LETHAL AND SUBLETHAL EFFECTS OF THREE INSECT GROWTH REGULATORS ON *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

by

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(Under the Direction of ASHFAQ AHMAD SIAL)

ABSTRACT

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) has emerged as a problematic pest for many soft-skinned fruits including blueberry. Due to extremely low tolerance for infested fruit in the market, broad-spectrum insecticides are often used as the primary means to control this pest. The risk of insecticide resistance development and other health and environmental concerns associated with broad-spectrum insecticides call for more target-specific and environmentally-friendly alternatives. Chemicals commonly known as insect growth regulators such as juvenile hormone (JH) analogs, ecdysteroid agonists, and chitin synthesis inhibitors may serve as an ideal alternative. My results showed that a JH analog, pyriproxyfen (1 to 100 ppm), and a chitin synthesis inhibitor, novaluron (0.01 to 10 ppm), effectively limited development of larvae continuously exposed in medium; whereas an ecdysteroid agonist, methoxyfenozide, was only effective at higher dosages (10 to 1000 ppm).

INDEX WORDS: Spotted-wing drosophila, mortality, development, methoxyfenozide, novaluron, pyriproxyfen

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DEDICATION

I dedicate this to my family.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Drosophila suzukii Matsumura (Diptera: Drosophilidae) commonly known as spotted-wing Drosophila (SWD) is an invasive species. It was first detected in California in 2008 and since then has spread throughout the United States. Females have a uniquely sclerotized and serrated ovipositor that is used to puncture the fruit surface and lay eggs inside blueberries and other small fruits. The developing larvae feed inside the fruit rendering it unmarketable (Mitsui et al. 2006). Due to extremely low tolerance for infested fruit in the market, broad-spectrum insecticides are used as the primary means to control this pest. Several broad-spectrum insecticides are registered for management that have toxic effects on non-target organisms and the environment (Walsh et al. 2011). Repeated applications of insecticides with similar modes of action can lead to resistance development in field populations of *D. suzukii* (Gress et al. 2018). The risk of resistance development combined with other health and environmental issues associated with broad-spectrum insecticides call for more target-specific and environmentally-friendly options to control this pest. In this situation, reduced-risk chemicals called "insect growth regulators" (IGRs) may serve as good alternatives. IGRs typically are more selective and non-target friendly and can be incorporated into integrated pest management (IPM) and insecticide resistance management (IRM) programs in order to effectively control *D. suzukii* on a more sustainable basis (Smagghe et al. 2012, Tunaz and Uygun 2004). IGRs are considered to be effective usually during the larval stages because they impair growth, but the effects occur more slowly compared to other pesticides (Nasr et al. 2010). IGRs consist of three main groups:

ecdysteroid agonists, chitin synthesis inhibitors and juvenile hormone analogs (Dhadialla et al. 2005).

Ecdysone receptor agonists

Methoxyfenozide

Steroid hormones in insects are called ‘ecdysteroids’ or ‘ecdysones’. Ecdysone was first identified as ‘molting hormone’, and it is converted into 20-hydroxyecdysone (20E) in several tissues. 20E is considered to be the more active form involved in the molting process. Ecdysone, 20E and other ecdysteroids are secreted by prothoracic glands at specific times during the larval stages (Pener and Dhadialla 2012). Prothoracicotropic hormone (PTTH), brain neuropeptide, stimulates to prothoracic glands to produce ecdysteroid hormone via Torso-receptor/extracellular-signal-regulated kinase (ERK) pathway (Loof et al. 2015). During the molting process, ecdysteroid hormone binds to ecdysteroid receptor (EcR) protein which creates a heterodimer with ultraspiracle (USP) protein. The ecdysteroid hormone-EcR-USP complex activates a cascade of ecdysone response genes to initiate the molting process (Ito-Harashima 2017).

Dibenzoylhydrazines (also known as bisacylhydrazines) are an effective class of pesticides because these chemicals and their analogs are the true agonists of 20E binding to the EcR. These compounds cause premature molting with deformed cuticle formation and also inhibit the feeding process in larval stage, so that eventually death occurs (Pener and Dhadialla 2012). Diacylhydrazines have been tested on at the larval and adult stages of least 16 different insect orders. The results showed that although diacylhydrazines were harmless for many orders, lethal effects were observed for lepidopteran, dipteran and coleopteran larvae (Dhadialla et al. 1998). After the discovery of the first ecdysteroid agonist 1, 2-dibenzoyl- 1 -tert-butylhydrazine

(RH-5849), others were released such as tebufenozide (RH-5992), halofenozide (RH-0345), and chromafenozide (ANS-118, CM-001). Subsequently, methoxyfenozide (RH- 2485) came to the market (Dhadialla et al. 1998, Smagghe et al. 2012).

Methoxyfenozide [N-tert-butyl-N'-(3-methoxy-o-toluoyl)-3,5-xylohydrazide] is an IGR in the group of diacylhydrazine insecticides (Carlson et al. 2001). Since methoxyfenozide, just as other molting hormone agonists, binds to EcR-USP complex, methoxyfenozide induces premature and lethal molting by mimicking 20E (Carlson et al. 2001, Ito-Harashima 2017). Once methoxyfenozide is applied on insects, it stimulates the transcription of genes that are dependent on the rising titer of 20E. On the other hand, genes activated by the decline or disappearance of 20E are not transcribed once methoxyfenozide exists in hemolymph (Dhadialla 2005). Ecdysis is the final step of molting, and neuropeptides, such as eclosion hormone, are released when 20E is cleared from the hemolymph. After the application of ecdysteroid agonists, ecdysis fails because release of these neuropeptides is disrupted, leading to precocious and incomplete molting (Dhadialla 2005). Ecdysone agonists also decrease egg production, cause ovicidal activity and inhibit spermatogenesis (Bengochea et al. 2012). Ecdysteroids play an important role in the regulation of yolk protein synthesis in some lepidopteran, coleopteran, and dipteran insects, and ecdysone agonists when applied can reduce egg production (Dhadialla et al. 1998).

Currently, methoxyfenozide is the most widely registered of all diacylhydrazine insecticides, and it is extensively used in more than 50 countries for different crops such as vegetables, forestry, and tea (Smagghe et al. 2012). Methoxyfenozide was discovered in 1996 (Le et al. 1996) and is commercially produced by Dow AgroSciences under the name of Intrepid 2F, which was registered with the Environmental Protection Agency (EPA) in 2000 for agricultural purposes (Anonymous 2000, Dhadialla 2005). Methoxyfenozide is modestly root

systematic in comparison to tebufenozide, and it is not significantly effective as a leaf-systemic (Carlson et al. 2001). It is most effective when it is digested and has contact and ovicidal activity (Dhadialla 2005).

Chitin synthesis inhibitors

Novaluron

Chitin is the major component of the cuticular exoskeleton and the peritrophic matrix (PM) of insects. Chitin is also an important component of many internal structures such as alimentary canal, tracheal system, genital ducts, and the ducts of the several dermal glands. Old cuticle and PM are degraded and replaced during the molting. Therefore, for the development of insects, biosynthesis, turnover, and modification of chitin are continuous and essential processes. Numerous units of N-acetyl-β-D-glucosamine (GlcNAc) linked by β-1,4 glycoside bonds are the main components of the polysaccharide polymer chitin, and the activated precursor for chitin biosynthesis is uridine diphosphate N- acetylglucosamine (UDP-N- acetylglucosamine) (Zhu et al. 2016).

Benzoylphenyl ureas are called chitin synthesis inhibitors (CSIs) because these chemicals block chitin synthesis (Tunaz and Uygun 2004). Despite the fact that the mode of action of CSIs has been investigated for decades, the precise mode of action has not been identified (Zhu et al. 2016). It has been proposed that CSIs are involved in the inhibition of chitin synthesis, inhibition of proteases and inhibition of UDP-N- acetylglucosamine transport through the membrane (Miyamoto et al. 1993). Nasonkin et al. (1999) identified a sulfonylurea receptor (Dsur) in *Drosophila melanogaster,* and this receptor is the homolog of mammalian SUR which belongs to the ABC-transporter family. This discovery was important in that the anti-diabetes drug glibenclamide, which targets human SUR1, blocks K^+ entry and subsequently increase cellular

Ca^{+2} concentration to trigger insulin release (Matsumura 2010). Abo-Elghar et al. (2004) reported that there is a similarity between glibenclamide and diflubenzuron (CSI). Intracellular vesicles prepared from *D. melanogaster* and *Blatella germanica* showed that SUR is likely to be the target side of diflubenzuron because both compounds, glibenclamide and diflubenzuron, inhibited chitin synthesis in both species. In this experiment, intracellular cuticular vesicles prepared from *B. germanica* showed that both compounds, glibenclamide and diflubenzuron, interfered with chitin synthesis by binding to the glibenclamide binding site of the SUR and inhibiting Ca^{2+} uptake effectively. Abo-Elghar et al. (2004) speculated that cuticular vesicles can contain SUR-type transporters that interfere with chitin synthesis. Diflubenzuron is likely to induce the depolarization of the vesicle membrane by binding the receptor (SUR), which alters its K^+ channel, causing inhibition of Ca^{+2} uptake, and this process subsequently interferes with the incorporation of N-acetylglucosamine into the insect chitin and resulting in the inhibition of chitin synthesis in *B. germanica*. Additionally, when *B. germanica* last instar nymphs were treated with both glibenclamide and diflubenzuron, both chemicals induced the same morphological problems such as molting malfunctions, adults with twisted wings and short abdomens (Matsumura 2010).

Novaluron, (±)-1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) phenyl]-3-(2,6-difluorobenzoyl) urea was discovered by Makhteshim-Agan Industries Ltd (Cutler 2007). Novaluron is considered as effective against the larvae of Lepidoptera (Pener et al. 2001), Coleoptera (Pener et al. 2001), Hemiptera (Pener et al. 2001), and Hymenoptera (Glowacka and Malinowski, 1994). It can act both through ingestion and contact (Ishaaya et al. 2001) and interferes with the synthesis of chitin required for molting or replacement of the PM (Cutler 2007). It is important to note that novaluron primarily causes mortality usually during the larval

stages similar to methoxyfenozide used in my project, whereas the juvenile hormone analog, pyriproxyfen, used in my project, usually leads to mortality during metamorphosis (Su et al. 2003). Timing is crucial for novaluron application, and results may not be observed until molting begins.

Juvenile hormone analogs

Pyriproxyfen

The development of *Drosophila* and other insects is regulated primarily by 20-hydroxyecdysone (20E), the active molting hormone, and the sesquiterpenoid juvenile hormone (JH), the status quo hormone (Riddiford et al. 2003). Regulation of JH secretion in corpus allatum is maintained by two neurohormones. Even though allatotropins stimulates to secretion of JH in corpus allatum, it is inhibited by allatostatins (Dhadialla 2005). The hormones are produced by different cells in the brain and ring gland in *Drosophila* larvae, but during metamorphosis, the gland dissociates with the JH producing cells becoming the paired corpora allata and the gonads and other tissues producing 20E in adults. The JH receptor encoded by the Methoprene-tolerant (Met) protein was first identified in the common fruit fly, *Drosophila melanogaster* (Meigen), and the receptor binds JH with high affinity and dimerizes with the related Germ cell-expressed (Gce) protein. Both are basic helix–loop–helix Per/Arnt/Sim (bHLH-PAS) proteins that as a dimer move into the nuclei of tissues targeted by JH and induce expression of the Kruppel homolog (Kr-h1) protein, which is a downstream transcription factor that blocks metamorphosis (Jindra 2015a,b). When larvae reach the 3rd and final instar, depletion of the JH titer ends its action, and metamorphosis is initiated by a small pulse of 20E, which activates expression of the Broad-complex protein that promotes metamorphosis (Kayukawa 2017).

Pyriproxyfen (2-[I-methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine), an IGR and JH agonist, was discovered in 1985 (Hatakoshi et al. 1986) and later registered for agricultural purposes. Pyriproxyfen acting as a JH analog prevents metamorphosis or disables adult emergence of mosquitoes and houseflies (Bensebaa et al. 2015, Sullivan and Goh 2008, Zhou and Riddiford 2008). Pyriproxyfen is a designated reduced-risk insecticide by the U.S. EPA with low risk of resistance and is non-toxic to bees, vertebrates and natural enemies of target pests (Sullivan and Goh 2008).

Subsequent studies showed that pyriproxyfen binds to Met-Gce and effectively maintains larval characteristics and interferes with pupal-adult molting in *D. melanogaster* (Charles et al. 2011, Jindra 2015a). Analogs of JH typically do not inhibit larval development of *Drosophila spp.* but do interfere with embryogenesis and metamorphosis. Pyriproxyfen when applied to some insects also interferes with fertility and fecundity. Reproduction in many adult insects is dependent on neuroendocrine hormones, 20E, and JH (Singh and Kumar 2015). Specifically, JH stimulates the synthesis of the major yolk protein, vitellogenin, in the fat body; separation of new egg follicles in the ovaries from the germarium; and uptake of yolk protein by oocytes. Wilson and Fabian (1986) reported that in *D. melanogaster* Met mutants vitellogenesis and oviposition were delayed. Therefore, it can be speculated that pyriproxyfen could interfere with reproductive processes in *D. suzukii*.

The above literature review shows that methoxyfenozide, novaluron, and pyriproxyfen are potential insecticides that can be incorporated into *D. suzukii* management. No studies to date have evaluated the insecticidal activity of these chemicals for control of *D. suzukii*. The objective of this research was to determine lethal and sublethal effects of methoxyfenozide, novaluron, and pyriproxyfen on progeny exposed to these pesticides. This research tried to answer whether these

pesticides would be as effective as conventional pesticides and which concentration is the most ideal for each insecticide. Secondly, this research tried to answer which stages of *D. suzukii* are most vulnerable to these insecticides. In this framework, my studies of each insecticide are covered in the following chapters: Chapter two - Lethal and sublethal effects of methoxyfenozide on *D. suzukii*, Chapter three - Lethal and sublethal effects of novaluron on *D.suzukii*, and Chapter four - Lethal and sublethal effects of pyriproxyfen on *D. suzukii*.

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CHAPTER 2

LETHAL AND SUBLETHAL EFFECTS OF METHOXYFENOZIDE ON *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

Introduction

Molting in insects is stimulated with the rise of 20-hydroxyecdysone (20E) and following this, 20E titer decreases (Dhadialla 2005). In order to complete molting cycle, 20E titers should be cleared in the hemolymph (Truman 1984). When 20E binds to ecdysone receptor, several classes of "early" and "late" genes are regulated. While activation of early genes is initiated by 20E-receptor complex, "late" genes are blocked (Dhadialla et al. 2005). Yao et al. (1993) showed that ecdysteroid hormone binds to ecdysteroid receptor (EcR) protein when EcR-Ultraspiracle (USP) protein receptor complex is formed. Ecdysteroid hormone-EcR-USP complex binds to the response elements of many genes and initiates transcription of mRNA. In response to rising 20E titer, apolysis (Separation of epidermis from old cuticle) takes place and molting fluid is released into ecdysial space. The molting fluid consists of inactive chitinolytic enzymes involved in the digestion of the old cuticle when chitinolytic enzymes are activated. At the same time, the epidermal cells are involved in the synthesis of protein and the secretion of the new epicuticle and cuticle (Dhadialla et al. 2005). When the level of 20E titer drops, old procuticle is digested by enzymes in the molting fluid. Following this, the resorption of molting fluid occurs and pre-ecdysial tanning of the new cuticle is completed. Finally, when the 20E titer decreases to a basal level, old cuticle is shed with the aid of peptides, eclosion hormone and ecdysis-triggering hormone so that molting is completed successfully (Dhadialla et al. 2005, Reynolds 1987).

Methoxyfenozide is an insect growth regulator in the class of non-steroidal ecdysone agonists and it mimics ecdysteroid hormone (Hamaidia et al. 2018). Once methoxyfenozide binds EcR-USP protein receptor complex, methoxyfenozide-EcR-USP complex interferes with expression of some genes and release of eclosion hormone (Smagghe et al. 2012). Similar to 20E, ecdysone agonists-EcR-USP complex initiate the expression of transcription factors which stimulates the expression of upregulated genes. Once larvae are intoxicated with methoxyfenozide, a nuclear hormone receptor E75 (Early gene) activated by 20E is expressed in *D. melanogaster*. E75 is important because "late" proteins such as DOPA decarboxylase (DDC) are depressed by E75. Normally, DDC protein is expressed when 20E is absent. However, once larvae are intoxicated with methoxyfenozide, DDC is not expressed since methoxyfenozide mimics 20E and activate early proteins such as E75. Absence of DDC leads to lack of tanning and hardening of new cuticle. Similarly, once larvae are intoxicated with methoxyfenozide, genes like those for the 14-kDa larval cuticle protein (LCP14) are not expressed since expression of these genes requires to clearance of 20E (Dhadialla et al. 2005, Smagghe et al. 2012). Subsequently, this process induces the premature apolysis, head capsule slippage and formation of pharate larvae (Soin et al. 2010).

High efficiency was observed with ecdysone receptor agonist methoxyfenozide when tested on lepidopterans (Carlson et al. 2001). On the other hand, it is important to indicate that a little information has been obtained with regard to the effect of methoxyfenozide on dipteran species (Hamaidia et al. 2018). The ecdysteroid agonist 1, 2-dibenzoyl- 1 -tert-butylhydrazine (RH-5849) was tested on the fruit fly, *Drosophila melanogaster* (Meigen) cells and it was shown that this compound is capable of acting like an ecdysteroid agonist at the cellular and biochemical levels. When RH-5849 was tested on *D. melanogaster* cells, proliferation was

blocked, and acetylcholinesterase levels increased (Wing 1988). Additionally, an in vitro study conducted with *D. melanogaster* Kc cultured-cell line showed that RH-5849 and tebufenozide replaced tritiated ponasterone A (PoA) from the cytosolic protein extracts obtained from *D. melanogaster* Kc (Mikitani K. 1996). In other words, dibenzoylhydrazines compete with tritiated PoA in order to bind ecdysone receptor sites (Wing 1988). Moreover, when RH-5849 and tebufenozide were tested in the epithelial cell line from midge, *Chironomus tentans* (Fabricius), it was reported that these chemicals led to differentiation and inhibition of cell growth and interfered with chitin metabolism because these chemicals increased the synthesis of chitinolytic enzymes. Additionally, treatment of RH-5849 and tebufenozide caused the formation of stratified columnar epithelium and the change of protein pattern when these compounds were tested on cell line from *C. tentans* (Quack et al. 1995).

Methoxyfenozide may be an ideal option for integrated pest management (IPM) because it is harmless to special beneficial insects or it is less toxic (Carlson et al. 2001). Methoxyfenozide is a useful pesticide because it has a new mode of action, low risk for ecotoxicology and mammalian profiles (Dhadialla 2005). Hence, methoxyfenozide can be incorporated into IPM strategies to obtain more desirable results for *D. suzukii* management. For these reasons, trials were initiated to determine whether methoxyfenozide would disrupt development and could be incorporated into *D. suzukii* management.

Material and Methods

Insects

D. suzukii were taken from a laboratory colony established from flies captured in Clarke County, GA in 2013. Flies were reared in 117 ml square bottom polypropylene bottles (Genesee Scientific, San Diego, CA) each containing 50 ml of standard fly diet (Jaramillo et al. 2015). A

pinch of active dried baker's yeast was sprinkled into each bottle. Bottles were capped with bonded dense-weave cellulose acetate plugs and placed on plastic trays in growth incubators (Model I36VLCB, Percival Scientific, Perry, IA) set at 24°C, 65% relative humidity, and a photoperiod of 14 h light:10 h dark. All experimental groups were housed in the same conditions.

Insecticide

The chemical used in this study was methoxyfenozide (Intrepid 2F, Insect Growth Regulator, EPA Reg. No. 62719-442, Dow Agro Sciences Indianapolis, IN.).

Adult mortality after exposure to methoxyfenozide-treated medium

The first test was designed to determine the mortality effects of methoxyfenozide in the media contacted or ingested by *D. suzukii* adults. Newly emerged virgin adults were separated based on sex using the dark spots on the male wings as a distinguishing characteristic (Walsh et al. 2011) and transferred to media treated with a wide dosage range of methoxyfenozide. The Intrepid 2F, methoxyfenozide formulation, was diluted to 1000 ppm with deionized water as a stock solution. Serial dilutions in water were prepared from the stock solution to 100 ppm, 10 ppm, 1 ppm, 0.1 and 0.01 ppm. For each concentration, six 16 oz plastic deli cups (Hofmann plastics-HT16) were filled with Instant ready blue media (20 g, Formula 4-24® Instant Drosophila Medium), yeast (3.2 mg, Fleischmann's active dry yeast), and 100 ml insecticide solution and mixed. Control cups were filled with the same mixture including water with no insecticide. The media cups without lids were covered with mesh and placed in the fume hood overnight to solidify. Afterwards, 15 males and 15 females were placed in each cup that was capped with a lid that had a 1.25 cm hole plugged with a moist dental cotton wick to provide

moisture source and multiple small pinholes to enable ventilation and prevent condensation. Five days later, the number of surviving adults was counted, and the results analyzed.

Puparia and adult development from eggs laid by methoxyfenozide-treated females in untreated medium

The dose-mortality information was used to set the dose range for experiments to determine whether post-embryonic development of flies was affected by methoxyfenozide treatment of females that oviposit in untreated medium or in medium with the same methoxyfenozide concentration as that used to treat females. Adults were treated as described above, and after five days, surviving adults were counted and half of the adults were transferred to new 16 oz cups with untreated media for 24 h. Flies were then removed, and ten eggs in the media of each cup were transferred by paintbrush to a new 2 oz cup containing approximately 25 cm^3 of untreated media. Ten replicates were set up for each concentration. Emergent puparia and adults were counted daily for 14 days, and adults removed immediately after counting so that they could not lay eggs.

Development of eggs laid by methoxyfenozide-treated females in methoxyfenozide-treated medium

The other half of the adults exposed to different concentrations of methoxyfenozide were transferred to new 16 oz cups containing media with the same methoxyfenozide dose for 24 h. Flies were then removed, and ten eggs in the media of each cup were transferred by paintbrush to a new 2 oz cup containing approximately 25 cm^3 of medium treated with the same dose of methoxyfenozide. Ten replicates were set up for each concentration. Emergent puparia and adults were counted daily for 14 days, and adults removed immediately after counting so that they could not lay eggs.

Data analysis

The analysis of data was conducted with R studio software version 3.5.2 (R Foundation for Statistical Computing; Vienna, Austria). To meet the assumptions of normality, data for adult mortality, pupation, adult emergence, time to pupation and time to adult emergence were analyzed by one-way ANOVA. Results were provided with means and ± standard errors (SE). Data were illustrated by the bar charts and tables, and treatments were assessed with regard to dose-dependent response. In addition to this, the means of each parameter were compared by Tuckey's honestly significant difference (HSD) test to evaluate the significant difference between means. During the statistical analysis, α=0.05 was used as the level of significance. Skewness and Kurtosis values were measured in order to determine if data is normally distributed. Skewness and Kurtosis values in all data were between -1.96 and + 1.96.

Results

Adult mortality after exposure to methoxyfenozide-treated medium

Adults continuously exposed to methoxyfenozide doses for 5 days did not exhibit significant mortality ($F= 0.811$; $df= 6$; $P= 0.0568$; Fig. 2.1), but females that survived that the 1000 ppm treatment laid few eggs.

Development of eggs laid by methoxyfenozide-treated females in untreated medium

The percentage of flies completing development from the egg stage to either the pupal or adult stage was significantly reduced by treatment of adults prior to oviposition with different concentrations of methoxyfenozide ($F= 19.05$; $df= 6$; $P < 0.001$; Fig. 2.2 and $F= 15.86$; $df= 6$; $P < 0.001$; Fig. 2.2). More pronounced effects were observed at concentrations 100 ppm and higher. Dead larvae with necrotic spots and adults with malformed wing were observed with 10

ppm and higher dosages. Ecdysis difficulties were also observed as larvae failed to molt and emergence was observed on the side of the larval exoskeleton rather than the head region.

Exposure of females to methoxyfenozide significantly prolongated puparia formation and adult emergence of their untreated progeny in a dose-responsive manner (pupariation: $F= 5.15$; $df= 6$; $P <0.001$; Table 2.1, and adult development: $F= 7.48$; $df=6$; $P <0.001$; Table 2.1). Untreated progeny that hatched from eggs whose adults were in control media and were treated up with 0.01 to 10 ppm took up approximately (~6 days) for puparia formation, but untreated progeny that hatched from eggs whose adults were in 100 and 1000 ppm took up approximately 6.5 days for puparia formation. On the other hand, untreated progeny that hatched from eggs whose adults were in control media and were treated up with 0.01 to 100 ppm took up approximately (~11 days) for adult emergence, but untreated progeny that hatched from eggs whose adults were in 1000 ppm took up approximately 11.5 days.

Development of eggs laid by methoxyfenozide-treated females in methoxyfenozide-treated medium

Treatment of females and their progeny with the same methoxyfenozide dose reduced the percentage of progeny that completed development to the pupal and adult stages in a concentration-dependent manner ($F= 170.4$; $df= 6$; $P < 0.001$; Fig. 2.3 and $F= 146.2$; $df= 6$; $P < 0.001$; Fig. 2.3). The percentage of puparia formation from media with the lower doses was similar to that of the control, but puparia formation from media treated with the 10 ppm dose was significantly reduced. No puparia were found in the 100 ppm and 1000 ppm treated media. A similar pattern was observed for adult emergence. Dead larvae with necrotic spots, larval-pupal intermediates, dead puparia, adults with malformed wings, and half enclosed adults were observed in the media with 0.1 ppm and higher doses. Ecdysis difficulties were also observed

such as larvae failed to molt, and emergence was observed on the side of the larval exoskeleton rather than the head region.

Methoxyfenozide exposure of females and their progeny significantly delayed puparia formation and adult emergence (pupariation: $F= 9.069$; $df= 4$; $P <0.001$; Table 2.2, and adult development: $F= 11.91$; $df= 4$; $P <0.001$; Table 2.2). Time to puparia formation and adult emergence by the progeny treated with the lower doses was similar to that of the control, but time to puparia formation and adult emergence by progeny treated with the 10 ppm dose was significantly higher. Most notably, time to pupation and adult emergence increased by approximately a day for progeny and females exposed to 10 ppm in comparison to those from control.

Discussion

To evaluate the efficacy of methoxyfenozide for control of *D. suzuki*, we first established that introduction of adults on methoxyfenozide-treated media did not cause mortality. This information was used to determine the effects of methoxyfenozide over a similar range on the development of progeny hatching from eggs oviposited by dosed adults in similarly dosed or untreated medium. In total, we found that the higher doses of methoxyfenozide (100 to 1000 ppm) used in all experiments had sub-lethal and lethal effects on the development of *D. suzukii.* No studies to date have evaluated the insecticidal activity of methoxyfenozide for control of *D. suzukii*.

Treatment of adults with high doses of methoxyfenozide prior to oviposition reduced pupariation and adult emergence of progeny deposited as eggs by the treated females in untreated medium. The highest dosage slightly delayed pupariation and adult emergence. Both males and females were treated with the methoxyfenozide, and higher doses appeared to have significant

effect on mating and fertilization of the oviposited eggs and the completion of embryonic and post-embryonic development, given the above data. These results stand in contrast to the greatly reduced and delayed pupariation and adult emergence of progeny hatching from eggs oviposited by *D. suzukii* females in medium treated with intermediate and higher doses of methoxyfenozide as were the adults. In this experimental situation, progeny hatching from the eggs were continuously exposed to methoxyfenozide in the medium both by feeding and integument penetration through the larval stages.

Our experiment showed that methoxyfenozide caused larval death with necrotic spots, ecdysis difficulties, larval-pupal intermediates, dead puparia, adult emergence with malformed wings and half enclosed adult emergence. Methoxyfenozide did not prevent puparia from turning into adults. Therefore, it can be speculated that methoxyfenozide can be a useful larvicide in contrast to pyriproxyfen which interferes with the development of the insect during pupal stage. However, these effects only can be obtained with high concentrations of methoxyfenozide on *D. suzukii*.

In addition, flies treated with 1000 ppm laid fewer eggs. Similar results were obtained when methoxyfenozide was tested on other dipterans. In one experiment, freshly laid eggs of the common house mosquito, *Culex pipiens L.* were treated with concentrations determined to be lethal (LC_{50}= 24.54 µg/L (0.024 ppm) and LC_{90}= 70.79 µg/L (0.07 ppm)). Gravid females were placed into a cage with methoxyfenozide treated water. Results showed that even though the LC_{50} dose caused 13.44% decrease in egg hatching, it was 46.99% with the LC_{90} dose. Larvae emerged from side of the exoskeleton rather than the head region, and larvae were observed with two heads or displaced eyes. Moreover, abnormal egg shell morphology and hatching patterns were noticed. Both concentrations prolonged development (Hamaidia and Soltani 2016).

Lawrence (1993) reported that when RH 5849 was topically applied to newly emerged caribbean fruit fly, *Anastrepha suspensa* (Loew) females at 1.0 g/fly in acetone, flies laid 60-75 % inviable eggs.

Experiments with other ecdysone agonists showed that although treated larvae initiate ecdysis, pesticide application results in the failure of this process by interfering with ecdysteroid titer level (Dhadialla et al. 1998). Diacylhydrazines including methoxyfenozide can cause toxicity in insects by binding ecdysone receptor (Ecr), just as 20-hydroxyecdysone (20E) (Soin et al. 2010). Even though methoxyfenozide is toxic to lepidopterans selectively, some exceptions have been observed with some dipteran insects. There are three main reasons methoxyfenozide can selectively work on different species even in the same order (Dhadialla et al. 1998, Dhadialla 2005). (1) A metabolic difference in detoxification is observed between insects in terms of susceptibility. (2) The pesticide is not transported to target site in non-susceptible insects. (3) Target sites can differ between susceptible and non-susceptible insects.

Similar results were obtained from other studies when methoxyfenozide is tested on dipterans and other orders. Darvas et al. (1998) reported that methoxyfenozide was approximately 10 times more potent than other ecdysteroid agonists when tested on yellow fever mosquito, *Aedes aegypti* (L.), larvae. In this experiment, 4^{th}-instar *A. aegypti* larvae were introduced into 100 ml of water treated with methoxyfenozide with a wide range of doses between (0.63, 0.31, 0.16, 0.08, 0.04, and 0.02 mg $^{kg-1}$). Methoxyfenozide led to the evagination of early imaginal discs and expansion of the larval thorax, which resulted in the formation of the larval-pupal intermediates. The swollen puparia-like thorax lacked anterior spiracles. Application of the 0.02 mg $^{kg-1}$ (0.02 ppm, lowest concentration) of methoxyfenozide led to these consequences. Moreover, molting failure was observed due to hanging larval exuvium or head

capsule slippage failure (Darvas et al. 1998). Beckage et al. (2004) reported similar results were obtained with methoxyfenozide tested on late 3rd instar larvae mosquito species such as *A. aegypti,* southern house mosquito*, Culex quinquefasciatus* Say, and african malaria mosquito, *Anopheles gambiae* Giles. After mosquito larvae were treated with methoxyfenozide, insects were unable to molt successfully. Even though a new cuticle was synthesized by larvae with normal sclerotization, larvae were unable to ecdyse and shed the exuvium. LC_{50} values of methoxyfenozide were 12.85 (0.12 ppm), 3.12 (0.03 ppm), and 2.75 (0.02 ppm) µg/100 ml for *A. aegypti, C. quinquefasciatus*, and *A. gambiae* in turn.

Hamaidia et al. (2018) reported that methoxyfenozide had insecticidal activity on common house mosquito, *Culex pipiens* L. and mosquito, *Culiseta longiareolata* Macquart, newly molted 4th larvae. After 24 hours exposure, methoxyfenozide blocked molting, and larval death was observed due to the exuviation difficulties. LC_{50} values were 25.67 (0.025 ppm) and 24.54 (0.024 ppm) µg L^{-1} for *C. longiareolata* and *C. pipiens* mortality, in turn. Hamaidia et al. (2018) also reported that methoxyfenozide reduced larval and pupal development time with a dose-response relationship. In addition to this, methoxyfenozide interfered with body volume and biochemical content, such as proteins, carbohydrates and lipids, in both *C. longiareolata* and *C. pipiens.* Some distinctive morphological abnormalities were observed such as curved larval body, distended larval head, deformed air tube, exuviations difficulties, curly wings. Trisyono and Chippendale (1997) also reported that when tebufenozide was tested on 4th instar European corn borer, *Ostrinia nubilalis* (Hubner) by feeding method with treated diets at 0.016 and 0.031 ppm concentrations*,* larval inhibition of growth, delayed pupation and decreased adult emergence were observed.

Methoxyfenozide can be useful in that it can be incorporated into IPM with the purpose of the suppression of *D. suzukii* populations. Additionally, methoxyfenozide can be incorporated into IPM programs to target *D. suzukii* specifically rather than using broad-spectrum insecticides killing nontarget beneficial insects. Nonetheless, methoxyfenozide should be examined extensively before it is incorporated into IPM and whether methoxyfenozide is harmless for the natural enemies of *D. suzukii* should be determined.

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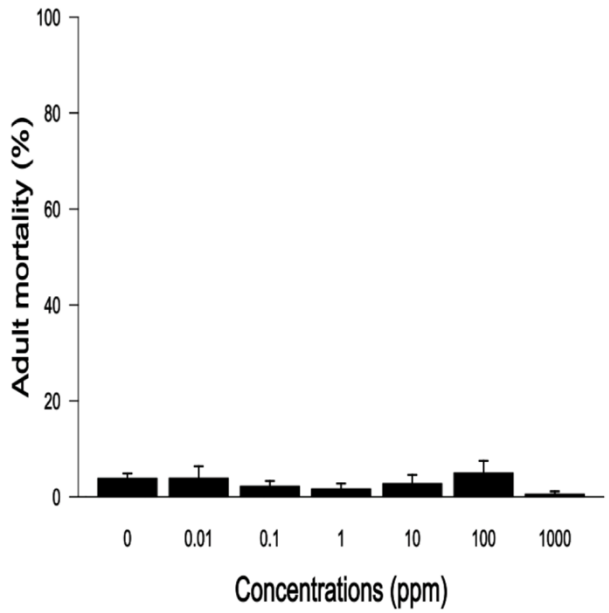

Fig. 2.1. Percent mortality of *D. suzukii* adults exposed to medium treated with methoxyfenozide doses (ppm) for five days (Mean ± SEM). Letters denote significantly different mean percentages of adult mortality among treatment groups at the 0.05 significance level (Tukey's HSD test). F= 0.811; df= 6; P= 0.0568.

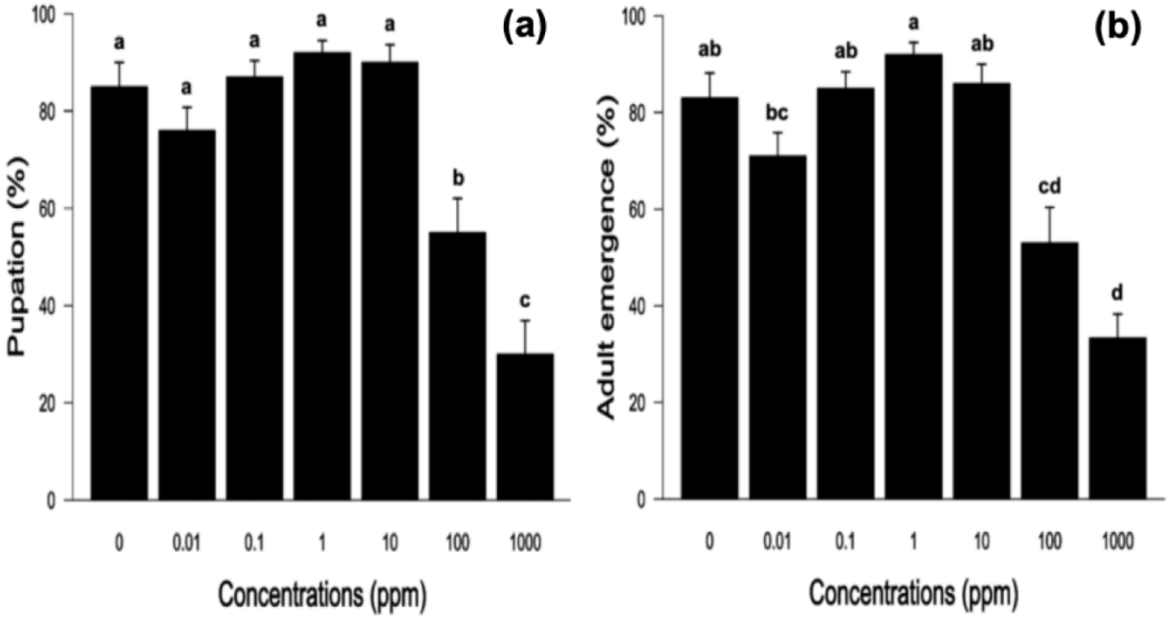

Fig. 2.2. Percent pupariation (a) and adult emergence (b) from eggs laid by *D. suzukii* females treated with methoxyfenozide doses (ppm) in untreated medium. Letters denote significantly different percentages of pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test). $F=19.05$; $df= 6$; $P < 0.001$, and $F= 15.86$; $df= 6$; $P < 0.001$.

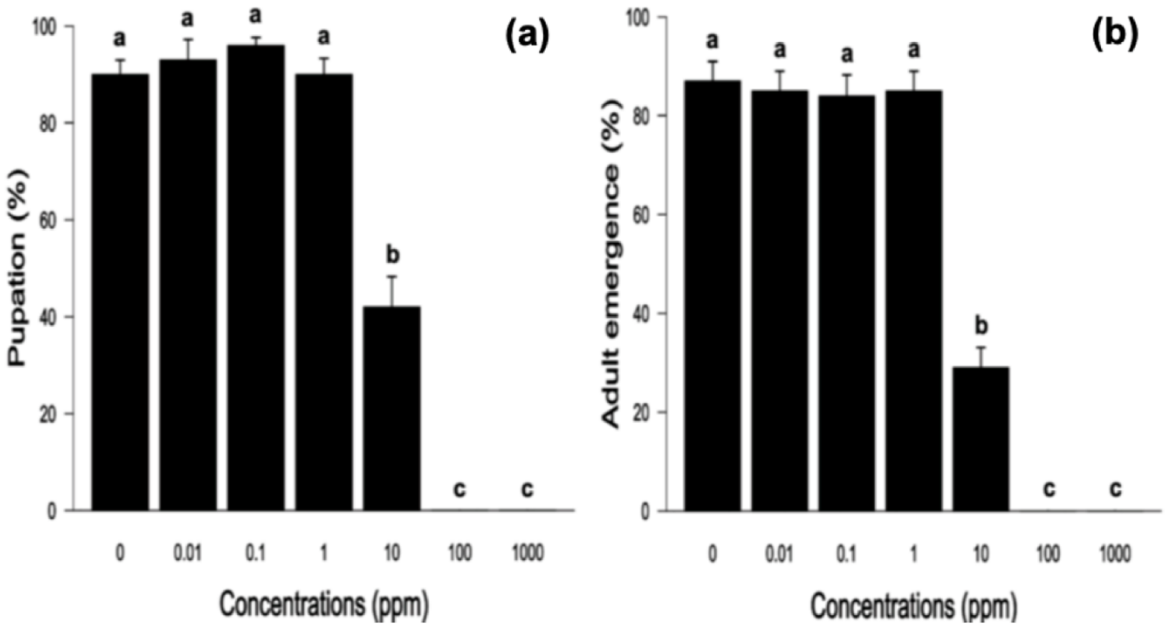

Fig. 2.3. Percent pupariation (a) and adult emergence (b) from eggs laid by methoxyfenozide-treated *D. suzukii* females in medium treated with the same methoxyfenozide dose (ppm). Letters denote significantly different percentages of pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test). $F= 170.4$; $df= 6$; $P < 0.001$ and $F= 146.2$; $df= 6$; $P < 0.001$.

Fig. 2.4. Dead larva with necrotic spot. Methoxyfenozide adult only exposure test (10 ppm in media).

Fig. 2.5. Dead adult with malformed wing. Methoxyfenozide adult only exposure test (10 ppm in media).

Fig. 2.6. Dead larva emerging from side of the larval exoskeleton. Methoxyfenozide adult only exposure test (100 ppm in media).

Fig. 2.7. Dead larva with necrotic spot. Methoxyfenozide adult only exposure test (100 ppm in media).

Fig. 2.8. Dead half enclosed adult. Methoxyfenozide continuous exposure test (0.1 ppm in media).

Fig. 2.9. Dead adult with malformed wing. Methoxyfenozide continuous exposure test (1 ppm in media).

Fig. 2.10. Dead larval-pupal intermediates. Methoxyfenozide continuous exposure test (10 ppm in media).

Fig. 2.11. Dead larva with necrotic spot. Methoxyfenozide continuous exposure test (10 ppm in media).

Fig. 2.12. Dead larva emerging from side of the larval exoskeleton. Methoxyfenozide continuous exposure test (10 ppm in media).

Fig. 2.13. Dead puparia. Methoxyfenozide continuous exposure test (10 ppm in media).

Table 2.1. Duration of puparia and adult development from eggs oviposited by methoxyfenozide-treated *D. suzukii* females in untreated medium

Methoxyfenozide dose for adults (ppm)	Days to pupation[a]	Days to adult emergence[b]
0	6.21 ± 0.06ab	11.17 ± 0.06abc
0.01	5.98 ± 0.05b	10.80 ± 0.08cd
0.1	6.05 ± 0.04b	10.76 ± 0.05d
1	6.02 ± 0.05b	10.93 ± 0.05bcd
10	6.16 ± 0.06ab	11.24 ± 0.08ab
100	6.40 ± 0.08a	11.26 ± 0.09ab
1000	6.42 ± 0.19a	11.40 ± 0.19a

Letters denote significantly delayed pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test).

[a] Number of days (mean ± SEM) from oviposition to pupation after treatment.

[b] Number of days (mean ± SEM) from oviposition to adult emergence.

Table 2.2. Duration of puparia and adult development from eggs oviposited by methoxyfenozide-treated *D. suzukii* females in methoxyfenozide-treated medium

Methoxyfenozide dose for adults and progeny (ppm)	Days to pupation[a]	Days to adult emergence[b]
0	5.90 ± 0.10b	10.84 ± 0.06b
0.01	5.74 ± 0.07b	10.62 ± 0.05b
0.1	5.80 ± 0.06b	10.83 ± 0.07b
1	5.74 ± 0.11b	10.93 ± 0.11b
10	6.71 ± 0.24a	11.56 ±0.17a
100	No pupae	No adult
1000	No pupae	No adult

Letters denote significantly delayed pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test).

[a] Number of days (mean ± SEM) from oviposition to pupation after treatment.

[b] Number of days (mean ± SEM) from oviposition to adult emergence.

CHAPTER 3

LETHAL AND SUBLETHAL EFFECTS OF THE INSECT GROWTH REGULATOR, NOVALURON, ON *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

Introduction

Chitin synthesis inhibitors (CSIs) are usually applied as larvicides to insects for pest control. Even though larvae treated with CSIs can survive until molting, these chemicals disrupt synthesis of new cuticle by inhibiting chitin synthesis. Due to the fact that larvae molt at every instar, CSI can be effective during all larval stages (Miyamoto et al. 1993). These compounds not only induce abortive molting but also abnormal endocuticular deposition (Mulder 1973). Younger instars are more sensitive to CSIs compared to older ones in most insect species (Grosscurt 1978).

Benzoylphenyl ureas are a class of CSIs used as larvicides, and they also disrupt the fertility and fecundity (Miyamoto et al. 1993). Importantly, inhibition of egg hatch can be observed when the female adults are treated both by oral uptake and contact exposure (Grosscurt 1978). Therefore, CSIs are both ovicidal and larvicidal for different insect pests (Ascher et al. 1987). Benzoylphenyl ureas also are antifeedant agents (Malinowski and Pawinska 1992). The authors reported that the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) larvae consumed less novaluron treated leaves compared to untreated leaves. However, CSIs have not been reported to be adulticide since these chemicals do not induce direct knockdown or mortality (Elek 1988a).

Novaluron is considered to be safe owing to the fact that it rarely causes negative effects on non-target beneficial species, because this chemical inhibits chitin formation species-specifically (Ishaaya et al. 2001). Therefore, adults of non-target species such as predators and parasitoids are rarely affected (Ishaaya et al. 2002). Novaluron is also an ideal chemical because it has low mammalian toxicity (LC-50 for rat - oral > 5000 mg/kg) (Ishaaya et al. 2001). In addition, novaluron did not cause cross-resistance between other pesticides such as pyriproxyfen and buprofezin when tested on whiteflies (Ishaaya et al. 2002). Because novaluron is a rainfast compound, this chemical is an ideal pesticide for rainy seasons (Ishaaya et al. 2001). In the new era of agriculture, biorational pesticides are needed that can eliminate targeted pest and also are harmless to non-target organisms (Cutler 2005). Because resistance has become an important concern, more environmentally friendly and target specific pesticides are considered to be beneficial for the development of agriculture (Ishaaya 2003). Therefore, incorporation of novaluron into IPM can be beneficial to achieve more desirable results. For these reasons, trials were initiated to determine whether novaluron would disrupt development and could be incorporated into *D. suzukii* management.

Material and Methods

Insects

D. suzukii were taken from a laboratory colony established from flies captured in Clarke County, GA in 2013. Flies were reared in 117 ml square bottom polypropylene bottles (Genesee Scientific, San Diego, CA) each containing 50 ml of standard fly diet (Jaramillo et al. 2015). A pinch of active dried baker's yeast was sprinkled into each bottle. Bottles were capped with bonded dense-weave cellulose acetate plugs and placed on plastic trays in growth incubators (Model I36VLCB, Percival Scientific, Perry, IA) set at 24°C, 65% relative humidity, and a

photoperiod of 14 h light:10 h dark. All experimental groups were housed in the same conditions.

Insecticide

Novaluron (Diamond® 0.83EC, Insect Growth Regulator, EPA Reg. No. 66222-35-400) was obtained from Chemtura Corporation, Middlebury, VT.

Adult mortality after exposure to novaluron treated medium

The first test was designed to determine the mortality effects of novaluron in the media contacted or ingested by *D. suzukii* adults. Newly emerged virgin adults were separated based on sex using the dark spots on the male wings as a distinguishing characteristic (Walsh et al. 2011) and transferred to media treated with a wide dosage range of novaluron. The Diamond, novaluron formulation, was diluted to 1000 ppm with deionized water as a stock solution. Serial dilutions in water were prepared from the stock solution to 100 ppm, 10 ppm, 1 ppm, 0.1 and 0.01 ppm. For each concentration, six 16 oz plastic deli cups (Hofmann plastics-HT16) were filled with Instant ready blue media (20 g, Formula 4-24® Instant Drosophila Medium), yeast (3.2 mg, Fleischmann's active dry yeast), and 100 ml insecticide solution and mixed. Control cups were filled with the same mixture including water with no insecticide. The media cups without lids were covered with mesh and placed in the fume hood overnight to solidify. Afterwards, 15 males and 15 females were placed in each cup that was capped with a lid that had a 1.25 cm hole plugged with a moist dental cotton wick to provide moisture source and multiple small pinholes to enable ventilation and prevent condensation. Five days later, the number of surviving adults was counted, and the results analyzed.

Puparia and adult development from eggs laid by novaluron-treated females in untreated medium

The dose-mortality information was used to set the dose range for experiments to determine whether post-embryonic development of flies was affected by novaluron treatment of females that oviposit in untreated medium or in medium with the same novaluron concentration as that used to treat females. Adults were treated as described above, and after five days, surviving adults were counted and half of the adults were transferred to new 16 oz cups with untreated media for 24 h. Flies were then removed, and ten eggs in the media of each cup were transferred by paintbrush to a new 2 oz cup containing approximately 25 cm^3 of untreated media. Ten replicates were set up for each concentration. Emergent puparia and adults were counted daily for 14 days, and adults removed immediately after counting so that they could not lay eggs.

Development of eggs laid by novaluron-treated females in novaluron-treated medium

The other half of the adults exposed to different concentrations of novaluron were transferred to new 16 cups containing media with the same novaluron dose for 24 h. Flies were then removed, and ten eggs in the media of each cup were transferred by paintbrush to a new 2 oz cup containing approximately 25 cm^3 of medium treated with the same dose of novaluron. Ten replicates were set up for each concentration. Emergent puparia and adults were counted daily for 14 days, and adults removed immediately after counting so that they could not lay eggs.

Data analysis

The analysis of data was conducted with R studio software version 3.5.2 (R Foundation for Statistical Computing; Vienna, Austria). To meet the assumptions of normality, data for adult mortality, pupation, adult emergence, time to pupation and time to adult emergence were analyzed by one-way ANOVA. Results were provided with means and ± standard errors (SE).

Data were illustrated by the bar charts and tables, and treatments were assessed with regard to dose-dependent response. In addition to this, the means of each parameter were compared by Tuckey's honestly significant difference (HSD) test to evaluate the significant difference between means. During the statistical analysis, $\alpha=0.05$ was used as the level of significance. Skewness and Kurtosis values were measured in order to determine if data is normally distributed. Skewness and Kurtosis values in all data were between -1.96 and + 1.96.

Results

Adult mortality after exposure to novaluron-treated medium

Continuous exposure of adults to novaluron for 5 days resulted in significant mortality with the 1000 ppm dose ($F= 7.622$; $df= 6$; $P < 0.001$; Fig. 3.1). Females that survived the 100 and 1000 ppm treatments laid few to no eggs.

Development of eggs laid by novaluron-treated females in untreated medium

Novaluron treatment of females significantly affected puparium formation and adult emergence of progeny in untreated medium ($F=33.58$; $df=4$; $P < 0.001$; Fig. 3.2 and $F=28.22$; $df=4$; $P < 0.001$; Fig. 3.2). The percentage of puparia formation and adults emerging from media with the lower doses was similar to that of the control media, but puparia formation and adult emergence from media treated with the 10 ppm dose was significantly reduced. Dead larvae with necrotic spots and puparia were observed with 0.01 ppm and higher dosages.

There was no significant delay in puparia formation and adult emergence of progeny hatching from eggs laid by treated adults (pupariation: $F=1.67$; $df= 4$; $P= 0.172$; Table 3.1 and adult development: $F=2.44$; $df= 4$; $P= 0.06$; Table 3.1).

Development of eggs laid by novaluron-treated females in novaluron-treated medium

Puparium formation was significantly inhibited in the progeny exposed to novaluron in medium over the same range as the parental adults ($F=41.44$; $df= 4$; $P < 0.001$; Fig. 3.3). The percentage of progeny that completed pupariation was significantly reduced with lower dosages, and no pupae were found in 1 ppm and 10 ppm dosages. Adult emergence was blocked by novaluron over the same range ($F=40.29$; $df=4$; $P < 0.001$; Fig. 3.3). Dead larvae with necrotic spots, dead puparia, and adults with malformed wings were observed with 0.01 ppm and higher dosages. Importantly, all progeny in the 10 ppm treated media died in the 1st instar stage.

Exposure of females and their progeny to novaluron significantly prolonged puparia formation and adult emergence in a dose-responsive manner ($F= 5.37$; $df= 2$; $P= 0.010$; Table 3.2 and $F= 6.87$; $df= 2$; $P= 0.003$; Table 3.2).

Discussion

To evaluate the efficacy of novaluron for control of *D. suzuki*, we first established that introduction of adults on novaluron treated-media caused mortality in a concentration-dependent manner. The mortality test showed that novaluron caused 20% adult mortality with the 1000 ppm dose within 5 days in our study, and when 5% mortality was observed for controls. This information was used to determine the effects of novaluron over a similar range on the development of progeny hatching from eggs oviposited by dosed adults in similarly dosed or untreated medium. In total, we found that the medium and higher doses of novaluron (10 to 1000 ppm) in the media had lethal and sub-lethal effects on the development of *D. suzukii.* No studies to date have evaluated the insecticidal activity of novaluron for the control of *D. suzukii*.

Treatment of adults with sublethal doses prior to oviposition did not affect pupariation and adult emergence of progeny deposited as eggs in untreated medium, but the highest dose10

ppm reduced both significantly. No dose significantly delayed pupation and adult emergence. These results stand in contrast to the greatly reduced pupariation and adult emergence of progeny hatching from eggs oviposited by treated *D. suzukii* females in medium treated with intermediate and lower doses of novaluron. In this experimental situation, progeny hatching from the eggs were continuously exposed to novaluron in the medium both by feeding and integument penetration through the larval stages.

Our experiment showed that novaluron caused larval and puparia death, and adults emerged with malformed wings. Females that survived the 100 and 1000 ppm treatments laid few to no eggs. This ovicidal effect can occur with either topical treatment to the eggs or by feeding of adult females in several species (Grosscurt 1978). In the adult exposure experiment, egg or progeny development may have been affected by transmission of toxicant to eggs during vitellogenesis, as suggested in studies of in several species (Elek 1998b, Ivie and Wright 1978). Another reason hatchability of eggs may be affected can be because cuticle secretion during embryogenesis could be impaired by novaluron. After Colorado potato beetle, *Leptinotarsa decemlineata* was treated with diflubenzuron, and examined by electron microscopy, it was proposed that the main reason larvae cannot emerge from the egg is because of the formation of an amorphous cuticule rather than a normal lamellate cuticle. Therefore, it is likely that this process can disable embryo use of muscles in order to leave eggs due to the lack of adequate rigidness of cuticle (Grosscurt 1978). Ovicidal effect of novaluron also was observed for dipteran species such as common house mosquito, *Culex pipiens,* when the chemical was tested on female adults. Results showed that mosquitos in the control group laid 1571 eggs and this number decreased to 1353 by the group treated with the LC_{50} dose of 0.33µg/l (0.00033 ppm) and to 1304 by group treated with the LC_{90} dose of 0.75 µg/l (0.00075 ppm). Similarly, although 96.84%

hatching was observed in control group, and it was 75.49% in the group treated with the LC_{50} dose and 55.02% in the group treated with the LC_{90} dose (Djeghader et al. 2014).

Larvae treated with novaluron can grow until molting, but death occurs during ecdysis owing to the lack of biosynthesis of chitin (Tunaz and Uygun 2004). The results of my study are consistent with reports that show novaluron interferes with the development of dipterans and other insect groups. Mulla et al. (2003) reported that novaluron inhibited adult emergence when novaluron was tested on 2nd and 4th instar yellow fever mosquito, *Aedes aegypti (L.)* larvae in water-storage containers. It was also reported that 2nd larvae were more susceptible than 4th instar larvae during this experiment. At the high concentration (1 ppb (0.001 ppm)), the vast majority of mortality occurred in the larval stage. However, malformed adults and dead puparia were observed with lower concentrations (<1 ppb (<0.001 ppm)) (Mulla et al. 2003). Djeghader et al. (2014) reported that novaluron caused mortality when it was applied to 4th instar common house mosquito, *Culex pipiens L*. The highest concentration of 0.9 µg/l (0.0009 ppm) induced more than 96% of mortality. However, the lowest concentration of 0.3 µg/l (0.0003 ppm) caused 40% mortality. Some morphological effects were observed such as precocious and incomplete emergence and adults that were unable to leave their exuvial cuticle completely. Novaluron prolonged larval stages and adult longevity when larvae were treated. Significant mortality was not observed when mosquito puparia were treated, but pupal development time increased significantly.

Other dipteran species are similarly affected by novaluron treatment. When novaluron was tested on eggs/larvae of housefly, *Musca domestica* (L.) high concentrations 10 (10 ppm) and 20 (20 ppm) mg active ingredient (ai)/kg caused >80 % larval mortality in both assays including dipping 0-2 hours old 1st instar larvae and feeding by larvae placed in media as eggs. In

this experiment, the feeding method caused more mortality compared to the dipping method. At sublethal doses, 10 (10 ppm) and 20 (20 ppm) mg ai/kg of novaluron, adult emergence with disabilities such as malformation of the wing, leg, head, thorax and abdomen were observed in both bioassays (Cetin et al. 2006). Bouaziz et al. (2011) also reported that novaluron was effective against mosquito, *Culiseta longiareolata* (Macquart) when 3rd and 4th stage larvae were tested. The LC_{50} doses were 0.51 (0.00051 ppm) and 0.91 (0.00091 ppm) µg/l for 3rd and 4th stage larvae in turn. During the experiment, metabolite analyses of treated insects showed that novaluron affected carbohydrate, lipid and protein. Even though increases were observed for carbohydrate and lipid amounts, the storage protein amount declined. In addition, novaluron application decreased body weight and development time. Su et al. (2003) also reported that novaluron was highly effective when it was tested on 2nd and 4th stage larvae of the southern house mosquito, *Culex quinquefasciatus* Say. Novaluron solutions were added to disposable waxed-paper cups with mosquito larvae. Inhibition of adult emergency (IE_{50}) was 0.159 ppb (0.000159 ppm) for 2nd stage larvae and 0.118 ppb (0.000118 ppm) for 4th stage larvae. Arredondo-Jiménez et al. (2006) also reported that novaluron was an effective larvicide for different mosquito species as 1st instar larvae. LC_{50} values were 18.99 (0.0018 ppm), 16.56 (0.0016 ppm), 15.36 (0.0015 ppm), 19.17 (0.0019 ppm) and 9.45 (0.0009 ppm) g a.i./L for the new world malaria mosquito, *Anopheles albimanus Wiedemann*, *Anopheles pseudopunctipennis* (T.), yellow fever mosquito, *Aedes aegypti (L.)*, asian tiger mosquito, *Aedes albopictus* (Skuse), and southern house mosquito *Culex quinquefasciatus* (Say) respectively.

The majority of pesticides are considered to be toxic because they cause multigenic, carcinogenic and teratogenic effects on human being and domestic animals. IGR's are considered to be a safe group of pesticides against non-target invertebrates, fish, birds, and other

wildlife (Tunaz and Uygun 2004). Because novaluron inhibits chitin formation selectively in insects, this chemical is considered safe owing to the fact that it rarely causes negative results on adults of non-target beneficial organisms such as predators and parasites (Ishaaya et al. 2002). The new era of agriculture needs biorational pesticides which can eliminate targeted pests, and which are also harmless to non-target organisms (Cutler 2005). Because insect resistance is an important concern, more environmentally friendly and target specific pesticides are considered to be beneficial for the development of agriculture (Ishaaya 2003). Therefore, stage-specific and environmentally friendly IGR's such as novaluron can be an excellent option of IPM strategies (Tunaz and Uygun 2004).

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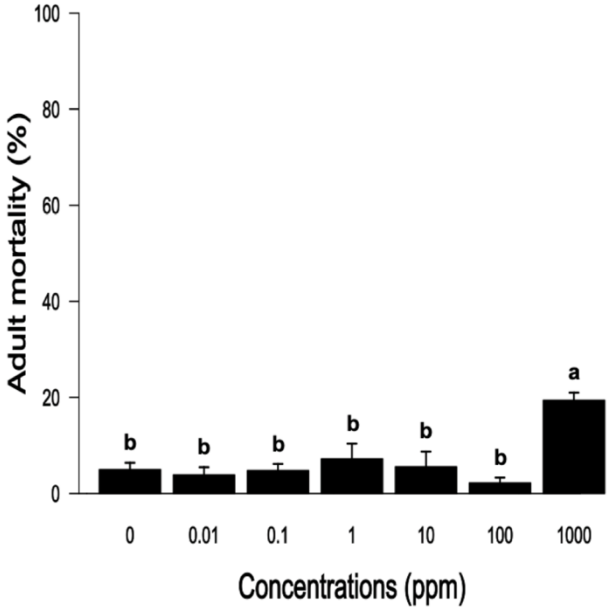

Fig. 3.1. Percent mortality of *D. suzukii* adults exposed to medium treated with novaluron doses (ppm) for five days (Mean ± SEM). Letters denote significantly different mean percentages of adult mortality among treatment groups at the 0.05 significance level (Tukey's HSD test). F= 7.622; df= 6; $P < 0.001$.

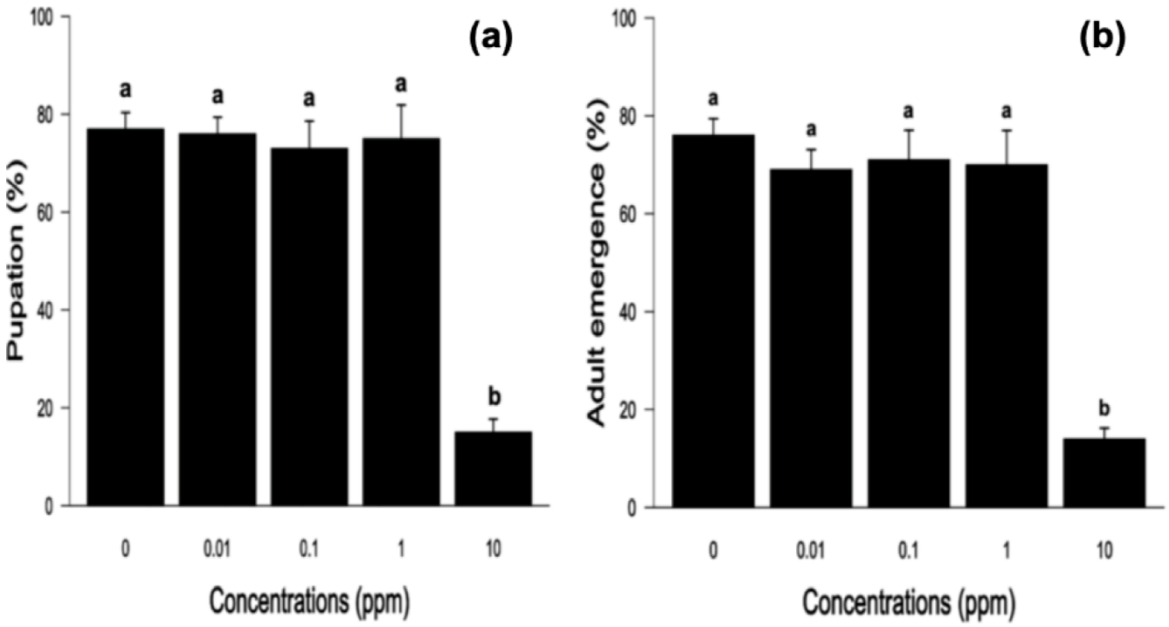

Fig. 3.2. Percent pupariation (a) and adult emergence (b) from eggs laid by *D. suzukii* females treated with novaluron doses (ppm) in untreated medium. Letters denote significantly different percentages of pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test). $F=33.58$; $df= 4$; $P < 0.001$ and $F=28.22$; $df=4$; $P < 0.001$.

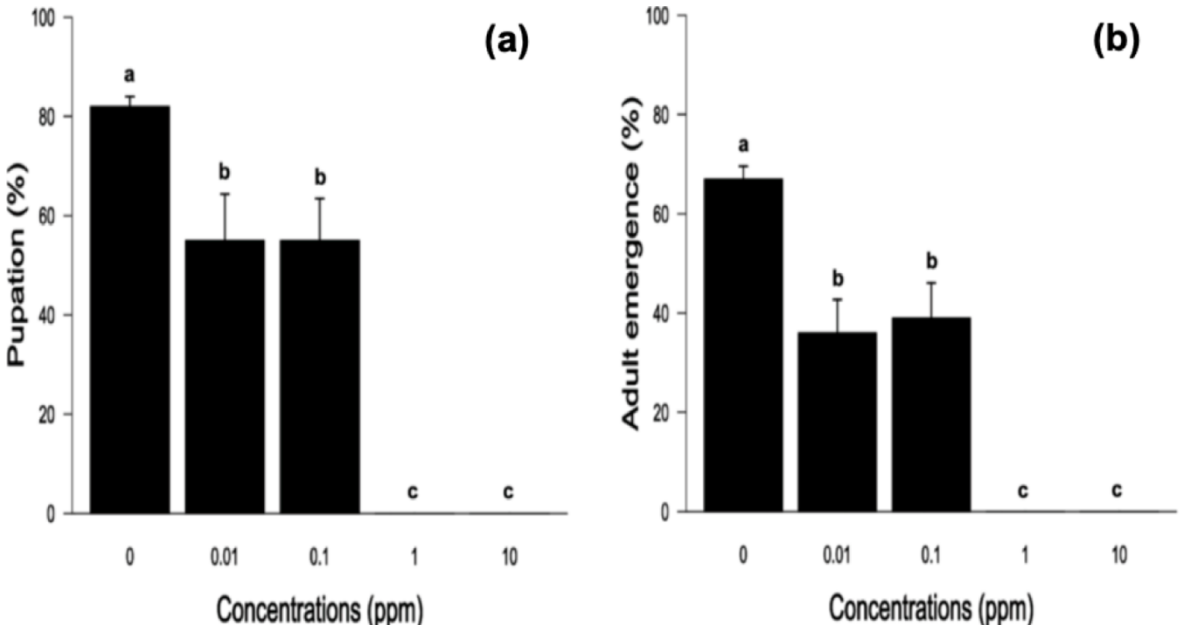

Fig. 3.3. Percent pupariation (a) and adult emergence (b) from eggs laid by novaluron-treated *D. suzukii* females in medium treated with the same novaluron dose (ppm). Letters denote significantly different percentages of pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test). $F=41.44$; $df= 4$; $P < 0.001$ and $F=40.29$; $df=4$; $P < 0.001$.

Fig. 3.4. Dead larva with necrotic spot. Novaluron adult only exposure test (0.01 ppm in media).

Fig. 3.5. Dead puparia. Novaluron adult only exposure test (0.01 ppm in media).

Fig. 3.6. Dead adult with malformed wing. Novaluron continuous exposure test (0.01 ppm in media).

Fig. 3.7. Dead larva with necrotic spot. Novaluron continuous exposure test (0.01 ppm in media).

Fig. 3.8. Dead puparia. Novaluron continuous exposure test (0.1 ppm in media).

Fig. 3.9. Dead larva with necrotic spot. Novaluron continuous exposure test (0.1 ppm in media).

Fig. 3.10. Dead 1st instar larva with necrotic spot. Novaluron continuous exposure test (10 ppm in media).

Table 3.1. Duration of puparia and adult development from eggs oviposited by novaluron-treated *D. suzukii* females in untreated medium

Novaluron dose for adults (ppm)	Days to pupation[a]	Days to adult emergence[b]
0	5.99 ± 0.03	11.43 ± 0.05
0.01	6.14 ± 0.08	11.76 ± 0.09
0.1	6.14 ± 0.06	11.77 ± 0.14
1	6.42 ± 0.40	11.60 ± 0.08
10	6.83 ± 0.39	11.88 ± 0.16

[a] Number of days (mean ± SEM) from oviposition to pupation after treatment.

[b] Number of days (mean ± SEM) from oviposition to adult emergence.

Table 3.2. Duration of puparia and adult development from eggs oviposited by novaluron-treated *D. suzukii* females in novaluron-treated medium

Novaluron dose for adults and progeny (ppm)	Days to pupation[a]	Days to adult emergence[b]
0	5.69 ± 0.10b	10.54 ± 0.06ab
0.01	6.29 ± 0.15a	10.81 ±0.09a
0.1	5.81 ± 0.13ab	10.36 ± 0.08b
1	No pupae	No adult
10	No pupae	No adult

Letters denote significantly delayed pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test).

[a] Number of days (mean ± SEM) from oviposition to pupation after treatment.

[b] Number of days (mean ± SEM) from oviposition to adult emergence.

CHAPTER 4

LETHAL AND SUBLETHAL EFFECTS OF THE INSECT GROWTH REGULATOR, PYRIPROXYFEN, ON *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

Introduction

Insect growth regulators (IGRs) are unique in that they are insect-specific and stage-specific. However, effects are observed to occur more slowly compared to other chemicals (Seccacini et al. 2014). Juvenile hormone analogs (JHAs) are IGRs that are mostly isolated from plants and coevolution of plants for defense against insects is considered to be a factor for the development of these chemicals in plants (Dhadialla et al. 2005). A few JHAs have been produced such as methoprene, hydroprene, kinoprene, fenoxycarb, and pyriproxyfen (Dhadialla et al. 2005).

Pyriproxyfen is a phenoxy analog and it is similar to fenoxycarb (Dhadialla et al. 2005). Pyriproxyfen is a JHA and has been used for agricultural purposes to control a range of arthropods since 1990s. It was firstly registered in 1991 to control mosquitos in Japan (Miyamoto et al. 1993). Pyriproxyfen is effective on different orders such as Diptera, Hemiptera, Dictyoptera, Lepidoptera, Hymenoptera, Siphonoptera and Orthoptera (Chen and Liu 2002). Importantly, pyriproxyfen is more effective when it is applied during last larval stage or embryogenesis (Tunaz and Uygun 2004). Pyriproxyfen is considered to be a biorational pesticide since it is an ideal alternative for organophosphates (Tunaz and Uygun 2004). Under normal circumstances, when an insect has reached critical weight in the last instar, production of juvenile hormone (JH) is blocked and metamorphosis is initiated by the release of ecdysteroid

hormones. However, pyriproxyfen acting as a JH analog with high stability effectively binds to JH receptors thereby preventing metamorphosis or disabling adult emergence of dipteran species and cause suppression of embryogenesis and sterility (Bensebaa et al. 2015, Miyamoto et al. 1993, Sullivan and Goh 2008, Zhou and Riddiford 2008).

Integrated pest management requires selective pesticides that can preserve the natural enemies (Chen and Liu 2002). The mode of action of pyriproxyfen suggests it likely has a low risk of resistance selection in insect pest populations, and it is generally non-toxic to bees, vertebrates and natural enemies of target pests (Sullivan and Goh 2008). Pyriproxyfen is also considered to be safe for mammalian health and environment by EPA (Sihuincha et al. 2005). For these reasons, trials were initiated to determine whether pyriproxyfen would disrupt development and could be incorporated into *D. suzukii* management.

Material and Methods

Insects

D. suzukii were taken from a laboratory colony established from flies captured in Clarke County, GA in 2013. Flies were reared in 117 ml square bottom polypropylene bottles (Genesee Scientific, San Diego, CA) each containing 50 ml of standard fly diet (Jaramillo et al. 2015). A pinch of active dried baker's yeast was sprinkled into each bottle. Bottles were capped with bonded dense-weave cellulose acetate plugs and placed on plastic trays in growth incubators (Model I36VLCB, Percival Scientific, Perry, IA) set at 24°C, 65% relative humidity, and a photoperiod of 14 h light:10 h dark. All experimental groups were housed in the same conditions.

Insecticide

Pyriproxyfen (Knack 0.86 EC, Insect Growth Regulator, EPA Reg. No. 59639-95) was obtained from Valent USA Corporation, Walnut Creek, CA.

Adult mortality after exposure to pyriproxyfen-treated medium

The first test was designed to determine the mortality effects of pyriproxyfen in the media contacted or ingested by *D. suzukii* adults. Newly emerged virgin adults were separated based on sex using the dark spots on the male wings as a distinguishing characteristic (Walsh et al. 2011) and transferred to media treated with a wide doses range of pyriproxyfen. The Knack, pyriproxyfen formulation, was diluted to 1000 ppm with deionized water as a stock solution. Serial dilutions in water were prepared from the stock solution to 100 ppm, 10 ppm, 1 ppm, and 0.1 ppm. For each concentration, six 16 oz plastic deli cups (Hofmann plastics-HT16) were filled with Instant ready blue media (20 g, Formula 4-24® Instant Drosophila Medium), yeast (3.2 mg, Fleischmann's active dry yeast), and 100 ml insecticide solution and mixed. Control cups were filled with the same mixture including water with no insecticide. The media cups without lids were covered with mesh and placed in the fume hood overnight to solidify. Afterwards, 15 males and 15 females were placed in each cup that was capped with a lid that had a 1.25 cm hole plugged with a moist dental cotton wick to provide moisture source and multiple small pinholes to enable ventilation and prevent condensation. Five days later, the number of surviving adults was counted.

Development of eggs laid by pyriproxyfen-treated females in pyriproxyfen-treated medium

The dose-mortality information was used to set the dose range for experiments to determine whether post-embryonic development of flies was affected by pyriproxyfen treatment of females that oviposit in treated medium with the same pyriproxyfen concentration as that used

to treat females. Adults were treated as described above, and after five days, surviving adults were counted. Adults exposed to different concentrations of pyriproxyfen were transferred to new 16 oz cups containing media with the same pyriproxyfen dose for 24 h. Flies were then removed, and ten eggs in the media of each cup were transferred by paintbrush to a new 2 oz cup containing approximately 25 cm^3 of medium treated with the same dose of pyriproxyfen. Ten replicates were set up for each concentration. Emergent puparia and adults were counted daily for 14 days, and adults removed immediately after counting so that they could not lay eggs.

Data analysis

The analysis of data was conducted with R studio software version 3.5.2 (R Foundation for Statistical Computing; Vienna, Austria). To meet the assumptions of normality, data for adult mortality, pupation, adult emergence, time to pupation and time to adult emergence were analyzed by one-way ANOVA. Results were provided with means and ± standard errors (SE). Data were illustrated by the bar charts and tables, and treatments were assessed with regard to dose-dependent response. In addition to this, the means of each parameter were compared by Tuckey's honestly significant difference (HSD) test to evaluate the significant difference between means. During the statistical analysis, $\alpha=0.05$ was used as the level of significance. Skewness and Kurtosis values were measured in order to determine if data is normally distributed. Skewness and Kurtosis values in all data were between -1.96 and + 1.96.

Results

Adult mortality after exposure to pyriproxyfen-treated medium

Adults exposed to pyriproxyfen in the medium for up to 5 days exhibited dose dependent mortality ($F= 91.78$; $df= 5$; $P <0.001$; Fig. 4.1). Adults exposed to the 100 ppm treatment had

high mortality, and the surviving females laid some eggs; whereas no adults survived 1000 ppm treatment.

Development of eggs laid by pyriproxyfen-treated females in pyriproxyfen-treated medium

Treatment of females and their progeny with the same pyriproxyfen dose reduced the percentage of progeny that completed development to the pupal and adult stages in a concentration-dependent manner ($F= 58.5$; $df= 4$; $P <0.001$; Fig. 4.2). No puparia were found in the media treated with 100 ppm. Few to no adults developed from the eggs exposed to any of the pyriproxyfen doses in media ($F= 1318$; $df= 4$; $P <0.001$; Fig. 4.2). Dead puparia and adults with malformed wings or partially out of the puparium were observed in the media treated with 0.1 ppm and higher dosages.

Exposure of females and their progeny to pyriproxyfen significantly delayed puparia formation in a dose-responsive manner ($F= 7.1873$; $df= 3$; $P <0.001$; Table 4.1). There was no apparent delay in adult emergence of progeny hatching from eggs in medium with the lowest dose, but no adults emerged from the media treated with the higher doses ($F= 1.1161$; $df= 3$; $P= 0.3156$; Table 4.1).

Discussion

To evaluate the efficacy of pyriproxyfen for control of *D. suzuki*, we first established that adults exposed to pyriproxyfen treated-media exhibited mortality in a concentration-dependent manner. Adults held with 10 or 100 ppm pyriproxyfen-treated media had 30% and 80% mortality respectively within 5 days, and 100% mortality at 1000 ppm. This information was used to determine the effects of pyriproxyfen over a similar range on the development of progeny hatching from eggs oviposited by dosed male and female adults in similarly dosed medium. In total, we found that the medium and higher doses of pyriproxyfen (10 to 1000 ppm) had lethal

and sub-lethal effects on the development of *D. suzukii*. These doses greatly reduced and delayed pupariation and adult emergence of progeny hatching from eggs oviposited by *D. suzukii* females in the treated medium. In this experimental situation, progeny hatching from the eggs were continuously exposed to pyriproxyfen in the medium both by feeding and integument penetration through the larval stages. We observed that *D. suzukii* developing in medium treated with the low pyriproxyfen doses remained in the puparia or half eclosed and some adults had malformed wings. As a JH analog, it likely promoted JH pathway signaling, which disrupted metamorphosis and ultimately adult development.

No studies to date have evaluated the insecticidal activity of pyriproxyfen for control of *D. suzukii*, although it is included in a list of short residual pesticides for this pest on small fruits by The Center for Agriculture, Food, and the Environment, University of Massachusetts (https://ag.umass.edu/print/11316). The results of our study are consistent with reports that pyriproxyfen interferes with pupal formation and adult emergence and survival of dipterans and other insect groups (Tunaz and Uygun 2004, Sullivan and Goh 2008).

The physiological effects of pyriproxyfen seen in our experiment agreed with the deterioration of metabolic mechanism in other holometabolous insects. Similar results have been obtained from other experiments when other dipterans were tested with pyriproxyfen. Topical application of pyriproxyfen doses to *D. melanogaster* 3rd instar larvae was shown to inhibit 50% of adult development at 0.29 ng/1µl (0.29 ppm) per larva. Similar to our results, delayed pupae formation and adult emergence were observed with 0.29 ng concentration (Bensebaa et al. 2015). Mortality of freshly emerged adults was reported for flesh fly, *Sarcophaga ruficornis* (L.), after female adults were topically treated with pyriproxyfen at 50 (10.000 ppm) and 100 (20.000 ppm) µg/5 µl per adult of pyriproxyfen using 10 µl syringe on the ventral surface of the abdomen. Both

concentrations caused 20% mortality within 24 h after treatment (Singh and Kumar 2015). When 50 (10,000 ppm) and 100 (20,000 ppm) µg of pyriproxyfen /5 µl doses of pyriproxyfen were applied to virgin female *S. ruficornis*, progeny of the treated adults completed development but emerged as adults with defects in wings, mouthparts and appendages that together resulted in ineffective mating (Singh and Kumar 2015). When 0.01 ppm pyriproxyfen was applied to late instar midge, *Polypedilum nubifer* larvae (Skuse), it caused 90% inhibition of adult emergence. Experiments were conducted in aquaria filled with 2 cm of washed sand and 8 L of each concentration (Trayler et al. 1994). In another experiment, 3rd instar larvae of the Asian tiger mosquito, *A. albopictus* and southern house mosquito, *Culex quinquefasciatus* (Say) were tested with pyriproxyfen. Pyriproxyfen inhibited adult emergence significantly, when 3rd instar of larvae were placed in 500 mL disposable cups filled with 100 mL of each concentration and 0.01 g of larval diet slurry. Importantly, the 0.05 mg/L (0.05 ppm) concentration caused total suppression of adult emergence (Khan et al. 2016).

In our experiment, we also observed that *D.* suzukii females treated with 100 ppm laid far fewer eggs than the control females. Similar results were obtained with other dipteran species. When the house fly*, Musca domestica* (L.) was treated topically with pyriproxyfen application, it interfered with fecundity and fertility. Three days old virgin females were treated with 0.5 µl acetone solution containing the test compound on the dorsal thorax of the insect. Following this, treated females were paired with untreated males. The results showed that females treated with 10 µg/0.5 µl (20, 000 ppm) per insect or 20 µg/0.5 µl (40, 000 ppm) per insect produced 17 times fewer eggs than untreated females. Similarly, hatchability was decreased to 5.8%, when females were treated with 20 µg/0.5 µl (40, 000 ppm) per insect (Kawada et al. 1992). Singh and Kumar (2015) also reported that decreased fecundity was exhibited by *S. ruficornis* treated with the

above pyriproxyfen doses. Suman et al. (2013) reported that high concentrations have an ovicidal effect when applied to eggs freshly laid by the Asian tiger mosquito, *Aedes albopictus* (Skuse) and yellow fever mosquito, *Aedes aegypti* (L.). A concentration of 1 ppm caused 80.6% hatching inhibition for *A. albopictus eggs* and 47.3% for *A. aegypti* eggs.

Similar results were gathered when pyriproxyfen was tested on other orders. Second instar larvae of the African cotton leafworm, *Spodoptera littoralis* (Boisduval) were fed by artificial diet mixed with pyriproxyfen concentrations. Pyriproxyfen prevented larvae from consuming diet as an antifeedant chemical. Results also showed that 21.33% of larvae treated with 75 mg active ingredient/kilogram (a.i)/kg (75 ppm) diet pyriproxyfen reached to pupal stage, but no adults were found. For controls, 100% pupariation and 96.30% emergence of adults were observed. Delayed larval and adult development were observed after treatment of the lowest concentration 3.25 mg (a.i)/kg (3.25 ppm) (Nasr et al. 2010). Another experiment was conducted with silverleaf whitefly, *Bemisia tabaci* (Gennadius). Cotton seedlings infested with 0-1-day-old eggs were dipped in pyriproxyfen solution. Results showed that total suppression of egg hatching was observed with 0.1 mg (ai)/liter (0.1 ppm) pyriproxyfen solution. Secondly, *B. tabaci* females were placed in cages for 48 hours in which treated cotton seedling were placed. Seedlings treated with 5 mg (ai)/liter (5 ppm) pyriproxyfen induced the oviposition of infertile eggs. Lastly, when 2nd instars were treated with 0.04-5 mg (ai)/liter (0.04-5 ppm) pyriproxyfen, larvae reached to the pupal stage at a similar percentage as the untreated control, but total suppression was observed for adult emergence. In this experimental design, cotton seedlings were treated with range of pyriproxyfen concentrations after being infested with 2nd instar *B. tabaci* (Ishaaya and Horowitz 1992). Oblique-banded leafroller, *Choristoneura rosaceana* (Harris), was reported to be sensitive to against pyriproxyfen. In this experiment, fifth-instar

larvae were fed leaf-disk treated with 10-µl droplets of pyriproxyfen in a small petri dish. For larvae were treated with the highest concentration (30 ppm), 6% of larvae reached the adult stage normally compared to controls for which 86% emergence occurred. Prolongation of pupation and adult emergence was observed with concentrations higher than 1 ppm. When a sublethal dose (0.3 ppm) was tested, it resulted in the weight gains, and interfered with both fertility and fecundity (Sial and Brunner 2010).

The results reported here suggest pyriproxyfen can be incorporated into IPM with the purpose of the suppression of *D. suzukii* populations. Its efficacy should be examined extensively in field studies before it is incorporated into IPM and whether pyriproxyfen is harmless for the natural enemies of *D. suzukii* should be determined.

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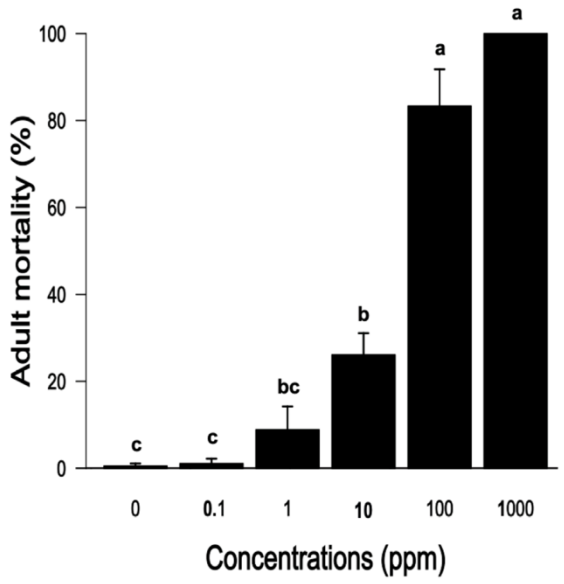

Fig. 4.1. Percent mortality of *D. suzukii* adults exposed to medium treated with pyriproxyfen doses (ppm) for five days (Mean ± SEM). Letters denote significantly different mean percentages of adult mortality among treatment groups at the 0.05 significance level (Tukey's HSD test). F= 91.78; df= 5; P <0.001.

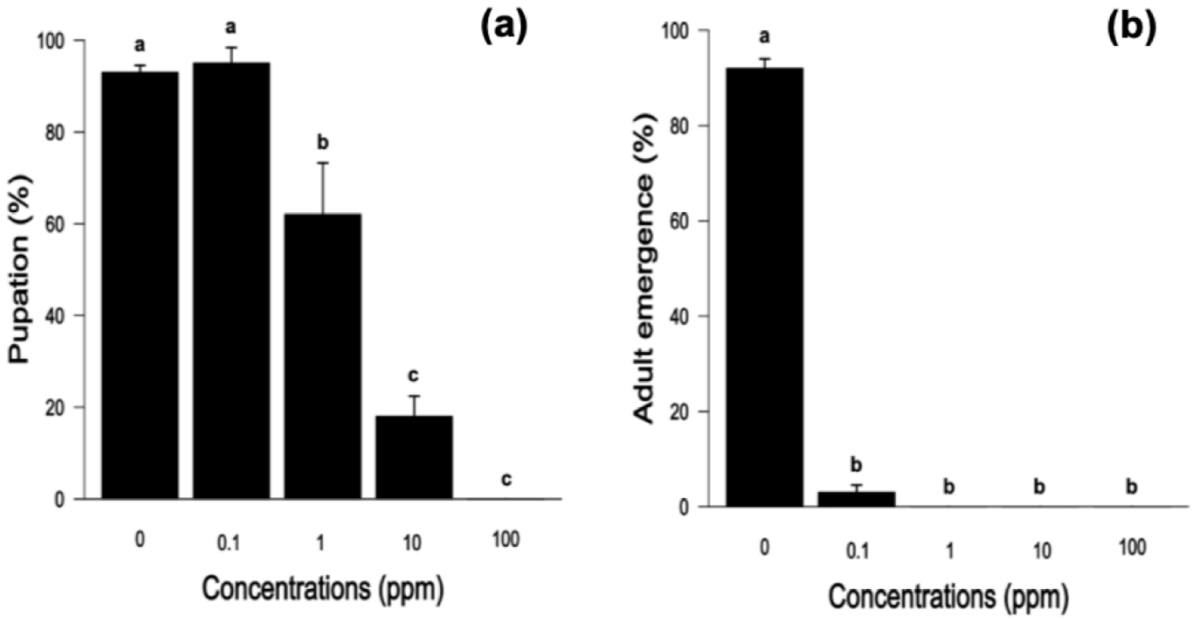

Fig. 4.2. Percent pupariation (a) and adult emergence (b) from eggs laid by pyriproxyfen-treated *D. suzukii* females in medium treated with the same pyriproxyfen dose (ppm). Letters denote significantly different percentages of pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test). $F= 58.5$; $df= 4$; $P <0.001$ and $F= 1318$; $df= 4$; $P <0.001$.

Fig. 4.3. Dead adult with malformed wing. Pyriproxyfen continuous exposure test (0.1 ppm in media).

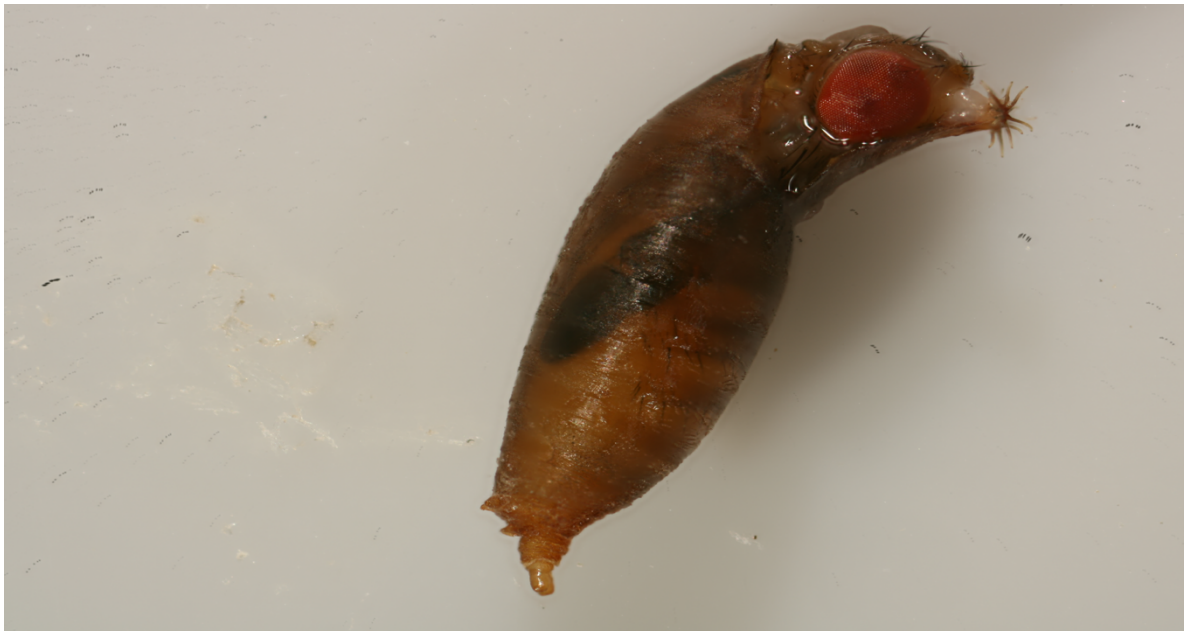

Fig. 4.4. Dead half enclosed adult. Pyriproxyfen continuous exposure test (0.1 ppm in media).

Fig. 4.5. Dead puparia. Pyriproxyfen continuous exposure test (1 ppm in media).

Table 4.1. Duration of puparia and adult development from eggs oviposited by pyriproxyfen-treated *D. suzukii* females in pyriproxyfen-treated medium

Pyriproxyfen dose for adults and progeny (ppm)	Days to pupation[a]	Days to adult emergence[b]
0	5.87 ± 0.14b	9.94 ± 0.02
0.1	5.75 ± 0.17b	10 ± 0
1	6.54 ± 0.22ab	No adult
10	7.26 ± 0.48a	No adult
100	No pupae	No adult

Letters denote significantly delayed pupation and adult emergence among treatment groups at the 0.05 significance level (Tukey's HSD test).

[a] Number of days (mean ± SEM) from oviposition to pupation after treatment.

[b] Number of days (mean ± SEM) from oviposition to adult emergence.

CHAPTER 5

CONCLUSIONS

This study showed that pyriproxyfen, novaluron and methoxyfenozide have insecticidal effects on the development of *D. suzukii*. This invasive species is a major pest in soft-skinned fruits of commercial important. The better understanding of how to manage this pest can enable us to improve more effective management strategies. It is crucial that incorporation of more selective insecticides can provide us more sustainable agriculture.

Firstly, we were able to evaluate lethal effect of pesticides. Pyriproxyfen and novaluron caused significant mortality with highest doses. Secondly, experiments showed that methoxyfenozide and novaluron are effective during larval stage and pyriproxyfen can prevent adults emerge from puparia. Thirdly, all insecticides interfered with fertility and fecundity. Lastly, experiments showed that methoxyfenozide and novaluron were transmitted to progeny when adults were exposed to insecticides for 5 days. These IGRs have not been tested on *D. suzukii* previously. Therefore, this study can provide preliminary results to develop more efficient management strategies. Further studies are needed to determine the most ideal concentration for these IGRs. In addition to this, field studies should be conducted to obtain more realistic results.