CULTURAL AND BIOLOGICAL CONTROL TACTICS FOR SPOTTED-WING DROSOPHILA IN ORCHARD SYSTEMS POST-HARVEST

By

Charlotte Mae Johanna Schuttler

A THESIS

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

Entomology – Master of Science

2023

ABSTRACT

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is an invasive, polyphagous vinegar fly with high fecundity and short generation time. *Drosophila suzukii*, or 'spotted-wing Drosophila' has spread quickly across the United States and has quickly become the main pest of concern in tart cherry production. Millions of dollars are spent each year on chemical and labor costs to address this invasive pest. Current management tactics rely upon multiple applications of broad-spectrum insecticides. As populations of *D. suzukii* resistant to spinosyns and pyrethroids have recently been found in California, alternative management strategies are needed to reduce dependency on chemical control. Recent releases of classical biological control agents have begun across the United States, but their ultimate establishment and potential impact is yet unknown. Innovative methods of cultural and biological control could help restore integrated pest management (IPM) of *D. suzukii* in cropping systems. This thesis investigates cultural control and biological control methods for managing *D. suzukii* infestation in post-harvest fruit waste as means of reducing overall populations within an area and potentially decreasing the need for other management methods. Orchard systems often manage disposal of post-harvest crops consisting of any ripe, overripe, and decomposing fruit which may function as a reproductive source for pests. Crushing fruit or adding 15% or 25% organic poultry manure by volume were identified as effective cultural control methods of decreasing the number of *D. suzukii* and non-suzukii drosophila on post-harvest cherry waste. Adding *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) larvae to *D. suzukii* infested fruit waste was found to limit *D. suzukii* infestation. These cultural and biological control methods can be integrated into existing management programs to help reduce in situ populations of *D. suzukii* without further addition of insecticides.

Dedicated to Carolyn Schuttler

ACKNOWLEDGEMENTS

I would like to thank Hannah Burrack for taking me as a student even though she had enough on her plate, for always lending an ear and a new way to think about things, and for encouraging a life-work balance. Thank you to Matthew Grieshop for giving me many opportunities to continue learning, and many tools to be successful. Thank you to Juan Huang for her assistance and mentorship over many years, without which I would be lost. Thank you to Holly Hooper for showing me how to be a scientist. I would like to thank the members of my committee (Matt Grieshop, Nikki Rothwell, Marianna Szucs, and Hannah Burrack) for their different perspectives, patience, understanding, and tenacity for research. Thank you to the many (past and present) members of the Organic Pest Management Lab, the Pollinator Lab, and the Tree Fruit Entomology Lab. I would like to thank the many technicians who assisted me in laboratory and field work, especially Maggie Foley and Robin Fisher. I want to thank the members of the Northwest Horticulture Research Center especially Nikki Rothwell, Karen Powers, Jen Zelinski, Myron Anderson, and Bill Kline for their hard work, dedication, and for being my ‘Up North’ home. I would like to thank my grower collaborators, Gene Garthe and Garthe Farms, and Cherry Bay. Thank you to Jim Koan and Almar Orchards, Amy Herbruck and Herbruck’s Poultry, Jonathan Cammack at Evo Conversion Systems, and Chelsea Holcomb and Jeffery Tomberlin at Texas A&M. Thank you to my funding sources, NCSARE and Project GREEEN, for making this research possible. Thanks to my mom, Carolyn Schuttler for always encouraging me. Thanks to my dogs for keeping me company. Finally, thank you to Nate Alto for being my babe.

TABLE OF CONTENTS

CHAPTER 1. FARM-SCALE ASSESSMENT FOR MANAGING *DROSOPHILA SUZUKII* INFESTATION OF POST-HARVEST FRUIT WASTE

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a vinegar fly of eastern Asian origin, which has recently undergone a dramatic global range expansion (Asplen et al. 2015). *Drosophila suzukii* prefer to lay eggs in soft-skinned fruit such as blueberries and caneberries but will utilize a range of crop hosts including strawberries, cherries, and damaged or spoiled fruit (Walsh et al. 2011, Burrack et al. 2013). Eggs are laid under the surface of ripe and ripening fruit, and larvae develop internally which can result in larval infestation in harvested fruit. Development from egg to adult can take as little as 8 days with adults living for up to two weeks (Lee et al. 2011). This quick development results in rapid population growth and multiple overlapping generations of *D. suzukii* each summer, making pest management difficult (Lee et al. 2011).

The arrival of *D. suzukii* to the United States has caused millions of dollars' worth of losses in multiple crops (Bolda et al. 2010). *Drosophila suzukii* was detected in Michigan in 2010 and quickly became the key pest of tart cherries (Wilson et al. 2019). In the Midwest United States, tart cherries (*Prunus cerasus*, Rosales: Rosaceae) are marketed from June 25th to August 15th (NASS 2023). The large, serrated ovipositor of the female *D. suzukii* means that cherries are at risk of infestation from the moment the fruit begins to gain color throughout harvest (Wilson et al. 2019). Michigan is the main U.S. state growing tart cherry, producing 43.8 million kilograms out of the United States total of 780,179 kilograms (~56%) in 2021 (NASS 2023). In 2021 there were 9307.8 hectares (23,000 acres) of tart cherries grown in Michigan producing a crop valued at $58 million dollars (NASS 2023). Prior to the establishment of *D. suzukii* in Michigan, the major tart cherry pests included the cherry fruit fly *Rhagoletis* *cingulate* (Loew) (Diptera: Tephritidae), black cherry fruit fly *Rhagoletis fausta* (Osten Sacken) (Diptera: Tephritidae), plum curculio *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), and the American plum borer, *Euzophera semifuneralis* (Walker) (Lepidoptera: Pyralidae), which were managed with detection based sprays from early May until pre-harvest at the end of July (Brown et al. 1989, Sirrine 2006, Michigan State University Extension 2004) As observed in other crops (Joshi et al. 2022), pre-harvest insecticide use in tart cherries has increased following *D. suzukii* establishment in Michigan to meet strict no-tolerance requirements for fruit infestation.

Organophosphates, pyrethroids, and spinosyns are among the most effective classes of insecticides against *D. suzukii*, and years of increased used has raised concern for resistance development (Fanning et al. 2018, Van Timmeren and Issacs 2013, Mishra et al. 2018). Resistance to spinosyns and pyrethroids has already been observed in California populations of *D. suzukii* (Gress and Zalom 2019, Ganjisaffar et al. 2022). Growers are interested in non-chemical control tactics to reduce populations of *D. suzukii* and decrease reliance on insecticides. Innovative cultural control techniques have the potential to limit additional insecticide use and could help restore integrated pest management in small fruit production. *Drosophila suzukii* are a mobile pest which will show flexible preference when whole fruit are not available (Kirkpatrick 2018, Kienzle et al. 2020). Management techniques which target other potential resources like fruit waste, could limit in situ populations.

Fruit waste may consist of infested fruit, damaged fruit, and unripe or overripe fruit. Massive amounts of fruit pomace left over from juice, cider, and wine production end up in landfills or composting sites (Dhillon et al. 2013). From 2021to 2022, approximately 0.2 million tons of apple pomace and 1.3 million tons of wine pomace were produced in United States, the physical equivalent of 214 thousand adult African elephants (Jackson et al. 2022, Ferrer-Gallego and Silva, 2022). Market conditions for tart cherries lead to the periodic disposal of large amounts of fruit which cannot be sold (Paggi and Nicholson 2013). Disposal of fruit waste may include piling in unused fields, composting, feeding to livestock, and landfilling (Esparza et al. 2020). Unprocessed fruit waste can provide late season resources for multiple drosophila species and other pest insects and create early season pressure the following season (Bal et al. 2017).

Treatment of post-harvest waste may also reduce other nuisance pests. Sap beetles (Coleoptera: Nitidulidae) are attracted to overripe, rotting fruit; are considered potential pests in strawberry, sweet corn, melon, peach, raspberry, blueberry, and cherry; and are a major pest of berries in the Northeastern United States (Loughner et al. 2007). Multiple sap beetle species are found in fruit and function as a pest complex (Powell 2015, Rondon et al. 1969). Sap beetle adults are attracted to fermentation odors and will directly feed and lay eggs in both rotting and sound fruit (Powell 2015). Sap beetle generation time is roughly four weeks, and more than one generation occurs per growing season (Emekci and Moore 2015). Larval sap beetles cause damage by feeding on fruit, contaminate fruit through their presence, and facilitate further infestation by secondary pests such as *Drosophila* spp. and phytopathogens (Rondon et al. 1969, Souza et al. 2019, Lin and Phelan 1991). Prompt removal of ripe fruit from fields reduces their attractiveness to sap beetles (Loughner et al. 2008).

Volatiles from ripe and fermenting fruit attract more than just sap beetles, particularly non-suzukii drosophila species such as *Drosophila melanogaster* (Meigen), *D. simulans* (Sturtevant), *D. hydei* (Sturtevant), *D. busckii* (Coquillet), and *D. robusta* (Sturtevant) (Sturtevant 1921, Band 1993). These non-suzukii drosophila species have a similar life span to *D. suzukii* but are unable to infest undamaged fruit pre-harvest, and while mostly a concern for damaged fruit post-harvest, can cause pathogen spread in fruit production and infest products during processing (Hubhachen et al. 2022). Previous research has found *D. melanogaster* populations in Michigan have variable levels of resistance to multiple classes of insecticides, highlighting the need for implementation of cultural management methods (Hubhachen et al. 2022).

My work seeks to develop strategies to reduce the ability of insect pests to utilize post-harvest fruit waste by comparing several possible management tactics. Crushing fruit post-harvest has been found to work at small scale to reduce *D. suzukii* infestation but has not previously been examined at a farm scale (N. Rothwell, personal communication). Previous research suggests that incorporating organic poultry manure into fruit waste at a rate of 25% by volume will inhibit *D. suzukii* adult emergence by 95% (Hooper and Grieshop 2021). I conducted a series of experiments comparing crushing, manure addition, or fertilizer addition under field conditions to assess potential reduction of in situ *D. suzukii* populations and possible impacts on other post-harvest pests. In addition to testing different concentrations of manure, I also tested the addition of urea as a synthetic alternative to manure. To the best of our knowledge this is the first study managing pest insects in post-harvest cherry waste, and we utilized multiple evaluation methods in order compare them for potential future research. Therefore, a secondary goal of this experiment was to evaluate sampling methods to determine which methods were most effective.

Materials and Methods

2021 Experiment

We selected four treatments to apply to cherries post-harvest: (1) crushing, (2) incorporation of 25% manure by volume; and (3) incorporation of 15% manure by volume compared to (4) an untreated control. Granulated organic chicken manure obtained from Herbruck's Poultry Ranch, Inc. (Saranac, MI) was used for manure containing treatments. Seven sites (Table B.1) were chosen in the Leelanau peninsula (Michigan, USA) and one replicate of each treatment was established per site. Cherries were harvested by cooperating growers and placed in selected sites. Due to unfavorable conditions during the 2021 field season, there was limited availability of tart cherries, so some locations utilized sweet cherries (Table B.1). At each location, cherries were measured by volume with 5-gal plastic buckets and dumped into four piles. Each pile consisted of 95 L of cherries (~73.5kg). Piles were spaced at least 100 m apart. For crushing treatments, cherries were crushed with tractor tires until 90% of fruit was macerated then fruit was raked back into a pile. For manure treatments poultry manure was measured and applied to waste piles. Treatments with 15% manure had 13.2 L of water added, and treatments with 25% manure had 26.5 L of water added. All other treatments had 13.2 L of water added to them. All waste piles were mixed for approximately 4 min with hand tools after treatments were applied to either incorporate manure or to maintain conditions comparable to manure incorporation.

Insects coming from fruit piles and those from around fruit piles were monitored. To capture insects emerging from fruit waste, each pile was capped with an emergence cage (Bugdorm, Talchung, Taiwan) measuring 60 cm L by 60 cm W by 60 cm H, secured at four points with ground stakes. The whole experiment took place between July 15th and August 24th, 2021, with all sampling starting on July 22nd, 2021. The inside of each emergence cage was vacuumed twice weekly using a handheld aspirator (BioQuip Products, Inc., Rancho Dominguez, CA). Aspirated samples were frozen for at least 24 h, after which the number of male *D. suzukii*, female *D. suzukii,* non-suzukii drosophila, and sap beetles (Coleoptera: Nitidulidae) were counted under a stereomicroscope.

To monitor insects on and around fruit waste piles, we used three yeast-baited cup traps (Huang et al. 2017). All traps were suspected from 0.91m stakes and placed either directly next to each fruit waste pile (trap E) or 10 m from the pile in opposite directions (traps N & S). Yeast trap contents were collected weekly, filtered, and *D. suzukii*, non-suzukii drosophila, and sap beetles were sorted and counted.

2022 Experiment

A second field season was completed in 2022 following similar methods to 2021. Plots were established at five locations (Table B.1). In 2022, tart cherries, cv. Montmorency were harvested from the Northwest Michigan Horticulture Research Station (Traverse City, MI), moved to each location, and divided into piles. In addition to the treatments compared in 2021, the following treatments were added during 2022: (5) A *D. suzukii* seeded control and (6) a urea fertilizer treatment. Urea was added with the intent to isolate the effect of nitrogen addition on *D. suzukii* infestation. *Drosophila suzukii* used to infest the seeded control were from a colony established in 2016 from flies collected from the Trevor Nichols Research Center (TNRC) at Michigan State University (Fennville, MI). Flies were reared on corn meal diet (Dalton et al. 2011) in 300 ml wide mouth glass mason jars (Ball Corporation, Broomfield, CO) and maintained in a growth chamber set at 21˚C, 70% relative humidity (RH), and a photoperiod of 16:8 (L:D) hours. Flies were anesthetized using CO_2 and separated by sex on a FlyStuff FlyPad (Genesee Scientific, San Diego, CA). Following separation, females were placed in new jars of diet for two days prior to field release. Flies were released into the center of emergence tents immediately after setting up and left in for the entirety of the experiment. Urea fertilizer (The Andersons, Inc., Maumee, OH) was measured and mixed with water and the solution was dumped onto piled fruit. All piles were mixed for approximately 4 min by shovel before tents were added.

The 2022 experiment took place between July 22-August 24th, 2022. Sampling began on July 25th, 2022. Yeast cup captures were used to assess insects near fruit piles following the same methods as in 2021. To measure insects emerging from piles within cages, vacuum samples were collected as in 2021, and in addition, a single yellow sticky card was hung inside each cage and was replaced weekly. Sticky cards were stored at -20°C after collection until the number of *D. suzukii*, non-suzukii drosophila, and sap beetles could be enumerated.

Statistical Analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Vacuum samples, cup trap captures, and yellow sticky card captures for all target insects were all compared via a mixed model ANOVA fitted to a logistic distribution via Proc GLIMMIX. A constant of 1.5 was added to response variables to retain zero values in the analysis. Site was included in models as a random effect, and treatment and week of experiment were considered fixed effects. In instances where there were significant effects of independent variables or their interactions, pairwise mean comparisons were conducted using the Tukey-Kramer adjustment with α=0.05. In cup traps insect counts from traps N and S were averaged and compared against the corresponding trap E and across treatments.

Results

Drosophila suzukii

Analyzed means of *D. suzukii* collected in vacuum samples during 2021 (Figure 1.1A) showed significant effects of treatment ($F_{3,138}$=4.29, p=0.0063), week ($F_{5,138}$=4.79, p=0.0005) and their interaction ($F_{15,138}$=2.03, p=0.0170). Capture was not significantly different between treatments from week 1 to week 3. From week 3 to week 6, significantly more *D. suzukii* were collected from the control treatment than in crushed, 15% manure, or 25% manure treatments. Highest overall capture occurred in week 4 of the experiment and an average of 143 ± 96, 2 ± 0.2, 3 ± 0.8, and 3 ± 1.4 adult *D. suzukii* were collected from the control, crushed, 15% manure, and 25% manure treatments, respectively.

Vacuum captures of *D. suzukii* in 2022 did not differ between treatments ($F_{5,111}$=1.42, p=0.2226), week ($F_{4,111}$=0.87, p=0.4852), and there was no interaction between week and treatment ($F_{20,111}$=0.68, p=0.8417). Sticky traps were added within emergence cages in 2022 as an additional form of data collection. Numerically, more *D. suzukii* were caught in sticky traps (2256) than in vacuum samples in 2022 (63). Based on catch from vacuum samples in 2021 (1685), it is likely that the addition of the sticky card inside the tent interacted with vacuum capture in 2022.

Analyzed sticky trap capture of *D. suzukii* in 2022 (Figure 1.1B), showed that treatment ($F_{5,88}$=14.64, p<0.0001), week ($F_{3,88}$=14.56, p<0.0001), and their interaction ($F_{15,88}$=2.66, p=0.0023) were significant. Similar to vacuum capture in 2021, for the first 2 weeks of the experiment there was no significant differences in capture between treatments, but in week 3 there were more *D. suzukii* caught in the control treatment than in all other treatments. In week 3, an average of 98 ± 40, 37.3 ± 11.3, 15.7 ± 7.9, 21.5 ± 7.5, 5 ± 1.9, and 3.1 ± 0.9, adult SWD were collected from the control, seed control, crushed, urea, 15% manure, and 25% manure treatments, respectively.

In 2021, the most *D. suzukii* per week were captured in cup traps associated with 15% manure (26.4 ± 0.2) treated piles as compared to crushed cherries (14.4 ± 0.2) or control (15.1 ± 0.2) treatments(treatment: $F_{2,234}$=3.90, p=0.0095; Figure 1.2A). The number of *D. suzukii* caught in 25% manure (18.7 ± 0.2) and 15% manure (26.4 ± 0.2) treatments were not significantly different from each other. *Drosophila suzukii* capture increased from week 1 to week 3, declined in week 4, and increased again in week 5 (week: $F_{4,234}$=57.62, p<0.0001). There were no interactions between experimental week and treatment ($F_{12,234}$=0.62, p=0.8240). In 2021, nearly twice as many *D. suzukii* were captured in cup traps placed on piles (10,532) than in traps placed 10m away (5896) from piles ($F_{1,234}$=4.14, p=0.0429) with no interaction between treatment and trap placement ($F_{3,234}$=1.80, p = 0.1486).

A similar pattern was observed in 2022 cup traps (Figure 1.2B) where treatments containing manure had the highest *D. suzukii* captures (15%: 65.8 ± 19.3 ; 25%: 65.3± 21.6). (treatment: $F_{5,180}$=16.23, p<0.0001). There was no significant difference between control (5.2 ± 0.9), seeded control (15.0 ± 3.8), crushed cherry (10.4 ± 2.4), and urea treatments (4.6 ± 1.1). *D. suzukii* capture increased from week 1 to week 3 and declined in week 4 (week: $F_{3,180}$=31.83, p<0.0001), but there was no interaction between week and treatment ($F_{15,180}$=0.87, p=0.6036). There was a significant interaction between trap placement and treatment in 2022 (location*treatment: $F_{5,180}$=3.70, p = 0.0032), but this only affected the 15% manure treatment in that more flies were captured in traps located on the pile. Traps placed on and off piles captured similar numbers of flies for all other treatments.

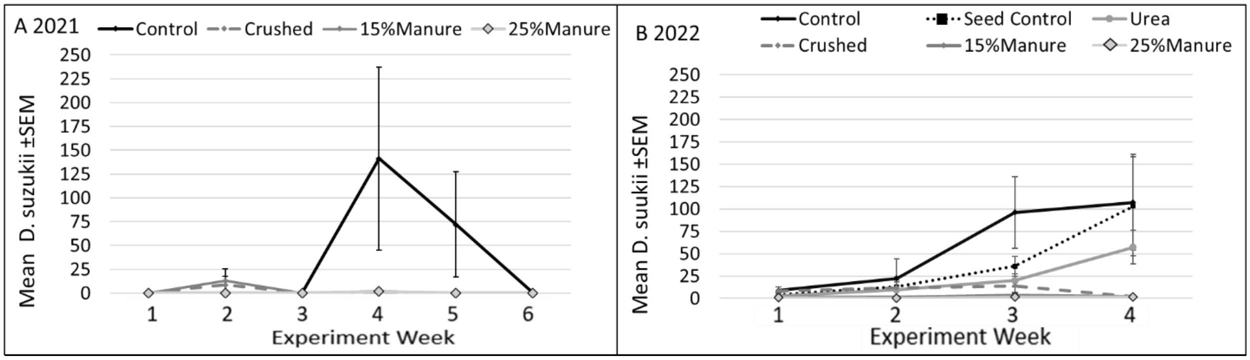

Figure 1.1 A) Mean *D. suzukii* (±SEM) captured per treatment collected via vacuum during 2021 field season. B) Mean *D. suzukii* (±SEM) captured per treatment captured via sticky card within emergence cages during 2022 field season.

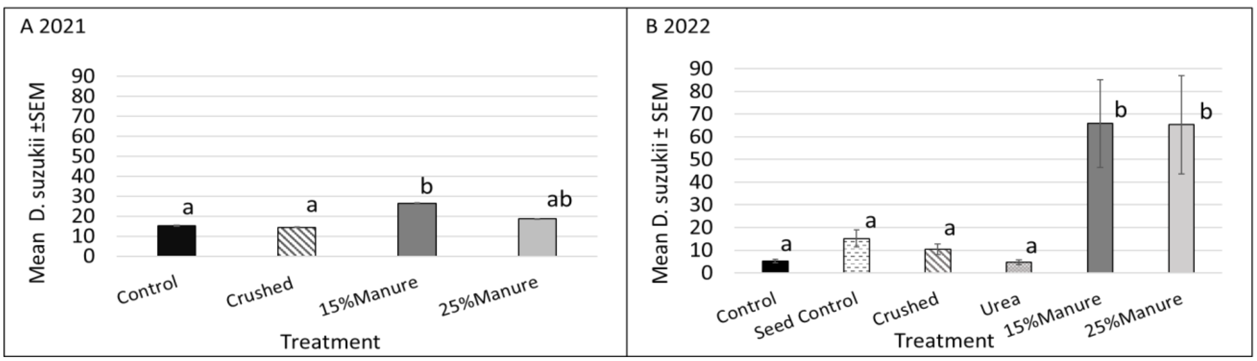

Figure 1.2 Adjusted mean *D. suzukii* (±SEM) collected per treatment per week via cup trap in (A) 2021 and (B) 2022 averaged across all three traps per replicate. All figures show significance with α=0.05.

Non-suzukii Drosophila

Other Drosophilidae species observed included *Drosophila melanogaster* (Meigen), *Drosophila simulans* (Sturtevant), *Drosophila hydei* (Sturtevant), *Drosophila busckii* (Coquillet), and *Drosophila robusta* (Sturtevant). Non-suzukii drosophila capture via vacuum in 2021 differed significantly by week ($F_{5,138}$=10.96, p<0.0001), treatment ($F_{3,138}$=22.09, p < 0.0001), and their interaction ($F_{15,138}$=2.27, p = 0.0069; Figure 1.3A). The control and crushed treatments had a similar number of *Drosophila* captured in the first and second week of the experiment. In weeks 3 and 4, the most non-suzukii drosophila were caught in the control treatment. Capture declined in week 5 at which point all treatments had a similar amount of non-suzukii drosophila.

Vacuum capture in 2022 of non-suzukii *Drosophila* spp. (Figure 1.3B) differed significantly between week ($F_{4,111}$=12.39, p<0.0001), treatment ($F_{5,111}$=74.36, p < 0.0001), and their interaction ($F_{20,111}$=4.01, p = 0.0069). In the first week of the experiment, there was no significant difference between treatments. By the second week, the control treatment had the most drosophila with numbers peaking in week 3 and decreasing in week 4, similar to capture in 2021. The urea treatment (83.5 ± 41.1) had less capture than the control treatment (192.9 ± 90.1) but more capture than the manure treatments (15%: 7.7 ± 5.2; 25%: 3.9 ± 1.4). Catch in the crush treatment increased from week 1 to week 2, but by week 3, declined to rates similar to those of manure treatments (crushed cherry week 3: 44.9 ± 16.6).

Sticky card capture in 2022 (Figure 1.3C) differed by treatment ($F_{5,88}$=57.16, p<0.0001), week ($F_{3,88}$=16.51, p<0.0001), and their interaction ($F_{15,88}$=3.81, p<0.0001). The control treatment and seeded control were not significantly different at any timepoint and had highest captures throughout the experiment. Capture in the urea experiment increased steadily throughout the experiment. The crushed treatment had a similar pattern to 2021 and 2022 vacuum samples with a decline in capture from week 2 to week 3, and low capture throughout the rest of the experiment, similar to capture in manure treatments. In week 3, an average of 83.6 ± 28.9, 58.4 ± 9.3, 24 ± 7.4, 51 ± 13.9, 12.8 ± 9.4, and 4.4 ± 0.5 non-suzukii drosophila were collected from the control, seed control, crushed, urea, 15% manure, and 25% manure treatments, respectively.

In 2021 means of non-suzukii *Drosophila* spp. from cup traps showed no significant treatment effect ($F_{3,234}$=1.47, p = 0.2245) and no interaction (week*treatment $F_{12,234}$=1.15, p = 0.3220). The number of non-suzukii drosophila did not steadily increase throughout the experiment, but more non-suzukii drosophila were collected during the final week of the experiment than earlier weeks across all treatments (week: $F_{4,234}$=3.40, p = 0.0100).

In 2022, significantly more non-suzukii drosophila were caught in cup traps associated with piles containing 25% manure (6.1 ± 1.0) than those treated with urea (2.4 ± 0.5) or left untreated (2.8 ± 0.6) (Figure 1.4; treatment: $F_{5,180}$=3.90, p=0.0022). Similar to vacuum samples in 2021 and 2022, cup trap captures from the crushed cherry treatment started high in week 1 (14.7 ± 4.8) but declined in week 2 (2.7 ± 0.8) and remained low throughout the rest of the experiment (treatment*week: $F_{15,180}$=2.02, p=0.0163).

In 2021 and 2022 twice as many non-suzukii drosophila were collected from cup traps located on piles, rather than from traps 10m away from piles (2021: $F_{1,234}$=7.93, p = 0.0053; 2022: $F_{1,180}$=4.59, p=0.0335). There was no interaction between treatment and location of the cup traps in 2021 ($F_{3,234}$=0.17, p = 0.9173) or 2022 ($F_{5,180}$=1.54, p = 0.1800).

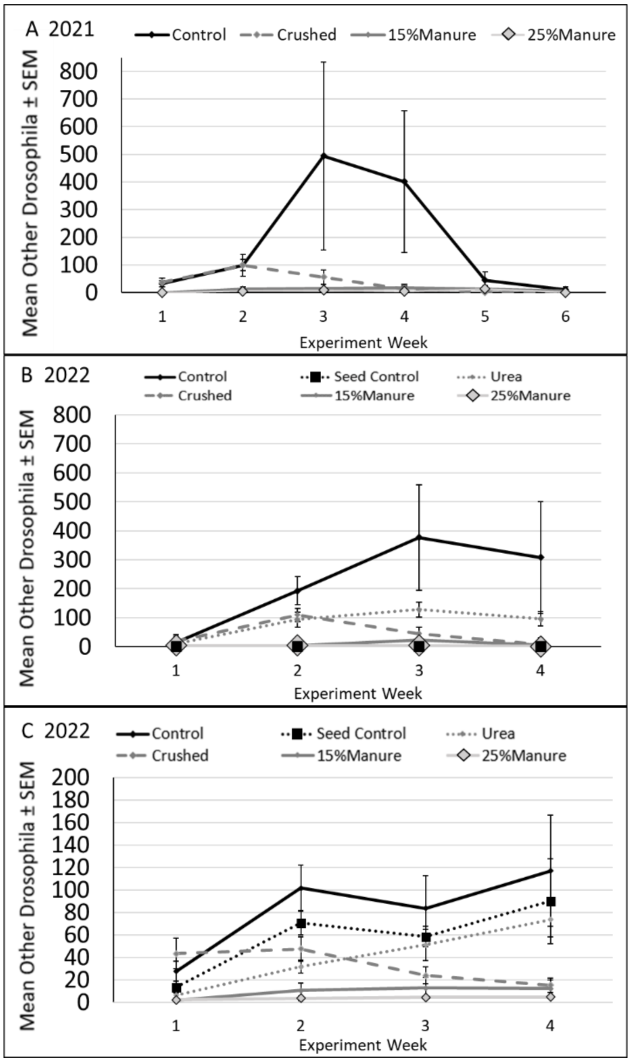

Figure 1.3 A) Mean non-suzukii drosophila (±SEM) capture via vacuum in 2021. B) Mean non-suzukii drosophila (±SEM) capture via vacuum in 2022. C) Mean non-suzukii drosophila (±SEM) capture via sticky card sampling 2022. Note scale difference for 1.3C.

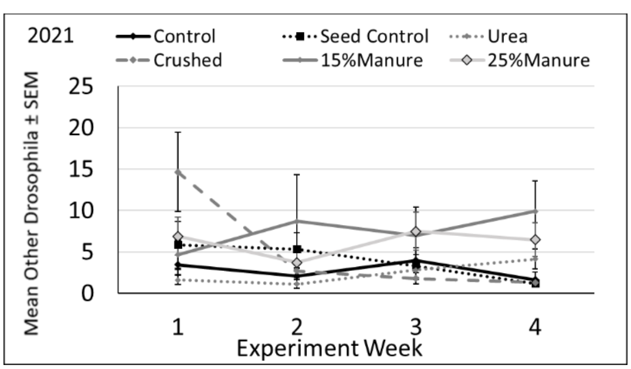

Figure 1.4 Mean non-suzukii drosophila (±SEM) capture via cup traps averaged across all three traps per replicate in 2022.

Sap Beetles

Two morphospecies of sap beetle were observed in trap captures and vacuum samples, and vouchers specimens of both have been deposited in A.J. Cook Arthropod Research Collection at Michigan State University.

More sap beetles were collected in vacuum samples from the control treatment (1.0 ± 0.4) as compared to the 15% manure (0.3 ± 0.4) treatment during 2021 (Figure 1.5A; $F_{3,138} = 3.06$, $p = 0.0304$). There was no significant difference between the control(1.0 ± 0.4), crushed cherry (0.6 ± 0.4), or 25% manure (0.4 ± 0.4) treatments. Sap beetle numbers increased as the experiment continued, peaking in weeks 4 and 5 ($F_{5,138}=7.12$, $p < 0.0001$). There was no interaction between week and treatment ($F_{15,138} = 0.68$, $p = 0.8009$).

In 2022, significantly fewer sap beetles were collected from vacuum samples from the seeded control (0.00005 ± 0.3) as compared to other treatments (control: 2.6 ± 0.3, crushed cherry:1.6 ± 0.3, urea: 2.5 ± 0.3, 15% manure:1.2 ± 0.3, 25% manure: 0.9± 0.3) ($F_{5,111} = 7.33$, $p < 0.0001$; Figure 1.5B). More beetles overall were caught in week 4 ($F_{4,111} = 7.62$, $p < 0.0001$) than in any other week, and there were no interactions between independent variables ($F_{20,111} = 0.89$, $p = 0.5950$).

The most sap beetles captured via sticky card in 2022 were caught in the crushed cherry (14.3 ± 0.2), 25% manure (12.7 ± 0.2), and control (6.3 ± 0.2) treatments compared to the seeded control (5.8 ± 0.2), urea (4.0 ± 0.2), and 15% manure (5.5 ± 0.2) treatments ($F_{5,88}=4.98$, $p=0.0005$; Figure 1.5C). In 2022, the second week of the experiment had the most beetles caught of all weeks ($F_{3,88}=5.38$, $p=0.0019$), and there was no interaction among independent variables ($F_{15,88}=1.01$, $p=0.4547$).

Sap beetles captures in cup traps in 2021 did not differ between treatments ($F_{3,234} = 2.38$, $p = 0.0703$), but did differ over time with significantly more beetles captured in the last (fifth) week of the experiment, mirroring 2021 vacuum capture (week: $F_{4,234} = 72.17$, $p < 0.0001$). There was no interaction between week and treatment ($F_{12,234} = 0.35$, $p = 0.9777$).

In 2021 ($F_{1,234} = 5.39$, $p = 0.0211$) and 2022 ($F_{1,180} = 21.74$, $p < 0.0001$) cup traps placed 10m away from piles captured 1.5 or 2.4 times as many beetles, respectively, as captured from traps near piles. There was no interaction between location and treatment in either year (2021: $F_{3,234} = 0.92$, $p = 0.4333$; 2022: $F_{5,180} = 0.44$, $p = 0.8226$). Cup trap capture associated with the untreated control (1.7 ± 0.4) caught significantly more sap beetles than in 15% (0.5 ± 0.4) or 25% (0.7 ± 0.4) manure treatments in 2022 ($F_{5,180} = 3.90$, $p = 0.0022$; Figure 1.6). In 2022, the first week of the experiment had the most beetles caught of all weeks ($F_{3,180} = 2.66$, $p = 0.0495$), and there were no interaction between week and treatment ($F_{15,180} = 0.66$, $p = 0.8231$).

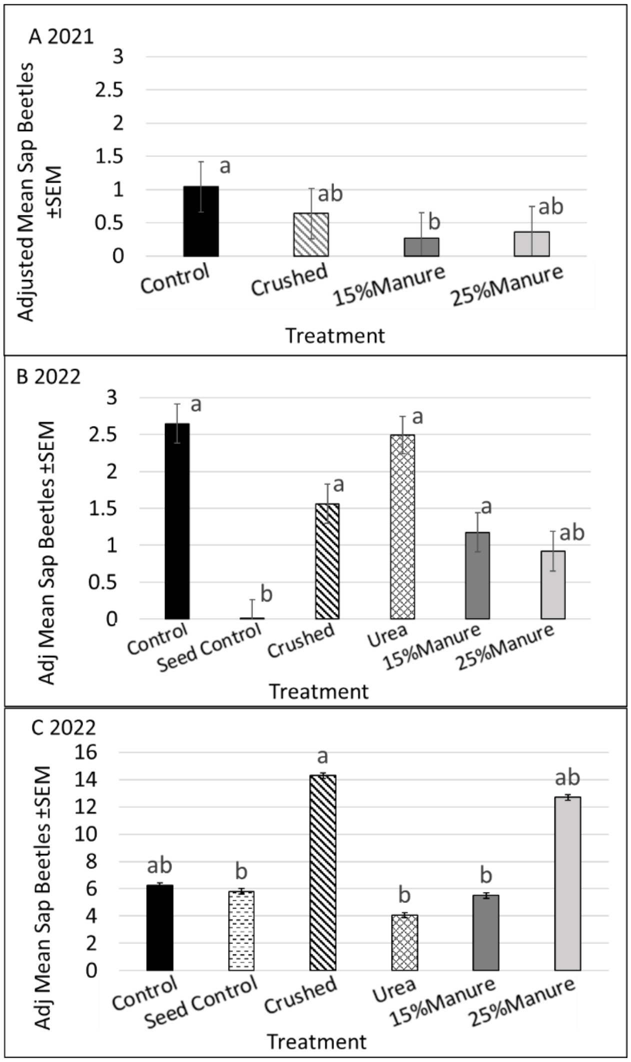

Figure 1.5 A) Mean number of sap beetles (±SEM) per treatment caught via vacuum sampling in 2021. B) Mean number sap beetles (±SEM) caught in vacuum sampling in 2022. C) Mean number of sap beetles (±SEM) caught via sticky card in 2022. Note scale difference for 1.5C. All figures show significance with α=0.05.

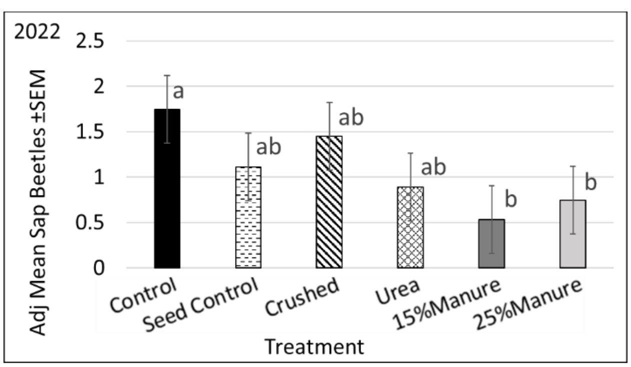

Figure 1.6 Mean number of sap beetles caught (±SEM) in cup traps in 2022, averaged across all three traps per replicate. Significance shown with α=0.05.

Discussion

This research supports previous studies suggesting incorporation of poultry manure or crushing as treatments to decrease *D. suzukii* emergence from post-harvest waste cherries (Hooper and Grieshop 2021, N. Rothwell personal communication). Concentration of 15% manure or 25% manure by volume added to waste cherries in the field were equally good at reducing *D. suzukii* and other non-suzukii drosophila emergence. The mechanisms underlying this reduction are currently unknown but could include direct or indirect fitness effects. High amounts of ammonia and urea have been shown to be toxic to *D. melanogaster* and can affect the number of eggs laid, egg viability, and overall development time, with *D. suzukii* showing greater sensitivity in lab trials (Belloni et al. 2016). Nutrient addition may also alter the microbes present in waste fruit, and microbial communities can influence *D. suzukii* host selection and performance (Bing et al. 2018, Hamby and Becher 2016) and those communities can in turn be influenced by *D. suzukii* diet (Nikolouli et al 2022). For example, yeasts and other microbes have been shown to affect larval development and affect oviposition preference in *D. suzukii* adults (Sato et al. 2021, Bellutti et al. 2018).

We hypothesized that increased nitrogen in poultry manure was responsible for the reduction of *D. suzukii.* To test this hypothesis, we used granulated soluble urea as a synthetic nitrogen source at an amount equivalent to the nitrogen provided by 20% poultry manure by volume. However, incorporation of urea fertilizer did not result in lower *D. suzukii*, other drosophila species, or sap beetles in waste fruit when compared to manure treatments or crushing. Future research should investigate other nitrogen-based fertilizers as a synthetic alternative for manure composting as well as seek to quantify microbial communities throughout fruit decomposition in treated and untreated fruit.

Crushing cherries was found to be as effective at reducing D*rosophila suzukii* and non-suzukii drosophila species as incorporating poultry manure at 15% or 25% by volume. Drosophila emergence from manure treatments was low throughout our experiments, but it took 2 to 3 weeks for crushed treatments to separate from untreated fruit. This delayed effect may be due to the time it took crushed cherries to break down to the point at which they were no longer able to support *Drosophila* spp. larvae. *Drosophila suzukii* pupate at a shallow depth underground similar to many *Tephritidae* spp., which are negatively impacted by dry soil conditions (Ballman et al. 2017, Hulthen and Clarke 2006, Montoya et al. 2008). Previous research in blueberries correlated low humidity with reduced pupal survival, though larvae located inside blueberries were not affected (Rendon et al. 2020). Crushing cherries likely exposes pupae and larvae to desiccation and more efficient predation (Lee et al. 2019). Future research could investigate larval and pupal exposure in crushed fruit and the likelihood of desiccation based on location of waste and local weather. Crushing cherries may provide a method of sanitation which targets multiple stages of *Drosophila spp.* and may be more cost effective than adding fertilizer to fruit waste if appropriate equipment is available on location.

None of the treatments we investigated reduced sap beetle populations. When comparing only between treatments, there is a general pattern over all sampling types and all years, with most sap beetles captured in either the crushed or control treatments and the least sap beetles captured in 15% manure treated piles. However, statistical analysis did not find consistent differences between treatments across sampling types and years (Figure 1.5,1.6). Sampling methods of Nitidulidae within this study are consistent with previously successful methods comprised of vinegar or sugar-based cup traps, yellow sticky traps, and direct samples from rotting fruit (Powell 2015, Williams et al. 1995, Peng and Williams 1991). Sap beetle generation time is roughly four weeks, and 1 to 4 generations can occur during Michigan growing seasons dependent on species (Emekci and Moore 2015). It is possible that insects caught in the first and second weeks of the experiment were present as adults in harvested fruit, while those caught in the fourth and fifth weeks may have been larvae at the time of harvest. Capture data shows that more beetles were caught in cup traps away from waste piles regardless of treatment and week, and our cup traps may have been attracting insects not associated with our waste fruit piles.

Our different sampling methods provided different types of information with respect to all our focal insects. Cup traps were placed directly on waste fruit piles and around piles. These trap captures provide information on insects attracted both to fruit waste and to the traps themselves. Cup trap captures of *D. suzukii* were higher in 15% and 25% manure treatments than in the untreated control, while vacuum samples and sticky traps captures of insects within emergence cages were lower for these treatments. This may suggest that insects are more attracted to the trap lure than the manure treated fruit waste when in close proximity. Commercial cup trap lures which produce similar volatiles to the yeast baited traps used here were shown to attract *D. suzukii* within 93m (Kirkpatrick et al. 2018). Therefore, it is also possible that our cup traps were capturing *D. suzukii* to the general area. With respect to monitoring insects emerging from waste piles within cages, sticky cards provided similar information to vacuum samples and were easier to use and assess.

Nonchemical control tools are critically needed for *D. suzukii.* Resistance to multiple classes of insecticides has already been found in *D. suzukii* and *D. melanogaster* in California and Michigan, respectively (Hubhachen et al. 2022, Gress and Zalom 2019, Ganjisaffar et al. 2022). There is increasing research showing sanitation works to reduce ambient populations of pest insects within agricultural production. The larvae of the navel orange worm, *Amyelois* *transitella* (Walker) (Lepidoptera: Pyralidae) have been shown to use almonds as an overwintering haven in California (Zalom et al. 1984). Similar to *D. suzukii, A. transitella* is not controllable without insecticides, but post-harvest sanitation has been shown to reduce infestation in future season when left over nuts are removed from the field (Higbee and Siegel, 2009, Zalom et al. 1984). When pigs were grazed in cherry plots plum curculio fruit damage was significantly lower and in pear plots codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) and oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) damage was significantly lower following the first year of the study (Buehrer and Grieshop, 2014). Research in raspberries has found that culled fruit should be removed or contained as *D. suzukii* can emerge and infest clean fruit (Leach et al. 2018). Solarization can limit *D. suzukii,* but that method is not practical for large amounts of fruit (Haye et al. 2016, Leach et al. 2018). Fruit waste burial reduces *D. suzukii* emergence, but fruit must be buried 24 cm which is also not practical for substantial amounts of fruit (Hooper and Grieshop 2020).

Our work investigates the treatment of post-harvest waste for instances when sanitation within an orchard is unreasonable or cannot be replicated due to the amount of fruit. The tart cherry market of the United States often requires the disposal of fruit to maintain market values. The United States produced a total of 78 million kilograms of tart cherry in 2021 with a utilized production value of $85.9 million dollars (NASS 2023). When considering fruit held back from market, rejected due to infestation or imperfections, as well as fruit lost during harvest, this leads to a lot of cherries in need of disposal. Of the 78 million kilograms of tart cherries produced in 2021, 136,077 kilograms were not sold (NASS 2023). Fruit waste may be fed to animals, composted, or landfilled, but often disposal consists of piling in unused fields (Esparza et al. 2020). Multiple drosophila species and other pest insects can use unmanaged fruit waste as late season resources for overwintering and create early season pressure the next year (Bal et al 2017).

Post harvest waste treatment may similarly contribute to an integrated management system for *D. suzukii.* Post-harvest management and sanitation have potential benefit for diversified farms growing multiple *D. suzukii* hosts by reducing ambient populations in early season crops and is part of the management actions taken by sweet cherry growers in Switzerland for *D. suzukii* (Schöneberg et al. 2021, Hennig and Mazzi 2018). Whether post-harvest treatment can effectively reduce populations the following year and thereby benefit large scale monoculture planting is less clear. In the northern United States, low winter temperatures cause significant *D. suzukii* mortality (Stockton et al. 2019), but not complete extirpation. In Oregon, Washington, and British Columbia, CA, *Drosophila suzukii* were detected overwintering in all regions and all years from 2010-2014, despite winter temperatures below -17°C (Thistlewood et al. 2018). *D suzukii* has also been shown to use fruit waste including dropped fruits, wild fruit, and fruit compost as a reproductive resource before, during, and after target crop season, but season to season population dynamics of *D. suzukii* remain poorly understood (Lee et al. 2015, Bal et al. 2017, Ballman and Drummond 2017).

All current methods commonly used to manage *D. suzukii* are unable to completely prevent yearly damage or eradicate this invasive pest. Incorporating sanitation of post-harvest waste as part of regular integrated pest management programs can remove reservoirs of off-season reproduction and reduce pressure on susceptible crops later in the season. The results of our work suggest that manure treatments and crushing reduce *D. suzukii* and other Drosophilidae in post-harvest cherry waste. These tactics may be useful for diversified growers with later season *D. suzukii* host in proximity to cherries, but additional research would be necessary to determine if significant benefits from post-harvest treatments are realized in subsequent growing seasons.

CHAPTER 2. NITROGEN BASED FERTILIZER APPLICATION TO LIMIT *DROSOPHILA SUZUKII* INFESTATION OF FRUIT AND FRUIT WASTE

Introduction

Drosophila suzukii is a globally significant invasive pest of soft-skinned fruit crops which spread throughout North American beginning in 2008 through 2012 (Asplen et al. 2015). During its approximately one-month lifespan, a female *D. suzukii* can lay over 400 eggs, with up to 13 generations annually depending on climate conditions (Walsh et al. 2011, Kanzawa 1939). Since its introduction to North America, *D. suzukii* has caused extensive damage to the small and stone fruit industries (Tait et al. 2021). The most common management response to *D. suzukii* has been aggressive pre-harvest application of pesticides, but resistance to the microbial insecticide spinosad and pyrethroid resistance have already been found in populations of *D. suzukii* in California, (Tait et al. 2021, Gress and Zalom 2019, Ganjisaffar et al. 2022). Increasing potential for insecticide resistance necessitates an increase in non-chemical control methods.

While most other drosophilids prefer fermented fruit, *D. suzukii* prefer ripe fruit, likely an ecological shift facilitated by the female *D. suzukii's* enlarged serrated ovipositor which allows it to target both immature and ripe fruit (Atallah et al. 2014). *Drosophila suzukii* is highly polyphagous and it has been documented feeding on over 25 plant-families (Elsensohn and Loeb, 2018). While *D. suzukii* has shown preference for raspberries, blackberries, and other small fruit, it will infest waste of fruits which it does not normally infest pre-harvest (Walsh et al. 2011, Burrack et al. 2013, Abraham et al. 2015, Bal et al. 2017). From 2021 to 2022, 175,350 metric tons of apple pomace and approximately 1.3 million tons of wine pomace were produced in the United States (Jackson et al. 2022, Ferrer-Gallego and Silva, 2022). Research is ongoing for secondary use of wine-pomace, but much post-harvest fruit waste ends up sitting near production sites, in-field, or in landfills worldwide (Bal et al. 2017, Marcos et al. 2023, Spinei and Oroian

2021). This waste can be infested by *D. suzukii* and related species and can provide a host when fresh fruit is no longer available (Bal et al. 2017).

Fertilizers used for other purposes in growing systems may also have applications as control tools for *D. suzukii*. Nitrogen, phosphorus, and potassium are manipulated in growing systems via granular or irrigation applications to enhance growth, crop yield, and even fruit color (Ali et al. 2012, Vargas and Bryla 2015, Prange and DeEll 1997, Jezek et al. 2018). Calcium based field treatments have been used to manipulate fruit firmness (Lee et al. 2016). Calcium nitrate can be used pre-harvest and post-harvest to prevent spoilage of litchi, guava, and Indian gooseberry (Alila and Achumi 2012, Singh and Mandal 2000, Goutam et al. 2010, Azam et al. 2021, Yadav and Singh 2002). Calcium nitrate and potassium nitrate can be used pre-harvest to increase shelf-life of seedless barberry (Hosseini et al 2022). In lab trials, female *D. suzukii* laid fewer eggs and fewer larvae survived in diets containing high concentrations of ammonia and urea when compared to *Drosophila melanogaster* (Belloni et al. 2016) and adding small amounts of high nitrogen chicken manure to apple pomace has been shown to reduce *D. suzukii* survival by 80-100% in lab and small field trials (Hooper and Grieshop 2021).

These experiments were conducted to investigate applying nitrogen-based fertilizers for *D. suzukii* management in fruit pre-harvest and post-harvest fruit waste. The goals of these experiments were 1) to examine if nitrogen-based fertilizers result in similar reductions in egg laying and larval survival as observed for high nitrogen poultry manure when incorporated into fruit waste, and 2) to determine if nitrogen fertilizers could be used as a *D. suzukii* infestation deterrent on fruit prior to harvest. If nitrogen is the main component affecting *D. suzukii* survival in manure treated waste fruit, then it is expected that nitrogen-based fertilizers will limit *D. suzukii* larval and egg survival in fertilizer treated fruits and fruit waste. If nitrogen fertilizers disrupt *D. suzukii* survival, it is possible that its presence will be a deterrent to laying *D. suzukii* females when applied to ripe fruit.

Materials and Methods

A colony of *D. suzukii* was established from flies collected at the Michigan State University Trevor Nichols Research Center (TNRC; Fennville, MI) in 2016. Flies were reared on corn meal diet (Dalton et al. 2011) in 300ml wide mouth glass mason jars (Ball Corporation, Broomfield, CO). The colony was maintained in a growth chamber (Percival Scientific, Perry, IA) set at 21˚C, 70% RH, 16:8 L:D. Flies were anesthetized using CO_2, separated by sex on a FlyStuff FlyPad (Genesee Scientific, San Diego, CA), and held in 50 ml polystyrene vials (Genesee Scientific, San Diego, CA) prior to lab experiments. Following transfer to experimental arenas, flies were monitored until moving about the arena to confirm survival.

Experiment 1- Nitrogen incorporation into fruit waste

Pomace from organically grown apples was obtained from a certified organic orchard and cidery in Flushing, MI. Prior to experiments, apple pomace was frozen at -20ºC for a minimum of one week prior to use to eliminate any previous arthropod infestation. Apple pomace was thawed to room temperature prior to use in experiments. Apple pomace mixed with poultry manure at 20% by volume in previous experiments had 1.5g N/ 250ml pomace, which is used as the ‘100%’ value in this experiment. To assess effects of nitrogen added to infested fruit waste, five soluble nitrogen fertilizers were each dissolved in 10ml deionized water at five different rates: 6%,12.5%,25%,50%, or 100% by volume (Table 2.1). Based on Hooper and Grieshop (2021), incorporation of 25% poultry manure by volume to apple pomace can reduce *D. suzukii* emergence from infested waste by 95%. Fertilizer was diluted to the desired concentration in 10 ml water, and solutions were then incorporated into 250 ml apple pomace over a period of 2 minutes. This volume of pomace was subdivided evenly into five 50 ml replications of each treatment. Granulated organic chicken manure (50 ml) from Herbruck's Poultry Ranch, Inc. (Saranac, MI) was brought to 25% moisture with distilled water before use. Experimental arenas consisted of a 118 ml plastic deli container (Deli-Serve, Chattanooga, TN) capped by a lid with a 2 cm hole, sealed with a foam plug ("flug", Bio-Serv, Flemington, NJ). Arenas were filled with 50 ml of apple pomace with fertilizer incorporated, manure incorporated, or left plain. Each treatment was replicated five times. Ten, seven-day old female *D. suzukii* from mixed-sex colony containers were placed in each arena for 48 h. Arenas were placed in a growth chamber set to the same parameters used for the *D. suzukii* colony and checked daily for fly emergence. Emerging flies were removed using an aspirator, enumerated, and sexed. The experiment concluded when no *D. suzukii* emerged from any container for seven consecutive days.

Table 2.1 Fertilizer sources, analysis, and dilution in 10ml water to achieve 1.5g N (100%).

Fertilizer	Manufacturer	Nitrogen (mg/L)	Phosphorus (mg/L)	Potassium (mg/L)	6.25% (g)	12.5% (g)	25% (g)	50% (g)	100 % (g)
Urea	Easy Peasy Plants (Alvin, IL)	46	0	0	0.20	0.41	0.82	1.63	3.26
Calcium Nitrate	Envy (Chicago, IL)	15.5	0	0	0.60	1.21	2.42	4.84	9.67
Potassium Nitrate	Fireworks Cookbook LLC (Kathleen, GA)	13	0	44	0.45	0.89	1.79	3.57	7.14
Magnesium Nitrate	Greenway Biotech, Inc. (Santa Fe Springs, CA)	11	0	0	0.85	1.70	3.41	6.82	13.63
Ammonium Sulfate	Pure Original Ingredients (Lindon, UT)	21	0	0	0.72	1.44	2.88	5.77	11.54
Poultry Manure	Herbruck's Poultry Ranch, Inc. (Saranac, MI)	4	3	2	--	--	--	--	4.93*

*Manure is measured as 20% by volume of total media (250ml)

Experiment 2- Oviposition following surface nitrogen application on whole fruit

Organically grown blueberries (North Bay Produce, Inc. Traverse City, MI) were put into 3L of deionized water and gently agitated periodically over a period of 4 hours before use to remove pesticide or other residues and then left on paper towel to dry. To assess the effect of a pre-harvest N application on fruit surfaces as opposed to incorporation into post-harvest waste, blueberries were then dipped in solutions containing 1.5 g N per 150 ml from of urea, calcium nitrate, or magnesium nitrate sources (Table 2.2) and swirled for 4 sec to ensure maximum coating. Berries in the control treatment were swirled in plain deionized water.

Table 2.2 Fertilizer grams per solution percentage.

Fertilizer	**Amount of fertilizer (g) per 250 ml, 1.5 g N**	**Amount of fertilizer (g) per 250 ml, 3.0 g N**
Urea	3.26	6.52
Calcium Nitrate	9.67	19.34
Magnesium Nitrate	13.63	27.26

Ten blueberries per treatment were placed in a 15cm petri dish (VWR International LLC, Radnor, PA), and a dish containing fruit with each treatment was placed on the floor of an emergence cage held within a grow tent (2.7mx1.2mx2m, Vivosun, Ontario, CA) at 21˚C 70%RH, 16:8 L:D. Treatment placement was randomized in each cage to avoid position effects. Three-day old female (100) and male (100) *D. suzukii* from mixed sex colony vials were placed within a 9 cm petri dish (VWR International LLC, Radnor, PA) with dampened filter paper (No. 1, qualitative filter paper, GE Healthcare Bio-Sciences, Pittsburgh, PA) and released within the center of the cage. This choice experiment was replicated five times. After 48 hours blueberries were removed and dissected using a stereomicroscope to count eggs and larvae.

A second round of comparisons in which N rates for three fertilizers were doubled to 3 g per 250 ml were conducted following initial results (Table 2.2). Blueberries were treated as in the previous experiment, and treatments for choice assays were arranged in cages following the same methods. Female (60) and male (60) 3-day old *D. suzukii* from mixed sex colony vials were placed in cages using the same methods as for the prior experiment. The experiment was replicated across five cages, which were held under the same conditions as the previous experiment. After 48 h, blueberries were removed and stored in a fridge at 4˚C before dissection under a stereo microscope to count eggs.

Experiment 3- Adult emergence following surface nitrogen application on whole fruit

This experiment was conducted using the same parameters and apparatus as Experiment 2 "*Oviposition following surface nitrogen application on whole fruit*". Fruits were removed from cages following 48 h of exposure to gravid female flies and placed in 473 ml deli containers (Deli-Serve, Chattanooga, TN) with modified lids (Figure 3.1). Containers were held in a plant growth tent (2.7mx1.2mx2m, Vivosun, Ontario, CA) at 21 ˚C 70%RH, 16:8 L:D. Adult *D. suzukii* were removed and counted daily via aspiration until no flies had emerged for seven days.

Statistical Analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Data was analyzed using a mixed model ANOVA via Proc GLIMMIX. Data from Experiment 1 were fitted to a log distribution, and a Satterwaithe adjustment was applied to control for unequal variances. In Experiments 2 and 3 data were fitted to a normal distribution. For all analyses, replicate was treated as a random variable and total number of *D. suzukii* eggs, larvae, or in Experiment 3 adult *D. suzukii,* was the response variable. Pairwise comparisons of treatment means were made using a Tukey-Kramer adjustment with α=0.05.

Results

Experiment 1- Nitrogen incorporation into fruit waste

There was a significant effect of treatment ($F_{25,100}$=9.73, p<0.0001) on the number of emerging *D. suzukii*. No *D. suzukii* emerged from treatments containing calcium nitrate, potassium nitrate, ammonium sulfate, or poultry manure (Table 2.3). Very small numbers of *D. suzukii* emerged from treatments containing magnesium nitrate which was significantly lower than emergence in the untreated control. Urea treatments, except for the 50% rate, had similar *D. suzukii* emergence to the untreated control. The highest urea concentration did not significantly differ from the untreated control.

Table 2.3 Mean emergence of *D. suzukii* (±SEM) from fertilizer treatments at 6.5%,12.5%, 25%, 50%, or 100%; control; or 20% poultry manure by pomace volume. Values indicated by the same letter are not significantly different, α=0.05.

Treatment	***Mean D. suzukii ± SEM***				
Control	8.8 ± 2.3 [a]				
Poultry Manure	0.0 ± 0.0 [c]				
	6.5%	**12.5%**	**25%**	**50%**	**100%**
Ammonium Sulfate	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]
Calcium Nitrate	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]
Magnesium Nitrate	0.2 ± 0.2 [bc]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]
Potassium Nitrate	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]
Urea	6.8 ± 5.1 [ab]	9.0 ± 4.2 [a]	8.4 ± 4.2 [a]	0.4 ± 0.4 [bc]	4.8 ± 1.2 [ab]

Experiment 2- Oviposition following surface nitrogen application on whole fruit

A similar number of *D. suzukii* eggs were laid across all treatments (Figure 2.1; $F_{3,9}$=1.25, p= 0.3486). Doubling nitrogen rate did not change oviposition in treated fruit compared to untreated fruit ($F_{3,12}$=0.6, p=0.625; Figure 2.2)

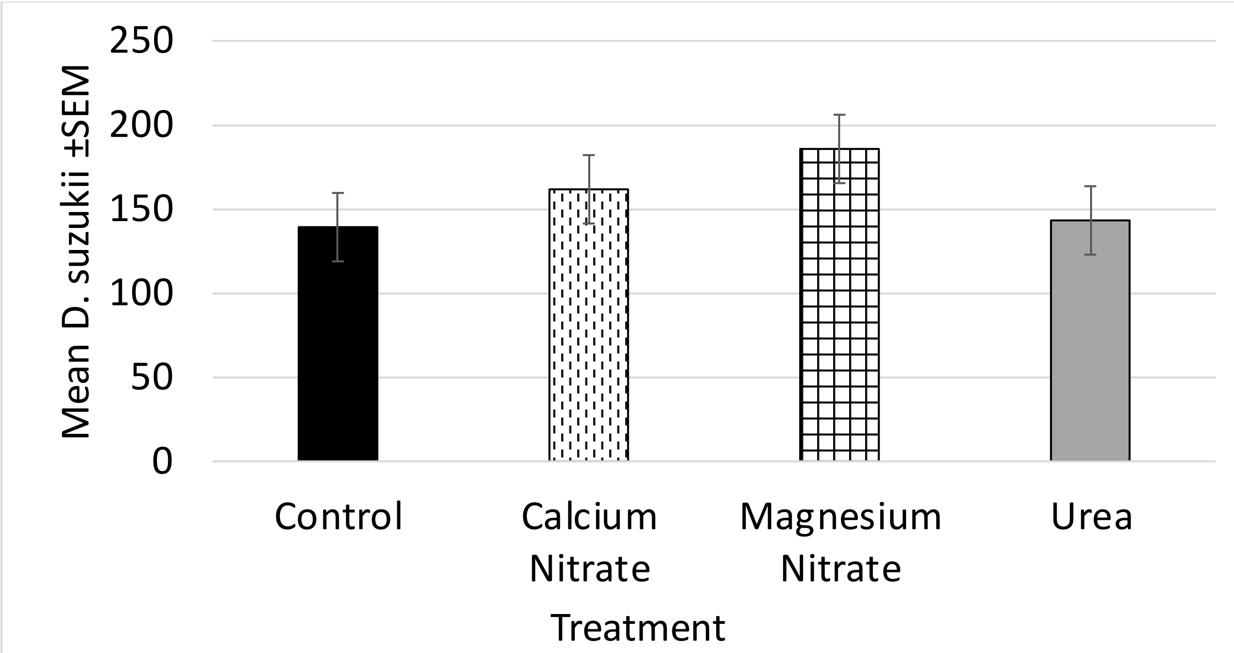

Figure 2.1 Mean number of *D. suzukii* eggs or newly hatched larvae (±SEM) per fertilizer treatment. Values did not significantly differ, α=0.05.

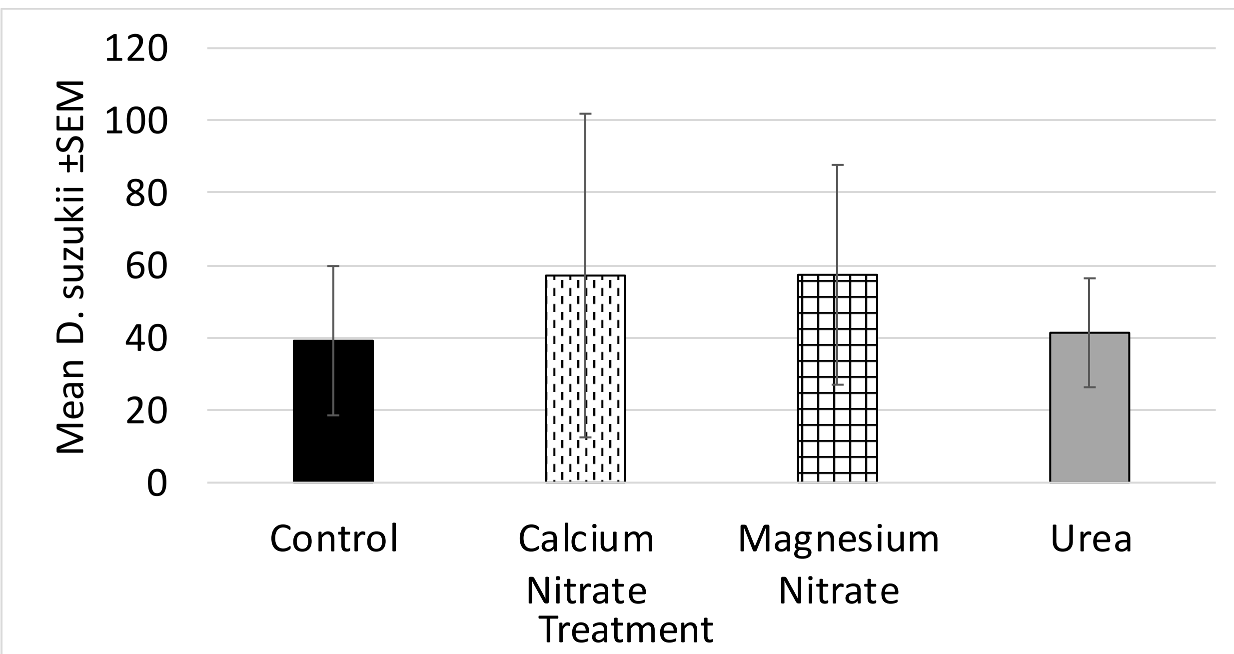

Figure 2.2 Mean number of *D. suzukii* eggs or newly hatched larvae (±SEM) per fertilizer treatment. Values did not significantly differ, α=0.05.

Experiment 3- Adult emergence following surface nitrogen application on whole fruit

Similar to observations in Experiment 2, the number of surviving adult *D. suzukii* did not differ between treatments dipped in different fertilizer solutions ($F_{3,9}$=1.58, p=0.2607; Figure 2.3).

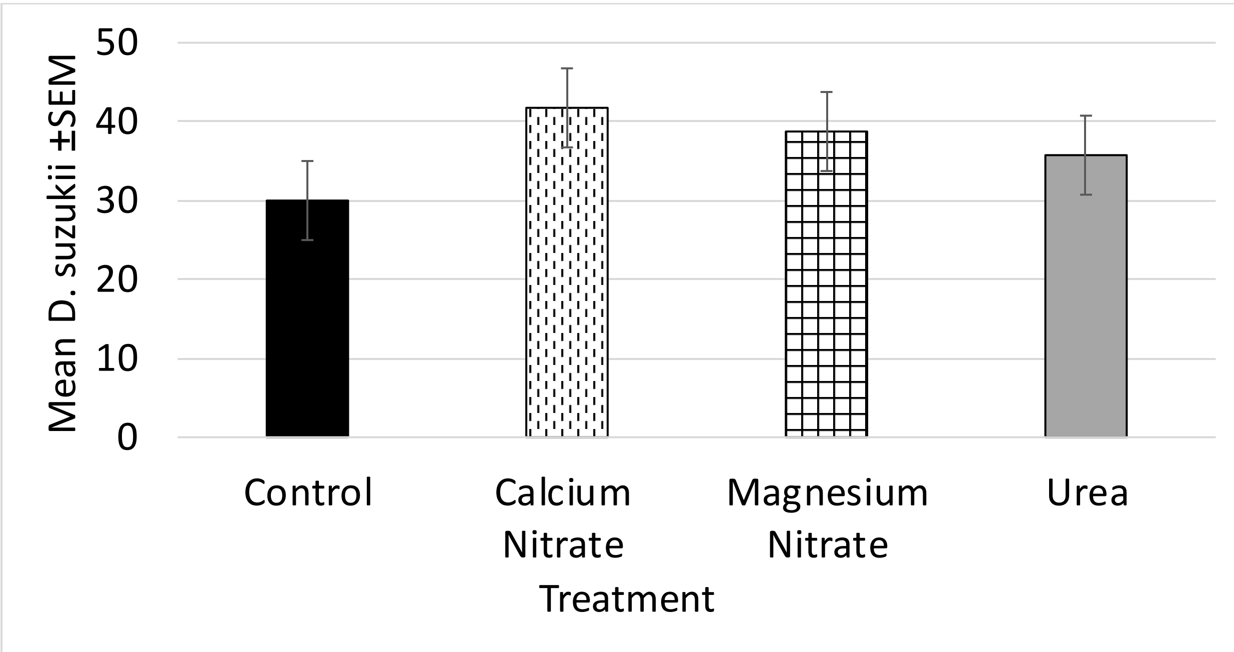

Figure 2.3 Mean number of emerging *D. suzukii* adults (±SEM) per fertilizer treatment. Values did not significantly differ, α=0.05.

DISCUSSION

Our laboratory experiments suggest that nitrogen containing fertilizers may be a useful tool for controlling *D. suzukii* when incorporated in post-harvest waste, but that the fertilizers evaluated do not reduce egg laying or survival of subsequent larvae when applied to whole fruit preharvest. One of the goals of this research was to examine if nitrogen-based fertilizers result in similar reductions in egg laying and larval survival as observed for high nitrogen poultry manure when incorporated into fruit waste. In lab and field trials, adding at least 15% organic poultry manure by volume reduced *D. suzukii* survival in post-harvest fruit waste (Hooper and Grieshop 2021, Chapter 1 this thesis). Ammonia and urea are known to be toxic to drosophilids at high levels and can affect the number of eggs laid, egg viability, and overall development time of *D. suzukii* (Belloni et al. 2016).

When certain nitrogen-based fertilizers (calcium nitrate, potassium nitrate, ammonium sulfate, and magnesium nitrate) were incorporated into apple pomace, there was a notable reduction in *D. suzukii* survival. However, when urea was added to apple pomace, there was no reduction of *D. suzukii* compared to the control at almost all levels of nitrogen evaluated. All fertilizers were added to pomace at rates scaled to nitrogen levels in organic poultry manure. It was expected that if nitrogen is the main component affecting *D. suzukii* reduction in manure treated waste fruit, nitrogen-based fertilizers incorporated with waste fruit should yield similar results. Because all fertilizers were added based on similar rates of nitrogen, but results differed between fertilizers, it can be assumed that nitrogen is not the sole component specifically causing *D. suzukii* reduction within treated fruit waste.

These experiments focused on nitrogen, but other macronutrients were not investigated and could impact management of *D. suzukii.* Phosphorus has been shown to strongly influence oviposition preference which could be utilized in attract-and-kill or push-pull pest management (Cloonan et al. 2018, Olazcuaga et al. 2019). Similarly, *D. suzukii* females invest adult-acquired nitrogen, carbon, and essential amino acids to eggs and somatic tissue (O'Brien et al. 2008). Adding excessive amounts of one macronutrient will alter the overall balance of macronutrients within the diet (Jang and Lee 2018). Further research to investigate addition of other macronutrients and how they might affect *D. suzukii* eggs, larvae, and oviposition is warranted.

This research suggests that fertilizer addition to waste impacted characteristics beyond nitrogen-toxicity. The internal *D. suzukii* microbiome is dependent on its environment and associated microbes can be harmful or beneficial (Bing et al. 2018). Yeasts are essential for *D.*

suzukii larval development and have been found to affect oviposition performance (Bellutti et al. 2018). Female *D. suzukii* can detect and show preference for lay materials based on volatiles, pH, sugar content, and firmness (Olazcuaga et al. 2019, Silva-Soares et al. 2017, Kim et al. 2023). Previous research indicates that microbial volatiles may be used to repel *D. suzukii* from reproductive materials or attract them in attract-and-kill scenarios (Hamby and Becher 2016, Sato et al. 2021, Rering et al. 2023). Tracking microbial change and physical characteristics over time was out of the scope of this research and could be investigated in future research. Similarly, it is likely that whatever differences in characteristics, chemical makeup, or microbiome were made by fertilizer incorporation were not replicated when fertilizers were applied to whole fruit.

A second goal of this research was to determine if nitrogen fertilizers could be used as a *D. suzukii* deterrent on fruit prior to harvest. Fertilizers applied to the surface of whole fruits application did not inhibit *D. suzukii* egg laying or reduce *D. suzukii* survival. It is likely that whatever physiological changes take place when nitrogen fertilizers are incorporated into fruit waste, are not replicated when fertilizers are applied to whole fruit. From this research, it can be concluded that application of these nitrogen-based fertilizers to whole fruit does not effectively manage *D. suzukii* infestation but may provide control when incorporated in post-harvest fruit wastes. Future research investigating if other fertilizers or percentages are suitably deleterious to *D. suzukii* reproduction as well as possible direct affectation to *D. suzukii* eggs and larvae is supported.

CHAPTER 3. SURVIVAL OF *DROSOPHILA SUZUKII* FOLLOWING *HERMETIA ILLUCENS* CO-INFESTATION

Introduction

Drosophila suzukii is an invasive fruit pest which was first reported in Michigan in 2010 (Asplen et al. 2015). In its current North American range three to nine overlapping generations of *D. suzukii* develop annually, creating the potential for exponential population growth (Walsh et al. 2011, Asplen et al. 2015). Fruit found to be infested pre- or post-harvest will be discarded due to a strict zero-tolerance policy of insects in marketed fruit (Van Timmeren and Issacs 2013). Intense and repetitive use of insecticides used for pre-harvest management of *D. suzukii* creates not only an environmental concern but also a concern of increased insecticide resistance (Van Timmeren and Issacs 2013, Fanning et al. 2018, Shaw et al. 2019, Jones 2020). Populations of *D. suzukii* showing resistance to commonly used pyrethroid insecticides and the only organically acceptable active ingredient against *D. suzukii*, spinosyn, have already been found in California (Gress and Zalom 2019, Ganjisaffar et al. 2022). Like many drosophila species, *D. suzukii* will oviposit in decomposing fruit late in the growing season, including fruits with tougher skin and tissue as they breakdown, as well as processing and post-harvest fruit waste (Bal et al. 2017).

In addition to rendering fruit unmarketable, *D. suzukii* infestation can also cause loss from secondary infections of bacterial and fungal pathogens (Molina et al. 1974, Louis et al.1996). Aside from fruit discarded due to insect infestation, fruit waste may also include damaged or overripe fruit discarded during processing and pomace left over from cider, juice, and wine production (Dhillon et al. 2013, Maicas and Mateo 2020, Buzby et al. 2014). Culled fruit and fruit waste provide resources for multiple *Drosophila* species which can lead to further infestation if left in and around orchards and contamination in processing areas (Bal et al 2017, Leach et al 2018). Fruit waste management may consist of piling in unused fields, composting, feeding animals, and landfilling (Esparza et al. 2020). Postharvest sanitation can be implemented to prevent further *D. suzukii* infestation of fruit wastes. Solarization of infested fruit in plastic bags is an option, but this is not feasible for the large quantities of waste typical in packing or processing operations (Haye et al. 2016, Leach et al 2018). Burial can effectively reduce emergence (Hooper and Grieshop 2020) but requires waste to be buried at least 24 cm which is also impractical for large quantities of fruit. Composting and crushing have been investigated with some success but would require extensive labor which may be costly for small operations with large amounts of fruit (Hooper and Grieshop 2021, N. Rothwell personal communication, Chapter 1 this thesis).

Current biocontrol agents for *D. suzukii* consists of a few North American Pteromalidae, and Diapriidae wasp species which parasitize pupae, generalist predators, and a few introduced Asian-native Braconidae and Figitidae wasp species (Lee et al. 2019, Beers et al. 2022, Wang et al. 2020). North American wasp parasitoids have been difficult to rear on *D. suzukii* and do not effectively target *D. suzukii* in situ (Lee et al. 2019, Huang et al. 2023). Generalist predators such as rove beetles, ants, spiders, and earwigs will remove pupated larvae in the orchard floor, but are unlikely to affect the eggs, larvae, and adult *D. suzukii* which infest fruits in the canopy (Woltz and Lee 2017, Gabarra et al 2015, Lee et. al 2019). Asian-native wasps are more specific *D. suzukii* parasitoids but are not currently commercially available within the United States and establishing rearing colonies is time consuming and would likely be cost prohibitive for smaller fruit producers (Seehausen et al. 2022, Fellin et al. 2023).

Hermetia illucens (Linnaeus) (Diptera: Stratiomyidae) is a wasp mimic with worldwide distribution and is active in the southeastern United States (Marshall et al. 2015). Like many soldier flies, *H. illucens* is often associated with decomposing substrates and eggs are typically deposited above or adjacent to decaying matter including fruit, carrion, and manure (James 1935, Tingle et al. 1975, Sheppard et al. 2002). Larvae feed for about two weeks and then become prepupae which can be collected as they leave food materials prior to pupation (Craig Sheppard et al. 1994, Tomberlin et al. 2002, Holmes et al. 2013). *Hermetia illucens* larvae have been used to compost wastes and are considered to be an under-utilized method of waste sanitation (Čičková et al. 2015, Horgan et al. 2023).

Hermetia illucens emerge as adults after about two weeks (Tomberlin et al. 2002). Adults do not bite, sting, or spread diseases (Oliveira et al. 2016). *Hermetia illucens* larvae are able to decompose food waste effectively because of their strong mouthparts and high gut enzymatic activity and have been shown to degrade antibiotics and pesticides during bioconversion (Kim et al. 2011, Tomberlin et al. 2002, Lalander et al. 2016). *Hermetia illucens* are already commonly used for household composting as the rearing process can be replicated inexpensively from home to commercial scale (Devic and Maquart 2015, Craig Sheppard et al. 1994;2002, Diener et al. 2011, Rayar et al. 2020). *Hermetia illucens* have been reared on poultry manure, swine manure, dairy manure, vegetable wastes, and food wastes (Newton et al. 2005, Rehman et al. 2017, Parra Paz et al. 2015, Salomone et al. 2016). Aside from waste management, *H. illucens* presence in media has been shown to limit *Musca domestica* (Linnaeus) (Diptera: Muscidae) reproduction (Sheppard 1983, Furman et al. 1959, Bradley and Sheppard 1984).

We set out to test if *H. illucens* could be used to limit or prevent *D. suzukii* infestation in waste fruit which would present a mode of cultural control that would be more accessible and cost effective than other current sanitation methods. Area-wide management of *D. suzukii* on post-harvest fruit wastes could reduce the total pest population within crop and non-crop areas (Haye et al. 2016). Managing *D. suzukii* infested fruit waste by adding *H. illucens* larvae would not require the addition of insecticides, and therefore may be beneficial to organic growers who have more limited pest control options. To assess the potential of *H. illucens* larvae to reduce *D. suzukii* development in post-harvest fruit waste, we compared a range of infestation scenarios with the expectation that the addition of *H. illucens* larvae to *D. suzukii* infested pomace would reduce emergence of *D. suzukii* adults.

Materials and Methods

Drosophila suzukii used in experiments were obtained from a laboratory colony sourced from the Trevor Nichols Research Center (TNRC) at Michigan State University (Fennville, MI) in 2016 and maintained on a cornmeal-based diet (Dalton et al. 2011) in 50 ml polystyrene vials (Genesee Scientific, San Diego, CA). *Drosophila suzukii* were reared in a growth chamber set at 22˚C, 70% relative humidity (RH), and a photoperiod of 16:8 h (L:D).

For experiments 1, 2, and 3 *H. illucens* were sourced from EVO Conversion Systems, LLC (College station, TX). For experiment 4, *H. illucens* were purchased from DubiaRoaches.com, LLC (Wichita, KS).Apple pomace was collected from a certified organic apple orchard and cidery in Flushing, MI and frozen at -20ºC for a minimum of 48 h prior to kill any arthropods present at the time of collection. Apple pomace was placed within a mesh cage to prevent infestation and brought to room temperature before use.

Experimental arenas used in Experiments 1, 2, and 3 consisted of a 473 ml plastic deli container (Deli-Serve, Chattanooga, TN) with a 23-gauge galvanized steel hardware cloth (Everbilt, Wilmington, DE) bottom to allow for drainage held within a 946 ml plastic deli container (Deli-Serve, Chattanooga, TN). A No. 1 qualitative filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA) was placed on top of the hardware cloth to keep adult *D. suzukii* within the upper portion of the arenas. 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).

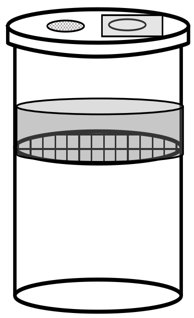

Figure 3.1 Experimental arena consisting of a 473 ml plastic deli container with a wire-mesh bottom set within a 946 ml plastic deli container. The smaller cup is lined with a piece of filter paper, on top of which fruit waste was placed. Arenas were capped by a lid with two 2 cm holes, one covered in fine mesh and the other covered by Parafilm.

Experiment 1- D. suzukii establishment in H. illucens infested substrates

Five infestation timing treatments were compared: simultaneous *D. suzukii* and *H. illucens* infestation, *D. suzukii* infestation 3 d after *H. illucens* infestation, *D. suzukii* infestation 7 d after *H. illucens* infestation, and *D. suzukii* alone. Each infestation timing was compared in both 250 ml (170g) organic apple pomace, or 90% organic apple pomace mixed with 10% organic poultry manure (Herbruck's Poultry Ranch, Inc., Saranac, MI, USA) by volume for a total of ten treatments. Treatments were replicated three times.

For treatments containing *H. illucens,* 3 g of approximately 12-day old (second instar) larvae were added to experimental arenas, and for *D. suzukii* containing treatments, ten seven-day-old presumed mated female flies from mixed sex colony vials were added to arenas. Arenas were held in a growth chamber (Percival Scientific, Perry, IA) at 22˚C, 70% RH, 16:8 L:D, and checked daily for *D. suzukii* emergence. Adult *D. suzukii* were aspirated daily and recorded until no emergence took place for seven days.

Experiment 2- Staggered H. illucens infestation of D. suzukii infested fruit waste

Drosophila suzukii infested apple pomace was generated by placing ten seven-day-old presumed mated females from mixed sex colony vials in cages with the substrate for 48 h, after which all adult *D. suzukii* were removed. Three different *H. illucens* infestation timing treatments were applied to 250 ml of *D. suzukii* infested pomace (Figure 3.2). Three grams of approximately 15- day old (second instar) *H. illucens* larvae (five insects) were added on the same day as *D. suzukii* infestation, three days after *D. suzukii* infestation, or seven days after *D. suzukii* infestation. Two controls consisting of just *H. illucens* or just *D. suzukii* added on the first day of the experiment were also compared for a total of five treatments. All treatments were replicated five times. Treatments were randomly arranged on trays and held within a growth chamber with controls set to 22˚C, 70% relative humidity (RH), and a photoperiod of 16:8 h (L:D). Arenas were checked daily and emerged *D. suzukii* were removed via aspiration daily and recorded, until seven days had passed since the last emergence. In instances where *D. suzukii* pupated outside of experimental arenas, pupae were removed to vials and kept standard to colony rearing methods and monitored for survival. Surviving *D. suzukii* were counted with those removed from arenas.

Experiment 3- Combination of D. suzukii infested fruit waste and H. illucens infested fruit waste

In order to determine if the presence of *H. illucens* larvae repel *D. suzukii* females, if larvae predate *D. suzukii* eggs and larvae, or both, this experiment combined *D. suzukii* infested pomace with pomace containing *H. illucens* larvae, pomace containing *D. suzukii* eggs and larvae, or plain pomace. Organic apple pomace was infested with adult *D. suzukii* for 48 hrs. *D. suzukii* adults were then removed. Infested pomace (250 ml) was added to an additional 250 ml of: (1) *D. suzukii* infested pomace, (2) pomace containing no insects, or (3) pomace infested with 3g of approximately 12-day old (second instar) *H. illucens* larvae (Figure 3.2). Arenas were checked daily until seven days past the last *D. suzukii* eclosion. Emerging *D. suzukii* were removed via aspiration and counted.

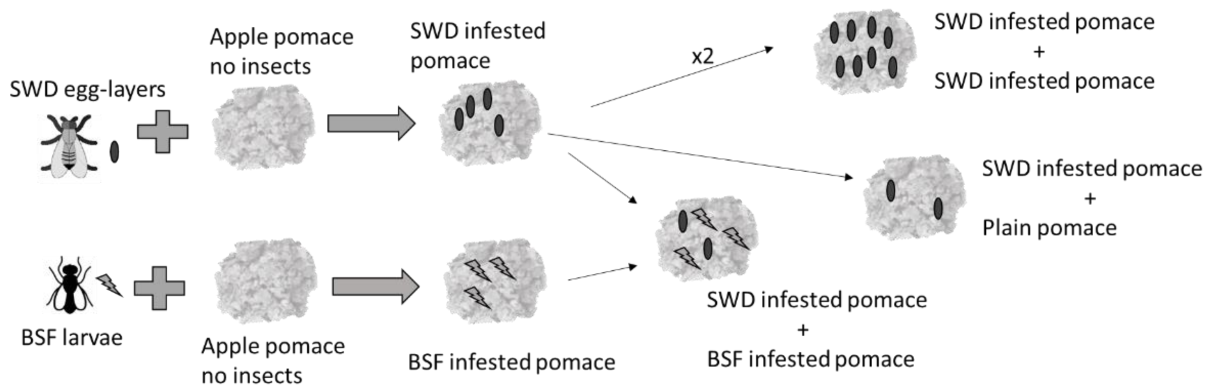

Figure 3.2 Apple pomace was infested with either *D. suzukii* (SWD) or *H. illucens* (BSF). SWD infested pomace was then mixed with either more SWD infested pomace, plain apple pomace, or BSF infested pomace.

Experiment 4- H. illucens infestation rate

This experiment was conducted to compare *D. suzukii* egg and larvae survival when exposed to different rates of *H. illucens* infestation. To do this, we provisioned three different volumes of pomace, 170g (250ml), 340g (500ml), 680g (750ml), into 946 ml plastic deli containers (Deli-Serve, Chattanooga, TN) and then infested with 15, 30, or 45 pre-mated female *D. suzukii* respectively for 48 h. Each container was capped with a corresponding lid with two 2 cm holes, one hole covered with 150-micron polyester mesh (The Cary Company, Addison, IL) to allow for ventilation, and one hole covered with Parafilm (Bemis Company, Inc., Neenah, WI) to allow for aspirator insertion.

Five approximately 8-day old *H. illucens* larvae were added to each experimental arena. Positive controls consisted of each volume of pomace infested with only *D. suzukii.* Negative controls consisted of each volume of pomace with only 5 *H. illucens* added, for a total of nine treatments. Treatments were replicated four times, and there were thirty-six experimental arenas in total. Arenas were placed in a grow tent (2.7mx1.2mx2m Vivosun, Ontario, CA) at 21℃, 70 RH with a photoperiod of 16:8 L:D for a period of three weeks. Following the start of *D. suzukii* emergence, all treatments were checked every other day, and *D. suzukii* were collected via aspiration every two days, frozen, sexed, and counted. The experiment concluded when all *H. illucens* larvae had pupated. *Hermetia illucens* pupae were then removed from all arenas and held to assess survival.

Statistical Analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). In all experiments, a general linear mixed model ANOVA was fitted to a logistic distribution via Proc GLIMMIX. The response variable was emergent *D. suzukii* and replicate was included in models as a random effect. In experiment 2 a Satterwaithe adjustment was applied to control unequal variances. In experiment 4 pomace volume and *H. illucens* infestation were considered fixed effects. In experiment 1, where significant effects were observed, nonparametric test of treatment effects were conducted via Proc NPAR1WAY using the Kruskal-Wallis test. In experiments 2-4, pairwise mean comparisons were conducted using the Tukey-Kramer adjustment with α=0.05 when significant effects were observed.

Results

Experiment 1- D. suzukii establishment in *H. illucens* infested substrates

Treatments were significantly different (X^2 = 22.8, p = 0.0018, df = 7; data not shown), with *D. suzukii* emerging only from the control treatment which did not contain *H. illucens*.

Experiment 2- Staggered H. illucens infestation of D. suzukii infested fruit waste

There were significant differences between treatments ($F_{2,12}$ = 45.14, p = < 0.0001). No *D. suzukii* adults emerged in treatments where *H. illucens* larvae were infested simultaneously with *D. suzukii* (Figure 3.3). When *H. illucens* were added day 7, 4 times more *D. suzukii* (39.4 ± 11.3) emerged than when *H. illucens* were added day 3 (9.8 ± 2.0). In treatments where *H. illucens* were added on day 3, *D. suzukii* were observed to pupate on the lids of arenas. Of the total 29 *D. suzukii* pupae collected from lids across all treatments, 5 survived to adulthood.

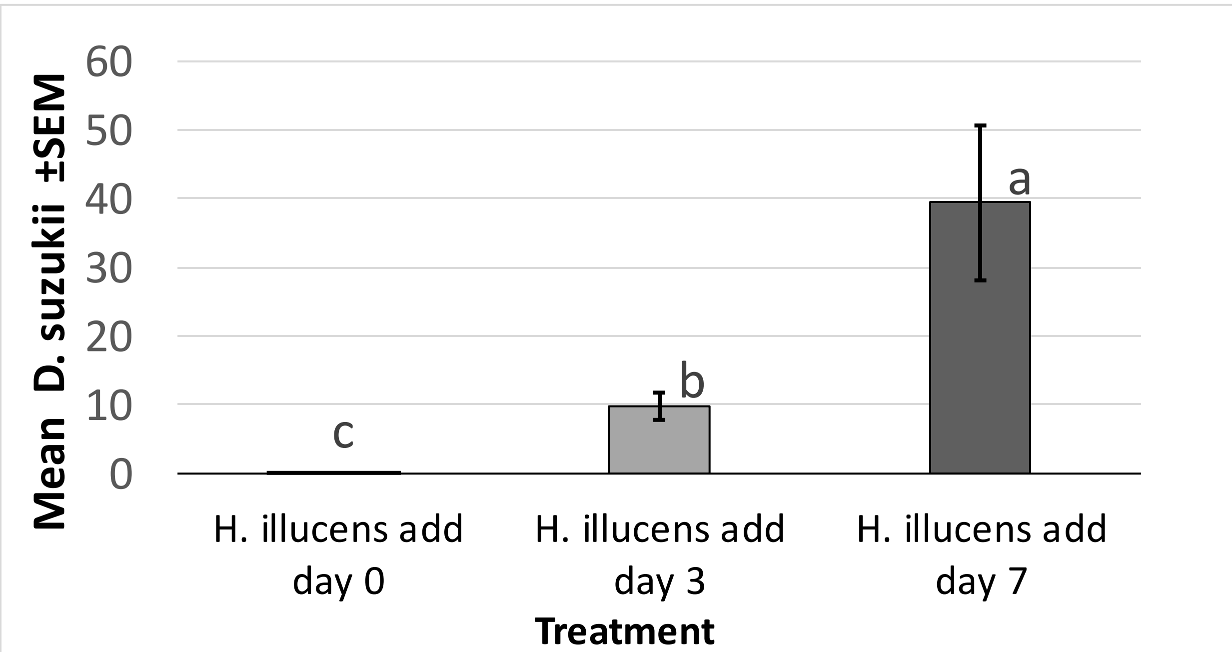

Figure 3.3 Mean *D. suzukii* (±SEM) collected in Experiment *2- Staggered H. illucens infestation of D. suzukii infested fruit waste*. Letters denote a significant difference between α=0.05.

Experiment 3- Combination of D. suzukii infested fruit waste and H. illucens infested fruit waste

When *D. suzukii* infested pomace was added to plain pomace, more *D. suzukii* reached adulthood per replicate (96 ± 14.4) than when *D. suzukii* infested pomace was added to *H. illucens* infested pomace (31 ± 3.6) per replicate ($F_{2,8}$=37.25, p<0.0001). *Hermetia illucens* infestation reduced *D. suzukii* emergence as compared to emergence from *D. suzukii* infested pomace added to either infested or plain pomace. Treatments with no *H. illucens* larvae did not have significantly different numbers of *D. suzukii* emerge (Figure 3.4).

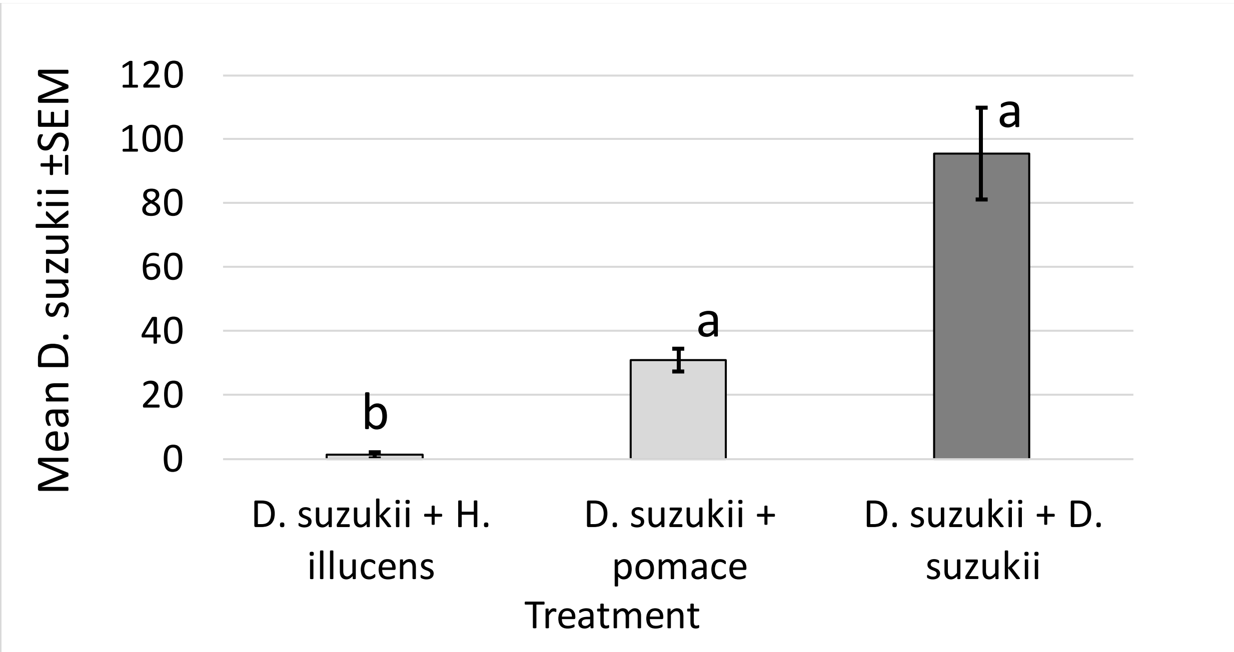

Figure 3.4 Mean emergence *D. suzukii* (±SEM) from each treatment where *D. suzukii* infested pomace was added to plain pomace, more *D. suzukii* infested pomace, or *H. illucens* infested pomace. Letters denote a significant difference between α=0.05.

Experiment 4- H. illucens infestation rate

In contrast with previous experiments, *H. illucens* infestation did not significantly reduce *D. suzukii* emergence regardless of volume of pomace in this experiment. More *D. suzukii* emerged as pomace volume increased ($F_{2,17}$=6.29, p=0.009). Treatments with 250 ml pomace had an average of 222 ± 42.5 adult *D. suzukii* emerge per replicate, 500 ml had an average of 335 ± 42.5 *D. suzukii,* and 750ml had an average of 435 ± 42.5 *D. suzukii* per replicate. The lowest volume of pomace (250ml) had significantly less *D. suzukii* than the highest volume of pomace (750ml).

Discussion

Here I show that adding black soldier fly larvae to apple pomace can reduce infestation by *D. suzukii*. However, the effectiveness of this method at controlling *D. suzukii* numbers depends on the timing of black soldier fly addition relative to *D. suzukii* infestation. When *D. suzukii* adults were added to infest substrate containing *H. illucens* larvae, *D. suzukii* offspring only survived to adulthood in control treatments where no *H. illucens* larvae were present. When *D. suzukii* were allowed to infest apple pomace for different time periods before *H. illucens* larvae addition, *D. suzukii* only emerged in treatments where *H. illucens* larvae were added following *D. suzukii* infestation. In laboratory settings, *D. suzukii* larvae will exit their diet to pupate and in field settings they are known to leave fruit to pupate in the soil to avoid intraspecific competition (Da Silva et al. 2019, Woltz and Lee 2017). When *H. illucens* larvae were added three days after *D. suzukii* infested pomace, *D. suzukii* larvae were observed crowding and pupating on arena lids (Figure 3.1). This atypical pupation behavior has never been observed in research utilizing this experimental design

Competition with closely related insects has been observed to affect *D. suzukii* behavior and performance. In laboratory trials, the presence of other *Drosophila* species in a substrate significantly reduced *D. suzukii* emergence and egg laying (Shaw et al. 2017). Intraspecific competition has been observed in tephritid flies (Averill and Prokopy, 1987; Nufio & Papaj, 2001) and *D. suzukii* females may similarly experience increased pressure to select uninfested hosts (Bezerra Da Silva et al. 2019). While other studies reference *H. illucens* larvae repelling other dipterans, there is little information about the mechanisms behind this behavior. *Hermetia illucens* are known to condition material with their own gut microflora, which might be a source of a deterrent or chemiosignal (Yu et al. 2011). Bradley and Shepard (1984) suggested that *H. illucens* larvae have an allomone which repels other dipterans from lay material. Further research should be completed to determine what signals *H. illucens* infestation creates, if any, to dissuade

D. suzukii females, which may further be exploited for oviposition deterrence and infestation management (Mitsui et al. 2006, Shaw et al. 2017, Tait et al. 2021).

When we added *H. illucens* larvae to substrate previously infested with *D. suzukii*, *D. suzukii* did not survive to adulthood. It is likely that *H. illucens* addition resulted in larval death, perhaps through predation, injury, or by rendering the substrate no longer a suitable food source. Current insects examined for biocontrol of *D. suzukii* rely heavily on parasitism or predation of larvae and pupae in preharvest fruit. As such, those biological control agents may also be exposed to pesticides or other management practices for *D. suzukii.* Incorporating *H. illucens* in post-harvest fruit waste sanitation provides a scenario in which the biocontrol insects are less likely to be exposed to pesticides used against *D. suzukii* (Lee et al. 2019, Tait et al. 2021). The information from these experiments presents another avenue for improved biocontrol using native species against invasive pests.

When *H. illucens* were added to different rates of *D. suzukii* infested pomace, there was no significant difference between treatments. This contrasted with our results from Experiments 1-3 where addition of *H. illucens* larvae were always shown to limit *D. suzukii* reproduction in apple pomace. The treatment which most successfully curtailed *D. suzukii* infestation was that in which *H. illucens* larvae were added shortly after *D. suzukii* infestation. It is possible that in our final experiment, there was no difference found between addition rates of *H. illucens* because *D. suzukii* had already had substantial time to infest the pomace and so were not affected by any microbial or volatile shifts due to *H. illucens*. More research is necessary to investigate optimal timing and density of *H. illucens* infestation as a post-harvest biological control agent.

Although all *H. illucens* larvae in all experiments were of a similar instar based on age in days, those used in our last experiment were observed to be smaller in mass and body size than those used in previous experiments which might have been the cause for resulting discrepancies. Although both sources of insects were reared on a mass rearing diet with similar ingredients, these diets were not identical. It has been suggested that differences in growth rates and bioconversion efficiency of later larval stages can be due time spent on substrate (Horgan et al. 2023, Sheppard et al. 2002*)*.

Post harvest fruit waste infestation with *H. illucens* may provide additional benefits beyond pest control. *Hermetia illucens* have been documented to reduce *Salmonella* spp, *Escherichia coli* O157:H7, antibiotics, and pesticides during bioconversion, and the resulting larvae may be utilized as livestock and other animal food (Erickson et al. 2004, Lalander et al. 2016). *Hermetia illucens* could be utilized in many different *D. suzukii* management systems as *H. illucens larvae* are more cost effective than other biocontrol insects currently available, process multiple waste streams willingly, and do not require the addition of any pesticides or materials restricted in organic production (Lee et. al 2019, Tait et al. 2021). *Hermetia illucens* larvae are readily available in the United States via native wild populations and commercial rearing operations. The addition of *H. illucens* to process fruit waste might also be more cost-effective when compared to cost of materials and/or labor required for solarization, burial, or composting with animal manures (Leach et al 2018, Hooper and Grieshop 2020;2021). Overall, this research supports that *H. illucens* larvae addition can impact the *D. suzukii* infestation process and has the potential to be an inexpensive and accessible addition to management of fruit waste infested with or at risk of infestation by *D. suzukii.*

LITERATURE CITED

Abraham, J., Zhang, A., Angeli, S., Abubeker, S., Michel, C., Feng, Y., Rodriguez-Saona, C., 2015. Behavioral and Antennal Responses of Drosophila suzukii (Diptera: Drosophilidae) to Volatiles from Fruit Extracts. Environmental Entomology 44, 356–367.

Ali, L., Alsanius, B.W., Rosberg, A.K., Svensson, B., Nielsen, T., Olsson, M.E., 2012. Effects of nutrition strategy on the levels of nutrients and bioactive compounds in blackberries. Eur Food Res Technol 234, 33–44.

Alila, P., Achumi, I., 2012. Pre-Harvest Chemical Treatments Affect Post-Harvest Quality of Litchi Fruit. Acta Hortic. 755–761.

Asplen, M.K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.-L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vétek, G., Vogt, H., Walton, V.M., Yu, Y., Zappalà, L., Desneux, N., 2015. Invasion biology of spotted wing Drosophila (Drosophila suzukii): a global perspective and future priorities. J. Pest Sci 88, 469–494.

Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., Kopp, A., 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in Drosophila suzukii and related species. Proceedings of the Royal Society B: Biological Sciences 281, 20132840.

Averill, A.L., Prokopy, R.J., 1987. Intraspecific Competition in the Tephritid Fruit Fly Rhagoletis Pomonella. Ecology 68, 878–886.

Azam, M., Ilahy, R., Shen, J., Qadri, R., Iqbal, M.A., Yasmin, Z., Haider, S.-T.-A., Khan, M.A., Jaskani, M.J., Malik, A.U., Hussain, S., 2021. Exogenous calcium nitrate application delays senescence, enhance nutraceutical properties and antioxidant defense system during storage. Emirates Journal of Food and Agriculture 33(10):852-862.

Bal, H.K., Adams, C., Grieshop, M., 2017. Evaluation of Off-season Potential Breeding Sources for Spotted Wing Drosophila (Drosophila suzukii Matsumura) in Michigan. J. Econ. Entomology 110, 2466–2470.

Ballman, E.S., Collins, J.A., Drummond, F.A., 2017. Pupation Behavior and Predation on Drosophila suzukii (Diptera: Drosophilidae) Pupae in Maine Wild Blueberry Fields. J. Econ. Entomology 110, 2308–2317.

Ballman, E.S., Drummond, F.A., 2017. Infestation of Wild Fruit by Drosophila suzukii Surrounding Maine Wild Blueberry Fields1. JAUE 33, 61–70.

Band, H.,1993. Drosophilidae (Diptera) Collected in Spring in Michigan. The Great Lakes Entomologist 26 (3).

Beers, E.H., Beal, D., Smytheman, P., Abram, P.K., Schmidt-Jeffris, R., Moretti, E., Daane, K.M., Looney, C., Lue, C.-H., Buffington, M., 2022. First records of adventive populations of the parasitoids Ganaspis brasiliensis and Leptopilina japonica in the United States. J. Hymenoptera Research 91, 11–25.

Belloni, V., Galeazzi, A., Bernini, G., Mandrioli, M., Versace, E., Haase, A., 2018. Evolutionary compromises to metabolic toxins: Ammonia and urea tolerance in Drosophila suzukii and Drosophila melanogaster. Physiology & Behavior 191, 146–154.

Bellutti, N., Gallmetzer, A., Innerebner, G., Schmidt, S., Zelger, R., Koschier, E.H., 2018. Dietary yeast affects preference and performance in Drosophila suzukii. J. Pest Sci 91, 651–660.

Bezerra Da Silva, C.S., Park, K.R., Blood, R.A., Walton,V.M.,2019. Intraspecific Competition Affects the Pupation Behavior of Spotted-Wing Drosophila (Drosophila Suzukii). Scientific Reports 9 (1):7775.

Bing, X., Gerlach, J., Loeb, G., Buchon, N., 2018. Nutrient-Dependent Impact of Microbes on Drosophila suzukii Development. mBio 9 (2).

Bolda, M.P., Goodhue, R.E., Zalom, F.G., 2010. Spotted Wing Drosophila: Potential Economic Impact of a Newly Established Pest. Giannini Foundation of Agricultural Economics 4.

Bradley, S.W., Sheppard, D.C., 1984. House fly oviposition inhibition by larvae of Hermetia illucens, the black soldier fly. J. Chem Ecol 10, 853–859.

Brown, S.K., Way, R.D., Terry, D.E.,1989. Sweet and Tart Cherry Varieties: Descriptions and Cultural Recommendations. New York Food and Life Sciences 127.

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

Buzby, J.C., Farah-Wells, H., Hyman, J., 2014. The Estimated Amount, Value, and Calories of Postharvest Food Losses at the Retail and Consumer Levels in the United States. USDA-ERS SSRN Journal 121.

Čičková, H., Newton, G.L., Lacy, R.C., Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Management 35, 68–80.

Cloonan, K.R., Abraham, J., Angeli, S., Syed, Z., Rodriguez-Saona, C., 2018. Advances in the Chemical Ecology of the Spotted Wing Drosophila (Drosophila suzukii) and its Applications. J. Chem Ecol 44, 922–939.

Craig Sheppard, D., Larry Newton, G., Thompson, S.A., Savage, S., 1994. A value added manure management system using the black soldier fly. Bioresource Technology 50, 275–279.

Dhillon, G.S., Kaur, S., Brar, S.K., 2013. Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. Renewable and Sustainable Energy Reviews 27, 789–805.

Diener, S., Zurbrügg, C., Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag Res 27, 603–610.

Elsensohn, J.E., Loeb, G.M., 2018. Non-Crop Host Sampling Yields Insights into Small-Scale Population Dynamics of Drosophila suzukii (Matsumura). Insects 9, 5.

Emekci, M., Moore, D., 2015. Sap Beetles, in: Wakil, W., Romeno Faleiro, J., Miller, T.A. (Eds.), Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges, Sustainability in Plant and Crop Protection. Springer International Publishing, Cham, pp. 205–235.

Erickson, M.C., Islam, M., Sheppard, C., Liao, J., Doyle, M.P., 2004. Reduction of Escherichia coli O157:H7 and Salmonella enterica Serovar Enteritidis in Chicken Manure by Larvae of the Black Soldier Fly. Journal of Food Protection 67, 685–690.

Esparza, I., Jiménez-Moreno, N., Bimbela, F., Ancín-Azpilicueta, C., Gandía, L.M., 2020. Fruit and vegetable waste management: Conventional and emerging approaches. Journal of Environmental Management 265, 110510.

Fanning, P.D., Grieshop, M.J., Isaacs, R., 2018. Efficacy of biopesticides on spotted wing drosophila, Drosophila suzukii Matsumura in fall red raspberries. Journal of Applied Entomology 142, 26–32.

Fellin, L., Grassi, A., Puppato, S., Saddi, A., Anfora, G., Ioriatti, C., Rossi-Stacconi, M.V., 2023. First report on classical biological control releases of the larval parasitoid Ganaspis brasiliensis against Drosophila suzukii in northern Italy. BioControl 68, 1–12.

Ferrer-Gallego, R., Silva, P., 2022. The Wine Industry By-Products: Applications for Food Industry and Health Benefits. Antioxidants 11, 2025.

Furman, D.P., Young, R.D., Catts, Paul.E., 1959. Hermetia illucens (Linnaeus) as a Factor in the Natural Control of Musca domestica Linnaeus. J. Econ. Entomology 52, 917–921.

Gabarra, R., Riudavets, J., Rodríguez, G.A., Pujade-Villar, J., Arnó, J., 2015. Prospects for the biological control of Drosophila suzukii. BioControl 60, 331–339.

Ganjisaffar, F., Demkovich, M.R., Chiu, J.C., Zalom, F.G., 2022. Characterization of Field-Derived Drosophila suzukii (Diptera: Drosophilidae) Resistance to Pyrethroids in California Berry Production. J. Econ. Entomology 115, 1676–1684.

Goutam, M., Dhaliwal, H.S., Mahajan, B.V.C., 2010. Effect of pre-harvest calcium sprays on post-harvest life of winter guava (Psidium guajava L.). J. Food Sci Technol 47, 501–506.

Gress, B.E., Zalom, F.G., 2019. Identification and risk assessment of spinosad resistance in a California population of Drosophila suzukii. Pest Management Science 75, 1270–1276.

Hamby, K.A., Becher, P.G., 2016. Current knowledge of interactions between Drosophila suzukii and microbes, and their potential utility for pest management. J. Pest Sci 89, 621–630.

Haye, T., Girod, P., Cuthbertson, A.G.S., Wang, X.G., Daane, K.M., Hoelmer, K.A., Baroffio, C., Zhang, J.P., Desneux, N., 2016. Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. J. Pest Sci 89, 643–651.

Hennig, E.I., Mazzi, D., 2018. Spotted Wing Drosophila in Sweet Cherry Orchards in Relation to Forest Characteristics, Bycatch, and Resource Availability. Insects 9, 118.

Higbee, B., and J. Siegel. 2009. New Navel Orangeworm Sanitation Standards Could Reduce Almond Damage. California Agriculture 63 (1): 24–28.

Holmes, L.A., Vanlaerhoven, S.L., Tomberlin, J.K., 2013. Substrate Effects on Pupation and Adult Emergence of Hermetia illucens (Diptera: Stratiomyidae). Environmental Entomology 42, 370–374.

Hooper, H., Grieshop, M.J., 2021. Composting susceptible fruit wastes reduces Drosophila suzukii (Diptera: Drosophilidae) reproductive habitat. Pest Management Science 77, 202–207.

Hooper, H., Grieshop, M.J., 2020. Postharvest Burial of Drosophila suzukii (Diptera: Drosophilidae) Infested Fruit Waste Reduces Adult Emergence. Environmental Entomology 49, 59–65.

Horgan, F.G., Launders, M., Mundaca, E.A., Crisol-Martínez, E., 2023. Effects of Intraspecific Competition and Larval Size on Bioconversion of Apple Pomace Inoculated with Black Soldier Fly. Agriculture 13, 452.

Hosseini, A., Moradinezhad, F., Khayyat, M., Aminifard, M., 2022. Bioactive Compounds and Quality Attributes of Fresh Seedless Barberry (Berberis vulgaris L.) at Cold Storage as Influenced by Multiple Spraying of Calcium Nitrate and Potassium Nitrate. Journal of Agricultural Sciences – Sri Lanka 17, 258.

Huang, J., Gut, L., Grieshop, M., 2017. Evaluation of Food-Based Attractants for Drosophila suzukii (Diptera: Drosophilidae). Environmental Entomology 46, 878–884.

Huang, J., Leach, H., Buffington, M., Rothwell, N., Wilson, J.K., 2023. Resident parasitoids associated with Drosophilidae in Michigan tart cherry orchards and woodland edges. Journal of Hymenoptera Research 96, 485–494.

Hubhachen, Z., Pointon, H., Perkins, J.A., Van Timmeren, S., Pittendrigh,B., Isaacs, R., 2022. Resistance to Multiple Insecticide Classes in the Vinegar Fly Drosophila Melanogaster (Diptera: Drosophilidae) in Michigan Vineyards." J. Econ. Entomology 115(6): 2020–28.

Hulthen, A.D., Clarke, A.R., 2006. The Influence of Soil Type and Moisture on Pupal Survival of Bactrocera Tryoni (Froggatt) (Diptera: Tephritidae). Australian Journal of Entomology 45 (1): 16–19.

Jackson, C., Shukla, V., Kolba, N., Agarwal, N., Padilla-Zakour, O.I., Tako, E., 2022. Empire Apple (Malus domestica) Juice, Pomace, and Pulp Modulate Intestinal Functionality, Morphology, and Bacterial Populations In Vivo (Gallus gallus). Nutrients 14, 4955.

Jang, T., Lee, K.P., 2018. Comparing the impacts of macronutrients on life-history traits in larval and adult Drosophila melanogaster: the use of nutritional geometry and chemically defined diets. Journal of Experimental Biology 221, jeb181115.

Jezek, M., Zörb, C., Merkt, N., Geilfus, C.-M., 2018. Anthocyanin Management in Fruits by Fertilization. J. Agric. Food Chem. 66, 753–764.

Joshi, N. K., Demchak, K., Biddinger,D., 2023. Seasonal Population of Drosophila Suzukii (Diptera: Drosophilidae) and Pesticide Use Pattern after Its Invasion in Caneberry Crops in Pennsylvania (USA). Arthropoda 1 (1): 25–34.

Kanzawa, T., 1939. Studies on Drosophila suzukii Mats. Studies on Drosophila suzukii Mats. pp.49

Kienzle, R., Groß, L.B., Caughman, S., Rohlfs, M., 2020. Resource Use by Individual Drosophila Suzukii Reveals a Flexible Preference for Oviposition into Healthy Fruits. Scientific Reports 10 (1): 3132.

Kim, H., Kim, Y., Roh, G.H., Kim, Y.H., 2023. Comparison of Preference for Chemicals Associated with Fruit Fermentation between Drosophila melanogaster and Drosophila suzukii and between Virgin and Mated D. melanogaster. Insects 14, 382.

Kim, W., Bae, S., Park, K., Lee, S., Choi, Y., Han, S., Koh, Y., 2011. Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). Journal of Asia-Pacific Entomology 14, 11–14.

Kirkpatrick, D., Gut, L., Miller, J., 2018. Estimating Monitoring Trap Plume Reach and Trapping Area for *Drosophila suzukii* (Diptera: Drosophilidae) in Michigan Tart Cherry, J. Econ. Entomology, 111, 1285–1289.

Lalander, C., Senecal, J., Gros Calvo, M., Ahrens, L., Josefsson, S., Wiberg, K., Vinnerås, B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. Science of The Total Environment 565, 279–286.

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

Lee, J. C., D. J. Bruck, A. J. Dreves, C. Ioriatti, H. Vogt, and P. Baufeld. 2011. In Focus: spotted wing drosophila, Drosophila suzukii, across perspectives. Pest Manag. Sci. 67: 1349–1351

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

Lee, J.C., Dreves, A.J., Cave, A.M., Kawai, S., Isaacs, R., Miller, J.C., Van Timmeren, S., Bruck, D.J., 2015. Infestation of Wild and Ornamental Noncrop Fruits by Drosophila suzukii (Diptera: Drosophilidae). Annals of the Entomological Society of America 108, 117–129.

Lee, J.C., Wang, X., Daane, K.M., Hoelmer, K.A., Isaacs, R., Sial, A.A., Walton, V.M., 2019. Biological Control of Spotted-Wing Drosophila (Diptera: Drosophilidae)—Current and Pending Tactics. Journal of Integrated Pest Management 10, 13.

Lin, H., Phelan, P.L., 1991. Identification of food volatiles attractive to dusky sap beetle, Carpophilus lugubris (Coleoptera: Nitidulidae). J. Chem Ecol 17, 1273–1286.

Louis, C., Girard, M., Kuhl, G., Lopez-Ferber, M., 1996. Persistance of *Botrytis cinerea* in its vector *Drosophila melanogaster*. Phytopathology 86 (9): 934-939

Loughner, R.L., Loeb, G.M., Demchak, K., Schloemann, S., 2007. Evaluation of Strawberry Sap Beetle (Coleoptera: Nitidulidae) Use of Habitats Surrounding Strawberry Plantings as Food Resources and Overwintering Sites. Environmental Entomology 36, 1059–1065.

Loughner, R.L., Loeb, G.M., Schloemann, S., Demchak, K., 2008. Evaluation of Cultural Practices for Potential to Control Strawberry Sap Beetle (Coleoptera: Nitidulidae). J. Econ. Entomology 101, 850–858.

Maicas, S., Mateo, J.J., 2020. Sustainability of Wine Production. Sustainability 12 (2): 559.

Marcos, J., Carriço, R., Sousa, M.J., Palma, M.L., Pereira, P., Nunes, M.C., Nicolai, M., 2023. Effect of Grape Pomace Flour in Savory Crackers: Technological, Nutritional and Sensory Properties. Foods 12, 1392.

Marshall, S.A.,Woodley, N.E., Hauser, M., 2015. The historical spread of the Black Soldier Fly, Hermetia illucens (L.) (Diptera, Stratiomyidae, Hermetiinae), and its establishment in Canada. Journal of the Entomological Society of Ontario 146, 51-54.

Michigan State University Extension, 2004. Michigan Fruit Management Guide E-154.

Mishra, R., Chiu, J.C., Hua, G., Tawari, N.R., Adang, M.J., Sial, A.A., 2018. High throughput sequencing reveals Drosophila suzukii responses to insecticides. Insect Science 25, 928–945.

Mitsui, H., Takahashi, K.H., Kimura, M.T., 2006. Spatial distributions and clutch sizes of Drosophila species ovipositing on cherry fruits of different stages. Population Ecology 48, 233–237.

Molina, J.J., Harrison, M.D., Brewer, J.W., 1974. Transmission of Erwinia carotovora var. atroseptica by Drosophila melanogaster Meig. I. Acquisition and transmission of the bacterium. American Potato Journal 51, 245–250.

Montoya, P., Flores, S., Toledo, J., 2008. Effect of Rainfall and Soil Moisture on Survival of Adults and Immature Stages of Anastrepha Ludens and A. Obliqua (Diptera: Tephritidae) under Semi-Field Conditions. Florida Entomologist 91 (4): 643–50.

Newton, L., Sheppard, C., Watson, D., Burtle, G., Dove, R., 2005. Using the Black Soldier Fly, Hermetia Illucens, as a Value-Added Tool for the Management of Swine Manure. North Carolina State University.

Nikolouli, Katerina, Hervé Colinet, Christian Stauffer, and Kostas Bourtzis, 2022. How the Mighty Have Adapted: Genetic and Microbiome Changes during Laboratory Adaptation in the Key Pest Drosophila Suzukii. Entomologia Generalis 42.

Nufio, C.R., Papaj, D.R., 2001. Host marking behavior in phytophagous insects and parasitoids. Entomologia Experimentalis et Applicata 99, 273–293.

O’Brien, D.M., Min, K.-J., Larsen, T., Tatar, M., 2008. Use of stable isotopes to examine how dietary restriction extends Drosophila lifespan. Current Biology 18, R155–R156.

Olazcuaga, L., Rode, N.O., Foucaud, J., Facon, B., Ravigné, V., Ausset, A., Leménager, N., Loiseau, A., Gautier, M., Estoup, A., Hufbauer, R.A., 2019. Oviposition Preference and Larval Performance of Drosophila suzukii (Diptera: Drosophilidae), Spotted-Wing Drosophila: Effects of Fruit Identity and Composition. Environmental Entomology 48, 867–881.

Oliveira, F., Doelle, K., List, R., O'Reilly, J.R., 2015. Assessment of Diptera: Stratiomyidae, genus Hermetia illucens (L., 1758) using electron microscopy. Journal of Entomology and Zoology Studies 3(5):147-152.

Paggi, M., Nicholson, C.F., 2013. Federal and State Marketing Orders. US Programs Affecting Food and Agricultural Marketing. Natural Resource Management and Policy 137–169.

Parra Paz, A.S., Carrejo, N.S., Gómez Rodríguez, C.H., 2015. Effects of Larval Density and Feeding Rates on the Bioconversion of Vegetable Waste Using Black Soldier Fly Larvae Hermetia illucens (L.), (Diptera: Stratiomyidae). Waste Biomass Valor 6, 1059–1065.

Peng, C., Williams R.N., 1991. Effect of Trap Design, Trap Height, and Habitat on the Capture of Sap Beetles (Coleoptera: Nitidulidae) Using Whole-Wheat Bread Dough. J. Econ. Entomology 84 (5): 1515–19.

Powell, G.S.,2015. A Checklist of the Sap Beetle (Coleoptera: Nitidulidae) Fauna of Indiana, with Notes on Effective Trapping Methods. *Insecta Mundi.*

Prange, R., DeEll, J., 1997. Preharvest Factors Affecting Postharvest Quality of Berry Crops. HortScience 32, 824–830.

Rehman, K. ur, Cai, M., Xiao, X., Zheng, L., Wang, H., Soomro, A.A., Zhou, Y., Li, W., Yu, Z., Zhang, J., 2017. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (Hermetia illucens L.). Journal of Environmental Management 196, 458–465.

Rendon, D., Hamby, K.A., Arsenault-Benoit, A.L., Taylor, C.M., Evans, R.K., Roubos, C.R., Sial, A.A., et al. 2020. Mulching as a Cultural Control Strategy for Drosophila Suzukii in Blueberry. Pest Management Science 76 (1): 55–66.

Rering, C.C., Quadrel, A., Urbaneja-Bernat, P., Beck, J.J., Ben-Zvi, Y., Khodadadi, F., Aćimović, S.G., Rodriguez-Saona, C., 2023. Blueberries infected with the fungal pathogen Colletotrichum fioriniae release odors that repel Drosophila suzukii. Pest Management Science 79 (12):4906-4920.

Rondon, S.I., Price, J.F., Cantliffe, D.J., 1969. Sap Beetle (Coleoptera: Nitidulidae) Management in Strawberries. University of Florida IFAS Extension 2004.

Sato, A., Tanaka, K.M., Yew, J.Y., Aya Takahashi, A., 2021. Drosophila Suzukii Avoidance of Microbes in Oviposition Choice. Royal Society Open Science 8 (1): 201601.

Schöneberg, T., Lewis, M.T., Burrack, H.J., Grieshop, M., Isaacs, R., Rendon, D., Rogers, M., Rothwell, N., Sial, A.A., Walton, V.M., Hamby, K.A., 2021. Cultural Control of Drosophila suzukii in Small Fruit-Current and Pending Tactics in the U.S. Insects 12, 172.

Seehausen, M.L., Valenti, R., Fontes, J., Meier, M., Marazzi, C., Mazzi, D., Kenis, M., 2022. Large-arena field cage releases of a candidate classical biological control agent for spotted wing drosophila suggest low risk to non-target species. J. Pest Sci 95, 1057–1065.

Shaw, B., Brain, P., Wijnen, H., Fountain, M.T., 2018. Reducing Drosophila suzukii emergence through inter-species competition. Pest Management Science 74, 1466–1471.

Shaw, B., Hemer, S., Cannon, M.F.L., Rogai, F., Fountain, M.T., 2019. Insecticide Control of Drosophila suzukii in Commercial Sweet Cherry Crops under Cladding. Insects 10, 196.

Sheppard, C., 1983. House Fly and Lesser Fly Control Utilizing the Black Soldier Fly in Manure Management Systems for Caged Laying Hens. Environmental Entomology 12, 1439–1442.

Sheppard, D.C., Tomberlin, J.K., Joyce, J.A., Kiser, B.C., Sumner, S.M., n.d. Rearing Methods for the Black Soldier Fly (Diptera: Stratiomyidae). J. Med. Entomology 39, 4.

Silva-Soares, N.F., Nogueira-Alves, A., Beldade, P., Mirth, C.K., 2017. Adaptation to new nutritional environments: larval performance, foraging decisions, and adult oviposition choices in Drosophila suzukii. BMC Ecol 17, 21.

Singh, J.P., Mandal, B.K., 2000. Role of wrappers and post-harvest application of calcium nitrate on the storage behaviour of sub-tropical litchi Cv. Manaraji. Journal of Applied Biology 10, 37–42.

Sirrine, R.J., 2006. Tart Cherry (Prunus Cerasus L.) Production in Northern Michigan: Agroecological and Social Issues. Ph.D., University of California, Santa Cruz.

Souza, Mireli Trombin de, Souza, Michele Trombin de, Rizzato, F.B., Zawadneak, M.A.C., Cuquel, F.L., 2019. Feeding of Lobiopa insularis (Coleoptera: Nitidulidae) on strawberries. Crop Protection 119, 180–184.

Spinei, M., Oroian, M., 2021. The Potential of Grape Pomace Varieties as a Dietary Source of Pectic Substances. Foods 10, 867.

Stockton, D., Wallingford, A., Rendon, D., Fanning, P., Green, C.K., Diepenbrock, L., Ballman, E., et al. 2019. Interactions Between Biotic and Abiotic Factors Affect Survival in Overwintering Drosophila Suzukii (Diptera: Drosophilidae). Environmental Entomology 48 (2): 454–64.

Sturtevant, A.H.,1921. The North American Species of Drosophila. Carnegie Institution of Washington.

Tait, G., Mermer, S., Stockton, D., Lee, J., Avosani, S., Abrieux, A., Anfora, G., Beers, E., Biondi, A., Burrack, H., et al. 2021. Drosophila suzukii (Diptera: Drosophilidae): A Decade of Research Towards a Sustainable Integrated Pest Management Program. J. Econ. Entomology 114, 1950–1974.

Thistlewood, H.M.A., Gill, P., Beers, E.H., Shearer, P.W., Walsh, D.B., Rozema, B.M., Acheampong, S., et al. 2018. Spatial Analysis of Seasonal Dynamics and Overwintering of Drosophila Suzukii (Diptera: Drosophilidae) in the Okanagan-Columbia Basin, 2010–2014. Environmental Entomology 47, (2): 221–32.

Tomberlin, J.K., Sheppard, D.C., Joyce, J.A., 2002. Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomyidae) Reared on Three Artificial Diets. Annals of the Entomological Society of America 95, 379–386.

USDA National Agricultural Statistics Service (dataset). 2023.

Van Timmeren, S., Isaacs, R., 2013. Control of spotted wing drosophila, Drosophila suzukii, by specific insecticides and by conventional and organic crop protection programs. Crop Protection 54, 126–133.

Vargas, O.L., Bryla, D.R., 2015. Growth and Fruit Production of Highbush Blueberry Fertilized with Ammonium Sulfate and Urea Applied by Fertigation or as Granular Fertilizer. Hort. Science 50, 479–485.

Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J., Bruck, D.J., Walton, V.M., O'Neal, S.D., Zalom, F.G., 2011. Drosophila suzukii (Diptera: Drosophilidae): Invasive Pest of Ripening Soft Fruit Expanding its Geographic Range and Damage Potential. J. Integr Pest Manag 2, G1–G7.

Wang, X., Daane, K.M., Hoelmer, K.A., Lee, J.C., 2020. Biological Control of Spotted-Wing Drosophila: An Update on Promising Agents. Drosophila Suzukii Management. Springer International Publishing pp. 143-167.

Williams, R., M. Ellis, G. Keeney. 2017. A Bait Attractant Study of the Nitidulidae (Coleoptera) at Shawnee State Forest in Southern Ohio. The Great Lakes Entomologist 27 (4).

Wilson, J., Gut, L., Rothwell, N., Haas, M., Pochubay, E., Powers, K., Whalon, M., Wise, J., 2019. Managing Spotted Wing Drosophila in Michigan Cherry. Michigan State University Extension.

Woltz, J.M., Lee, J.C., 2017. Pupation behavior and larval and pupal biocontrol of Drosophila suzukii in the field. Biological Control 110, 62–69.

Yadav, V.K., Singh, H.K., 2002. Effect of preharvest application of calcium nitrate, Topsin-M and Bayleton on postharvest life of aonla (Emblica officinalis Gaertn.) fruit. J. Appl. Hort. 4 (2): 83–86.

Zalom F, Weakley C, Hendricks L, Bentley W, Barnett W, Connell J. 1984. Cultural management of the navel orangeworm by winter sanitation. Calif Agr 38(3):28

APPENDIX A. RECORD OF DEPOSITION OF VOUCHER SPECIMENS

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: **2023-09**

Author and Title of thesis: **Charlotte Mae Johanna Schuttler**

CULTURAL AND BIOLOGICAL CONTROL TACTICS FOR SPOTTED-WING DROSOPHILA IN ORCHARD SYSTEMS POST-HARVEST

Museum(s) where deposited:

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

Deposited: October 24, 2023

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Drosophilidae*	Drosophila suzukii	adult	10	pinned
Drosophilidae **	Drosophila suzukii	adult	10	pinned
Nitidulidae		adult	12	pinned
Stratiomyidae	Hermetia illucens	larva	10	alcohol

*Michigan State University Organic Pest Management (OPM) Laboratory Colony

** Michigan State University Tree Fruit Entomology (FTE) Laboratory Colony

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

[signature] 11/28/23

Curator Date

APPENDIX B. CHAPTER 1 SUPPLEMENTARY MATERIAL

Table B.1 GPS coordinates accurate within 3.8m for each experimental replicate per location per year including cherry cultivar. Logged using GPS Logger ver. 3.2.1 (BasicAirData, Paris, FR) and processed with GPX Viewer ver. 1.41.2 (Vectura Games OU, Tallinn, EE).

Location code	Treatment	GPS coordinates	Year	Cherry cultivar
A	Control	45.009864, -85.644006	2021	Montmorency
A	Crushed	45.007657, -85.641816	2021	Montmorency
A	15% Manure	45.008375, -85.642650	2021	Montmorency
A	25% Manure	45.009173, -85.643197	2021	Montmorency
B	Control	44.884993, -85.670074	2021	Emperor Francis
B	Crushed	44.884126, -85.670087	2021	Emperor Francis
B	15% Manure	44.885091, -85.668656	2021	Emperor Francis
B	25% Manure	44.884039, -85.668776	2021	Emperor Francis
BB	Control	44.883220, -85.668487	2021	Emperor Francis
BB	Crushed	44.882486, -85.669206	2021	Emperor Francis
BB	15% Manure	44.883393, -85.669801	2021	Emperor Francis
BB	25% Manure	44.882542, -85.667831	2021	Emperor Francis
C	Control	44.883223, -85.680222	2021	Montmorency
C	Crushed	44.883362, -85.679106	2021	Montmorency
C	15% Manure	44.882482, -85.678079	2021	Montmorency
C	25% Manure	44.882441, -85.680421	2021	Montmorency
D	Control	44.896264, -85.678967	2021	Montmorency
D	Crushed	44.896276, -85.674789	2021	Montmorency
D	15% Manure	44.896295, -85.677473	2021	Montmorency
D	25% Manure	44.896340, -85.676330	2021	Montmorency
G	Control	45.136641, -85.650824	2021	Blushing Gold
G	Crushed	45.135776, -85.650503	2021	Blushing Gold
G	15% Manure	45.138618, -85.651807	2021	Blushing Gold
G	25% Manure	45.137589, -85.651276	2021	Blushing Gold
GG	Control	45.135792, -85.648505	2021	Blushing Gold
GG	Crushed	45.136753, -85.648963	2021	Blushing Gold
GG	15% Manure	45.137838, -85.649377	2021	Blushing Gold
GG	25% Manure	45.140211, -85.650452	2021	Blushing Gold
A	Control	45.009673, -85.643796	2022	Montmorency
A	Crushed	45.010409, -85.644731	2022	Montmorency
A	15% Manure	45.008292, -85.642503	2022	Montmorency
A	25% Manure	45.007595, -85.641761	2022	Montmorency
A	Seed Control	45.009152, -85.643008	2022	Montmorency
A	Urea	45.010927, -85.645219	2022	Montmorency

Table B.1 (cont'd)

Location code	Treatment	GPS coordinates	Year	Cherry cultivar
B	Control	44.882652, -85.669950	2022	Montmorency
B	Crushed	44.885185, -85.670049	2022	Montmorency
B	15% Manure	44.884408, -85.668486	2022	Montmorency
B	25% Manure	44.885130, -85.688784	2022	Montmorency
B	Seed Control	44.882740, -85.667927	2022	Montmorency
B	Urea	44.883648, -85.667954	2022	Montmorency
C	Control	44.885206, -85.678844	2022	Montmorency
C	Crushed	44.885226, -85.680407	2022	Montmorency
C	15% Manure	44.885225, -85.681789	2022	Montmorency
C	25% Manure	44.885205, -85.683614	2022	Montmorency
C	Seed Control	44.884990, -85.684727	2022	Montmorency
C	Urea	44.885207, -85.677429	2022	Montmorency
D	Control	44.896278, -85.678779	2022	Montmorency
D	Crushed	44.896276, -85.676167	2022	Montmorency
D	15% Manure	44.896282, -85.675038	2022	Montmorency
D	25% Manure	44.896202, -85.673941	2022	Montmorency
D	Seed Control	44.896283, -85.680490	2022	Montmorency
D	Urea	44.896260, -85.677485	2022	Montmorency
E	Control	44.882534, -85.680609	2022	Montmorency
E	Crushed	44.881796, -85.680661	2022	Montmorency
E	15% Manure	44.881839, -85.679559	2022	Montmorency
E	25% Manure	44.881957, -85.678771	2022	Montmorency
E	Seed Control	44.883338, -85.680547	2022	Montmorency