IMPROVING MONITORING OF SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*, DIPTERA: DROSOPHILIDAE) IN MICHIGAN FRUIT CROPS

By

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ABSTRACT

IMPROVING MONITORING OF SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*, DIPTERA: DROSOPHILIDAE) IN MICHIGAN FRUIT CROPS

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The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is currently one of the most important invasive insects with a global distribution. Methods for detection and trapping *D. suzukii* have not yet been optimized for this devastating pest of berry crops and cherries. Laboratory assays quantifying alightment on sticky, odorless disks of various colors, *D. suzukii* consistently alighted most on red, purple, and black disks. Under field conditions, baited, red sticky sphere traps consistently captured 3-6x more *D. suzukii* than clear deli-cup traps baited with the same lure. In another test, baited, red sphere traps captured significantly more *D. suzukii* than deli-cup traps baited with a lure or with yeast sugar bait, and baited red panel traps captured significantly more *D. suzukii* than deli-cup traps baited with a lure in cherry orchards. In raspberry high tunnels, baited red sphere traps captured significantly more *D. suzukii* than deli-cup traps baited with the same lure. In cherry orchards and raspberry high tunnels, baited red panel traps and combination panel plus sphere traps captured significantly more *D. suzukii* than deli-cup traps and yellow panel traps when all traps were baited with the same lure. Baited red traps consistently captured more *D. suzukii*, demonstrating traps integrating both visual and olfactory cues are superior monitoring tools and a simple, dry trap requires far less labor and maintenance than deli-cup traps containing liquid bait. Central-trap, multiple release-recapture experiments used to interpret *D. suzukii* captures in a monitoring trap in tart cherry orchards revealed the plume reach was short (< 3 m) and the maximum dispersive distance for *D. suzukii* was about 90 m, yielding a trapping area of 2.7 ha. Capturing one *D. suzukii* in a monitoring trap translates to approximately 192 *D. suzukii* per trapping area, indicating that control actions should be implemented if the fruit is at a vulnerable stage. These data provide the first information about the dispersal distance and monitoring trap efficacy, and capture data per single monitoring trap can now be used to estimate absolute pest density in cherries. Between two seasonally induced morphs, responses of female *D. suzukii* to six volatiles were evaluated separately for electroantennogram (EAG) and behavioral assays. Isoamyl acetate, acetic acid and geosmin elicited significantly different responses from summer morphs compared with those of winter morphs, and winter morphs exhibited a reduced antennal response to the volatiles overall. As determined by scanning electron microscopy, summer morphs had more basiconic sensilla, but not statistically so. Geosmin and bornyl acetate elicited significantly different behavioral responses between the two morphs in no-choice tests. T-maze assays with geosmin further revealed significantly different responses between the morphs with summer morphs showing aversion and winter morphs no aversion to geosmin. Overall, these studies demonstrated the responses of two seasonally induced morphs of *D. suzukii* are different, and future studies are justified to further understand how these differences contribute to pest management for the two seasonal *D. suzukii* morphs.

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CHAPTER 1: BIOLOGY, ECOLOGY, AND MANAGEMENT OF *DROSOPHILA SUZUKII*

INTRODUCTION

History and distribution of *Drosophila suzukii.*

The spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), endemic to Japan, is also present in the eastern part of China (Peng 1937), Taiwan (Lin et al. 1977), North and South Korea (Kang and Moon 1968), Thailand (Okada 1976), and India (Parshad and Duggal 1965). *Drosophila suzukii* was collected for the first time in the United States in Oahu, Hawaii in 1980, where it was reported on several of the Hawaiian Islands but did not cause any damage (Hauser 2011). The North American invasion was first recorded August 2008 (Bolda 2010) when a sample drosophilid fly collected from a raspberry field in Santa Cruz County, California was sent to the Entomology Department of the Plant Pest Diagnostics Center of the California Department of Food and Agriculture (CDFA) in Sacramento (Hauser 2011). This sample was quickly identified as a member of the genus *Drosophila*, and since no members of this species were considered to be pests, the specimen was not identified to species (Hauser 2011). However, at the time the sample was submitted, the raspberry and strawberry crop were infested with *D. suzukii* larvae that subsequently caused significant crop damage (Hauser 2011). In the spring of 2009, the CDFA received several records of larvae found in healthy cherries. The infestation was blamed on the western cherry fruit fly (*Rhagoletis indifferens*), and the larvae inside the cherry that were clearly drosophilids were thought to be secondary invaders (Hauser 2011). After numerous samples of drosophilid-infested fruit were submitted to the CDFA, suspicion was raised that a normally harmless *Drosophila* species was the primary cause for the infestation (Hauser 2011). Adult specimens were finally turned in to the CDFA laboratory, and with the aid of morphological characters, the specimens were identified to species level as *Drosophila suzukii* (Hauser 2011).

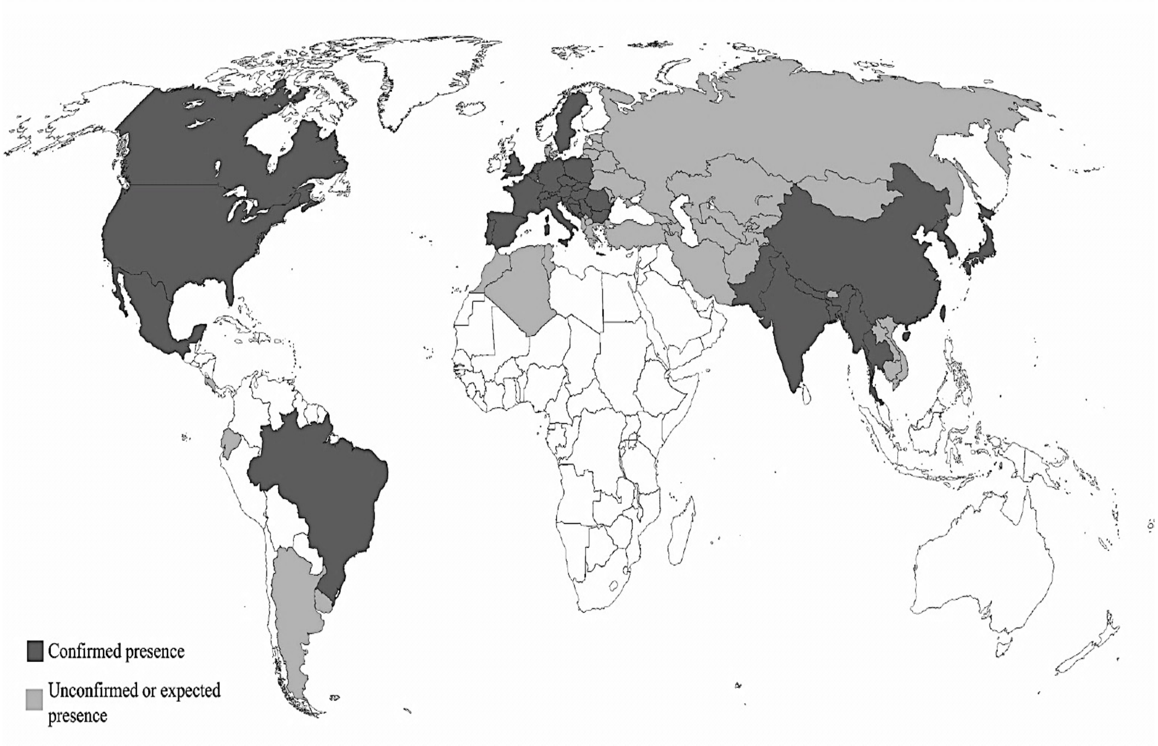

Figure 1.1. Current worldwide *D. suzukii* distribution map (as of May 2015). Countries are indicated as follows: (1) *D. suzukii* presence has been confirmed (dark gray), and (2) *D. suzukii* unconfirmed or expected presence because of geographic proximity, or because presence has not been confirmed after initial record (light gray) (Asplen et al. 2015).

By 2009, *D. suzukii* had spread to more than 20 California counties, and was also found in Oregon, Washington, and British Columbia (Canada), as well as in Florida (Hauser 2011). As of 2012, it has been detected in a total of 39 US states (Lee et al. 2012), and has rapidly spread across Europe (Fig. 1.1). As of 2012, it has been detected in Spain, Italy, France (Calabria et al. 2012), Switzerland, Austria, Germany (Asplen et al. 2015), Belgium (Mortelmans et al. 2012), Croatia (Milek et al. 2011), and Slovenia (Seljak 2011). *Drosophila suzukii* has also been detected in Mexico and South America as of 2012 (Deprá et al. 2014) (Fig 1.1).

Biology of *Drosophila suzukii.*

Identification, lifecycle and reproduction. Adult *D. suzukii* are 2-3 mm long, have red eyes, a pale brown or yellowish brown thorax, and black stripes on the abdomen (Cini et al. 2012). As pictured below in Figure 1.2, key morphological characteristics for identifying *D. suzukii* males are a conspicuous dark spot on the leading edge of each wing along with two sets of black tarsal combs on the first and second tarsal segments (Fig. 1.2) (Hauser 2011). These two characteristics are readily observable and make identification of males relatively easy. Females possess a large serrated ovipositor that is 6-7 times as long as the spermatheca (Hauser 2011), and is the identifying feature that separates them from other species of Drosophila.

The pre-oviposition period for females ranges from 1-3 d. When a female reaches maturity and begins egg laying, the ovipositor of the female (Fig 1.2) penetrates the skin of the ripening fruit and eggs are deposited, develop, and hatch within the fruits (Walsh et al. 2011). A female lays about 1-3 eggs per oviposition site, averaging 380 eggs in her lifetime (Walsh et al. 2011). Larvae develop through three instars while feeding upon the host fruit until they exit to pupariate, which occurs partially or fully outside of the fruit (Asplen et al. 2015). Development is temperature-dependent; total time from egg to adult can range anywhere from 10-79 days (Tochen et al. 2014). Depending on the environmental conditions, up to 13 generations can be completed per year, with generations often overlapping (Tochen et al. 2014).

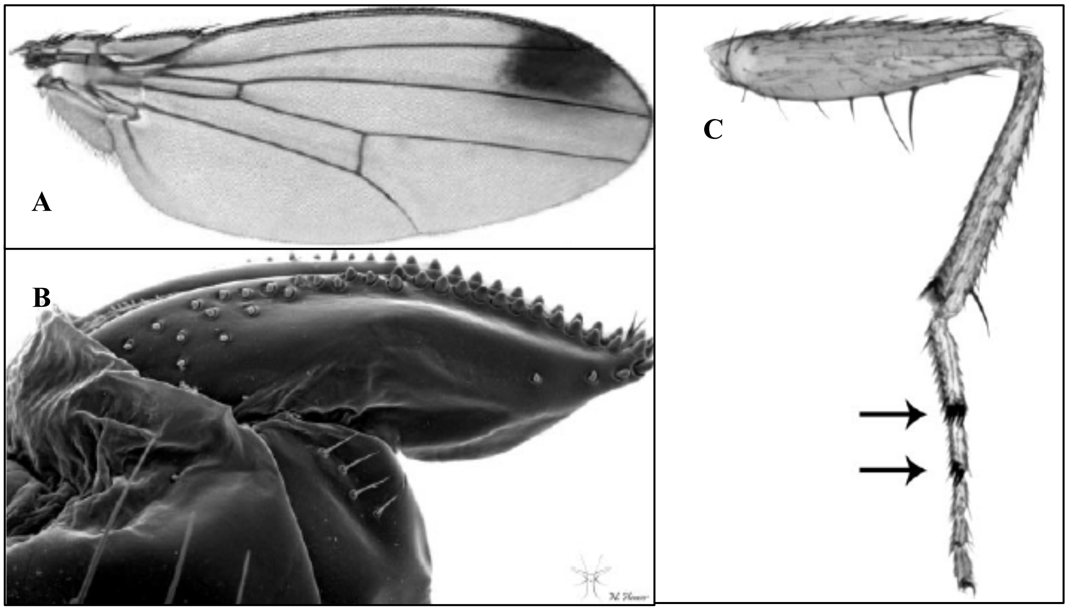

Figure 1.2. Key characteristics for identification of *Drosophila suzukii* (Hauser 2011): (A) black spot on leading edge of male wings, (B) female serrated ovipositor and (C) male tarsus with combs.

Overwintering biology and seasonally-induced morph. Insects have evolved a number of physiological mechanisms for coping with the effects of low temperature, such as reproductive diapause, cold hardiness, and improved tolerance to environmental stressors such as extreme cold (Kimura 1988; Hoffman et al. 2003; Teets and Denlinger 2013). Many species of *Drosophila* are believed to overwinter in cold climates as adults under leaf litter where they are protected from extreme cold by a layer of snow (Hoffman et al. 2003; Stephens et al. 2015). Their overwintering strategy may include reproductive diapause, limiting their reproduction to favorable conditions and directing their resources to winter survival (Hoffman et al. 2003; Wallingford and Loeb 2016). Additionally, environmental signals such as shortening day lengths and gradually decreasing temperatures can trigger seasonal cold-hardening adaptations such as rapid cold-hardening or gradual acclimation experienced during development (Lee et al. 1987; Teets and Denlinger 2013). Developmentally acclimated flies would be the most prepared to survive the starvation and freeze stress associated with overwintering (Wallingford and Loeb 2016). *Drosophila suzukii*, has a well-described winter morph that has a darker pigmented body and longer wings than the summer morph (Stephens et al. 2015; Shearer et al. 2016). In the laboratory, this morph occurs when larvae are subjected to colder temperatures and shorter day length (Shearer et al. 2016). Additionally, *D. suzukii* winter morphs have various up-regulated and down-regulated genes compared to *D. suzukii* summer morphs that are responsible for female diapause, synthesis of cryoprotectants and chitin, and metabolic processes, among others (Shearer et al. 2016; Wallingford and Loeb 2016).

Our current knowledge on this morph is limited to physiological changes and oviposition capacity (Stephens et al. 2015; Jakobs et al. 2016; Shearer et al. 2016; Wallingford and Loeb 2016); little is known and understood about the overwintering behavior and capabilities of *D. suzukii*. Despite predictions that *D. suzukii* would not be able to survive in colder regions of northern latitudes such as those of Michigan, Oregon, and Canada (Kimura 2004; Dalton et al. 2011), *D. suzukii* has proven to be a successful and established pest in a wide range of environments including the harsh climates of northern regions (Isaacs et al. 2010; Shearer et al. 2016).

Feeding habits. *Drosophila suzukii* is a highly polyphagous pest of small fruits, stone fruits, and a variety of wild, ornamental, and uncultivated hosts (Walsh et al. 2011; Lee et al. 2015). Fruits are generally considered susceptible to *D. suzukii* when they begin to ripen and color, with adults preferring ripe to unripe crop hosts (Lee et al. 2011b; Lee et al. 2015); however *D. suzukii* can develop in unripe fruits (Walsh et al. 2011). It is likely that non-crop hosts play a vital role when cultivated hosts are unavailable (Diepenbrock et al. 2016).

Thin-skinned berries such as cane berries, blueberries, strawberries, and grapes (wine and table, depending on cultivar) are common crop hosts for *D. suzukii*. Michigan is one of the top producers of blueberries; in 2016, 110 million pounds of blueberries were harvested on 20,300 acres contributing nearly $130.4 million to the state's economy (Michigan Department of Agriculture – MDA, 2016). The raspberry and blackberry industry in Michigan is fairly small, but consumers still enjoy these fruits purchased at retail stores, you-pick farms, farmers markets, or at Michigan restaurants. Michigan has 13,100 acres of vines with about 3,050 acres devoted to wine grape production at more than 100 commercial wineries making Michigan the eight-largest grape producing state in the nation (MDA, 2016).

In the United States, raspberries appear to be the preferred host for *D. suzukii* (Bellamy et al. 2013; Burrack et al. 2013), whereas some other fruits such as cranberries and peaches are unsuitable unless previously damaged (Steffan et al. 2013; Stewart et al. 2014). Some apples, pears, persimmons, greenhouse mandarins and tomatoes can also be infested if previously split or damaged, but *D. suzukii* is not considered a primary pest of these crops (Lee et al. 2011a).

Stone fruits such as cherries, apricots, and plums are also considered hosts for *D. suzukii* (Asplen et al. 2015). Michigan produces more cherries than any other state, and provides 75 percent of the tart cherries and 20 percent of the supply of sweet cherries produced in the United States (MDA, 2016). Michigan is the largest producing region in the world for Montmorency tart cherries, the variety known as “America’s super fruit,” (MDA, 2016).

Integrated pest management of *Drosophila suzukii.*

Integrated pest management (IPM) programs emphasize ecosystem-based strategies that provide economical long-term solutions to pest problems utilizing a variety of tools together, such as behavioral manipulation, cultural practices, biological control, and chemical practices, to suppress a pest population below an economic injury level. Integrated pest management practices may include: use of mating disruption, the disruption of pest habitat, adjusting cultivation or sanitation techniques, and other strategies that encourage natural enemies and limit pest populations. Insecticide treatments alone may not always be economically optimal or ecologically acceptable. Methods of pest control require a precise understanding of the target insect’s biology, physiology, and behavior, among others. Shifting management practices from relying solely on insecticides towards IPM necessitates the combination of different methods together that minimize harmful effects on the environment and economic losses to growers (Pedigo and Rice 2014).

Monitoring for pests in crops. Basic tactics of integrated pest management have been developed to defend crop plants against the ravages of pests (Newsom 1980). Early and accurate detection of pests is a cornerstone of management programs and a common monitoring approach is to use traps to sample and collect pests; trap captures informs when pests are present. If the number of pests captured in traps exceeds a predetermined expert judgment-based injury level, then control methods should be taken to prevent damage or unacceptable infestation in crops.

Traps are traditionally devices that limit the displacement of previously free-ranging insects in space through time (Miller et al. 2015). A key feature of trapping is the intersection of a trap with the moving insects that are foraging in the environment. Typically, traps are stationary and the moving insects that are foraging for resources must meet the trap after a chance encounter with attractive cues from the trap. Once the foraging insects encounter cues from the trap that guide them to the trap, they must then be retained by the trap for the trap to be considered efficient. Trap retention must match the behavior of the responders to ensure they remain trapped, and is often the easiest mechanism of the trap to modify and improve.

There are many environmental factors that influence the probability of an insect finding the trap. For example, the plume reach for olfactory attractants varies with wind speed, and there are a number of environmental factors that can influence an insects flight behavior and willingness to search and forage for resources such as temperature, humidity, wind speed, and time of day. If environmental conditions permit an insect to respond and arrive at a trap, they are likely to remain favorable for the short time period it takes for an insect to engage the trapping mechanism (Miller et al. 2015). However, efficiencies across trap types vary with the degree to which their engineering matches the tendency of the responder to engage the capture mechanism (Muirhead-Thomson 1991).

Understanding the trap efficiency, maximum dispersal distance for the insect, and the trapping area over which the trap is sampling from is key. One method for doing this is to use a mark-release-capture method, where the insects are uniquely marked to correspond to a release point some distance away from a central trap. According to the analytical methods of Miller et al. (2015), the proportion of marked insects that are captured in the central trap can be plotted on a graph against the release distance to derive information about the plume reach.

An effective trap for monitoring should be specialized for that specific pest insect. To attract the target species, the trap has to have optimal visual cues by being the most attractive color, size, and shape. Moreover, the trap must retain the insects once ensnared and due to trap specificity, should catch few nontarget insects. Retention of insects in traps is accomplished through: 1) the use of dry collection traps of various designs that allow entry into the trap, but prevent the insect from exiting the trap, 2) sticky adhesives that prevent the insect from leaving once they alight on the material, 3) drowning solutions, 4) toxicant sources inside the traps that cause death of the insects that enter the traps.

Chemical lures and attractants. Olfactory cues from fruit play important roles in resource recognition for polyphagous insects like *D. suzukii*. They are attracted to fermented sweet materials such decomposing fruits, but also wines, vinegars, and fermentation volatiles such as acetic acid and ethanol (Cha et al. 2012). A chemical lure in an appropriate trap provides growers with an improved means to monitor their crops and to determine the presence and changes in populations, and make management decisions such as when to apply insecticide sprays. Early recommendations for trapping of *D. suzukii* included protocols that used apple cider vinegar or grape wine as an attractant in traps (Beers et al. 2010). Both bait types were successful in luring *D. suzukii* to traps, but improvements needed to be made in the bait or lure used to improve detection efforts and increase trap selectivity.

Cha et al. (2013) found that out of the 13 antennally active chemicals, acetoin, ethyl lactate, and methionol increased *D. suzukii* response to a mixture of acetic acid and ethanol in field trapping experiments. A five-component blend of acetic acid, ethanol, acetoin, ethyl lactate and methionol was as attractive as the starting mixture of wine and vinegar in field tests conducted in Oregon and Mississippi (Cha et al. 2013). These results indicate that acetic acid, ethanol, acetoin, and methionol are the key olfactory cues for *D. suzukii*, and a blend of these four components could be used in a chemical lure for monitoring and management (Cha et al. 2013). The four-component chemical lure was more selective for *D. suzukii* compared with fermentation baits or food baits as attractants, and reduced the number of nontarget drosophilid flies, muscid flies, cutworm and armyworm moths, and pest yellow jackets captured in traps (Cha et al. 2014).

Burrack et al. (2015) compared four noncommercial bait mixtures and two configurations of a commercially available four component synthetic lure across 10 locations in 10 states. The baits they selected, which were apple cider vinegar, a yeast and sugar mixture (active dry yeast, sugar, water, and unscented dish soap), a fermenting bait cup (water, whole wheat flour, sugar, apple cider vinegar, ethanol, and unscented dish soap), and Droskidrink (apple cider vinegar, red wine, and sugar) had not previously been directly compared to one another, but were commonly recommended for use in monitoring programs in the United States and Europe (Burrack et al. 2015). Across all locations, the fermenting bait cup and the synthetic lure suspended over apple cider vinegar captured the most *D. suzukii* overall, but the synthetic lure suspended over an unscented drowning solution generally captured a higher proportion of *D. suzukii* to non-target drosophilids and was most closely related to the rate of fruit infestation than other attractants (Burrack et al. 2015). Abraham et al. (2015) investigated the behavioral responses to volatiles from blueberry, cherry, raspberry, and strawberry fruit extracts, and identified antennally active compounds from the most attractive fruit extract. The attractiveness of the fruit extracts ranked raspberry as the most attractive extract, followed by strawberry, blueberry, and cherry (Abraham et al. 2015). In GC-EAD experiments, Abraham et al. (2015) found 11 raspberry extract volatiles that consistently elicited antennal responses in *D. suzukii*. This is the first report of a study that identified a blend of fruit volatiles that are attractive for *D. suzukii*, raising the possibility of improved monitoring tools and synthetic lures. To further improve *D. suzukii* attractants, olfactory cues such as pheromones specific to *D. suzukii* should be explored to determine if these types of olfactory cues can be incorporated into a lure for use in a trap to improve trap selectivity and decrease non-target captures in traps.

Chemical control. The zero tolerance of the fresh and processed fruit markets for insect infestation of fruit, coupled with high populations of *D. suzukii* found in and around crop fields, have resulted in growers taking a proactive approach to protecting their crops. Detection of adult *D. suzukii* flies in monitoring traps when fruit becomes susceptible to infestation initiates repeated applications of foliar insecticides (Van Timmeren and Isaacs 2013). Current management practices for *D. suzukii* rely on spraying broad-spectrum insecticides starting when fruit becomes susceptible to infestation by *D. suzukii* and continuing through harvest. These current practices are unsustainable, harmful to beneficial arthropods, and expensive for growers. Alternatives to chemical insecticides are necessary and a greater understanding of behavior is critical to developing management strategies that might contribute to the sustainable management of this devastating pest.

Objectives.

The overall goal of this research was to develop behavioral manipulation methods that would lead to improved management of *D. suzukii* populations in fruit crops. To investigate this broad question, I undertook a set of investigations into the behavior and development of monitoring tools for *D. suzukii*. The first objective was to determine the visual responses of *D. suzukii* to color in the laboratory, and response to various trap designs within crops to develop a trap optimized for capturing *D. suzukii*. If this approach were successful, an optimized trap for capturing *D. suzukii* while decreasing captures of non-target insects would further improve monitoring efforts and improve reliability of monitoring traps. The second objective aimed at understanding movement of *D. suzukii*, the plume reach and trapping area for a monitoring trap, and how captures in a monitoring trap can be used to determine absolute pest density within that trapping area. If successful, this knowledge would be indispensable for developing a threshold that growers could use reliably use to determine when control actions should be taken based on captures in traps. My third objective was to understand differential behaviors and responses between two seasonally induced morphs of *D. suzukii* to ecologically relevant volatiles. If successful in understanding the differences, a monitoring trap could be optimized for capturing the overwintering morph of *D. suzukii* in the winter and early spring. There are strong management implications for increased knowledge on the winter morph of *D. suzukii*, particularly because we expect that the surviving populations of this pest are quite low given low spring adult captures. This provides a unique opportunity to exploit their winter or spring resources and further reduce the surviving population by targeting the winter morph for control after the population bottleneck.

CHAPTER 2: ALIGHTMENT OF SPOTTED WING DROSOPHILA (DIPTERA: DROSOPHILIDAE) ON ODORLESS DISKS VARYING IN COLOR

INTRODUCTION

Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a recently introduced and highly invasive pest in the United States that destroys soft-skinned fruits (Hauser 2011). A serrated ovipositor allows females to deposit eggs into ripening fruits prior to harvest (Lee et al. 2011). Such *D. suzukii* injury facilitates infestation by other *Drosophila* species (Walsh et al. 2011) and rapid decomposition of fruit. Left unmanaged, *D. suzukii* is estimated to cause annual losses of 860 million dollars total to black berries, raspberries and cherries in the Western United States (Hamby et al. 2012). Similar rates of loss are spreading across all USA small fruit production. Effective management of insect pests like *D. suzukii* requires efficient monitoring usually via trapping (Beers et al. 2011, Cha et al. 2012, Landolt et al. 2012a, Lee et al. 2012). Early and accurate detection will be the cornerstone of *D. suzukii* management.

Understanding a fly's basic behavioral traits can facilitate improvements in trapping. For example, spherical shape and red or dark color provided the best visual target for the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Prokopy 1968). By contrast, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is maximally attracted to black or yellow spheres (Nakagawa et al. 1978). Shape, color, and odor of fruits are expected to be key determinants of ovipositional site selection by *D. suzukii*, as they are for many other insects (Fletcher 1987, Harris and Miller 1988, Renwick and Chew 1994).

The ability of many insects to discriminate between different colors has been established beyond a doubt (Burkhardt 1964). Menzel (1979), Briscoe and Chittka (2001), and Stavenga (2002), among others, have reviewed the evolution of color vision in insects and the ability of invertebrates to perceive color, including reds. Although many moths lack photoreceptors sensitive to longer wavelengths, most day-active insects possess photoreceptors that cover the whole spectrum sensed by humans (Wakakuwa et al. 2004); as many as six different receptor types can be found in some species of Diptera (Hardie 1986).

The present investigation primarily answers the question of what type of visual cue best promotes *D. suzukii* alightment on a small sticky surface with the applied aim of optimizing trap color. This is our first step in a systematic investigation of the optimal sensory parameters for *D. suzukii* trapping. Here the color of a visual target of arbitrarily chosen shape emitting no host odor was varied with the goal of establishing the most and least effective colors for promoting alightment on targets at close range in the laboratory. The hypotheses tested were: 1) certain colors strongly promote *D. suzukii* alightment while others do not, 2) *D. suzukii* responds differently to visual targets varying in color (reflected wavelength composition) and not just brightness of a black and white image, and 3) certain colors are preferred irrespective of whether the background shifts from light to dark.

MATERIALS AND METHODS

***Drosophila suzukii* colony.**

Only lab-reared *D. suzukii* were used in these experiments. Our colony was split from a large colony maintained at the Trevor Nichols Research Center of Michigan State University (6237 124th Avenue, Fennville, MI) arising from stock field-collected on the grounds of this fruit research station. Our colony was maintained on the *D. suzukii* solid food diet of Dalton et al. (2011) in 50 mL polystyrene vials (Genesee Scientific, San Diego, CA) and held in a growth chamber at 24°C, 45% RH and a photoperiod of 16:8 (L:D). Adults used in the experiments were 1-2 wk posteclosion. All flies were removed from their vials containing the solid food diet, anesthetized with CO_2, sorted by sex, and counted prior to the experiment. The flies entered the experiment within 1 h of being removed from the vials containing the solid food. They were not provisioned with food or water while in the bioassay cages so as to promote responsiveness to visual targets. The life span of such flies rarely exceeded 2 d under the bioassay conditions.

Experimental protocols.

For all experiments, 5-cm-diameter paper disks were printed on white office paper using an HP Color LaserJet CP4025 laser ink-jet printer. Disks were then glued to a second identical disk to create a double-sided colored disk. Coloration was designated according to the calibration specifications in Microsoft PowerPoint 2010 Version 14.0 (Table 1). Both sides of a disk were coated with transparent Tangle-Trap glue (Tanglefoot Company, Grand Rapids, MI) and then suspended 15 cm below the ceiling of a 60- by 60- by 60-cm white insect cage (BugDorm-2120). The cages were placed in four rows of

3 cages in a room with a 2.5-m ceiling held at 22°C and ca. 63% RH. Full spectrum lighting for insects (Shields 1989) was provided by four fluorescent tubes (Lumichrome F40W 1 x C 5000 k, Lumiram Germany) affixed 18 cm from the room ceiling, and four LED fixtures (Smart Electrician™, Intertek) each emitting 1050 lumens. The distance between the lights and the sticky disks was ca. 1 m. All lights were installed on timers to yield a common photoperiod of 16:8 (L:D). Flies were released at the bottom center of the cage at ca. 1100 h, and each test ran for only 24 h. Unless otherwise specified, the experimental design was randomized complete block, i.e., one replicate of each treatment and condition was present for a given block and such blocks were accumulated across time, always using a fresh batch of flies. Assignment of treatments to cages (no-choice tests) or positions of treatments within a cage (choice tests) was always randomized, but with the restriction that treatments could rarely occur in the same position twice. Unless otherwise stated, count data required no transformations and were analyzed by multi-way ANOVA in JMP 10 (SAS Institute, Cary, NC, USA, 2012); means were separated by Tukey's HSD tests. The statistical power inherent in these experiments was deemed too low for meaningful specification of interactions. These experiments were conducted from December 2014 to May 2015. A weakness of this analysis of choice-test results was our lack of knowledge about whether or not treatments exerted their effects independently. But, comparing choice and no-choice outcomes was helpful in identifying treatment dependencies within the choice tests.

Table 2.1. Microsoft PowerPoint settings used to generate colored disks.

	Numerical Value under Microsoft PowerPoint Settings					
	RGB			HSL		
Color Output	Red	Green	Blue	Hue	Saturation	Luminosity
Red	255	0	0	0	255	128
Orange	255	102	0	17	255	128
Yellow	255	255	0	42	255	128
Green	0	128	0	85	255	64
Blue	0	0	255	170	255	128
Purple	102	0	255	213	255	51
White	255	255	255	170	0	255
Black	0	0	0	170	0	0

Experiment 1: Choice tests of colored disks.

Drosophila suzukii response to various colors was first examined by a choice test using white cages held in a room with white walls and ceiling. The colors used were: red, orange, yellow, green, blue, purple, white, and black. Additionally, a checkered black and white disk was created using the pattern fill option in Microsoft PowerPoint; each square measured 1 mm on a side. A clear disk was fashioned from a clear 3M acetate sheet (3M™ Clear Gloss Acetate Label Product FA01B). One disk of each color was suspended from the top of the cage in a 25-cm-diameter circle of evenly spaced disks separated by ca. 5 cm. One hundred flies were released per cage (50:50 M:F). Eight blocks were accumulated through time.

Experiment 2: No-choice tests of colored disks.

No-choice tests were conducted by suspending a single disk of a particular color in the center of a cage 15 cm from the ceiling. Colors used were: red, orange, yellow, green, blue, purple, white, black, and a checkered black and white disk. Fifty *D. suzukii* were released per cage (25:25 M:F). Eight replicates were accumulated through time.

Experiment 3: Response to colored disks by gender.

Experiments 1 and 2 were conducted without regard to the gender of responders. To test whether male and female *D. suzukii* responded differently to colors, a no-choice experiment was set up as per Experiment 2, but using only: black, purple, red, yellow, blue and white. One hundred males and one hundred females were released into individual cages, each receiving a particular color. Six blocks were accumulated through time. The data were analyzed by three-way ANOVA.

Experiment 4: Response to color vs. equivalent gray scale.

Claims that an insect perceives and responds to color require evidence that the equivalent response cannot be obtained by presenting just the gray scale (brightness) equivalent of a given color (Burkhardt 1964). Accordingly, we presented *D. suzukii* with yellow and red disks alongside those of corresponding gray scale created by printing a "color" using the gray scale setting in the printer preferences. Each cage had one such pairing of disks. One hundred *D. suzukii* were released into each cage (50:50 M:F). We accumulated 12 replicates for each pairing. Data were analyzed using a Student's paired t-test (JMP 10). These count data required transformation to $\sqrt{x+0.5}$ to establish normality.

Experiment 5: Robustness of color responses in black cages.

Response to color was examined in a black cage to test if there were any differences in color response due to background against which targets were presented. Black fabric was wrapped around the outside of a cage made of black netting. The top of the cage was left open and a light source mounted below a black ceiling. To rule out that failure to discriminate among colors might be explained simply by dimness, light intensity was increased to the level of the white-background experiments by adding an additional row of fluorescent lighting to compensate for the greater absorbance of the black background. Choice tests were conducted by suspending one disk of each color from the top of the cage as per Experiment 1. Disks were red, purple, black, blue, yellow, and white. Fifty *D. suzukii* were released into each cage (25:25 M:F). Eight replicates were accumulated through time. These count data required transformation to $\sqrt{x+0.5}$ to establish normality.

Experiment 6: Choice tests of fluorescent disks.

Response to various fluorescent colors was first quantified by a choice test (as per Experiment 1) using 5-cm-diameter white paper disks painted with “Americanca Neons” (DecoArt, Stanford, KY) fluorescent acrylic pigments: Fiery Red (DHS4), Thermal Green (DHS5), Sizzling Pink (DHS3), Electric Blue (DHS6), Torrid Orange (DHS2), and Scorching Yellow (DHS1). The paint dried at room temperature for 24 h and then was baked in an oven for 1 h at 50°C to further remove volatiles. One hundred *D. suzukii* were released into each cage (50:50 M:F). Eight replicates were accumulated through time.

Experiment 7: Choice tests of fluorescent red vs. non-fluorescent red.

A two-choice test was conducted to quantify rates of alightment of *D. suzukii* to nonfluorescent red and fluorescent red color. A fluorescent red disk was created by painting a 5-cm-diameter disk with Fiery Red neon paint as per Experiment 6, and one regular red disk was created as per Experiment 1. One disk of each pigment was hung ca. 10 cm apart and 15 cm from the ceiling of the cage. One hundred *D. suzukii* were released into each cage (50:50 M:F). Eight replicates were accumulated through time. Data were analyzed using a Student's paired t-test (JMP 10).

Experiment 8: No-choice tests of fluorescent red vs. non-fluorescent red.

Because fluorescent paint might activate flies from greater distances, no-choice tests were conducted to detect possible differences between choice and no-choice outcomes. One disk of either fluorescent red or non-fluorescent red was suspended centrally 15 cm below the cage ceiling. Fifty *D. suzukii* were released into each cage (25:25 M:F). Fourteen replicates were accumulated through time. Data were analyzed using a Student's paired t-test (JMP 10). To establish normality, count data required transformation to $\sqrt{x + 0.5}$.

RESULTS

Experiment 1: Choice tests of colored disks.

About 40% of the flies released in this choice experiment were recovered from the disks, suggesting that the collective set of disks near the cage ceiling stimulated many flies to visit. Numerically, white disks captured the fewest *D. suzukii* (Fig. 1). Significantly more *D. suzukii* landed on red, purple, and checkered disks than on white disks ($F_{16, 79}$ = 3.51, P = 0.0002). The highest mean *D. suzukii* captures occurred on red and purple disks; however, preference for these colors was not significantly different from many of the other colors (Fig. 2.1).

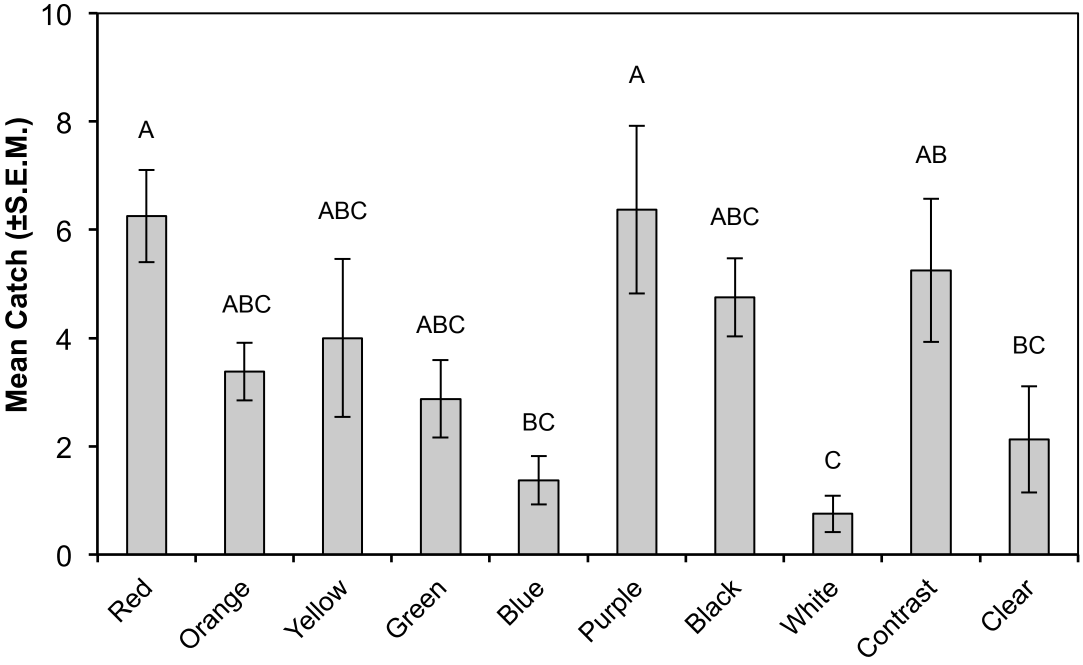

Figure 2.1. Numbers of *Drosophila suzukii* captured on colored disks in a choice test. Bars topped with a common letter within a given panel do not differ significantly at the 0.05 level. Vertical lines indicate SEM.

Experiment 2: No-choice tests of colored disks.

The overall pattern of *D. suzukii* captures in the no-choice context (Fig. 2.2) was similar to that for choice (Fig. 2.1). Significantly more *D. suzukii* landed on red, orange, green, purple, and black disks than on white disks ($F_{16, 79}$ = 8.35, P = 0.0001), which again captured the fewest *D. suzukii*. Highest mean captures occurred on purple, red, orange, and black disks. However, the differences in responsiveness to all colored disks were less pronounced in the no-choice context than in the choice context, perhaps because direct competition between colors was eliminated. All of the colored disks captured only between 14-22% of the total *D. suzukii* released into a cage, suggesting that the overall size of a visual target or group of targets influenced the outcomes (40% were captured by a cluster of targets). Experiments 1 and 2 confirm Hypothesis 1 – targets of certain colors are preferred over others when presented against a white background.

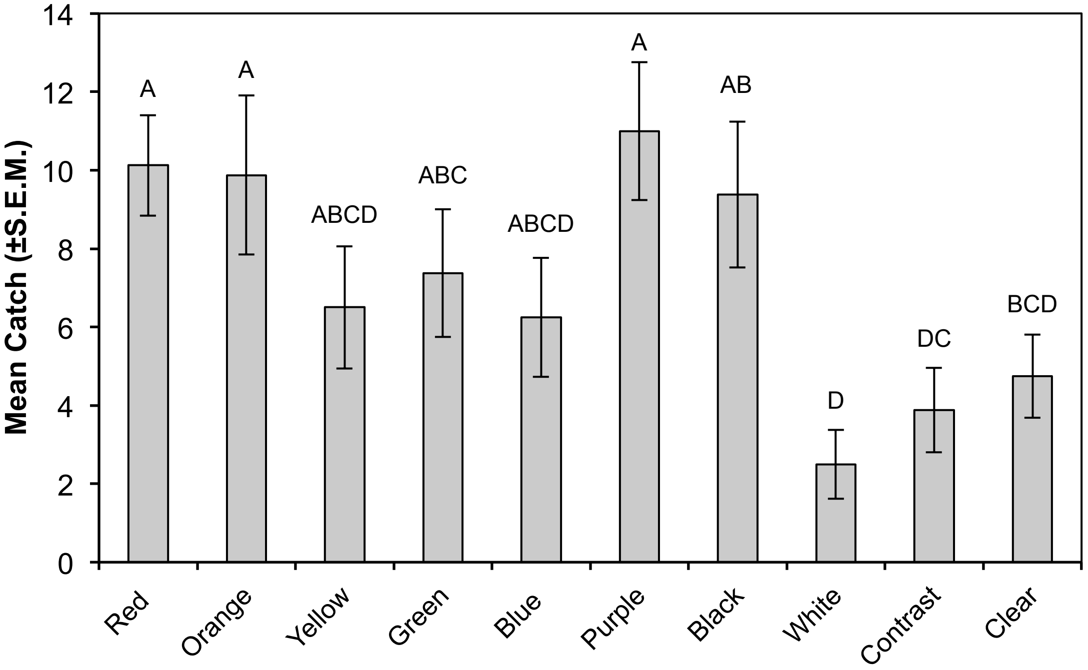

Figure 2.2. Numbers of *Drosophila suzukii* captured on colored disks in a no-choice test. Bars topped with a common letter within a given panel do not differ significantly at the 0.05 level. Vertical lines indicate SEM.

Experiment 3: Response to colored discs by gender.

No evidence was found for a gender effect in this six-choice test ($F_{5, 25} = 0.50$**,** P = 0.4821) (Figure 2.3). The pattern in response to colors seen above and Hypothesis 1 were further confirmed here.

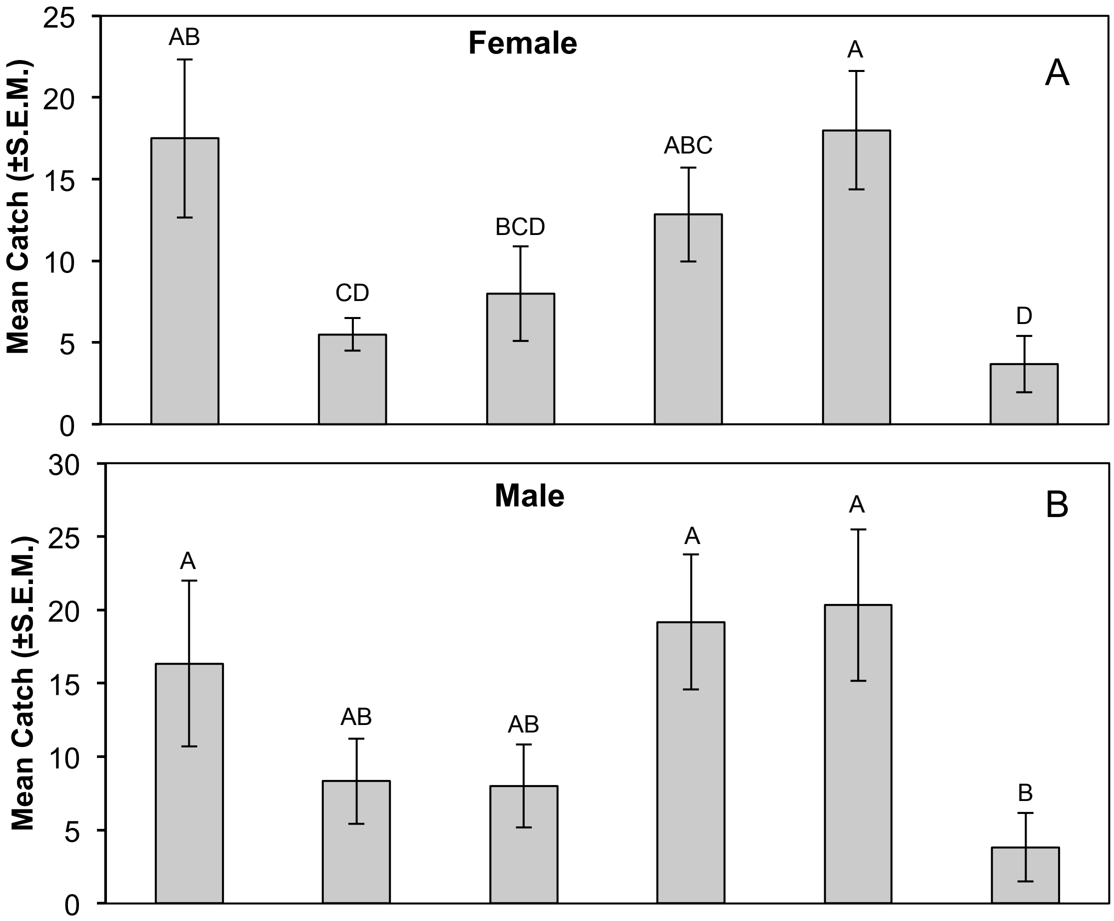

Figure 2.3. Numbers of *Drosophila suzukii* female (A) and male (B) captured on colored disks presented as a choice test. Bars topped with a common letter within a panel do not differ significantly at the 0.05 level. Vertical lines indicate SEM.

Experiment 4: Response to color vs. equivalent gray scale.

Mean *D. suzukii* capture rates on a red disk vs. one of the equivalent gray scale were 12.8 ± 2.2 vs. 5.4 ± 0.7, respectively (difference significant at P = 0.0013). Capture rates for a yellow disk vs. one of the equivalent gray scale were 7.0 ± 0.8 vs. 3.8 ± 0.8, respectively (difference significant at P = 0.0006). Thus, Hypothesis 2 (that *D. suzukii* perceives color) is confirmed. This conclusion also held when the data were analyzed separately by gender.

Experiment 5: Robustness of color responses in black cages.

Against a black background, red and yellow disks captured the most *D. suzukii*, while blue disks captured the fewest (Fig. 2.4). However, none of the colored disks captured significantly more *D. suzukii* than any other ($F_{5, 47} = 1.71$, $P = 0.1543$). The pattern of response to the colored disks did not follow the pattern seen in previous experiments. Experiment 5 refuted Hypothesis 3 - certain colors will be preferred irrespective of whether the background shifts from light to dark.

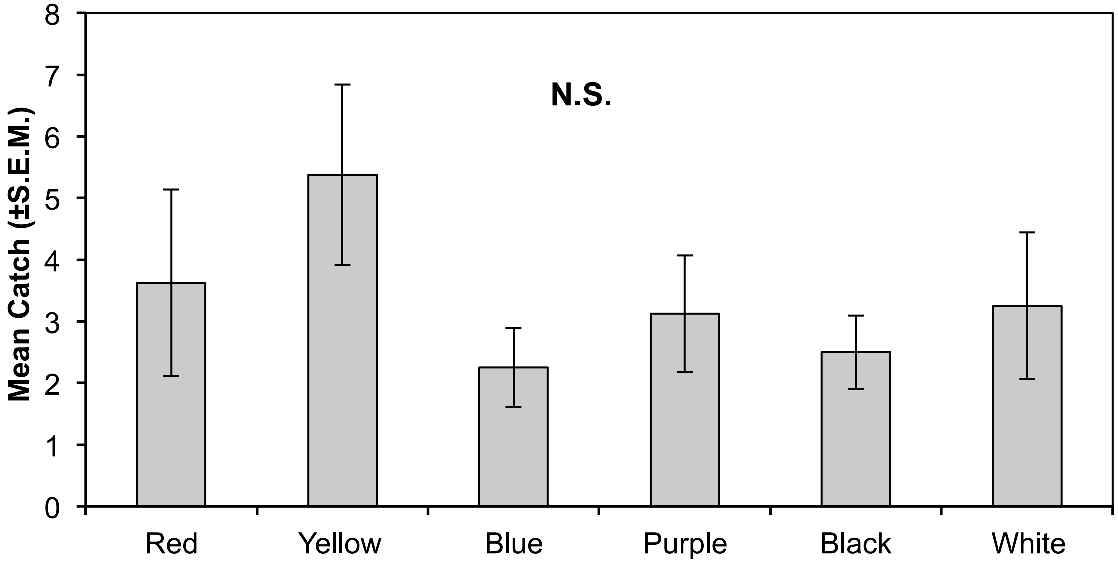

Figure 2.4. Numbers of *Drosophila suzukii* captured on colored disks presented as a choice test with a black cage background. Bars topped with a common letter within a given panel do not differ significantly at the 0.05 level. Vertical lines indicate SEM.

Experiment 6: Choice tests of fluorescent disks.

About 47% of the flies released were recovered from the collective set of disks. Of these, 18% were captured on the fluorescent red disk, whereas the other colors each captured 6% or fewer, Numerically, fluorescent green and fluorescent yellow captured the fewest flies. Significantly more flies landed on fluorescent red disks ($F_{5,42} = 6.53$, P = 0.0001) than all other florescent disk colors (Fig. 2.5). The pattern in response to colors seen in Experiment 1 and Hypothesis 1 were further confirmed here.

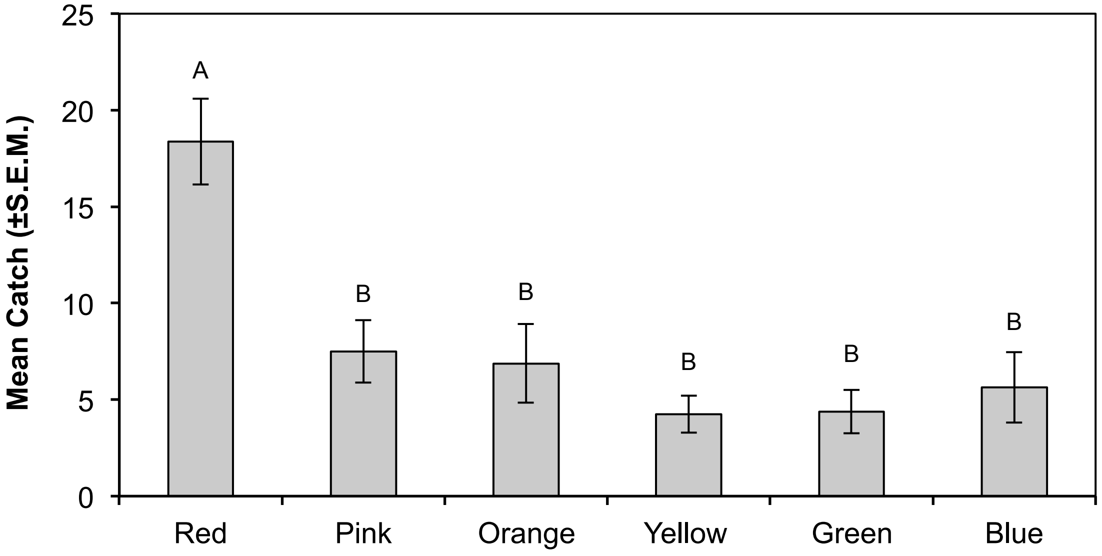

Figure 2.5. Numbers of *Drosophila suzukii* captured on fluorescent colored disks presented as a choice test. Bars topped with a common letter within a given panel do not differ significantly at the 0.05 level. Vertical lines indicate SEM.

Experiment 7: Choice tests of fluorescent red vs. non-fluorescent red.

Sticky disks painted fluorescent and nonfluorescent red caught equivalently (11.8 ± 2.5 vs. 10.9 ± 1.7); t(14) = -0.28595, P = 0.7797.

Experiment 8: No-choice tests of fluorescent red vs. non-fluorescent red.

However, the no-choice test revealed a significant difference in the alightment on fluorescent red disks and nonfluorescent red disks (11.2 ± 1.1 vs. 7.5 ± 0.9); t(26) = 2.85, P = 0.0085. Thus, fluorescent red emerged as the pigment most strongly promoting *D. suzukii* visitation and alightment.

DISCUSSION

This study has added to basic understanding of *D. suzukii* behavior by proving that both genders: 1) are capable of perceiving color, and 2) respond identically to the range of colors presented either in the choice or no-choice test. Such congruence is fortunate because a trap presenting optimal color cues for one gender will be also optimized for the other. Such an outcome cannot be taken for granted. For example, Katsoyannos and Kouloussis (2001) found that the optimal color for capturing olive fruit fly males was orange, while that for females was red. Further support that spotted wing drosophila traps will not need to be tuned by gender comes from the finding of Landolt et al. (2012b) that catch patterns across a range of odors was identical for males vs. females. Similar response across both color and odor cues suggests that both genders benefit equally by responding to the same cues that might signal equally beneficial food resources. Alternatively, male responses may have been selected to match those of females so as to optimize mate finding. Many drosophilids are known to mate at ovipositional sites (Spieth 1974, Markow and Grady 2008).

Receptors maximally absorbing in the red (~650 nm) are documented to have appeared multiple times independently in the Odonata, the Hymenoptera, and the Coleoptera (Briscoe and Chittka 2001). The spectral range covered by a given red receptor can vary even at the species level (Briscoe and Chittka 2001). For example, *Pieris rapae crucivora* has a unique visual pigment expressed in the red and another in the deep red (Wakakuwa et al. 2004). Such differences can also influence foraging behaviors. For example, an island population of *Bombus terrestis* responded significantly more strongly to red artificial flowers than did several mainland populations (Skorupski et al. 2007). Likewise, *D. suzukii* responded strongly to red disks suggesting they are endowed with long-wavelength receptors whose inputs they respond to more strongly than many other wavelengths.

This study also has practical significance. We postulate that red, and particularly fluorescent red, will be the best pigment for monitoring traps. While no difference between red and fluorescent red was measured in the choice test, a clear and significant difference occurred in the no-choice test. Perhaps the fluorescent red disk was better at attracting *D. suzukii* from a distance, but once nearby, flies alighted on any red object. Additionally, purple and black strongly promote *D. suzukii* alightment (Figs. 1 and 2). This is not surprising given that host fruits for *D. suzukii* larvae span red (strawberries, cherries, and raspberries), purple (blueberries), and black (blackberries).

The current study provides a foundation for further research addressing questions like: 1) what is the optimal shape for visual targets having a consistent color?, 2) does odor influence preference for color and shape?, 3) do the cues originating across respective sensory modalities interact additively or synergistically?, and 4) do natural largely green backgrounds and contrast influence preference for color?

CHAPTER 3: IMPROVING MONITORING TOOLS FOR SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII*

INTRODUCTION

Spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), is a major invasive pest of small and stone fruits and has rapidly expanded its global range over the past decade. This invasive species was first detected in California in 2008 (Hauser, 2011) and as of 2012 has been detected throughout the United States, Europe, Canada, Mexico (Cini et al., 2012; Asplen et al., 2015), and South America (Deprá et al., 2014). This rapid range expansion has been accompanied by significant crop losses in blueberries, cherries, raspberries, and strawberries (Walsh et al., 2011). Effective management programs are urgently needed and must be implemented to combat this devastating invasive pest. Current management practices rely on numerous insecticide applications beginning as fruit ripens and continuing through harvest, disrupting IPM programs that have been developed for other pests (Haye et al., 2016). Improvements in monitoring for *D. suzukii* will facilitate accurate pest treatment thresholds and timing of insecticide applications.

Many trap-and-lure systems have been developed and tested for *D. suzukii* monitoring, yet efforts have proven less than adequate for early detection, and inconsistent performance makes traps not yet reliable as a guide for management decisions (Lee et al., 2012, 2013). Recent studies have evaluated the attractiveness of various types of alcohols and vinegars, including apple cider vinegar, rice wine vinegar, Merlot wine (Landolt et al., 2012a, b; Cha et al., 2012, 2014), and also yeast baits consisting of a yeast and sugar mixture (Iglesias et al. 2014). In addition to homemade baits, commercial lures have been developed using blends of compounds that have been found to be attractive to *D. suzukii* (Cha et al., 2014).

Improvements in trap design should maximize attraction and retention of the targeted species; these might include: trap color, size, shape, selectivity for target insect, ease of handling, and retention (Miranda et al., 2001). Sphere traps effectively capture insect pests such as apple maggot (Prokopy, 1975), blueberry maggot (Liburd et al., 1998), Mediterranean fruit fly (Katsoyannos & Papadopoulos, 2004), and others, and given their success for Tephritidae, they should be evaluated for Drosophilidae. Most recent studies on *D. suzukii* monitoring have focused on cup trap designs and attractants where traps generally are comprised of a translucent plastic cylinder with small entry holes, a bait, and a liquid drowning solution to retain flies (Lee et al., 2012, 2013; Iglesias et al., 2014; Burrack et al., 2015). Components of the trap that have been evaluated include: the number and size of entry holes (Lee et al., 2013), trap color (Renkema et al., 2014; Kirkpatrick et al., 2015; Rice et al., 2016), addition of visual stimuli such as color (Renkema et al., 2014; Addesso et al., 2015), shape and size (Rice et al., 2016), and various drowning solutions inside the trap to prevent escape (Iglesias et al., 2014). Still, trapping remains less than optimal for *D. suzukii* because captures in traps are poorly correlated to fruit infestation, and traps are not selective for *D. suzukii* (Lee et al. 2012; Cha et al. 2013; Iglesias et al. 2014; Renkema et al. 2014; Burrack et al. 2015; Frewin et al. 2017).

The following five experiments were conducted in an effort to improve *D. suzukii* monitoring. First, a field evaluation of colored sphere traps measured the response of adult *D. suzukii* to different colored plastic sphere traps baited with commercial synthetic lures in raspberry high tunnels. Second, a comparison of small and large red sphere traps to a deli-cup trap was conducted in raspberry high tunnels; all traps were baited with the same type of commercial lure. Third, a commercial lure and yeast bait comparison study was conducted using the deli-cup trap for monitoring *D. suzukii*, deployed in Michigan cherry orchards. Fourth, the same bait and lure comparison was conducted in large enclosed field cages with known densities of laboratory-reared *D. suzukii*. Lastly, baited deli-cup traps were compared to baited sphere traps to determine the overall attractiveness of a red plastic sphere trap baited with a synthetic lure, compared to the deli-cup trap baited with either the same synthetic lure or with yeast bait.

MATERIALS AND METHODS

Drosophila suzukii **colony.**

Laboratory-reared *D. suzukii* were used in field cage experiments. Our laboratory colony was sourced from a colony maintained at the Trevor Nichols Research Center of Michigan State University (6237 124th Avenue, Fennville, MI 49408) originating from flies field-collected at this site. The laboratory colony was maintained on the *D. suzukii* solid food diet (Dalton et al., 2011) in 50 mL polystyrene vials (Genesee Scientific, San Diego, CA) and held in a growth chamber at 24°C, 45% r.h. and L16:D8 photoperiod. All flies used in the experiments were 3-7 days old and sexually mature. Flies were lightly anesthetized with CO_2 to facilitate handling, sorting by sex, and counting. Flies were starved for 2 h prior to use in experiments.

Experiment 1: field evaluation of colored sphere traps.

To evaluate *D. suzukii* response to color in the field, plastic sphere traps were created using 7-cm-diameter whiffle balls (Easton Sports, Inc., Rantoul, IL) with 26 holes (1 cm diameter). These devices were spray-painted one of eight colors: Apple Red (249124), Sun Yellow (249092), Brilliant Blue (249120), Grape (249113), Meadow Green (249100), White (249090), Black (249122), and Hot Fluorescent Red (2264838) (Rust-oleum Corporation, Vernon Hills, IL). All spray formulations used identical solvents and the pigments were not volatile. The sphere traps contained a Scentry *D. suzukii* commercial lure inside the sphere (Scentry Biologicals, Billings, MT), and were coated with a thin layer of insect Tangle-trap (Tanglefoot Company, Grand Rapids, MI).

Response to trap color in the field was examined in September 2015 in two different Haygrove high tunnels with organic raspberry plantings (cvs. Himbo Top, Joan J, and Polka) on sandy loam soil located at the MSU Horticulture Farm (3291 College Road, Holt, Michigan 48842). Each high tunnel was 8 m wide and 60 m long, with north-south orientation, and covered with Luminance THB plastic. Three rows were 2 m apart and plants were approximately 1.5 m tall and supported with trellis wires. Berries were at different stages of ripening and wild *D. suzukii* were present throughout the duration of this trapping study as evidenced by constant captures. One sphere trap of each color was suspended on the bottom trellis wire of the raspberry planting 1 m from the ground, and spheres were spaced 2 m apart. One trap of each color was present in each tunnel with a total of eight traps per tunnel, and two total tunnels used. Traps were hung within the raspberry foliage and the leaves and canes were pruned to provide 5 cm of clearance on all sides. Traps were visible to the human eye from ca. 5 m away.

The design of Experiment 1 was randomized complete block with 7 replicates accumulated at weekly intervals in each of the two high tunnels from July 22, 2015 until August 19, 2015. The responses of different fly populations were being sampled each week; thus, each week's capture in a high tunnel constituted a replicated block. Traps were collected weekly and brought to the laboratory for careful counts of the number and sex of *D. suzukii* recorded for this and the four other studies that follow. Once captures were quantified, flies were removed from the spheres in the laboratory, sticky material refreshed, and traps were returned to the field where their positions within blocks was re-randomized. Seven replicates were accumulated through time.

Experiment 2: comparison of differently sized sphere traps to a deli-cup trap.

The performance of two sizes of red baited plastic sphere traps was directly compared to the deli-cup trap. Red plastic sphere traps of 7-cm-diameter (1437 cm^3) vs. 8-cm-diameter (2145 cm^3) were constructed as above. The deli-cup trap consisted of a translucent plastic 32 oz. deli-cup (Fabri-Kal Corporation, Kalamazoo, MI) perforated with twelve 0.5-cm holes near the lip of the deli-cup leaving a 3-to 4-cm section without holes to facilitate pouring out the liquid, and containing 150 ml of aqueous drowning solution. Unscented dish soap (Seventh Generation, Inc., Burlington, VT) was added to the water in deli-cup traps to break the surface tension of the water. To prevent entering flies from escaping, a 7.5 cm x 9 cm yellow, double-sided sticky card was hung on a paper clip from the lid on the inside of each deli-cup. All traps were baited with a Scentry *D. suzukii* commercial; the lure was clipped to the side of the deli-cup so that 4 cm of space prevented the yellow sticky card from sticking to the lure, and lures were placed inside the sphere traps. Trap deployments, experimental design, and data collection paralleled those of Experiment 1. Sixteen weekly replicates were accumulated between August 21, 2015 and September 4, 2015.

Experiment 3: bait and lure comparison study in Michigan cherry orchards.

Captures of wild *D. suzukii* in response to different baits and lures were compared using deli-cup traps (constructed as above) in Michigan cherry orchards from June through August 2015. Two liquid baits were tested: 1) a yeast bait (1 tablespoon active dry yeast (Red Star, Lesaffre Yeast Corporation, Milwaukee, WI), 4 tablespoons sugar (Meijer, Walker, Michigan), and 12 oz of distilled water) and 2) the Suzukii bait, a commercial blend (BioIberica, Spain). Liquid soap was not added to the commercial Suzukii bait or the yeast bait because it was not required to break the surface tension of these two liquid baits. Three commercial lures were tested: 1) the Trécé strip (Trécé Inc., Adair, OK), 2) the Scentry plastic satchel (Scentry Biologicals, Billings, MT) and 3) the Alpha Scents black pouch (Alpha Scents, Inc., West Linn, OR). The drowning solution in the deli-cup traps consisted of soapy water for the three lure treatments, and the liquid bait itself for the two bait treatments. A 7.5 cm x 9 cm yellow sticky card was clipped under the lids of these traps to enhance fly retention.

Traps were hung in the perimeter row of trees from a shaded branch in the bottom of the canopy approximately 2 m from the ground, and spaced 20 m apart. The experimental design was randomized complete block, with one replicate of each treatment present in each of ten cherry blocks (5 sites in Southwest MI [3 in Berrien County and 2 in Allegan County], and 5 in Northwest MI [1 in Benzie County, 1 in

Antrim County, 1 in Grand Traverse County, and 2 in Leelanau County]). Trap positions were rotated clockwise weekly over the ten-week trial. The soapy water in the traps and yeast baits were replaced weekly, while the Suzukii bait and lures were changed after five weeks.

Experiment 4: semi-field bait and lure comparison study.

Three octagonal domed field cages (2.5 m high and 4.3 m wide) were constructed in an unmowed grassy field at the MSU Entomology Farm (4301 Collins Rd, Lansing, MI 48910). Cage frames were constructed of PVC pipe and enclosed with clear plastic sheeting (Polyethylene sheeting, Model CFHD0610C, HDX, Seattle, WA). Sixteen white polyester netting (Utility fabric #10173292, JoAnn Fabrics, Hudson, OH) windows covering approximately 80% of the surface areas were added to each cage for ventilation preventing overheating. Total volume of the field cages was 42 m^3. Eight 1 m tall evergreen shrubs (*Thuja occidentalis*) were planted inside each cage to provide shelter for known numbers of lab-reared *D. suzukii* released therein. Environmental conditions inside the cages were similar to environmental conditions outside the cages.

This study, conducted from August 11, 2015 to September 29, 2015, compared captures in a deli-cup trap baited with: 1) the yeast bait, 2) the Alpha Scents lure, or 3) an unbaited negative control deli-cup trap (constructed as per Experiment 2). The yeast bait and Alpha Scents lure were selected because they were two of the best attractants from Experiment 3. Here, two baited deli-cup traps and a negative control trap having no lure were compared in no-choice tests where each field cage received one trap. Traps were hung by monofilament line at the cage center and 0.3 m above the ground. Four hundred (sex ratio 1:1) 3-7 day old sexually mature *D. suzukii* from the laboratory colony were released into each field cage. The number and proportion of released flies captured were quantified after 1 week. Each cage received a new treatment after each replicate to balance any possible cage bias. Four replicates were accumulated through time.

Experiment 5: comparison of yeast- and lure-baited deli-cup traps with lure-baited sphere traps.

D. suzukii captures on a red sphere trap baited with the Scentry lure (constructed as per Experiment 1) were directly compared with deli-cup traps (constructed as per Experiment 2) baited with either a Scentry lure or yeast bait. Traps were deployed from September 21, 2015 to October 5, 2015 in a raspberry high tunnel at the MSU Horticulture Farm following procedures of Experiment 1. Eight replicates were accumulated through time.

Data analysis.

All data were analyzed via ANOVA (JMP 10). In order to meet assumptions of normality, Experiments 2 – 5 were log-transformed. Experiment 1 did not require transformation for analysis. Where significant effects were observed, means were separated using the Tukey's HSD means separation test. For experiment 3, catch data was pooled across the season, and the variables site, lure treatment and sex were included in the model.

RESULTS

Experiment 1: field evaluation of colored sphere traps.

Red sphere traps captured significantly more *D. suzukii* than green and white sphere traps, and black sphere traps captured significantly more *D. suzukii* than white sphere traps ($F_{7, 55} = 3.72$, $P = 0.003$) (Figure 3.1). Fluorescent red sphere traps captured almost as many *D. suzukii* numerically as red and black sphere traps, but were not significantly different than any other color tested (Figure 3.1).

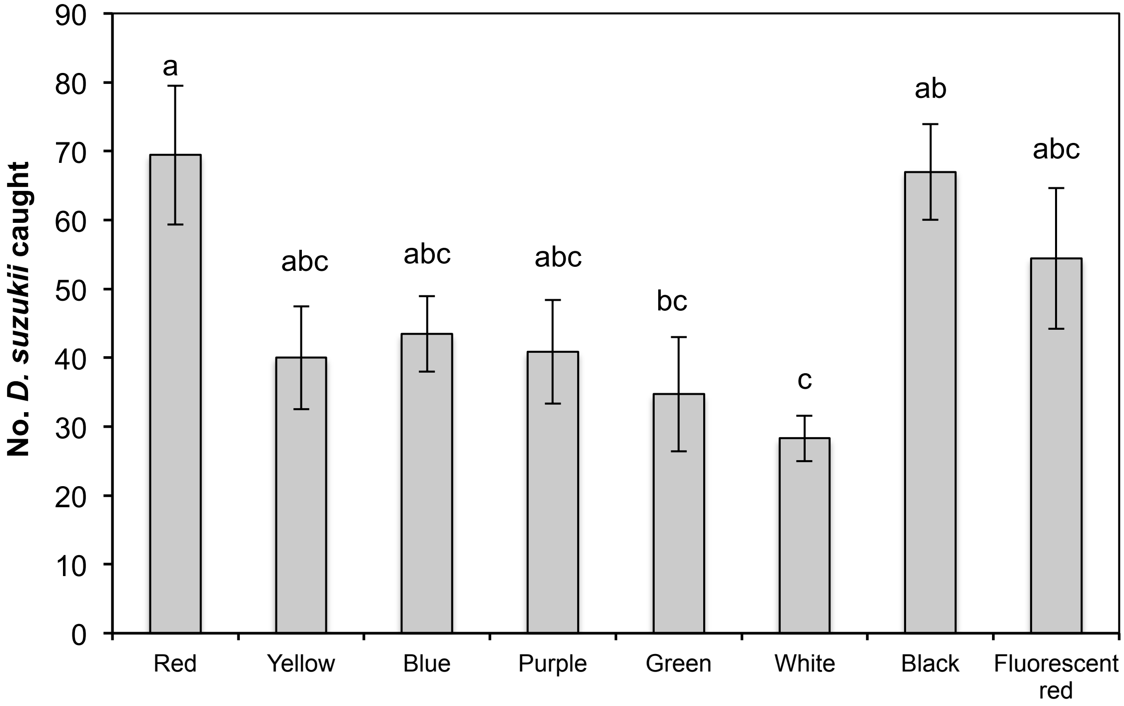

Figure. 3.1. Mean (± SEM) number of *Drosophila suzukii* captured on colored 7-cm-diameter sphere traps presented as a choice test in in raspberry high tunnels. Means capped with different letters are significantly different (Tukey’s HSD test: P<0.05).

Experiment 2: comparison of differently sized sphere traps to a deli-cup trap.

Significantly more *D. suzukii* ($F_{2, 47} = 24.8$, $P < 0.0001$) were captured on the 7-cm-diameter and 8-cm-diameter red sphere traps than in the deli-cup trap (Table 3.1).

Both sizes of red sphere traps captured equivalently and 3-4 times more flies than the deli-cup trap (Table 3.1).

Table 3.1. Summary of results of experiments 2-5: mean (± SEM) number of *Drosophila suzukii* captured (experiment 4: mean proportion) and output of ANOVA.

Exp.	Treatment	*D. suzukii* captures	ANOVA
2	Small Sphere	76.69 ± 14.11a	$F_{2, 47} = 24.8$, $P < 0.0001$
	Large Sphere	90 ± 14.53a	
	Deli-cup Trap	16.88 ± 4.75b	
3	Yeast	662.95 ± 223.45a	$F_{13, 49} = 12.908$, $P < 0.0001^1$
	Scentry	548.35 ± 151.69a	
	Alpha Scents	379.25 ± 108.25a	
	Trécé	79.15 ± 17.24b	
	BioIberica	121.3 ± 43.84b	
4	Yeast	0.274 ± 0.046a	$F_{2, 9} = 26.06$, $P = 0.001$
	Alpha Scents	0.064 ± 0.015b	
	Negative Control	0.004 ± 0.003c	
5	Sphere + Scentry	67.13 ± 11.5a	$F_{2, 23} = 23.765$, $P < 0.0001$
	Deli-cup + Yeast	26.5 ± 2.43b	
	Deli-cup + Scentry	10.63 ± 2.03b	

Means within an experiment followed by different letters are significantly different (Tukey's HSD test: P<0.05).

[1] Treatment: F = 23.078, P < 0.0001; Site: F = 8.388, P < 0.0001

Experiment 3: bait and lure comparison study in Michigan cherry orchards.

Significantly more *D. suzukii* ($F_{14, 99} = 18.256$, $P < 0.0001$) were captured in deli-cup traps containing the yeast bait, the Scentry lure, or the Alpha Scents lure than in deli-cup traps containing the BioIberica Suzukii bait or the Trécé lure (Table 3.1). Site ($P < 0.0001$) and lure treatment ($P < 0.0001$) were significant variables (Table 3.1). There was no significant difference in numbers of males and females captured in traps ($P = 0.337$).

The highest captures were recorded for traps baited with the Scentry lure or the yeast bait, and they attracted 4 to 8 times more flies than the Suzukii bait or the Trécé lure (Table 3.2). However, none of the baits or lures tested was selective for *D. suzukii*, and the majority of the captures were non-target flies (Table 3.2). The percentage of total captures in traps ranged from 31-39% for *D. suzukii*, and 60-68% for non-target flies for the baits and lures tested; but there was no statistical significance in selectivity for *D. suzukii* ($F_{4, 24} = 0.47$, $P = 0.757$) for any of the baits or lures tested (Table 3.2). The yeast bait yielded the highest percentage of *D. suzukii* captures (Table 3.2).

Table 3.2. Mean (± SEM) total number of *Drosophila suzukii* and proportion of non-target drosophilid captures for baited deli-cup traps in Southwest Michigan cherry orchards.

	D. suzukii		Proportion Non-
Treatment	Male	Female	target captures
Trécé	610 ± 23.7	415 ± 17.6	0.65
Scentry	4577 ± 184.3	3426 ± 169.3	0.68
Alpha Scents	3208 ± 385.9	2571 ± 122	0.65
Yeast	5498 ± 778.5	4984 ± 246.2	0.61
BioIberica	1035 ± 208.5	704 ± 44.2	0.63

Captures of *Drosophila suzukii* or captures of non-targets were not significantly different among treatment (Tukey's HSD: P > 0.05).

Experiment 4: semi-field bait and lure comparison study.

Significantly more *D. suzukii* were captured in the deli-cup trap baited with the yeast bait ($F_{2, 9} = 26.06$, $P = 0.001$) than in the deli-cup trap baited with the Alpha Scents lure or in the negative control deli-cup trap (Table 3.1). Significantly more *D. suzukii* were captured in the deli-cup trap baited with the Alpha Scents lure than in the negative control deli-cup trap (Table 3.1). Captures of *D. suzukii* in the yeast baited deli-cup trap, the deli-cup trap baited with the Alpha Scents lure, and the negative control deli-cup trap were 27.0, 6.0, and 0.4%, respectively (Table 3.1).

Experiment 5: comparison of yeast- and lure-baited deli-cup traps with lure-baited sphere traps.

Significantly more *D. suzukii* were captured on the red sphere trap baited with the Scentry lure than in both a deli-cup trap baited with the Scentry lure and a deli-cup trap baited with the yeast bait ($F_{2, 23} = 23.765$, $P < 0.0001$). The red sphere trap captured 3 times more *D. suzukii* than the deli-cup trap baited with the yeast bait, and 6 times more *D. suzukii* than the deli-cup baited with the Scentry lure (Table 3.1).

DISCUSSION

In raspberry high tunnels, sticky-baited red sphere traps consistently captured *D. suzukii* in greater numbers than baited translucent deli-cup traps. A trap integrating both visual and olfactory cues attracts more flies than typical deli-cup traps relying only on olfactory cues. One hypothesis for increased captures is that the shape of the sphere traps is more attractive to *D. suzukii* because they resemble the host fruits. Rice et al. (2016) document that spherical traps capture more *D. suzukii* than other trap shapes, and spheres of 15.2 and 25.4 cm diameter captured more flies than spheres of 2.5 cm diameter. Similar results with sphere traps have been obtained for other dipteran pests, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Nakagawa et al., 1978) and the cherry fruit fly, *Rhagoletis indifferens* (Mayer et al., 2000).

Another reason for increased captures on the sphere trap compared with deli-cup traps is that some *D. suzukii* entering the deli-cup trap are not retained. Flies entering the deli-cup trap may walk along the inside surface searching for resources and never encounter either the drowning solution at the bottom of the trap or the yellow sticky card hanging in the middle of the trap. This behavior of entering and exiting the trap has been documented in laboratory and field trapping studies. Hampton et al. (2014) showed that only 10-30% of the flies visiting traps enter the traps and drown, and many flies visit the exterior surface of the trap, enter the trap, and later leave the trap.

Previous studies in laboratory, semi-field, or field conditions have shown *D. suzukii* prefer darker colors, such as red and black (Lee et al., 2013; Kirkpatrick et al., 2015; Rice et al., 2016). This sphere trap color study conducted in raspberry high tunnels shows a similar response to color under field conditions, where red and black sphere traps consistently captured the most wild *D. suzukii*. These results corroborate those of Rice et al. (2016) who demonstrated greater captures of wild populations of *D. suzukii* on red and black spheres deployed in peach orchards. Our results strongly indicate that adult *D. suzukii* utilize visual cues as an aspect of their foraging behavior in raspberry high tunnels.

The greater effort required to service sticky sphere traps is justified by their superior trapping efficiency making them a more reliable monitoring tool than the deli-cup trap. Male *D. suzukii* can be identified on the sphere traps in the field, but identifying female *D. suzukii* from other drosophilids requires the flies to be removed from the sphere and identified under a microscope, a task that is labor intensive and time consuming, and requires the ability to identify and distinguish *D. suzukii* from other drosophilids captured that are similar in size and appearance. Deli-cup traps are easier to refresh, but require the liquid to be transported to the laboratory so the insects can be sorted under a microscope, presenting the opportunity for the liquid to spill during transport. The contents of deli-cup traps must be checked and refreshed weekly, a task that is very labor intensive. Moreover, the contents of deli-cup traps can develop a very foul odor after aging in the field, something that is not experienced with sphere traps.

Similar to deli-cup traps, sticky sphere traps may capture large numbers of non-targets. Yeast and cider baits are all generalist attractants for many insects and not specific for *D. suzukii*, although the former is reported to capture greater numbers of *D. suzukii* in baited traps (Landolt et al., 2012; Lee et al., 2013; Kleiber et al., 2014). The yeast bait also captured significant numbers of *D. suzukii*, but selectivity with the yeast bait was not different than the other baits or lures tested, and is still less than 40%. Future research on traps should optimize preferential capture of *D. suzukii* to non-target species, specifically other drosophilids, to facilitate easier identification while in the field.

The data on proportion of *D. suzukii* recaptured in the field cages are highly unique because virtually nothing is known about how to relate captures in a monitoring trap to absolute density of these flies; no precise action thresholds for *D. suzukii* have been developed to date. Our finding that a single baited trap in a 4 m diameter field cage captured 27% of *D. suzukii* released therein is an important first step in the process of translating capture number for this pest into an estimate of absolute density, as has recently been accomplished for codling moth, *Cydia pomonella*, (Miller et al., 2015; Adams et al., 2017). Given that a *D. suzukii* trap in a substantially smaller cage than that used for *C. pomonella* captured only 27% of released flies, we predict its sampling power in the field may be remarkably weak and that any level of capture in such a trap may signal an already very high *D. suzukii* population.

Red baited plastic sphere traps consistently capture *D. suzukii* over time, and captures are higher compared to a baited deli-cup trap. This study has practical significance, and we postulate that red sphere traps will be useful monitoring tools for *D. suzukii* in the future. The current study provides a foundation for further research addressing questions like 1) are other sticky trap designs, such as red colored panels, effective for capturing *D. suzukii*? And 2) can refinement of visual and olfactory cues be optimized specifically for *D. suzukii* while reducing captures of non-target drosophilids?

CHAPTER 4: IMPROVING MONITORING TRAPS FOR *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) BY INCORPORATING VISUAL AND OLFACTORY CUES

INTRODUCTION

Drosophila suzukii Matsumura (Diptera: Drosophilidae) has spread rapidly throughout all major fruit production regions of the United States, Europe, Canada, Mexico (Cini et al. 2012; Asplen et al. 2015), and South America (Deprá et al. 2014; Asplen et al. 2015). This pest is fast becoming a severe worldwide problem. In contrast to other species of *Drosophila* that only attack overripe or decaying fruit, *D. suzukii* females have a prominent serrated ovipositor that injects eggs into ripening fruit; resulting internal feeding by larvae renders fruit unmarketable (Hauser et al. 2011; Walsh et al. 2011). Current control programs for *D. suzukii* rely solely on insecticides (Haviland and Beers 2012; Van Timmerren and Isaacs 2013; Dipenbrock et al. 2016) that disrupt integrated pest management programs (IPM) put in place for other pests (Haye et al. 2016). Considerable research has gone into developing potent trapping systems for detecting *D. suzukii* and eventually setting thresholds for if and when to spray insecticides. Despite those efforts, a threshold for *D. suzukii* has yet to be developed and implemented.

Many trap-and-lure systems have already been tested for *D. suzukii*. Physical features such as trap size, shape, color, among others, have been studied both in the laboratory and the field for a diversity of trap models (Lee et al. 2012, 2013; Iglesias et al. 2014; Renkema et al. 2014; Kirkpatrick et al. 2015; Rice et al. 2016; Kirkpatrick et al. 2017). Despite some variability due to crop type, trap position in the crop, and weather conditions, red traps (Rice et al. 2016; Kirkpatrick et al. 2017; Rice et al. 2017) are proving to be more effective than the clear plastic cups first used to monitor this pest. Moreover, synthetic baits are replacing liquid yeast-based or apple cider vinegar lures (Cha et al. 2013; Burrack et al. 2015). However, the potency of all *D. suzukii* traps and lures developed to date needs improvement because capture of a single fly means an already high population is present (Kirkpatrick et al. 2018). Due to short generation time, the population can build rapidly and if fruit are susceptible cause economic injury before any action can be taken. Only when monitoring traps become more reliable can their captures drive pest management decisions.

The following experiments were conducted to compare novel trap designs and synthetic lures to that of the standard *D. suzukii* deli-cup trap in an effort to further improve monitoring.

MATERIALS AND METHODS

Experiment 1: Comparison of colored paned traps in cherry orchards.

Rectangular (20 x 30 cm) plastic panel traps (hereafter called the panel trap) were created in four colors (red, white, yellow, and green) from stock purchased from Great Lakes IPM (Vestaburg, MI). Both sides of each panel were covered with Tanglefoot glue (Marysville, OH) for capture and retention of insects. A 1 cm hole in each corner of a panel enabled it to be hung in the field and a similar hole in the center of the trap allowed the lure to be attached with a twist-tie. Inspired by the Ladd trap used for trapping apple maggot flies (Kring 1970), a sphere and panel combination panel trap (hereafter called the combination trap), was created using a yellow panel trap (constructed as above) and a disposable red plastic sphere (OL-100SS12-100, Great Lakes IPM, Vestaburg, MI) cut in half so that one hemisphere could be centered on each side of the yellow panel trap. The red plastic spheres were then coated with a thin layer of Tanglefoot. The deli-cup trap was constructed of a plastic 32 oz. deli cup (Fabri-Kal, Kalamazoo, MI) perforated with twelve 0.5 cm holes near the lip of the cup leaving a 3-to 4-cm section without holes to facilitate pouring out the liquid. To prevent flies from escaping, a 7.5 cm x 9 cm yellow, double-sided sticky card was hung on a paper clip from the lid on the inside of each cup. Traps were baited with the Scentry *D. suzukii* commercial lure hung from the side of the cup on a paper clip so that 4 cm of space separated the yellow sticky card and the lure so the two did not stick to each other. The deli-cup trap baited with the Scentry *D. suzukii* commercial lure contained 150 ml of soapy water drowning solution. Unscented dish soap (Seventh Generation, Inc., Burlington, VT USA) was added to the water to create a soapy water drowning solution in the deli-cup traps.

Response to trap designs was compared in Michigan cherry orchards from June – August 2017. Treatments for this experiment included: a red panel trap, green panel trap, white panel trap, yellow panel trap, a combination trap, and a deli-cup trap; all traps were baited with the Scentry *D. suzukii* commercial lure. Traps were hung in the perimeter row of trees from a shaded branch in the bottom of the canopy approximately 2 m from the ground, and spaced approximately 20 m apart. The experimental design was randomized complete block, with one replicate of each treatment present in each of the four cherry blocks (2 sites in Berrien County and 2 sites in Allegan County) used for this study. Trap position was rotated clockwise weekly over the ten-week trial. New panel traps and combination traps were used for each replicate. The soapy water in the traps was replaced weekly, while the Scentry commercial lures were changed after five weeks. In all experiments, traps were transported to the laboratory for accurate counts under a dissecting microscope of the number of male and female *D. suzukii*.

Experiment 2: Comparison of colored panel traps in raspberry high tunnels.

Captures of *D. suzukii* were compared using various trap types (constructed as per Experiment 1) in Haygrove high tunnels with organic raspberry plantings (cultivars Himbo Top, Joan J, and Polka) located at the Michigan State University Horticulture Farm (Holt, Michigan). Rows were spaced 2 m apart and plants were ca. 1.5 m tall and supported with a trellis wire. Berries were at different stages of ripening and wild *D. suzukii* were present throughout this study as evidenced by constant captures. Treatments for this experiment included: a red panel trap, green panel trap, white panel trap, yellow panel trap, one cup trap, and one combination trap; all traps were baited with the Scentry *D. suzukii* commercial lure. Traps were hung on a trellis wire of the raspberry plantings 1.5 m from the ground, spaced 2.5 m apart, and were hung within the raspberry foliage with the leaves and canes pruned from around the trap to ensure the traps were visually apparent to humans from ca. 5 m.

The design of Experiment 2 was randomized complete block with fifteen replicates accumulated at weekly intervals in each of the three high tunnels from July 31 – August 28, 2017. The responses of fly populations in the three tunnels were measured each week; thus, each week's capture in a high tunnel constituted a replicated block. New traps were used each week, and their position within blocks was re-randomized each week.

Experiment 3: Comparison of trap types in cherry orchards.

Captures of wild *D. suzukii* were compared using a red panel trap baited with a Scentry lure and deli-cup trap (constructed per Experiment 1) baited with either a Scentry lure or a yeast-and-sugar bait (1 tablespoon active dry yeast (Red Star, Lesaffre Yeast Corporation, Milwaukee, WI), 4 tablespoons sugar (Meijer, Walker, Michigan), and 12 oz of distilled water) containing 150 ml of liquid drowning solution. Included in this experiment was a sphere trap created using a 7-cm-diam whiffle ball (Easton Sports, Inc., Rantoul, IL USA) with 26 holes (1 cm diam) that were spray-painted Apple Red (249124) (Rust-oleum Corporation, Vernon Hills, IL). A small cut at the equator of the trap allowed insertion of the commercial lure. The sphere was then coated on the outside with a thin layer of Tanglefoot glue to facilitate capture and retention of trapped insects on the outside of the trap. Traps were deployed from June – August 2016 in Michigan cherry orchards (3 sites in Berrien County and 2 sites in Allegan County) following procedures of Experiment 1 for the ten-week trial.

Experiment 4: Comparison of trap types in raspberry high tunnels.

Drosophila suzukii captures were compared using a red sphere trap (constructed as per Experiment 3) baited with a Scentry lure, a red panel trap baited with a Scentry lure (constructed as per Experiment 1), a deli-cup trap baited with a Scentry lure and a deli-cup trap baited with a yeast and sugar solution (constructed as per Experiment 1). Traps were deployed from June – August 2016 in raspberry high tunnels at the Michigan State University Horticultural Farm following procedures of Experiment 2. Ten replicates were accumulated through time.

Experiment 5: Captures by gender on red panel traps in cherry orchards.

Drosophila suzukii male and female captures were compared with a red panel trap (constructed as per Experiment 1) baited with a Scentry lure. Traps were deployed from June 20 – August 2017 in eight Michigan cherry orchards in southwest and northwest Michigan (2 sites in Berrien County, 2 sites in Allegan County, 3 sites in Leelanau County, and 1 site in Manistee County) following procedures of Experiment 1.

Data analysis.

All data were analyzed using ANOVA (JMP v.12; SAS Institute, Cary, NC). Data from experiments 1 and 3 were pooled across the season and satisfied assumptions of normality. To meet assumptions of normality, data from experiments 2, 4, and 5 were log-transformed. Where significant effects were observed, means were separated with Tukey's honestly significant difference (HSD) means separation test. For Experiment 5, a two-way ANOVA was used to analyze the data and the variables gender, date, and the interaction gender*date were included in the model.

RESULTS

Experiment 1: Comparison of colored panel traps in cherry orchards.

Significantly more flies ($F_{5, 23} = 5.42$, $P = 0.003$) were captured on the red panel trap and the combination panel trap than the yellow panel trap (Fig. 4.1). Adding a sphere to the panel trap did not improve catch. Numerically, fewer flies were captured in the deli-cup, green panel and white panel traps than on the red panel or combo panel traps (Fig. 4.1).

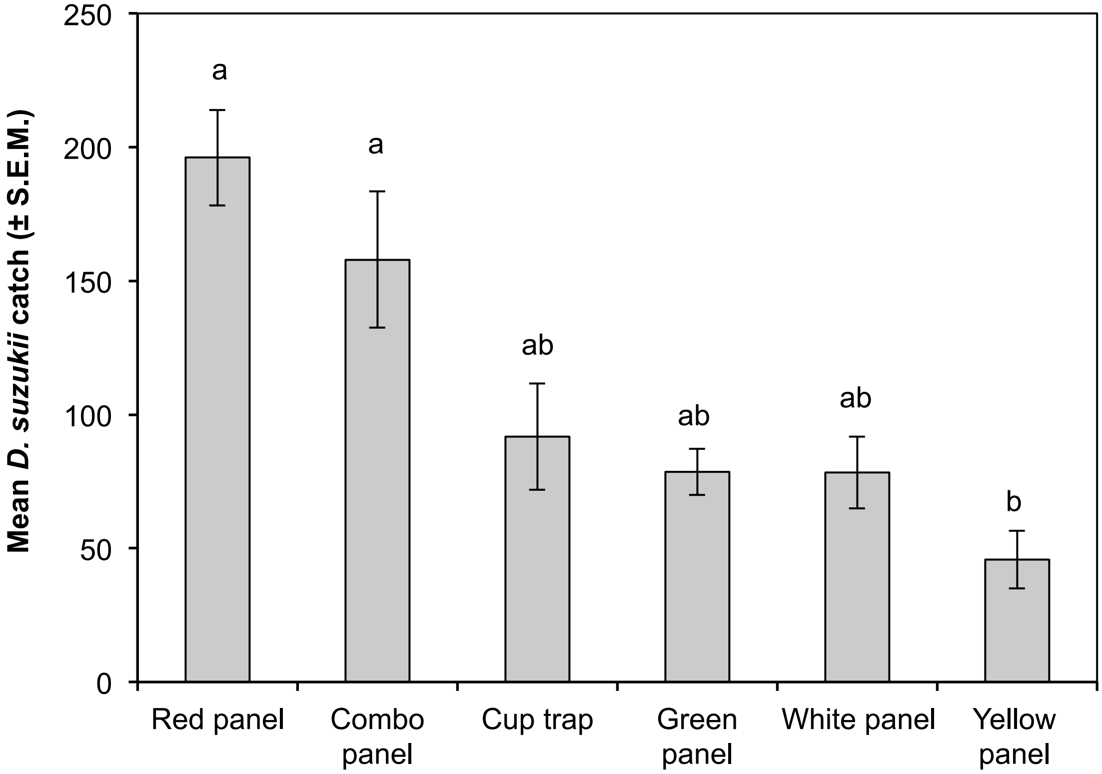

Figure 4.1. Mean (± S.E.M.) number of *Drosophila suzukii* captured on panel traps or a cup trap in cherry orchards. Means capped with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

Experiment 2: Comparison of colored panel traps in raspberry high tunnels.

Significantly more flies ($F_{5, 89} = 5.33$, $P < 0.0003$) were captured on the combination panel trap than in the deli-cup trap or on the yellow panel trap, and significantly more flies were captured on the white panel trap than on the yellow panel trap (Fig. 4.2).

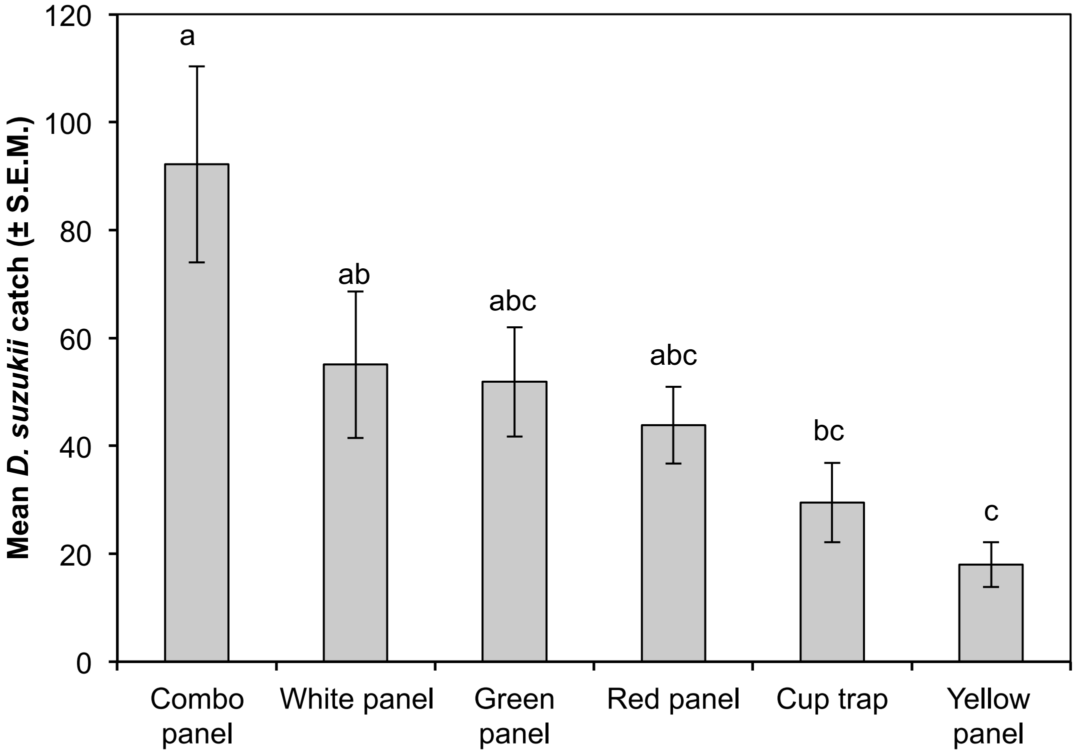

Figure 4.2. Mean (± S.E.M.) number of *Drosophila suzukii* captured on panel traps or a cup trap in raspberry high tunnels. Means capped with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

Experiment 3: Comparison of trap types in cherry orchards.

Significantly more flies ($F_{3,19} = 14.791$, $P < 0.0001$) were captured on the sphere trap with the Scentry lure than in the deli-cup trap with the yeast solution or the deli-cup trap with the Scentry lure, and significantly more flies were captured on the panel trap with the Scentry lure than the deli-cup with the Scentry lure (Fig. 4.3).

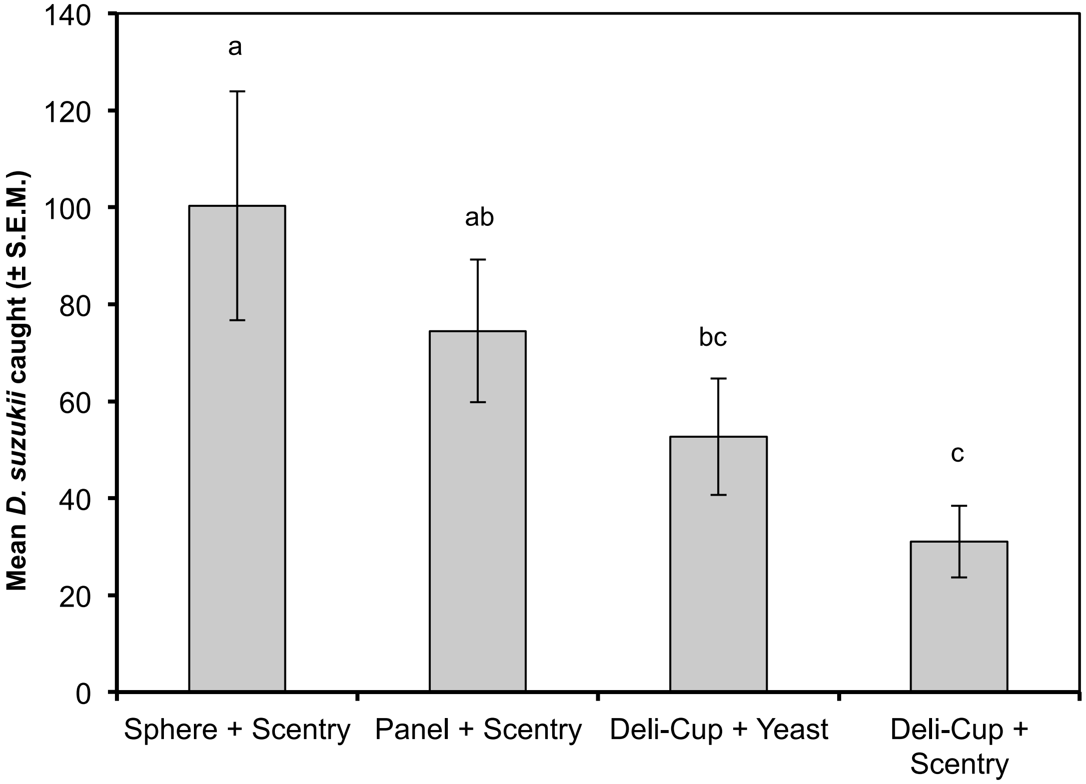

Figure 4.3. Mean (± S.E.M.) number of *Drosophila suzukii* captured with traps in cherry orchards. Means capped with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

Experiment 4: Comparison of trap types in raspberry high tunnels.

Significantly more flies ($F_{3, 40} = 3.1$, $P = 0.0384$) were captured on the sphere trap with the Scentry lure than in the deli-cup trap with the Scentry lure (Fig. 4.4). Numerically, more flies were captured in the deli-cup baited with the yeast solution and red panel trap baited with the Scentry lure than in the deli-cup trap baited with the Scentry lure (Fig. 4.4).

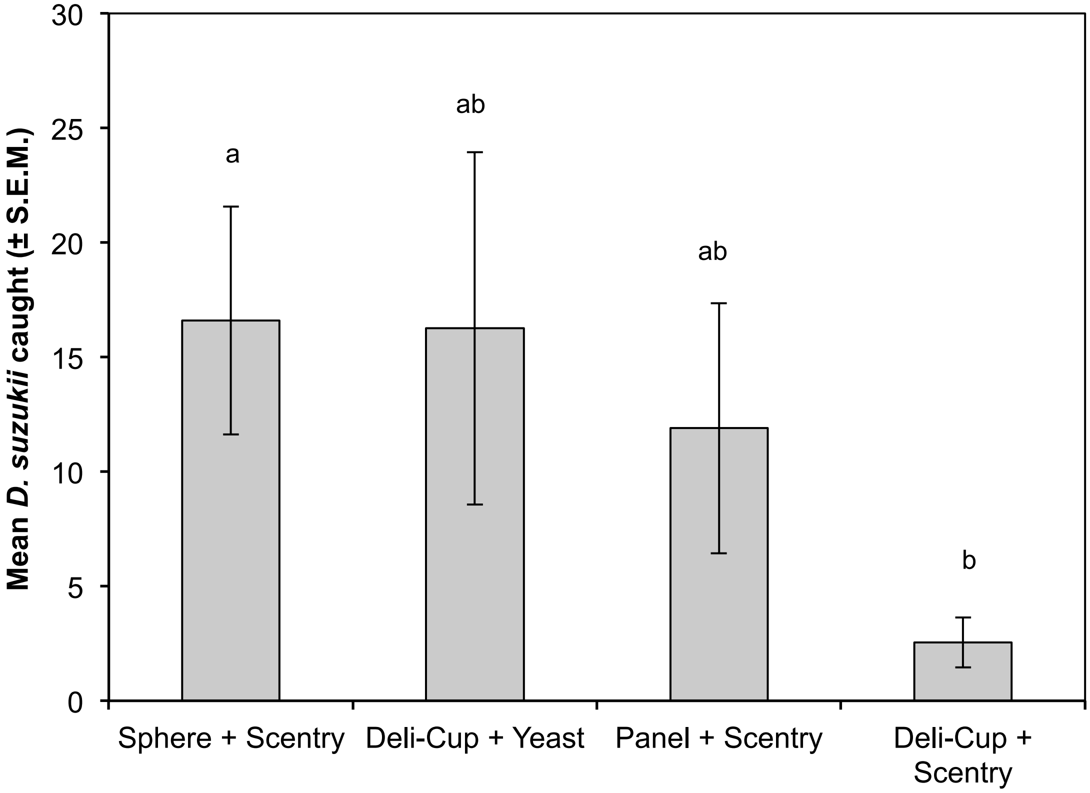

Figure 4.4. Mean (± S.E.M.) number of *Drosophila suzukii* captured with traps in raspberry high tunnels. Means capped with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

Experiment 5: Captures by gender on red panel traps in cherry orchards.

The effect for captures on the red panel trap by week yielded a significant difference ($F_{49, 13} = 12.16$, $P < 0.001$). Captures by gender ($P = 0.5001$) and the interaction of gender x week ($P = 0.5162$) were not significant factors in the model (Fig. 4.5).

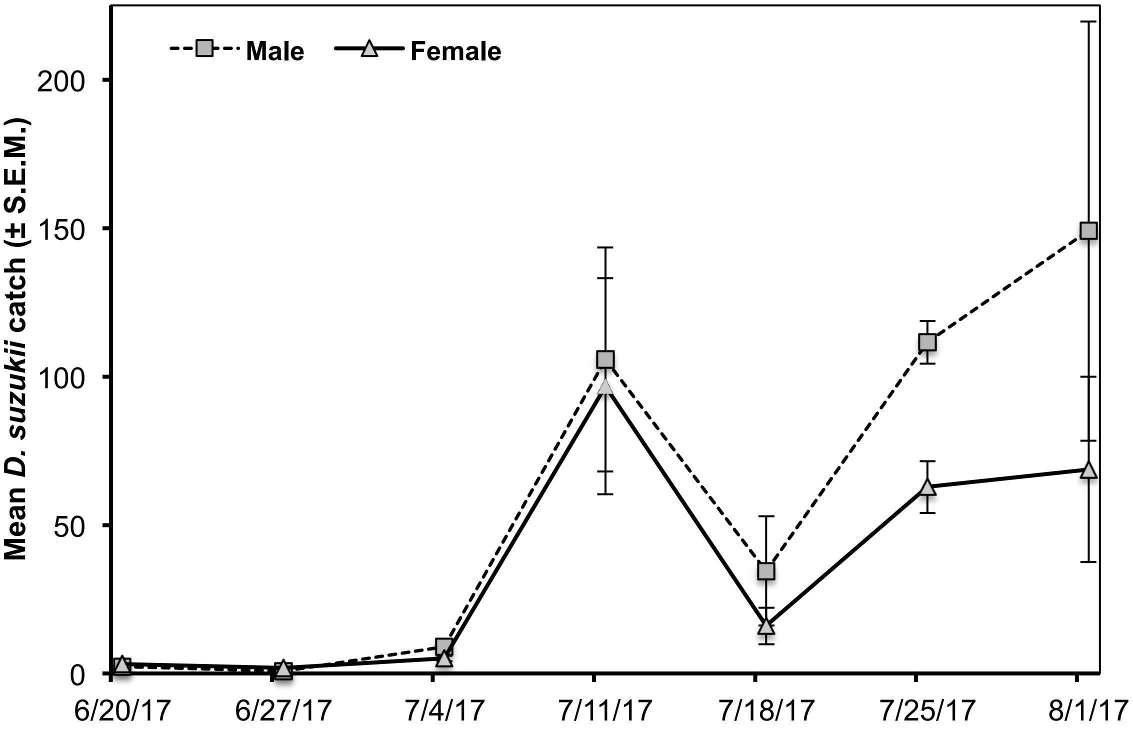

Figure 4.5. Mean (± S.E.M.) number of *Drosophila suzukii* males (dashed line) and females (solid line) captured with red panel traps in cherry orchards over the growing season. Means capped with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

DISCUSSION

In cherry orchards and raspberry high tunnels, red traps baited with a commercial lure consistently captured more *D. suzukii* than clear deli-cup traps baited with the same commercial lure. A trap that integrates both visual and olfactory cues captures more flies than traps utilizing olfactory cues alone.

A deli-cup trap baited with a yeast and sugar solution captured more flies than a deli-cup trap that was baited with the commercial Scentry lure. Furthermore, deli-cup traps baited with liquid solutions are messy, have a foul odor, and need to be transported to the laboratory to check the contents of the traps, presenting the potential for the trap contents to spill during transport. Using a dry sticky trap, such as a panel trap or sphere trap baited with a commercial lure is an improvement over using a deli-cup trap; there is no liquid bait or solution in the traps and thus no spillage. The traps still need to be transported to the laboratory to confirm identity of female *D. suzukii* captured on traps with the aid of a microscope. Using the panel trap allows for the trap to go directly under the microscope and identify female *D. suzukii* captured on the traps, and doesn't require the contents of the trap to be sorted prior to checking the contents to confirm identity.

Fly captures by gender from this study show that catch changes over time as the population builds throughout the season, but there was no significant difference in captures of males and females in traps over time. Even though the first captures in traps are usually females in the early spring, males are captured in traps along with females the week following first capture of females. These early-season captures in traps typically occur in spring before the fruit is at a stage vulnerable to infestation by *D. suzukii*.

The presence of male *D. suzukii* captured on red panel traps can be visually confirmed in the field, greatly reducing time and effort for identifying presence of *D. suzukii*. Male *D. suzukii* can be identified in the field directly on the trap by visually confirming the presence of spots on the wings, removing the need to transport the traps to the laboratory to confirm captures of *D. suzukii* with the aid of a microscope. This makes monitoring efforts easier and less time consuming when using the panel trap and allows for monitoring efforts to move away from using the deli-cup trap and having to sort through liquid contents to identify *D. suzukii*.

This study has practical significance, and we postulate that red panel traps will be useful monitoring tools for *D. suzukii* in the future. This study provides a foundation for further research addressing how further improvements in trap design are justified. While this trap design improves ease of trap use and being able to identify males captured on traps in the field, this trap design still falls short of being a monitoring tool that enables detection of *D. suzukii* while populations are at or below the economic injury level. Using any of the monitoring traps currently available and capturing a single *D. suzukii* signals an already high population that can build quickly and cause economic losses (Kirkpatrick et al. 2018). Further research should be done examining how a threshold could be developed based on the percentage of traps within a growing region capturing *D. suzukii.* For example, the current pattern of low captures of *D. suzukii* for about four weeks in Michigan cherry orchards early in the season is not enough to trigger action based on low numbers of flies captured in those traps because fruit is not yet ripe enough to be susceptible to infestation by *D. suzukii*. When populations begin to build and a percentage of traps within a region, for example if 10% of traps are capturing *D. suzukii* within a growing region, those captures together could be enough to decide when a treatment is needed. Additionally, some measure of fruit susceptibility, such as fruit color or firmness, should be incorporated into the decision-making process. Despite not being able to develop an action threshold for *D. suzukii* with the improved monitoring traps, the increased captures with red traps and ease of use of the traps in the field by using a dry, sticky red trap are significant contributions to the improvement of monitoring tools for this pest and further research using the red, sticky traps is justified to develop action thresholds in the future.

CHAPTER 5: ESTIMATING MONITORING TRAP PLUME REACH AND TRAPPING AREA OF *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) IN MICHIGAN TART CHERRY

INTRODUCTION

Research by Miller et al. (2015) on practical applications of random walk theory and confirmatory field experiments (Adams et al. 2017 a,b) using codling moth (*Cydia pomonella*) have revitalized the notion that capture numbers from insect monitoring traps can be translated into useful estimates of absolute pest density so as to sharpen management decisions. The current research extends this approach to estimating absolute pest density to *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) with dual aims of improving management of this invasive pest and expanding validation to this new methodology for more informed pest management. Specific aims of the current research were to quantify *D. suzukii* monitoring trap plume reach, trapping area, and overall trapping probability per trapping area (T_{fer}), as well as to associate a pest density estimate with a particular catch number.

Drosophila suzukii is a highly invasive pest originating from Asia that infests small and stone fruit growing regions of North America, Europe, and South America (Goodhue et al. 2011; Calabria et al. 2012; Cini et al 2012; Deprá et al. 2014; Asplen et al. 2015) and has a broad host plant range that includes cultivated crops as well as wild and native fruiting plants (Lee et al. 2011, 2015; Walsh et al. 2011; Bellamy et al. 2013). Unlike most *Drosophila* species, female *D. suzukii* have a large, serrated ovipositor that enables oviposition into ripening fruit; the resulting larval feeding renders the fruit unmarketable (Lee et al. 2011, Walsh et al. 2011, Burrack et al. 2013). Because of the broad host range and ability to target small and stone fruit as it ripens, *D. suzukii* is a serious economic threat to commercial fruit production (Goodhue et al. 2011). Current management practices rely on an unsustainable frequency and cost of insecticide applications and raise the potential risk for insecticide resistance (Haviland and Beers 2012; Van Timmeren and Isaacs 2013). Many commercial and homemade traps have been used to capture *D. suzukii*. However, nothing is known about how capture numbers relate to absolute density for *D. suzukii*. This is the first attempt to translate catch of *D. suzukii* in a monitoring trap into estimates of absolute adult density.

MATERIALS AND METHODS

Drosophila suzukii **Colony.**

Our laboratory colony was sourced from a colony maintained at the Trevor Nichols Research Center of Michigan State University (6237 124th Avenue, Fennville, MI) originating from flies field collected on the grounds of this research station. Flies were reared and maintained on the *D. suzukii* solid food diet (Dalton et al. 2011) in 50-mL polystyrene vials (Genesee Scientific, San Diego, CA) and held in a growth chamber at 24°C, 45% relative humidity (RH) and a photoperiod of 16:8 (L:D) h. Adults flies used in the experiments were 3-7 d old and sexually mature.

Marking methods for ***D. suzukii.***

Flies were lightly anesthetized with CO_2 to facilitate handling, sorting by sex, and counting. Glass powder insufflators with polypropylene plastic tops (Model 119, DeVilbiss Healthcare, Port Washington, NY) were used to apply micronized fluorescent dusts to the flies for release (Crumpacker 1974). Insufflators permitted many flies at a time to be marked quickly and well. Fluorescent dusts have been used in previous mark-capture studies for marking various species of *Drosophila*, including *D. suzukii*, and no differences in the survival have been found between marked or unmarked individuals (Crumpacker and Williams, 1973; Crumpacker 1974; McKenzie 1974; Rice et al. 2017). Flies for each release distance were uniquely marked with a different color of fluorescent dust (DayGLO Color Corporation, Cleveland, OH) with the same color for marking always used for the same distance in each replicate. The specific colors used here were: Horizon Blue (ECO19), Signal Green (ECO18), Aurora Pink (ECO11), Blaze Orange (ECO15), and Saturn Yellow (ECO17). One powder insufflator was used for each separate color of fluorescent dust. Two "puffs" of the powder (ca. 6 mg) were applied over groups of 100 (50 male: 50 female) anesthetized flies held in a glass beaker. Marked flies were then transferred to a ventilated 237 ml plastic deli-cup (Fabri-Kal Corporation, Kalamazoo, MI) containing a moist 9 cm diam filter paper and 3 cm of dental wick moistened with 20% sucrose solution. Deli-cups containing marked flies were held for up to 18 hrs in a growth chamber under rearing settings prior to release in orchards.

Orchards.

The first study area was a 0.9 ha experimental tart cherry orchard block located at the Trevor Nichols Research Center of Michigan State University (Fennville, MI). The second was a 30 ha commercial tart cherry orchard (Lawrence, MI). No insecticides were sprayed in either orchard for the duration of the studies. Marked *D. suzukii* were released in the commercial tart cherry orchard only after cherry harvest had been completed to avoid exposure of marked flies to insecticides applied by growers.

Single-trap, multiple release tests.

The trials at the Trevor Nichols Research Center were conducted from May 23 to June 19 2016, and those in the commercial tart cherry orchard from July 4 to August 21 2017. At Trevor Nichols Research Center, release points were flagged in the four cardinal directions from the central monitoring trap (Fig. 1) at 10, 20, 30, 40, and 50 m for five replicates. At the commercial orchard, release points were flagged in the four cardinal directions from the central trap (Fig. 5.1) at 10, 20, 30, and 40 m for three replicates, and at 40, 80, 120, 160, 200 m for one replicate. Longer distances for one replicate of 120, 160, and 200 m were used to confirm maximum dispersive distance of the insect. In the nine replicates, the number of marked flies released at each point started with 400 flies at the closest distance, and was increased by 400 flies per distance to account for increasing annulus area as distance increased from the central monitoring trap. For all replicates, flies were released from 10:00 – 11:00 am. To release flies from the deli-cups, the tops of the deli-cups were removed and the flies flew out on their own or were gently dumped from the deli-cup onto the leaves of the trees. Approximately 6,200 total marked *D. suzukii* were released per replicate.

A single 20 x 30 cm double-sided, sticky, red panel trap (Great Lakes IPM, Vestaburg, MI) baited with a commercial *D. suzukii* lure (Scentry Biologicals, Billings, MT) was placed in the bottom third of the canopy of a cherry tree near the center of the block. A 1.5 cm hole in each corner of a panel enabled it to be hung from a tree branch with twist ties and a similar hole in the center of the trap allowed the lure to be attached with a twist-tie. This trap incorporated a red visual cue (Kirkpatrick et al. 2015) and was easily placed under the microscope to identify *D. suzukii* from other non-target drosophilids. Field studies have documented that this trap was reliable in capturing *D. suzukii* and performed similarly to other trap types used for this pest (D. Kirkpatrick, unpublished data). Traps were checked daily and the sticky panels with any captured flies were replaced. Panels with captures were examined in the laboratory under a combination of UV illumination: 22 W fluorescent Circline BL #2851L and 15 W fluorescent tube BL #2806 (BioQuip products, Rancho Domingues, CA), and 32 W UV LED retrofit bulb (Battery Junction, Old Saybrook, CT) in an ML300L 3-cell D flashlight (Mag Instruments, Ontario, CA) to determine color of the powder marks (Adams et al. 2017a). One commercial lure was used for 1 wk and changed weekly for each new replicate.

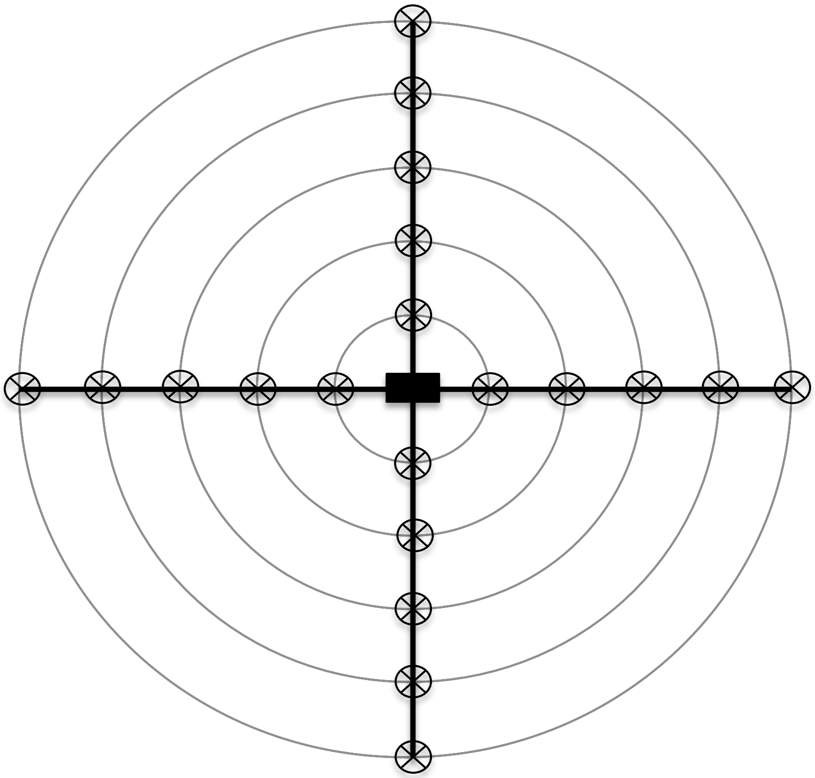

Figure 5.1. Spatial pattern of *Drosophila suzukii* releases (depicted by circled x) around one central monitoring trap (depicted by rectangle).

Data Analysis.

Terminology for data analysis follows Miller et al. (2015). Plume reach indicates the distance from the trap that insects respond to the plume. Likewise, combined data from two years were graphed in three ways: 1) an untransformed plot of proportion of *D. suzukii* recaptured vs. distance of release from central trap, 2) 1/proportion of *D. suzukii* recaptured vs. distance of release from central trap (MAG plot), and 3) annulus area x proportion of *D. suzukii* recaptured vs. distance of release from central trap (Miller plot).

Areas of trapping annuli under even trap spacing were calculated as per Miller et al. (2015). The untransformed plot provided confirmation that the release distances were appropriately spaced, and confirms random movement by *D. suzukii* when the line fitted to the graph is smoothly concave and approaches the x-axis asymptotically. For random walkers, the MAG plot will be linear over the close release distances; its slope can be used to determine plume reach of the monitoring trap using the standard curve of Miller et al. (2015), Fig. 4.12. A second-order polynomial was fitted to the Miller plot data; the line was forced through the origin such that the point at which the line crosses the x-axis estimates the maximum dispersive distance for 95% of the responding population (Adams et al. 2017a). T_{fer} (average proportion caught out of all insects in the full trapping area) for the cardinal direction release experiments was calculated by dividing the mean of the proportion caught at a specific distance (spT_{fer}) x annulus area by the mean annulus area (Mean (spTfer x Annulus Area) / Mean Annulus Area) (Eq. 5.2, Miller et al. 2015).

RESULTS

Cardinal-direction releases.

Less than 2% of released flies were recaptured on average. Catch decreased smoothly with distance from the central trap and approached the x-axis asymptotically (Fig. 2) as expected for random walkers. The mean proportion caught at the closest distance of 10 m from the central trap was 0.017 ± 0.004 (mean ± SEM), and mean proportion caught decreased to 0.0006 at the furthest distance of 80 m. The MAG plot produced a straight line with a slope of 0.88 (Fig. 3), which using the standard curves of Miller et al. (2015) equates to a plume reach of < 3 m. The Miller plot of data projects the maximum dispersive distance for 95% of the population at approximately 90 m (Fig. 4). Using these data, the resulting trapping radius of 93 m equates to a trapping area of 2.7 ha for a red, sticky lure-baited monitoring trap in tart cherry. The mean T_{fer} was 0.0052 (n = 9).

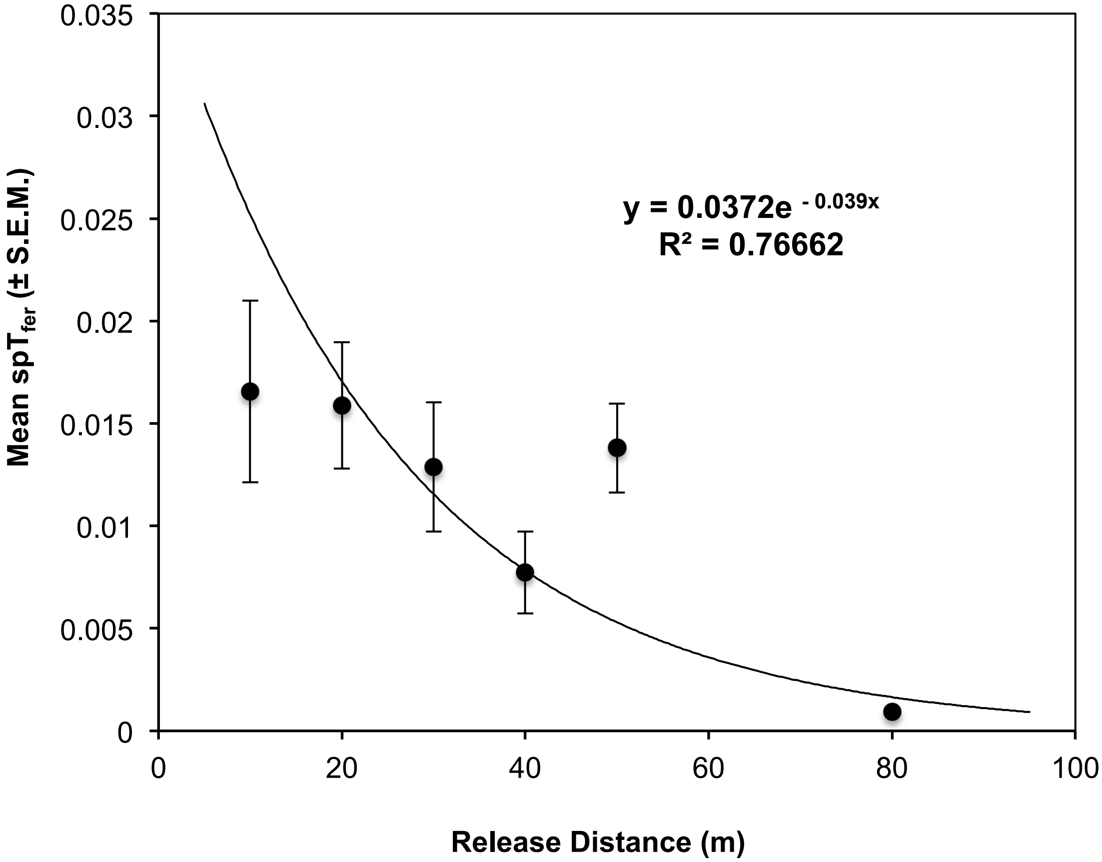

Figure 5.2. Mean probability of catch at a specific distance (spT$_{fer}$) versus distance (m) of release of *Drosophila suzukii* from a single, central trap.

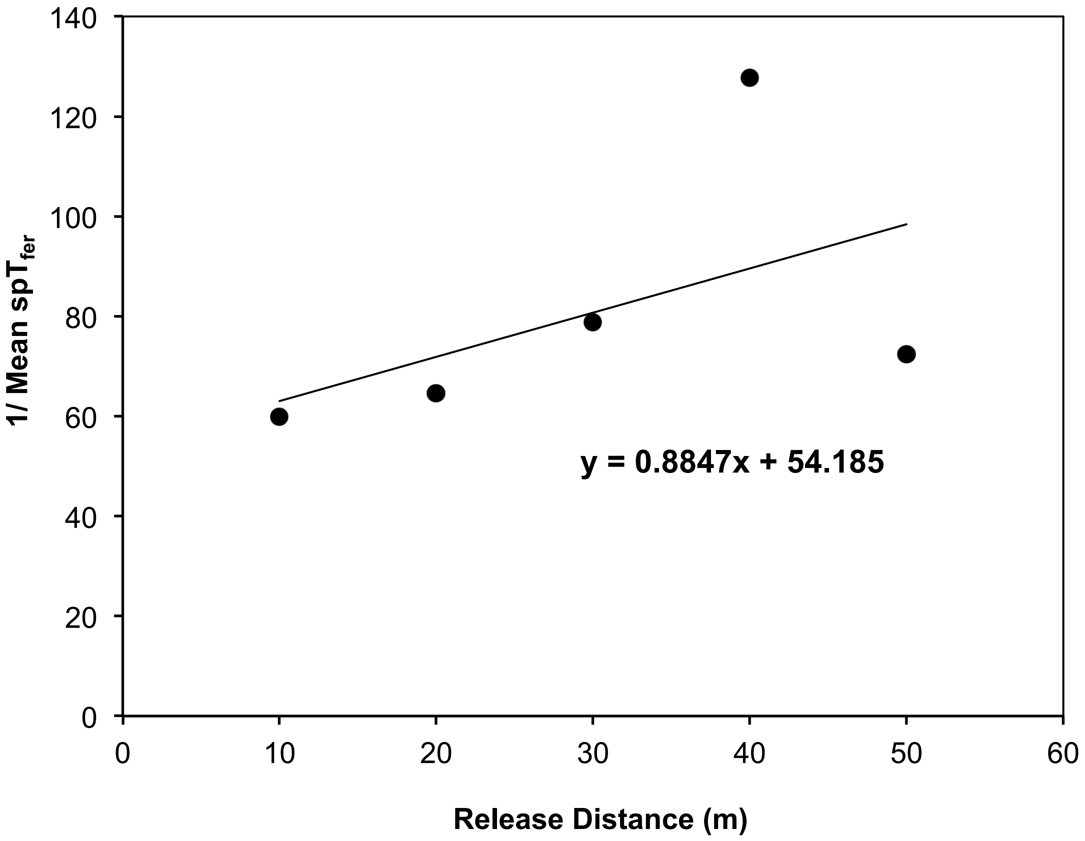

Figure 5.3. MAG plot of *Drosophila suzukii* mean spT_{fer} data.

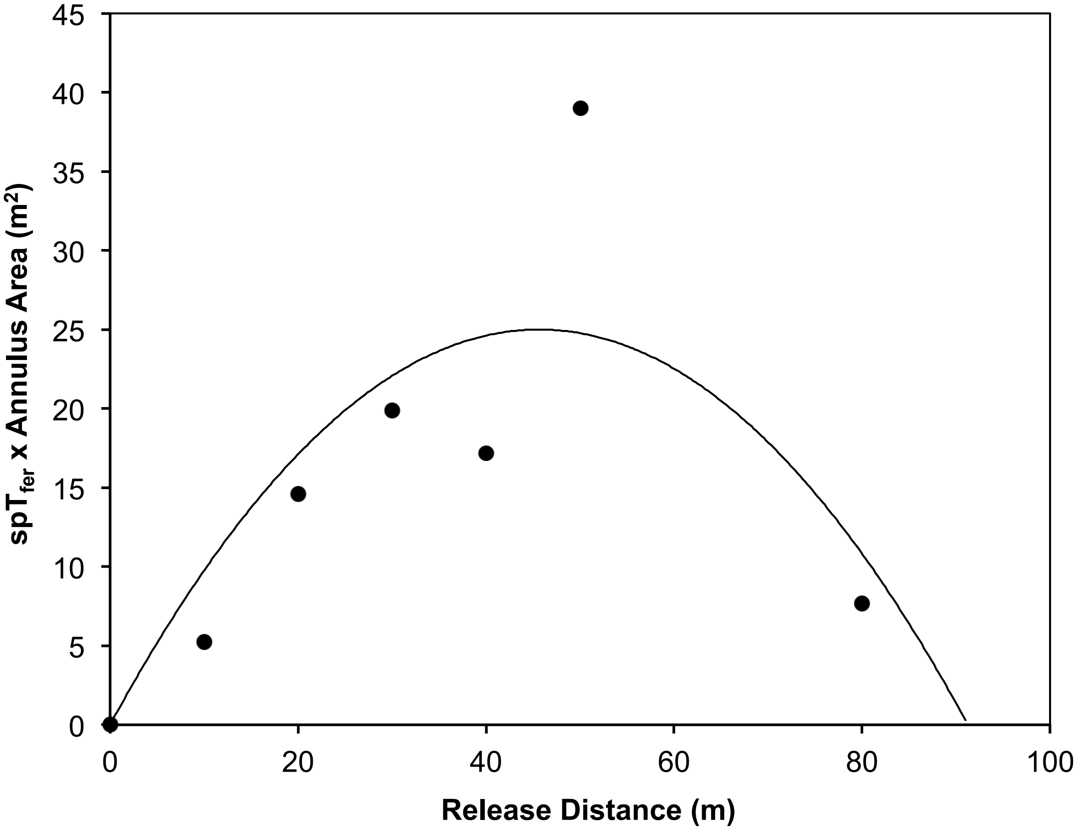

Figure 5.4. Miller plot transformation for the *Drosophila suzukii* releases.

DISCUSSION

Plume Reach, Maximum Dispersion, Trapping Area, and T_{fer}.

Our estimate of plume reach for the lure-baited monitoring trap in tart cherry was short (< 3 m) but similar to that for a standard sex pheromone-baited monitoring trap for codling moth (< 5 m) in commercial apple (Adams et al. 2017a). With such a short plume it is even possible that this trap functions more to arrest (Miller et al. 2009) *D. suzukii* than to attract them from a distance. Short plumes are also associated with volatiles of low molecular weight that evaporate readily but are more difficult for antennae to collect from air when in low concentration (Gut et al. 2004). Since their lure compositions are very similar, we postulate that the plume reaches for other types of *D. suzukii* monitoring traps would be similar to that measured for the current trap. Although its reach is very short, the plume still plays a very important role in delimiting dispersion and guiding the foraging insect into the trap.

Maximum net dispersive distance for 95% of the released *D. suzukii* was estimated at 90 m and that dispersal took place over only several days, as limited by the relatively short lifespan of the released flies in the field. Adding maximum dispersive distance (90 m) to plume reach (3 m) yields a trapping radius of 93 m (Miller et al. 2015); and trapping area is then calculated at 2.7 ha.

For the current study in commercially managed tart cherry, the average probability of capture for flies in the trapping area (T_{fer}) was 0.0052. This value may seem tiny, but is similar to that measured for Western corn rootworm (0.006) and codling moth (0.008); all T_{fer} values measured over an entire trapping area turn out to be small (Table 5.8 of Miller et al. 2015).

Replicates of our experiment spanned a variety of growing conditions from early season when the fruit was beginning to ripen, throughout the growing season in different stages of ripening, and post-harvest when unharvested fruit on the trees was ripe. These replicates also spanned a variety of environmental conditions such as varying temperature, humidity, sunlight, and resource abundance as seasonality changed throughout the replicates. Despite the differences in environmental conditions and fruit stages, the data fit well together across the two seasons and provide important insights of dispersal patterns for *D. suzukii* regardless of time of season or stage of fruit, and provide the first account of *D. suzukii* dispersive capabilities, plume reach, and trapping radius for a *D. suzukii* monitoring trap. This new information about plume reach of a monitoring trap can be used to determine optimal spacing of traps used for attract-and-kill studies and can provide suggestions about what density of monitoring traps within an orchard is acceptable and most cost efficient for growers.

Converting Monitoring Trap Catch to Absolute Pest Density.

Absolute density of *D. suzukii* per trapping area (2.7 ha) is calculated by dividing catch number by T_{fer} (0.0052) (Miller et al. 2015; Adams et al. 2017a). Captures in a monitoring trap of 1, 50, 100, 500, and 1,000 equate to ca. 192, 9,615, 19,230, 96,154, and 192,300 *D. suzukii* per trapping area of 2.7 ha. Assuming the sex ratio for *D. suzukii* is 1:1 (as supported by laboratory studies by Emiljanowicz et al. (2014)) and that both sexes respond equally to traps (as supported by laboratory studies of Kirkpatrick et al. (2015) and field studies by Landolt et al. (2012), Cha et al. (2012), and Kirkpatrick et al. (2017)), then half the absolute pest density within the trapping area can be taken as female and half male.

The mean number of eggs laid by a female *D. suzukii* over her lifespan is estimated ca. 380, and approximately 1-3 eggs are laid per oviposition site (Walsh et al. 2011). These data, coupled with short generation times, high reproductive potential, overlapping generations during the growing season, suggest *D. suzukii* has the potential to reach very high population levels so as to generate extremely high levels of infestation within a trapping area over a single growing season in the absence of insecticide sprays. When a single fly is captured in a monitoring trap (suggesting there are ca. 192 flies within that trapping area and half of those are females with the potential to lay 380 eggs), in the next generation within that trapping area there will be ca. 36,000 flies if all the eggs successfully develop into adult flies. In the generation following, if half of those flies are females each with the potential to lay an average of 380 eggs, then in the generation that follows there will be upwards of seven million flies within that trapping area alone in only three generations, with the possibility of another ten generations remaining in that growing season depending on environmental conditions. This example shows how, in the absence of control tools, *D. suzukii* populations have the ability to quickly build to very high numbers if action is not taken to control that population.

Probability of Damage when *D. suzukii* Catch per Trap is Zero.

Given the highly stochastic nature of trapping random walkers (Miller et al. 2015; Adams et al. 2017a), a catch of zero pests in a single trap should not be interpreted as zero infestation. For a *D. suzukii* population at a damaging level of 100 individuals per ha, we can (as per Adams et al. 2017b) use the binomial equation and the average catch probability measured here of 0.0052 to calculate that the probability of catching zero flies per one trap under the current experimental conditions; it is an unacceptable 0.26. One tactic for increasing sampling power and accuracy is to deploy multiple traps so as to record mean catch per trap (Adams et al. 2017b) rather than a single trapping datum. As documented for codling moth (Adams et al. 2017b), five traps were successfully spaced only 4 m apart in a line so as to reduce cost by minimizing travel between sampling sites. This approach could also be useful for *D. suzukii* to increase the sampling power and accuracy of a trap given that the short plume reach of its monitoring trap predicts very low trap competition at distances exceeding twice the plume reach (Miller et al. 2015).

Applying These Methods to Other Pests.

The Miller et al. (2015) methods for interpreting catch in a monitoring trap, first validated on codling moth using a pheromone baited trap (Adams et al. 2017a), were now successfully applied to a drosophilid pest for which this method has never been applied, using a vastly different lure than a sex-pheromone lure. Many types of traps and lures are used to monitor *D. suzukii*, and until the current study nothing was known about relating captures in traps to the absolute population. Moreover, once a single *D. suzukii* is captured in a single trap, the population density is already high. If the fruit are at a vulnerable stage, management steps should be immediately implemented. These analyses methods are now broadly recommended for any randomly foraging pest, as well as for beneficial insect populations, and should be applicable in diverse crops. They offer a clear and practical pathway for substantial improvement in quantitative pest management.

CHAPTER 6: COMPARATIVE ANTENNAL AND BEHAVIORAL RESPONSES OF SUMMER AND WINTER MORPH *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) TO ECOLOGICALLY RELEVANT VOLATILES

INTRODUCTION

First discovered on mainland USA in 2008, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) has rapidly spread to become a devastating global pest of berry and cherry crops in the Americas and Europe (Walsh et al. 2011; Cini et al. 2014; Deprá et al. 2014; Asplen et al. 2015). Unlike other species of *Drosophila*, female *D. suzukii* have a large, serrated ovipositor that enables them to penetrate the skin of undamaged and ripening fruit and lay eggs inside (Walsh et al. 2011; Atalla et al. 2014). The feeding larvae degrade the fruit, causing significant crop losses (Bolda et al. 2010; Farnsworth et al. 2017). Current management programs rely primarily on regular applications of conventional broad-spectrum insecticides (Van Timmeren and Isaacs 2013; Haye et al. 2016; Diepenbrock et al. 2017), which are not only unsustainable, but also have negative impacts on beneficial arthropods and disrupt integrated pest management programs (Desneux et al. 2007; Biondi et al. 2012). Moreover, little is understood about the seasonal biology and behavior of *D. suzukii*, particularly during cold periods (Asplen et al. 2015), knowledge that is necessary to effectively implement control strategies.

To adapt and survive in certain temperate climates, insects have evolved a number of physiological mechanisms for coping with the effects of low temperature, such as reproductive diapause, cold hardiness, and improved tolerance to environmental stressors such as extreme cold (Kimura 1988; Hoffman et al. 2003; Teets and Denlinger 2013). Several species of *Drosophila* overwinter in cold climates as adults under leaf litter where they are protected from extreme cold temperatures by a layer of snow (Hoffman et al. 2003; Stephens et al. 2015). Their overwintering strategy may include reproductive diapause, limiting their reproduction to favorable conditions, and directing their resources to winter survival (Hoffman et al. 2003; Wallingford and Loeb 2016). Additionally, environmental signals such as shorter day lengths and decreasing temperatures can trigger seasonal adaptations such as rapid cold-hardening or gradual acclimation experienced during development (Lee et al. 1987; Teets and Denlinger 2013). Developmentally acclimated flies would be the most prepared to survive the starvation and freeze stress associated with overwintering (Wallingford and Loeb 2016).

Drosophila suzukii has a wide climatic range and is now found on nearly every continent (Calabria et al. 2012; Deprá et al. 2014; Asplen et al. 2015). Phenotypic plasticity in response to seasonal changes produces separate morphs of *D. suzukii* that survive in a range of temperatures and environmental conditions, including low or freezing temperatures (Stephens et al. 2015; Jakobs et al. 2016; Shearer et al. 2016). This winter morph has a darker pigmented body and longer wings than the summer morph (Stephens et al. 2015; Shearer et al. 2016). In the laboratory, this morph can be induced when larvae are subjected to colder temperatures and shorter day lengths (Shearer et al. 2016). Additionally, *D. suzukii* winter morphs have various up-regulated and down-regulated genes compared to *D. suzukii* summer morphs that are responsible for female diapause, synthesis of cryoprotectants and chitin, and metabolic processes, among others (Shearer et al. 2016; Wallingford and Loeb 2016).

Our current knowledge of the winter morph of *D. suzukii* is limited to physiological changes and oviposition capacity (Stephens et al. 2015; Jakobs et al. 2016; Shearer et al. 2016; Wallingford and Loeb 2016). As temperatures in northern regions begin to decline in the late fall and winter months, captures of *D. suzukii* in traps decline until captures are extremely low or zero. After January, typically no winter morph *D. suzukii* are captured in traps, and the first summer morphs are not captured in traps in late May or June in Michigan and northern regions (D. Kirkpatrick, unpublished data). Understanding differences in response to cues between the two morphs could potentially lead to improved monitoring during the winter and early spring periods and inform studies of early season biology.

While attractive volatiles for *D. suzukii* summer morphs for use in trapping have been identified (Cha et al. 2012; Cha et al. 2013; Revadi et al. 2015), there is a lack of information regarding volatiles that might be important for overwintering behavior. To better understand the influence of volatiles between these morphs, we evaluated 1) antennal responses to various odorants using electroantennogram (EAG), 2) morphology and number of olfactory sensory neurons using a scanning electron microscope (SEM), and 3) behavioral responses in no-choice and T-maze bioassays to determine if there was a differential response to volatiles between *D. suzukii* summer morphs and winter morphs. Differential response to odorants may help optimize traps that are morph-dependent, but also allow us to better understand the overwintering behavior of *D. suzukii*.

MATERIALS AND METHODS

***Drosophila suzukii* colonies.**

Laboratory-reared *D. suzukii* female adults were used in all experiments. The laboratory colony was initially obtained from field-collected flies in 2015 and was maintained as summer morphs on the *D. suzukii* solid food diet (Dalton et al., 2011) in 50 mL polystyrene vials (Genesee Scientific, San Diego, CA) and held in a growth chamber at 24°C, 45% R.H. and 16:8 (L:D) photoperiod.

The *D. suzukii* winter morph colony was reared from the summer morph colony. Thirty adult summer morphs were held in vials with diet in a growth chamber at 24°C for 3 days. The adult flies were then removed from the vials, and the vials containing eggs and young larvae were placed in another growth chamber at 10°C, 45% R.H. and 12:12 (L:D) photoperiod (Shearer et al. 2016). Winter morph flies eclosed from pupae in these vials after an average of 42 days.

All flies used in the experiments were 3-7 days old and sexually mature. Flies were lightly anesthetized with CO_2 to facilitate handling, sorting by sex, and counting.

Odorants.

Odorants were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of the highest purity available (>95%). Odorants used for experiments were isoamyl acetate (W205508-SAMPLE-K), geosmin (G5908-1ML), methionol (318396-5G), linalool (51782-1ML), bornyl acetate (45855-1ML-F), and acetic acid (64-19-7). Paraffin oil (18512-1L) was used as a solvent for all chemicals except acetic acid, where deionized water (DI) water was used as a solvent. Test odorants isoamyl acetate, geosmin, methionol, linalool, and bornyl acetate were diluted in paraffin oil to obtain three concentrations (10^{-1}, 10^{-2}, and 10^{-3}) and the control solution was paraffin oil alone. The test odorant acetic acid was diluted in DI water to obtain three concentrations (10^{-1}, 10^{-2}, and 10^{-3}) and the control solution for this odorant was DI water alone. Acetic acid, methionol, and isoamyl acetate were selected for their known attraction to summer morph *D. suzukii* (Cha et al. 2012; Revadi et al. 2015). Both bornyl acetate (derived from pine) and linalool (derived from flowers) were evaluated because of their potential as a sugar source when fruit is not available (sap from pines in the fall, and nectar from flowers in the spring). Geosmin (derived from soil-borne bacteria) was selected because it had previously been evaluated for *D. suzukii* summer morphs as a potential deterrent (Wallingford et al. 2016) and due to the potential for soil and leaf litter to be an overwintering site for *D. suzukii.*

Electroantennogram recordings.

Antennal receptivity of adult female *D. suzukii* to synthetic compounds was determined by electroantennogram (EAG). The procedure for EAG is similar to that previously described for *D. melanogaster* by Ayer and Carlson (1992). Flies were held during recording in a 200 µl pipette tip. The fly was inserted into the pipette tip using a mouth aspirator with the head of the fly towards the small end of the tip. The small end of the tip was trimmed such that the antennae and head emerged from the end of the tip, and a second cut was made to trim the pipet tip approximately 1 mm behind the fly. To prevent the fly from crawling out backwards, clay was placed in the large end of the cut tip so that it touched the posterior end of the fly. The fly was then placed on a microscope slide with the head next to a coverslip that was mounted on the microscope slide. The antennae were maneuvered so that they lay flat on the stack of the coverslip. To stabilize one of the antennae further, a glass capillary tube was used to hold the second antennal segment in place. The third antennal segment was centered under a stereomicroscope

(Nikon SMA645, Japan) that was mounted on a vibration isolation table. Reference and recording electrodes were glass capillaries (World Precision Instruments, 1B100F-4), pulled such that tips were ≤ 1 μm in diameter, and filled with Drosophila Ringer's solution (in mM): NaCl 100, KCl 5, $MgCl_2$ 20, $CaCl_2$ 0.15, HEPES 5, Sucrose 115, Trehalose 5. The reference electrode was inserted into the contralateral eye. Using a stereomicroscope (Nikon SMA645, Japan), the recording electrode was then brought into contact with the posterior aspect of the third antennal segment and advanced until stable electrical contact was established. The electrodes made electrical contact with a high impedance amplifier (World Precision Instruments, DAM 50) via silver/silver-chloride wires. The output signal of the amplifier was fed into a computer via a digitizer (Axon CNS molecular devices, Digidata 1440A). The electrical signal was collected using Axoscope 10.4 software and measured and analyzed by using Clampfit 10.4 software. Odor stimuli were presented from Pasteur pipettes holding solutions of chemicals in paraffin oil or DI water on filter paper. An aliquot of 50 μl of solution was dropped on a 0.5-inch filter paper strip placed in the shaft of a Pasteur pipette. The tip of a Pasteur pipette was placed through a hole in a tube that carried a humidified air stream over the fly. Compressed air was controlled by a solenoid that created puffs of odorant through the pipette. A minimum of ten females of each morph was used in these tests. Flies were starved for 2 h prior to use in experiments. All EAG experiments were performed from 10:00 to 14:00 h at 21-22°C and 45-60% R.H.

Scanning electron microscope photos.

Summer and winter morph flies were placed in a -80 °C freezer for 5 minutes. After defrosting, the flies' heads were cut off with a small syringe and then fixed at 4°C for 1-2 hours in 4% glutaraldehyde buffered with 0.1 M sodium phosphate at pH 7.4. Following a brief rinse in the buffer, samples were dehydrated in an ethanol series (25%, 50%, 75%, 95%) for 45 min at each gradation. Samples were critical point dried in a critical point dryer (EM CPD300, Leica Microsystems, Vienna, Austria) using carbon dioxide as the transitional fluid. The heads were mounted on aluminum stubs with heads facing up using carbon tape (Ted Pella, Inc., Redding, CA) and were then coated with osmium (≈10 nm thickness) in a NEOC-AT osmium chemical vapor deposition (CVD) coater (Meiwafosis Co., Ltd., Osaka, Japan). Samples were examined and photos were taken in a JEOL 6610LV (tungsten hairpin emitter) scanning electron microscope (JEOL Ltd., Tokyo, Japan).

Using the 250x magnification SEM photos of the third antennal segment, the total antennal area using was calculated by tracing the circumference of the antennae using ImageJ. The number of ab4 and ab6 small basiconic sensilla were counted (de Bruyne et al. 2001) within a 40 x 60 μm area (approximately where the recording electrode was placed for the EAG studies) on the anterior of region 4 in the third antennal segment using the 600x magnification photos (Fig. 2). The ab4 basiconic sensilla are responsible for detection of the geosmin compound (Stensmyr et al. 2012), but it is difficult to visually distinguish from the ab6 basiconic sensilla (Venkatesh and Singh 1984); therefore all small basioconic sensilla were counted and considered together.

No-choice test laboratory behavioral bioassays.

No-choice tests were conducted to elucidate behavioral responses of *D. suzukii* to volatiles. Traps for behavioral assays were 20 ml glass scintillation vials with a 3-D printed (Ultimaker 3, Cambridge, MA) plastic lid with a 1 cm diameter opening in a funnel shape to facilitate capture of the flies. Bioassay arenas were 3.79 L glass jars held in a room with 45-60% R.H. and 22°C, covered with a piece of mesh material to facilitate airflow. Each bioassay arena contained a piece of filter paper in the bottom moistened with water to provide humidity inside the arenas, and a vial trap containing either 1 ml of solution (concentration 10^{-3}) or the control solution. A yeast and sugar solution (83% DI water, 14% sugar, 3% baker's yeast) was added to the study as a positive control, because it is routinely used as bait for trapping *D. suzukii* (Burrack et al. 2015). Twenty female flies were held in the assay room to acclimate to the conditions in a Petri dish with a moist piece of filter paper in the bottom and were starved for 2 h before each experiment. Petri dishes were placed in the bottom of the glass arena, and flies emerged from Petri dishes on their own. Traps were assessed after a 24 h period by counting the number of flies caught in each of the vial traps.

T-maze laboratory behavioral bioassays.

T-maze assays were used to further elucidate stimulatory or deterrent effects of geosmin on *D. suzukii* summer and winter morphs. The T-maze assay was adapted from a previously described assay with some modifications from Stensmyr et al. (2012). Two 1000 µl pipette tips were cut 2.5 cm from the narrow end and 0.5 cm from top, and were inserted into the bottom of two 1.5 ml microcentrifuge tubes with 2 mm removed from the tapered end. These two assembling units were connected using 4-cm of tubing (Tygon, E-3603, Akron, OH). In the middle of the tubing, a small hole was made and a 1000 µl pipette tip (cut 0.5 cm from the narrow end) was inserted into the tubing via the opening. A 0.8 mm × 3.2 mm piece of filter paper was inserted in between the pipette tip and the tip of microcentrifuge tube so flies in the tubing could not make contact with the filter paper. A solvent or test compound (5 µl) was applied to the filter paper after the assembly. After the compound was applied, ten female flies were gently introduced into the long pipette tip. In both microcentrifuge tube lids, a small hole was made using a syringe needle to facilitate airflow to maintain a concentration gradient for each tested compound. Trials were run in a room with 45-60% R.H. and 22°C and the numbers of flies entering the trap were counted after 24 h. The response index (RI) was calculated following the equation of Stensmyr et al. (2012) and calculated as (O-C)/T, where O is the number of flies in the baited arm, C is the number of flies in the control arm, and T is the total number of flies used in the trial. The resulting index ranges from -1 (complete avoidance) to 1 (complete attraction) (Stensmyr et al. 2012).

Statistical analyses.

EAG response data were normalized with response subtracted from the respective control solution and analyzed using a two-way analysis of variance. The antennal area and number of small basiconic sensilla satisfied normality assumptions using a Levene's test and were compared using analysis of variance. Data from both behavioral experiments satisfied assumptions of normality using a Levene's test and were analyzed using a two-way analysis of variance. Data from the no-choice tests were analyzed using a generalized linear mixed model with a Poisson distribution. The T-maze data were analyzed using the response index calculation with a generalized linear model. Tukey's HSD was used for all post-hoc comparisons. Data were analyzed using R (3.3.3, R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Electroantennogram recordings.

Using dose response curves, we determined the appropriate concentration for each odorant tested to be 10^{-2}, except for acetic acid, where a 10^{-1} concentration was also included. We found that summer morph response was elevated compared to winter morphs across four of the volatiles tested (Fig. 6.1). This was significantly different for geosmin ($F_{1, 18} = 4.9$, $P = 0.03$), isoamyl acetate ($F_{1, 18} = 4.5$, $P = 0.04$), and acetic acid 10^{-1} ($F_{1, 18} = 14.8$, $P = 0.001$). Acetic acid 10^{-2} response was higher for summer morphs, but not significantly different ($F_{1, 18} = 4.1$, $P = 0.05$). Likewise, methionol elicited a larger response in summer morphs, but not significantly so ($F_{1, 18} = 3.5$, $P = 0.07$). There were no differences found between morphs for either bornyl acetate ($F_{1, 18} = 0.06$, $P = 0.8$) or linalool ($F_{1, 18} = 0.04$, $P = 0.8$).

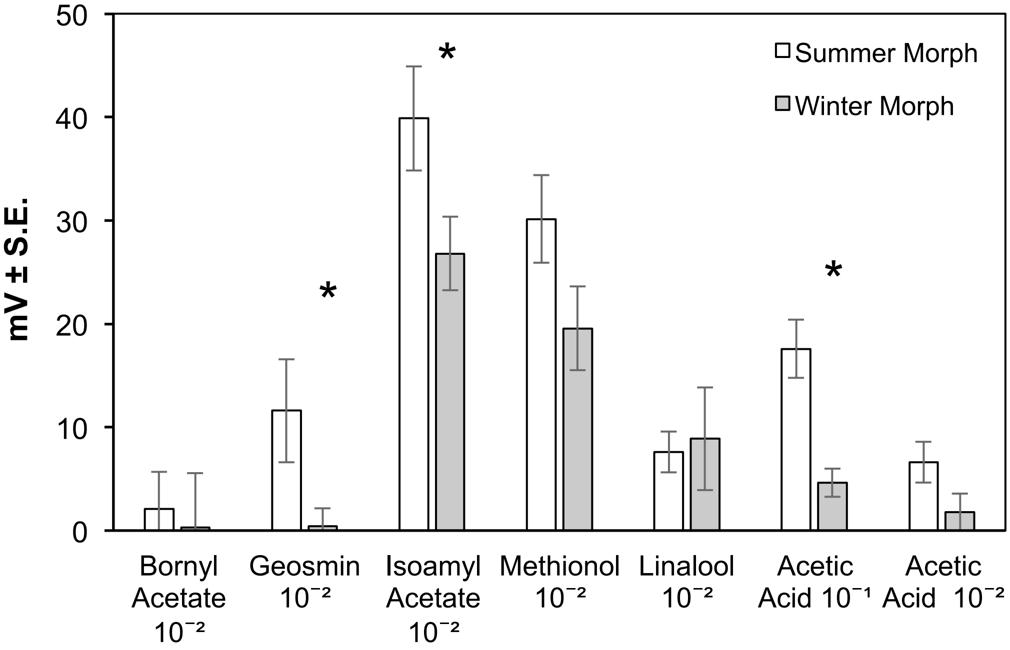

Figure 6.1. Mean *Drosophila suzukii* (± S.E.M.) summer morph (white bars) and winter morph (dark gray bars) response to volatiles in electroantennogram assays. Asterisks indicate a significant difference ($P < 0.05$).

Scanning electron microscope photos.

The area of the third antennal segment was similar between summer morphs (13.1 ± 0.9 mm) and winter morphs (13.2 ± 0.8 mm) ($F_{1,7} = 0.001$, $P = 0.98$). There were numerically more small basiconic sensilla (Fig. 6.2) found on summer morph antennae (8.6 ± 1.2) compared to winter morph antennae (6.2 ± 0.6), but these were statistically similar ($F_{1,7} = 3.36$, $P = 0.1$).

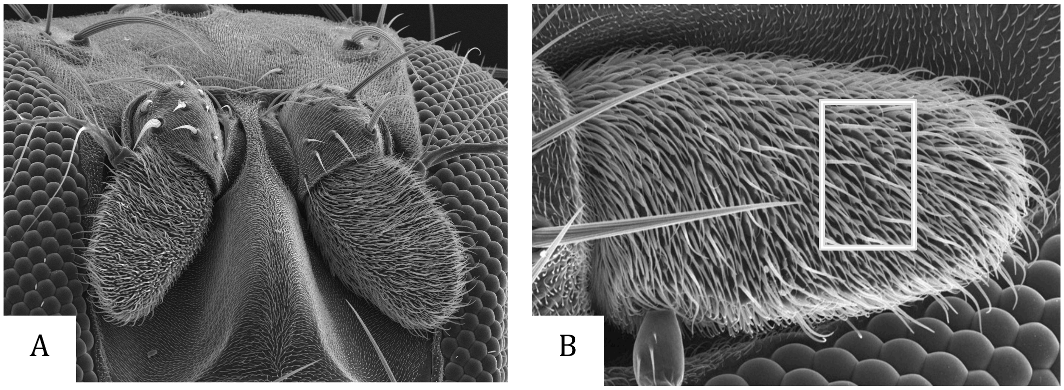

Figure 6.2. Scanning electron microscope (SEM) photos of the third antennal segments of *Drosophila suzukii*. Photo (A) shown at 250x and photo (B) at 600x magnification. Photo (B) shows the 40 x 60 µm area (white box) used to count the number of small basiconic sensilla on the third antennal segment of summer and winter morphs.

No-choice test laboratory behavioral bioassays.

Winter morph *D. suzukii* exhibited a significantly higher response to the volatiles geosmin ($F_{1, 14}$ = 9.36, P = 0.008) and bornyl acetate ($F_{1, 14}$ = 4.9, P = 0.04) compared with summer morphs in no-choice behavioral bioassays (Table 6.1). There was no significant difference between the morphs across any of the other tested volatiles.

Table 6.1. Average response (± S.E.) and associated statistical values by winter and summer morph *Drosophila suzukii* in a no-choice test to nine different volatiles. Bolded p-values indicate significantly different means between the two morphs.

Treatment	Summer morph mean	Winter morph mean	F-value	P-value
Water	22.2 ± 5.4%	27.2 ± 7.7%	$F_{1,14}$ = 0.28	P = 0.61
Paraffin Oil	6.7 ± 1.7%	15.6 ± 4.4%	$F_{1,14}$ = 3.82	P = 0.07
Yeast Sugar	47.2 ± 7.5%	38.3 ± 7.8%	$F_{1,14}$ = 0.68	P = 0.42
Geosmin	17.2 ± 3.2%	35.6 ± 4.9%	$F_{1,14}$ = 9.36	**P = 0.008**
Bornyl Acetate	10 ± 1.9%	20.6 ± 4.5%	$F_{1,14}$ = 4.90	**P = 0.04**
Linalool	9.4 ± 3.7%	7.2 ± 1.5%	$F_{1,14}$ = 0.37	P = 0.55
Acetic Acid	17.2 ± 5.5%	20.5 ± 6.6%	$F_{1,14}$ =0.17	P = 0.68
Methionol	20.5 ± 4.1%	10.5 ± 2.8%	$F_{1,14}$ = 4.23	P = 0.05
Isoamyl acetate	18.3 ± 3.7%	17.2 ± 5.2%	$F_{1,14}$ =0.03	P = 0.86

T-maze laboratory behavioral bioassays.

The summer morph flies exhibited a weak avoidance and the winter morph flies showed a slight attraction to geosmin (Fig. 6.3). When the response index was compared to zero (no response), summer morphs had a significant avoidance to geosmin ($F_{1,11} = 7.3$, $P = 0.01$). However, response from winter morphs was not significantly different from zero ($F_{1,11} = 0.01$, $P = 0.95$).

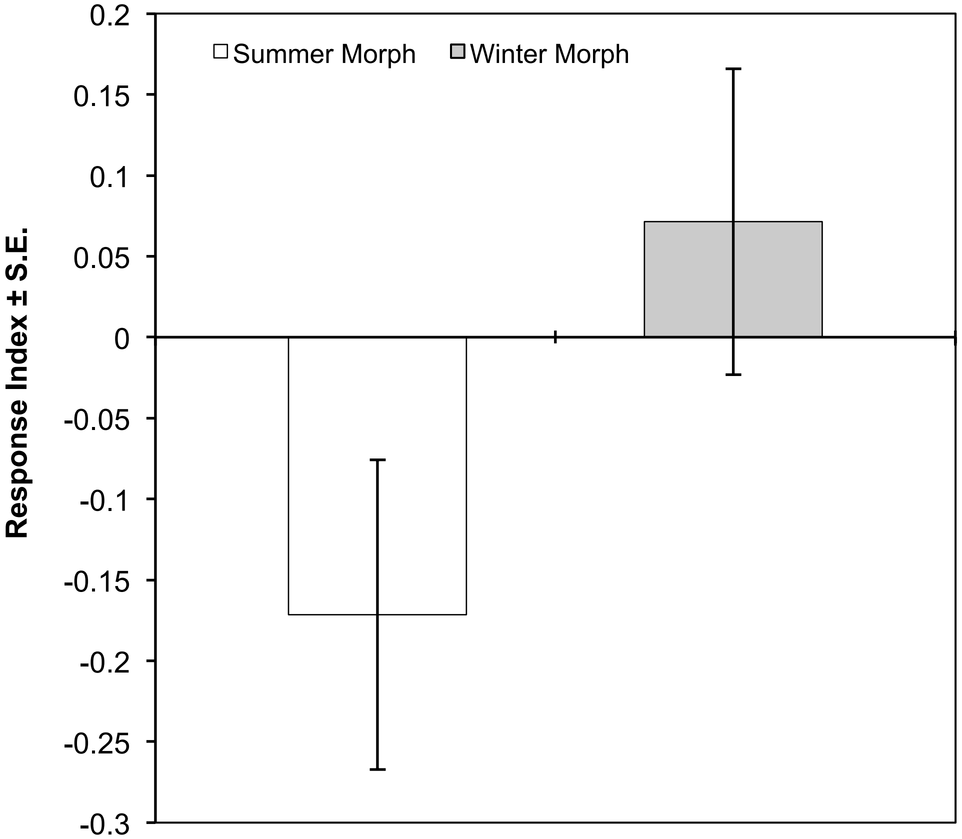

Figure 6.3. Response Index (RI) of summer morph (white bar) and winter morph (gray bar) flies in a T-maze assay with a choice between geosmin (concentration 10^{-3}) and a paraffin oil control. Error bars represent S.E.

DISCUSSION

Electroantennography and the recording from receptor cells is a commonly used technique that has been previously used to explore *D. suzukii* antennal responses to individual volatile chemicals (Cha et al. 2012; Revadi et al. 2015). The current study reports the use of electroantennogram (EAG) for screening volatile chemicals that mediate attraction and drive behaviors between two different seasonally induced morphs of *D. suzukii*. EAG has also been used to previously compare several types of *Drosophila* mutants (Störtkuhl et al. 1999; Kain et al. 2008), but to our knowledge, this is the first time that differences in response to volatiles between seasonally induced insect morphs have been evaluated using any type of electrophysiological technique.

In our EAG study, both summer and winter morphs showed an antennal response to all volatiles tested, suggesting that both morphs have the same type of olfactory sensory neurons and receptors present for these compounds. However, winter morphs had a consistently reduced response to most volatiles when compared with summer morphs, which could provide insights into behavioral strategies used between the two morphs for locating resources and successful overwintering sites. Winter morphs are thought to be quiescent during the winter, and a reduction in the capacity to respond to environmental stimuli and cold winter stress would likely benefit their survival during this period. EAG is excellent for quickly assessing the receptive range of an insect's antenna and evaluates the sum of the electrical response of the activated olfactory sensory neurons on the entire antenna (Olsson and Hansson 2013). Despite being a commonly used electrophysiological technique for evaluating olfactory reception, EAG has potential shortcomings and is subject to change depending on the connection strength, insect vitality, and position of the electrode (Olsson and Hansson 2013). For a more quantitative measurement of the olfactory response, single-sensillum recording (SSR) is an electrophysiological technique that can target specific receptor sites and could be used in the future to further evaluate the difference in response to volatiles between the two morphs of this species.

Geosmin is a volatile compound produced by a select number of fungi, bacteria, and cyanobacteria (Stensmyr et al. 2012). It is typically recognized as a warning sign for the presence of toxic compounds for insects, and negatively affects behaviors such as attraction, feeding, and oviposition (Becher et al. 2010; Stensmyr et al. 2012). Geosmin is a known repellent for some species of *Drosophila* (Stensmyr et al. 2012), and has been previously evaluated as a repellent for *D. suzukii* summer morphs (Wallingford et al. 2016; Wallingford et al. 2017). We found that while the number of ab4 and ab6 small basiconic sensilla, where the ab4 and ab6 olfactory sensory neurons are located in other *Drosophila* (De Bruyne et al. 2001), were numerically lower on winter morph antennae compared to summer morphs, there was no statistical difference in the number of basiconic sensilla between morphs. This small reduction could be responsible for the change in EAG response, and further exploration is needed on the effect of the possible reduction of these basiconic sensilla. In our EAG study and the no-choice behavioral assay, there was a significant difference in response to geosmin between the two morphs. Thus, geosmin was the only volatile further evaluated in the T-maze behavioral bioassay to evaluate potential repellency to geosmin. In the T-maze assay, we found that there was a slight aversion to geosmin by summer morphs, and no significant response (aversion or attraction) to geosmin by winter morphs (Fig. 3). In a study by Wallingford et al. (2017), it was found that geosmin did not repel summer morphs and did not reduce the density of *D. suzukii* on treated fruit, but acted as an ovipositional deterrent. This is one possible explanation for why a higher aversion to geosmin was not seen from summer morphs in either type of behavioral bioassay, despite a strong relationship between other species of *Drosophila* and repellency, which is typically highly conserved among flies (Stensmyr et al. 2012). Other types of avoidance behaviors, such as avoidance to CO_2, are not conserved in all *Drosophila* species and have been adapted to suit *D. suzukii* (Pham and Ray 2015). Low aversion to geosmin may also help explain why some summer morphs were found in vials containing geosmin in the no-choice assays, though still numerically less than winter morphs. Certain behaviors could be further adapted to suit *D. suzukii* winter morphs and could offer a distinct evolutionary advantage for successful overwintering for *D. suzukii* winter morphs by not avoiding volatiles that can potentially indicate overwintering sites. The landscape and fruit present when summer morphs are active and searching for suitable hosts is very different compared to the landscape for a winter morph *D. suzukii* searching for a suitable overwintering site. Further studies evaluating differential responses between the two morphs are warranted, particularly with geosmin.

Our no-choice behavioral assay indicated a significantly higher response from winter morphs to bornyl acetate compared with summer morphs (Table 1). No difference with bornyl acetate was found in our EAG studies and in fact, very low response to this compound was observed with both morphs (Fig. 1). Interestingly, field studies have found high populations of *D. suzukii* in pine trees during the fall, but we are not currently aware of what drives this attraction. While there was a reduced response from winter morphs to many of the compounds tested using EAG, fewer differences were noted in our behavioral studies. We expect that *D. suzukii* behavior is highly plastic dependent on climate, resource availability, and other factors, so we would expect to see greater difference between morphs when compared in their natural environmental settings. Other insects that produce a distinct seasonal morphs for winter survival also exhibit shifts in behavior, such as increased takeoff and flight capacity of winter morph cabbage whitefly, *Aleyrodes proletella* (Iheagwam 1977), longer wings for greater dispersal from orchards in the fall of winter morph pear psylla, *Cacopsylla pyricola* (Oldfield 1970), and migration from peaches and interrupted parthenogenesis of the overwintering green peach aphid, *Myzus persicae* (Blackman 1974).

Additional studies could further elucidate preference for specific resource signals within and between morphs with two-choice or multi-choice experimental behavioral assays. Lower temperatures experienced by winter morphs in the field may also be important for observing representative behavior in behavioral bioassays. We also emphasize the need for further evaluation of the behavioral responses to these compounds in a field or semi-field study, relating spatial and temporal dependent factors to these morphs. Moreover, these morphs were lab-reared under static temperature and photoperiod conditions, which could have implications for the physiological and behavioral responses to environmental stimuli.

There are important management implications for increased knowledge on the winter morph of *D. suzukii*, particularly because we expect that the surviving populations of this pest are quite low given low spring adult captures, similar to orchards with pear psylla populations where peak spring densities were often considerably lower than peak fall densities of the overwintering form (Horton et al. 1992). This provides a unique opportunity to exploit their winter or spring resources and further reduce the surviving population by targeting the winter morphs for control after the population bottleneck. Our findings indicate seasonally induced morphs of *D. suzukii* have behavioral adaptations that likely help them to locate appropriate resources for successful overwintering in northern climates. Further defining the overwintering requirements for this pest will allow us to better understand their seasonal phenology and make appropriate management decisions.

CHAPTER 7: CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The research presented in this thesis has provided new information in the study of *D. suzukii* with relevance for improved monitoring systems as a part of pest management for this devastating pest. There is an increased need for alternative methods of pest control for *D. suzukii* to reduce the reliance on insecticide use in agricultural production systems. Ultimately, successful pest control will be the result of reduced damage to crops and an overall reduction of economic losses related to this damage. Although I have been able to improve monitoring traps in the field by the addition of color as a visual cue and a sticky surface to ensnare flies, future research should investigate the relationship of trap captures to insecticide spray timing, and develop a reliable action threshold that informs efficient chemical control. Current management practices consist of insecticide sprays sometimes unrelated to actual pest pressure that are highly unsustainable, detrimental to beneficial arthropod populations, and expensive for growers. It is a challenge to achieve control of *D. suzukii* without chemical control methods because of this pest's ability to utilize many different resources for feeding and development, and the ability of the populations to reach such high levels because of the many overlapping generations throughout the growing season. However, the incorporation of behavioral manipulation into current management practices could contribute to suppressing population levels and achieve zero infestation of crops. Results of this thesis indicate that for *D. suzukii*, there is a possible target for behavioral manipulation through development of a trap with visual and olfactory cues incorporated that are specific to this pest to create an optimized monitoring trap.

I have investigated *D. suzukii* response to color in an effort to add a visual cue to monitoring traps specific to *D. suzukii* (Chapter 2), possibly increasing captures and potentially reducing the captures of non-target insects. In laboratory assays, *D. suzukii* consistently alighted on red, purple, and black odorless, colored, sticky disks (Chapter 2), and in field studies red traps consistently captured more *D. suzukii* than clear deli-cup style traps (Chapters 3 and 4). This work demonstrates that a trap integrating both visual and olfactory cues together increases captures for *D. suzukii*. However, non-target captures were not reduced, and further evaluation and improvement of liquid baits and commercial lures should be studied in the future to improve olfactory attractants specifically for attracting *D. suzukii*.

An optimized trapping system includes: optimized olfactory attractants, optimized visual attractants, and an optimized retention process to match the target insect, and should be easy and convenient to use. Various novel trap designs were tested in semi-field and field experiments in an effort to maximize attraction and retention of *D. suzukii* (Chapters 3 and 4). The first trap used for capturing *D. suzukii* after it was detected was a clear cup trap style design with liquid bait inside. This early trap design did not utilize any visual cues and used food based bait that was a general attractant for many insects. Utilizing what was learned from laboratory studies examining response to color (Chapter 2), a red visual cue was incorporated with a spherical shape in the design of a novel sticky trap design. In a field study in raspberry high tunnels and in cherry orchards, red sphere traps consistently captured more *D. suzukii* than clear deli-cup traps when both were baited with the same lure (Chapters 3 and 4). However, it was difficult to examine captures on sticky sphere traps in the laboratory to confirm captures of female *D. suzukii,* so a red panel trap was designed to remedy to this problem (Chapter 4). The biggest advantage over spherical traps is that they can easily be placed under the microscope to identify *D. suzukii* from other species of *Drosophila*. Red panel traps were tested in field studies in cherry orchards and raspberry high tunnels, and it was shown that red panel traps captured significantly more *D. suzukii* than clear deli-cup traps when both traps were baited with the same lure (Chapter 4). Also, there was no statistical difference in the number of male and female *D. suzukii* captured on a red panel trap in cherry orchards. Using a red panel trap allows for identification of male *D. suzukii* in the field, removing the need to bring the traps back to the lab for identification of *D. suzukii* from other *Drosophila* captured on the trap (Chapter 4). In multiple field studies, it was demonstrated that red sphere or red panel traps integrating both visual and olfactory cues were superior tools for monitoring *D. suzukii* over the deli-cup traps. Furthermore, a dry sticky trap is easier to use in the field and is less time consuming than a deli-cup style trap utilizing messy liquid baits. Further studies to improve the red panel trap are necessary to further improve ease of use of the traps and allow the commercials lures to be hung inside the trap and out of the sticky material on the outside.

Through the use of single-trap, multiple release-capture experiments, captures in a monitoring trap were related to absolute pest density (Chapter 5). It was found that the plume reach for a red panel trap baited with a commercial lure in tart cherry orchards was short (< 3 m) and the dispersive distance for *D. suzukii* was found to be about 90 m; yielding a trapping area of 2.7 ha. These data were consistent across two growing seasons, and provided the first information about dispersal distance and monitoring trap efficacy for *D. suzukii* in a fruit crop setting. Catch data per monitoring trap was used to estimate absolute pest density in cherries, and it was found that catching a single *D. suzukii* in a monitoring trap translates to approximately 192 *D. suzukii* per trapping area of 2.7 ha. Measured values may be different in other fruit crops systems, and future research is justified to calculate these values and compare them with what was found in cherries.

Differences in antennal and behavioral responses compared between summer and winter morph *D. suzukii* to volatiles (Chapter 6) provided valuable information about comparative differences between the two seasonally induced *D. suzukii* morphs. Little is known and understood about the winter biology of *D. suzukii* or what resources are utilized when fruit is not available. Six ecologically relevant volatiles were tested separately in electroantennogram (EAG) and behavioral assays between the two morphs. In EAG studies, there was a significant difference between the morphs to the volatiles isoamyl acetate, acetic acid, and geosmin, and overall there was a consistent reduced response from winter morphs. In laboratory behavioral assays, geosmin and bornyl acetate elicited significantly different behavioral responses from the two morphs. T-maze behavioral assays were used to further explore the differences between the two morphs to the volatile geosmin, and revealed that summer morphs had a significant aversion to geosmin, while winter morphs had no significant aversion to geosmin. Overall, it was demonstrated that the responses of the two seasonally induced morphs to environmental stimuli are different, and future studies are justified to further understand how these physiological and behavioral differences may contribute to pest management of *D. suzukii* by targeting the overwintering population.

By combining the knowledge of *D. suzukii* biology with non-chemical methods such as trapping, advancements in monitoring for these systems and improved monitoring traps was achieved. Combining the information learned in Chapters 2, 3, and 4 with the information learned in Chapter 5, a monitoring trap optimized for capturing *D. suzukii* and with an increased ease of use in the field and a known trapping area can be developed. The following steps would be to implement use of the novel trap design in the field and have growers and pest management professionals evaluate the trap versus the current available trap designs. Ultimately, the novel trap design could be used to develop a reliable action threshold for growers and pest management professionals to use to determine the best timing for when chemical control programs should be initiated and for implementation of successful integrated pest management programs.

APPENDIX

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____2018-01_____________

Author and Title of thesis:
Danielle M. Kirkpatrick
Improving monitoring of spotted wing drosophila (*Drosophila suzukii*, Diptera: Drosophilidae) in Michigan fruit crops

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

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Drosophilidae	*Drosophila suzukii*	adult	10	pinned
Drosophilidae	*Drosophila suzukii*	adult	10	alcohol

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