TOWARD BETTER MANAGEMENT OF SPOTTED-WING DROSOPHILA (DROSOPHILA SUZUKII) IN MICHIGAN CHERRY ORCHARDS

By

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ABSTRACT

TOWARD BETTER MANAGEMENT OF SPOTTED-WING DROSOPHILA (DROSOPHILA SUZUKII) IN MICHIGAN CHERRY ORCHARDS

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Spotted-wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is an invasive species that has impacts worldwide. Current monitoring methods and decision-making protocols are unreliable indicators of *D. suzukii* population and propensity to infest a crop. The aim of this research was to develop behavior-based tools that would lead to improved management of *D. suzukii* populations in Michigan cherry. The commercial Scentry® lure provided higher *D. suzukii* attractiveness than other commercially available lures. Sticky panels tested with a variety of colors and patterns showed that most *D. suzukii* are captured on a green panel or a light-colored panel with a dark contrasting sphere in the center, as well as panel traps with a large trap surface are. Studies aimed at understanding the relationships between fruit development and *D. suzukii* infestation revealed that over all the varieties of sweet and tart cherries tested, softer, riper fruit were more susceptible to infestation than unripe fruit. There were strong positive relationships between *D. suzukii* larval infestation and the change in color and the change in the amount of force required to puncture the skin of the cherry fruit. There also was a good relationship between Growing Degree Days (base 4°C) post bloom and larval infestation, with fruit at a low risk of infestation by *D. suzukii* prior to about 600 GDD's. This research provides information on creating a risk of infestation model that uses fruit ripeness stage based on Growing Degree Days, combined with effective monitoring tools, to provide options for improved decision-making in the management of *D. suzukii*.

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INVASION HISTORY AND BIOLOGY

Spotted-wing Drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is native to southeast Asia, where it is often regulated by natural biological control agents (Cloonan et al. 2018; Daane et al. 2016; Girod et al. 2018; Ideo et al. 2008). The biology and invasion history of *D. suzukii* has been extensively reviewed by Asplen et al. (2015). This invasive species emerged as a pest in the US mainland in 2008, where it was discovered in California berry crops and rapidly spread across the continent (Asplen et al. 2015; Cloonan et al. 2018; Enriquez and Colinet 2017). It was detected in Michigan in 2010 (Isaacs 2011) and is now well established in all fruit production regions (Wilson et al. 2019). Males are distinguished from other *Drosophila* flies by a dark spot on the distal end of the leading wing, and females are identified by a large serrated ovipositor (Asplen et al 2015). Female *D. suzukii* use their ovipositor to lay eggs into ripening fruit, whereas other *Drosophila* species lay eggs on overripe and decaying fruit (Asplen et al. 2015; Bellutti et al. 2017; Cloonan et al. 2018; Tochen et al. 2014). Females can lay over 300 eggs, requiring a minimum of 10 days to develop to adults (Asplen et al. 2015; Leach et al. 2018). Under typical summer temperatures in Michigan, *D. suzukii* may complete over a dozen generations.

Environmental requirements

Temperature and humidity greatly affect the physiology, survival, fecundity, reproductive status, and behavior of *D. suzukii* and other Drosophilids (Enriquez and Colinet 2017; Tochen et al. 2016). Despite thriving in regions where temperatures fall below freezing, *D. suzukii* suffers significant mortality following exposure to freezing temperatures (Dalton et al. 2011). Adults are also sensitive to high temperatures. For example, oviposition and egg viability are reduced at higher temperatures (Walsh et al. 2011).

Due to their small size, Drosophilids, including *D. suzukii*, are especially sensitive to heat stress and water loss (Tochen et al. 2016). To avoid desiccation, *D. suzukii* adults move to locations of more favorable temperature and humidity conditions (Enriquez and Colinet 2017; Tochen et al. 2016). Temperature and humidity likely interact to affect *D. suzukii* behaviors. Studies have shown that an increase in humidity increases adult captures in traps and *D. suzukii* activity in general, however an increase in temperature alone had no effect (Enriquez and Colinet 2017; Tochen et al. 2016). Temperature and humidity readings from on-site or off-site locations may not accurately reflect the microclimate in the canopy of the orchard (Enriquez and Colinet 2017; Tochen et al. 2016). It is currently unknown how flies cope with heat stress in an orchard environment (Enriquez and Colinet 2017),

Humidity appears to be an especially important factor in *D. suzukii* development. Female *D. suzukii* reproductive status is greatest between 82 and 94% relative humidity (Tochen et al. 2016). In the field, low humidity levels corresponded with low catch in traps (Tochen et al. 2016). Cultural practices to decrease humidity levels in the field may lead to less *D. suzukii* infestation (Tochen et al. 2016). Data have not yet been published on how humidity affects development and population growth in *D. suzukii* (Tochen et al. 2016). Low humidity could be an important cultural practice for controlling *D. suzukii*. At humidity levels below 20 percent, *D. suzukii* did not survive and reproduce, and greater RH levels led to higher reproductive potential (Tochen et al. 2016). Understanding how *D. suzukii* adults respond to changes in humidity is critical to monitoring as well. Placing traps within high humidity regions of the orchard may improve early capture of *D. suzukii* (Tochen et al. 2016).

Economic impacts

Spotted-wing drosophila can have a dramatic impact on the production of thin-skinned berry and stone fruit crops. The larvae feed and defecate inside infested fruit, making it unmarketable (Asplen et al. 2015; Tochen et al. 2014). In addition, the oviposition scar leaves the fruit susceptible to secondary infection and bacteria (Asplen et al. 2015; Cloonan et al. 2018). Economic losses from *D. suzukii* in raspberries, blackberries, blueberries, strawberries, and cherries in the western US were estimated to be up to $500 million annually (Asplen et al. 2015; Farnsworth et al. 2016; Goodhue et al. 2011; Tochen et al. 2014). Complete crop losses have been noted in organic strawberries, raspberries, and cherries in Europe (Cini et al. 2012, Weydert and Mandrin 2013). In the coastal regions of California, mild weather allows *D. suzukii* to be active all year round, making management of this pest particularly challenging. Cherries are one of the most affected crops (Leach et al 2018). Tart cherries are important to Michigan agriculture, which produces about 75 percent of the nation's total domestic production (Lagoudakis et al. 2019; Lang 2017).

Characteristics of preferred hosts

D. suzukii has a wide host range that includes caneberries, blueberries, strawberries, cherries, apricots, and plums (Asplen et al. 2015; Wiman et al. 2016). When given a choice, SWD show preference for ovipositing in raspberries over all other cultivated fruits, and will choose cherries over blueberries (Tochen et al. 2014). Cultivar type has been shown to influence oviposition preference in blackberries, blueberries, cherries, raspberries, and wine grapes (Cloonan et al. 2018). In addition to the many cultivated crops, *D. suzukii* can utilize a wide variety of wild host plants which makes it difficult to control (Asplen et al 2015; Cloonan et al. 2018; Lee et al. 2011). Although *D. suzukii* can oviposit in unripe fruit, oviposition increases as fruit become riper and pH and Brix increase. Larval development also increases in fruits with higher sugar content (Cloonan et al. 2018; Lee et al. 2011; Lee et al. 2016).

There are several factors associated with fruit ripening that influence host suitability. Early season developing fruit is firmer than ripe fruit, and as many fruits ripen (blueberry, cherry, and raspberry) they become darker in color (Lee et al. 2016). Higher fruit firmness is associated with less oviposition, with female flies preferring softer fruit (Burrack et al. 2013; Cloonan et al. 2016). Females prefer ripening fruit because ripening fruit give off more CO_2 than ripe fruit (Cloonan et al. 2018). They cannot lay eggs and develop on cranberries and peaches because they are too firm (Asplen et al 2015; Bellamy et al. 2013, Cloonan et al. 2018). When calcium silicate is applied to blueberries, the puncture pressure of the fruit increases and less oviposition is observed (Lee et al. 2016). Fruit puncture force is typically measured using a small, handheld portable penetrometer, with a high variation of human error when used (Jantra et al. 2018).

Many host fruits share several ubiquitous volatiles, and *D. suzukii* may rely on the relative amounts of volatiles to determine oviposition site (Abraham et al. 2015; Revadi et al. 2015). Gravid *D. suzukii* females and other fruit attacking flies may use fermentation volatiles such as ethanol, acetic acid, and β-phenylethanol to find feeding sites, but they have little effect on oviposition (Abraham et al. 2015; Revadi et al. 2015). Ripening, undamaged cherries do not give off acetic acid and ethanol like other host fruits, but cherries are reported as a primary host fruit for *D. suzukii* (Revadi et al. 2015). One argument for the poor response of *D. suzukii* to artificial lures is that in the field the natural background odor masks the effect of the synthetic compounds and other overlapping cues (Revadi et al. 2015).

Management

Zero tolerance for *D. suzukii* infestation has led to heavy insecticide use as the principle control option (Beers et al 2011). In California, infestation in raspberry has almost been eliminated through aggressive chemical management strategies, but this is not a sustainable option for long term control and this approach is not possible for organic growers (Asplen et al. 2015; Leach et al. 2018). To manage *D. suzukii* in Michigan, current recommendations are to treat with registered insecticides at a minimum 7-day spray interval and minimize any delay in harvest (Wilson et al. 2019). Intensive use of insecticides has led to other pest management concerns such as insecticide resistance, risks to natural enemies and, secondary pest outbreaks (Asplen et al. 2015; Cloonan et al. 2018; Leach et al. 2018; Tochen et al. 2014; Tochen et al. 2016; Van Timmeren and Isaacs 2013).

Cultural controls can work well in conjunction with other treatment methods to control *D. suzukii*, such as increasing harvest frequency in fruits with long harvest times like raspberries (Leach et al. 2018). Because of the challenges this pest presents for fresh fruit growers, some have explored alternative methods to mitigating the inevitable infestation of harvested fruit.

For example, it has been shown that leaving fruit in a sealed container for 2-3 days in direct sun, will kill *D. suzukii* larvae (Leach et al. 2018). Freezing fruit is another method to kill larvae that may be present in harvested fruit (Asplen et al. 2015; Leach at al. 2018). Unfortunately, these methods do not allow the fruit to be sold as fresh market produce and can cause damage to the fruit. The investigations into these post-harvest sanitation methods demonstrates how challenging it is to prevent infestation.

Decision-making models have yet to be developed for *D. suzukii*. Because of the high reproductive potential when conditions are favorable, the short generation time, and high generational overlap, modeling based on temperature alone may not be adequate. In addition, the micro-climate of wild hosts located inside woodlands and the variations in local temperatures can dramatically impact the survival and reproductive rate of this pest (Asplen et al. 2015; Tochen et al. 2014), making predictions quite difficult. A useful model for *D. suzukii* management may be one that incorporates fruit temperature and fruit phenology. Research has shown that in cherries at temperatures below 10°C and above 30°, no eggs were laid, and motor function of adults decreased (Asplen et al. 2015; Enriquez and Colinet 2017; Tochen et al. 2014). Larval development of *D. suzukii* stops at 31.5°C, and 50% of adult flies have been shown to die (Lt50) at 37°C (Asplen et al. 2015; Enriquez and Colinet 2017). While pupae have been shown to be more tolerant than adults to prolonged high temperatures (Enriquez and Colinet 2017), alterations to the in-crop micro-climate could potentially impact both adult and larval survival and ultimately fruit infestation.

TRAPS, LURES, AND MONITORING

Since *D. suzukii* was first detected in North America, there has been considerable interest in developing effective traps and baits. Current methods to monitor *D. suzukii* prior to fruit damage are inadequate for decision making (Kirkpatrick et al. 2017). Widely used vinegar and yeast baits are neither efficient nor selective, and there is not yet a commercially available bait more attractive to *D. suzukii* than volatiles given off by ripening fruit (Abraham et al. 2015, Burrack et al. 2013). The current method for monitoring *D. suzukii* presence is a clear deli cup coupled with a synthetic lure and a drowning solution (Kirkpatrick et al. 2018a, Landolt et al. 2012). An issue with this method is that once one flies are captured, the populations in the field are already high due to the rapid generation time and high generational overlap of *D. suzukii* (Wiman et al. 2014, Kirkpatrick et al. 2018a). The risk of oviposition and infestation by *D. suzukii* is what growers find more important than presence in the field (Kirkpatrick et al 2018a,b, Lee et al. 2016). Despite these efforts, trapping remains an unreliable means of determining when an insecticide treatment should be applied (Abraham et al. 2015, Kirkpatrick et al. 2016).

Trap design

The most widely used trap design is a deli-cup with holes or mesh for insect entrance containing a liquid drowning solution baited with an attractant (Lee et al. 2012). The baited deli-cup trap is inexpensive and easy to deploy. The main drawbacks of this design are that the bait is not specific to *D. suzukii* and requires frequent maintenance to replace the drowning solution (Lee et al. 2013). Additionally, identifying and counting flies in the liquid can be difficult and time-consuming. Kirkpatrick et al. (2018) found that a dry sticky panel or sphere trap required less maintenance and captured more flies than a deli-cup trap. However, identifying and counting flies, especially females, remains an obstacle to use of sticky traps for monitoring *D. suzukii*.

The size of the entrance or entrapment area can influence trap efficiency. For cup traps, flies must locate the holes and enter the trap to be retained in the drowning solution. Not surprisingly, cup traps with larger entry points captured more *D. suzukii* that traps with smaller holes (Cloonan et al. 2018; Lee et al. 2012; Lee et al. 2013). Thus, the greater efficiency of sticky traps may result from a greater likelihood of capturing flies that are attracted to the trap. This is consistent with the finding that larger spheres captured more *D. suzukii* than smaller spheres (Rice et. 2016). It follows that larger panels or panels with more of their surface covered with adhesive should capture more flies.

Baits and lures

Volatiles given off by plants are important cues for foraging, mating, and oviposition by many insect species. *D. suzukii* appears to use fermentation and yeast odors like other vinegar flies to locate hosts (Cloonan et al. 2018). Mated females, unmated females, and males may all use different chemical cues given off by a plant depending on their situation (Cloonan et al. 2018). In the lab, a single compound may elicit an antennal response, but in the wild it may have no effect (Cloonan et al. 2018; Bruce et al. 2005). Commercial lures for *D. suzukii* currently use blends of compounds rather than a single compound, as combining the compounds have synergistic effects on fly attractiveness (Asplen et al. 2015; Cloonan et al. 2018).

The standard baits used for monitoring *D. suzukii* are apple cider vinegar or a yeast sugar solution (Lee et al. 2012). Apple cider vinegar is a byproduct of acetic acid bacterial metabolism and is attractive to *D. suzukii* adults (Abraham et al. 2015, Cloonan et al. 2018). Yeast is necessary for adult and larval development (Cloonan et al. 2018) and its effects on oviposition response in various susceptible crops has been studied (Bellutti et al. 2017; Cloonan et al. 2018).

Synthetic lures have gradually been replacing liquid baits (Burrack et al. 2015). The commercial lures currently used (Scentry and Trécé) are loaded with a combination of 4 components identified by Cha et al. (2014): acetic acid, ethanol, acetoin, and methionol. All four components are from microbial metabolism (yeasts) (Abraham et al. 2015, Cloonan et al. 2018). This attractant blend is not specific to *D. suzukii* and traps baited with these lures capture an average of 35 percent non-target drosophilids, as all drosophilids are attracted to microbial volatiles (Asplen et al. 2015; Cloonan et al. 2018, Lee et al. 2013). In large multi-state comparisons of different commercial lures, the best lure was the Pherecon® *D. suzukii* lure suspended over apple cider vinegar, but the non-target drosophilid capture rate was more than half of all flies captured (Abraham et al. 2015, Cloonan et al. 2018; Burrack et al. 2015). A combination of 11 antenally active volatiles from homogenized raspberry extract were developed into a synthetic kairomone lure but it was not more attractive than the raspberry extract itself, which led the authors to conclude that there was something else that stimulates *D. suzukii* host-finding or oviposition behavior (Abraham et al. 2015). No sex pheromones have been found to be associated with *D. suzukii* and identifying pheromones that influence *D. suzukii* behavior is likely key to developing a better lure (Cloonan et al. 2018; Lee et al. 2012).

Trap color

Visual cues are another important sensory element that Drosophilid flies, including *D. suzukii*, use to locate a host. *Drosophila melanogaster* show a strong response to short-wavelengths withing the visible spectrum (ultraviolet to green) and less sensitivity to long-wavelength colors (red to infrared) (Paulk et al. 2013). Similarly, Little et al (2019) observed greater sensitivity of *D. suzukii* to light in the blue-green range compared to red. Kirkpatrick et al. (2015) found that *D. suzukii* male and female flies prefer to land on odorless disks that are red, purple, or black. This is not surprising as most host fruits for *D. suzukii* are red (cherries), purple (blueberries), and black (blackberries) (Asplen et al. 2015; Kirkpatrick et al. 2018). In field studies, red, black, and purple traps captured more *D. suzukii* than clear traps and white traps (Basoalto et al. 2013; Kirkpatrick et al. 2016). Lee et al. (2012) found that a red cup trap baited with apple cider vinegar captured more target insects than a clear cup trap with the same bait. Red traps overall have been consistently more effective at monitoring *D. suzukii*. However, stage and color of the crop that traps are placed in may affect which color is best for trapping *D. suzukii* (Lee et al. 2013).

Contrast appears to be an important factor in *D. suzukii* host finding. Kirkpatrick et al. (2018) created a modified yellow panel trap with a red sphere in the center of the trap baited with a commercial Scentry ® lure. This trap captured more flies than red panels baited with the same lure in raspberry high tunnels (Kirkpatrick et al. 2018). Providing contrast between background and foreground may be key to color discrimination by *D. suzukii* (Little et al. 2019). Moreover, color combinations pairing green as a background against a longer wavelength color, such as purple, was especially attractive to *D. suzukii*. They proposed that reflectance from the contrasting colors likely mimics the natural setting of fruit against foliage that flies encounter in the field (Little et al 2019).

Future needs

D. suzukii likely employs both odor and visual cues to find a host (Kirkpatrick et al. 2016; Cloonan et al. 2018). *D. suzukii* show a preference toward darker colors when odor is not a factor (Kirkpatrick et al. 2016), but these results may vary depending on the crop type that the traps are deployed in (Lee et al. 2013). Red traps with a commercial Scentry® Lure captured more *D. suzukii* than a clear cup trap with the same lure in cherry fields (Kirkpatrick et al. 2017), and yellow traps captured the most flies in black/purple fruit crops (Lee et al. 2013). These results suggest that *D. suzukii* utilizes visual cues combined with olfactory cues to determine the best location for feeding and oviposition.

There remains a lack of monitoring lures and traps that are specific for *D. suzukii* and efficient enough to accurately predict fruit infestation in most USA fruit growing regions (Abraham et al. 2015, Asplen et al. 2015; Cloonan et al. 2018). Once a single female has been captured in a trap, the fruit is often already infested, and researchers have been unable to quantify the relationship between adult trap capture and larval infestation in fruit (Asplen et al. 2015; Cloonan et al. 2018; Kirkpatrick et al. 2018). The development of reliable and easy to use commercial traps and lures would allow growers to apply insecticide treatment more efficiently and effectively (Abraham et al. 2015, Cloonan et al. 2018). Traps must target only *D. suzukii*, capture a majority of insects that come to the trap, provide early detection, and correlate capture with fruit infestation (Cloonan et al. 2018). Lure efficiency depends on the crop. For example, in northern latitudes where winter can reduce the *D. suzukii* population, traps in blueberry field have been found to detect *D. suzukii* 1-5 weeks before infestation (Cloonan et al. 2018). In warmer climates were *D. suzukii* is present all year, these lures do not predict infestation in crops (Cloonan et al. 2018). Decision-making for *D. suzukii* management should combine both an efficient trapping system with other means of predicting infestation, including fruit phenology (Kirkpatrick et al. 2018).

RESEARCH OBJECTIVES

The overall aim of this research was to develop behavioral tools that would lead to improved management of *D. suzukii* populations in cherry. To achieve this goal, two areas were investigated: 1) refinement of sticky panel traps and lures as tools for monitoring *D. suzukii* and 2) investigation of relationships between the development of sweet and tart cherry fruits and *D. suzukii* infestation. The first objective was to determine the effectiveness of commercially available *D. suzukii* lures and sticky red panel traps. The second objective was to develop *D. suzukii* panel traps with contrasting light and dark colors and determine their effectiveness and specificity for capturing *D. suzukii*. The goal was to identify an optimized trapping system for capturing *D. suzukii* while decreasing captures of non-target insects to improve reliability of monitoring traps. The third objective was to understand the relationships between cherry fruit development and *D. suzukii* infestation. Specifically, determine cultivar differences in susceptibility and the relationship between two fruit developmental characteristics; change in color and firmness, as well as the potential for *D. suzukii* to infest the fruit. The research was designed as a first step in developing models for predicting the risk of *D. suzukii* infestation in Michigan cherry.

CHAPTER 2: TOWARD OPTIMIZATION OF TRAP DESIGNS FOR SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*) IN MICHIGAN CHERRY ORCHARDS

INTRODUCTION

Spotted wing drosophila (*Drosophila suzukii* Matsumura) is a major invasive pest of soft skinned fruits, most notably cherries and various berries (Lee et al. 2012, Asplen et al. 2015). It has caused extensive losses to these crops since its first discovery in California in 2008 (Asplen et al. 2015). Unlike other Drosophilidae, *D. suzukii* females are capable of ovipositing directly into ripening fruit where hatched larvae feed on the flesh inside, making the fruit unmarketable (Asplen et al. 2015). In addition, its rapid reproductive output, wide host range from wild to cultivated plants, and its presence in virtually all fruit production regions in the US make this invasive species a very challenging pest to manage.

Detecting the presence of an insect pest and assessing population density as the season progresses are essential components of an integrated pest management program. Monitoring pest activity often is accomplished by trapping but reliability of this approach requires the availability of efficient and selective systems. Although considerable effort has been directed toward developing baits and traps for monitoring *D. suzukii*, current monitoring tools are not yet optimized (Kirkpatrick et al. 2016, 2018a). Trapping systems often capture a large number of non-target insects, making identification of *D. suzukii* difficult and time consuming. Moreover, trapping data has not been a reliable indicator of the risk of fruit infestation (Kirkpatrick et al. 2016, Wiman et al. 2014, Lee et al. 2012). Without reliable monitoring and trapping systems, fruit growers are unable to make informed decisions on whether and when to apply insecticides; instead the best they can do is to begin their spray schedule when SWD are first trapped in their area (Isaacs et al. 2013, Wiman et al. 2014, Van Timmeren and Isaacs 2013). Improving our ability to detect and monitor *D. suzukii* population would improve control decisions (Lee et al. 2015), and likely reduce the number of sprays necessary to produce salable fruit (Van Timmeren & Isaacs 2013). Therefore, more efficient traps and attractive baits are in need to implement effective and economical management programs for this devasting insect pest.

D. suzukii appears to use fermentation-based stimuli and yeast odors like other vinegar flies to locate their hosts (Cloonan et al. 2018). The baits used most frequently for monitoring *D. suzukii* are apple cider vinegar or a yeast-sugar solution. Apple cider vinegar, a byproduct of acetic acid bacterial metabolism, is attractive to *D. suzukii* adults (Abraham et al. 2015, Cloonan et al. 2018). Yeast-sugar mixtures containing baker's yeast are currently the most attractive and reliable fermentation bait but attract many other flies in addition to *D. suzukii*. A wine-vinegar mixture acted synergistically in attraction of *D. suzukii* compared to either product alone (Landolt et al. 2012). Synthetic lures based on odors from a wine-vinegar mixture was as attractive as the wine-vinegar mixture (Cha et al. 2012, 2014) and have gradually replaced liquid baits for trapping *D. suzukii*. The commercial lures currently produced by Scentry and Trécé are loaded with a combination of 4 components identified by Cha et al. (2014): acetic acid, ethanol, acetoin, and methionol. However, these lures were found to be less effective for capturing *D. suzukii* than a fermenting bait consisting of whole wheat flour, sugar, apple cider vinegar and active dry yeast in most test locations (Burrack et al. 2015).

The most widely used trap design is a deli-cup with holes or mesh to allow insects entrance and a drowning solution baited with an attractant (Lee et al. 2012). The baited deli-cup trap is inexpensive and easy to deploy. The main drawbacks of this design are that the bait is not specific to *D. suzukii* and requires frequent maintenance to replace the drowning solution (Lee et al. 2013). Additionally, identifying and counting flies in the liquid can be difficult and time-consuming. Kirkpatrick et al. (2018) found that a dry sticky panel or sphere trap required less maintenance and captured more flies than a deli-cup trap. However, identifying and counting flies, especially females, remains an obstacle to use of sticky traps for monitoring *D. suzukii*.

Visual cues also are used by Drosophilid flies, including *D. suzukii*, to locate hosts (Aluja & Prokopy 1993, Borst 2009, Bruce et al. 2005, Hardie 1986, Katsoyannos and Kouloussis 2001, Kirkpatrick et al. 2016). Color vision in *Drosophila melanogaster*, a sister species of *D. suzukii*, has been studied extensively (Borst 2009, Little et al. 2019). Drosophila species have a high level of sensitivity to the ultraviolet region (350 nm), blue to blue-green (450-490 nm), and green to yellow region (520-600 nm) (Shields 1989); but are less sensitive to light of longer wavelengths such as orange, red, and infrared (Kelber & Henze 2013, Menne & Spatz 1977). Similarly, Little et al. (2019) observed greater sensitivity of *D. suzukii* to light in the blue-green range compared to red. Previous research has demonstrated that trap color significantly affects *D. suzukii* capture. Lee et al. (2012) found that a red cup trap baited with apple cider vinegar captured more target insects than a clear cup trap with the same bait. Later, Lee et al. (2013) found that a combination of yellow and red traps enhanced *D. suzukii* capture. Kirkpatrick et al. (2018) found that red, purple, and black traps caught more *D. suzukii* than other colored traps in the field. This is not surprising as most host fruits for *D. suzukii* are red (cherries), purple (blueberries), and black (blackberries) (Asplen et al. 2015; Kirkpatrick et al. 2016).

Contrast appears to be another important factor in *D. suzukii* host finding. Kirkpatrick et al. (2018) created a modified yellow panel trap with a red sphere in the center baited with a commercial Scentry® lure. This trap captured more flies than red panels baited with the same lure in raspberry high tunnels (Kirkpatrick et al. 2018). Providing contrast between background and foreground may be key to color discrimination by *D. suzukii* (Little et al 2019). This research team observed that color combinations pairing green as a background against a longer wavelength color, such as purple, was especially attractive to *D. suzukii*. They proposed that reflectance from the contrasting colors likely mimics the natural setting of fruit against foliage that flies encounter in the field (Little et al 2019).

Additionally, male and female flies may respond to color differently depending on their biological state at the time they encounter a trap. In some species, females are more likely to be attracted to colors that mimic preferred oviposition sites compared with males responding to colors that mimic feeding sites (Katsoyannos and Kouloussis 2001, Hardie 1986). For example, yellow and orange spheres captured the most male *Bactrocera oleae* (olive fruit fly), but more females of the same species were drawn to red and black spheres (Katsoyannos and Kouloussis 2001). *Mucosa domestica* (common housefly) males have a region of photoreceptors that are specialized for the analysis of polarized light in the sky which is used to find females in flight. This may explain differences in male and female captures in traps (Hardie 1986). The apparent attraction to purple and black colors by female *D. suzukii*, may be due to their similarity to the color of preferred oviposition sites (Takahara and Takahashi 2016, Kirkpatrick et al. 2016).

The aim of this study was to identify an optimized trapping system for capturing *D. suzukii* while decreasing captures of non-target insects to improve monitoring and trapping efficiency. The specific objectives were 1) compare the effectiveness of three sticky red panel traps designed for monitoring *D.* suzukii, 2) develop *D. suzukii* panel traps with contrasting light and dark colors and determine their effectiveness and specificity for capturing *D. suzukii* and 3) to compare the effectiveness of commercially available lures that could be used in combination with a dry, color-based trap.

MATERIALS AND METHODS

Experiment 1: Comparison of various sticky red panel traps to a standard cup trap

This study was conducted in the summer of 2018 over a period of 10 weeks from May 27 to August 5. Traps were placed in 12 tart cherry orchards, 6 in southwest and 6 in northwest Michigan. Four types of traps were place in each orchard, 3 sticky red panel traps that differed in hue and a standard deli-cup trap. The three red sticky panels were 1) a 23 cm long and 14 cm wide red panel with an adhesive area of 17 cm long by 10 cm wide in the center (standard red), 2) the same red panel but with additional adhesive added to the existing sticky area using sprayable Tanglefoot® (Tanglefoot® Company, Grand Rapids, Michigan) 3 cm from each side (enhanced red), and 3) a 23 cm long and 14 cm wide burgundy panel (Scentry® Biologicals Inc., Billings, Montana) that is coated with an adhesive in the central 10 cm x 15 cm area that remains tacky at temperatures down to 0°F (burgundy) (Fig. 1). Cup traps were clear plastic deli cups (473 mL, Gordon Food Service, Grand Rapids, Michigan) with 12-0.5cm holes drilled around the upper rim of the cup and contained 100mL unscented soapy water (0.1%, Seventh Generation Natural Dish Liquid, Seventh Generation, Inc.) in the bottom of the cup as a drowning solution. All traps were baited with a Scentry® Spotted Wing Drosophila Lure (Scentry® Biologicals Inc.) either attached to the upright short side of the panel or hung above the drowning solution. The six traps were randomly placed along the edge of the orchard in the second row from the wood edge with two or three trees between each trap type depending on tree spacing (at least 10 m apart). Traps were hung ca. 2 meters from the ground at the base of the canopy of cherry trees surrounded by foliage and fruit, but not touching the trap. Panel traps were replaced weekly and the drowning solution in the cup traps was replaced weekly. Traps were transported back to the laboratory and the number of male and female *D. suzukii* flies were counted under a dissecting scope.

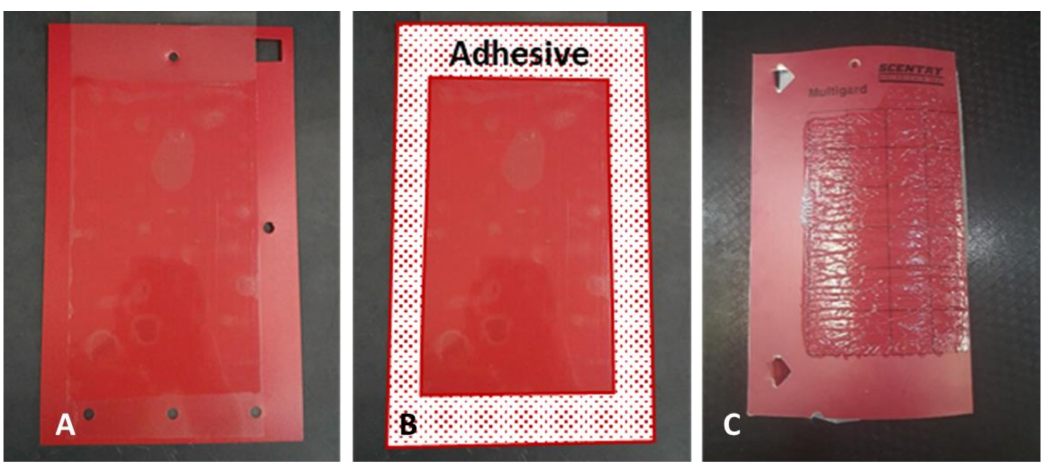

Figure 2.1. Three different red hued sticky panel style traps. Standard red panel style sticky trap (A), enhanced red panel style sticky trap (B), both measuring 23 cm long and 14 cm wide. Burgundy panel style sticky trap (C) with cool temperature adhesive measuring ca. 23 cm x 14 cm with adhesive (ca. 15 cm x 10 cm) centered toward one side.

Experiment 2: Comparison of 3 commercially available D. suzukii lures

This experiment was conducted in the summer of 2018 over a period of 10 weeks from May 29 to August 1. Traps were placed in the same 12 tart cherry orchards, 6 in southwest and 6 in northwest Michigan as used for the panel comparison. Standard deli-cup traps as described above were baited with one of three different lures hung inside using a paperclip glued to the lid (Fig. 2). The three lures were 1) a Scentry® Spotted Wing Drosophila Lure (Scentry® Biologicals Inc.), 2) a Trécé PHEROCON® SWD Spotted Wing Drosophila Broad Spectrum Lure (Dual) and 3) a Trécé PHEROCON® SWD Peel-Pak Lure (Trécé Inc., Adair, Oklahoma) (Gel) (Fig. 2). Traps were serviced by removing and replacing soapy water weekly, and trap contents were transported to the laboratory where the number of male and female *D. suzukii* were counted under the dissecting scope.

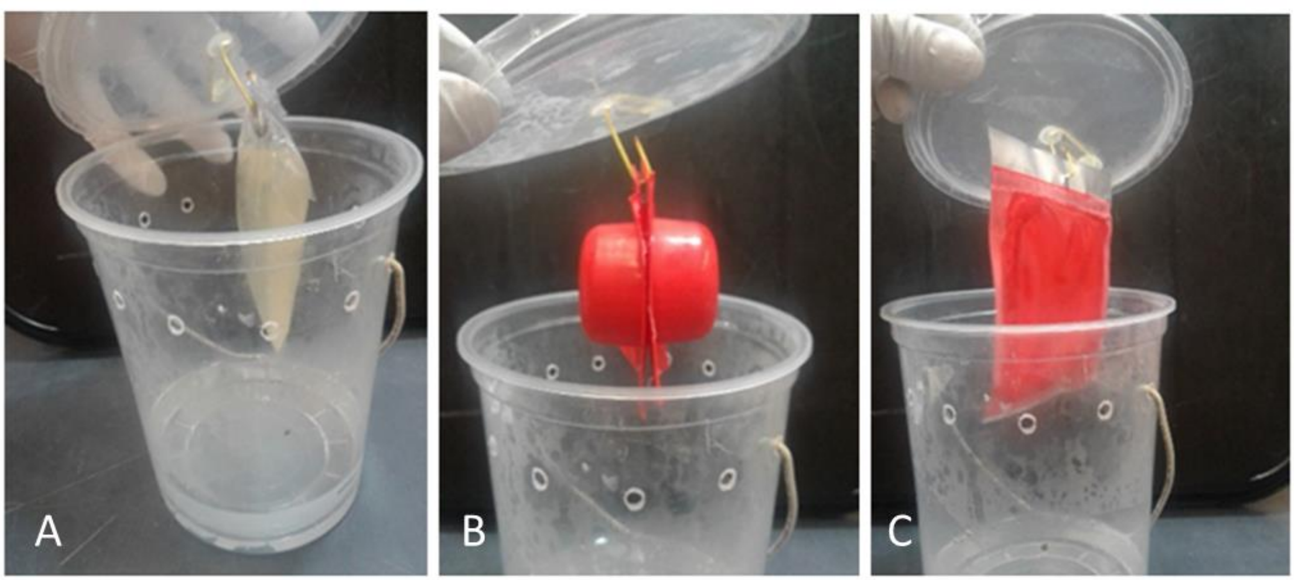

Figure 2.2. Three different commercially available *D. suzukii* lures tested in cup-style insect traps. Each trap was baited with one of the three different types of lures: A) Scentry Spotted Wing Drosophila Lure (Scentry®), B) Trécé PHEROCON® SWD Spotted Wing Drosophila Broad Spectrum Lure (Dual), and C) Trécé PHEROCON® SWD Peel-Pak Lure (Gel).

Experiment 3: Comparison of sticky panel traps varying in color and design

In order to investigate the color and pattern preference of *D. suzukii* in the field, sticky panel traps were custom-made in various colors and patterns and compared for their ability to capture flies in an experiment conducted from June 5 to Aug 13, 2019. The seven rectangular (13.2 x 19.8 cm) plastic traps tested were a red panel (Red), green panel (Green), yellow panel (Yellow), green and purple checkered panel with unit squares of 6.6 x 6.6 cm (G & P Checkered), yellow and red checkered panel (Y & R Checkered), yellow with a red circle in the center (7.5 cm diam.) (Y & R Circle), and green with a purple circle in the center (7.5 cm diam.) (G & P Circle) (Fig. 3). Both sides of each panel had the same design and were coated with Tanglefoot glue (Tanglefoot® Company, Grand Rapids, Michigan) to ensnare insects. The seven sticky panel traps along with a standard clear plastic cup trap were baited with Scentry® *D. suzukii* lures and deployed in a randomized complete block design with each of the eight traps replicated in six tart cherry orchards in Southwest Michigan. Traps were hung in the perimeter row of trees from a shaded branch in the bottom of the canopy approximately 2 m from the ground, spaced at least 10 m apart. Each panel and cup trap were replaced weekly and transported to the laboratory for accurate counts of male and female *D. suzukii* and non-target Drosophilidae under a dissecting scope. Scentry® commercial lures were changed every 4 weeks. Due to frequent chemical sprays disrupting the experiment, all the traps were taken down after three weeks on 3 June prior to harvest and deployed again for three weeks after harvest on 24 June. Trap data before harvest was considered as early season capture; while trap data after harvest was presented as late season capture while unharvested cherries remained on the trees.

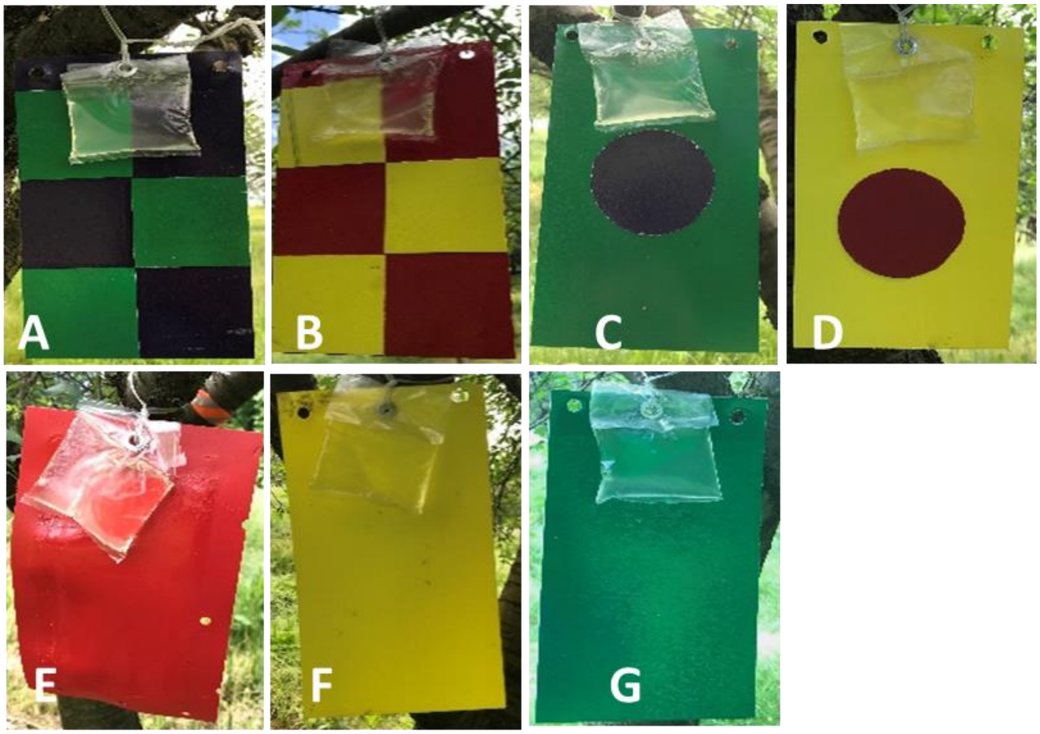

Figure 2.3. Contrasting Color Traps. Plastic cards measuring ca. 14 cm x 24 cm, coated with insect adhesive and painted in contrasting colors in checker-board pattern (check) (A and B) or with single circular dot (circle) (C and D) in the center. Squares of color measured ca. 7 x 8 cm. and circle diameter 7 cm. Single color sticky cards in E) red, F) yellow, and G) green. All panels were deployed with short side up and Sentry® lure affixed at the top edge.

DATA ANALYSIS

All statistical analyses were performed in R Studio R-3.5.3 (R Studio Team 2015, Manufacturer, Boston, Massachusetts, United States of America). Trap data from 2019 were split and analyzed separately as early (June 5 to July 3) and late season captures (July 31 to August 13), due to lack of data because of frequent insecticide sprays during the mid-season and the growers desire to not have traps present during harvest. Due to violations of the assumption of normality, the data were modeled with generalized linear modeling ("glm") function in R. Tests of primary effects were conducted using Analysis of Deviance with a Gaussian model function and identity link functions. Means separation was conducted using a modified Tukey's for generalized hypothesis testing ("glht") function in the R package "multicomp") (Hothorn et al., 2008). Results of analytical tests were considered statistically significant at $\alpha = 0.05$.

RESULTS

Experiment 1: Comparison of various sticky red panel traps to a standard cup trap

There were significant differences in total *D. suzukii* trap captures among the burgundy panel, standard red panel, enhanced red panel, and the cup style trap ($df = 3, 44$; $F = 2.672$; $p = 0.059$) (Fig. 2.4). No significant difference was detected between total *D. suzukii* captured on the standard red panel compared to the burgundy panel ($p = 0.471$) and the standard red panel versus the enhanced red panel ($p = 0.649$). There was a significant difference between total *D. suzukii* capture on the enhanced red panel versus the burgundy panel ($p = 0.045$). The three panel traps and the cup trap all captured similar numbers of male *D. suzukii* ($df = 3, 44$; $F = 2.199$; $p = 0.102$). There was a significant difference in female *D. suzukii* captures among the three panel traps and the cup style trap ($df = 3, 44$; $F = 5.912$; $p = 0.002$). No significant difference was detected between female *D. suzukii* captured on the enhanced red panel compared to the burgundy panel ($p = 0.052$), standard red panel ($p = 0.342$), and the cup style trap ($p = 0.560$). There was significantly more female *D. suzukii* captured in the cup style trap compared to the burgundy panel ($p < 0.001$) and the standard red panel ($p = 0.016$).

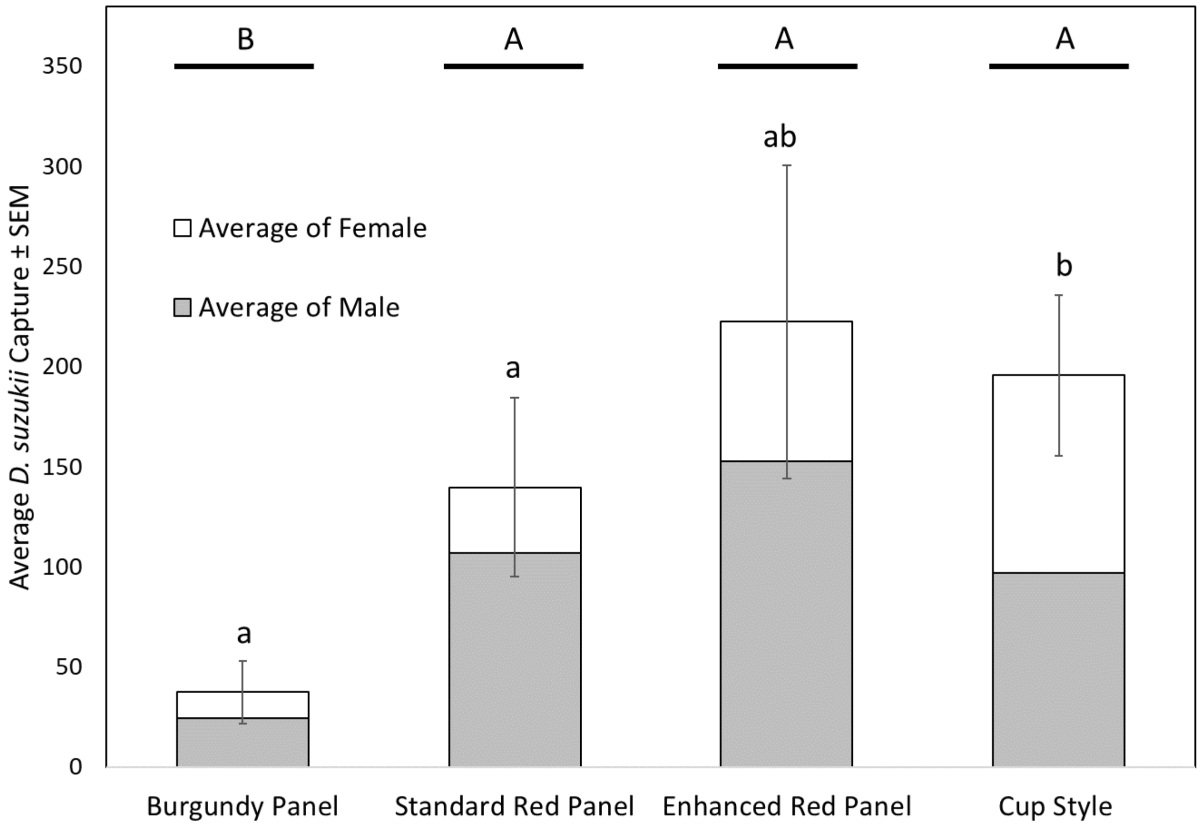

Figure 2.4. Mean (± standard error of the mean) *D. suzukii* captures over 10 weeks on 3 different types of *D. suzukii* panels (burgundy, standard red panel, enhanced red panel) and cup traps baited with a Scentry® lure. Bars topped with common capital letters do not vary significantly between total *D. suzukii* capture, and bars topped with common lower-case letters do not vary significantly between female *D. suzukii* capture ($\alpha < 0.05$).

Experiment 2: Comparison of 3 commercially available D. suzukii lures

There were significant differences in the total *D. suzukii* captures among the commercial lures tested in this experiment (df = 2, 33; F = 4.700; p = 0.016) (Fig. 2.5). Similarly, statistically significant differences were found among the tested lures in male captures (df = 2, 33; F = 5.034; p = 0.013) (Fig. 5), but not in female captures (df = 2, 33; F = 3.048; p = 0.061). The commercial Scentry® lure attracted significantly more male *D. suzukii* over the course of this experiment compared to the dual lure (p = 0.004) and the gel lure although the latter relationship was not statistically significant (p = 0.143). The commercial Scentry® lure also attracted more female *D. suzukii* over the course of this experiment compared to dual lure (p = 0.036) and the gel lure, however only the former was statistically significant (p = 0.369). The gel lure caught more male *D. suzukii* (p = 0.414) and more female *D. suzukii* than the dual lure (p = 0.503) (Fig. 5) but these differences were not statistically significant.

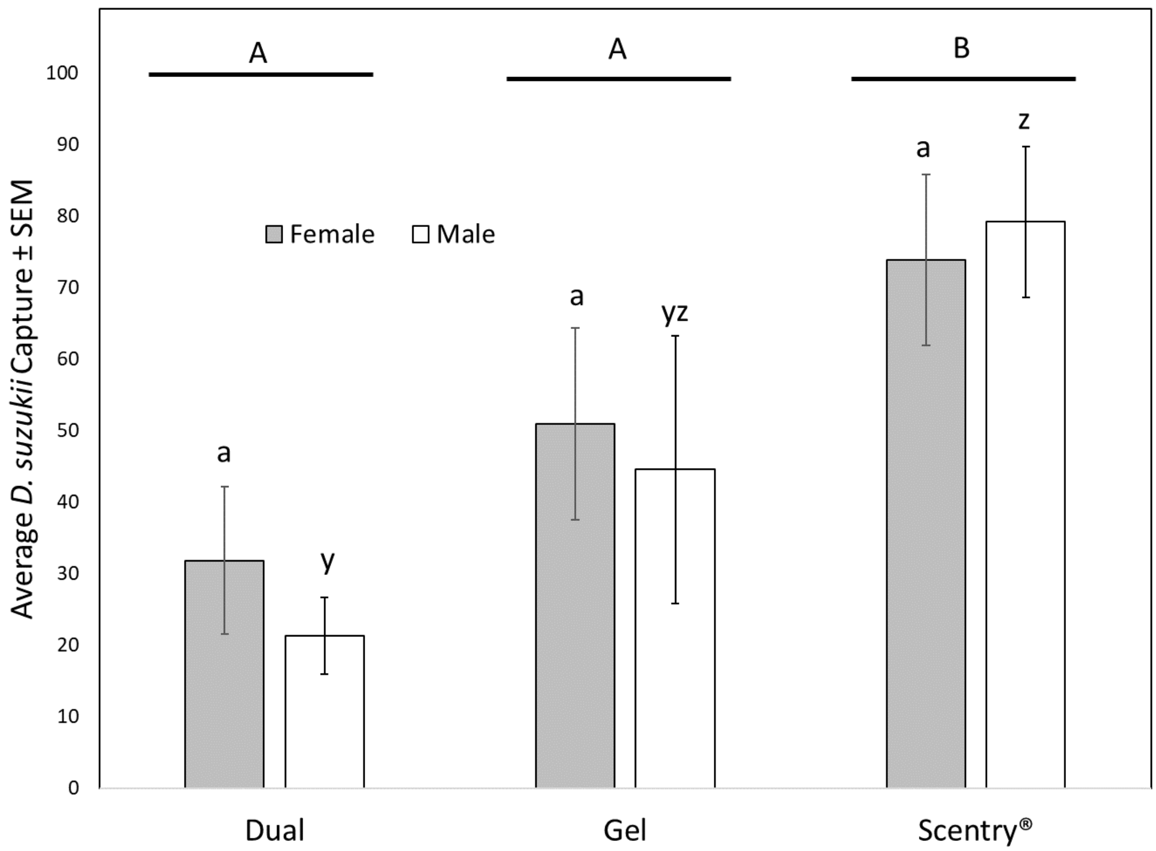

Figure 2.5. Average *D. suzukii* (± SEM) captured over 10 weeks of trapping at 12 different sites with three different types of lures: Scentry®, Trécé® PHERECON SWD Broad Spectrum Lure (Dual), and Trécé® PHERECON SWD Peel-Pak Lure (Gel). Bars topped with common letter do not vary significantly ($\alpha < 0.05$). Capital letters indicate differences in total catch, while lowercase letters indicate differences in capture of each sex.

Experiment 3: Comparison of sticky panel traps varying in color and design

Significant differences were detected in total fly captures among the trap designs during the early season (*df* = 7, 220; *F* = 2.997; *p* = 0.005) (Fig. 2.6). A total of 31 *D. suzukii* were captured over the course of the early trapping period (June 5 to July 3) with the first fly captured on the yellow panel during the week of June 12. The cup trap captured significantly more flies than all of the different panel trap designs during this early season portion of the experiment (Fig. 6). The green panel with a purple circle in the center and solid yellow or green panel captured numerically more *D. suzukii* than the other panel traps, but the difference in catch was not statistically significant. The red panel trap captured zero *D. suzukii* in the early season.

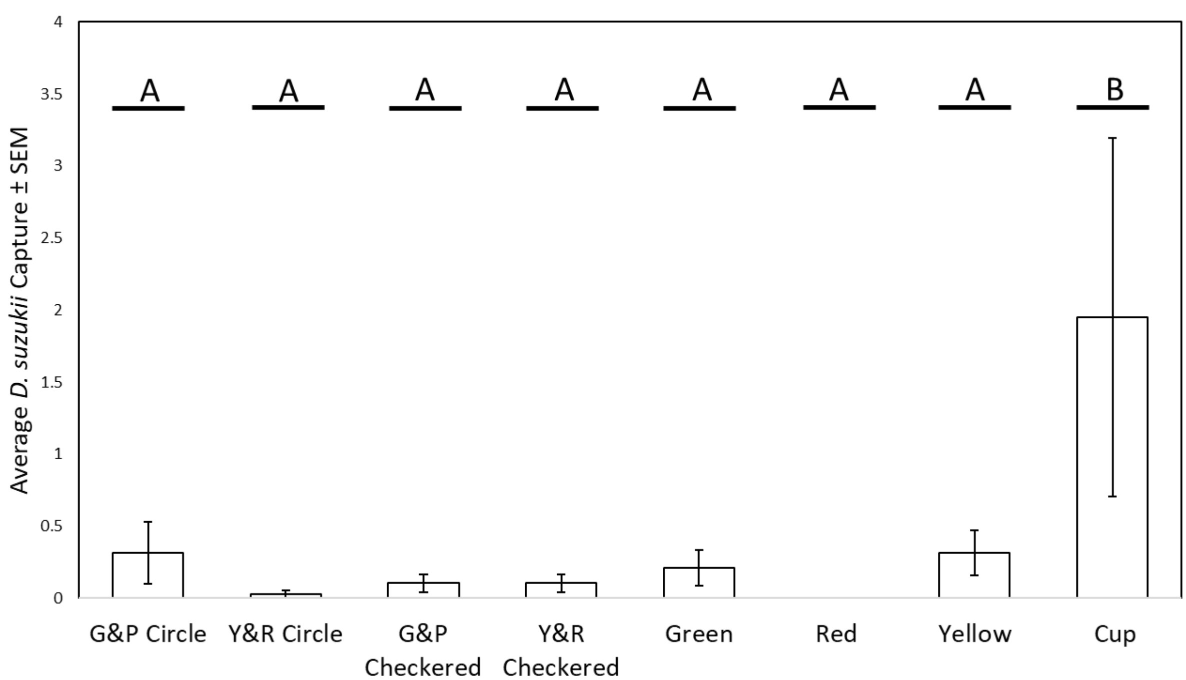

Figure 2.6. Total mean number of *D. suzukii* captured by panel and cup traps during the early season (June 5 to July 3) (G = Green, P= Purple, Y= Yellow, R = Red). Bars topped with same letter are not significant at α = 0.05.

Significant differences were detected in total fly captures among the trap designs during the late season (*df* = 4, 172; *F* = 10.441; $p < .001$) (Fig. 7). A total of 28,841 *D. suzukii* were captured during the late season trapping period (June 24-July 15). Statistically the trap designs that captured the most during the late season were the solid green, solid yellow, Y & R circle, and cup trap, however there no significant differences among these treatments ($p > 0.05$). The lowest number of flies were captured on solid red, yellow and red checkered, and G & P circle, but again there was no significant differences among these treatments.

Significantly more flies were captured on the green panel trap than on the red panel ($p < 0.001$), the G & P circle and both checkered traps (G & P circle, $p < 0.001$; G & P checkered, $p < 0.001$; Y & R checkered, $p = 0.022$) (Fig. 7). Significantly more flies were captured on the yellow panel trap than on the red panel and the G & P circle (red, $p < 0.001$; G & P circle, $p = 0.011$). Captures in the cup trap were statistically equivalent to captures in all the panel traps. There were no significant differences in *D. suzukii* captures between any of the patterned traps (checkered or circle). We noticed a strong male bias in trap capture with males accounting for 83% of *D. suzukii* capture and females accounting for 17% of capture.

The average capture of *D. suzukii* when proportioned by the area covered by a particular color is presented in Fig. 2.8. We detected significant differences in *D. suzukii* capture per square centimeter between panel trap color patterns (*df* = 6, 178; *F* = 5.811; $p < 0.001$) (Fig. 8). Examining captures in this manner revealed that significantly more *D. suzukii* were captured on the red circle portion of the Y & R circle panel than the yellow portion ($p = 0.037$). In contrast, similar numbers of flies were captured on the green panel and purple circle portions of the G & P circle trap ($p = 0.243$). For the checkered traps, there also were no significant differences in catches in the areas covered by each color (G & P checkered, p = 0.738; Y & R checkered, p = 0.265).

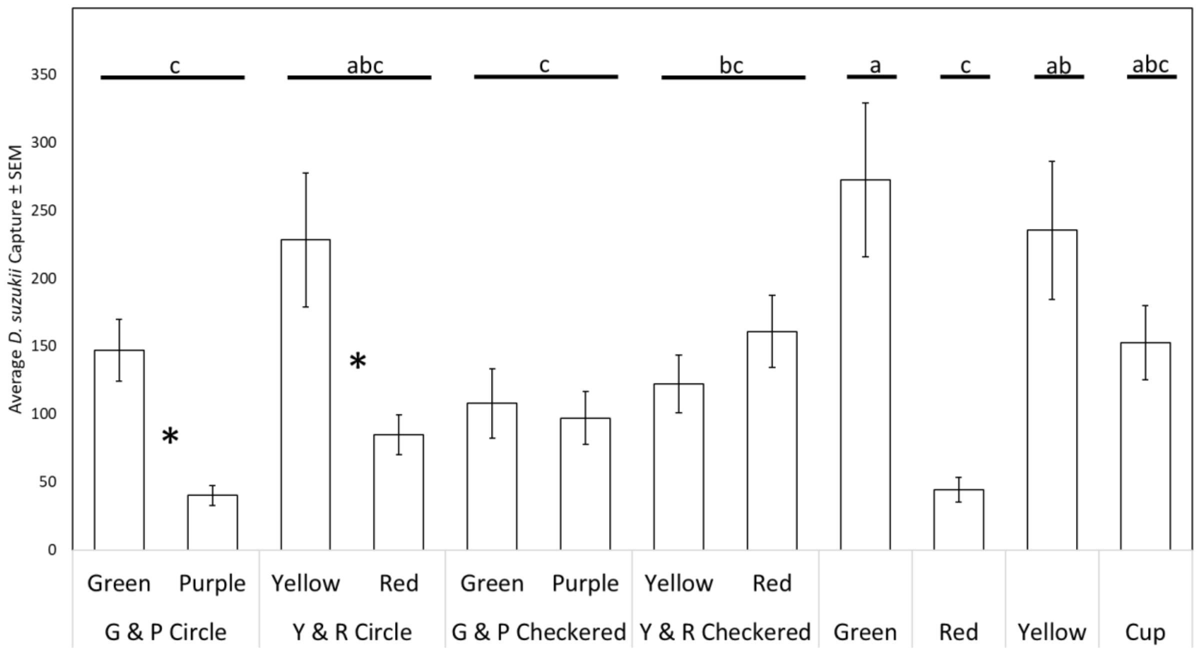

Figure 2.7. Average (± SEM) number of *D. suzukii* captured on colored panels and cup traps in a field experiment during the late season. Bars topped with a common letter do not vary significantly (p = 0.05), and bars topped with * vary within trap color capture.

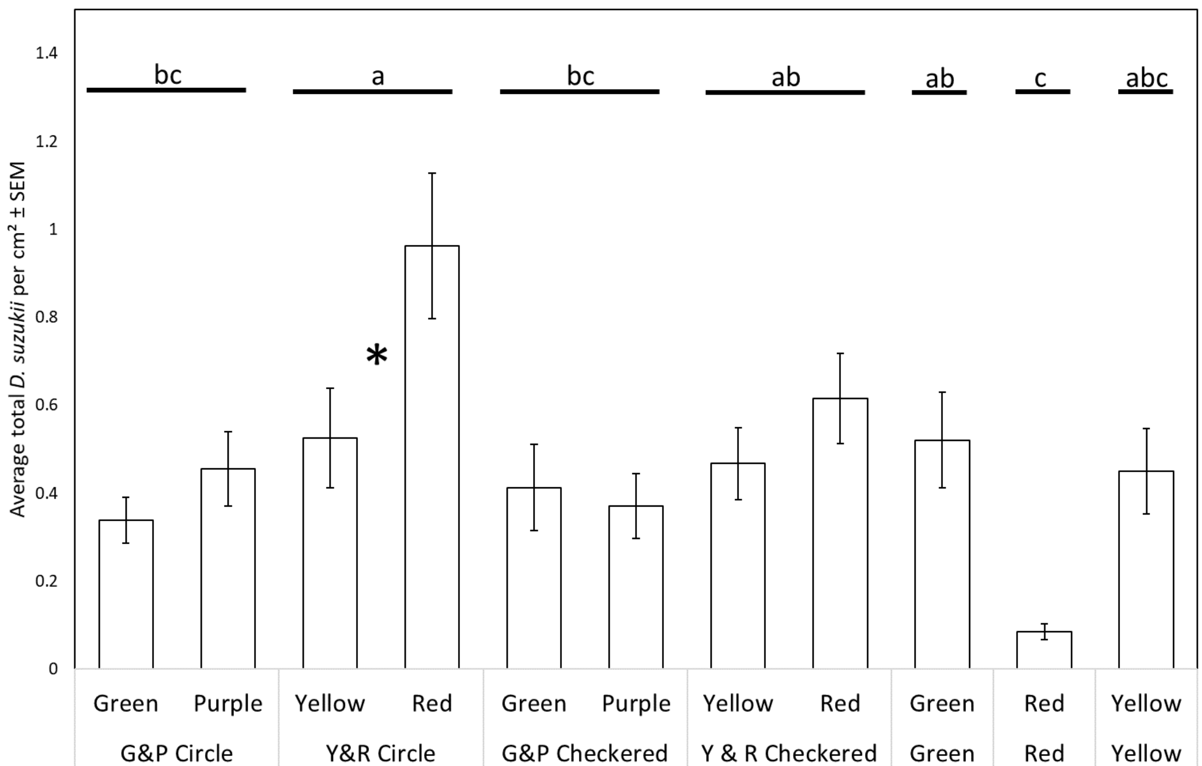

Figure 2.8. Average number of *D. suzukii* per cm^2 of each color on seven different colored panels in a field experiment over a three-week period after cherry harvest. Bars topped with a common letter do not vary significantly (p = 0.05), and bars topped with * vary within trap color capture.

In the 2019 experiment, I also assessed the number of non-target drosophila and similar looking flies captured on the various traps (Fig. 2.9). Significant differences were found among trap types in the early season (df = 7, 275; F = 9.628; p < 0.001) as well as during the post-harvest trapping period (df = 7, 195; F = 3.457; p = 0.002). There were significantly more non-target Drosophila species captured in early season than the late season (p = 0.003). No significant differences were detected in non-target flies captured between any of the trap types during the early season. Cup traps captured significantly more non-targets during the later season than any of the panel traps tested (p = 0.013).

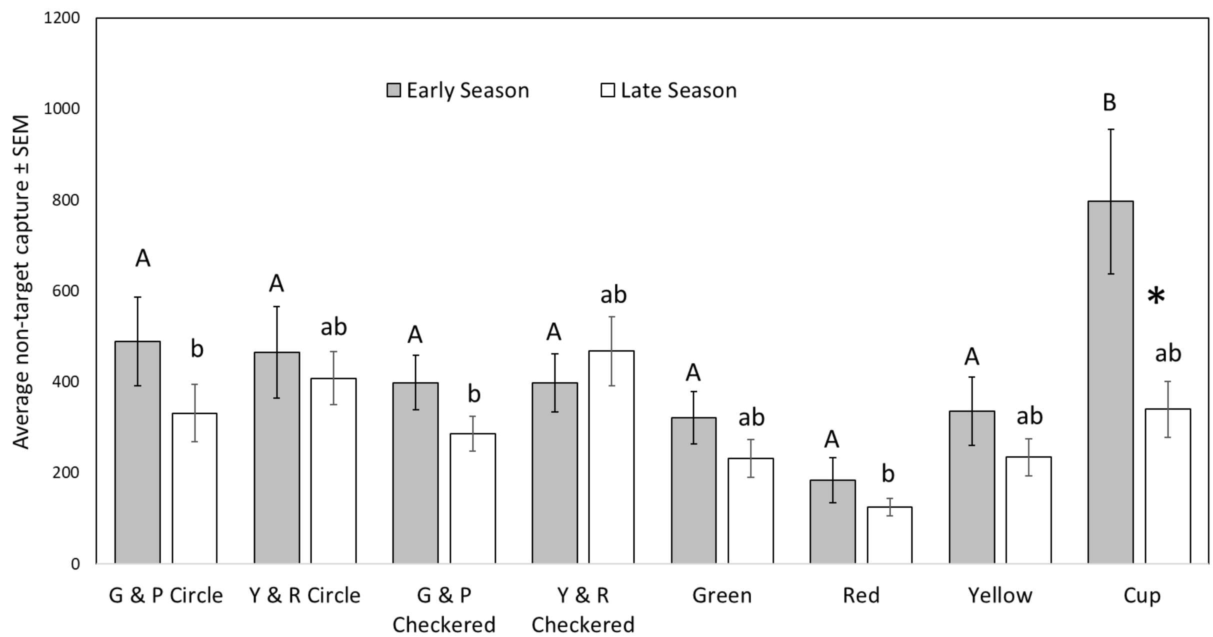

Figure 2.9. Average (± SEM) number of non-target Drosophila species captured early and late season on colored panels and in cup traps. Bars topped with common letter do not vary significantly (p = 0.05). Early season variance is defined by upper case letters, and late season variance is characterized by lower case letters. Bars topped with an * differ within a trap treatment vary between early and late season captures.

DISCUSSION

Although a single "best" *D. suzukii* trapping system was not identified, these results provide valuable insights that should advance the development of effective dry traps for this highly problematic invasive pest. In 2018, the enhanced red panel trap captured numerically more flies than the standard red panel or the deli-cup trap. Thus, the efficiency of a sticky red panel trap was enhanced when the entire surface rather than just the central portion of the trap was coated with adhesive. Previous research by Lee et al. (2012), also found that that traps with a greater surface area captured more flies. However, there were drawbacks to the red panel trap fully coated with adhesive that should be considered when developing a commercial sticky panel trap. The enhanced red panel was more difficult to handle and adding adhesive to the outer edges by hand was time-consuming. Additionally, in the absence of any non-sticky areas the lure tended to become tangled on the trap, making it difficult to replace the panel each week. I suggest that a commercial trap should cover most of the trap with adhesive, but there should be a small area near the top that is free of adhesive.

Identifying flies stuck to the trap was a major drawback of all models of sticky panel traps tested during both seasons. Positively identifying *D. suzukii* flies was difficult and time-consuming, especially during hot periods when the flies desiccated more quickly. Males were easier to find than females, as one could readily see the dark spot on the wing and identification could be done in the field. Females were very difficult to identify in the field. Positive identification generally required examining the panel trap with the aid of a dissecting microscope and manipulating flies on the trap with an insect pin to locate the serrated ovipositor. Searching for male *D. suzukii* on panel traps typically required less time than it did to search for them in the drowning solution used to snare flies in the cup trap. While *D. suzukii* flies captured in the deli-cup trap had to be filtered out of the liquid, sorted and identified underneath a microscope, male flies trapped on the panel trap usually could be located and identified with a hand lens in the field. It also took more time to count *D. suzukii* on the sticky panels because there were also many non-target insects that had been unintentionally captured, since the lures and traps were not specific to *D. suzukii*.

A major difference between the panel style traps and the cup style traps that influences their trapping efficiency is the ease by which insects can be retained. While insects landing anywhere on the panel coated with adhesive are likely to be captured, only flies entering the small holes (<0.5 cm) at the top of the cup have a chance of being captured. Retaining insects in the cup trap requires that they find the entry point and fall into the liquid in the bottom of the trap.

Commercial lures based on the Cha blend (Cha et al. 2014) present the best option for being used in combination with a colored panel trap to monitor *D. suzukii*. The Scentry® *D. suzukii* lure captured substantially more flies than the two other commercial lures tested. One possible explanation for the higher catch may be a higher release of attractant volatiles. As mentioned by Lee at al. (2012), traps with larger volatile release capture more flies. The Scentry lure releases volatiles from the entire surface of the lure, whereas the Trécé® PHERECON SWD Broad Spectrum Lure (Dual) only released from the center of the lure, and Trécé® PHERECON SWD Peel-Pak Lure (Gel) volatiles only released from one side of the lure. The components which make up the commercial lures are from microbial metabolism (yeasts), and the combination of these components has a synergistic effect on fly attractiveness (Abraham et al. 2015; Asplen et al. 2015; Cloonan et al. 2018). Unfortunately, high number of non-targets were attracted to all the lures. More research is needed to find the volatiles that are specific to *D. suzukii*.

Based on the finding of Little et al. (2019) that contrast appears to be an important factor in *D. suzukii* host finding, panel traps with contrasting colors and patterns in an effort to improve trap performance were tested. None of these traps captured significantly more flies than single color panel traps. This is contrary to the findings of previous research examining the potential of contrast for enhancing *D. suzukii* captures in traps. A modified yellow panel trap with a red sphere in the center of the trap baited with a commercial Scentry® lure captured more flies than red panels baited with the same lure in raspberry high tunnels (Kirkpatrick et al.

2018). Little et al. (2019) tested several color combinations and found that pairing green as a background against purple was especially attractive to *D. suzukii*.

How the contrasting cues are presented appears to be important. The checkered pattern did not increase captures of *D. suzukii*. However, a yellow background with a central red circle did show promise for increasing trap performance. When fly captures in this study were expressed on a per unit area basis, a significant number of *D. suzukii* were captured in the red portion of the trap. The flies apparently were able to recognize the central dark circle. Unfortunately, this area only represented a small portion of the total trapping area. Thus, total captures on the trap were not significantly elevated. In the study conducted by Kirkpatrick et al (2018) the red sphere in the center of the yellow panel trap was large, comprising nearly the same trapping area as the solid yellow panel. Future traps that combine a panel and central area with contrasting colors should be designed such that the two parts of the traps are nearly equal in size.

We concur with Little et al. (2019) that reflectance from the contrasting colors likely mimics the natural setting of fruit against foliage that flies encounter in the field. The colored and patterned panel traps caught very few flies early in the season when fruit and foliage were green, and flies were likely not searching for hosts to oviposit in. As cherries ripen, the fruit turns from green to red, while the leaves in the background remain green. *D. suzukii* uses volatile cues from the yeasts that grow on the ripening fruit to find preferential oviposition sites, as they are necessary for larval development (Cloonan et al. 2018). The trapping results late in the season showed that *D. suzukii* were attracted to the red circle against the yellow panel. The contrast likely mimicked that of the dark ripening fruit against the lighter-colored foliage.

Since *D. suzukii* show high sensitivity to short wavelength colors, it is unsurprising that the most were captured on the plain green sticky panel than any of the other traps. Similar to the results of Lee et al. (2013) and Kirkpatrick et al. (2016), many *D. suzukii* also were captured on the yellow panel. These results support the conclusion of Little et al (2019) that *D. suzukii* flies are responding to the wavelength of light reflected off the traps rather than color. In this study the red panel traps captured the fewest *D. suzukii*, which contradicts the high captures in the red panel trap reported by Kirkpatrick et al. (2016, 2018). *D. suzukii* does show low sensitivity to long-wave colors, which may be why captured on red panels was so low. Additionally, the red panel traps used and those tested by Kirkpatrick et al (2017) were not produced with the same red color. It is possible that the traps used by Kirkpatrick et al. (2017) reflected a low wavelength of light and were thus attractive to *D. suzukii*. Similar reasoning may explain why the purple panel with a green central circle did not capture the highest number of flies as was predicted by the results of Little et al (2019). In this case, the purple used to construct this trap may not have reflected the low wavelength needed to contrast against the higher wavelength given off by the yellow panel. Future studies should be sure to measure the UV wavelengths given off by the various traps.

Finding the best trap to monitor for *D. suzukii* can lead to better spray timing and save growers money by eliminating the need for unnecessary insecticide applications (Van Timmeren and Isaacs 2013). Determining the best color for the trap depending on crop type, specificity of the lure, and ease of *D. suzukii* identification are a few of the next steps toward managing *D. suzukii*. For now, *D. suzukii* can be detected reliably in the field using a bright colored sticky panel with a dark circle in the middle or a cup style trap with a commercial Scentry® lure about as well as a deli-cup trap with a bait incorporated into the drowning solution. However, until a threshold can be determined that relates trap numbers to fruit infestation, fruit should be collected periodically and tested for *D. suzukii* larvae to determine whether the management program being used is effective (Van Timmeren and Isaacs 2013). Creating a model that uses fruit ripeness stage and bloom date combined with trapping and monitoring efforts may be the best option for growers to time treatments and defend against *D. suzukii*.

CHAPTER 3: SUSCEPTIBILITY OF SWEET AND TART CHERRY CULTIVARS TO INFESTATION BY *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

INTRODUCTION

Spotted-wing drosophila, *Drosophila suzukii,* Matsumura has become a significant pest of cherries and cane berries in the US since its introduction in 2008 (Asplen et al. 2015). It was first discovered in California and quickly spread throughout the US including Michigan in 2010 (Lee et al. 2016). It has been estimated that this invasive pest cost US fruit industries approximately $421.5 million in damages in 2013 as a result of up to 50% yield losses (Farnsworth et al. 2017). In Michigan in 2010, *D. suzukii* cost small fruit growers an estimated $25 million in losses (Jones and Rothwell 2018). Losses of 20-25% of the tart cherry crop were reported in Michigan in 2016-2019 (N. Rothwell, unpublished). Michigan sweet cherries incurred substantial losses due to *D. suzukii* infestation for the first time in 2019.

Unlike its close relative *Drosophila melanogaster*, *D. suzukii* can attack and feed on undamaged, ripening fruit. Female *D. suzukii* has a serrated ovipositor not found in other vinegar fly species, which can puncture the skin of soft fruit to facilitate oviposition. Female *D. suzukii* can lay up to 25 eggs a day under favorable conditions, and up to 400 eggs in her lifetime (Asplen et al. 2015, Hamby et al. 2016). *D. suzukii* damage occurs when larvae feed inside fruit, while the oviposition scar may leave the fruit vulnerable to invasion by bacteria and other pathogens (Asplen et al. 2015).

Sweet cherries (*Prunus avium*) and tart cherries (*Prunus cerasus*) are among the fruit crops that *D. suzukii* likes to attack. Indeed, previous research has shown that *D. suzukii* has a higher reproductive rate on sweet cherries than on blueberries (Lee et al. 2011). Michigan is a major producer of both tart and sweet cherries. The state is the nation's leader in tart cherry production, generating about 60% of the nation's total domestic production valued at $59M annually (USDA NASS, June 2019). The vast majority of tart cherries grown in Michigan and elsewhere in the United States are the high-yielding 'Montmorency' cultivar. 'Montmorency' [WJ1]trees yield a large crop in most years but are susceptible to spring frost and cherry leaf spot, a disease that can shorten their lifespan (Iezonni 2005). The other tart cherry cultivar grown in Michigan, 'Balaton', was developed in the Michigan State University breeding program with the aim of developing a high-quality tart cherry that was less sensitive to spring frost and diseases (Iezonni 2005). Michigan ranks 4th in sweet cherry production, with about 6,700 bearing acres generating $13.M in production value annually. Sweet cherries in Michigan are primarily grown for the processing market. Three of the principal cultivars are 'Gold', 'Ulster' and 'Emperor Francis'. 'Gold' is a white fleshed variety with no red pigment, which makes it a good candidate for brining, and a ripening date of mid-July (Brown et al. 1989). 'Ulster' is a dark, nearly black cherry when ripe and ripens around the same time as 'Gold' (Brown et al. 1989). 'Emperor Francis' is a bright red and firm cherry when ripe, ripening 3 to 5 days earlier than the other cultivars (Brown et al. 1989).

With a zero tolerance for larval presence in marketable fruit, Michigan cherry growers heavily rely on insecticide sprays to protect their valuable crops from *D. suzukii* infestation. The current management strategies are to monitor for this invasive pest and once it is found, chemical applications are initiated. Currently, the recommendations are to spray an insecticide every 7 days when the weather is dry, and then spray again after rain (Wilson et al. 2019). Van

Timmeren and Isaacs (2013) showed that even with high levels of adult mortality from sprays, the larvae are still able to grow and develop inside blueberry fruit. The later the cherries are harvested the more likely they are to be attacked by *D. suzukii*. The challenge to control *D. suzukii* is exacerbated by insecticide pre-harvest intervals; insecticide applications cannot be made within certain days of cherry harvest, and thus as harvest approaches fruit can be vulnerable to *D. suzukii* infestation. The continued use of insecticides as the only option for *D. suzukii* control is not a sustainable long- term option (Wiman et al. 2014, Yeh et al. 2020). Intensive use of insecticides is not only costly but has led to other pest management concerns such as insecticide resistance, risks to natural enemies and, and secondary pest outbreaks (Asplen et al. 2015; Cloonan et al. 2018; Leach et al. 2018; Tochen et al. 2014; Tochen et al. 2016; Van Timmeren and Isaacs 2013).

A concerted effort has been directed toward developing monitoring tools to assess *D. suzukii* activity in susceptible crops with the aim of improving management programs. Unfortunately, current methods to monitor *D. suzukii* prior to fruit damage have proved ineffective for decision making as no threshold that relates to fruit infestation has been determined. Food-based baits for *D. suzukii* are neither efficient nor selective, and there is not yet a commercially available bait more attractive to *D. suzukii* than volatiles given off by ripening fruit (Abraham et al. 2015, Burrack et al. 2013). The most widely used *D. suzukii* trap, a clear deli cup coupled with synthetic lures and a drowning solution, is inexpensive, but labor intensive to use (Kirkpatrick et al. 2018, Landolt et al. 2012). The major issue with trapping *D. suzukii* adults to make management decisions is that once counts begin to surge, the populations in the field are often already high due to the rapid generation time and high generational overlap of *D. suzukii* (Wiman et al. 2014, Kirkpatrick et al. 2018).

Assessing the risk of oviposition and infestation by *D. suzukii* as the season progresses may be a more useful means of determining the timing of controls than trapping (Kirkpatrick et al 2018, Lee et al. 2016). Different cherry varieties and ripeness levels have been associated with the degree of fruit susceptibilities to *D. suzukii* oviposition and development (Lee et al. 2011; Tochen et al. 2014). In no choice bioassays, 'Montmorency', 'Balaton', 'Carmine Jewel', and 'Kántorjánosi' tart cherry cultivars were all susceptible to *D. suzukii* oviposition at some point during their development, and 'Kántorjánosi' cherries produced more *D. suzukii* larvae and adults at the ripening stage than the other tart varieties tested (Kamiyama and Guédot 2019). The apparent susceptibility of sweet cherry also varies by cultivar. Although a similar number of eggs were laid on ripe fruit of each of five sweet cherry cultivars, fewer developed to adulthood on 'Bing' compared to the others (Lee et al. 2011).

A variety of fruit characteristics that accompany fruit development have been associated with susceptibility to *D. suzukii* oviposition and larval development (Lee et al. 2016). Change in color and firmness of the skin as fruit ripen have received the most attention as characteristics that influence susceptibility. Small fruits are typically measured by changes in color as they ripen, and riper, darker, fruit have been shown to be more susceptible to *D. suzukii* oviposition (Lee et al. 2011; Lee et al. 2016). By classifying the ripeness stages of blackberries, blueberries, cherries, grapes, raspberries and strawberries based on transition from green to blush to red or blue, Lee et al. (2011) found that fruits generally increased in susceptibility once fruit began to color. A recent study by Lee et al. (2016) characterized blueberry susceptibility to *D. suzukii* oviposition based on firmness and color. As skin penetration force of blueberry decreased and the blueberry color changed from green to bluish/pink, the probability of oviposition increased. However, there was not a clear threshold for predicting when oviposition would occur.

The overall aim of the research presented herein was to develop the information needed to improve the decision-making process for managing *D. suzukii* in the principal tart and sweet cherry cultivars grown in Michigan. To achieve this goal, the seasonal progression of cherry susceptibility to infestation by *D. suzukii* was assessed with four main objectives. The first was to compare the susceptibility of 'Montmorency' and 'Balaton' tart cherries throughout the progression of fruit ripening using no choice and choice laboratory bioassays to assess relative larval infestation. In the second objective, the susceptibility of 'Ulster', 'Gold' and 'Emperor Francis' sweet cherries was compared throughout the progression of fruit ripening using no choice and choice laboratory bioassays to assess relative larval infestation. The third objective was to address the relationship between the seasonal progression of sweet cherry infestation by *D. suzukii* and fruit developmental characteristics, most notably color and the pressure required to puncture the cherry skin. The final objective was to address the relationship between fruit development based on Growing Degree Days (base 4 °C) and infestation by *D. suzukii*.

MATERIALS AND METHODS

Drosophila suzukii colony

Laboratory reared *D. suzukii* used for these experiments were reared and maintained in 50 mL polystyrene vials (Genesee Scientific, San Diego, CA) containing 5 mL standard Drosophila diet (Dalton et al. 2011) at Michigan State University (578 Wilson Road, East Lansing, MI 48824). The colony was held in a growth chamber at 24°C, 70% RH, and a photoperiod of 16:8 (L:D). Adults used for these experiments were less than 1-week old. Flies were anesthetized with CO_2 and separated by sex.

2018 choice bioassays

Experiments were conducted in 2018 to assess the preference of *D. suzukii* for different cultivars of tart and sweet cherry and different stages of fruit ripeness. 'Ulster', 'Gold', and 'Montmorency' cherry fruit were collected from orchards located at the Northwest Michigan Horticulture Research Station (Traverse City, MI), and 'Balaton' cherry fruit were collected from a commercial cherry orchard in Northport, MI. 'Montmorency' cherries were collected from well-established cherry orchards, and 'Ulster' and 'Gold' sweet cherries were collected from small, high density experimental plots. Collected cherries of each cultivar were divided into two sub-samples. One sample was used for an ovipositional experiment, while the other was used to determine fruit firmness as described below.

Choice bioassays were used to assess the effect of cherry cultivar and ripeness of fruit on oviposition by lab reared, two-day-old, mated *D. suzukii*. Four binary choice-tests were conducted: (1) ripe 'Montmorency' versus ripe 'Balaton', (2) ripe 'Ulster' versus ripe 'Gold', (3) ripe ‘Montmorency’ versus unripe ‘Montmorency’ and (4) ripe ‘Balaton’ versus unripe ‘Balaton’. Choice tests were conducted in dome cages (60 × 60 × 60 cm, BugDorm, MegaView Science Co., Taichung, Taiwan), with 5 replicates or cages for each of the four tests. A total of 60 cherries (30 of each cultivar or ripeness stage) were evenly spread on the bottom of the cage. Ten female and nine male flies were carefully released into each cage and allowed to oviposit for 48 h. A cotton ball soaked in 10% (w/v) sugar water was provided as a food source. During the 48-hour period, cages were held in the laboratory at the Northwest Michigan Horticulture Research Center at room temperature (20-25 °C). Afterwards, each fruit was inspected under a dissecting scope to count the pairs of thread-like breathing tubes on the fruit surface; this number was used to estimate the number of eggs laid in each fruit.

To assess the relationship between fruit firmness and ovipositional preference, the grams of force required to puncture the fruit skin was measured on randomly selected ripe ‘Montmorency’ (n=150), ‘Balaton’ (n=150), ‘Ulster’ (n=30), and ‘Gold’ (n=30) cherries, as well as another 150 unripe ‘Montmorency’ cherries using a benchtop Agrosta Fruit Texture Analyzer (Agrosta 2018, Agrosta Ltd, Serqueux, France). The texture analyzer was programed to gradually press the tip of a $0.5 cm^2$ plunger against the outer surface of a single cherry until the tip broke the skin of the fruit and entered the flesh. Firmness measures were not taken for ‘Balaton’, due to limited availability of fruit.

2019 seasonal progression of larval infestation

No choice bioassays

The seasonal progression of tart and sweet cherry susceptibility to *D. suzukii* larval infestation was assessed through no-choice bioassays conducted in the summer of 2019. 'Montmorency' cherries were collected from high-density trees with a Mahaleb rootstock at the Northwest Michigan Horticultural Research Center. 'Balaton' tart cherries were collected from an orchard in Northport, Michigan. 'Ulster', 'Gold' and 'Emperor Francis' sweet cherries were collected from fully grown trees planted in alternative rows in an experimental block of sweet cherries at the Northwest Michigan Horticultural Research Center. All fruit was hand-picked to ensure that the stems and fruit remained intact. Trees where fruit were collected were treated with fungicides but not insecticide during the growing season. About two gallons of fruit from each cultivar were collected every 2-4 days starting after pit hardening and continuing through harvest. Fruit were placed in one-gallon resealable plastic bags, brought back to the laboratory and divided into subsamples for use in no choice bioassays and measurements of fruit developmental characteristics conducted the same day.

For no-choice bioassays, five pairs of two-day old *D. suzukii* were removed from a laboratory colony and placed in a 16 oz plastic cups (Gordon Food Service, Grand Rapids, Michigan) containing 5-10 cherries. A cotton ball soaked in sugar water was also placed in each cup as a food source. Bioassays were initially replicated 5-6 times on each collection date. All cups were placed on trays on baking racks and left at lab temperature for 48 hours. Afterwards, all the flies and cotton balls were removed, and fruit were held in cups in the laboratory for an additional 9-10 days. In the set of bioassays assessing weekly infestation in 'Balaton', 'Ulster' and ‘Emperor Francis’, the 5 fruit from each cup were subjected to the Washington State Department of Agriculture brown sugar method for extracting larvae from fruit. The fruit were gently crushed to break up the skin and inner flesh, and then covered with a solution of 889 g brown sugar per one liter of water. After a minimum of 15 minutes, the fruit and solution mixture were filtered first through a coarse strainer (6 mm holes) into a large bowl to remove most of the fruit solids, and then strained through a coffee filter (holes < 1mm), allowing water to pass but retaining eggs and larvae on the filter. The contents of the coffee filter were examined under a dissecting microscope and the number of eggs, larvae and pupae were counted. In the set of bioassays assessing infestation of ‘Balaton’, ‘Montmorency’, ‘Ulster’ and ‘Gold’, the 10 fruit from each of the six cups containing each cultivar were combined into a single sample for each cultivar and subjected to the brown sugar method larval extraction, which led to a lack of replication for this portion of the study. In all bioassays, the empty cups were carefully inspected for leftover larvae or pupae that had already exited the fruit.

Natural field infestation of ‘Montmorency’, ‘Balaton’, ‘Ulster’ and ‘Gold’ cherries was also assessed on each sample date. Sub-samples of 60 fruit from each cultivar were subjected to the brown sugar test on the same day they were collected to determine background *D. suzukii* infestation in the field.

Fruit development characteristics

To measure fruit color on each sample date, cherries were placed on a metal tray (46 cm x 66 cm) and arranged in an order from the least- to the most- ripe based on human vision and color perception under laboratory lighting. Fruit was separated into a maximum of 5 groups, labeled as “-2”, “-1”, “0”, “+1”, and “+2” from the least- ripe to the most- ripe fruit, with the middlemost ripeness rating labeled as “0”. For each group of fruit, color was quantified using a spectrophotometer (Konica Minolta CR-400 Chroma Meter). A single cherry was placed under the lens of the spectrophotometer and the CIE L*a*b* numerical values for color were recorded. In this color scale, L defines lightness (scale of 0-100), a* defines the green-red component (scale of -128 – 128), and b* defines the blue-yellow component (scale of -128 – 128). Color measurements were taken for 10 cherries from each group, with two measurements for each cherry by measuring each side of the cherry for a total of 20 color measurements.

Fruit firmness was assessed for each color subgroup using a benchtop Agrosta Fruit Texture Analyzer (Agrosta 2018, Agrosta Ltd, Serqueux, France). To measure firmness, the texture analyzer was programed to gradually press the tip of a 0.5cm^2 plunger against the outer surface of a single cherry until the tip broke the skin of the fruit and entered the flesh. A single reading of grams of force required to puncture the skin was taken on 20 cherries for each color subgroup. Color and puncture analyses were completed on the middlemost ripeness reading (“0”).

Growing degree days

Daily minimum and maximum temperatures were used to calculate Growing Degree Days (GDD) using the Baskerville and Emin method (Baskerville and Emin 1969). A base temperature of 4 °C was selected based on the previous work of Zavalloni et al. (2006) who documented a tight relationship between the accumulation of GDD (base 4 °C) from full bloom and ‘Montmorency’ tart cherry fruit growth, a model developed to be able to predict harvest. A tool on the MSU Enviroweather website (https://enviroweather.msu.edu/) was used to calculate GDD based on data recorded by weather stations located at the Northwest Michigan Horticultural Research Center or at the Garthe Orchard in Northport, Michigan.

Data analysis

All statistical analyses were performed in R Studio R-3.5.3 (R Studio Team 2015, Manufacturer, Boston, Massachusetts, United States of America). A generalized linear model (“glm” in R) with an analysis of variance (ANOVA) was conducted on the egg laying choice bioassay experiment. Puncture pressure bioassay results were analyzed using a Welch’s two-sided t-test. Generalized linear modeling with means separation conducted using a modified Tukey’s for generalized hypothesis testing (“glht” function in the R package “multicomp”) was conducted to assess the infestation by seasonal progression results (Hothorn et al., 2008). Results of analytical tests were considered statistically significant at $\alpha = 0.05$. Fruit color as the season progressed was expressed as the proportion of a*/b*, with the value increasing as fruit turned from green to blush to red or decreasing as the fruit turned from green to golden. Puncture force was expressed as the grams of force/cm^2 required to penetrate the fruit skin. Relationships for number of larvae successfully developing in cherries and GDD accumulation were assessed using a power regression. Logarithmic (base 10) regressions were run to assess the significance of the relationship between a*/b* or puncture force and the number of larvae successfully developing in cherries as the season progressed. For all relationships, larval count data was transformed using ‘n+1’ so the data meets the qualifications to run power and logarithmic regressions. To run the logarithmic relationships, color data was transformed using '$\log_{10}(|a^*/b^*|)$', and the puncture pressure was transformed using '$\log_{10}(n)$'.

RESULTS

2018 choice bioassays

In the choice assay comparing oviposition on ripe 'Montmorency' or 'Balaton' cherries, *D. suzukii* did not show a preference for either cultivar (Fig. 3.1; df = 1, 9; F = 0.03; p = 0.86). Female flies deposited an average of about 2.2 eggs per cherry.

Figure 3.1. Mean (± S.E.) number of eggs laid in 30 ripe 'Montmorency' and 'Balaton' tart cherry cultivars in a choice test bioassay.

In the choice assay comparing oviposition on ripe 'Ulster' or 'Gold' sweet cherry varieties, *D. suzukii* deposited significantly more eggs in 'Ulster' compared to 'Gold' cherries (Fig.3.2A; df = 1,9; F = 3.64; p = 0.022). While an average of nearly 1.4 eggs were laid in each 'Ulster' cherry, fewer than 0.1 eggs were deposited in 'Gold' cherries. Overall, females only deposited two eggs in the 150 'Gold' cherries presented to them in the bioassay, whereas they laid more than 200 eggs in the 'Ulster' cherries.

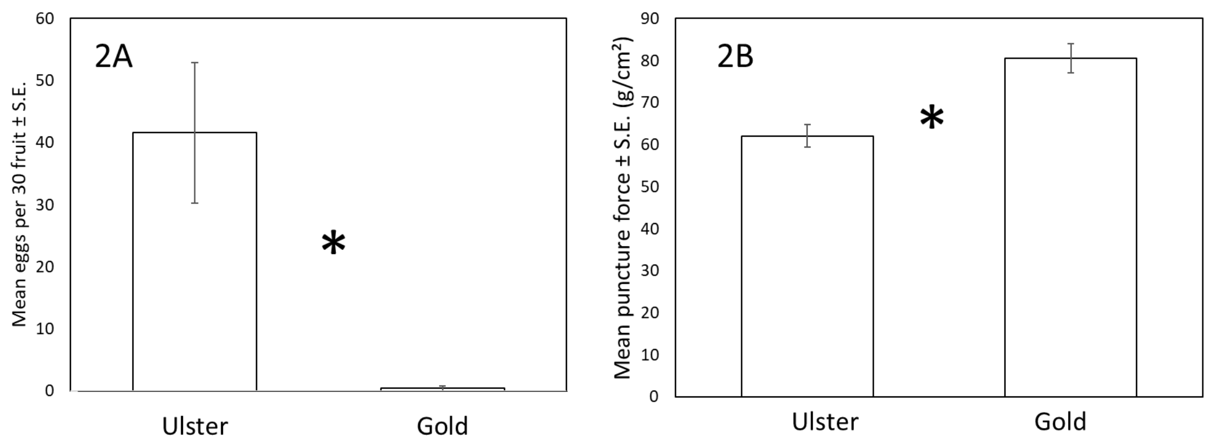

Figure 3.2. 2A) Mean (± S.E.) number of eggs inserted by *D. suzukii* females in five replicates of 30 ripe 'Ulster' and 'Gold' sweet cherry varieties presented in a two-choice test. An asterisk indicates a significant difference in means at the 0.05 level. 2B) Average (± S.E.) firmness of 'Ulster' and 'Gold' cherries as measured by a benchtop Agrosta Fruit Texture Analyzer. An asterisk indicates a significant difference in means at the 0.05 level.

The firmness of ripe 'Gold' sweet cherries was significantly greater than that recorded for ripe 'Ulster' cherries (Fig. 3.2B; $df = 1, 29$; $F = 4.23$; $p < 0.001$). It required about 20% more force to puncture 'Gold' compared to 'Ulster' fruit.

In the choice assay comparing oviposition on ripe 'Montmorency' or unripe 'Montmorency' cherries, *D. suzukii* deposited significantly more eggs in the ripe fruit (Fig. 3.3A; $df = 1, 9$; $F = 24.40$; $p = 0.007$). Females laid nearly 3 times as many eggs on ripe compared to unripe 'Montmorency' cherries.

The firmness of unripe cherries was significantly greater than that recorded for ripe 'Montmorency' cherries (Fig. 3.3B; $df = 1, 149$; $F = 15.72$; $p < 0.001$). It required over three times the force to puncture unripe compared to ripe fruit.

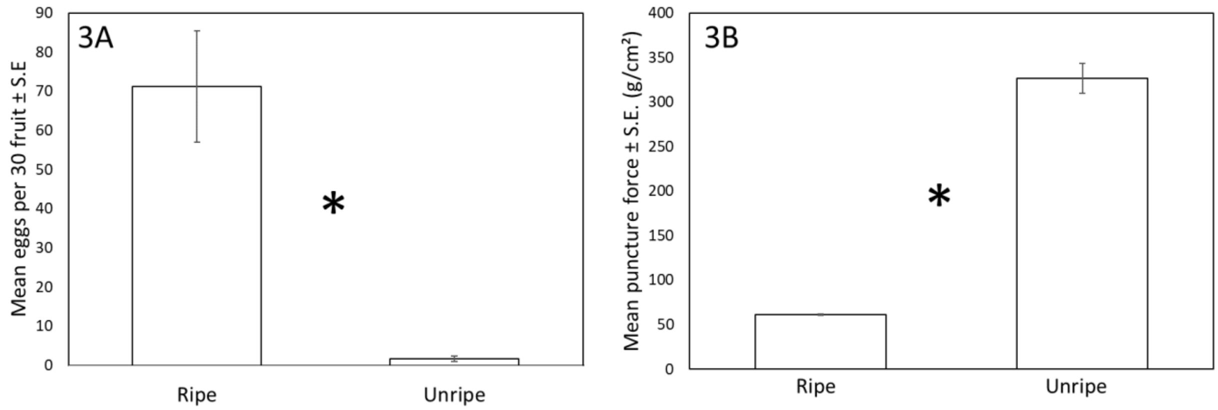

Figure 3.3. A) Mean (± S.E.) number of eggs inserted by *D. suzukii* females in five replicates of 30 ripe and unripe 'Montmorency' cherry fruit presented in a two-choice test. An asterisk indicates a significant difference in means at the 0.05 level. B) Average firmness of 150 ripe and unripe 'Montmorency' cherry fruit as measured by a benchtop Agrosta Fruit Texture Analyzer. An asterisk indicates a significant difference in means at the 0.05 level.

The choice test comparing ripe 'Balaton' and unripe 'Balaton' provided no data, as all the flies died within 24 h, possibly due to insecticide residue on the fruit or a bad batch of colony flies.

2019 seasonal progression of larval infestation

The seasonal progression in susceptibility of sweet and tart cherries to larval infestation by *D. suzukii* is presented in Figures 3.4 and 3.5. For all cultivars, larvae and pupae were recovered from fruit exposed in the bioassays and naturally in the field. However, the timing and extent of *D. suzukii* infestation varied by time of season, cultivar and type of exposure to fruit. In no choice bioassays, the initial infestation of both 'Ulster' and 'Gold' sweet cherries occurred around July 9 but natural infestation was not recorded until about a week later. For all cultivars, infestation increased as the season progressed and infestation under no choice condition was much higher than natural infestation in the field. Larval infestation was about 4 times higher in ripe 'Ulster' compared to ripe 'Gold' cherries, 350 eggs/60 cherries vs 87 eggs/60 cherries. Larval infestation occurred much earlier in 'Montmorency' than in 'Balaton' tart cherries in this experiment (Fig. 3.5). 'Balaton' cherries were unique among the 4 cherry cultivars in that susceptibility to *D. suzukii* was very low until late July, just prior to harvesting the ripe fruit. However, infestation of ripe tart cherries on July 29 was over 6 times higher in 'Balaton' compared to 'Montmorency' cherries, 80 eggs/60 cherries vs 12 eggs/60 cherries. The failure of larvae to develop in 'Montmorency' cherries collected on July 23, 25, 27 is contrary to previous no choice bioassays with this cultivar and was likely due to an issue with colony flies or a mistaken insecticide application in the plot.

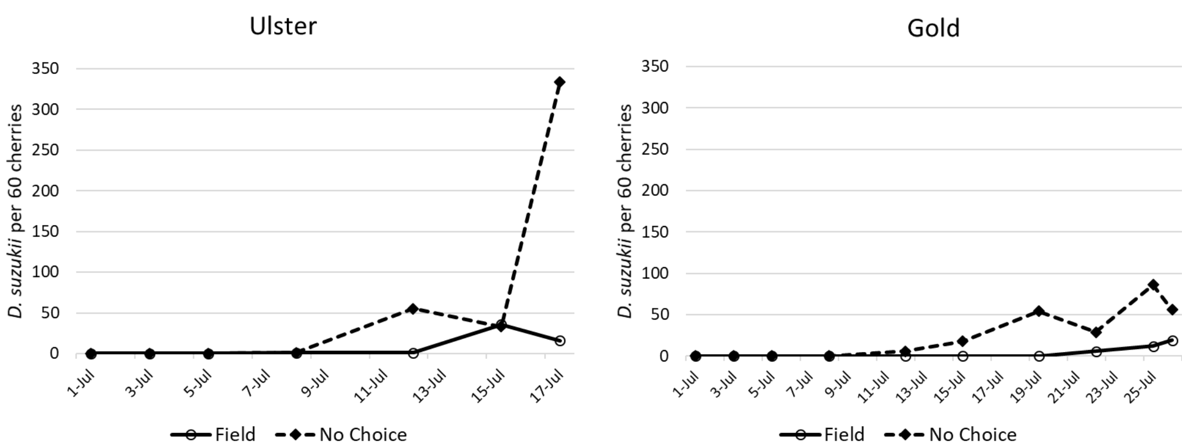

Figure 3.4. Seasonal progression of *D. suzukii* infestation in 'Ulster' and 'Gold' sweet cherry varieties. Solid line represents natural field infestation and the dashed line represents no-choice bioassay results.

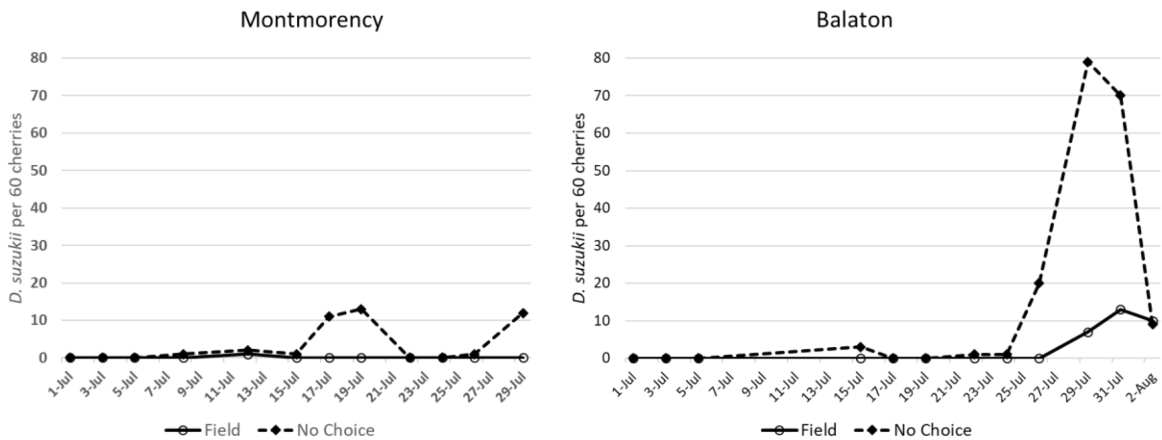

Figure 3.5. Seasonal progression of *D. suzukii* infestation in 'Montmorency' and 'Balaton' tart cherry varieties. Solid line represents natural field infestation and the dashed line represents no-choice bioassay results.

No choice bioassays

In no choice bioassays, larvae were unable to develop in 'Balaton', 'Emperor Francis' or 'Ulster' cherries in the earliest sample of unripe or green fruit (Table 3.1). As the season progressed and the cherries colored, larvae were detected in the fruit of all cultivars on each sample date. However, significantly fewer larvae completed development in fruit collected on the earliest sample dates compared to the final sample date. The highest levels of *D. suzukii* infestation were recorded in the ripest fruit and all showed a similar level of susceptibility, with 41 to 52 larvae recovered from the 25-fruit assayed.

Fruit	Sample Date	Ripeness (color)	Mean larvae ± S. E.
Balaton	25-Jun	green	0.00 ± 0.00 **a**
Balaton	2-Jul	green-yellow	0.50 ± 0.34 **ab**
Balaton	9-Jul	red-purple	7.17 ± 2.47 **abc**
Balaton	16-Jul	purple	19.83 ± 3.32 **c**
Balaton	23-Jul	dark purple	44.17 ± 6.77 **d**
ANOVA			$F_{4,4}$ = 16.62; p = 0.03
Emperor Francis	18-Jun	green	0.00 ± 0.00 **a**
Emperor Francis	25-Jun	15-25% blush	0.67 ± 0.49 **a**
Emperor Francis	2-Jul	50% blush	2.83 ± 2.64 **a**
Emperor Francis	9-Jul	95% blush	10.50 ± 3.41 **a**
Emperor Francis	16-Jul	95% blush	51.67 ± 7.74 **b**
ANOVA			$F_{4,4}$ = 5.95; p = 0.09
Ulster	18-Jun	green	0.00 ± 0.00 **a**
Ulster	25-Jun	red	3.33 ± 1.82 **a**
Ulster	2-Jul	purple	4.50 ± 1.34 **a**
Ulster	9-Jul	dark purple	10.33 ± 6.26 **a**
Ulster	16-Jul	dark purple	40.67 ± 12.49 **b**
ANOVA			$F_{4,4}$ = 7.33; p = 0.07

Table 3.1. Mean (± S.E.) number of *D. suzukii* larvae collected from five replicates of 5 fruit for ‘Balaton’, ‘Emperor Francis’, and ‘Ulster’ cherry varieties, determined by a no-choice bioassay. Data marked with common letters do not have significance at the 0.05 level.

Fruit development characteristics

For the two dark-skinned cultivars, ‘Ulster’ and ‘Balaton’, the a*/b* color proportion gradually increased as the fruit ripened. This measure of change in color from green to red as fruit matured was positively correlated with the number of larvae that developed in cherries exposed to *D. suzukii* in no-choice bioassays (Fig. 3.6). The relationship was logarithmic, with a sharp rise in infestation as the fruits reached their ripest state.

For the light-skinned cultivar, ‘Gold’, the a*/*b proportion gradually declined in a negative fashion as the fruit turned from green to gold. This relationship was also logarithmic, with a change in color correlating to the number of larvae that developed in cherries exposed to *D. suzukii* in no-choice bioassays (Fig. 3.6).

The mean force required to puncture the cherry skin as the season progressed also was correlated with successful development of *D. suzukii* larvae in no-choice bioassays (Fig. 3.7). The relationship for all three cherry cultivars tested was logarithmic, with a sharp rise in infestation as the fruits reached their ripest state. Very few larvae developed in fruit with puncture pressures around 150-200 grams/cm^2, but infestation increased substantially once the force required to penetrate the skin dropped below 100 grams/cm^2.

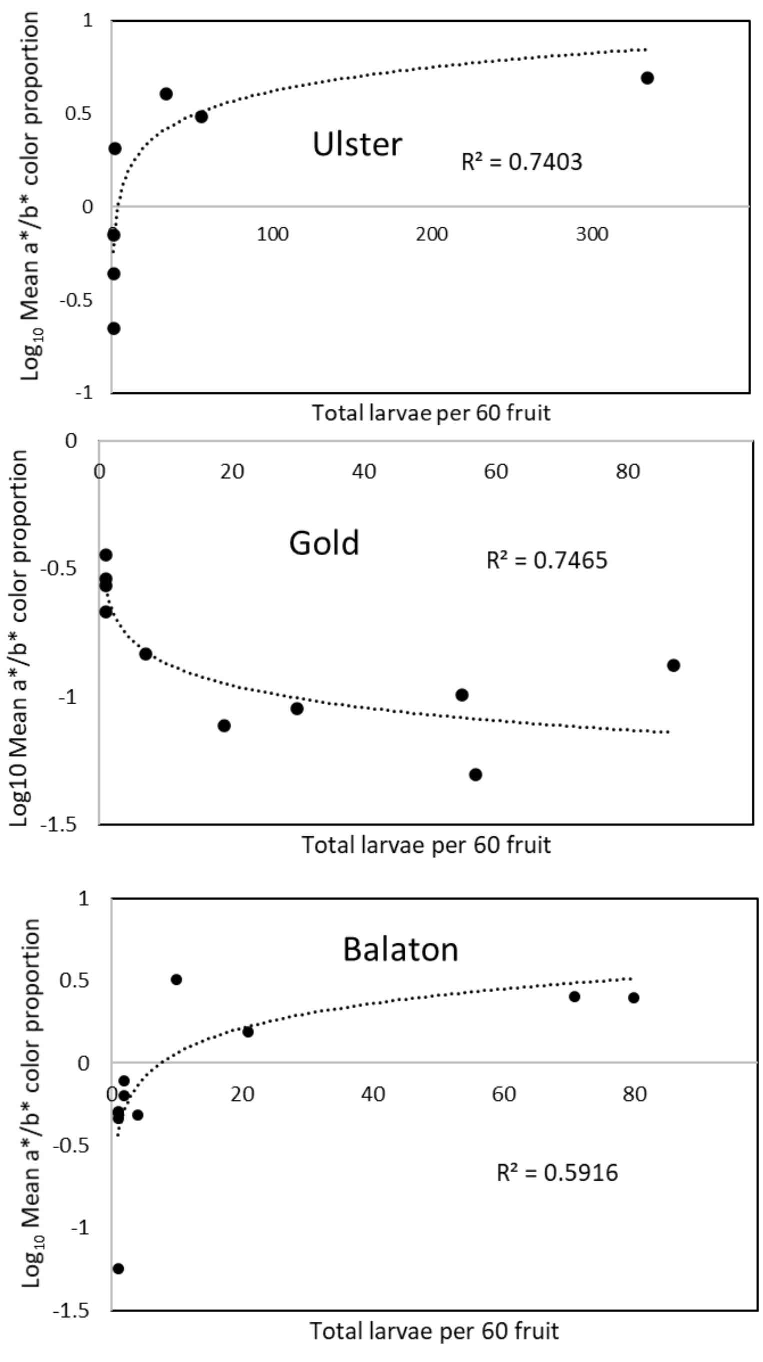

Figure 3.6. Logarithmic relationship between change in fruit color as they ripen (based on the CIE L*a*b* lightness scale) and infestation of 60 ripening fruit 'Ulster', 'Gold', and 'Balaton' cherry cultivars by *D. suzukii*.

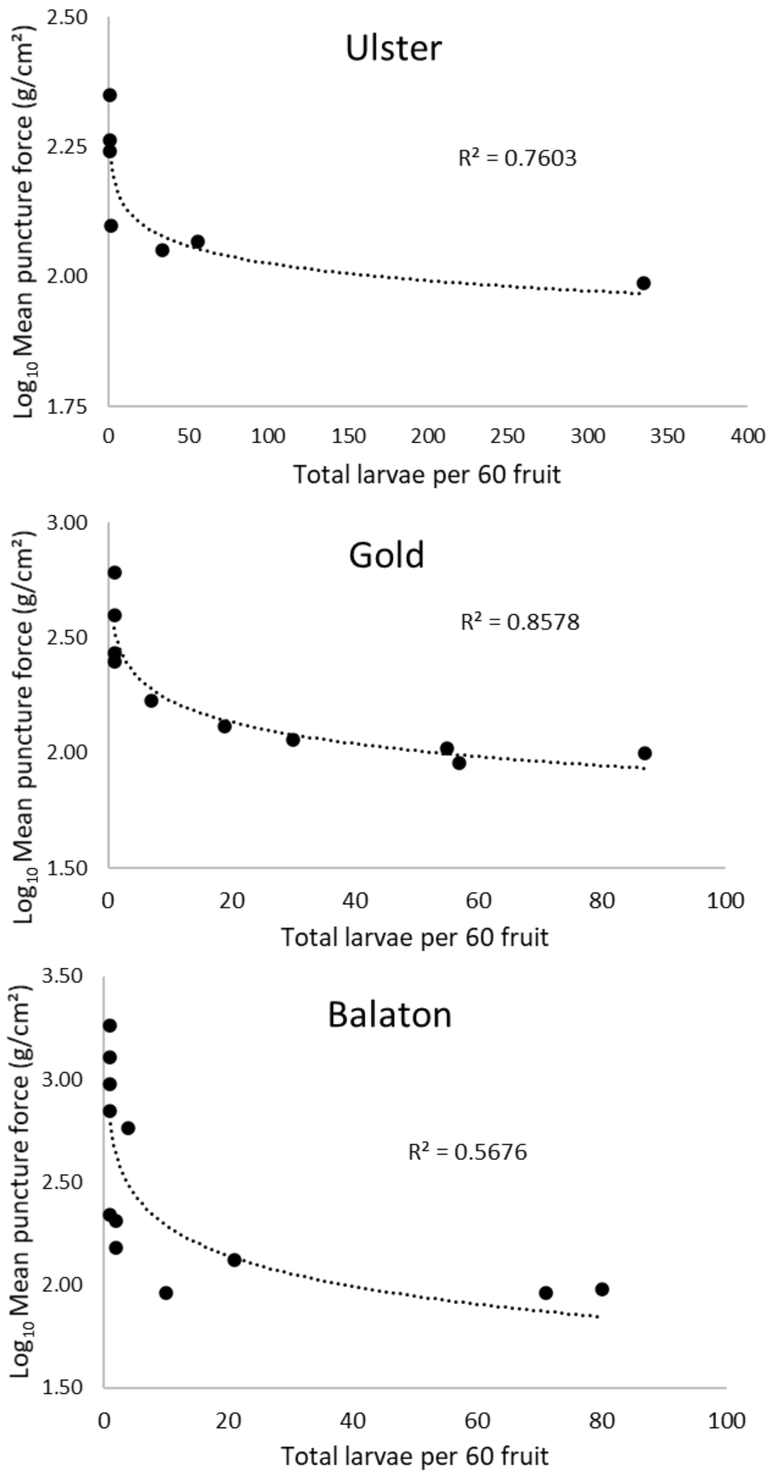

Figure 3.7. Logarithmic relationship between change in change in pressure required to puncture the skin of 20 cherries and infestation of ripening fruit from 'Ulster', 'Gold', and 'Balaton' cherry cultivars by *D. suzukii*.

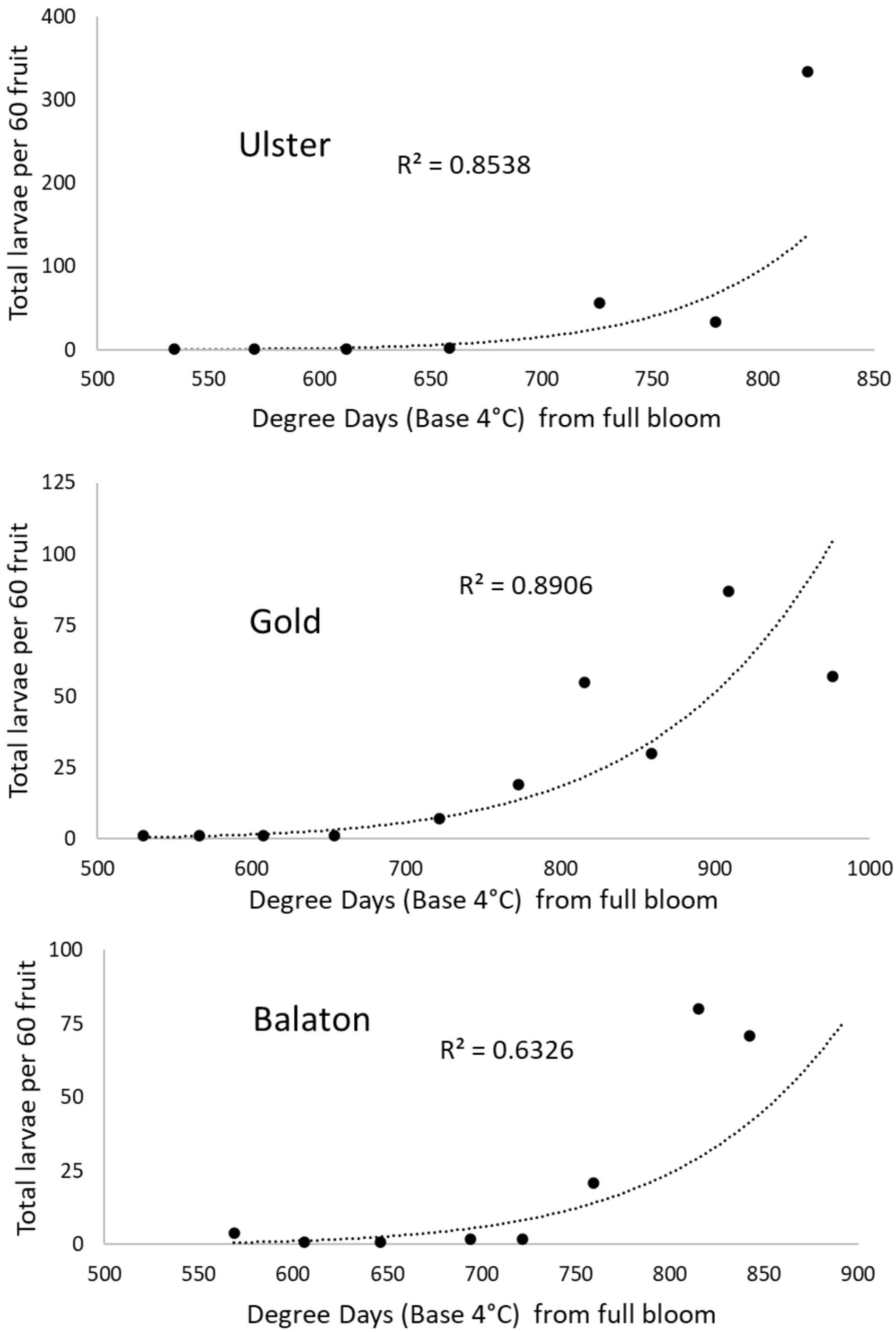

Figure 3.8. Power relationship between cumulative GDD base 4°C and infestation of ripening fruit from 'Ulster', 'Gold', and 'Balaton' cherry cultivars by *D. suzukii*.

Growing degree days

The seasonal progression in susceptibility of 'Ulster', 'Gold', and 'Balaton' cherry to *D. suzukii* was positively correlated with the accumulation of GDD (base 4 °C) from full bloom (Fig. 3.8). The relationship for 'Ulster' and 'Gold' cherry cultivars tested was a power relationship, with a sharp rise in infestation as the fruits reached their ripest state. There was a weak power relationship of larval infestation and GDD accumulation for 'Balaton' cherries. For all varieties tested, very few larvae developed in fruit until about 600 GDD had been reached and infestation increased substantially once accumulated GDD reached about 750.

DISCUSSION

Overall, my results showed that 'Montmorency' and 'Balaton' tart cherries, and 'Ulster', 'Gold' and 'Emperor Francis' sweet cherries were quite susceptible to *D. suzukii*. For all cultivars, the level of infestation increased as the season progressed, and fruit ripened. Similar findings have been previously reported for several tart and sweet cherry cultivars. In no choice bioassays, 'Montmorency', 'Balaton', 'Carmine Jewel' and 'Kántarjánosi' tart cherries were all susceptible to *D. suzukii* infestation beginning at the ripening stage of fruit development (Kamiyama and Guédot 2019). In no choice tests, *D. suzukii* deposited eggs and completed development in ripe 'Bing', 'Black Tartarian', 'Brooks', 'Early Burlat' and 'Tulare' sweet cherries (Lee et al. 2011).

While a number of laboratory studies have quantified the potential for *D. suzukii* to attack various fruit and ripeness stages, this study is the first to directly compare the extent of infestation in laboratory no choice bioassays versus natural infestation in the field. For all four cultivars tested, larval infestation was consistently higher under no choice conditions than natural infestation in the field. This has important ramifications for the management of this pest. Laboratory bioassays are good indicators of the potential for infestation and its relationship to fruit ripening. However, it does not mean that fruit that could be infested actually will be infested. Thus, applying insecticides early in the season because fruit have begun to color may be unnecessary in some cases. *D. suzukii* population density in a particular orchard certainly impacts the timing and degree of infestation but this has proved difficult to measure. Adult captures in monitoring traps appear to be unreliable for determining the need to treat, likely due to the rapid generation time and high generational overlap of *D. suzukii* (Wiman et al. 2014, Kirkpatrick et al. 2018). Environmental conditions influence the extent to which *D. suzukii* deposit eggs in the fruit and how successfully larvae develop. Temperature and humidity can greatly affect the behavior and fecundity of *D. suzukkii* (Tochen et al. 2016). Female reproductive status was greatest between 82 and 94% relative humidity and low humidity levels corresponded with low adult activity (Tochen et al. 2016). Oviposition and egg viability are also reduced at higher temperatures (Walsh et al. 2011). Advancing the understanding of how these factors affect *D. suzukii* infestation in the field would greatly improve our ability to manage this prolific pest.

No choice and choice bioassays revealed some key differences in the suitability of 'Ulster' and 'Gold' sweet cherries to attack by *D. suzukii*. When given a choice between ripe fruit of the two cultivars, females overwhelming preferred to insert their eggs in 'Ulster'. Indeed, in the choice bioassay they only deposited two eggs in the 150 'Gold' cherries presented to them, whereas they laid more than 200 eggs in the 'Ulster' cherries. Difference is the suitability of the two cultivars was less pronounced in the no choice bioassay. 'Ulster' and 'Gold' cherries were initially infested at about the same ripeness stage and larval development in fruit remained at similar, low levels until fruit were ripe and nearing harvest.

Some pronounced differences in physical characteristics of the two sweet cherry cultivars likely contributed to differences in the patterns of infestation by *D. suzukii.* 'Ulster' is a dark, deep red cherry at the ripe stage, while 'Gold' is a golden cherry when ripe. *D. suzukii* showed a clear preference for the dark-skinned fruit. Dark colors, such as purple, appear to be especially attractive to female *D. suzukii*, most likely because these are close to the color of preferential oviposition sites (Takahara and Takahashi 2016, Kirkpatrick et al. 2016). Contrast is also an important visual cue when it comes to *D. suzukii* host finding (Little et al. 2019). The no choice bioassay was conducted in a dome cage with a white background. In this setting the dark 'Ulster' fruit may have been more apparent to searching females than the light-skinned 'Gold' fruit. In a no choice scenario and in the field, 'Ulster' and 'Gold' cherries were both susceptible to *D. suzukii* infestation. In very ripe fruit at harvest, however, 'Ulster' cherries sustained over 3 times the infestation compared to 'Gold' cherries. The high susceptibility of 'Ulster' to *D. suzukii* also may be related to their being a softer fruit at harvest. It required about 20% more force to penetrate the skin of 'Gold' compared to 'Ulster' cherries. It has previously been found that higher firmness leads to less oviposition (Burrack et al. 2013; Lee et al. 2016), and female flies have been shown to prefer softer blueberries (Kinjo et al. 2013). Fruit sugar content also has been associated with increased susceptibility of sweet cherries to successful attack by *D. suzukii,* with the numbers of eggs inserted in fruit increasing with increasing °Brix (Lee et al. 2011). Although I did not measure sugar content, 'Ulster' cherries are known as a very sweet cherry.

Choice and no choice bioassays also provided some valuable insights into the relative vulnerability of 'Montmorency' and 'Balaton' tart cherries to *D. suzukii*. Infestation levels were nearly equivalent when given a choice between the two fruits (Fig. 1). However, the no choice bioassays indicated that 'Balaton' cherries become extremely susceptible to *D. suzukii* when the fruit reach the final stage of ripeness (Fig. 5). Nearly six times more larvae developed in highly ripe 'Balaton' compared to 'Montmorency' cherries. The apparent discrepancy in the two bioassays was likely associated with the timing of the bioassays. The no choice assay was timed to coincide with the ripening of 'Montmorency' cherries. This cultivar ripens about a week before 'Balaton' cherries. Thus, the 'Balaton' cherries in the no choice assay conducted in 2018 may not have reached the stage of ripeness when they were the most vulnerable to *D. suzuki*i.

My results are consistent with other studies showing that *D. suzukii* females generally do not attack unripe tart cherries or other small fruits, but readily infested fruits soon after they start to color (Lee et al., 2011, Kamiyama and Guédot 2019). When given a choice between ripe and unripe fruit, the vast majority of eggs were deposited in ripe fruit. However, females did lay a few eggs in green 'Montmorency' cherries. Kamiyama and Guédot (2019) found that in no choice bioassays a few eggs were laid in unripe tart cherry fruits but in all cases failed to develop to adulthood. This is consistent with the results of my no choice assays, in which no larvae emerged from unripe fruit tested in the first three sample period, all of which occurred prior to fruit beginning to color.

The results of this study also are unique in that they represent the first quantification of the relationship between the risk of *D. suzukii* susceptibility and changes in color and the toughness of the skin; two fruit characteristics that accompany the ripening process. Previous studies have taken a qualitative approach to examining the relationship between fruit ripening and *D. suzukii* egg deposition or larval development. Grouping fruit into general categories based on visual color changes, e.g., green, blush, pink and red, revealed that very few larvae developed in green fruit, but successfully attacked fruit as soon as it started to color (Lee et al. 2011, 2016). In contrast, I assigned a value to the change in color as fruit ripened based on the proportion of a*/b* on the CIE L*a*b* lightness scale. The approach generated a curvilinear relationship between change in color and infestation for the two cultivars that turn from green to red as they ripen, However, the approach was less useful for quantifying the change in color for the 'Gold' cultivar, likely due to the fruit retaining a light skin color up until harvest.

Measuring the firmness of different types or ripeness stages of fruit has previously demonstrated that oviposition in soft-skinned small fruits increased as the force required to penetrate the skin decreased (Kinjo et al. 2013, Burrack et al. 2013, Ioriatti et al. 2015, Lee et al. 2016). Fruit firmness has often been measured with handheld devices resulting in high variation due to human error (Jantra et al. 2018). Using a tabletop penetrometer with a small diameter probe to break the fruit skin resulted in very consistent measurements of penetration force. Furthermore, by measuring puncture pressure every 2-3 days as fruit developed, I was able to confirm strong relationships between increasing force required to penetrate the skin of several cherry cultivars and increasing larval development in the fruit.

For Michigan cherry growers, insecticides targeting *D. suzukii* generally are applied weekly beginning once adult flies have been detected in traps and fruit has started to color. The 4-6 preventive sprays required to keep *D. suzukii* in check is a serious economic hardship for cherry growers. Some of these sprays may be unnecessary in a given season, but without a more precise means of identifying when the crop is at risk, growers must take a cautionary approach to protecting their livelihood.

A great deal of effort has been directed toward establishing sampling methods that could refine the decision-making process for managing *D. suzukii*. Despite progress in developing trapping systems to monitor this invasive pest, adult captures in traps has not proved to be a reliable means of determining when or if controls are warranted. Assessing fruit infestation is another option that has been explored for guiding management decisions. This strategy, however, seems highly risky as once infested fruit are found it is probably too late to mitigate crop losses. Assessing fruit infestation also may be impractical given the large sample size likely required for early detection.

Focusing on the susceptibility of the fruit rather than the presence and density of flies shows promise as a viable approach for improving the decision-making process for managing *D. suzukii*. Previous research found that degree day accumulations were an excellent means of modeling 'Montmorency' tart cherry fruit development (Zavalloni et al. 2006). Using this work as a foundation, I have shown that the accumulation of GDD's (base 4°C) is also a very good predictor of the potential for infestation of 'Balaton', 'Ulster' and 'Gold' cherry cultivars by *D. suzukii*. For these cultivars, fruit were at low risk of infestation by *D. suzukii* prior to about 600 GDD's. This finding represents a key step in developing a tool that predicts the risk of *D. suzukii* infestation in cherry, enabling growers to more precisely time control tactics. Future research should focus on developing risk models that take into account both fruit phenology and environmental variables, most notably temperature and humidity.

CHAPTER 4: CONCLUSIONS AND FUTURE RESEARCH OBJECTIVES

The research provided in this thesis has provided new information in the study of *D. suzukii* with relevance for improved pest management in Michigan cherry orchards. There is an increased need for better methods to monitor *D. suzukii,* as well as improve decisions on when to apply insecticide. Successful monitoring of the pest will help to reduce the use of insecticidal sprays and can lead to a reduction of economic losses related to the damage that this pest causes. Although it has been shown that the current methods to monitor *D. suzukii* are still applicable, future research should work toward improving the efficiency of baits and colored sticky panel traps. Current management practices of treating with insecticide prophylactically are not sustainable, expensive for growers, and detrimental to beneficial arthropods. Since *D. suzukii* has an incredibly broad host range and unique biology, it is a challenge to achieve complete control of this pest in an orchard environment. However, the incorporation of behavior-based manipulation into current management strategies could lead to significant reductions in damage and infestation of cherry crops. Results of this thesis indicate that *D. suzukii* is a target for behavior-based manipulation strategies though development of a trap with visual cues, as well as the development of a risk model that uses the growing degree days of the target crop.

I have investigated how *D. suzukii* responds to commercially available traps and lures, as well as different colored and patterned stick panel traps, all while decreasing captures of non-target insects to improve monitoring and trapping efficiency (Chapter 2). In field experiments, a dry sticky panel trap with a high trap capture surface area increased *D. suzukii* capture, and the standard Scentry® lure performed better than the other commercially available *D. suzukii* lures tested. Plain green, plain yellow, and yellow panel with a contrasting red circle sticky panel traps with a Scentry® lure captured the most *D. suzukii* than the other patterned and solid color panels tested. Unfortunately, there was still a very high number of non-target Drosophila captured on all traps tested, and this led to difficulty of examining traps to find *D. suzukii*. This work demonstrates that a trap incorporating visual cues, as well as reflectance of color increases the capture of *D. suzukii* in traps. Further evaluation and improvement of dry sticky panel traps and commercial lures should be studied in the future to improve visual and olfactory attractants for attracting *D. suzukii.*

I have also investigated the susceptibility of key varieties of sweet and tart cherries to determine their susceptibility to *D. suzukii* throughout the growing season (Chapter 3). While a number of laboratory studies have quantified the potential for *D. suzukii* to attack various fruit and ripeness stages, this study is the first to directly compare the extent of infestation in laboratory no choice bioassays versus natural infestation in the field. As cherry fruit was collected during the season, changes and color and changes in skin toughness of the cherry fruit was measured. In choice test bioassays, *D. suzukii* preferred darker, softer fruit over lighter, tougher fruit. For all four cultivars tested, larval infestation was consistently higher under no choice conditions than natural infestation in the field. However, it does not mean that fruit that could be infested actually will be infested. By measuring puncture pressure every 2-3 days as fruit developed, I was able to confirm strong relationships between increasing force required to penetrate the skin of several cherry cultivars and increasing larval development in the fruit. Previous research found that degree day accumulations were an excellent means of modeling

‘Montmorency’ tart cherry fruit development, and using this work as a foundation, I have shown that the accumulation of GDD’s (base 4°C) is also a very good predictor of the potential for infestation of cherries by *D. suzukii*. With the cherry varieties tested, the fruit were at low risk of infestation by *D. suzukii* prior to about 600 GDD’s. This finding represents a key step in developing a tool that predicts the risk of *D. suzukii* infestation in cherry, enabling growers to more precisely time control tactics.

From this work, a monitoring trap optimized for *D. suzukii* combined with the timing of fruit susceptibility could be utilized to develop a reliable threshold for growers to determine the best timing for initiating a successful integrated pest management program targeting this important cherry pest.

APPENDIX

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens 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: ____2020-05____

Author and Title of thesis:
Sarah R. Dietrich
Toward better management of spotted wing Drosophila (*Drosophila suzukii*) in Michigan cherry orchards

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Drosophilidae	*Drosophila suzukii*	adult	20	pinned/pointed

REFERENCES

REFERENCES

Abraham, J., A. Zhang, S. Angeli, S. Abubeker, C. Michel, Y. Feng, and C. Rodriguez-Saona. 2015. Behavioral and antennal responses of *Drosophila suzukii* (Diptera: Drosophilidae) to volatiles from fruit extracts. Environ. Entomol. 44: 356–67.

Aluja, M. & R. Prokopy. 1993. Host odor and visual stimulus interaction during intratree host finding behavior of *Rhagoletis pomonella* flies. J. Chem. Ecol. 19: 2671-2696.

Asplen, M. K., G. Anfora, A. Biondi, D. Choi, D. Chu, K. M. Daane, P. Gibert, A. P. Gutierrez, K. A. Hoelmer, W. D. Hutchison, et al. 2015. Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. J. Pest Sci. 88:469–494.

Basoalto, E., R. Hilton, and A. Knight. Factors affecting the efficacy of a vinegar trap for *Drosophila suzukii* (Diptera: Drosophilidae). J. Appl. Entmol. 137: 561-570.

Beers E.H., R.A. Van Steenwyk, P.W. Shearer, W.W. Coates and J.A. Grant. 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag Sci 67:1386–1395.

Bellamy, D. E., M. S. Sisterson, and S. S. Walse. 2013. Quantifying host potentials: indexing postharvest fresh fruits for spotted wing Drosophila, *Drosophila suzukii*. PLOS ONE. 8: e61227.

Bellutti, N., A. Gallmetzer, G. Innerebner, S. Schmidt, R. Zelger, E. H. Koschier. 2017. Dietary yeast affects preference and performance in *Drosophila suzukii*. J. Pest Sci. 91: 651-660.

Borst, A. 2009. *Drosophila*'s view on insect vision. Curr. Biol. 19: 36-47

Brown, S. K., R. D. Way, and D. E. Terry. 1989. Sweet and tart cherry varieties: descriptions and cultural recommendations. New York Food Life Sci. Bull. No 127

Bruce, T., L. Wadhams, and C. Woodcock. 2005. Insect host location: a volatile situation. Trends Plant Sci. 10: 269-274.

Burrack, H. J., G. E. Fernandez, T. Spivey, and D. A. Kraus. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: Drosophilidae), an invasive frugivore. Pest Manag. Sci. 69: 1173–1180.

Burrack, H. J., M. Asplen, L. Bahder, J. Collins, F. Drummond, C. Guédot, R. Isaacs, D. Johnson, A. Blanton, J. Lee, et al. 2015. Multistate comparison of attractants for monitoring

Drosophila suzukii (Diptera: Drosophilidae) in blueberries and caneberries. Environ. Entomol. 44: 704-712.

Cha, D. H., T. Adams, H. Rogg, and P. J. Landolt. 2012. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing Drosophila, *Drosophila suzukii.* J. Chem. Ecol. 38: 1419–31.

Cha D. H., C. T. Adams, B. Werle, B. J. Sampson, J. J. Adamczyk, H. Rogg, and P. J. Landolt. 2014. A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace. Pest Manag. Sci. 70: 324–331.

Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insectol. 65: 149-160.

Cloonan, K. R., J. Abraham, S. Angeli, Z. Syed, and C. Rodriguez-Saona. 2018. Advances in the chemical ecology of the spotted wing Drosophila (*Drosophila suzukii*) and its applications. J. Chem. Ecol. 44: 922-939.

Daane, K. M., X. Wang, A. Biondi, B. Miller, J. C. Miller, H. Riedl, P. W. Shearer, E. Guerrieri, et al. 2016. First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential biological agents. J. Pest Sci. 89: 823-835.

Dalton, D. T., V. M. Walton, P. W. Shearer, D. B. Walsh, J. Caprile, R. Isaacs. 2011. Laboratory survival of Drosophila suzukii under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manag. Sci. 67: 1368-1374.

Enriquez, Thomas, and H. Colinet. 2017. Basal tolerance to heat and cold exposure of the spotted wing Drosophila, *Drosophila suzukii*. PeerJ. 5: e3112.

Farnsworth, D., K. A. Hamby, M. Bolda, R. E. Goodhue, J. C. Williams, and Frank G. Zalom. 2016. Economic analysis of revenue losses and control costs associated with the spotted wing Drosophila, *Drosophila suzukii* (Matsumura), in the California raspberry industry. Pest Manag. Sci. 73: 1083-1090.

Girod, P., O. Lierhmann, T. Urvois, T. C. J. Turlings, M. Kenis, and T. Haye. 2018. Host specificity of Asian parasitoids for potential biological classical biological control of *Drosophila suzukii.* J. Pest Sci. 91: 1241-1250

Goodhue, R. E., M. Bolda, D. Farnsworth, J. C. Williams, and F. G. Zalom. 2011. Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest. Manag. Sci. 67: 1396-1402.

Hamby, K. A., D. E. Bellamy, J. C. Chiu, J. C. Lee, V. M. Walton, N. G. Wiman, R. M. York, and A. Biondi. 2016. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. J. Pest Sci. 89: 605–19.

Hardie, R. C. 1986. The photoreceptor array of the dipteran retina. Trends Neurosci. 9: 419–423.

Hothorn, T., Bretz, F., & Westfall, P. 2008. Simultaneous inference in general parametric models. Biom J. 50: 346-363.

Ideo, S., M. Watada, H. Mitsui, and M. T. Kimura. 2008. Host range of *Asobara japonica* (Hymenoptera: Braconidae), a larval parasitoid of drosophild flies. 2008. Entomol. Sci. 11: 1-6.

Ioriatti, C., V. Walton, D. Dalton, G. Anfora, A. Grassi, S. Maistri, and V. Mazzoni. 2015. *Drosophila suzukii* (Diptera: Drosophilidae) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. J. Econ. Entomol. 108: 1148–55.

Isaacs R. 2011. First detection and response to the arrival of Spotted Wing Drosophila in Michigan. Newsl. Mich. Entomol. Soc. 56:10–12.

Isaacs, R., B. Tritten, S. Van Timmeren, J. Wise, C. Garcia-Salazar, and M. Longstroth. 2013. Spotted wing drosophila management recommendations for Michigan raspberry and blackberry growers. Michigan State University Extension. https://www.canr.msu.edu/ipm/uploads/files/SWDManagement-MichiganRaspberryBlackberry-Aug-2013.pdf

Jantra, C., D. C. Slaughter, J. Roach, and S. Pathaveerat. 2018. Development of a handheld precision penetrometer system for fruit firmness measurement. Postharvest Bio. Technol. 144: 1-8.

Jones, D., and N. Rothwell. 2018. Cultural management strategies to reduce orchard suitability for spotted wing Drosophila. Michigan State University Extension. https://www.canr.msu.edu/news/cultural-management-strategies-to-reduce-orchard-suitability-for-spotted-wing-drosophila

Kamiyama, M. and C. Guédot. 2019. Varietal and developmental susceptibility of tart cherry (*Prunus cerasus*) to *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 112: 1789-1797.

Katsoyannos, B. I., and N. A. Kouloussis. 2001. Captures of the olive fruit fly *Bactrocera oleae* on spheres of different colours. Entomol. Exp. Appl. 100: 165–172.

Kelber, A. & M. J. Henze. 2013. Colour vision: parallel pathways intersect in Drosophila. Curr. Biol. 23: R1043-R1045.

Kinjo H, Y. Kunimi, T. Ban and M. Nakai. 2013. Oviposition efficacy of *Drosophila suzukii* (Diptera: Drosophilidae) on different cultivars of blueberry. J Econ Entomol 106: 1767–1771.

Kirkpatrick, D. M., P. S. McGhee, S. L. Hermann, L. J. Gut, and J. R. Miller. 2016. Alightment of spotted wing Drosophila (Diptera: Drosophilidae) on odorless disks varying in color. Environ. Entomol. 45: 185–191.

Kirkpatrick, D. M., P. S. McGhee, L. J. Gut, and J. R. Miller. 2017. Improving monitoring tools for spotted wing Drosophila, *Drosophila suzukii*. Entomol. Exp. Appl. 164: 87-93.

Kirkpatrick, D. M., L. J. Gut, and J. R. Miller. 2018a. Development of a novel dry, sticky trap design incorporating visual cues for *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 111: 1775–79.

Kirkpatrick, D. M., L. J. Gut, and J. R. Miller. 2018b. Estimating monitoring trap plume reach and trapping area for *Drosophila suzukii* (Diptera: Drosophilidae) in Michigan tart cherry. J. Econ. Entomol. 111: 1285–1289.

Lagoudakis, A., B. Behe, and T. Malone. 2019. Market Segments in the Fresh 'Balaton' Tart Cherry Market in Michigan. Michigan State University. Report No. 1099-2019-1634.

Landolt, P. J., T. Adams, and H. Rogg. 2012. Trapping spotted wing Drosophila, *Drosophila suzukii* (Mastsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. J. Appl. Entomol. 136: 148-154.

Lang, G. A. 2017. The cherry industries in the USA: current trends and future perspectives. VIII International Cherry Symposium. 1235: 119-132.

Leach, H., J. Moses, E. Hanson, P. Fanning, R. Isaacs. 2018. Rapid harvest schedules and fruit removal as non-chemical approaches for managing spotted wing Drosophila. J. Pest. Sci. 91: 219-226.

Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing Drosophila, *Drosophila suzukii*. Pest Manag. Sci. 67: 1358-1367.

Lee, J. C., H. J. Burrack, L. D. Barrantes, E. H. Beers, A. J. Dreves, K. A. Hamby, D. R. Haviland, et al. 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. J. Econ. Entomol. 105: 1350–1357.

Lee, J. C., P. W. Shearer, L. D. Barrantes, E. H. Beers, H. J. Burrack, D. T. Dalton, A. J. Dreves, et al. 2013. Trap designs for monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 42: 1348–1355.

Lee J.C., A. J. Dreves, A.M. Cave, S. Kawai, R. Isaacs, J.C. Miller, S. van Timmeren S, and D. J. Bruck. 2015. Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Ann Entomol Soc Am. 108: 117-129.

Lee, J. C., D. T. Dalton, K. A. Swoboda-Bhattarai, D. J. Bruck, H. J. Burrack, B. C. Strik, J. M. Woltz, and V. M. Walton. 2016. Characterization and manipulation of fruit susceptibility to *Drosophila suzukii.* J. Pest Sci. 89: 771–780.

Little, C. M., A. R. Rizzato, L. Charbonnneau, T. Chapman, and N. K. Hillier. 2019. Color preference of the spotted wing Drosophila, *Drosophila suzukii.* Sci. Rep. 9: 16051. Doi: https://doi.org/10.1038/s41598-019-52425-w

Menne, D. and H.C. Spatz. 1977. Colour vision in *Drosophila melanogaster.* J. Comp. Physiol. 114: 301-312.

Paulk, A., S. S. Millard and B. van Swinderen. 2013. Vision in Drosophila: seeing the world through a model's eyes. Ann Rev Entomol 58: 313–332.

Rice, K., B. Short, S. Jones, T. Leskey. 2016. Behavioral responses of *Drosophila suzukii* (Diptera: Drosophilidae) to visual stimuli under laboratory, semifield, and field conditions. Environ. Entomol. 45: 1480-1488.

RStudio Team. 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/

Shields, E. J. 1989. Artificial light: experimental problems with insects. Bull. Ent. Soc. Am. 35: 40–45.

Takahara, B. and K. H. Takahashi. 2016. Associative learning of color and firmness of oviposition substrates in *Drosophila suzukii*. Entomol. Exp. Appl. 162: 13-18.

Tochen, S., D. T. Dalton, N. Wiman, C. Hamm, P. W. Shearer, and V. M. Walton. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ. Entomol. 43: 501–510.

Tochen, S., J. M. Woltz, D. T. Dalton, J. C. Lee, N. G. Wiman, and V. M. Walton. 2016. Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. J. Appl. Entomol. 140: 47-57.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, S. D. O'Neal, and F. G. Zalom. 2011. Drosophila suzukii (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integr. Pest Manag. 2: G1‹G7

Weydert, C. and J-F. Mandrin. 2013. Le ravageur emergent *Drosophila suzukii* situation en France et conniassances en verger (2éme partie). Infos CTIFL. 292: 32-40.

Wiman, N. G., D. T. Dalton, G. Anfora, A. Biondi, J. C. Chiu, K. M. Daane, B. Gerdeman et al. 2016. *Drosophila suzukii* population response to environment and management strategies. J. Pest Sci. 89: 653-665.

Wiman, N. G., V. M. Walton, D. T. Dalton, G. Anfora, H. J. Burrack, J. C. Chiu, K. M. Daane, et al. 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLOS ONE. 9: e106909.

Wilson, J. L. Gut, N. Rothwell, M. Haas, E. Pochubay, K. Powers, M. Whalon and J. Wise. 2019. Managing spotted wing drosophila in Michigan cherry. https://www.canr.msu.edu/ipm/uploads/files/MISWDGuideCherryJuly2019.pdf

Wilson, J. R., R. Isaacs, and L. Gut. 2019. Michigan spotted wing Drosophila update – July 30, 2019. https://www.canr.msu.edu/news/michigan-spotted-wing-drosophila-update-july-30-2019

Van Timmeren, S. and R. Isaacs. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop programs. J. Crop Prot. 54: 126-133.

Yeh, D. A., F. A. Drummond, M. I. Gómez, and X. Fan. 2020. The economic impacts and management of spotted-wing Drosophila (*Drosophila suzukii*): the case of wild blueberries in Maine. J. Econ. Entomol. Doi: https://doi.org/10.1093/jee/toz360

Zavalloni, C., J.A. Andresen and J.A. Flore. 2006. Phenological models of flower bud stages and fruit growth of 'Montmorency' sour cherry based on growing degree-day accumulation. J. Amer. Soc. Hort. Sci. 131(5): 601–607.