

**EXPLORING ECOLOGICAL PARAMETERS OF WINTER MORPH SPOTTED WING
DROSOPHILA, *DROSOPHILA SUZUKII* (MATSUMURA) (DIPTERA:
DROSOPHILIDAE)**

By

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ABSTRACT

EXPLORING ECOLOGICAL PARAMETERS OF WINTER MORPH SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII* (MATSUMURA) (DIPTERA: DROSOPHILIDAE)

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Drosophila suzukii is a destructive vinegar fly that causes hundreds of millions of dollars in economic losses per year. Monitoring traps are an important tool for estimating *D. suzukii* activity. Understanding differences in summer and winter morph *D. suzukii* behavior and response to trap placement could improve trapping programs. I found a 3.5°C difference in temperature where 25% of *D. suzukii* walked for summer and winter morphs when evaluating locomotion temperature thresholds. When evaluating trap position preferences in the laboratory, I found that placement of trap was the determining factor for where winter morph *D. suzukii* were captured and winter morph were 6 times more likely to be captured in a floor trap than an aerial trap. In the field, I found that aerial cup traps were most effective for summer trapping and cup traps placed on the ground were most effective for winter trapping. However, during mid-August I observed significantly more summer morph male flies captured in dome traps placed on or under leaf litter. Additionally, the aerial cup traps were the least selective for *D. suzukii*, capturing 89 times more non-target drosophilids than *D. suzukii*. My results provide insight into the behavioral differences between summer and winter morph *D. suzukii*. Furthermore, my data shows how season influences preferred trap position, and could lead to improved monitoring of *D. suzukii*.

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CHAPTER 1: Literature Review of *Drosophila suzukii*

Economic/agricultural importance

Drosophila suzukii (Matsumura, Diptera: Drosophilidae) or spotted-wing drosophila, is an invasive vinegar fly native to Asia, first observed in 1931 in Japan (Hauser 2011). *Drosophila suzukii* is in a subgroup from *Drosophila melanogaster* (Meigen) group of the subgenus *Sophophora*. They are a pest of berries and soft fruits in North America, South America, Asia, Europe, and Africa (Asplen et al. 2015; Kwadha et al. 2021) (Fig. 1.1) and are estimated to have caused hundreds of millions of dollars in annual economic loss in small fruits (Bolda et al. 2010; Onstad and Crain 2019). For example, Farnsworth et al. (2017) projected the annual economic losses to the California raspberry industry to be \$40 million. What makes this pest so detrimental to agriculture is the female's serrated ovipositor, which allows females to deposit eggs into ripening fruit. In contrast, most other vinegar flies can only lay eggs in overripe or decomposing fruit.



Asplen et al. 2015

Figure 1.1: The geographic distribution of *D. suzukii* in 2015, where confirmed presence is represented by dark grey and unconfirmed or expected presence in light grey. However, recent reports have shown confirmed presence in Africa (Asplen et al. 2015; Kwadha et al. 2021).

Host Range

Drosophila suzukii can infest a wide range of fruits including: raspberries, blackberries, strawberries, blueberries, sweet cherries, tart cherries, peaches, and plums (Cha et al. 2013). Fresh fruit host range has been hypothesized to be limited by skin thickness, for instance, Lee et al. (2011) found in a laboratory study that *D. suzukii* were more likely to lay eggs on ripe, softer fruits compared to growing fruit that had a harder skin. Furthermore, *D. suzukii* eggs were more likely to develop on ripe fruit compared to unripe fruit. Walton et al. (2019) reported similar findings, suggesting that firmness of fruits influenced *D. suzukii*. Thin skinned fruits will have a larger percentage of infested fruit compared to thicker skinned ones (Burrack et al. 2013; Hamby et al. 2016).

Drosophila suzukii also readily reproduces in fruit waste, such as dropped fruits and fruit pomace (Bal et al. 2017). This includes waste from fruits that are not usually associated with *D. suzukii* reproduction (e.g., apples and pears) once the fruit skins have been compromised. Furthermore, Stockton et al. (2019), demonstrated that *D. suzukii* will reproduce on decaying fungal tissue and Schuttler and Grieshop (unpublished) have successfully reared *D. suzukii* on a variety of non-fruit vegetable wastes. With a wide range of host crops and potential breeding resources, this pest can rapidly establish in many habitats. Humans also play a role in the quick establishment of this pest; for instance, when infested fruits are transported across the country for consumption (Calabria et al. 2012).

Geographic Range/Habitat Requirements

Drosophila suzukii was first described in Japan in 1939 (Kanzawa 1939), then found on the island of Oahu, Hawaii in 1980, and in the central coast region of California, USA in 2008 (Hauser 2011). *Drosophila suzukii* rapidly expanded its range by the time writing could be found (Hauser 2011). It was first found in Michigan in 2010.

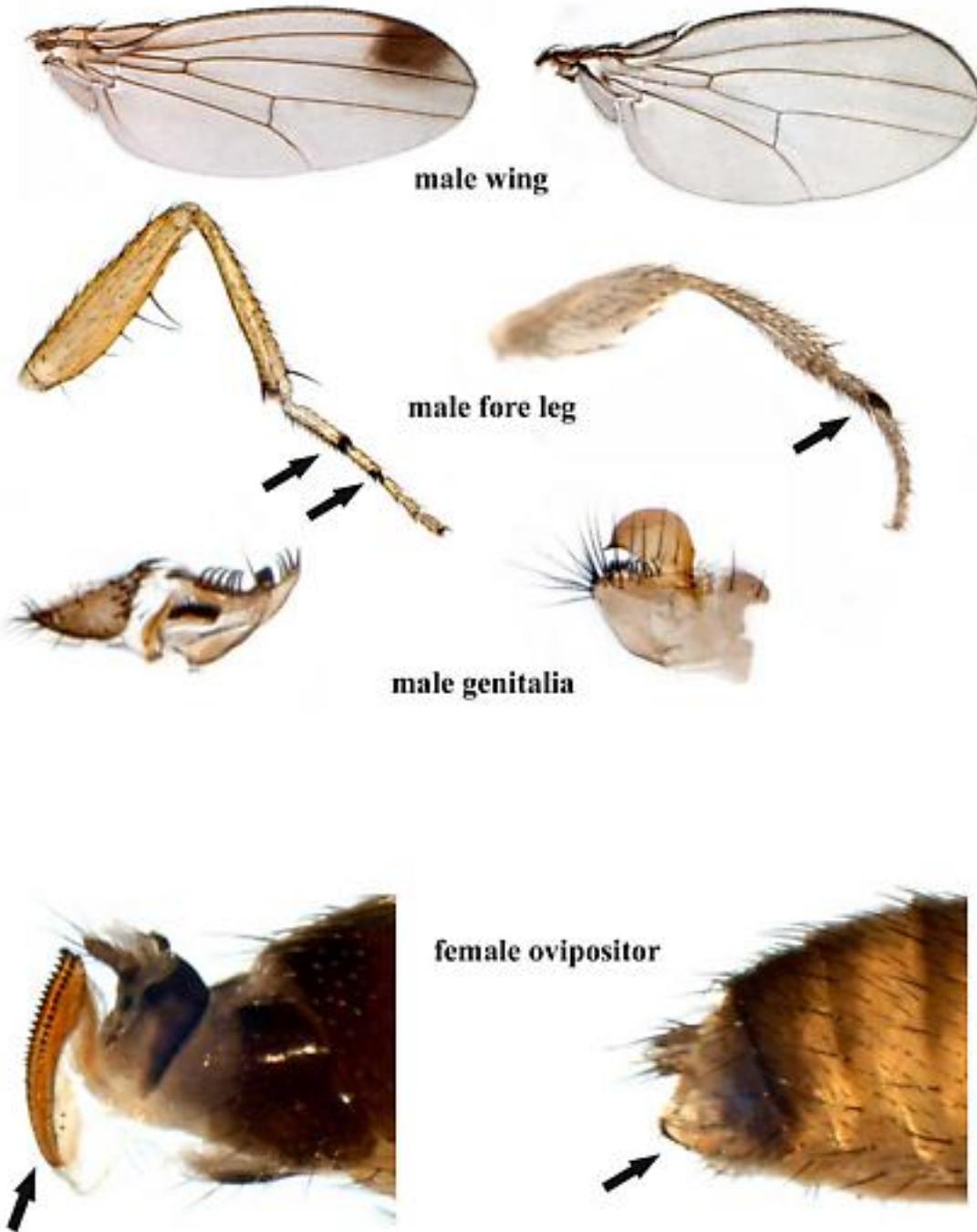
The broad reproductive range of *D. suzukii* means that it can reproduce throughout the summer, when developing fruit is available, as well as during the early spring and summer on decaying material. Thus, it is important to monitor factors that could influence their survival during the winter and early spring. Research suggests that consistent temperatures below freezing (0°C) can have a substantial effect on survival of overwintering *D. suzukii* (Jakobs et al. 2015; Ryan et al. 2016; Stockton et al. 2019). To avoid these lethal temperatures, *D. suzukii* most likely take refuge in various shelters. These shelters could be under leaf litter or snowpacks, inside pomace and fruit

wastes, or structural debris; anything that creates a buffer between the fly and harsh temperatures. Stockton et al. (2019) suggests that *D. suzukii* most likely leave these refuge areas during milder days of winter, when they can tolerate the temperature and search for other resources they can feed and reproduce on. Other factors that may influence survival during extreme cold periods are life stage, morph, and sex of *D. suzukii*.

Morphology and Identification

Drosophila suzukii has a variety of distinctive morphological characteristics that make them easy to differentiate from other *Drosophila spp.* Both sexes are a light yellow/brown color with red eyes. Males have distinctive wing spots that give the insect its common name and two black combs on their forelegs. Females lack wing spots, but have a distinctive bladed ovipositor with two rows of sclerotized teeth and often protrudes from the body when specimens are stored in alcohol (Hauser 2011; Asplen et al. 2015). Dissection of *D. suzukii* genitalia is more dependable for correct identification, with the female's serrated ovipositor typically six to seven times longer than the spermatheca, whereas in other *Drosophila spp.* it is only two to five times longer (Hauser 2011) (Fig. 1.2).

Drosophila suzukii (Matsumura, 1931) *Drosophila simulans* Sturtevant, 1919



Bugguide / Martin Hauser <https://bugguide.net/node/view/336306>

Figure 1.2: *Drosophila suzukii* have several characteristics that make identification simple. Males have a spot on their wings and two black combs on their forelegs. Females have a serrated ovipositor that is much larger compared to other drosophilids.

Life Cycle and Phenology

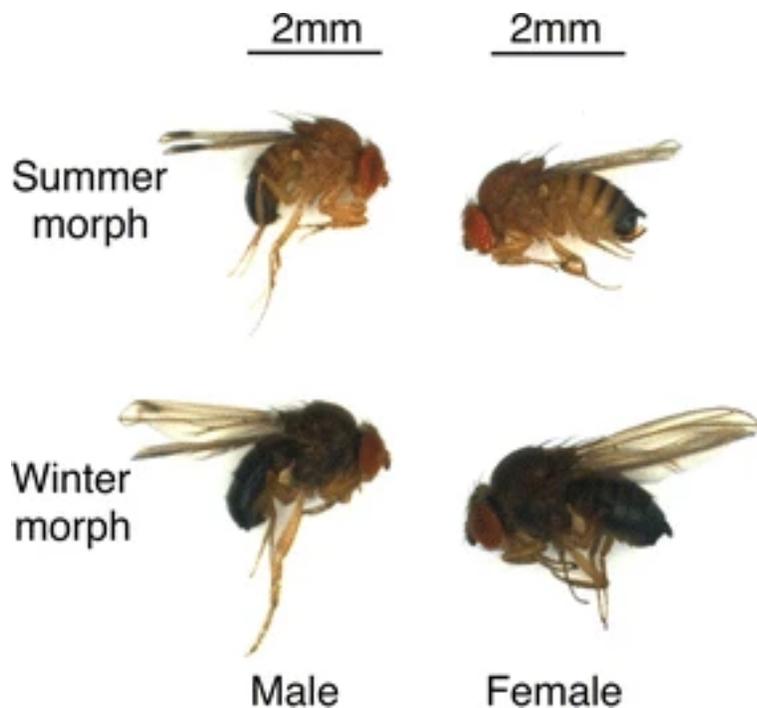
Female *D. suzukii* lay their eggs in ripening fruit, or other host resources, with a single female capable of producing over 300 eggs over her lifetime (Dreves et al. 2014). After emergence, larvae develop through three instars and then pupate. Larva can either develop into pupa inside the host substrate or emerge and pupate on or beneath the soil (Asplen et al. 2015; Woltz and Lee 2017). Following eclosion, female *D. suzukii* undergo a 1-3 day pre-oviposition period after which she can begin laying 25 or more eggs a day under optimal temperatures (Kinjo et al. 2014; Asplen et al. 2015)

Drosophila suzukii have a rapid life cycle, typically ranging from 7-15 days from egg to adult (Walsh et al. 2011). This rapid life cycle results in numerous generations during the growing season which can lead to major fruit losses. While the optimal temperature for reproduction is 22°C, *D. suzukii* can reproduce below and above this threshold with a theoretical temperature range of 14-28°C (Tochen et al. 2014). The mean adult development time is 1050 degree days (DD) (base 5.975°C) or 70 days at 21°C (Asplen et al. 2015). However, development time can vary from 8-79 days depending on the temperature each life stage is exposed to (Lee et al. 2011; Asplen et al. 2015). Using the lower development base threshold of 5.975°C, *D. suzukii* requires 19.025 DD for eggs to develop, 121.76 DD for larva to develop, and 93.22 DD for pupa to develop. Tochen et al. (2014) found that *D. suzukii* summer morphs have a lower threshold of 7.2°C and an upper threshold of 42.1°C, whereas Asplen et al. (2015) reported 5.98°C and 31.5°C for lower and upper thresholds, respectively.

In addition to favorable temperatures, *D. suzukii* females require proteins that are obtained from yeast and other microbes and carbohydrates for successful egg

production and maturity (Tochen et al. 2014; Plantamp et al. 2017). After nutrition, temperature and humidity are the most influential factors for *D. suzukii* population abundance (Guédot et al. 2018). Understanding their physiology, what habitats they utilize, what pheromones are attractive to them, and discovering the best methodology to monitor them will help growers and researchers minimize damage seen on crops.

Due to its distribution in largely temperate climates, where some portion of the year is too cold to support their physiology, *D. suzukii* exhibit a reproductive diapause (Dalton et al. 2011; Jakobs et al. 2015; Stephens et al. 2015; Wallingford et al. 2016; Shearer et al. 2016). This adaptation is coupled with a seasonal dimorphism, known as a “summer morph” and “winter morph.” In addition to exhibiting a reproductive diapause, winter morph flies typically have longer wings, larger bodies and darker pigmentation (Stephens et al. 2015; Jakobs et al. 2015; Shearer et al. 2016) (Fig. 1.3).



(Shearer et al. 2016)

Figure 1.3: *Drosophila suzukii* have a seasonal dimorphism to enhance their survival during cold periods in temperate climates, known as a “summer morph” and “winter morph” (Shearer et al. 2016). Summer morph *D. suzukii* are on the top row and winter morph are on the bottom. Males are on the left and females are on the right.

Winter Morphs and Reproductive Diapause

Developing *D. suzukii* larvae exhibit adult winter morph characteristics when they experience temperatures below 10°C and a 12 hour, or less, light photoperiod (Toxopeus et al. 2016; Shearer et al. 2016; Grassi et al. 2018; Enriquez and Colinet 2019). While overwintering, these winter morph *D. suzukii* will suppress ovarian development (Terhzaz et al. 2018; Rendon et al. 2018), resulting in reproductive diapause, increase energy reserves by making metabolic adjustments (Dalton et al. 2011; Alford et al. 2019; Enriquez and Colinet 2019), and are more resistant to desiccation (Rajpurohit et al. 2008; Ramniwas et al. 2013; Fanning et al. 2019). Winter morph *D. suzukii* in reproductive diapause can restart after 59 DD (Grassi et al.

2018). They also found that female *D. suzukii* can lay eggs after 3 DD at base 7.2°C. Finally, Stockton et al. (2019) found that winter morph *D. suzukii* can survive short intervals of temperatures as low as -7.5°C. Little is known about where winter morph *D. suzukii* overwinter and trapping studies during the winter are usually unsuccessful with extremely low catch (Harris et al. 2014; Pelton et al. 2016; Rossi-Stacconi et al. 2016; Wallingford et al. 2018).

Previous research has examined fecundity, cold tolerance, desiccation resistance, and survival rates between summer and winter morph *D. suzukii*. Shearer et al. (2016) evaluated gene expression of these morphs and found that winter morph *D. suzukii* have an upregulation of carbohydrates and alter ion transportation to enhance their survival in cold temperatures. They also provided the first molecular evidence of reproductive diapause in winter morphs, finding a decrease in the transcripts used for oogenesis and DNA replication.

Winter morph *D. suzukii* can suppress ovarian development and have increased cold tolerance compared to summer morph (Toxopeus et al. 2016). 80% of winter morph *D. suzukii* didn't enter a chill coma until -5.5°C, compared to summer morph at -0.5°C. They also reported that it could take up to 30 days for winter morph females to reach full ovarian maturity when reared at cooler temperatures. Fanning et al. (2019) found winter morphs survived longer in drier conditions compared to summer morph flies, suggesting that winter morph *D. suzukii* are better adapted to retaining water content.

While an increasing amount of research has been conducted on differences in fecundity and environmental tolerances between summer and winter morph *D. suzukii*,

comparatively little attention has been paid to behavioral differences between these two phenotypes. Given differences in minimum thermal tolerances between the two phenotypes, it would be useful to know whether these differences extend to both ground and aerial locomotion. Understanding the relative likelihood of movement of the two phenotypes may expand our ability to interpret monitoring efforts for the two morphs.

Pest Management

Integrated Pest Management (IPM) of *D. suzukii* has been difficult due to the pest's broad host range, damage characteristics, and rapid life cycle. In temperate climates such as the Great Lakes Region, *D. suzukii* populations rapidly increase in mid-summer (late July at the time of writing), at which point grower's only option is to prevent adults from ovipositing in fruit. This is most commonly accomplished through the repeated applications of broad spectrum, contact insecticides. In some cases, flies can be kept from fruit with protective structures (e.g., screen and plastic coverings). Biological and cultural control tactics have also been researched, but with limited success.

Chemical control of *D. suzukii* relies on numerous insecticides from the organophosphate, pyrethroid, spinosyn, and neonicotinoid classes (Van Timmeren and Isaacs 2013; Haye et al. 2016). *Drosophila suzukii* is a zero-tolerance pest, meaning growers often make weekly applications to maintain control, resulting in numerous insecticide sprays to host crops (Timmeren et al. 2018). With frequent insecticide sprays, growers and researchers need to closely monitor populations for signs of insecticide resistance. Gress and Zalom (2019) demonstrated that *D. suzukii* collected from two orchards in California, USA had LC50 values 4.3-7.7 times higher in resistance

to spinosad, a common insecticide used to manage *D. suzukii*. In Michigan, USA, researchers found a significant increase in LC50 and LC90 values for the insecticide spinetoram over three years (Timmeren et al. 2019). Resistance ratios for spinetoram were up to 4.1. However, an evaluation of resistance from Northern Italy populations found that *D. suzukii* had LC50 values 12.7-21.0 and 3.4-5.8 times higher for deltamethrin and cyantraniliprole treatments, respectively, compared to control populations (Civolani et al. 2021). Spinosad was effective for managing both treated and untreated populations.

Rotating classes of insecticides can help reduce the risk of insecticide resistance. Timmeren et al. (2018) suggested that growers should immediately stop insecticide sprays after harvest, allowing late season *D. suzukii* to reproduce. Late season reproduction should mitigate resistant alleles from persisting in populations. Additionally, it has been suggested that having unmanaged areas surrounding orchards can also help maintain susceptibility of *D. suzukii* (Timmeren et al. 2019). Unmanaged areas have populations that potentially haven't been exposed to any insecticides, thus having exposed individuals mating with unexposed helps mitigate resistant alleles.

Biological control of *D. suzukii* has been attempted with living biopesticides and Entomopathogenic Nematodes (EPN), and efforts have been made to identify parasitoids and predators. Recently, Lee et al. (2019) reviewed current and pending tactics of biological control for *D. suzukii*. For a fungal pathogen to be effective, it needs to not only affect mortality with direct exposure, but indirect contact as well (Lee et al. 2019). Two fungal strains of *Beauveria bassiana* are commonly used and have demonstrated residual effects on *D. suzukii* (Woltz et al. 2015). For nematodes to be an

effective tool of control, they must be released in large amounts, but studies have been in laboratory settings only. All species of nematodes tested had low infection rates on *D. suzukii* larvae with infection reduced further on pupating individuals. Natural predators are another tool for controlling pest populations. *Drosophila suzukii* have several known predators: earwigs (Dermaptera:Forficulidae), damsel bugs (Hemiptera:Nabidae), spiders (Araneae), ants (Hymenoptera), and pirate bugs (Hemiptera:Anthocoridae), with up to 40% of the collected specimens containing *D. suzukii* DNA in their guts (Wolf et al. 2018). Finally, parasitoids are another option for biological control. *Trichopria drosophila* and *Pachycrepoideus vindemmiae* are common parasitoids of *D. suzukii* (Wang et al. 2018), but they also attack other drosophilids and only result in less than 10% of parasitism in *D. suzukii* (Lee et al. 2019).

Cultural and physical control of *D. suzukii* includes pruning, the use of weed mats to intercept pupating flies, and management of crop loads and fruit wastes (sanitation). These are methods that can be used in place of insecticides or to reduce application of sprays. Schöneberg et al. (2021) reviewed current and pending tactics for control of *D. suzukii*. They suggested that growers should plant crop cultivars that differ in time to ripen, the fruits physical and chemical properties (firmness, texture, volatile organic compounds), and pH to help mitigate *D. suzukii* damage. Another method is using fine mesh to cover crops; however, the timing must be planned out for it to be an effective tool. Population levels need to be known as well for determining how effective net coverage can be. Leach et al. (2016) found that exclusion netting with insecticide sprays on raspberries had the biggest impact on *D. suzukii* populations. The combined methods delayed the first observed larval infestation by 10 days, and reduced the total

number of eggs, larvae, and adults in fruits up to 5 times. They also reported that exclusion netting did not affect raspberry size or quality.

Other approaches for managing *D. suzukii* are growers altering the crop microclimate, to make the crop environment unfavorable for *D. suzukii* (Schöneberg et al. 2021). For example, pruning, trellising, mulching, and irrigation are effective tools for changing the microclimate. Rendon et al. (2019) reported that weed mats prevent *D. suzukii* larva from reaching an optimal underground habitat for pupation. Harvest management, like removing dropped fruits, fruit wastes, and sanitation practices help minimize surrounding habitats that *D. suzukii* could utilize. Leach et al. (2018) demonstrated that harvesting fruit every 1-2 days significantly reduced the number of *D. suzukii* larvae compared to harvesting every 3 days. Sanitation practices, like clearing leaf litter can also be beneficial (Haye et al. 2016). This takes away potential overwintering habitats for *D. suzukii* and fallen leaves can provide a barrier from the harsh temperatures. Furthermore, burying infested fruit 24cm after harvest can decrease *D. suzukii* emergence by 97% (Hooper and Grieshop 2020a; 2020b). As overwintering *D. suzukii* have been found to be the source for upcoming season's first infestation, it is vital to remove any habitats *D. suzukii* can use during the off season (Panel et al. 2018).

While *D. suzukii* management is largely dependent on the frequent application of broad-spectrum insecticides, using multiple strategies to contain a pest is preferred. Integration across these strategies is most feasible if growers have some understanding of local *D. suzukii* densities, thus monitoring for *D. suzukii* is extremely important.

Monitoring *D. suzukii*

Monitoring programs, including the use of insect traps, are one of the foundations of IPM. Monitoring allows pest managers to know when and where to apply insecticides to achieve optimal effect. Insect traps range from pitfall traps, sticky cards, toxic apparatuses, pheromone/lure traps, and more (Epsky et al. 2005; Flint 2012; Miller et al. 2015). Traps can provide information on what insect populations are present in the area as well as how their populations fluctuate over time. An effective trap should primarily capture the target pest, with little bycatch.

Development of a cost effective and reliable monitoring trap for *D. suzukii* continues to be an important goal. Lee et al. (2012) compared seven different trap types in nine fruit crops. Each trap type varied in volume/area, number of entry holes, type of entry holes (open or mesh), and rain tent coverings. Selectivity of traps was observed, and they found that none of the traps were selective to *Drosophila* species, only ~30% of the *Drosophilids* caught were *D. suzukii*. The Haviland trap, a Rubbermaid container with a mesh lid, entry holes on the side, and a rain tent covering the trap, caught the most *D. suzukii*. Haviland trap catch was positively correlated with the number of mesh openings on the trap body.

Over a two-year study, Iglesias et al. (2014) compared a clear 0.95L cup trap with 10 0.64cm holes near the upper rim, to other cup traps that had sticky cards within the cup trap, and to a yellow sticky card. In the first year all traps were baited with apple cider vinegar, however for the second year they used a yeast and sugar bait and removed the sticky card in an attempt to improve captures. After the two-year study, they found there were no differences in *D. suzukii* capture between any of the apple

cider vinegar baited traps, but the yeast and sugar baited trap had the highest capture. Overall, the yeast and sugar baits captured more nontarget drosophilids and *D. suzukii* than any of the vinegar baited traps. Similarly, researchers in California, USA, found when monitoring *D. suzukii* in raspberry plots, the yeast sugar bait captured more flies in the fall harvest compared to the apple cider vinegar bait (Hamby et al. 2014). They also discovered that both baits may be more attractive to female *D. suzukii*, as they captured more females than males while monitoring. Harris et al. (2014) went on to evaluate how season and plant type influenced *D. suzukii* capture. They found that deciduous fruit plants had high trap capture from the spring to midsummer and in the fall. Whereas evergreen citrus plants only had high trap capture during the winter season. They concluded that fruit availability did not play a role in trap capture, as deciduous and evergreen plants had high trap captures when fruit was available or absent. This data suggests that season, or temperature, plays an important role in where *D. suzukii* will be captured.

Visual stimuli have also been evaluated for *D. suzukii* trapping. In laboratory assays, Kirkpatrick et al. (2016) evaluated odorless sticky disks in multiple colors. Red, purple, and black colored disks had the highest capture of *D. suzukii* when against a white background. They determined that fluorescent red was the best trap color, and white or clear traps performed the lowest. In a black background, all non-fluorescent colors had equal performance, suggesting background color is important for choosing an optimal trap for monitoring. Kirkpatrick et al. (2017) demonstrated that traps with visual and olfactory cues combined captured more *D. suzukii* than other traps. In a field experiment, colored sticky spheres were compared to the clear deli cup trap, and they

discovered red and black spheres captured more *D. suzukii* compared to white spheres. The red sphere with a Scentry lure captured 3-6 times more flies than the clear deli cup trap with the Scentry lure as well. These findings were supported by another field experiment, in cherry orchards and raspberry high tunnels (Kirkpatrick et al. 2018). They found once again that the sticky red sphere trap with the same lure in a clear deli cup trap, consistently captured more *D. suzukii* than the deli cup trap. They also noted that dry sticky traps are more cost effective since there is less labor associated with changing the drowning solution in a deli cup trap.

Overview of *D. suzukii* lures

The development of effective olfactory lures for *D. suzukii* has been widely researched. One group, Landolt et al. (2012) compared the efficacy of single to multiple component lures for trapping *D. suzukii*. They found when comparing vinegar, wine, acetic acid and ethanol, combinations between vinegar and wine and acetic acid and ethanol captured more flies together than any of the baits did alone. Cha et al. (2013) went on to evaluate a four-component lure (made from acetic acid, ethanol, acetoin and methionol) and fermented food baits to compare attractiveness for *D. suzukii* in the United States and Germany. They also compared the performance of two trap types, a dome trap and various cup traps, and how nontarget capture varied between traps and baits. Cha et al. found that the four-component chemical lure was the most attractive bait and caught the most *D. suzukii* for both sexes, capturing over three times more than other baits. They also found that traps that had a wider opening, like the dome traps, captured more *D. suzukii* compared to traps that had smaller openings, like the cup traps.

Iglesias et al. (2014) not only evaluated trap design, but also how bait type and age of bait influenced capture of *D. suzukii*. They compared the attraction of variations of yeast and vinegar baits and observed how long baits were effective in the field before needing replacement. The yeast baits were the most attractive to *D. suzukii* compared to the vinegar baits. However, it was noted that although yeast baits had the highest capture, the ingredients also made it harder to identify *D. suzukii* in the cloudy mixture. They also concluded that when the bait was over 14 days old it greatly decreased the capture of both *D. suzukii* and non-target insects, likely due to changes in the olfactory cues produced as yeast colonies began to decay. As researchers evaluate bait preference for summer morph *D. suzukii*, physiological and behavioral differences should also be observed for winter morph *D. suzukii* as well. Kirkpatrick et al. (2018) demonstrated that winter morph had lower antennal responses when exposed to several volatiles. Furthermore, summer morphs strongly avoided geosmin, and winter morphs had no preference. Summer morph *D. suzukii* females had increased olfactory responses compared to winter morph females.

Karageorgi et al. (2017) determined that *D. suzukii* prefer ripe fruit for oviposition and rotten fruit for feeding. Wong et al. (2018) found similar results, stating fruit-based lures would be more attractive for gravid females. Additionally, Jaffe et al. (2018) demonstrated that baits with a single component lure were more attractive to females ready to lay eggs, compared to a multi-component lure.

Bait preference was compared in raspberry and cherry fields within the USA and Europe (Larson et al. 2021). Previous research suggested using a quinary chemical component blend for effective *D. suzukii* monitoring. Researchers compared the quinary

blend to apple cider vinegar bait. The quinary blend captured the first *D. suzukii* before the apple cider vinegar trap and found the blend to be more selective for *D. suzukii*. Higher selectivity makes analyzing trap capture quicker and easier when the trap isn't full of other non-target drosophilids.

Overview of Placement

The placement, i.e., positioning, relative crops, and height of traps has not received as much attention as trap and lure performance. Rice et al. (2017) demonstrated that *D. suzukii* prefer low-hanging fruit in raspberry plots. They found that traps at the shortest height of 60cm, on average captured 4 flies per day, while traps higher up at 110 and 135cm only averaged 1-1.5 flies per day. Tonina et al. (2018) evaluated the dispersal of *D. suzukii* from the forest edge into cherry orchards and preferred flying height by placing traps 1-8m above the ground. They found that the higher the trap, the less *D. suzukii* captured. When orchard cherries weren't ripe, *D. suzukii* preferred being closer to the forest edge and flying at lower heights, whereas when cherries were ripe, *D. suzukii* would fly further into the fruit orchard and fly higher. However, other researchers have suggested that time of day may influence where *D. suzukii* are in the orchards or plantings. During the early day *D. suzukii* were more active around the bottom of the crop, and during the evening the most activity was observed towards the top half of the crop (Jaffe and Guédot 2019). This preference could be crop dependent, as Drummond et al. (2019) found that in wild blueberries trap efficiency was significantly improved by placing the trap 1.2m above the ground and above the top of the blueberry canopy, compared to traps placed within the crop

canopy. Traps placed 1.2m above the canopy caught up to 4 more times *D. suzukii* than traps placed less than 0.25m above the ground.

Trap captures of *D. suzukii* are very inconsistent and can be unreliable. Considering this, Singh et al. (2021) assessed how host fruit presence could compete with monitoring traps in a laboratory setting. They found when fruit was easily accessible, trap re-capture decreased by 64-88%, whereas when fruit was less accessible re-capture was reduced by 0-51%. Another study found similar results, where host fruit abundance influenced *D. suzukii* preference in bait (Huang and Gut 2021). They found that a higher presence of raspberries and blackberries caused a decrease in attraction to a symbiotic yeast lure. This data supports the hypothesis that accessible fruit can out-compete monitoring traps.

With variable results for summer morph preference on trap height and vertical space occupied, it would be useful to determine preferences for winter morph *D. suzukii* as well. Many researchers have reported little to no capture of *D. suzukii* during cold periods, which could be explained by misguided trap placement (Harris et al. 2014; Pelton et al. 2016; Rossi-Stacconi et al. 2016; Wallingford et al. 2018; Leach et al. 2019). Improving trap placement to specifically monitor winter morphs has the potential to accurately monitor their population dynamics during the off-season. This knowledge will also help growers and researchers prepare for the coming fruit season's infestation levels, and hopefully decrease populations of any overwintering *D. suzukii*.

Summary and Objectives

Although it has been a decade since *D. suzukii* appeared in Michigan, sustainable control of this pest has not been achieved. Insecticide sprays are the main

tactic for managing population levels. Monitoring traps can be used as a tool to make educated decisions on insecticide application sprays, making traps a vital component for management. Researchers have suggested that weather conditions can influence pest captures and should be further evaluated (Harris et al. 2014). It has been determined that catching one *D. suzukii* in a monitoring trap means there are about 192 flies per 2.7 hectares (Kirkpatrick et al. 2018). Thus, improving trapping methodologies and understanding the biology of this pest is vital for management. The specific objectives of this study are to:

Determine at what temperature terrestrial locomotion is inhibited for summer and winter morph *D. suzukii*. *Hypotheses:* a) *Drosophila suzukii* will show a significant decline in the proportion that walk at temperatures below 5°C, and b) the morph of *D. suzukii* will influence the thermal threshold, with winter morph flies walking at cooler temperatures than summer morph.

Determine winter and summer morph *D. suzukii* response to monitoring trap height. *Hypothesis:* The two morphs will respond differently to monitoring traps, with summer morph favoring aerial traps and winter morphs favoring terrestrial traps.

CHAPTER 2: Investigating the lower thermal locomotion thresholds of summer and winter morph *Drosophila suzukii*

Introduction

Drosophila suzukii (Matsumura, Diptera: Drosophilidae) or spotted-wing drosophila, is an invasive vinegar fly that has rapidly become one of the most serious pests of soft fruits in Asia, the Americas, and Europe. Females possess a unique serrated ovipositor that allows them to lay eggs in ripening fruit. *Drosophila suzukii* is estimated to cause hundreds of millions of dollars in annual economic loss in small fruits (Bolda et al. 2010; Onstad and Crain 2019). Major damage occurs during the growing season in plantings, however, *D. suzukii* has a wide host range and can take advantage of additional resources. For example, they also reproduce on fruit waste and decaying fungal tissues (Bal et al. 2017; Stockton et al. 2019). Understanding this pest's behaviors and physiology is critical for the development of a deeper understanding of *D. suzukii*.

Drosophila suzukii exhibits a reproductive diapause that helps it succeed in temperate climates where some portion of the year is too cold to support their physiology (Dalton et al. 2011; Jakobs et al. 2015; Stephens et al. 2015; Wallingford et al. 2016; Shearer et al. 2016). This adaptation is coupled with a seasonal dimorphism, known as a "summer morph" and "winter morph." Winter morph flies typically have longer wings, larger bodies and darker pigmentation as well (Stephens et al. 2015; Jakobs et al. 2015; Shearer et al. 2016; Tran et al. 2020). Shearer et al. (2016) were the first to provide molecular evidence of reproductive diapause in winter morphs, finding a decrease in the transcripts used for oogenesis and DNA replication. Toxopeus et al. (2016) found that 80% of winter morph entered a chill coma at -5.5°C, whereas summer

morph went into a chill coma at -0.5°C . They also reported that summer morph females reached full ovarian maturity 10 days after eclosion, and winter morph took up to 30 days after eclosion. Winter morph *D. sukii* also have been reported to survive longer in drier conditions compared to summer morph, suggesting winter morphs retain water better than summer morphs (Fanning et al. 2019).

Researchers have also explored behavioral differences between the two morphs for improving trapping methodologies. Kirkpatrick et al. (2018) found that winter morph *D. sukii* had decreased olfactory responses to volatiles compared to summer morph. Furthermore, winter morph had no preference to geosmin, whereas summer morph strongly avoided it. Additionally, they demonstrated that summer morph *D. sukii* had higher antennal responses than winter morph when exposed to several volatiles. Developmental thresholds for *D. sukii* have also been evaluated, ranging from $5.9 - 7.2^{\circ}\text{C}$ for the lower end and $31.5-42.1^{\circ}\text{C}$ for the upper developmental threshold. Another behavioral avenue that could be evaluated is the morphs response to temperature and how it influences locomotion. This information could provide insight into when to monitor *D. sukii* during the cold season (Tochen et al. 2014; Asplen et al. 2015).

When trapping *D. sukii* during cold periods, usually very few flies are captured (Harris et al. 2014; Pelton et al. 2016; Rossi-Stacconi et al. 2016; Wallingford et al. 2018). Determining the temperature at which *D. sukii* stops terrestrial locomotion could be used to inform researchers and growers at which temperatures monitoring is unlikely to be useful. My goals were to determine at what temperature terrestrial locomotion is inhibited and if morph of *D. sukii* influences those thermal thresholds. I hypothesized that *D. sukii* will show a significant decline in the number of flies that

walk at temperatures below 5°C. I also hypothesized that morph would influence thermal thresholds, with winter morph *D. suzukii* walking at cooler temperatures than summer morph.

Materials and Methods

I evaluated how temperature influenced walking behavior of summer and winter morph *D. suzukii* in a walk-in growth chamber.

Drosophila suzukii colony

Drosophila suzukii were collected from a colony established from wild flies captured in a tart cherry orchard at the Trevor Nichols Research Center of Michigan State University (MSU; Fennville, MI, USA) in 2018. Summer morph flies were reared in a growth chamber at 23°C, 77% RH, and a 18L:6D photoperiod. The summer morph colony was maintained with 90mL of solid cornmeal diet (Dalton et al. 2011) in 16oz wide mouth Ball mason jars. A small piece of fine mesh covered the top of the jar. The winter morph colony was maintained with the same methodology described above until January 2021. Rearing then switched to 5mL of the cornmeal diet in 50mL polystyrene vials (Gene-see Scientific, San Diego, CA, USA). Winter morph colonies are in growth chambers longer than summer morph colonies, and the fine mesh used with mason jars allowed more air flow, and the diet would dry out too fast. Thus, switching back to vials decreased air flow and increased humidity within vials to preserve diet and immature life stages of *D. suzukii*. To establish a winter morph colony, adult summer morph flies were left in a summer condition growth chamber, described above, to lay eggs for 2 days.

The adults were then removed and vials with eggs were placed in a growth chamber set at 10°C, >60% RH, and a 9L:15D photoperiod.

Experiment 1: Evaluation of Terrestrial Locomotion - Walking

Temperature treatments for summer and winter morph were 0, 2, 2.5, 5, 10, 15°C and -1.5, -0.5, 0, 5, 10, 15°C, respectively. The selected temperatures were chosen based on preliminary observations on temperatures that resulted in 0% and 100% movement for each morph, so the lower thresholds vary between summer and winter morph *D. sukuzii*. For each temperature/morph/sex combination 5 groups of 10 flies were assayed per cohort with 3 cohorts run over the total experiment, thus 150 flies were evaluated in total for each temperature/morph/sex combination. Flies were placed into an arena consisting of a clear 946mL polystyrene deli-cup (Fabri-Kal, Kalamazoo, MI, USA) with 2 1.5cm holes on the sides of the cup. Masking tape was used to cover holes while flies were acclimating to prevent them from escaping.

Experiments were conducted in a walk-in growth chamber at the MSU Entomology Dept. Insectary. Flies used in experiments were collected 1-3 d or 1-14 d after eclosion from summer and winter morph colonies, respectively, and less than 3 hr before trials. Flies were anesthetized with CO₂ prior to collection and stored in their respective colony chambers while the walk-in chamber equilibrated. Flies were acclimated to walk-in chamber temperatures for 10 min prior to initiating experiments. To begin trials, flies were chilled for 5 min in a cooler with ice packs. Flies were removed from the cooler and placed into arenas for 10 min to acclimate to the temperature in the walk-in chamber.

After the acclimation period described above, a paint brush was used to force movement. The brush was inserted into one of the holes on the side of the cup, and each fly was lightly tapped on their abdomen. A laser infrared thermometer (Amazon, ASIN: B07XG3P7R9) was used to monitor the temperature of the arena to ensure the temperature was similar to the set temperature of the walk-in chamber. The actual temperatures in the chamber were used for the analysis, not the set temperatures mentioned above, for improved accuracy. Flies would be tapped up to 10 times before concluding if the fly moved or not, resulting in a binary behavior of walking or not walking. The number of brush taps was recorded and if the fly walked or didn't walk. Once all 10 flies had been examined, an aspirator was used to place them into 70% ethanol for storage. From each cohort, an additional 10 flies were used as a control group that were subjected to the same tapping process at 23°C, 25-35% RH to confirm that the cohorts were in good health.

The number of flies that walked or didn't walk for all the temperatures were compared using the 'drc' package, R version 3.6.1 (R Core Team, 2015). Prior to running the log-logistic regression, I ran a Pearson's Chi-squared Test to compare behavioral responses of female and male *D. sukuzii* for summer and winter morph. Based on those results, I determined that female and male *D. sukuzii* respond similarly, so I only ran 2 log-logistic models, comparing summer and winter morph behavioral responses. The binary data were fitted to a two-parameter binomial log-logistic function with parameters c (lower limit) and d (upper limit) constrained to 0 and 1, respectively, while the b (slope) and e (LT 50) parameters were calculated. The log logistic equation for a four-parameter model is described below:

$$f(\text{dose}) = c + d - c1 + (\text{dose}/e)^b$$

Temperatures were converted into Kelvin, as the method does not allow negative “dose” values. After the analysis, I transformed the output of values back to Celsius. Locomotion Thresholds (LT) values for 1, 10, 25, 50, 75, 95, and 99 were compared using 95% confidence intervals between summer and winter morph *D. sukikii*. Reported values were rounded to two significant figures to represent the relative accuracy of LT estimates.

Results

Experiment 1: Evaluation of Terrestrial Locomotion

Summer morph *D. sukikii* willingness to walk was modeled for temperatures at 0, 2, 2.5, 5, 10, 15°C. Sex of *D. sukikii* summer morphs did not impact behavioral responses, so their behavioral responses were analyzed together (Chi² Value = 0.897, D.F. = 5, p = 0.970). In general, the number of individuals willing to walk decreased as the temperature decreased (Fig. 2.1A). The LT50 (±SEM) estimate for summer morph was 4.9°C ± 0.1 (t=3887.198, p<0.001) (Fig. 2.1A) (Table 2.1). The LT1 (±SEM) estimate for summer morph was 0.9°C ± 0.2 and the LT10 (±SEM) estimate was 3.0°C ± 0.01. The LT25 (±SEM) estimate for summer morph was 3.9°C ± 0.1 and the LT75 (±SEM) estimate was 5.8°C ± 0.1. The LT95 (±SEM) estimate for summer morph was 7.4°C ± 0.2 and the LT99 (±SEM) was 8.9°C ± 0.2 (Table 2.1).

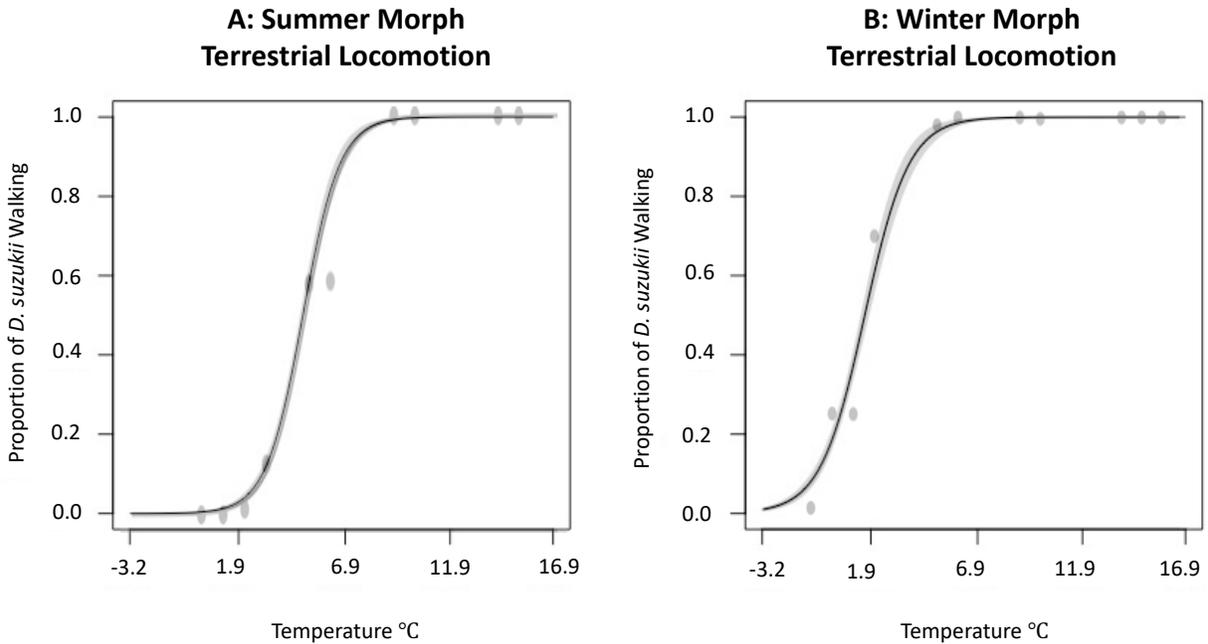


Figure 2.1: Locomotion temperature (°C) curves for summer (A, left) and winter (B, right) morph *D. sukuzii*. 95% confidence intervals are shaded in gray.

Table 2.1: The LT 1, 10, 25, 50, 75, 95, and 99 (\pm SE) estimates of summer and winter morph *D. sukuzii*. ‘*’ indicates significant differences in temperature for a given LT value between the two morphs.

Estimated Proportion for Locomotion Thresholds	Summer Morph <i>D. sukuzii</i> (\pm SE)	Winter Morph <i>D. sukuzii</i> (\pm SE)
<i>b</i> (slope)	-322 ± 17.0	-266 ± 16.9 *
<i>e</i> (LT50)	$4.9^{\circ}\text{C} \pm 0.1$	$1.6^{\circ}\text{C} \pm 0.1$ *
LT1	$0.9^{\circ}\text{C} \pm 0.2$	$-3.1^{\circ}\text{C} \pm 0.3$ *
LT10	$3.0^{\circ}\text{C} \pm 0.01$	$-0.7^{\circ}\text{C} \pm 0.1$ *
LT25	$3.9^{\circ}\text{C} \pm 0.1$	$0.4^{\circ}\text{C} \pm 0.1$ *
LT75	$5.8^{\circ}\text{C} \pm 0.1$	$2.7^{\circ}\text{C} \pm 0.2$ *
LT95	$7.4^{\circ}\text{C} \pm 0.2$	$4.6^{\circ}\text{C} \pm 0.3$ *
LT99	$8.9^{\circ}\text{C} \pm 0.2$	$6.3^{\circ}\text{C} \pm 0.4$ *

Winter morph *D. sukuzii* willingness to walk was modeled for temperatures at -1.5, -0.5, 0, 5, 10, 15°C. Sex of *D. sukuzii* winter morphs did not impact behavioral responses, so their behavioral responses were analyzed together (Chi² Value = 10.6, D.F. = 5, p = 0.06). In general, the number of individuals willing to walk decreased as the temperature decreased (Fig. 2.1B). The LT50 (±SEM) estimate for winter morph was 1.6°C ± 0.1 (t=2709.0, p<0.001) (Fig. 2.1B) (Table 2.1). The LT1 (±SEM) estimate for winter morph was -3.1°C ± 0.3 and the LT10 (±SEM) was -0.7°C ± 0.1. The LT25 (±SEM) estimate for winter morph was 0.4°C ± 0.1 and the LT75 (±SEM) estimate was 2.7°C ± 0.2. The LT95 (±SEM) estimate for winter morph was 4.6°C ± 0.3 and the LT99 (±SEM) estimate was 6.3°C ± 0.4 (Table 2.1). However, there was a discrepancy found in the model, I found 0% movement at -1.5°C, whereas the model predicted 1% movement at -3.1°C.

Discussion

As temperature decreased, the proportion of *D. sukuzii* that walked also decreased (Fig. 2.1A-B). This information will provide insight to pest management professionals monitoring *D. sukuzii* and deciding optimal temperatures for trapping and placement of traps. The pattern of this reduction in locomotion was affected by the “morph” of *D. sukuzii*. There was up to a 3.5°C difference between summer and winter morph *D. sukuzii* willingness to walk at various temperature thresholds. This supports my hypothesis that the morph of *D. sukuzii* influences thermal thresholds of the pest, with winter morphs walking at colder temperatures.

There are other reports of temperature shifts between *D. sukuzii* morphs. Toxopeus et al. (2016) reported a 5°C shift between summer and winter morph *D.*

suzukii when entering a chill coma, at -0.5°C and -5.5°C , respectively. I found similar results for *D. suzukii* willingness to walk in colder temperatures. For example, I found 50% of winter morph *D. suzukii* were willing to walk 3.3°C cooler than 50% of summer morph, 1.6°C and 4.9°C , respectively. Additionally, I found 25% of winter morph *D. suzukii* were willing to walk 3.5°C cooler than 25% of summer morph, 0.4°C and 3.9°C , respectively. It is intriguing to note that the temperature shift between summer and winter morph *D. suzukii* has been reported for developmental stages and now shown here in locomotion as well. There are clear differences between the morphs that should be further examined.

A decrease in compliance in performance of tasks when exposed to cold temperatures is a common trend within *Drosophila spp.* Dillon and Frazier (2006) evaluated how temperature and air pressure influenced walking and flight in *Drosophila melanogaster* (Meigen). They discovered that temperature was the most influential factor for the pace *D. melanogaster* walked, and air pressure did not affect walking speed until it was the lowest air pressure, 34kPa, which is unlikely to be observed in the wild. However, air pressure massively impacted flight performance. Flies showed a reluctance to fly when exposed to low air pressure, and this behavior was more pronounced when in cold temperatures as well. Nyamukondiwa and Terblanche (2009) investigated how age, fed status, and sex of *Ceratitits rosa*, affected the critical thermal minimum and maximum. They found that flies up to 14 days old were the most tolerant of temperatures, and fed flies had a higher tolerance as well. Garcia and Teets (2019) found when evaluating climbing performance in *Drosophila melanogaster*, the willingness to climb and height climbed both decreased with increased cold exposure.

Additionally, it has been shown that laboratory diet can influence the cold tolerance in *D. melanogaster* (Littler et al. 2021). While some diets did not influence cold hardiness, other diets impacted the fly's relative cold hardiness by up to 70%. I did not analyze starved vs. fed *D. suzukii*, but flies had access to laboratory diet during the entire experiment. It would be beneficial if other researchers explored how fasting impacts terrestrial locomotion in *D. suzukii*. It would also be valuable to evaluate thermal thresholds of flight for both morphs of *D. suzukii* as well, but these were past the scope of this paper.

There was a discrepancy for LT1 estimated values for winter morph *D. suzukii*. The model predicted the flies would withstand colder temperatures than what was observed. A potential explanation is under sampling, each temperature was only replicated three times. Additionally, the 0% movement I found at -1.5°C was observed for all 3 replicates, meaning there wasn't a range of data for the line of best fit to follow.

In conclusion, I suggest that if it is cooler than -3.1°C traps do not need to be deployed for monitoring *D. suzukii* winter morph. I found a 2.2°C difference between summer and winter morphs for their lower thermal threshold, 2.5°C difference for the upper thermal threshold and a 3.3°C difference in LT50. This data would suggest that the morph of *D. suzukii* does indeed influence the temperature they can walk at for terrestrial locomotion. Additionally, it shows that winter morph *D. suzukii* are capable of walking at temperatures below 5°C , different from my hypothesis. However, this hypothesis was supported by summer morph *D. suzukii*, with over 50% unwilling to walk below 5°C . This information is important for researchers and growers monitoring *D. suzukii* during the colder season, informing them that if it's below -3.1°C they do not

need traps in the field. This could also lead to influencing trap placement during the off-season as temperatures fluctuate (Hernandez (Ch. 3). Researchers and growers can use my data to make informed decisions about pre and post growing season monitoring of *D. suzukii*.

CHAPTER 3: Exploring how trap position influences spotted-wing drosophila (*Drosophila suzukii*)

Introduction

Drosophila suzukii (Matsumura, Diptera: Drosophilidae) or spotted-wing drosophila, is an invasive vinegar fly that has rapidly become a major pest in agriculture. *Drosophila suzukii* attack berries and soft fruits using the female's serrated ovipositor to deposit eggs in ripening fruit. They are estimated to have caused hundreds of millions of dollars in annual economic losses (Bolda et al. 2010; Onstad and Crain 2019). For raspberries in California, annual economic losses due to *D. suzukii* can be up to \$40 million (Farnsworth et al. 2017). With a devastating impact on stone fruits, *D. suzukii* is a zero-tolerance pest.

Chemical control is heavily relied on to manage *D. suzukii* populations. Timmeren et al. (2018) reported that growers typically have weekly insecticide applications to control population levels. Although, with frequent insecticide use, the probability of insecticide resistance increases. Researchers have found *D. suzukii* resistant populations to spinosyns. Resistant fly populations had LC50 values 4.3-7.7 times higher than a susceptible population (Gress and Zalom 2019). Resistance has also been discovered against other insecticides as well; LC50 values were 12.7-21.0 and 3.4-5.8 times higher in resistant populations for deltamethrin and cyantraniliprole, respectively (Civolani et al. 2021). With resistant populations of *D. suzukii* steadily growing, other management tactics should be implemented.

Monitoring pests is crucial to the judicious application of insecticides. Monitoring traps can provide information on what insect populations are present and how populations fluctuate over time. Traps for *D. suzukii* need to be cost effective

and reliable. Lee et al. (2012) compared several different traps that varied by volume and entry holes. They found that none of the traps evaluated were selective; of the drosophilids captured, only 30% were *D. suzukii*. After they evaluated trap capture, selectivity, and cost, a clear 0.95L cup trap with 10 0.64cm holes near the upper rim became a commonly used trap. Although, other studies have reported that red sticky spheres were more effective than the clear deli cup trap (Kirkpatrick et al. 2017; Kirkpatrick et al. 2018).

Drosophila suzukii have a seasonal dimorphism, known as a “summer morph” and “winter morph.” The management strategies discussed above majorly target summer morph populations. Winter morphs occur when immature flies experience temperatures below 10°C and a 12 hour, or less, light photoperiod (Toxopeus et al. 2016; Shearer et al. 2016; Grassi et al. 2018; Enriquez and Colinet 2019). These flies are characterized by darker, larger bodies and longer wings (Stephens et al. 2015; Jakobs et al. 2015; Shearer et al. 2016). Winter morph *D. suzukii* exhibit a reproductive diapause (Dalton et al. 2011; Jakobs et al. 2015; Stephens et al. 2015; Wallingford et al. 2016; Shearer et al. 2016), and are hypothesized to have better overwintering capacity (Enriquez and Colinet 2019).

Winter morph *D. suzukii* have higher energy reserves, increased desiccation resistance, and increased survival at low temperatures compared to the lighter colored, smaller summer morphs (Rajpurohit et al. 2008; Dalton et al. 2011; Ramniwas et al. 2013; Shearer et al. 2016; Alford et al. 2019; Enriquez and Colinet 2019; Fanning et al. 2019). It is likely that the winter morph of *D. suzukii* are under leaf litter, which creates an environment of higher humidity and temperature, making it more suitable for *D.*

suzukii to overwinter (Kirkpatrick et al. 2018; Wallingford et al. 2018). These cold acclimated flies are the source for the upcoming spring's population of summer morph that will damage fruit during the growing season (Wallingford et al. 2018). As these overwintering populations create the next generation, monitoring and trapping the population at their most vulnerable stage is crucial.

Few *D. suzukii* are captured during the winter (Harris et al. 2014; Pelton et al. 2016; Rossi-Stacconi et al. 2016; Wallingford et al. 2018). The clear deli cup trap mentioned above, is used for both summer and winter morphs of *D. suzukii*, without literature supporting that the trap was optimized for both morphs. To my knowledge, there has been no supporting evidence for identical protocols for trapping two different morphs. Although, with winter morph taking refuge under the leaf litter, a ground trap may be more suited for winter trapping.

Placement of *D. suzukii* monitoring traps has not been thoroughly investigated. Rice et al. (2017) demonstrated that *D. suzukii* were captured up to 4 times more at a trap 60cm above the ground compared to 110 or 135cm in raspberry plantings in the southern USA. Tonina et al. (2018) found similar results, placing traps 1-8m above the ground and concluding the higher the trap was, the less *D. suzukii* that were captured. However, time of day and the ripening stage of fruit may influence trap captures (Tonina et al. 2016; Jaffe and Guédot 2019). Additionally, the type of fruit may also influence trap height preference. Drummond et al. (2019) found in blueberry fields that traps 1.2m above the ground captured up to 4 times more *D. suzukii* than traps less than 0.25m above the ground. While research has covered various trap heights, to our knowledge there has not been any research that places monitoring traps directly on the ground.

Ground traps are an unexplored option that have the potential to greatly impact and improve overwintering monitoring of *D. suzukii*.

The objectives of this study were to determine summer and winter morph *D. suzukii* response to monitoring trap height. I hypothesized that the two morphs would respond differently to monitoring traps, with summer morphs favoring aerial traps, and winter morphs favoring ground traps.

Materials and Methods

I evaluated the effect of trap height on the capture of both summer and winter morph *D. suzukii* in a laboratory and summer and fall/winter field experiments.

Drosophila suzukii colony

Drosophila suzukii used in the laboratory experiment were sourced from a colony established from wild flies captured in a cherry orchard at the Trevor Nichols Research Center of Michigan State University (MSU; Fennville, MI, USA) in 2018. Summer morph flies were reared in a growth chamber at 20°C, 60% RH, and a 18L:6D photoperiod. The colony was maintained with 5mL of solid cornmeal diet (Dalton et al. 2011) in 50mL polystyrene vials (Gene-see Scientific, San Diego, CA, USA). Winter morphs were reared at 10°C, >60% RH, and a 9L:15D photoperiod. Summer morph adults were starved for 2 hours prior to use in experiments with damp filter paper. Winter morph adults were not starved, but were kept in the cold chamber until the start of each experiment with dry filter paper.

Experiment 1: Laboratory comparison of trap height

I evaluated the effect of trap height in a series of 4 two-choice trapping trials, that were each replicated 5 times. A cup style trap fabricated from a clear 946mL polystyrene deli-cup (Fabri-Kal, Kalamazoo, MI, USA) perforated with 12 0.5cm holes around the rim of the cup and a commercial StorGard (Item #: ST/K0-3567-01 OR (TRECE #051934-OK-001)) dome trap designed to capture stored product beetle pests on the floor were compared. The specific choices evaluated were: a) Dome (Floor) vs. Cup (Hanging); b) Cup (Floor) vs. Cup (Hanging); c) Cup (Floor) vs. Dome (Hanging); or d) Dome (Floor) vs. Dome (Hanging) (Fig. 3.1). In each trial, a trap was either hung at 43cm or placed on the floor.

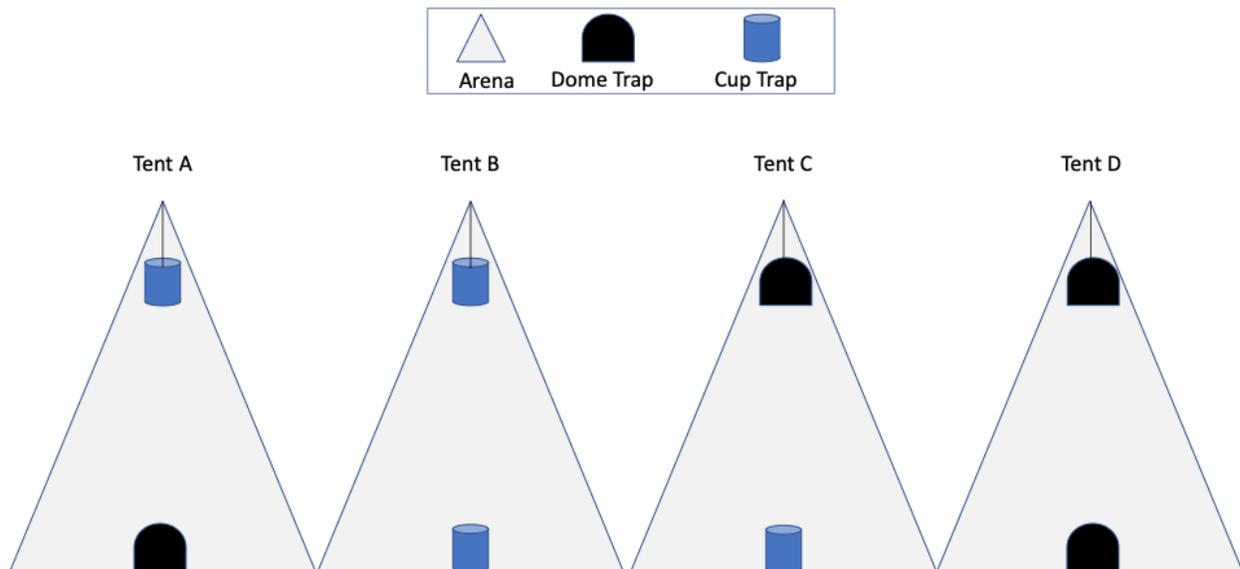


Figure 3.1: A visual representation of the arenas used in Experiment 1. Tent A represents the dome centered on floor versus the cup hanging from the top of the insect cage. Tent B represents cup floor versus cup hanging. Tent C represents cup floor versus dome hanging. Tent D represents dome floor versus dome hanging.

Experiments were carried out in insectary rooms with average relative humidity and temperatures of 50% and 25°C, respectively. Each two-choice test was conducted in a 60x 60x60cm white insect cage (BugDorm-2120, BioQuip Products, Rancho Dominguez, CA, USA) lighted by two 5000 Lumen LED Work Light ((Smart Electrician Menards, Holt, MI, USA). Traps were oriented so that they were centered in the cage and baited with 150mL (cup) and 20mL (dome) of a 24 h old sugar-yeast solution of 355mL of distilled water, 15g of activated yeast and 60g of granulated white sugar. Each cage had 4 petri dishes (10cm x 1.5cm) in the corners of the cage floor with 30 males and 30 females of either summer morph or winter morph - 120 flies of each morph. Trials were ran for 24 h, then each trap was stored in a freezer to kill any remaining live flies at the end of the experiment. Insects were then counted by morph and sex.

Experiment 2: Comparison of trap height in field August 2019

Cup and dome traps were tested at 4 commercial tart cherry orchards in Southwest Michigan. The 2 treatment groups: Ground Cup vs. Hanging Cup and Ground Dome vs. Hanging Dome were examined over 3 weeks in August 2019. The cup traps that were placed on the ground were buried 8cm into the ground for stability. Chicken wire (1.2cm x 1.2m x 15.2m 19 Gauge Hardware Cloth Model # CA19-4X50MF12, Home Depot, Okemos, MI) was placed over the traps on the ground to avoid tampering from animals. Each treatment group was 191m away from the other. The hanging traps were placed 13cm below the lowest branch on the tree. Hanging dome traps had a 0.5cm hole drilled into the lid for a twist tie to go through and hang the

dome from a branch. Lids of the petri dishes previously mentioned, were hot glued to the bottom of the dome trap to prevent insects from interacting with the bottom of the trap, considering when the dome is on the ground the bottom of the trap is not accessible to them. Traps were checked weekly for data collection.

Experiment 3: Comparison of trap height in Fall/Winter Field Experiment

The cup traps mentioned above, and a modified dome trap were tested at three woodlots divided into 6 experimental replicates in East Lansing, MI, from June 10, 2020 to December 16, 2020. The modified dome had 0.227kg plastic jars, with a 6.35cm diameter (Amazon, ASIN: B07DKT7FZY) glued to the bottom of the dome so that it could retain equal amounts of bait to the cup trap. A woodlot replicate consisted of an 7.3m by 7.3m square block that had one of the following trap positions and types randomly chosen: Cup:Hanging, Cup:Ground (buried 3in into the ground for stability), Dome:Ground (modified plastic jar buried into the ground and the dome level with the ground), or Dome:Leaf litter (same as dome ground placement except leaf litter was placed over the dome covering the trap with 1cm of leaf litter). The square blocks were 22.9m in from the edge of the woodlot and were at least 45.7m away from each other if there were multiple replicates in a woodlot. These distances were chosen to minimize competition among replicates.

All traps were baited with 150mL of 10% saltwater and a Scentry Spotted Wing *Drosophila* Lure (GL/SC-5100-12, Great Lakes IPM, Vestaburg, MI). All ground traps were covered with the same chicken wire mentioned previously. Lures were replaced every 3 weeks. From April 2020 to July 2020, traps were checked 3 times per week.

From July 2020 to December 2020 traps were checked weekly. From December 2020 to March 2021 traps were checked based on the predicted average daily temperature. Traps were put out in the woodlots if the average daily temperatures were predicted to be $>0^{\circ}\text{C}$. From March 2021 to May 2021 traps were checked weekly. From May 2021 to June 2021 traps were checked biweekly. Captured flies were transported back to the lab and evaluated using a stereo dissecting microscope. Specimens were first classified as *D. suzukii*, non-target *Drosophila* spp., or other non-target arthropods. *Drosophila suzukii* were further classified as male or female and then as either summer morph or winter morph. To determine morph of *D. suzukii*, specimens from laboratory colonies were used as a baseline. If wild specimens were similar in coloration to laboratory summer morphs, they were classified as summer morphs. If wild specimens were any darker than the summer morph laboratory colony, they were classified as winter morphs.

Data Analysis

Experiment 1: Laboratory comparison of trap height

Proportions of trap capture were analyzed using a randomized complete block design ANOVA test with morph, sex, trap, and interactions as the evaluated fixed factors using the 'aov' function in R version 3.6.1 (R Core Team, 2015). Data was arcsine transformed to normalize data. Significant main effects at $p \leq 0.05$ were followed by a Tukey's HSD comparison.

Experiment 2: Comparison of trap height in field August 2019

Proportions of trap capture were analyzed using a randomized complete block design ANOVA test with morph, sex, trap, and interactions as the evaluated fixed factors using the 'aov' function in R version 3.6.1 (R Core Team, 2015). Data was log (x+1) transformed, to normalize data. Significant main effects at $p \leq 0.05$ were followed by a Tukey's HSD comparison.

Experiment 3: Comparison of trap height in Fall/Winter Field Experiment

Mean female and male *D. sukuzii* captures per day were analyzed by repeated measures ANOVA using the SAS GLM procedure, with the trap type as the subject factor and time (trapping period/week) as the within subject factor (SAS Institute, 2021). Mean separations were performed via Tukey's HSD test ($\alpha = 0.05$). Trapping periods were broken into two peaks of high capture, the first peak was during June 10, 2020 to September 9, 2020. Only summer morphs were captured during this time, so morph was not a factor in the model. The second peak was from September 16, 2020 to December 16, 2020, with summer and winter morph *D. sukuzii* captured during this time. I ran this peak as two models, one for summer morph captures, and one for winter morph captures, so morph was not a factor in the models. I elected to use the Greenhouse-Geissler correction as epsilon values were consistently lower than 0.75.

Additionally, I analyzed non-target drosophilid capture, defined as any non *D. sukuzii* *Drosophila spp.*, over two time periods: May 13, 2020 to September 16, 2020 and November 11, 2020 to December 16, 2020. These analyses employed similar models as those used for *D. sukuzii* with the exception that they did not include a "sex" model factor.

Results

Experiment 1: Laboratory comparison of trap height

In the Dome (Floor) vs. Cup (Hanging) arena, morph of *D. sukuzii* had an effect on which trap they were captured in, with winter morphs being approximately 2 times more likely captured in the floor trap compared to the hanging trap. Whereas summer morphs were 46% less likely to be captured in the floor trap ($F_{1,12}=6.78$, $p=0.02$). The number of *D. sukuzii* captured were similar regardless of trap position ($F_{1,12}=0.60$, $p=0.81$), but the interaction between morph and position was significant with summer morphs being captured more in the Cup (Hanging) trap, whereas the winter morphs were captured more in the Dome (Floor) trap ($F_{1,12}=11.29$, $p<0.01$) (Fig. 3.2A).

In the Cup (Floor) vs. Cup (Hanging) arena, there was a significant blocking effect ($F_{1,32}=2.88$, $p<0.04$). Morph ($F_{1,32}=22.34$, $p<0.001$), sex ($F_{1,32}=8.87$, $p<0.01$), and position ($F_{1,32}=18.96$, $p<0.001$) all had a significant effect on the number of *D. sukuzii* captured, with winter morphs being over 3 times more likely captured in the Cup (Floor) trap compared to the Cup (Hanging) trap, and with summer morphs being 61% less likely to be captured in the floor trap (Fig. 3.2B).

In Cup (Floor) vs. Dome (Hanging) arena, there was a significant block effect ($F_{1,32}=5.39$, $p=0.001$). Morph ($F_{1,32}=54.36$, $p<0.001$), sex ($F_{1,32}=7.49$, $p=0.01$), and position ($F_{1,32}=119.36$, $p<0.001$) all had a significant effect on the number of *D. sukuzii* captured, with winter morphs being nearly 16 times more likely captured in the Cup (Floor) trap, whereas summer morphs were 5 times less likely to be caught in the Cup (Floor) trap (Fig. 3.2C).

In Dome (Floor) vs. Dome (Hanging) arena, there was a significant blocking effect ($F_{1,31}=6.29$, $p<0.001$). Morph ($F_{1,31}=17.93$, $p<0.001$), sex ($F_{1,31}=4.78$, $p<0.04$), and position ($F_{1,31}=37.97$, $p<0.001$) all had a significant effect on the number of *D. suzukii* captured. The interaction between morph and position was significant ($F_{1,31}=19.72$, $p<0.001$) with winter morphs being 5 times as likely captured in the floor dome trap whereas summer morphs were 19% less likely to be captured in the floor trap (Fig. 3.2D). There was a general trend of winter morphs being more likely to be captured in a floor trap, while summer morphs could be caught in either the floor or hanging position of traps (Fig. 3.2A-D).

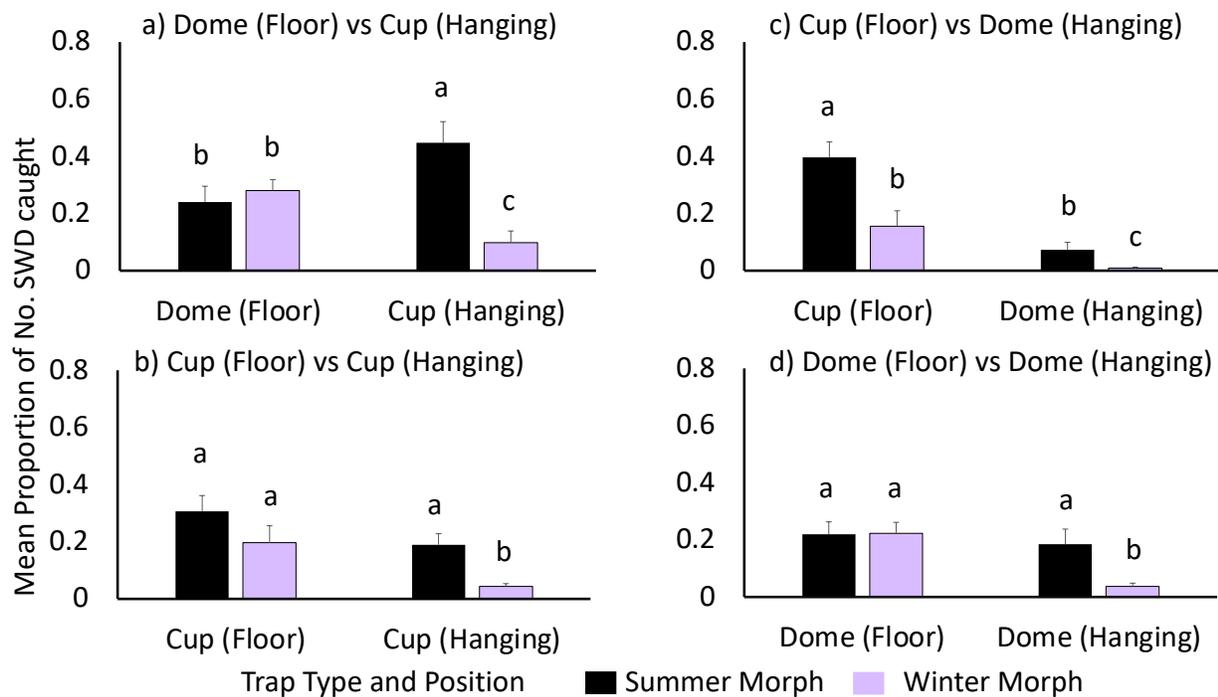


Figure 3.2: Lab bioassay of two choice tests between two different trap heights, replicated five times. a) Mean proportions (\pm SEM) of *Drosophila suzukii* captured per trap in either a dome trap placed in the center of the floor of cage (Dome (Floor)) or a cup trap hanging from the top of the insect cage (Cup (Hanging)). b) Mean proportions (\pm SEM) of *Drosophila suzukii* captured per trap in either a cup trap placed in the center of the floor of cage (Cup (Floor)) or a dome trap hanging from the top of the insect cage (Dome (Hanging)). c) Mean proportions (\pm SEM) of *Drosophila suzukii* captured per trap in either a cup trap placed in the center of the floor of cage (Cup (Floor)) or a cup trap

Figure 3.2 (cont'd) hanging from the top of the insect cage (Cup (Hanging)). d) Mean proportions (\pm SEM) of *Drosophila suzukii* captured per trap in either a dome trap placed in the center of the floor of cage (Dome(Floor)) or a dome trap hanging from the top of the insect cage (Dome (Hanging)). Means with different letters are significantly different (Tukey's HSD test; $p \leq 0.05$), summer morph comparisons are indicated with capital letters and winter morph comparisons are indicated with lower case letters.

Experiment 2: Comparison of trap height in field August 2019

There was a significant blocking effect ($p < 0.001$) for the number of wild *D. suzukii* captured at each site. Trap position had a significant effect on the amount of wild *D. suzukii* captured with cup traps being more than 28 times likely to catch *D. suzukii* compared to the dome traps ($F_{1,10} = 443.71$, $p < 0.001$) (Fig. 3.3). Of the total mean number of flies captured, the hanging cup trap caught 83.2%, the ground cup trap 13.8%, the hanging dome 2.8%, and the ground dome 0.2%, respectively. Position influenced the amount of wild *D. suzukii* captured with hanging traps being 29 times more likely to capture flies compared to the ground traps ($F_{1,10} = 158.86$, $p < 0.001$) (Fig. 3.3). Sex did not significantly differ between trap positions ($F_{1,10} = 443$, $p > 0.05$). Overall, the dome trap seemed to be ineffective at capturing *D. suzukii* at field sites compared to the cup trap (Fig. 3.3).

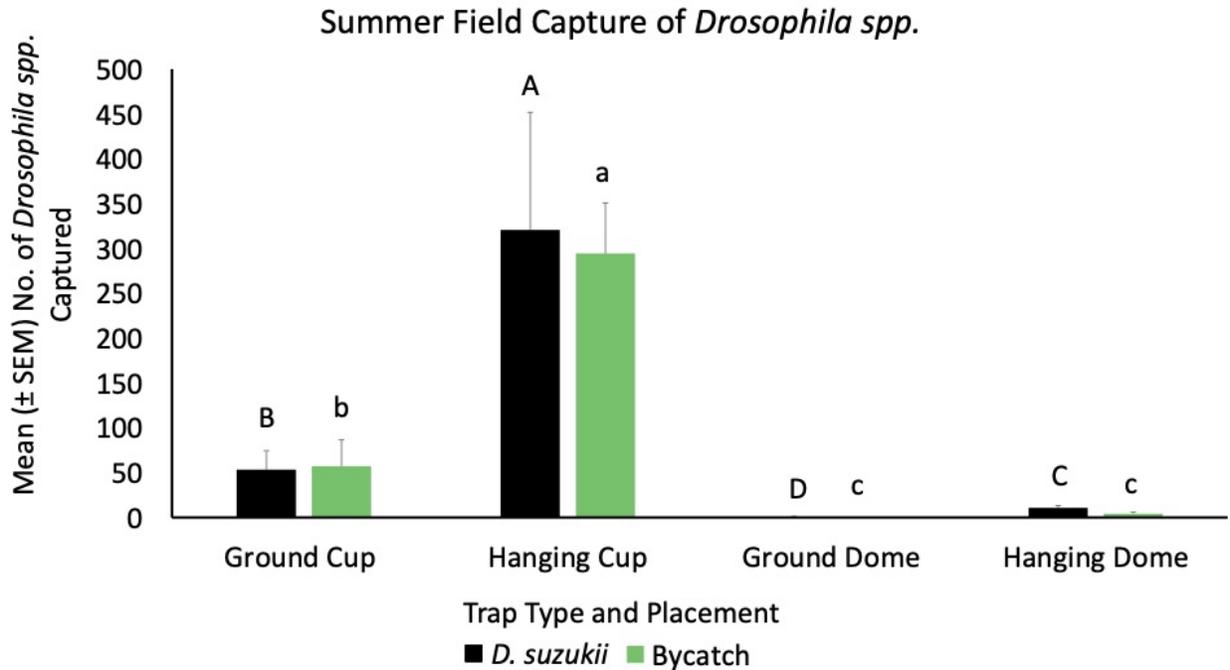


Figure 3.3: Mean (\pm SEM) number of wild summer morph *D. suzukii* preferences of trap position in a field experiment at four sites in Southwest MI. Means with different letters are significantly different (Tukey's HSD test: $p \leq 0.05$), *D. suzukii* comparisons are indicated with capital letters and bycatch comparisons are indicated with lower case letters.

Experiment 3: Comparison of trap height in Fall/Winter Field Experiment

*First Peak: June 10 – September 9, 2020 (Summer Morph *D. suzukii* Capture)*

My overall repeated measures model for the June 10, 2020 to September 9, 2020 data provided a significant time, time and block, time and trap position, time and sex, and time and position and sex interactions ($F_{17,595}=66.53$, $p < 0.0001$; $F_{85,59}=2.50$, $p=0.0011$; $F_{51,595}=6.60$, $p < 0.0001$; $F_{17,595}=6.75$, $p < 0.0001$; $F_{51,595}=2.24$, $p=0.0138$, respectively). Block, position, and sex main effects were significant across time ($F_{5,35}=2.83$, $p=0.0300$; $F_{3,35}=2.96$, $p=0.0457$; $F_{1,35}=9.90$, $p=0.0034$, respectively), as well

the two-way interaction of trap position and sex ($F_{3,35}=5.18$, $p=0.0046$). Post hoc Tukey's HSD output for model parameters at each time point are presented below.

No significant main effects or interactions were detected for trap captures on June 10 to July 16, August 12, and September 2, 2020 (Table 3.1, Fig. 3.4). On July 23, 2020, there was a significant blocking effect ($F_{5,35}=6.10$, $p=0.0004$). However, on July 30, August 5, August 19, August 26, and September 9, 2020, trap position and/or sex main effects and/or trap position and sex interactions were significant, with males captured more frequently in the Dome:Ground or Dome:Leaf litter traps compared to females. For July 30, 2020, only the sex factor was significant ($F_{1,35}=10.36$, $p=0.0028$), with 2-3 times more males captured than females, except in the Cup:Hanging traps. On August 5, 2020, the trap position main effect ($F_{3,35}=7.34$, $p=0.0006$) and the interaction between trap position and sex was also significant ($F_{3,35}=6.88$, $p=0.0009$) with capture ranging from 0.75 ± 0.52 for males in the Cup:Ground to 4.47 ± 0.54 for females in the Cup:Hanging. On August 19, 2020, the main effect sex was significant ($F_{1,35}=13.42$, $p=0.0008$) and the interaction between trap position and sex ($F_{3,35}=3.38$, $p=0.0288$), with capture ranging from 3.38 ± 0.66 for females in the Dome:Ground traps to 11.67 ± 1.57 for males in the Dome:Ground traps. For August 26, 2020, main effects block, trap position and sex were significant ($F_{5,35}=4.52$, $p=0.0028$; $F_{3,35}=15.91$, $p<0.0001$; $F_{1,35}=11.48$, $p=0.0018$, respectively). The interaction between trap position and sex ($F_{3,35}=4.47$, $p=0.0093$) was also significant, with trap capture ranging from 1.76 ± 0.3 for females in the Cup:Hanging to 21.79 ± 5.84 for males in the Dome:Ground. On September 9, 2020, farm and trap position main effects were significant ($F_{3,35}=5.87$, $p=0.0005$; $F_{3,35}=9.34$, $p=0.0001$, respectively) and the interaction between trap position

and sex ($F_{3,35}=2.92$, $p=0.0476$), with trap captures ranging from 1.5 ± 0.57 for males in the Cup:Hanging to 4.95 ± 0.93 for males in the Dome:Leaf litter traps.

Table 3.1: Mean \pm SEM *D. suzukii* summer morphs captured per day in the four trap conditions between 6/10/20 and 9/9/20. There were interactions found in all bolded time points, except for 7/30/20, which is marked and described below.

Date	Dome:Leaf litter		Dome:Ground		Cup:Ground		Cup:Hanging	
	Male	Female	Male	Female	Male	Female	Male	Female
6/10/20	0 \pm 0	0 \pm 0	0 \pm 0	0.08 \pm 0.08	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
6/12/20	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0.08 \pm 0.08	0.17 \pm 0.11	0.33 \pm 0.25
6/15/20	0 \pm 0	0.06 \pm 0.06	0 \pm 0	0.06 \pm 0.06	0.06 \pm 0.06	0.22 \pm 0.22	0 \pm 0	0.11 \pm 0.07
6/17/20	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0.5 \pm 0.32	0.08 \pm 0.08	0 \pm 0
6/19/20	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
6/22/20	0 \pm 0	0.11 \pm 0.05	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
6/25/20	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0.06 \pm 0.02
7/2/20	0 \pm 0	0 \pm 0	0 \pm 0	0.02 \pm 0.01	0.02 \pm 0.01	0 \pm 0	0 \pm 0	0 \pm 0
7/9/20	0.07 \pm 0.03	0 \pm 0	0.05 \pm 0.02	0.1 \pm 0.04	0.14 \pm 0.06	0.14 \pm 0.06	0.29 \pm 0.12	0.02 \pm 0.01
7/16/20	0.29 \pm 0.12	0.36 \pm 0.15	0.26 \pm 0.11	0.43 \pm 0.17	0.48 \pm 0.19	0.86 \pm 0.35	0.5 \pm 0.2	0.81 \pm 0.33
7/23/20	1.26 \pm 0.52	1.38 \pm 0.56	1.79 \pm 0.73	2.26 \pm 0.92	1.45 \pm 0.59	1.29 \pm 0.52	0.76 \pm 0.31	1.74 \pm 0.71
7/30/20	5.69 \pm 1.01a ∞	2.24 \pm 0.68b ∞	3.79 \pm 1.19a ∞	0.93 \pm 0.33b ∞	4.17 \pm 1.7a ∞	1.71 \pm 0.5b ∞	3.48 \pm 1a ∞	3.74 \pm 0.5b ∞
8/5/20	3.31 \pm 0.65ab	2.22 \pm 0.73abc	1.31 \pm 0.23bc	1.08 \pm 0.43bc	0.75 \pm 0.52c	1.67 \pm 0.61bc	1.33 \pm 0.39bc	4.47 \pm 0.54a
8/12/20	5.71 \pm 2.33	2.69 \pm 1.1	5.76 \pm 2.35	2.74 \pm 1.12	5.38 \pm 2.2	5.1 \pm 2.08	7.07 \pm 2.89	7.26 \pm 2.96

Table 3.1 (cont'd)

Date	Dome:Leaf litter		Dome:Ground		Cup:Ground		Cup:Hanging	
	Male	Female	Male	Female	Male	Female	Male	Female
8/19/20	9.74 ± 1.67ab	3.93 ± 1.1b	11.67 ± 1.57a	3.38 ± 0.66b	6.81 ± 2.13ab	5 ± 1.65ab	6.07 ± 1.14ab	6.45 ± 1.44ab
8/26/20	12 ± 3.3ab	5.24 ± 1.41b	21.79 ± 5.84a	8.21 ± 1.94b	2.4 ± 1.11b	2.74 ± 1.08b	2.38 ± 0.28b	1.76 ± 0.3b
9/2/20	7.43 ± 3.03	3.5 ± 1.43	7.33 ± 2.99	4.19 ± 1.71	2.64 ± 1.08	3.81 ± 1.56	3.62 ± 1.48	4.43 ± 1.81
9/9/20	4.95 ± 0.93a	3.71 ± 0.63ab	3.24 ± 0.82ab	1.62 ± 0.48b	1.62 ± 0.41b	2.5 ± 0.73ab	1.5 ± 0.57b	2.14 ± 0.64ab

Bold text indicates significant treatment effect and different letters following means within a date indicate significant differences among treatments within that date (Tukey HSD alpha = 0.05).

* Indicates a blocking effect for that given time period.

∞ Indicates no significant interaction between trap position and sex during analysis.

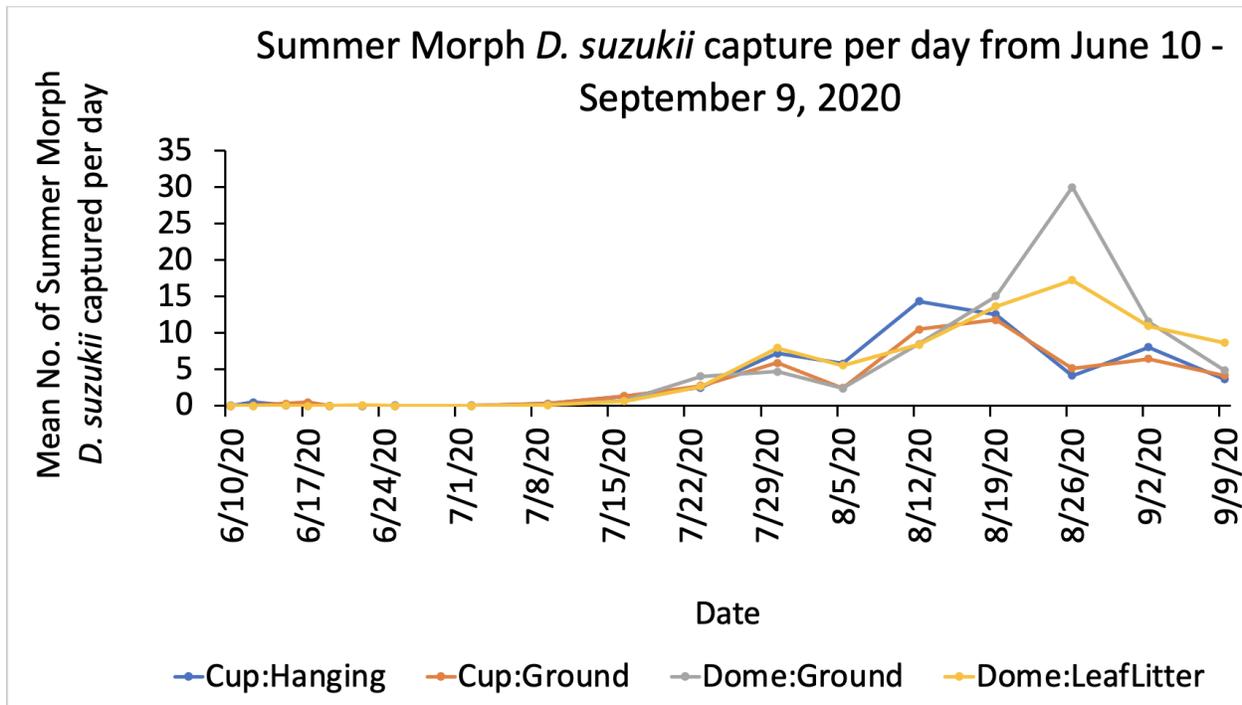


Figure 3.4: Mean number of summer morph *D. sukukii* capture per day in six woodlots in East Lansing, MI, from June 10 - September 9, 2020.

Second Peak: September 16 – December 16, 2020 (Summer Morph D. suzukii Capture)

My overall repeated measures model for summer morph captures between September 16, 2020 to December 16, 2020, provided significant time, time and block, time and trap position, and time and sex interactions ($F_{13,455}=79.56$, $p<0.0001$; $F_{65,455}=4.46$, $p<0.0001$; $F_{39,455}=2.54$, $p=0.0057$; $F_{13,455}=6.92$, $p<0.0001$, respectively). Block and sex main effects were significant across time ($F_{5,35}=5.33$, $p=0.0010$; $F_{1,35}=24.00$, $p<0.0001$, respectively). No significant interactions were observed. Post hoc Tukey's HSD output for model parameters at each time point are presented below.

No significant main effects or interactions were detected for trap captures on September 23 and November 4 to December 16, 2020 (Table 3.2, Fig. 3.5). However, on September 16 and 30, October 7, 14, 21, and 28, 2020, trap position and/or sex main effects were significant, with the Cup:Hanging traps capturing more *D. suzukii* compared to other traps, and generally, males were captured almost 2 times more than females during this time period. For September 16, 2020, block and trap position were significant ($F_{5,35}=4.01$, $p=0.0056$; $F_{3,35}=6.65$, $p=0.0011$, respectively), with trap captures ranging from 1.06 ± 0.58 for the Dome:Ground traps to 4.67 ± 1.5 in the Cup:Hanging traps. On September 30, 2020, only trap position was significant ($F_{3,35}=6.69$, $p=0.0011$), where the Cup:Hanging and Dome:Leaf litter traps captured up to 3 times more *D. suzukii* compared to the other traps. For October 7, 2020, block, trap position and sex were significant main effects ($F_{5,35}=3.09$, $p=0.0206$; $F_{3,35}=3.73$, $p=0.0200$; $F_{1,35}=8.52$, $p=0.0061$, respectively), with male *D. suzukii* captured nearly 2 times more than females, and the Cup:Hanging traps captured 2 to 3 times more flies. On October 14, 2020, block and sex were significant ($F_{5,35}=8.22$, $p<0.0001$; $F_{1,35}=12.39$, $p=0.0012$,

respectively), with male *D. sukuzii* captured nearly 2 times more than females. For October 21, 2020, block, trap position and sex were significant main effects ($F_{5,35}=4.46$, $p=0.0030$; $F_{1,35}=4.83$, $p=0.0065$; $F_{1,35}=15.23$, $p=0.0004$, respectively), where males were captured over 2 times more frequently than females and the Cup:Hanging and Dome:Leaf litter traps captured over 2 times more than the other traps. On October 28, 2020, trap position was the only significant main effect ($F_{1,35}=9.86$, $p<0.0001$), where the Cup:Hanging traps captured up to 10 times more flies than the other traps.

Table 3.2: Mean \pm SEM *D. suzukii* summer morphs captured per day in the four trap conditions between 9/16/20 and 12/16/20. There were no interactions found during this time period, thus, the table presents main effects.

Date	Dome:Leaf litter	Dome:Ground	Cup:Ground	Cup:Hanging	Male	Female
9/16/20 *	2.48 \pm 0.43 ab	1.06 \pm 0.58 b	2.3 \pm 0.82 b	4.67 \pm 1.5 a	2.78 \pm 0.57	2.17 \pm 0.51
9/23/20	4.63 \pm 1.05	4.82 \pm 1.51	5.14 \pm 0.69	4.33 \pm 1.31	5.96 \pm 0.69	3.51 \pm 0.42
9/30/20	0.89 \pm 0.16 ab	0.3 \pm 0.07 c	0.44 \pm 0.14 bc	0.93 \pm 0.24 a	0.74 \pm 0.09	0.54 \pm 0.07
10/7/20 *	2.89 \pm 1.11 ab	1.37 \pm 0.22 b	2.29 \pm 0.89 ab	4.69 \pm 1.45 a	3.87 \pm 0.96 †	1.75 \pm 0.36
10/14/20 *	6.87 \pm 2.19 a	7.32 \pm 1.5 a	7.71 \pm 1.69 a	10.99 \pm 3.46 a	10.48 \pm 2.4 †	5.97 \pm 1.46
10/21/20 *	5.65 \pm 0.91 a	2.88 \pm 0.88 b	1.96 \pm 0.33 b	4.04 \pm 1.88 ab	5.05 \pm 1.01 †	2.22 \pm 0.52
10/28/20	0.17 \pm 0.06 b	0.1 \pm 0.02 b	0.44 \pm 0.1 b	1.05 \pm 0.34 a	0.48 \pm 0.07	0.4 \pm 0.11
11/4/20	3.07 \pm 0.89	2.02 \pm 0.28	2.12 \pm 0.46	1.14 \pm 0.31	2.76 \pm 0.25	1.42 \pm 0.13
11/11/20	2.25 \pm 0.92	2.11 \pm 0.38	1.65 \pm 0.39	1.65 \pm 0.57	2.43 \pm 0.44	1.4 \pm 0.24
11/18/20	0.01 \pm 0.01	0.04 \pm 0.04	0.08 \pm 0.05	0.08 \pm 0.04	0.04 \pm 0.03	0.07 \pm 0.02
11/25/20	0.33 \pm 0.2	0.26 \pm 0.11	0.21 \pm 0.07	0.24 \pm 0.13	0.32 \pm 0.11	0.21 \pm 0.09
12/2/20	0.02 \pm 0.02	0.01 \pm 0.01	0.01 \pm 0.01	0.02 \pm 0.02	0.02 \pm 0.01	0.01 \pm 0.01
12/9/20	0 \pm 0	0 \pm 0	0.01 \pm 0.01	0 \pm 0	0 \pm 0	0.01 \pm 0
12/16/20	0.02 \pm 0.02	0 \pm 0	0.05 \pm 0.04	0 \pm 0	0.02 \pm 0.01	0.02 \pm 0.01

Bold text indicates significant treatment effect and different letters following means within a date indicate significant differences among treatments within that date (Tukey HSD alpha = 0.05).

* Indicates a blocking effect for that given time period.

Table 3.2 (cont'd)

∞ Indicates no significant interaction between trap position and sex during analysis.
 † Indicates that sex was a significant main effect in the model and is corresponded with the sex that had a higher capture per day.

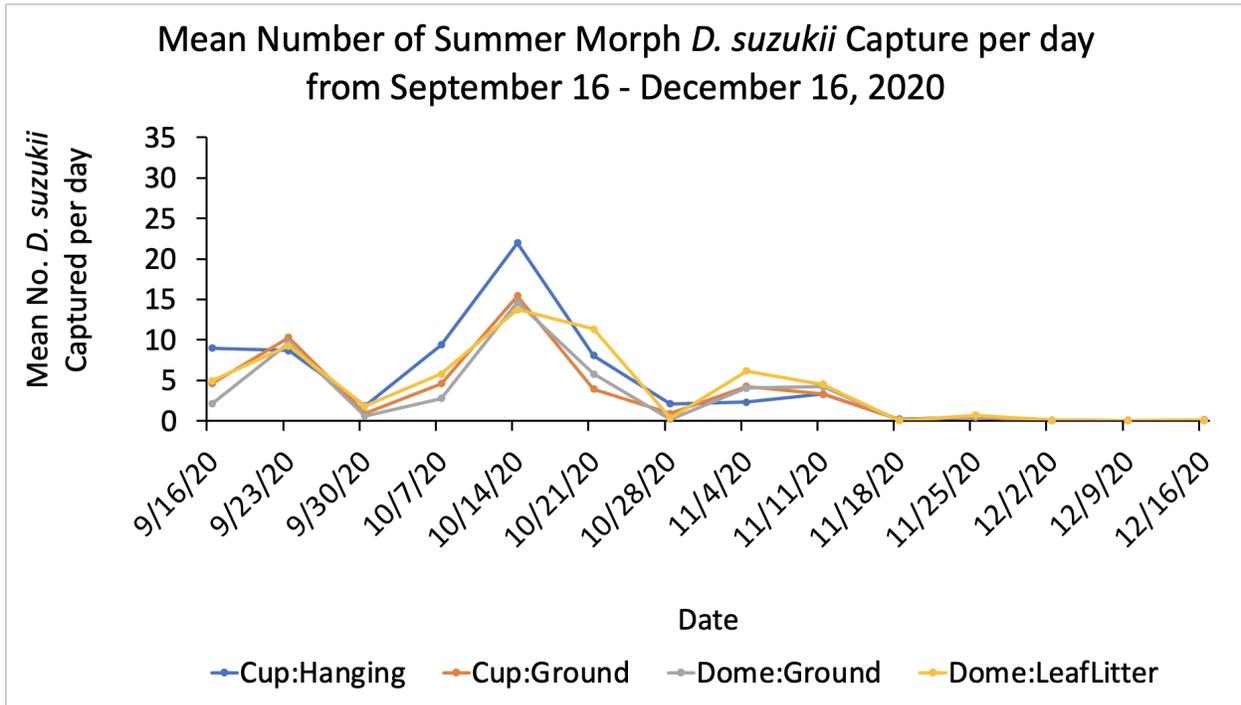


Figure 3.5: Mean number of summer morph *D. sukukii* capture per day in six woodlots in East Lansing, MI, from September 16 - December 16, 2020.

*Second Peak: September 16 – December 16, 2020 (Winter Morph *D. sukukii* Capture)*

My overall repeated measures model for winter morph captures on September 16, 2020 to December 16, 2020, provided a significant time and time and block ($F_{13,455}=60.93, p<0.0001$; $F_{65,455}=3.08, p=0.0076$, respectively). Block main effect was significant across time ($F_{5,35}=3.64, p=0.0094$). Post hoc Tukey’s HSD output for model parameters at each time point are presented below.

No significant main effects or interactions were detected for trap captures on September 23 and 30, November 4, 11, and 25, and December 9, 2020 (Table 3.3, Fig.

3.6). However, on October 14, 2020, there was a significant blocking effect ($F_{5,35}=5.91$, $p=0.0005$). On September 16, October 7, 21, 28, November 18, and December 2 and 16, 2020, trap position, block and/or sex main effects and/or trap position and sex interactions were significant, where females were captured approximately 2 times more than males, and the Cup:Hanging traps captured 2-17 times more *D. sukikii* in the fall (September/October), and the Cup:Ground traps captured 2-32 times more in winter months (November/December). For September 16, 2020, trap position and sex main effects were significant ($F_{3,35}=5.07$, $p=0.0051$; $F_{1,35}=4.48$, $p=0.0414$, respectively), with trap captures ranging from 0 in the Dome:Leaf litter traps to 0.17 ± 0.09 in the Cup:Hanging traps and female *D. sukikii* captured over 4 times more than males. On October 7, 2020, block and sex were significant main factors ($F_{5,35}=3.56$, $p=0.0104$; $F_{1,35}=5.65$, $p=0.0230$, respectively), where females were captured 2.5 times more frequently. For October 21, 2020, trap position and sex were significant main effects ($F_{3,35}=3.00$, $p=0.0438$; $F_{1,35}=4.67$, $p=0.0377$, respectively), with females captured approximately $\frac{1}{3}$ times more frequently and trap captures ranging from 0.62 ± 0.15 in the Dome:Ground traps to 1.17 ± 0.11 in the Dome:Leaf litter traps. On October 28, 2020, trap position was the only significant factor ($F_{3,35}=14.43$, $p<0.0001$), with trap captures ranging from 0.02 ± 0.02 in the Dome:Leaf litter traps to 1.21 ± 0.36 in the Cup:Hanging traps. For November 18, 2020, block and trap position were significant main effects ($F_{5,35}=6.11$, $p=0.0004$; $F_{3,35}=3.24$, $p=0.0337$, respectively), with trap captures ranging from 0.02 ± 0.02 in the Dome:Leaf litter traps to 0.31 ± 0.14 in the Cup:Ground traps. On December 2, 2020, block, trap position and sex were significant main effects ($F_{5,35}=11.03$, $p<0.0001$; $F_{3,335}=6.77$, $p=0.0010$; $F_{1,35}=5.34$, $p=0.0269$,

respectively), with trap captures ranging from 0.02 ± 0.02 in the Dome:Ground traps to 0.21 ± 0.06 in the Cup:Ground traps. Additionally, females were captured nearly 2 times more than males. For December 16, 2020, block, trap position and sex main effects ($F_{5,35}=2.70$, $p=0.0362$; $F_{3,35}=17.65$, $p<0.0001$; $F_{1,35}=18.19$, $p=0.0001$, respectively) and the interaction between trap position and sex was also significant ($F_{3,35}=9.60$, $p<0.0001$), with capture ranging from 0 for males in the Cup:Hanging to 0.55 ± 0.01 for females in the Cup:Ground traps.

Table 3.3: Mean \pm SEM *D. suzukii* winter morphs captured per day in the four trap conditions between 9/16/20 and 12/16/20. There was only one interaction found during this time period, thus, the top portion of the table presents main effects. On 12/16/20 there was an interaction that is presented at the bottom of the table.

Date	Dome: Leaflitter	Dome: Ground	Cup: Ground	Cup: Hanging	Male	Female
9/16/20	0 \pm 0 c	0.01 \pm 0.01 bc	0.13 \pm 0.04 ab	0.17 \pm 0.09 a	0.02 \pm 0.02	0.11 \pm 0.03 †
9/23/20	0.07 \pm 0.04	0.07 \pm 0.05	0.13 \pm 0.06	0.17 \pm 0.07	0.07 \pm 0.03	0.15 \pm 0.04
9/30/20	0.01 \pm 0.01	0.01 \pm 0.01	0 \pm 0	0.02 \pm 0.02	0.01 \pm 0	0.02 \pm 0.02
10/7/20 *	0.15 \pm 0.14 a	0.12 \pm 0.06 a	0.18 \pm 0.07 a	0.33 \pm 0.11 a	0.11 \pm 0.04	0.28 \pm 0.09 †
10/14/20 *	0.82 \pm 0.3	1.05 \pm 0.36	1.3 \pm 0.35	1.37 \pm 0.47	0.96 \pm 0.25	1.31 \pm 0.35
10/21/20	1.17 \pm 0.11 a	0.62 \pm 0.15 a	0.82 \pm 0.21 a	0.64 \pm 0.23 a	0.65 \pm 0.12	0.97 \pm 0.16 †
10/28/20	0.02 \pm 0.02 b	0.08 \pm 0.03 b	0.68 \pm 0.09 a	1.21 \pm 0.36 a	0.44 \pm 0.11	0.56 \pm 0.11
11/4/20	4.64 \pm 1.83	2.71 \pm 0.36	3.67 \pm 0.61	2.62 \pm 0.49	3.02 \pm 0.38	3.8 \pm 0.68
11/11/20	4.44 \pm 2.45	2.86 \pm 0.22	3.55 \pm 0.69	1.95 \pm 0.48	3.26 \pm 0.88	3.14 \pm 0.7
11/18/20 *	0.02 \pm 0.02 b	0.23 \pm 0.16 ab	0.31 \pm 0.14 a	0.23 \pm 0.11 ab	0.18 \pm 0.09	0.21 \pm 0.08
11/25/20	0.88 \pm 0.36	0.88 \pm 0.14	1.12 \pm 0.14	0.6 \pm 0.26	0.83 \pm 0.15	0.91 \pm 0.17
12/2/20 *	0.11 \pm 0.05 ab	0.02 \pm 0.02 b	0.21 \pm 0.06 a	0.15 \pm 0.09 a	0.09 \pm 0.04	0.16 \pm 0.06 †
12/9/20	0.05 \pm 0.04	0 \pm 0	0.11 \pm 0.07	0 \pm 0	0.03 \pm 0.02	0.05 \pm 0.02

Table 3.3 (cont'd)

Date	Dome:Leaf litter		Dome:Ground		Cup:Ground		Cup:Hanging	
	Male	Female	Male	Female	Male	Female	Male	Female
12/16/20 *	0.02 ± 0.02 b	0.1 ± 0.07 b	0.05 ± 0.03 b	0.07 ± 0.05 b	0.1 ± 0.05 b	0.55 ± 0.1 a	0 ± 0 b	0.02 ± 0.02 b

Bold text indicates significant treatment effect and different letters following means within a date indicate significant differences among treatments within that date (Tukey HSD alpha = 0.05).

* Indicates a blocking effect for that given time period.

∞ Indicates no significant interaction between trap position and sex during analysis.

† Indicates that sex was a significant main effect in the model and is corresponded with the sex that had a higher capture per day.

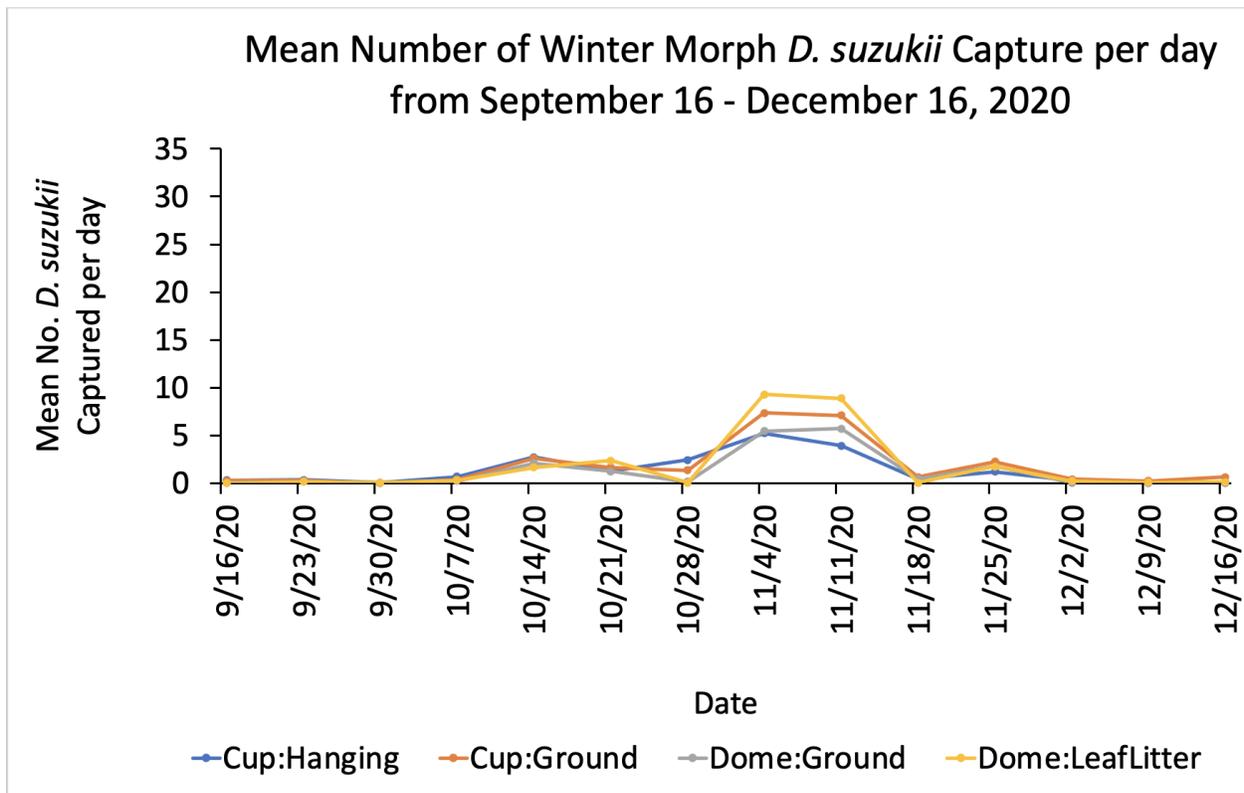


Figure 3.6: Mean number of winter morph *D. sukuzii* capture per day in six woodlots in East Lansing, MI, from September 16 - December 16, 2020.

Third Peak: May 13 – September 16, 2020 (Non-target Drosophilid Capture)

My overall repeated measures model for non-target drosophilid captures on May 13 to September 16, 2020, provided a significant time and time and position of trap ($F_{30,450}=10.11$, $p<0.0001$; $F_{90,450}=3.51$, $p=0.0002$, respectively). Trap position main effect was also significant across time ($F_{3,15}=19.89$, $p<0.0001$). Post hoc Tukey's HSD output for model parameters at each time point are presented below.

No significant main effects or interactions were detected for trap captures from May 13 to May 25, June 3, July 2, and September 9, 2020 (Table 3.4, Fig. 3.7). However, on May 27 to June 1, June 5 to June 25, July 9 to August 26, and September 16, 2020, trap position main effect was significant, where the Cup:Hanging traps captured more non-target drosophila compared to the other traps consistently through the above trapping intervals. During the time periods where trap position was significant, the Cup:Hanging traps capture ranged from 0.98 ± 0.19 to 33.58 ± 11.42 , Cup:Ground ranged from 0.42 ± 0.16 to 12.11 ± 5.13 , Dome:Ground ranged from 0.21 ± 0.08 to 5.93 ± 1.63 , and Dome:Leaf litter ranged from 0.25 ± 0.17 to 8.62 ± 0.77 . For example, on May 29, 2020, the Cup:Hanging traps captured significantly more non-target drosophila ($F_{3,15}=5.60$, $p=0.0088$), with trap capture ranging from 0.33 ± 0.17 in the Dome:Ground to 11.42 ± 4.56 in the Cup:Hanging traps. Additionally, on June 12, 2020, the Cup:Hanging traps again captured the most other *Drosophila* spp. ($F_{3,15}=7.17$, $p=0.0033$) with trap capture ranging from 1.75 ± 0.36 in the Dome:Leaf litter to 33.58 ± 11.42 in the Cup:Hanging traps. This pattern continues on July 16, 2020, with trap captures ranging from 4.74 ± 1.15 to 24.4 ± 6.35 in the Cup:Hanging traps ($F_{3,15}=6.26$, $p=0.0057$). On August 19, 2020, again I observed a significant difference in the

Cup:Hanging trap capture compared to the other traps ($F_{3,15}=14.6$, $p=0.0001$), with trap captures ranging from 1.6 ± 0.49 in the Dome:Leaf litter to 9.1 ± 1.8 in the Cup:Hanging traps.

Table 3.4: Mean \pm SEM non-target *Drosophila* spp. captured per day in the four trap conditions between 5/13/20 and 9/16/20.

Date	Dome:LeafLitter	Dome:Ground	Cup:Ground	Cup:Hanging
5/13/20	0 \pm 0	0 \pm 0	0.5 \pm 0.26	0.33 \pm 0.17
5/15/20	0.17 \pm 0.11	0.08 \pm 0.08	0.75 \pm 0.38	2.67 \pm 1.08
5/18/20	0.11 \pm 0.07	0.28 \pm 0.22	0.78 \pm 0.4	2.61 \pm 1.09
5/20/20	0.42 \pm 0.24	0.25 \pm 0.17	1.75 \pm 1.46	3.83 \pm 1.4
5/22/20	0.42 \pm 0.27	0 \pm 0	1.08 \pm 0.71	2.17 \pm 0.77
5/25/20	0.06 \pm 0.06	0.11 \pm 0.07	0.06 \pm 0.06	2.72 \pm 1.56
5/27/20	0.25 \pm 0.17 b	0.25 \pm 0.11 b	0.42 \pm 0.16 b	4.67 \pm 1.79 a
5/29/20	0.67 \pm 0.17 b	0.33 \pm 0.17 b	1.25 \pm 0.59 b	11.42 \pm 4.56 a
6/1/20	1.22 \pm 0.52 b	0.22 \pm 0.11 b	1.5 \pm 0.55 ab	3.17 \pm 0.88 a
6/3/20	0.42 \pm 0.2	0.75 \pm 0.46	3.25 \pm 1.88	5.67 \pm 3.3
6/5/20	0.42 \pm 0.42 b	1.17 \pm 0.36 b	1.5 \pm 0.87 ab	11.33 \pm 5.19 a
6/8/20	3.61 \pm 2.09 b	2 \pm 0.62 b	5.22 \pm 2.14 b	27.72 \pm 7.62 a
6/10/20	5.92 \pm 3.94 b	0.5 \pm 0.26 b	5.67 \pm 2.72 b	22.33 \pm 5.53 a
6/12/20	1.75 \pm 0.36 b	2.5 \pm 0.72 b	6 \pm 2.32 b	33.58 \pm 11.42 a
6/15/20	1.61 \pm 0.58 b	1.06 \pm 0.45 b	12.11 \pm 5.13 ab	19.17 \pm 4.38 a
6/17/20	2 \pm 0.8 b	0.25 \pm 0.11 b	4 \pm 1.33 ab	22.58 \pm 10.07 a
6/19/20	0.42 \pm 0.2 b	0.92 \pm 0.44 b	0.83 \pm 0.38 b	9.5 \pm 3.13 a
6/22/20	0.28 \pm 0.16 b	0.5 \pm 0.22 b	2.61 \pm 1.58 b	20.83 \pm 9.14 a
6/25/20	1.06 \pm 0.37 b	1.11 \pm 0.47 b	5.94 \pm 2.09 b	21.5 \pm 6.04 a
7/2/20	2.5 \pm 0.58	3.19 \pm 0.78	2.69 \pm 0.82	12.38 \pm 6.1
7/9/20	2.88 \pm 0.69 b	3.24 \pm 0.94 b	4.12 \pm 1.91 b	18 \pm 3.72 a
7/16/20	4.74 \pm 1.15 b	5.26 \pm 1.8 b	9.12 \pm 2.86 b	24.4 \pm 6.35 a
7/23/20	8.62 \pm 0.77 b	5.86 \pm 1.87 b	7.45 \pm 3.01 b	16.88 \pm 3.13 a
7/30/20	8.48 \pm 1.5 b	5.93 \pm 1.63 b	5.43 \pm 2.6 b	17.79 \pm 3.16 a
8/5/20	1.92 \pm 0.6 b	0.83 \pm 0.25 b	1.78 \pm 0.74 b	7.36 \pm 1.23 a
8/12/20	3.14 \pm 0.88 b	2.43 \pm 0.38 b	2.07 \pm 0.62 b	9.62 \pm 2.4 a
8/19/20	1.6 \pm 0.49 b	3.05 \pm 0.66 b	2.17 \pm 0.79 b	9.1 \pm 1.8 a
8/26/20	1.64 \pm 0.41 b	1.79 \pm 0.47 b	1.02 \pm 0.25 b	4.62 \pm 0.84 a
9/2/20	0.76 \pm 0.25	1.14 \pm 0.32	2.38 \pm 1.11	4.83 \pm 1.45
9/9/20	0.31 \pm 0.14	0.62 \pm 0.33	0.29 \pm 0.05	1.33 \pm 0.33
9/16/20	0.4 \pm 0.21 ab	0.21 \pm 0.08 b	0.62 \pm 0.16 ab	0.98 \pm 0.19 b

Table 3.4 (cont'd)

Bold text indicates significant treatment effect and different letters following means within a date indicate significant differences among treatments within that date (Tukey HSD alpha = 0.05).

* Indicates a blocking effect for that given time period.

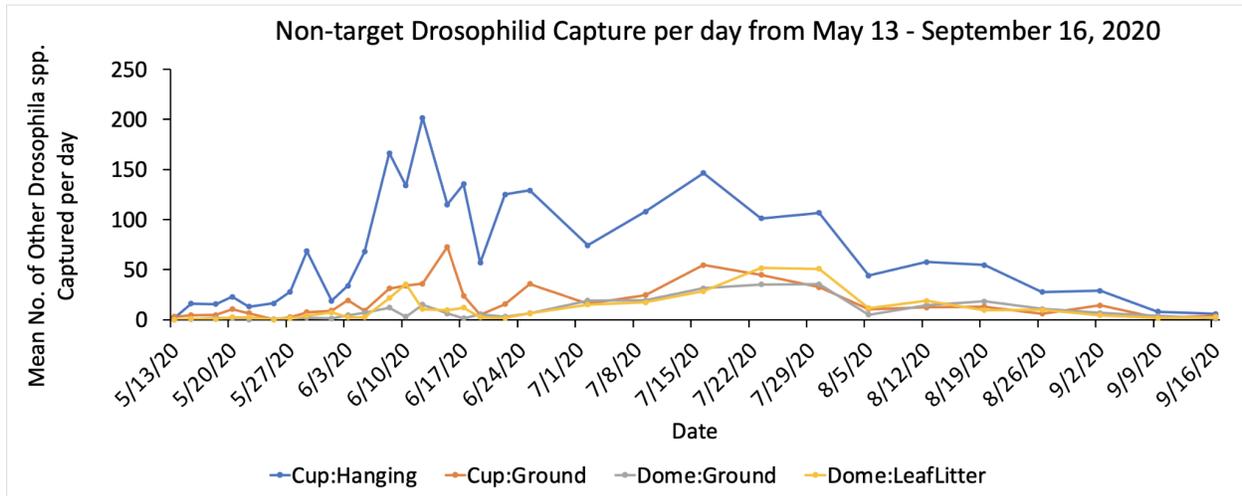


Figure 3.7: Mean number of other *Drosophila spp.* captured per day in six woodlots in East Lansing, MI, from May 13 to September 16, 2020.

Fourth Peak: November 11 – December 16, 2020 (Non-target Drosophilid Capture)

My overall repeated measures model for non-target drosophilid captures on November 11 to December 16, 2020, provided a significant time and time and position of trap ($F_{3,15}=10.53, p=0.0006$; $F_{15,75}=3.89, p=0.0081$, respectively). Trap position main effect was also significant across time ($F_{3,15}=19.89, p<0.0001$). Post hoc Tukey’s HSD output for model parameters at each time point are presented below.

No significant main effects or interactions were detected for trap captures on November 18 and December 16, 2020 (Table 3.5, Fig. 3.8). However, on November 11, 2020, block main effect was significant ($F_{5,15}=3.54, p=0.0258$). On November 25, and December 2 and 9, 2020, trap position main effect was significant, where the

Cup:Ground traps captured more non-target drosophila than the other traps. For November 25, 2020, trap position main effect was significant ($F_{3,15}=11.39$, $p=0.0004$), with captures ranging from 0.55 ± 0.19 in the Dome:LeafLitter traps to 4.1 ± 0.68 in the Cup:Ground traps. On December 2, 2020, trap position main effect was significant ($F_{3,15}=4.27$, $p=0.0229$), with trap captures ranging from 0.12 ± 0.04 in the Dome:LeafLitter traps to 5.95 ± 2.77 in the Cup:Ground traps. For December 9, 2020, trap position main effect was significant ($F_{3,15}=10.23$, $p=0.0006$), with trap captures ranging from 0.4 ± 0.16 in the Dome:LeafLitter traps to 4.45 ± 0.98 in the Cup:Ground traps.

Table 3.5: Mean \pm SEM non-target *Drosophila* spp. captured per day in the four trap conditions between 11/11/20 and 12/16/20.

Date	Dome:LeafLitter	Dome:Ground	Cup:Ground	Cup:Hanging
11/11/20 *	0.79 ± 0.19	1 ± 0.22	1.88 ± 0.67	1.57 ± 0.33
11/18/20	0.12 ± 0.09	0.05 ± 0.03	0.21 ± 0.15	0.45 ± 0.15
11/25/20	0.55 ± 0.19 c	1.17 ± 0.38 bc	4.1 ± 0.68 a	2.93 ± 0.51 ab
12/2/20	0.12 ± 0.04 b	0.93 ± 0.55 ab	5.95 ± 2.77 a	1.69 ± 0.81 ab
12/9/20	0.4 ± 0.16 b	1.21 ± 0.18 b	4.45 ± 0.98 a	0.98 ± 0.46 b
12/16/20	0.1 ± 0.05	0.29 ± 0.13	0.05 ± 0.03	0.17 ± 0.04

Bold text indicates significant treatment effect and different letters following means within a date indicate significant differences among treatments within that date (Tukey HSD alpha = 0.05).

* Indicates a blocking effect for that given time period.

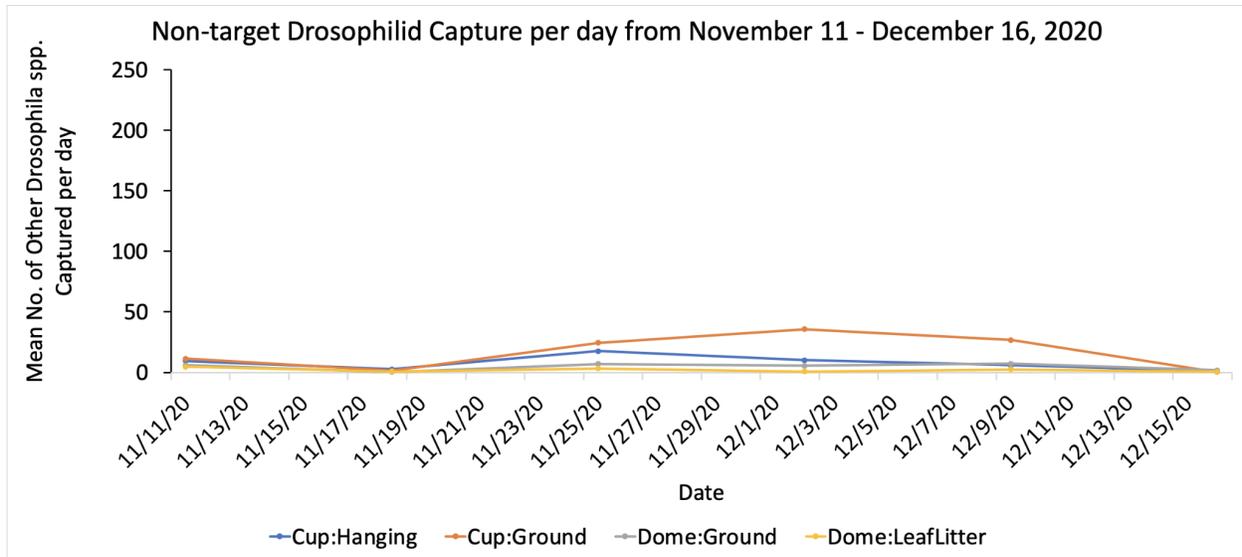


Figure 3.8: Mean number of other *Drosophila spp.* captured per day in six woodlots in East Lansing, MI, from November 11 to December 16, 2020.

Discussion

I hypothesized that a greater number of winter morph *D. sukii* would be captured in the traps positioned on the ground, while summer morphs would have higher capture in traps positioned in the air. From my fall/winter field data, I concluded that winter morph *D. sukii* were caught more in the Cup:Ground traps compared to other traps by a factor of 56, supporting my hypothesis (Table 3.4, Fig. 3.6). However, for summer morph *D. sukii*, traps in the air were not the only traps that had high capture. Dome traps on or under leaf litter were also attractive to summer morph, especially males, partially supporting my hypothesis for summer morph *D. sukii* (Table 3.2, Fig. 3.4). Finally, I discovered that the Cup:Hanging traps that are commonly used to monitor *D. sukii*, were the least selective traps (Table 3.4 and 3.5, Fig. 3.7-8). The Cup:Hanging traps captured more non-target drosophilids by a factor of 89.

Improved trap position may provide important information for developing a better understanding of where *D. suzukii* are located when entering and leaving winter seasons. My laboratory experiment provided support for my hypothesis of ground traps capturing more winter morph *D. suzukii* compared to hanging traps (Fig. 3.2). My 2019 summer field experiment validated my laboratory experiment for summer morph *D. suzukii*, with hanging cup traps capturing the most (Fig. 3.3). My fall/winter 2020 field experiment also supported my hypothesis that ground traps capture more winter morph *D. suzukii* (Table 3.3, Fig. 3.6). Additionally, I discovered that sex and morph of *D. suzukii*, along with season also influence trap captures.

Experiment 1: Comparison of trap height in laboratory

My laboratory experiment showed that regardless of trap height, the placement of the trap had the biggest impact on winter morph *D. suzukii* recapture. Traps placed on the floor were on average 6 times more likely to capture winter morphs than a hanging trap (Fig. 3.2). Although there was a difference in bait volumes between the dome trap (20mL of bait) and the cup trap (150mL of bait), it did not influence the amount of *D. suzukii* winter morphs captured, with both the dome and cup traps having similar capture rates when placed on the floor. In contrast, summer morphs were four times more likely to be captured in a hanging trap compared to a ground trap (Fig. 3.2). This coincides with the common practice of monitoring by hanging traps in the target plant canopy (Lee et al. 2012; Landolt et al. 2012; Cha et al. 2013; Iglesias et al. 2014). This suggests that sugar yeast baits are attractive to *D. suzukii* summer and winter morphs,

but that placement of traps may have a large influence on capture by morph (Landolt et al. 2012; Cha et al. 2013).

Experiment 2: Comparison of trap height in field August 2019

My summer field experiment in August 2019 supported the conclusion of my laboratory experiment with wild *D. suzukii* summer morphs showing a preference for hanging cup traps, and few flies were caught in dome traps. In fact, over 80% of the flies caught were in the hanging cup trap (Fig. 3.3) and both ground and hanging domes caught significantly less flies compared to the cup traps. This could be due to a trap with lower amounts of bait, resulting in lower volatiles, and a reduced ability to stand out from competing fruit volatiles in the orchards (Singh et al. 2021; Huang and Gut 2021). The dome traps captured less *D. suzukii* in the laboratory study as well; however, I did not observe as drastic of a difference between the cup and dome traps. This could be due to a controlled laboratory setting, whereas in the field setting there were competing fruit volatiles in the background.

Experiment 3: Comparison of trap height in Fall/Winter Field Experiment

My woodlot trapping experiment showed that trap capture fluctuated by season, trap position, and morph and sex of *D. suzukii*. During the first trapping period, June 10 to September 9, 2020, the dome traps (Dome:Ground and/or Dome:Leaf litter) captured the most summer morph *D. suzukii*, with up to 12 times more males captured than females (Table 3.1, Fig. 3.4). During the second trapping period for summer morphs, September 16 to December 16, 2020, the Cup:Hanging traps captured the most

summer morph *D. sukuzii*, with up to 2 times more males captured (Table 3.2, Fig. 3.5). For the second trapping period for winter morphs, season affected trap captures. In the fall for Michigan, the Cup:Hanging traps captured the most winter morph *D. sukuzii* and in the winter, the Cup:Ground traps captured the most (Table 3.3, Fig. 3.6). Up to 27 times more winter morph females were captured compared to males during this trapping period.

First Peak: June 10 – September 9, 2020 (Summer Morph D. sukuzii Capture)

During the first trapping period, June 10 to September 9, 2020, I found that summer morph *D. sukuzii* trap preference differed between sex, morph, and season in Michigan woodlots. I observed trap type and position preferences varied from females to males as well. The Dome:Ground and Dome:Leaf litter traps were preferred by summer morph male *D. sukuzii* (up to 12 times more males than females), in August 2020, whereas summer morph females were captured in higher quantities in the Cup:Hanging traps (up to 5 times more females than males) (Table 3.1, Fig. 3.4).

One potential explanation for male *D. sukuzii* capture increasing in the dome traps during the fall months, is that smaller bodied males were seeking higher humidity environments (under the leaf litter) to conserve body moisture. Previous research has demonstrated that males desiccate more quickly than females (Fanning et al. 2019; Savage et al. 2021). Alternatively, this shift in trap preference could also be explained by an unknown behavioral sexual dimorphism. Previous research has shown that female *D. sukuzii* are deterred by geosmin, an earthy/soil volatile, but data for males is

lacking (Wallingford et al. 2016; Kirkpatrick et al. 2018). It would be useful for other researchers to evaluate *D. suzukii* preference for geosmin for both females and males.

Second Peak: September 16 – December 16, 2020 (Summer Morph D. suzukii Capture)

During the second trapping period for summer morph *D. suzukii*, September 16 to December 16, 2020, I found that trap position influenced trap captures for 5 of the 6 significant time points within that time period (Table 3.2, Fig. 3.5). In September and October, the Cup:Hanging traps outcompeted the other traps frequently and captured up to 4 times more than the other traps. I also found that sex influenced trap capture, where I caught up to 2 times more summer morph males than females during this trapping period (Table 3.2, Fig. 3.5). As seasons changed from fall to winter, in November and December, trap capture among the four traps was comparable for summer morph *D. suzukii*. It is interesting to note that in warmer seasons the Cup:Hanging traps outcompeted the other traps, but in cooler seasons traps had no significant differences in capture. This would suggest that the Cup:Hanging traps are not the most effective trap during colder periods.

Second Peak: September 16 – December 16, 2020 (Winter Morph D. suzukii Capture)

During the second trapping period for winter morph *D. suzukii*, September 16 to December 16, 2020, trap position and/or sex of fly influenced trap captures for 7 of 8 significant time points within that period (Table 3.3, Fig. 3.6). Initially, I observed the Cup:Hanging and Cup:Ground traps performed similarly with higher capture compared to the dome traps, then as seasons changed from fall to winter, at least one of the ground traps had higher capture compared to the Cup:Hanging traps. In September and

October 2020, the Cup:Hanging traps captured up to 60 times more winter morph *D. suzukii* than the dome traps. The Cup:Hanging traps captured up to 2 times more winter morph *D. suzukii* than the Cup:Ground traps. In November and December 2020, the Cup:Ground traps captured up to 56 times more winter morph *D. suzukii* than the Cup:Hanging traps. Usually, I captured more females than males from September to December 2020. It is interesting to note, when observing summer morph data, generally I captured more males than females, and when observing winter morph data, I typically captured more females than males.

One potential explanation for the Cup:Hanging traps outcompeting the other traps in the fall, but not in the transition to winter, could be energy reserves. It has been reported that *D. suzukii* winter morphs have an increased energy reserve compared to summer morphs (Dalton et al. 2011; Alford et al. 2019; Enriquez and Colinet 2019). With summer morphs lacking this increase in energy reserves, saving their energy, and only going into traps that are easily accessible is a logical hypothesis. Alternatively, morph of *D. suzukii* could also influence trap attractiveness. Previous researchers have found evidence for summer morph *D. suzukii* exploring various trap heights in orchards, so it is possible the same concept could be applied to woodlots (Tonina et al. 2016; Rice et al. 2017). Time of day could also potentially influence what height strata *D. suzukii* are exploring as well (Jaffe and Guédot 2019). Furthermore, Toxopeus et al. (2016) reported a 5°C shift for developmental thresholds in summer and winter morphs. Hernandez (Ch. 2) found movement thresholds for summer and winter morph *D. suzukii*, where only 1% of summer morph would walk at $0.9^{\circ}\text{C} \pm 0.2$, and for 1% of winter morph at $-3.1^{\circ}\text{C} \pm 0.3$. Lastly, I could have captured more females than males

because they are bigger in body mass, potentially increasing their longevity through the winter.

Third Peak: May 13 – September 16, 2020 and Fourth Peak: November 11 – December 16, 2020 (Non-target Drosophilid Capture)

Like *D. sukuzii*, non-target drosophilid capture varied by trap height and seasonal period. Non-target drosophilid capture from May 13 to September 16, 2020 was significantly higher for the Cup:Hanging traps compared to one or more of the ground traps by a factor of 2-89 times (Cup:Ground, Dome:Ground, Dome:Leaf litter) (Table 3.4, Fig. 3.7). Furthermore, the two dome traps typically caught the least non-targets during this period. The highest capture I observed during this time period was 33.58 ± 11.42 mean non-target drosophilids captured per day on June 12, 2020, in the Cup:Hanging traps. During November 11 to December 16, 2020, the Cup:Ground traps had the highest non-target capture ranging from 1.33-49 times more and differed significantly from at least one of the other traps (Cup:Hanging, Dome:Ground, Dome:Leaf litter) at every time point (Table 3.5, Fig. 3.8). The highest capture I observed during this time period was 5.95 ± 2.77 mean non-target drosophilids captured per day on December 2, 2020, in the Cup:Ground traps. Thus, during warmer seasons in Michigan, the Cup:Hanging traps were the least selective, and during cold seasons, the Cup:Ground traps were the least selective. However, the magnitude of capture was far greater in the Cup:Hanging traps compared to the Cup:Ground traps.

A potential explanation for the seasonal differences in non-target captures is that temperature influences locomotion of *Drosophila spp.*, and reduced temperatures may

alter their behavior. Dillon and Frazier (2006) demonstrated that temperature was the most influential factor for the walking pace of *Drosophila melanogaster* (Meigen). Additionally, others have shown that as exposure to cold temperatures increased, willingness to climb surfaces also decreased in *D. melanogaster* (Garcia and Teets 2019). Alternatively, as I suggested for *D. suzukii* above, other *Drosophila spp.* may explore different height strata, and that could vary with temperature as well.

Conclusions

Overall, I observed differences in trap captures between summer and winter morph *D. suzukii* and between females and males. From each time period it is evident that the Cup:Hanging traps were not the most effective trap during Michigan's early winter months, and this trend was clear for both summer and winter morph *D. suzukii*. In November and December 2020, I found that the Cup:Hanging traps performed similarly among the other traps for summer morph *D. suzukii* capture and that the Cup:Ground traps outcompeted the Cup:Hanging traps for winter morph *D. suzukii* capture. Furthermore, during my entire trapping period, I captured more male summer morphs and more female winter morphs in total. Further research is needed to explore how sex and morph affect trap preference. Researchers should also explore the influence humidity has on *D. suzukii* trap placement preference. Lastly, I found that the Cup:Hanging traps are the least selective trap for non-target drosophilids for the majority of each year, capturing up to 33.58 ± 11.42 flies per day. In early winter months, the Cup:Ground traps captured the most non-target drosophilids, at the much lower rate of 5.95 ± 2.77 flies per day. It would be beneficial if other researchers

evaluated these trapping methodologies outside of Michigan, to see if these behavioral trends are similar in other states or countries.

In conclusion, my experiments revealed that although the standard cup trap, “Cup:Hanging”, used for wild *D. suzukii* capture may be preferred for its simple assembly and low-cost materials (Lee et al. 2012), its performance is likely affected by seasonal conditions and the morph being targeted. For example, beginning in mid-August, traps placed on the ground, or even under leaf litter, may be more suitable for monitoring *D. suzukii* population levels. In the laboratory, winter morphs were 6 times more likely to be captured in a floor trap than a hanging trap even under warm (24°C) conditions. In woodlots in early winter months (November and December in Michigan), the Cup:Ground traps captured 56 times more winter morph *D. suzukii* than the other traps. Additionally, the Cup:Hanging traps were the least selective for *D. suzukii* and captured up to 89 times more non-target drosophilids compared to the Cup:Ground, Dome:Ground and Dome:Leaf litter traps. Optimal placement of traps would provide growers and researchers a more accurate estimate of population levels of *D. suzukii*. This information could lead to better integrated pest management strategies, and a deeper understanding of behavioral differences between summer and winter morph *D. suzukii*.

Chapter 4: Conclusions and Future Directions

Drosophila suzukii is an invasive vinegar fly that rapidly became a major pest in small fruit crops in the United States including Michigan. For my thesis, I described the thermal thresholds for terrestrial locomotion in summer and winter morph *D. suzukii* and investigated how trap placement influences monitoring of *D. suzukii*. While numerous studies have examined effective monitoring practices for summer morph *D. suzukii*, trapping winter morphs has been neglected. Because winter morph *D. suzukii* are a likely source for subsequent seasons' infestation (Panel et al. 2018), improving monitoring during colder seasons may improve our ability for time management actions in regions with a temperate climate. Furthermore, knowing the temperature ranges when *D. suzukii* is active, leads to understanding how trap captures translate to population activity.

In Chapter 2, I observed thermal thresholds for terrestrial locomotion in summer and winter morph *D. suzukii*. My specific objectives for this chapter were to determine at what temperature terrestrial locomotion was inhibited for summer and winter morph *D. suzukii*. I hypothesized that *D. suzukii* would show a significant decline in the proportion that walk at temperatures below 5°C. Furthermore, I hypothesized that morph would influence thermal thresholds, with winter morph *D. suzukii* walking at cooler temperatures compared to summer morph. My research showed that as temperature decreased, *D. suzukii*'s reluctance to walk increased (Table 2.1, Fig. 2.1A-B). I found that morph of *D. suzukii* does influence thermal thresholds, as there was up to a 3.5°C difference between willingness to walk at several temperatures. Furthermore, the biggest difference observed was for 25% movement of summer and winter morph *D.*

suzukii. Only 25% of summer morph would walk at 3.9°C, compared to winter morph at 0.4°C. Interestingly, other research has shown a 5°C shift in developmental thresholds for *D. suzukii* (Toxopeus et al. 2016). Here I found a 3.5°C shift for thermal thresholds, which shows clear behavioral differences between the two morphs of *D. suzukii*. However, there was a discrepancy found in the model for winter morph *D. suzukii*. I found 0% movement at -1.5°C, whereas the model predicted 1% movement at -3.1°C for winter morphs. A potential explanation for this discrepancy is under sampling, each temperature was only replicated 3 times. Additionally, the 0% movement I found at -1.5°C was observed for all 3 replicates, meaning there wasn't a range of data for the line of best fit to follow.

Overall, my research shows that there are behavioral differences between summer and winter morph *D. suzukii*, and differences between the two should be further investigated to expand understanding of this detrimental pest. A potential avenue to explore would be discovering the thermal thresholds for summer and winter morph *D. suzukii* in flight. Previous research with *Drosophila melanogaster* has shown that diet can influence their cold tolerance (Littler et al. 2021). With this knowledge in a species close to *D. suzukii*, it would also be beneficial to evaluate how fasting and different diets influences their thermal tolerance.

In Chapter 3, I observed both laboratory reared and wild *D. suzukii* response to monitoring traps. Specifically, my objective was to determine how summer and winter morph *D. suzukii* respond to monitoring trap height. I hypothesized that the two morphs would respond differently to monitoring traps, with summer morph favoring aerial traps and winter morphs favoring terrestrial traps. I began in the laboratory, with choice tests

between a cup and a dome trap baited with sugar yeast (Ch. 3, Hernandez, 2021). I found that morph of *D. suzukii* did influence trap capture, with winter morph being on average 6 times more likely to be captured in a ground trap, whereas summer morphs were approximately 49% less likely to be captured in a ground trap (Fig. 3.2). My summer field experiment validated my laboratory experiment, where over 80% of the flies captured were in the hanging cup trap (Fig. 3.3). The ground and hanging dome traps captured significantly less flies, which could be explained by a difference in bait volumes between the domes and cup traps.

In my fall/winter field experiment, I found time periods where I did not capture any *D. suzukii*. From January to June, in both 2020 and 2021, I did not capture any *D. suzukii*. However, I consistently captured other *Drosophila* species since the traps were deployed in June 2020. From June to August 2020, summer morph *D. suzukii* were typically captured in aerial cup traps (Table 3.1). However, in mid-August 2020 I observed a shift, where the dome traps on or under leaf litter were capturing more *D. suzukii* than the other traps (Fig. 3.4). This shift was caused by a significant increase in male capture. Up until that point I had considered removing them from the study because of low capture. The shift towards ground traps could be explained by body mass. Male *D. suzukii* are smaller in body size compared to females, so they could be seeking higher humidity environments to conserve body moisture. Future research should explore this hypothesis.

I found that season and morph were the most influential factors for trapping *D. suzukii*. For summer morph *D. suzukii*, aerial cup traps captured up to 4 times more flies in late fall, and in early winter all traps performed similarly (Table 3.2, Fig. 3.5). For

winter morph *D. sukii*, during the early winter months in Michigan, ground cup traps caught 56 times more winter morph *D. sukii* compared to the other traps (Table 3.3, Fig. 3.6). Thus, for warm seasons in Michigan, May to August, an aerial cup trap should be used, with mainly summer morphs being captured. In mid-August, traps placed on the ground or under leaf litter could give more accurate population estimates. During cold seasons in Michigan, November and December, ground cup traps should be used, and summer and winter morphs will be captured. Growers and researchers can use this information to improve overwintering trapping of *D. sukii*.

When evaluating non-target drosophilid capture in warm seasons, I found that the aerial cup trap was most frequently the least selective trap, having higher non-target captures compared to *D. sukii* capture (Table 3.4-5, Fig. 3.7-8). The aerial cup traps captured more non-targets by a factor of 89. Traps on the ground (ground cup, ground dome, and leaf litter dome) typically captured fewer non-target drosophilids. Lee et al. (2012) also reported that aerial cup traps were not selective, with only ~30% of *Drosophila spp.* captured being *D. sukii*. During cold seasons, the ground cup trap had the most non-target drosophilids, although fewer were captured in cold seasons than warm. For example, aerial cup traps highest mean capture per day was 33.58 ± 11.42 and ground cup traps was 5.95 ± 2.77 for non-target drosophilids.

Overall, my research suggests that there are clear differences between summer and winter morph *D. sukii*. Behaviorally, I found a 3.5°C shift for a lower locomotion temperature threshold between summer and winter morph *D. sukii*. Additionally, I found differences in preference of trap position, with summer morph *D. sukii* generally being captured more in aerial cup traps, and winter morphs being captured more in

ground cup traps. Based on my data, when trapping for overwintering *D. suzukii*, a cup trap placed on the ground will be the most effective and will capture more summer and winter morphs. Lastly, I found that the aerial cup traps are least selective for *D. suzukii* during warm seasons. My research helps the scientific community understand this detrimental pest more thoroughly and could improve overwintering trapping methodologies.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museums 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: 2021-06

Author and Title of thesis:

Author: Ariana Hernandez

Title: Exploring ecological parameters of winter morph spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae)

Museum(s) where deposited:

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

Specimens

Family	Genus-species	Life Stage	Quantity	Preservation
Drosophilidae	<i>Drosophila-suzukii</i>	adult	5 Female	70% EtOH
Drosophilidae	<i>Drosophila-suzukii</i>	adult	5 Male	70% EtOH
Drosophilidae	<i>Drosophila-suzukii</i>	adult	5 Female	Pinned on a point
Drosophilidae	<i>Drosophila-suzukii</i>	adult	5 Male	Pinned on a point

LITERATURE CITED

LITERATURE CITED

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