on female ovaries and basal oocytes after adult exposure (Soltani and Soltani-Mazouni 1992); thus there may be multiple mechanisms at work.

Novaluron field spray timing should still be based upon the direct ovicide or larvicide when targeting obliquebanded leafroller. However, the contribution of sublethal activity (reduced egg viability) should be considered in relation to overall population management. This is relevant when targeting obliquebanded leafroller, and also when novaluron is used against other pome fruit pests like codling moth, when obliquebanded leafroller adults may be present. The ability of novaluron to retain its potency after an artificial rain treatment of 40 mm/h at 5 and 24 h allows for control at wetter climates (Ishaaya et al. 2001). The sublethal effects, along with minimal effects towards natural enemies (Suh et al. 2000, Cloyd and Dickinson 2006), make novaluron a suitable organophosphate alternative in an IPM program. The pending cancelation of organophosphates, growing resistance to these compounds, and concerns over ecological impacts of the broad spectrum chemistries have increased impetus to develop newer chemistries to replace the heavily relied upon organophosphates. Education into the most appropriate use and timing of these new chemistries is crucial for proper and effective control over pests.

Results from this experiment have demonstrated that transovarial transmission is a likely mechanism for the observed sublethal effects. However, further research is needed to fully understand the physiological pathway that occurs with transovarial transmission which novaluron takes after adults have been exposed. Research is needed to investigate

whether novaluron is present in the proteins of the chorion surrounding the egg or in the egg itself to determine if novaluron is transovarially transmitted. Also, research into effective timing of a field application is needed to fully optimize the effects of novaluron, using both its larvicidal/ovicidal, and transovarial effects to control this pest and possibly coincide with other pest species

CHAPTER 5

EFFECTS OF NOVALURON ON *TRICHOGRAMMA PLATNERI* (HYMENOPTERA: TRICHOGRAMMATIDAE), A PARASITOID OF CODLING MOTH (LEPIDOPTERA: TORTRICIDAE)

1. INTRODUCTION

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a major pest of apple production throughout the United States (Barnes 1991). This key pest has generally been controlled using broad-spectrum insecticides like organophosphates (Barnes and Moffitt 1963, Croft and Hoyt 1983, Riedl et al. 1983, Howitt 1993, Wise et al. 2008). However, with the implementation of the Food Quality Protection Act (FQPA 1996), organophosphates have been slated for cancelation by 2012 (US EPA 2006). With the impending phase-out and also signs of resistance to organophosphates (Varela et al. 1993, Knight et al. 1994, Dunley and Welter 2000, Reuveny and Cohen 2004, Mota-Sanchez et al. 2007, Whalon et al. 2008), new tactics to control this pest have been developed.

Novaluron, a chitin synthesis inhibitor, is among the newer chemistries that have been developed to combat this pest. Chemistries in this class prevent proper chitin synthesis after the molting process (Post et al. 1974). However, the exact step where inhibition occurs, whether at the polymerization stage or in chitin precursor transport, is still unclear (Hajjar and Casisa 1978, Nakagawa and Matsumura 1994). Timings of novaluron applications for codling moth control have primarily been based on the compounds ovicidal and lavicidal properties (Wise et al. 2008). However, recent studies have demonstrated sublethal activity in the form of reduced egg viability, after adult exposure to this compound in Coleoptera and Lepidotpera (Kostyukovsky and Trostanetsky 2006, Wise et al. 2007, Alyokhin et al. 2008, Gökçe et al. 2009). This sublethal activity, seen with novaluron and studies demonstrating minimal effects

towards adult natural enemies (Suh et al. 2000, Cloyd and Dickinson 2006), allows for novaluron to be a suitable fit into an IPM program.

Along with new chemistries, the use of biological controls, including entomopathogenic nematodes, granulovirus, and parasitoids, to suppress codling moth have been studied as well. Of the many types of biological control used for codling moth, *Trichogramma* wasps have been mass reared and released in efforts to successfully control codling moth (List and Davis 1932, Webb and Alden 1940, Dolphin et al. 1972, Hassan et al. 1988, Yu et al. 1984, Bloem et al. 1998, Cossentine and Jensen 2000, Mansfield and Mills 2002). These wasps are a generalist, gregarious endoparasitoid of many Lepidopteran. The main species prevalent in apple orchards belong to the *Trichogramma minutum* complex, including *Trichogramma minutum* Riley and *Trichogramma platneri* Nagarkatti (Pinto 1998).

A concern with release of *Trichogramma platneri* is the potential negative impact pesticides may have on these natural enemies. Historically, negative pesticide impacts on natural enemies have been documented with the use of broad-spectrum insecticides (Croft and Brown 1975, Croft 1990, Desneux et al. 2007). Although novaluron has minimal direct effects on natural enemies, studies conducted with novaluron and Dimlin, another chitin synthesis inhibitor, have demonstrated adverse effects towards Honey bees (*Apis mellifera* L.), spined soldier bug (*Podisus maculiventris* (Say)), *Trichogramma pretiosum* Riley, and lacewings (*Chysoperla carnea* (Stephens)) (Medina et al. 2002, Thompson et al. 2005, Bastos et al. 2006, Cutler et al. 2006).

The objectives of this study are to determine if a) sublethal activity, in the form of reduced viability seen from studies stated above are seen in *Trichogramma platneri*, b)

parasitism of eggs laid from treated codling moths results in decreased wasp emergence, and c) parasitism of codling moth eggs laid onto surfaces treated with novaluron decreases wasp emergence.

2. MATERIALS AND METHODS

2.1. Chemical Material

For exposure of codling moth eggs and adults, Rimon 0.83EC was prepared to the recommended field rate of 0.155 g L^{-1} AI equivalent to labeled field rate of 145 g AI ha^{-1}, and Latron alone served as an untreated control. Since Rimon 0.83EC (Chemtura U.S.A. Corporation, Middlebury, CT) (novaluron formulation for apples) leaves an oily residue on petri dishes, another novaluron formulation Diamond 7.5 WG (Chemtura U.S.A. Corporation, Middlebury, CT), was used as a replacement for *Trichogramma platneri* adult exposure bioassays. Diamond 7.5 WG was prepared according to the equivalent field rate of Rimon (0.155 g L^{-1} AI equivalent to labeled field rate of 145 g AI ha^{-1}). Due to the inability of novaluron to fully homogenize in water, Latron B-1956 (a spreader and sticker by Dow Agro Sciences LLC, Indianapolis, IN) was added at 0.038:1 liter of solution to the Rimon and control treatments.

2.2. Insect material

Trichogramma platneri were obtained from Rincon-Vitova Insectaries (Ventura, CA) as pupae in eggs of *Ephestia* spp. Cards of *Trichogramma* were placed into a petri dish and sealed with parafilm to allow for adult emergence. Codling moth pupae were obtained from the Yakima Agricultural Research Laboratory (Wapato, Washington), reared on artificial diet, and conditioned in constant light for 24 – 48 h at 21 °C and 60% RH. Male and female pupae were separated according to their abdominal structure into 1-

liter plastic containers (Peterson 1965). They were incubated at 21 °C, 60% RH, and 16:8 h (L:D) photoperiod until adult eclosion. After adult emergence, adult moths were transferred into 1-liter containers lined with wax paper for egg deposition.

2.3. *Trichogramma platneri* adult exposure

Petri dishes were coated with 2 ml of novaluron solution (1 ml lid and 1 ml on bottom dish) and allowed to fully dry. After petri dishes had fully dried, 24 – 48 h old wasps were placed into the dishes in excess of 300 wasps/container and incubated for 24 h. After the exposure period, two wasps were transferred to 50 ml scintillation vials (RPI Corp Mt. Prospect, IL) that contained ten 1 - 2 d old codling moth eggs laid onto wax paper. The treated wasps were incubated with the codling moth eggs for 24 h and then were removed. Parasitized eggs were further incubated 7 d at 23 ± 1 °C, 60% RH, and 16:8 (L:D) h photoperiod to allow for pupal development and adult emergence. An egg was deemed as parasitized if it elicited the "black egg" color, which is the wasp pupae forming inside the parasitized egg. For adult emergence, one exit hole/parasitized egg was labeled as adult emergence. Wasps were visually observed to ensure proper detection and parasitism of codling moth eggs. Controls for this experiment consisted of petri dishes treated with distilled water. Each treatment was replicated 8 times.

2.4. Exposure to eggs laid by novaluron treated adults

To test the effects eggs laid by treated adult codling moths have on wasps' parasitism and subsequent adult emergence, adult codling moths were exposed to novaluron and control through both contact and ingestion as described by Gökçe et al. (2009). A piece of wax paper (33.5 cm × 13 cm) was sprayed until drip with the novaluron solution and allowed to fully dry. Once dry, the paper was used to line the

sides and bottom of a 1-liter plastic container. Newly emerged codling moth adults (1-3 d old) were placed into the exposure container for 3 days, then transferred to clean 1-liter containers with wax paper for egg deposition. After adult wasps had emerged, ten 1 – 2 d old codling moth eggs and 2 wasps were placed inside a 50 ml scintillation vial. The vials were incubated for 24 h to allow for wasps to parasitize the eggs. After the parasitization period, wasps were removed and the eggs were further incubated as described above. Each treatment was replicated 7 times.

2.5. Exposure of *Trichogramma* to codling moth eggs laid onto treated surfaces

Codling moth adults were held in 1–liter plastic containers lined with wax papers as the egg deposition substrate that had been treated with novaluron for the treatment, or with Latron for the control. Ten 1 – 2 d old eggs were cut from the wax papers and placed into a 50 ml scintillation vial along with 2 wasps. The vials were incubated for parasitization for 24 h and then adult wasps were removed. Parasitized eggs were further incubated as described above.

2.6. Statistical Analysis

For all three experiments, the number of parasitized eggs was compared with the controls using one way analysis of variance and the Tukey's multiple comparison test (PROC MIXED, SAS 2002). Also the percent hatch of wasps was normalized using arcsine square root transformation before analysis of variance and Tukey's multiple comparison test.

3. RESULTS

3.1. *T. platneri* adult exposure

Exposure of *T. platneri* adults to novaluron had a variable effect on the number of codling moth eggs parasitized and percent adult emergence after parasitization (Figure 5.1, 5.2). There was no difference seen in the number of codling moth eggs parasitized after adult exposure between treatment and control. Data collected for adult emergence once eggs were parasitized showed a significant reduction in egg hatch from 99 ± 1% in the control to 73 ± 8% in the treatment ($p < 0.001$, $df = 14$, $F = 17.81$).

3.2. Exposure to eggs laid by novaluron treated codling moth adults

T. platneri offered codling moth eggs laid by adults previously exposed to novaluron showed significant reductionsin both of the number of eggs parasitized and percent of adults emerging from parasitized eggs (Figure 5.1, 5.2). There was a significant difference between treatment and control in the number of eggs parasitized ($p < 0.0001$, $df = 12$, $F = 63.03$). There was also a significant difference seen in the percent of adult emergence once eggs were parasitized ($p < 0.0001$, $df = 12$, $F = 264.34$).

3.3. Exposure of *T. platneri* to codling moth eggs laid onto treated surfaces

Results from this experiment have shown significant differences in both the number of eggs parasitized and percent of adult emergence (Figure 5.1, 5.2). There was a significant decrease in the number of eggs parasitized by *T. platneri* when compared to the control ($p = 0.0035$, $df = 10$, $F = 14.44$). The complete lack of adult emergence (0%) once eggs were parasitized was significantly lower than the >80% emergence recorded in the control ($p < 0.0001$, $df = 10$, $F = 116.93$).

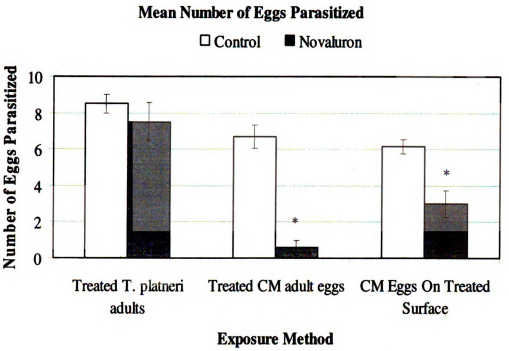

Figure 5.1. Mean (±SEM) number of eggs parasitized by *T. platneri* from three different exposure methods to novaluron. Bars with * indicate significant difference from the control (Tukey's, $p < 0.05$).

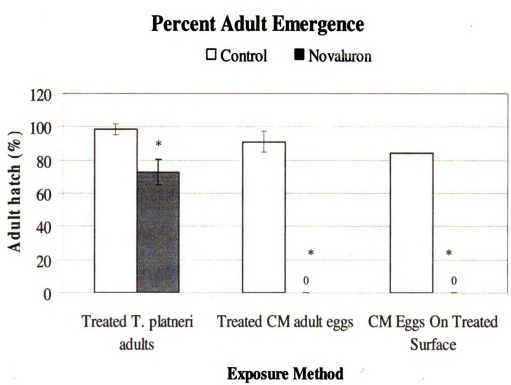

Figure 5.2. Mean (±SEM) percent of adult *T. platneri* emerged from parasitzed codling moth eggs ,from three different exposure methods to novaluron. Bars with * indicate significant difference from the control (Tukey's, $p < 0.05$).

4. DISCUSSION

The presented study has demonstrated that exposure of *T. platneri* to novaluron causes sublethal effects, decreased percent adult emergence and egg parasitism. Observations made during the experiment ensured that wasps were able to locate eggs and oviposit. Results from the *T. platneri* adult exposure did not show a dramatic decrease in adult emergence, but showed high significance. This can be a result of insufficient exposure time to achieve a lower emergence rate. The experiment where eggs laid from treated adult codling moths were exposed to *T. platneri* demonstrates that enough novaluron is present in the egg to prevent adult wasps from emerging. Reductions in the number of eggs parasitized occurred in only two of the experiments, those with

eggs laid from treated codling moth and codling moth eggs laid onto treated surfaces. Observations of parasitized eggs for these two trials didn't demonstrate the typical "black egg" which arises when the *T. platneri* larvae pupates within the host egg. However, in the trial when adult *T. platneri* were exposed to novaluron there was no difference in the number of parasitized eggs. However, a study conducted by Bastos et al. (2006) demonstrated that parasitization rates were not significantly affected after exposing *T. pretiosum* Riley to eggs dipped in novaluron. The results obtained in this study were similar to results with *T. pertiosum* when adult emergence was observed. The significant decrease in adult emergence demonstrates that exposure of *T. platneri* to novaluron causes detrimental effects on the parasite larvae and/or eggs developing within the host egg.

The data collected from codling moth eggs deposited onto novaluron treated surfaces demonstrated similar results as with eggs deposited by treated adults. The significant decrease in the number of parasitized codling moth eggs and also reductions in adult wasp emergence from both these trials demonstrates that novaluron is present in the eggs. The egg deposited onto the treated surface simulates the recommended ovicidal mechanism of this compound, novaluron penetrating into the egg causing non-viable eggs. However, the eggs deposited after adult codling moth exposure elicited similar results, suggesting that transovarial transmission can be a possible mechanism of the sublethal effects documented by Gökçe et al. (2009) for codling moth.

Although novaluron has no known direct toxicity to exposed adults, sublethal effects are observed once adults are exposed. The results in this experiment along with experiments conducted on *T. pretiosum*, *Podisus maculiventris,* and *Bombus terrestris*

have demonstrated negative effects on reproduction (Bastos et al. 2006, Cutler et al. 2006, Mommaerts et al. 2006). The effect observed after adult exposure for *Podisus maculiventri* and *Bombus terrestris* resulted in reduced egg viability. However, results from this experiment demonstrate that *T. platneri* adults exposed to novaluron demonstrate a reduced pupal viability rather than reduced egg viability. The categorized "black egg" of host eggs simulates a parasitoid pupa inside the host egg. This "black egg" demonstrated that the eggs deposited by the parasitoid are viable and able to develop into the pupal stage. The "black egg" was not observed in the other two experiments, suggesting that the eggs deposited by the wasps were unable to develop into pupae.

Novaluron field applications should still be based on the ovicidal and larvicidal activities when targeting codling moth. However, the results from this experiment and from laboratory work conducted by Gökçe et al. (2009) demonstrating the reduced egg viability should be considered when developing a spray program. The impacts on natural enemies need to be considered when using novaluron as an organophosphate replacement/alternative in fruit IPM programs. This insect growth regulator has shown minimal direct toxicity towards adult natural enemies, but the sublethal effects, reduced pupal viability and egg viability, observed are an important consideration for augmentative biological control. The use of *T. platneri* to control codling moth should still be a viable control option; however, proper timing of releases is necessary to ensure proper and effective control.

Further research into the exact stage in parasitoid development this compound takes effect after adult exposure should be considered. Also, the bioassays presented where run under laboratory conditions resulting in maximum dose exposure, future

research with this compound and these wasps should be include field aged residues to fully understand the effects that can occur under the conditions wasps are likely to encounter in agricultural settings.

CHAPTER 6

CONCLUSION

The codling moth (Linnaeus) and obliquebanded leafroller (Harris) are major pests in apple production throughout the United States. Codling moth damage if left uncontrolled can result in 90% crop loss (Caprile and Vossen 2005). While codling moth is an internal feeder of apples that attacks a high percentage of the crop, the surface damage caused by the leafroller can range from 3 – 20% crop loss if control measures aren't taken (Angello et al. 1996, Ho 1996, Lawson et al. 1996). With the devastation these two pests can cause, several control tactics have been implemented to minimize populations and damage. The most commonly used control tactic has been the use of organophosphates, principally azinphosmethyl (Guthion), which are broad spectrum insecticides. However, with the passage in the US of the Food Quality Protection Act (FQPA, 1996) the use of broad spectrum chemicals is under reregistration to determine the effects on the environment and human safety. This intense scrutiny has caused this chemistry to be slated for cancellation by 2012 (EPA, 2006). With the current phase out of organophosphate use, coupled with signs of resistance to OP's in populations of both codling moth and leafrollers (Reissig et al. 1986, Varela et al. 1993, Knight et al. 1994, Carriére et al. 1996, Lawson et al. 1997, Dunley and Welter 2000, Ahmad et al. 2002, Smirle et al. 2002, Reuveny and Cohen 2004, Mota-Sanchez et al. 2007, Whalon et al. 2008) novel control tactics are being developed. Insect Growth Regulators (IGR) are among the new chemistries being adopted by the fruit industries as they provide promising control measures along with lower environmental and human health impacts

(US EPA 1997). Yet, broad adoption of this IGR may require rather precise timing of applications to be effective.

Within the IGR group lie the chitin synthesis inhibitors. Of the many chitin synthesis inhibitors on the market, novaluron has shown promise as a control measure for both of the key apple pests. Currently, novaluron is used as an ovicide and larvicide. However, studies conducted in the previous chapters and by others have shown sublethal activity after adult exposure, i.e. reduced egg viability (Cutler et al. 2005, Kostyukosky and Trostanetsky 2006, Wise et al. 2007, Gökçe et al. 2009). Research presented in the previous chapters has shown that once adults are exposed to novaluron, the eggs laid by these treated females yields a significantly reduced hatch percent when compared to the control. These reduced egg viability results were seen in laboratory trials as well as trials involving field aged residues on leaves or fruit. From the field residue trials, reduced egg viability was recorded up to 21 d after the initial treatment, depending on plant substrate. The residue analysis conducted with OBLR eggs has demonstrated that novaluron is present in the egg and is not significantly transferred to the egg by tarsal contact of females. The experiments conducted have shown that after adult exposure, the compound is transport by the female into the eggs before they are laid. Experiments conducted by treating different sexes only have shown similar reduced egg viability results. Although male treatment alone showed a significant decrease in egg viability, there was a significant difference in the effect on percent hatch between female treated alone vs. male treated alone. The extent male exposure has on the transfer of this compound has yet to be studied, although contact with females through copulation is the likely method of transfer or physiological damage to spermatheca (Moore et al. 1978).

This new mechanism for sublethal effects, transovarial, along with the established direct ovicidal and larvicidal effects allow for several effective means of using novaluron in fruit IPM programs. However, the transovarial activity seen in both codling moth and OBLR have been seen in some beneficial insects. Although novaluron is safer for adult natural enemies, the research conducted on *Podisus maculiventris* (Say) and *Trichogramma peritosum* Riley have demonstrated the reduced viability of eggs after adult exposure to novaluron (Bastos et al. 2006, Cutler et al. 2006). The same results were seen in the experiments conducted for this thesis against *Trichogramma platneri* Nagarkatti. Reduced egg viability was seen following for two of the methods of exposure; parasitism of eggs laid by adults treated with novaluron, and codling moth eggs laid onto surfaces treated with novaluron. Reduced pupal viability was observed after treated *T. platneri* adults were given codling moth eggs for parasitism, suggesting that for parasitoids, the sublethal effect after exposure is at the pupal stage instead of the egg stage.

Although adverse affects were seen in *Trichogramma platneri*, the use of novaluron as a tool in the IPM strategy should still be considered. With proper education and timing, the use of novaluron to control these two economically devastating pests can be potential replacements for the previously reliable organophosphates. Applications of novaluron when trees are in bloom should be avoided since affects towards bumblebees have been seen as well (Mommaerts et al. 2006). The effects delineated by experiments presented in this thesis, along with those conducted by others, should provide guidance in determining proper spray timings where the best window to control these pests is targeted without detrimental impacts on natural enemies.

The experiments conducted with novaluron on codling moth and OBLR have shown that once adults are exposed, the compound is transovarially transferred to the eggs laid by the females. The methods of exposures tested have all resulted in a significant decrease in egg fertility along with effects seen by treating different sexes. Also the residue analysis of OBLR eggs has demonstrated that the compound is in the egg. However, further studies need to be conducted to answer questions created after completing these experiments. Some of these areas that need to be studied to fully understand the physiological method and/or movement of this compound are; 1) determine the location in the egg novaluron is present (inside the chorion vs. vitellogenin), 2) determine male influence in transmission, 3) any resistance development after prolonged exposure, and 4) the stage at which novaluron effects parasitoid eggs inside the host egg.

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2009-04

Title of thesis or dissertation (or other research projects):

Novaluron Sublethal Activity in Codling Moth, *Cydia pomonella*, via Transovarial Transmission and Effects on Natural Enemies

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Soo-Hoon Samuel Kim

Date July 8, 2009

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:						Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	
Cydia pomonella (Linnaeus)	USA, Washington, Yakima CO. Yakima ARS Lab Reared from Lab colony June 24, 2009 Soo-Hoon Kim coll.					10	10		MSU
Choristoneura rosaceana (Harris)	USA, Michigan, Ingham CO. Michigan State University Reared from lab colony June 19, 2009 Soo-Hoon Kim coll.					10	10		MSU
Trichogramma platneri Nagarkatti	USA, California, Ventura CO. Rincon-Vitova Beneficial Insect Supplier Reared from lab colony June 24, 2009 Soo-Hoon Kim coll.					10	10		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Soo-Hoon Samuel Kim

Date July 8, 2009

Voucher No. 2009-04
Received the above listed specimens for deposit in the Michigan State University Entomology Museum

Curator Date 9 July 2009

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