Evaluating Host Plant use by Spotted Wing Drosophila, *Drosophila suzukii*, in Minnesota.

A Thesis SUBMITTED TO THE FACULTY OF UNIVERSITY OF MINNESOTA BY GRACE SWARD

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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May 2017

Acknowledgements

I am grateful to Dr. Christopher Philips, Dr. William Hutchison, Dr. Robert Koch, and Dr. Mary Rogers for their guidance, time, and support with this study. I am also grateful to the cooperating farmers and the Three Rivers Parks District for allowing sampling to be done on their sites. A big thanks to Vincent Belsito and Liz Sampson for their hard work and assistance in this project. I would also like to extend my gratitude to the UMN entomology graduate students and the staff at North Central Research and Outreach center. Finally, I am grateful to my funding sources; the MAES rapid agriculture response fund and North Central SARE graduate student grant.

Dedication

I would like to humbly dedicate this thesis to Ms. Karen Marshall and my mother Kristie Sward. First, Ms. Marshall, your belief in me and ability to help me find the right tools to succeed has given me the confidence to face the odds and persevere. To my mom, I would have never followed this path if you didn't tell me that a "bug studier" is a real career, encouraged me my entire life, and put up with bugs in your freezer far longer than most mothers would. The two of you have had an enormous impact on my success. For that, I can never thank you enough.

Abstract

Spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is newly established in North America and become one of the most devastating pests for small fruit crops. With a broad host range, thin-skinned berries are particularly susceptible to infestation, and fruit producers are desperate for methods to control this pest. One of the keys to developing an integrated management program for SWD is to understand host preference, varietal susceptibility of crop hosts, and overall susceptibility and use of non-crop hosts. In this thesis, the infestations levels and host plant growth stages were recorded over time during growing seasons of one day in 2014, and various sampling dates in 2015 and 2016 in Minnesota. Both crop and non-crop host plants were examined for their phenological and overall susceptibility.

The first research chapter was performed working in replicated small plots, experiments were conducted to evaluate differences in varietal susceptibility on cold hardy red primocane fruiting raspberries. Significant differences were observed in both the level of infestation and in the average number of larvae per berry by variety. In addition, it appears that there is a significant negative correlation between the level of infestation and the number of larvae per fruit with the time of sampling, but only an interaction of variety and time for the number of larvae per berry.

The second research chapter was performed using nine field sites of either farm or non-farm wild locations separated by a minimum of 400 meters. Crop hosts: raspberry, blueberry, and elderberry, as well as non-crop host plants were examined and sampled for fruit ripening stages an infestation rates. Significant differences were observed in

infestations within non-crop host plants and crop host plants. Crop hosts sustained infestations starting in the green phenological stage. These data provide a better understanding of the biology and ecology of this pest which is critical in refining current management practices. Knowledge of these interactions can aid in optimizing control strategies such as fine-tuning spatial and temporal control measures, which may be particularly important for early season infestations.

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Chapter 1

Introduction and Literature Review

Drosophila suzukii - Arrival of a new invader

Currently there are over 500 introduced insect pests of crops in the U.S. that cost growers approximately \$13 billion in crop losses and another \$500 million in control costs (Pimentel et al. 2005). Among the recent invasive species in Minnesota, the spotted wing drosophila, *Drosophila suzukii* Matusumura, has emerged as the most devastating pest of soft fruits including blackberry, raspberry, blueberry, cherry, peach, grape, and strawberry (Walsh et al. 2011). *Drosophila suzukii* females oviposit primarily in ripening fruits, presenting a major threat to U.S. fruit industries (Asplen et al. 2015).

Drosophila. suzukii was first discovered in the United States in Hawaii in the 1980's, but was not reported to cause any damage which is attributed to extremely low fruit and berry agricultural production in Hawaii (Kaneshiro, 1983). Extensive damage was noted when this insect was found in 2008 in California, the first continental U.S. state to report infestations (Bolda et al. 2009; Walsh et al. 2011). Since its first detection in California, *D. suzukii* has spread throughout the U.S., arriving in Minnesota in 2012 (Burrack et al. 2012). Feeding damage from this pest causes significant yield losses (often reaching 100%), at an estimated cost of approximately \$1 billion annually from 20% feeding damage and increasing pesticide use by growers (Bolda et al. 2009; Walsh et al. 2011; Burrack et al. 2012; Asplen et al. 2015).

The invaded range of *D. suzukii* includes more than North America. In 2008, the same year it was reported in California, infestations resulting in yield loss were also reported from Spain and Italy (Lee et al. 2011; Abraham et al. 2015; Chireceanu et al. 2015; Kiss et al. 2016). Infestations of *D. suzukii* have been reported in more than 20 countries in North America, Central America, Europe, and Asia (Lee et al. 2011; Piotrowski et al. 2013; Depra et al. 2014; Chireceanu et al. 2015; Asplen et al. 2015; Kiss et al. 2016). In these countries, *D. suzukii* has infested numerous crop hosts, such as cherry, blueberry, and raspberry, and non-crop host plants, including *Prunus mahaleb L.*, *Sambucus nigra L.*, and *Rubus ulmifolius* Schott (Asplen et al. 2015; Arnó et al. 2016). This broad host range has undoubtedly facilitated its global spread (Yu et al. 2013; Harris et al. 2014; Haviland et al. 2014; Hardin et al. 2015; Lee et al. 2015; Arnó et al. 2016; Kenis et al. 2016) Reports on the impact of *D. suzukii* from different countries range from a nuisance pest to causing major yield and economic losses (Asplen et al. 2015).

In Minnesota, the presence of *D. suzukii* can be viewed as a "game changer" to small fruit production, as these crops historically required very little insecticide use, but now incur the highest infestation rates of small and thin-skinned fruit crops (Lee et al. 2011; Burrack et al. 2013b). In addition, the sudden arrival of a new invasive species in a growing system where chemical management techniques are uncommon has led many regional growers to discontinue the production of raspberry and blueberry crops (Minnesota Fruit and Vegetable Growers Association Conference, personal correspondence 2015). A successful Integrated Pest Management (IPM) program is dependent on understanding where and when pests occur before acting. However, a major

challenge with developing an IPM program for *D. suzukii* is our lack of fundamental knowledge about its biology and ecology. A better understanding of the biology and ecology of invasive species is critical in refining current management practices for these pests.

D. suzukii life cycle and development

pests. There are approximately 1,600 described species of *Drosophila* (Brake & Bachili, 2008). The majority of *Drosophila* flies are associated with rotten or over-ripened fruits; however, *D. suzukii* shows a preference for oviposition in ripening or ripe fruit. Unlike most *Drosophila* species, female *D. suzukii* can access a previously unutilized food niche using a large, highly sclerotized ovipositor to break the skin of ripening thin skinned fruits and berries (Lee et al. 2011). Eggs are deposited under the oviposition scar, with larvae progressing through three instars feeding on internal fruit tissues. Pupation typically occurs partially or fully outside of infested fruit. When *D. suzukii* is exposed to cold weather as larvae, their exoskeleton becomes highly sclerotized, darker, and the adults from these larvae go into reproductive arrest in preparation for winter diapause (Hamby et al. 2016; Toxopeus et al. 2016; Jakobs et al. 2017). It's still not understood where or how *D. suzukii* overwinters, merely that there is potential for overwintering.

The rate of development is temperature dependent, with total development time ranging from 10 to 79 days (Kanzawa, 1939; Lee et al. 2011; Tochen et al. 2014).

Depending on the weather conditions, oviposition rates can exceed 25 eggs per day and

there can be as many as 13 generations per year (Kanzawa 1939; Kinjo et al. 2014; Tochen et al. 2014). The short generation time coupled with high reproductive rates causes rapid population growth and increasing pest pressure over time (Wiman et al. 2014). The highest level of adult activity occurs between 16.7 and 22.2°C for winter and summer morph flies respectively, and activity decreases at temperatures above and below this range (Hamby et al. 2013).

Degree days and development

Due to the impact of temperature on *D. suzukii*, a predictive degree day models have been developed (Wiman et al. 2014). The model designed by Wiman et al. (2014) is based off of developmental data as well as average trap capture data. This degree day model can be used to predict the initial arrival of *D. suzukii* with relative accuracy to the early fly captures in monitoring traps (Wiman et al. 2014). The authors indicate the later season inaccuracy of their model is likely due to the high fecundity and large generational overlap that occurs in the field (Wiman et al. 2014). Host choice and competition inside of berries may also be contributing to the unpredictability of *D. suzukii* population numbers later in the season (Hardin et al. 2015; Bahder et al. 2016). For example, it has been shown that larvae develop at a significantly different timeframe depending on berry type as well as temperature (e.g. blueberry vs. mulberry) (Yu et al. 2013; Wiman et al. 2014; Harris et al. 2014). Moreover, larval competition along with nutritional value of diet larvae feed on significantly impacted larval survivorship and the length of time in development (Stafford et al. 2012; Hardin et al. 2015).

Overwintering and migration in northern climates

In some areas of the United States D. suzukii can be found throughout the year; however, in other areas, such as Minnesota, it is still not clear if this pest reestablishes every year, or if they are overwintering (Kimura 2004; Mitsui et al. 2010; Harris et al. 2014, Stephens et al. 2015, Pelton et al. 2016; Toxopeus et al. 2016). Migratory behaviors are well documented among insects, though large-scale migration has not been reported for D. suzukii (Dingle 1972; Mitsui et al. 2010). Overwintering, however, has been recorded, and D. suzukii has the capability of producing what has been termed a "wintermorph" (Stephens et al. 2015; Hamby et al. 2016; Toxopeus et al. 2016). This wintermorph has a larger, darker, and highly sclerotized exoskeleton compared to its summer counterpart (Hamby et al. 2016). Holding D. suzukii at 10°C is along with a lower photoperiod is enough to produce winter-morph flies (Tochen et al. 2014). Winter-morph flies have been shown to have significantly improved cold survivorship compared to the summer morphs (Toxopeus et al. 2016). However, it is unlikely that they can survive the winters in northern regions (e.g. Minnesota and Canada) due to winter temperatures often falling below 10°C before ample subnivium layer of snow has accumulated. In these northern regions, D. suzukii are likely immigrating, which is an area for further exploration.

Phenology and host susceptibility

The host range of *D. suzukii* is broad, with thin-skinned berries (e.g., caneberries, blueberries, strawberries) and stone fruits (e.g., cherries, peaches, apricots, plums) being

particularly susceptible to infestation (Bellamy et al. 2013). In the US, raspberries appear to be particularly preferred hosts (Bellamy et al. 2013; Burrack et al. 2013a). In addition to cultivated fruits, many wild plants can serve as potentially important hosts (Mitsui et al. 2010; Cini et al. 2012; Poyet et al. 2014; Lee et al. 2015). It is not known to what extent these non-crop host plants are utilized, and the role they play in facilitating infestations of crops later in the growing season.

It is imperative to know when and where a pest occurs to develop an IPM strategy. Most phenology data for D. suzukii is based on baited trap counts of D. suzukii spread out across the United States, mostly in the southern and western regions. Per these studies, D. suzukii will typically have one to two population peaks in winter and spring during the fruiting period of various crops (Haviland et al. 2014; Bahder et al. 2015; Bahder et al. 2016; Thomas et al. 2016). In Minnesota, D. suzukii is characterized by significant population growth from mid-July through late September (Sward et al. 2016). Degree days coupled with IPM practices are hypothesized to be a factor in these population fluctuations in different states and time periods in the season (Wiman et al. 2016). Evidence shows, however, that flies caught in or attracted to baited traps are physiologically different than flies vacuum sampled in fields, indicating the accuracy of current population estimates may be inaccurate or untimely (Diepenbrock and Burrack 2016). Female flies caught in traps carried fewer eggs than females caught in the field by sweep net sampling (Diepenbrock and Burrack 2016). Confirmation of D. suzukii after an individual is caught in a trap can take up to a week to collect, identify, and confirm presence before the information is disseminated to the growers. Greater understanding of

timely and accurate population levels is desperately needed by growers to implement their control strategies.

One of the keys to the development of integrated pest management management programs for *D. suzukii* is to improve our understanding of preference, varietal susceptibility, and phenology of preferred alternative and crop hosts. Crop hosts of greatest concern include: raspberry, blueberry, blackberry, cherry, peach, strawberry, and grape (Bellamy et al. 2013). Infestations are not consistent in these crops. Intensity of infestation fluctuates regionally and by country (Goodhue et al. 2011; Burrack et al. 2013a; Tochen et al. 2014; Lee et al. 2015;). Reports from countries around the world specify raspberry, blueberry, and strawberry as the most dramatically impacted crops (Asplen et al. 2015). If specific mechanisms of host use and varietal susceptibility as well as plant-insect phenology can be determined, we can provide berry producers with methods to continue sustainable production and maintain profitability. For example, growers may be able to select specific varieties that provide a phenological mismatch to population presence thus providing an environmentally sound approach, providing growers with a significant and sustainable improvement over the frequent use of insecticide applications currently recommended.

Host susceptibility and selection

Berries become susceptible beginning at the blush fruit stage for many berry types. Multiple studies have found a positive correlation with % Brix and pH while simultaneously a negative correlation to fruit skin thickness (Burrack et al. 2013a;

Hamby et al. 2016; . Lee et al. 2016; Lee et al. 2011; Little et al. 2016). Berries like cranberries have skin that is too thick for the *D. suzukii* ovipositor to penetrate, however *D. suzukii* will readily infest and develop in damaged cranberry fruit and may be able to in other fruit similar to cranberry (Steffan et al. 2013). Raspberries, blackberries, and honeysuckle on the other hand have extremely thin skinned berries allowing easy access; raspberries and honeysuckle consequently incur high rates of infestation (Lee et al. 2015; . Lee et al. 2016; Little et al. 2016).

Identifying oviposition hosts by *D. suzukii* involves attraction or repulsion from various volatiles given off by the plants or berries. In addition to characteristics of the host fruit itself, bacteria and other microorganisms on the berries may also have an attractive or repulsive effect (Hamby & Becher, 2016; Mazzetto et al. 2016). Host selection for *D. suzukii* may include olfactory cues, color, brix, or pH (Lee et al. 2011; Keesey et al. 2015; Lee et al. 2013, 2016; Little et al. 2016; Yu et al. 2013). *Drosophila suzukii* has been found to be more sensitive than other drosophilids at detecting volatiles given off by the plant during the blush and ripe stages (Abraham et al. 2015; Arnó et al. 2016; Keesey et al. 2015). As the fruit ripens, the Brix level and pH both increase while the skin thickness decreases.

Insects may select hosts through visual or olfactory cues. The way in which D. suzukii selects its hosts is notably different than other Drosophilids. D. suzukii had a significantly greater response to the chemical β -cyclocitral, a leaf compound found earlier in the season, compared to its closest relatives: D. melanogaster and D. biarmipes (Keesey et al. 2015). Drosophila suzukii and D. biarmipes, are both significantly more

sensitive to volatile chemicals given off by plants than *D. melanogaster* (Keesey et al. 2015). These differences in response to early-season cues indicate that *D. suzukii* has evolved to seek out fresh fruit more than overripe or decaying fruit. In addition, while many drosophilids are known to tolerate a relatively high naturally occurring alcohol percentage which is not the case for *D. suzukii*, further demonstrating a niche preference of *D. suzukii* for ripening fruit (Sampson et al. 2015). From its differences in attraction to earlier plant volatiles and its sensitivity to alcohols, it's likely that *D. suzukii* and *D. melanogaster* have evolved to inhabit different niches in the same system (Kleiber et al., 2014). *Drosophila. suzukii* may create more available niche space for *D. melanogaster* through damaging the fruit, though no instances have been recorded.

Currently, there is little information on the susceptibility of cold-hardy berry varieties or the potential to use plant phenology as a cultural control for *D. suzukii* infestations. In addition, the limited information available on *D. suzukii* management is based on studies from other regions with drastically different climates than central and northern Minnesota. If specific mechanisms on host use and varietal susceptibility as well as plant-insect phenology can be identified, documented, and disseminated, we can provide berry producers methods to continue sustainable production and maintain profitability.

Alternative Hosts

The ability of an invasive insect to establish in a novel environment and become a major pest depends, in part, on its dispersal ability and its ability to utilize available host

plants. Knowledge of these interactions can aid in optimizing control strategies such as fine-tuning spatial and temporal control measures. Spatial optimization of control may be particularly important when early season infestations are likely to occur due to the emigration of individuals from other areas. Dispersal and movement from crop and non-crop hosts remains one of the most challenging variables to characterize, particularly for insects of small size in natural settings. Such hosts could serve as sources for *D. suzukii* populations, especially during the early season, when few cultivated hosts are available (Steffan et al. 2013). Identifying and quantifying the effect of non-crop hosts on *D. suzukii* infestation can be used to predict local-scale risk of infestation. Alternate hosts of *D. suzukii* may play an important role as host reservoirs that result in earlier and more severe infestations as well as serve as a refuge for *D. suzukii* from insecticide applications. Therefore, we must determine to what extent non-crop plants are utilized as hosts, when and to what extent these hosts are exploited, and how these hosts influence *D. suzukii*.

Movement of a pest in the environment plays a critical role in the ability of populations to reach densities large enough to cause problems in crop hosts. *Drosophila suzukii*'s ability and propensity to infest a wide range of hosts makes management increasingly difficult (Lee et al. 2015). Field margins as well as adjacent woodland retained higher numbers of *D. suzukii* trap captures early in the season which decline once the crop hosts ripen (Haviland et al. 2014; Klick et al. 2015). Initial exploration identified multiple potential ornamental fruiting hosts found in the surrounding environment. Alternative fruiting hosts recorded to have *D. suzukii* infestations include

pokeweed, buckthorn, elderberry, honeysuckle, dogwood, and mulberry (Lee et al. 2015). Alternative hosts can be used as a source-sink for future infestations into crop hosts later in the summer or to avoid pesticide applications (Steffan et al. 2013). Unlike most drosophilids, *D. suzukii* does not have strong host fidelity (Yu et al. 2013; Diepenbrock et al. 2016). Therefore, since adult *D. suzukii* tend to be captured at consistently higher numbers in woodland and field margins early in the season, it is likely that alternative hosts are playing a key role in in early season population increases.

In addition, because the use of non-crop host plants could serve as sources for *D. suzukii* populations, we must also identify and quantify the extent to which non-crop hosts influence *D. suzukii* infestation in host crops. Acquiring this information is of critical importance for non-crop hosts which fruit during the early season, when few cultivated hosts are available. For *D. suzukii* to thrive in the environment like it does, it must be able to survive at times when very few host options are available. Plants such as honeysuckle, mulberry, and more than likely a variety of others should be monitored for early season infestations by *D. suzukii*. In warmer climates, *D. suzukii* may well be able to overwinter through using later season host plants. Teasing out which alternative hosts are being utilized will go a long way in predicting and ultimately controlling *D. suzukii*.

Finally, the ability of *D. suzukii* to utilize any host will depend on the overlap in phenology. For example, if *D. suzukii* populations can overwinter in Minnesota there needs to be both early and late season hosts available for populations to persist and grow (Hamby et al. 2016; Toxopeus et al. 2016). A lapse in between host availability of as little as two weeks can cause an end to their population growth. Likewise, if *D. suzukii* is

migrating into Minnesota there needs to be an overlap in phenology of host plants to allow it to move north from warmer climates (Dingle, 1972; Mitsui et al. 2010). Although *D. suzukii* is more likely to succeed through migration phenology, it is unclear to what extent and where hosts are utilized in facilitating infestation spread.

Current Management

Insecticides

Since an effective IPM strategy has not yet been established for *D. suzukii*, most of the control recommendations to date are limited to insecticide applications (Van Timmeren & Isaacs, 2013). Currently management of *D. suzukii* in Minnesota small fruit management consists of insecticide applications on a 4-5-day schedule once *D. suzukii* is caught in a monitoring trap and susceptible fruit are present in the field (Lee et al. 2011; Van Timmeren & Isaacs, 2013).

As is common with invasive insects, most research on *D. suzukii* to date has focused on the identification and evaluation of effective insecticides. Most available chemistries are broad-spectrum, conventional insecticides including synthetic pyrethroids, carbamates, and organophosphates, making them incompatible with organic production. Organic producers are much more limited in their chemical control options and must therefore rely more on alternative control approaches. Our lack in fundamental understanding of natural biological controls, proper cultural management practices, and the limited availability understanding of effective chemical management make

available for organic farmers: most often used are a pyrethrin and spinosad. Of which the spinosad insecticide has the most consistent control in organic systems.

Whether conventional or organic, one of the most efficacious chemical class for control of *D. suzukii* is spinosyn. Because of its efficacy, this active ingredient is a staple in most growers' management programs. Applications are typically made on a calendar-based schedule. Organic insecticides are applied on a 3 to 5 day schedule while conventional insecticides are applied on a 5 to 7 day schedule. Such rapid calendar-based spray programs and the wide-spread use of a single active ingredient provides tremendous selection pressure for the development of insecticide resistance and a breakdown in *D. suzukii* management for growers.

Such intense chemical application method severely impacts the surrounding environment with high economic cost to growers (Bolda 2009). Resistance development is an issue when pesticide applications are so widely used. It is likely that resistance has the potential to become a wide spread problem because of a few factors. These factors include: the short life cycle of *D. suzukii*, high fecundity, and wide insecticide use with few chemical classes available; however, the proclivity for Drosophilid flies to develop insecticide avoidance and non-crop host availability may mitigate the speed at which resistance is developed (Smirle et al. 2016)

Cultural controls

Management strategies other than insecticide applications include exclusion, sanitation, pruning, solarization, burying, and mass trapping of *D. suzukii* (Cormier et al. 2015; Mazzetto et al., 2016; Tochen et al., 2016). Mass trapping, is not likely to be a viable long-term management option for *D. suzukii* given the rapid population growth of this pest (Mazzetto et al. 2016). Use of traps is effectively used as a scouting tool and early warning system option (Heath et al. 1995; Lee et al. 2012; Harris et al. 2014). Both sanitation and plant pruning have been shown to be effective management strategies (Goodhue et al. 2011; Tochen et al. 2016; Jakobs et al. 2017). Sanitation involves removal of all susceptible fruit as often as possible, as well as removal of leaf litter below the plants that may be used to escape chemical applications or aid in overwintering (Cormier et al. 2015; Mazzetto et al., 2016; Tochen et al., 2016). Drosophila suzukii is frequently found amongst shade and humidity of host canopies, to produce an inhospitable environment for D. suzukii pruning plants allows light and air-flow to produce an environment inhospitable to *D. suzukii* (Diepenbrock & Burrack 2016; Tochen et al. 2016). Solarization, burying, and freezing harvested fruit kills larvae and pupae in the fruit (Goodhue et al. 2011; Lee et al. 2011). Though 100% control is not likely to be achieved, cultural control options can reduce infestations within crop hosts outside of chemical intervention.

Summary

In developing an IPM program it is imperative to understand where and when the pest is active. Thus far, researchers have uncovered the early stages to answering these questions. The body of knowledge is increasing about how color, volatiles, potential deterrents, and host potentials influence *D. suzukii* (Kleiber et al. 2014; Lee et al. 2013; Abraham et al. 2015; Renkema et al. 2016). For a pest, such as *D. suzukii*, with a short lifespan and a reported plethora of host plants, timing is important.

Currently, there is little information on the susceptibility of cold-hardy berry varieties or the potential to use plant phenology as a cultural control tactic for *D. suzukii* management. In addition, the limited information that is available on *D. suzukii* management is based on studies from other regions with different climates than that of Minnesota. If specific mechanisms on host use and varietal susceptibility as well as plant-insect phenology can be identified, documented, and disseminated, we can provide berry producers methods to continue sustainable production and maintain profitability.

For this reason, this research focused on:

- 1. Evaluating the phenology of *D. suzukii* infestations and the impact of different varieties of cold-hardy red raspberries on *D. suzukii*.
- 2. Identifying and evaluating non-crop host plant use by *D. suzukii* and correlate seasonal phenology of both crop and non-crop hosts with *D. suzukii* infestations.

Until we answer these questions, insecticide applications will continue to be the predominate strategy used by growers to control these pests. Initiation of control strategies and understanding where *D. suzukii* is in the environment is crucial knowledge for applications or other management methods to be effectively implemented. The current insecticide application schedule will undoubtedly lead to unintended environmental and ecological consequences and result in insecticide resistance or non-target impacts.

Chapter 2

The phenology of infestations and the impacts of different varieties of cold hardy red raspberries on *Drosophila suzukii*.

Introduction

Spotted wing drosophila, *Drosophila suzukii* (Matsumura), is a significant worldwide pest of berries and stone fruits causing economic losses across the globe (Lee et al. 2011; Calabria et al. 2012; Deprá et al. 2014; Kinjo et al. 2014; Asplen et al 2015). Unlike most *Drosophila* species, *D. suzukii* females oviposit primarily in ripening fruits, presenting a major threat to U.S. fruit industries. Since its first detection in California during the 2008 growing season, *D. suzukii* has spread throughout the U.S. causing significant yield losses, often reaching 100%. Current *D. suzukii* management consists of insecticide applications on a four to five-day schedule. Increased chemical inputs add substantial new costs to growing operations and increased risks to surrounding ecosystems, leading to numerous growers abandoning these crops. These dramatic increases in pesticide use with combined yield losses are leading to estimated cost approaching \$1 billion annually (Bolda et al. 2010; Walsh et al. 2010; Lee et al. 2011; Burrack et al. 2012; Burrack et al. 2013).

Drosophila suzukii possesses a broad host range, with thin-skinned berries (e.g., caneberries, blueberries, strawberries) and stone fruits (e.g., cherries, peaches, plums) are particularly susceptible to infestation (Bellamy et al. 2013). In Japan, where *D. suzukii* biology has been studied since the 1930s, Kanzawa (1939) reported *D. suzukii* -related

damage on various fruit crops with subsequent authors reporting its occurrence on various wild fruits as well (Kimura et al. 1977; Nishiharu 1980; Mitsui et al. 2010). In the US, raspberries appear to be particularly preferred hosts for *D. suzukii*), while some other small fruits are only suitable when damaged (Burrack et al. 2013b; Steffan et al. 2013).

Successful integrated pest management (IPM) is dependent on understanding where and when pests occur before taking action. However, a major challenge with the rapid arrival of invasive species such as D. suzukii is our lack of fundamental knowledge about their biology and ecology. Without a firm understanding of some of these basic questions including potential geographic range and invasiveness management attempts are likely to fail (Gutierrez & Ponti 2013). Recurring questions from fruit growers include how, where, and when cultural or chemical control methods should be applied. A better understanding of the biology and ecology of this species is critical in refining current management practices for this pest. Spatial optimization of control may be particularly important when early season infestations are likely to occur due to the emigration of overwintering individuals from other areas (Kimura 2014; Mitsui et al. 2010; Shearer et al. 2016). Dispersal and movement from crop and non-crop hosts remains one of the most challenging variables to characterize, particularly for insects of small size in natural settings (Burrack et al. 2013; Gutierrez & Ponti 2013; Haviland et al. 2014; Diepenbrock et al 2016; Klick et al 2016). Nevertheless, it is also one of the more important variables in designing monitoring and control programs for invasive pests.

One of the keys to development of an IPM program for *D. suzukii* remains understanding host preference and varietal susceptibility of preferred host crops. Currently, there is little information on the susceptibility of cold hardy berry varieties or the potential to use plant phenology as a cultural control for *D. suzukii* infestations. In addition, the limited information that is available on D. suzukii management is based on studies from other regions with drastically different climates (Mitsui et al. 2010; Haviland et al. 2014). If specific mechanisms on host use and varietal susceptibility as well as plant-insect phenology can be identified, documented, and disseminated, we can provide berry producers methods to continue sustainable production and maintain profitability. Selecting varieties that provide a phenological mismatch with key pests is an environmentally-sound approach, providing growers with a significant and sustainable improvement over the frequent use of insecticide applications currently recommended. Therefore, we evaluated susceptibility of six varieties of cold hardy raspberries to D. suzukii over two years. Given its high potential for rapid spread and economic impact, it is imperative that we improve our understanding of the underlying processes that shape D. suzukii distributions.

Materials and Methods

Infestation Rates

The potential differences in *D. suzukii* infestation rates between species and varieties within species were measured from 2014 to 2015 at the North Central Research and Outreach Center in Grand Rapids, MN. A total of six varieties (Table 1) grown in a

replicated trial established in 2010 were observed on one sample date in 2014 and 12 sample dates each in 2015. The 2014 sample was collected on 9 September, and in 2015, samples were collected throughout the fruiting period, beginning on 10 August and ending on 28 August. Varieties were replicated in four blocks and between three and ten fruit, depending on availability, were collected per plot at each harvest from each variety.

Following collection, samples were placed in 30-ml to 89-ml plastic cups (Dart Container Corporation, Mason, MI, 48854) depending on fruit size. Cups were sealed with a screened lid to reduce fungal growth. In some cases, a small cotton swab or sand layer was added to the bottom of the container to absorb moisture. Cups were placed in the laboratory at approximately $21\pm1^{\circ}$ C. Fruit were immediately placed individually in a sugar water solution (Dreves et al. 2014) and macerated. The percent of fruit with emerging *D. suzukii* ([number of infested fruit/total number of fruit] X 100) and the total number of larvae present per fuit were recorded.

Abundance and damage

Data on larval abundance and percentage damaged fruit were analyzed using the general linear hypothesis within the mixed model ANOVA framework, correcting for heteroscedasticity as necessary for lack of normality using JMP®, Pro 11 (SAS Institute Inc., Cary, NC, 1989-2007) (SAS 2013). The model included the factors: variety, sample date (time), and the variety x time interaction. If no interaction was determined, a one-way analysis of variance with a Tukey's HSD at the P < 0.05 level of significance was used to separate differences in means. The relationship between the number of larvae

present and the percent of infested fruit were examined using correlation in JMP®, Pro 11(SAS Institute Inc., Cary, NC, 1989-2007). Variables for these analyses consisted of the total larval abundance per fruit and the average percent of damaged fruit and the date the sample was collected. Analyses were performed on data from both in 2014 and 2015.

Results

Infestation rates

Plant variety did significantly alter the percentage of infested fruit (F=5, 76 = 36.67 p < 0.0001); (Fig. 1) as did the time of sample (F=1, 76 = 3.39 p < 0.0081), but there was no interaction between variety and time (F=5, 76 = 1.75 p < 0.134). There was a significant negative correlation between the percent of infested fruit and sample date (F=1, 86 = 42.71 p < 0.001; r2 = 0.33) with significantly fewer berries infested with later samples (Fig 2).

Abundance and damage

Plant variety also had a significant impact on the average number of larvae per fruit (F=10, 370 = 48.81 p < 0.0001) (Fig 3); however, we also found that there was a significant impact of sample date (F=3, 370 = 2.83 p < 0.038) as well as an interaction between sample date and variety (F=30, 370 = 2.39 p < 0.0001) (Fig4). When each sample date was evaluated, significant differences were detected between varieties on seven sample dates (Fig 4). Finally, there was a significant negative correlation between the

average number of larvae per fruit and sample (F=1, 412 = 183.80 p < 0.0001; r2 = 0.33) with significantly fewer larvae per berry in later samples (Fig 5).

Discussion

There is no doubt that the host range of *D. suzukii* is vast (Kimura et al. 1977; Nishiharu 1980; Mitsui et al. 2010; Burrack et al. 2013; Lee et al. 2015). Nevertheless, *D. suzukii* appears to exhibit some level of varietal preference within host species, or certain varieties may be more suitable, leading to increased adult populations. However, there may be other factors that are influencing the infestation patterns observed. Consistent with other studies, we found that there are higher levels of infestations in some varieties, and that some varieties produced more larvae (Lee et al. 2011; Burrack et al. 2013; Kinjo 2013). We also observed a significant negative correlation in both factors as the harvest season progressed.

Somewhat puzzling was the observed decrease in infested fruit and larvae over time even at a time when the number of adults observed in baited traps was increasing (*CRP unpublished data*). This trend could indicate dispersal of *D. suzukii* to neighboring crop hosts or alternative hosts in the environment. One possible explanation for this could be due to changes in host that modify a key biological trigger that reduces the olfactory attractiveness of the natal food source. Numerous studies have shown that many different species of insects, including some Drosophilids, use olfactory cues to locate and ultimately utilize host plants (Hoffman 1985; Yu et al 2013; Keesey et al. 2015; Diepenbrock et al 2016). In addition, different varieties of crops contain differing

amounts of resources, such as soluble sugar content (Brix) which likely affects varietal preference (Burrack et al. 2013; Yu et al 2013; Diepenbrock et al 2016) (Table 1). Slight changes in fruit chemistry can cause greater attractiveness to female *D. suzukii*, through volatiles released or via better nutrition for developing larvae.

Timing is important when dealing with agricultural pests, and local climactic conditions or microclimates may also influence *D. suzukii* population dynamics (Tochen et al. 201; Rogers et al. 2015; Wiman et al 2016). Pest development time and plant phenology play an essential role in the severity of an infestation. In addition to understanding how climate impacts nutritional values and attractiveness of varieties, this information may also be useful in evaluating the potential to provide a phenological mismatch between host and pest. Further exploration into varietal susceptibility is needed to determine what characteristic might make varieties more or less susceptible to *D. suzukii* infestations.

What remains to be determined constitutes optimal development for *D. suzukii*. Previous assumptions describe fitness for *D. suzukii* as larger, heavier individuals, faster development time, and greater numbers of eggs laid. It has not been thoroughly explored if fitness truly is defined by those parameters. For example, greater egg numbers may not be the more fit option if few of them survive. The varietal differences observed though fits with the ecological concept of ideal free distribution (Fretwell & Lucas 1970; Fretwell 1972). A less optimal environment may be selected by the adult female if the competition in the more optimal environment yields detrimental characteristics (such as competition) to the survival of offspring. Changes in host preference and decreasing

larval populations, while trap populations increase (*CRP unpublished data*), support this idea. As yet, the ideal free distribution has not been explored nor discussed in previous research as it relates to *D. suzukii*. Future studies should focus on evaluating ideal free distribution for *D. suzukii* including: determining impacts of host plant and variety on fitness, the effects of competition on fitness, and the combination of these two factors.

Conclusions

Currently the greatest focus for research and extension efforts on *D. suzukii* has been about the identification of effective insecticides. The majority of these compounds are broad-spectrum insecticides. As growers continue to rely on pesticides for control, this will undoubtedly lead to unintended environmental and ecological consequences and may result in insecticide resistance.

Unfortunately, there is a lack of information on the biology and management of many invasive pests. Successful IPM is dependent on understanding where and when pests occur before taking action. However, a major challenge with invasive species such as *D. suzukii* is our lack of fundamental knowledge about their biology, and ecology, which is critical in refining adequate implementation of effective monitoring and management practices.

The results of this project allow us to begin to identify mechanisms of varietal susceptibility and plant-insect phenology, through which we can provide berry producers with methods to continue sustainable production and maintain profitability. Selecting varieties that provide a phenological mismatch with key pests is an environmentally-

sound approach, providing farmers with a significant and sustainable improvement over the frequent use of insecticide applications currently recommended. Broadening our understanding of these fundamental knowledge gaps is critical to implementing and refining sufficient and effective monitoring and management practices for this pest.

One of the keys to development of an IPM program for *D. suzukii* remains understanding host preference and varietal susceptibility of preferred host crops. For cold hardy raspberries, it is clear that there is a varietal preference occurring. Understanding the host selection and varietal preference opens the door to further understand the biology and ecology of this pest. Key questions remain unanswered, but additional studies can add to our understanding of where and when outbreaks and infestations will occur which will ultimately allow a more integrated approach in managing *D. suzukii*.

3. Figures and Tables

Table 1. Berry quality ratings for varieties of Red Primocane-Fruiting raspberries evaluated for Drosophila suzukii infestations in 2014 and 2015.

Variety	Hardiness	Harvest	Productivity	Fruit Size	Attractiveness	Firmness	Flavor	Freezing
Autumn Bliss	fair	early	very good	large	very good	good	very good	very good
Polana	good	early	excellent	medium	very good	fair	fair	good
Autumn Britten	fair	early	good	large	very good	very good	excellent	good
Caroline	fair	mid late	good	large	very good	good	very good	good
Joan J	excellent	mid	very good	large	excellent	very good	very good	very good

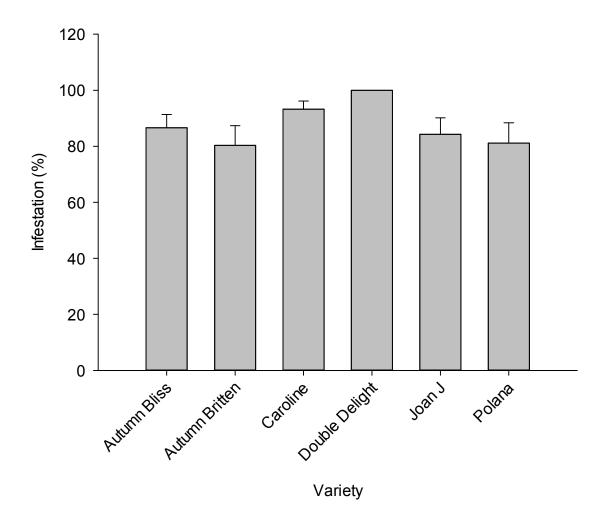


Fig 1. Varieties of Red Primocane-Fruiting raspberries evaluated for *Drosophila suzukii* infestations by percent of infested berries in 2014, Grand Rapids, MN.

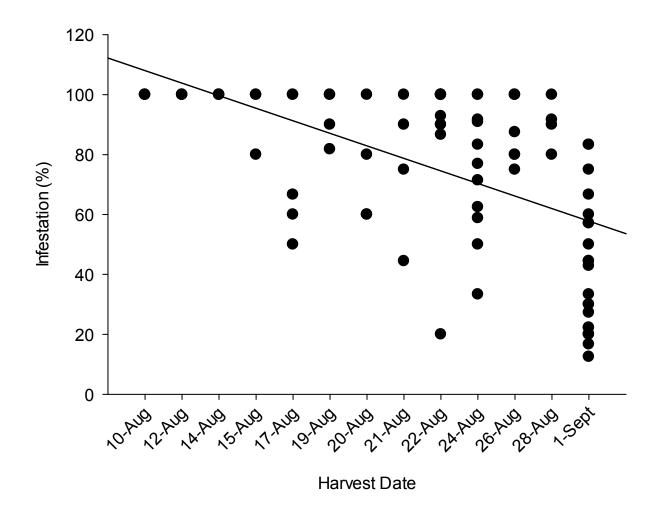


Fig 2. Regression of percent infested fruit over time for the varieties of Red Primocane-Fruiting raspberries evaluated for *Drosophila suzukii* infestations in 2015, Grand Rapids, MN. Percent infestation = 112.264 - (4.186 * Time), R² = 0.33, p<0.001.

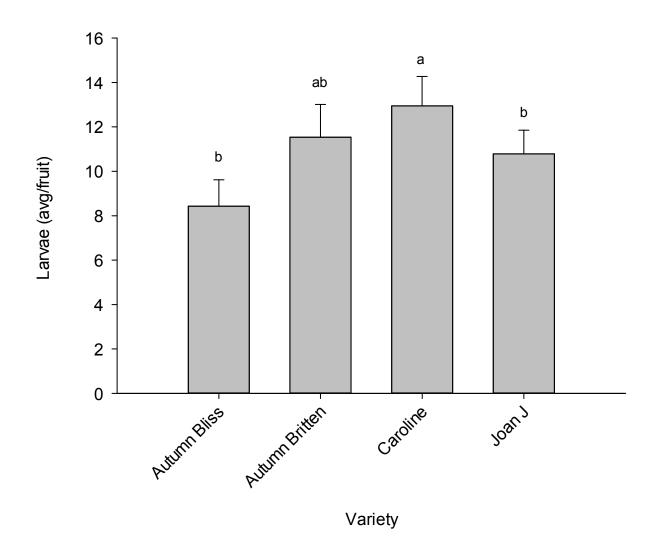


Fig 3. Average larvae per fruit for each variety of Red Primocane-Fruiting raspberries evaluated for *Drosophila suzukii* infestations in 2015, Grand Rapids, MN.

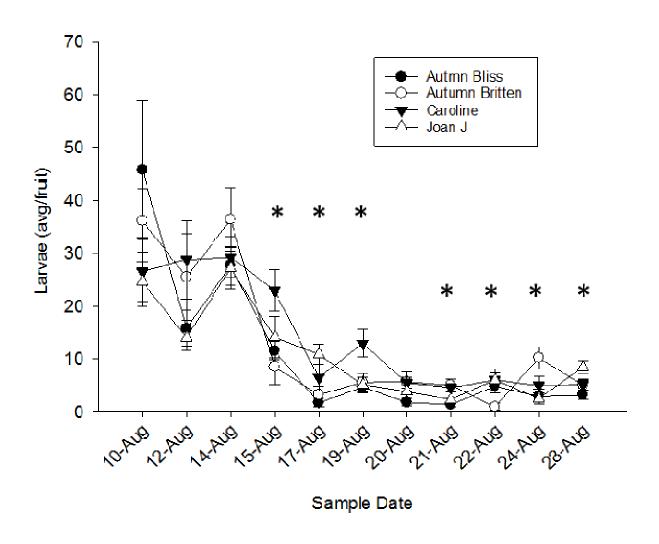


Fig 4. Mean number (+/- SEM) of larvae per fruit over time by varieties of Red Primocane-Fruiting raspberries evaluated for *Drosophila suzukii* infestations in 2014 and 2015. Dates marked with an asterisk are significantly different.

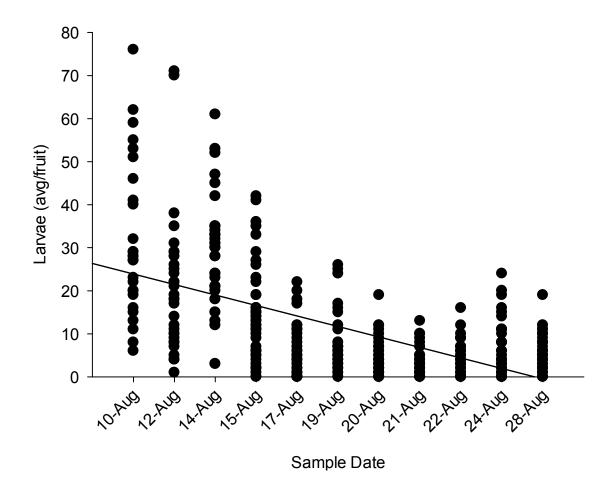


Fig 5. Regression of the average number per berry over time for the varieties of Red Primocane-Fruiting raspberries evaluated for *Drosophila suzukii* infestations in 2015, Grand Rapids, MN. Larvae = 26.337 - (2.438 * Time), R² = 0.33, p<0.001.

Chapter 3

Phenology of Spotted Wing Drosophila, *Drosophila suzukii*, Infestations within Crop and Non-Crop Hosts

Introduction

Spotted wing drosophila (SWD), *Drosophila suzukii* (Matusumura), is a major worldwide pest of berry and stone fruits (Lee et al. 2011; Loeb et al. 2013; Piotrowski et al. 2013; Depra et al. 2014; Asplen et al. 2015; Chireceanu et al. 2015; Kiss et al. 2016). Since its first detection in California in 2008, *D. suzukii* has spread throughout the U.S., North and South America, Europe, and Asia causing significant yield losses, often reaching 100% (Bolda et al. 2009; Burrack et al. 2013b; Walsh et al. 2011). Unlike other *Drosophilids*, *D. suzukii* is attracted to and oviposits in undamaged, intact, ripe berries, utilizing a previously inaccessible niche to other *Drosophilids* (Walsh et al. 2011). This is possible due to a heavily sclerotized and serrated ovipositor which female flies use to cut through fruit skin in order to lay eggs within(Swoboda-Bhattarai & Burrack 2016).

This pest was first described in 1934 by Kanzawa et al. in Japanese cherries. Since then, *D. suzukii* has been reported to infest a plethora of berries and stone fruits including, raspberries, blueberries, cherries, apricots, peaches, plums, and grapes to name a few (Kimura et al. 1977; Nishiharu 1980; Burrack et al. 2014). A few of these crops, such as raspberries and blueberries, have historically required little pest intervention leaving growers with little experience to rely on to manage *D. suzukii* infestation. It is considered a "zero tolerance" pest, and the presence of one individual in a harvest causes rejection of the entire shipment, leading to 100% crop loss (Bruck et al. 2011).

Current *D. suzukii* management recommendations involve insecticide applications on a 5- to 7-day rotation. Financial losses from an estimated 20% feeding damage alone costs growers approximately \$1 billion (Bolda et al. 2009; Burrack et al. 2013b; Walsh et al. 2011). Increased chemical applications add substantial financial losses as well and increased damage to the surrounding environment. Implementing an effective IPM management strategy involves knowing when and where a pest occurs. However, a major challenge is our lack of fundamental knowledge about their biology, and ecology in their new environment (Pedigo and Higley 1992; Pedigo 1996). The ability of an invasive insect to establish as a major pest in a novel environment depends, in part, on its dispersal ability and its ability to utilize available host plants (Pedigo 1996). A potentially important aspect to add to our toolbox of early detection and management for *D. suzukii* is its possible use of early fruiting non-crop host plants.

Drosophila suzukii has been reported to utilize several non-crop alternative hosts within field margins and in adjacent woodlots (Arnó et al. 2016; Klick et al. 2015). Such alternative hosts include invasive honeysuckle (Lonicera spp.), pokeweed (Phytolacca americana, L.), and buckthorn (Rhamnus cathartica L.) (Diepenbrock et al. 2016; Lee et al. 2015). How D. suzukii moves within the environment, specifically between crop and non-crop host plants is not well understood, and thus requires further exploration. Early fruiting host plants may act as a source for D. suzukii populations when few other host options are available. Thus allowing population densities to build prior to the availability of crop hosts (Steffan et al. 2013). Improving our understanding of D. suzukii host use patterns can aid in the development of effective management programs. Management

implemented when early fruiting non-crop hosts begin to ripen may well provide growers with an additional approach to managing this devastating pest.

Herein, I report the results of a two-year study designed to determine what non-crop host plants *D. suzukii* utilizes in Minnesota, to what extent is *D. suzukii* using these non-crop hosts of both the proportion of berries infested and average number of individuals per berry. Further, to determine the role of plant phenology on infestations and whether such phonology cycles can be used within management. Non-crop host plants were evaluated in field margins adjacent to commercial raspberry (*Rubus ideaus*), and high bush blueberry (*Vaccinium corumbosum*) fields as well as in wild areas within parks and nature preserves. All sites were within a maximum of 70 miles away from Minneapolis, MN. It is vital that we improve our understanding of *D. suzukii* dispersal in the environment considering the virulence and economic impact of this pest to small fruit growers.

Materials and Methods

Plants Sampled

Nine sampling locations were selected around Minneapolis and St. Paul, MN. Starting in May 2015, *D. suzukii* monitoring traps were placed at the nine locations, with two traps per location. Plants were located and identified within 50 m of monitoring traps. Potential alternative non-crop hosts were determined using methods similar to Lee et. al. (2015). Potential alternative non-crop hosts identified near sample sites included: gray dogwood (*Cornus racemosa*), Morrow's honeysuckle (*Lonicera morrowii*),

Tartarian honeysuckle (*Lonicera tatarica*), common buckthorn (*R. cathartica l.*), wild black raspberry (*Rubus occidentalis*), wild strawberry (*Fragaria vesca*), wild plum (*Prunus americana*), and wild gooseberry (*Ribes spp.*). Farm locations contained crop hosts including: red raspberry (*R. ideaus*), blueberry (*V. corumbosum*), and elderberry (*Sambucus canadensis*).

Phenology data

From May to August in 2015 and 2016 plants were visited weekly in a repeated measures fashion. Every visit, each plant species growth stage was defined as either budding, flowering, or fruiting. Once plants reached the fruiting stage and fruit sampled, each fruit was used to evaluate infestation levels was also visually assessed by their developmental stage, recorded as green, blush, ripe, or overripe. This was done each week for both crop and non-crop hosts.

Farm locations

Five farm locations were selected based on the presence of known host crops, florican red raspberry (*R. ideaus*), highbush blueberry (*V. corumbosum*), and potential alternative non-crop hosts. To maximize the distance between sample locations, all sites was separated by a minimum of 400 meters. Two farm locations also grow elderberry (*S. canadensis*) as a crop host. To determine larval infestation rates, fruit were collected from plants within 50 m of a monitoring trap in the field sites and incubated in the laboratory. In 2015, at each sample date 30 fruit per plant were collected at each site on a weekly

basis. In 2016, 10 fruit per host plant were collected at each site each week. The sampling number was different between the years to allow for more efficient and accurate processing of berries. All farm sites contained a potential crop host where *D. suzukii* infestations have been reported previously. If sampling of one host occurred at multiple sites, every effort was made to collect those samples on the same day. All fruiting plant species were sampled within a 50-m radius of a Pherocon SWD lures and traps (Trécé, Inc.). Data collected from each site included date, location, number of fruits or flowers collected per plant species, and the condition of the fruit (green, blush, ripe, or overripe).

Park locations

Sampling and identification of alternative non-crop hosts were similar to what is described in the "Farm plots" section. However, the locating fruit and sampling procedure was different than the "Farm plots" section. Fruiting non-crop hosts were identified within a 50-m radius of a Pherocon SWD lure and haphazardly sampled within arm's reach. Three natural areas were selected based on the presence of potential alternative hosts, and to maximize the distance between sample locations. Data collected from each site was the same as data collected at farm location sites.

In laboratory

After collection, individual fruit were brought to the laboratory and placed in 1 oz. soufflé cups covered with plastic ventilated lids. Cups were kept in the laboratory at $21\pm1^{\circ}\mathrm{C}$ for approximately 18 days to allow time for development, and examined for the

presence of adults. The percent of fruit with emerged adult *D. suzukii* ([number of infested fruit/total number of fruit] X 100) and numbers of alive or deceased larvae, pupae and adults were recorded.

D. suzukii adult populations were monitored at all sites using commercially available Pherocon SWD lures and traps (Trécé, Inc.). A minimum of two traps were set up at each location and were collected weekly. Traps consisted of dual lures hanging from the lid of a Pherocon trap baited with 150 ml of apple cider vinegar and 0.2 ml of unscented dish soap (Seventh Generation, Inc.). Bait solution was replaced and trap contents removed when traps were serviced. Trap contents were processed in the laboratory using a Zeiss Stemi 508 Zoom stereomicroscope, and the number of male and female D. suzukii recorded for each trap. Lures were replaced every 4 weeks, according to the manufacturer recommendations (Trécé, Inc.).

Data analysis

Unless otherwise noted, all analyses were performed using JMP®, Pro 13 (SAS Institute Inc., Cary, NC, 1989-2007). A one-way analysis of variance (ANOVA) in the fit x by y framework was used to evaluate the total number of individuals per berry by plant type. The mean individuals per berry was used per plant type for each week, then the overall mean from that per plant type was used to perform the ANOVA. The same was done for the average number of individuals per berry by host fruit stage. If significant p – value was recorded, the means were separated using a tukey's HSD with a Bonferroni correction. A t-test was performed between the mean number of individuals per berry.

Comparisons for the t-test tested between crop and non-crop host plants as well as using only non-crop host plants comparing between location types (farm or wild).

To compare proportions of infested fruit, a logit binomial multiple logistic regression model was used in the fit model framework. Proportion of infestations were analyzed with the factors: *host species, week, location type, host type,* and *ripeness stage* in multiple combinations of interactions. The best two models were chosen by using the lowest AICc value.

Results

Crop hosts sampled were *R. idaeus*, *V. corumbosum*, and *S. canadensis*. Of the crop hosts sampled, all sustained infestations over the growing seasons 2015 and 2016. Potential non-crop hosts identified and sampled were: *L. tatarica*, *L. morrowii*, *R. occidentalis*, *Fragaria vesca*, *Prunus americana*, *C. racemosa*, *Rhamnus cathartica l.*, and *Ribes spp*. Of these potential non-crop hosts, five were infested at some amount over the course of the years 2015 and 2016. These hosts being: *L. tatarica*, *L. morrowii*, *R. occidentalis*, *C. racemosa*, and *R. cathartica l. Rhamnus cathartica l.* sustained extremely low infestations at a rate not significantly different than the non-infested non-crop hosts that it was excluded from the rest of the analysis. *Lonicera tatarica* was only identified in 2016, therefore it was also excluded from analysis, however it did sustain infestations.

Significant differences were found for the infestations between plant types for the average number of larvae per berry ($F_{5,190}$ =6.369, P<0.0001) (Fig 6). Tukey's HSD

yielded significant results in with three means separation levels. *R. idaeus* (M = 0.736) sustained the greatest amount of infestation followed by *L. morrowii* (M = 0.429). *Cornus racemosa* was the least infested plant species (M = 0.032).

Results from the ANOVA between fruit ripeness stages yielded significant results $(F_{5,190}=7.2821, P<0.0001)$ (Fig 7 and 8). When analyzing the average number of individuals per berry, the ripe fruit stage sustained highest numbers of infestation (M = 0.679). The over ripe berry stage sustained the second most infestation numbers (M = 0.439). Least infested were bud and flower stages (M = 0). Green fruit stage did sustain infestations in *R. idaeus* and *V. corumbosum*.

Results from the logistic regression first determined whether more specific factors (host species) or more general factors (host type or location type) best fit the model. The first model which fit the least included the factors: host type, week, and the host type and week (AICc = 3951.5616). The next model which did not have the best fit included the factors: location type, week, and the location type and week interaction (AICc = 3932.3374). The equation which fit best included the factors: host species, week, and the host species and week interaction. This model was the best fit over the more general models (AICc = 3721.5709). All six host species were significant factors in predicting proportion of infestation ($p \le 0.0037$). Host species and week were significant interactions ($p \le 0.0238$), except L. morrowii × week (p = 0.4063) (Fig 7 and 10).

The second equation used was a more complex model with the factors: *host* species, week, ripeness stage, the host species and week interaction, the host species and

ripeness stage interaction, and the week and ripeness interaction (AICc = 3481.596). Lonicera morrowii was the only significant host factor with only host comparisons (p=0.0311). Host by week interaction yielded two hosts significant factors: C. racemosai and R. idaeus (p = 0.0001 and p < 0.0001 respectively).

Trap counts were visually different by locations. Farm location trap counts for 2015 and 2016 can be found at fruitedge.umn.edu (Fruitedge website). Trap captures were earlier in wild locations than farm location (Fig 11). Populations in traps peaked in the wild locations before farm locations while. Conversely, populations reached higher numbers in farm locations than in wild locations.

Significant differences were found when analyzing crop vs non-crop hosts with average number of individuals per berry. Ripe fruit were infested more in the crop hosts than non-crop hosts (Fig 12) (t = 2.0962, P = 0.0204). No significance was found comparing non-crop host plants between location types (Fig 13) (t = 0.5605, p = 0.5801)

Discussion

To what extent are SWD using alternative hosts?

Data from the current study do not reveal a clear pattern for crop and non-crop host use, nor do they identify a clear link that non-crop host may be acting as a source for later season crop host infestations. Nevertheless, these data provide valuable information improving our understanding of how *D. suzukii* utilize crop and non-crop hosts in the environment, which will aid in improving management of this devastating pest. There is

no shortage of information highlighting the fact that *D. suzukii* has a large host range and many alternative hosts (Arnó et al. 2016; Burrack et al. 2013b; Diepenbrock et al. 2016; Kenis et al. 2016; Lee et al. 2015). However, what was lacking is understanding how *D. suzukii* utilizes these hosts given the unique Minnesota climate. Results of the current study show that *D. suzukii* utilize the non-crop hosts: honeysuckle (*L. morrowii* and *L. tatarica*), wild black raspberry (*R. occidentalis*), dogwood (*C. racemosa*), and to a significantly lesser extent buckthorn (*R. cathartica l.*) in Minnesota. It is important to note that these species may not be the only alternative hosts utilized in Minnesota's landscape, they were simply the only potential hosts present at sampling locations. For example, mulberry is a prevalent early-fruiting plant in Minnesota which has also been identified as a host for *D. suzukii*, however it was not included in this study (Harris et al. 2014; Lee et al. 2015; Yu et al. 2013). Using specific species to estimate or record infestations is imperative to demonstrate an accurate picture of what hosts *D. suzukii* is utilizing in the environment based on these data.

There are several methods insects use to locate hosts and eventually oviposit. These methods consist of using various types of cues, or signals, as guides to locate a suitable host. Slight variations in some of these cues could have significant impacts on which plants *D. suzukii* utilize for hosts. Therefore variation in infestation rates across berry types may be driven by things such as berry color, brix (% of soluable sugars), volatiles given off by the plants, or most likely fruit skin thickness (Lee et al. 2011; Keesey et al. 2015; Lee et al. 2013; 2016; Little et al. 2016; Yu et al. 2013). Another possible influence on host choice is volatiles detected by the fly's antennae. *D. suzukii*

can register volatiles from fruit stages as early as blush and from bacteria found on the fruit (Abraham et al. 2015; Bellamy et al. 2013; Keesey et al. 2015; Mazzetto et al. 2016). Unlike other *Drosophila* species, *D. suzukii* does not show a strong host fidelity (Diepenbrock et al. 2016; Sward et al. 2016; Yu et al. 2013). It is clear that host plant selection and ultimately utilization is a complex process. Future studies should focus on determining if *D. suzukii* are seeking out specific host or simply using what is available. Moreover, they should strive to identify the specific mechanism that drive *D. suzukii* host plant selection, location, and ultimately oviposition.

A few studies have been done on fruit ripeness stage preference. Those have, so far, discerned that ripening fruit are susceptible to infestation and green fruit may be a target (Arnó et al. 2016; Lee et al. 2016; Yu et al. 2013). From this study, green fruit did sustain natural infestation almost exclusively in crop host berries (Fig 8). Further, an overall view of what infestations look like in the field and ecosystem, both collection of the proportion of berries infested and average number of individuals per berry is needed. The over ripe fruit from viewed at an average individual per berry perspective yields no difference between crop and non-crop host infestations. We can see differences though in the proportion, there is greater dispersion through the over ripe berries in non-crop hosts than in crop hosts.

Infested non-crop hosts were discovered at both wild and farm locations. Though a population increase is observed as the months progress, the proportion of infested berries increase at different rates depending on host species (Fig 7). In recent years, many growers have begun removal of potential non-crop host plants from their properties in an

effort to manage this pest. *D. suzukii* populations in non-crop host plants was not significantly different between wild and farm locations leads to the conclusion that wild locations could act as a source for infestations. Coupled with the ability of *D. suzukii* to travel long distances in a day, it is likely that while this approach may delay initial infestations it is unlikely that removal of non-crop host plants will greatly impact overall infestation in crop hosts (Iglesias et al. 2014).

Do crop and non-crop hosts differ in their phenologies?

Developing an integrated approach to managing *D. suzukii* will rely on knowing when and where a pest occurs before taking action. Time of day has been indicated to correspond to *D. suzukii* activity in the field (Diepenbrock et al. 2016). To date most phenology work, has focused on when *D. suzukii* populations occur with little exploration into the role plant phenology plays. Most of this has been done in an effort to correlate adult trap catch with field level infestation (Heath et al. 1995; Iglesias et al. 2014; Lee et al. 2012). However, predictive models and monitoring traps are not as accurate as desired and do not provide much information on where infestation may occur (Heath et al. 1995; Kleiber et al. 2014; Lee et al. 2012; Wiman et al. 2014). More information is needed as far as what and where hosts are being utilized in the environment. The results from this study support mounting evidence that alternative hosts are used by *D. suzukii*, but goes one step further by beginning to incorporate both crop and non-crop host plant phenology into predictive toolbox.

Understanding what constitutes host fruit susceptibility and when it occurs in the field will be crucial in order to develop a management strategy for *D. suzukii*. A greater knowledge base is needed on the plants *D. suzukii* uses before we can know the best course of action in managing this pest. Not only the fruit phenology, but the berry differences (brix, skin thickness, micro nutrients etc.). This study demonstrated the fruit susceptibility is slightly different between crop and non-crop host plants. Further, accurately quantifying infestations requires a two-pronged approach if we are to effectively provide growers with information. A grower may be less concerned if the number of individuals per berry is high but the percent of berries infested low than a grower who has low numbers of individuals per berry but high percent of their berries are infested. Currently quantifying infestation is a broad per berry number which may or may not be an accurate depiction of what is going on in the field.

Early season infestations within non-crop hosts, may provide much needed insight into how *D. suzukii* is able to invade crop fields so quickly. Little is known about how *D. suzukii* moves in the environment, it is speculated that non-crop hosts in field margins facilitate later crop infestations (Arnó et al. 2016; Klick et al. 2015). Every year, *D. suzukii* appears suddenly in monitoring traps and grows rapidly. These data show that *D. suzukii* appears in early season non-crop hosts at farm locations and wild locations then later gives way to higher infestations in crop hosts at farm locations (Fig 9 and 10). This revelation indicates that non-crop hosts are likely contributing to the buildup of populations that lead to future crop infestations. It is likely that in order for management

programs for *D. suzukii* to succeed, monitoring and management may have to begin outside of grower fields to deter future infestations inside the crop hosts.

Because the same infestation rates were detected in hosts on farm plots and non-crop hosts in wild locations, this suggests that while field margins may act as a refuge for moving into the crop hosts, *D. suzukii* is still prevalent in unmanaged landscapes, and that these populations may act as a source for future infestations. Although management in the wild locations may not be an option, using these areas and non-crop hosts as an early warning system may provide growers an easy and accurate way to know when *D. suzukii* is present and that they should initiate their management program.

Another significant discovery were infestations in green fruit of crop hosts. The current management recommendation is to begin management when susceptible fruit are present. To date it was believed that susceptible fruit were those beginning in the blush stage. *D. suzukii* demonstrates a willingness to infest earlier stage crop host fruit that is not observed in non-crop hosts (Fig 9 and 10). Given these new findings and the potential for flies moving in from non-crop hosts, the current recommendations to begin management program at blush fruit in susceptible crops may be too late for effective management. Infestations consistently appeared at the beginning of blush and ripe fruit presence, except for *V. corumbosum* and *C. racemosa* (Fig 9 and 10). There are several reasons this may occur including fruit thickness or overall attractiveness; however, if these hosts are not as attractive to *D. suzukii*, research designed to evaluate the mechanism driving attractiveness would be a step in the right direction to providing better management strategies.

Conclusion

Managing *D. suzukii* within non-crop host plants is unlikely to be an effective strategy. Insecticide applications are unavailable for use on non-crop host plants.

Sanitation in field margins may prove ineffective given understory growth and no set placement of hosts. Further, *D. suzukii* is prevalent throughout the environment and may be able to move into a farm field from wild areas far away from the farm.

Since infestations were recorded in the green fruit of two crop hosts, it is important to update the timing to begin management for *D. suzukii*. Farmers are used to using environmental cues as a factor in their decision processes. *L. morrowii* was consistently ripening and infested earlier than any other host. Because of this, it may be useful as an early warning system. Collecting the ripe fruit and checking infestations in a brown sugar water bath could be what's needed for a timely warning to begin management strategies. More work should also focus on natural infestations, especially in green berries. Management thus far may have been implemented too late to have a large effect on natural infestations within crop hosts. When examining infestations, more complex generalized linear models should be used, such as specific plant species.

While researchers are working diligently to find answers to this pest, key gaps in our understanding remain. Broadening our understanding of these knowledge gaps is extremely important in refining environmentally sound management strategies. Key areas where more research is needed include: migration, host preference, and key non-chemical management methods. Filling these gaps will go a long way in developing an IPM

strategy. While it appears that *D. suzukii* moves between alternative and crop hosts in a specific manor and time, additional studies are needed to answer key questions to determine when and where *D. suzukii* occurs. Nevertheless, this study is a step in the right direction to producing a more integrated methodology to management.

Graphs and Tables

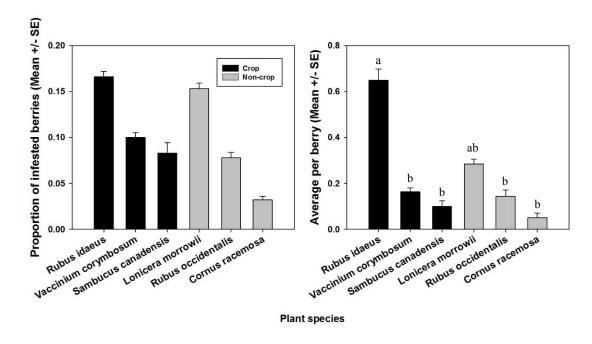


Fig 6. Average infestation rates \pm -SE for plant species. The graph on the left demonstrates results from an ANOVA and Tukey-kramer HSD with significantly different average number of individuals per berry $F_{5,190}=6.369$, P<0.0001.

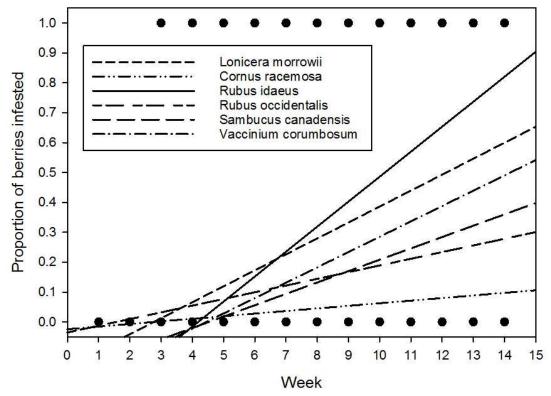


Fig 7. Regression of the average proportion of infested berries by plant species over time. Evaluated for *Drosophila suzukii* in 2015 and 2016.

L. $morrowii = -0.1475 + (0.05339*Week), R^2 = 0.10, p < 0.4603$

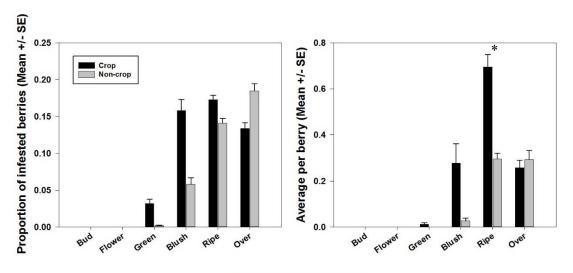
C. $racemose = -0.02394 + (0.008673*Week), R^2 = 0.03, p=0.0012$

R. idaeus = -0.3494 + (0.08359*Week), $R^2 = 0.17$, p=0.0006

R. occidentalis = -0.03513 + (0.02242*Week), $R^2 = 0.03$, p=0.0238

S. canadensis = -0.1722 + (0.03806*Week), $R^2 = 0.18$, p=0.0134

V. corumbosum = -0.2281 + (0.03806*Week), $R^2 = 0.17$, p<0.0001



Plant phenological stage

Fig 8. Average infestations (+/- SE) for each fruit ripeness stage by host type (crop or non-crop). The graph on the left demonstrates results from an ANOVA and Tukey-kramer HSD with significantly different average number of individuals per berry $F_{5,190}$ =7.2821, P<0.0001.

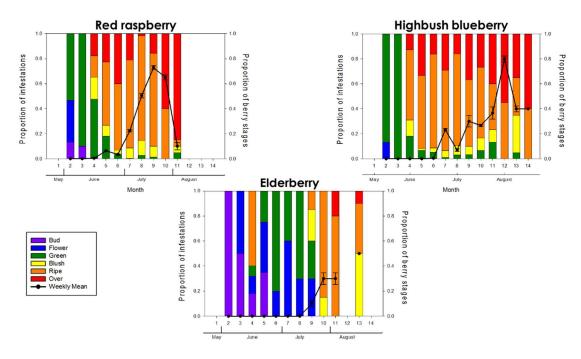


Fig 9. Comparison between the berry ripening phenology of each alternative host plant (lower bars) and infestation percent for their respective fruit over time (+/- SE). Weeks indicate sampling day groups on a weekly basis starting in May and ending in August.

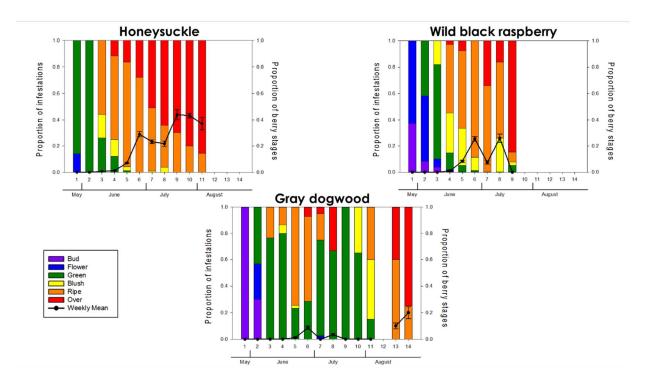


Fig 10. Comparison between the berry ripening phenology of each crop host plant (lower bars) and infestation percent for their respective fruit over time (+/- SE). Weeks indicate sampling day groups on a weekly basis starting in May and ending in August.

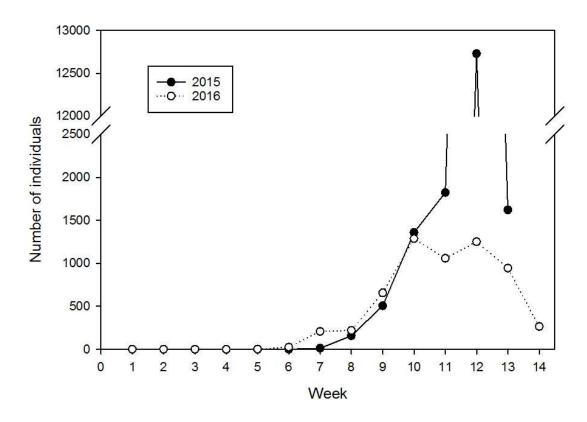


Fig 11. Number of individuals caught in traps in wild locations around the Minneapolis, MN in 2015 and 2016. Farm location data can be found at www.fruitedge.umn.edu

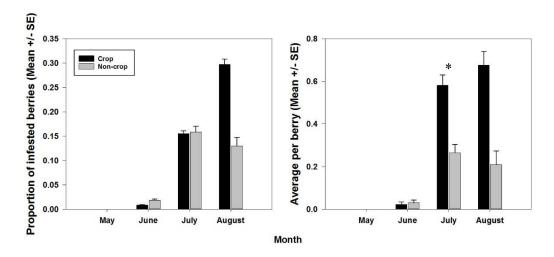


Fig 12. Average infestations (\pm -SE) by month at each location type by host type. Significance from t-test was found for ripe fruit in the average per berry t = 2.0962, P = 0.0204.

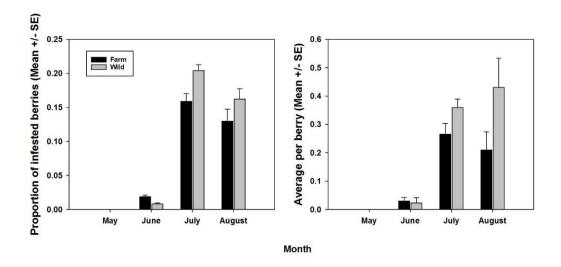


Fig 13. Average infestations (\pm -SE) by month by location type with non-crop host plants. No significance was found for the average number per berry in the t-test between location types t = 0.5605, p = 0.5801.

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Appendix 1

Insecticide timing trial

Introduction

Spotted wing drosophila (SWD), *Drosophila suzukii*, has emerged as a devastating worldwide pest of berry and stone fruit crops. Infestations occur from the females retention of a large, heavily sclerotized, serrated ovipositor which she uses to cut through fruit skin and lay eggs inside (Swoboda-Bhattarai & Burrack, 2016). Since its first detection in Japan and Southeast Asia in 1939, SWD has spread to over 20 countries worldwide causing significant economic damage (Asplen et al. 2015; Kanzawa, 1939). Presently, the most effective management recommendation is chemical applications on a 5 to 7-day spray schedule. Economic losses incurred by SWD damage alone totals approximately \$1 billion annually in the United States leading many growers to abandon these crops (Bolda et al. 2009; Burrack et al. 2013b; Walsh et al. 2011). Further, for many of the insecticides, the cost is not feasible for many small-scale growers.

Optimization of the timing of insecticide applications is imperative to reduce overall economic injury to the growers. We compared the efficacy of the different spray schedules with the control, the current practice (rotation of spinosad and pyrethroid), and the high and low rate of a new diamide for the percent of berries infested and the number of larvae per berry. The goal of this study was to first, determine the best time of applications for highest control, and second, to assess whether the new diamide pesticide is comparable to the current chemistries available.

Materials and Methods

This study was set up in a randomized complete block design at the University of Minnesota North Central Research and Outreach Center in Grand Rapids, MN.

Treatments included an untreated control, current spray practice (pyrethroid/spinosyn rotation), and the high and low rate of a new formula (Harvanta powered by cycloprene).

Plots were sprayed on either a 5 or 7-day spray schedule. The 5-day schedule was sampled with 10 berries per treatment every 3 and 5 days after treatment (DAT). The 7-day schedule was sampled with 10 berries per treatment every 3, 5, and 7 DAT. Berries were placed in individual cups and held for 3 days. A brown sugar water solution was made as described by Beers et. al. (date) and larvae were counted and recorded. A portion of the berries were held for 2 weeks to confirm SWD identification. An analysis of variance (ANOVA) was performed in JMP statistical software.

Results

Two objectives for this study were to determine differences in the timing of insecticide sprays in the percent and number of larvae of SWD infestations and to evaluate the new diamide chemical with regards to current practices. In terms of the timing efficacy for infested berries, no significant differences were observed, however between the treatments there was a difference. For the number of larvae per berry, significant differences were observed. Both the current practice and diamide high rate was more effective at the 7 day spray schedule with p values of 0.0001 and 0.0004 respectively. No differences were observed between 5 and 7 day sprays for the diamide

low rate, however it was very consistent. The control had significantly more larvae per berries than the other treatments.

Discussion

Since SWD arrived in the United States, growers have been relying on chemical intervention for small berry production, more than ever. Chemical sprays using frequent calendar based schedules greatly increase production costs and is not a sustainable option in the long run (Lee et al. 2011). In this study, significant differences were found between the 5 and 7-day spray schedules for percentage of berries infested (Fig 1). Different spray schedules did have a significant impact on the number of larvae per berry; the 7-day spray schedule was more effective than the 5-day spray schedule in both the current practice and the high rate of Harvanta (Fig 2). Such a difference is promising for growers. Spraying at a longer schedule could achieve the best control, reducing costs of chemical inputs and lowering environmental risk.

Harvanta appears to be a comparable option to the current practice. It had a comparable percentage of infested berries to current practices (Fig 1). The high rate of Harvanta showed a similar decrease in the number of larvae per berry as the current practice, going from higher infestation numbers in the 5-day spray schedule and lower infestation numbers in the 7-day spray schedule (Fig 2). Further, even at low rates, Harvanta is consistent in its control for SWD (Fig 1 & 2).

Infestations were noticeably high in all treatments. A few potential explanations are as follows. Control groups could have acted as refuges from insecticide applications

and infested the treatment groups. A row of choke cherry trees and plot of unsprayed blueberries were located close to the raspberry plot which could have inundated the environment with high levels of flies. There may be hot spots for SWD in Minnesota and one of these may have been where the raspberry plot was located. Regardless of the high SWD numbers, control was observed through chemical intervention.

Conclusions

Timing is everything when agricultural pest control is concerned. Recurring questions from growers include when and where control should be implemented. Since SWD is a no-tolerance pest, control recommendations centered around calendar based insecticide applications. Growers begin insecticide applications on a 5 to 7 day spray schedule when the first SWD is caught in a monitoring trap and there are susceptible berries in the field (Cormier et al. 2015; Thomas et al. 2016). Such application schedule is unsustainable and adds substantial costs for growers.

Key components to successful IPM strategies involve knowing when and where control measures should take place. Our lack of fundamental knowledge about when and where SWD occurs is a stopping block to developing management strategies. For example, in this study our infestations were higher than normal while we may only speculate why such numbers occurred. From refuge-type infestations to potential hot spots, how SWD moves in the environment is a large knowledge gap in need of exploration.

The results from this study allow us to begin to refine current chemical management strategies in regard to the timing of insecticide applications. Applications utilized on a 7 day spray schedule provide higher rates of control for the number of larvae per berry than a 5 day spray schedule, saving farmers money and reducing the impact of control on the environment. With few chemical options available, another insecticide class is imperative to stave off resistance development. Though just a stepping stone towards an effective IPM strategy, it is clear that timing of insecticide applications can have an impact on larval numbers per berry and that Harvanta may be a viable option to add into chemical control rotation.

Figures and Tables

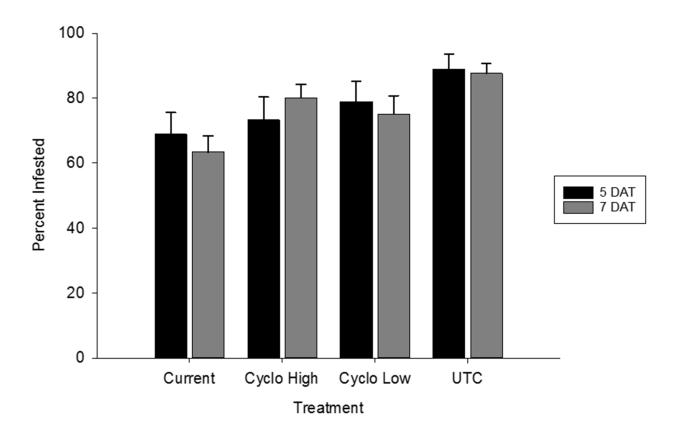


Fig 1. Comparison of percent of berries infested between spray schedule timing (5 and 7 day) for the current practice, Harvanta (Cyclo), and untreated control (UTC). P-values > 0.05

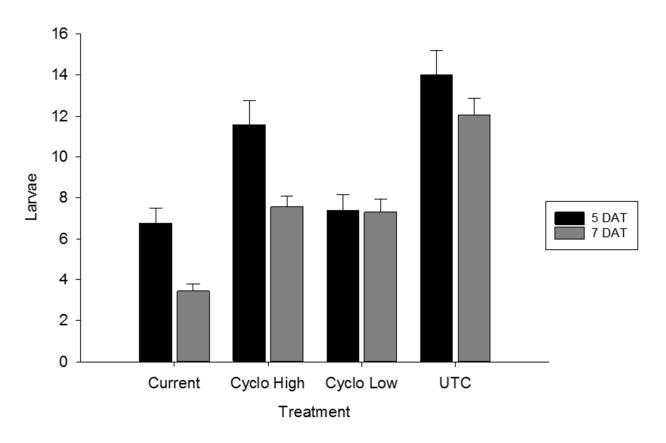


Fig 2. Comparison of the average number of larvae per berry between spray schedule timing (5 and 7 day) for the current practice, Harvanta (Cyclo), and untreated control (UTC). Current: P-value<0.05 High=0.0004 Low>0.05 UTC>0.05

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