ABIOTIC AND LANDSCAPE FACTORS THAT AFFECT SPOTTED-WING-DROSOPHILA (*DROSOPHILA SUZUKII*) POPULATIONS IN OKLAHOMA

By

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ABIOTIC AND LANDSCAPE FACTORS THAT AFFECT SPOTTED-WING-DROSOPHILA (*DROSOPHILA SUZUKII*) POPULATIONS IN OKLAHOMA

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Spotted wing drosophila (SWD), *Drosophila suzukii*, is a species of fruit fly native to East Asia that has become a serious invasive pest around the world. SWD females possess a large sclerotized ovipositor that can puncture ripe or ripening fruit, on which eggs are laid, resulting in significant damage. SWD was first detected in the continental U.S. in 2008 in California and has since spread to almost every state. Crop damage caused by SWD in the western U.S. alone is estimated to be nearly $500 million annually (Cuthbertson et al. 2014). SWD is thus considered a serious economic threat to U.S. fruit production and research into SWD ecology and life history is needed to improve population management. In 2013, SWD was first recorded in Oklahoma in Tulsa County, threatening the state's soft-fruit production (Lee 2014). SWD population monitoring was conducted using deli cup traps during the 2015 and 2016 blackberry growing season to determine population trends. Abiotic and biotic factors such as temperature, humidity, and habitat types were compared to SWD trap counts. To determine vegetation preference by SWD, traps were deployed in two different habitat types, tree lines or cropland, at each site. Our results showed that decreases in humidity negatively affect SWD populations and SWD occurs in higher numbers in tree lines than adjacent blackberry cropland. To determine alternative host plants of SWD, soft fruits from multiple plants were collected at the field sites, adult SWD allowed to emerged in the lab. Wild blackberry, pokeweed, and red mulberry were found to be hosts. SWD collected from deli traps in the field were also used for a separate genetic study using microsatellite markers to determine where Oklahoma populations originated from. The study determined multiple, genetically variable populations of SWD have been introduced into Oklahoma. The insight gained from this research will aid Oklahoma soft fruit producers in incorporating more effective tree line trapping and seasonal monitoring strategies into their integrated pest management plans to control SWD.

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CHAPTER I

INTRODUCTION

Information is needed about spotted-wing drosophila (SWD), *Drosophila suzukii*, populations in Oklahoma. No previous studies have been conducted in Oklahoma to determine what effects Oklahoma landscape and climate have on SWD populations. Small-scale blackberry and blueberry orchards can be found in the central and eastern part of the state. The presence of SWD threatens production of soft-fruit crops and minimizes profits for growers. Almost nothing is known about how SWD was introduced into the state and its biology and impact on production in Oklahoma blackberry orchards. This study was designed to provide information about SWD population history and biology for growers. They can use this information to build a solid foundation to manage this invasive pest more effectively in the future.

OBJECTIVES

Abiotic and Landscape Factors that Affect Spotted-wing Drosophila Populations

1) Determine where SWD trapping is most effective.
2) Determine if temperature and humidity are correlated with SWD abundance.
3) Determine which Oklahoma blackberry cultivars are susceptible to SWD infestation.
4) Determine if there are any native Oklahoma plant species serving as alternative hosts for SWD.
5) Determine if vegetation composition surrounding susceptible crops affects SWD abundance.

Invasion History of Spotted-wing Drosophila in Oklahoma via Genetic Analysis

1) Determine if there is genetic variability among various SWD populations in Oklahoma

2) Determine if SWD presence in Oklahoma is due to a single or multiple introductions

CHAPTER II

LITERATURE REVIEW

Family Drosophilidae

Spotted-wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is an invasive fruit fly from Southeast Asia (Emiljanowicz et al. 2014). SWD belongs to the subgenus *Sophophora*, which is divided into multiple species groups (Hahn 2007). *Drosophila* species vary widely in morphology, ecology, and behavior within the genus. They are a highly cosmopolitan taxa found in almost every continent and habitat type (Clark et al. 2007). Because of their abundance and relatively small genomes, *Drosophila* spp. have played a vital role in the study of animal genetics for more than a century (Chiu 2013). Even though the majority of the *Drosophila* genome has been sequenced (Signor 2013), the resulting genetic knowledge has not been applied to pest management because *Drosophila* spp. have not been considered to be agricultural pests (Hahn 2007). SWD is the outlier of this relatively harmless genus, costing fruit producers millions of dollars in damage each year (Asplen et al. 2015).

History of Invasion

Spotted wing drosophila is a native fruit fly of Southeast Asia that has a high capability for dispersal (Cini et al. 2012). Their successful dispersal has resulted in them being found in almost every continent of the world (Figure 1). The spread of this invasive fruit fly has been economically devastating to soft skin fruit growers all over the world. (Hauser 2011, Lee 2011, Cini et al. 2012, Asplen et al. 2015). Unlike other species of fruit flies, SWD requires pristinely ripe fruit for oviposition instead of rotting fruit (Cini et al. 2012. Stewart et al. 2014). This means SWD can compromise ripe, non-damaged, non-harvested and harvested products, unlike most fruit flies that are only a threat to damaged fruits that have fallen off the vine, tree, or bush.

This new fruit fly species was first observed damaging Japanese-grown cherries in 1916. By 1930, infestations were so severe that buyers began rejecting cherries because of damage (Lee et al. 2011a). According to Kanzawa (1939) SWD was first described as a species in Japan in 1931. It is possible that SWD is not native to Japan, but was introduced at the turn of the century from an unknown area of Asia (Asplen et al. 2015). SWD has also been identified in other Asian countries including China, Thailand, North and South Korea, Pakistan, Myanmar, Thailand, eastern Russia, and India. (Hauser 2011, Cini et al. 2012, Asplen et al. 2015).

Europe

The first occurrence of SWD in Europe was reported in 2008 in Rasquera, Spain, where 12 adults were collected (Lee et al. 2011b). Calabria et al. (2012) sampled for SWD at eight different locations in Europe over the course of three years. This study found that SWD was present in two locations in Spain, three locations in France, and two locations in Italy from 2007 and 2009. The only significant fruit damage from SWD was reported in France and Italy in 2010 (Hauser 2011; Lee et al. 2011b). Recent surveys have shown that the species has been recorded in most of the Mediterranean countries in Europe and has continued to spread north and east (Cini et al. 2012). According to Asplen (2015) SWD was recorded in southern England for the first time in 2012. The most recent European countries to detect SWD in fruit include Germany, Belgium, Austria, Switzerland, the Netherlands, Hungary, Poland, Greece, Croatia, and the Czech Republic (Asplen et al. 2015; Bjelis et al. 2015).

Hawaii and Central and South America

The Hawaiian Islands were the first documented area to host SWD outside of Asia. SWD was identified in Oahu in 1980 and subsequently dispersed to the other islands (Cini et al. 2012). Specimens were claimed to have been collected from Costa Rica and Ecuador in the late 1990's, but this claim has not been confirmed (Deprá 2014; Calabria et al. 2012). In 2014, SWD was detected in various locations in Brazil (Deprá 2014; Gabarra et al. 2014; Vilela and Mori 2014). Deprá et al. (2014) collected 156 adults in five different locations in southern Brazil, confirming that SWD is expanding its geographical range in South America.

North America and Continental USA

Initial detections of SWD in the continental United States were reported from strawberries and caneberries in California in 2008 (Hauser 2011, Lee et al. 2011a, Cini et al. 2012, Kinjo 2013). From 2009 to 2011, SWD was recorded in Oregon, Washington, Alberta, British Columbia, Manitoba, Ontario, Quebec, Utah, Michigan, Wisconsin, Louisiana, North Carolina, South Carolina, and Florida (Dreves 2011, Hauser 2011, Dean et al. 2013). Between 2012 and 2013, SWD was detected in Arkansas, Colorado, Idaho, Illinois, Indiana, Iowa, Kansas, Missouri, Nebraska, Oklahoma, South Dakota, Texas, and Wyoming (Asplen et al. 2015). Since 2008, SWD has been detected in almost every state.

Life Cycle of SWD

According to Lee et al. (2011b), females become sexually mature an average of 1 day and 23 hours after pupal emergence. Males then court females by fanning their wings and tapping their legs. The mating ritual and process can last from 2 minutes to 1 hour and 25 minutes. After mating, the female lays eggs inside ripe fruit, cutting into the skin with the heavily sclerotized, serrated ovipositor and placing eggs under the skin (Walsh 2010, Isaacs et al. 2010).

Each female can lay up to 100 eggs per day (Issacs et al. 2010), and on average, 563 eggs in a lifetime (Lee et al. 2011b). Females lay 1 to 3 eggs per fruit in as many as 16 individual fruits per day (Cini et al. 2012). Larvae hatch within 2 to 72 hours following oviposition. After feeding inside the fruit and completing three larval instars within 5 to 7 days, larvae pupate and emerge as adults within 3 to 15 days (Cini et al. 2012, Dreves et al. 2014).The entire life cycle from egg to adult can vary from as short as 10 days to as long as 79 days and is largely dependent on temperature (Asplen et al. 2015). Depending on temperature, *D. suzukii,* on average*,* can complete 10 generations per year (Caprile 2011).

Thermal Tolerance

The thermal range for *Drosophila* species is quite variable depending on location and type of habitat. Temperate species showed a higher tolerance for cold climate but were more sensitive to extreme heat than tropical species (David et al. 2005, Dalton et al. 2011). Males of *Drosophila* species have shown sterility in both extreme high and low temperatures (David et al. 2005. Dalton et al. 2011). However, the discovery of a summer and winter morph of SWD has led to more cold tolerance studies. The studies concluded that summer morphs were unable to survive at a temperature of 10°C for three months, while the winter morphs could survive at 1°C for several months (Dalton et al. 2011, Stephens et al. 2015).

Optimal development temperatures from egg to adult stage was 28.2 °C. LT50 was found to be 4.88 °C in lab setting (Ryan et al. 2016). Adults exposed to temperatures lower than 10 °C experienced increased mortality. Adults that emerged at 10 °C underwent reproductive diapause in contrast to those that acclimated to the low temperatures.

Adults are the only life stage known to overwinter, hibernating in protected areas such as soil or leaf litter by heated buildings (Kaçar et al. 2015). For colder areas such as Michigan, the Pacific Northwest, and the Alps in northern Italy, overwintering seems to be vital to the persistence of SWD populations (Asplen et al. 2015, Tonina et al. 2016). However, Jakobs et al. (2015) exposed SWD adults to overwintering conditions below leaf litter and found that none survived the lowest temperature of -14° C for more than 4 hours. More information about the threshold temperature for sterile males could be useful in defining geographical populations (David et al. 2005). Wang et al. (2016) found that the mean number of mature eggs per female was positively correlated to minimum daily temperature. Females caught in April to September had larger egg loads than overwintering females caught from November to March. A large proportion of overwintering females sampled were void of eggs. There is overwhelming evidence that suggests adult females undergo reproductive diapause when host fruit is not available (Rossi-Stacconi et al. 2016; Wang et al. 2016; Zhai et al. 2016). Ryan et al. (2016) recently observed females that were mated before being exposed to cold temperatures and observed how fertility is affected by cold. 38% of females were observed to lay eggs that were viable after being exposed to a 42 day cold treatment. This data suggests that females may have the ability to storm sperm during overwinter and lay eggs in the spring (Ryan et al. 2016).

Identification

Often, SWD adults are mistaken for other fruit flies. Identification is important because of the economic damage these flies cause compared with other species. Adult male SWD are relatively easy to identify; they exhibit one dark spot on the tip of each of their forewings, and two dark sex combs on the first and second tarsomeres of the foreleg (Walsh et al. 2010). Unlike males, females do not have dark spots on their wings; the most distinguishing physical characteristic that defines them from other *Drosophila* species is their large, serrate ovipositor (Van Timmeren et al. 2012) (Figure 2). The abdomen of the female can be pressed gently to expose the unique ovipositor (Walsh et al. 2010). For identification, males should be viewed under 10x magnification and females under 50x magnification. For the examination of genitalia for both sexes, magnification should be set at 200x for successful identification (Mulder et al. 2013). Both sexes have brown to light yellow bodies and have dark unbroken bands around the abdominal segments. Adults are known to display wide phenotypic variation across individuals (Beers et al. 2010)

SWD eggs are white, oval, and measure 0.6 mm long by 0.18 mm wide. The eggs also have two protruding filaments on one end, which likely are used for gas exchange as they maintain contact with the environment outside the infested fruit. Currently, the milky white SWD larvae cannot be distinguished from other *Drosophila* spp. larvae based on morphological characteristics (Johnson and O'Neil, 2013). SWD exhibits three larval instars; the first instar is less than 2 mm long, the second instar is 2 to 3.5 mm long, and the third instar is 3.5 to 5 mm long (Dreves et al. 2014).

Damage

SWD continues to spread rapidly, causing extreme economic losses in fruit-producing regions of the world (Cini et al. 2012). SWD causes more damage than other fruit fly species because of its preference for ripe fruit its invasive nature, and rapid reproduction (Bellamy et al. 2013, Asplen et al. 2015). Adult females target soft-skinned and stone fruits such as strawberry, blackberry, raspberry, blueberry, grape, peach, and cherry (Lee et al. 2015). Fruit damage can be both direct and indirect. Oviposition and larval feeding cause direct damage, leading to deterioration of fruit tissue (Renkema et al. 2015). Indirect damage occurs when fruit wounds are exposed to secondary pathogens like bacteria and yeast, which cause further deterioration of fruit and can increase the fruit's susceptibility to attack by other *Drosophila* species (Johnson and O'Neil 2013, Renkema et al. 2015).

Beers et al. (2011) found that infestation rates increased as soon as fruit began to ripen. Larval survival was also higher in ripe fruits, compared to fruits that were severely under-ripe. According to Kinjo (2013), female SWD clearly tend to oviposit more eggs in softer fruits than in firmer fruits. This study suggests that firmer or thicker-skinned fruits are less susceptible to SWD infestation than thinner-skinned fruits. Due to greater susceptibility, thin-skinned fruits (e.g., blueberries, raspberries, blackberries, strawberries, cherries) are most affected by SWD infestation.

In the United States, estimated yield losses from SWD range from 20-40% for blueberries, cherries, raspberries, and caneberries (Gabarra et al. 2014). According to Goodhue (2011), California's gross revenues would decrease by 37% for raspberries and 20% for strawberries if SWD was not managed. Soft fruit growers in the eastern United States estimate the damage was approximately $27.5 million in 2013 (Bruck et al. 2011). Also, without adequate control measures, damage from SWD can result in up to $500 million in annual losses in Western US production areas (Cuthbertson et al. 2014). Furthermore, in a single region of Italy, yield losses from strawberry, raspberry, blueberry, blackberry, and cherry were estimated to be more than 3.3 million Euros per year (Goodhue 2011).

Another reason why SWD causes economic damage worldwide is its invasive success. SWD is extremely fecund and can produce up to 13 generations per year under ideal weather conditions (Bruck et al. 2011, Asplen et al. 2015). These pests also have an extremely high potential for dispersal. Seven years after the original documentation of SWD in the U.S., it has been found in most of the 50 states (Stewart et al. 2014). Accidental passage through infested fruit is probably the main cause of such rapid global spread of SWD (Cini et al. 2012; Mulder et al. 2013). Furthermore, according to Gabarra et al. (2014), SWD's lack of natural enemies in newly invaded areas allows for more successful dispersion and colonization (Chabert 2012, Gabarra et al. 2014).

Population Dynamics

Populations of SWD are greatly influenced by temperature and humidity (Wiman et al. 2016). Throughout the northeast United States, populations likely are very low in the spring as a result of the few overwintering adults, and then gradually increase (Langille et al. 2016). Even if the temperature is too cold for SWD to overwinter in certain regions, flies may be introduced every spring by infested fruit shipped into the area. *Drosophila suzukii* populations have the ability to disperse (Hauser 2011). SWD are small enough that they could be carried long distances in the wind, and if able to fly high enough, they could travel even greater distances in wind currents (Briem et al. 2016)

Alternative Host Plants

Researchers are just beginning to explore the effect that landscapes have on SWD populations and infestation rates in agricultural fields. Klick et al. (2016) using mark and recapture methods concluded that when there are no host plants present in the surrounding vegetation, trap counts for SWD in raspberry crops were significantly lower compared to fields with an abundance of host plants. Also, SWD have been found to be highly abundant in woodland landscapes, resulting in early season crop risk (Pelton et al. 2016). Although research and extensive monitoring have been done on cultivated crops to explore susceptibility to SWD, potential, non-crop and ornamental hosts have not been frequently studied (Hauser 2011). Lee et al. (2015) surveyed field sites in Michigan and Oregon that were at least 50 meters away from known SWD infested crops. Approximately 104 species of ornamental and wild plants are associated with adult SWD, suggesting these species are viable host plants (Kenis et al. 2016, Lee et al. 2015, Poyet et al. 2015). Although an extensive list of alternative hosts exists for native western plant species, interior states have had limited surveys. Currently, there are no studies published on the potential native host plants in Oklahoma. Determining alternative host plants in different ecoregions is vital to help create management programs for growers across the U.S. and the world (Cini et al. 2012, Lee et al. 2015).

Monitoring

A successful integrated pest management (IPM) system for SWD includes monitoring, identification, and control. Future management and control rely initially on implementing successful monitoring techniques (Isaacs et al. 2010). Monitoring traps are used to measure the distribution and seasonal activity of SWD (Lee et al. 2013). Commercial trap brands include the multi-lure trap (Better World Manufacturing Inc. Fresno, CA), Droso-trap (Biobest, Belgium), CAPtiva (Marginal Designs, Oakland, CA), Spotted wing drosophila trap (Contech Enterprises Inc., Victoria, Canada), Victor fly trap (Woodstream Corp., Lititz, PA), and various McPhail-type traps (Agrisense Ltd., Pontypridd, United Kingdom) (Lee et al. 2013). According to Oklahoma State University's Spotted Wing Drosophila Monitoring Program (Mulder et al. 2013), monitoring traps should be placed at fruit level three weeks before ripening. Traps can be made from ~950-ml, clear plastic containers with lids. The containers should be altered to have

8-cm diameter holes around the upper side of the container to allow SWD to enter the trap. Cup trap designs catch significantly more SWD than sticky plate traps (Iglesias et al. 2014).

There are various bait recipes recommended from different studies (Walsh et al. 2010, Johnson and O'Neil 2013, Dreves et al. 2014), but two effective ingredients in the majority of the studies are apple cider vinegar and a few drops of unscented dish soap (Isaacs et al. 2010, Walsh et al. 2010, Johnson and O'Neil, 2013). Trécé lures (Trécé Inc. Adair, OK) have been found to be effective and should be suspended above the drowning solution in the trap for best results (Isaacs et al. 2010). Growers should monitor and change the traps consistently every week (Walsh et al. 2010). To process contents of the trap, liquid should be strained through cheesecloth to separate liquid from captured insects; a 20X hand lens should be used to identify SWD adults (Johnson and O'Neil 2013). Volatile compounds from a mixture of merlot grape wine and rice vinegar proved to be highly attractive to SWD (Cha et al. 2012). The researchers suggested that incorporating these naturally attractive volatiles into SWD lures would be highly effective for monitoring populations.

Kleiber et al. (2014) tested multiple compounds for attractiveness to SWD. The authors screened 17 compounds that were structurally related to fermentation products, including acetic acid, ethanol, ethyl acetate, and 2-phenethyl alcohol. The authors concluded that adding these compounds to apple cider vinegar traps did not increase the number of flies captured. However, compounds present in wine and vinegar such as methanol, ethanol, acetic acid, and ethyl acetate were less deterrent than other compounds tested. Hamby and Becher (2016) suggest that adding specific microbial volatile constituents that are attractive to dipteran species could potentially improve selectiveness for trapping and monitoring populations of *D. suzukii.*

To monitor for larvae, 30 fruits are selected that would be ideal for consumption. Selected fruit is submerged in a container with 240 ml of water and 60 ml of salt for 30 minutes. If the fruit is infested, white larvae will crawl out of the fruit and can be seen using 20x magnification (Isaacs et al. 2010, Dreves et al. 2014). Fruit growers should start management programs immediately after detection of SWD when fruit is susceptible. Fruit is susceptible to infestation when it is beginning to ripe or is already ripe (Johnson and O'Neil 2013, Dreves et al. 2014).

Some studies altered physical aspects of the traps to see which designs were more effective at attracting SWD adults. Lee at el. (2013) compared the efficiency of different colors, bait surface areas, and top and side position entry points. Yellow traps caught the most adults compared with black, red, white, and clear traps. Traps with a bait surface area of 90 cm^2 and side entry points caught the most SWD adults. Another study suggests that traps with dark red colors catch more SWD females than yellow and green (Basoalto et al. 2013).

Insecticides

Conventional insecticides have been evaluated in field and lab trials for efficacy againstSWD adults. Multiple studies found that pyrethroids (bifenthrin, beta-cyfluthrin, permethrin, zeta-cypermethrin), organophosphates (malathion, diazinon) and spinosyns (spinosad, spinetoram) were highly effective at managing SWD adults, with applications resulting in almost 100% mortality (Beers et al. 2011, Bruck et al. 2011, Van Timmeren and Isaacs 2013). The insecticides remained effective for 5 to14 days. The performance of these products was consistently effective over various crops, sites, and growing conditions. Neonicotinoids (acetamiprid, thiamethoxam) and other insecticides (chlorantraniliprole, abamectin) did not produce mortality rates as high as the previously listed insecticides and only lasted 1-3 days before SWD appeared again in monitoring traps (Beers et al. 2011, Bruck et al. 2011). Since there is currently zero tolerance for infested fruit at processing facilities, Bruck et al. (2011) do not recommend using the chemistries acetamiprid, thiamethoxam, chlorantraniliprole, or abamectin for SWD management. Insecticide treatments should be reapplied every 7 days and immediately after a rain event (Isaacs et al. 2010). Johnson and O'Neil (n.d.) recommend using insecticides that have very short pre-harvest intervals and restricted entry intervals. Furthermore, Cowles et al. (2015) showed that adding sucrose to effective insecticides increased mortality rates in adults and larvae. Sucrose attracted SWD and encouraged them to feed on insecticide-treated fruit. Additionally, adding cane sugar alone or in combination with yeast (*Saccharomyces cerevisiae* or *Aureobasidium pullulans*) significantly improves efficacy of insecticide treatments (Knight et al. 2015). Growers should also consider rotating various compounds to decrease the chance for genetic resistance to develop given the fast generation time of SWD (Bruck et al. 2011, Asplen et al. 2015).

According to Isaacs et al. (2010) organic insecticide options for SWD control are limited. There are two spinosad formulations (Entrust® 80WP, Entrust® SC, Dow AgroSciences LLC, Indianapolis, IN), that seem to work better against *D. suzukii* than other products like PyGanic. Managers recommend alternating Entrust® with organic pyrethrum insecticides to achieve control and manage for insecticide resistance in target populations.

Cultural Control

Cultural control methods being evaluated include tillage to bury infested fruit, physical exclusion with netting, fruit cooling, irradiation, and post-harvest sorting (Asplen et al. 2015). According to Hampton et al. (2014), early ripening blueberry cultivars including ‘Bluetta’, ‘Earliblue’, and ‘Collins’ can be harvested before SWD are active, minimizing SWD damage and cost of control. One example of alternative to chemical control is the use of volatile repellents, which have shown success in field and lab and could be considered to be part of successful and efficient integrated management programs for SWD in the future (Krause Pham and Ray 2015. Renkema et al. 2016, Wallingford et al. 2016).

Krause Pham and Ray (2015) evaluated how SWD olfactory behavior can be manipulated to deter flies from fruit production areas. The authors substituted diethyltoluamide **(**DEET) a formula that has proven effective for deterring SWD infestation, for naturally occurring repellents such as butyl anthranilate, methyl N,N- dimethylanthranilate, and ethyl anthranilate. The authors showed that SWD avoided traps containing a 10% solution of these compounds. These compounds have pleasant smells and are safe for human consumption. This could give conventional and organic fruit producers better control and protection from SWD in the future (Krause Pham and Ray 2015).

Removing any possible host plant from either outside or inside the fruit production area is vital to minimize possible re-infestation (Isaacs et al. 2010, Lee et al. 2015). Any fruit remaining in the field after harvest can be used as food or as a breeding site for remaining flies. Therefore, non-harvested fruits should be removed and disposed of properly so SWD does not persist in the production area (Dreves et al. 2010, Johnson and O’Neil 2013). Composted fruit could potentially increase the severity of an infestation since the eggs and larvae are not rapidly destroyed (Cini et al. 2012). Walsh et al. (2010) suggest many techniques for proper disposal such as insecticide treatment, disposal in closed containers, solarization, or bagging and burying the fruit. These cultural control methods should be applied to every small-scale grower's sanitation practices in order to prevent re-infestation (Beers et al. 2011, Cini et al. 2012, Issacs et al. 2010). However, sanitation and cultural control methods are mostly preventative practices. If a persistent population of SWD is detected, aggressive pest management methods (i.e., chemical control) should be applied to prevent further fruit damage (Walsh et al. 2010).

Biological Control

Until 2015, the only species that has been found parasitizing SWD in the United States and Italy is *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) (Rossi-Stacconi et al. 2013). Recently, Gabarra et al. (2014) found that *Orius laevigatus* (Fieber) (Hemiptera: Anthochoridae) feed on SWD eggs, and a soil predator, *Labidura riparia* Pallas (Dermaptera: Labiduridae), consumed SWD larvae.

Toledo et al. (2006) examined the use of entomopathogenic fungi for biological control of other fruit fly species (*Anastrepha ludens* (Loew) and *Ceratitis capitat* (Wied)). Fungi that showed the greatest potential for infecting and killing adult fruit flies were *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae.* These fungi were shown to enter the host through the skin or via the digestive tract after ingestion. A study conducted by Woltz et al. (2015) tested these fungal biocontrol agents and found that *M. anisopliae* was the only pathogen effective in decreasing SWD survival in a field setting. However, it had low residual activity and did not negatively affect SWD fecundity.

Mechanical Control

Exclusion of adult flies using nets has proven to be a highly effective management solution for fruit growers (Cini et al. 2012, Cormier et al. 2015). Mesh should be added around the perimeter of the fruit production area, entirely enclosing the crop. Mesh should be 1 x 0.6 mm in size or smaller in order to successfully exclude SWD adults (Cormier et al. 2015). Fruit production areas that are equipped with high tunnels for manipulating harvest dates and reducing disease spread have shown lower SWD infestation rates (Rogers et al. 2016, Iglesias et al. 2014. Cormier et al. 2015, Rogers et al. 2016). These structures also provide a site for drape netting around the perimeter of fruit crops to exclude SWD (Rogers et al. 2016, Asplen et al. 2015).

Challenges of IPM to Manage SWD

Development of comprehensive IPM management systems for SWD is hindered by lack of knowledge about this pest. Many biological and ecological aspects about SWD remain unknown, including overwintering behavior (Asplen et al. 2015). However, biological information could potential play a major role in timing of chemical and other management application strategies for growers in the future. Currently, the most effective management strategies for SWD is the application of rotated chemical insecticides to protect crop hosts (Haye et al. 2016). However, there is limited information about how SWD develops resistance to commonly used insecticides (Asplen et al. 2015). Researchers are trying to find other avenues of successful control for SWD other than just chemical. Studies have researched alternative methods such as, semiochemicals, trapping, biological control, sanitation, landscape management, and post-harvest treatments. Simple “good husbandry” practices such as sanitation and vegetation removal, at this time, are considered to be the most important factors for SWD control around the world (Haye et al. 2016). Continued research and results should provide entomologists and growers a better understanding of the biology and conquest of this prolific pest. Biological information will lead to more integrated and successful management programs for *D. suzukii* in soft fruit crops. However, studies completed thus far, can conclude that incorporating multiple strategies into management practice will implement the most efficient control economically and environmentally.

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TABLES AND FIGURES

Figure 1. The worldwide distribution of SWD as of 2015 (Asplen et al. 2015).

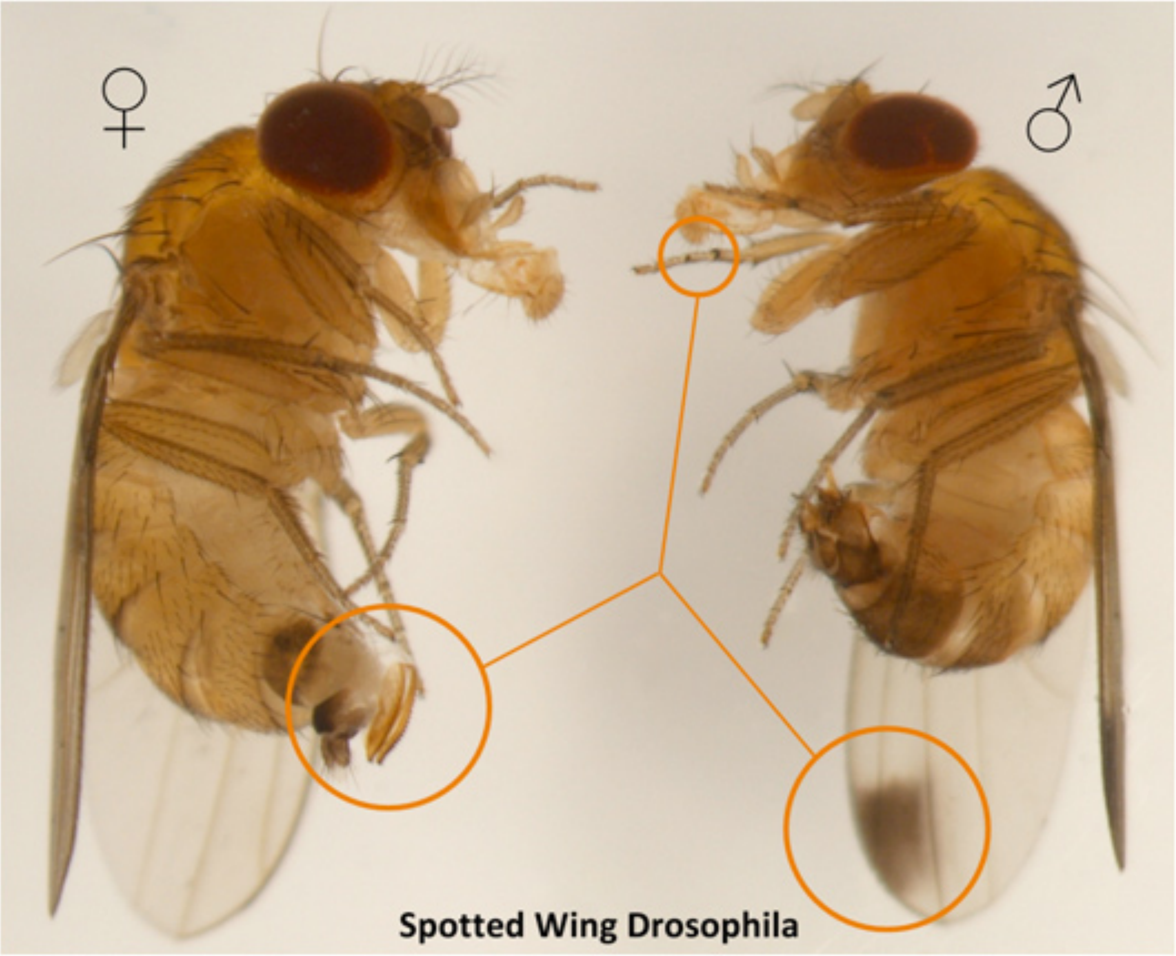

Figure 2. Unique morphological characteristics of male and female SWD used for identification (Rick Grantham)

CHAPTER III

OBJECTIVE I: ABIOTIC AND LANDSCAPE FACTORS THAT AFFECT SPOTTED-WING DROSOPHILA POPULATIONS

INTRODUCTION

Environmental conditions and landscape ecology play a large role in the life history and population ecology of spotted-wing drosophila (SWD), *Drosophila suzukii* (Matsumura) (Klick et al. 2014). Because SWD is adapted to a temperate climate, the main abiotic factors that limit its growth and reproduction are temperature and humidity (Shearer 2016, Tochen 2016). Food source and habitat characteristics also influence SWD reproduction. Understanding how these and other factors drive population dynamics of *D. suzukii* is a challenge to developing effective integrated pest management (IPM) strategies for this key pest of small fruits.

Physiological data can be used to explain mechanisms for successful reproduction and survival of invasive species and their response to environmental change (Plantamp et al. 2016). Calculation of degree days has become a standard method in determining optimal timing for IPM techniques on insect pests, both native and exotic (Wiman et al. 2016).

Wiman et al. (2016) illustrated a clear relationship between reproductive potential and physiological time. Using a lower threshold of 7.2 °C and an upper of 30 °C, they determined that in a field setting, *D. suzukii* oviposition occurred between 50 and 800 degree days (DD_{50}). These results suggest that females can reproduce early in the season under mild temperatures, but host plants may not be available for oviposition. Reproductive potential was positively correlated with temperature, supporting other studies that show SWD prefers a temperate climate for reproduction and oviposition, whereas cold temperatures hinder reproductive potential (Arno 2016, Harris et al. 2014). Also, SWD populations decrease during the hottest months of the year, but increase in autumn and spring (Arno 2016, Harris et al. 2014, Wang et al. 2016, Wiman 2014). In Oklahoma, ideal temperatures for SWD reproduction and survival occur in early summer, where the average temperature is 21°C in May, and 25.5 °C in June. In July, the average temperature increases to 28.3°C, close to the SWD upper threshold of 30°C (U.S. Climate Data, 2017). Thus, blackberries grown in Oklahoma are susceptible to SWD because blackberries ripen in late May and June with most production complete by mid-July. Growing early- or late-season cultivars can help minimize infestation rates (Hampton et al. 2014). However, there have not been any studies done on the susceptibility of Oklahoma blackberry varieties to SWD infestation.

Extreme heat and cold are detrimental to *D. suzukii*. Recently, this fruit pest was found to be chill susceptible, and pupae have been shown to be more chill susceptible than adults (Ryan et al. 2016, Jackobs et al. 2015). Laboratory studies have shown that SWD adults, especially females, are capable of surviving at extreme cold temperatures. The LD50 for females at -4°C was 24 hours, 0°C was 3 days, and 2°C was 5 days. Winter morphs of SWD are darker and able to survive for several months at 1 °C (Wallingford and Loeb 2016, Wiman 2016). Consistently, females have been found to be more cold tolerant than males. If strong, winter morph females survive, their fertility is not affected by cold temperatures (Plantamp et al. 2016, Ryan et al. 2016, Tochen et al. 2014). This supports the hypothesis that only a small number of females are capable of overwintering in extreme temperatures (Stephens et al. 2015, Wallingford and Loeb 2016). *Drosophila suzukii* is thought to overwinter outside of agricultural fields in forest hedges, the same places they use for alternative food sources and refuge in the spring, summer, and fall (Briem et al. 2016).

The heavily sclerotized ovipositor of SWD females allows them to infest a variety of wild and agricultural host plants (Grant and Sial 2016). There is greater potential for economic damage to crops adjacent to tree lines that contain non-crop plants because these alternative hosts elongate the reproductive season of SWD (Klick et al. 2014, Little et al. 2016, Pelton et al. 2016). Non-crop host plants benefit SWD populations by providing alternative food sources, refugia from pesticide application, shelter from extreme heat or cold, and a suitable overwintering habitat (Klick et al. 2014). Approximately 104 species of ornamental and wild plants are associated with adult SWD, suggesting these species are viable host plants (Kenis et al. 2016, Lee et al. 2015, Poyet et al. 2015). This expansive list of hosts indicates that SWD is an extremely polyphagous pest species. In several studies, some plant species had eggs present in the fruit, but the eggs could not develop further into larvae for unknown reasons (Kenis et al. 2016, Lee et al. 2015, Poyet et al. 2015).

Non-crop host plants play a vital role in the life cycle and population ecology of this invasive fruit pest. Additional attention should be focused on natural habitats surrounding agricultural sites that harbor the majority of alternative host plant species. It is no surprise that SWD has been observed in tree lines adjacent to crops. These nearby habitats are home to plant species that provide food, shelter, and thermal cover. Tree lines can provide refuge from extreme summer heat and shelter from strong and cold winter winds of the Midwest (Capel 1988), improving winter survival. Since SWD is a temperature-sensitive pest, these areas could provide protection from the extreme heat of the Great Plains (Gardner 2009, Capel 1988). Tree hollows, leaf litter, and detritus also provide favorable microclimates (O'Connel and Keppel 2016), which are thought to be a key aspect to overwintering survival. Although tree lines and wooded areas are important to SWD survival, it is still unknown how much time these flies spend in woodlands and how they use these resources compared to agricultural areas.

The objective of this study is to provide vital information about population dynamics of *D. suzukii* in Oklahoma. Detailed analysis of how SWD populations are affected by environmental and landscape factors will provide useful information for developing future management plans. A consistent trapping technique was applied for two growing seasons to monitor population trends of SWD throughout the summer and fall. I hypothesize that more SWD are associated with tree lines compared to blackberry orchards because surrounding woodlots offer more resources to SWD than orchards, such as refuge from extreme temperatures and pesticide pressure, and alternative host plants (O'Connel and Keppel 2016). To demonstrate what factors affect SWD populations, trap counts were correlated with local temperature, humidity, and vegetation composition data. I hypothesize that temperature and humidity affect SWD populations throughout the summer and fall. Six Oklahoma blackberry cultivars were examined for SWD infestation, which was determined by collecting field samples of each cultivar and counting the number of eggs per berry. I hypothesize that early cultivars are more susceptible to SWD infestation than late-season cultivars based on previous studies on blueberry cultivars (Hampton et al. 2014). Lastly, potential alternative host plants were identified by collecting fruits from wild plants at each location and observing for emergence of SWD adults in the lab. Based on the polyphagous nature of SWD and existing literature, I hypothesize that SWD are using native plants as well as preferred crop hosts.

MATERIALS AND METHODS

Fly Populations

During 2015, SWD adults were monitored from June through October for seven sampling dates. In 2016, monitoring took place from May through November, resulting in fourteen sampling dates. In 2015, monitoring occurred at four field locations: Perkins, Stillwater, Owasso, and Sapulpa, Oklahoma. In 2016, one sampling location was added in Mounds, Oklahoma and a new Stillwater location replaced the location monitored in 2015. Each location is a privately owned “U pick” blackberry orchard with the exception of the Perkins site, which is the Cimarron Valley Research Station, owned and operated by the Oklahoma Agricultural Experiment Station, and the Stillwater location in 2015, which is a local vineyard. Acreage for blackberry or grape production varied by location, ranging from 2000- 40,000 m^2 (Table 1).

Deli cup bait traps were used to capture SWD adults (Beers et al. 2010). The deli cups were 946-ml Reditainer Deli Containers (Clear Lake Enterprises, Reditainer, Port Richey, FL). Various trap designs have been shown to catch more *D. suzukii* than sticky cards (Iglesias et al. 2014). Each trap contained 480 ml of apple cider vinegar at 5% acidity (Great Value Apple Cider Vinegar, Wal-Mart Stores Inc., Bentonville, AR). Approximately 29 ml of unscented dish soap (Seventh Generation Natural Dish Liquid, Free & Clear, Burlington, VT) was added to the apple cider vinegar (Isaacs et al. 2010, Walsh et al. 2010). A small hole was cut into the center of the deli cup lid and a paper clip was threaded through the hole. A lure (Trécé Inc., Adair, OK) was attached to the paper clip and suspended above the liquid contents of the trap (Iglesias et al. 2010, Isaacs et al. 2014, Walsh et al. 2010). Ten holes, each measuring 1 cm in diameter, were drilled along the top sides of the cup so flies could access the trap and encounter the trap solution (Beers et al. 2010).

At each sampling location, two traps were placed inside the blackberry crop and another two traps were placed in the surrounding tree line. Traps were hung at a height of 1 meter on a metal post inserted into the ground. Trap contents were collected every two weeks and were replaced by a fresh deli cup with the same contents. However, the same lids and lures were kept on the post until the lures needed to be changed every 4 weeks. Collected trap contents were processed in the lab.

Samples were processed by straining the contents of the collected trap through a cheesecloth to catch all insects. Insects were transferred onto a Petri dish and viewed under 20X magnification to be identified and sexed. Bycatch drosophilids and other species were not counted when examined under the microscope. After numbers of SWD males and females were recorded, they were stored in glass vials containing 70% ethanol. Storage vials were separated by location, trap site, and date of collection. African fig fly (AFF), *Zaprionus indianus* Gupta, another invasive fruit fly, was identified in traps and counted along with SWD. It should be noted that biweekly trap catch numbers cannot be used to provide a proper population estimate due to differences among locations, landscape effects, and management practices. Thus, the intent of this objective is to report a seasonal trend of SWD population activity across months in Oklahoma.

Repeated measures analysis was used to evaluate trap catch data for the 2015 and 2016 seasons (PROC MIXED, SAS 9.4). Response variables were trap counts for females, males, and both sexes combined for each year of sampling, and main effects were location, site, and time. Location represents the orchard or vineyard sampled and site represents placement of traps in the surrounding tree line or within the crop. There were four unique sampling locations in 2015, and five in 2016. Insect abundance data were square root (x) transformed prior to analysis. Significance was determined at $\alpha=0.05$.

Temperature and Humidity

Local temperature and humidity data were correlated with SWD trap catches to identify population trends that may be related to these abiotic factors over time. Local climate data were retrieved from the closest Mesonet weather station to each sampling location (Brock et al 1995, McPherson et al 2007). In 2015, three out of the four sampling locations were located in Creek, Tulsa, and Payne Counties. In 2016, all five sites were located in the same counties from 2015. Average daily temperature was calculated from the start until the last day of each two-week sampling period. These temperature and humidity values were then averaged across all sampling locations for each sampling period. These data were then correlated with total SWD trapped in each sampling period.

Pearson's Correlation Coefficient analysis (PROC CORR, SAS 9.4) was used to examine the relationship among total fly counts for each sample date in 2015 and 2016 to corresponding temperature and humidity data. Average temperature and humidity were calculated for all location for each sample date during the 2015 and 2016 seasons by taking the average daily temperature and humidity for all locations and dividing by the number of trapping locations. These values were correlated to total number of flies trapped for each sample date.

Cultivar Preference and Seasonality of Infestation

This study was conducted in 2015 at the Cimarron Valley Research Station, Perkins, OK. This location is unique because six different, commonly grown blackberry varieties are under cultivation: Chickasaw, Apache, Natchez, Ouachita, Triple Crown, and Tupy. This sampling location did not receive any pesticide applications during the entire trial, thereby ensuring oviposition and fruit selection were not affected by management practices. Every 3 days, 10 replications consisting of 10 ripe berries each were collected from blackberry plants. Berry samples were placed in marked plastic bags and transported back to the lab and examined under the microscope at 30X and the number of SWD eggs were counted per berry. The total number of eggs per rep were recorded and then combined to compare the mean number in all cultivars. To determine blackberry cultivar susceptibility, total number of eggs per rep were analyzed with ANOVA in the program JMP (Version 11) SAS Institute, Cary NC. This analysis was done for each separate cultivar per sample date.

The seasonality of infestation was studied May through July 2016. The same sampling locations that were used for monitoring SWD populations were also used for this study. Two 20’ x 20’ poly tarps (ALL IN SAFETY Supplies Corp., Brooklyn, NY) measuring 6 mm thick were supplied to growers to cover 3-4 consecutive plants of an early- and late-ripening cultivar when preventative pesticide applications were applied. This procedure ensured that there would be ripe berries that were susceptible to SWD oviposition. The Perkins location did not have any pesticide management, so the tarp was not needed to protect the berries from pesticide application. Only three plants were sampled in Perkins to keep the sampling options consistent among sampling locations. Twenty berries were collected from the non-treated plants biweekly at each of the five locations. Since the 2015 study determined that all Oklahoma cultivars are susceptible, all available cultivars at the blackberry orchards were sampled. However, most of the cultivars sampled were Natchez, which are commonly grown because they produce large and succulent berries. Berry samples were then taken back to the lab and the number of eggs were counted using a microscope at a 30X magnification. Due to extremely hot temperatures in the summer, blackberry growing season was shorter than usual for Oklahoma. As a result, a maximum of four blackberry replications were sampled at each location for the 2016 season. Mounds only had two sampling dates. Since the data were not as robust as planned, statistical analysis could not be performed. Recorded egg numbers from collected blackberries were used to determine which locations experienced the highest infestation rates.

Alternative Host Plants

The same blackberry orchards that were used to monitor SWD adults were also used to sample potential alternative host plants. At each field location, 50 meters were measured from the edges of the blackberry crop in all four cardinal directions (Lee et al. 2015). Within this area, any herbaceous or woody plants and trees that bore soft fruit or flowers were considered a suitable host plant for SWD (Diepenbrock 2016). Fruits or fleshy flower buds were considered because of their potential to be used for feeding and oviposition. Plant species were collected biweekly to allow new plants to emerge in the landscape. Surrounding tree lines on the property, adjacent or parallel to the crop, were examined for possible alternative hosts. During the 2015 sampling season, 89 native Oklahoma and ornamental species were collected. Since no previous alternative host studies had been conducted for SWD in Oklahoma, collection of flowering and fruiting plants was not selective in 2015. In 2016, sampling for alternative host plants was more selective based on results from 2015. Only eight new potential host species were added to the list of collected species for a total of 97 species collected over both seasons. Sampling included only fruiting plant species that had similar characteristics to preferred agricultural hosts of *D. suzukii*, and species that had previously been recorded as alternative hosts (Kenis et al. 2016, Lee et al. 2015).

Collected plant species were brought to the lab to rear SWD from eggs oviposited in fruit or flower buds. Each species was placed in a plastic deli jar organized by location and date. Mesh fabric was placed over the top and secured with a rubber band. Plants were kept at room temperature for 2 weeks, which allowed enough time for SWD eggs to develop into adults (Asplen et al. 2015). All species collected were identified and recorded with their common name, scientific name, date of collection, and location. If SWD adults emerged from any species successfully, it was recorded as a positive host plant.

Landscape Heterogeneity

Landscape heterogeneity was determined for each location using data from ArcGIS® software. The GPS coordinates of all six trapping locations from 2015 and 2016 were acquired from Google Earth. The GPS coordinates located in the center of the blackberry crops were chosen to represent the sampling location in ArcGIS®. The location and GPS information were then inserted into ArcMaps by creating a point shape file. The x field was represented by the longitude and the y field was represented by the latitude. Then the "GCS_WGS_1984" projection was selected to match Google Earth's projection, after which the points were exported to a shape file. The shape file was then reprojected into the "Albers_Conical_Equal_Area" to make sure it had similar projections compared to other data sets.

Landscape mapping information was obtained through the Oklahoma Department of Wildlife Conservation's Oklahoma Ecological System Mapping website ("Oklahoma Ecological System Mapping" 2017). This mapping tool provides all of the landscape and ecoregion classification for the entire state of Oklahoma. Once the website was accessed, the Raster Dataset (.ZIP) file was downloaded. Once the download was complete, the raster was opened as a new file in ArcMaps.

Once all of the data were imported, individual trapping locations were analyzed for vegetation composition by using the Raster dataset layer coupled with the imported GPS points. This study was designed to determine vegetation composition within a 100-meter radius from the blackberry crop for each trapping location. Individual buffer layers were created using a buffer tool for each location. The buffer layer radius was set at 100 meters. Once each location had a buffer layer, each location was selected individually using an identification number in the attribute table. With a new layer added, data were exported to connect the information to the newest layer. This process was completed for all six trapping locations. The "Extract by Mask" tool was selected for each location, which clipped the vegetation information in the raster data within the allotted 100-meter radius and calculated the percentage of habitat cover at each trapping location. Each 100-meter clip contained approximately 331 pixelated squares. Habitat percentage data were calculated by the number of pixels out of 331 that contained a certain type of vegetation cover (Figure 3). The habitat type percentages were calculated and simplified into three different habitat types: woodlot (Habitat 1), prairie/row crops (Habitat 2), and urban (Habitat 3). If there were different types of tree cover classifications at a location, they were grouped together to simplify the vegetation analysis.

After percentage of land cover was determined for each location, these data were analyzed using Pearson's Correlation Coefficients (PROC CORR, SAS 9.4) to compare total number of SWD captured at each trapping location with habitat type. Comparisons were made for each year of data separately and both years combined. Hab1Pct, Hab2Pct, and Hab3Pct represent the percentage of habitats 1, 2, and 3, respectively. The designation Hab12Pct is the percentage in either habitat 1 or 2, Hab23Pct is the percentage in either habitat 2 or 3, and Hab13Pct is the percentage in either habitat 1 or 3.

Early SWD trap abundance was also compared to all of the sampling locations in 2016 to determine if higher percentage of tree cover influenced the earlier emergence of SWD from overwintering habitat in the tree lines. Therefore, data sampling started at the end of April 2016 so the first SWD emergence date could be observed by identifying SWD in traps. The data generated from ArcView GIS was used to calculate the percentage of tree cover at each sampling locations. The number of SWD caught from all four traps were combined to calculate total SWD abundance per sampling date at each location. Traps were deployed at all locations on April 11 and traps were deployed for a month before they captured any SWD adults. The first two sampling dates where traps caught SWD were counted as the first two sample dates. The first two sampling dates combined spanned a total of 4 weeks, which occurred from May 5 to May 23, 2016.

RESULTS

Fly Populations

Results were consistent for repeated measures analysis of variance for females and both sexes combined for 2015 trap data (Table 2). Site represented traps that were located in blackberry crop and traps located in the tree line, the sites were compared in the analysis. For females, Site and Time were significant ($P = 0.0052$ and $P < 0.0001$, respectively) and the Site*Time interaction was not significant ($P = 0.0823$). For males, only Time was significant ($P < 0.0001$). For both sexes combined, Site and Time were significant ($P = 0.0076$ and $P < 0.0001$, respectively) and the Site*Time interaction was not significant ($P = 0.2916$). Similar results were obtained from repeated measures analysis of variance for 2016 data. For females, Site and Time were significant ($P = 0.0019$ and $P < 0.0001$, respectively) and the Site*Time interaction was not significant ($P = 0.8577$). For males, Site and Time were significant ($P = 0.0009$ and $P < 0.0001$, respectively) and the Site*Time interaction was not significant ($P = 0.7520$). For both sexes combined, Site and Time were significant ($P = 0.0011$ and $P < 0.0001$, respectively) and the Site*Time interaction was not significant ($P = 0.8666$). Overall, the effects of Site and Time were consistent for both sampling seasons even though fly populations, sampling locations, and number of samples were different each season. Tree lines traps caught more SWD than blackberry traps for the 2015 and 2016 sampling seasons (Figures 4,5).

Temperature and Humidity

In 2015 there was no correlation between humidity and SWD abundance (Pearson's correlation coefficient = -0.138, $P < 0.7582$). There was a significant correlation between humidity and SWD abundance in 2016 (Pearson's correlation coefficient = -0.875, $P < 0.0001$). For both sampling years combined, there was a correlation between humidity and SWD abundance (Pearson's correlation coefficient = -0.653, $P < 0.0013$). There was not a significant correlation between temperature and SWD abundance in 2015 (Pearson's correlation coefficient = 0.427, $P < 0.3393$) . For 2016, there was not a significant correlation between temperature and SWD abundance, however, a weak relationship was found (Pearson's correlation coefficient = -0.438, $P < 0.1174$). For both sampling years combined, there was not a significant correlation between temperature and SWD abundance (Pearson's correlation coefficient = -0.234, $P < 0.3072$) (Table 3)

Cultivar Preference and Seasonality of Infestation

All six cultivars of blackberries sampled at the Cimarron Valley Research Station in Perkins were susceptible to SWD infestation (Figure 6). The highest mean egg counts were found in Apache, Ouachita, Natchez, Triple Crown, and Chickasaw with averages of 13.1, 11.9, 10.5, 9.9, and 9.8 eggs per berry, respectively. Ouachita, Natchez, and Chickasaw are considered early-ripening cultivars, producing blackberries until the end of June. Apache and Triple Crown are considered late-ripening cultivars, producing blackberries until the end of July. These results suggest that SWD does not have a preference for early- or late-season cultivars in Oklahoma. It is important to note that in late June when early- and late-season cultivars are available, Chickasaw, Natchez, and Ouachita experienced higher egg loads than Triple Crown and Tupy. However, when Chickasaw, Natchez, and Ouachita were no longer producing fruit, Apache had the highest egg load with an average of 13 eggs per berry.

Perkins experienced the heaviest egg loads out of all five sampling locations (Figure 7), for three out of the four sampling dates. The rep collected on June 7, 2016 had 166 eggs. Eggs were present July 5, 2016 and on July 18, 2016 there were 102 eggs present on the berries. Of the remaining sampling locations, the highest egg loads were 127, 93, 74, and 7 at Stillwater, Sapulpa, Mounds, and Owasso. It is important to note that due to unusually hot temperatures, the blackberry season at Mounds was cut short by 4 weeks, so the data set was less robust than the other locations.

Alternative Host Plants

Of the 97 native and ornamental species sampled in Oklahoma, only three plants were found to be viable hosts for SWD based on adult emergence: pokeweed (*Phytolacca americana* L.*),* wild blackberry (*Rubus flagellaris* L.H. Bailey), and red mulberry (*Morus rubra* L.). When harvesting sand plum for observation of SWD emergence, an African fig fly (AFF) *Zaprionus indianus* Gupta was observed emerging from the fruit. This was the first record of this invasive fruit fly for Oklahoma. In 2015 and 2016, AFF was recovered from sand plum fruit. In 2016, they were also identified multiple times in the SWD deli traps.

Landscape Heterogeneity

Spotted-wing drosophila abundance was compared to percentage of habitat types for each location. No significant correlations were found between overall SWD abundance and the vegetation composition at each location.

Orchards that had the highest woodlot cover percentages had higher trap number for the first two sampling dates. For 2016, the Sapulpa, Mounds, and Stillwater locations had the most woodlot cover within the 100-meter radius out of all five sampling locations, with woodlot composition percentages of 77.7%, 49%, and 30%, respectively. Mounds had the highest abundance of SWD for the first two sampling dates, with a total count of 713 adults. Stillwater had the second highest abundance with 281, and Sapulpa had the third highest abundance with 197 SWD adults with the first two sampling dates. Perkins and Owasso both contained 0% woodlot vegetation within the 100-meter radius and they had far less SWD emerge within the first two sampling dates. Perkins yielded 28 SWD adults and Owasso traps contained 23 SWD adults (Table 4). Although no statistical analysis was performed on these comparisons, there seems to be a positive relationship between woodlots and higher abundance of SWD early in the season.

DISCUSSION

Abiotic factors such as temperature and humidity affect the behavior of arthropods (Stack Whitney et al. 2016). Data from Oklahoma orchards suggest that time and trap site affect SWD abundance. The high significance of time influencing SWD trap catches was expected, considering insect pest populations fluctuate and alter dispersal patterns in response to limiting factors such as temperature, intraspecific competition, humidity, and food resource availability (Bong et al. 2014, Murphy et al. 2014, Silva and Elliot 2016). Resources are affected by summer and winter months, summer months being more agriculturally and ecologically productive than winter months in North America (Liang et al. 2017). Since seasonal fluctuations of abiotic factors are inevitable, organisms have evolved different physiological or behavioral responses to cope with changes in temperature and humidity that are not ideal for survival and reproduction (Koštál 2016, Murphy et al. 2014). Spotted-wing drosophila is no exception, consistently showing response to temperature and humidity by population fluctuations (Arno 2016, Tochen et al. 2014, Tochen et al. 2016). My results show that there is an inverse relationship between humidity and SWD abundance, suggesting that increasing humidity levels negatively affect SWD populations. However, Tochen et al. (2016) studied the effects of relative humidity on SWD survival and found that increasing humidity increases the survivorship and reproductive capacity of SWD in a lab setting. Although the results from Tochen et al. (2016) are the opposite of mine, no one has studied the effects of humidity on SWD in the field. My results suggest that there are many other factors besides relative humidity levels that are impacting populations. Increasing temperatures were associated with reduced SWD abundance, although this relationship was weak. These results support my hypothesis that SWD populations will fluctuate in response to changes in temperature and humidity in Oklahoma. Tochen et al. (2014) found that SWD reproduction ceased at 30°C in a lab setting. Although I did not find any significant correlation to temperature in 2015, it is important to note that the data set in 2015 included half the number of sampling dates than 2016. Thus, the data from 2015 were not as robust and could be responsible for a loss of fidelity in comparing temperature to SWD abundance in 2015 and both years combined.

The data suggest that Oklahoma SWD populations seem to be most productive towards the end of April through the beginning of July, coinciding with peak availability of blackberries and blueberries in Oklahoma. Increased availability of food resources coupled with mild temperatures are an ideal combination for rapid increases in SWD populations. Although increasing temperatures result in an increase in physiological aspects of most insect species including SWD, exceedingly high temperatures can negatively impact populations just as much as low temperatures (Arno 2016, Harris et al. 2014, Wang et al. 2016, Wiman 2014). Traps catch significantly less SWD from the middle of July through October, suggesting that field populations are reduced due to high heat and humidity. Also, SWD has less access to oviposition sites since blackberry and blueberry fruits are no longer in season. During 2016, however, Oklahoma had an abnormally hot fall and winter and did not experience a freeze until late November. Once temperatures cooled down in November, another spike in SWD populations occurred. It is still unknown what SWD are feeding on in October and November, since all positive host plants observed in this study are done fruiting at this point. Since they have an increase in population during late fall, this proves that information is still lacking about their fall feeding and pre-overwintering behaviors. Similar population trends have been observed for SWD elsewhere, with populations dropping during the hottest months and then spiking when temperatures cool down with the onset of winter (Arno 2016, Harris et al. 2014, Wang et al. 2016, Wiman 2014). Proper timing of traps should coincide with expected peaks in SWD abundance, which is early spring through mid-summer (i.e., April through early July) in Oklahoma.

Significantly more SWD were caught along tree lines surrounding orchards compared to the blackberry crop. Results of repeated measures analysis supported the hypothesis that more flies would be caught in tree line traps than blackberry traps (Figs. 1 and 2). Similarly, Klick et al. (2015) found higher numbers of SWD in tree lines adjacent to raspberry orchards. Other arthropod pests and natural enemies have been shown to exploit resources available in habitats adjacent to crop fields (Bianchi 2006, Stack Whitney et al. 2016). Deploying multiple traps along tree lines surrounding susceptible crops could drown more adult SWD and minimize reproductive females from attacking crops. My data suggest that integrating multiple traps along tree lines surrounding orchards may be an effective strategy for capturing a high proportion of the SWD population. Hampton et al. (2014) found that mass trapping in blackberry fields resulted in an increase in infestation of fruit that were close to traps compared to fruit with no nearby traps. This surprising result suggests that deploying traps specifically attractive to SWD near susceptible crops could actually do more harm than good. Traps have attractive odors and colors that mimic host characteristics, adding to the attractiveness of the soft fruit crops. Clusters of attractants give SWD more incentive to visit the locations of the traps because more resources are potentially available, which results in more SWD contacting susceptible fruit. Trap-and-kill technology could be an important component of an IPM program for SWD management in berry orchards if implemented in an effective way (Hampton 2014). Oklahoma trapping data suggest that tree line trapping is most effective in catching a significant proportion of the population. Although SWD utilize the tree lines, it is not recommended that growers spray pesticides into the entire tree line. Targeted herbicide and insecticide sprays would be more effective at eliminating SWD and host plants,, while reducing negative impacts on beneficial species and tree line habitat.

Frequent application of pesticides is a common response to an emerging invasive pest (Roubos et al. 2014). Since SWD is relatively new to Oklahoma, effective integrated pest management methods have yet to be developed for this pest. Five of six sampling locations incorporated chemical rotation into their SWD management strategy. Frequent pesticide applications could cause SWD to seek refuge in surrounding woodlots and tree lines edges (Klick et al. 2015, Madeira et al. 2016). In addition to refuge from pesticides, woodlots may provide suitable microclimates for SWD survival during periods of high heat and humidity (O'Connel and Keppel 2016). Although my findings suggest tree lines harbor more SWD adults than blackberry orchards, eliminating tree line habitat is not a feasible option. Natural habitats are considered important areas to conserve natural enemies and beneficial insects (Landis et al. 2000, Madeira et al. 2016). Although no potential SWD parasitoids or predators have been observed or studied in Oklahoma, natural enemies may play an important role in regulating SWD populations in the future as their populations become more established in North America. Until 2014, there was only one species that had parasitized SWD in the United States, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) (Rossi- Stacconi et al. 2013). However, researchers have conducted more research to find other North American native parasitoids in attempt to control this rapidly spreading pest. In 2014, Gabarra et al. (2014) discovered that *Orius laevigatus* (Fieber) (Hemiptera: Anthochoridae) feeds on SWD eggs. Fungi such as *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* have shown limited success (Toledo et al. 2006). In addition to providing habitat for potential natural enemies, tree lines also provide habitat and vertical structure for predatory birds and spiders, thermoregulation, wind protection, and travel corridors. Proper management of these systems can increase overall biodiversity and long-term survival of natural enemies (Dix 1995).

Tree lines also harbor native host plants that are used by SWD, which can increase infestation risk to crops surrounded by these habitats. Adjacent woodlots and tree line edges show increased productivity as the growing season progresses, which attract an abundance of arthropods, including pests, into surrounding habitats (Madeira et al. 2016, Tscharntke et al. 2005). In these areas, reproductive SWD adults have access to a wider variety of resources and food when preferred soft-fruit crops are unavailable, ensuring reproductive success and extending the amount of time populations are still active (Pelton 2016). Thus, blackberries in orchards surrounded by woodlots may be susceptible to SWD for longer periods than blackberries in orchards that have no alternative food sources for SWD. Other agricultural pests such as soybean aphid have been observed alternating between crop and wild alternative host plants (Stack Whitney et al. 2016). During my study, SWD adults were reared from three species of wild host plants, all of which were found in the woodlots adjacent to crop fields. *Phytolacca americana* (American pokeweed) was found in relatively high abundance at five of six sampling locations. American pokeweed is a very common weedy plant found in Oklahoma that does well in disturbed agricultural areas and field margins. SWD were observed emerging from 20 separate collections of pokeweed in the lab. This study provides evidence that SWD is successfully completing its life cycle on three native Oklahoma plant species commonly found within forested habitat. This information can be useful to growers as an additional integrated pest management strategy. Elimination of wild reservoirs for insect pests is one cultural control option for (Madeira et al. 2016). However, using these alternative hosts as trap crops could be a more effective and sustainable option to growers. Trap cropping is a successful strategy for managing a variety of pests in many different agroecosystems (Heikki and Hokkanen 1991).

Since American pokeweed fruit ripens in mid to late summer, it could be used alongside late-season blackberry cultivars if pokeweed is more attractive to SWD.

No correlation was found between vegetation composition and total number of flies caught at each location. This relationship could possibly be different if a radius larger than 100 meters was used. A sampling radius of 1000 meters may provide more information on the influence of surrounding habitat to SWD. Although total SWD abundance was not correlated with vegetation composition, early SWD abundance did differ among trapping locations. The three sampling locations with the highest woodlot percentage had the largest populations for the first two sampling dates. Perkins and Owasso both had 0% woodlot composition within 100 meters of the center of the blackberry crop. Very few SWD were recovered at these locations early in the season. Locations with a higher percentage of woodlot had the substantially larger numbers of SWD adults caught during the first two sampling dates. Compared to the Perkins location, there was a seven-fold, ten-fold, and twenty-five-fold increase in SWD abundance during the first two sampling periods at Sapulpa, Stillwater, and Mounds, respectively. Similar increases were observed when comparing these three locations to Owasso. Similarly, Pelton et al. (2016) found that overall SWD abundance for the entire sampling season was not correlated with vegetation composition. However, earlier SWD activity was observed in locations surrounded by woodlots. My study shows that early generations of SWD are more abundant in locations with higher percentages of woodland habitats. These results suggest surrounding woodlots provide more overwintering habitat and shelter to SWD than sites that have fewer trees. Ultimately, this could result in a larger proportion of adults surviving winter and recolonizing crops during the spring and summer. Although Perkins had the 0% wood cover in the surrounding 100 meters, it contained the highest overall abundance of SWD compared to all other locations. This can be explained because Perkins was the only location that had absolutely no insecticide or fungicide application to the berries. All other sampling locations were "U- pick" blackberry orchards, so they were managed with pesticide application to protect the crop from SWD. This limited the population growth because the SWD could not oviposit on the berries. Perkins had so many susceptible berries, it allowed the SWD population to increase exponentially.

All six commonly grown Oklahoma blackberry cultivars are susceptible to SWD. Although some early-ripening cultivars such as Ouachita and Natchez experienced higher egg loads than others, all cultivars were attacked successfully by SWD. This suggests that SWD do not have a preference for specific cultivars. Spotted-wing drosophila oviposits and feeds on whatever suitable host is available. As mentioned previously, wooded landscapes adjacent to soft-fruit crops allow SWD populations to establish earlier than areas without trees (Pelton et al. 2016), making early-ripening cultivars more susceptible to infestation.

To compare infestation rates among sampling locations, tarps were used to shield specific plants from pesticide application, thereby making berries at all locations equally susceptible to SWD. The data suggest that these attempts were not successful. Perkins was the only location that had no insecticides nor fungicides applied to the blackberries, resulting in extremely high egg loads. Even though three blackberry plants were shielded from pesticide application at the other locations, very few SWD eggs were recovered from berries sampled at these locations. Owasso had one of the more intense management plans, involving heavy pesticide applications with rotation and persistent cultural management and upkeep. As a result, almost no eggs were found on the non-treated berry samples, even though a large amount of SWD were found in tree line traps. These results suggest that rigorous management may force SWD into surrounding vegetation to seek refuge from pesticides. The management plans at Stillwater, Mounds, and

Sapulpa fall in between the two extremes at Owasso and Perkins. Coincidentally, egg loads fell in the middle range at these locations.

The results obtained from this study will aid Oklahoma growers with more tools to combat this invasive pest. Changing the current trapping methods will be useful for growers wanting to monitor populations and implement mass trapping techniques. Growers should be aware that placing SWD traps next to blackberry crops could actually increase the amount of infestation in nearby fruit. Traps that are previously set up in the crops should be moved to the surrounding tree line. This study also showed that alternative host plants are being utilized by SWD in the surrounding natural vegetation and tree lines. Alternative hosts could be a key aspect to consider when managing resident populations of SWD. Although they are harboring the pest, physical or chemical removal of hosts from the property are only short term solutions for SWD management. Preliminary tests should be done to plant native hosts in a trap cropping system adjacent to crops to see if this can mitigate the infestation rates in blackberry crops. If the host plants are observed to be successfully luring SWD away from the agricultural crops, this could potentially be incorporated into an integrated pest management plan. All of the information obtained from this research project gave more insight into the life history of SWD in Oklahoma, and this information can be utilized for grower recommendations in the future

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TABLES AND FIGURES

Table 1. Trapping locations used for the 2015 and 2016 sampling seasons and specific crop and acreage information for each location.

Location	**Acreage**	**Crop**
Owasso	5 acres	Blackberries
Sapulpa	3 acres	Blackberries
Mounds	3 acres	Blackberries
Stillwater 2015	10 acres	Grapes
Stillwater 2016	0.5 acres	Blackberries

Table 2. Results of repeated measures analysis of variance for spotted-wing drosophila abundance in response to site and time. Statistical significance for main effects and the interaction term were determined at P ≤ 0.05

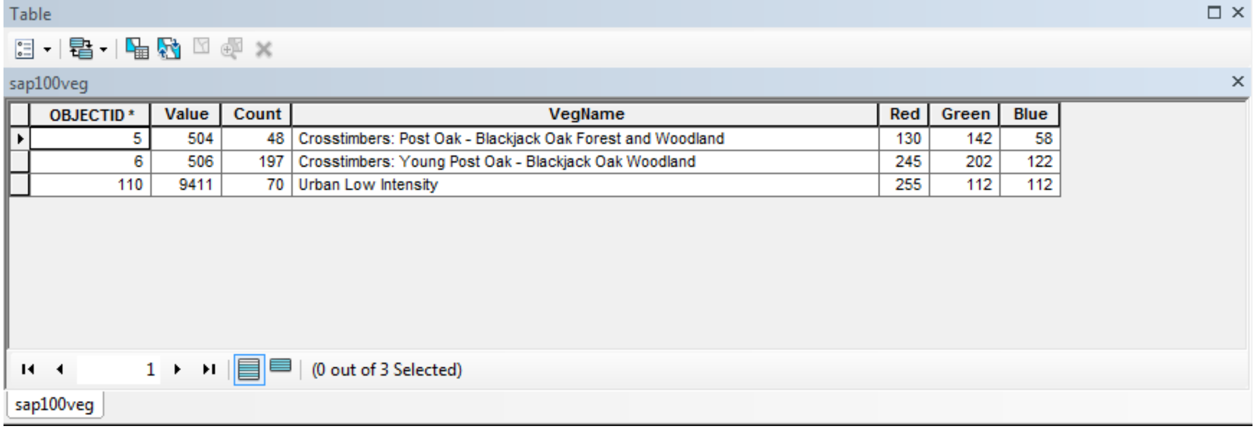
Year	**Sex**	**Effect**	**Num DF**	**Den DF**	**F Value**	**Pr > F**
2015	Female	Site	1	33.4	8.96	0.0052
		Time	6	40.2	37.22	< 0.0001
		Site * Time	6	40.2	2.04	0.0823
2015	Male	Site	1	30.3	0.09	0.7631
		Time	6	61.4	14.74	< 0.0001
		Site * Time	6	61.4	0.57	0.7545
2015	M & F	Site	1	37.4	7.97	0.0076
		Time	6	45.3	47.92	< 0.0001
		Site * Time	6	45.3	1.27	0.2916
2016	Female	Site	1	28.9	11.7	0.0019
		Time	13	186	10.71	< 0.0001
		Site * Time	13	186	0.59	0.8577
2016	Male	Site	1	37.9	12.99	0.0009
		Time	13	169	13.3	< 0.0001
		Site * Time	13	169	0.71	0.7520
2016	M & F	Site	1	30.7	13.02	0.0011
		Time	13	182	13.14	< 0.0001
		Site * Time	13	182	0.58	0.8666

Table 3. Temperature and humidity correlation with total fly counts during the 2015 sampling season, 2016 sampling season, and both sampling seasons combined (Pearson's correlation, P ≤ 0.05).

	Year	**SWD**	**Humidity**	**Temperature**
Coefficient	2015	1	-0.13786	0.427
P-value			0.7682	0.3393
Coefficient	2016	1	-0.87493	-0.43785
P-value			<0.0001	0.1174
Coefficient	2015-2016	1	-0.65294	-0.23406
P-value			0.0013	0.3072

Table 4. Sampling location woodlot percentages compared to abundance of SWD emerged in the first two sampling dates (Pearson correlation, $P \leq 0.05$).

		5/10/16	**5/24/16**	**5/10- 5/24/16**
Location	**Woodlot %**	**SWD Total**	**SWD Total**	**SWD Total for Both Sample Dates**
Perkins	0%	0	28	28
Sapulpa	77.7%	8	189	197
Stillwater	30%	25	256	281
Owasso	0%	4	19	23
Mounds	49%	145	568	713



OBJECTID *	Value	Count	VegName	Red	Green	Blue
5	504	48	Crosstimbers: Post Oak - Blackjack Oak Forest and Woodland	130	142	58
6	506	197	Crosstimbers: Young Post Oak - Blackjack Oak Woodland	245	202	122
110	9411	70	Urban Low Intensity	255	112	112

Figure 3. Vegetation classification generated from ArcView GIS after analysis. Count is the number of pixels out of 331 in a 100-m radius that represented a specific vegetation cover. Count was divided by 331 to get a vegetation cover percentage for the specific sampling site.

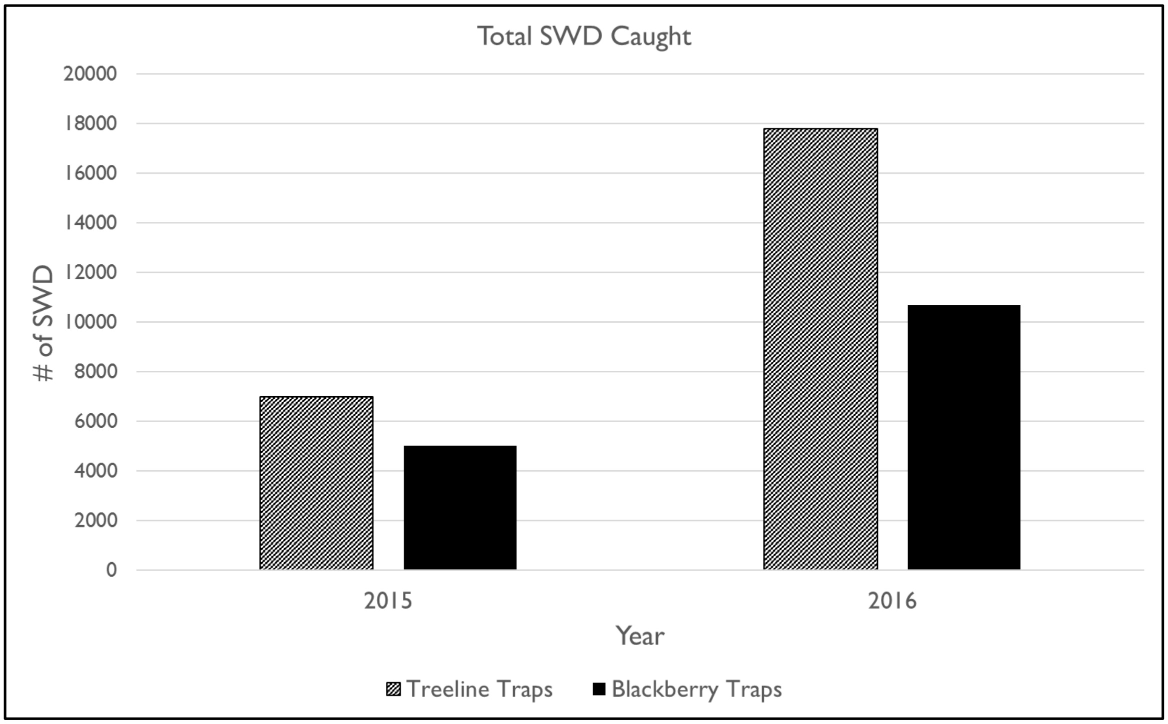


Figure 4. Sum of total *D. suzukii* trapped in traps located in tree lines compared to blackberry traps for the 2015 and 2016 sampling seasons.

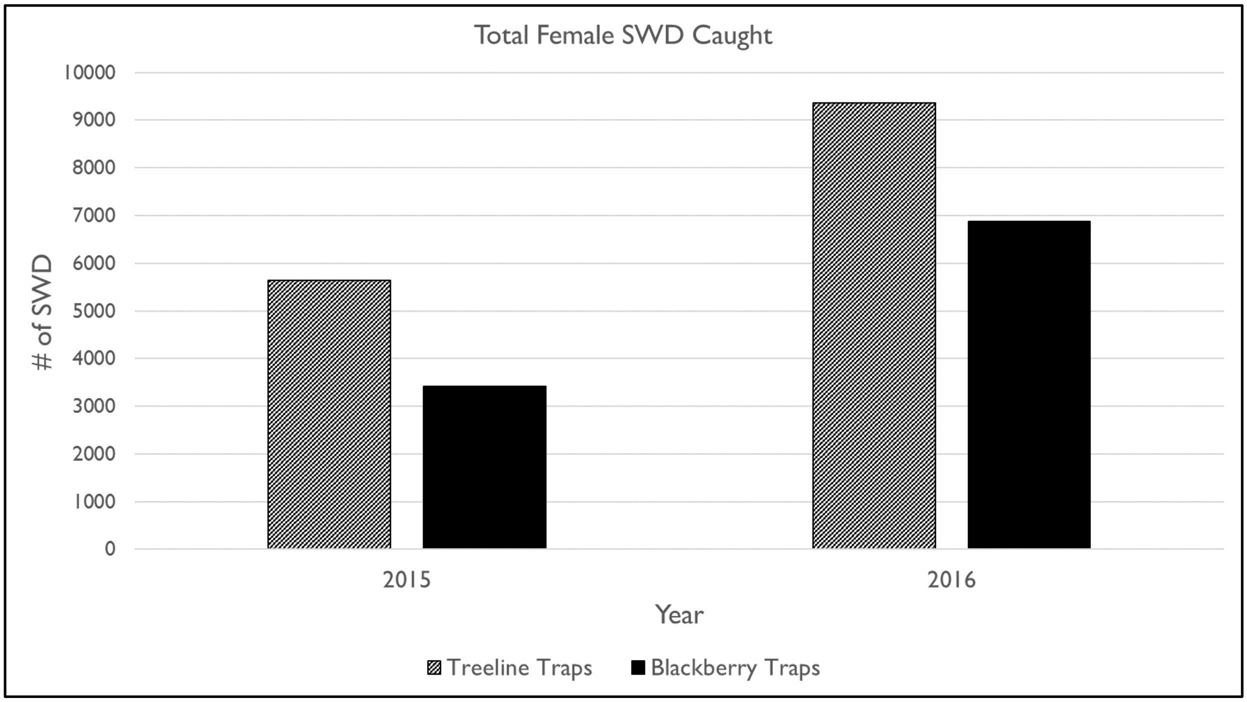


Figure 5. Sum of total female *D. suzukii* in traps located in tree lines compared to blackberry traps for the 2015 and 2016 sampling seasons.

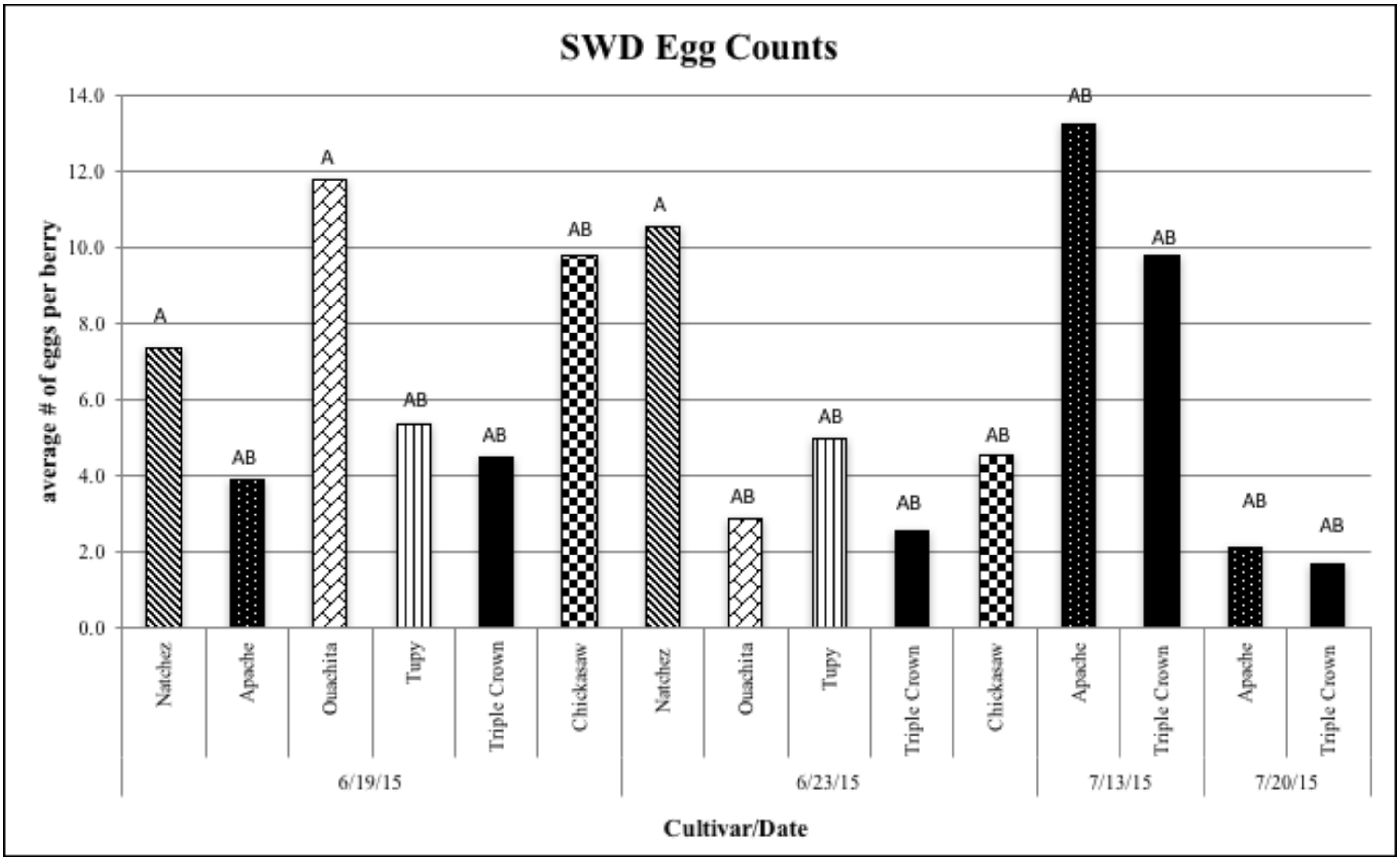


Figure 6. Graph of SWD egg count averages per berry over 10 replications of blackberry cultivar. Cultivars not included if berries were not ripe enough to be collected. Treatments followed by the same letter are not significantly different (ANOVA, $P \leq 0.05$).

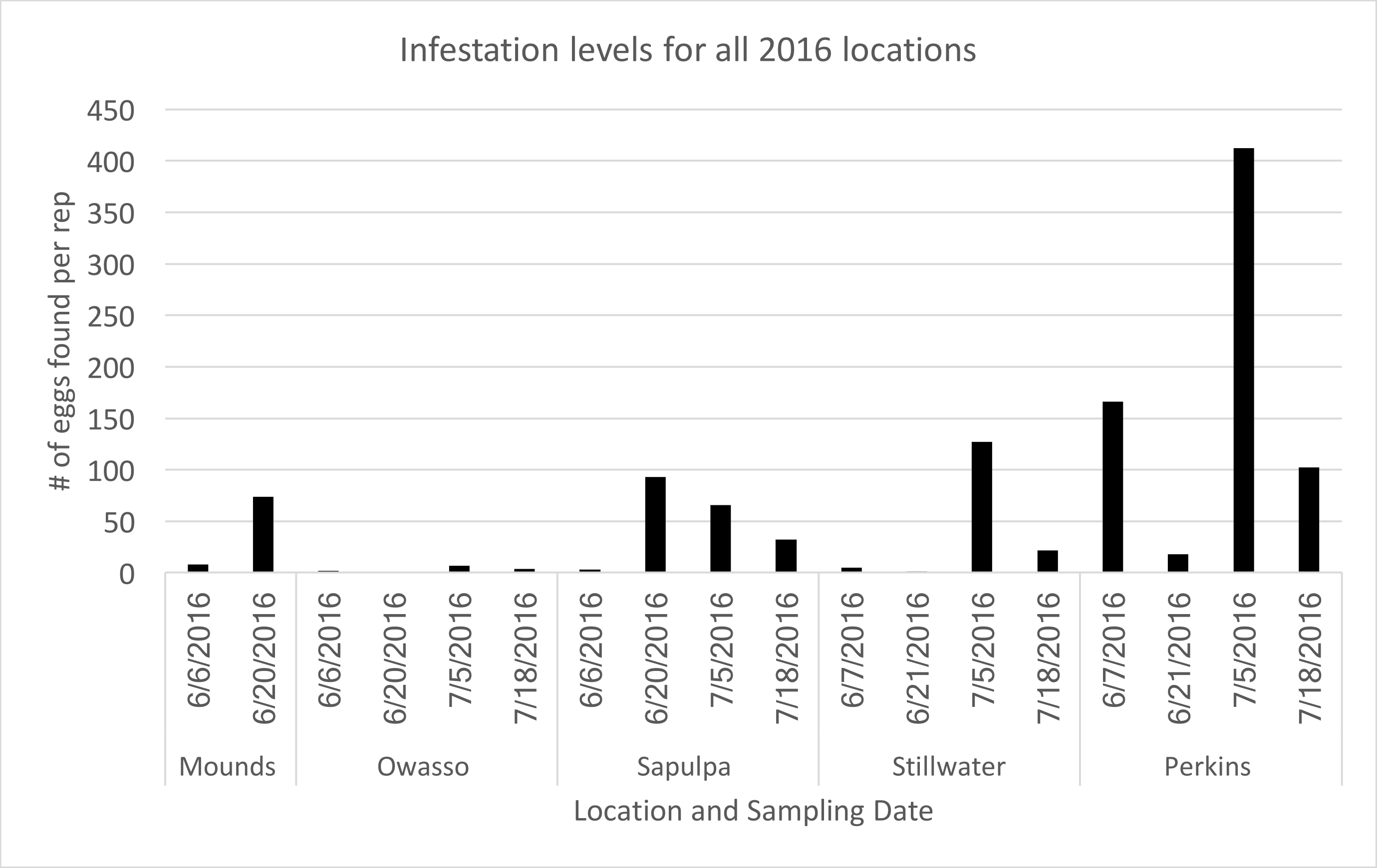


Figure 7. Egg infestation rates of berry reps taken from all 2016 sampling locations. Each sample date is one rep. One rep contained 20 ripe, untreated, blackberries.

CHAPTER IV

OBJECTIVE II: INVASION HISTORY OF SPOTTED-WING DROSOPHILA IN OKLAHOMA VIA GENENTIC ANALYSIS

INTRODUCTION

Globalization has facilitated travel and made trade easier for humans. It has allowed easier access to food from all over the world that was previously unavailable because of lack of transportation and technology. The amount of food the U.S. imports continues to increase each year. In 2013, the U.S. imported 635 billion pounds of food (USDA ERS - Import Share of Consumption 2017). Although many countries benefit from global trade, there are some drawbacks including an increased number of invasive species entering the U.S. each year (Hulme 2009, Pimentel et al. 2005). Many invasive species are accidently introduced with produce and other imported goods through shipping containers (Hulme et al. 2008). In 2006, more than 90% of global trade was carried by cargo-carrying ships (Hulme 2009). The frequency of trade and amount of food shipped through these cargo ships creates many opportunities for species to enter the U.S. undetected. An estimated 50,000 exotic species have been introduced and have become established in the U.S. Many more species than the documented 50,000 are introduced, but not all species can establish and survive. Only 4,300 species of the 50,000 (9%) have become invasive species in the U.S. (Pimentel et al. 2005). Invasive species have to overcome many challenges such as population bottleneck effects, from being rapidly introduced to a new environment (Fraimout et al. 2017). Species that are introduced into fragmented areas characterized by low biodiversity are more likely to survive, whereas established ecosystems present more of a challenge for successful establishment of exotic species (Sakai et al. 2001). In the U.S., invasive species are common in agriculture systems, which often consist of disturbed and fragmented landscapes that are simplified and ecologically compromised (BenDor et al. 2009).

Invasive species have severe economic impacts on agriculture, recreation, and forestry (Pimentel et al. 2005). Lee (2002) estimated that invasive species cause $137 million in damage and control expenses each year in the United States. They also create environmental problems by compromising existing ecosystems and outcompeting native species (Sakai et al. 2001). In 2005, 400 of the 958 species listed under the Threatened or Endangered Species Act were considered at risk because of being outcompeted by invasive species (Pimental et al. 2005).

SWD has become a very successful invasive species because of their adaptable traits and reproductive potential. Many insects have great potential to become invasive because of their high reproductive capacity and ability to evolve quickly under novel environmental pressure (Estoup and Guillemaud 2010, Sakai et al. 2001). As of 2005, 4,500 exotic arthropod species were introduced to the United States, of which 95% were introduced accidentally (Pimental et al. 2005). The small size of insects and other arthropods make it easy for them to stow away undetected on planes, cars, boats, and luggage.

In addition to small size, spotted-wing drosophila (SWD), *Drosophila suzukii*, is a successful invader because it is multivoltine and polyphagous, attacking 84 different host species (Gutierrez et al. 2016). Their small size limits their potential for natural dispersal, suggesting their global spread is mostly likely the result of shipment of infested fruit from Asia and Europe (Asplen et al. 2015).

Spotted-wing drosophila is native to East Asia and is a serious pest of soft fruit. Females possess a heavily sclerotized ovipositor that can cut into ripe or ripening soft fruit to lay their eggs. Oviposition and larval feeding are extremely damaging to infested fruit, which are further compromised by subsequent infection from bacteria and pathogens. Since its discovery in Japan in 1912 (Kawanza), it has spread to almost every continent. The invasive success of SWD has made it a global problem in fruit production areas. The spread of this pest is likely facilitated by movement of infested fruit, and increasing global temperatures (Haye et al. 2016). Analyzing pathways of SWD movement can help determine modes of transportation and introduction of this invasive species into new areas (Adrion et al. 2014). Understanding the introduction and colonization history of invasive pests can help identify prevention tactics for future spread of these harmful organisms (Fraimout 2015).

Although SWD is a large threat to fruit production around the world, not much is known about its colonization history. A rough outline of its invasion history can be determined by tracing back documented observations of SWD in new areas. Spotted-wing drosophila was first observed outside of Asia in Hawaii in 1980 (Adrion et al. 2014). Damage was first seen in Europe in 2009 in Italy (Cini et al. 2014). In 2008, SWD was found in California and Spain for the first time (Calabria et al. 2012, Hauser 2011). Since its initial discovery in the continental United States in 2008, it has spread to 41 states, southern Canada, and most of Europe (Cini et al.

2014). The invasive success of SWD in a wide variety of locations and climates provides evidence that it is a highly adaptable species. This is interesting considering SWD evolved in temperate climates in Asia and has been very successful in the Pacific Northwest, which is typified by high humidity and mild temperatures. Also, SWD has been observed successfully overwintering and reproducing in the northern United States and Canada where extremely cold winter temperatures are normal. In contrast, SWD has successfully invaded southern states, like Oklahoma, where summer months are extremely hot.

Invasive species that successfully establish in a new area exhibit genetic traits of plasticity and tolerance (Estoup and Guillemaud 2010, Lee 2002, Pimentel et al. 2005), and SWD is no exception. Population genetics can be used to determine invasion history of SWD and other pests. High genetic variability within a population suggests that the population is comprised of individuals introduced from different areas; hence, the population resulted from multiple introductions to the same location. Variation can also be a good indicator of genetic stability of reproductive populations (Adrion et al. 2014).

Spotted-wing drosophila belongs to the subgenus *Sophophora* and is closely related to *Drosophila melanogaster*, the model organism in animal genetics (Chiu 2013). There are 15 closely related species to SWD, but their genetic relationship has yet to be determined. Sequencing of the COI gene region and identification via morphological characteristics are both acceptable methods for differentiating SWD from similar species (Atallah et al. 2014).

Adrion et al. (2014) were among the first to attempt to understand the invasion history of SWD using genetic techniques by analyzing six X-chromosomal gene fragments from 246 individual flies collected from the eastern United States to determine genetic variability. Their results showed high nucleic diversity, suggesting that recent colonization was due to independent demographic colonization events. Fraimont et al. (2015) designed a set of 28 polymorphic microsatellite markers that could be used to determine invasion history of SWD. Using sample populations from Hawaii and France, they found these two populations could be differentiated genetically by these microsatellites.

Following Fraimont et al. (2015), I conducted a study to determine if SWD populations in Oklahoma were the result of multiple introductions through analysis of genetic variation among flies collected from three different sampling locations. Samples exhibiting missing microsatellite markers would confirm variation in population genetics. This will suggest that SWD populations from various Oklahoma locations were derived from multiple introductions over time rather than a single introduction with subsequent spread to other locations. I hypothesized that SWD populations in Oklahoma are the result of multiple introductions instead of a single introduction.

MATERIALS AND METHODS

For this study, 3 microsatellite markers identified by Fraimont et al. (2015) were used to determine the degree of genetic variation among SWD collected from Perkins, Stillwater, and Sapulpa, Oklahoma. These populations were selected because they were widely separated geographically. Thus, it was assumed that these populations were reproductively isolated and so did not exchange genes. Ten individual flies were selected from each location for genetic analysis. All individuals used for genetic analysis were collected from monitoring traps for the 2015 sampling season and subsequently stored in labeled collection tubes containing 70%

ethanol. All individuals were verified as SWD using morphological characters, which are commonly used and reliable traits for identifying adults in the field (Atallah et al. 2014).

Individuals selected for genetic analysis underwent DNA extraction for polymerase chain reaction (PCR) tests. A DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) was used to extract DNA. All of the buffers and solutions needed for DNA extraction were provided in the DNeasy Kit. Fly DNA was extracted following protocol from the kit, but slightly modified for a few steps. A single fly was placed in an individual 1.5-ml test tube that was labeled by sample location and a unique designation number. Tissue was prepared by pouring liquid nitrogen in the test tube and simultaneously grinding the fly into a pulp with a plastic pestle. Once the tissue was finely ground, 180 µL of Buffer ATL and 20 µL of Proteinase K were added to the tube. The sample was then placed into a 55 ºC water bath and held overnight. Following immersion, the sample was vortexed for 15 seconds to ensure all of the buffer and tissue were thoroughly combined. Once the sample was vortexed, 200 µL of Buffer AL was added. The sample was vortexed again for another 15 seconds. The sample was placed in a 70 ºC water bath for 10 minutes, then it was removed and 200 µL of chilled 100% ethanol were pipetted into the sample and vortexed for 15 seconds. All of the liquid solution in the tube was then pipetted out of the tube and pipetted into a labeled 2 mL Qiagen collection tube. The collection tube was then centrifuged at 8 x 1000 rpm for 1 minute. Once finished, the collection tube was then removed from the spin column and discarded. The spin column was placed in a new collection tube and 500 µL of Buffer AW1 was added. The sample was then centrifuged at 8 x 1000 rpm for 1 minute. Once removed from the centrifuge the spin column was placed in a new collection tube and 500 µL of Buffer AW2 was added. The sample was centrifuged at 13.2 x 1000 rpm for 3 minutes to dry the membrane. Once the sample was centrifuged the collection tube was tossed and the spin column was placed in a labeled 1.5-mL tube. Twenty µL of autoclaved water was then pipetted directly on the spin column and placed into the centrifuge for 1 min at 8 x 1000 rpm. This last step was repeated twice, resulting in a sample of 40 µL of DNA. The labeled 1.5-mL tube was then placed in a -20 ºC freezer to preserve the DNA product. This protocol was followed for all 30 samples.

The quality and quantity of the DNA product was tested using a NanoDrop 3300 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The DNA reader was cleaned with autoclaved water to ensure that there was no preexisting DNA on the reader. Then 1 µL of water was pipetted from the SWD samples onto the reader and measured as a blank. Samples were only used for PCR analysis once the reader determined each sample had enough DNA of sufficient quality.

Multiplex PCR was used to amplify DNA from all samples. Microsatellite marker primers were selected from Fraimont et al. (2015): DS26, DS07, and DS15 (Integrated DNA Technologies, Coralville, IA, USA). Autoclaved water was added to each primer to achieve 5 µM concentration. To determine the ideal melting temperature of all three primers, a gradient PCR was conducted for each primer to adjust the thermocycler settings to an optimal temperature. A temperature range of 50-60 ºC was tested against the primers. The results of our gradient PCR determined that 55 ºC was the optimal temperature to produce the strongest PCR bands. Once the optimal temperature was determined, a primer pool of all 6 forward and reverse primers was mixed so they could be all pipetted in the DNA samples at the same time. Fifteen DNA samples were processed at one time. A master mix product that equaled 17 samples was created in order to incorporate a negative sample and to account for pipetting error. For the primer pool, 17 µL of forward and 17 µL of reverse primers were added to the pool for all three primers. The master mix consisted of 34 µL of primers, 93.5 µL of autoclaved water, and 212.5 µL of Taq polymerase (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Five µL of DNA from each sample were added to a 0.6-mL PCR tubes labeled with the DNA samples and contained 5 µL of the respective DNA, then 20 µL of master mix was pipetted into the DNA and mixed with the pipet tip for a total product of 25 µL. The 15 finished samples were then transferred into a thermocycler set at 95 ºC for 5 minutes, 95 ºC for 15 minutes, 55 ºC for 20 minutes, 72 ºC for 30 minutes, and 72 ºC for one hour, and an idle temperature of 16 ºC once all 40 cycles were complete. While the thermocycler was running, a gel was prepared using 1.5% agarose and 3 µL of SYBR safe added to stain the gel. Once the gel was prepared, it was covered with foil and left to sit for 15 minutes. After the gel set, it was added into a gel electrophoresis tray with 1% TAE. Once the thermocycler was finished, the tubes were removed and set in a PCR tube holder and held at room temperature. A 1-kb ladder was pipetted into the first well of the gel, then each of the 15 PCR products were pipetted into individual wells, and the negative was pipetted into the last well. Electrophoresis was set to run for 60 minutes at 90 volts. Once the bands migrated through the gel, they were analyzed with Bio-Rad Image Lab$^{\text{TM}}$.

RESULTS

Polymorphism was found in microsatellite markers for 6 individuals out of the 30 tested. In the first 15 samples tested, Perkins 2 was missing one band, DS15. The Sapulpa population showed even more variation. Sapulpa 6, 8, and 10 were missing one band, DS15, and Sapulpa 7 was missing two bands, DS15 and DS07 (Figure 8). In the second set of 15 samples, Stillwater 6 had one missing band, DS15 (Figure 9).

DISCUSSION

This study was designed to be a preliminary examination of polymorphism across genetic markers among individuals collected from different locations in Oklahoma. No previous studies have been conducted on the genetic variation of SWD populations in Oklahoma or surrounding states. Oklahoma populations of SWD appear to have a high degree of genetic variability, given that a high proportion (20%) of individuals sampled exhibited genetic variation using only 3 markers. These results indicate there have been multiple introductions of SWD into the state. Multiple introductions of SWD are likely the result of importation of infested soft fruit into new areas (Haye et al. 2016).

Currently, the state of Oklahoma is not taking measures to prevent further introductions of SWD. This contrasts with protocol established to prevent the spread of other destructive plant pests both regionally and nationally. For example, the U.S. Domestic Japanese Beetle Harmonization Plan includes a prevention plan to reduce the artificial spread of the beetles to high risk states (APHIS 1998). One of the issues addressed by the Harmonization Plan is regulating movement of nursery stock among states. Protocol requires that all nursery plants be produced in greenhouses that are free of Japanese beetle or chemically treated for Japanese beetle prior to shipment out of state. Additionally, all nursery shipments must be authorized by Canadian Food Inspection Agency inspectors before being moved over the Canadian border. A similar USDA APHIS federal quarantine (7 CFR 301.81) helps control the spread of invasive red imported fire ants (RIFA) in nursery stock, hay, and earth-moving equipment. Currently, twenty southern Oklahoma counties are on the quarantine list to prevent further spread of RIFA into northern Oklahoma (USDA APHIS: IFA Interactive Map 2017). Similar protocols could be established to prevent spread of SWD into new areas via infested fruit. For example, in California check points are established along state lines where imported fruits and vegetables are subject to inspection for invasive arthropod pests and pathogens. Produce harboring any suspicious pests or disease organisms are confiscated (California Border Protection Stations 2017).

California, Oregon, and Washington produce the majority of soft fruit crops in the U.S., so preventing the spread of SWD within these states is vital. These three states stand to lose $500 million dollars each year resulting from SWD damage (Langille et al. 2016). Oklahoma does not produce the same volume of soft fruit crops commercially as these western states. However, "U-pick" blackberry farms are common in Oklahoma because blackberries grow so well in Oklahoma (Carroll 2017). Most states east of the Rocky Mountains have similar small-scale growing operations, with the exception of commercially produced fruit in Florida and North Carolina (strawberries), Michigan, New Jersey, North Carolina, Georgia, and Florida (blueberries) (Asplen 2015). The presence of SWD has threatened the fruit production of family-owned farms in these states. Most of these locally owned soft fruit producers are pressured to produce sustainably and/or organically grown berries for consumers (Asplen et al. 2015).

A shift in management plans has been observed throughout the U.S. in small scale and commercial producers in order to manage the pest effectively (Asplen et al. 2015). Growers who have been producing blackberries for many years have never faced a pest as devastating as SWD. Because SWD is a relatively new pest to the U.S., there is a knowledge gap about SWD and methods needed for its control. A lack of knowledge coupled with "pesticide free" expectations from "U-pick" consumers have created a dilemma for growers. Local producers now have to budget for weekly or biweekly pesticide applications in order to have undamaged berries. Some growers are limited to using only pesticides approved by the Organic Materials Review Institute (OMRI) to market their product as organic. Profit margins can be impacted severely because of the amount of pesticide needed to control SWD effectively (Iglesias and Liburd 2016). Although pesticides are the only widely accepted method of control for SWD, frequent use of pesticides will likely result in resistance evolving in SWD populations (Haye et al. 2016).

For future work, I plan to increase the number of microsatellite markers tested to 5 in an attempt to detect more genetic variability in the sampled SWD populations. High Resolution Melting will also be tested on PCR band products to determine the loci where genetic variability is occurring in target populations. This is determined through melt curve analysis, where curves can detect small differences in melting temperature, indicating variations in nucleic acid sequences (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

These current and future genetic studies will give insight into the invasion pathways of SWD and inform development of future control measures in Oklahoma. Oklahoma is a unique state because it is positioned in the middle of the western and eastern states of the U.S. This could play a role in the genetic diversity of SWD populations in Oklahoma because SWD are being introduced from both sides of the U.S. Coastal eastern states like Florida are most likely where SWD were introduced by fruit shipments (Walsh et al. 2014), then spreading to surrounding states, eventually making their way to Arkansas. On the opposite side of the country, California was the first mainland state to identify SWD (Bolda et al. 2010). The populations quickly began to spread to nearby states such as Oregon, Idaho, Colorado and Montana. In 2012, SWD was found in Arkansas and Colorado (Johnson and O’Neil 2013). The spread to Arkansas is most likely due to populations of SWD in Tennessee being accidently introduced into the state by humans or SWD being carried by wind. Arkansas and Colorado border Oklahoma, and could have been the source of introduced SWD populations in Oklahoma. The Arkansas border is around 86 miles east Tulsa, OK where 3 of my sample locations were. Stillwater locations were located about 155 miles west of Arkansas. Transportation of infested fruit from Arkansas to Tulsa could possibly be more frequent than transportation of infested fruit to Stillwater because of the distance. It is possible that SWD could have been introduced to Oklahoma from Colorado as well, although travelers are most likely travel through Kansas for the shortest route. SWD was detected in Kansas in 2013, the same year SWD was detected in Oklahoma (Everman et al. 2015). Northern central Oklahoma locations, such as Stillwater, most likely have SWD as the result of Kansas and Colorado populations being transported accidently.

There are currently 76 local farmer's markets across the state of Oklahoma (OK Grown, 2017). Although all produce sold at these markets is grown in Oklahoma, there are no laws in place that prohibit consumers from transporting potentially infested berries throughout and outside of the state. Buying fruit from markets that are seemingly undamaged, may be harboring unhatched SWD eggs. The accidental transport of the eggs aid in the spread of SWD to areas where it is not already established. Ideally, federal quarantine plans similar to those developed for Japanese beetle and RIFA could be established to help reduce risk of human-assisted movement of SWD. Since SWD populations are constantly being reintroduced into new areas across the U.S., determining pathways of transport is vital to protect susceptible crops grown in areas not infested with SWD. Currently there is a zero tolerance policy for larvae in fruit shipment. If a single fruit is determined to be infested in a whole shipment, all of the fruit will be rejected (Aly 2017). Dumping rejected fruit shipments into the environment does not provide a solution to the spread of this pest, it only allows the eventual release of SWD adults into the environment. These rejected fruit shipments should be disposed of properly to prevent further SWD spread. Infested fruit should be placed in plastic bags or submerged in salt water to kill potential emerging larvae. Irradiation treatments for fresh fruit shipments have been proposed since the treatments have been proven effective in a lab setting (Follett 2014). Cold storage of fruit has also been shown to reduce survival of immature SWD (Aly 2017). These methods could potentially be used as future control plans to prevent the spread of this invasive pest across the United States and the world.

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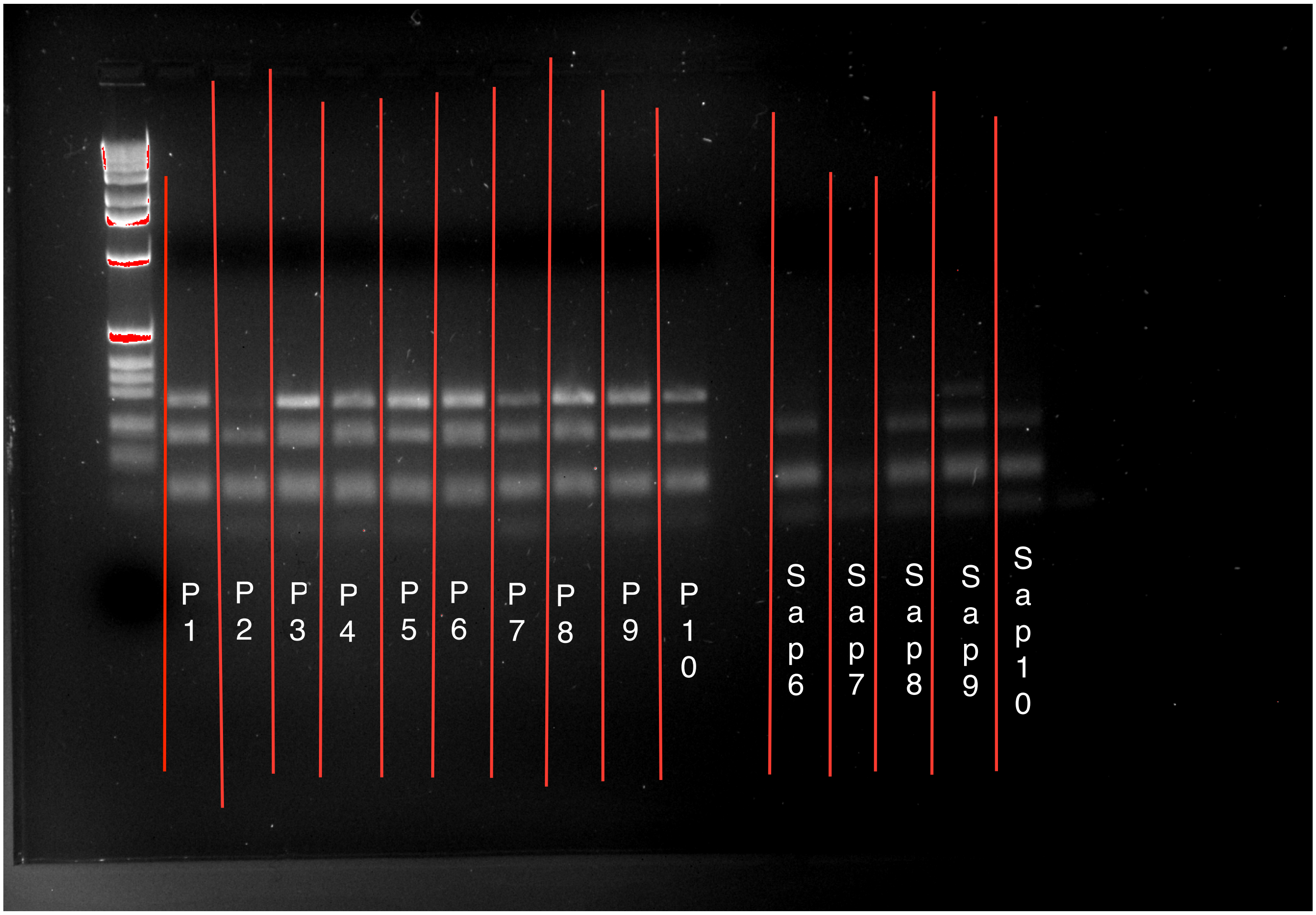

Figure 8. Electrophoresis results for individuals 1-10 from Perkins, and individuals 6-10 from Sapulpa.

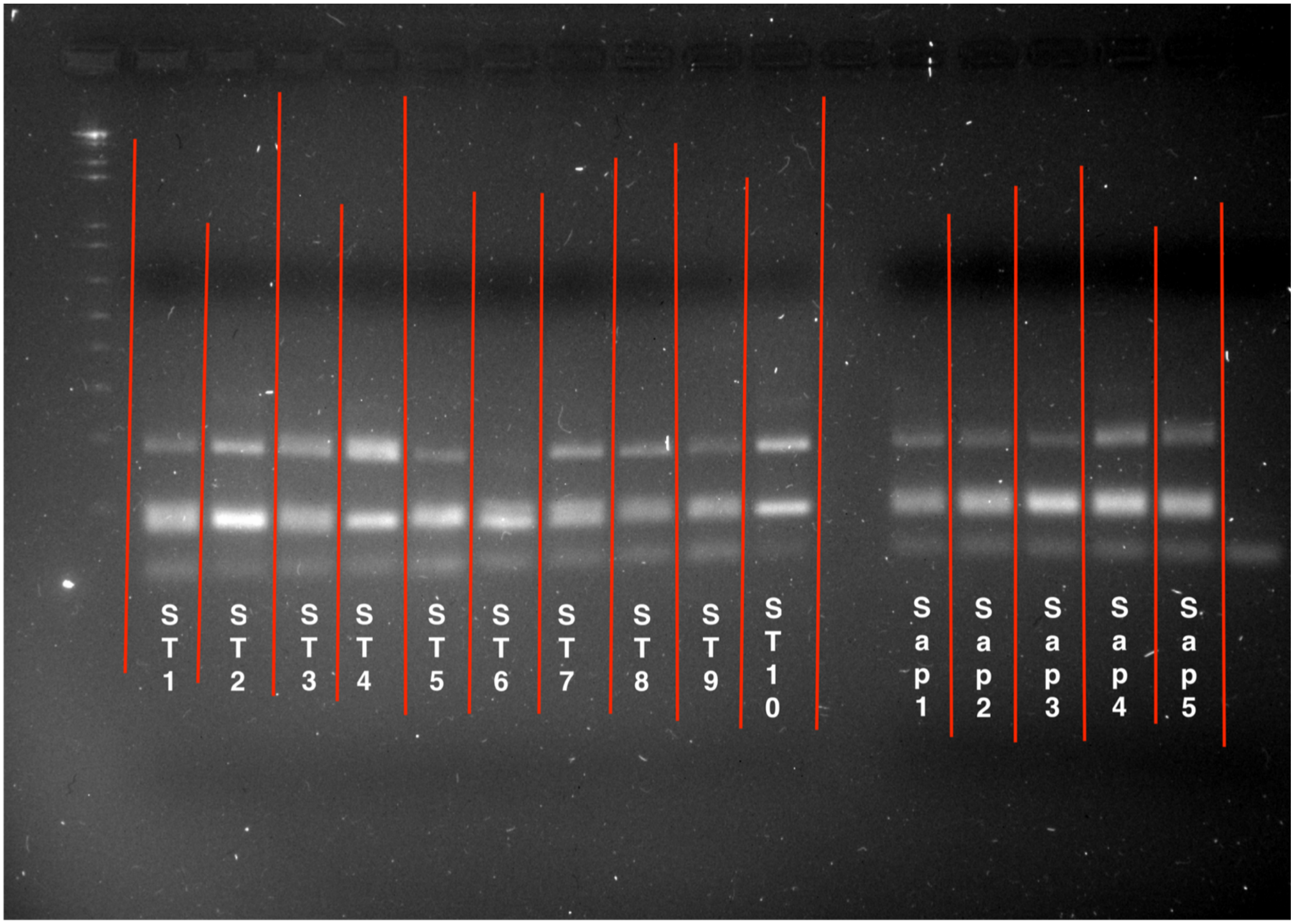

Figure 9. Electrophoresis results for individuals 1-10 from Stillwater, and individuals 1-5 from Sapulpa.

CHAPTER V

MANAGEMENT IMPLICATIONS

Spotted-wing drosophila is a threat to blackberry and blueberry production in Oklahoma (Mulder et al. 2013). The objective of this research was to gain a more comprehensive view of Oklahoma SWD populations and the biology of this economically important pest of soft fruits. Thus, comprehensive population monitoring was conducted and trap counts were compared to abiotic factors to see if any relationships existed. Oklahoma blackberry cultivars were also examined to determine which cultivars were the most susceptible to SWD infestation. Vegetation composition and alternative host species were observed to see how SWD uses the surrounding vegetation, which may affect its population size. Information obtained from this study will be used to help soft fruit growers in Oklahoma more effectively manage this pest.

My data suggest that monitoring traps placed in tree lines surrounding orchards capture more SWD than traps placed within blackberry crops. Significantly more SWD were caught in tree line traps compared to blackberry traps over the course of two

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sampling seasons. This suggests that SWD spends the majority of its time in surrounding wooded habitat, only leaving tree cover to oviposit in nearby blackberries and other crops as they ripen. Hampton et al. 2014 found that deploying SWD traps in soft fruit crops attracts more SWD to the crop, resulting in increased infestation of fruit near the traps.. Thus, an attract-and-kill strategy could be used against SWD by deploying numerous traps in tree lines, reducing infestation of susceptible crops. Spotted wing drosophila are most likely using tree lines adjacent to soft fruit crops for food, shelter, and overwintering sites (O'Connell and Keppel 2016).

Although there was no statistically significant relationship between temperature and SWD abundance, lower trap counts were observed during the hottest days of the summer. The smaller sample size in 2015 may have accounted for lack of correlation between temperature and SWD populations, but an increase in humidity was significantly correlated with decreasing SWD abundance. Spotted wing drosophila populations are known to be affected by fluctuating temperature and humidity. When temperature and humidity increase, flies seek out tree lines and vegetation cover for refuge (Arno 2016). This behavior is evident when analyzing my trap counts from 2015 and 2016. No significant correlation was found between habitat type and overall SWD numbers trapped at sampling locations, consistent with results reported by Pelton et al. (2016). However, my results suggest that tree cover provides suitable overwintering habitat for SWD, which can produce a higher population density of flies early in the season. Thus, growers of early-season blackberry cultivars surrounded by at least 30% tree cover should be aware that they will most likely experience greater numbers of early SWD generations compared to sites with less tree cover. Higher numbers of flies earlier in the season could result in a greater rate of infestation in early-season fruit. Growers that produce late-season cultivars such as Triple Crown and Apache shouldn't worry about early infestation due to tree cover, but should be prepared for infestation rates to increase later in the season, considering these are the last blackberry cultivars left for SWD to use. Even though blackberry orchards with little to no tree cover are less likely to see large numbers of early SWD generations, growers should still monitor for SWD due to increasingly warm spring temperatures.

All six blackberry cultivars tested (i.e., Chickasaw, Apache, Natchez, Ouachita, Triple Crown, and Tupy) were susceptible to SWD infestation. Chickasaw, Natchez, Ouchita, and Tupy are considered early-season cultivars, and Apache and Triple Crown are considered late-season cultivars. My results suggest there is no difference in susceptibility to SWD between early- and late-season blackberry cultivars. Extremely high egg loads were observed in early-season cultivars when fruit was at a susceptible stage of ripening. Similarly, egg loads were high in late-ripening cultivars as early-season fruits were done producing. The ripening times of these cultivars may be delayed if grown in the Upper Midwest and other northern states because of colder temperatures (Pelton et al. 2016). Rhode Island has seen success in growing early-season blueberry cultivars before SWD populations are at their peak, greatly reducing the rate of infestation (Hampton et al. 2014). Since Oklahoma has high spring and summer temperatures, SWD are observed as early as the beginning of May. Unfortunately, there are no blackberry cultivars grown in Oklahoma that produce in March and April as most early-season cultivars start producing during the first week of June.

Unlike blackberries, grapes produced in Oklahoma do not appear to be susceptible to SWD. In 2015, 10 different grape cultivars were sampled from July 20 to September 11 to determine if SWD were infesting grapes. Every week, 200 grapes from various cultivars were examined for SWD egg presence, and none were found to have SWD eggs. This was surprising because SWD adults were found in monitoring traps placed within two Oklahoma vineyards.

This could be due to the skin thickness of grape cultivars grown in Oklahoma. Thus, Oklahoma viticulture does not appear to be under threat from SWD, although wine and table grape producers should take care to reduce damage to fruit during harvest or from birds and insects such as green June beetle, thereby minimizing susceptibility to SWD and other fruit flies.

Surrounding woodlots support several native alternative host plants. I found pokeweed (*Phytolacca americana* L.*),* wild blackberry (*Rubus flagellaris* L.H. Bailey), and red mulberry (*Morus rubra* L.) to be viable host plants for SWD. Availability of alternative host plants is an aspect of management that should not be overlooked. Physical removal and herbicide applications can target these plants if growers have access and authority to control vegetation in surrounding landscapes. Although I sampled 97 host plant species, the list of alternative host plants used by SWD in Oklahoma is not exhaustive, and further research is required to elucidate all potential hosts of SWD. Additionally, alternative host plants could be used as trap crops to reduce SWD infestation in the desirable crop. Integrating trap crops into a blackberry orchard would be labor intensive, but the potential benefit to reducing crop damage may be worth further investigation. Trap crops could reduce pesticide applications targeting SWD within the crop and provide habitat for beneficial insects (Heikki and Hokkanen 1991).

Managing native host plants and modifying trapping methods could be key aspects to a comprehensive IPM program for SWD. Implementing monitoring techniques is the most important aspect for effectively controlling SWD in Oklahoma. Deploying monitoring traps early in the season around the last week of March or the first week in April is recommended because the first SWD generation was observed in traps deployed on May 10, 2016. Traps should be checked weekly during these months to help determine a more accurate emergence date. Due to the very mild winter of 2017, it is likely that SWD will be emerging even earlier than 2016.

Growers should be one step ahead of the pest to ensure initial populations are detected. Once the initial detection occurs, growers should be prepared to apply their selected chemical control plan with rotation to prevent insecticide resistance developing in target populations of SWD. Monitoring traps equipped with Trécé lures (Trécé Inc., Adair, OK) and apple cider vinegar should be applied in the surrounding tree lines and not within crops. As of now, alternative host plants could potentially be sprayed with herbicide or removed for a short-term remedy to reduce resources available to emerging flies. However, SWD will probably find additional hosts that have yet to be determined as alternative hosts. If future studies show that trap cropping with alternative host plants is an effective management tool, it could be recommended as a potential cultural management approach for Oklahoma growers.

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VITA

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Master of Science

Thesis: ABIOTIC AND LANDSCAPE FACTORS THAT AFFECT SPOTTED-WING-DROSOPHILA (*DROSOPHILA SUZUKII*) POPULATIONS IN OKLAHOMA

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Haley E. Butler grew up in the suburbs of Washington D.C. until she was 11 and her family moved to the small town of Ottawa, KS. As a young girl she was always fascinated in animals. After graduating from high school in 2011, Haley attended Oklahoma State University to pursue a B.S. degree in Wildlife Ecology and Management. Every summer of her undergraduate career, Haley held internships positions relating to her major to broaden her knowledge and experiences in research and conservation. After interning as a biological technician for the U.S. Fish and Wildlife Service, she was offered an undergraduate research position in the Department of Entomology and Plant Pathology. She was hired by Dr. Ali Zarrabi to help with research on aphid resistant crop varieties. Her research opportunity motivated her to pursue a Master's in Entomology at OSU in 2015. She gave multiple presentations around the country during her two-year Master's program, including the 2016 International Congress of Entomology in Orlando, Florida. After obtaining her Master's degree, Haley is leaving for Paraguay in September 2017 to join the Peace Corps as a Protected Areas Management volunteer.

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