

POST-HARVEST CROP SANITATION STRATEGIES IN *DROSOPHILA SUZUKII*  
SUSCEPTIBLE CROPPING SYSTEMS

By

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## ABSTRACT

### POST-HARVEST CROP SANITATION STRATEGIES FOR *DROSOPHILA SUZUKII* SUSCEPTIBLE CROPPING SYSTEMS

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*Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), or spotted wing Drosophila, is an invasive, polyphagous vinegar fly that has rapidly spread across the United States. They have caused significant damages to the berry and stone fruit industries due to the female's ability to oviposit directly into mesocarp of soft-skinned fruit. Control is primarily obtained through calendar spray programs composed of broad spectrum insecticides, which are applied at weekly or shorter intervals. These intensive insecticide regimens paired with *D. suzukii*'s high fecundity and short generation time has introduced the potential of insecticide resistance, especially in organic cropping systems where the number of insecticide classes are severely restricted. While there is an emphasis on grower training to ensure chemical rotation, the options for effective control are so limited that resistance management is difficult. Thus, this thesis explores alternative *D. suzukii* management strategies to reduce the current dependency on chemical management. Post-harvest crop sanitation strategies are a form of cultural control that involve the disposal of infested fruits and any ripe, overripe, and decomposing fruit that can act as a reproductive host. Burying and composting were identified as effective disposal methods for *D. suzukii* susceptible fruits. Burying *D. suzukii* infested fruit wastes 24 cm underground reduced adult emergence by 97%. Likewise, composting fruit waste with 25% chicken manure reduced *D. suzukii*'s reproductive success > 95%. Integrating these cultural control tactics into existing management programs will help to restore IPM in *D. suzukii* susceptible cropping systems.

Dedicated to Burnelle Bucher.

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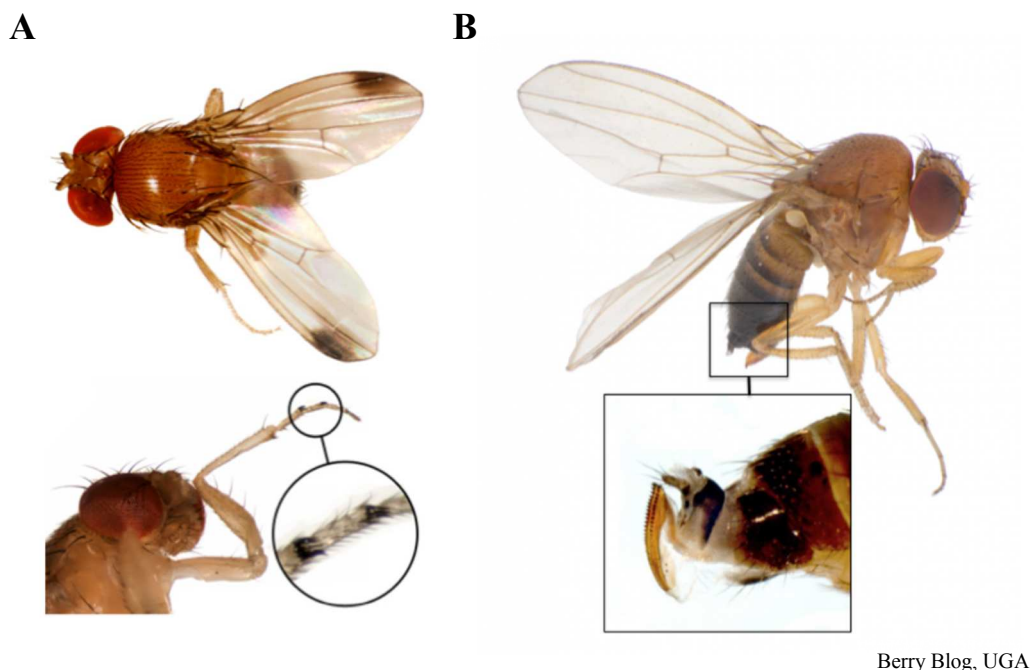
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## CHAPTER 1. SPECIES UNDER STUDY: *DROSOPHILA SUZUKII*

### Introduction

#### *Biology*

*Drosophila suzukii* (Diptera: Drosophilidae), or spotted wing *Drosophila*, belong to the subgenus *Sophophora* within the species group of *D. melanogaster* (Hauser 2011). The species group of *D. melanogaster* is comprised of 6 species subgroups that are native to eastern Asia (Hauser 2011, Asplen et al. 2015). Adult *D. suzukii* males can be identified by a dark spot on the leading edge of each wing, and by two sets of black tarsal combs on the first and second tarsal segments of the fore leg (Hauser 2011) (Figure 1. 1A). The dark spots can take 2 days post-eclosion to fully develop on the wings (Hauser 2011). Adult *D. suzukii* females can be identified by a large and highly sclerotized serrated ovipositor (Asplen et al. 2015) (Figure 1. 1B).



Berry Blog, UGA

**Figure 1. 1** Adult *D. suzukii* males are distinguished by a black spot on the leading edge of each wing and two sets of black tarsal combs on the first and second tarsal segments of the fore leg (A), and adult *D. suzukii* females are characterized by a large ovipositor with dark, sclerotized teeth (B).

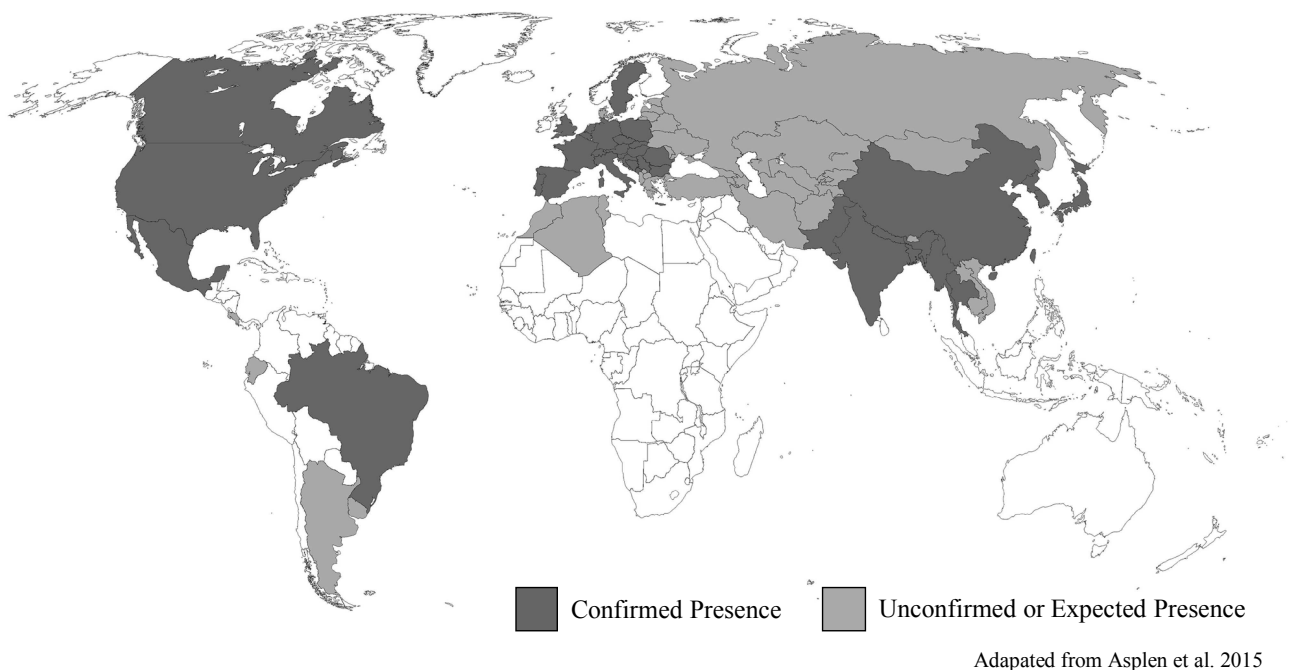
Post-eclosin, females have a pre-oviposition period of 1 – 3 days (Asplen et al. 2015). Once reproductively active, females deposit an egg singly or in small clutches into the mesocarp of soft-skinned fruit by use of their serrated ovipositor (Mitsui et al. 2006). Upon hatching, the larvae undergo three molts (Asplen et al. 2015). The 1<sup>st</sup> and 2<sup>nd</sup> instar larvae feed on fruit tissue and supplementary yeasts, while the 3<sup>rd</sup> instar larvae cease feeding and search for a pupation site (Asplen et al. 2015, Woltz and Lee 2017). Pupation occurs primarily outside of the fruit, with 3<sup>rd</sup> instar larvae dropping to the ground from hanging fruit or exiting fruit that has previously dropped to the ground to pupate underneath the soil's surface (Woltz and Lee 2017). Generation time from egg to adult can take from 8 – 79 days depending on the temperature (Lee et al. 2011, Asplen et al. 2015). Temperatures below 10°C and above 30°C severely impair juvenile development (Kirk Green et al. 2019).

Females can lay more than 25 eggs per day, with highest reproduction recorded on cherry at 22°C (Asplen et al. 2015). The majority of adult activity occurs at dawn or dusk, and minimal to no activity occurs on extremely hot or cold days (Hamby et al. 2016). Adult lifespan was found to be  $86.1 \pm 4.25$  days on average in the laboratory, and is an estimated 1 month in the wild (Emiljanowicz et al. 2014, Tochen et al. 2014). The combination of their relatively long lifespan and short generation time can result in up to 13 generations of *D. suzukii* per year depending on weather conditions (Tochen et al. 2014). Many of these generations over-lap, which can lead to exponential population growth (Asplen et al. 2015).

#### *Native Range and Rapid Spread*

*D. suzukii* were first described by Matsumura in 1931 in Japan, and he was the first to publish records of the damage caused by this pest on cherries and blueberries in 1936 and 1939

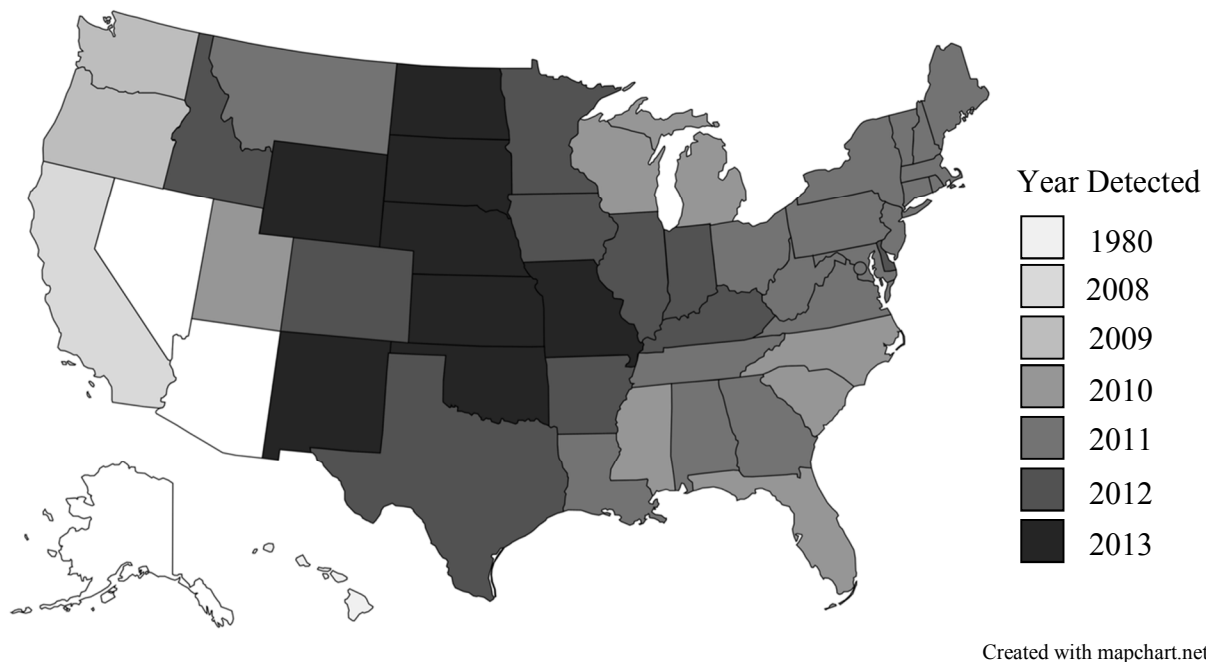
(Hauser 2011). However, there are earlier reports of *D. suzukii* in Japan dating back to 1916, and it is unclear if *D. suzukii* was native to Japan or was introduced during the early 1900s (Hauser 2011). In addition to Japan, there are records of *D. suzukii* in the eastern part of China, North Korea, South Korea, the Kashmir region of India, Thailand, Taiwan, Myanmar, the Russian Far East, and Pakistan prior to its spread to Europe and the Americas (Cini et al. 2012). *D. suzukii* are now present on every continent but Antarctica and parts of Oceania (Figure 1. 2).



**Figure 1. 2** Map of global *D. suzukii* distribution as of May 2015. Countries denoted by dark gray have confirmation of *D. suzukii* presence. Countries denoted by light gray are expected to have *D. suzukii* presence due to geographic proximity to countries with confirmed presence and/or have initial records that have yet been confirmed.

In North America, *D. suzukii* was reported in the Hawaiian Islands on Oahu, but was not considered a pest (Hauser et al. 2011). They first appeared on the mainland in 2008 along the coastal, berry producing regions of California, but were misidentified as *Drosophila biarmipes* (Malloch) (Diptera: Drosophilidae) (Hauser 2011, Asplen et al. 2015). It took until 2009 to

correctly identify *D. suzukii*, and by then it had then spread to 20 counties in California, as well as Oregon, Washington, and Florida (Hauser 2011). Its identification precipitated intensive monitoring throughout the rest of the country, and subsequent reports confirmed *D. suzukii*'s presence in Wisconsin, North Carolina, South Carolina, Mississippi, and Utah by 2010 (Asplen et al. 2015). Currently, *D. suzukii* are found in 47 states within the United States (Figure 1. 3).



**Figure 1. 3** Map of United States *D. suzukii* distribution. States are denoted by color with year of first confirmed *D. suzukii* presence.

### *Overwintering*

It is uncertain if *D. suzukii* overwinter locally in regions that experience harsh winters or if they migrate to areas with milder weather because *D. suzukii* captures drop to zero between December and May in the Midwest (Guédot et al. 2018). Their native range has a temperate climate that is marked by seasonal and regional fruit availability, so it is plausible that *D. suzukii*

are capable of migrating between high and low latitudes to avoid drastic fluctuations in temperature (Asplen et al. 2015). If *D. suzukii* are not migrating, their survival over the winter is dependent on their ability to adapt to colder temperatures or to find refuge in insulated areas, either natural or man-made (Asplen et al. 2015). Woodland and leaf-litter are potential protected microclimates for *D. suzukii*, and farms that have an abundance of woodland adjacent to their crop experience *D. suzukii* captures approximately one week earlier in the spring and significantly later in the fall compared to farms that lack woodland (Pelton et al. 2016).

Only a small percentage of *D. suzukii* were able to survive winter temperatures in the laboratory (Pelton et al. 2016). The majority of the surviving adults were in the winter morph, which have a darker coloration, smaller bodies, and longer wings compared to the summer morph (Stephens et al. 2015). Winter morphs appear when temperatures drop below 10°C and the photoperiod shortens, and their activity becomes restricted to the warmest part of the day (Hamby et al. 2016). Mortality in both morphs occur before the temperature at which their body fluids freeze, i.e. the super-cooling point, making *D. suzukii* a chill-intolerant species. However, the adult winter morphs have a significantly lower lethal temperature compared to summer morphs (Stephens et al. 2015). They can tolerate a temperature of -7.5°C for a short duration and a temperature of 1°C for several months (Stephens et al. 2015, Stockton et al. 2019).

*D. suzukii* adults that undergo eclosion at temperatures below 10°C are thought to be in a state of reproductive diapause, and adults that undergo eclosion prior to temperatures dropping below 10°C are hypothesized to be reproductively active (Dalton et al. 2011). Mated females are capable of storing sperm throughout the winter, which allows them to lay viable eggs the following spring (Guédot et al. 2018). Thus, a reduced number of overwintering females could delay the onset and infestation of early fruit crops the following growing season (Rossi-Stacconi

et al. 2016). *D. suzukii* must select an overwintering location that has a food supply to survive the winter, or they must retain the ability to forage (Wallingford et al. 2018). Fruit wastes, tree sap, and nectar from early flowering blossoms have been suggested as overwintering carbohydrate sources (Bal et al. 2017, Wallingford et al. 2018).

### *Affected Crops and Diet*

*D. suzukii* utilize numerous fruit crops as a reproductive resource, such as ripening or ripe blackberries, blueberries, cherries, peaches, raspberries, strawberries, and select cultivars of grapes (Lee et al. 2011). While *D. suzukii* are primarily known for targeting healthy fruit, they will also oviposit into decomposing fruits in the late growing season (Bal et al. 2017). This includes fruit that initially has tougher skin and tissue, such as apples and pears, and post-harvest and processing fruit wastes (Bal et al. 2017). Additionally, *D. suzukii* utilize alternative hosts, which includes wild, ornamental, and uncultivated fruits (Lee et al. 2015). For example, *Lonicera spp.* act as a carbohydrate resource to *D. suzukii* at the beginning of the growing season before cultivated fruit crops become available in Michigan (Leach et al. 2018).

Carbohydrates obtained from sugars are necessary for adult survival, and proteins are necessary for egg maturation (Plantamp et al. 2017). *D. suzukii*'s feeding and oviposition sites do not typically overlap because adults can only feed on the drop of fruit juice that exits the oviposition scar after ovipositing into healthy fruit (Plantamp et al. 2017). The nutrients gained from this drop of fruit does not enable females to produce mature eggs or equate the energy cost of oviposition (Plantamp et al. 2017). To fulfill their nutritional requirements, adults are potentially obtaining food from leaf surfaces or floral nectaries (Plantamp et al. 2017). Overall, a low protein to carbohydrate diet is optimal for adult *D. suzukii* survival and reproduction, and

result in *D. suzukii* reaching peak fecundity at a later age compared to a high protein to carbohydrate diet (Rendon et al. 2018). In contrast, a high protein to carbohydrate diet is advantageous to *D. suzukii* larvae survival (Silva-Soares et al. 2017).

Microbes associated with fruit decomposition are the primary source of proteins for drosophilids (Jaramillo et al. 2015). However, the nutritional benefits gained from microbes can be species specific. For example, the yeast *Hanseniaspora uvarum*, which is available during the early stages of fruit decomposition, has been found to enhance larval development and survival (Bellutti et al. 2017). *D. suzukii* are hypothesized to have a mutualistic association with *H. uvarum*, and it has consistently been found to dominate the *D. suzukii* gut community (Hamby et al. 2012, Bellutti et al. 2017, Lewis et al. 2019). In contrast, the bacterium *Enterococcus faecalis* has a negligible effect on *D. suzukii* larval development due to its inability to colonize in the gut (Bing et al. 2018). The presence of microbes can also be deleterious to *D. suzukii* larvae on a high protein diet, although this antagonistic interaction has yet to be explored (Bing et al. 2018).

### *Impact within the United States*

Blueberries, raspberries, blackberries, and cherries are the primary crops economically affected in the Western United States, with *D. suzukii* being reported from southern California to British Columbia, Canada (Asplen et al. 2015). Patterns of infestation varies according to location. *D. suzukii* can be found year-round in the milder climactic regions of California (Asplen et al. 2015). In contrast, central San Joaquin Valley experiences high populations during the both the early season and late growing season, with populations declining mid-season when temperatures rise above 30°C and again when temperatures drop in December (Kaçar et al. 2016). These production regions can suffer an annual estimated loss of up to \$500 million in *D.*

*suzukii*-related damages when left unmanaged (Asplen et al. 2015). Economic losses are a result of direct crop damage and an increase in labor and materials necessary for management, primarily from an increase in insecticide applications, netting, and monitoring (Lee et al. 2011).

The Eastern and North Central United States berry and cherry fruit industries are comprised of a network of small fields ranging from 0.1 to 5 acres, as well as localized areas of mid-to-large fields of commercial plantings of berry and cherry crops (Asplen et al. 2015). This includes tart cherries in Michigan, strawberries in Florida and North Carolina, blueberries in Michigan, New Jersey, North Carolina, Georgia, and Florida, and *Rubus* species in North Carolina and New York (Asplen et al. 2015). In response to consumer demand, many of these growers follow sustainable or organic guidelines (Asplen et al. 2015). Historically, limited applications of insecticides were effective in the Eastern United States due to low pest pressure (Asplen et al. 2015). With the arrival of *D. suzukii*, farms in these regions are facing drastic economic losses from decreased yields and increased production costs (Asplen et al. 2015). It was estimated that the Eastern United States suffered a loss of \$27.5 million due to *D. suzukii* in 2013 (Asplen et al. 2015). Additionally, many farms in the North Central region of the United States suffer a lack of regular fruit removal due to the prevalence of the “pick-your-own” harvest model, which contributes to *D. suzukii* pest pressure (Asplen et al. 2015).

Michigan is ranked #1 in both cherry and highbush blueberry production within the United States (MDA, 2014). The sweet and tart cherry industries are valued at \$25.8 and \$72.9 million, respectively, with over 34,000 acres of cherry orchards across the state (MDA, 2014). Additionally, Michigan is the largest producer of Montmorency tart cherries in the world, with over 90,000 tons of Montmorency cherries harvested each year (MDA, 2014). In 2015, *D. suzukii* infestations peaked during Montmorency cherry harvest, which resulted in several load

rejections by the cherry processor in northern Michigan (Rothwell, unpublished). According to the North American Blueberry Council, the Michigan highbush blueberry industry is valued \$55.4 million and produces an annual 75 – 110 million pounds of blueberries on approximately 20,000 acres. The Michigan raspberry and blackberry industries are comparatively small, being 600 acres of crop land, although fall-fruited berries have been recently developed to extend the berry season (MDA, 2014). Infestations in Michigan's berry crops are drastic when *D. suzukii* is left unmanaged, with losses in 2012 exceeding \$26 million (R. Isaacs, unpublished).

### **Current *D. suzukii* Management**

#### *Monitoring Programs and Attractants*

Detecting a pest early is the foundation of any successful pest management program, which is typically done through monitor trapping (Cini et al. 2012). Monitor trapping alerts growers on initial pest presence and tracks population flux throughout the growing season (Kirkpatrick et al. 2017). Once populations exceed a predetermined action threshold, growers can initiate additional control measures to prevent damage to their crop (Kirkpatrick et al. 2017). The action threshold for *D. suzukii* is a single fly capture because it equates to approximately 192 flies within a trapping area of 26 acres (Kirkpatrick et al. 2018). As traps are stationary, insects need to approach monitoring traps by chance or by being lured via a chance encounter with an attractive cue emitted by the trap (Miller et al. 2015).

In nature, *D. suzukii* utilize many olfactory cues to locate food and reproductive resources. Among blueberry, cherry, raspberry, and strawberry fruit extracts, *D. suzukii* were attracted to raspberry and strawberry more so than blueberry and cherry based on their antennal activity (Abraham et al. 2015). However, *D. suzukii* do not display a preference for ripening fruit

volatiles although they are primarily associated with fresh fruit (Keesey et al. 2015). They are most attracted to decomposing fruits, wines, vinegars, and fermentation volatiles, such as acetic acid and ethanol (Cha et al. 2018). Thus, initial recommendations for *D. suzukii* trapping included protocols that incorporated apple cider vinegar or grape wine (Beers et al. 2011).

These materials are still popular along with a yeast-sugar mix and Scentry Biological's (Scentry Biologicals, Inc., Billings, MT) commercial lure, which are used in a variety of commercial and homemade monitoring traps. Cup trap designs are most commonly used for monitoring, which consist of a translucent plastic cylinder with multiple small entry holes, a bait, and a liquid drowning solution (Cini et al. 2012). Multiple studies have shown that red and black colored traps capture significantly higher numbers of *D. suzukii* compared to the translucent cup traps, which suggests that monitoring traps that have both visual and volatile cues are more effective (Cini et al. 2012, Kirkpatrick et al. 2017).

### *Current Chemical Management*

Insecticide applications have significantly increased due to *D. suzukii*'s arrival. This is a result of the high pest pressure growers face from *D. suzukii* and the zero-tolerance policy regarding insect infestation for the fresh and processed berry markets (Van Timmeren and Isaacs 2013). Once fruit begins to ripen, captures of adult flies in monitoring traps precipitates an additional 5 – 7 applications of insecticides per growing season until harvest is complete (Van Timmeren and Isaacs 2013). Control is primarily gained through pyrethroid, organophosphate, and spinosyn classes, and methomyl in the carbamate class (Asplen et al. 2015). Organic growers rely heavily on spinosyn due to the limited classes permitted in organic cropping systems, and azadirachtin and organic pyrethrins do not offer economic control (Asplen et al. 2015). A recent

study by Fanning et al. (2017) highlights that the biopesticides sabadilla alkaloids and *Chromobacterium subtsugae* can be used in conjunction and/or rotation with spinosyn for effective *D. suzukii* control.

The aforementioned insecticides provide differential levels of residual protection, which vary between 5 – 14 days in laboratory bioassays (Van Timmeren and Isaacs 2013). However, insecticidal efficacy and durability is impacted by weather, and many insecticides rapidly break down after extended exposure to rainfall and ultraviolet light (Van Timmeren and Isaacs 2013). Furthermore, these measures do not account for larval infestation post-application. Certain insecticides, including the organophosphate phosmet, the spinosyn spinetoram, and neonicotinoids imidacloprid, acetamiprid, and thiamethoxam, have been shown to display lethal action post-infestation due to their ability to penetrate plant tissue, i.e. curative activity (Wise et al. 2015). The curative activity of these compounds contributes to their overall efficacy by killing *D. suzukii* eggs and/or larvae post-infestation (Wise et al. 2015).

### *Current Biological Management*

Currently, there are no effective biological control agents for *D. suzukii* in the United States (Asplen et al. 2015). Ants, spiders, and predatory hemipterans, such as *Miridae* and *Orius* species, have been observed to predate on immature *D. suzukii* in the field (Woltz and Lee 2017). Additionally, a few native parasitoids, including *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae), have been reared from field collected *D. suzukii* pupae (Gabarra et al. 2015). While these species consume *D. suzukii*, they do not effectively reduce field populations, and, thus, the majority of biological control research is focused on classical biological control programs (Woltz and Lee

2017, Renkema and Cuthbertson 2018). Currently, *Ganaspis brasiliensis* (Ihering) and *Leptopilina japonica* (Novković & Kimura) (Hymenoptera: Figitidae), two parasitoids from *D. suzukii*'s native range, are being evaluated in a California quarantine for introduction within the United States (Wang et al. 2018).

Additionally, inter-species competition could contribute to biocontrol in the field. Previous laboratory studies have shown that exploitative competition occurs when *D. suzukii* are forced to compete for reproductive resources with *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Dancau et al. 2017). *D. suzukii* populations were dramatically reduced in both pairwise contests and group competitions with *D. melanogaster* (Dancau et al. 2017). Furthermore, *D. suzukii* were hesitant to oviposit into media pre-inoculated with *D. melanogaster* eggs in choice experiments (Shaw et al. 2017). This suggests that the population reduction observed via inter-species competition is partly due to female oviposition choice (Shaw et al. 2017). If fruit that has been previously infested is aversive to egg-laying females, then the hetero-specific signals emitted from infested fruit could potentially be exploited as a method of biocontrol or developed into a synthetic egg-laying repellent (Mitsui et al. 2006, Shaw et al. 2017).

### *Current Cultural Management*

Plastic covering or netting in blueberry, raspberry, and cherry cropping systems are effective against *D. suzukii* (Asplen et al. 2015, Rogers et al. 2016). Berries grown in high tunnels covered by plastic or fitted with netting with a mesh size of less than 0.98 mm significantly reduces *D. suzukii* infestations without sacrificing crop yield or quality (Rogers et al. 2016). High tunnels covered with plastic tend to have greater success than those covered with

netting due to it increasing the high tunnel's internal temperature above 30°C, which impairs *D. suzukii* development (Rogers et al. 2016). Furthermore, physically excluding *D. suzukii* can reduce insecticide applications by delaying infestation. Leach et al. (2016) recorded a delay of three weeks in raspberries grown in netted high tunnels. Netting in cherry can be applied to high density plantings as a whole or in single rows at the time of ripening (Dallabetta, unpublished). While netting offers effective control, the initial investment is expensive (Leach et al. 2016). In berry crops, netting for one acre of 122-meter tunnels would cost approximately \$6,100, while in cherry one acre of netting to cover a field in single row netting would cost approximately \$38,000 – \$43,000 (Dallabetta, unpublished, Leach et al. 2016).

Alternatively, changing harvest frequencies in fruits that ripen over a long period of time can preserve or increase economic profit for growers while simultaneously reducing *D. suzukii* infestation levels. Harvesting on a 2-day schedule instead of a 3-day schedule significantly reduced infestation of 3<sup>rd</sup> instar larvae (Leach et al. 2018). Additionally, the yield per plant was found to be highest on a 2-day schedule, with a single 10 m row of cv. 'Himbo Top' generating an estimated \$807 in revenue compared to an estimated \$530 and \$538 from 1-day and 3-day schedules, respectively (Leach et al. 2018).

There are additional cultural control strategies that are in the early stages of development within the United States, including in-field cultivation, fruit cooling, irradiation, and post-harvest sorting (Asplen et al. 2015, Iglesias and Liburd 2017). A promising area of *D. suzukii* management is focused on the manipulation and/or removal of suitable habitat. *D. suzukii* prefer cool, humid microhabitats within crops, with dry, warm conditions reducing *D. suzukii* activity and egg viability (Haye et al. 2016, Diepenbrock and Burrack 2017). Thus, management tactics such as pruning fruit canopies, using drip irrigation, increasing plant spacing, or using dark

mulches between rows could contribute to greater light penetration, increased airflow, and heightened temperatures, which could aid in decreasing host suitability for *D. suzukii* (Haye et al. 2016, Rendon and Walton 2019).

### *Current Post-Harvest Crop Sanitation Strategies*

Culled fruit and fruit wastes need to be removed from crop fields and disposed of because flies can continue to emerge from compromised fruit and use it as reproductive habitat (Bal et al. 2017, Leach et al. 2018). Not implementing a crop sanitation strategy against *D. suzukii* can lead to clean fruit becoming infested and pest pressure intensifying as the growing season progresses (Leach et al. 2018). There are currently few recommendations given to growers on how to effectively dispose of *D. suzukii* susceptible fruit wastes. Recent work by Leach et al (2018) found that bagging and solarizing infested berries for 32 hours reduced *D. suzukii* larvae survival by 99%. There was no significant difference in larvae mortality after 32 hours between clear, white, and black bags, although fruit in the clear bags reached the highest internal temperatures and remained at temperatures lethal to *D. suzukii* for the longest periods of time (Leach et al. 2018). While this method of disposal is effective against culled berries, it is difficult to translate to stone fruit and large quantities of fruit wastes (Haye 2016). Alternative options for fruit waste disposal include burning, freezing, use as animal feed, burial, and composting, although there is a current lack of research to support these recommendations.

## **Alternative Management Strategies**

### *Cultivation, Burial, and Impacts on Insect Pest Management*

Cultivation is a floor management tactic that has historically been tied to growing crops via preparing seedbeds, incorporating organic matter and fertilizers into soil, and suppressing weeds (Stinner and House 1990). There are a variety of cultivation practices and technologies, which can be divided into three main sub-categories: conventional tillage, reduced tillage, and no-tillage. Conventional tillage is the most widespread approach to cultivation within mechanized agriculture. A moldboard plow is used to invert the top 20 – 25 centimeters of soil, which leaves behind a bare soil surface (Stinner and House 1990). Reduced tillage leaves behind a comparatively large quantity of plant residue by use of diskers, chisels, and sweeps that loosen the soil (Stinner and House 1990). No-tillage, also known as direct-drill and zero tillage, is a further reduced form of tillage, and uses specialized equipment to create shallow grooves on the soil's surface for depositing seeds (Stinner and House 1990). This approach to cultivation leaves behind almost all plant residue (Stinner and House 1990). Both reduced-tillage and no-tillage fall under the umbrella term of conservation tillage, which requires 30% of plant residue to be left on the soil's surface (Stinner and House 1990).

Cultivation, applying herbicides, laying mulch, and propane flame burning are management tactics used to control weeds within orchard cropping systems (Stefanelli et al. 2009, Rowley et al. 2011). In recent years, the Swiss Sandwich System (SSS) has largely replaced conventional tillage as a more sustainable tillage practice (Stefanelli et al. 2009). The SSS is a reduced-tillage practice that only tills underneath tree rows, which leaves behind a strip of permanent floor vegetation (Stefanelli et al. 2009). In addition to retaining soil structure and drainage compared to conventional tillage, this practice increases the availability of plant-based

resources like pollen, nectar, alternative prey, and shelter (Fiedler et al. 2008). The presence of these otherwise limited-resources can improve the reproduction and searching ability of natural enemies, which can lead to lower pest populations in the crop as demonstrated by studies in blueberry, cabbage, wheat, and tomato (Olson and Wäckers 2007, McCabe et al. 2017).

Cultivation can also be used to directly reduce the survival of insect pests through mechanical damage and disturbance (Stinner and House 1990). Some orchard pests that spend part of their life-cycle near or in the soil are susceptible to this cultural control strategy, such as the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), and the grape berry moth, *Paralobesia viteana* (Clemens) (Lepidoptera: Tortricidae) (Baughman et al. 2015, Matlock et al. 2017). Codling moth did not survive a burial depth of 1 cm in sand when buried as either a larvae or pupae in the laboratory (Baughman et al. 2015). Similarly, diapausing grape berry moth survival was significantly reduced at a burial depth of 1 cm in sand in the laboratory, with field trials demonstrating emergence interference was the mechanism behind reduced survival rather than mechanical injury (Matlock et al. 2017). Two hypotheses were proposed to explain emergence interference: 1) the soil's weight pinned the pupa into place, and 2) soil abrasion inflicted lethal injuries on the pupa as they moved through the soil (Matlock et al. 2017).

Using burial as a method of emergence interference has been successful with the dogwood borer, *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae) and the melon fly, *Bacrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) (Klungness et al. 2005, Gut et al. 2005). Mounding soil 5 cm above the graft union of apple rootstocks was found to reduce the survival of dogwood borer larvae by 76 – 96%, which offered control equal to or higher than insecticidal trunk sprays (Gut et al. 2005). A burial depth of 46 cm prevented all melon fly emergence from infested fruit (Klungness et al. 2005). In contrast, a burial depth of 15 cm improved melon fly

survival compared to infested fruit left on the soil's surface (Klungness et al. 2005). The melon fly typically pupates underneath the soil's surface, which suggests that self-burying behavior should be considered before implementing burial as a pest management strategy because it could impact the burial depth required to prevent adult emergence (Back and Pemberton, 1914).

### *Composting and Impacts on Insect Pest Management*

Composting is the process of biological decomposition that transforms organic matter into a homogeneous, plant available product, i.e. compost (Azim et al. 2018). This process is primarily microbial, with different microbial groups characterizing the different stages of the composting process (Azim et al. 2018). Oxygen availability is critical to these microbes for aerobic respiration, which subsequently generates heat as they decompose organic matter (Azim et al. 2018). Temperatures increase to 40°C during the initial, mesophilic stage of the composting process (Azim et al. 2018). As temperatures continue to rise, the mesophilic microbes are replaced by thermophilic microbes (Azim et al. 2018). Thermophilic decomposition slows as temperatures reach 60°C, and is eventually replaced by enzymatic decomposition at 70°C (Azim et al. 2018). During the cooling and subsequent maturation phases, mesophilic microbes re-colonize the composting materials (Azim et al. 2018). The composting process is complete when heat is no longer produced after turning the compost pile, the compost does not become anaerobic while stored, and the compost does not draw nitrogen from the soil post-amendment (Azim et al. 2018).

In addition to temperature, the microbial community composition and abundance is dependent on the moisture content, initial C:N ratio, feedstock materials, and operating system (Azim et al. 2018). The feedstock materials should ideally have a carbon to nitrogen ratio of 1:25

(de Bertoldi et al. 1983). Higher carbon to nitrogen ratios slow decomposition because additional generations of microbes are needed to oxidize the excess carbon (Azim et al. 2018). Lower carbon to nitrogen ratios result in an atmospheric loss of nitrogen via the volatilization of ammonia (Tiquia and Tam 2000). Feedstock materials within the range of 3 – 11 pH can be composted, although a pH between 5.5 – 8 is optimal (de Bertoldi et al. 1983). The pH drops during the mesophilic stage of the composting process due to organic acid formation, which is later neutralized during the thermophilic stage once ammonia is released (Azim et al. 2018). Feedstock materials can be composted in closed or open systems, with open-air windrows being the most common method due their low cost, versatility, and highly predictable results (de Bertoldi et al. 1983). The resulting compost is stable and hygienic, and can be added to field soils to improve soil health (Litterick et al. 2004).

Regular additions of compost to field soils can benefit soil structure, water drainage, nutrient availability, carbon sequestration, biodiversity, and the complexity of soil food webs (Litterick et al. 2004, Martínez-Blanco et al. 2013). Crop growth and yield can be directly improved from an increase in nutrient availability, and indirectly improved from an increase in root growth caused by the modified physical characteristics of the soil (Leroy et al. 2008). Pest suppression can also be indirectly improved due to the increased biotic complexity of the soil environment (Litterick et al. 2004). The majority of studies have focused on compost amendments reducing pathogen and plant parasitic nematode incidence (Litterick et al. 2004). There are limited studies that demonstrate compost's effects on insect pest suppression, although Brown and Tworkoski (2004) found that there was a greater abundance of predators, primarily Carabid and Staphylinid beetles, in apple orchards that received regular compost amendments compared to control apple orchards.

Alternatively, composting could be used as a method of waste disposal to directly reduce insect pest incidence. Food, manure, and fish wastes are used as reproductive habitat to a multitude of Dipteran pests, such as flies in the Drosophilidae, Muscidae, and Calliphoridae families (Rubasinghe et al. 2013). Rubasinghe et al. (2013) determined that compost mixes containing 50% food waste and 50% garden waste had optimal composting efficacy and minimal fly emergence. Fly emergence was further reduced when compost mixes were covered in a layer of clean garden waste and constructed under a roof cover. Additionally, Rubasinghe et al. (2013) observed that adult flies and larvae were most abundant between weeks 1 – 4 of the composting process, after which their numbers gradually decreased. Furthermore, they observed that larvae were almost exclusively located on the outer layers of the compost piles. Fly reduction was attributed to the reproductive resources being degraded over time and the high temperatures generated by the thermophilic stage of decomposition (Rubasinghe et al. 2013).

## **Summary and Objectives**

Despite *D. suzukii* arriving to the United States over a decade ago, pest populations are largely controlled by applications of broad spectrum insecticides. A recent report from Gress and Zalom (2019) has confirmed that populations of *D. suzukii* near Watsonville, CA demonstrated resistance to the bio-insecticide spinosad, which intensifies the need for non-chemical *D. suzukii* management strategies. While the risk of spinosad resistance is amplified in areas of high organic production, conventional growers also heavily rely on spinosyn-based insecticides for *D. suzukii* control (Gress and Zalom 2019). To mitigate the development of insecticide resistance, research on alternative, cost-efficient management strategies are urgently needed so that crop and revenue losses do not exceed their current levels.

Approximately 92% of *D. suzukii* are eggs, larvae, or pupae in the field, so management tactics that target juvenile *D. suzukii* have great potential to reduce localized *D. suzukii* populations (Emiljanowicz et al. 2014). Post-harvest crop sanitation strategies are currently an under-utilized area of *D. suzukii* management that target the immature stage of this pest. The overarching goals of this study are to generate recommendations to growers on effective methods of fruit waste disposal for *D. suzukii* infested fruits, and to provide a culturally based integrated pest management (IPM) strategy that reduces the current dependence on chemical management. The specific objectives of this study are to:

1. **Determine the potential of larval/pupal burial as a means to interfere with adult emergence.** *Hypotheses:* a) *D. suzukii* will have reduced survival at deeper burial depths, and b) burial of infested fruit will result in reduced *D. suzukii* survivorship.
2. **Determine the potential of composting fruit wastes as a means to reduce *D. suzukii* reproductive habitat.** *Hypotheses:* a) *D. suzukii* will have reduced fecundity in compost treatments with lower amounts of fruit wastes, and b) *D. suzukii* will have differential fecundity in compost treatments created with animal manure or plant based feedstocks.

## CHAPTER 2. BURIAL OF *DROSOPHILA SUZUKII* INFESTED FRUIT WASTE REDUCES ADULT EMERGENCE

### Introduction

The invasive vinegar fly spotted wing *Drosophila*, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), has rapidly spread across the continental United States since its first detection in 2008 (Asplen et al. 2015). *Drosophila suzukii* females have a unique, serrated ovipositor, which allows them to penetrate and lay eggs into soft-skinned fruit prior to harvest (Lee et al. 2011). Larvae hatch and develop within the fruit, which leads to discoloration and eventual collapse of fruit tissues (Asplen et al. 2015). The ability of females to exploit immature and ripe fruit has caused extensive damage to the small and stone fruit industries, with berry and cherry growers suffering the highest losses of marketable fruit (Lee et al. 2011). A female *D. suzukii* can lay over 400 eggs within her estimated adult lifetime of 1 month in the wild (Hamby et al. 2016, Tochen et al. 2014). Development from egg to adult can take as little as 8 days, which results in multiple overlapping generations of *D. suzukii* each summer (Lee et al. 2011). The combination of these factors leads to exponential population growth and severe pest pressure in the later growing season.

The high damage potential and population growth parameters of *D. suzukii* have resulted in widespread adoption of calendar-based spray programs of broad spectrum insecticides (Van Timmeren and Isaacs 2013). A single capture of *D. suzukii* within a monitoring trap prompts an additional five to eight insecticide applications per season, often beginning when fruit starts to ripen and persisting until harvest is complete (Kirkpatrick et al. 2017, Van Timmeren and Isaacs 2013). These additional insecticide applications paired with *D. suzukii*'s high fecundity and generation turnover presents the risk of insecticide resistance, which is especially concerning in

certified organic cropping systems where growers rely almost exclusively on spinosad for control (Van Timmeren and Isaacs 2013, Gress and Zalom, 2019). Thus, there is an urgent need to evaluate non-chemical management tactics to mitigate these intensive insecticide regimens.

Post-harvest crop sanitation strategies are an under-researched area of *D. suzukii* management with the potential to decrease the availability of *D. suzukii* reproductive habitat and, consequently, reduce localized *D. suzukii* populations. While *D. suzukii* have most often been associated with pre-harvest fruit, they also reproduce in decomposing materials like other drosophilids (Bal et al. 2017). This includes fruits that are not suitable “fresh hosts” due to tougher skin and tissue (e.g. apples and pears) and post-harvest and processing fruit wastes (Bal et al. 2017). Failure to implement a sanitation strategy has been shown to intensify pest pressure as the growing season progresses, but there is little guidance given to growers on how to dispose of susceptible fruit wastes (Leach et al. 2018). Solarizing infested berries in plastic bags effectively kills immature *D. suzukii*, but this tactic is less feasible for stone fruit or the large quantities of fruit wastes typical of post sorting or processing operations (Haye et al. 2016, Leach et al. 2018). Burial is another potential approach to fruit sanitation.

Burial has been successful in reducing the survivorship of other fruit pests, such as codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and grape berry moth, *Paralobesia viteana* (Clemens) (Lepidoptera: Tortricidae) (Baughman et al. 2015, Matlock et al. 2017). In contrast, a shallow burial depth of 2.5 cm improved the survivorship of plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), which pupate underneath the soil’s surface like *D. suzukii* (Baughman et al. 2015, Woltz and Lee 2017). This suggests that self-burying behavior must be taken into account when considering using burial as a sanitation strategy because it may influence the depth needed to prevent adult emergence.

The overall goal of this study was to evaluate burial of fruit waste as a cultural *D. suzukii* management tactic. The following four experiments were conducted to meet this goal: 1) a laboratory investigation of the depth *D. suzukii* larvae prefer to pupate in two soil textures, 2) a laboratory comparison of the same two soil textures on *D. suzukii* pupae survivorship at varying burial depths, 3) a field evaluation investigating the potential of burying *D. suzukii* infested fruit as an effective method of fruit waste disposal, and 4) a laboratory evaluation comparing *D. suzukii* lipid concentrations between *D. suzukii* emerging from infested apple pomace buried at varying depths.

## **Materials and Methods**

### *D. suzukii* Colony

*D. suzukii* used in experiments were maintained in a laboratory colony originally sourced from the Trevor Nichols Research Center (TNRC) at Michigan State University (Fennville, MI) in 2015. The colony was reared on a corn meal based solid food diet (Dalton et al. 2011) in 50 ml polystyrene vials (Genesee Scientific, San Diego, CA). Insects were reared in a growth chamber set at 25°C, 70% relative humidity (RH), and a photoperiod of 16:8 h (L:D).

### *Soil Collection and Analysis*

Bulk soil samples were collected from TNRC (TNRC1 and TNRC2) and the Michigan State University Entomology Research Farm (ENT Farm) to use in laboratory bioassays. Soils were sifted through a 2 mm wire mesh sieve (Hubbard Scientific, Fort Collins, CO) and subsamples were sent to Midwest Laboratories (Omaha, NE) for a textural and chemical analysis (Table 2. 1 and Appendix B). The TNRC 1 soil and the TNRC 2 soil are henceforth referred to as

Sandy Soil and Loamy Soil, respectively. Additionally, percent moisture was recorded by drying a 40 g subsample of each soil type at 105°C for 24 hours in a drying oven and reweighing the subsample (Table 2. 1).

| Soil     | Sand | Silt | Clay | % Moisture |
|----------|------|------|------|------------|
| Sandy    | 74%  | 20%  | 6%   | 8%         |
| Loamy    | 58%  | 30%  | 12%  | 9%         |
| ENT Farm | 60%  | 34%  | 6%   | 14%        |

**Table 2. 1** Texture analysis by Midwest Laboratories (Omaha, NE) and percent moisture of the three soil samples collected from Trevor Nichols Research Center (Fennville, MI) and Entomology Farm (East Lansing, MI) at Michigan State University.

#### *Experiment 1 – Larval Depth Selection for Pupation*

To determine *D. suzukii* pupation depth in two soil textures, two soils collected from TNRC were evaluated. This yielded 2 treatments replicated ten times. Ten male and 10 female 7 d old *D. suzukii* adults were placed in new diet vials for 48 hours, and then were lightly anesthetized with CO<sub>2</sub> and removed to allow eggs to develop. After 5 additional days, 3<sup>rd</sup> instar larvae were removed from the vials containing the solid food diet and were immediately transferred to experimental arenas filled with either the Sandy Soil or the Loamy Soil.

Experimental arenas were constructed from Schedule 40 PVC pipe with a 5 cm internal diameter mounted to a 10 x 10 cm piece of corrugated plastic sign-board (Coroplast, Vanceburg, KY). A 0.5 cm hole was punched into the bottom for drainage and was lined with a piece of 150-micron polyester mesh (The Cary Company, Addison, IL). Each arena measured 11 cm in height, with the PVC pipe split into sections 5 cm, 7 cm, and 9 cm from the base. All PVC pipe sections were rejoined with electrical tape and filled with the appropriate soil. A 1 cm space was left at the top of the PVC pipe (Figure 2. 1A).

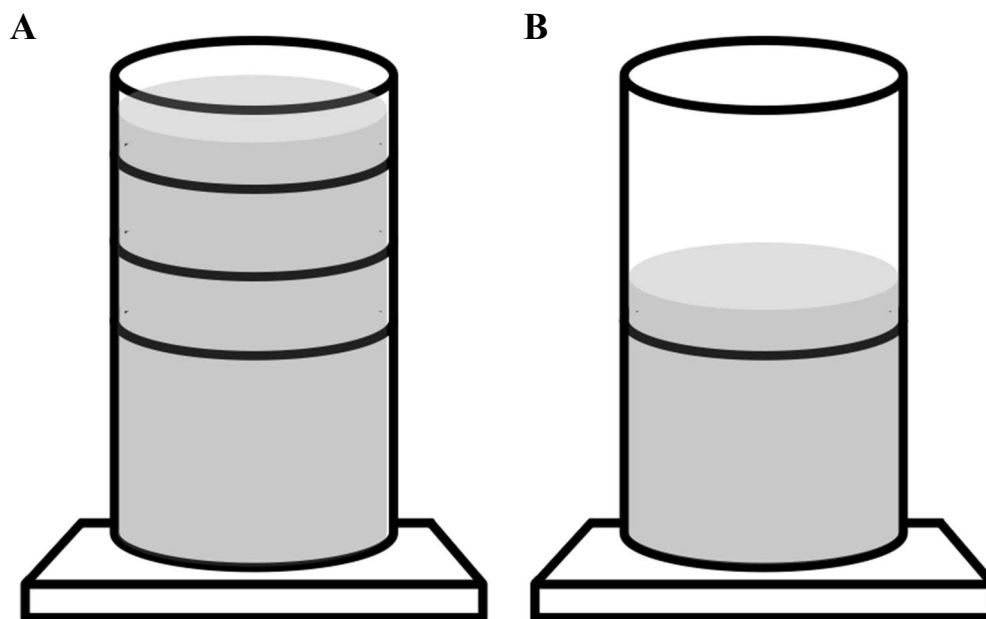
Ten 3<sup>rd</sup> instar *D. sukii* larvae were randomly arranged on the top of the soil surface of each arena, and the arenas were capped with 150-micron polyester mesh secured with a rubber band. Arenas were placed in a growth chamber (25°C, 75% RH, 16:8 L:D). After 24 hours, the surface of the soil was checked for any larvae that pupated and the arena was disassembled section by section, beginning at the top and proceeding to the bottom. Each layer was evaluated by removing a PVC section, gently scraping the soil into a 9 cm Petri dish and counting the number of pupae present. Depth selection was determined by the proportion of pupae in each experimental unit that were found in each PVC pipe section. Pupae present at each depth was used as the response variable. To test treatment effects, a two-way analysis of variance was conducted in R version 3.4.0. (R Foundation for Statistical Computing, Vienna, Austria) by using the GLM function with family set to a binomial distribution. Layers 3 to 5 cm and 5 to 10 cm were excluded from the analysis because zero pupae present at these depths across all replicates. Where significant effects were observed, means were separated by Tukey's Honest Significant Difference for post hoc comparisons with  $\alpha = 0.05$ .

### *Experiment 2 – Pupal Burial and Survival*

To determine *D. sukii* emergence in two different soil textures when buried as pupae at varying burial depths, seven burial depths (0, 1, 3, 5, 7, 9, and 11 cm) were evaluated using either the Sandy Soil or the Loamy Soil. This yielded 14 treatments replicated five times. Ten male and 10 female 7 d old adult *D. sukii* were placed in new diet vials for 48 hours, and then were lightly anesthetized with CO<sub>2</sub> and removed to allow eggs to develop. After 8 additional days, pupae were removed from the vials containing the solid food diet and were immediately transferred to experimental arenas.

Experimental arenas were constructed as described above, but modified to be 21 cm in height. The PVC pipe was split into two sections, with lengths determined by burial treatment. The upper portion of the pipe had an 0.3175 cm hole drilled 2 cm from the top to allow for the insertion of a FlyStuff CO<sub>2</sub> Blowgun (Genesee Scientific, San Diego, CA). The PVC pipe affixed to the base was filled with the appropriate soil, and 10 pupae were randomly distributed on the soil surface. The corresponding upper portion of the PVC pipe was secured to the lower portion of the tube with electrical tape, and was filled with an additional layer of soil, which left a 5 cm space at the top of the PVC pipe (Figure 2. 1B). The arenas were capped by a piece of 150-micron polyester mesh secured with a rubber band and placed in a growth chamber (25°C, 75% RH, 16:8 L:D).

The arenas were checked daily for emergence until no emergence was detected for 7 consecutive days. Survivorship was determined by the proportion of pupae in each experimental unit that emerged over the duration of the experiment. Pupal survival was used as the response variable. To test treatment effects, a two-way analysis of variance was conducted in R version 3.4.0. (R Foundation for Statistical Computing, Vienna, Austria) by using the GLM function with family set to a binomial distribution. Where significant effects were observed, means were separated by Tukey's Honest Significant Difference for post hoc comparisons with  $\alpha = 0.05$ . A contrast statement was used to determine the significance of burial depth within a soil texture.



**Figure 2.1** Natural pupation arena (A) and pupal burial arena (B) constructed from Schedule 40 PVC pipe with a 5 cm internal diameter mounted to a 10 x 10 cm piece of corrugated plastic sign-board, which had a 0.5 cm mesh-lined hole in the bottom for drainage. Natural pupation arenas measured 11 cm in height, which split into layers that exposed soil in layers of 0 to 1, 1 to 3, 3 to 5, and 5 to 10 cm. Burial arenas measured 21 cm in height, split into two sections with lengths corresponding to burial treatments of 0, 1, 3, 5, 7, 9, and 11 cm.

### *Experiment 3 – Field Burial and Adult Emergence*

To determine the potential of burying *D. suzukii* infested fruit waste as an effective method of fruit waste disposal, five burial depths (0, 12, 24, 36, 48 cm) were evaluated in a randomized complete block design. This yielded 5 treatments replicated five times. Ten male and 10 female adult *D. suzukii* were placed in new diet vials post eclosion for 7 days to ensure mating, and then were lightly anesthetized with CO<sub>2</sub> and placed on a FlyStuff FlyPad (Genesee Scientific, San Diego, CA). Flies were separated by sex, and 25 females were placed within a 1242 ml plastic container (Rubbermaid®, High Point, NC) containing 500 g of organic apple pomace. Containers were capped by a lid that had a 10 cm hole lined with a piece of 150-micron polyester mesh and placed in a growth chamber (25°C, 75% RH, 16:8 L:D). After 48 hours, all

egg-laying females were removed. Immature *D. suzukii* were allowed to develop on the apple pomace in the growth chamber for an additional 5 days before being transferred to the field.

Field experiments were conducted along the perimeter of a deer fenced apple orchard located at Michigan State University's ENT Farm in a plot measuring 4.5 m by 36.5 m. The plot within the orchard was situated on flat land that had been cultivated one week after an application of glyphosate at the rate of 3 fl oz/acre. Burial depth treatments were deployed randomly in a Latin Square design in holes dug within 3 m by 3 m blocks that were spaced 3 m apart in a line. A container with 500 g of *D. suzukii* infested organic apple pomace was placed within the hole at the various depths, and holes were refilled with soil to be surface level. This area was capped by an emergence cage (Bugdorm, Talchung, Taiwan) measuring 60 cm L by 60 cm W by 60 cm H. Emergence cages were secured to the ground, and then baited with a modified red sphere trap (Great Lakes IPM, Vestaburg, MI) (Figure 2. 2A). Each trap had eighteen exterior 0.5 cm holes, contained a Scentry *D. suzukii* lure (Scentry Biologicals, Billings, MT), and was covered with Tangle-Trap (Tanglefoot Company, Grand Rapids, MI). Blocks were then covered with a blue-topped canopy tent (Caravan Canopy Sports, La Mirada, CA) measuring 3 m by 3 m to provide shade and lower the internal temperature within the emergence cage (Figure 2. 2B). Soil moisture and temperatures under the canopy tent and inside of the emergence cages were recorded (Appendix B).

Once F1 emergence began, the emergence cages were vacuumed daily with a hand-held aspirator (BioQuip Products, Inc., Rancho Dominguez, CA) to actively capture any emerging *D. suzukii*. Captured flies were frozen and counted in the laboratory. This procedure continued until no emergence was detected for 7 consecutive days, after which the traps were removed and all passively captured *D. suzukii* were counted. F1 adult emergence was used as the response

variable. To test treatment effects, a one-way analysis of variance was conducted in R version 3.4.0. (R Foundation for Statistical Computing, Vienna, Austria) by using the GLM function with a negative binomial distribution. Where significant effects were observed, means were separated by Tukey's Honest Significant Difference for post-hoc comparisons with  $\alpha = 0.05$ .



**Figure 2. 2** The modified red sphere trap (A) had eighteen exterior 0.5 cm holes, contained a Scentry *D. suzukii* lure, and was covered with Tangle-Trap, which was placed within emergence cages underneath blue-topped canopy tents (B) located at the Entomology Research Farm at Michigan State University (East Lansing, MI).

#### *Experiment 4 – Infested Pomace Burial and Adult Emergence*

To determine *D. suzukii* emergence when buried with fruit waste at varying burial depths and if a reduction in emergence was due to a depletion of lipid reserves, five burial depths (0, 3, 6, 12, 24 cm) were evaluated using the ENT Farm Soil. This yielded 5 treatments replicated five times. Apple pomace was infested as above, but reduced to 40 g of organic apple pomace placed within a 118 ml deli cup (Solo Cup Co., Highland Park, IL). The deli cups were capped by a lid that had a 5 cm hole lined with a piece of 150-micron polyester mesh and placed in a growth chamber (25°C, 75% RH, 16:8 L:D).

Experimental arenas were replicas of the arenas used in the pupal burial and survival experiment, but the PVC's internal diameter was increased to 10 cm and arenas were modified to be 40 cm in height. The PVC pipe affixed to the base was filled with soil, and the 40 g of *D. suzukii* infested organic apple pomace was placed on the soil's surface. The corresponding upper portion of the PVC pipe was secured to the lower portion of the tube with electrical tape, and then was filled with an additional layer of soil, which left a 5 cm space at the top of the pipe. The arenas were capped by a piece of 150-micron polyester mesh secured with a rubber band, and held in a growth chamber (25°C, 75% RH, 16:8 L:D).

The arenas were checked daily for emergence until no emergence was detected for 7 consecutive days. Any emerging flies were counted, and individually sorted by date and sex into 1.7 ml micro-centrifuge tubes (Denville Scientific, Holliston, MA) that were frozen at - 20°C for the lipid assay. F1 adult emergence was used as the response variable. To test treatment effects, a one-way analysis of variance was conducted in R version 3.4.0. (R Foundation for Statistical Computing, Vienna, Austria) by using the GLM function with a negative binomial distribution. Where significant effects were observed, means were separated by Tukey's Honest Significant Difference for post-hoc comparisons with  $\alpha = 0.05$ .

The lipid assay protocol was adapted from Olson et al. (2000) that was originally used for parasitic wasps. Sodium Sulfate (50  $\mu$ L) was added to tubes containing individual flies. Flies were crushed with a plastic pestle that had been soaked in a 10% bleach solution, which was subsequently rinsed into the tube with Chloroform Methanol (450  $\mu$ L). The tubes were vortexed and centrifuged for 3 minutes at 13,000 rpm, and then 235  $\mu$ L of the solution was removed and placed into a separate tube. This solution was evaporated at room temperature, leaving behind a lipid precipitate. Sulfuric Acid (40  $\mu$ L) was added to the precipitate and heated to 90°C for 2

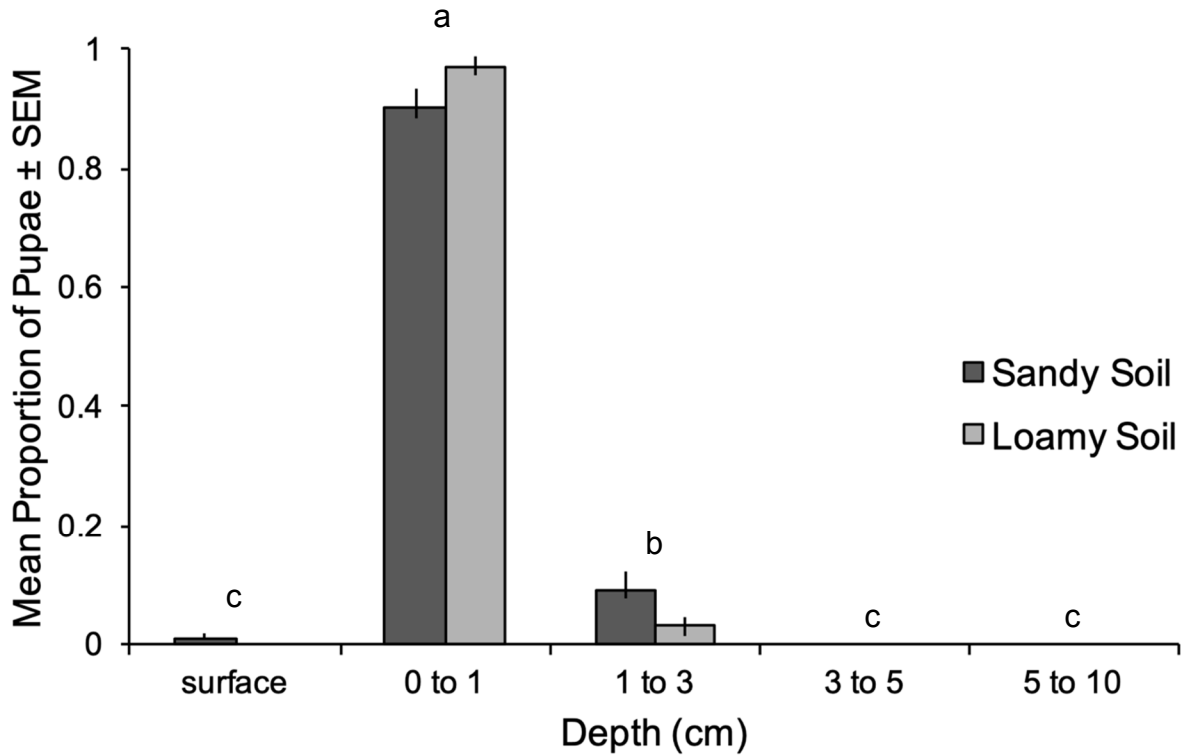
minutes. After being heated, the tubes were immediately cooled on ice, and a Vanillan reagent (480  $\mu$ L) was added. This solution was allowed to react for 25 minutes at room temperature before 200  $\mu$ L of solution per fly sample was transferred to a microplate well in a 96 well plate (Corning, Inc., Corning, NY).

To calibrate the standard, 0, 1, 5, 10, 25, 50, 75, and 100  $\mu$ g of soybean oil were used in the protocol described above, and the relationship between the absorbance value and lipid concentration was determined with linear regression. Absorbance was recorded at 490 nm with a spectrophotometer (BioTek, Winooski, VT), and lipid concentrations were estimated using the linear equation calculated by the lipid standard. Lipid data was normalized with a square root transformation, and treatment effects were tested with a two-way analysis of variance by using the GLM function. Where significant effects were observed, means were separated by Tukey's Honest Significant Difference for post-hoc comparisons with  $\alpha = 0.05$ . All analyses were conducted in R version 3.4.0. (R Foundation for Statistical Computing, Vienna, Austria).

## **Results**

### *Experiment 1 - Larval Depth Selection for Pupation*

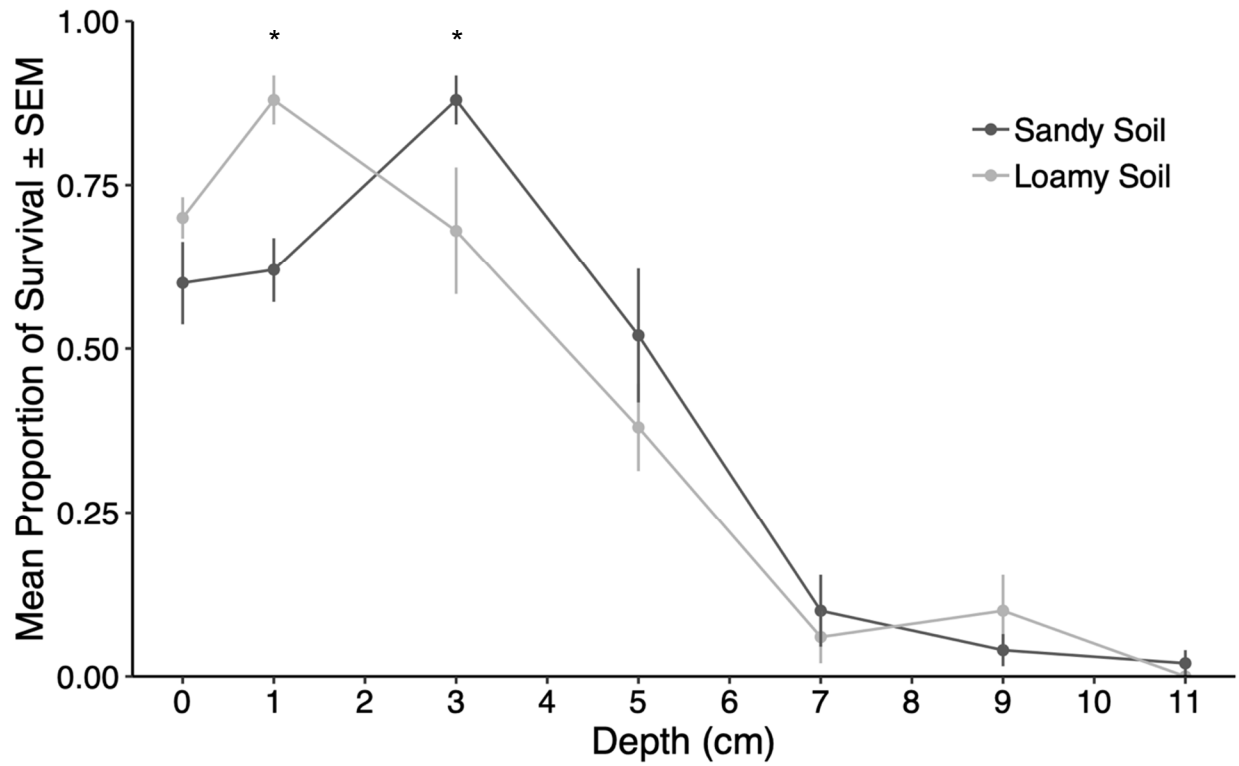
There was a significant effect for burial depth ( $X^2$  (2, N = 60) = 0.45,  $p = < 0.001$ ), but there was no significant effect for soil texture nor the interaction between soil texture and burial depth. The majority of pupae were recovered from layer 0 to 1 cm in Sandy Soil (mean  $0.90 \pm$  SEM 0.03) and Loamy Soil (mean  $0.97 \pm$  SEM 0.01). No larvae were buried deeper than 3 cm in either soil texture (Figure 2. 3).



**Figure 2. 3** Mean proportion of *D. sukikii* larvae ( $\pm$  SEM) that pupated per layer, with layers being surface, 0 to 1, 1 to 3, 3 to 5, and 5 to 10 cm in two soils that were collected from Trevor Nichols Research Center at Michigan State University (Fennville, MI). Letters denote significance between soil layers at  $\alpha = 0.05$ .

#### *Experiment 2 - Pupal Burial and Survival*

There was no significant effect for soil texture, but there was a significant effect for burial depth ( $X^2$  (6, N = 70) = 0.77,  $p = < 0.001$ ) and the interaction between soil texture and burial depth ( $X^2$  (6, N = 70) = 2.06,  $p = 0.001$ ). In Sandy Soil, highest survivorship (88%) was at a depth of 3 cm. In Loamy Soil, highest survivorship (88%) was at a depth of 1 cm (Figure 2. 4). Survival was lowest in both Sandy Soil and Loamy Soil at a depth of  $\geq 7$  cm (Table 2. 2).



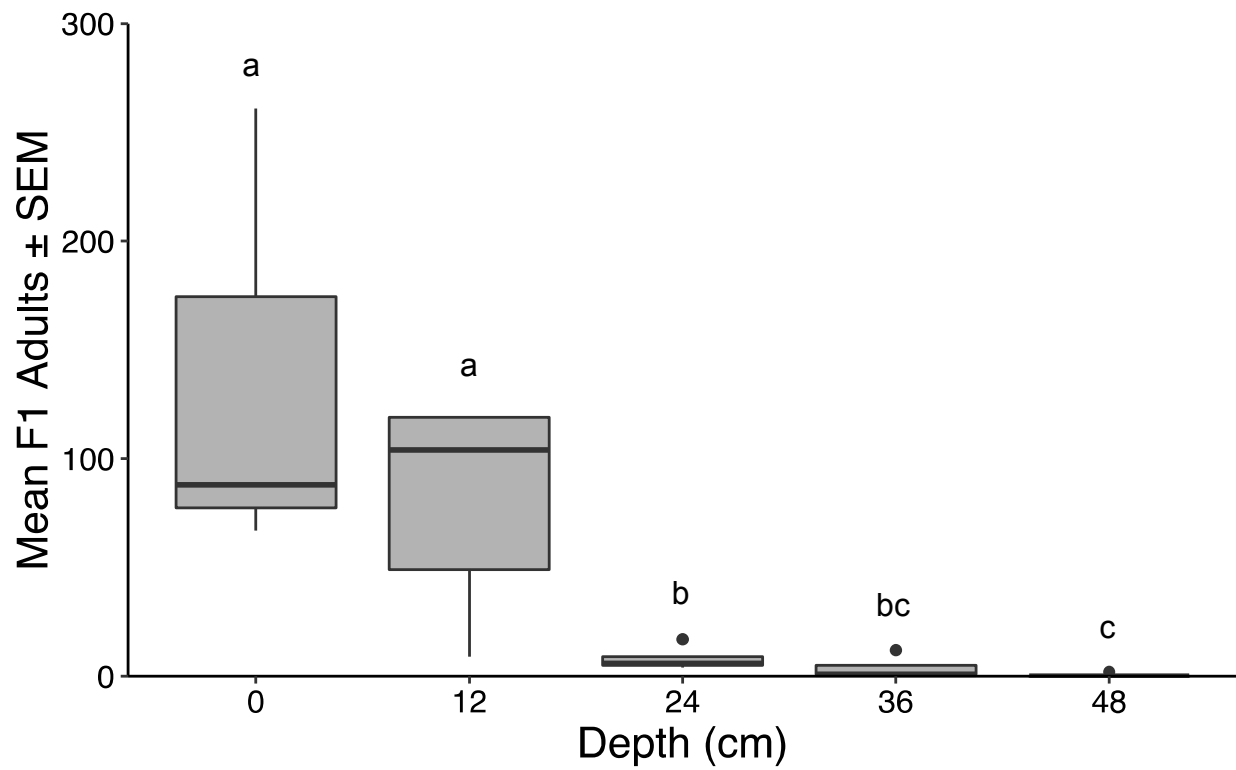
**Figure 2. 4** Mean proportion of *D. suzukii* pupae ( $\pm$  SEM) ( $n=10$ ) that survived to adulthood after being buried at depths of 0, 1, 3, 5, 7, 9, and 11 cm deep in Sandy Soil or Loamy Soil. Asterisks (\*) denote significance between soil textures at  $\alpha = 0.05$ .

| Treatment | Sandy Soil           | Loamy Soil           |
|-----------|----------------------|----------------------|
| surface   | $0.60 \pm 0.07^{ab}$ | $0.70 \pm 0.07^{ab}$ |
| 1 cm      | $0.62 \pm 0.07^{ab}$ | $0.88 \pm 0.05^a$    |
| 3 cm      | $0.88 \pm 0.05^a$    | $0.68 \pm 0.07^{ab}$ |
| 5 cm      | $0.52 \pm 0.07^b$    | $0.38 \pm 0.07^b$    |
| 7 cm      | $0.10 \pm 0.04^c$    | $0.06 \pm 0.03^c$    |
| 9 cm      | $0.04 \pm 0.03^c$    | $0.10 \pm 0.04^c$    |
| 11 cm     | $0.02 \pm 0.02^c$    | $0.00 \pm 0.00^c$    |

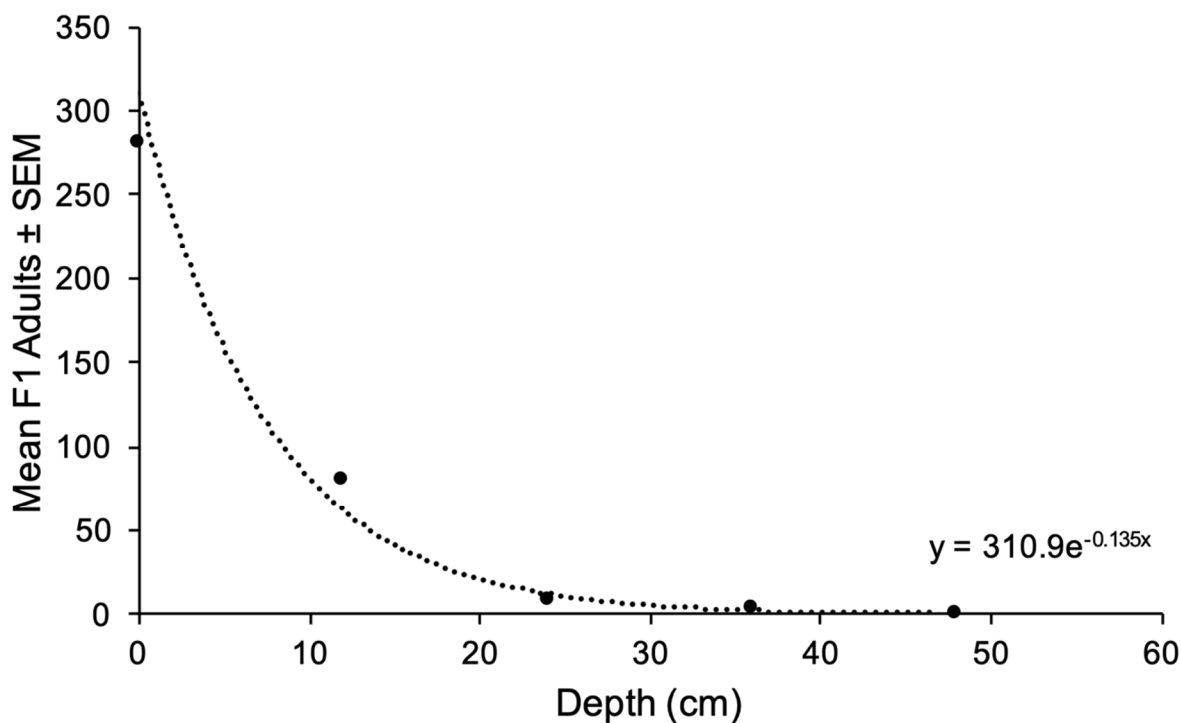
**Table 2. 2** Mean adult emergence ( $\pm$  SEM) of pupae that survived to adulthood out of 10 pupae that were placed within burial arenas 0, 1, 3, 5, 7, 9, and 11 cm deep underneath the soil's surface in two soils that were collected from Trevor Nichols Research Center at Michigan State University (Fennville, MI). Values marked with different letters are significantly different within columns at  $\alpha = 0.05$ .

### *Experiment 3 - Field Burial and Emergence*

There was a significant effect for burial depth ( $F_{4, 20} = 28.60$ ,  $p = < 0.001$ ). F1 adults emerged from all burial depths, with emergence decreasing exponentially with deeper burial depths (Figure 2. 5). Compared to the unburied control ( $280.8 \pm 94.35$ ), mean  $\pm$  SEM adult emergence was reduced by 71.5, 97.0, 98.6, and 99.9% from depths of 12 ( $80 \pm 21.93$ ), 24 ( $8.2 \pm 2.35$ ), 36 ( $3.8 \pm 2.23$ ), and 48 cm ( $0.4 \pm 0.40$ ), respectively (Figure 2. 6).



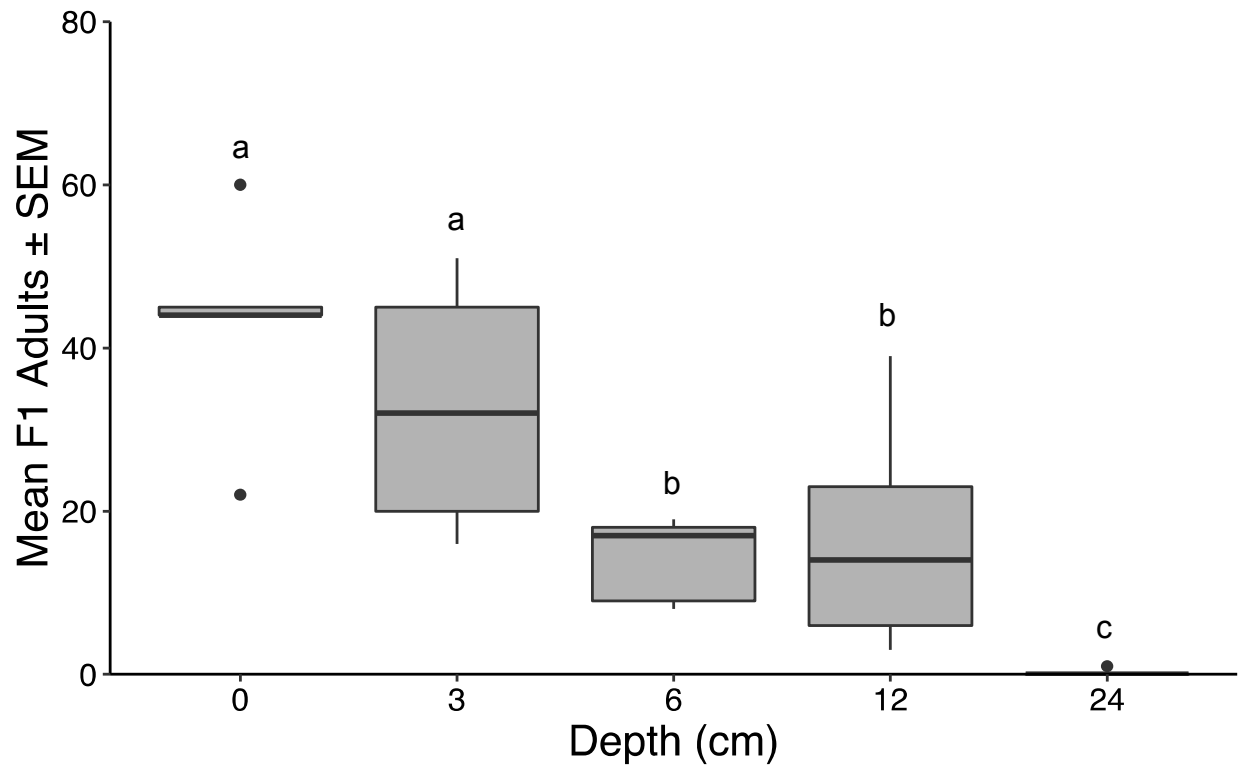
**Figure 2. 5** Mean emergence of *D. suzukii* F1 adults from burial depths of 0, 12, 24, 36, and 48 cm from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI). Letters denote a significant difference between burial depths at  $\alpha = 0.05$ .



**Figure 2. 6** Exponential equation of *D. suzukii* F1 adult emergence of from burial depths of 0, 12, 24, 36, and 48 cm from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI).

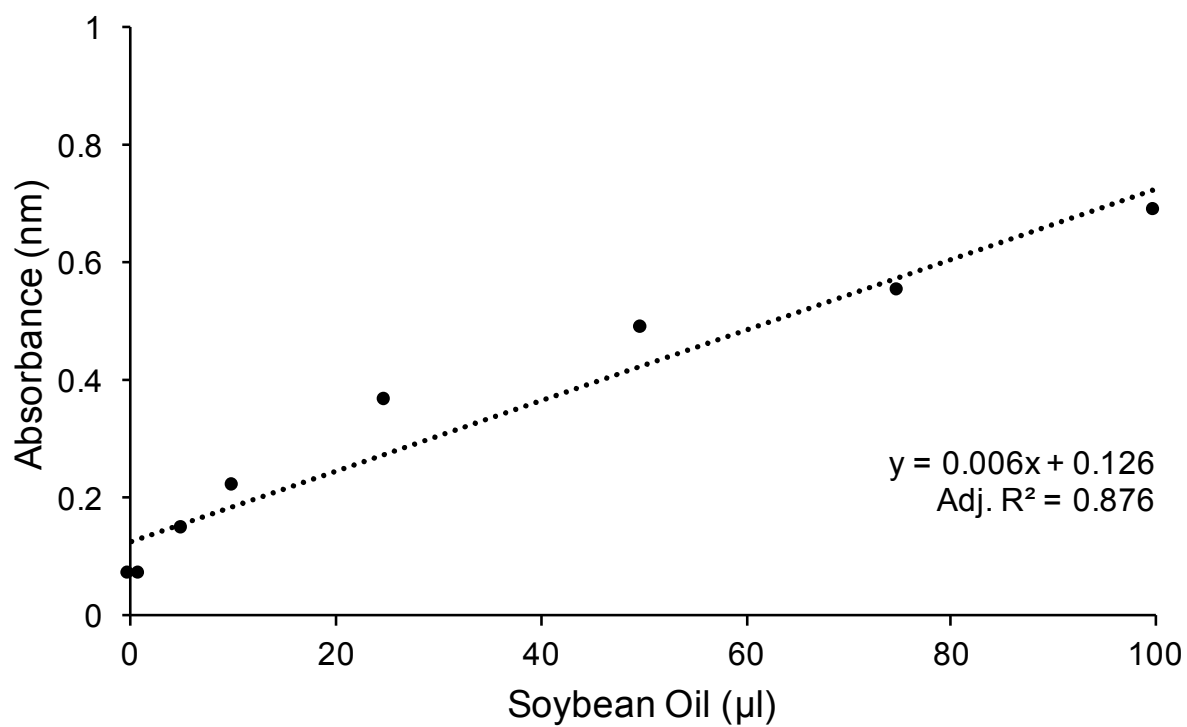
#### *Experiment 4 - Infested Pomace Burial and Survival*

There was a significant effect for burial depth ( $F_{4, 20} = 21.24$ ,  $p = < 0.001$ ). F1 adults emerged from all burial depths, with a decrease in emergence at deeper burial depths. Compared to 0 cm ( $43.0 \pm 6.07$ ), mean  $\pm$  SEM adult emergence was reduced by 23.7, 67.0, 60.5, and 99.5% from depths of 3 ( $32.8 \pm 6.81$ ), 6 ( $14.2 \pm 2.35$ ), 12 ( $17.0 \pm 6.50$ ), and 24 cm ( $0.2 \pm 0.20$ ), respectively (Figure 2. 7).

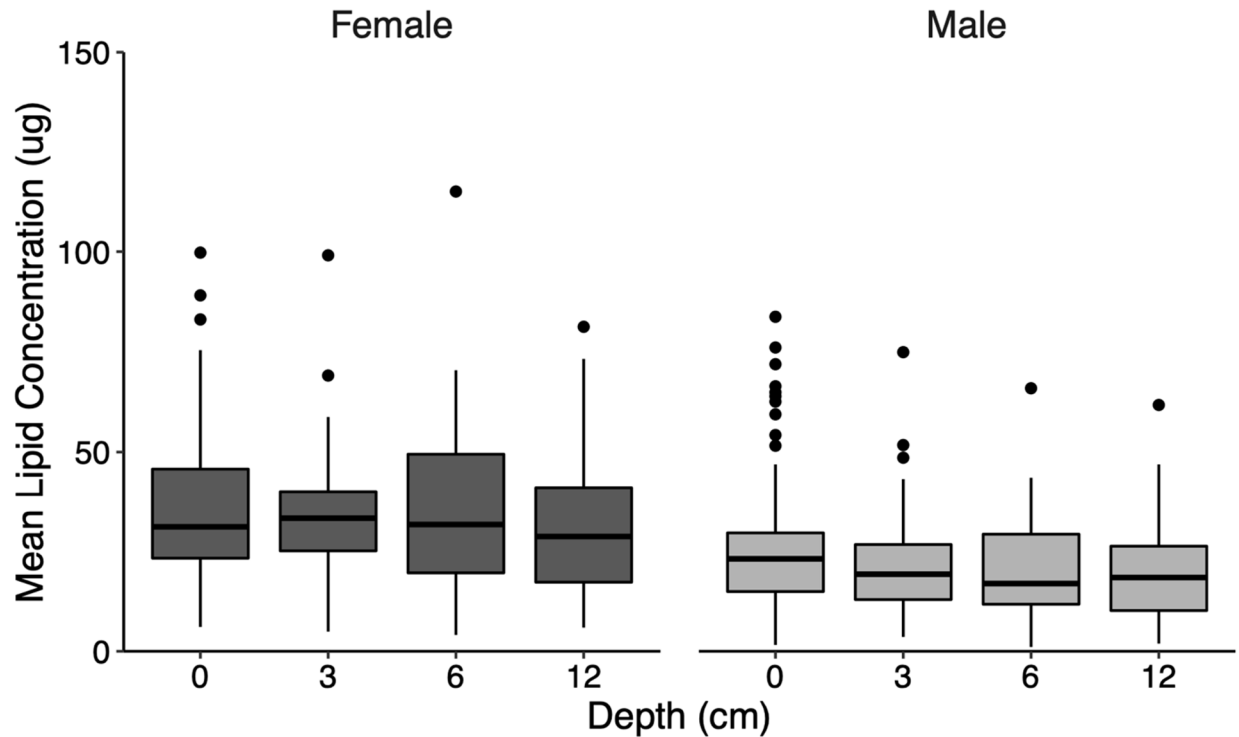


**Figure 2. 7** Mean emergence of *D. suzukii* F1 adults ( $\pm$  SEM) from burial depths of 0, 3, 6, 12, and 24 cm from soil that was collected from the Entomology Farm at Michigan State University (East Lansing, MI). Letters denote a significant difference between burial depths at  $\alpha = 0.05$ .

The linear equation of the lipid standard ( $F_{7,16} = 24.18$ , Adj.  $R^2 = 0.876$ ,  $p = < 0.001$ ) was used to calculate the equation  $y = 0.006x + 0.1265$  (Figure 2. 8). Only one female fly emerged from a burial depth of 24 cm, and, thus, was excluded from the subsequent statistical lipid analysis. Lipid concentration was not different between flies of varying burial depths, but was significant between male and female flies ( $F_{1,537} = 73.36$ ,  $p = < 0.001$ ). Across burial depths, estimated lipid content was 36% higher for females (mean  $34.3 \pm \text{SEM } 1.1$ ) ( $n = 291$ ) compared to males (mean  $22.1 \pm \text{SEM } 0.9$ ) ( $n = 251$ ) (Figure 2. 9).



**Figure 2. 8** Linear equation of the lipid standard, which was calculated by reacting 0, 1, 5, 10, 25, 50, 75, and 100 μg of soybean oil with a Vanillan reagent (480 μL).



**Figure 2. 9** Estimated mean lipid concentrations of *D. suzukii* F1 adults ( $\pm$  SEM) that emerged from burial depths of 0, 3, 6, 12 cm from soil that was collected from the Entomology Farm at Michigan State University (East Lansing, MI). There was a significant difference between male and female flies ( $p = < 0.001$ ) at  $\alpha = 0.05$ .

## Discussion

*D. suzukii* larvae immediately buried themselves upon making contact with the soil, with 93.5% of larvae preferring to pupate above a depth of 1 cm and never exceeding a depth of 3 cm for both soil textures evaluated. These results are consistent with reports by Renkema and Devkota (2016), who found that 93 – 100% of larvae pupated between 0.1 – 0.6 cm below the soil's surface in a sandy soil at 7.5 – 12.5% moisture. However, soils with very different textures (i.e. with a high percentage of clay), compaction, or water holding capacity might yield different results. For example, self-burying larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), were found to pupate at shallower depths in soils of high compaction

compared to soils of low-compaction (Hennessey 1994). Bulk density and water saturation also influenced how deep the Mediterranean fruit fly, *Ceratitis capitata* (Wiedermann) (Diptera: Tephritidae) chose to pupate (Eskafi and Fernandez 1990). In order to extrapolate outside of the tested soils, future studies should assess the influence of a broader diversity of soil characteristics on *D. suzukii* pupation depth preferences.

While *D. suzukii* larvae preferred a shallow burial depth to pupate, they could extract themselves from dramatically deeper depths. Zero emergence was never obtained in the field, with flies being able to unbury themselves from as deep as 48 cm. This depth provided the best suppression rate, being 99.9% lower than pomace left on the soil's surface. These results are similar to those of Klungness et al. (2005), who found that a burial depth of 46 cm was required to prevent the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), from emerging from infested fruit waste. However, burying fruit wastes 48 cm underground may not be a feasible option for growers due to the subsurface layers of soil having higher compaction, lower organic matter, and less aggregation compared to the surface layers of soil (Voroney 2007). Thus, a burial depth of at least 24 cm is suggested because it provides a 97.0% reduction in *D. suzukii* emergence compared to pomace left on the soil's surface.

However, this optimal burial depth may change depending on soil texture or other physical characteristics as evidenced by experiment 2. There was a significant interaction between soil texture and burial depth, with emergence differing between soil textures at depths of 1 and 3 cm. It is possible that soils with greater porosity have a negative impact on *D. suzukii* at shallower depths. In Sandy Soil, 30% less *D. suzukii* emerged from a depth of 1 cm compared to Loamy Soil. Sand has a significantly larger particle size than silt and clay, which creates larger open spaces between particles in the soil (Voroney 2007). Soil water retention is usually

inversely related to pore size, with sandy soils having the lowest water retention, followed by silt, then soils with a high clay concentration (Voroney 2007). Thus, it is possible that Sandy Soil lost water at a faster rate than Loamy Soil, which may have increased *D. suzukii* susceptibility to desiccation at shallower depths.

Dry soil conditions have been shown to reduce larval survival by up to 40% in the laboratory and alter *D. suzukii* behavior (Renkema and Devkota, 2016). The susceptibility of immature *D. suzukii* to desiccation may also help to explain the deeper depths they were able to emerge from in experiment 4 compared to experiment 2. In experiment 4, the buried pomace remained moist throughout the study period, which may have provided a more optimal microhabitat for *D. suzukii* development. In contrast, individual pupae were put directly in contact with the soil in experiment 2, which potentially exposed them to an enhanced rate of desiccation and, thus, reduced survivorship at comparatively shallower burial depths. There is an abundance of literature suggesting that the emergence of multiple *Tephritidae spp.*, which exit fruit hosts to pupate underground like *D. suzukii*, is reduced by dry soil conditions (Hou et al. 2006, Hulthen and Clarke 2006, Montoya et al. 2008). To better understand how soil moisture affects soil-dwelling *D. suzukii*, future studies should investigate how soil moisture levels influence *D. suzukii* pupal development and survival.

How burial interferes with emergence, however, remains unclear, as burial depth did not affect the lipid concentrations of emerging *D. suzukii* adults. However, the lipid assay did detect a difference between male and female *D. suzukii*, which is similar to the results of Tochen et al. (2016). This suggests that all emerging *D. suzukii* had sufficient long-term energy reserves to reach the soil's surface. Thus, four alternative hypotheses that could explain reduced *D. suzukii* emergence from deeper burial depths are – 1) an increase in desiccation from pro-longed soil

exposure, 2) an accumulating quantity of soil pinned them into place, 3) a decreased probability that they could find their way to the soil's surface, and 4) an increase in injury due to abrasion caused by moving through the soil column.

In summary, culled fruit or fruit wastes should be removed from crop fields and disposed because *D. suzukii* utilizes them as a reproductive resource (Leach et al. 2018, Bal et al. 2017). These results suggest that burying fruit 24 cm below the soil's surface can reduce adult emergence from infested wastes by over 95%. Incorporating this practice into existing *D. suzukii* management programs may help alleviate late-season pest pressure and reduce the number of insecticide applications required in a growing season. Future studies should seek to demonstrate the level of population reduction attributable to burial of different fruits at the farm scale to better understand its feasibility and cost. Such information would allow growers to make informed decisions on whether and when to adopt this *D. suzukii* management tactic.

### **CHAPTER 3. COMPOSTING SUSCEPTIBLE FRUIT WASTES REDUCES *DROSOPHILA SUZUKII* REPRODUCTIVE HABITAT**

#### **Introduction**

Spotted wing *Drosophila*, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a polyphagous vinegar fly native to East Asia that infests a wide variety of wild and cultivated soft-skinned fruits by means of a large, serrated ovipositor (Asplen et al. 2015). In susceptible crops, such as blueberries, cherries, raspberries, and strawberries, direct damage from larval feeding causes hundreds millions of dollars of damage each year (Asplen et al. 2015, Haye et al. 2016). Despite *D. suzukii* arriving to the United States over a decade ago, management still largely depends on applications of broad spectrum insecticides applied at weekly or shorter intervals to ripening or ripe fruits (Van Timmeren and Isaacs 2013, Asplen et al. 2015). These frequent insecticide applications have disrupted integrated pest management (IPM) programs and increased the risk of insecticide resistance, especially when paired with the high fecundity and short generation time of *D. suzukii* (Haye et al. 2016).

Although they have an ovipositional preference for fresh fruits, *D. suzukii* also exploit decomposing fruit as a reproductive resource. They oviposit into a wide range of decomposing fruit, including fruits that have thick skins and tough tissues while fresh (e.g. apples and pears) and post-harvest and processing fruit wastes (Bal et al. 2017). This plasticity has allowed *D. suzukii* to exploit a diverse nutritional niche, being both early colonizers of the first available fruit crops in the spring and late colonizers of decomposing fruits in the fall (Jarmamillo et al. 2015). The availability of late season reproductive habitat has implications for the subsequent growing season, as spring *D. suzukii* populations are hypothesized to be founded by surviving overwintering adults (Rossi-Stacconi et al. 2016). Thus, eliminating susceptible fruit wastes from

crop fields can potentially delay the onset and decrease the severity of early season *D. suzukii* in addition to decreasing pest pressure in the fall.

There is limited information available on disposal methods for *D. suzukii* susceptible fruits despite the high volume of fruit wastes produced each year. An estimated 16-36% of fruit crops are lost due to mechanical, microbial, or physiological reasons (Maiti et al. 2018). Additionally, the left-over pomace produced by wineries and cideries can account for up to 20% and 25% of the harvested fruit mass, respectively (Roberts et al. 2008, Shalini and Gupta 2010). Solarization has been demonstrated as an effective method of disposal for *D. suzukii* infested raspberries, with 99% of *D. suzukii* larvae killed after fruit was bagged and solarized for 32 hours (Leach et al. 2018). Unfortunately, this practice is difficult to translate to stone fruits and is impractical for large fruit processing operations (Haye et al. 2016). Composting is another potential method for disposing of *D. suzukii* infested fruits that is feasible for a diversity of fruit crops and large quantities of fruit wastes.

Composting is the accelerated decomposition of organic matter into a homogenous, sanitary product, i.e. compost, which is mediated by aerobic bacteria, fungi, and other organisms in the presence of sufficient moisture and temperature (Azim et al. 2018). Composting has successfully eliminated fruit waste as reproductive habitat for other dipteran pests associated with decomposing fruit, such as the house fly, *Musca domestica* (L.) (Diptera: Muscidae) and vinegar fly, *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Rubasinghe et al. 2013). On exposed compost piles, adult flies and larvae were predominant during the early stages of the composting process and gradually decreased as fruit waste decomposed over time (Rubasinghe et al. 2013). The rate of decomposition is largely dependent on the initial C:N ratio of the starting materials, i.e. feedstock, with the optimal carbon to nitrogen ratio being 25:1

(Azim et al. 2018). Higher C:N ratios slow decomposition because additional generations of microbes are needed to oxidize the excess carbon, and lower C:N ratios result in nitrogen loss via the volatilization of ammonia (Tiquia and Tam 2000, Azim et al. 2018).

The overall goal of this study was to determine if composting could eliminate fruit waste as *D. suzukii* reproductive habitat, which would provide growers with a cultural control tactic to build into their pre-existing *D. suzukii* management programs. To meet this goal, compost treatments containing different quantities of fruit waste and feedstock materials were compared in the laboratory and in the field. A leaf-woodchip blend and chicken manure were chosen as feedstock materials because they are considered farm compost ingredients that are easily accessible to growers (D'Hose et al. 2012). It was hypothesized that *D. suzukii* reproduction would decrease on compost treatments containing smaller amounts of fruit waste, and that reproduction would be differential between compost treatments made with plant based feedstock materials versus manure based feedstock materials.

## **Materials and Methods**

### *D. suzukii* Colony

A *D. suzukii* colony was established from flies collected at the Trevor Nichols Research Center (TNRC) at Michigan State University (Fennville, MI) in 2015. Flies were reared on a corn meal based solid food diet (Dalton et al. 2011) in 50 ml polystyrene vials (Genesee Scientific, San Diego, CA). The colony was maintained in a growth chamber set at 25°C, 70% relative humidity (RH), and a photoperiod of 16:8 (L:D) hours. To ensure mating, 10 male and 10 female adult *D. suzukii* were held in diet vials post eclosion for 3 days prior to laboratory experiments and 7 days prior to the field experiment. Flies were then lightly anesthetized with

CO<sub>2</sub>, placed on a FlyStuff FlyPad (Genesee Scientific, San Diego, CA), and separated by sex. Following collection, female flies were immediately transferred to experimental arenas.

### *Compost Feedstocks and Analysis*

The feedstock materials used to create compost treatments included a leaf-woodchip blend, organic chicken manure, and organic apple pomace. The leaf-woodchip blend was created from a 3:2 volumetric ratio of leaves to pine bark woodchips (Royal Pines, Reynoldsburg, OH) that was homogenized in a lab blender (Vitamix Turboblend 2-speed, Cleveland, OH). The organic chicken manure was obtained as dried pellets from Herbruck's Poultry Ranch, Inc. (Saranac, MI), and was brought to 25% moisture before use. For laboratory experiments, the pellets were pulverized in the lab blender prior to being rehydrated. The apple pomace was obtained from an organic apple orchard and cidery located in Flushing, Michigan. Samples of all feedstock materials were sent to A&L Great Lakes Laboratories (Fort Wayne, IN) for chemical analysis (Appendix C).

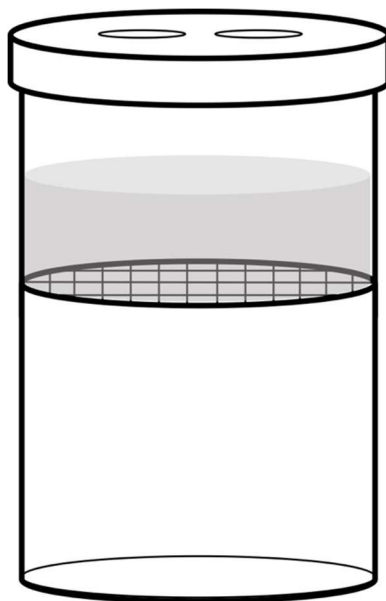
### *Compost Feedstock Comparison Experiments*

To determine *D. suzukii* reproduction on compost mixes containing different quantities of fruit waste and different feedstock materials, five ratios of apple pomace to feedstock (100:0, 70:30, 30:70, 10:90, and 0:100) and two feedstocks (a leaf-woodchip blend and chicken manure) were evaluated. This yielded 9 treatments replicated five times. Compost treatments were created by weighing the composting materials into plastic bins and homogenizing them with a whisk (Winco, Lodi, NJ) for 2 minutes. Apple pomace used in laboratory experiments was frozen at -

20°C for a minimum of one week prior to use to eliminate any arthropods that had previously infested the apple pomace.

Experimental arenas consisted of a 473 ml plastic deli container (Deli-Serve, Chattanooga, TN) with a 23 gauge galvanized steel hardware cloth (Everbilt, Wilmington, DE) bottom held within a 946 ml plastic deli container (Deli-Serve, Chattanooga, TN) to allow for drainage. A No. 1 qualitative filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA) was placed on top of the hardware cloth to keep flies within the upper portion of the arenas. Arenas were filled with 200 ml of compost and inoculated with 10 previously mated *D. suzukii* females. Arenas were then capped by a lid that had two 2 cm holes. One hole was covered with 150 micron polyester mesh (The Cary Company, Addison, IL) to allow for ventilation, and the other hole was covered with Parafilm (Bemis Company, Inc., Neenah, WI) to allow for the insertion of an aspirator hose (Figure 3. 1). Arenas were placed in a growth chamber (25°C, 75% RH, 16:8 L:D), and after 48 hours all flies were removed. Arenas were checked daily for emergence of the next generation of flies, which were collected using an aspirator. The experiment was terminated once no emergence was detected for 7 consecutive days.

Following completion of the first experiment, a subsequent experiment was completed to determine if *D. suzukii* could reproduce on compost treatments containing smaller proportions of chicken manure. Five new ratios of apple pomace to chicken manure (100:0, 99:1, 95:5, 90:10, and 80:20) were evaluated. This yielded 5 treatments replicated five times. The same experimental apparatus and general procedures described above were used.



**Figure 3. 1** Experimental arena consisted of a 473 ml plastic deli container with a wire-mesh bottom perched within a 946 ml plastic deli container. The smaller cup was lined with a piece of filter paper, on top of which 200 ml of a compost treatment was placed. Arenas were capped by a lid with two 2 cm holes, one covered in fine mesh and the other covered by Parafilm.

#### *Field Evaluation of Manure Based Composts*

To determine the potential of composting *D. suzukii* susceptible fruit as an effective method of fruit waste disposal, five ratios of apple pomace to chicken manure (100:0, 90:10, 75:25, 50:50, and 0:100) were evaluated in a randomized complete block design. This yielded 5 treatments replicated five times. Compost treatments were created by weighing feedstock materials into plastic buckets (Leaktite, Leominster, MA), and homogenizing in a cement mixer (Central Machinery, Camarillo, CA and Multiquip, Carson, CA). The pelletized chicken manure was mixed with water for 2 minutes, and then the proportional quantity of apple pomace was added and mixed for an additional 6 minutes. The large quantity of apple pomace used in this experiment could not be frozen to eliminate incidental arthropod presence. Thus, prior to the experiment, twenty 200 ml samples were taken from the apple pomace, and placed within

experimental arenas described above (Figure 3. 1). Arenas were held in a growth chamber (25°C, 75% RH, 16:8 L:D) to allow for any immature *Drosophila* to develop. After 10 days, all emerged flies were collected using an aspirator to provide an estimate of infestation prior to experimental use.

Field experiments were conducted at a site located at Michigan State University's ENT Farm described in Chapter 2 (Hooper, 2019). For each treatment, An LDP-recycled plastic stock tank (Tuff Stuff, Terra Bella, CA) was filled with 100 l of compost, and was capped by an emergence cage (Bugdorm, Talchung, Taiwan) measuring 60 cm L by 60 cm W by 60 cm H. The emergence cage was secured to the stock tank with bungee-cords (Winston Products, Cleveland, OH), and blocks were covered by blue-topped canopy tents (Caravan Canopy Sports, La Mirada, CA) measuring 3 m by 3 m (Figure 3. 2A). The canopy tents provided shade to help lower the internal temperature of the emergence cages, with temperatures under the canopy tents and within the emergence cages recorded (Appendix C).

Emergence cages with the various treatments were placed in blocks that measured 3 m by 3 m and were spaced 3 m apart in a line with treatments randomly arranged in a Latin Square. One-hundred previously mated *D. sukii* females were released inside each emergence cage, and after 10 days, emergence cages were vacuumed with a hand-held aspirator (BioQuip Products, Inc., Rancho Dominguez, CA) to actively capture any emerging *D. sukii*. Each emergence cage was baited with the modified red sphere trap described in Chapter 2 (Figure 3. 2B) (Hooper, 2019). Emergence cages were vacuumed daily for a total of 35 consecutive days, with all captured flies frozen, identified, and counted in the laboratory. On day 35, the traps were removed and all passively captured *D. sukii* were counted.



**Figure 3. 2** Emergence cages covering 100 l of compost within an LDP-recycled plastic stock tank, which was placed underneath blue-topped canopy tents (A) and baited with a modified red sphere trap (B) located at the Entomology Research Farm at Michigan State University (East Lansing, MI). Modified red sphere traps had eighteen exterior 0.5 cm holes, contained a Scentry lure, and was covered with Tangle-Trap.

### *Statistical Analyses*

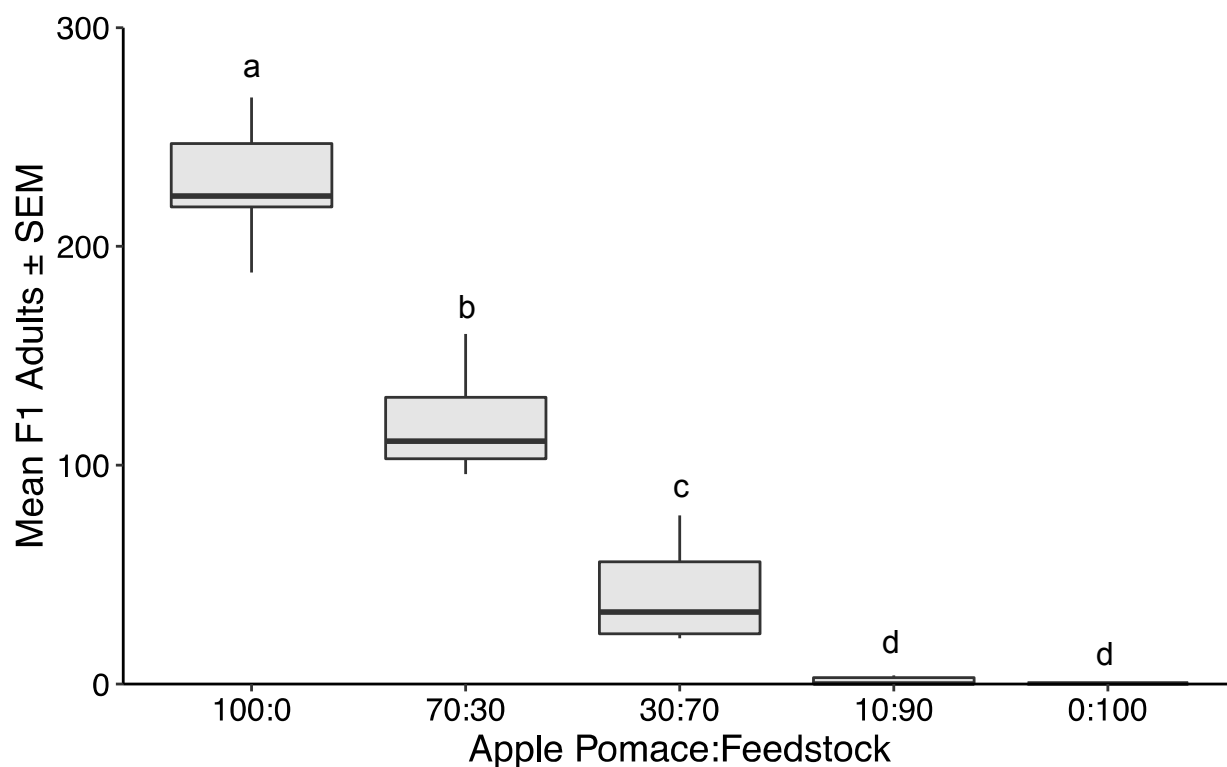
To test treatment effects on total *D. suzukii* reproduction, laboratory and field emergence data was analyzed with a one-way analysis of variance. The GLM function was used with a negative binomial distribution. *D. suzukii* emergence was the response variable. The same analysis and model was used to test treatment effects on total *D. melanogaster*, other *Drosophila*, and non-target organism emergence from the field experiment. To test for treatment effects on *D. suzukii* development times, field emergence data was normalized and analyzed with a two-way analysis of variance. The GLM function was used, with family set to a binomial distribution. Normalized *D. suzukii* emergence was used as the response variable. In all analyses, means were separated by Tukey's Honest Significant Difference for post-hoc comparisons with  $\alpha = 0.05$  where significant effects were observed. Due to the high numbers of *D. melanogaster* in the field experiment, a linear regression was performed on the *D. suzukii* and *D. melanogaster* emergence data to test for correlation across treatments. *D. suzukii* and *D. melanogaster* emergence data was

normalized with a log-transformation. All analyses were conducted in R version 3.4.0. (R Foundation for Statistical Computing, Vienna, Austria).

## Results

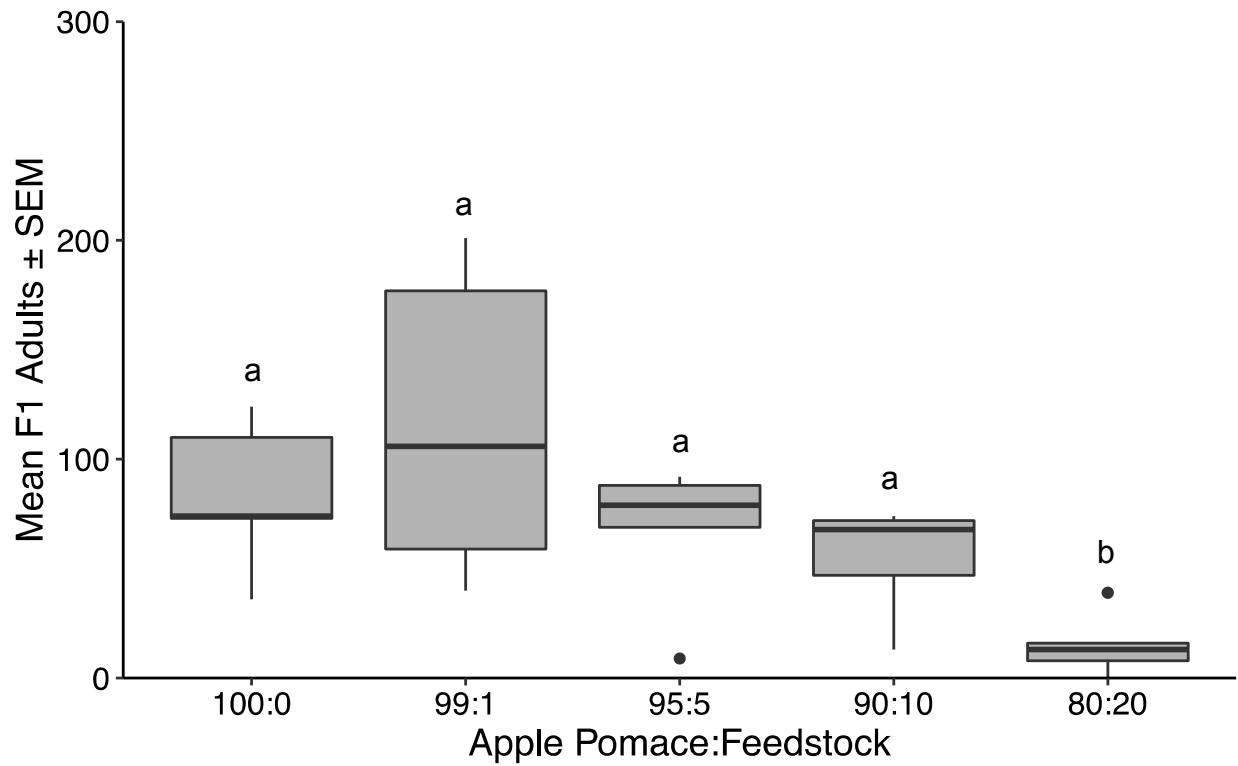
### *Compost Feedstock Comparison Experiments*

There was a significant effect for the ratio of apple pomace to feedstock ( $F_{4, 20} = 115.14$ ,  $p < 0.001$ ) on *D. suzukii* reproduction. *D. suzukii* reproduced on all of the leaf-woodchip based compost treatments containing apple pomace, with a decrease in reproduction correlated to an increase in the proportion of the leaf-woodchip feedstock. Compared to the 100:0 ( $228.8 \pm 13.57$ ) compost treatment, mean  $\pm$  SEM F1 adult emergence decreased by 47.5, 81.6, and 99.4% from 70:30 ( $120.2 \pm 11.55$ ), 30:70 ( $42.0 \pm 10.73$ ), and 10:90 ( $1.4 \pm 0.87$ ) compost treatments, respectively (Figure 3. 3). In contrast, no F1 adults emerged in any compost treatments containing chicken manure, although limited juvenile development was observed.



**Figure 3. 3** Mean emergence of *D. suzukii* F1 adults ( $\pm$  SEM) from 100:0, 70:30, 30:70, 10:90, and 0:100 apple pomace to a leaf-woodchip blend compost treatments. Treatments marked with different letters are significantly different at  $\alpha = 0.05$ .

In the subsequent experiment evaluating smaller proportions of chicken manure, there was a significant effect for the ratio of apple pomace to feedstock ( $F_{4, 20} = 6.25$ ,  $p = 0.002$ ) on *D. suzukii* reproduction. Mean  $\pm$  SEM F1 emergence was 81.8% lower in the 80:20 ( $15.2 \pm 6.54$ ) compost treatment compared to the 100:0 ( $83.4 \pm 31.68$ ) compost treatment. Although not statistically significant, mean  $\pm$  SEM emergence was 28.5% higher in the 99:1 ( $116.6 \pm 15.49$ ) compost treatment compared to the 100:0 ( $83.4 \pm 31.68$ ) compost treatment (Figure 3. 4). The microbial community present was qualitatively different between compost treatments across replicates (Figure 3.5).



**Figure 3. 4** Mean emergence of *D. suzukii* F1 adults ( $\pm$  SEM) from 100:0, 70:30, 30:70, 10:90, and 0:100 apple pomace to chicken manure compost treatments. Treatments marked with different letters are significantly different at  $\alpha = 0.05$ .



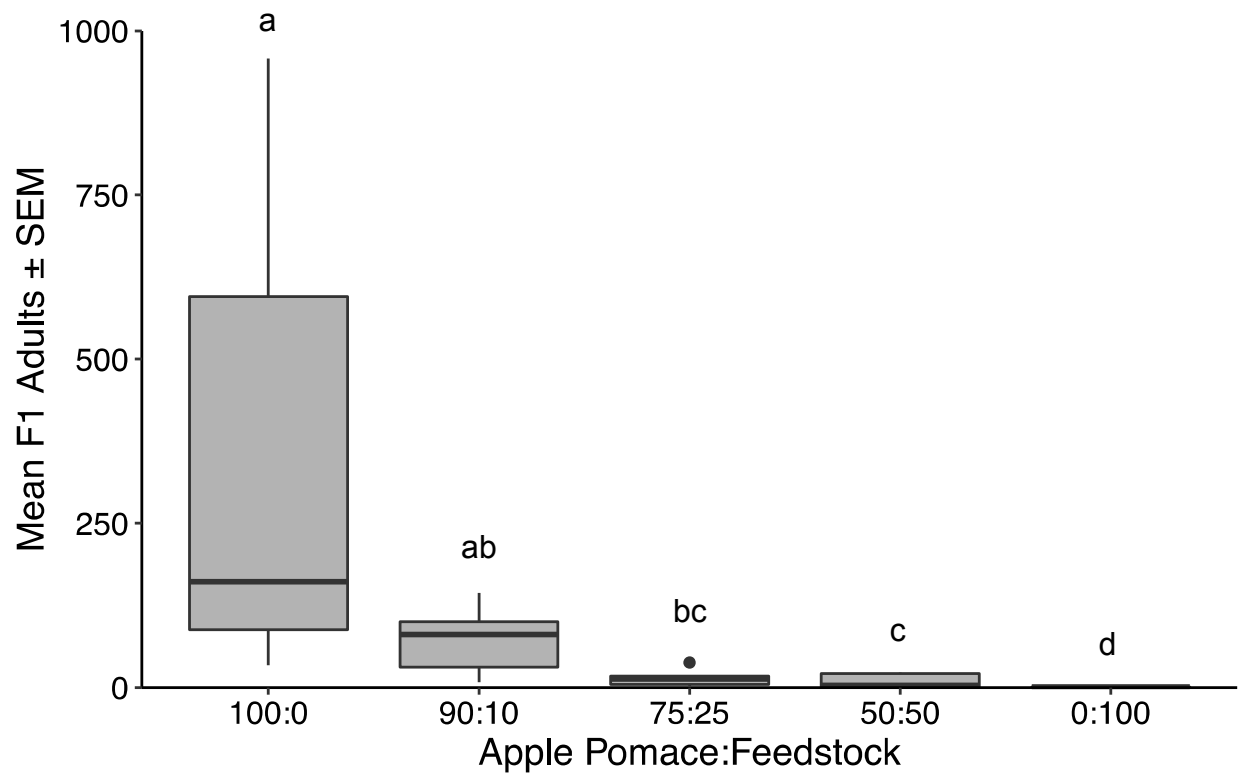
**Figure 3. 5** Images showing the visual appearance of the microbial communities present on 100:0 (A), 99:1 (B), 95:5 (C), 90:10 (D), and 80:20 (E) apple pomace to chicken manure compost treatments on the first day of F1 *D. suzukii* emergence.

### Field Evaluation of Manure Composts

Apple pomace used in the field experiment was pre-infested by drosophilids, with *D. melanogaster* making up 96% of the emerging *Drosophila* species. (Table 3. 1). There was a significant effect for the ratio of apple pomace to feedstock ( $F_{4, 20} = 22.27$ ,  $p = < 0.001$ ) on *D. suzukii* reproduction. *D. suzukii* reproduced on all of the chicken manure based compost treatments, with *D. suzukii* emergence decreasing exponentially with an increasing proportion of chicken manure. Compared to the 100:0 compost treatment, mean  $\pm$  SEM adult emergence decreased by 76.3, 95.0, 95.9, and 99.4% from 90:10, 75:25, 50:50, and 0:100 compost treatments, respectively (Figure 3. 5 and Table 3. 2).

| <i>D. suzukii</i> | <i>D. melanogaster</i> | Other <i>Drosophila</i> | Non-targets     |
|-------------------|------------------------|-------------------------|-----------------|
| 0.20 $\pm$ 0.12   | 8.55 $\pm$ 3.55        | 0.15 $\pm$ 0.08         | 0.00 $\pm$ 0.00 |

**Table 3. 1** Mean emergence of *Drosophila spp.* and non-target arthropods from 200 ml samples of apple pomace obtained from an organic apple orchard and cidery located in Flushing, MI, which was subsequently used in the field experiment located at the Entomology Farm at Michigan State University (East Lansing, MI).

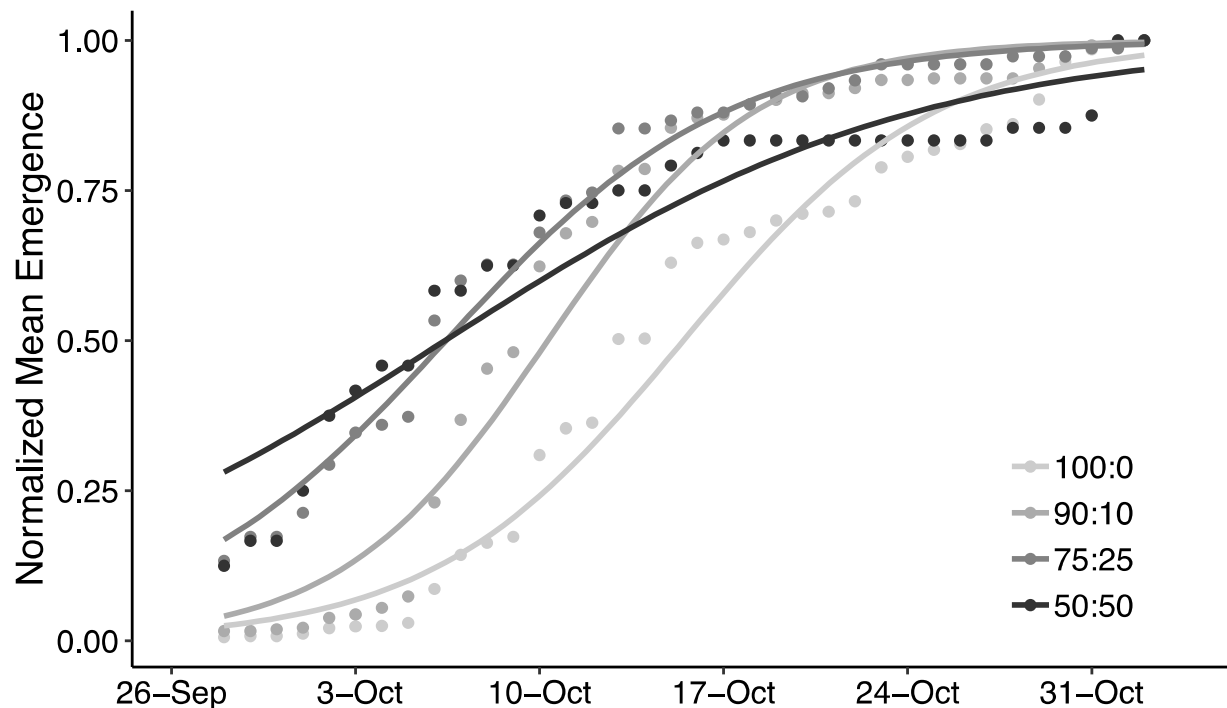


**Figure 3. 6** Mean emergence of *D. suzukii* adults ( $\pm$  SEM) from 100:0, 90:10, 75:25, 50:50, and 0:100 apple pomace to chicken manure compost treatments from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI). Treatments marked with different letters are significantly different at  $\alpha = 0.05$ .

| Treatment | <i>D. suzukii</i>           | <i>D. melanogaster</i>      | Other <i>Drosophila</i>      | Non-targets                  |
|-----------|-----------------------------|-----------------------------|------------------------------|------------------------------|
| 100:0     | 391.4 ± 188.27 <sup>a</sup> | 951.6 ± 334.12 <sup>a</sup> | 66.0 ± 22.47 <sup>bc</sup>   | 94.4 ± 15.78 <sup>b</sup>    |
| 90:10     | 92.6 ± 32.31 <sup>ab</sup>  | 368.0 ± 90.00 <sup>ab</sup> | 873.4 ± 811.74 <sup>a</sup>  | 1646.6 ± 826.85 <sup>a</sup> |
| 75:25     | 19.4 ± 7.89 <sup>bc</sup>   | 122.6 ± 50.93 <sup>b</sup>  | 462.0 ± 427.79 <sup>ab</sup> | 1846.2 ± 946.14 <sup>a</sup> |
| 50:50     | 15.8 ± 6.69 <sup>c</sup>    | 17.6 ± 8.87 <sup>c</sup>    | 36.8 ± 12.47 <sup>cd</sup>   | 2054.4 ± 891.67 <sup>a</sup> |
| 0:100     | 1 ± 0.0 <sup>d</sup>        | 8.4 ± 4.52 <sup>c</sup>     | 6.6 ± 3.30 <sup>d</sup>      | 597.4 ± 457.40 <sup>a</sup>  |

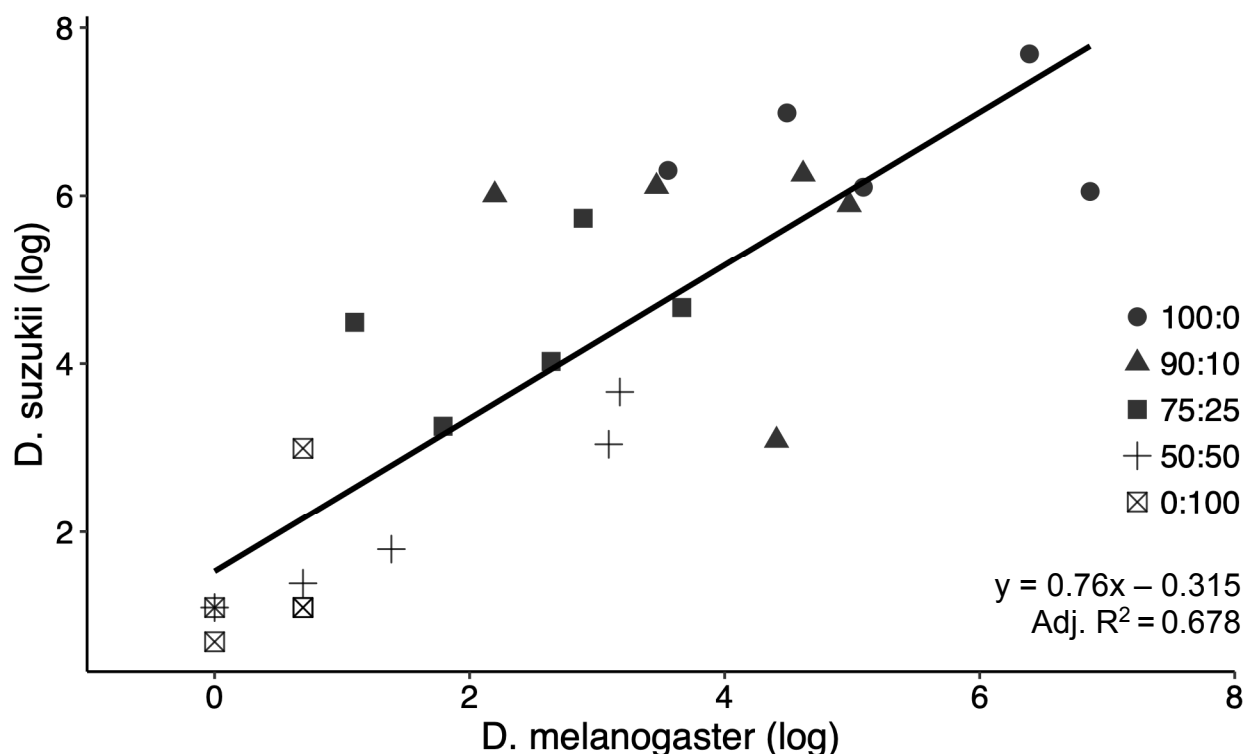
**Table 3. 2** Mean emergence ( $\pm$  SEM) of *D. suzukii*, other *Drosophila*, and non-target organisms from 100:0, 90:10, 75:25, 50:50, and 0:100 apple pomace to chicken manure compost treatments from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI). Values marked with different letters are significantly different within columns at  $\alpha = 0.05$ .

Only three *D. suzukii* emerged from the 0:100 compost treatment, and, thus, were excluded from the development analysis. There was a significant effect for the ratio of apple pomace to feedstock ( $F_{3, 712} = 3.49$ ,  $p = 0.015$ ), time ( $F_{1, 712} = 827.84$ ,  $p = < 0.001$ ), and the interaction between the ratio of apple pomace to feedstock and time ( $F_{3, 712} = 36.93$ ,  $p = < 0.001$ ) on normalized *D. suzukii* emergence. *D. suzukii* emerging from 50: 50 compost treatments emerged sooner than those from 100:0, 90:10, or 75:25 compost treatments (Figure 3. 6).



**Figure 3. 7** Normalized mean emergence of *D. suzukii* adults from 100:0, 90:10, 75:25, 50:50, and 0:100 apple pomace to chicken manure compost treatments over time from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI). There was a significant interaction between the ratio of apple pomace to chicken manure and day ( $p < 0.001$ ) at  $\alpha = 0.05$ .

Additionally, *D. melanogaster*, other *Drosophila spp.*, and non-target organisms were collected. Despite initial populations not being standardized across treatments, distinct emergence patterns were observed. Mean *D. melanogaster* emergence decreased exponentially with an increasing proportion of chicken manure ( $F_{4, 20} = 17.17$ ,  $p < 0.001$ ), and was correlated with *D. suzukii* emergence ( $F_{1,23} = 51.56$ , Adj.  $R^2 = 0.678$ ,  $p < 0.001$ ) (Table 3. 2 and Figure 3. 7). Mean other *Drosophila* emergence was highest in 90:10 compost treatments ( $F_{4, 20} = 6.54$ ,  $p = 0.001$ ) (Table 3. 2). Mean non-target arthropod emergence was higher in compost treatments containing chicken manure compared to pure apple pomace ( $F_{4, 20} = 4.08$ ,  $p = 0.014$ ) (Table 3. 2).



**Figure 3. 8** Relationship between *D. melanogaster* and *D. suzukii* that emerged from 100:0, 90:10, 75:25, 50:50, and 0:100 apple pomace to chicken manure compost treatments from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI). *D. melanogaster* emergence was correlated with *D. suzukii* emergence ( $p < 0.001$ ).

## Discussion

As the proportion of apple pomace decreased in leaf-woodchip based compost treatments, *D. suzukii* reproduction decreased proportionally. This suggests that composting fruit waste with a plant based feedstock does not impair the reproductive quality of the apple pomace beyond a dilution effect. However, assessing the reproductive quality of leaf-woodchip compost treatments beyond the initial days of the composting process may yield different results. As organic matter decomposes, the availability of carbohydrates decreases as microbes proliferate (Silva-Soares et al. 2017). A carbohydrate-poor diet has been found to increase the development time and reduce the fecundity of *D. suzukii* (Young et al. 2018). Thus, negative impacts on reproduction could be observed on subsequent generations of *D. suzukii* as the feedstock materials continue to degrade

over time, and future studies should monitor *D. suzukii* reproduction throughout the entirety of the composting process.

Alternatively, the presence of chicken manure in compost treatments was very deleterious to *D. suzukii*. Although *D. suzukii* were able to reproduce on all chicken manure based compost treatments in the field, reproductive success was reduced by > 75% and > 95% with the addition of 10% and 25% chicken manure, respectively. Thus, growers or processors seeking to reduce the reproductive potential of fruit wastes to *D. suzukii* should consider mixing them with 25% or more manure. This method of disposal may be especially beneficial for farms that have attached cideries and wineries because composting fruit wastes with chicken manure may also help to reduce *D. melanogaster* populations. *D. melanogaster* is a nuisance to processing facilities and is a major pest of grapes due to its ability to transmit the grapevine disease sour rot (Rombaut et al. 2017). Reproduction of *D. melanogaster*, like *D. suzukii*, showed a non-linear negative response to increasing concentrations of chicken manure.

In addition to reducing total *D. suzukii* emergence, high quantities of chicken manure altered *D. suzukii* development time. There was a significant interaction between the ratio of apple pomace to chicken manure and time, with flies from treatments containing 50% chicken manure emerging sooner than those containing < 50% chicken manure. While not yet explored in *D. suzukii*, *D. melanogaster* larvae can pupate prematurely when starved after reaching a critical weight, i.e. bail-out response (Koyama and Mirth 2018). The bail-out response has been documented in a variety of insect species, including the dung beetle, *Onthophagus taurus* (Schreber) (Coleoptera: Scarabaeidae), and the mason bee, *Osmia lignaria* (Say) (Hymenoptera: Megachilidae) (Shafiei et al. 2001, Helm et al. 2017). Due to the ephemeral quality of the apple pomace, it is possible that *D. suzukii* larvae reached critical weight prior to the apple pomace

rotting completely in treatments containing 50% manure. Once the decomposed apple pomace became an unsuitable nutritional resource, it potentially triggered a bail-out response in *D. suzukii* larvae that had reached a sufficient size.

These results suggest that composting fruit waste with a manure based feedstocks directly diminishes the reproductive quality of the apple pomace to *D. suzukii*. Two hypotheses that could explain the decrease in *D. suzukii* reproduction are – 1) the carbon to nitrogen ratio of the composting materials supported microbial life that rapidly metabolized carbohydrates necessary for *D. suzukii* development, and 2) the decomposing chicken manure exuded high levels of nitrogenous wastes that caused acute toxicity in *D. suzukii*. The negative effects on *D. suzukii* reproduction were observed beginning 10 days from the initiation of laboratory experiments while the compost treatments were likely in mesophilic stage of decomposition (Parr et al. 1994). If so, then carbohydrates would have still been readily available to *D. suzukii*, although they were being actively degraded by microbes (Azim et al. 2018). Therefore, hypothesis 2 is the most likely explanation.

Chicken manure is high in nitrogen, and diets high in nitrogen have been shown to decrease oviposition and egg viability of *D. suzukii* and *D. melanogaster* (Joshi et al. 1997, Belloni et al. 2018). The total nitrogen found in chicken manure is comprised of 70% uric acid and 30% undigested protein (Nahm 2005). As chicken manure decomposes, uric acid forms urea, which subsequently forms ammonia (Nahm 2005). Belloni et al. (2018) determined that high concentrations of dietary urea and ammonia reduced *D. suzukii* oviposition by 70% and 60%, respectively. Furthermore, they determined that expression of ornithine aminotransferase and glutathione-S-transferase, the enzymes responsible for nitrogen metabolism and stress response, were suppressed in *D. suzukii* compared to *D. melanogaster*. Thus, while *D. melanogaster* can

undergo a developmental delay and adapt their feeding rate to regulate nitrogen intake, *D. suzukii* do not have any such detoxification or excretory mechanisms (Belloni et al. 2018).

However, when fruit waste was composted with 1% chicken manure in the laboratory, *D. suzukii* reproduction increased by 28.5% compared to pure apple pomace. The nitrogenous wastes exuded from this minute quantity of chicken manure may have been too low to cause acute toxicity in *D. suzukii*, but it potentially permitted an accelerated growth of microbes beneficial to *D. suzukii* development. It has been hypothesized that the evolutionary shift of *D. suzukii* onto protein-poor fresh fruit from protein-rich decomposing fruit caused *D. suzukii* to supplement their diet with microbes (Jarmamillo et al. 2015, Bing et al. 2018). Yeasts have been suggested as the primary source of protein for *D. suzukii*, with an increase in microbes corresponding to an increase in larval fitness (Hardin et al. 2015, Silva-Soares et al. 2017). Thus, adding 1% chicken manure to apple pomace potentially enhanced beneficial microbial growth and, subsequently, increased the availability of proteins to *D. suzukii*, which resulted in an increase in reproduction.

Another interesting outcome of this study was the observation that *D. suzukii* readily co-existed with *D. melanogaster* in the field experiment. Previous laboratory studies have demonstrated that *D. suzukii* populations are dramatically reduced when forced to compete for reproductive resources with *D. melanogaster* (Dancau et al. 2017). The high captures of both *D. suzukii* and *D. melanogaster* from the field experiment demonstrate that *D. suzukii* are not exploitatively out-competed as previously suggested. Furthermore, apple pomace used in the field experiment was pre-infested by multiple *Drosophila* species, including *D. suzukii*. This contrasts with prior laboratory studies that suggested *D. suzukii* are hesitant to oviposit into media pre-inoculated with *D. melanogaster* eggs (Shaw et al. 2017). This study suggests that

female oviposition choice may not be as strongly influenced by the presence of heterospecifics as previously hypothesized. As *D. melanogaster* populations were not standardized in this study, future research should explore the relationship between *D. suzukii* and *D. melanogaster* in a field setting to investigate how *D. melanogaster* influences the reproductive success of *D. suzukii* and how these two species partition reproductive resources.

In summary, culled fruit or fruit wastes can act as important late season reproductive reservoirs to *D. suzukii* and should be eliminated from crop fields (Bal et al. 2017). These results suggest that composting fruit waste with 25% chicken manure can reduce *D. suzukii* reproduction by 95% during the initial stages of the composting process. Integrating this control tactic into existing *D. suzukii* management programs may help to reduce the need for insecticide applications by alleviating late-season pest pressure and inhibiting the onset and severity of overwintering populations for the following year. Future studies should investigate the level of *Drosophila* population reduction due to composting fruits at the farm scale. This would inform growers on whether and when they should adopt this management strategy, including growers who suffer from *D. melanogaster*. Additionally, future studies should explore how nitrogenous by-products, microbe abundance, and interspecific competition impact *D. suzukii* reproduction on compost treatments containing fruit wastes.

## CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

*Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), or spotted wing Drosophila, is an invasive, polyphagous vinegar fly that has spread into all major fruit production regions of the United States (Asplen et al. 2015). They have caused severe damages to the berry and stone fruit industries due to the female's ability to penetrate and lay eggs into soft-skinned fruit prior to harvest (Asplen et al. 2015). Control is almost exclusively gained through calendar spray programs composed of broad spectrum insecticides that are applied at weekly or shorter intervals (Van Timmeren and Isaacs 2013, Asplen et al. 2015). These intensive insecticide regimens paired with *D. suzukii*'s high fecundity and short generation time has introduced the potential of insecticide resistance, especially in organic cropping systems where the number of insecticide classes are severely restricted (Asplen et al. 2015, Woltz and Lee 2017). Resistance to spinosyn has already developed in *D. suzukii* populations near Watsonville, CA, which has intensified the need for non-chemical management strategies (Gress and Zalom 2019).

A successful IPM program balances chemical control with a variety of suppressive tactics that mitigate disturbance to agro-ecosystems (Ehler 2006). Post-harvest crop sanitation strategies are a form of cultural control that involve the disposal of infested fruits and any ripe, overripe, and decomposing fruit that can act as a reproductive host (Walsh et al. 2011). Growers are encouraged to dispose of *D. suzukii* susceptible fruit wastes, which includes a variety of decomposing fruits and post-harvest and processing fruit wastes (Walsh et al. 2011, Bal et al. 2017). In the above work, burial and composting were identified as effective fruit waste disposal methods. Integrating these cultural control tactics into existing *D. suzukii* management programs may help to reduce the need for insecticide applications by alleviating late-season pest pressure and inhibiting the establishment of spring *D. suzukii* populations the following year.

When infested apple pomace was buried at depths of 0, 12, 24, 36, and 48 cm in the field, emergence of F1 adults exponentially decreased. A burial depth of 24 cm reduced *D. suzukii* survivorship by 97%. This burial depth could be achieved with a tractor's front loader or similar implement. However, soil texture could potentially affect this optimal burial depth because emergence differed between soil textures in the laboratory. Survival decreased by 30% from a depth of 1 cm in Sandy Soil compared to Loamy Soil. The Sandy Soil had greater porosity than Loamy Soil, and potentially lost moisture faster than Loamy Soil. Therefore, differential emergence may have been due to *D. suzukii* desiccating. Multiple *Tephritidae spp.*, which pupate a shallow depth underground like *D. suzukii*, are negatively impacted by dry soil conditions (Hou et al. 2006, Hulthen and Clarke 2006, Montoya et al. 2008). Thus, future studies should investigate how soil moisture levels influence *D. suzukii* pupal development and survival. This information could subsequently be used to improve the accuracy of population estimation models by factoring in rainfall events or irrigation practices.

Surprisingly, lipid concentration did not vary between flies that emerged from burial depths of 0, 3, 6, 12, and 24 cm in the laboratory. These results indicated that *D. suzukii* had sufficient long-term energy reserves to unbury themselves from all burial depths, which leaves the mechanism behind decreased emergence at deeper burial depths unclear. Four hypotheses that could explain reduced *D. suzukii* survival from deeper burial depths are – 1) pro-longed soil exposure increased desiccation, 2) the increasing weight of the soil pinned the flies into place, 3) the probability that flies could find their way to the soil's surface decreased, and 4) there was a greater chance of lethal injury caused by abrasion from the soil particles.

When apple pomace was composted with chicken manure at ratios of 100:0, 90:10, 75:25, and 0:100 in the field, *D. suzukii*'s reproductive success was reduced by > 95% in 75:25

compost treatments. Furthermore, *D. suzukii* emerged sooner from 50:50 compost treatments in what could be a bail-out response, i.e. the premature pupation of an insect larva due to starvation (Koyama and Mirth 2018). These results indicate that chicken manure is deleterious to *D. suzukii* reproduction and development. Two hypotheses that could explain the negative impact chicken manure has on *D. suzukii* emergence are – 1) the C:N ratio of the composting materials supported microbial life that rapidly degraded carbohydrates required for *D. suzukii* development, and 2) the decomposing chicken manure exuded high levels of nitrogenous wastes that were toxic to *D. suzukii*. In the laboratory, a negative impact on *D. suzukii* reproduction was observed while the compost treatments were likely in the mesophilic stage of decomposition (Parr et al. 1994). Carbohydrates still would be accessible to *D. suzukii* during this stage, which provides the most support to hypothesis 2.

In contrast to other drosophilids, *D. suzukii* are not typically exposed to high levels of nitrogenous wastes due to their utilization of fresh fruit hosts (Belloni et al. 2018). As a result, they lack efficient detoxification and excretory mechanisms of nitrogenous wastes (Belloni et al. 2018). Thus, exposure to high levels of urea or ammonia, which are both products of chicken manure decomposition, potentially caused acute toxicity in *D. suzukii* (Nahm 2005). Future studies should isolate different nitrogenous by-products produced by decomposing chicken manure, and test how they influence *D. suzukii* reproductive success when applied to a fruit host. The resulting information could potentially be exploited as a synthetic alternative to composting, which would be a more feasible option for large farming operations.

However, an addition of 1% manure to apple pomace in the laboratory increased *D. suzukii* reproduction by 28.5% compared to pure apple pomace. While this increase was not statistically significant, it may be of biological importance. The presence and relative abundance

of specific microbe species can have differential effects on *D. suzukii* fitness (Bing et al. 2018). For example, the yeast *Hanseniaspora uvarum* enhances larval development and survival, while the bacterium *Enterococcus faecalis* has a negligible effect on *D. suzukii* larval fitness (Bellutti et al. 2017, Bing et al. 2018). While the differing nutritional environments present in the different compost treatments likely favored the growth of different microbes, quantifying the microbial community present was outside the scope of this study (Bing et al. 2018). Thus, future studies should make quantitative species comparisons between compost treatments throughout the process of fruit decomposition and evaluate their associated fitness benefits to *D. suzukii*.

Additionally, this study highlights the need for research pertaining to *D. suzukii*'s potential competition with *D. melanogaster*. Previous studies conducted in the laboratory have suggested that *D. suzukii* are exploitatively out-competed by *D. melanogaster*, and that *D. suzukii* oviposition choice is strongly influenced by the presence of heterospecifics (Dancau et al. 2017, Shaw et al. 2017). Although *D. melanogaster* populations were not standardized, both species demonstrated high fecundity on shared reproductive resources in the field. Additionally, apple pomace used in the field experiment was pre-infested with both *D. melanogaster* and *D. suzukii*. These observations indicate that these two species can readily share a reproductive resource, which refutes the hypothesis that sanitizing fields creates a competitor free space in which *D. suzukii* could excel (Dancau et al. 2017).

In summary, the arrival of *D. suzukii* to the United States has caused widespread abandonment of IPM programs, and require non-chemical management strategies to be restored. Burial and composting are effective disposal methods of *D. suzukii* susceptible fruits that can be used to improve *D. suzukii* control. The efficacy of these methods could vary upon the host crop, operation size, and harvest frequency, and, thus, future studies should be done at the farm scale

to better understand the feasibility and cost of implementation under different contexts. Furthermore, investigating burial and composting at the farm scale would determine the *Drosophila spp.* population reduction attributable to these methods. Grower adoption of the cultural control tactics outlined in this thesis may decrease the number of insecticide applications required per year by reducing localized *D. suzukii* populations within and between growing seasons. This is significant for insecticide resistance management, minimizing secondary pest outbreaks, and improving the long-term environmental sustainability of *D. suzukii* susceptible cropping systems.

## **APPENDICES**

## Appendix A. 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: 2019 - 03

Author and Title of thesis: Holly Hooper. "Post-Harvest Crop Sanitation Strategies in *Drosophila suzukii* Susceptible Cropping Systems."

Museum(s) where deposited:

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

**Table A. 1** List of voucher specimens deposited in the Albert J. Cook Arthropod Research Collection, Michigan State University (MSU).

| Family        | Genus-Species             | Life Stage | Sex    | Quantity | Preservation |
|---------------|---------------------------|------------|--------|----------|--------------|
| Drosophilidae | <i>Drosophila suzukii</i> | Adult      | Female | 5        | Pinned       |
| Drosophilidae | <i>Drosophila suzukii</i> | Adult      | Male   | 5        | Pinned       |
| Drosophilidae | <i>Drosophila suzukii</i> | Adult      | Female | 5        | 75% EtOH     |
| Drosophilidae | <i>Drosophila suzukii</i> | Adult      | Male   | 5        | 75% EtOH     |

## Appendix B. Chapter 2 Supplementary Material

**Table B. 1** Chemical analysis by Midwest Laboratories (Omaha, NE) of the two soil samples collected from Trevor Nichols Research Center (TNRC) at Michigan State University (Fennville, MI) and the single soil sample collected from the Michigan State University Entomology Research Farm (ENT Farm) (East Lansing, MI).

|                                   | <b>TNRC 1</b> | <b>TNRC 2</b> | <b>ENT FARM</b> |
|-----------------------------------|---------------|---------------|-----------------|
| <b>Phosphorus (P<sub>1</sub>)</b> | 23 ppm        | 9 ppm         | 11 ppm          |
| <b>Phosphorus (P<sub>2</sub>)</b> | 45 ppm        | 23 ppm        | 17 ppm          |
| <b>Potassium (K)</b>              | 170 ppm       | 133 ppm       | 95 ppm          |
| <b>Magnesium (Mg)</b>             | 284 ppm       | 133 ppm       | 118 ppm         |
| <b>Calcium (Ca)</b>               | 1430 ppm      | 1453 ppm      | 1278 ppm        |
| <b>Sodium (Na)</b>                | 14 ppm        | 12 ppm        | 12 ppm          |
| <b>Nitrate (N)</b>                | 22 ppm        | 16 ppm        | 27 ppm          |
| <b>Sulfur (S)</b>                 | 15 ppm        | 14 ppm        | 9 ppm           |
| <b>Zinc (Zn)</b>                  | 2.8 ppm       | 2.2 ppm       | 1.0 ppm         |
| <b>Manganese (Mn)</b>             | 4 ppm         | 4 ppm         | 8 ppm           |
| <b>Iron (Fe)</b>                  | 28 ppm        | 16 ppm        | 45 ppm          |
| <b>Copper (Cu)</b>                | 0.9 ppm       | 0.9 ppm       | 0.5 ppm         |
| <b>Boron (B)</b>                  | 0.6 ppm       | 0.6 ppm       | 0.6 ppm         |
| <b>Organic Matter</b>             | 3.3%          | 2.4%          | 2.4%            |
| <b>Cation Ex. Capacity</b>        | 10 meq/100 g  | 9.9 meq/100 g | 9.0 meq/100 g   |
| <b>Soluble Salts</b>              | 0.2 mmhos/cm  | 0.3 mmhos/cm  | 0.2 mmhos/cm    |
| <b>pH</b>                         | 7.2           | 7.7           | 6.5             |

**Table B. 2** Daily temperature (°C) under the canopy tent and inside of the emergence cage averaged across blocks, and degree day accumulation inside of the emergence cage averaged across blocks for *D. sukuzii* from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI).

| <b>Date</b> | <b>Canopy (°C)</b> | <b>Cage (°C)</b> | <b>Degree Day</b> |
|-------------|--------------------|------------------|-------------------|
| 21–Aug      | 25.28              | 27.91            | 15.28             |
| 22–Aug      | 21.57              | 23.56            | 26.85             |
| 23–Aug      | 20.36              | 22.83            | 37.21             |
| 24–Aug      | 19.53              | 21.07            | 46.74             |
| 25–Aug      | 22.25              | 22.82            | 58.99             |
| 26–Aug      | 25.19              | 26.71            | 74.19             |
| 27–Aug      | 26.65              | 28.02            | 90.84             |
| 28–Aug      | 25.53              | 26.49            | 106.37            |
| 29–Aug      | 21.86              | 23.03            | 118.23            |
| 30–Aug      | 18.68              | 20.81            | 126.91            |
| 31–Aug      | 21.49              | 24.33            | 138.41            |
| 01–Sep      | 23.55              | 25.07            | 151.95            |
| 02–Sep      | 25.81              | 27.32            | 167.77            |
| 03–Sep      | 26.31              | 28.21            | 184.07            |
| 04–Sep      | 26.58              | 28.64            | 200.65            |
| 05–Sep      | 27.04              | 29.12            | 217.69            |
| 06–Sep      | 20.97              | 22.12            | 228.66            |
| 07–Sep      | 17.27              | 18.87            | 235.94            |
| 08–Sep      | 15.69              | 16.59            | 241.63            |
| 09–Sep      | 14.34              | 15.28            | 245.97            |
| 10–Sep      | 15.34              | 16.10            | 251.31            |
| 11–Sep      | 18.81              | 21.57            | 260.12            |

**Table B. 3** Daily soil moisture (%) reading under the canopy tent from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI).

| <b>Date</b> | <b>Block 1</b> | <b>Block 2</b> | <b>Block 3</b> | <b>Block 4</b> | <b>Block 5</b> |
|-------------|----------------|----------------|----------------|----------------|----------------|
| 21-Aug      | 50             | 68             | 62             | 60             | 55             |
| 22-Aug      | 51             | 52             | 54             | 61             | 65             |
| 23-Aug      | 60             | 52             | 53             | 55             | 70             |
| 24-Aug      | 69             | 54             | 49             | 60             | 50             |
| 25-Aug      | 46             | 43             | 58             | 100            | 57             |
| 26-Aug      | 73             | 79             | 54             | 100            | 100            |
| 27-Aug      | 60             | 75             | 70             | 75             | 60             |
| 28-Aug      | 70             | 75             | 70             | 70             | 80             |
| 29-Aug      | 80             | 75             | 90             | 100            | 90             |
| 30-Aug      | 95             | 80             | 80             | 92             | 75             |
| 31-Aug      | 75             | 100            | 80             | 75             | 70             |
| 01-Sep      | 70             | 76             | 65             | 78             | 70             |
| 02-Sep      | 92             | 85             | 81             | 85             | 100            |
| 03-Sep      | 90             | 90             | 80             | 91             | 89             |
| 04-Sep      | 89             | 85             | 74             | 75             | 89             |

## Appendix C. Chapter 3 Supplementary Material

**Table C. 1** Chemical analysis by A&L Great Lakes Laboratories (Fort Wayne, IN) of the organic apple pomace, organic chicken manure, and leaf-woodchip blend.

|                                              | <b>Apple Pomace</b> | <b>Chicken Manure</b> | <b>Leaf-Woodchip</b> |
|----------------------------------------------|---------------------|-----------------------|----------------------|
| <b>Moisture</b>                              | 86.19%              | 9.44%                 | 29.88%               |
| <b>Solids</b>                                | 13.81%              | 90.56%                | 70.12%               |
| <b>Ash</b>                                   | --                  | 25.12%                | --                   |
| <b>Organic Matter</b>                        | 12.59%              | 65.44%                | 68.45%               |
| <b>Organic Carbon (C)</b>                    | 6.28%               | 37.95%                | 34.23%               |
| <b>Carbon: Nitrogen Ratio (C:N)</b>          | 45.5:1              | 7.7:1                 | 72.9:1               |
| <b>Nitrogen, Total (TKN)</b>                 | 0.14%               | 5.415%                | 0.47%                |
| <b>Nitrogen, Ammonium (NH<sub>4</sub>-N)</b> | --                  | 0.330%                | --                   |
| <b>Nitrogen, Organic (N)</b>                 | --                  | 5.085%                | --                   |
| <b>Phosphorus (P)</b>                        | --                  | 1.612%                | --                   |
| <b>Potassium (K)</b>                         | --                  | 2.516%                | --                   |
| <b>Sulfur (S)</b>                            | --                  | 0.37%                 | --                   |
| <b>Magnesium (Mg)</b>                        | --                  | 0.53%                 | --                   |
| <b>Calcium (Ca)</b>                          | --                  | 7.77%                 | --                   |
| <b>Sodium (Na)</b>                           | --                  | 0.27%                 | --                   |
| <b>Aluminum (Al)</b>                         | --                  | 639 ppm               | --                   |
| <b>Copper (Cu)</b>                           | --                  | 75 ppm                | --                   |
| <b>Iron (Fe)</b>                             | --                  | 690 ppm               | --                   |
| <b>Manganese (Mn)</b>                        | --                  | 406 ppm               | --                   |
| <b>Zinc (Zn)</b>                             | --                  | 677 ppm               | --                   |

**Table C. 2** Daily temperature (°C) under the canopy tent and inside of the emergence cage averaged across blocks, and degree day accumulation inside of the emergence cage averaged across blocks for *D. sukuzii* from the experimental plot located at the Entomology Farm at Michigan State University (East Lansing, MI).

| <b>Date</b> | <b>Canopy (°C)</b> | <b>Cage (°C)</b> | <b>Degree Day</b> |
|-------------|--------------------|------------------|-------------------|
| 18-Sep      | 28.30              | 30.51            | 18.30             |
| 19-Sep      | 23.08              | 25.85            | 31.38             |
| 20-Sep      | 21.63              | 23.81            | 43.01             |
| 21-Sep      | 23.25              | 24.61            | 56.26             |
| 22-Sep      | 15.58              | 20.33            | 61.84             |
| 23-Sep      | 15.49              | 20.30            | 67.33             |
| 24-Sep      | 14.90              | 16.00            | 72.23             |
| 25-Sep      | 21.95              | 23.01            | 84.19             |
| 26-Sep      | 16.69              | 18.74            | 90.88             |
| 27-Sep      | 12.04              | 14.04            | 92.92             |
| 28-Sep      | 12.09              | 12.94            | 95.01             |
| 29-Sep      | 11.23              | 13.77            | 96.24             |
| 30-Sep      | 8.13               | 9.02             | 96.24             |
| 01-Oct      | 11.41              | 11.84            | 97.66             |
| 02-Oct      | 16.10              | 16.60            | 103.76            |
| 03-Oct      | 20.35              | 21.41            | 114.11            |
| 04-Oct      | 19.62              | 22.54            | 123.72            |
| 05-Oct      | 9.52               | 10.25            | 123.72            |
| 06-Oct      | 17.78              | 18.45            | 131.50            |
| 07-Oct      | 13.35              | 14.04            | 134.85            |
| 08-Oct      | 20.61              | 21.86            | 145.46            |
| 09-Oct      | 23.93              | 25.77            | 159.40            |
| 10-Oct      | 20.59              | 21.15            | 169.99            |
| 11-Oct      | 11.17              | 11.68            | 171.16            |
| 12-Oct      | 6.06               | 6.83             | 171.16            |

**Table C. 3 (cont'd)**

| <b>Date</b> | <b>Canopy (°C)</b> | <b>Cage (°C)</b> | <b>Degree Day</b> |
|-------------|--------------------|------------------|-------------------|
| 13-Oct      | 7.77               | 10.70            | 171.16            |
| 14-Oct      | 9.81               | 12.90            | 171.16            |
| 15-Oct      | 7.76               | 9.35             | 171.16            |
| 16-Oct      | 6.93               | 9.31             | 171.16            |
| 17-Oct      | 5.82               | 7.05             | 171.16            |
| 18-Oct      | 4.73               | 8.34             | 171.16            |
| 19-Oct      | 8.65               | 9.09             | 171.16            |
| 20-Oct      | 7.61               | 8.73             | 171.16            |
| 21-Oct      | 3.40               | 3.99             | 171.16            |
| 22-Oct      | 8.30               | 11.04            | 171.16            |
| 23-Oct      | 8.27               | 11.35            | 171.16            |
| 24-Oct      | 4.06               | 8.14             | 171.16            |
| 25-Oct      | 3.81               | 5.99             | 171.16            |
| 26-Oct      | 6.77               | 7.48             | 171.16            |
| 27-Oct      | 6.20               | 6.73             | 171.16            |
| 28-Oct      | 5.11               | 5.48             | 171.16            |
| 29-Oct      | 6.80               | 7.78             | 171.16            |
| 30-Oct      | 7.42               | 8.18             | 171.16            |
| 31-Oct      | 11.17              | 12.67            | 172.33            |
| 01-Nov      | 6.44               | 6.86             | 172.33            |
| 02-Nov      | 5.52               | 6.46             | 172.33            |

## **LITERATURE CITED**

## LITERATURE CITED

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