INTEGRATED MANAGEMENT STRATEGIES FOR SPOTTED WING DROSOPHILA, *DROSOPHILA SUZUKII*, IN SOUTHERN HIGHBUSH BLUEBERRIES

By

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To my mom who gave me strength
To my dad for continuous encouragement
Y para mi amor, que me hizo sonreír en cada paso de este viaje

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Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), an invasive pest threatening Florida's blueberry industry, causes direct injury to healthy fruit by ovipositing eggs under the fruit skin where larvae develop. A survey was conducted in 9 and 8 blueberry growing counties in 2012 and 2013, respectively, using a clear cup trap baited with apple cider vinegar (ACV). Spotted wing drosophila was found in all counties in both years except for the southernmost DeSoto County. Oviposition preferences were investigated using the two Florida-grown blueberry species, southern highbush and rabbiteye, and on blueberry ripening stages. Spotted wing drosophila appears to prefer southern highbush over rabbiteye, and blue fruit over other stages of fruit development. However, all stages of fruit development were susceptible to SWD infestation. In order to develop effective monitoring techniques for SWD, different traps with and without a yellow visual stimulus, baited with ACV were evaluated in blueberries. Results indicated that adding a yellow band (visual stimulus), odorless dish detergent, and/or a yellow sticky card inside the trap, did not increase captures. A clear cup trap baited with yeast-sugar-water was more attractive to SWD than ACV traps. In a subsequent study, four bait treatments were evaluated to investigate the attraction of

SWD in blueberries. Treatments included 1) ACV, 2) yeast-sugar-water, 3) yeast-sugar-water with whole wheat flour, dish detergent, and ACV, and 4) rice vinegar and red grape wine with dish detergent. The two yeast baits captured significantly more SWD than the vinegar baits. Finally, a field-based laboratory bioassay was used to identify chemical tools for managing SWD in blueberries. Seven treatments including 1) Belay® (high and low rate), 3) Danitol® (high and low rate), 5) Mustang Max®, 6) Delegate®, and 7) a water-treated control were evaluated against SWD. Belay® (both rates) was ineffective at reducing SWD adult activity throughout the 14 day experiment. Danitol® (both rates), Mustang Max®, and Delegate® were equally effective at reducing adult activity up to seven days. Danitol® (both rates) also reduced larval emergence. Successful tactics will be integrated into an IPM program for management of SWD in southern blueberries.

CHAPTER 1
INTRODUCTION

The Blueberry Industry in the United States and Florida

The United States is the largest producer of blueberries in the world, producing over 196,000 kg in 2011 (FAO-STAT 2013). The U.S. blueberry market includes both cultivated and wild species and is currently valued at approximately 880 mil USD annually, an increase of 37 percent from 2010 (644 mil USD) and 371 percent since 2001 (187 mil USD) (NASS-USDA 2013). United States fresh blueberries first reach the global market as early as late March to early-April and continue through October.

Florida is a major producer of early-season blueberries from March to May, producing approximately 80 percent of the national total. Although the acreage is considered small at about 2,300 ha in 2012, the industry is greatly valued due to high market prices when berries begin ripening as early as March. The average price for fresh blueberries in Florida at 7.18 USD kg^{-1} is well above the national average of just 4.87 USD kg^{-1}, and has remained steady since 2008 (ERS-USDA 2012b). Revenues for blueberries in Florida increased from 47 mil USD in 2010 to approximately 70 mil USD in 2011 (ERS-USDA 2012a). Florida also has a growing organic blueberry industry with over 43,545 kg of certified organic blueberries produced in 2008 (NASS-USDA 2012).

Blueberry Production in Florida

Blueberry (*Vaccinium* spp.) is one of the only cultivated crops native to North America and is grown as a deciduous crop. The two most commonly grown species of blueberry in Florida are the southern highbush (SHB) and the rabbiteye (RE). Southern highbush, *Vaccinium corymbosum* L. x *V. darrowi* Camp, is a cross between highbush and a lowbush evergreen species native to Florida (Lyrene and Ballington 2006). The SHB differs from the northern highbush species primarily due to the lower chill requirements which allow it to thrive in warm southeastern winters (Darnell 2006). Rabbiteye, *Vaccinium virgatum* Aiton, is a native species to the southeastern U.S. making it hardier than SHB and more tolerant to drought and soils with low organic matter. Rabbiteye also flowers in late spring so is less susceptible to freezes. Although RE is more adapted to climate in Florida, it has a longer fruit development time of 60 to 135 days (Birkhold et al. 1992) whereas SHB is much shorter ranging from 55 to 60 days (Maust et al. 1999). As a result, SHB from Florida are the first domestic berries on the U.S. market ripening in early March and continuing through May. Rabbiteye however, do not begin producing ripe fruit until May when market prices are much reduced. The reduced fruit development time for SHB is part of the reason why it accounts for more than 75 percent of the blueberries grown in Florida.

Blueberries are grown in either single or double, raised rows. Beds are raised to provide well-drained medium for the short root-system of the blueberry bushes in many of the low-lying locations in Florida. A layer of pine bark is either incorporated into the bed soil or added as a top layer to increase moisture retention, increase soil organic matter, and maintain optimum pH of 4.2 to 5.2 (Williamson et al. 2006). Southern highbush growers generally space their bushes 0.6 to 1.2 m apart with 2.7 to 3.5 m between rows, whereas the vigor of RE requires a spacing of 1.2 to 1.8 m between bushes and 3.0 to 3.7 m between rows (Williamson et al 2006).

Insect Pests of Blueberry in Florida

Since blueberries are a deciduous crop, pest issues can occur throughout the year before, during and after the season. Principal arthropod pests of blueberries in

Florida include blueberry gall midge, *Dasineura oxycoccana* (Johnson), Florida flower thrips, *Frankliniella bispinosa* (Morgan), blueberry leaf beetle, *Colaspis pseudofavosa* Riley, and the new invasive spotted wing drosophila, *Drosophila suzukii* (Matsumura). The blueberry maggot, *Rhagoletis mendax* Curran is problematic only for growers who live in the counties north of Live Oak, Florida.

Blueberry gall midge is an early-season pest of the buds of blueberries, *Vaccinium* spp. It was previously referred to as cranberry tipworm outside of the southern U.S. but recent evidence suggests that these are cryptic species determined by reproduction isolation and differences in mitochondrial genes (Cook et al. 2011, Mathur et al. 2012). Females oviposit within the vegetative and floral buds where the larvae hatch and feed on the bud tissues (Sarzynski and Liburd 2003, Dernisky et al. 2005). Lyrene and Payne (1992) found that feeding can cause yield loss of up to 80 percent in fields of severe infestation in southeastern blueberries.

Until recently, the Florida flower thrips, *Frankliniella bispinosa* (Morgan) was the key arthropod pest in SHB blueberry production (Liburd and Arévalo 2006, Arévalo and Liburd 2007). Thrips feeding and oviposition results in scarring of the fruit, rendering it unmarketable (Arévalo 2006). Monitoring for thrips consists of hanging white sticky traps within the canopy and collecting flower buds in alcohol (Arévalo and Liburd 2007, Liburd et al. 2009).

The blueberry leaf beetle, *Colaspis pseudofavosa* Riley, is the primary post-harvest pest in SHB (Nyoike and Liburd 2009). Adults prefer to feed on summer (new) growth foliage boring ‘shot holes’. Leaf beetles are known to be attracted to the black weed fabric that is commonly in southern blueberry production (Krewer et al. 2009). A high population of leaf beetles can interfere with plant (bush) vigor the following year and severely affect marketable yield.

The blueberry maggot, *Rhagoletis mendax* Curran, is found in the eastern U.S. from Nova Scotia, Canada, south to northern Florida in highbush and rabbiteye blueberries (Payne and Berlocher 1995). In Florida and Georgia, adults emerge in late May and continue through July. When they reach sexual maturity, adult female flies oviposit into ripening berries where larvae hatch and feed, rapidly degrading the fruit and rendering it unmarketable (Liburd et al. 1998).

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is a new invasive pest threatening the blueberry industry. Spotted wing drosophila females oviposit into the ripening fruit leaving a blemish on the skin of the berry (Mitsui et al. 2006). Eggs hatch inside the berry where the larvae develops, causing the berry to become soft and degrade rapidly (Walsh et al. 2011). Consequently, the berries become unmarketable. Additionally, berries found containing larvae are immediately rejected upon inspection. Estimated crop loss due to SWD injury in Florida blueberries in 2012 was 10 to 15 percent, a value of 7.8 mil to 11.7 mil USD (eFly 2012).

Objectives

Since SWD is quickly becoming an important pest in Florida, more information is needed on the extent of its infestation, fruit susceptibility to oviposition, monitoring, and control tools. The first objective of this study was to determine the magnitude and level of SWD infestation in Florida blueberries by conducting a statewide survey in the major blueberry growing regions of Florida. The second objective was to develop and refine monitoring programs for SWD, including trap design and bait attraction. Third, we wanted to determine if oviposition host selection by SWD varied by blueberry species or fruit maturity stage. Finally, we wanted to identify tools that growers can use for managing SWD in Florida blueberries.

Justification

Spotted wing drosophila has a wide host range and given Florida's near year-round growing season, SWD has the potential to spread rapidly throughout the state (Lee et al. 2011, Walsh et al. 2011). A survey was conducted in the major blueberry growing regions in Florida to help determine the extent to which SWD is present in blueberries. This information will help growers understand the potential threat SWD may pose to their crops.

Growers are recommended to monitor for SWD prior to implementing control tactics as part of an integrated pest management (IPM) program. However, since SWD is a new pest, monitoring techniques are still being developed. Commonly recommended techniques include a plastic cup trap made from a deli container with a lid and multiple entry holes along the upper rim (Wu et al. 2007, Beers et al. 2011, Birmingham et al. 2011, Lee et al. 2012,Walsh et al. 2011, Cini et al. 2012). Some modifications of this trap include features to prevent SWD from escaping such as a yellow sticky card hanging inside of the trap. Since SWD is in the family Drosophilidae, also known as the vinegar or fruit flies, many of the recommended baits are vinegars, alcohols, fruits, yeast mixtures and combinations of the like (Kanzawa 1939, Steck et al. 2009, Walsh et al. 2011, Landolt et al. 2012a, 2012b). One of the goals of our research was to evaluate the effectiveness of new and commonly recommended trap designs and baits for capturing SWD in blueberry fields. Effective monitoring programs that detect SWD early in the season will help to determine when to implement control actions and evaluate the effectiveness of control techniques. In addition to effectiveness (trap captures), the ease of implementing the trap and lure system will be discussed.

An effective monitoring program is dependent upon knowledge of the pest biology, ecology, and behavior. Some host plants of SWD are more preferred for oviposition than others. Burrack et al. (2013) found that SWD preferred to lay eggs on different fruits in the following order from most preferred to least: blackberries, raspberries, strawberries, blueberries, and grapes. Factors that play a role in oviposition site selection have yet to be determined but larval presence, texture, firmness, brix levels, and pH have been suggested (Chess and Ringo 1985, Lee et al. 2011, Burrack et al. 2013). Major injury to blueberries occurs after the eggs hatch and the larvae begin to develop in the berry. Therefore, control techniques must be implemented prior to oviposition. In order to investigate SWD oviposition behavior, we examined SHB and RE species to determine the most preferred host. The first objective of our oviposition study was to determine whether the two most commonly grown species of blueberries in Florida (SHB and RE) were suitable oviposition hosts that supported larval development. Secondly, we aimed to evaluate SWD preference for different blueberry maturity (ripening) stages for oviposition. Results from these studies will help to determine where and when to establish traps for an effective SWD monitoring program.

It is general practice for growers to use insecticides for SWD control. Insecticides target the adult stage since the immature stages occur within the berry. Common effective conventional chemicals are in the classes of organophosphates and pyrethroids, reduced-risk chemicals in the spinosyn (spinetoram) class, and organic chemicals in the pyrethrin and spinosyn (spinosad) classes (Liburd and Iglesias 2013). These chemicals have shown effectiveness in the lab and the field (Bruck et al. 2011). However, the limited number of control tools available results in increasingly more applications of the same chemicals. Given the rapid development time of SWD, it is a highly potential candidate for pesticide resistance. Therefore, it is important for growers to have multiple effective tools available for SWD control. One of the objectives of this study was to compare the efficacy of reduced-risk tools to commonly used tools for SWD control. New tools provide growers with alternatives options for rotational control programs, aid in resistance management, and reduce risk to worker and environmental health.

CHAPTER 2
LITERATURE REVIEW

Spotted Wing Drosophila

The spotted wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is an invasive pest species native to parts of East Asia (Kanzawa 1939, Markow and O'Grady 2006, Ometto et al. 2013). The first records of SWD are in Japan in 1916 and it was described by Matsumura in 1931 (Kanzawa 1939). Spotted wing drosophila was first recorded in the western hemisphere in Hawaii in 1980 (Kaneshiro 1983) and detected in California in 2008 (Bolda et al. 2010). Subsequent captures occurred in Washington, Oregon, and Florida in 2009, and Wisconsin, Michigan, Utah, Kentucky, Louisiana and the Carolinas in 2010 (Steck et al. 2009, Walsh et al. 2011). By the end of 2012, SWD had spread to 37 states in the U.S. Spotted wing drosophila has also been found in parts of Canada and Europe (BCMA 2012, Calabria et al. 2012). Since its first record in Hillsborough County, Florida in 2009, SWD has quickly spread throughout Florida becoming a concern for all berry growers in the state (Steck et al. 2009).

Identification

The family Drosophilidae is also known as the vinegar, fruit, or pomace flies. Spotted wing drosophila has several similar morphological characteristics to the common vinegar fly, *Drosophila melanogaster* Meigen that frequents over-ripe and damaged fruits. The abdomen of SWD is round, light brown to pale yellow and has unbroken, horizontal bands on the dorsal side (Figures 2-1, 2-2). They have large, red compound eyes and a posteromedial ocellar triangle (Triplehorn and Johnson 2005, Markow and O'Grady 2006). In addition, SWD has sponging mouthparts with which they suck up their food.

The male flies can be easily distinguished from most other vinegar flies by the single dark spot at the distal end of the R_{2+3} vein on the wings (Figure 2-2). The wing spots can be seen with the naked eye or a hand lens for easy identification in the field. The males of the sister species, *Drosophila biarmipes*, and close relative *D. subpulchrella*, also have the wingspots which can make distinguishing between them difficult (Takamori et al. 2006, Ometto et al. 2013). *Drosophila biarmipes* however, does not pose a threat to ripe fruit due to the female's short, weakly sclerotized ovipositor, which only allows her to oviposit in overripe or damaged fruit. *Drosophila suzukii* can be distinguished from *D. biarmipes* by the two rows of sex combs on the forelegs, one on the first tarsal segment and one on the second, whereas *D. biarmipes* has both rows of sex combs on the first tarsal segment (Hauser 2011). The set of two sex combs appear as two black horizontal stripes on the forelegs when using a hand lens for identification.

Female SWD are slightly larger than the males and do not possess the dark wingspots (Figure 2-2). Females look very similar to other drosophilids except for the large, dark, and heavily serrated ovipositor that is use to pierce the skin of unripe and ripe host fruits (Figure 2-3). When ovipositors of other drosophilids are compared to that of SWD, they appear rounded, faintly colored, and blunt with small, light teeth, limiting oviposition to soft, overripe, or damaged host fruits only.

Biology

The lifecycle of SWD includes an egg, larva, pupa, and adult. The eggs are laid under the skins of thin-skinned fruits that will hatch in 1 to 3 d inside the berry (Kanzawa 1939). Eggs are milky white, oblong and approximately 0.5 mm by 0.2 mm (Kanzawa

1939). The eggs have two long respiratory spiracles that protrude from the skin of the blueberry. Larvae of SWD are thin, white, and soft-bodied with pointed anterior and posterior ends (Fig. 2-4). The mouthparts appear black on the anterior end. The three larval instars generally develop in 4 to 5 d inside of the berry (Kanzawa 1939). Spotted wing drosophila pupae are oblong and range from light brown to dark brown as they develop through three stages (Fig. 2-5). Spiked spiracles are located at the anterior end whereas pointed caudal spiracles at the posterior end. The red eyes and wing pads of the adult fly can be seen through the pupal case in the final stage. Pupation can occur in the soil, inside or outside the fruit and generally occurs within 5 to 7 d from egg hatch (Kanzawa 1939, Walsh et al. 2011).

Kanzawa (1939) observed that SWD lifecycle can be completed in 10 to 24 d, whereas a recent study showed that the lifecycle can be completed in 12 to 15 d at 18.3°C (Walsh et al. 2011). Spotted wing drosophila has been shown to have as many as 15 generations per year when observed in captivity (Kanzawa 1939). It has been predicted to complete 3 to 9 generations in the western U.S. and Canada based on degree-day models (Walsh et al. 2011).

The spotted wing drosophila prefers temperature between 20 and 25°C (Kanzawa 1939). It is tolerant to temperatures as low as -1.6°C for females and -0.1°C for males and as high as 32.6°C for females and 32.2°C for males (Kimura 2004). Adult male SWD can survive for 88 d at a constant 10°C. Even when exposed to a 7-d freeze (-0.2°C), adults were predicted to survive up to 103 d at 10°C (Kimura 2004). In addition, SWD overwinters or enters into a state of reduced activity during periods of severe cold temperatures and short daylength, allowing it to continue its lifecycle the following season (Kimura 2004, Dalton et al. 2011). Males show signs of sterility above 30°C (Kanzawa 1939). Whereas the lower sterility threshold for SWD males has not been studied, *D. melanogaster* shows signs of sterility at 12 and 30°C (Pe´tavy et al. 2001, Chakir et al. 2002, Markow and O'Grady 2006). The broad range of temperature tolerance in SWD is one reason why SWD has become such a damaging pest throughout North America from Canada, south to Florida.

Spotted Wing Drosophila as a Pest of Blueberries

Spotted wing drosophila has become a major threat to the blueberry industry in Florida and the world (Walsh et al. 2011). Injury from SWD results in depressed scars on the fruit left upon insertion of the ovipositor. The more economically important injury occurs as a result of larval development inside the berries, causing rapid deterioration and softening that render the fruit unmarketable. In addition, the fruit becomes more susceptible to subsequent infection by fungal or bacterial pathogens (Walsh et al. 2011). Finally, there is a zero-tolerance from consumers for larvae infested fruit in the fresh blueberry market. Economic data analyses in California showed yield losses from SWD in strawberries and raspberries accounted for 20 and 50 percent of crop value, respectively (Goodhue et al. 2011) and 40 percent for blueberries (Bolda et al. 2010). Furthermore, a recent SWD working group in the eastern U.S. evaluated potential and current loss estimates in the east for the first time. Potential loss in blueberries for 2012 from reporting eastern states was estimated at 138.7 mil USD (eFly 2012). Estimated crop loss in Florida blueberries in 2012 was 10 to 15 percent, a value of 7.8 to 11.7 mil USD (eFly 2012). Spotted wing drosophila certainly requires attention for the protection of the blueberry industry.

Unlike most vinegar flies that typically prefer damaged or decaying fruit, female SWD lays eggs in healthy, ripening fruit. Female SWD lays 1 to 3 eggs per oviposition site, with an average of 380 eggs throughout her lifetime (Kanzawa 1939, Mitsui et al. 2006). Multiple female SWD can lay eggs on a single fruit resulting in several larvae injuring one berry. Recent research investigating oviposition as a factor of fruit maturity stage has shown that females prefer to lay eggs in ripe blueberries over unripe, green berries and that a greater number of SWD developed in the ripe berries over the unripe stages in both blueberries and blackberries (Lee et al. 2011). However, in the same study, no significant differences were found in eggs laid or larvae developed among the remaining stages. Higher brix levels seemed to have a positive correlation with oviposition and development whereas firmness had a negative correlation. Penetration force may also be a possible contributing factor to host selection. When greater force was needed to penetrate the host, fewer eggs were laid or fewer larvae developed on multiple different berry crops (Burrack et al. 2013).

Management Practices of Spotted Wing Drosophila

Most *Drosophila* spp. are not agricultural pests but rather nuisance pests in human habitats. As a result, few management techniques for control of SWD were available for the U.S. before 2008. As SWD has become an increasingly damaging pest to the U.S. berry industry research on management tools and techniques is developing rapidly.

Monitoring

Monitoring for SWD is a crucial integrated pest management step to determine where and when populations entered a field and when to begin control. Trap designs used for capturing SWD are based on other commercial fruit fly traps. The standard

SWD trap that is used in blueberries consists of a plastic deli container or jar with a lid and entry holes along the sides (Wu et al. 2007, Beers et al. 2011, Birmingham et al. 2011, Lee et al. 2012,Walsh et al. 2011, Cini et al. 2012). Commercial traps are also being used including a dome trap (Trappitt trap, Agrisense Ltd., Pontypridd, United Kingdom), cylindrical gate trap (Fruit Fly Trap, Contech Enterprises Inc., Victoria, Canada), and the CAPtiva or “Red Zorro" trap (Marginal Design, Oakland, CA). Dome traps have shown slightly better results compared to a cup trap with 4 holes along the rim however, the large unmeshed entry hole in the bottom of the dome traps allows a greater number of larger non-target captures that must be sifted through for SWD identification (Landolt et al. 2012). A recent study showed that the related *Drosophila melanogaster* is more attracted to darker colors such as black and red, especially in high light conditions (Kagawa et al. 2012). Similarly, Edwards et al. (2012) showed that black and red were more attractive to SWD when used in the field. Other studies in the western U.S. showed that the color of the trap had less of an impact but rather the greater the total entry size the greater the number of SWD captured in the field (Lee et al. 2012, Basoalto et al. 2013). One disadvantage is that larger entry size increases the potential for water to enter the trap via rain or irrigation. Traps with shades or roofs have been slightly more successful at capturing SWD but ineffective in windy conditions as the shades were blown over and bait spilled (Lee et al. 2012). Yellow sticky traps hung inside the trap are thought to increase captures by helping to prevent SWD escape and are commonly used in the field.

Traps are generally baited with a liquid that acts as a drowning solution. *Drosophila* are also called vinegar flies because they are attracted to overripe, decaying fruit, yeasts and other volatiles of fermentation (Hunter et al. 1937, Becher et al. 2010). Kanzawa (1939) tested many different vinegars and wines including molasses, rice wine, grape wine, fruit juices, and in numerous combinations. In the U.S., apple cider vinegar (ACV) was the first successful bait recommended for use in SWD traps (Steck et al. 2009, Walsh et al. 2011). Apple cider vinegar is clear and allows for easy identification, has longevity in the field and is inexpensive when compared to other baits such as wine (personal experience). Additional baits used include yeast-sugar-water mixture, wines, other vinegars, fruit purees, and volatile components of the above compounds such as acetic acid and ethanol (Walsh et al. 2011). Adding dish soap to any of the above baits is thought to decrease surface tension and increase fly capture (Walsh et al. 2011). In addition, combinations of various vinegars with wines have shown synergistic effects on SWD attraction (Landolt et al. 2012a, 2012b).

Control

Cultural control techniques include sanitation to reduce areas for SWD reproduction and short harvest intervals to prevent larval infestation in marketable fruit. Netting with mesh 0.98 mm or smaller, will prevent *D. suzukii* from reaching the berries entirely (Kawase and Uchino 2005). However, installation of netting may not be an economically feasible control technique for growers with large operations.

The most effective control techniques are insecticides that target the adult stage. There are no insecticides that target the larval stage inside of the berries. The most common chemicals used by conventional blueberry growers for SWD control are Delegate® 25 WG (spinetoram, Dow AgroSciences LLC, Indianapolis, IN), Mustang Max® (zeta-cypermethrin, FMC Corp, Philadelphia, PA), and Malathion® 8 EC (malathion, Arysta LifeScience North America, LLC, Cary, NC). A direct spray of all these chemicals resulted in 100 percent mortality of adult SWD (Bruck et al. 2011). However, not all SWD will be killed on contact in a blueberry field. Mustang Max® provided 100 percent adult mortality after 10 d of residual activity, Malation® provided significant adult mortality up to 7 d, whereas Delegate® provided control up to 3 d (Bruck et al. 2011). Organic growers have a limited number of chemicals available to them including Entrust® (spinosad, Dow AgroSciences LLC, Indianapolis, IN) and Pyganic® (pyrethrin, McLaughlin Gormley King Co., Minneapolis, MN). Very few tools are available for chemical control resulting in multiple applications of the same chemicals as SWD populations reach damaging levels. Furthermore, SWD is at a high risk of pesticide resistance due to its rapid development time.

Figure 2-1. Male spotted wing drosophila. Photo courtesy of L. E. Iglesias.

Figure 2-2. Female spotted wing drosophila. Photo courtesy of L. E. Iglesias.

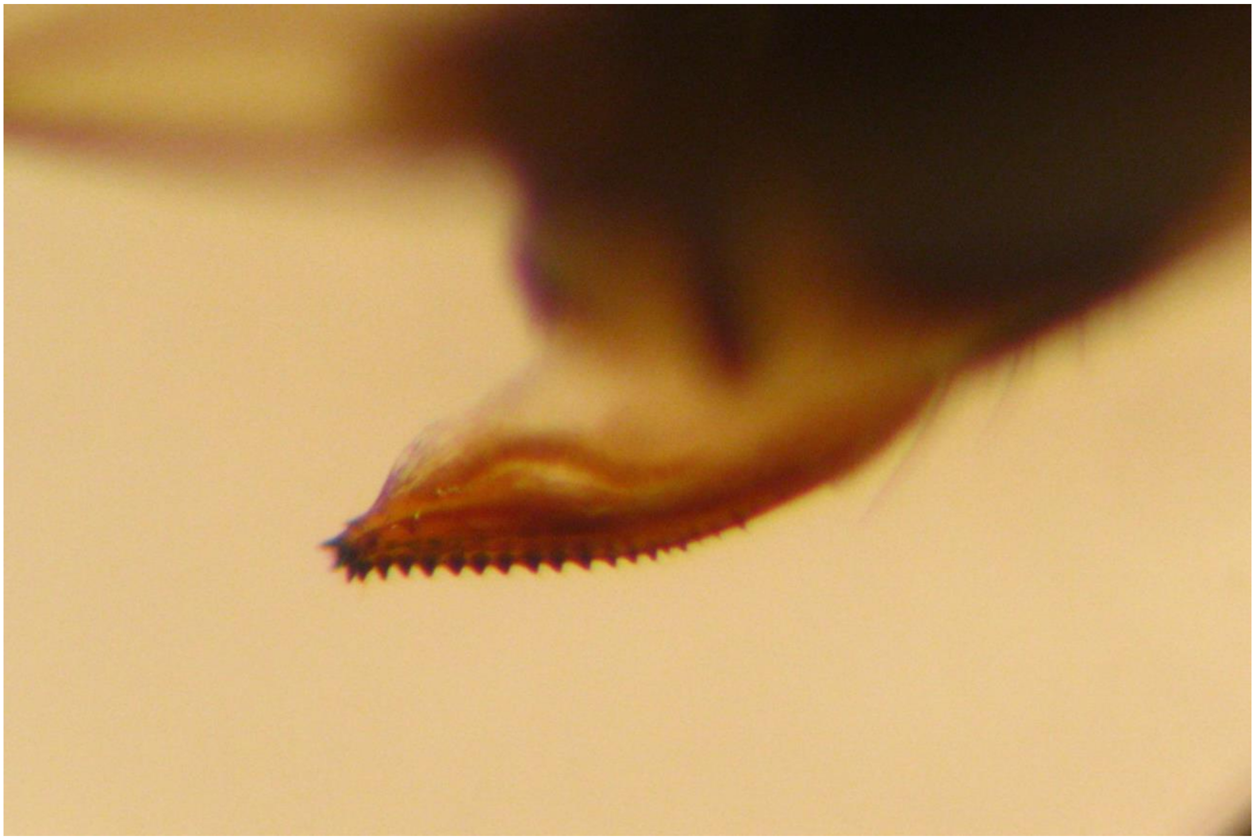

Figure 2-3. Serrated ovipositor of the female spotted wing drosophila. Photo courtesy of L. E. Iglesias.

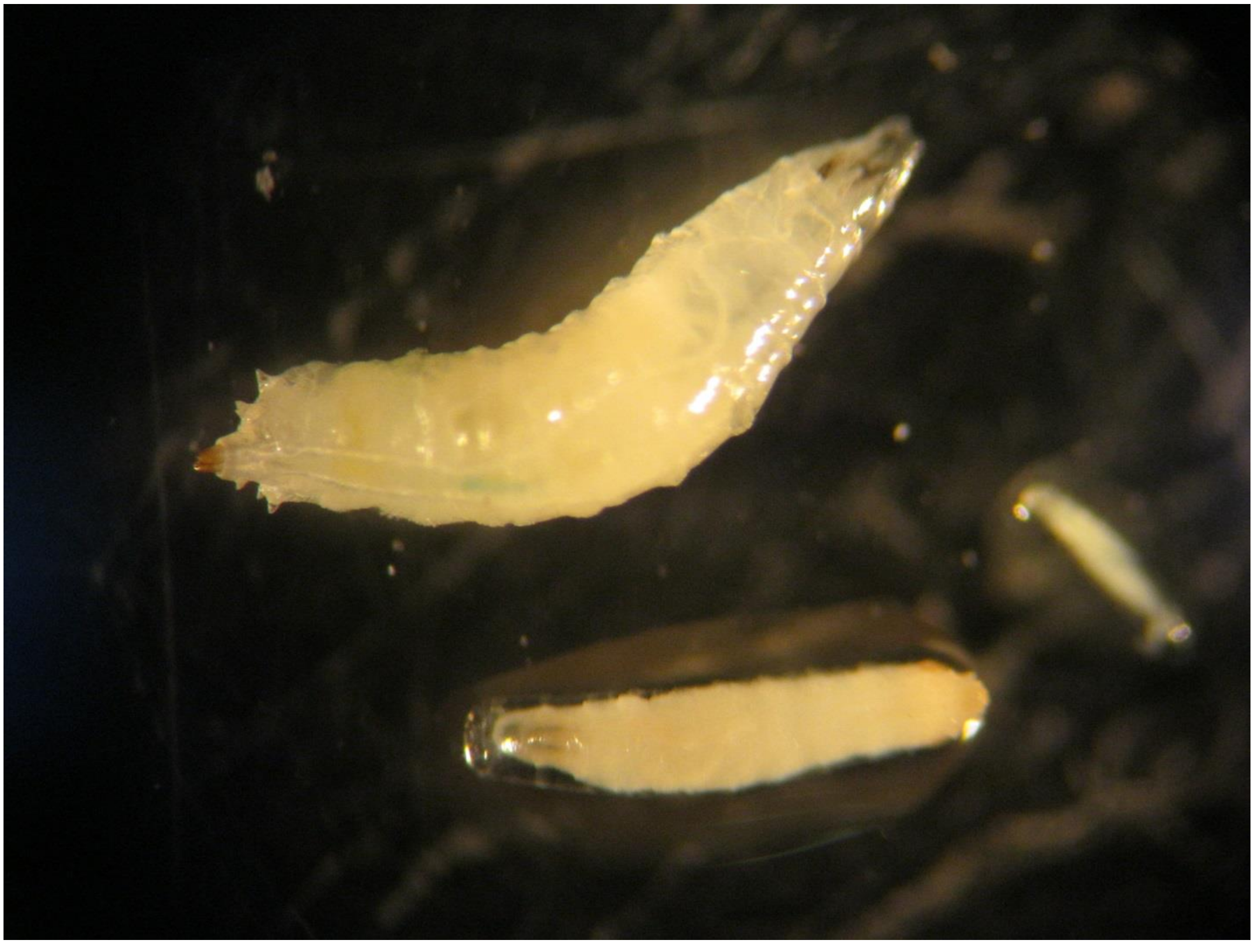

Figure 2-4. SWD larval instars. Clockwise from right, first, second, and third. Photo courtesy of L. E. Iglesias.

Figure 2-5. Three pupal instars. From left, first, second, and third instars. Photo courtesy of L. E. Iglesias.

CHAPTER 3
SURVEY FOR SPOTTED WING DROSOPHILA IN FLORIDA BLUEBERRIES

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is an invasive fruit fly pest from East and Southeast Asia (Kanzawa 1935, Markow and O'Grady 2006, Ometto et al. 2013). It was first described in Japan by Dr. Shounen Matsumura in 1931 as *Leucophenga suzukii* then amended by Kanzawa to *Drosophila suzukii*, cherry fruit fly (Kanzawa 1935). Since then, SWD has been recorded in Korea, Canada, Thailand, Russia, China, India, Spain, and Italy (Hauser et al. 2009, Calabria et al. 2012, Walsh et al. 2011). In the U.S., SWD has been established in Hawaii since at least 1980 and its first continental appearance was in California in 2008 (Kaneshiro 1983, Steck et al. 2009, Walsh et al. 2011). It has since reached 37 U.S. states.

The ecology of SWD and the climate of Florida may contribute to the potential threat of SWD to the blueberry industry. The SWD has a wide host range that includes all thin-skinned fruits and some stone fruits (Walsh et al. 2011). Given Florida's warm, humid climate and occasional hard freezes in the north and north-central regions, commercial SWD hosts grow throughout most of the year. Strawberry (*Fragaria ananassa* Duchesne) harvest extends from December through March, blueberry season is late March through July, peaches and nectarines (*Prunus* spp.) from April until late May or early June, blackberries (*Rubus* spp.) are May through July, and grapes [*Vitis* spp. (bunch and muscadine)] occur June through September. Additionally, potential alternative non-crop hosts may serve as hosts between cropping seasons including wild blackberry, nightshade (*Solanaceae* spp.), and wild holly (*Ilex* spp.).

Weather conditions during highbush blueberry season are ideal for SWD reproduction. In mid-April as the southern highbush blueberry harvest season begins to peak in north Florida, average temperatures were 20 and 23°C in Alachua County in 2012 and 2013, respectively (FAWN 2013). An earlier report indicated that SWD was found to be most active at 20°C (Kanzawa 1935). The SWD also has a rapid development time and can complete its lifecycle in 12 to 15 d at 18.3°C. Development time can be even shorter averaging 9 d 15 h at 25°C (Kanzawa 1939, Walsh et al. 2011).

Early studies reported up to 13 generations per year for SWD when observed in captivity (Kanzawa 1939). The SWD is predicted to complete 3 to 9 generations in the western U.S. and Canada based on degree-day models (Walsh et al. 2011). Given Florida's ideal climate, SWD could potentially experience up to 10 or more generations as predicted in California (Walsh et al. 2011).

There have been multiple confirmed and unconfirmed reports of SWD throughout Florida in blueberries and other fruits since its first detection in Florida in 2009 (Steck et al. 2009). In a recent monitoring study, 409 SWD adults were captured in six Florida counties in blueberry plantings during the 2011 blueberry growing season (Liburd 2011 unpublished). An extensive survey in the major blueberry growing regions will better help understand the potential risk posed by SWD to the Florida blueberry industry. Furthermore, effective monitoring for SWD will guide control actions and protects the crop during the production season. The ultimate goal of this study was to determine the extent to which SWD populations were present in commercial Florida blueberry plantings. The specific objective was to survey for SWD in commercial and non-commercial blueberry plantings using the currently recommended trap and bait system.

Materials and Methods

Survey Sites

The survey was conducted over two consecutive blueberry growing seasons, 2012 and 2013. In 2012, we surveyed nine Florida counties from north to south including, Suwannee, Alachua, Putnam, Marion, Citrus, Lake, Orange, Polk, and DeSoto (Figure 3-1).

During 2013, we surveyed eight counties. The counties included all of those surveyed for 2012 except DeSoto County. This county is the southernmost county surveyed and it was excluded from the 2013 survey because no SWD was found in 2012.

The counties selected represented greater than 70 percent of harvested blueberry acreage in Florida in 2007 according to the most recent survey (ERS-USDA 2012). A total of 28 survey sites were selected in the various counties during 2012 and 2013 (14 in each year). Among the 28 sites there were 24 conventionally managed sites, 2 organically managed, and 2 with separate plantings dedicated to both conventional and organic management onsite (Table 3-1). Sites in the southernmost counties were chosen where farm managers or extension agents offered assistance in sample collection to help reduce travel required for the weekly sample collections. Training on proper sample collection techniques was provided to all farm managers and extension agents to eliminate variance due to procedural differences and to minimize errors in the data.

Site Characteristics

Blueberry bushes at survey sites were between 4 and 8 y old and approximately 1.5 m high. In the northern region of the state (Suwannee, Alachua, Putnam, and

Marion counties), sites were surrounded mostly by rural residential or small farm lands. All locations in Putnam and Alachua counties were on low-lying lands prone to flooding as a result of heavy rains and were equipped with freeze protection. Suwannee and Marion county sites were at research centers and were bordered by forest or agricultural plots. In the central region (Citrus, Lake, and Orange counties) surrounding areas were small berry or vegetable farms or natural landscapes consisting of oak hammocks. Many of the sites were within 1 km of a lake or low-lying wetlands. Sites in the southern region of the survey (Polk and DeSoto counties) in general were surrounded by rural, non-agricultural residential areas with mostly landscape vegetation.

Monitoring Traps

Since SWD causes injury to berries as a result of larval development and upon insertion of the ovipositor into the skin of the berry, traps were set in each field during the full green fruit stage just as they were changing to pink. Lee at al. (2011) showed that SWD had a higher percentage of larval development in the fully ripe blueberries than in the earlier stages. Initial trap set date for each site for both seasons varied based on the ripening stages of the blueberry fruit. During 2012, the first traps were set on 7 February and trap setup continued until all traps were set by 11 April 2012. In 2013, the first traps were set on 6 February and continued until all traps were set at the field sites by 17 April 2013. Traps remained in the field through the peak harvest period and were removed by 31 May in 2012 and 29 May in 2013.

Two to six traps were established at each site with at least one trap set in the center of the planting and the remaining along the perimeter. The number of traps assigned to the perimeter or the center was dependent on the size of the planting. Sites that were greater than 20.2 ha had at least two traps placed within the center of the planting. Perimeter traps were hung within 3.1 m of the edge of the field. Traps were hung in the shade in the center of the blueberry bush using a twist tie, since Walsh et al. (2011) showed traps in shady areas perform better than traps placed in the direct rays of the sun.

Traps were made with 0.95-L clear plastic cups with lids (Solo®, Urbana, IL) with eight to ten 6.35-mm holes along the upper rim of the cup (Liburd and Iglesias 2013). The trap used in 2012 included a yellow band (visual stimulus) inserted inside the trap whereas the trap for the 2013 survey did not (Figures 3-2, 3-3). The yellow band was thought to increase visual attraction to the trap. However, our trapping study in year one (Chapter 5) showed that the yellow band did not increase captures of SWD. Each trap contained 150 mL of apple cider vinegar (ACV) [5% acetic acid (Winn Dixie, Jacksonville, FL)]. The apple cider vinegar was used as bait since previous research had shown it to be attractive to SWD (Steck et al. 2009, Walsh et al. 2011). Odorless, colorless dish soap (Palmolive Pure and Clear, Colgate-Palmolive Company, New York, NY) was added to the bait to act as a surfactant to help reduce the surface tension of the ACV and prevent fly escape.

Sample Collection

Samples were collected weekly and transported back to the University of Florida Small Fruit and Vegetable IPM (SFVIPM) laboratory in Gainesville, Florida for processing. Sample collection consisted of pouring all liquid bait into a collection container in the field and refilling the trap with fresh bait to the 150 mL level pre-marked on the trap. Male and female SWD were counted and identified using keys from Triplehorn and Johnson (2005), Markow and O'Grady (2006), and Vlach (2010).

Data Analysis

Data were square root transformed before analysis to stabilize the variances. A one-way analysis of variance (ANOVA) was used to determine whether there were differences among means of SWD captured for each county per year. Mean comparisons were evaluated using Tukey's HSD (JMP, SAS Institute 2013). Means were considered significant when $P \leq 0.05$. Welch's t-test was used to compare mean differences for female, male, and total SWD captured between 2012 and 2013 (JMP, SAS Institute 2013). Welch's t-test was used because variances were considered unequal by Levene's test for equal variances among female, male, and total SWD captured for each year of the survey (JMP, SAS Institute 2013).

Results

The mean SWD captured in 2012 was significantly higher than in 2013 ($t = 3.95$; df = 1000.9; $P < 0.0001$, Table 3-3). There were a total of 844 and 498 SWD captured in 2012 and 2013, respectively (Table 3-2).

There were significant differences in SWD captures among counties in 2012 ($F = 22.02$; df = 8, 584; $P < 0.0001$) and 2013 ($F = 2.52$; df = 7, 645; $P < 0.0001$, Table 3-4). Citrus County had the highest mean captures in 2012 (4.81 ± 0.31). Marion County (1.44 ± 0.37) and Alachua County (1.34 ± 0.20) were significantly higher than Orange (0.53 ± 0.17) and Suwannee County (0.05 ± 0.49) in 2013. DeSoto County did not have any captures during the 2012 season and therefore was not included in the 2013 survey. Slight differences were found when evaluating differences between years for each county. Citrus County had significantly higher SWD captured in 2012 ($t = 7.46$; df = 144.3; $P < 0.0001$) compared with 2013. Alternatively, in Marion County there were significantly more SWD captured in 2013 compared with 2012 ($t = 2.81$; df = 44.3; $P =$

0.0074) (Figure 3-4). All other counties had similar captures between the two years in the study.

Twice as many females as males were captured throughout the study. There were a total of 911 females and 431 males captured throughout the 2-y study (Table 3-2). The mean number of females captured in 2012 was significantly greater than the number captured in 2013 (t = 3.85; df = 1002.8; P = 0.0001, Table 3-3). Likewise, the mean number of males captured in 2012 were also higher than in 2013 (t = 3.01; df = 991.4; P < 0.0027). The overall ratio of female to male SWD was 2.06 (568:276) in 2012 and 2.21 (343:155) in 2013.

In 2012, there were significantly more females captured than males in Alachua (t = 3.83; df = 260; P = 0.0002), Putnam (t = 3.72; df = 86.8; P = 0.0016), and Citrus counties (t = 3.34; df = 196; P = 0.0010) (Figure 3-5). A similar trend was seen in the 2013 survey year (Figure 3-6). Significantly more females were captured in Alachua (t = 4.34; df = 177; P < 0.0001), Putnam (t = 3.47; df = 58.9; P = 0.0010), Citrus (t = 2.41; df = 284; P = 0.0167), and Polk counties (t = 1.99; df = 91.6; P = 0.0493).

Discussion

Our results showed that SWD is present in the major blueberry producing regions of Florida. The differences between SWD population in 2012 and 2013 may be the result of a number of factors including temperature differences between the years and growers' management programs. Weekly mean temperatures averaged below 20°C until the first week in May 2013; conversely during the early part of 2012 temperatures averaged between 20 and 23°C. The SWD has shown decreased activity at temperatures below 20°C and above 25°C (Kanzawa 1935, Kimura 2004).

When the survey began in 2012 many growers were hearing about SWD for the first time or were unaware of its presence in damaging numbers in Florida. As a result, growers were not implementing management programs for this pest. As we obtained weekly capture data the growers were notified and management actions were taken based on our recommendations. As the 2013 season began, blueberry growers implemented control plans for SWD early in the season especially if SWD was recorded at the site during the previous year. This shift in awareness and proactive management approach of growers may have contributed to the decrease in the numbers of SWD during 2013. Further statewide surveys will help to answer whether the current management programs are effective at reducing SWD populations to the next season.

Spotted wing drosophila captures varied by county throughout the state. The highest mean captures were in the central (Citrus Co.) and north-central (Alachua Co.) parts of the state. Citrus County is a unique location with many factors that can contribute to higher numbers of SWD. Citrus County has a large number of organically managed blueberries. The tools available for control of SWD for organic blueberry growers are extremely limited. Chemical control options are only limited to Entrust® (Spinosad, Dow AgroSciences, Indianapolis, IN) and Pyganic® (pyrethrin, MGK, Minneapolis, MN). Entrust® has been shown to be an effective tool against SWD adults in lab and field trials (Bruck et al. 2011). Alternatively, lower activity on SWD has been recorded for the insecticide Pyganic®. In fact only direct-sprays against SWD adults resulted in reduced activity without any form of residual effectiveness in field trials (Bruck et al. 2011). Many of these organic growers resorted to cultural techniques including sanitation methods such as removing culls and stripping the bushes entirely of ripe fruit, with only partially successful results.

Secondly, there are several growers in Citrus County who grow strawberries adjacent to blueberries. Strawberries are a known host of SWD and the crop seasons overlap to a limited extent in the field. Blueberries begin harvesting in March and the strawberry season climaxes in March/ April depending on market price, temperature and pest pressure (Nyoike and Liburd 2013). Spotted wing drosophila is an opportunistic species and moves from one fruit crop to another as the season progresses allowing population numbers to increase rapidly.

Alachua County had the second highest mean SWD captures in both years of our study and has the largest number of blueberry growing hectares in the state. The greater the number of hectares, the greater risk Alachua is for SWD losses. In addition, Alachua County has a large number of blueberries being grown under high tunnels. High tunnels cover the blueberries during the winter for protection from freezes and cold temperatures. The tunnels act as greenhouses, creating artificial warm environments that promote SWD development. Blueberries grown in high tunnels ripen much sooner than field berries and as a result are the first U.S. berries on the market each season.

Early ripening varieties of host fruits have been shown to be at a greater risk to injury by SWD (Burrack et al. 2013); however, it is unclear whether this is the case for Florida. Spotted wing drosophila populations are higher in the tunnels than in the field early in the season, potentially increasing the risk for injury. If these populations are not managed they may pose a greater risk to field berries if and when they migrate toward new resources (Coyne et al. 1982).

Marion County had the highest mean SWD captures in 2013 but was among the lowest counties in 2012 (Table 3-4). The field site located in Marion County was at the University of Florida Plant Science Research and Education Unit in Citra, Florida. This location is not a commercially managed planting and is used for research purposes only. During 2012, these plantings had severe problems with cedar wax wing (*Bombycilla cedrorum* Vieillot) pests. As a result many of the berries were lost to feeding injury or as fallen culls. In 2013, however, the cedar wax wings were not a concern and there was a large amount of fruit on the bushes throughout the season. Therefore, there was a much greater amount of food and reproductive resources available to SWD in 2013 than in 2012. This may explain the significantly higher number of SWD in Marion County in 2013 than in 2012.

DeSoto and Suwannee Counties had the lowest SWD captured during the survey, which may have been influenced by climate. DeSoto was the southernmost county in the study and as such experienced the warmest temperatures earlier in the year compared with other sites. High temperatures in DeSoto typically reach 30ºC in early to mid-April (FAWN 2013). Conversely, the survey sites located in Suwannee County grew mostly rabbiteye blueberries. Rabbiteye blueberries ripen during the summer months when temperatures are much warmer. The spotted wing drosophila is most active at 20ºC (Kanzawa 1935). In addition, SWD males are believed to become sterile in temperatures above 30ºC (Kanzawa 1935); however, more research is needed to determine whether this is valid in Florida conditions.

More females than males were captured during 2012 and 2013. One possible explanation for this trend is that the apple cider vinegar bait used in the traps for the survey is more attractive to females than males. Becher et al. (2012) found that female *D. melanogaster* were more attractive to food-related odors, ethyl acetate and balsamic vinegar, than were their male counterparts. Furthermore, female flies require more food than males due to egg production and are constantly searching for oviposition sites from which the food related odors originate (Becher et al. 2012). It is possible that using food-related odors as baits for SWD such as apple cider vinegar may result in a greater number of female flies captured.

The results of this study indicate that SWD is present in 8 or the 9 major blueberry growing counties in Florida. Given its rapid development and Florida's year-round growing season, SWD is likely to become a concern for blueberry growers in the seasons to come. Growers are encouraged to continue to monitor for this invasive pest to remain current on the possible threat to their blueberries and to be proactive in their management programs for this pest.

Table 3-1: Counties and sites included in 2012 and 2013 survey for SWD, including number of traps deployed at each site.

2012	County	Number of Sites Surveyed	Total Traps Deployed
	Suwannee	1	7
	Alachua	3	16
	Putnam	1	6
	Marion	1	5
	Citrus	2	13
	Lake	2	8
	Orange	1	5
	Polk	2	12
	DeSoto	1	3
2013			
	Suwannee	1	3
	Alachua	3	17
	Putnam	1	6
	Marion	1	3
	Citrus	2	12
	Lake	2	6
	Orange	2	7
	Polk	2	12
	DeSoto	0	0

Table 3-2. Total number of SWD captured in 2012 and 2013 survey seasons.

Year	Samples	Female	Male	F:M Ratio	Total SWD
2012	593	568	276	2.06	844
2013	653	343	155	2.21	498
Total	1246	911	431	2.11	1342

Table 3-3. Mean male, female, and SWD capture per sample for year 2012 and 2013 of SWD survey.

		Year		
		2012	2013	
Female	Mean ± SEM	0.96 ± 0.10	0.53 ± 0.06	
	t value			3.85
	df			1002.8
	P value			0.0001*
Male	Mean ± SEM	0.47 ± 0.06	0.24 ± 0.04	
	t value			3.01
	df			991.4
	P value			0.0027*
SWD Total	Mean ± SEM	1.42 ± 0.01	0.76 ± 0.09	
	t value			3.95
	df			1000.9
	P value			<0.0001*

Table 3-3. Mean number of SWD captured in each county in the 2012 and 2013 survey study.

	Mean ± SEM*	
County	2012	2013
Suwannee	0.29 ± 0.47c	0.05 ± 0.49b
Alachua	1.65 ± 0.25b	1.34 ± 0.20a
Putnam	0.47 ± 0.41bc	0.41 ± 0.33ab
Marion	0.21 ± 0.44cA	1.44 ± 0.37aB
Citrus	4.81 ± 0.31aA	0.90 ± 0.19abB
Lake	0.20 ± 0.46c	0.41 ± 0.27ab
Orange	1.23 ± 0.48bcA	0.53 ± 0.17bB
Polk	0.16 ± 0.31cA	0.33 ± 0.31abB
DeSoto	0 ± 0.68c	---[†]

*SEM = standard error of the mean.
[†]No survey sites in DeSoto County in the 2013 survey season. Means with the same letter are not significantly different ($P \leq 0.05$). Lowercase letters represent differences within columns. Capital letters represent differences across rows.

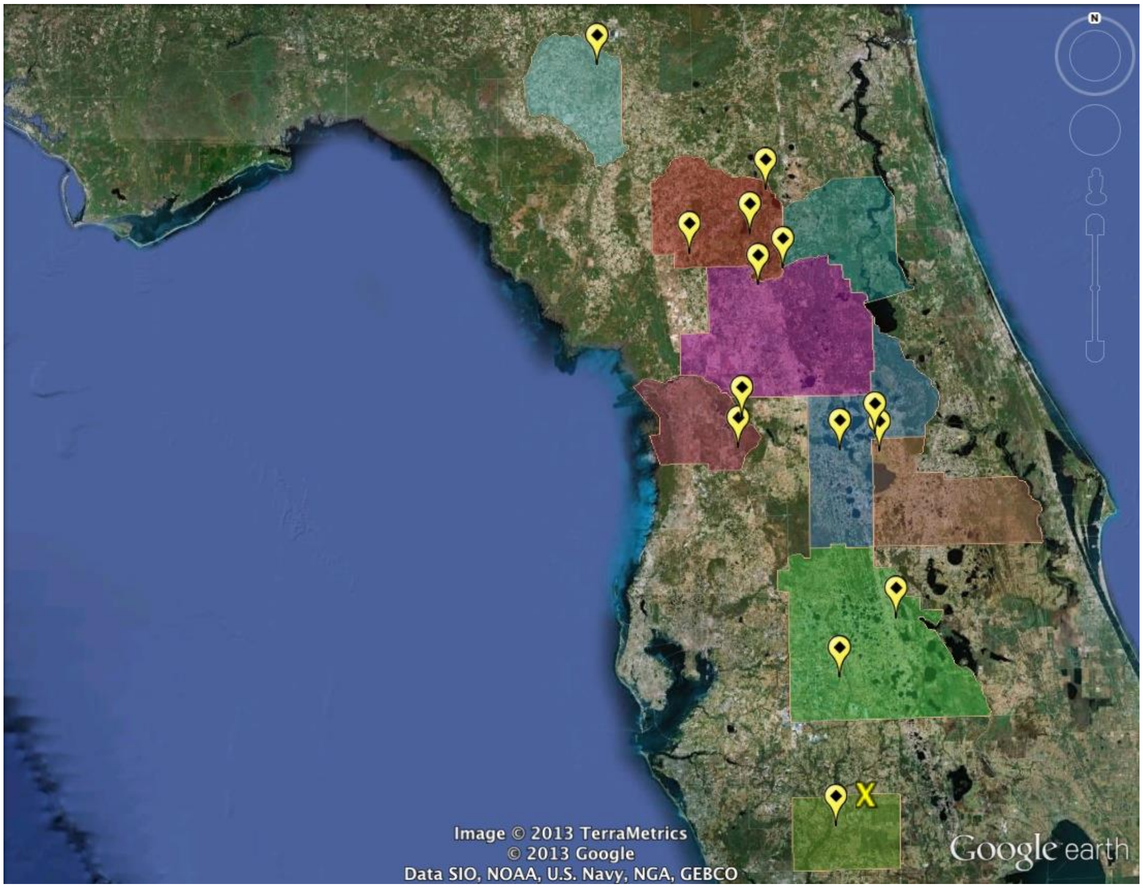

Figure 3-1. Survey sites and counties for 2012-2013 blueberry seasons. Yellow place marks denote survey sites for 2012 and 2013. Yellow "X" is DeSoto County that was only surveyed in 2012, not 2013. Color overlays are surveyed Florida counties.

Figure 3-2. Plastic cup trap with yellow stimulus used during the 2012 survey year. Photo courtesy of L. E. Iglesias.

Figure 3-3. Plastic cup trap without yellow stimulus used for 2013 survey. Photo courtesy of L. E. Iglesias.

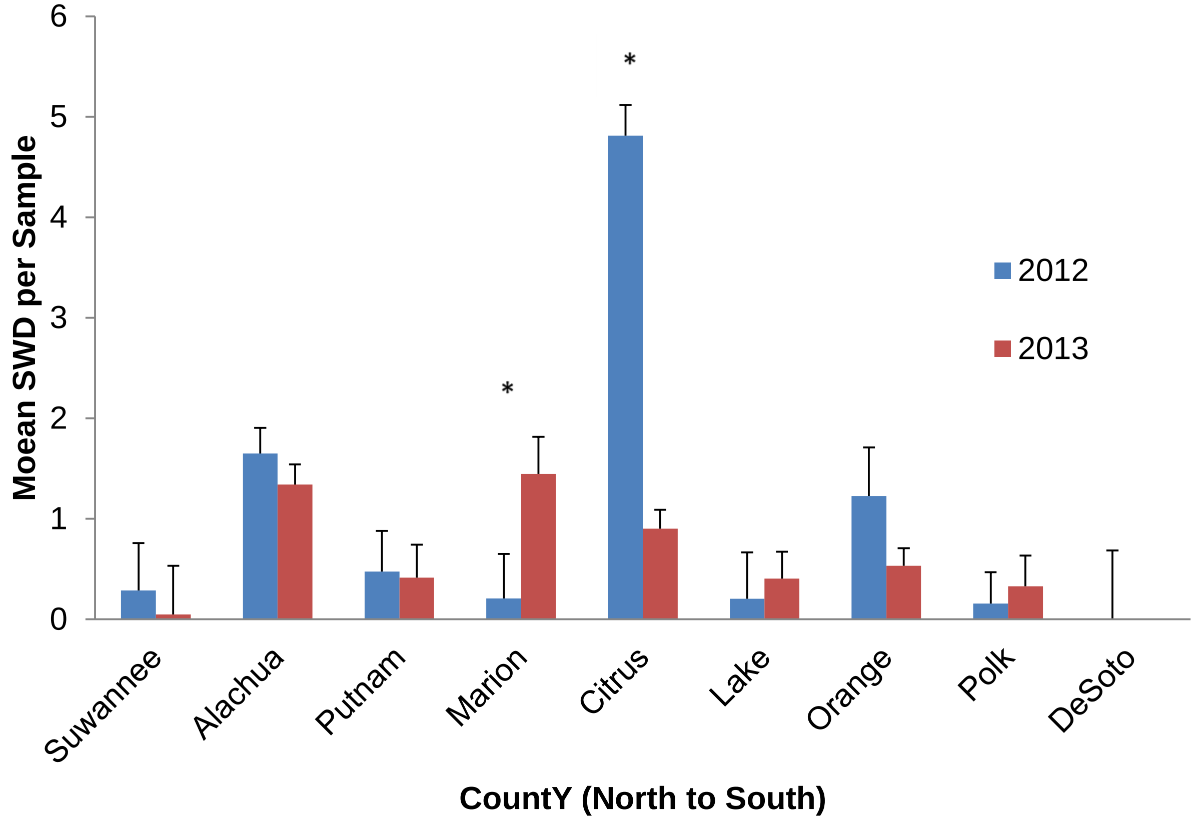

Figure 3-4. Mean SWD captured by county for 2012 and 2013 survey years. Asterisk (*) indicates significant differences at $P \leq 0.05$ among survey years.

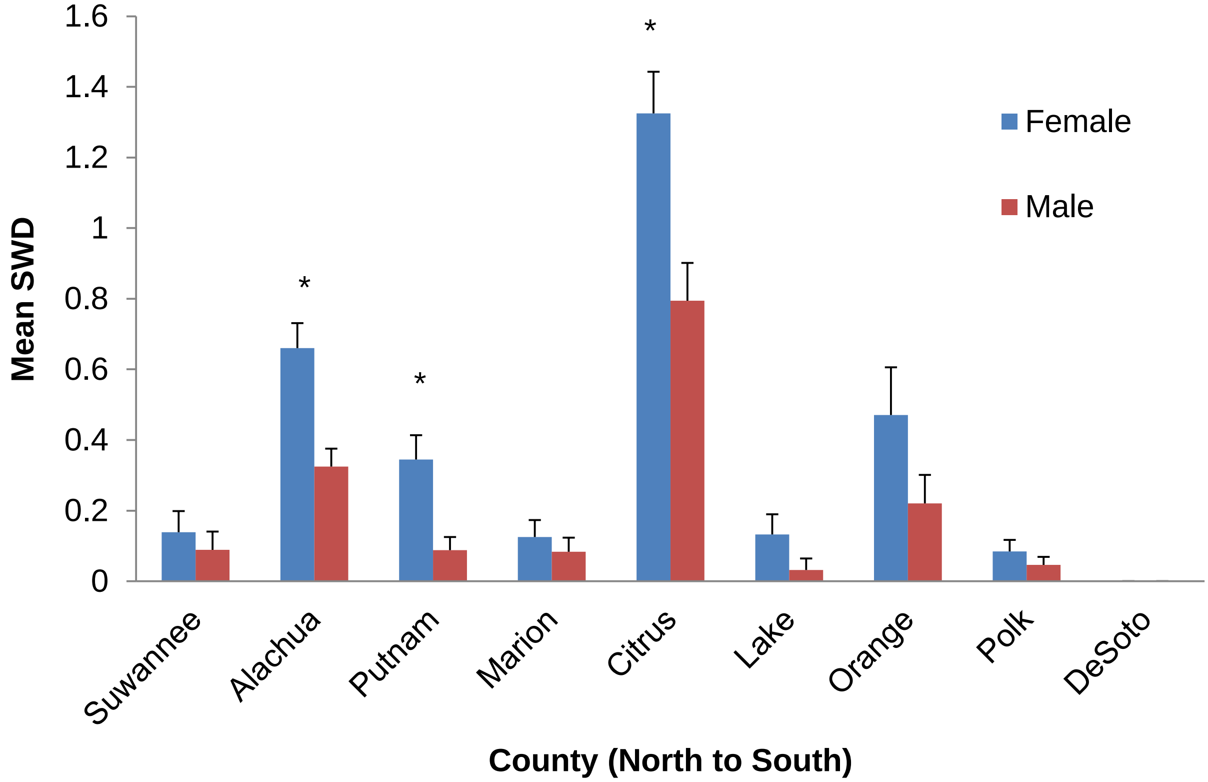

Figure 3-5. Mean female and male SWD captured in each county for 2012. Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

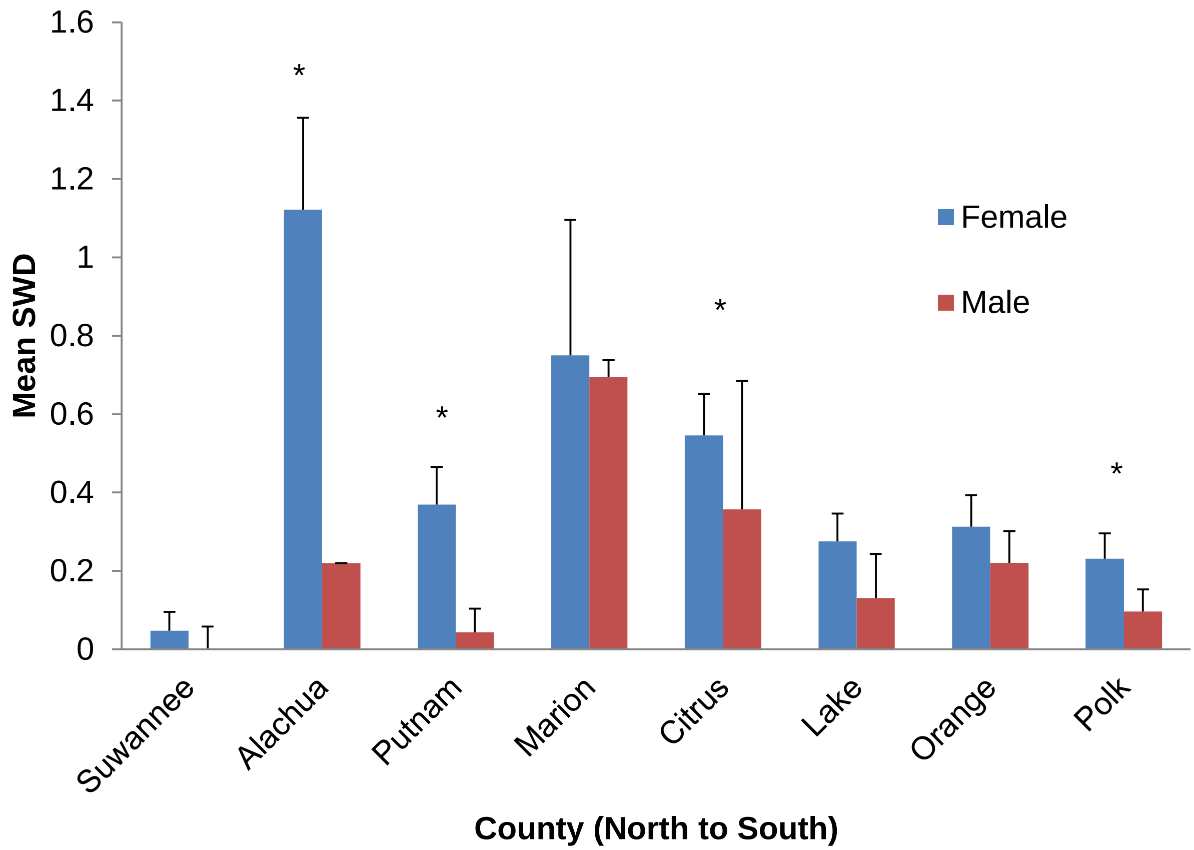

Figure 3-6. Mean female and male SWD captured in each county for 2013. Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

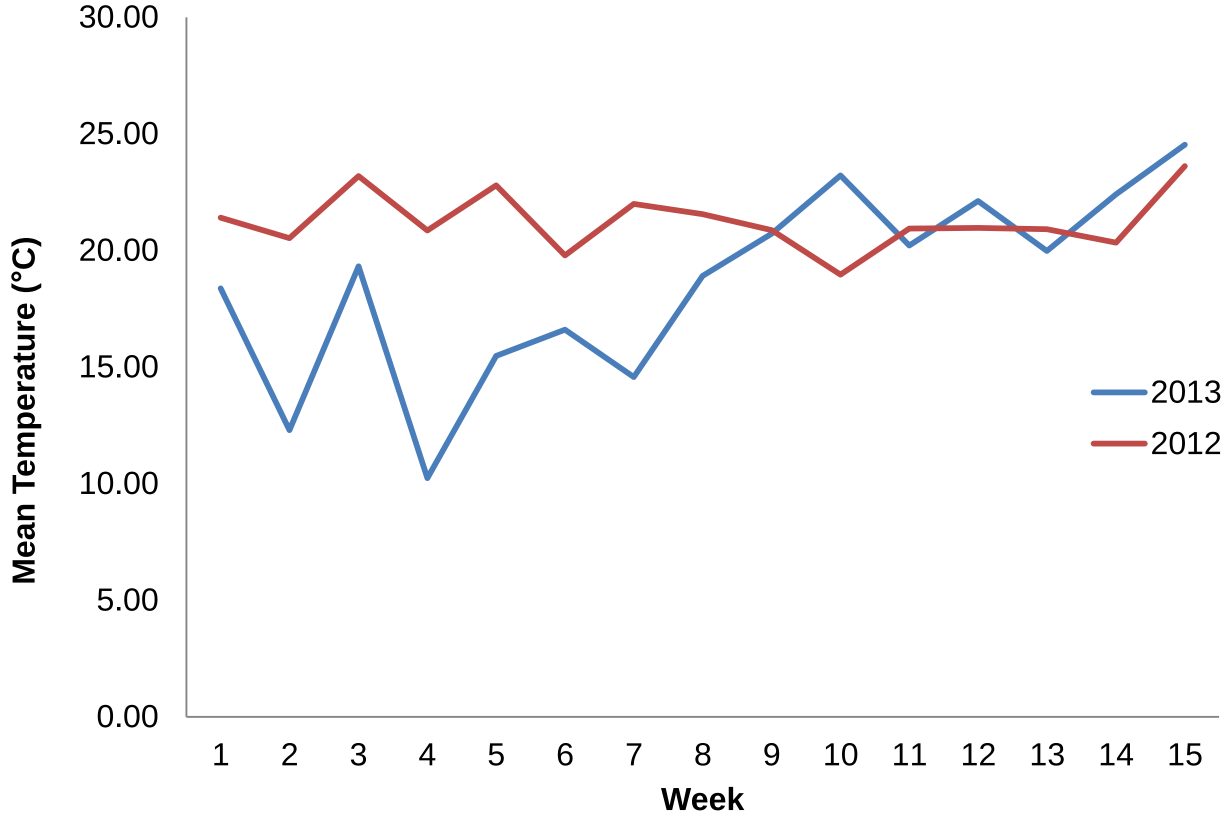

Figure 3-7. Mean temperatures in survey areas for 2012 and 2013. Week number begins February 1, 2012 to June 1, 2012.

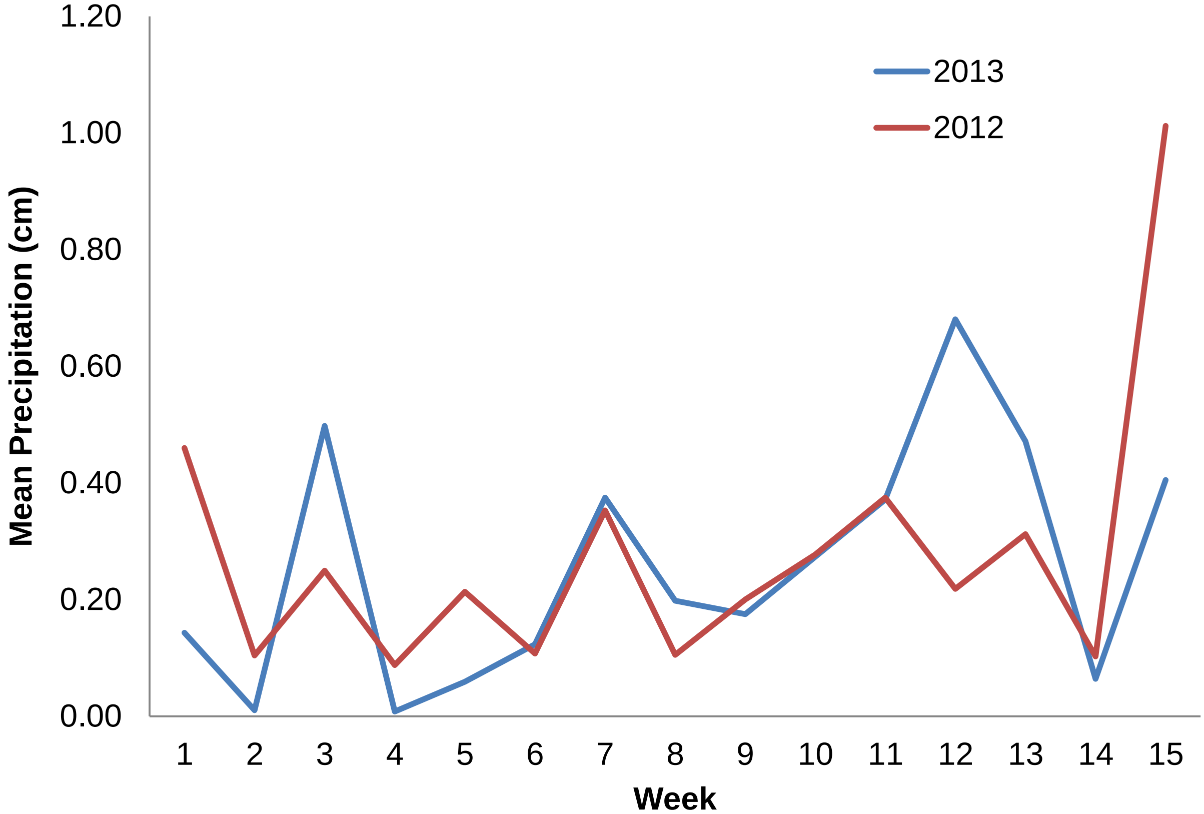

Figure 3-8. Mean precipitation in survey areas in 2012 and 2013. Week number begins February 1, 2013 to June 1, 2013.

CHAPTER 4
OVIPOSITION PREFERENCE OF SPOTTED WING DROSOPHILA IN SOUTHERN HIGHBUSH AND RABBITEYE BLUEBERRIES

The ability to appropriately time insecticide applications is critical for the effective management of a key pest. Therefore, determining the stage when the spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), will oviposit into blueberries will help to improve the timing of pesticide applications against adults. The SWD is known to oviposit in ripening fruits (Kanzawa 1935, Lee et al. 2011, Walsh et al. 2011, Burrack et al. 2013, Liburd and Iglesias 2013); however, information on the stage of ripening in blueberries when SWD will oviposit is still unclear.

The SWD has a wide host range that includes thin-skinned fruits such as blueberries (*Vaccinium* spp.), strawberries (*Fragaria ananassa* Duchesne), caneberries (*Rubus* spp.), grapes (*Vitis* spp.), and cherries (*Prunus* spp.) as well as some known stone fruits like peaches (*Prunus persica* L.), nectarines [*P. persica* (L.) Batsch], and plums (*Prunus salicina* Lindl.) (Steck et al. 2009, Walsh et al. 2011, Burrack et al. 2013). It is a frugivore pest that utilizes host fruits for feeding and reproduction. Frugivores generally lay their eggs in locations that provide the best opportunity for larval survival.

Lee et al. (2011) found that SWD preferred to oviposit in blackberries, blueberries, cherries, raspberries, and strawberries over grapes in laboratory choice tests. Further investigations show that host preference for SWD increased as brix level rises (Lee et al. 2011). In a recent study with no-choice tests, Burrack et al. (2013) found no differences in eggs laid in various blackberry maturity stages. We hypothesized that SWD will show host preference to fully ripe blueberries over green and pink fruit.

We chose to conduct assays mainly on southern highbush (SHB) blueberry because it has a higher risk than rabbiteye (RE) species of loss due to injury by SWD in Florida. Rabbiteye has characteristics dissimilar to SHB varieties, such as a grittier texture, larger seeds, and tougher skins (Lyrene and Moore 2006). Some potential factors influencing host preference are brix levels, pH, skin thickness, and firmness (Lee et al. 2011, Burrack et al. 2013). Given the characteristics of RE, we predict it will be less susceptible to SWD than SHB. In addition, RE ripens later in the summer months in Florida than SHB and may be less at risk for infestation by SWD.

Berry stage preference helps determine when pest management tactics need to be implemented including monitoring for SWD and application of pesticides. The objectives of the study were to: 1) evaluate the suitability of RE and SHB blueberry species (at different berry maturity stages) for SWD larval development and 2) to evaluate SWD oviposition preferences for different ripening stages of SHB blueberries. To address these objectives we conducted no-choice and choice laboratory bioassay experiments.

Materials and Methods

Source of Flies

Spotted wing drosophila flies were obtained from a laboratory colony reared in an environmental chamber (Model I36VL, Percival Scientific, Inc., Perry, IA) at the University of Florida, Small Fruit and Vegetable IPM (SFVIPM) laboratory in Gainesville, Florida. Chamber conditions were maintained at 23°C with 16:8 h light:dark cycle and RH ~65%. Flies were reared on Formula 4-24® (Carolina Biological Supply, Burlington, NC) in 0.25-L polypropylene bottles (Applied Scientific, Kalamazoo, MI) with foam stoppers (Jaece, North Tonawanda, NY). Flies used in both experiments were sexually mature (4 to 7 d old). They were removed from polypropylene bottles using an air pump and placed into 50-mL plastic vials with screw caps. The plastic vials were placed in the freezer for 90 s to immobilize the flies before they were transferred to bioassay chambers.

Blueberry Bushes

The blueberries used in both studies were selected from a blueberry planting located at the University of Florida, Plant Science Research and Education Unit (PSREU) in Citra, Florida. Blueberry branches were transported back to the SFVIPM laboratory in Gainesville, Florida. There were four blocks of SHB blueberry bushes and four blocks of RE blueberry bushes (approximately 4 to 6 years old and 1 m tall) planted 1 m apart. Blocks were blocked by surrounding vegetation. Each block contained 8 rows each with 25 blueberry bushes. Each row had 5 different varieties each with 5 bushes. Pine bark was used as mulch for blueberry rows. Blueberries were watered daily with drip irrigation, and no other chemicals had been used in the plantings for pest management.

Prior to the start of laboratory bioassays, larval tests were performed in salt solution to ensure that berries were maggot-free (Hueppelsheuser 2010). Larval salt tests were completed by randomly collecting at least 30 ripe berries from each block and placing them in a resealable plastic bag. Berries were lightly crushed in the bag and covered with a salt solution (68.25 g salt to 0.95 L water). The fruit were allowed to sink to the bottom of the bag for approximately 10 to 15 min and larvae, if present, to float to the top of the bag. None of the berries tested were infested with larvae.

Suitability of Rabbiteye and Southern Highbush Blueberry Species for SWD Larval Development

A no-choice laboratory bioassay was used to determine the suitability of RE and SHB blueberry species for SWD larval development. The experiment was a two-way factorial design with 4 replicates. Blueberries species and berry maturity stage were the effects. The two blueberries species tested were southern highbush (*Vaccinium corymbosum* L. x *V. darrowi* Camp) and rabbiteye (*Vaccinium virgatum* Aiton). The 4 berry maturity stages evaluated were: 1) green, 2) green-pink, 3) pink, and 4) blue.

Thirty-two berries were randomly selected from each of RE and SHB species from a planting at the PSREU in Citra, Florida. Two berries were selected from each of the 4 maturity stages. Maturity stages and species were separated into individual bioassay containers (32 containers with 2 berries each). Ten mated females were introduced into the chambers using the freezing method described above. The bioassay chamber consisted of a 0.5-L clear plastic deli container (Solo®, Urbana, IL) with a mesh lid (Figure 4-1). A 30-mL container with a cotton wick was filled with 15 mL of 1 M of sugar water to feed the flies and placed in the treatment container. Bioassay chambers were placed on the laboratory bench under grow lights on a 16:8 h light:dark cycle at a mean temperature of 22.8°C. Female flies remained in the bioassay chamber for 96 h before they were removed. Berries were kept for 14 d after flies were removed to check for emergence. Suitability of host was measured by the number of adult SWD that emerge from the berries.

The mean number of emerged SWD per berry was calculated. A two-way analysis of variance (ANOVA) was used to determine effects of blueberry species, maturity stage and their interaction on SWD emergence if any. Significant means were separated using Tukey's HSD (JMP, SAS Institute 2013).

Berry Maturity Stage

A choice laboratory bioassay was used to evaluate SWD oviposition preferences for different ripening stages of SHB blueberries. The experimental was completely randomized design with 5 treatments and 24 replicates. Treatments represented berry maturity stages and included: 1) blue, 2) pink-blue, 3) pink, 4) green-pink, and 5) green.

Forty-eight branches were randomly selected from two SHB blueberry varieties, Emerald and Jewel. Branches were selected that had stems at least 7.6 cm long and at least one berry for each treatment stage. Branches were placed in resealable plastic bags in an ice cooler, and transported back to the SFVIPM laboratory in Gainesville, Florida. Two branches, one branch from each variety were placed into a bioassay chamber. Two varieties were used in this study to provide SWD with an alternative in case females had a preference for one variety over another and to ensure enough berries were available for the study.

Each bioassay chamber consisted of a 1-L clear plastic deli container and a mesh lid (Figure 4-2). The chambers had a 35-mL plastic vial filled with tap water in which a foam stopper and two branches were placed. The vial was secured in a 30-mL container to prevent movement within the chamber and risk killing flies. A 30-mL container with a cotton wick was filled with 15 mL of 1 M sugar water to feed the flies and placed in the treatment container. Bioassay chambers were placed on the laboratory bench under grow lights on a 16:8 h light:dark cycle at a mean temperature of 22.8°C.

Once the branches were secured in the bioassay chamber, 10 mated females were introduced following freezing procedures detailed above. Females remained in the chambers for 72 h. Oviposition events were measured by observing and counting the number of females ovipositing on berries. Females were observed at least two times daily for 5 min. One oviposition event was counted when the abdomen of the female was curled and her ovipositor was extended into the skin of the blueberry. A female was recorded as having oviposited multiple times during one observation only if she moved to a different berry before ovipositing again.

The mean number of oviposition events per treatment was calculated per observation. A one-way ANOVA was used to evaluate mean differences among berry maturity stages and Tukey's HSD was used to separate means (JMP, SAS Institute 2013).

Results

Suitability of Rabbiteye and Southern Highbush Blueberry Species for SWD Larval Development

The mean number of SWD that emerged from the RE blueberry species was 0.63 ± 0.18 per berry compared with 1.06 ± 0.18 from SHB blueberries (Figure 4-3). Although SHB blueberry appeared almost two times more suitable than RE, the data were not significantly different at $P \leq 0.05$ ($F = 3.02$; df = 1, 24; $P = 0.10$). The effect of berry maturity (ripening) stage was highly significant ($F = 9.25$; df = 3, 24; $P = 0.0003$) (Figure 4-4). The mean emergence from blue berries was significantly higher than all other stages. No differences were found among the other stages. Additionally, color*blueberry species interaction effects were not significant ($F = 0.36$; df = 3, 24; $P = 0.79$).

SWD Oviposition Events and Preferences for Different Ripening Stages of Southern Highbush Blueberries

Overall, the mean number of oviposition events per observation for all treatments was low (Table 4-1) but there were significant differences among treatments (F = 3.38; df = 4, 325; P = 0.0098). There were a significantly higher number of oviposition events on blue berries than there were on pink-blue berries (Figure 4-5). More than half of the events (50%) occurred on blueberries, 38% on green-pink berries, 6% on both pink and green berries, and zero events took place on pink-blue berries (Figure 4-6).

Discussion

There were no significant differences between number of emerged SWD from the RE and SHB blueberry species at $P \leq 0.05$. However, the mean number of emerged SWD from SHB was two times greater than that of RE. Considering the high degree of variability among biological organisms the difference between species at P = 0.10 could be considered biologically significant. These results suggest that the characteristics of RE blueberry that make it different from SHB (grittier texture, firmer skin, and larger seeds) may play a role in host suitability. Similarly, results from Burrack et al. (2013) found in choice and no-choice assays with agar medium, SWD would not oviposit at the highest tested firmness (in their study penetration force) of 52.00 cN. These results may suggest that SWD will reject a host if the firmness is too high. Neither firmness nor other possible characteristics were quantified in this study and therefore cannot necessarily be assumed to be different between species. The firmness and brix content of these two species will need further research before firm conclusions can be drawn. Subsequent experiments should include quantifying possible related factors.

The number of female flies to berries in the bioassay chambers does not mimic the conditions in a blueberry field, where the number of female SWD is likely to be much lower than the number of blueberries present. Intraspecific competition plays a key role in oviposition site selection in some *Drosophila* species (Chess and Ringo 1985) and may have played a similar role in this study. In a study by Chess and Ringo (1985) *Drosophila melanogaster* and *Drosophila simulans* laid significantly more eggs on media that had not been pre-conditioned with *Drosophila* larvae, suggesting that these species prefer oviposition sites that have not been oviposited on previously. In addition, the same study showed that larval survival was significantly lower in media with larvae present. Due to the high female to berry ratio in my study, it is possible that more than one larva could have been developing inside the berries. As discussed by Chess and Ringo (1985) it is possible that the survival of larvae may have been reduced due to intraspecific larval competition for resources.

In the choice tests, female flies preferred to oviposit on fully-ripe blue berries over pink-blue berries however, no clear pattern was present among ripening stages. Conversely, in no-choice tests significantly more flies emerged from blue blueberries than all other stages. These findings suggest that though oviposition preference may not differ drastically among stages, suitability of each stage for larval development might. In a study by Lee et al. (2011), the mean number of oviposition events was only slightly lower for green-pink blueberries than it was for blue berries. Furthermore, the same study found no significant differences between eggs laid on blue berries and green-pink berries. Since larval development may vary depending on medium and competition, larval survivorship should be included in subsequent studies.

The results of our study suggest that both species of blueberries that are grown in the southeastern U.S. are susceptible to larval infestation by SWD. Southern highbush blueberries appear 50% more suitable than RE blueberries ($P = 0.10$) Additionally, SWD will oviposit on earlier ripening stages including mature green berries. Blue berries appear to be the most susceptible stage but growers should begin their management programs (monitoring and spraying) early in the season when berries are full green since flies are capable of ovipositing in these berries. It will be important for growers to manage both RE and SHB in a similar fashion until more information becomes available.

Table 4-1. Mean oviposition events per observation on different berry maturity stages in choice study.

Stage	Mean ± SEM*
Green	0.02 ± 0.03ab
Green-Pink	0.10 ± 0.03ab
Pink	0.02 ± 0.03ab
Pink-Blue	0 ± 0.03b
Blue	0.12 ± 0.03a

* Means followed by the same letters are not significantly different at $P \leq 0.05$.

Figure 4-1: Bioassay chamber for no-choice experiment. Photo courtesy of L. E. Iglesias.

Figure 4-2. Bioassay chamber for choice experiment. Photo courtesy of L. E. Iglesias.

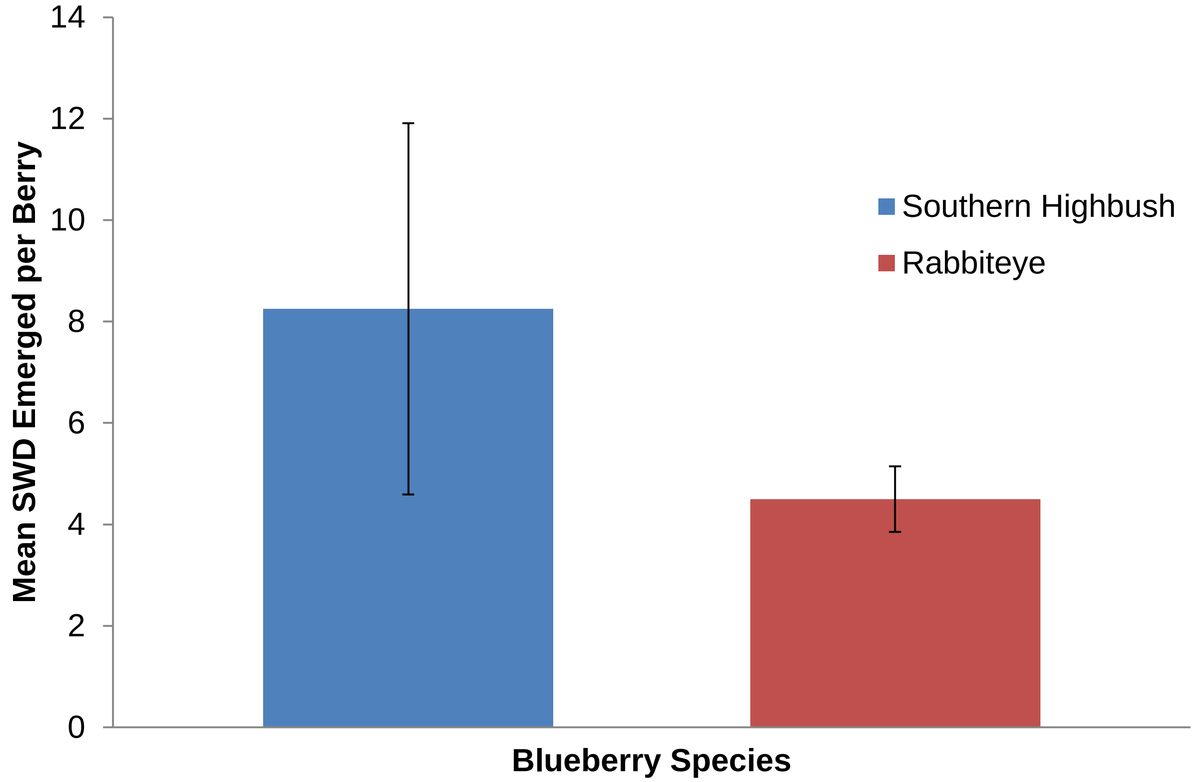

Figure 4-3: Mean SWD emerged from southern highbush and rabbiteye species in no-choice bioassay. Bars with the same letters are not significantly different at $P \leq 0.05$.

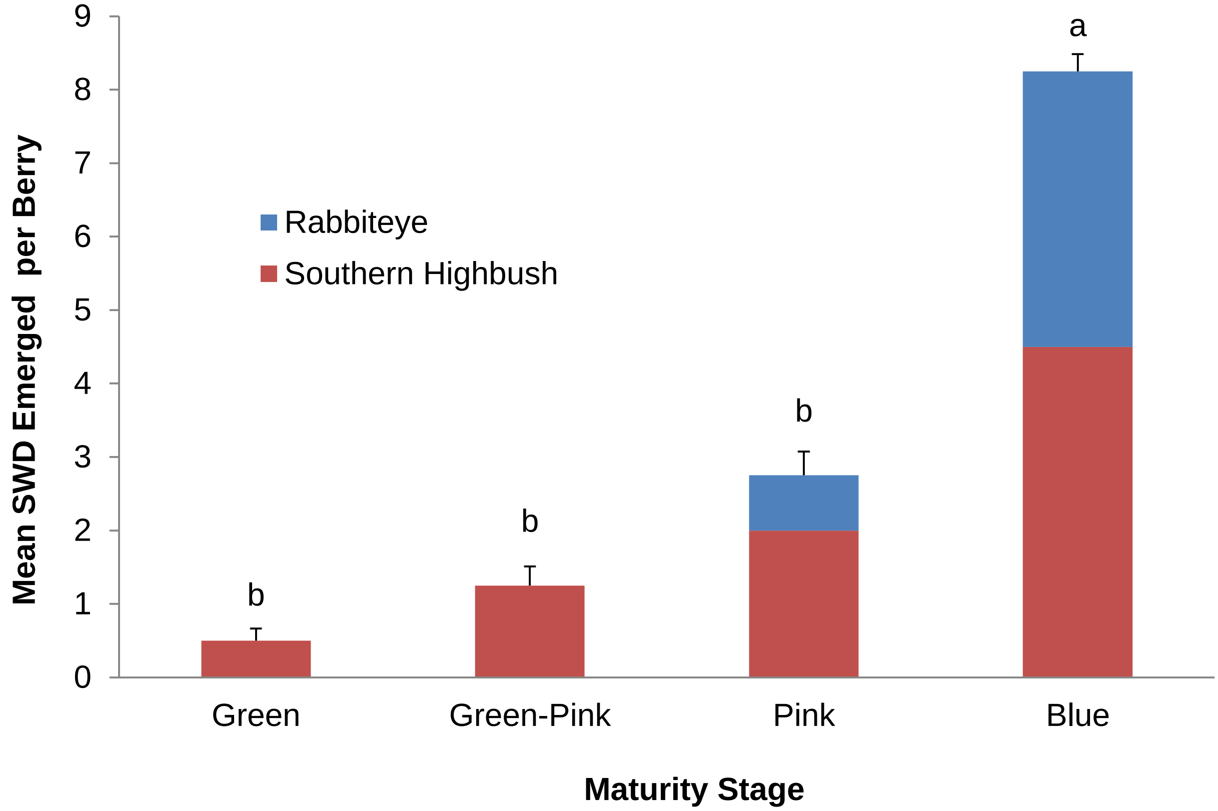

Figure 4-4. Mean SWD emergence from different berry maturity stages in no-choice tests. Bars with the same letters are not significantly different at $P \leq 0.05$.

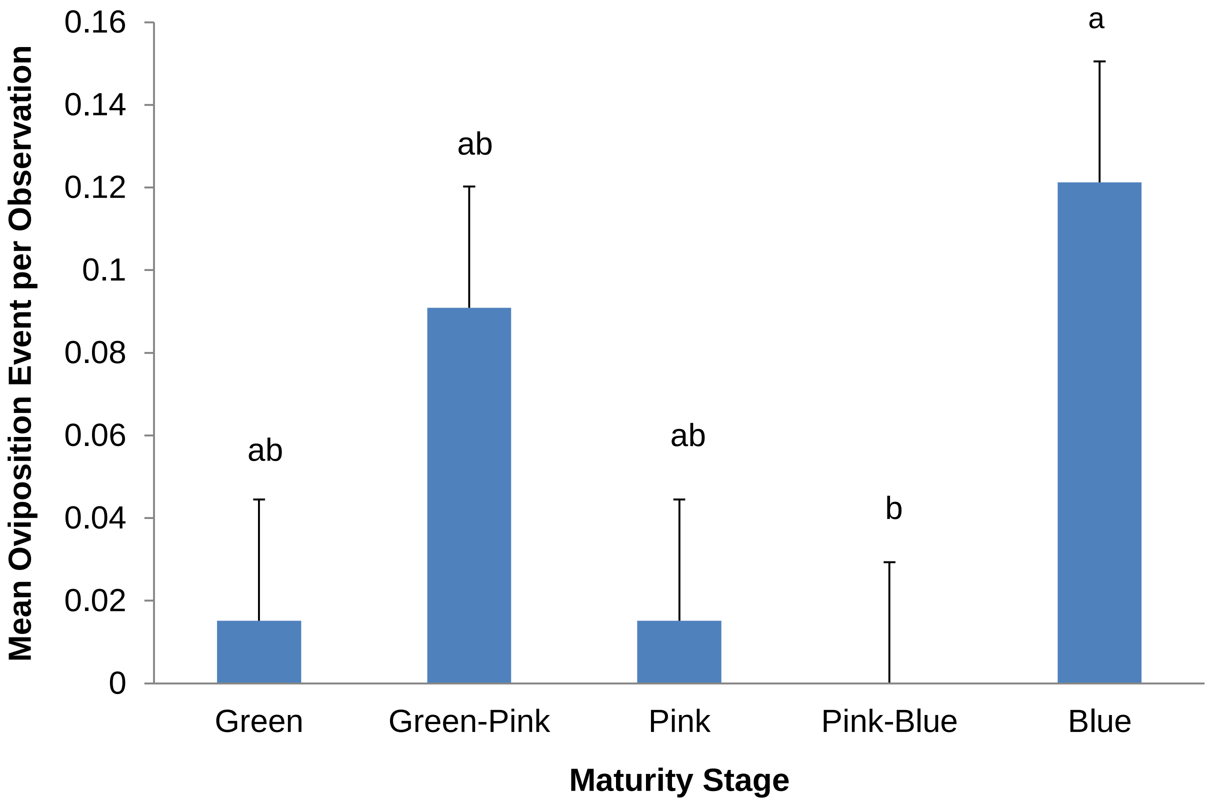

Figure 4-5. Mean oviposition events per observation on different maturity stages in choice study. Bars with the same letters are not significantly different at $P \leq 0.05$.

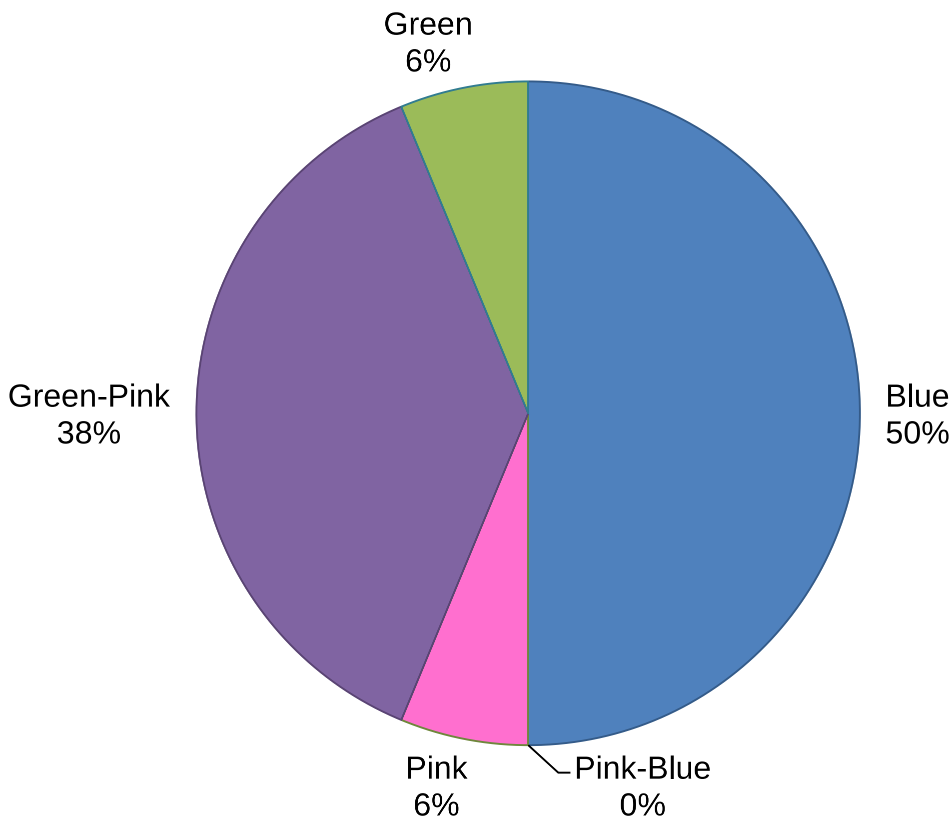

Figure 4-6. Percent oviposition on different stages of blueberry maturity stages in choice bioassays.

CHAPTER 5
EVALUATION OF TRAPS AND BAITS FOR MONITORING OF SPOTTED WING DROSOPHILA IN BLUEBERRIES

Effective monitoring is the cornerstone for a successful integrated pest management (IPM) program for a key invasive pest. Monitoring involves collecting biological information on pests and beneficials, as well as data on climate and crop phenology (Flint and Gouveia 2001). Traps are important tools that are frequently used in pest management programs to guide management actions. The effectiveness of a trap is dependent on several factors including attractant, size, color, and ease of handling. The most effective traps detect pest populations before the action threshold is reached so that growers can implement control measures before economic damage occurs.

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an invasive pest, causing devastating economic losses in blueberries up to 40 percent in California in 2008 (Bolda et al. 2010) and 10 to 15 percent in Florida in 2012 (eFly 2012). Due to its rapid development time, its populations can increase quickly (Walsh et al. 2011). Therefore, effective management programs must be developed to combat this invasive pest.

Current monitoring methods for SWD employ the use of a trap-and-lure system. Trap designs have been based on traps for other *Drosophila* spp. and generally use a container with a lid, entry holes, and a liquid bait that also acts as a drowning solution. In the past, several types of traps have been tested for SWD including handmade plastic cups with 2 to 10 entry holes, traps with mesh entries, and plastic cups with tents to provide shade and prevent water from entering (Kanzawa 1939, Wu et al. 2007, Lee et al. 2012). Dome traps and commercial “spice” jar traps have also been evaluated (Basoalto et al. 2013) with varying degrees of success.

Insects are attracted to colors that have the same or similar spectral reflectance patterns as their host (Prokopy and Owens 1983). Differences in the attractiveness of colors for some insects are related to their reflectance values and the ability of the insect to perceive reflected light at certain wavelengths (Roessingh and Stadler 1990). Color is an easily manipulated feature of a trap that could potentially affect trap attractiveness. Recent studies investigating differences in color preference of SWD showed a higher affinity toward darker colors such as red and black (Lee et al. 2012, Basoalto et al. 2013). However, these studies also indicated that clear was just as attractive as colored traps. Therefore, we chose to employ clear traps for our trap comparison studies.

Yellow has been shown to be attractive for many insects including plant-feeding dipterans because it depicts a foliage-type band (Prokopy and Owens 1983). Many traps used for monitoring SWD populations employ a yellow sticky card inside of the trap to act as a visual stimulus and prevent flies from escaping (Beers et al. 2011, Walsh et al. 2011, Klick et al. 2012, Bellamy et al. 2013). Preliminary trapping studies by Landolt et al. (2012a) indicated that a dome-type trap with a yellow bottom captured more SWD than a clear trap. We wanted to determine whether the color yellow would act as a visual stimulus and improve trap captures for SWD.

Drosophilids are able to walk on the surface of a liquid due to special structures on the distal tarsal segments (Walker 1992, Triplehorn and Johnson 2005). Odorless dish detergent is added to liquid baits to break the surface tension, causing the flies to drown. Adding dish detergent could be an inexpensive and easy method for increasing captures (if effective). In our treatment design, we included a trap baited with apple cider vinegar with and without a drop of odorless dish detergent to test the effect of the dish detergent on SWD captures.

Baits developed for SWD have been based on previous studies on the attractiveness of fermentation products such as ethanol, acetic acid, and methanol to other *Drosophila* spp. (Reed, 1938, Becher et al. 2010, Lebreton et al. 2012). The first recommended traps used for SWD monitoring in the U.S. were vinegars and wines. However, there is variability between different kinds of vinegars and wines (Kanzawa 1935; Landolt et al. 2012a, 2012b). Kanzawa (1935) found that molasses mixed with a home-brewed red wine was more attractive than the two components on their own as well as cherry juice, rice wine, sugar water, and a variety of botanical oils. In a recent study, Landolt et al. (2012a) found that apple cider vinegar was more attractive than red grape wine (Merlot). Combinations of wines and vinegars showed promise when used as bait for SWD. For instance, rice vinegar and red wine vinegar when added to a red grape wine (Merlot) were significantly more attractive than the red grape wine alone (Landolt et al. 2012b). We chose to include the most attractive combination of rice vinegar and red grape wine (Landolt's mix) in our study.

Drosophila spp. has been associated with multiple yeast species found during gut dissection. Drosophila feed on the yeasts of fermenting food sources as well as the juices from the source itself. Hamby et al. (2012) found that *Drosophila suzukii* also shows this association with yeasts from fermenting berries. In addition, results from Becher et al. (2012) suggest that SWD is attracted to the yeasts associated with fermentation, not the fermentation volatiles themselves. Recent baits for SWD have been a yeast-sugar-water mixture and one with the addition of whole wheat flour and ACV (Rich's mix) both of which we have included in this bait study. Apple cider vinegar remains a common bait because it is clear, has longevity in the field, preserves specimens, and is inexpensive. The objective of this study was to determine the relative attractiveness of various types of trap designs and commonly recommended baits in blueberry fields. Additionally, the ease SWD identification and servicing associated with each bait is discussed.

Materials and Methods

Experimental Design

The experimental sites were located within the center of commercial blueberry plantings. Experimental designs were completely randomized with 4 replicates (rows). Each replicate consisted of one double-planted row approximately 1 m wide and at least 60 m long. A buffer zone of at least 15 m separated each replicate. Each row was planted with multiple varieties of southern highbush (SHB) blueberries. Pine bark was used as mulch for blueberry rows. Blueberries were watered daily with drip or overhead irrigation. Bushes were planted approximately 1 m apart and the height ranged from 1 to 1.5 m.

Trap Comparison Study

Two experiments were conducted during the 2012 trapping study. Experiment 1 was conducted in high tunnels at a conventional blueberry operation located in Alachua County. The experiment ran from 25 January until 10 April 2012. Experiment 2 was conducted from 19 April to 10 May 2012 in the fields of an organically managed planting located in Citrus County.

Two experiments were conducted for the 2013 trapping study (experiments 3 and 4). Experiment 3 was conducted in a field planting at a conventional blueberry operation located in Alachua County. The experiment ran from 18 April to 23 May 2013. Experiment 4 was conducted at the same organically managed planting in Citrus County as the 2012 study and took place from 3 May to 22 May 2013. All locations in 2012 and 2013 were included in our survey study (Chapter 3) and were being monitored closely for SWD presence. The trapping studies began when SWD populations were high and the berries were fully ripe and therefore starting dates differed at each location.

In 2012, five trap designs were evaluated, all baited with apple cider vinegar (ACV). Four of the traps were made from 0.95-L clear plastic deli containers with lids (Solo®, Urbana, IL) and ten 0.64-cm holes along the upper rim. Two of the cup treatments had yellow visual stimuli (30-cm wide foamboard) placed around the rim of the cup, one of which had odorless, colorless dish soap (Palmolive Pure and Clear, Colgate-Palmolive Company, New York, NY) added to the bait to help reduce the surface tension of the ACV and prevent fly escape. The last two cup treatments were one with a small yellow sticky card (7.6 x 7.6 cm) hanging inside and a standard transparent cup (without the yellow sticky card). The final trap was a standard yellow sticky card (15.2 x 20.3 cm) used for monitoring flying insects (Great Lakes IPM, Vestaburg, MI), acting as the control. Plastic cup traps were baited with 150 mL of ACV that also acted as a drowning solution. The yellow sticky card had a 7.4-mm vial containing ACV attached as a lure.

Based on results from the 2012 trapping study, 4 trap designs were evaluated in 2013. Three of the traps used in 2012 were re-evaluated in 2013: basic cup, cup with yellow band around the rim and soap, and cup with yellow sticky card inside. The cup trap with the yellow band around the rim (without soap) and the yellow sticky card were excluded from the treatments (based on 2012 results). A fourth trap similar to the one used in 2012 with the yellow sticky card inside was adopted, however this trap was baited with a 150 mL yeast-sugar-water mixture. The yeast bait was made with 14.8 g baker's yeast, 39.4 g of white sugar, and 710 mL of tap water (Table 5-1). With the exception of the fourth trap, all other traps were baited with 150 mL ACV.

Bait Study

The bait study was conducted at an organically managed blueberry planting located in Citrus County, FL. The study ran from May 8 to May 27, 2013. There were 4 bait treatments evaluated using the standard plastic cup trap (0.95-L clear plastic deli containers with lids and ten 0.64-cm holes along the upper rim). Treatments evaluated were: 1) ACV plus dish detergent, 2) yeast-sugar-water mixture, 3) Rich's mix, and 4) Landolt's mix (Table 5-1).

Sample Collection

Samples were collected weekly and transported back to the University of Florida Small Fruit and Vegetable IPM (SFVIPM) laboratory in Gainesville, Florida for processing. Sample collection consisted of pouring all liquid bait into a collection container in the field and refilling the trap with fresh bait to the 150-mL level pre-marked on the trap. Yellow sticky cards in the trapping studies were replaced weekly with fresh cards. Traps were rotated each week to prevent positional bias. Male and female SWD were identified and counted for each sample collected (Triplehorn and Johnson 2005, Markow and O'Grady 2006, Vlach 2010). Since the baits used were not specific to

SWD, other *Drosophila* spp. were collected in the traps. These specimens were also recorded.

Data Analysis

In the trapping studies, a one-way analysis of variance (ANOVA) was used to determine whether there were differences among means of the SWD captured for each treatment for each experiment. Mean comparisons were evaluated using Tukey's HSD (JMP, SAS Institute 2013). Data were square root transformed to standardize the variances. Means were considered significantly different when $P \leq 0.05$. Results from each experiment are presented separately for the 2012 and 2013 trapping studies.

The data from the bait study were square root transformed to standardize the variances. A one-way ANOVA and Tukey's HSD were used to compare the means of SWD captured (JMP, SAS Institute 2013).

Welch's t-test was used to compare mean differences among female and male SWD captured in all experiments (JMP, SAS Institute 2013). Welch's t-test was used because variances were considered unequal by Levene's test for equal variances (JMP, SAS Institute 2013). Means were considered significantly different when $P \leq 0.05$.

Results

Trap Comparison Study

In 2012, experiments 1 and 2 showed similar trends for mean SWD captured among treatments (Figures 5-1, 5-2). The yellow sticky card (control) did not capture any SWD throughout the duration of the experiments. The basic cup, cup with the yellow band, and cup with the yellow band and the soap captured significantly more SWD than the yellow sticky card in experiment 1 ($F = 6.28$; df = 4, 95; $P = 0.0002$). The cup with the yellow sticky card was not significantly different than the other cup traps or the sticky card. In experiment 2, all of the cup treatments captured significantly more SWD than the yellow sticky card ($F = 6.28$; df = 4, 95; $P = 0.0002$). There were no significant differences among the four cup treatments in either experiment 1 or 2.

The number of females was significantly greater than the number of males in the basic cup ($t = 1.9$, df = 37.1, $P = 0.0617$), cup with the yellow band ($t = 2.7$, df = 42.1, $P = 0.0097$), and cup with the yellow band and the dish soap ($t = 2.2$, df = 47.8, $P = 0.0321$) in experiment 1 (Figure 5-3). In experiment 2, no significant differences were found among number of female and male SWD captured for each treatment (Figure 5-4).

In 2013, the results from both experiments were similar (Figures 5-5, 5-6). The commonly recommended trap design with the yellow sticky card and the yeast mixture captured significantly greater number of SWD in both experiments ($F = 10.12$; df = 3, 108; $P < 0.0001$). The yeast trap captured a mean of 1.44 and 10.83 in experiments 1 and 2, respectively. There were no differences among any of the cup traps baited with ACV in both experiments. The trap with the yellow band did not capture any SWD during experiment 3.

No significant differences ($P \leq 0.05$) in number of females and males captured were observed in any of the traps in 2013 (Figures 5-7, 5-8).

Bait Study

Results of the bait study showed significant differences among the bait treatments ($F = 11.7$; df = 3, 43, $P < 0.0001$) (Figure 5-9). Both Rich's mix (mean = 12.91) and the yeast-sugar-water bait (mean = 6.36) had significantly greater mean captures than the ACV bait (mean = 0.75) and Landolt's mix (mean = 0.67). None of the treatments captured different numbers of females and males (Figure 5-10).

The bait treatments also attracted other dipteran species, specifically other *Drosophila* spp. and the recent invasive fig fruit fly, *Zaprionus indianus* Gupta (Figure 5-11). The number of *Drosophila* spp. captured were significantly different (F = 10.3; df = 3, 43, P < 0.0001). Rich's mix captured the highest number of *Drosophila* spp. (mean = 59.17). Significant differences were found in *Z. indianus* (F = 7.32; df = 3, 43, P = 0.0005) per trap with Rich's mix capturing significantly greater numbers of *Z. indianus* (mean = 21.33) than either the ACV bait or Landolt's mix.

Discussion

The results of our 2012 trapping study support previous findings that the color yellow does not increase trap captures of SWD (Lee et al. 2011 and Basoalto et al. 2013). The ACV traps with the yellow sticky card inside were not significantly different from other ACV traps (without the yellow sticky card). The trap with the yellow sticky card and the yeast captured significantly more SWD than the same trap with ACV. This may suggest that the bait is a more important factor in attracting SWD flies than the visual stimulus of the yellow sticky card. The yellow sticky card may catch a few SWD; however, this tends to complicate matters since identification of SWD can be difficult when flies land on the sticky card. We noticed that specimens tend to desiccate rapidly, requiring immediate identification.

The results of the bait study showed that the yeast baits (Rich's and the yeast-sugar-water mixes) were significantly more attractive than the vinegar baits (ACV and Landolt's mix). Adults and larvae of SWD and other drosophilids will feed upon the associated yeasts and bacteria as well as the fruit material from damaged or fallen berries (Markow and O'Grady 2008, Hamby et al. 2012). This may explain the attractiveness of the yeast baits. In addition, odors of fermenting fruit baits such as wine or vinegar may be masked by the odors from the surrounding fruit.

All baits come with their advantages and disadvantages. The vinegar baits are generally clear for easy identification in the field or lab, have longevity in the field, and act as decent preservatives for collected specimens. The wine baits may be clear if using white wine but red wines will quickly stain the flies. The yeast-sugar-water mixtures are cloudy and have sediments that make identification difficult and timely. The whole wheat flour in Rich's mix made identifying and counting SWD very difficult and timely because it was the same color as the flies and the grains were too large to be sifted out.

In addition, none of the baits tested in the study are specific to SWD but to many other species of insects attracted to sweet or fermenting odors (Landolt 1995). Other drosophilids are especially attracted to the odors of fermenting fruit and yeasts (Reed 1938, Zhu et al. 2003, Budick and Dickenson 2006, Markow and O'Grady 2008, Becher et al. 2010, Becher et al. 2012, Landolt et al. 2012a, Landolt et al. 2012b, Lebreton et al. 2012, Steck et al. 2012). Rich's mix was especially attractive to *Drosophila* spp. with a mean of 59.17 per trap. Traps with high numbers of non-target species make identifying SWD difficult and timely. The size of the entry holes can be designed small enough to prevent entry of larger insects such as hemipterans and lepidopterans but unless a specific bait for SWD is developed, other vinegar flies will likely be found in SWD traps.

The effectiveness of the trap and bait system for capturing SWD is more dependent upon the attractiveness of the bait than the cup trap modifications evaluated in SHB blueberry fields. Therefore the basic cup trap would be recommended as it requires less time to construct and is less expensive than its modified counterparts. An effective bait is not only attractive to the target pest but also promotes quick and easy construction, handling, and pest identification. Benefits also include longevity of the bait in the field and preservation of specimens. Due to the highly destructive potential of SWD, intense monitoring programs in blueberries and other thin-skinned fruits are recommended. Growers must consider whether they value a highly-attractive bait such as a yeast-sugar-water mixture or Rich's mix, or a bait that will make handling and identification quick and easy such as apple cider vinegar or Landolt's mix.

Table 5-2: Bait study treatments and mixtures.

Bait	Ingredient	Manufacturer	Amount	Trap amount
ACV	ACV	5% acetic acid, Winn Dixie, Jacksonville, FL	150 mL	150 mL
(ACV)	Dish detergent	Palmolive Pure and Clear, Colgate-Palmolive Company, New York, NY	drop	
Yeast-Sugar-Water	Yeast	Fleischmann's RapidRise™ Yeast, ACH Food Companies, Inc., Cordova, TN	14.8 g	150 mL
(YSW)	Sugar	white granulated, Publix, Lakeland, FL	39.4 g	
	Water	tap	710 mL	
Rich's Mix (Rich)	Yeast	Fleischmann's RapidRise™ Yeast, ACH Food Companies, Inc., Cordova, TN	29.6 g	150 mL
	Sugar	white granulated, Publix, Lakeland, FL	118.3 g	
	Wheat flour	King Arthur Flour Co., Inc., Norwich, VT	59.1 g	
	ACV	5% acetic acid, Winn Dixie, Jacksonville, FL	29.6 mL	
	dish detergent	Palmolive Pure and Clear, Colgate-Palmolive Company, New York, NY	few drops	
	water	tap	710 mL	
Landolt's Mix	Rice Vinegar	25% acetic acid, Korea	280 mL	150 mL
(Land)	Red Grape Wine	Merlot 12% ethanol, Carlo Rossi, Modesto, CA	420 mL	
	Dish detergent	Palmolive Pure and Clear, Colgate-Palmolive Company, New York, NY	few drops	

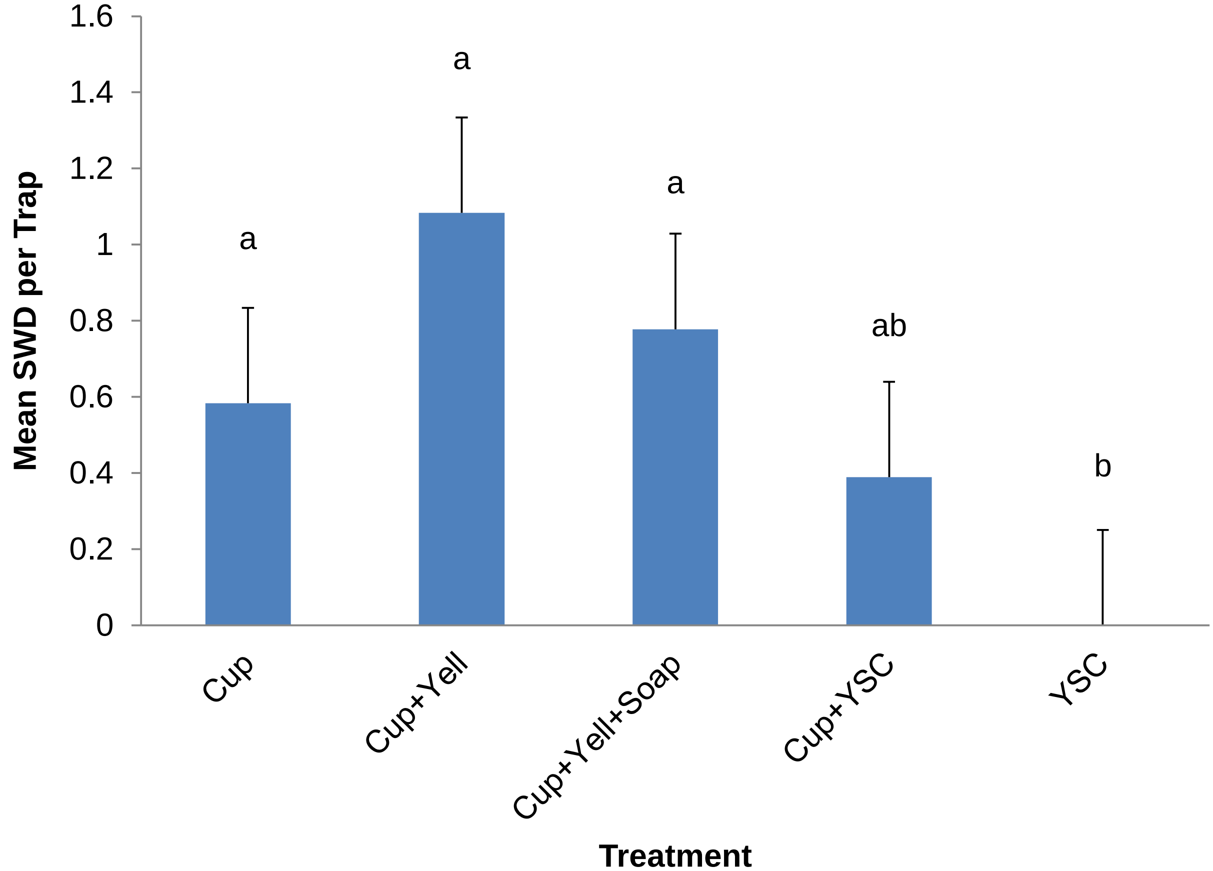

Figure 5-1: Mean spotted wing drosophila (SWD) captured per trap in 2012 trapping study, experiment 1. Treatments included the basic cup trap (cup), cup trap with a yellow band (cup+yell), cup trap with yellow band and odorless dish soap (cup+yell+soap), cup trap with a yellow sticky card (cup+YSC), and a yellow sticky card (YSC). Bars with the same letters are not significantly different at $P \leq 0.05$.

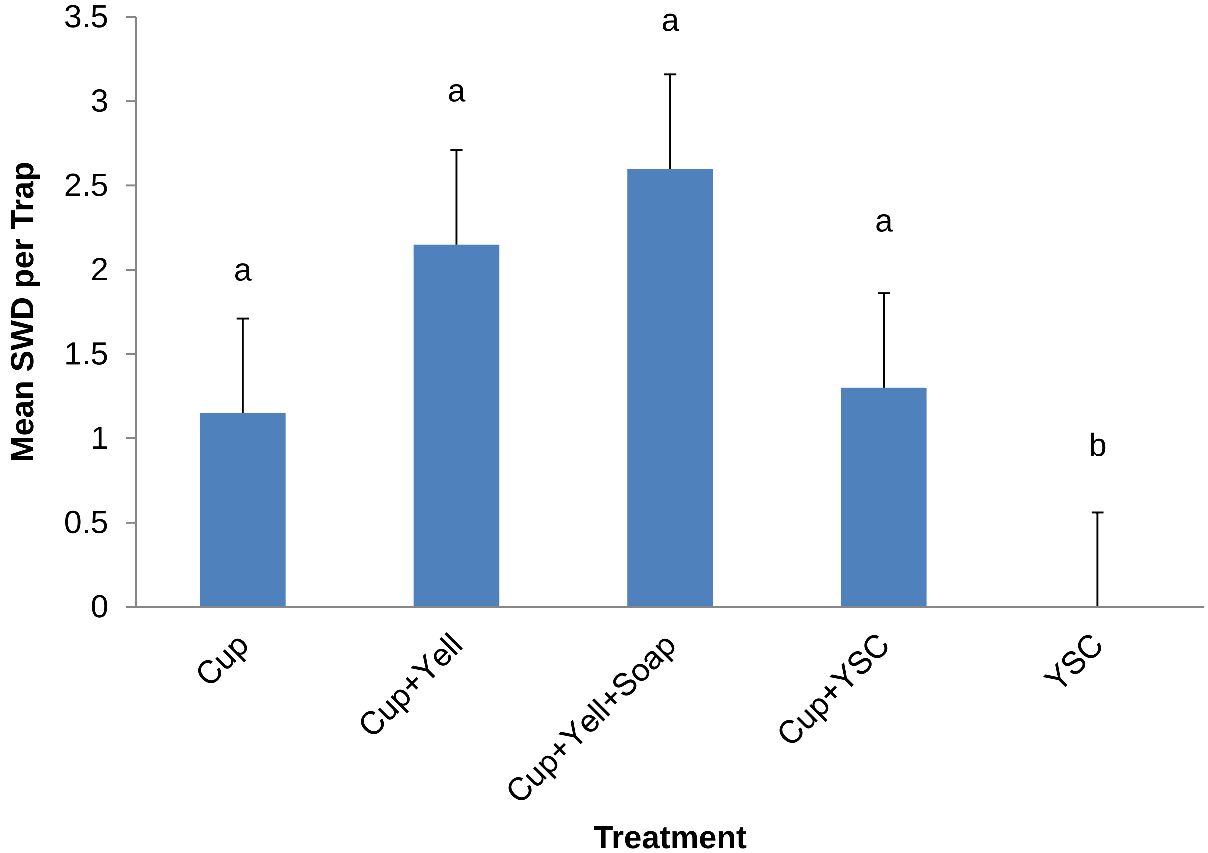

Figure 5-2: Mean spotted wing drosophila (SWD) per trap in 2012 trapping study, experiment 2. Treatments included the basic cup trap (cup), cup trap with a yellow band (cup+yell), cup trap with yellow band and odorless dish soap (cup+yell+soap), cup trap with a yellow sticky card (cup+YSC), and a yellow sticky card (YSC). Bars with the same letters are not significantly different at $P \leq 0.05$.

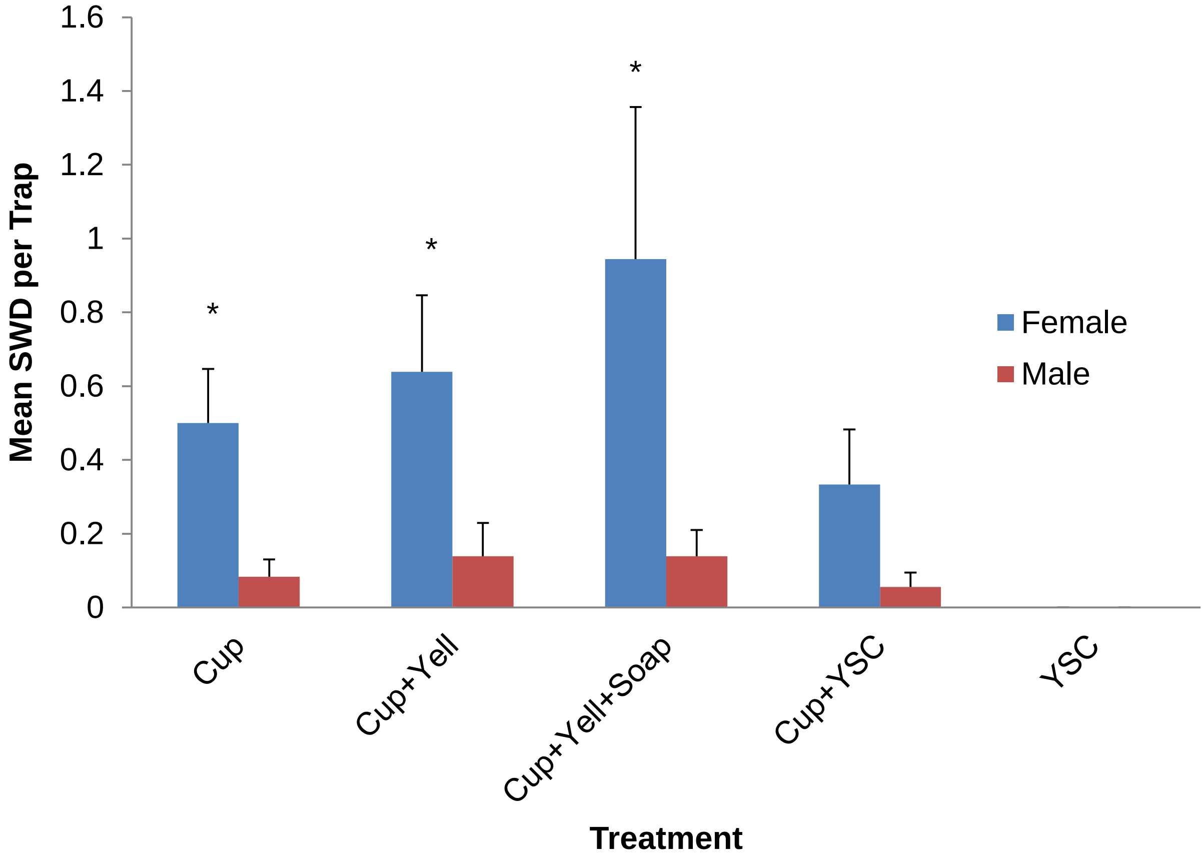

Figure 5-3: Mean male and female spotted wing drosophila (SWD) captured in 2012 trapping study, experiment 1. Treatments included the basic cup trap (cup), cup trap with a yellow band (cup+yell), cup trap with yellow band and odorless dish soap (cup+yell+soap), cup trap with a yellow sticky card (cup+YSC), and a yellow sticky card (YSC). Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

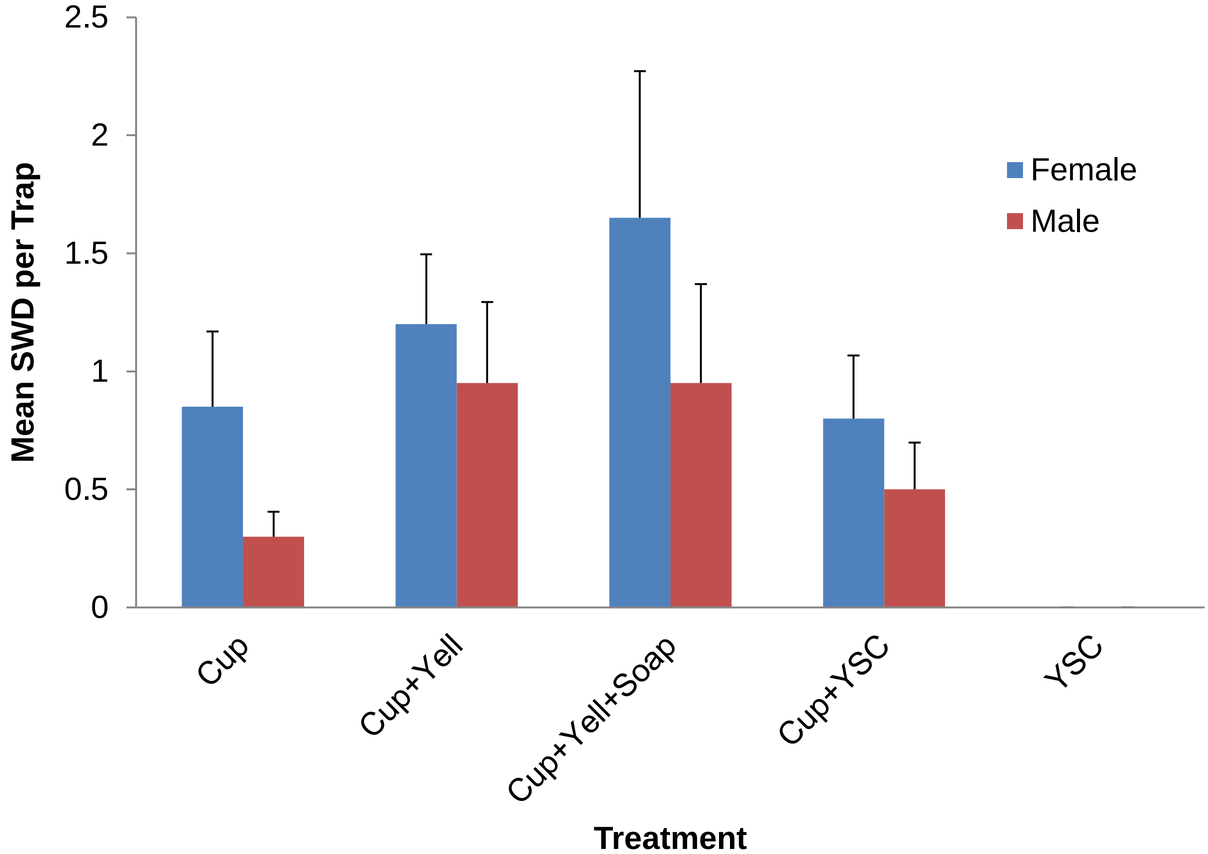

Figure 5-4: Mean male and female spotted wing drosophila (SWD) captured in 2012 trapping study, experiment 2. Treatments included the basic cup trap (cup), cup trap with a yellow band (cup+yell), cup trap with yellow band and odorless dish soap (cup+yell+soap), cup trap with a yellow sticky card (cup+YSC), and a yellow sticky card (YSC). Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

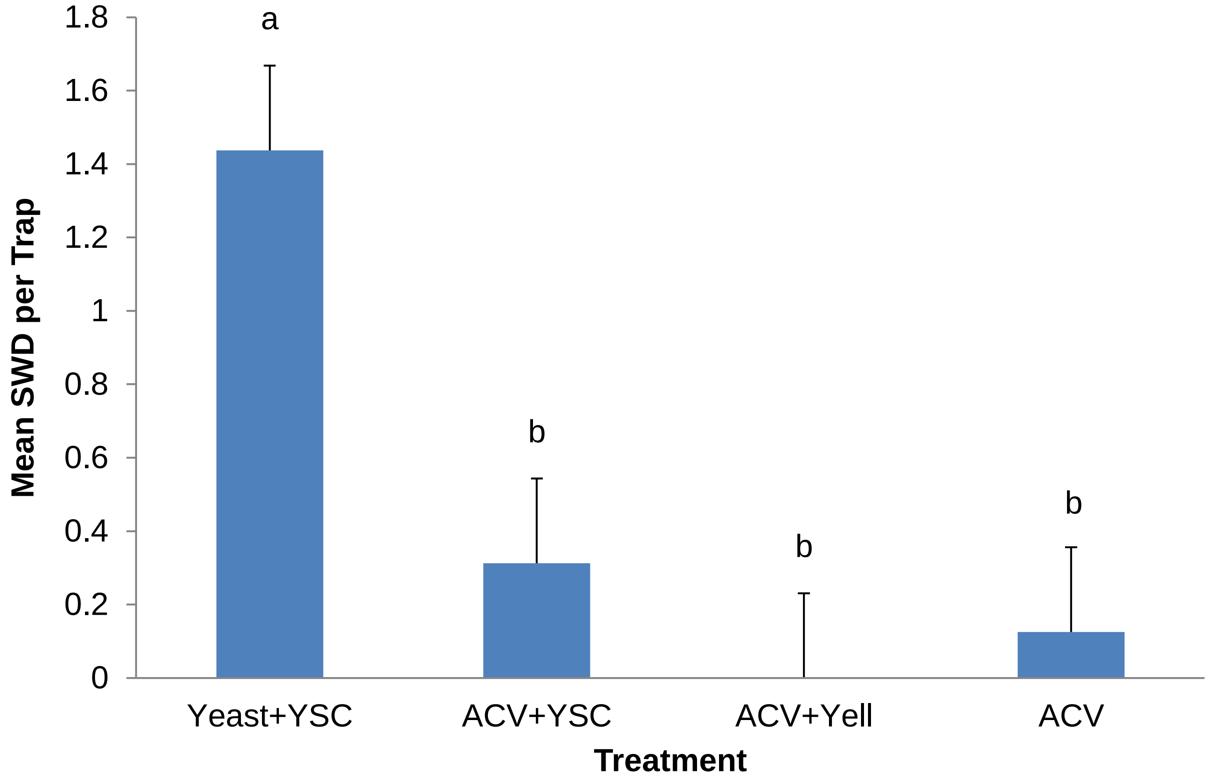

Figure 5-5: Mean spotted wing drosophila (SWD) captured per trap in 2013 trapping study, experiment 3. Treatments included the basic cup trap with a yellow sticky card baited with a yeast-sugar-water mixture (yeast+YSC), cup trap with a yellow sticky card with apple cider vinegar bait (ACV+YSC), cup trap with a yellow band with ACV and odorless dish soap (ACV+yell), and a basic cup trap with ACV and odorless dish soap (ACV). Bars with the same letters are not significantly different at $P \leq 0.05$.

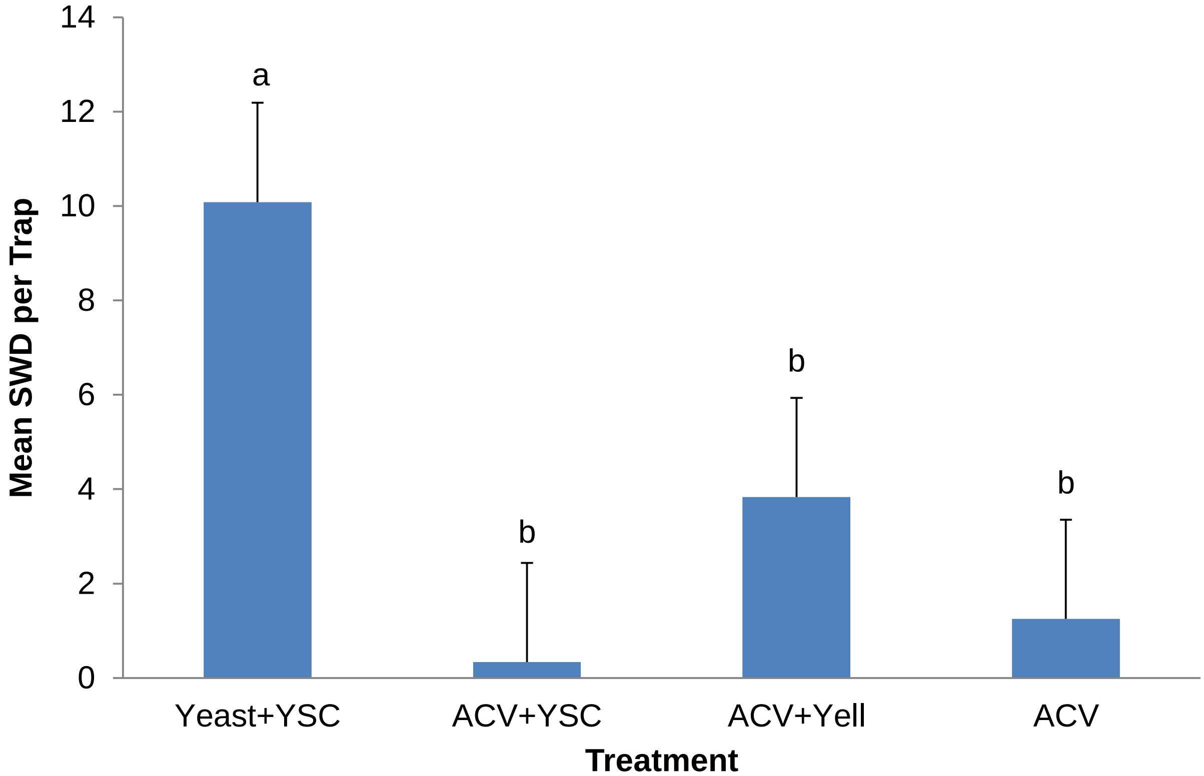

Figure 5-6: Mean spotted wing drosophila (SWD) captured per trap in 2013 trapping study, experiment 4. Treatments included the basic cup trap with a yellow sticky card baited with a yeast-sugar-water mixture (yeast+YSC), cup trap with a yellow sticky card with apple cider vinegar bait (ACV+YSC), cup trap with a yellow band with ACV and odorless dish soap (ACV+yell), and a basic cup trap with ACV and odorless dish soap (ACV). Bars with the same letters are not significantly different at $P \leq 0.05$.

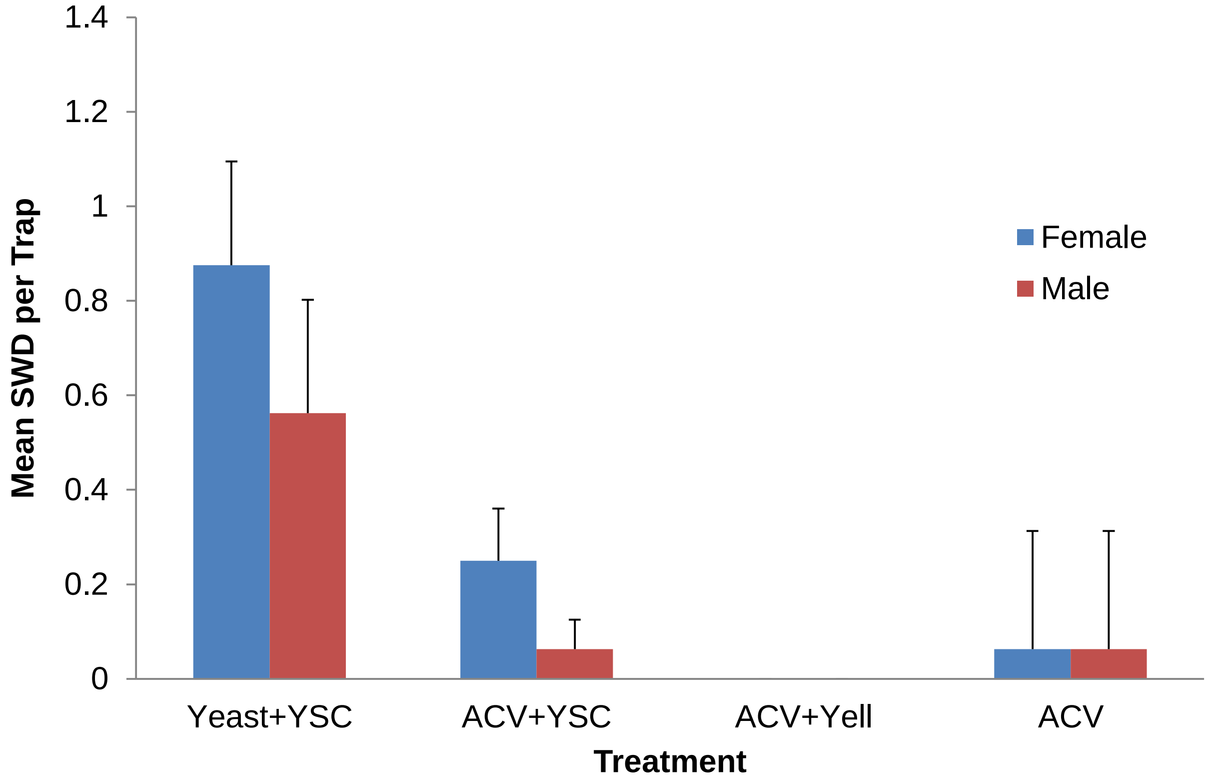

Figure 5-7: Mean male and female spotted wing drosophila (SWD) captured per trap in the 2013 trapping study, experiment 3. Treatments included the basic cup trap with a yellow sticky card baited with a yeast-sugar-water mixture (yeast+YSC), cup trap with a yellow sticky card with apple cider vinegar bait (ACV+YSC), cup trap with a yellow band with ACV and odorless dish soap (ACV+yell), and a basic cup trap with ACV and odorless dish soap (ACV). Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

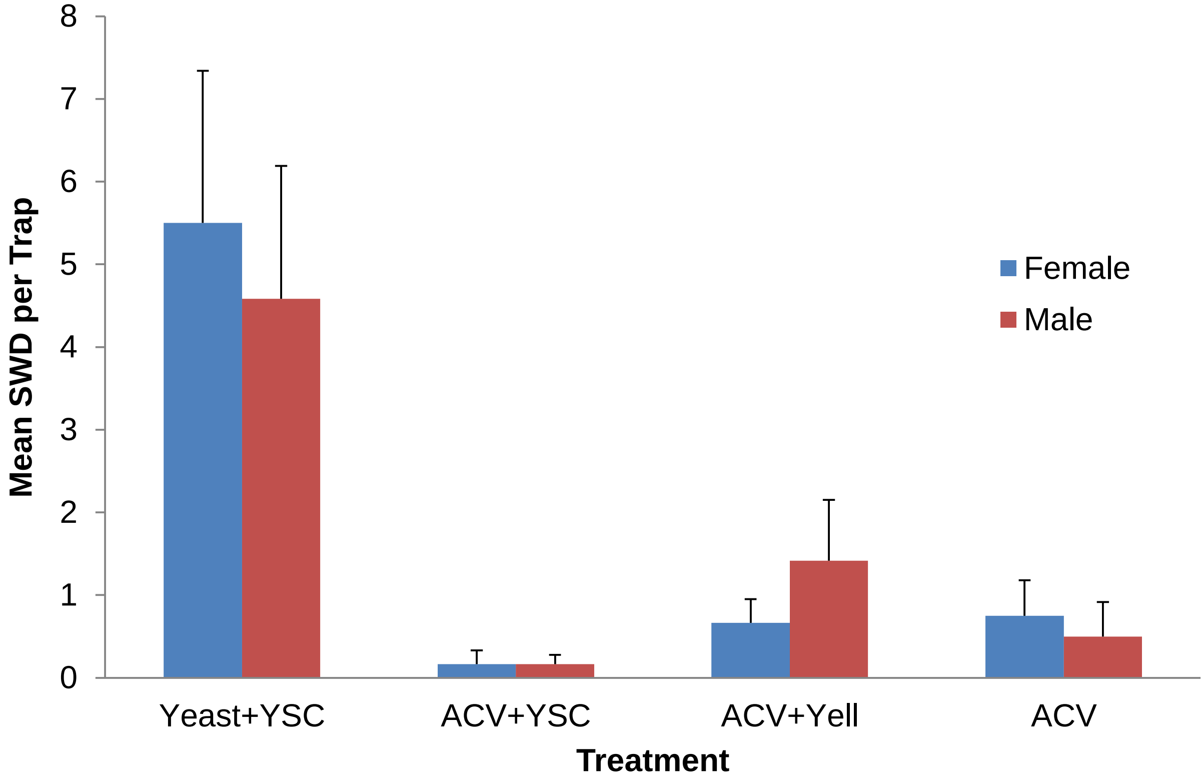

Figure 5-8: Mean male and female spotted wing drosophila (SWD) captured per trap in the 2013 trapping study, experiment 4. Treatments included the basic cup trap with a yellow sticky card baited with a yeast-sugar-water mixture (yeast+YSC), cup trap with a yellow sticky card with apple cider vinegar bait (ACV+YSC), cup trap with a yellow band with ACV and odorless dish soap (ACV+yell), and a basic cup trap with ACV and odorless dish soap (ACV). Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

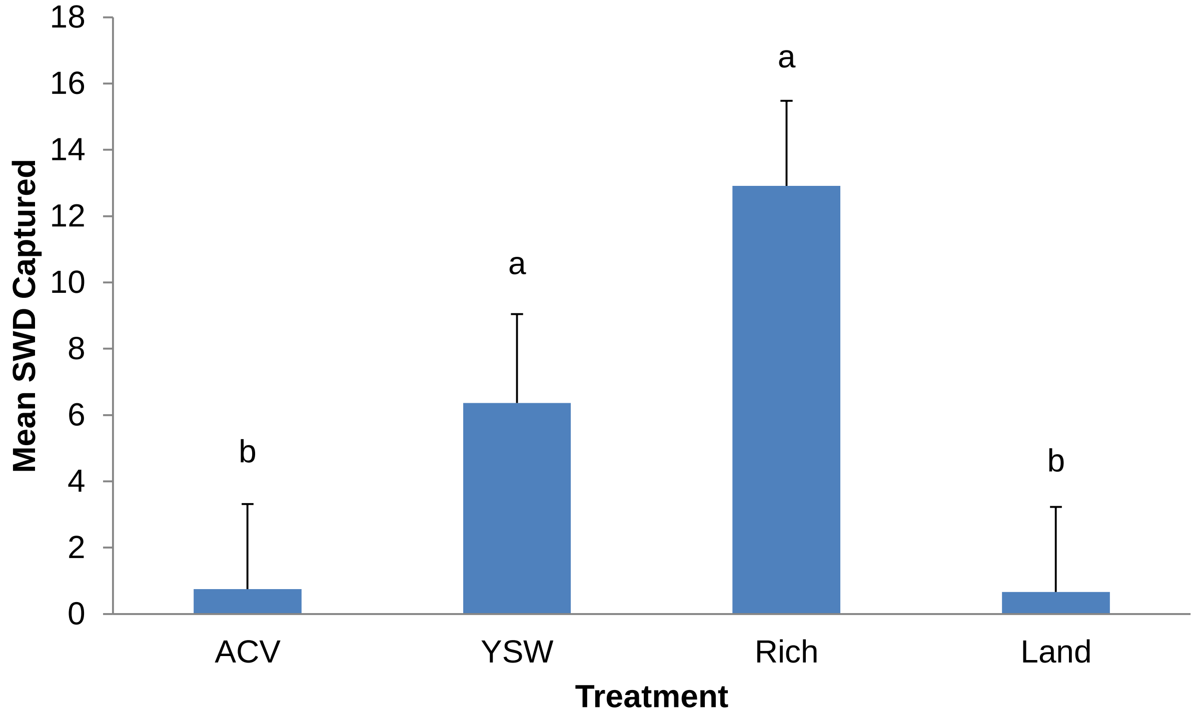

Figure 5-9: Mean spotted wing drosophila (SWD) captured during 2013 bait study. All treatments used the basic cup trap design. Treatments included apple cider vinegar (ACV), yeast-sugar-water mixture (YSW), Rich's Mix (Rich), and Landolt's Mix (Land). Bars with the same letters are not significantly different at $P \leq 0.05$.

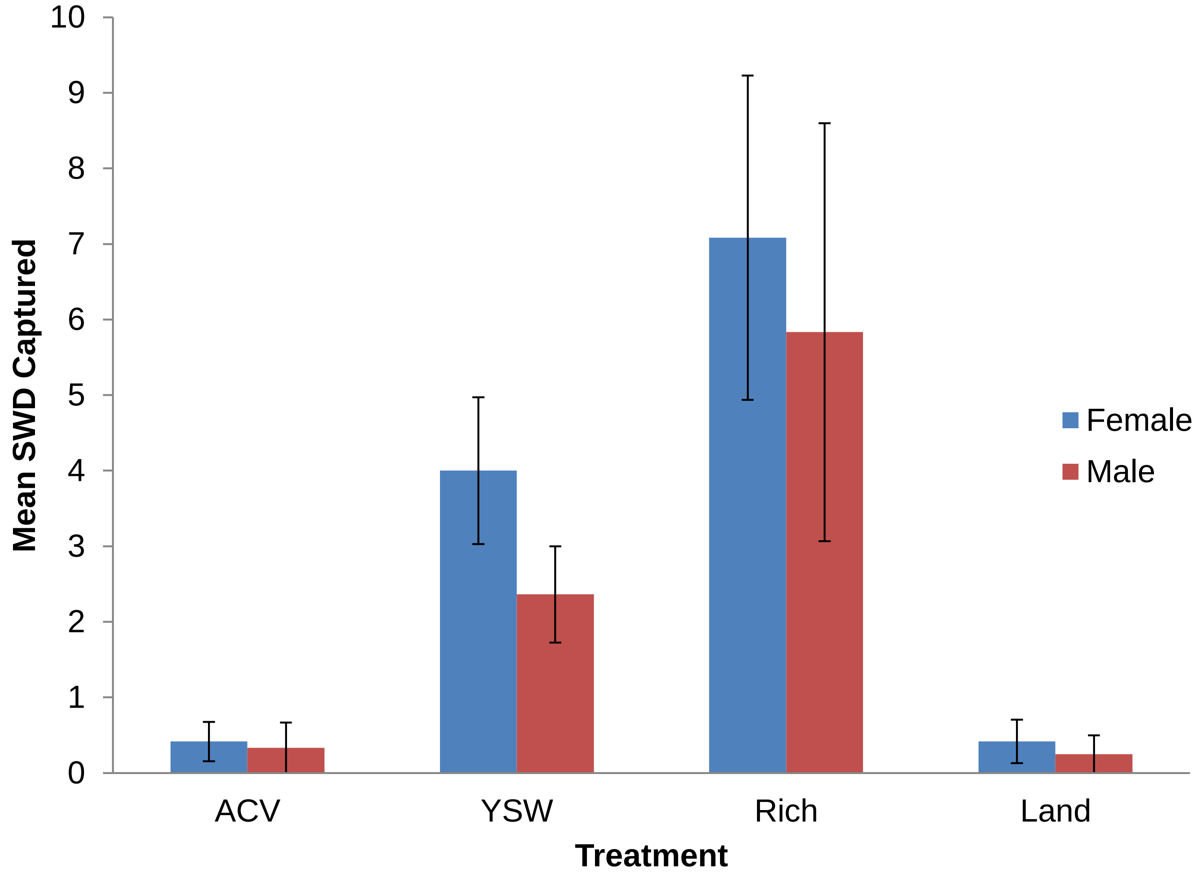

Figure 5-10: Mean female and male spotted wing drosophila (SWD) captured during 2013 bait study. Treatments included apple cider vinegar (ACV), yeast-sugar-water mixture (YSW), Rich's Mix (Rich), and Landolt's Mix (Land). Asterisk (*) indicates significant differences at $P \leq 0.05$ among female and male SWD captured.

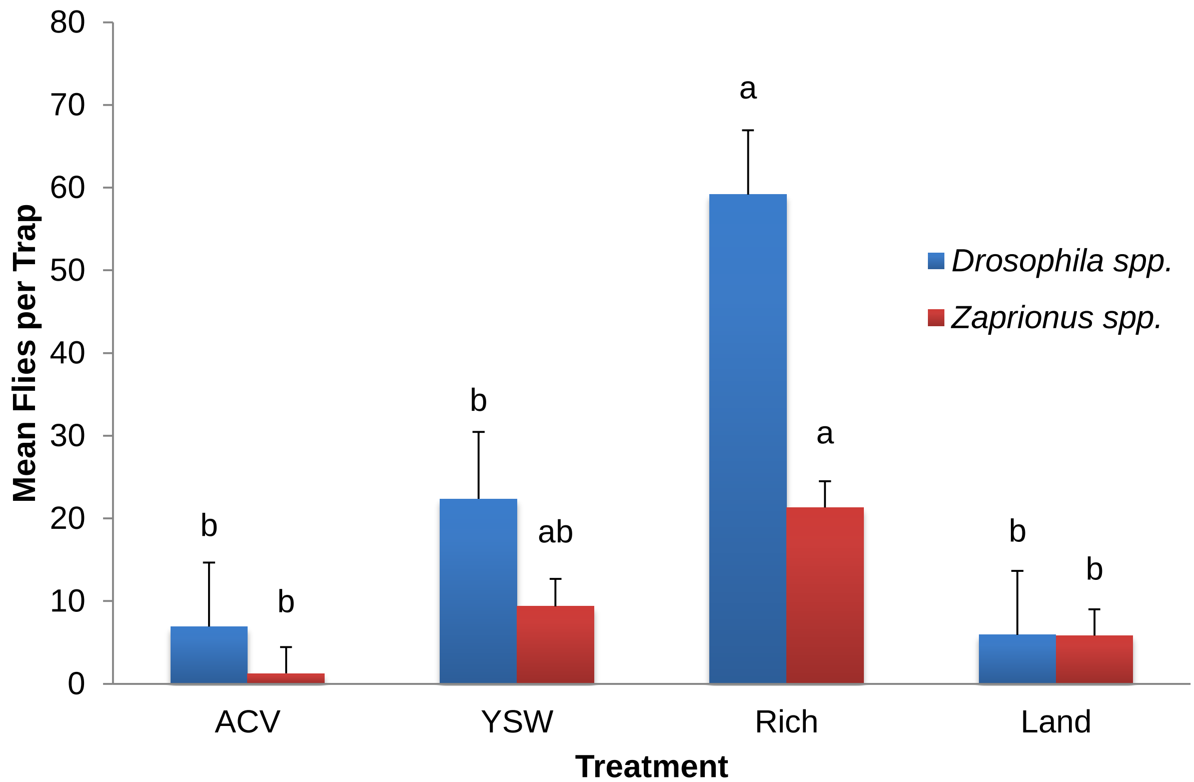

Figure 5-11: Mean *Drosophila* spp. and *Zaprionus* spp. captured per trap in the 2013 bait study. Treatments included apple cider vinegar (ACV), yeast-sugar-water mixture (YSW), Rich's Mix (Rich), and Landolt's Mix (Land). Bars with the same letters are not significantly different among treatments at $P \leq 0.05$.

CHAPTER 6
EVALUATING ALTERNATIVE CHEMICAL TOOLS FOR CONTROL OF SPOTTED WING DROSOPHILA

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), causes direct injury to blueberry plants by scarring the berry upon insertion of the ovipositor and by the rapid deterioration of berries as a result of larval development. Yield losses in Florida blueberries in 2012 due to SWD injury have been estimated at 7.8 to 11.7 mil USD (eFly 2012). Therefore, developing an effective management program is essential for growers to protect their crops and their livelihoods.

An integrated pest management (IPM) program is comprised of a number of strategies and techniques based on knowledge of the pest, specific site characteristics, and market values. Currently Florida blueberry growers have several cultural control techniques available for SWD management. Sanitation by removing fallen, damaged or overripe berries from the fields and destroying them reduces areas for SWD reproduction. Short harvest intervals help to keep ripe fruit off of the bushes during the time of the season when SWD populations are likely to increase. In addition, exclusion netting with mesh size less than 0.98 mm can prevent SWD entry by 100 percent (Kawase and Uchino 2005). However, cultural controls have their limitations as they can be costly and timely for large blueberry operations and are most effective when coupled with chemical control strategies.

Chemical controls that target the adult stage of SWD are currently the most effective technique for controlling SWD populations. Conventional growers most commonly use Delegate® 25 WG (spinetoram, Dow AgroSciences LLC, Indianapolis, IN), Mustang Max® (zeta-cypermethrin, FMC Corp, Philadelphia, PA), and Malathion® 8 EC (malathion, Arysta LifeScience North America, LLC, Cary, NC) (Liburd and

Iglesias 2013). A direct spray of all these chemicals in laboratory tests resulted in 100 percent mortality of adult SWD (Bruck et al. 2011). However, some flies will not be in contact with a direct spray in the field. Tests on residual activity showed that Mustang Max® provided 100 percent adult mortality after 10 d, Malation® provided significant adult mortality up to 7 d, and Delegate® provided control up to 3 d (Bruck et al. 2011).

A chemical toolbox full of available alternatives will give a grower the flexibility to choose the best option for their operation by taking into consideration chemical cost, the chemical class to prevent resistance by rotating chemicals, reentry and pre-harvest intervals and worker, consumer, and environmental safety. The purpose of this study was to provide growers with additional tools to use for SWD control. To do this we conducted a field-based laboratory bioassay to evaluate the residual effects of various chemicals on 1) adult SWD mortality as a result of contact on blueberry branches and 2) larval survival to the next generation in blueberries.

Materials and Methods

A field-based laboratory bioassay was conducted during the spring 2012. Various insecticides from different classes were applied at manufacturer's recommended rate to blueberries in the field. Blueberry branches with fruit were transported back to the laboratory. Spotted wing drosophila adults were exposed to treated branches in bioassays chambers and adult mortality and larval emergence were recorded.

Source of Flies

Drosophila suzukii flies were obtained from a laboratory colony reared in an environmental chamber (Model I36VL, Percival Scientific, Inc., Perry, IA) at the University of Florida, Small Fruit and Vegetable IPM (SFVIPM) laboratory in Gainesville, Florida. Environmental conditions were maintained at 23°C with 16:8 h light:dark cycle and RH ~65%. Flies were reared on Formula 4-24® instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC) in 0.25-L polypropylene bottles (Applied Scientific, Kalamazoo, MI) with foam stoppers (Jaece, North Tonawanda, NY). Spotted wing drosophila used in both experiments were 4- to 7-d old. Flies were transferred from culture bottles using an air pump into plastic vials. Flies were immobilized by placing the plastic vials in the freezer for 90 s and then introduced into the bioassay chambers.

Field Setup

The experimental field plot was located at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, Florida. The experimental area was 62.2 by 59.4 m with four blocks, blocked by surrounding vegetation. Each block consisted of 8 rows 27.4 m long and 1 m wide. A 1.9 m wide grass buffer zone was established between each row, a 7.32 m buffer between the north and south blocks, and 13.72 m buffer between the east and west blocks. Each row had 25 bushes planted 1 m apart. Each row was made up of 5 different varieties of southern highbush (SHB) blueberries each with 5 bushes. Bushes were approximately 4 to 6 years old and 1 m tall. Pine bark was used as mulch for blueberry rows. Blueberries were watered daily with drip irrigation, and no other chemicals had been used in the plantings for pest management.

The experiment was conducted in two phases between 10 April and 23 May 2012. The experimental field design was a randomized complete block with four replicates and seven treatments. Treatments were formulated insecticide products registered for use on small fruits Florida (Table 6-1). Treatments were applied using a back-pack sprayer.

All treatments were randomly assigned to a row in each block. There was an additional buffer row with no treatment between the control and the other treatments. The additional buffer row was included adjacent to the control to prevent any risk of spray drift between treatment rows and the control row.

Prior to the start of laboratory bioassays, larval tests were performed to ensure that berries were free from infestation (Hueppelsheuser 2010). First, larval salt tests were completed by randomly collecting at least 30 ripe berries from each block and placing them in a resealable plastic bag. The berries were lightly crushed in the bags and some salt solution (59 mL salt to 0.95 L water) was added. The fruit were allowed to sink to the bottom of the bag for approximately 10 to15 min. No larvae floated to the top of the solution and were therefore considered uninfested by the salt test.

Laboratory Setup

One day after insecticide application, two branches were selected from blueberry varieties (Emerald and Jewel) in each treatment row, placed in resealable plastic bags in an ice cooler, and transported back to the SFVIPM laboratory in Gainesville, Florida. Branches were selected that had stems of at least 7.6 cm long and at least five berries at different ripening stages. One branch from each variety was placed into a bioassay chamber for males and the other two branches into a chamber for females. This procedure was repeated for all treatments 3, 7, and 14 d after insecticide application.

Bioassay chamber consisted of 1-L transparent plastic deli container with a mesh lid (Figure 6-1). Each treatment container had a 35-mL plastic vial filled with tap water in which a foam stopper and two branches were placed. The vial was secured in a 30-

mL container to prevent movement within the chamber and risk killing flies. A 30-mL container with a cotton wick was filled with 1 M of sugar water placed in the treatment container to feed the flies. Bioassay chambers were placed on the laboratory bench in a completely randomized design under grow lights with a 16:8 h light:dark cycle at a mean temperature of 22.8°C.

Once the branches were secured in the bioassay chamber 10 mated females and 10 males were introduced into separate containers following freezing procedures detailed above. Flies remained in the chambers for 72 h.

Data Collection and Analysis

Mean daily temperature (°C), relative humidity (%), and total rainfall (cm) were collected using FAWN (Florida Automated Weather Network, Gainesville, FL) for the duration of the study (Figures 6-2, 6-3).

Adult activity

Adult activity measurements were recorded 72 h after the flies were introduced into the bioassay containers, just prior to removal. Data were taken by picking up the container and gently tapping the sides to elicit an activity response from the flies. Fly activity was measured on a scale of 0 to 3, using methods described in Liburd et al. (2003). A score of 3 indicated unaltered fly activity (fly in its natural state). A score of 2 indicated decreased responsiveness to tapping. A score of 1 indicated no responsiveness to tapping and a general inverted, twitching appearance. Fly death was designated a score of 0. Flies that died unrelated to the insecticides such as drowning in sugar solution, vial movement, or berry drop, were omitted from analysis. Containers were observed for 5 min.

The total number of flies in each activity category (0 through 3) was weighted based on their categorical number. The weighted total was then averaged by the number of responding flies in each container (total flies minus omitted flies). Data were square root transformed to standardize the variances. Means were separated using analysis of variance (ANOVA) and Dunnet's Method (JMP, SAS Institute 2013). Differences were considered significant when $P \leq 0.05$.

Larval survival (emergence)

Larval survival (emergence) was measured by counting the number of flies that emerged from the berries in each chamber containing female flies. Emergence was recorded 48 h after the first fly emerged in each container. Therefore, the start of the 48 h emergence period differed slightly for each container. The total number of emerged flies was averaged by the number of berries in each container. Data were square root transformed to account for unequal variances. Analysis of variance (ANOVA) was used to compare emerged flies per berry and means were separated using a Tukey's HSD (JMP, SAS Institute 2013). Differences were considered significant when $P \leq 0.05$.

Results

Adult Activity

There was a significant treatment effect on SWD activity on 1 d ($F = 8.14$, df = 6, 47; $P < 0.0001$), 3 d ($F = 2.21$; df = 6, 47; $P = 0.05$), and 7 d post-treatment ($F = 2.49$; df = 6, 47; $P = 0.04$). On 1 d, Delegate®, Mustang Max®, Danitol® high, and Danitol® low significantly reduced SWD activity level below the control whereas no significant differences were found between Belay® high, Belay® low and the control. On 3 d post-treatment, Danitol® high reduced activity more than Belay® low and the control. Additionally, Danitol® high, Danitol® low, and Mustang Max® significantly reduced

SWD activity below the control. Treatment effects were significant whereas gender effects were not (F = 1.61; df = 1, 47; P = 0.21) on 7 d post-treatment. Both Danitol® treatments reduced activity significantly more than Delegate®, Belay® low and the control. On 14 d post-treatment, neither gender (F = 1.28; df = 1, 47; P = 0.26) nor treatment effects (F = 0.89; df = 6, 47; P = 0.51) were significant.

When evaluating means among days within treatments neither Belay® treatments showed any significant differences. Both Danitol® treatments reduced activity on 1 d and 3 d more than 14 d post-treatment whereas 1 d was lower than 3 d post treatment (Figure 6-4). Mustang Max® and Delegate® reduced activity on 1 d post-treatment more than all other days. Only Danitol® low had significant treatment differences between gender (F = 12.86; df = 1, 24; P = 0.0015); activity was reduced more for males than it was for females. On 1 d post-treatment, Danitol® low, Danitol® high, and Mustang Max® reduced male activity greater than female activity. On 3 d post-treatment, no significant differences were found. Danitol® low reduced male activity significantly greater than female activity on 7 d post-treatment. Belay® high showed a significant reduction in male activity on 14 d post-treatment.

Larval Survival

On 1 d post-treatment, all treatments had lower number of emerged flies per berry than did the control (F = 3.47; df = 6, 20; P = 0.02). There were no significant differences between treatments and the control on 3 d (F = 0.57; df = 6, 21; P = 0.75), 7 d (F = 1.38; df = 6, 21; P = 0.27), or 14 d post-treatment (F = 1.29; df = 6, 21; P = 0.31).

Discussion

Our study identified effective insecticides for SWD management. Results indicate that Mustang Max®, Delegate®, and Danitol® high and low rates were effective at reducing SWD activity up to 3 d residual. After 7 d residual, Danitol® at the high and low rates was more effective than Mustang Max® and Delgate®. There were slight differences between the effect of the chemicals on female and male activity. On 1 d post-treatment, Danitol® at both rates, Mustang Max®, and Delegate® reduced male more than female activity and on 7 d post-treatment only Danitol® at the low rate reduced male more than female activity. The efficacy of Danitol® in our study supports recent findings (Beers, et al. 2011, Bruck et al. 2011) that Danitol® exhibits residual control up to 7 d. In addition, no significant differences were found in SWD activity between the high and low rates of Danitol® suggesting that the low rate would be an effective alternative tool for controlling SWD in the field.

Danitol® is a pyrethroid in class 3A like Mustang Max®, which is one of the most commonly used insecticides against SWD. Growers are recommended to rotate chemical application by chemical class to prevent pest resistance to effective chemicals. Pesticide resistance occurs when resistant individuals survive and produce resistant offspring. Rotating to a different chemical class will help to ensure no resistant individuals in the population survive to carry on the resistant gene. Although Danitol® and Mustang Max® are in the same chemical class, they differ in the preferred timing of application within the season due to their preharvest intervals (PHI). Mustang Max® has a PHI of only 1 d, making it attractive to growers during peak harvest when harvest intervals are 3 to 4 d. In contrast, Danitol® has a 3 d PHI and would be most effective during early season before harvest when SWD populations are beginning to increase.

Delegate® and Belay® are labeled as reduced-risk chemicals by the EPA because they have lower toxicity to non-target organisms, short residual activity, and are slower acting. Delegate® has been shown to be effective at reducing SWD populations up to 3 d on treated cherry leaves (Beers et al. 2011) and up to 7 d on blueberries (Bruck et al. 2011). Delegate® also has a 3 d PHI which may limit its use during peak harvest. Belay® is a class 4A insecticide, a neonicotinoid. Neonicotinoids are systemic synthetic chemicals based on the chemical nicotine and are absorbed into the insect's body by consumption. Neonicotinoids mimic acetylcholinesterase, the enzyme that breaks down acetylcholine, blocking the binding cites and allowing acetylcholine to build up in the insect's body, leading to twitching and death. Though clothianidin [Belay®] has not been evaluated on SWD prior to our study, other neonicotinoids have shown to be less effective than pyrethroids [Danitol® and Mustang Max®], organophosphates [Malathion®], or spinosyns [Delagate®] by knockdown (Bruck et al. 2011) and by residual exposure (Beers et al. 2011).

Overall, Mustang Max® and Delegate® remain effective tools for control of SWD up to 3 d in the field. Previous studies suggest their effectiveness up to 7 d (Beers et al. 2011, Bruck et al. 2011). Mustang Max® is suggested for use during peak harvest as it has a 1 d PHI. In addition, Danitol® at the low rate of 0.75 L ha^{-1} is an effective tool for control during the early season due to its 3 d PHI. Belay® appears to be an ineffective tool for managing SWD. Future studies may investigate more effective methods of application such as chemigation (the application of pesticides through overhead irrigation systems), aerial applications or applications baited with a sweet lure such as sugar.

Table 6-1. Insecticide treatments for efficacy study 2012.

Trade Name	Treatment Code	Chemical	Manufacturer	AI Rate (h^{-1})
Belay® Insecticide	Bel lo	clothianidin	Valent U.S.A Corporation, Walnut Creek, CA	0.29 L
Belay® Insecticide	Bel hi	clothianidin	Valent U.S.A Corporation, Walnut Creek, CA	0.44 L
Danitol® 2.4 EC	Dan lo	fenpropathrin	Valent U.S.A Corporation, Walnut Creek, CA	0.75 L
Danitol® 2.4 EC	Dan hi	fenpropathrin	Valent U.S.A Corporation, Walnut Creek, CA	1.17 L
Mustang Max®	Mus	zeta-cyperpethrin	FMC Corp., Philadelphia, PA	0.29 L
Delegate® 25 WG	Del	spinetoram	Dow AgroSciences LLC, Indianapolis, IN	0.42 g
Control	Cont	-	-	-

Table 6-2. Mean activity level per fly between treatments within each day after application.

	Bel4	Bel6	Dan10.3	Dan16	Mus4	Del6	Cont
Day 1	2.5 ± 0.2	2.2 ± 0.2	1.2 ± 0.2*	1.2 ± 0.2*	1.0 ± 0.2*	0.9 ± 0.2*	2.7 ± 0.2
Day 3	2.5 ± 0.2	2.3 ± 0.2	1.9 ± 0.2*	1.8 ± 0.2*	2.1 ± 0.2*	2.1 ± 0.2*	2.6 ± 0.2
Day 7	2.7 ± 0.2	2.6 ± 0.2	2.2 ± 0.2*	2.2 ± 0.2*	2.5 ± 0.2	2.6 ± 0.2	2.8 ± 0.2
Day 14	2.7 ± 0.2	2.4 ± 0.2*†	2.7 ± 0.2	2.6 ± 0.2	2.3 ± 0.2	2.6 ± 0.2	2.9 ± 0.2

Asterisk (*) indicates significant differences when compared to the control at $P < 0.05$.

†Low activity due to replicate losing all male flies to the freezing process.

Table 6-3. Mean activity level per fly for female SWD between treatments within each day after application.

Trt	Bel4	Bel6	Dan10.3	Dan16	Mus4	Del6	Cont
Day 1	2.6 ± 0.1	2.4 ± 0.0	1.8 ± 0.1*	1.7 ± 0.1*	1.7 ± 0.1*	1.0 ± 0.4*	2.7 ± 0.1
Day 3	2.4 ± 0.1	2.5 ± 0.1	2.2 ± 0.1*	2.2 ± 0.1*	2.0 ± 0.2*	2.4 ± 0.1	2.8 ± 0.1
Day 7	2.7 ± 0.0	2.7 ± 0.1	2.5 ± 0.1*	2.3 ± 0.1*	2.6 ± 0.1	2.5 ± 0.0*	2.9 ± 0.0
Day 14	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.0	2.9 ± 0.0	2.6 ± 0.1*	2.6 ± 0.0	2.9 ± 0.1

Asterisk (*) indicates significant differences when compared to the control at $P < 0.05$.

Table 6-4: Mean activity level per fly for male SWD between treatments within each day post-treatment.

Trt	Bel4	Bel6	Dan10.3	Dan16	Mus4	Del6	Cont
Day 1	2.6 ± 0.1	2.3 ± 0.1	1.0 ± 0.2*	1.0 ± 0.2*	0.8 ± 0.2*	1.5 ± 0.2*	2.9 ± 0.1
Day 3	2.6 ± 0.1	2.4 ± 0.1	2.3 ± 0.2*	2.0 ± 0.1*	2.5 ± 0.1	2.1 ± 0.2*	2.8 ± 0.1
Day 7	2.7 ± 0.1	2.6 ± 0.1	2.0 ± 0.2*	2.3 ± 0.2	2.4 ± 0.1	2.8 ± 0.1	2.7 ± 0.1
Day 14	2.8 ± 0.1	1.4 ± 0.4*[†]	2.7 ± 0.1	2.6 ± 0.1	2.4 ± 0.2	2.7 ± 0.1	3.0 ± 0.0

Asterisk (*) indicates significant differences when compared to the control at $P < 0.05$.
[†]Low activity due to replicate losing all male flies to the freezing process.

Table 6-5: Mean emergence per berry.

Trt	Bel4	Bel6	Dan10.3	Dan16	Mus4	Del6	Cont
Day 1	0.4 ± 0.3b	0.2 ± 0.3b	0.1 ± 0.3b	0.0 ± 0.3b	0.2 ± 0.3b	0.6 ± 0.3b	1.4 ± 0.3a
Day 3	0.5 ± 0.2a	0.4 ± 0.2a	0.3 ± 0.2a	0.2 ± 0.2a	0.2 ± 0.2a	0.5 ± 0.2a	0.3 ± 0.2a
Day 7	2.3 ± 0.5a	1.0 ± 0.5ab	0.4 ± 0.5b	0.5 ± 0.5b	0.8 ± 0.5ab	0.9 ± 0.5ab	1.2 ± 0.5ab
Day 14	2.9 ± 0.9a	1.1 ± 0.9a	0.4 ± 0.9a	1.2 ± 0.9a	0.7 ± 0.9a	1.5 ± 0.9a	2.5 ± 0.9a

Asterisk (*) indicates significant differences when compared to the control at $P < 0.05$.

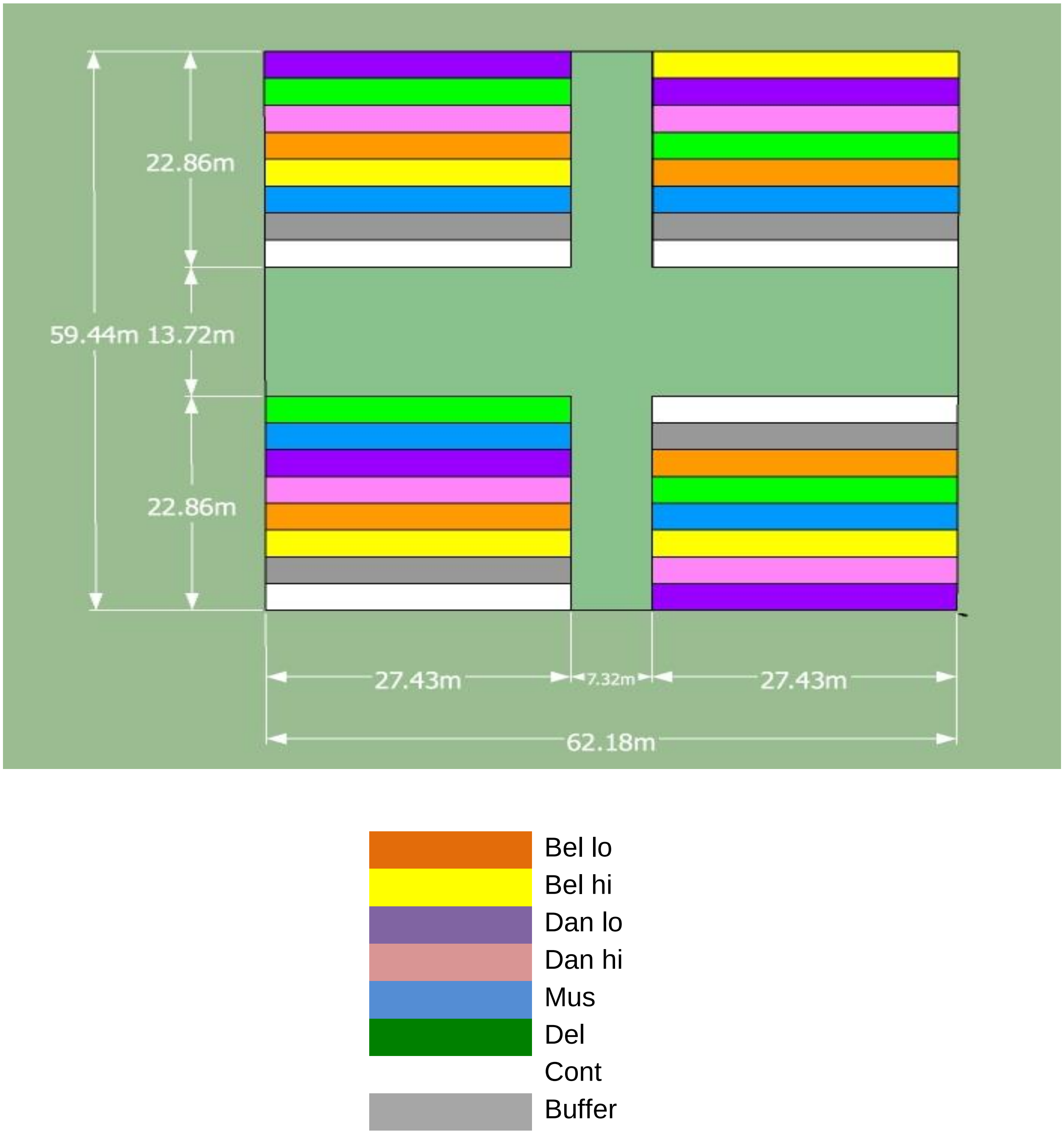

Figure 6-1: Field setup at UF-IFAS PSREU in Citra, Florida

Figure 6-2. Bioassay chamber for efficacy study. Photo courtesy of L. E. Iglesias.

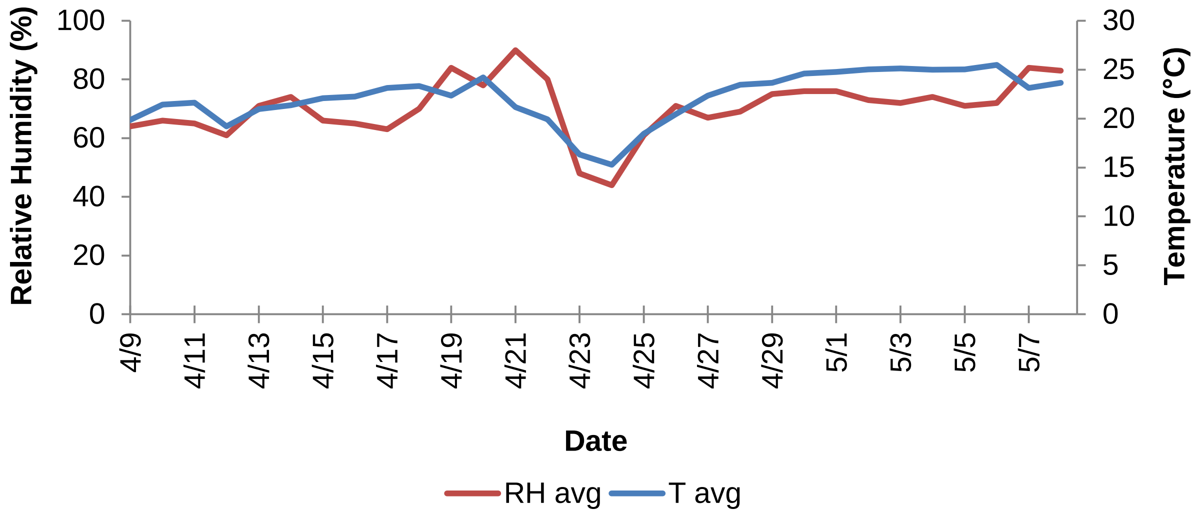

Figure 6-3: Daily mean relative humidity (%) and temperature (°C) at the UF-IFAS PSREU in Citra, Florida for during of pesticide efficacy study 2012.

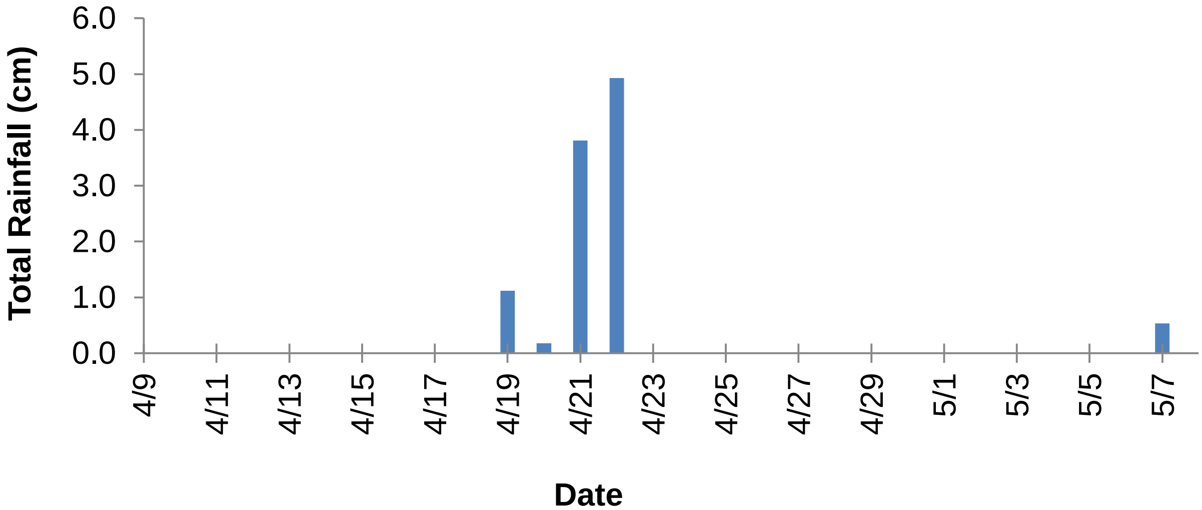

Figure 6-4: Total daily rainfall (cm) at the UF-IFAS PSREU in Citra, Florida for duration of pesticide efficacy study 2012.

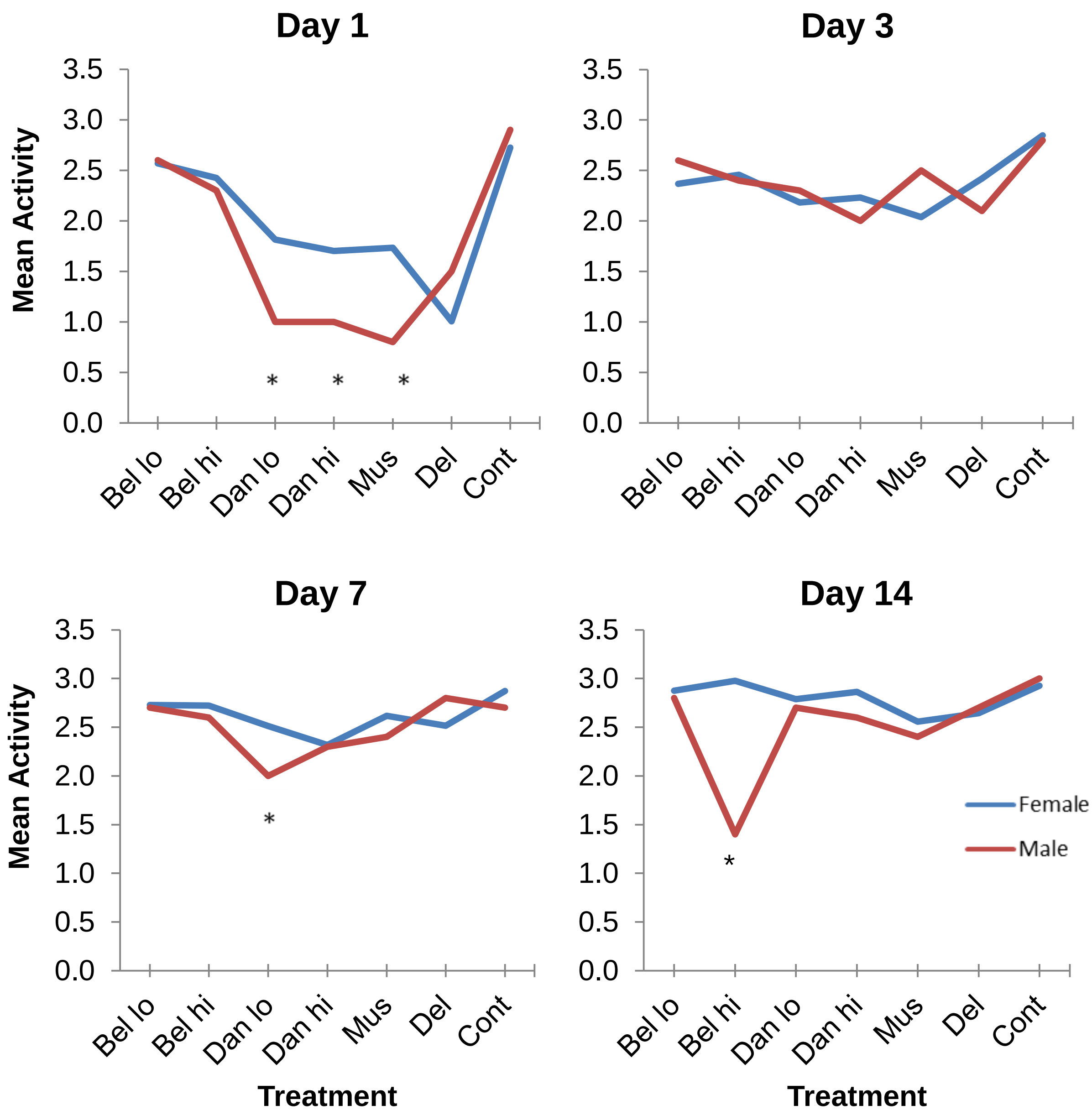

Figure 6-5: Average Female and Male Activity per Day by Treatment. Asterisks (*) indicate significant differences at $P < 0.05$.

CHAPTER 7
CONCLUSION

This study confirms the establishment of spotted wing drosophila (SWD) *Drosophila suzukii* (Matsumura), in Florida blueberries and emphasizes the importance of implementing effective monitoring programs to prevent economic loss. Spotted wing drosophila was found in 8 of 9 major blueberry-producing counties in Florida in a 2012 and 2013 survey, from Suwannee County in the north to Polk County in the south. Spotted wing drosophila was not detected in DeSoto County suggesting that high daily temperatures early in the season many play a role in preventing establishment of populations in more southern areas. Citrus County, which consists of a large number of organic blueberries and farms that produce other multiple SWD host crops, had the highest SWD captured per trap in 2012 (4.81 ± 0.31). Marion County (1.44 ± 0.37) which also grows multiple crops and has organic blueberries, and Alachua County (1.34 ± 0.20) which uses high tunnel production systems, captured significantly higher numbers of SWD than Orange (0.53 ± 0.17) and Suwannee counties (0.05 ± 0.49) in 2013. These results suggest that locations with limited chemical tools available for SWD control, produce multiple host crops in succession, or employ high tunnel systems that support a longer growing season may be at a higher risk of SWD infestation, but additional research is needed to test these conclusions.

The study confirmed that SWD will oviposit in both species of blueberries grown in Florida, the southern highbush (SHB) and rabbiteye (RE). The mean number of SWD that emerged from SHB was two times greater than that of RE and could be considered biologically significant. The findings indicate that the characteristics of RE blueberry that make it different from SHB (grittier texture, firmer skin, and larger seeds) may play a role in host suitability. However, more research on oviposition preferences and host is needed before firm conclusions are drawn. In addition, SWD prefer to oviposit on ripe blue fruit 50% of the time, 6% on pink, 38% on green-pink, and 6% on full green fruit. These data suggest that monitoring should begin much earlier in the season when berries are in the green-pink stage.

Trapping SWD with clear plastic cup traps baited with apple cider vinegar (ACV) performed was better more effective than a yellow sticky card baited with a vial of ACV. Modifications of the cup trap baited with ACV included the addition of a yellow band, yellow sticky card hanging inside of the cup, or dish detergent in the bait. These trap modifications did not appear to significantly increase trap captures. When the modified cup traps were compared with the standard plastic cup trap with a yellow sticky card inside baited with a yeast-sugar-water mixture, the yeast-baited trap captured significantly more SWD than the ACV cup traps. This suggests that the bait contributed more to the capture of SWD than the design of the trap itself.

The results of the bait study showed that the yeast baits (Rich's and the yeast-sugar-water mixes) were significantly more attractive to SWD than the vinegar baits (ACV and Landolt's mix) when used in blueberry fields. This attraction may be a result of adults and larvae of SWD and other drosophilids feeding upon the associated yeasts and bacteria as well as the fruit material from damaged or fallen berries (Markow and O'Grady 2008, Hamby et al. 2012). In addition, odors of fermenting fruit baits such as wine or vinegar may be masked by the odors emitted from the surrounding fruit. However, effective baits are also dependent upon ease of handling and SWD identification. Vinegar or wine baits are generally tinted but clear for easy identification in the field or laboratory, have longevity in the field, and act as decent preservatives for collected specimens. The yeast-sugar-water mixtures are cloudy and have sediments that make identification difficult and timely. Additionally, the yeast baits were especially attractive to other *Drosophila* spp. and with high numbers of non-target species SWD identification can be difficult and timely. When planning a monitoring program for SWD, growers must consider whether they value a highly attractive bait such as a yeast-sugar-water mixture or Rich's mix, or a bait that will make handling and identification quick and easy such as ACV or Landolt's mix.

Mustang Max® and Delegate® remain effective tools for control of SWD up to 3 d in the field. Previous studies suggest their effectiveness up to 7 d (Beers et al. 2011, Bruck et al. 2011). Mustang Max® is suggested for use during peak harvest due to its 1 d PHI. In addition, Danitol® at the low rate of 0.75 L ha^{-1} is an effective tool for SWD control and is recommended for use during the early season due to its 3 d PHI. Belay® appears to be an ineffective tool for managing SWD. Future studies should investigate the mechanisms behind the ineffectiveness of neonicotinoids on SWD as well as more effective methods of application such as chemigation (the application of pesticides through overhead irrigation systems), aerial applications or applications baited with a sweet lures such as sugar.

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BIOGRAPHICAL SKETCH

Lindsy Iglesias is a master's student in the Interdisciplinary Ecology program with a concentration in entomology at the University of Florida. She graduated with her B.S. in Environmental Science with a minor in Sustainability Studies from the University of Florida in 2010. Her undergraduate career also included 2 years of study in architecture at the University of South Florida where she focused on green building design. She spent the summer of 2010 interning at the Bioenergy Research and Education unit at the University of Florida conducting research on the bioremediation of human waste with algae to produce non-potable water and fertilizer. She began her master's program with Oscar Liburd in December of 2011 at the Small Fruit and Vegetable IPM Laboratory at the University of Florida. Her program began at a critical time when an invasive pest was causing major losses in small fruits. After she receives her master's degree she will continue towards her Ph.D. with Dr. Liburd researching monitoring methods for management of SWD to help protect the blueberry industry and the livelihoods of its growers.