

CURATIVE AND RAINFASTNESS CHARACTERISTICS OF
INSECTICIDES USED TO CONTROL SPOTTED WING DROSOPHILA (MATSUMURA)
IN TART CHERRY PRODUCTIONS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology—Master of Science

2018

ABSTRACT

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Spotted wing drosophila (*Drosophila suzukii* Matsumura) (SWD) is an invasive pest originated from East Asia, which has caused devastating damaged to soft-skinned fruit productions globally and an increase of growers' reliance on insecticide to meet market standards. However, the intensive use of insecticides raises community concerns regarding environmental health, human health, and the risk of SWD population to grow resistances. Therefore, exploring other activity modes of insecticides registered against SWD besides adulticide action may provide information to refine existing insecticide programs. The data from these experiments provide insight of the curative and rainfastness of insecticides registered against SWD in tart cherry productions. The residue data in these experiments compliment the biological data and provide better understanding on how these insecticides work against SWD.

ACKNOWLEDGEMENTS

The completion of this thesis and my Master programs would not be possible without the help of others. Therefore, I would like to thank my major advisor, Dr. John Wise for the guidance, opportunity, and encouragement. I thank my committee members, Dr. Rufus Isaacs and Dr. Nikki Rothwell for their guidance and advices. Chris Vandervoort and Tom Garagvalia for their help and expertise in analyzing the residue samples. Thank you to my fellow graduate students and colleagues, Anthony VanWoerkem, Celeste Wheeler, Charles and Jennifer Coslor for support and help during my field and lab work. I would also like to thank the late Jason Seward and farm crew of the Michigan State University Trevor Nichols Research Center for providing equipment and technical support. This work was supported in part by FFAR ROAR [2017 grant #544804, 2018 grant #606394 project title Sustainable Control Tactics for Spotted Wing *Drosophila* in Tart Cherry] and USDA Specialty Crop Block Grant [2016 grant#791N7700188, project title Refining Spotted Wing *Drosophila* Management Practices in Michigan Cherries]. A special thanks to my family and friends in Indonesia that supported me.

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KEY TO ABBREVIATIONS

ANOVA – analysis of variances

DAI – days after infestation

df – degrees of freedom

MRL – maximum residue limit

PHI – pre harvest interval

PIC – plant-insect-chemical

ppm – parts per million

CHAPTER 1: INTRODUCTION

Insecticides in Agricultural Insect Pest Management

Insects have a significant role in agriculture as pollinators, biocontrol agents, decomposers, and also as pests (Pimentel and Edwards 1982, Khan et al. 2007). Numerous insect species are listed as pests and affect farmers' profits by inflicting direct or indirect damage to crops, compromising yield quantity and quality. Therefore, farmers consider an array of integrated pest management (IPM) practices to protect their crops from such damage, including the use of genetically modified crop varieties, conserving natural habitats and refuge sites to increase biocontrol agent populations and effectiveness, cultivation practices, and insecticides (Gurr et al., 2004; Pimentel et al., 1993; Romeis et al. 2018). When growing high value specialty crops, insecticides are a frequent and reliable choice due to the need to meet high market standards.

The progression of insecticide discovery, use and regulation has been driven by many factors, including environment and human health concerns and new pest challenges. In 1874, Othmar Tseidler first synthesized DDT and this was frequently used to control insect-borne diseases, such as malaria and typhus. Due to its high toxicity to these medical pests, DDT was soon used in agricultural systems and thus boosted yield production and was widely adopted around the globe because it was inexpensive (Zacharia and Tano 2011). However, its high toxicity to non-target organisms, such as mammals, birds, and reptiles, raised concerns and caused strict DDT use to only mosquitos control in malaria epidemic areas. The United States and Europe have banned DDT use in agricultural systems (Casida and Quistad 1998). In the second half of the 20th century, broad-spectrum neurotoxins such as organophosphates,

carbamates, and pyrethroids replaced the use of DDT in many countries. By the 21st century diamides, spinosyns, insect growth regulators, and neonicotinoids began replacing many of the older classes, and these newer insecticide chemistries are considered as more selective or possessing novel modes of action (Oberemok et al. 2015). These are categorized as reduced risk compounds by EPA, which are considered to have lower impact to human health, lower toxicity to non-target organisms (birds, fish, and plants), lower potential for groundwater contamination, and low pest resistance potential, compared to older insecticide classes (Berkett and Cromwell, 2009).

While older insecticide classes relied on lethal effects and direct toxicity to a wide range of insect life stages, newer classes often possess multiple modes of activity specific to certain life stages. Modes of activities are referred as field-assessable symptoms of insecticide action towards targeted organisms that are responsible for control (Wise & Whalon, 2009). Examples of insecticides' modes of activity besides adulticide action are sublethal effects, repellency, oviposition deterrence, antifeedant, and curative action which can be valuable to crop protection (Bostanian et al., 2012; Hulbert et al., 2011; Nansen et al., 2016; Wise et al., 2009). Sublethal effects do not cause direct mortality to the exposed target life-stages, however the effects are seen in the subsequent generation. These effects decrease the number of offspring and/or their fitness causing lower crop damage. A study on spotted wing drosophila (*Drosophila suzukii*) demonstrated female flies when treated with diflubenzuron and novaluron, which are both insect growth regulators, showed significantly lower total oviposition, oviposition per female, and emerging larval percentages (Whitener et al., 2018). A series of studies on codling moth (*Cydia pomonella*) and obliquebanded leafroller (OBLR) (*Choristoneura rosaceana*) showed a reduction in egg and egg viability following exposure of female adults to novaluron (Kim et al.,

2014; Soo-hoon S Kim, Wise, Gökçe, & Whalon, 2011). Transovarial refers to the movement of compounds from treated adults to eggs, and novaluron residues were recovered from eggs laid by treated females showing this to be the probable mechanism (Kim et al. 2014). Research on cotton aphid (*Aphis gossypii*) treated with sublethal dosage of imidacloprid demonstrated symptoms such as decrease of body weight, feeding intensity, longevity, and fecundity (Shi et al. 2011).

Insecticides with repellency activity causes insect pests to actively avoid treated crops while antifeedant and oviposition deterrence causes insect to select untreated plant tissues for oviposition and as food source. Spinetoram, spinosad, and zeta-cypermethrin can cause repellency in spotted wing drosophila (Van Timmeren et al. 2017). Diamondback moth (*Plutella xylostella*) females avoid ovipositing on treated chinese cabbage leaves and its larvae actively avoid leaf portions treated with spinetoram and gamma-cyhalothrin, which is another example of repellency and oviposition deterrence (Nansen et al. 2016). Plum curculio (*Conotrachelus nenuphar*) showed similar oviposition deterrence when exposed to thiamethoxam, acetamiprid, and thiacloprid, all neonicotinoids, in apples (Hoffmann et al. 2010). Another study demonstrated that thiacloprid, thiamethoxam, and imidacloprid surface residues were highly correlated with plum curculio oviposition deterrence on apples even after a period of field aging (Wise et al. 2006). Japanese beetles (*Popillia japonica*) showed different antifeedant effects when exposed to carbaryl and phosmet, but on different crops (Hulbert et al. 2011, 2012). Antifeedant and oviposition deterrence actions are most common in insects like plum curculio that feed before ovipositing (Hoffmann et al. 2010).

Curative activity is the lethal effect on pests that occurs post-infestation due to transitory penetration of the insecticide into plant tissue (Wise and Whalon 2009). Studies show that neonicotinoid and organophosphate insecticides possess curative activity against plum curculio

on tart cherries and apples, apple maggots (*Rhagoletis pomonella*) in apples, spotted wing drosophila and blueberry maggot (*Rhagoletis mendax*) on blueberries (Wise, et al. 2007, Hoffmann et al. 2009, Wise et al. 2009, 2015). In addition to these insecticide groups, other groups demonstrated limited curative activity, such as spinetoram against blueberry maggot and spotted wing drosophila on blueberries and novaluron against plum curculio on tart cherries (Hoffmann et al. 2009, Wise et al. 2015). As curative activity is dependent on by insecticide penetration into fruit subsurface tissue, fruit structure may affect levels of curative control. Therefore, there is merit to understand the insecticides' modes of activities on different crop systems.

Combining information regarding the target pest, crop, chemical and their interactions is the base to an effective insecticide application program. These interactions can be simplified into the Plant-Insect-Chemical (PIC) Triad model (Wise and Whalon 2009). Numerous research themes on interactions between insects and chemicals have been established, while interactions between insects and plants has been growing in the past decade (Wise and Whalon 2009). However, limited research regarding interactions between chemicals, in this case insecticides, and plants is available. In order to be effective, the insecticide must persist on or inside plant tissue to result in the necessary exposure to the target insect pest. Insecticide persistence and movement within plants may behave differently based on plant morphological and chemical characteristics causing different pest toxicity (Chowdhury et al. 2005). Microroughness, wax content, existing microstructures on leaf surfaces, and chemical characteristics, such as K_{ow} values and the molecular size, affect insecticide penetration into leaf structures leading to possible different penetration behavior between plant species or organs (Hoffmann et al. 2009). Studies show that spinosad penetrates differently in blueberries and apples (Wise et al. 2009,

2015). Thus, this dynamic system may cause insufficient amounts of active ingredient to reach the targeted pest. Research on how insecticides behave on plants may result in understanding of how they can be optimally presented to the insect pest at their different life stages. Combining this information with the insect and plant element will provide a broader understanding to discover new possible pest management strategies or even additional information for new chemicals.

Environmental conditions variably affect interaction within the PIC Triad, influencing insecticide effectiveness. Insecticide deposits on the plant must stand against wind, dew formation, exposure to UV and rain (Thacker and Young 1999, Burrows et al. 2002). Rainfall greatly affects insecticide persistence by washing off pesticide residues from plant surfaces causing lower protection (Taylor and Matthews 1986, Gautam et al. 2016, Wise et al. 2016). Growers are faced with decisions on whether to reapply insecticides or not, following a rainfall event. Unnecessary reapplication may not only increase environmental risk, but also increase unnecessary expenditures for crop protection, while not reapplying may result in devastating pest damage. Wash off and control levels may differ between crops even for the same target pest. Leaf defoliation patterns by Japanese beetle were different between blueberries and grapes both treated with phosmet at the same simulated rainfall rates (Hulbert et al. 2011, 2012). Other studies demonstrated that rainfall significantly reduced adult spotted wing drosophila mortality and repellency (Van Timmeren and Isaacs 2013, Gautam et al. 2016, Van Timmeren et al. 2017). Residue wash-off differs between plant species as it was higher in apple leaves than grape leaves for the compound phosmet at the same simulated rainfall rate (Hulbert et al. 2011, Wise et al. 2016). This implies that rainfall effects differ between plant species when plant physiological

attributes vary. Thus, understanding it can provide additional information for refining management strategies.

To summarize this section, numerous studies from many disciplines have been conducted to understand insecticide activity and environmental fate. Field efficacy trials, resistance development within populations, alternating compound groups within spraying programs, insecticide residue profiles and runoffs, are other topics that researchers can cover to understand insecticides and ensure the insecticide registered are effective and safe. Therefore, comprehensive information on existing compounds in their ecosystems is needed to refine spraying programs and maximize pest control.

Spotted Wing Drosophila

Spotted wing drosophila (*Drosophila suzukii*) has become a global pest for soft skin fruit since its invasion into temperate zone production systems. In 2008, the first SWD detection was found in Europe and the United States mainland starting respectively from Spain and California (Hauser 2011). Since then, subsequent reports stated that SWD was found in USA's northern states, and South America, including Uruguay, Brazil, Chile, and Argentina (Hauser 2011, Cini et al. 2012, Van Timmeren and Isaacs 2014, Asplen et al. 2015, Andreazza et al. 2017). Female SWD infest a wide range of ripe and ripening soft skin fruit crops, wild berry fruit, and even decaying durable fruit, such as apple and pear (Lee, Dreves, et al. 2015, Kenis et al. 2016, Bal et al. 2017). Therefore, SWD is difficult to manage due to season-long oviposition host availability.

Different from most other drosophilids, female SWD can oviposit eggs into unripe and ripening fruit using their sclerotized ovipositors, threatening pre-harvest crops (Keeseey et al. 2015). Gravid females will lay eggs as fruit reaches certain firmness level (Lee et al. 2011). Eggs

quickly develop into adults within one to two weeks and together with female's ability to lay up to 141 eggs depending on host type, humidity, and temperature this poses a threat of rapid population growth and multiple generations existing at one time (Lee et al. 2011, Tochen et al. 2014, 2016, Wiman et al. 2016). Adults overwinter as quiescent reproductive melanized winter morphs in November/December (Gutierrez et al. 2016, Shearer et al. 2016, Wiman et al. 2016). Due to this overwintering behavior, SWD populations may be active in fields as warmer weather occurs.

Management Practices

Several IPM practices have been and are in development to manage SWD populations. These practices include sanitary measures, surveys for potential biocontrol agents from SWD origins, trapping using fruit volatiles and pheromones for monitoring, and insecticide applications (Schetelig et al. 2017). Netting, pruning, mowing, rapid harvesting, removing damaged are some sanitary measures used to reduce SWD damage (Leach et al. 2018).

Monitoring using traps have been less successful in determining early populations due to the activity of lures and baits in comparison to the attractiveness of fruit (Kirkpatrick et al. 2018, Wong et al. 2018). Available baits do not correlate with fruit infestation and are not specific to *Drosophila suzukii*, making the sorting process challenging while fruit injury may have already occurred when SWD are captured in traps (Cloonan et al. 2018).

Insecticides are currently the main control practice for SWD. Compared with the IPM program in use before introduction of this invasive pest, growers spray more frequently using a range of insecticide classes to manage SWD populations, such as organophosphates, pyrethroids, and spinosyns, as soon as fruits are susceptible (Bruck et al. 2011). Besides these three classes, researchers are exploring new compounds and different adjuvants as alternatives control

methods. Novaluron and diflubenzuron, both insect growth regulators, while not lethal to adults, significantly reduced oviposition and emerging flies in laboratory settings (Whitener et al. 2018). *Chromobacterium substugae* is a biopesticide which showed positive results in reducing larval infestation on blueberries when rotated with spinosad (Fanning et al., 2017). Researchers have also attempted to add phagostimulants and adjuvants to insecticide mixtures to increase protection (Cowles et al. 2015, Gautam et al. 2016, Fanning, VanWoerkom, et al. 2018). Even though with several managing options, high market standards have forced growers to rely on insecticides for control due the fast and reliable results.

Curative action is a mode of activity to consider in SWD insecticide research besides mortality and oviposition deterrence. In order to help control SWD populations, targeting larvae inside infested fruit may be useful. Research in blueberries have shown that phosmet, spinetoram, fenprothrin, and several neonicotinoids have curative activity (Wise et al. 2015). Neonicotinoid is an insecticide classes known to have limited adulticide activity compared to the other main classes; however they still may be able to contribute to effective control (Beers et al. 2011, Wise et al. 2018) Thus, combining neonicotinoids into spraying programs might help control SWD population in fields and decrease further infestation. However, these results cannot be generalized. Another report has indicated that pyrethroids displayed minimum SWD curative control in sweet cherries (Shawer et al. 2018). Therefore, there is a risk of estimating curative activity when over-generalizing among insecticide classes, active ingredients, and crop systems. Minimal work has been done on the interaction between the chemical used to control SWD, with its host plants, and environmental aspects in tart cherry production systems. These studies are essential to understand the system in which SWD, their host, and the insecticides interact. This

understanding will help provide information about existing chemicals or new ones that can refine existing SWD management practices to comply with consumer and market demands.

Tart Cherry Production

Tart cherry is a commodity with economic importance in the United States, including Michigan as the tart cherry highest producer. In 2015, nationally marketed tart cherries reached 114.76 million kg with values of \$1.38 million (NASS, 2016). Fresh tart cherries circulated in the market have zero damage tolerance for diseases, post-harvest, shipping treatment, or insect pests. The market demands has drove Integrated Pest Management (IPM) to heavily rely on insecticides because of the low economic threshold (Wise and Whalon, 2009).

Tart cherry fruit are highly susceptible to SWD. Since its introduction, revenue lost due to SWD in West US cherry productions is estimated to reach \$100 million (Bolda et al. 2011). In order to protect their crops, growers spray using insecticides registered for tart cherries, including organophosphates, pyrethroids, neonicotinoid, diamides, and spinosyns (Wilson et al. 2015). These intensive spraying programs drive concerns of SWD populations developing resistances to existing chemicals. Current SWD insecticide control research focuses on blueberries, raspberries, and sweet cherries. As these crops have different morphological characters, such as wax amounts, firmness levels, leaf and fruit surface architecture; control levels using insecticide may differ between them and tart cherries (Bukovac and Petracek 1993, Chowdhury et al. 2001, 2005).

Growers rely on intensive insecticide applications to control SWD in soft-skinned fruit productions (Bruck et al. 2011). However, consumer and society concern regarding the negative effects of insecticides on human health, pollinators, beneficial arthropods, and the environment

(Daane et al. 2016) have resulted in maximum application times, international market's maximum residue level (MRL) regulation, and insecticide group limitation (Haviland and Beers 2012). Intensive insecticide applications without rotation between modes-of-action may cause resistance development within SWD populations and reports show SWD resistances against spinosad and minor shifts against malathion and spinetoram (Smirle et al. 2017, Gress and Zalom 2018, Van Timmeren et al. 2018). As these current spraying programs continue, there is a possibility for resistance against other major insecticide classes to occur. Therefore, understanding existing insecticide compounds characteristics in controlling SWD is beneficial to refine spraying programs to comply with growers and consumer's needs of information regarding to reapplication after weather events. To date, little is known about insecticide rainfastness on tart cherries. Again, even though studies have shown rainfastness characteristic of several insecticides on different crop species, over-generalizing may cause misestimating protection level on tart cherries and help prevent over application or decrease crop losses.

The objectives of this thesis are to explore key characteristics of insecticides used to control SWD on tart cherries. The first major objective is to examine curative activity of these insecticides on tart cherries. The second objective is to examine the rainfastness attributes of insecticides on tart cherry. The third objective is to examine the different insecticide penetration profiles at different fruit development stages.

CHAPTER 2: CURATIVE ACTIVITY OF INSECTICIDES USED TO CONTROL SPOTTED WING DROSOPHILA (DIPTERA: DROSOPHILIDAE) IN TART CHERRY

Abstract

Tart cherry (*Prunus cerasus* L.) fruit were infested with spotted wing drosophila over three days. After the infestation period, insecticides were applied 1 and 3 days later. Small larvae, large larvae, pupae, and total individuals were counted 9 days after first infestation. Insecticide treated tart cherries were subjected to residue analysis. Phosmet, spinetoram, acetamiprid, and zeta-cypermethrin reduced live SWD counts compared with control by > 50% at all life stages and insecticide application times, whereas cyantraniliprole showed moderate curative control, and *Chromobacterium subtsugae* demonstrated no curative action. Residue analysis demonstrated that zeta-cypermethrin residues mostly remained on fruit surface. Small portions of phosmet, spinetoram, and cyantraniliprole were able to penetrate fruit surfaces and move into subsurface tissues. Acetamiprid was the only compound for which >47% penetrated into the fruit subsurface consistently across two years. Curative activity demonstrated in this study can provide valuable additional control mechanism for management of *D. suzukii* in cherry IPM programs.

Introduction

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is recognized as a pest on a broad range of soft bodied fruit crops globally as well in the United States since its introduction to the mainland through California in 2008 (Hauser 2011, Asplen et al. 2015). Spotted wing drosophila has spread throughout the mainland and is a pest of Michigan blueberries, raspberries, and cherries since its detection in 2010 (Van Timmeren and Isaacs

2014). Percent revenue loss from SWD infestation has been estimated to reach 37% for raspberries and 20% for processed strawberries in California (Goodhue et al. 2011). The market's zero tolerances to insect infestation on fruit causes a threat of severe economic loss; therefore, growers rely on insecticides as their main option to control SWD due to fast and reliable results.

Insecticides remain critical in SWD management although numerous control practices have been developed and used to control SWD in orchards (Haye et al. 2016). Weekly insecticide sprays are started as soon fruit are susceptible and SWD adults are detected with monitoring tools. This early spraying program has contributed to an increase of total insecticide use in susceptible crops (Diepenbrock et al. 2017). Frequent use of several insecticide classes recognized for SWD control, including organophosphates, pyrethroids, and spinosyns; poses a risk for the SWD population to develop resistance (Beers et al. 2011, Van Timmeren and Isaacs 2013, Van Timmeren et al. 2018). Besides programs to assess the risk of developing resistance, insecticide applications are complicated by seasonal application limitations and global market maximum residue limits (MRL) (Haye et al. 2016, Diepenbrock et al. 2017). Thus, additional information is needed to refine existing insecticide application programs to comply with environmental, economic, and international trade concerns.

Effective SWD control using insecticides is based on broad knowledge of Integrated Pest Management (IPM) and the interactions between plants, insects, and chemicals within these systems. This approach can be simplified to a model called the Plant-Insect-Chemical (PIC) Triad (Wise and Whalon 2009). The chemical elements of the PIC Triad provide information on how chemicals behave in order to optimize pest control. In relation with the *Plant* element of this model, information may cover how certain chemicals may penetrate different plant tissues, how

they are transported, or how long residue can be detected in these tissues over time under environmental pressures (Wise et al. 2006, 2015, Hoffmann et al. 2009, Hulbert et al. 2012).

Curative activity is the lethal action to pest post-infestation resulting from the transitory penetration of insecticide into plant tissues (Bostanian et al. 2012). Neonicotinoids and organophosphates have demonstrated curative activity against plum curculio (*Conotrachelus nenupha*) in tart cherries, apple maggots (*Rhagoletis pomonella*) in apples, and SWD in blueberries whereas pyrethroids seem to vary in curative control (Hoffmann et al. 2009, Wise et al. 2009, 2015). Other studies have shown insecticide deposition varies between plant species (Chowdhury et al. 2001); therefore, this difference may as well affect curative activity towards certain pests. Curative activity alone might not be the first approach for a successful SWD management program, because reducing the female flies is also an important goal for insecticides. However, this information can help provide insight on how these chemicals may still help suppress SWD population after infestation, and which insecticides should be selected in different situations. Understanding insect-plant-chemical interactions is essential in predicting how IPM practices will perform in agricultural systems, including insecticide applications.

The objectives of this study were to 1) investigate curative activity of different insecticides on SWD, 2) determine the effect of immediate and delayed timing on immature life stages, and 3) document the associated residue penetration profiles for each compound in tart cherry fruit.

Materials and Methods

Field plots were located at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC) in Fennville, MI (42°35'40.9"N 86°09'19.9"W) in orchards of tart

cherry trees, *Prunus cerasus*, cv. Montmorency. Fruit were collected on June 28 2017 and June 22 2018. Fruit were then stored in a walk-in cooler at temperature of 2.7°C to be processed 1-2 days after. Insecticide doses were based on labeled rates applied in 935 liters/ ha (100 gallons per acre) of diluent. These doses were applied in 750 ml sprayer bottles (Lansing Sanitary Supplies Inc., Lansing, MI) set to a mist type spray (Table 1).

Bioassays

Ten undamaged ripe berries were placed into 946 ml plastic containers (WNA Upscale Disposable, Chattanooga, TN). A disc containing 1 ml of diet was placed on the bottom of container to maintain healthy adult flies (cornmeal diet, Drosophila Species Stock Center, San Diego, CA). Six male and six female SWD were added to each container and removed after 72 hours to allow for mating and egg deposition. Plastic container lids were perforated ($\varnothing < 1$ mm) to facilitate air circulation and to reduce condensation.

Infested fruits were sprayed with treatment compounds at 1 (immediate) and 3 (delayed) days after adult SWD were removed, with 5 replications for each treatment combination. Approximately 1.4 ml of solution was sprayed onto fruit of each experimental unit. Experimental units were assessed 9 days after first infestation, and small (< 2 mm) larvae, large (≥ 2 mm) larvae, and pupa were recorded. Tart cherries were moved into 946 ml re-closable bags (Gordon Food Service, Grand Rapids, MI). Cherries were crushed, allowing brown sugar water to enter the fruit. Two hundred ml of brown sugar water with a ratio of 172 gram of brown sugar per 1 liter of tap water was inserted into each plastic bag (Michigan State University Extension, 2017). After an hour, the berry mixture was poured over a mesh tray with mesh size of 8.38 mm for larvae and water to run through, and cleaned using a sprayer bottle. The remaining liquid was

then poured into a reusable coffee filter and small larvae, large larvae, and pupae were counted under a stereomicroscope.

Statistical Analysis

Toxicity was determined by separately comparing the total number of small larvae, large larvae, pupae, and total of individuals recovered between each insecticide treatment and application time combinations at each year using a two-way ANOVA in PROC Mixed (SAS, 2009). Data were tested for normality and homogeneity assumptions by using Shapiro-Wilk and Levene's test respectively. Transformation was done to data if necessary to meet assumption requirements. Ranked test was conducted on data that could not be normalized. Required transformations used in each stage are listed in the results. Mean separations between insecticide treatments and application time combinations were done using Tukey's HSD test. All tests were run with $\alpha=0.05$ and done using SAS 9.4 (SAS Institute, 2009).

Table 1. Active ingredients, insecticide groups, formulation brand, manufacture, dose, and field rate of used formulation

Active Ingredient	Insecticide Group	Trade name	Manufacture	Field Rate	Amount of product per 750 ml of water
phosmet	organophosphate	Imidan 70W	Gowan Corporation, Yuma, AZ	1,680 g AI/ha	1.91 g
<i>Chromobacterium subtsugae</i>	biopesticide	Grandevo DF	Marrone Bio Innovations, Inc., Davis, CA	1,008 g AI/ha	2.70 g
cyantraniliprole	diamide	Exirel 10SE	DuPont, Wilmington, DE	100.6 ml AI/ha	0.76 ml
acetamiprid	neonicotinoid	Assail 30SG	United Phosphorous Inc., Abingdon, VA	111.3 g AI/ha	0.34 g
zeta-cypermethrin	pyrethroid	Mustang Maxx .8EC	FMC Corp., Philadelphia, PA	28 g AI/ha	0.22 ml
spinetoram	spinosyn	Delegate 25WG	Dow AgroSciences LLC, Indianapolis, IN	105 g AI/ha	0.34 g
*2-Hydroxy-1,2,3-Propanetricarboxylic Acid	adjuvant	Tri-fol	Wilbur-Ellis Company LLC, Fresno, CA	11.83 ml	0.44 ml

Table 2. Ion monitored in mass spectrometer and the limit of detection (LOD) and limit of quantitation (LOQ) for each treatment compound in 2017 and 2018 residue analysis

Compound	M+H (m/z)	Qualifier (m/z)	LOD ($\mu\text{g/g}$)	LOQ ($\mu\text{g/g}$)
phosmet	161	160	0.015	0.05
zeta-cypermethrin	209	163	0.005	0.010
acetamiprid	223	152	0.015	0.05
spinetoram	784.5	142.4	0.121	0.40
cyantraniliprole	475	286	0.005	0.010

Insecticide Residue Analysis

Surface and Subsurface Residue

Insecticide treated fruits were collected for residue analysis and stored in a -10°C freezer. Thirteen tart cherry fruits were collected and tested for surface and subsurface residues. Fruit were placed into 60 ml of acetonitrile and sonicated for 30 seconds to obtain surface residues. Fruit were then move into a new glass jar, grounded and 4 g of magnesium sulfate, 1 g of sodium chloride, and 60 ml of dichloromethane (EMD Milipore Chemicals, Inc., Billerica, MA) were added to each sample to extract the remaining sub-surface residues.

Surface residue solvent was decanted through 12 g of sodium sulfate placed in Whatman filter paper \varnothing 12.5 cm to remove water (Tisch Scientific, North Blend, OH). Subsurface residues solvents were placed into 250 ml separatory funnels. Solvents were shook vigorously and left for phases to separate. Dichloromethane was run through 12 g of sodium sulfate placed in Whatman filter paper \varnothing 12.5 cm (Tisch Scientific, North Blend, OH). Two additional separations were done and rinsed with 20 ml of clean dichloromethane. Collected solvent was evaporated and 2 ml of acetonitrile was added for HPLC or GC analysis. Samples were sonicated for 1 minute to collect remaining residues. Remaining particulates were removed by passing samples through a 0.45- μ m 25-mm syringe filter (Pall, East Hills, NY).

Samples were analyzed for spinetoram residues using a 2690 separator module HPLC, with a 2487 dual-wavelength absorbance detector (Waters, Milford, MA). A C18 reserved-phase column with 4.6-mm bore and 5-mm particle size was used. Flow rates was set at 0.3 ml/minute. The mobile phase was started at 90:10 water: acetonitrile with formic acid (0.1%) and reduced to 70:30 between 12 and 13 minutes at 20°C. The detectors was set to monitor 745.86 m/z for spinetoram. Acetamiprid and phosmet were analyzed using GC/MSD (Agilent 6890 Gas

Chromatograph with a 5973 N Mass Spectra Detector (MSD); Agilent Technologies, Santa Clara, CA) that was equipped with a Zebron ZB-5ms 30m, 0.25mm I.D. and a 0.25µm film thickness. For the GC/MSD analysis settings, the oven was held at 115°C for five minutes with a ramp of 9°C per minute to 280°C, followed by a ramp of 30°C per minute to 310°C. The MSD transfer line was held at 285°C. The mass spectrometer were set to monitor for ions according to table 2. The injector were rinsed three times with acetone and three times with dichloromethane between and also before each injection. All compounds were quantified against a standard curve, and recovery data recorded as µg of AI per gram (ppm) of plant substrate.

Results

Curative Activity on Spotted Wing Drosophila

2017 Bioassays

In 2017, the number of live SWD differed significantly among insecticide treatments for the life stages of large larvae (Table 3). The insecticide application timing also significantly affected individual counts at the life stages of small larvae and pupae, but did not affect large larvae and the total individuals. Treatments that included zeta-cypermethrin showed significantly higher pupae counts at insecticide application 3 day after infestation (DAI). Significant interaction between insecticide treatments and application time only occurred for the small larvae count. Individuals recovered from cherries treated with phosmet, zeta-cypermethrin, acetamiprid, or spinetoram were significantly lower than the controls at all developmental stages besides small larvae, whereas cyantraniliprole demonstrated significant lower counts than the control at 1 DAI application time at all developmental stages except small larvae. Individuals recovered from *C. substugae* treatments were not significantly different from the control at all developmental

stages. Large larvae collected from all insecticide treatments applied on 1 DAI were >60% lower than the control. Numbers of large larvae collected from phosmet, zeta-cypermethrin, acetamiprid, or spinetoram applied at 3 DAI treatments were >70% lower than in the control. Both spinetoram and phosmet showed the lowest large larvae counts in 1 and 3 DAI application times, respectively. The number of pupae collected from cherries treated with phosmet, zeta-cypermethrin, acetamiprid, spinetoram, or cyantraniliprole applied at 1 DAI were > 60% lower than control while the same insecticides applied at 3 DAI were only > 49% lower than control. Phosmet-treated fruit showed the lowest pupae counts at both 1 and 3 DAI application times.

Total individuals collected from all insecticide treatments, except *C. subtsugae*, applied at 1 DAI were significantly lower compared with the control, while in the 3 DAI application only phosmet, zeta-cypermethrin, acetamiprid, and spinetoram-treated fruit resulted in significantly lower SWD than the control. At 1 DAI, total individuals collected from zeta-cypermethrin and *C. subtsugae* were significantly higher than phosmet, acetamiprid, and spinetoram while individuals collected from cyantraniliprole were in-between these two groups. At 3 DAI, total individuals collected from *C. subtsugae* and cyantraniliprole were non-significant compared with the control. In addition, individuals collected from zeta-cypermethrin and acetamiprid were significantly higher than phosmet and spinetoram.

2018 Bioassays

In the 2018 trial, insecticide treatments had significant effects to the individual counts at all life stage and total counts (Table 3). The insecticide application time after infestation period only significantly affected individual counts at the life stages of large larvae and the total individuals counted in this experiment. Small larvae counts from all treatments were not significantly different from the control at both 1 and 3 DAI application times. At 1 DAI

application time, zeta-cypermethrin showed the highest recovery of small larvae followed by cyantraniliprole. Phosmet and spinetoram-treated fruit resulted in the lowest small larvae counts. Small larvae counts from acetamiprid-treated fruit was not significantly different from the counts from phosmet and zeta-cypermethrin, but also not from cyantraniliprole. Large larvae and total individuals counts were significantly higher when phosmet and spinetoram was applied at 3 DAI compared to 1 DAI. Interactions between insecticide treatments and application time occurred only for the large larvae count. Individuals recovered from cherries treated applied with phosmet and spinetoram were significantly the lowest groups among all life stage counts and total individuals at all application times. Individual counts from *C. subsugae* were constantly non-significant compared to the control among all life stage counts and total individuals at all application times. Zeta-cypermethrin resulted in individual counts that were non-significant at the life stage of small larvae at both 1 and 3 DAI application periods compared to the control. Acetamiprid showed significantly lower individual counts compared to control at small larvae on 1 DAI application time, large larvae on 1 DAI application time, and the total individuals recovered from 1 and 3 DAI application times. Cyantraniliprole showed non-significant count differences compared to control among all life stages and total individual counts on 3 DAI application time. Total individual counts showed that acetamiprid was not significantly different compared to zeta-cypermethrin and spinetoram at both 1 and 3 DAI application time.

Insecticide Surface and Subsurface Residues

Residue analysis demonstrated different penetration profiles among insecticides. Residue evaluation showed spinetoram and cyantraniliprole have higher surface than subsurface residues, with subsurface residue being <27% from total residue recovered at both 2017 and 2018 (Table 4). Zeta-cypermethrin residues did not penetrate into the subsurface areas at all from the 2017

trials while 15% of total residue recovery was from the subsurface tissues. Acetamiprid showed the highest proportion of subsurface residue levels with >47% moving to subsurface tissue across both years. Phosmet recovery from 2017 trials showed 0.02% of total residue to move into subsurface tissue, whereas 55.6% of total residue moved into subsurface tissue in 2018.

Table 3. Untransformed mean \pm SE number of *Drosophila suzukii* small larvae (<2mm), large larvae (>2mm), pupae, total individuals recovered from samples after 9 days since infestation. Treatments were applied 1 and 3 days after infestation using six male and six female flies for each sample. Mean separation was done using Tukey test. Different lowercase letters indicates significant differences within a column. Different capital letters indicates significant differences between 1 and 3 DAI within a row. All test were done at $\alpha=0.05$

Compound	Small Larvae (<2mm)		Large Larvae (>2mm)	
	1 DAI	3 DAI	1 DAI	3 DAI
2017				
untreated	2.6 \pm 1.4aA	0 \pm 0.0aB	54.2 \pm 16.9aA	31.2 \pm 5.2aA
phosmet	0 \pm 0.0ab	0 \pm 0.0aA	6.4 \pm 1.9deA	5.4 \pm 2.8cA
zeta-cypermethrin	0.8 \pm 0.8abA	0 \pm 0.0aA	15.4 \pm 2.0bcA	14.8 \pm 3.0abcA
acetamiprid	0 \pm 0.0bA	0 \pm 0.2aA	6.6 \pm 1.2dA	9 \pm 1.6bcA
spinetoram	0.25 \pm 0.25abA	0 \pm 0.0aA	4 \pm 1.5eA	6.6 \pm 1.4cA
cyantraniliprole	0.6 \pm 0.2abA	0 \pm 0.0aB	11 \pm 1.6bcdA	20.6 \pm 4.7abA
<i>C. substugae</i>	0.4 \pm 0.4abA	0 \pm 0.0aA	21.2 \pm 1.6abA	24.2 \pm 2.0aA
transformation	Rank		Rank	
insecticide (F; df; P)	2.10; 1, 55; 0.0681		30.77; 6, 55; <.0001	
day (F; df; P)	13.16; 1, 55; 0.0006		1.91; 1, 55; 0.1722	
ins x day (F; df; P)	3.07; 1, 55; 0.0115		1.03; 6, 55; 0.4160	
2018				
untreated	1.4 \pm 0.75abcA	0.2 \pm 0.20abA	36.2 \pm 7.17aA	46.4 \pm 7.82aA
phosmet	0 \pm 0.00cA	0 \pm 0.00bA	0.8 \pm 0.37cA	16.6 \pm 4.27bB
zeta-cypermethrin	4.4 \pm 0.68aA	3.6 \pm 1.03aA	10.4 \pm 0.81bcA	16.8 \pm 2.35bA
acetamiprid	1.0 \pm 0.77bcA	1.6 \pm 0.93abA	12.2 \pm 1.07bcA	16.4 \pm 2.32aA
spinetoram	0.6 \pm 0.23cA	2.8 \pm 0.80aA	4.0 \pm 0.41cA	16.0 \pm 2.35cB
cyantraniliprole	3.2 \pm 0.86bA	3.6 \pm 1.47aA	12.2 \pm 3.54bA	23.8 \pm 4.00abA
<i>C. substugae</i>	0.2 \pm 0.20aA	0 \pm 0.00aA	46.8 \pm 1.66aA	42.6 \pm 5.73aA
transformation	Rank		Rank	
insecticide (F; df; P)	12.47; 6, 56; <.0001		24.16; 6, 56; <.0001	
day (F; df; P)	0.00; 1, 56; 1.000		32.39; 1, 56; <.0001	
ins x day (F; df; P)	1.68; 6, 56; 0.1442		2.36; 6, 56; 0.0421	

Table 3. (cont'd)

Compound	Pupae		Total	
	1 DAI	3 DAI	1 DAI	3 DAI
2017				
untreated	4.4 ± 1.03abA	7 ± 2.5abA	61.2 ± 16.42aA	38.2 ± 6.56aA
phosmet	0.2 ± 0.2cA	0.4 ± 0.2cA	6.6 ± 1.10deA	5.8 ± 2.78dA
zeta-cypermethrin	0.2 ± 0.2cA	1.2 ± 0.4bcB	16.4 ± 2.14bcA	16 ± 1.86bcdA
acetamiprid	0.6 ± 0.4cA	3.6 ± 1.3abcA	7.2 ± 1.36deA	12.8 ± 2.69cdA
spinetoram	0.25 ± 0.25cA	0.8 ± 0.4cA	4.5 ± 1.55eA	7.4 ± 1.44dA
cyantraniliprole	1.8 ± 0.9bcA	1.8 ± 0.6abcA	13.4 ± 2.50cdA	22.4 ± 4.82abcA
<i>C. substugae</i>	6 ± 0.8aA	8.2 ± 1.5aA	27.6 ± 1.57abA	32.4 ± 0.68abA
transformation	Rank		Rank	
insecticide (F; df; P)	18.72; 6, 55; <.0001		36.30; 6, 55; <.0001	
day (F; df; P)	9.25; 1, 55; 0.0036		3.02; 1, 55; 0.0881	
ins x day (F; df; P)	0.99; 6, 55; 0.4429		1.46; 6, 55; 0.2088	
2018				
untreated	8.4 ± 3.70abA	8.4 ± 2.16aA	46 ± 7.89aA	55 ± 9.63aA
phosmet	0 ± 0.00cA	0 ± 0.00bA	0.8 ± 0.37cA	16.6 ± 4.27cB
zeta-cypermethrin	0.6 ± 0.60cA	0.4 ± 0.24bA	15.4 ± 1.50bcA	20.8 ± 2.87cA
acetamiprid	5 ± 1.30abA	3.8 ± 0.73aA	18.2 ± 2.27bcA	21.8 ± 3.06bcA
spinetoram	0.6 ± 0.23cA	0 ± 0.00bA	5.2 ± 0.50bcA	18.8 ± 2.52cB
cyantraniliprole	1.4 ± 0.40bcA	3 ± 0.89aA	16.8 ± 4.49bA	30.4 ± 4.78abcA
<i>C. substugae</i>	8.8 ± 2.03aA	3.8 ± 1.02aA	55.8 ± 1.74aA	46.4 ± 6.07abA
transformation	log(x+0.9)		Rank	
insecticide (F; df; P)	25, 47; 6, 56; <.0001		26.69; 6, 56; <.0001	
day (F; df; P)	0.42; 1, 56; 0.5193		19.54; 1, 56; <.0001	
ins x day (F; df; P)	1.31; 6, 56; 0.2661		2.00; 6, 56; 0.0804	

Table 4. Amount of active ingredients (AI) recovered from tart cherry fruit surface and subsurface from 2017 and 2018 trials

Treatment	Active Ingredient Recovered (ppm)			
	2017		2018	
	Fruit Surface	Fruit Subsurface	Fruit Surface	Fruit Subsurface
phosmet	41.33 ± 0.4	0.81 ± 0.03	31.27 ± 12.22	17.38 ± 1.35
zeta-cypermethrin	0.99 ± 0.14	0 ± 0	20.95 ± 1.53	3.7 ± 0.07
acetamiprid	0.08 ± 0.01	1.45 ± 0.33	1.29 ± 0.22	0.61 ± 0.06
spinetoram	125.19 ± 40.12	0.68 ± 0.2	2.3 ± 0.52	0.3 ± 0.02
cyantraniliprole	78.58 ± 2.08	0.48 ± 0.01	75.18 ± 44.75	19.91 ± 1.15

Discussion

This study demonstrates various levels of curative activity between major and alternative insecticide compounds used to control SWD in cherries. Phosmet, zeta-cypermethrin, spinetoram, and acetamiprid provided strong curative control compared to the untreated, whereas cyantraniliprole provide moderate curative action. *Chromobacterium subtsugae* did not provide curative control based on this study. Insecticide application timing (immediate post-infestation versus delayed) influenced the effectiveness of zeta-cypermethrin, spinetoram and phosmet.

Zeta-cypermethrin demonstrated lethality to SWD larvae in fruit compared to the water control, even though residue data suggests <18% sub-surface penetration. Zeta-cypermethrin possess a high log P (log K_{ow}) value (6.6) which implies its hydrophobic characteristics to stay in wax structures (Thurston County Health Department, 2012). These residue results were similar to another study in blueberries, where only <1% of residues were recovered from subsurface (Wise et al 2015). Although zeta-cypermethrin possess general characteristics as a surface contact insecticide, filament structures on SWD egg and oviposition holes likely serve as additional insecticide entry and exposure route, allowing zeta-cypermethrin to possess lethal action on SWD eggs and/or larvae (Wise et al. 2015). Pupae recovered from the 2017 bioassay resulted in significantly more pupae numbers when applied at 3 DAI. These count shifts implies that a late application may result in missing susceptible life stages. Similarly, the Asian lady beetles (*Harmonia axyridis*) and green house white fly (*Trialeurodes vaporarium*) LC_{50} variably shifted by >3 fold from eggs to later life stages when treated with pyrethroids (Wang et al. 2003, Youn et al. 2003). Thus, for zeta-cypermethrin the post-infestation lethality is short lived.

Cyantraniliprole demonstrated moderate curative activity indicated by the total individuals collected during this study. Cyantraniliprole has a log P (log K_{ow}) value lower than

zeta-cypermethrin (cyantraniliprole = 1.94) which explains the residue recovery from fruit subsurface. Even though being able to penetrate fruit surface and being present to SWD larvae and eggs, results were still weaker than most other compounds. There is a variation of positive and negative results within the literature regarding to performance of cyantraniliprole in semi-field and laboratory trials (Beers et al. 2011, Diepenbrock et al. 2017, Shawer et al. 2018). Diepenbrock et al. (2017) found that cyantraniliprole had lower adulticide effects than other insecticides used to manage SWD, but was still able to show lower infestation than the untreated control. The low adulticide action is supported by a baseline study which demonstrated that LC_{50} and LC_{90} of cyantraniliprole is relatively higher than phosmet, malathion and spinetoram (Smirle et al. 2017, Van Timmeren et al. 2018). Other studies have shown adding adjuvants or phagostimulants increased adult mortality (Cowles et al. 2015, Gautam et al. 2016, Fanning, VanWoerkom, et al. 2018). Adding sugar demonstrated an increase of adult mortality when treated with cyantraniliprole (Cowles et al. 2015). Although these studies were not designed to demonstrate lethality to eggs and larvae, the increase of adult mortality due to adjuvant additions might correlate with better ovicidal and larvacidal action. Therefore, cyantraniliprole is likely to provide moderate curative action when being used as a rotational compound in insecticide programs, but is not an optimal choice if specifically needing curative action.

Acetamiprid results demonstrated strong curative activity and consistent performance even under delayed application times, even though previous research has shown neonicotinoids are ineffective adulticides for SWD (Beers et al., 2012; Bruck et al., 2011). These results were consistent with others studies for imidacloprid, acetamiprid, and thiamethoxam curative activity against SWD (Wise et al. 2015, Shawer et al. 2018). Acetamiprid residue profiles indicated that large portions penetrated into fruit subsurface, allowing for toxic exposure to SWD eggs and

larvae (Table 4). Acetamiprid log P (log K_{ow}) is the lowest from all compound tested for residues (0.8). Neonicotinoids are systemic insecticides that have been effective for control in homopteran and beetle pest (Mota-Sanchez et al. 2006, Kliot and Ghanim 2012, Van Timmeren et al. 2012). These results imply that there might be a benefit in combining acetamiprid into a spraying program as is able to control population growth in infested orchards. In blueberries, growers use mixed spraying tanks containing neonicotinoids and pyrethroids (P. Fanning, personal communication). However, there has been no reports of this tank-mixing in tart cherry production.

Phosmet and spinetoram demonstrated curative activity in both years. Phosmet results were consistent with previous studies where several organophosphates demonstrated effective curative control, such as phosmet against SWD in blueberries, apple maggots in apples, and azinphosmethyl against plum curculio in apples (Hoffmann et al. 2009, Wise et al. 2009, 2015). Whereas, spinetoram results were consistent with curative activity against SWD in blueberries, spinosyns' curative activity varies within the literature depending on the target pest and host (Wise et al. 2009). The 2018 bioassay results showed delaying application of these compounds resulted in a significant increase of large larvae and total living SWD. These compounds may have missed susceptible life stages or their application have extended developmental time of earlier life stages. Developmental time from egg to adults of Asian lady beetles and pupal period of old world bollworm (*Helicoverpa armigera*) were observed on individuals treated with spinosyns (Galvan et al. 2005, Wang et al. 2009). Phosmet and spinetoram both demonstrated penetrative properties into fruit subsurface with a large portion remaining on fruit surface, consistent with results from previous studies (Table 4) (Hoffmann et al. 2009, Wise et al. 2015). These insecticides both have positive log P (log K_{ow}) values (phosmet = 2.95; spinetoram = 2.44

– 4.82 depending on isomer structure and pH of the environment) (National Center for Biotechnology Information; California Department of Pesticide Regulation, 2007). These values alone indicate that these compounds are hydrophobic and suggest affinity to the fruit surface. Although only a small portion moved through fruits surface, it is enough to cause mortality as studies have showed low SWD LC₉₀ to spinetoram (Van Timmeren et al. 2018). Insecticide penetration does not solely depend on log K_{ow} value. Other factors have been mentioned to affect chemical penetration into plant tissue, such as wax thickness, epidermal thickness, environment humidity and temperature, and molecule size of chemicals may have influenced phosmet and spinetoram penetration into fruit (Baur et al. 1997, Knoche and Bukovac 2000, 2001).

Chromobacterium subtsugae, in general, did not provide curative activity. Bioassay results indicated non-significant result compared to the control at all developmental stages and application times. Field and semi-field trials indicated that this biopesticide provides good control when compared to the untreated check (Wise et al. 2017, Fanning et al. 2018). Therefore, it is not clear why it fails to provide curative action. Unfortunately, residue data could not be measured for this compound to help explain whether *C. subtsugae* has the ability to penetrate fruit tissues or not. Further research is needed to understand the mobility of *C. subtsugae* in plant tissues.

Different application times provided an insight for certain compounds, like acetamiprid, where curative activity is reliable even when sprays are delayed. For other compounds like zeta-cypermethrin, phosmet, and spinetoram, delayed application is likely to result in diminished curative action on the target pest. These results suggest that the delayed insecticide applications can miss susceptible developmental stages (Wang et al. 2003).

Current SWD control programs rely on intensive insecticide applications. Continued use at this rate may cause negative environmental effects and insecticide effectiveness loss due to SWD resistance (Smirle et al. 2017, Van Timmeren et al. 2018). It requires great cost to develop new insecticides; therefore, there is merit to maintain existing products' effectiveness.

Understanding the Plant-Insect-Chemical (PIC) Triad in this system might provide additional information for effective pest management strategies. Previous studies have explored relation between SWD oviposition with fruit characteristics associated with fruit development stages in blueberries, strawberries, cherries, and black berries (Lee et al. 2011, Burrack et al. 2013, Lee, Dalton, et al. 2015). They found that fruit hardness significantly affects SWD oviposition and infestation. Therefore, studies to explore compounds that may increase fruit firmness and effect fruit composition may lead to field practices for growers. Fruit firmness changes, which may occur because of these compounds, however, may adversely affect insecticide penetration into fruit and curative control. Therefore, it is important to understand the tradeoffs and interactions that might occur.

Tart cherry spotted wing drosophila IPM programs would not solely suggest curative activity as their main strategy. It does not prevent SWD infestation. However, understanding registered insecticide's curative activity at their field rates provides some practical implications and additional information for insecticide programs. Spinetoram and phosmet curative activity and residue profile helps explain why these compounds are effective in field trials. However, excessive used of these compounds may lead to population resistance or market rejection due exceeding MRL limits. Acetamiprid, which was considered by some as not effective for SWD management, possess curative activity, which may provide an option to suppress SWD populations more broadly in multi-crop orchard environments. Further research focused on

understanding insecticide penetration within different developmental stages of fruit or fruit types would be valuable to refine curative activity and insecticide programs. Combination of this new knowledge will be essential in refining and improving SWD management programs.

CHAPTER 3: RAINFASTNESS OF INSECTICIDES USED TO CONTROL SPOTTED WING DROSOPHILA IN TART CHERRIES

Abstract

Tart cherry production is challenged by precipitation events that may reduce crop protection against spotted wing drosophila (*Drosophila suzukii*). Semi-field bioassays were used to assess simulated rainfall effects on adult mortality, immature survival, and residue wash-off from different plant tissues. Tart cherry shoots containing 5 leaves and 5 fruits were taken and treated with 0, 12.7, or 25.4 mm of simulated rainfall. Shoots were infested with spotted wing drosophila for five days. Adult mortality was recorded on 1, 3, and 5 days after shoots were infested. Small larvae, large larvae, pupae, and total individuals were counted 9 days after the first infestation day. All insecticide demonstrated higher adult mortality and lower immature survival compared to the untreated control at 0 mm of rainfall. Adult mortality caused by phosmet, zeta-cypermethrin, spinetoram, and cyantraniliprole were adversely affected by simulated rainfall. Immature survival increased in samples treated with phosmet, zeta-cypermethrin, spinetoram, and *Chromobacterium subtsugae* as rainfall amount increased. In all bioassays, acetamiprid was the least affected by simulated rainfall. Phosmet and spinetoram residues were the most sensitive to wash-off. This study provides information on rainfall effects on SWD insecticide management and for informed decision-making on whether reapplication is required.

Introduction

Spotted wing drosophila (*Drosophila suzukii* Matsumura) (SWD), a multivoltine polyphagous invasive species originated from East Asia, has become a major fruit pest globally

(Asplen et al. 2015). In 2008, SWD invaded Europe and the United States mainland starting from Spain and California. Since then, reports have stated that SWD has been found in South America, including Uruguay, Brazil, Chile, and Argentina (Cini et al. 2012, Andreatza et al. 2017). Unlike most other drosophila, female SWD are able to oviposit into the ripening fruit stages of various cultivated soft skinned fruit, wild berry fruits, or even decayed durable fruit, such as apples or pear using their sclerotized ovipositors (Lee, Dreves, et al. 2015, Kenis et al. 2016, Bal et al. 2017). Therefore, intensive insecticide spraying programs are required to maintain high value crops to fulfill market standards (Haviland and Beers 2012).

Currently, organophosphates, spinosyns, and pyrethroids are the main insecticide classes used by growers to control SWD (Beers et al. 2011, Van Timmeren and Isaacs 2013). Integrated pest management programs recommend spraying programs to start once the target pest is caught in traps at or above a certain threshold level. However, existing traps are impractical and not sufficiently selective to SWD, and also lose competitive attraction as adjacent fruit ripens (Kirkpatrick et al. 2018). This can result in fruit being infested before flies are detected in monitoring traps. Therefore, growers often begin weekly sprays as soon fruit are susceptible. This intensive spraying program may lead to detrimental effects to natural enemies, the environment, and possibility developing insecticide resistance within SWD populations. In addition, while growers must comply with seasonal application limitations and global market maximum residue limits, they also have to respond to weather conditions that may interfere with management programs (Haye et al. 2016, Diepenbrock et al. 2017).

For effective pest control, insecticides must be persistent on or in plant tissues and be able to withstand weather events, such as rainfall, UV light, and temperature (Thacker and Young 1999, Katagi 2004). Rainfall can have detrimental effects on insecticide performance by

dislodging insecticide deposits from the plant surface, drawing insecticide concentration from within plant tissues, and reducing overall insecticide bioavailability to nonlethal dosages (Taylor and Matthews 1986). Studies have shown adult SWD mortality decreased as increased rainfall amount treated to various insecticides on blueberries (Gautam et al. 2016). Studies have also reported insecticide residues to be affected by rainfall amount (Hulbert et al. 2012, Melo et al. 2015, Wise et al. 2016). These studies suggest that the impact of rainfall on insecticide performance is influenced by the amount of rain, the inherent toxicity of the compound on the target pest, drying time post-application, affinity of the compound to the plant surface and penetrative capacity, and the physiological attributes of the crop plant. In addition to compromised protection levels, pesticide wash off may adversely affect the environment (Casida and Quistad 1998).

Michigan is largest tart cherry producing state in the USA. Michigans' primary tart cherry production counties experience an average precipitation amount of 77.6 mm during the growing season (NRCS USDA). Growers are regularly faced with a decision of whether or not to reapply insecticides following a precipitation event. Unnecessary reapplication will lead to increased production cost and risk of detrimental effects to the environment, plus the risk of residues exceeding MRL values if fruit are being exported. Not spraying may lead to an unprotected crop and SWD infestation at harvest. There are currently no published reports of the impact of rainfall on pesticides used to control pests of tart cherries. The objectives of this study were to investigate the impact of various amounts of rainfall on 1) the performance of insecticides in controlling SWD in tart cherries, both in terms of reducing adult mortality and survival of immature stages, and 2) surface and sub-surface insecticide residues from cherry leaves and fruit.

Materials and Methods

Field Plots and Insecticide Application

Field plots were located at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC) in Fennville, MI (42°35'40.9"N 86°09'19.9"W). Each treatment plot consisted of one tart cherry tree, *Prunus avium*, cv. Montmorency, surrounded by eight buffer trees (6 m × 4.5 m spacing). Each treatments consisted of 5 replicated experimental plots. Insecticide applications were made on July 11 2017 and July 2 2018 between 09:00-12:00 am with an average air temperature of 21°C, 88% humidity, and 1.29 km/h wind speed. The selected insecticides represented six chemical classes and treatment concentrations were based on labeled field rates (Table 5). Insecticides were applied using an FMC 1029 air blast sprayer (Jonesboro, AK) sprayer calibrated to deliver material and water diluent in 935 liters/ ha (100 gallons per acre) of diluent.

Table 5. Active ingredients, insecticide groups, formulation brand, manufacture, rate, and field rate of used formulation

Active Ingredient	Insecticide Group	Trade name	Manufacture	Rate	Field rate
phosmet	organophosphate	Imidan 70W	Gowan Corporation, Yuma, AZ	1,680 g AI/ha	2.125 lb/acre
<i>Chromobacterium substugae</i>	biopesticide	Grandevo DF	Marrone Bio Innovations, Inc., Davis, CA	1,008 g AI/ha	3 lb/acre
cyantraniliprole	diamide	Exirel 10SE	DuPont, Wilmington, DE	100.6 ml AI/ha	13.5 fl oz/acre
acetamiprid	neonicotinoid	Assail 30SG	United Phosphorous Inc., Abingdon, VA	111.3 g AI/ha	5.3 ounce/acre
zeta-cypermethrin	pyrethroid	Mustang Maxx .8EC	FMC Corp., Philadelphia, PA	28 g AI/ha	4 fl oz./acre
spinetoram	spinosyn	Delegate 25WG	Dow AgroSciences LLC, Indianapolis, IN	105 g AI/ha	6 oz./acre
*2-Hydroxy-1,2,3-Propanetricarboxylic Acid	adjuvant	Tri-fol	Wilbur-Ellis Company LLC, Fresno, CA	0.62-2.5 ml/liter	0.5-2 pint/100 gal

Table 6. Ion monitored in mass spectrometer and the limit of detection (LOD) and limit of quantitation (LOQ) for each treatment compound in 2017 and 2018 residue analysis

Compound	M+H (m/z)	Qualifier (m/z)	LOD ($\mu\text{g/g}$)	LOQ ($\mu\text{g/g}$)
phosmet	161	160	0.015	0.05
zeta-cypermethrin	209	163	0.005	0.010
acetamiprid	223	152	0.015	0.05
spinetoram	784.5	142.4	0.121	0.40
cyantraniliprole	475	286	0.005	0.010

Semi-field Bioassays

Cherry shoots containing 5 fruit and 5 leaves were collected approximately 4 hours after treatment and stored in a 2.7°C walking cooler. Cherry shoots were placed in water soaked OASIS floral foam bricks and sorted into a Generation 3 Research Sprayer Track (DeVries Manufacturing; Hollandale; Minnesota; United States of America). The rainfall simulator was set up with the AI 11008VS nozzle (TeeJet Technologies, Wheaton, IL), run at 69 kPa (10 PSI) and 0.8 kilometers/hour, and distance between nozzle and shelf of 100.3 cm. Shoots were run through 12.7 mm, and 25.4 mm of simulated rain. Controls (0 mm) were not placed in the rain simulator. Three rain gauges were placed inside the rainfall simulator to measure uniformity and amount of simulated rainfall.

Air dried cherry shoots were then placed into 0.95 L plastic containers with floral foam on the bottom and food quality wax to insure shoot steadiness. Six female and six male spotted wing drosophila adults were added into containers with a 1 ml diet disc to maintain a healthy fly conditions. Adult fly mortality were recorded 1, 3 and 5 days after fly exposure. After 5 days, flies were removed and cherry shoots held for an additional 4 days until assessment for survival of small larvae (<2 mm), large larvae (≥ 2 mm), and pupae. Fruit were placed into a 0.95 L closable bags (Gordon Food Service; Grand Rapids; MI; United States of America). Berries were crushed to allow brown sugar water to enter fruit. One hundred and fifty milliliters of brown sugar water with a ratio of 172 g of brown sugar per 1 L of tap water was added to each plastic bag. After an hour, the berry mixtures were then poured over mesh tray with hole sizes of 8.38 mm (SE GP2-14 stackable sifting pan) for larvae and water to run through and cleaned using a sprayer bottle. Liquid were stored in a 0.95 L plastic container and stored in a walking cooler to be accessed the next day. The next day, liquid was poured into a reusable coffee filter and small,

large larvae, and pupae were counted under stereomicroscope. Lethality was determined by comparing total of small larvae, large larvae, pupae, and total individuals found between each insecticide treatment. The bioassay in 2018 was run twice in order to increase the replication.

Insecticide Residue Analysis

A parallel set of shoots as used in the bioassay were run in the rainfall simulator for residue analysis. Surface and subsurface residues for both leaves and fruit tissue were measured to determine the degree of wash off due to simulated rain. Approximately 20 leaves and 10 fruit from each set of treatments were placed in respectively 120 ml and 60 ml of acetonitrile high performance liquid chromatographers (HPLC)-grade (EMD Milipore Chemicals, Inc., Billerica, MA). Samples were sonicated for 30 s to obtain surface residues. Plant tissues were moved into new sample jars and 120 ml and 60 ml of dichloromethane (VWR Analytical, Radnor, Pennsylvania) were added to respectively leaf and fruit samples. Fruit samples were ground to increase contact surface with solvent. Subsequently, 4 g of magnesium sulfate and 1 g of sodium chloride were added to leaf and fruit samples. All samples were stored in a 4°C cooler until laboratory processing.

Surface Residues

Samples were sonicated for 30 seconds and acetonitrile was decanted through 12 g of sodium sulfate (EMD Chemicals Inc.) placed in Whatman filter paper \varnothing 11.25 cm to remove water (Tisch Scientific, North Blend, OH) to remove water. Sodium sulfate columns were rinsed twice with clean 10 ml of acetonitrile to collect remaining residues. Solvent was evaporated under a fume hood and 2 ml of acetonitrile for HPLC or GC analysis was added. Samples were sonicated for 1 minute to collect suspected remaining residues. Remaining particulates were

removed by passing samples through a 0.45- μm 13-mm syringe filter (Pall, East Hills, New York, United States of America).

Subsurface Residues

Sample extracts were passed through 12 g of sodium sulfate (EMD Chemicals Inc.) placed in Whatman filter paper \varnothing 11.25 cm to remove water (Tisch Scientific, North Blend, OH) to remove remaining water. Filtering was repeated until remaining water is collected. Sodium sulfate columns were rinsed twice using 10 ml of dichloromethane between each repetitions. Solvents were evaporated and 2 ml of acetonitrile were added for HPLC or GC analysis.

Samples were analyzed for spinetoram residues using a 2690 separator module HPLC, with a 2487 dual-wavelength absorbance detector (Waters, Milford, MA). A C18 reserved-phase column with 4.6-mm bore and 5-mm particle size was used. Flow rates was set at 0.3 ml/minute. The mobile phase was started at 90:10 water: acetonitrile with formic acid (0.1%) and reduced to 70:30 between 12 and 13 minutes at 20°C. The detectors was set to monitor 745.86 m/z for spinetoram. Acetamiprid and phosmet were analyzed using GC/MSD (Agilent 6890 Gas Chromatograph with a 5973 N Mass Spectra Detector (MSD); Agilent Technologies, Santa Clara, CA) that was equipped with a Zebron ZB-5ms 30m, 0.25mm I.D. and a 0.25 μm film thickness. For the GC/MSD analysis settings, the oven was held at 115°C for five minutes with a ramp of 9°C per minute to 280°C, followed by a ramp of 30°C per minute to 310°C. The MSD transfer line was held at 285°C. The mass spectrometer were set to monitor for ions according to table 6. The injector were rinsed three times with acetone and three times with dichloromethane between and also before each injection. All compounds were quantified against a standard curve, and recovery data recorded as μg of AI per gram (ppm) of plant substrate.

Statistical Analysis

Semi-field Bioassays

Mean adult mortality were compared using a repeated-measure ANOVA on square-rooted arcsine-transformed data $((\arcsin(x))^{1/2})$ to meet normality and homogeneity assumptions. Analysis was performed using PROC MIXED, with the Kenward-Rogers degree of freedom calculation method. Data from 5 days of exposure were exclude from this analysis due to the inability to meet assumptions. The factor exposure time (1 or 3 d) was treated as the repeated measure and each plastic container served as an experimental unit and subject to this repeated-measure class. The factor run was treated as a random factor in 2018 data analysis. The two error terms for the 2018 model were an interaction between run, insecticide, and rainfall amount, and an interaction between run, insecticide, rainfall amount, and observation day. Post hoc Tukey's HSD test to assess pairwise comparison between adult mortality at different rainfall intensities at an insecticide treatment, pairwise between different exposure days of an insecticide and rainfall treatment, and pairwise differences between adult mortality at an insecticide, rainfall amount, and observation treatment combination with the appropriate untreated control. All test were done using $\alpha=0.05$.

All eggs, larvae, and pupae that were found between insecticide and simulated precipitation rates treatment combinations were analyze using ANOVA. Toxicity was determined by separately comparing total of small larvae, large larvae, pupa, and total of individuals recovered between each insecticide and rainfall treatment combinations at each year using a two-way ANOVA in PROC MIXED. The two error terms for the 2018 model were an interaction between run, insecticide, and rainfall amount, and an interaction between run, insecticide, rainfall amount, and observation day. Data were tested for normality and

homogeneity assumptions by using Shapiro-Wilk and Levene's test respectively. Square root transformation were done to data if necessary to meet assumption requirements. Data set that did not meet homogeneity assumption were then ran with the REPEATED command in PROC MIXED with testing every main effect and their interactions. Model was than chosen based on the lowest Akaike information criterion (AIC) value (Anonim, 2018). Kenward-Rogers degree of freedom calculation test was used to correct for the possibility of artificial inflations. Ranked test was done to data that could not be normalized. Required transformation used in each stages are listed in the results. Mean separation between insecticide treatment and application time combinations were done using Tukey's HSD test. Each run were treated as a random factor in all analysis. Immature stage survival data from 2017 were not include due to high natural infestation which occurred before bioassays. All tests were run with $\alpha=0.05$ and done using SAS software 9.4 (SAS Institute, 2009).

Residue Analysis

Data were checked for normality and homogeneity using Shapiro-Wilk and Levene's test. Transformation was done to data to meet normality and homogeneity assumptions. Rainfall effects on fruit surface and subsurface residues were analyzed using a one-way ANOVA separately between rainfalls at each insecticide treatment and plant organ. Multi comparison was done using a Dunnett's test to determine differences between rainfall treatments and the control (0 mm rainfall). Analyses were separated by year. All tests at $\alpha=0.05$ and performed using SAS software 9.4 (SAS Institute, 2009).

Results

Bioassay

2017 Semi-field Bioassay

Adult Mortality

The repeated measure ANOVA results showed that only rainfall amount and observation days significantly affected adult mortality while insecticides, insecticide \times rainfall, insecticide \times observation day, rainfall \times observation day, and insecticide \times rainfall \times observation day did not significantly affect adult mortality (Table 7). Based on our multi comparisons, no significant differences were found between any insecticide, rainfall amount, and observation day treatment combination comparisons (Figure 1). There was strong evidence that fruit were infested with SWD before the study began and condensation in bioassay chambers may have caused unwanted adult mortality and wash-offs, thus being responsible for the non-significant results.

Immature Stages Survival

Based on the two-way ANOVA, insecticide and insecticide \times rainfall amount were not significant at all life stages. However, rainfall was significant at all life stages. Based on our multi comparisons, no significant differences were found between any insecticides or rainfall treatment comparisons at any life stage (Table 8). Due to the high population of this season, we believe that fruit were infested with SWD before the study began and condensation in bioassay chambers may have caused wash-offs, thus being responsible for the non-significant results.

Table 7. Statistical variables of fixed effects and nested interaction for repeated measure analysis of adult mortality observed 1, 3, and 5 days after various insecticides were treated with 0, 12.7, and 25.4 mm of simulated rainfall from 2017 trial

Effect	Numerator df	Denominator df	F Value	Pr > F
insecticide	6	84	0.8	0.5741
rainfall	2	84	14.87	<.0001
insecticide × rainfall	12	84	0.57	0.8618
observation day	1	84	58.69	<.0001
insecticide × observation day	6	84	1.52	0.1813
rainfall × observation day	2	84	0.79	0.4553
insecticide × rainfall × observation day	12	84	1.02	0.437

Table 8. Mean \pm SE of *Drosophila suzukii* small larvae (<2mm), large larvae (>2mm), pupae, total individuals recovered from samples after 9 days since infestation with 6 females and 6 males at 2017 trials. Shoots were treated with simulated rainfall (0, 12.7, and 25.4 mm). Mean separation was done using Tukey's HSD test. Different lowercase letters indicates significant differences within a column whereas different capital letters indicates significant differences within a row at a developmental stage. All test were done at $\alpha=0.05$. Data shown are untransformed values

Treatment	Large larvae			Pupae		
	0	12.7	25.4	0	12.7	25.4
untreated	15.6 \pm 4.65aA	28.8 \pm 8.33aA	36.6 \pm 7.75aA	18 \pm 0.58aA	2.6 \pm 1.29aA	3.6 \pm 1.03aA
phosmet	23 \pm 14.08aA	30 \pm 13.16aA	6.25 \pm 6.25aA	2.6 \pm 1.78aA	5.2 \pm 3.81aA	5 \pm 1.79aA
zeta-cypermethrin	15 \pm 7.57aA	37.75 \pm 13.26aA	20.2 \pm 6.92aA	7.6 \pm 4.20aA	14.25 \pm 7.49aA	4.8 \pm 2.85aA
acetamiprid	10 \pm 6.95aA	11.8 \pm 2.42aA	12.4 \pm 4.19aA	12 \pm 0.50aA	2.2 \pm 0.8aA	8.8 \pm 3.48aA
spinetoram	11 \pm 5.30aA	33.4 \pm 18.41aA	13 \pm 5.85aA	2.0 \pm 0.84aA	3 \pm 1.79aA	9.25 \pm 5.11aA
cyantraniliprole	7.6 \pm 5.49aA	15.4 \pm 2.84aA	16 \pm 6.88aA	0.6 \pm 0.4aA	2 \pm 0.45aA	0.4 \pm 0.25aA
<i>C. substugae</i>	13 \pm 7.99aA	14.2 \pm 6.08aA	47.6 \pm 12.06aA	0.8 \pm 0.37aA	6.4 \pm 5.90aA	4.6 \pm 2.66aA
transformation	sqrt(x), unequal variance by ins*rain			log(x+1)		
insecticide (F; df; P)	1.79; 6, 19.5; 0.1531			2.18; 6, 82; 0.0529		
rainfall (F; df; P)	5.58; 2, 27.6; 0.0092			3.45; 2, 82; 0.0365		
insecticide x rainfall (F; df; P)	0.78; 12, 15.9; 0.6606			0.84; 12, 82; 0.6084		

Table 8. (cont'd)

Treatment	Total		
	0	12.7	25.4
untreated	17.4 ± 4.66aA	31.4 ± 9.22aA	40.2 ± 8.08aA
phosmet	25.6 ± 15.70aA	35.2 ± 16.57aA	21.6 ± 7.97aA
zeta-cypermethrin	22.6 ± 11.66aA	52 ± 18.93aA	25 ± 9.61aA
acetamiprid	11.2 ± 7.25aA	14 ± 2.43aA	21.2 ± 6.19aA
spinetoram	13 ± 5.38aA	36.4 ± 20.07aA	22.25 ± 6.14aA
cyantraniliprole	8.2 ± 5.88aA	17.4 ± 2.80aA	16.4 ± 7.06aA
<i>C. substugae</i>	13.8 ± 8.35aA	20.6 ± 11.93aA	52.2 ± 14.19aA
transformation	sqrt(x), unequal variance by ins		
insecticide (F; df; P)	1.94; 6, 32; 0.1050		
rainfall (F; df; P)	5.68; 2, 67.1; 0.0053		
insecticide x rainfall (F; df; P)	0.49; 12, 39; 0.9104		

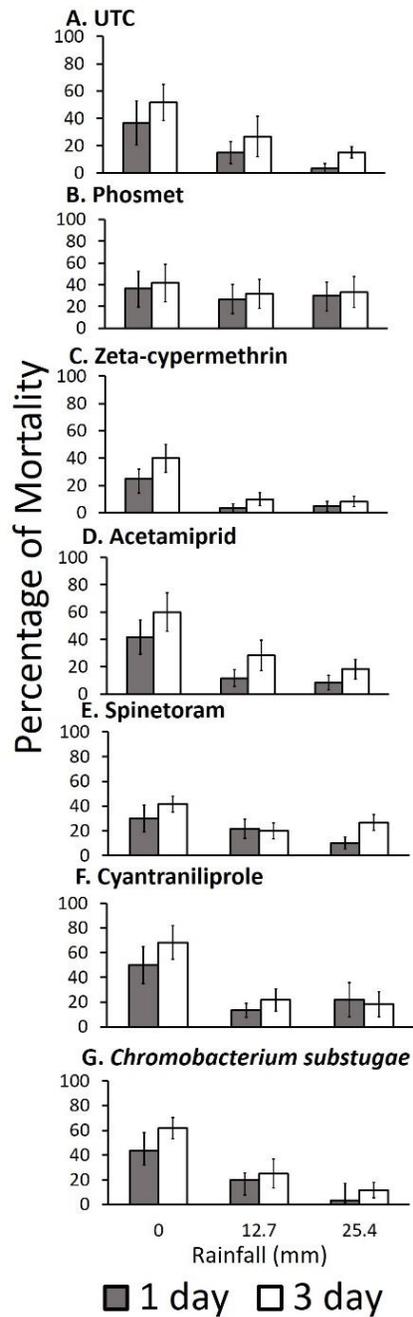


Figure 1. Mean \pm SEM of adult mortality percentage at after each insecticide and rainfall treatment combination on each observation day from 2017's trial. Data were analyzed using repeated measure three-way ANOVA. No significant differences were discovered from multi comparisons using Tukey's HSD test at $\alpha=0.05$.

2018 Semi-field Bioassay

Adult Mortality

Adult mortality depended on the insecticide, rainfall amount, and observation day. Our repeated measure ANOVA indicated significant effects of insecticide, rainfall amount, observation day main effects, and insecticide \times rainfall amount, insecticide \times observation day, rainfall amount \times exposure day, and insecticide \times rainfall amount \times observation on the percentage of adult mortality (Table 9).

The several pairwise comparison showed differences between adult mortality between rainfall at an insecticide treatment, between exposure day at an insecticide and rainfall treatment combination, and between insecticide \times rainfall amount \times exposure day combinations with the parallel untreated controls. Adult mortality at all insecticide and exposure days of 0 mm rainfall was significantly higher than the untreated control (Figure 2). Phosmet and cyantraniliprole showed significantly higher adult mortality after 12.7 and 25.4 mm rainfall at 1 day of exposure. Acetamiprid showed significant higher mortality only on 25.4 mm rainfall amount after 3 days of exposure whereas zeta-cypermethrin and *C. substugae* did not demonstrate significant differences in adult mortality at any observation days after 12.7 and 25.4 mm rainfall compared to the untreated control. Adult mortality of flies treated with phosmet, zeta-cypermethrin, spinetoram, and cyantraniliprole treated with 12.7 and 25.4 mm were significantly lower when compared to 0 mm at all periods of exposure days. On the other hand, only *C. substugae* treated with 25.4 mm rainfall demonstrated significantly lower adult mortality compared to 0 mm rainfall. Flies treated with phosmet and cyantraniliprole demonstrated significantly higher adult mortality at 12.7 and 25.4 mm rainfall after 3 days of exposure (Figure 2B and F) whereas

spinetoram caused adult mortality at all rainfall treatments to be significantly higher after 3 days of exposure (Figure 2E).

Immature Stages Survival

Insecticides, rainfall amount, and their interaction affected number of all life SWD immature life stages (Table 10). The total numbers of SWD immature life stages depended on interaction between insecticide and rainfall amount. Numbers of small larvae recovered from samples were affected by rainfall only in the cherries treated by phosmet and *C. subtsugae*. At 0 mm of simulated rainfall, phosmet was the only treatment to demonstrate significantly lower counts compared to the untreated control, although other insecticides were not significantly higher than phosmet. Small larvae counts were not significantly lower than the untreated control at 12.7 mm of simulated rainfall, whereas at 25.4 mm of simulated rainfall, spinetoram was the only treatment to have significantly lower numbers of small larvae compared to the untreated control.

Rainfall caused significantly higher large larvae to be recovered from samples treated with phosmet, zeta-cypermethrin, spinetoram, and *C. subtsugae* as rainfall amount increases. Large larvae numbers in samples treated with phosmet and zeta-cypermethrin were affected by 12.7 mm of simulated rain, whereas numbers in spinetoram and *C. subtsugae* were only significantly higher at 25.4 mm of simulated rain compared to the untreated control. The increase of large larvae numbers were >1.5 fold than 0 mm rainfall. At 0 mm of simulated rainfall, all samples treated with insecticides were significantly lower by 54-92% than the untreated control. Lowest numbers of large larvae were found at samples treated with phosmet followed by zeta-cypermethrin, cyantraniliprole, spinetoram, acetamiprid, and *C. subtsugae*. Large larvae collected from acetamiprid-treated shoots were not significantly lower compared to zeta-

cypermethrin and spinetoram. At 12.7 mm of simulated rainfall, all insecticide treatments besides *C. subtugae* were significantly lower than the untreated control, although reductions were only 40-58% lower. Phosmet demonstrated the lowest large larvae numbers compared to other insecticide treatments followed by spinetoram, cyantraniliprole, acetamiprid, and zeta-cypermethrin. Large larvae numbers were significantly lower at all insecticides besides *C. subtugae* treated at 25.4 mm of simulated rainfall. Large larvae numbers decreased 45-68% compared to the untreated control. Acetamiprid demonstrated the lowest large larvae number counts, although not significantly different among all insecticides treated with 25.4 mm of simulated rainfall.

Phosmet was the only treatment where the number of pupae was significantly affected by simulated rainfall. Pupae counts on samples treated with phosmet increased significantly after 12.7 mm of simulated rainfall. Pupae counts among insecticide treatments were not significantly lower compared to the untreated control at 0 and 12.7 mm of simulated rainfall. At 25.4 mm of simulated rainfall, only acetamiprid and spinetoram were significantly lower compared to the untreated control, although still not significantly lower compared to all insecticide treatments. Pupae counts were the lowest in samples treated with spinetoram followed by acetamiprid.

Simulated rainfall significantly affected shoots treated with phosmet, zeta-cypermethrin, and *C. subtugae* total numbers of life SWD immature stages counts. Total survival numbers increased as these samples were treated with 12.7 mm of simulated rainfall. At 0 mm of simulated rainfall, all insecticide treatments were significantly lower than the untreated control. Phosmet demonstrated the lowest count numbers, followed by zeta-cypermethrin, cyantraniliprole, spinetoram, acetamiprid, and *C. subtugae*. At both 12.7 and 25.4 mm of simulated rainfall, all insecticide treatment, besides *C. subtugae* resulted in significantly lower

counts compared to the untreated control. At 12.7 mm of simulated rainfall, spinetoram demonstrated the lowest total counts followed by cyantraniliprole, acetamiprid, zeta-cypermethrin, and phosmet whereas at 25.4 mm of simulated rainfall, acetamiprid demonstrated the lowest total counts followed by cyantraniliprole, spinetoram, phosmet, and zeta-cypermethrin.

Insecticide Residues Analysis

2017 Residue Profiles

Phosmet residues recovered from leaf surfaces and subsurface were significantly lower after both rainfall treatments than 0 mm of simulated rainfall (Table 11). Phosmet residues recovered from leaf subsurface was only significantly lower by >91% than the control at 12.7 mm (Figure 3). Both fruit surfaces and subsurface phosmet residues were significantly lower than 0 mm of simulated rainfall by >89% at all rainfall treatments.

Zeta-cypermethrin residues recovered from leaf surface and subsurface were both not significantly different compared the 0 mm rainfall treatment. Similarly, zeta-cypermethrin recovered from fruit surface and subsurface were not significantly different within all rainfall treatments. Although statistical analysis did not show significant differences, there was a numerical decrease of residues collected from leaf surface, leaf subsurface, and fruit surface tissue.

Table 9. Statistical variables of fixed effects and nested interaction for repeated measure analysis of adult mortality observed 1, 3, and 5 days after various insecticides were treated with 0, 12.7, and 25.4 mm of simulated rainfall from 2018 trial

Effect	Numerator df	Denominator df	F Value	Pr > F
insecticide	6	185	25.4	<.0001
rainfall	2	185	67.13	<.0001
insecticide × rainfall	12	185	5.43	<.0001
observation day	1	186	251.99	<.0001
insecticide × observation day	6	186	6.91	<.0001
rainfall × observation day	2	186	6.67	0.0016
insecticide × rainfall × observation day	12	186	1.85	0.0438

Table 10. Mean \pm SE of *Drosophila suzukii* small larvae (<2mm), large larvae (>2mm), pupae, total individuals recovered from samples after 9 days since infestation with 6 females and 6 males at 2018 trials. Shoots were treated with simulated rainfall (0, 12.7, and 25.4 mm). Mean separation was done using Tukey's HSD test. Different lowercase letters indicates significant differences within a column whereas different capital letters indicates significant differences within a row at a developmental stage. All test were done at $\alpha=0.05$. Data shown are untransformed values

Treatment	Small Larvae			Large Larvae		
	0	12.7	25.4	0	12.7	25.4
untreated control	1.80 \pm 0.76aA	2.30 \pm 0.99aA	2.33 \pm 0.80aA	37.90 \pm 3.71aA	37.50 \pm 2.20aA	44.78 \pm 6.46aA
phosmet	0.00 \pm 0.00bB	0.30 \pm 0.17aAB	1.00 \pm 0.60abA	2.80 \pm 0.76dB	15.70 \pm 4.07bA	18.00 \pm 3.82bcA
zeta-cypermethrin	0.40 \pm 0.27abA	0.22 \pm 0.21aA	0.60 \pm 0.22abA	7.30 \pm 1.73cdB	22.22 \pm 2.87bA	24.50 \pm 4.90abcA
acetamiprid	1.10 \pm 0.46aA	2.10 \pm 0.94aA	1.20 \pm 0.33aA	10.90 \pm 2.65bcA	19.10 \pm 2.06bA	13.90 \pm 3.76cA
spinetoram	0.20 \pm 0.13abA	0.30 \pm 0.21aA	0.00 \pm 0.00bA	10.60 \pm 1.20bcB	16.00 \pm 1.83bAB	20.30 \pm 2.77bcA
cyantraniliprole	0.50 \pm 0.40abA	0.60 \pm 0.31aA	0.80 \pm 0.33abA	9.80 \pm 2.77bcA	16.90 \pm 3.12bA	15.90 \pm 3.32bcA
<i>C. substugae</i>	0.10 \pm 1.07abB	2.00 \pm 0.63aA	2.63 \pm 1.38aA	17.40 \pm 5.09bB	26.70 \pm 3.28aAB	32.00 \pm 3.65abA
transformation insecticide (F; df; P)	Rank			sqrt(x), unequal variance by rain		
rainfall (F; df; P)	8.69; 6, 184; <.0001			27.11; 6, 156; <.0001		
insecticide x rainfall (F; df;, P)	5.47; 2, 184; 0.0049			29.23; 2, 118; <.0001		
	1.61; 12, 184; 0.0927			1.66; 12, 150; 0.0809		

Table 10. (cont'd)

Treatment	Pupae			Total		
	0	12.7	25.4	0	12.7	25.4
untreated control	4.30 ± 1.04aA	5.80 ± 1.34aA	6.78 ± 1.62aA	44.00 ± 4.34aA	45.60 ± 2.70aA	53.89 ± 6.67aA
phosmet	2.00 ± 0.86aB	6.30 ± 1.57aA	6.00 ± 1.45abA	4.80 ± 1.62cB	29.40 ± 4.85bA	25.00 ± 3.75bcA
zeta-cypermethrin	3.00 ± 1.09aA	3.56 ± 0.98aA	4.00 ± 0.86abA	10.70 ± 2.34bcB	26.00 ± 2.82bA	29.00 ± 4.86bcA
acetamiprid	3.80 ± 1.28aA	2.80 ± 0.49aA	2.40 ± 0.68bA	15.80 ± 3.83bA	24.00 ± 2.82bA	17.50 ± 3.99cA
spinetoram	3.40 ± 1.09aA	4.70 ± 1.41aA	2.10 ± 0.72bA	14.20 ± 2.49bA	21.00 ± 1.43bA	22.40 ± 2.38bcA
cyantraniliprole	2.10 ± 0.48aA	3.60 ± 1.18aA	3.10 ± 0.81abA	12.40 ± 2.70bcA	21.10 ± 2.90bA	19.80 ± 3.71cA
<i>C. substugae</i>	3.60 ± 1.28aA	5.70 ± 1.86aA	4.63 ± 1.39abA	21.10 ± 2.45bB	34.40 ± 4.94abAB	39.25 ± 4.47abA
transformation	sqrt(x), unequal variance by ins*rain			sqrt(x), unequal variance by rain		
insecticide (F; df; P)	2.75; 6, 66.5; 0.0191			24.75; 6, 173; <.0001		
rainfall (F; df; P)	3.55; 2, 102; 0.0324			29.43; 2, 119; <.0001		
insecticide x rainfall (F; df;, P)	0.99; 12, 57.5; 0.4719			2.04; 12, 152; 0.0244		

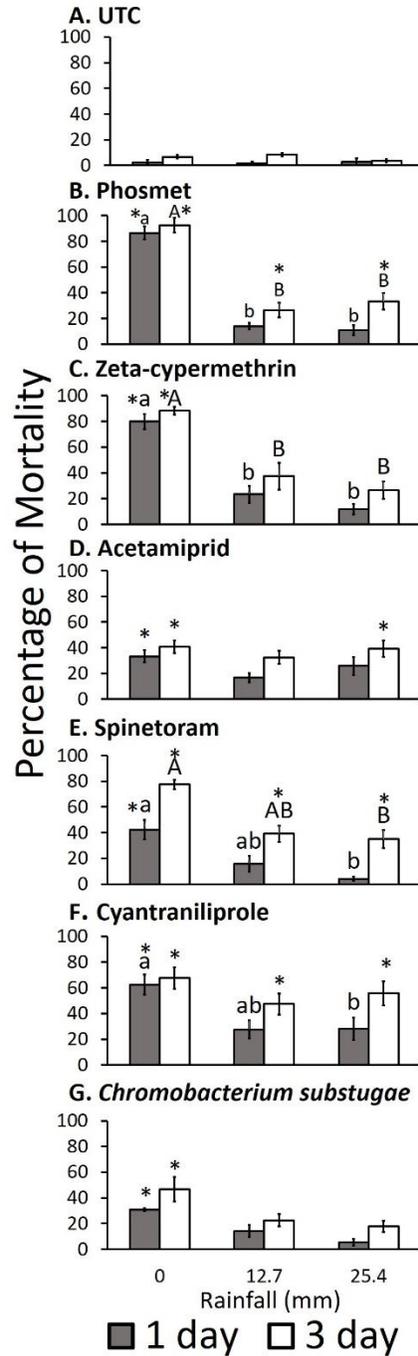


Figure 2. Mean \pm SEM of adult mortality percentage at after each insecticide and rainfall treatment combination on each observation day from 2018's trial. Data were analyzed using repeated measure three-way ANOVA. Different lower case letters indicate significant differences on 1 day observation while capital letters indicate significant differences on 3 day observations. Asterisks shows significant differences between insecticide with observation day and rainfall treatment combination and the untreated with the appropriate observation day and rainfall treatment combination using a Tukey's HSD post-hoc test (*). All test were done at $\alpha=0.05$.

Rainfall treatment did not cause significant differences in acetamiprid recovered from leaf surfaces. Acetamiprid residues from leaf subsurface was only significantly lower at 25.4 mm than 0 mm by ~58%. Acetamiprid recovered from fruit surface was not significantly different between all rainfall treatments, but acetamiprid recovered from fruit subsurface was significantly lower at 12.7 and 25.4 mm compared to 0 mm by 27.1 and 28.8% respectively.

Spinetoram residues recovered from leaf surface were not significantly different after rainfall compared to 0 mm of rainfall based on the Dunnett's test. However, residue collected from shoots treated with 12.7 and 25.4 mm of rainfall were lower numerically by >86%. Spinetoram residues recovered from leaf subsurface was significantly lower at all rainfall treatments compared with 0 mm rainfall by >98%. Spinetoram residues recovered from fruit surface were not significantly different after simulated rainfall. Spinetoram residues recovered from fruit subsurface were significantly lower at all simulated rainfall rates compared to 0 mm by >97%.

Cyantraniliprole residues collected from leaf surface were not significantly different. No cyantraniliprole residues were able to be collected from leaf subsurface. Cyantraniliprole residues from fruit surface and subsurface were not significantly different at all rainfall treatments.

Table 11. Statistical variables of ANOVA fixed effects for residues recovered from leaf surface, leaf subsurface, fruit surface, and fruit subsurface after 0, 12.7, and 25.4 mm of simulated rainfall from 2017 trial

Treatment		Leaf Tissue		Fruit Tissue	
		Leaf Surface	Leaf subsurface	Fruit surface	Fruit subsurface
phosmet	(F; df; P)	24.98; 2, 6; 0.0012	5.57; 2, 6; 0.0429	10.68; 2, 6; 0.0106	5.45; 2, 6; 0.0447
zeta-cypermethrin	(F; df; P)	1.33; 2, 6; 0.3335	3.38; 2, 6; 0.1039	1.15; 2, 6; 0.3791	χ^2 : 0.1255; 2; 0.9392a
acetamiprid	(F; df; P)	3.32; 2, 6; 0.1122	6.28; 2, 6; 0.0338	37.09; 2, 6; 0.0004	28.04; 2, 6; 0.0009
spinetoram	(F; df; P)	3.75; 2, 6; 0.0878	39.76; 2, 6; 0.0003	4.85; 2, 6; 0.0557	112.86; 2, 6; <.0001
cyantraniliprole	(F; df; P)	0.19; 2, 6; 0.8293	-b	0.17; 2, 6; 0.8457	2.61; 2, 6; 0.1530

^a Due to the inability for data to meet normality and homogeneity assumptions, data was analyzed using a Kruskal-Wallis test at $\alpha=0.05$

^b No residues were detected

Table 12. Statistical variables of ANOVA fixed effects for residues recovered from leaf surface, leaf subsurface, fruit surface, and fruit subsurface after 0, 12.7, and 25.4 mm of simulated rainfall from 2018 trial

Treatment		Leaf Tissue		Fruit Tissue	
		Leaf Surface	Leaf subsurface	Fruit surface	Fruit subsurface
phosmet	(F; df; P)	5.11; 2, 6; 0.0506	32.35; 2, 6; 0.0006	5.61; 2, 6; 0.0423	102.53; 2, 6; <.0001
zeta-cypermethrin	(F; df; P)	1.93; 2, 6; 0.2253	0.73; 2, 6; 0.5192	0.17; 2, 6; 0.8507	2.80; 2, 6; 0.1387
acetamiprid	(F; df; P)	0.97; 2, 6; 0.7062	0.38; 2, 6; 0.38	0.58; 2, 6; 0.5874	0.82; 2, 6; 0.4857
spinetoram	(F; df; P)	1.36; 2, 6; 0.3264	3.60; 2, 6; 0.0941	0.97; 2, 6; 0.4313	ND ^a
cyantraniliprole	(F; df; P)	0.86; 2, 6; 0.4697	0.09; 2, 6; 0.9114	1.70; 2, 6; 0.2608	0.07; 2, 6; 0.9351

^a No residues were detected

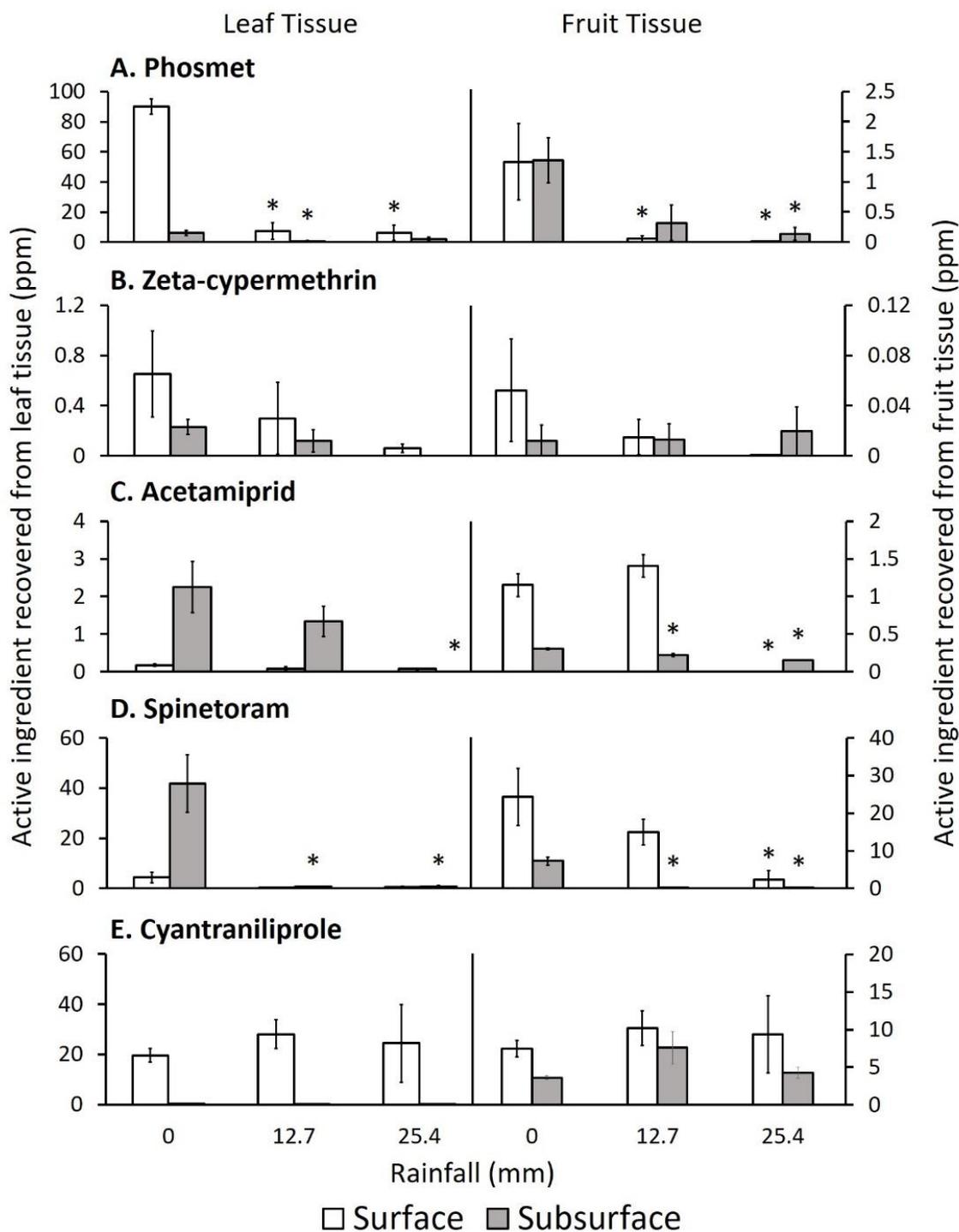


Figure 3. Mean \pm SEM of residues collected from tart cherry leaf and fruit surface and subsurface at each rainfall treatment in 2017 trials. Data were analyzed using ANOVA and data that showed significant difference were then test using a Dunnett's test. Asterisks (*) shows significant differences between rainfall treatment with no rainfall (0 mm) at $\alpha=0.05$.

2018 Residue Profiles

Phosmet recovered from leaf surface were not significantly different after rainfall compared to 0 mm of rainfall (Table 12). However, post-hoc tests indicated leaf surface residues recovered from 25.4 mm of rainfall was significantly lower than the untreated control (0 mm) by ~66%. Residues recovered from leaf subsurface at all rainfall amounts were significantly lower than 0 mm by >66%. Phosmet residues collected from fruit surface were significantly lower after 25.4 mm of rainfall compared to 0 mm by 96%. Although, phosmet residues collected from fruit surface after 12.7 mm of rainfall were numerically lower by 93%, the post-hoc test showed were marginally not significant ($t = -2.79$ $df_{\text{denum}} = 6$; $P = 0.0548$). Residues collected from fruit subsurface were significantly lower at all rainfall treatments compared to 0 mm by >88%.

Zeta-cypermethrin residues recovered from rainfall treatments were not significantly different compared to 0 mm of rainfall at all plant parts. Cyantraniliprole residues were not significantly different between residues recovered from samples treated with rainfall compared to 0 mm from all plant parts. However, residues recovered from leaf surface showed a numerical decrease after simulated rainfall. Spinetoram residues recovered from sample treated with simulated rainfall were not significantly different compared to 0 mm from all plant parts based on the statistical analysis. However, residues collected from leaf surface, leaf subsurface, and fruit surface showed a numerical decrease after treated with rainfall.

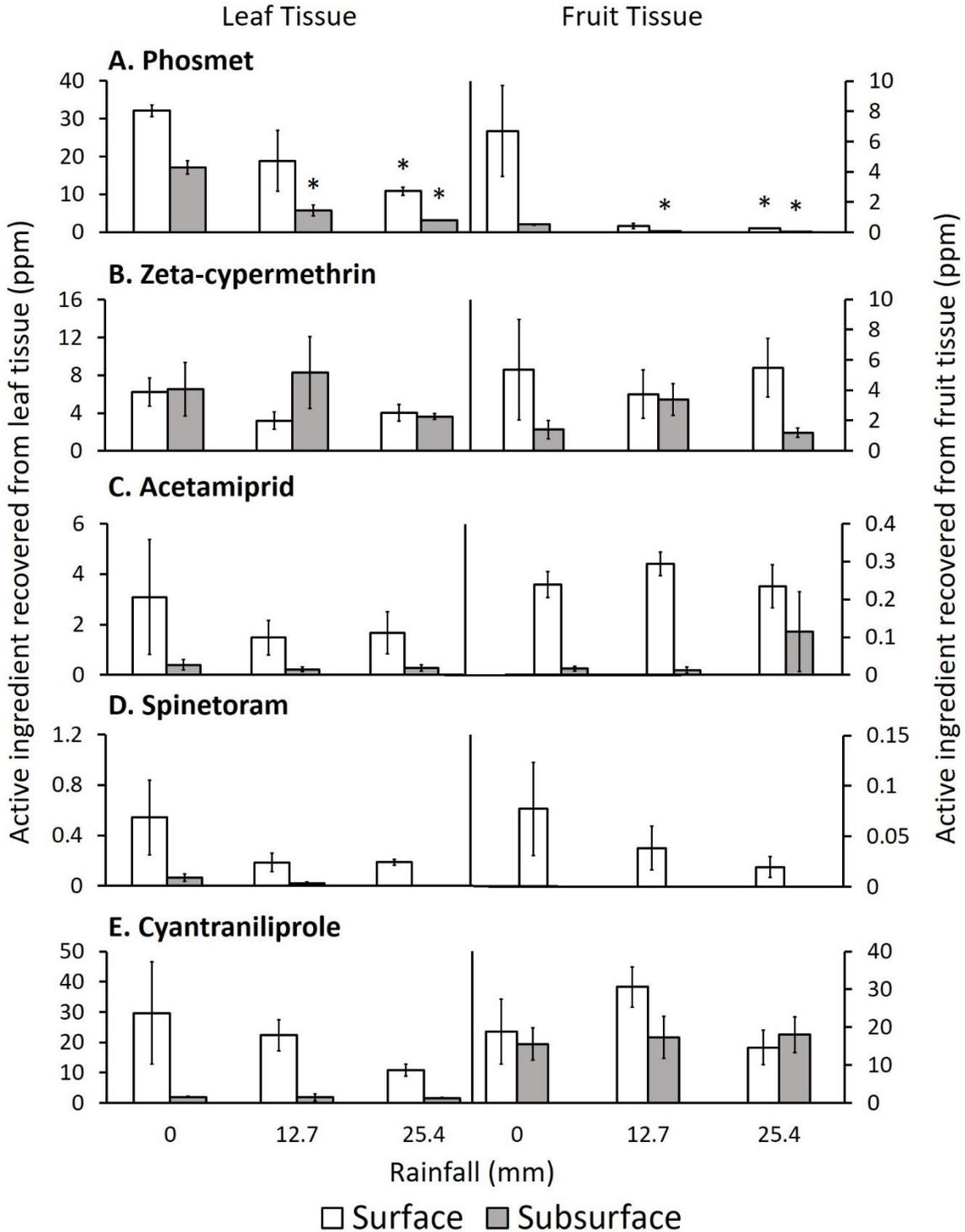


Figure 4. Mean \pm SEM of residues collected from tart cherry leaf and fruit surface and subsurface at each rainfall treatment in 2018 trials. Data were analyzed using ANOVA and data that showed significant difference were then test using a Dunnett's test. Asterisks (*) shows significant differences between rainfall treatment with no rainfall (0 mm) at $\alpha=0.05$.

Discussion

This study demonstrates varying effects of rain on the residues of insecticides on tart cherry fruit and leaves, and their ability to cause SWD adult mortality and immature development. Some of the most commonly used insecticides, such as phosmet, zeta-cypermethrin and spinetoram demonstrated that their adulticide activity is highly sensitive to the effects of rainfall.

Acetamiprid was the most resistant to wash-off from simulated rain based on the residue results and low SWD immature stage survival. Acetamiprid's low adulticide action was consistent with previous results against SWD (Beers et al. 2011). In addition, low neonicotinoid adulticide results were consistent with other studies done on codling moth (*Cydia pomonella*) and Japanese beetles (*Popillia japonica*); however, the neonicotinoids demonstrated other forms of control than just mortality, such as feeding or oviposition deterrence (Hulbert et al. 2011, 2012, Wise et al. 2016). Acetamiprid log P (log K_{ow}) is the lowest of all compounds tested for residues (0.8), suggesting high penetrative potential in plant tissues. Acetamiprid's residues in fruit and leaf subsurfaces likely serves as a protected refuge from the negative effects of rainfall. These fruit subsurface residues also provide toxic exposure to SWD larvae and eggs, likely explaining the noticeable SWD control even though high amounts of simulated rainfall. In addition, previous studies have shown acetamiprid to possess curative action against SWD in tart cherries (Andika, unpublished data). Thus, it may be beneficial to consider including acetamiprid in spray programs during periods of high risk of rainfall when controlling this pest.

Simulated rainfall adversely affected SWD control by zeta-cypermethrin and *C. subsugae*. Zeta-cypermethrin adulticide levels were different from previous studies, which demonstrated high inherent toxicity even after rainfall (Hulbert et al. 2012, Gautam et al. 2016).

Residue results indicated that active ingredients were less sensitive to wash-off by simulated rainfall; however, spatial distribution of the compound may have been affected, thus decreasing toxicity to the target pest. Although adult mortality from *C. subtsugae* samples were not affected by simulated rainfall, immature survival was adversely affected. Unfortunately, residue samples for *C. subtsugae* could not be analyzed, thus it is not clear whether these control decreases are caused by heavy reduction of active ingredient, low inherent toxicity, or low larvacidal activity.

Although phosmet control and residues were sensitive to rainfall, phosmet still demonstrated better SWD control compared to the untreated control. This high inherent toxicity was consistent with previous studies on Japanese beetles and codling moth, which demonstrated leaf defoliation and living larvae to be lower than the untreated control even after simulated rainfall (Hulbert et al. 2011, Wise et al. 2016). It is noteworthy that phosmet is most effective in acid water (pH = 5) and is hydrolysis in base pH causing its effectiveness to be compromised and have shorter half-time periods; thus this process may help explain phosmet's effectiveness were compromised and less residues were recovered (Schilder, 2008). However, azinphos-methyl, another organophosphate, half-time periods were less sensitive to neutral pH (7) than phosmet and were the same at pH=9 (Schilder, 2008). Thus, active ingredient behavior to rainfall within this insecticide class should not be generalized.

Simulated rainfall moderately affected cyantraniliprole. Adulticide action was affected by simulated rainfall; however, its effects on immature stages and the residue results were less affected by rainfall. Adulticide results were consistent from previous studies, which demonstrated that rainfall adversely affected cyantraniliprole SWD adulticide action on blueberries (Gautam et al. 2016). However, another study demonstrated codling moth control by cyantraniliprole to be less sensitive to simulated rainfall (Wise et al. 2016). Based on its residue

profiles, cyantraniliprole may possess other control activity besides adulticide action, such as oviposition deterrence, ovicidal, or larvacidal activity. However, to minimize the effect of rainfall, other studies have suggested adding an adjuvant may help increase cyantraniliprole adulticide action when rainfall occurs (Gautam et al. 2016).

Simulated rainfall adversely affected spinetoram's adulticide action and residues, but its lethality to SWD immature stages was less affected. Although spinetoram residues from our 2018 studies were not significantly affected by rainfall, numerically there was a noticeable decline. Spinetoram's inherent toxicity against SWD was consistent with a previous study with codling moth causing less larvae survival and SWD adulticide action even after simulated rainfall (Gautam et al. 2016, Wise et al. 2016). Spinetoram's inherent toxicity may have to do with its high toxicity based on its LC_{50} and LC_{90} compared to other compounds (Van Timmeren et al. 2018). These results imply that beside adulticide activity, spinetoram possessed ovicidal and larvacidal activity even at lower concentrations. It is noteworthy that adult mortality increased over time after spinetoram, phosmet, and cyantraniliprole samples were treated with simulated rainfall. Thus, continuous exposure to lower residues may still provide control against SWD.

Precipitation effects on chemical crop protection are complex and not yet fully understood. To date, most studies associated with insecticides and SWD control have focused on the effects of precipitation on adult mortality without directly measuring residue losses from the plant or considering impacts on immature life stages (Gautam et al. 2016). This study provides immature survival and residue results beside adult mortality; thus, it helps provide additional information on other modes of activity that may occur after precipitation events. It is noteworthy that precipitation's effects on crop protection systems may extend beyond the direct effects on

pesticide residue and the target pest. A recent study demonstrated rainfall to affect tree volatiles associated with repellency and attractant of codling moth in apples (Vallat et al. 2005). Thus, the complicated interchanges among plant volatiles may increase crop preferences to herbivores. In addition, insecticide application have been reported to decrease phytohormone or organic compounds concentration associated with plant defense (Wu et al. 2004, Szczepaniec et al. 2013). Due to the dynamics among plant, insects, chemical, precipitation and interactions among these systems, precipitation's effects to crop protection may be more than simply washing off insecticides from crops.

Results from this study provides information on how different insecticide compounds at labelled field rates behave differently under various rainfall amounts. It also provides additional emphasis that other mode of activity besides mortality may contribute to SWD management programs after precipitation. Thus, tart cherry growers may use these results as a base for informed decisions on insecticide application and reapplication before and after rainfall events.

CHAPTER 4: CONCLUSION

To date, insecticides remain a key part of Integrated Pest Management (IPM) for managing spotted wing drosophila (*Drosophila suzukii*) in fruit orchards. Due to the risk of these intensive spraying programs to the environment, human health, and farm economics, additional information to refine IPM programs is required. The two studies in this thesis were designed to deliver new knowledge in relation to characteristics of insecticides used to manage SWD in tart cherry orchards. The questions chosen in this thesis were ones containing practical aspects of insecticide applications in SWD management programs, but also to expand fundamental knowledge of how pesticides interface with plants, arthropods and the environment.

Current published research regarding SWD control using insecticides has focused on adulticide activity or field control, while less exploration has been done towards mortality of immature stages and using residue profiles to understand these mortality events. My first experiments provides new insight to insecticides' residue penetration profiles and curative activity on tart cherries. These results helps explain how insecticides may provide control after infestation against SWD immature stages located inside fruit. Thus, understanding curative action of insecticides registered for SWD in tart cherries provides insight to refine spraying programs. Based on the results, growers may consider acetamiprid, phosmet, or spinetoram to spray in orchards when there is risk that infestation has already occurred. Growers can adjust spraying programs to spray active ingredients with high curative activity later in the season while still complying with regulated PHI and maximum spraying amounts. Another reduced-risk insecticide with moderate curative action, cyantraniliprole, may need further research on whether or not addition of adjuvant may add curative activity of this compound. Exploration of reduced-

risk compounds other than the compounds tested in this experiment will add more options to SWD management. Community concerns of insecticide use in food products and their adverse effects to the environment will continue to shape IPM, insecticides use, and insecticide regulations. This chapter delivered new knowledge on how products behave in fruit tissues and the subsequent suppression of SWD population by targeting the immature stage of this pest.

In the second study, I asked how rainfall affects SWD spray residues and their efficacy. We specifically asked questions on how rainfall affected insecticide performance in terms of adult mortality, immature survival, and residue levels. In contrast to much of the existing literature regarding rainfall effects to pest control, which has focused on residue wash off or direct mortality solely, this study provides another aspect on how rainfall may affect immature stages survival. Immature survival was asked specifically to elicit whether insecticide residues may still manage SWD population even after precipitation events. These results provide insight that although adulticide activities were adversely affected, toxicity to other life stages might not as other modes of activity may occur. Residue analysis also provided new insight on mode of activities which may occur after rainfall. However, replication of this experiment using the same compound may strengthen these preliminary conclusions. Rainfall adversely affected adult mortality and immature survival of *C. substugae*. Unfortunately, residue samples for *C. substugae* were not able to be taken which limits our understanding on why its control against SWD were affected. Again, acetamiprid's positive results based on this study may contribute to SWD management programs. These results may also suggest other applied questions, such as how curative activity is affected by rainfall.

Acetamiprid had noticeable curative control and was less sensitive to wash off according our studies although previous studies have shown its low adulticide properties. These beneficial

properties might be worth considering when integrating acetamiprid into existing spraying programs. Considering SWD to be abundant in orchards and always seem to manage to infest fruit even with intensive spraying programs, spraying a different chemical to post-infestation or just to protect during high precipitation periods might be an option for acetamiprid. Obtaining product options is essential to SWD management programs. It is worth remembering that tart cherries have to withstand other pest infestation, such as plum curculio (*Conotrachelus nenuphar*) and obliquebanded leaf roller (*Choristoneura rosaceana*), before fruit are susceptible to SWD. There are several effective compounds between these three pests that overlap, including phosmet, zeta-cypermethrin, spinetoram, and cyantraniliprole. In addition, most of these compounds have spraying amount limits in a season causing restrictions to compounds after a number of applications. However, inserting acetamiprid into a spraying program would have to comply with market's maximum residue limits (MRL) and pre-harvest intervals (PHI).

Pest management is dynamic in agroecosystems with many interrelated components. The plant-insect-chemical model, suggested by Wise et al. (2009), explains some components related to the outcomes of insecticide applications. Results from these new studies suggest other forms of the insect-chemical interaction besides adult mortality. Other forms, such as curative activity or toxicity against other life stages besides adults, may contribute to SWD management in agroecosystems and provides insight to refine spraying programs. In addition, these studies as well add to the chemical-plant interaction component of SWD management by providing information on how different activity ingredients behave under rainfall on different plant parts. However, other components, such as abiotic and biotic factors, may be included in this model elicit more information, but still simple to provide a basic framework. Studies have shown plant volatiles associated with crop defense mechanisms and pest attraction to be affected by

insecticide application and abiotic factors, such as weather conditions to affect plant volatiles (Vallat et al. 2005). Other weather conditions, such as UV light, may also be explored to understand insecticide degradation over time in different cropping systems (Leach et al. 2017). The exploration of these new topics, confirms the dynamic complexity of plant protection in agroecosystems.

Insecticides will continue to be important in modern agriculture. It has allowed our community to have more certainty of food supply for a period. However, its intensive use, if careless, may cause adverse effects in the future. Thus, continuous effort in exploring new aspects of insecticide application and the basic knowledge associated with it may complement other knowledge fields about agroecosystems and therefore refine existing spraying programs to comply with current or future needs.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

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

Voucher Number: 2018-07

Author and Title of Thesis:

Ignatius Putra Andika

“Curative and Rainfastness Characteristics of Insecticides Used to Control Spotted Wing *Drosophila* (Matsumura) In Tart Cherry Productions”

Museum(s) where deposited:

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

Table 13. List of voucher specimens

Family	Genus/Species	Life Stage	Quantity	Preservation
Drosophilidae	<i>Drosophila suzukii</i>	Adult	10♀ 10♂	Ethanol

REFERENCES

REFERENCES

- Andreazza, F., Bernardi, D., dos Santos, R. S. S., Garcia, F. R. M., Oliveira, E. E., Botton, M., & Nava, D. E. (2017). *Drosophila suzukii* in Southern Neotropical Region: Current Status and Future Perspectives. *Neotropical Entomology*, *46*(6), 591–605. <http://doi.org/10.1007/s13744-017-0554-7>
- Asplen, M. K., Anfora, G., Biondi, A., & Choi, D. (2015). Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, *88*, 469–494. <http://doi.org/10.1007/s10340-015-0681-z>
- Bal, H. K., Adams, C., & Grieshop, M. (2017). Evaluation of Off-season Potential Breeding Sources for Spotted Wing *Drosophila* (*Drosophila suzukii* Matsumura) in Michigan. *Journal of Economic Entomology*, *110*(6), 2466–2470. <http://doi.org/10.1093/jee/tox252>
- Baur, P., Buchholz, A., & Schönherr, J. (1997). Diffusion in plant cuticles as affected by temperature and size of organic solutes: Similarity and diversity among species. *Plant, Cell and Environment*, *20*(8), 982–994. <http://doi.org/10.1111/j.1365-3040.1997.tb00675.x>
- Beers, E. H., Van Steenwyk, R. A., Shearer, P. W., Coates, W. W., & Grant, J. A. (2011). Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. *Pest Management Science*, *67*(11), 1386–1395. <http://doi.org/10.1002/ps.2279>
- Bostanian, N. J., Wise, J. C., & Isaacs, R. (2012). Pesticides for Arthropod Control in Vineyards. In *Arthropod Management in Vineyards: Pests, Approaches, and Future Directions* (pp. 139–157). <http://doi.org/10.1007/978-94-007-4032-7>
- Bruck, D. J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., Defrancesco, J., Beverly, G., Spittler, H. (2011). Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. *Pest Management Science*, *67*(11), 1375–1385. <http://doi.org/10.1002/ps.2242>
- Bukovac, M. J., & Petracek, P. D. (1993). Characterizing pesticide and surfactant penetration with isolated plant cuticles. *Pesticide Science*, *37*(2), 179–194. <http://doi.org/10.1002/ps.2780370212>
- Burrack, H. J., Fernandez, G. E., Spivey, T., & Kraus, D. A. (2013). Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an invasive frugivore. *Pest Management Science*, *69*(10), 1173–1180. <http://doi.org/10.1002/ps.3489>
- Burrows, H. D., Canle L, M., Santaballa, J. A., & Steenken, S. (2002). Reaction pathways and mechanisms of photodegradation of pesticides. *Journal of Photochemistry and*

- Photobiology B: Biology*, 67(2), 71–108. [http://doi.org/10.1016/S1011-1344\(02\)00277-4](http://doi.org/10.1016/S1011-1344(02)00277-4)
- Casida, J. E., & Quistad, G. B. (1998). Golden age of insecticide research: past, present, or future? *Annual Review of Entomology*, 43, 1–16. <http://doi.org/10.1146/annurev.ento.43.1.1>
- Chowdhury, A. B. M. N. U., Jepson, P. C., Howse, P. E., & Ford, M. G. (2001). Leaf surfaces and the bioavailability of pesticide residues. *Pest Management Science*, 57(5), 403–412. <http://doi.org/10.1002/ps.311>
- Chowdhury, A. B. M. N. U. M. N. U., Jepson, P. C., Ford, M. G., Geoff, K., & Frampton, G. K. (2005). The role of cuticular waxes and surface roughness in determining the insecticidal efficacy of deltamethrin and dimethoate applied as emulsifiable concentrates to leaf surfaces. *International Journal of Pest Management*, 51(4), 253–263. <http://doi.org/10.1080/09670870500404674>
- Cini, A., Ioriatti, C., & Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology*, 65(1), 149–160.
- Cloonan, K. R., Abraham, J., Angel, S., Syed, Z., & Rodrigues-Saona, C. (2018). Advances in the Chemical Ecology of the Spotted Wing *Drosophila* (*Drosophila suzukii*) and its Applications. *Journal of Chemical Ecology*.
- Cowles, R. S., Rodriguez-Saona, C., Holdcraft, R., Loeb, G. M., Elsensohn, J. E., & Hesler, S. P. (2015). Sucrose Improves Insecticide Activity Against *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, 108(2), 640–653. <http://doi.org/10.1093/jee/tou100>
- Daane, K. M., Vincent, C., Isaacs, R., & Ioriatti, C. (2016). Entomological Opportunities and Challenges for Sustainable Viticulture in a Global Market, (January). <http://doi.org/10.1146/annurev-ento-010715-023547>
- Diepenbrock, L. M., Hardin, J. A., & Burrack, H. J. (2017). Season-long programs for control of *Drosophila suzukii* in southeastern U.S. blueberries. *Crop Protection*, 98, 149–156. <http://doi.org/10.1016/j.cropro.2017.03.022>
- Fanning, P. D., Grieshop, M. J., & Isaacs, R. (2018). Efficacy of biopesticides on spotted wing drosophila, *Drosophila suzukii* Matsumura in fall red raspberries. *Journal of Applied Entomology*, 142(1–2), 26–32. <http://doi.org/10.1111/jen.12462>
- Fanning, P. D., VanWoerkom, A., Wise, J. C., & Isaacs, R. (2018). Assessment of a commercial spider venom peptide against spotted-wing *Drosophila* and interaction with adjuvants. *Journal of Pest Science*, 91(4), 1279–1290. <http://doi.org/10.1007/s10340-018-1016-7>
- Galvan, T. L., Koch, R. L., & Hutchison, W. D. (2005). Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera:

- Coccinellidae). *Biological Control*, 34(1), 108–114.
<http://doi.org/10.1016/j.biocontrol.2005.04.005>
- Gautam, B. K., Little, B. A., Taylor, M. D., Jacobs, J. L., Lovett, W. E., Holland, R. M., & Sial, A. A. (2016). Effect of simulated rainfall on the effectiveness of insecticides against spotted wing drosophila in blueberries. *Crop Protection*, 81, 122–128.
<http://doi.org/10.1016/j.cropro.2015.12.017>
- Goodhue, R. E., Bolda, M., Farnsworth, D., Williams, J. C., & Zalom, F. G. (2011). Spotted wing drosophila infestation of California strawberries and raspberries: Economic analysis of potential revenue losses and control costs. *Pest Management Science*, 67(11), 1396–1402.
<http://doi.org/10.1002/ps.2259>
- Gress, B. E., & Zalom, F. G. (2018). Identification and risk assessment of spinosad resistance in a California population of *Drosophila suzukii*. *Pest Management Science*.
<http://doi.org/10.1002/ps.5240>
- Gurr, G. M., Wratten, S. D., & Altieri, M. A. (2004). Ecological Engineering for Pest Management. *Plant Protection Quarterly*, 244. <http://doi.org/10.1111/j.1442-9993.2005.01456.x>
- Gutierrez, A. P., Ponti, L., & Dalton, D. T. (2016). Analysis of the invasiveness of spotted wing Drosophila (*Drosophila suzukii*) in North America, Europe, and the Mediterranean Basin. *Biological Invasions*, 18(12), 3647–3663. <http://doi.org/10.1007/s10530-016-1255-6>
- Hauser, M. (2011). A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Management Science*, 67(11), 1352–1357.
<http://doi.org/10.1002/ps.2265>
- Haviland, D. R., & Beers, E. H. (2012). Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. *Journal of Integrated Pest Management*, 3(2), 1–6. <http://doi.org/10.1603/IPM11034>
- Haye, T., Cuthbertson, P. G. A. G. S., & Daane, X. G. W. K. M. (2016). Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. *Journal of Pest Science*, 89(3), 643–651. <http://doi.org/10.1007/s10340-016-0737-8>
- Hoffmann, E. J., Vandervoort, C., & Wise, J. C. (2009). Curative Activity of Insecticides Against Plum Curculio (Coleoptera: Curculionidae) in Tart Cherries. *Journal of Economic Entomology*, 102(5), 1864–1873. <http://doi.org/10.1603/029.102.0517>
- Hoffmann, E. J., Vandervoort, C., & Wise, J. C. (2010). Plum Curculio (Coleoptera: Curculionidae) Adult Mortality and Associated Fruit Injury After Exposure to Field-Aged Insecticides on Tart Cherry Branches. *Journal of Economic Entomology*, 103(4), 1196–1205. <http://doi.org/10.1603/EC10017>

- Hulbert, D., Isaacs, R., Vandervoort, C., & Wise, J. C. (2011). Rainfastness and residual activity of insecticides to control Japanese beetle (Coleoptera: scarabaeidae) in Grapes. *J Econ Entomol*, *104*(5), 1656–1664. <http://doi.org/10.1603/EC11077>
- Hulbert, D., Reeb, P., Isaacs, R., Vandervoort, C., Erhardt, S., & Wise, J. C. (2012). Rainfastness of Insecticides Used to Control Japanese Beetle in Blueberries. *Journal of Economic Entomology*, *105*(5), 1688–1693. <http://doi.org/10.1603/EC11412>
- Katagi, T. (2004). Photodegradation of pesticides on plant and soil surfaces. *Reviews of Environmental Contamination and Toxicology*, *182*, 1–189. <http://doi.org/10.1007/978-0-387-71724-1>
- Keeseey, I. W., Knaden, M., & Hansson, B. S. (2015). Olfactory Specialization in *Drosophila suzukii* Supports an Ecological Shift in Host Preference from Rotten to Fresh Fruit. *Journal of Chemical Ecology*, *41*, 121–128. <http://doi.org/10.1007/s10886-015-0544-3>
- Kenis, M., Tonina, L., Eschen, R., van der Sluis, B., Sancassani, M., Mori, N., Haye, T., Helsen, H. (2016). Non-crop plants used as hosts by *Drosophila suzukii* in Europe. *Journal of Pest Science*, *89*(3), 735–748. <http://doi.org/10.1007/s10340-016-0755-6>
- Khan, Z. R., Midega, C. A. O., Wadhams, L. J., Pickett, J. A., & Mumuni, A. (2007). Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push-pull strategy. *Entomologia Experimentalis et Applicata*, *124*(2), 201–211. <http://doi.org/10.1111/j.1570-7458.2007.00569.x>
- Kim, S. S., Vandervoort, C., Whalon, M. E., & Wise, J. C. (2014). Transovarial transmission of novaluron in *Choristoneura rosaceana* (Lepidoptera : Tortricidae), *353*, 347–353. <http://doi.org/10.4039/tce.2013.78>
- Kim, S. S., Wise, J. C., Gökçe, A., & Whalon, M. E. (2011). Novaluron Causes Reduced Egg Hatch After Treating Adult Codling Moths, *Cydia pomonella* : Support for Transovarial Transfer. *Journal of Insect Science*, *11*(126), 1–10. <http://doi.org/10.1673/031.011.12601>
- Kirkpatrick, D. M., Gut, L. J., & Miller, J. R. (2018). Development of a Novel Dry, Sticky Trap Design Incorporating Visual Cues for *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, (June), 1–5. <http://doi.org/10.1093/jee/toy097>
- Kliot, A., & Ghanim, M. (2012). Fitness costs associated with insecticide resistance. *Pest Management Science*, *68*(11), 1431–1437. <http://doi.org/10.1002/ps.3395>
- Knoche, M., & Bukovac, M. J. (2000). Finite dose diffusion studies: I. Characterizing cuticular penetration in a model system using NAA and isolated tomato fruit cuticles. *Pest Management Science*, *56*(12), 1005–1015. [http://doi.org/10.1002/1526-4998\(200012\)56:12<1005::AID-PS188>3.0.CO;2-Y](http://doi.org/10.1002/1526-4998(200012)56:12<1005::AID-PS188>3.0.CO;2-Y)

- Knoche, M., & Bukovac, M. J. (2001). Finite dose diffusion studies : III . Effects of temperature , humidity and deposit manipulation on NAA penetration through isolated tomato fruit cuticles †, 742(November 2000), 737–742. <http://doi.org/10.1002/ps.351>
- Leach, H., Moses, J., Hanson, E., Fanning, P., & Isaacs, R. (2018). Rapid harvest schedules and fruit removal as non-chemical approaches for managing spotted wing *Drosophila*. *Journal of Pest Science*, 91(1), 219–226. <http://doi.org/10.1007/s10340-017-0873-9>
- Leach, H., Wise, J. C., & Isaacs, R. (2017). Reduced ultraviolet light transmission increases insecticide longevity in protected culture raspberry production. *Chemosphere*, 189, 454–465. <http://doi.org/10.1016/j.chemosphere.2017.09.086>
- Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. *Pest Management Science*, 67(11), 1358–1367. <http://doi.org/10.1002/ps.2225>
- Lee, J. C., Dalton, D. T., Swoboda-Bhattarai, K. A., Bruck, D. J., Burrack, H. J., Strik, B. C., Woltz, J. M., Walton, V. M. (2015). Characterization and manipulation of fruit susceptibility to *Drosophila suzukii*. *Journal of Pest Science*, 89(3), 771–780. <http://doi.org/10.1007/s10340-015-0692-9>
- Lee, J. C., Dreves, A. J., Cave, A. M., Kawai, S., Isaacs, R., Miller, J. C., Bruck, D. J. (2015). Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). *Annals of the Entomological Society of America*, 108(2), 117–129. <http://doi.org/10.1093/aesa/sau014>
- Melo, A. A., Usano-Aleman, J., Guedes, J. V. C., & Hunsche, M. (2015). Impact of tank-mix adjuvants on deposit formation, cuticular penetration and rain-induced removal of chlorantraniliprole. *Crop Protection*, 78, 253–262. <http://doi.org/10.1016/j.cropro.2015.09.021>
- Mota-Sanchez, D., Hollingworth, R. M., Grafius, E. J., & Moyer, D. D. (2006). Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Pest Management Science*, 62(1), 30–37. <http://doi.org/10.1002/ps.1120>
- Nansen, C., Baissac, O., Nansen, M., Powis, K., & Baker, G. (2016). Behavioral Avoidance - Will Physiological Insecticide Resistance Level of Insect Strains Affect Their Oviposition and Movement Responses ?, 1–12. <http://doi.org/10.1371/journal.pone.0149994>
- Oberemok, V. V., Laikova, K. V., Gninenko, Y. I., Zaitsev, A. S., Nyadar, P. M., & Adeyemi, T. A. (2015). A short history of insecticides. *Journal of Plant Protection Research*, 55(3), 221–226. <http://doi.org/10.1515/jppr-2015-0033>
- Pimentel, D., & Edwards, C. A. (1982). Pesticides and Ecosystems, (August), 595–600.

- Pimentel, D., McLaughlin, L., Zepp, A., Lakitan, B., Kraus, T., Kleinman, P., Fabius, V., Roach, W. J., Graap, E., Keeton, W. S., Selig, G. (1993). Environmental and economic effects of reducing pesticide use in agriculture. *Agriculture, Ecosystems & Environment*, 46(1–4), 273–288. [http://doi.org/10.1016/0167-8809\(93\)90030-S](http://doi.org/10.1016/0167-8809(93)90030-S)
- Romeis, J., Naranjo, S. E., Meissle, M., & Shelton, A. M. (2018). Genetically engineered crops help support conservation biological control. *Biological Control*, (July), 1–19. <http://doi.org/10.1016/j.biocontrol.2018.10.001>
- Schetelig, M. F., Lee, K.-Z., Otto, S., Talmann, L., Stökl, J., Degenkolb, T., Vilcinskas, A., Halitschke, R. (2017). Environmentally sustainable pest control options for *Drosophila suzukii*. *Journal of Applied Entomology*, (September), 3–17. <http://doi.org/10.1111/jen.12469>
- Shawer, R., Tonina, L., Tirello, P., Duso, C., & Mori, N. (2018). Laboratory and field trials to identify effective chemical control strategies for integrated management of *Drosophila suzukii* in European cherry orchards. *Crop Protection*, 103, 73–80. <http://doi.org/10.1016/j.cropro.2017.09.010>
- Shearer, P. W., West, J. D., Walton, V. M., Brown, P. H., Svetec, N., & Chiu, J. C. (2016). Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. *BMC Ecology*, 16(1), 1–18. <http://doi.org/10.1186/s12898-016-0070-3>
- Smirle, M. J., Zurowski, C. L., Ayyanath, M. M., Scott, I. M., & MacKenzie, K. E. (2017). Laboratory studies of insecticide efficacy and resistance in *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) populations from British Columbia, Canada. *Pest Management Science*, 73(1), 130–137. <http://doi.org/10.1002/ps.4310>
- Szczepaniec, A., Raupp, M. J., Parker, R. D., Kerns, D., & Eubanks, M. D. (2013). Neonicotinoid Insecticides Alter Induced Defenses and Increase Susceptibility to Spider Mites in Distantly Related Crop Plants. *PLoS ONE*, 8(5). <http://doi.org/10.1371/journal.pone.0062620>
- Taylor, N., & Matthews, G. A. (1986). Effect of different adjuvants on the rainfastness of bendiocarb applied to Brussels sprout plants. *Crop Protection*, 5(4), 250–253. [http://doi.org/10.1016/0261-2194\(86\)90058-X](http://doi.org/10.1016/0261-2194(86)90058-X)
- Thacker, J. R. M., & Young, R. D. (1999). The effects of six adjuvants on the rainfastness of chlorpyrifos formulated as an emulsifiable concentrate. *Pesticide Science*, 55(2), 197–218. [http://doi.org/10.1002/\(SICI\)1096-9063\(199902\)55:2<198::AID-PS867>3.0.CO;2-R](http://doi.org/10.1002/(SICI)1096-9063(199902)55:2<198::AID-PS867>3.0.CO;2-R)
- Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). Temperature-Related Development and Population Parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. *Environmental Entomology*, 43(2), 501–510. <http://doi.org/10.1603/EN13200>

- Tochen, S., Woltz, J. M., Dalton, D. T., Lee, J. C., Wiman, N. G., & Walton, V. M. (2016). Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. *Journal of Applied Entomology*, *140*(1–2), 47–57. <http://doi.org/10.1111/jen.12247>
- Vallat, A., Gu, H., & Dorn, S. (2005). How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. *Phytochemistry*, *66*(13), 1540–1550. <http://doi.org/10.1016/j.phytochem.2005.04.038>
- Van Timmeren, S., Horejsi, L., Larson, S., Spink, K., Fanning, P., & Isaacs, R. (2017). Diurnal activity of *Drosophila suzukii* (diptera: Drosophilidae) in Highbush Blueberry and Behavioral response to irrigation and application of insecticides. *Environmental Entomology*, *46*(5), 1106–1114. <http://doi.org/10.1093/ee/nvx131>
- Van Timmeren, S., & Isaacs, R. (2013). Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. *Crop Protection*, *54*, 126–133. <http://doi.org/10.1016/j.cropro.2013.08.003>
- Van Timmeren, S., & Isaacs, R. (2014). *Drosophila suzukii* in Michigan vineyards, and the first report of *Zaprionus indianus* from this region. *Journal of Applied Entomology*, *138*(7), 519–527. <http://doi.org/10.1111/jen.12113>
- Van Timmeren, S., Mota-Sanchez, D., Wise, J. C., & Isaacs, R. (2018). Baseline susceptibility of spotted wing drosophila (*Drosophila suzukii*) to four key insecticide classes. *Pest Management Science*, *74*(1), 78–87. <http://doi.org/10.1002/ps.4702>
- Van Timmeren, S., Wise, J. C., & Isaacs, R. (2012). Soil application of neonicotinoid insecticides for control of insect pests in wine grape vineyards. *Pest Management Science*, *68*(4), 537–542. <http://doi.org/10.1002/ps.2285>
- Wang, D., Gong, P., Li, M., Qiu, X., & Wang, K. (2009). Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pest Management Science*, *65*(2), 223–227. <http://doi.org/10.1002/ps.1672>
- Wang, K. Y., Kong, X. B., Jiang, X. Y., Yi, M. Q., & Liu, T. X. (2003). Susceptibility of immature and adult stages of *Trialeurodes vaporariorum* (Hom., Aleyrodidae) to selected insecticides. *Journal of Applied Entomology*, *127*(9–10), 527–533. <http://doi.org/10.1111/j.1439-0418.2003.00778.x>
- Whitener, A. B., Smytheman, P., & Beers, E. H. (2018). Toxicity of Insect Growth Regulators to Spotted-Wing Drosophila and Their Offspring by Three Routes of Exposure , 2017. *Arthropod Management Tests*, *43*(August), 1. <http://doi.org/10.1093/amt/tsy017>
- Willis, G. H., Smith, S., McDowell, L. L., & Southwick, L. M. (1996). Carbaryl washoff from soybean plants. *Archives of Environmental Contamination and Toxicology*, *31*, 239–243
ST–Carbaryl washoff from soybean plants.

- Wilson, J., Gut, L., Rothwell, N., & Haas, M. (2015). Managing Spotted Wing *Drosophila* in Michigan Cherry, 1–8.
- Wiman, N. G., Dalton, D. T., Anfora, G., Biondi, A., Chiu, J. C., Daane, K. M., Gerdeman, B., Gottardelo, A., Hamby, K. A., Isaacs, R., Grassi, A., Ioriatti, C., Lee, J. C., Betsey, M., Stacconi, M. V. R., Shearer, P. W., Tanigoshi, L., Wang, X., Walton, V. M. (2016). *Drosophila suzukii* population response to environment and management strategies. *Journal of Pest Science*, 89(3), 653–665. <http://doi.org/10.1007/s10340-016-0757-4>
- Wise, J. C., Coombs, A. B., Vandervoort, C., Gut, L. J., Hoffmann, E. J., & Whalon, M. E. (2006). Use of Residue Profile Analysis to Identify Modes of Insecticide Activity Contributing to Control of Plum Curculio in Apples. *Journal Economic Entomology*, 99(6), 2055–2064. <http://doi.org/10.1603/0022-0493-99.6.2055>
- Wise, J. C., Hulbert, D., & Vandervoort, C. (2016). Rainfall influences performance of insecticides on the codling moth (Lepidoptera: Tortricidae) in apples. *The Canadian Entomologist*, 11, 1–11. <http://doi.org/10.4039/tce.2016.40>
- Wise, J. C., Vanderpoppen, R., & Vandervoort, C. (2009). Curative activity of insecticides on *Rhagoletis pomonella* (Diptera: Tephritidae) in apples. *Journal of Economic Entomology*, 102(1998), 1884–1890. <http://doi.org/10.1603/029.102.0519>
- Wise, J. C., Vanderpoppen, R., Vandervoort, C., O'Donnell, C., & Isaacs, R. (2015). Curative activity contributes to control of spotted-wing drosophila (Diptera: Drosophilidae) and blueberry maggot (Diptera: Tephritidae) in highbush blueberry. *Canadian Entomologist*, 147(1), 109–117. <http://doi.org/10.4039/tce.2014.36>
- Wise, J. C., VanWoerkom, A. H., Wheeler, C. E., & Isaacs, R. (2018). Control of Spotted Wing *Drosophila* in Blueberry, 2017. *Arthropod Management Tests*, 43(1), 1–2. <http://doi.org/10.1093/amt/tsy066>
- Wise, J. C., VanWoerkom, A., & Isaacs, R. (2017). Control of Spotted Wing *Drosophila* in Blueberries, 2016. *Arthropod Management Tests*, 42(1), 4–5. <http://doi.org/10.1093/amt/tsx064>
- Wise, J., Kim, K., Hoffman, E. J., Vandervoort, C., Gokce, A., & Whalon, M. E. (2007). Novel life stage targets against plum curculio, *Conotrachelus nenuphar* (Herbst), in apple integrated pest management. *Pest Management Science*, 63(December 2006), 737–742. <http://doi.org/10.1002/ps>
- Wise, J., & Whalon, M. (2009). A Systems Approach to IPM Integration, Ecological Assessment and Resistance Management in Tree Fruit Orchards. In A. R. H. I. Ishaaya (Ed.), *Biorational Control of Arthropod Pests* (pp. 325–346). Springer. <http://doi.org/10.1007/978-90-481-2316-2>

- Wong, J. S., Wallingford, A. K., Loeb, G. M., & Lee, J. C. (2018). Physiological status of *Drosophila suzukii* (Diptera: Drosophilidae) affects their response to attractive odours. *Journal of Applied Entomology*, (February). <http://doi.org/10.1111/jen.12497>
- Wu, J. C., Qiu, H. M., Yang, G. Q., Liu, J. L., Liu, G. J., & Wilkins, R. M. (2004). Effective duration of pesticide-induced susceptibility of rice to brown planthopper (*Nilaparvata lugens* Stål, Homoptera: Delphacidae), and physiological and biochemical changes in rice plants following pesticide application. *International Journal of Pest Management*, 50(1), 55–62. <http://doi.org/10.1080/09670870310001630397>
- Youn, Y. N., Seo, M. J., Shin, J. G., Jang, C., & Yu, Y. M. (2003). Toxicity of greenhouse pesticides to multicolored Asian lady beetles, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control*, 28(2), 164–170. [http://doi.org/10.1016/S1049-9644\(03\)00098-7](http://doi.org/10.1016/S1049-9644(03)00098-7)
- Zacharia, & Tano, J. (2011). Identity, Physical and Chemical Properties of Pesticides. In *Pesticides in the Modern World - Trends in Pesticides Analysis* (Vol. 1873). <http://doi.org/10.5772/17513>