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## Seasonality and Management of Spotted Wing Drosophila on Berry Crops and Wild Hosts in Arkansas

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Seasonality and Management of Spotted Wing Drosophila on Berry Crops and Wild Hosts in  
Arkansas

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Entomology

by

Lizabeth Herrera  
Tarleton State University  
Bachelor of Science in Wildlife Management, 2015

December 2017  
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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## Abstract

Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) is a serious invasive pest of small fruit production in North and South America and Europe since 2008. The primary control method is to apply insecticides every 5-7 days. Therefore, it is necessary to develop control tactics that are less chemical dependent to enhance an integrated approach for SWD management. The objectives of this study were to monitor SWD populations in different crop systems and adjacent landscape habitats; identify wild hosts of SWD; evaluate the effectiveness of insect exclusion netting in tunnels to prevent blackberry and blueberry infestations, and compare effects of netted tunnels on temperature and fruit quality. The majority of seasonal averages of SWD were lower in the traps placed in fruit crop plots than in the perimeter traps located next to a refuse pile of culled fruit and mulch than the traps located in host crop species. Of the potential wild fruit hosts sampled in Arkansas, these 12 had SWD infested fruit: wild blackberry and dewberry (*Rubus* spp.), American pokeweed (*Phytolacca americana* L.), black cherry (*Prunus serotina* Ehrh.), Carolina buckthorn (*Frangula caroliniana* (Walter) A. Gray), porcelain berry (*Ampelopsis glandulosa* var. *brevipedunculata* (Maxim.) Momiy), amur honeysuckle (*Lonicera maackii* (Rupr.) Herder); autumn olive (*Elaeagnus* spp.); elderberry (*Sambucus* spp.); mulberry (*Morus* spp.); native honeysuckle (*Lonicera* spp.); and Carolina moonseed (*Cocculus carolinus* (L.) DC.). These SWD hosts ripened from early June into October. Tunnels with insect exclusion netting excluded SWD fly entry and prevented fruit infestations in 2016 and delayed SWD infestations in 2017. For 2016 and 2017, the seasonal total number of hours of SWD lethal temperatures inside the tunnel treatments (netted high tunnel = 69.4, 50.8, plastic low tunnel = 58.6, 68.0 and netted low tunnel = 54.6, 41.7) were slightly warmer than in the uncovered plot (53.4, 32.6). Low percentages of relative humidity

(<65%) appeared to play an important role in the differences in fruit quality among the treatments. Netted low tunnel blackberries had significantly lower fruit firmness (6.9) than all other treatments (8.3, 7.7, 7.4). The uncovered plot had a significantly higher Brix (11.52) compared to the other treatments (9.31-9.68). It appears that the tunnels slightly lowered Brix levels with the recommended quality range being 10-12%. All netted blackberries in high (0.62) and low tunnels (1.11, 0.86) had significantly lower titratable acidity than the uncovered plot (1.49) indicating a slight reduction in fruit quality. There is potential for exclusion to be an effective control method against SWD, but additional studies should be conducted to explore modifications as well as the economics of building and implementation of exclusion netting into a management program. Understanding the seasonal phenology of alternative hosts and when SWD exploit them can help predict when and where populations may establish.

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## **Dedication**

I would like to dedicate this to my mother, Lupe Herrera

I love and miss you every day

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## CHAPTER 1: Literature Review on Biology and Control of Spotted Wing *Drosophila*

### History

*Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), more commonly known as spotted wing drosophila (SWD), is an introduced invasive species that has caused significant damage in the production of soft-bodied fruits in the continental U.S., Canada, Europe and Asia (Hauser 2011). Originating from Southeast Asia, the earliest records of this pest date back to 1916 in Japan. However, according to Kanzawa (1936), there is a possibility that spotted wing drosophila was introduced into Japan at the turn of the century. It wasn't until 1931 that *D. suzukii* was first described in Japan (Honshu: Kyoto and Aomori) by Matsumura, with reports of damages published just a few years later (Kanzawa 1936, 1939).

The first detection of *D. suzukii* in the United States was in Oahu, Hawaii, in 1980 and soon after, it was recorded in a few other Hawaiian Islands with no reports of substantial damage (Kaneshiro 1983, Nishida 1997, Beardsley et al. 1999, O'Grady et al. 2002). In 2008, the first mainland record was made in Santa Cruz County, California, collected in brambles and strawberries (Bolda et al. 2010). However, at the time of collection, the specimen was only identified to family (Drosophilidae), since no member of this group had been considered a pest except for the African fig fly, *Zaprionus indianus* Gupta. In 2009, there was an accumulation of reports of massive infestations of *Drosophila* larvae in cherries. The report raised concerns that the previously perceived harmless genus (*Drosophila*) may have been the primary cause and resulted in correctly identifying this species by using morphological characteristics based on the abundance of adult samples. By this point in time, *D. suzukii* had spread to over 20 counties in California and could also be found in Oregon, Washington, British Columbia, and Florida, exemplifying this species ability to disperse (Hauser 2011). As of 2015, spotted wing drosophila

has established itself across most of the contiguous United States, except in Arizona and Nevada due to the hot dry climate (Beers et al. 2010, Hadi 2013, Carroll and Peterson 2014, Grasswitz 2015). Globally SWD has expanded worldwide with records in Asia, North America, South America and Europe. This rapid expansion is mainly due to the cryptic nature of the larvae within the global fresh fruit trade, along with the lack of regulation of imported *Drosophila* species (Claudio et al. 2013). A model developed by dos Santos et al. (2017) predicts potential future invasions in Africa and Australia due to the environmental suitability of these areas (dos Santos et al. 2017).

## **Identification**

*Drosophilidae* is a family of flies referred to as vinegar flies that have two or fewer wing spots compared to the “true” fruit flies in the family *Tephritidae* that have colorful wing markings. Vinegar flies lay eggs on ripening, ripe, fermented or damaged fruits and vegetables while *Tephritids* lay eggs inside green to overripe fruit (Jacobs 2013). Of the estimated 1,500 species of *Drosophila*, *D. suzukii* is one of two unique species (*D. pulchrella* Tan being the other) known to oviposit in ripe (healthy) fruit. The oviposition behavior is unlike that of other vinegar flies that lay eggs on overripe or damaged fruit (Sasaki and Sato 1995, 1996). Adults are small (2-3 mm), have light yellow to brown bodies and red eyes. Their abdomens are rounded with dark unbroken bands across the abdominal segments. The wing cross veins of SWD are sharp and distinct compared to a “cloudy” appearance seen in other *drosophilid* species (Walsh et al. 2011, Van Timmeren et al. 2012). Both sexes also have distinct features from North American *Drosophila* species that make for simple identification.

The female SWD is equipped with a long sclerotized serrated ovipositor, allowing the utilization of more firm (pre-ripe) fruits for oviposition. The serrated ovipositor is a quick and

easy identifier. However, care should be taken while identifying SWD because other *Drosophila* species located in other countries share similar ovipositors with the possibility of also being invasive to North America. The most accurate method for confirming identification of female SWD is by removing and clearing the abdomen in KOH for the comparison of the spherical, mushroom-cap-shaped spermathecae to the size of the ovipositor. While most other *Drosophila* species have relatively small proportions, SWD has an ovipositor that is roughly 6-7 times as long as one spermatheca (Hauser 2011).

Male SWD have two main identifying characteristics; one conspicuous black spot on the first costal vein of each wing tip and two sets of black tarsal sex combs each with single row of three or four teeth (Van Timmeren et al. 2012). With teneral specimens, the black spot may not have developed yet or in rare instances small adults may not possess the spot at all. There are related species in the *suzukii* subgroup that share similar qualities that could be mistaken for *D. suzukii*. *Drosophila subpulchrella* Takamori and Watabe has a similar black spot on wing tip but has two transverse rows of sex combs (Hauser 2011).

While the adults are the easiest to identify, eggs can also act as an indicator of their presence. SWD eggs are translucent and milky-white with two straight respiratory filaments on one end, which provide oxygen to the egg during development inside the fruit (Hauser 2011, Walsh et al. 2011). Other drosophilid species such as *Zaprionus indianus* have four or more straight filaments while *Drosophila melanogaster* has two clubbed filaments. Species identification of larval and pupal stages is difficult since each closely resembles related species. However, if drosophilid larvae are inside undamaged fruit, they will most likely be SWD (rearing to adult is required to confirm species identification) (Walsh et al. 2011).

## **Life Cycle**

According to the original study by Kanzawa (1939), SWD completed its life cycle in 8-11 days. That study showed the egg stage lasted 1-3 days, the three larval stages lasted 3-13 days and the pupal stage averaged 4.5 days (Kanzawa 1939). Adults can live for 3-9 weeks, but can also overwinter for many months (Walsh et al. 2011). Development also varies depending on temperature and on the type of fruit (Walsh et al. 2011, Tochen et al. 2014, Hamby et al. 2016). Adult SWD can reach sexual maturity and begin mating one to two days after emergence. A female can lay 1-3 eggs per oviposition site up to 380 eggs in a lifetime. Multiple females can lay eggs in the same fruit (Walsh et al. 2011, Cini et al. 2012). Crepuscular hours are the preferred egg-laying period for SWD when temperatures are low and relative humidity is high (Wallingford et al. 2017).

The optimal temperature range for SWD varies between 20-27°C, but recent observations have shown development occurring at temperatures as low as 11.4°C and as high as 30°C. However, development periods decrease as temperatures reach lower and upper development thresholds (Tochen et al. 2014, Hamby et al. 2016). High humidity has shown to be more suitable for SWD longevity and reproduction, with the highest rate of increase recorded at 94% relative humidity. SWD survive longer at high levels of relative humidity and lay significantly more eggs. This relationship with relative humidity may be due to the effects moisture has on nutrient availability (Tochen et al. 2016). It is clear that temperature and relative humidity play a key role in SWD behavior and development.

## **Degree Days**

Insect development patterns can be predicted due to the consistent amount of heat accumulation required for an insect to reach specific life stages. The effect temperature has on

development was incorporated into degree-days models. Degree-days are a measurement of heat units over time, calculated by taking the average daily temperature (daily max temperature + daily minimum temperature divided by 2) minus the lower developmental threshold (Murray 2008). For SWD, the lower developmental threshold is 10°C and the upper being 31°C. SWD also displays an increased level of reproductive maturity as degree days accumulate in a potential reproductive range from 50-800-degree-days in the field. This model estimates the physiological timing of SWD egg laying and aids in timing treatment in an integrated pest management (IPM) program (Murray 2008, Tochen et al. 2014, Wiman et al. 2016).

## **Hosts**

Being an extremely polyphagous frugivore is a contributing factor to the spread of SWD. SWD utilizes a wide range of cultivated and wild hosts (Lee, Bruck, Dreves, et al. 2011, Cini et al. 2012). Soft-bodied fruits are the most susceptible to SWD infestation as studies have shown that ripening or ripe fruit with lower surface penetration force (softer skin) and high sugar content (Brix) are two of the preferred host qualities for SWD development (Burrack et al. 2013, Little et al. 2017). The following is a list of confirmed hosts of SWD: strawberry, caneberries, marionberry, sweet cherry, blueberry, buckthorn, Surinam cherry, orange jasmine, Chinese bayberry, honeysuckle (*Lonicera* spp), *Berberis aquifolium* Pursh, Oregon grape; *Cornus* spp., dogwood; *Cotoneaster lacteus* W.W. Smith, milkflower cotoneaster; *Elaeagnus umbellata* Thunberg, Autumn olive; *Frangula purshiana* (de Candolle) A. Gray, cascara buckthorn; *Lindera benzoin* (L.) Blume, spicebush; *Lonicera caerulea* L., blue honeysuckle; *Morus* sp., mulberry; *Phytolacca americana* L., pokeweed; *Prunus avium* (L.) L., wild cherry; *Prunus laurocerasus* L., cherry laurel; *Prunus lusitanica* L., Portuguese laurel; *Rubus armeniacus* Focke, Himalaya blackberry; *Rubus spectabilis* Pursh, salmonberry; *Sambucus nigra* L., black

elderberry; *Sarcococca confusa* Sealy, sweet box; *Solanum dulcamara* L., bittersweet nightshade; and *Symphoricarpos albus* (L.) S.F.Blake, snowberry (Kanzawa 1939, Walsh et al. 2011, Cini et al. 2012, Lee et al. 2015). However, damaged, dropped or split fruit that has a higher penetration such as apple, apricot, currant, fig, grape, hardy kiwi, peach, persimmon, plum, and tomato allow SWD entry without the need to oviposit under the skin (Lee et al. 2011a). Mixed crop settings of susceptible fruit can increase populations exponentially by providing greater host resources and staggered growing seasons (Grant and Sial 2016).

While fruit crops are the most important economically, alternative wild hosts also play a significant role in the population dynamics of SWD. Landscape habitats near field crops possibly contain alternative hosts and support SWD populations (Lee et al. 2015, Klick et al. 2016). SWD dispersal between fruit plantings and surrounding wild host plants in borders with a variety of ripening times provides SWD a refuge from insecticides applied to fruit crop and alternative food source before and after the crop harvest period. Many pests are dependent on both the crop and landscape habitat to sustain populations (Ricci et al. 2009, Mitsui et al. 2010, Klick et al. 2016). Alternate hosts also provide resources for overwintering populations. Furthering our understanding of the dynamics between crop and adjacent wild host plants could be beneficial in the design and implementation of long-term integrated pest management strategies.

## **Damage**

With the help of an inconspicuous life cycle, the initial infestation of SWD is often difficult to detect. The third instar is the most significant larval stage and causes the most damage through fruit collapse and leaking (Leach et al. 2016a). Oviposition can also physically damage the fruit, allowing for the introduction of secondary infections of pathogens, fungi, yeasts, and bacteria that result in accelerated decay and overall yield loss. This rapid

deterioration also looks similar in appearance to natural decay, making it difficult to associate SWD as the cause (Cini et al. 2012, Leach et al. 2016a).

Fresh and processed fruit are held to strict quality standards and, as such, presence of or damage caused by SWD can result in the rejection of an entire shipment of fruit as there is a zero-tolerance for this pest. SWD infestations can be economically detrimental to small fruit production not only in yield loss but also increased labor and pest control costs. Other countries have also experienced heavy losses due to SWD (Goodhue et al. 2011, Follett et al. 2014, Hampton et al. 2014, Farnsworth et al. 2017). In 2010, southern France suffered losses of up to 80% in strawberry crops and 30-40% loss of essential crops were experienced in Italy (Lee et al. 2011b).

Walsh et al. (2011) and Bolda et al. (2010) were the first studies done in North America to estimate the economic effects of SWD. The first thing to consider was the observations and evaluated yield loss of strawberry, blueberry, raspberry, blackberry and cherry in the significant fruit production areas (California, Oregon, and Washington) where the first introductions of SWD occurred. In 2011, the studies estimated a 20% yield loss in those significant fruit production areas. Excessive damage could result in a combined yearly revenue loss of US \$33.4 million for strawberries, US \$56.7 million for blueberries, US \$156.6 million for caneberries and US \$174.8 million for cherries (Goodhue et al. 2011, Walsh et al. 2011, Farnsworth et al. 2017).

### **Conventional Insecticides**

Currently, insecticide applied every five to seven days is the primary control method for SWD in the United States. These sprays prevent egg laying and are synchronized with the presence of SWD captured in baited traps and fruit ripening until the end of harvest (Beers et al. 2010, Bruck et al. 2011, Asplen et al. 2015). There are several classes of contact insecticides that

are effective against SWD. Systemic insecticides have little efficacy on SWD adults. Organophosphates (inhibit acetylcholinesterase) have been shown to be the most effective against SWD (Smirle et al. 2017), pyrethroids (sodium channel modulator), carbamates (acetylcholinesterase inhibitor) and spinosyns (nicotinic acetylcholine receptor allosteric modulator) are also effective and commonly applied. Since adults utilize plant canopies for refuge, good spray coverage is critical in managing SWD (Tochen et al. 2014, Schattman et al. 2015). When developing a spray program, compounds are rotated with different modes of action to delay or prevent the development of resistance to insecticides. There is confirmation that SWD can develop resistance due to increased detoxification and insensitivity of target sites. Conventional insecticide recommendations pose a problem for IPM programs because they are mainly broad-spectrum insecticides that growers rely on to produce a marketable yield. Frequent sprays are also costly, however other IPM tactics are not as well developed to supplement or eliminate chemical treatments (Bruck et al. 2011, Van Timmeren et al. 2012).

### **Organic Insecticides**

To date, biological control has not provided adequate reductions of SWD using parasitoids since parasitism rates are low (Fisher 2014). Instead, organic fruit production relies heavily on one effective insecticide, Entrust™ (spinosad). Pyrethrins are also used but aren't as effective, requiring more frequent applications with no residual control, compared to spinosads that provide 5-14 days of residual control. The dependency of spinosads in organic control could quickly lead to resistance due to the lack of organically approved insecticides that possess different modes of action for rotation (Bruck et al. 2011a, Schattman et al. 2015).

Since SWD populations can be supported in surrounding habitats, a border insecticide application has been evaluated as an additional IPM strategy. Timing is critical when planning



border spray programs, and could be most effective at the beginning of the season as flies begin to disperse from overwintering sites into the field. Early season applications can prolong the use of the more efficient insecticides that have limited applications for when the need for applications is higher, such as peak harvest when SWD pressure is highest. Border sprays have the potential to reduce the overall cost of management and a number of organic insecticides used in the field while also decreasing SWD populations (Iglesias and Liburd 2017).

### **Cultural Control**

One aspect of control that should be implemented across conventional and organic management is cultural control. With the demand for natural products rising comes the need for organic-approved methods to be developed to incorporate into an efficient and sustainable IPM program (Van Timmeren and Isaacs 2013, Pelton et al. 2017). Cultural control is modifying a pest's environment or habitat as a method of control (Meyer 2016). Cultural control can begin as early as selecting a cultivar that completes harvest before SWD populations begin to build (Hampton et al. 2014). Other horticultural practices such as pruning are essential because studies have shown that high infestations of SWD can be found in the canopy of the plant. Pruning opens up the canopy and reduces favorability by facilitating airflow and humidity around the crop (Fisher 2014, Diepenbrock and Burrack 2017).

Sanitation is one of the most critical methods of cultural control for SWD. It consists of removing any fruit that can be utilized by the pest to sustain a population. Frequent harvest reduces the number of susceptible fruit exposed to SWD to reduce populations and contain outbreaks. SWD prefer to oviposit in the lower hanging fruit within the plant canopy. Therefore thorough harvesting within the canopy is as important as harvesting the easily accessible fruit (Liburd and Iglesias 2013, Diepenbrock et al. 2016, Tochen et al. 2016, Rice et al. 2017). Along

with ripened fruit, collecting and disposal of overripe or damaged fruit off the ground reduces potential resources that SWD needs for development and reproduction. Ways of disposing of culled fruit is to solarize under plastic or inside plastic bags. Burying the fruit waste in sealed containers at least 30cm deep is another option that physically removes SWD from the crop area (Liburd and Iglesias 2013, Fisher 2014). Incorporating cultural control tactics with the extended residual control of chemical applications help protect fruit for extensive periods of time during harvest (Bruck et al. 2011).

## **Exclusion**

Another alternative to chemical applications is through physical exclusion, more specifically with insect exclusion netting. Exclusion nets have been incorporated into agricultural practices since the middle of the 20<sup>th</sup> century and used commonly since the 1990s against whiteflies in greenhouses (Chouinard et al. 2016). There are practical IPM programs for a variety of pests that have incorporated exclusion. Not only are insects physically excluded, but they are also prevented from transmitting plant pathogens. This method has received more attention as the search for organic non-chemical controls continues (Alnajjar et al. 2017). In North America, there have been exclusion studies done that show promise in the reduction of SWD populations in small-scale plantings (Link 2014, Daniel Cormier et al. 2015, Schattman et al. 2015, Chouinard et al. 2016, Leach, Van Timmeren, et al. 2016, Rogers et al. 2016, Alnajjar et al. 2017).

To exclude SWD, the size of the netted mesh openings need to be smaller than 1.0 mm, as the size of SWD adults range from 0.70 mm-1.24 mm (Kawase and Uchino 2005, Schattman et al. 2015). This exclusion netting can be incorporated into a tunnel structure that would physically deny SWD access to the crop. A limitation to horticulture tunnels is the potential for

altering the microclimate within and its effects on production, fruit quality and SWD survivability (Chouinard et al. 2016, Alnajjar et al. 2017). A few studies have observed possible differences in temperature and relative humidity inside high tunnels, factors that directly affect SWD populations (Tochen et al. 2014, 2016). These factors are variable depending on the material used in the tunnels; plastic covered tunnels have warmer temperatures compared to netted tunnels or uncovered plots. This heating either had no negative impact or slightly increased marketable yield and quality. Higher temperatures inside the tunnels also acted to suppress infestations inside tunnels due to the reproductive rate of SWD declining as temperatures surpassed the lethal limit (30°C) (Cormier et al. 2015, Rogers et al. 2016). While there are potential benefits of insect exclusion netting, multiple studies were unable to produce 100% exclusion of SWD, but did delay infestation. This delay could be incorporated with chemical control by reducing the cost of many sprays and risk of insecticide resistance. It has been shown that combining these methods result in lower infestations of SWD than using just one control alone (Leach et al. 2016, Rogers et al. 2016).

The use of exclusion netting is still a relatively new practice and as such raises some grower concerns about feasibility. Financially, exclusion has a lot of high costs upfront for material and labor needed to build structures, it is estimated to cost \$6,100/acre of the high tunnel for insect netting. These values have been estimated to be higher than yearly pesticide use, even when amortized over the seven-year lifespan of the netting (Schattman et al. 2015, Leach et al. 2016). Primary concerns of growers with exclusion tunnels are the cost and intensive labor for installment and maintenance as well as the potential interference of tunnels with equipment used in horticultural practices (mowers, sprayers, etc.). Insect exclusion netting is also susceptible to degradation that increases the more the screen is handled, requiring careful maintenance and

frequent replacement (Link 2014). More research needs to be done to make exclusion tunnels more feasible for grower use. Exclusion netting allows for the production of susceptible crops where SWD presence is known. Some growers have already begun designing less expensive tunnels modified with available materials ((Pullano 2015, Leach et al. 2016)).

## **Post-Harvest**

The management of SWD doesn't cease once the fruit is harvested. Infested fruit can externally appear to be undamaged during a small window of time between the fruit being susceptible and harvest. Therefore precautions need to be taken post-harvest to compensate. Wholesale berry crops are typically stored post-harvest at cold temperatures to increase the shelf life for shipment and double as a control measure for other pests. Depending on the temperature and duration of storage, cold storage can reduce survival and increase development time of immature SWD. Temperatures of at least 1.67°C will likely cause SWD development to halt at its current stage, the longer the cold storage session, the more likely the egg or larva will die (Aly et al. 2016).

Irradiation is another post-harvest quarantine treatment that can be an option for exported fruits and vegetables. At low doses, it is useful in treating for insect pests and does not reduce fruit quality (Follett et al. 2014). SWD has been tested for tolerance to radiation, in which tolerance increased with increasing age and developmental stage. The late-stage pupa was the most radiation-tolerant stage that may be found in fruit. However, most stages would likely be affected by the radiation. Despite the potential of irradiation against SWD, few countries currently allow irradiated fresh agriculture products. Instead, several countries like Australia regulate SWD as a quarantine pest and require a post-harvest fumigation with methyl bromide to decrease the risk of introduction (Follett et al. 2014).

## **Integrated Pest Management of Spotted Wing Drosophila**

Each of the control strategies mentioned is designed to target a particular aspect about the pest in question. However, these tactics aren't efficient or sustainable enough to be used as a "silver bullet" method against SWD but could be incorporated into a larger management plan. Exclusion tunnels have the potential to be a beneficial tool in excluding pests but also having more control of the crop itself. The combination of tunnels and chemical sprays could help protect the plant when exposed to an infestation. Unfortunately, the upfront costs and maintenance are what discourages this practice from being used more often.

If two or more control tactics were synchronized together with the behavior of SWD, each could contribute to reducing the dependency on one control and maintaining an extended management program. For example, applying border sprays at the beginning of the season targets overwintering SWD dispersing from the perimeter into the crop field. Not only would this control early season populations but delay and lessen number of insecticide sprays per season. This is where cultural control can supplement sprays. Both sanitation and pruning reduce the favorability of the crop that could push SWD back to the surrounding vegetation. Then finally post-harvest treatments act as a fail-safe system, especially during peak infestation. The objectives of this study is to evaluate the effectiveness of insect exclusion netting, determine the effects of increased temperatures in netted tunnels on SWD and fruit quality in the warmer southern USA and identify local fruiting plants being used a resource for egg laying by SWD.

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## **CHAPTER 2: Comparing Spotted Wing Drosophila Populations in Crop and Perimeter Habitats**

### **Abstract**

Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) has become a serious pest in small fruit production in North and South America and Europe. Frequent insecticidal applications (5-7 days) are the primary control method, therefore it is important to explore other effective strategies to incorporate into a more sustainable management plan. Factors that affect population dynamics need to be taken into consideration when implementing management tactics. Studies have shown that many pests are dependent on both the crop and landscape habitat to sustain populations throughout the year. With a wide host range, these unmanaged habitats could have an impact on SWD populations, especially with landscapes near crop plots. The main objective of this study was to monitor SWD populations in different crop systems and compare to landscape habitats at five Arkansas locations: the AAREC-Fayetteville organic farm (Farm 1, 2016-2017); a Berryville organic farm (Farm 2, 2016); a commercial fruit and vegetable farm in Springdale (Farm 3, 2016); a commercial berry farm in north Fayetteville (Farm 4, 2017) and a West Fork organic farm (Farm 5, 2017). The results showed factors that may play a role in supporting SWD populations, such as resource availability and preferable microhabitats. At Farm 3, the seasonal average of SWD was highest in the perimeter trap (14 flies) that was located next to a refuse pile (culled fruit, vegetables and wood mulch) compared to within the crop (6 flies). At Farms 2 and 5, traps that were within the perimeter tree canopy (4 and 53 flies) had higher seasonal averages than in-crop (1 and 6 flies) because shade can provide lower temperatures and higher relative humidity. SWD also appeared to disperse and exploit the more desirable resources between the field crops and the potential wild hosts within the surrounding habitats. Landscape habitat is important in providing a refuge for SWD during chemical

applications, temperature extremes and winter. More research is needed to determine how this dynamic can be exploited to enhance a management program for SWD.

**Key words:** spotted wing drosophila, landscape habitat, wild hosts, microhabitat

## Introduction

*Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is an invasive fruit pest that has caused significant damage in small fruit production in the continental U.S., Canada and Europe (Hauser 2011, Lee, Bruck, Curry, et al. 2011a, Asplen et al. 2015). The female is equipped with a serrated ovipositor that allows oviposition underneath the skin of soft-bodied fruit (Goodhue et al. 2011, Diepenbrock et al. 2016). Developing larvae consume fruit flesh and degrade fruit quality, deeming infested fruit unmarketable in fresh and processed markets (Tochen et al. 2014). Many attributes such as high fecundity, broad host range, tolerance of a variety of climates, and high dispersal potential have contributed to the success of spotted wing drosophila as an invasive species (Cini et al. 2012). Infestations have dramatically impacted commercial fruit production of caneberries, blueberries, cherries, grapes, and strawberries, due to intensive management of populations which contribute to an increased cost in production (Bolda et al. 2010, Goodhue et al. 2011, Diepenbrock et al. 2016).

Conventional insecticide applications are the primary control tactic against spotted wing drosophila (SWD). Current spray programs target ripening susceptible crops, but non-cultivated landscapes are often overlooked despite acting like a pest population sink (Ricci et al. 2009, Beers et al. 2010, Bruck et al. 2011). Despite intensive control of pest populations within a crop, pest numbers are not only dependent on the commodity but the qualities of wild hosts in the surrounding landscape as well. These landscape habitats provide pests like SWD with a preferable refuge that could increase potential risks of crop infestation (Ricci et al. 2009, Klick et al. 2016). SWD is a highly mobile and opportunistic pest, dispersing to more favorable habitats when resources are low or densities exceed carrying capacity (Mitsui et al. 2010, Klick et al. 2016).

With an extensive host range, these unmanaged habitats could have an impact on SWD densities, especially with landscapes containing alternative hosts near crop plots (Cini et al. 2012, Klick et al. 2016). To develop a more efficient IPM program for SWD, factors that affect the population dynamics such as seasonal movement and landscape habitats need to be considered to improve treatment synchronization (Ricci et al. 2009, Wang et al. 2016). The primary objective of this study was to compare SWD densities in different crop systems (blueberries, blackberries, and strawberries) versus adjacent landscape habitats during the summers of 2016 and 2017 in northwest Arkansas.

## **Materials and Methods**

### *Site Locations*

For this study there were five sites that ranged from conventional to organic practices with a variety of crops for the 2016 and 2017 seasons. Each site had either perimeter vegetation or adjacent unmanaged areas (**Table 1**). **Figures 1-3** show the layout of the crop plots and landscape habitats for each site that was being compared.

### *Monitoring*

The traps were designed to attract and kill adult SWD, and consisted of a one-liter clear plastic deli cup with about 20 (5 mm diameter) holes punctured in the upper half to two-thirds of the way around the container (**Fig. 4A**). Red and black duct tape strips each 2.5 cm wide were placed horizontally on the trap on either side of the holes to increase visual attractiveness to SWD (Lee et al. 2013). One Scentry SWD bag lure was attached to the underside of the lid to act as an attractant. Apple cider vinegar (300 ml) was added to each trap serving both as a drowning solution and an attractant. The traps were checked weekly by sieving the flies from the apple cider vinegar and placed into vials to be counted in the laboratory (**Fig. 4B**). The lures were



replaced once a month to maintain attractiveness to the fly traps. In crop trap placement was level to the fruit and perimeter traps varied between exposed and within tree lines (**Fig. 5-6**).

## **Results**

In 2016 and 2017, the perimeter traps at Farm 1 consistently had higher mean counts of SWD flies per trap compared to the in-crop traps (**Fig. 7**). The seasonal average of SWD in perimeter and in-crop traps were 14 and 6 flies in 2016 and 42 and 12 flies in 2017. At Farms 2, 3 and 5, the perimeter trap (4, 96 and 53 flies) almost consistently had higher mean counts of SWD compared to within the crop (1, 19 and 6 flies) (**Fig. 8A, Fig. 9B**). Farm 4 had higher mean counts of SWD within the crop compared to the perimeter. The seasonal total within the crops were 51 flies and 23 flies in the perimeter (**Fig. 9A**).

## **Discussion**

The high consistent catch in the perimeter of Farm 2 can be associated with the culled fruit, vegetable and wood mulch refuse pile located close to the trap (**Fig. 5A**). Damaged or overripe fruit can support SWD populations, which could explain why there were high catches in that area of the farm (Lee, Bruck, Curry, et al. 2011a). According to Diepenbrock and Burrack (2017), the plant canopy can reduce temperatures due to shade and high levels of relative humidity, in which create a microhabitat able to promote SWD activity. These microhabitats could explain the perimeter trap catches at the Farms 3 and 5 since the traps were placed within the shaded tree canopy.

The higher catch within the field crop traps at Farm 4 may be due to the resource abundance of that area compared to the perimeter. Despite the perimeter traps being near a woodland landscape, the total trap catches did not differ much compared to the crop interior. In the northwest corner, three of the traps were in raspberry plots, which were ranked number one

in a host potential index developed by Bellamy et al. (2013). The perimeter traps were near blackberry and blueberry plots, which ranked third and sixth on the host potential ranking. Numerous potential hosts, e.g., honeysuckle spp., wild blackberries, wild cherries and porcelain vine, had been found in the adjacent woodland areas to the west and northeast of the raspberry plots. Being highly mobile and opportunistic allows SWD the ability to disperse and exploit better resources (Mitsui et al. 2010). A similar type of observation was made at Farm 1 in 2016 and 2017 (**Fig. 10**). The adjacent unmanaged block (341 m away) had a few potential hosts including American pokeweed, wild blackberries and honeysuckle species. There is a possibility for dispersal between the unmanaged block and blackberry plots as SWD have been observed to travel distances of 67-87 m (J.C. Lee, unpublished, Klick et al. 2016).

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Table 1. Arkansas farm site descriptions noting farm # corresponding to each farm site, management type, city, crop, cultivars (if known) and the number of spotted wing drosophila (SWD) traps in crop and perimeter habitats by year.

Year	Farm #	Farm site	Management Type	City	Crop	Cultivars	Number of SWD traps	
							In crop	In perimeter
2016-2017	Farm 1	AAREC	Organic	Fayetteville	Blackberry	Prime-Ark®45 Prime-Ark®Freedom Prime-Ark®Traveler	1-2	2
2016	Farm 2	Dickey Farm	Conventional	Springdale	Strawberry	Chandler San Andreas	3	1
2016	Farm 3	Dripping Springs Garden	Organic	Berryville	Blueberry	Blueray Bluecrop Bluejay	3	6
2017	Farm 4	Sta-N-Step Farm	Conventional	Fayetteville	Raspberry, Blackberry, Blueberry, Raspberry/ Blackberry hybrids	-----	6	2
2017	Farm 5	Henderson Farm	Organic	West Fork	Blackberry	-----	1	2

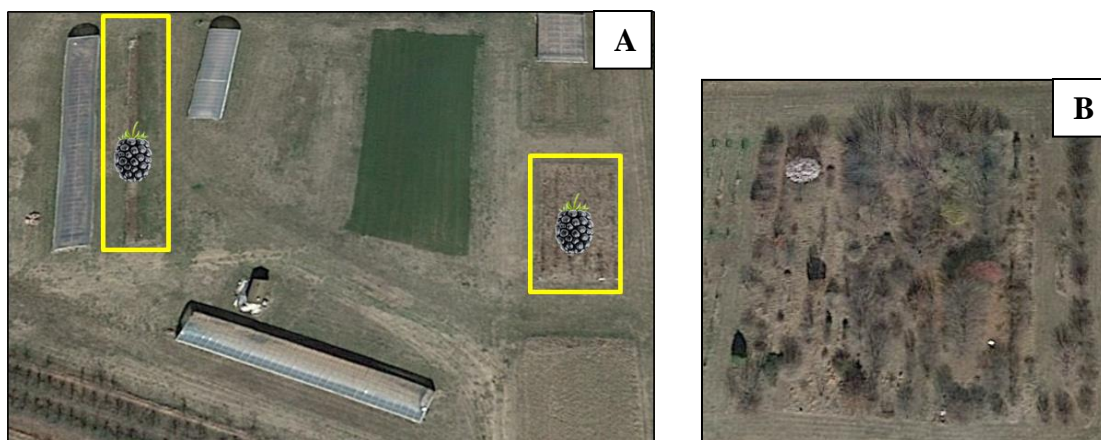


Figure 1. Site layout of Farm 1: A) Blackberry plot that includes a 55 m row (left) and an adjacent block (right); B) Ornamental block 341 m southeast of blackberry plot (2016-2017).

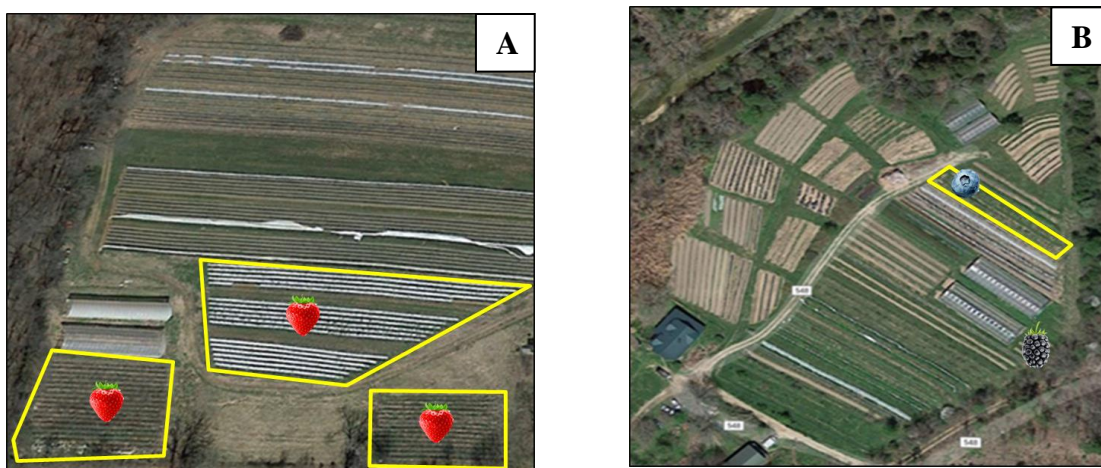


Figure 2. Site layout: A) Farm 2 (0.41 ha total, strawberry); B) Farm 3 (two 65 m blueberry rows) (2016).

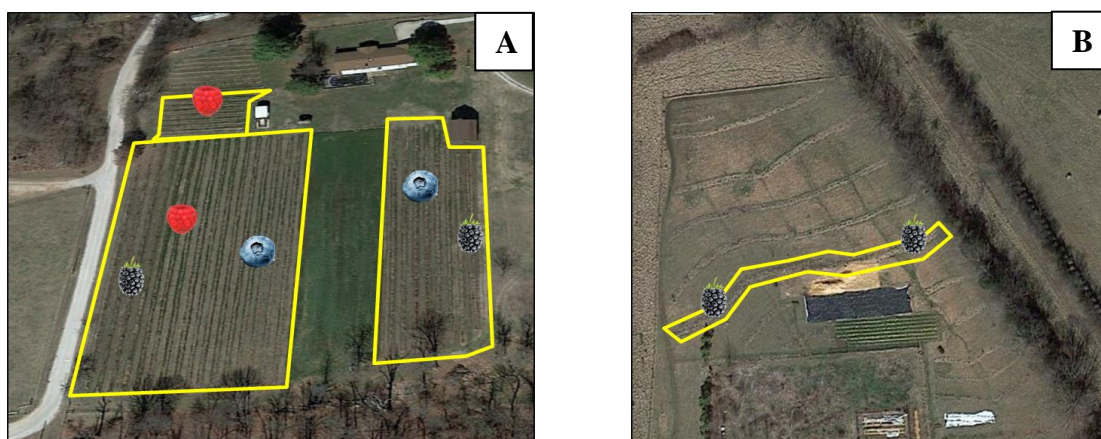


Figure 3. Site layout: A) Farm 4 (mixed fruit system); B) Farm 5 (blackberry) (2017).





Figure 4. A) Example of a spotted wing drosophila trap with apple cider vinegar drowning solution; and B) tools used in transfer fly from vinegar.

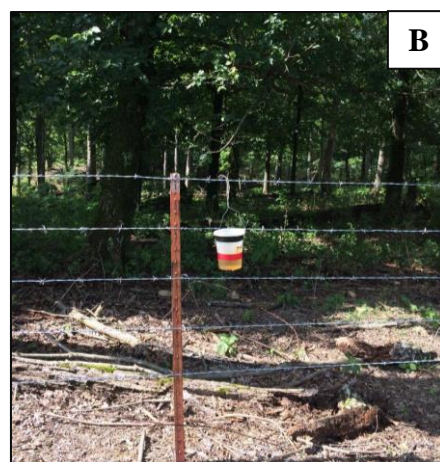


Figure 5. A) Gathering sample from perimeter trap next to refuse pile (Farm 2); and B) Farm 4 trap placement along wooded perimeter.

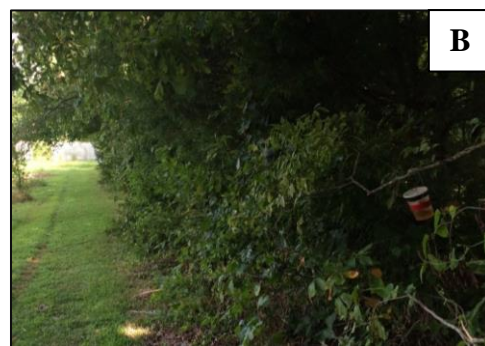


Figure 6. A) Trap within blackberry crop; and B) wooded perimeter trap in organic farm in West Fork, AR (2017).

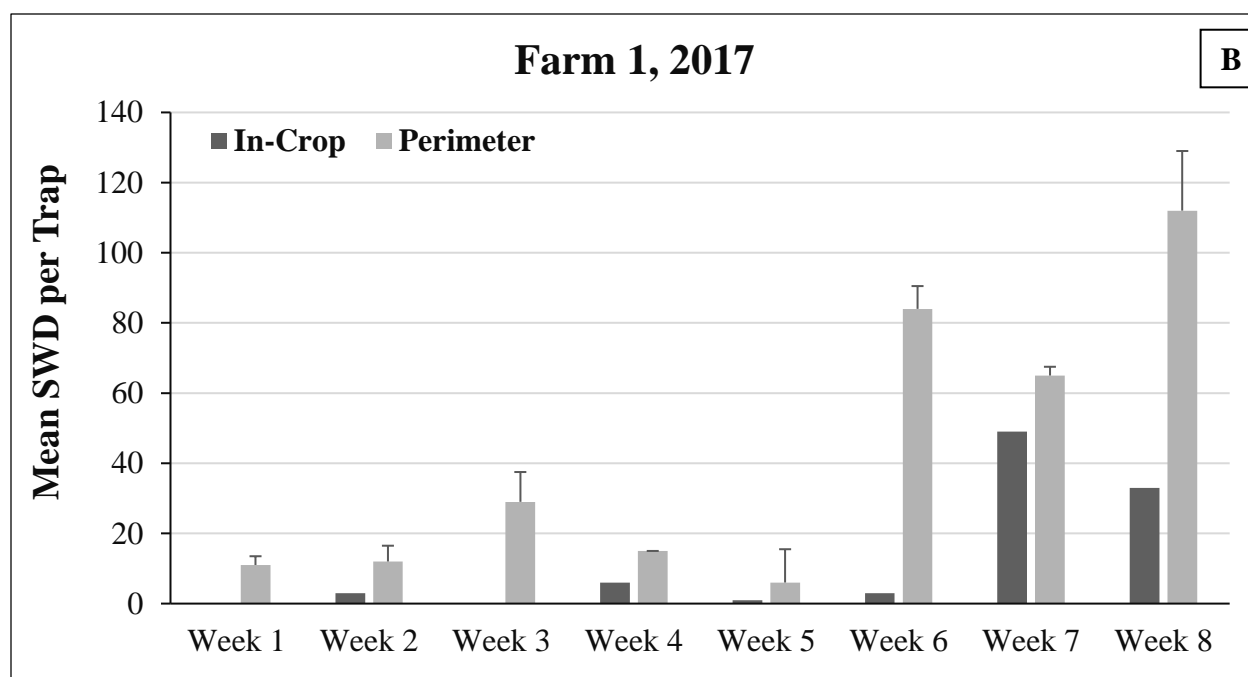
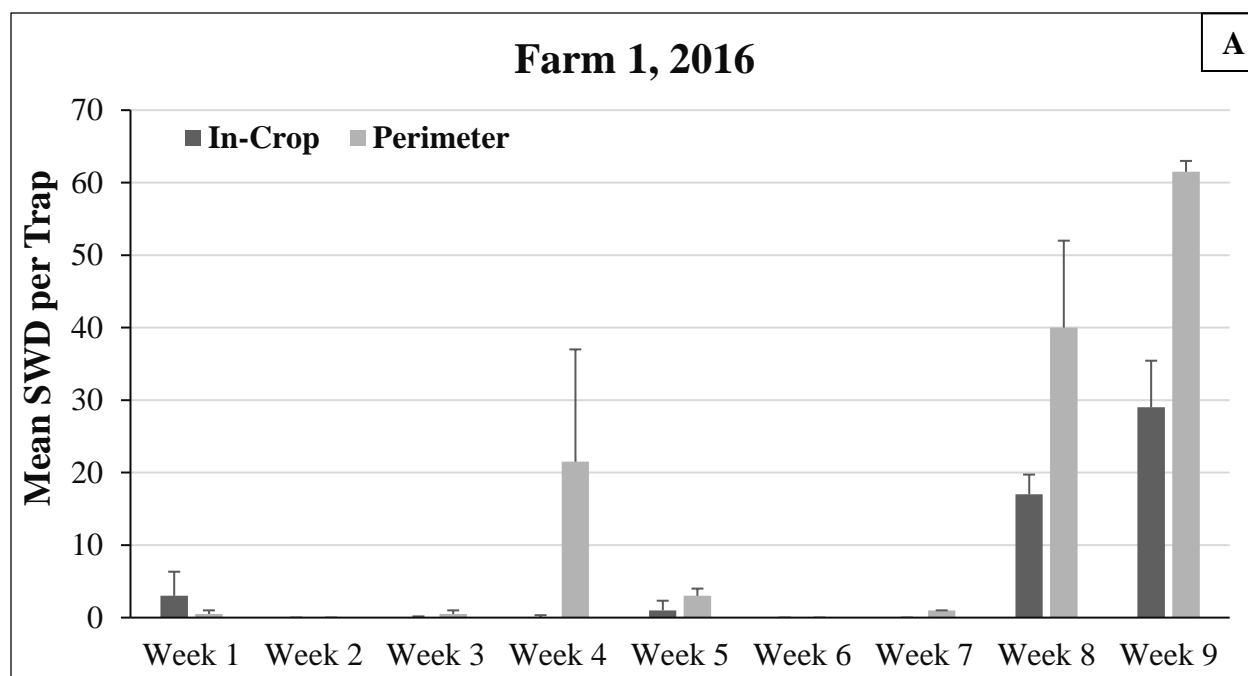


Figure 7. Mean number of spotted wing drosophila (SWD) adults per trap at Farm 1: A) 2016 and B) 2017 (*Means and standard errors calculated using SAS 9.4*)



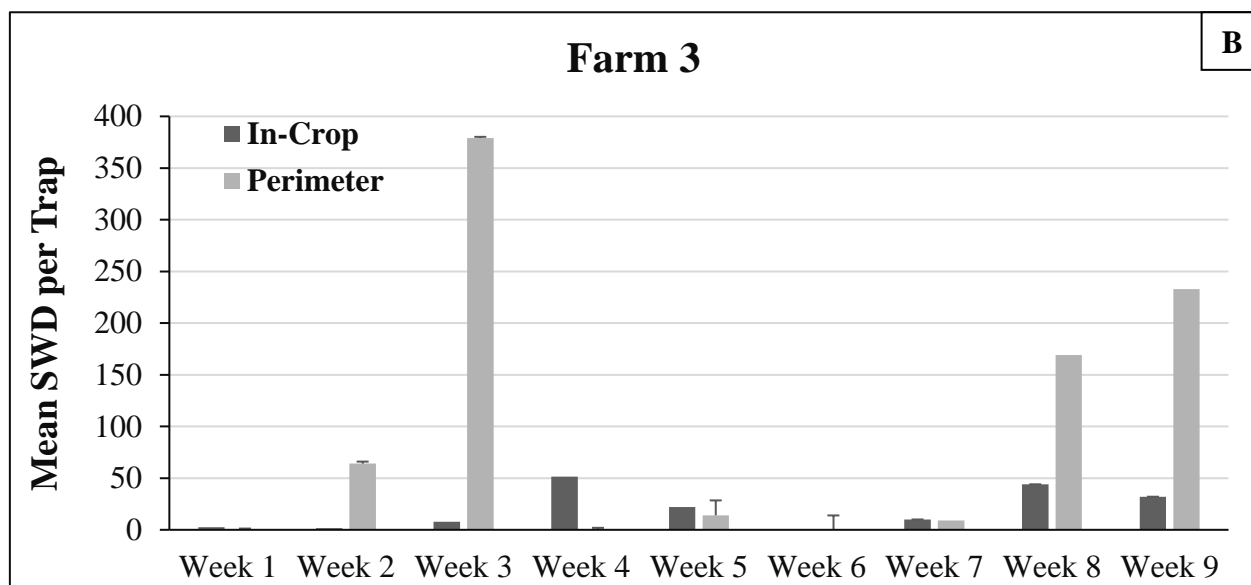
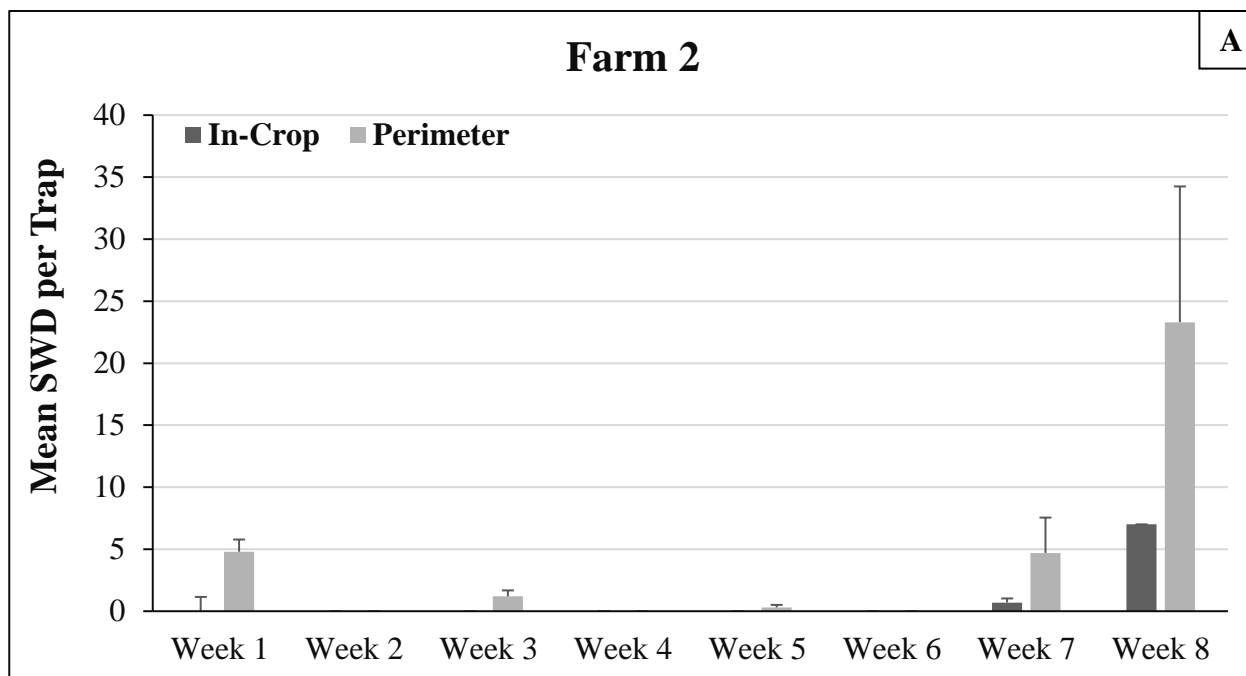


Figure 8. Mean number of spotted wing drosophila (SWD) adults per trap at: A) Farm 2 and B) Farm 3 (Means and standard errors calculated using SAS 9.4) (2016).

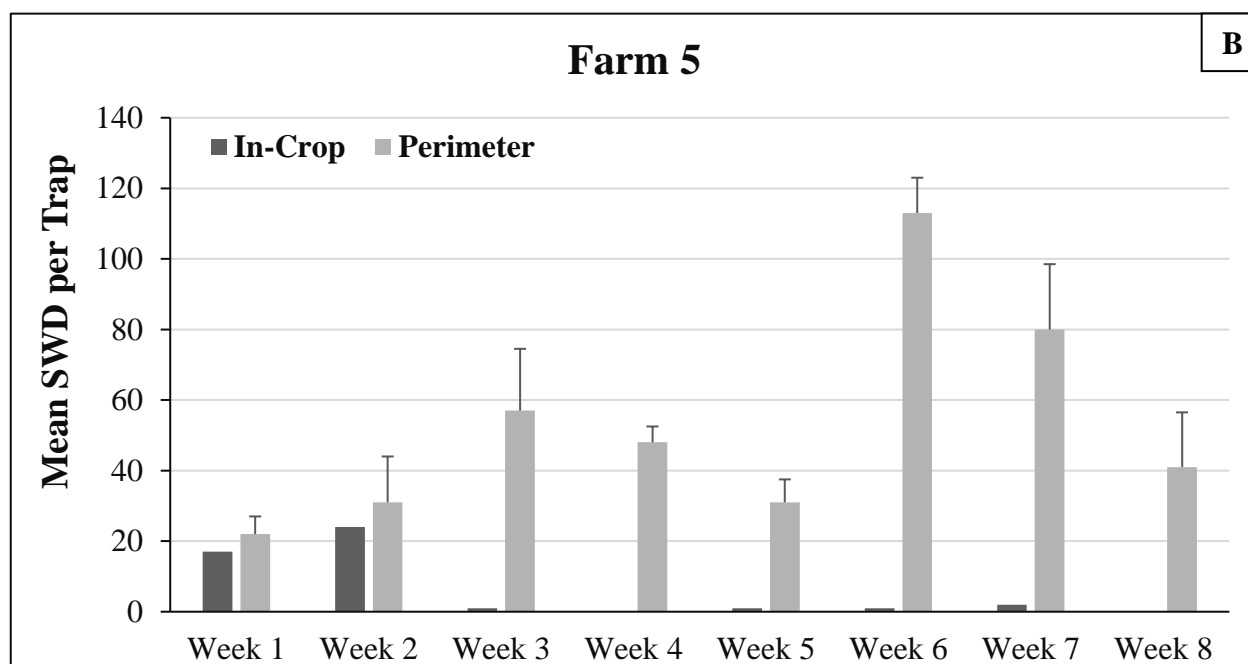
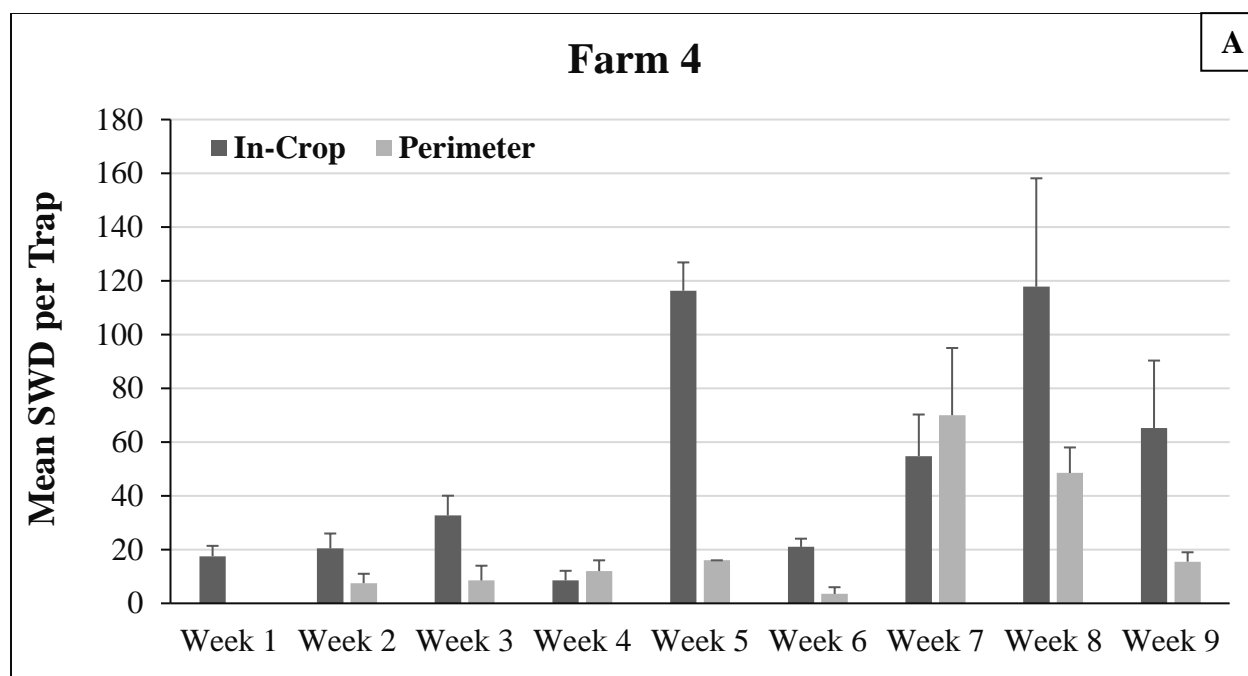


Figure 9. Mean number of spotted wing drosophila (SWD) adults per trap at: A) Farm 4; B) Farm 5 (Means and standard errors calculated using SAS 9.4) (2017).

### CHAPTER 3: Seasonal Phenology of Landscape Hosts of Spotted Wing Drosophila in Arkansas

#### Abstract

Spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura) has become a serious pest of small fruit production in North and South America and Europe. Being an extremely polyphagous frugivore is a contributing factor to the spread of SWD, which utilizes a wide range of cultivated and wild hosts. The purpose of this study was to further confirm or contribute to the rapidly growing list of alternative hosts identified in North America. Of the potential wild fruit hosts sampled, 12 had SWD infested fruit in Arkansas: wild blackberry and dewberry (*Rubus* spp.), American pokeweed (*Phytolacca americana* L.), black cherry (*Prunus serotina* Ehrh.), Carolina buckthorn (*Frangula caroliniana* (Walter) A. Gray), porcelain berry (*Ampelopsis glandulosa* var. *brevipedunculata* (Maxim.) Momiy), amur honeysuckle (*Lonicera maackii* (Rupr.) Herder); autumn olive (*Elaeagnus* spp.); elderberry (*Sambucus* spp.); mulberry (*Morus* spp.); native honeysuckle (*Lonicera* spp.); and Carolina moonseed (*Cocculus carolinus* (L.) DC.). These SWD hosts ripened from early June into October. The variety of host ripening times provides SWD a refuge from insecticides applied to fruit crop and alternative food source before and after the crop harvest period. Understanding the seasonal phenology of alternative hosts and when SWD exploit them may provide insight to develop and refine management tool for growers including monitoring and treating areas with alternative hosts in addition to current control methods.

**Key words:** spotted wing drosophila, alternative hosts, seasonal phenology

## Introduction

*Drosophila suzukii* Matsumura, commonly known as spotted wing drosophila (SWD), is an invasive fruit pest from Southeast Asia. First discovered in California in 2008, the distribution of SWD has reached the central small fruit production areas of North America and Europe (Hauser 2011, Lee, Bruck, Curry, et al. 2011a, Asplen et al. 2015). Unlike most vinegar flies (Drosophilidae) that feed on the overripened material, SWD is unique in that feeding and development occur in ripe soft-bodied fruit. The female uses a serrated ovipositor to lay eggs underneath the skin and larvae continue their development within the fruit (Hauser 2011, Walsh et al. 2011). Since its arrival, SWD has become a serious threat to fruit production with records of damage resulting in 80% of the main fruit crops in California, Oregon and Washington (strawberry, blueberry, caneberries and cherries) (Dreves et al. 2009, Lee et al. 2011b, Walsh et al. 2011) and up to 100% infestation in Arkansas blackberries (Johnson et al. 2016). Susceptible host crops such as caneberries require constant protection once the fruit begins to ripen. As a result, chemical control remains the primary method against SWD. This poses a challenge for growers who have to spray every five to seven days during ripening and harvest periods. This is a significant addition cost to production since SWD appeared. Organic growers currently have only one effective insecticide, spinosad (eFly Working Group 2014, Swoboda-Bhattarai and Burrack 2016).

Current spray programs target ripening susceptible crops, but non-cultivated landscapes are often overlooked, despite providing a refuge for SWD populations (Ricci et al. 2009, Klick et al. 2016). SWD is an extremely generalist frugivore with the ability to develop a wide host range of both cultivated and wild fruits with the list constantly growing (Kanzawa 1939, Walsh et al. 2011, Cini et al. 2012, Lee et al. 2015). These hosts are believed to play a huge role in

overwintering, as SWD has been observed feeding on overripe or damaged fruit such as persimmons, figs and fallen rotting apples during the winter months in Oregon (Lee et al. 2015).

Understanding the phenology of these alternative hosts could play an essential role in developing integrated pest management programs by reducing a food source for SWD populations and synchronizing treatments with ripening times (Ricci et al. 2009, Lee et al. 2015). SWD are highly mobile and have been observed to disperse to more favorable habitats containing alternative hosts during the growing season and to protected habitats to overwinter. These adjacent habitats with alternative hosts help maintain reproduction while fruit crops are being treated with insecticides or in-between ripening (Mitsui et al. 2010, Lee et al. 2015, Klick et al. 2016). This study aims to identify SWD hosts in Arkansas that may contribute to the rapidly growing list of alternative hosts identified in North America.

### **Materials and Methods**

Several wild plants that produce fruit in woodlots adjacent to commercial fruiting crops were suspected to be hosts for SWD in northwest Arkansas. From May to September 2016, these suspect wild hosts were visited to delimit fruit ripening periods and quantify several fruit infested by SWD (Lee et al. 2015). Samples of 30 ripening and ripened fruit from potential wild hosts were collected from the wild and areas adjacent to known SWD-infested berry plantings. Host plants were identified to the highest classification possible with the available resources (personal communication with Dr. Garry McDonald, Clinical Assistant Professor, University of Arkansas). These field sampling procedures were conducted weekly due to the female's ability to lay 7-16 eggs per day (Pfeiffer 2015).

Each fruit sample was then scanned under a stereomicroscope for presence of SWD eggs and dissected for presence of larvae and pupae. In 2016, infested fruit samples were transferred

to a convex 6.3 mm mesh metal screen in a rearing jar to prevent insect drowning (**Fig. 1A**). In 2017, infested fruit samples were placed in 473 ml deli cup on moist sand to help prevent insect drowning and mold growth (**Fig. 1B**) and rearing jars were held at 23°C and 65% RH in the laboratory (Lee et al. 2015). Emerging flies were restricted to rearing jars with a fine cloth cover. The rearing jars were monitored for fly emergence for up to 14 days. Flies were removed, and identified to species (Van Timmeren et al. 2012). Numbers of SWD adults and immatures per sample were recorded.

## Results

The SWD hosts collected in Arkansas ripened from early June into October (**Table 1**). The following sampled hosts were infested with SWD in the northwest region of Arkansas: *Rubus* spp., *Elaeagnus* spp., *Morus* spp., *Sambucus* spp., *Lonicera* spp., *Phytolacca Americana* L., *Prunus serotina* Ehrh., *Frangula caroliniana* (Walter) A. Gray, *Ampelopsis grandulosa* var. *brevipedunculata* (Maxim.) Momiy, *Lonicera maackii* (Rupr.) Herder, and *Cocculus carolinus* (L.) DC. **Figure 2** shows a seasonal phenology of when SWD oviposition occurs in a potential alternative host. Six species had presence of SWD only at a particular time: elderberry (*Sambucus* spp.), Carolina buckthorn (*Frangula caroliniana* (Walter) A. Gray), American pokeweed (*Phytolacca americana* L.), porcelain berry (*Ampelopsis glandulosa* var. *brevipedunculata* (Maxim.) Momiy) and black cherry (*Prunus serotina* Ehrh.). Other plant species that produced soft fruit that have host for SWD in Arkansas but were free of SWD included: red mulberry (*Morus* spp.), green dragon (*Arisaema dracontium* (L.) Schott), eastern red cedar (*Juniperus virginiana* L.), sumac (*Rhus* spp.), and spicebush (*Lindera benzoin* (L.) Blume). However, two of these sampled species, elderberry (*Sambucus* spp.) and dogwood (*Cornus* spp.), were reported as SWD hosts in other states but these fruit were SWD-free in this

study. At least one SWD adult successfully emerged from nine out of the twelve species sampled (Fig. 2).

## Discussion

The timing of SWD fruit infestations varied by fruit ripening periods of the SWD hosts collected. There are a few factors that could explain the timing of oviposition in these samples. As the fruit ripens, the chemical composition (sugars, acidity, etc.) can change, which alters the attractiveness to SWD (Bellamy et al. 2013). Infestations are dependent on a variety of factors such as the timing of collection, the age of the plant and relative attractiveness of other hosts nearby (Lee et al. 2015). It is also most likely that SWD utilizes some of these hosts outside of the time frame observed in this study.

The potential for SWD to find a host is relatively high, as many cultivated and wild hosts have been shown to support SWD. The following is a list of confirmed hosts of SWD: strawberry (*Fragaria* spp.), caneberries (*Rubus* spp.), marionberry (*Rubus* spp.), sweet cherry (*Prunus avium*), blueberry (*Vaccinium* spp.), buckthorn (*Rhamnus* spp.), Surinam cherry (*Eugenia uniflora* L.), orange jasmine (*Murraya paniculata*), Chinese bayberry (*Myrica rubra* (Lour.) Siebold. and Zucc.), honeysuckle (*Lonicera* spp), Oregon grape (*Mahonia aquifolium* (Pursh) Nutt.), dogwood (*Cornus* spp.), milkflower cotoneaster (*Cotoneaster lacteus* W.W. Smith), autumn olive (*Elaeagnus umbellata* Thunberg), cascara buckthorn (*Frangula purshiana* (DC.) A. Gray), spicebush (*Lindera benzoin* (L.) Blume), blue honeysuckle (*Lonicera caerulea* L.), mulberry (*Morus* spp.), American pokeweed (*Phytolacca americana* L.), wildcherry (*Prunus serotina* Ehrh.), cherry laurel (*Prunus laurocerasus* L.), Portuguese laurel (*Prunus lusitanica* L.), Himalaya blackberry (*Rubus armeniacus* Focke), salmonberry (*Rubus spectabilis* Pursh), black elderberry (*Sambucus nigra* L.), sweet box (*Sarcococca confusa* Sealy), bittersweet nightshade

(*Solanum dulcamara* L.) and snowberry (*Symphoricarpos albus* (L.) S.F.Blake) (Kanzawa 1939, Walsh et al. 2011, Cini et al. 2012, Lee et al. 2015). Even firmer fruits such as apple and peaches can be infested if the fruit is damaged, making the potential to find a suitable host less of a challenge.

Alternative hosts play a crucial role in sustaining populations throughout the year (Lee et al. 2015, Klick et al. 2016). Hosts in surrounding landscape can provide SWD with a refuge when resources are depleted in a crop field. The variety of host ripening times provides SWD a refuge from insecticides applied to fruit crop and alternative food source before and after the crop harvest period. (Ricci et al. 2009, Mitsui et al. 2010). Understanding when SWD utilizes these hosts could provide growers enhanced monitoring and implementing controls for SWD before, during and after the season.



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Table 1. List of potential host species for spotted wing drosophila sampled in the field from May to October of 2016 and 2017 in Arkansas.

Family	Scientific Name	Common Name
Acanthaceae	<i>Juniperus virginiana</i> L.	Eastern Red Cedar
Adoxaceae	<i>Sambucus</i> spp.	Elderberry
Anacardiaceae	<i>Rhus</i> spp.	Sumac
Araceae	<i>Arisaema dracontium</i> (L.) Schott	Green Dragon
Caprifoliaceae	<i>Lonicera maackii</i> (Rupr.) Herder	Amur Honeysuckle
	<i>Lonicera</i> spp.	Other native honeysuckle
Cornaceae	<i>Cornus</i> spp.	Dogwood
Elaeagnaceae	<i>Elaeagnus</i> spp.	Autumn Olive
Lauraceae	<i>Lindera benzoin</i> (L.) Blume	Spicebush
Menispermaceae	<i>Cocculus carolinus</i> (L.) DC.	Carolina Moonseed
Moraceae	<i>Morus rubra</i> L.	Red Mulberry
Passifloraceae	<i>Passiflora edulis</i>	Yellow Passion Fruit
Phytolaccaceae	<i>Phytolacca Americana</i> L.	American Pokeweed
Rhamnaceae	<i>Frangula caroliniana</i> (Walter) A. Gray	Carolina Buckthorn
Rosaceae	<i>Rubus</i> spp.	Dewberry
	<i>Prunus serotina</i> Ehrh	Wild Black Cherry
	<i>Rubus</i> spp.	Wild Blackberries
Vitaceae	<i>Ampelopsis glandulosa</i> var. <i>brevipedunculata</i> (Maxim.) Momiy	Porcelain Berry

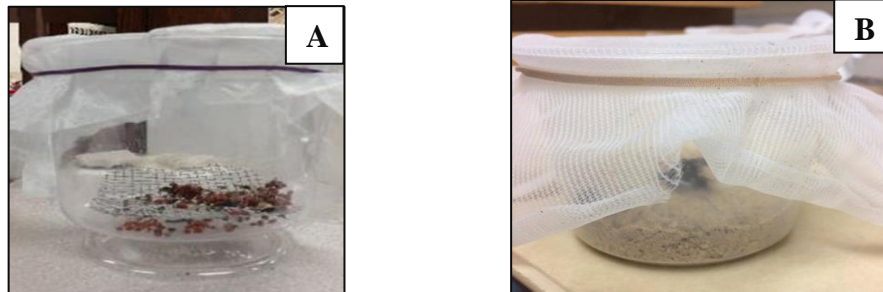


Figure 1: Rearing jars with ripe fruit from wild host containing spotted wing drosophila: A) on screen to prevent drowning (2016); and B) on moist sand to prevent desiccation and drowning (2017).

Plant Name	May	June	July	Aug	Sept	Oct	Nov
Wild Blackberry ( <i>Rubus</i> )		██████████	██████████				
American Pokeweed ( <i>Phytolacca Americana</i> L.)			██████████	██████████			
Black Cherry ( <i>Prunus serotina</i> Ehrh.)			██████████				
Carolina Buckthorn ( <i>Frangula caroliniana</i> (Walter) A. Gray)					██████████		
Porcelain Berry ( <i>Ampelopsis glandulosa</i> )*				██████████			
Amur Honeysuckle ( <i>Lonicera maackii</i> )					██████████		
Autumn Olive ( <i>Elaeagnus</i> )*				██████████			
Elderberry ( <i>Sambucus</i> )*				██████████			
Native Honeysuckle ( <i>Lonicera</i> )			██████████			██████████	
Carolina Moonseed* ( <i>Cocculus carolinus</i> )						██████████	
Dewberry ( <i>Rubus</i> )		██████████					
Mulberry ( <i>Morus</i> )		██████████					

Figure 2: In Arkansas in 2016 and 2017, fruit phenology of host species samples with spotted wing drosophila eggs present (\*no adult SWD emergence).

## CHAPTER 4: Efficacy of Insect Exclusion Netting Against Spotted Wing *Drosophila*

### Abstract

*Drosophila suzukii* Matsumura, commonly known as spotted wing drosophila (SWD), is an invasive fruit pest that has emerged as a threat to small fruit production in North and South America and Europe. Frequent insecticide applications every 5-7 days are the primary control method, therefore there is a strong need to reduce these costly applications for conventional growers. Also with the increase in demand for organic produce comes the need for approved tactics to organically control SWD, as there is only one effective organic insecticide, Entrust™ (spinosad). Insect exclusion screen is being evaluated in several regions of the USA as a non-chemical, organic means of preventing SWD from infesting fruit. However, there are few studies that have shown the effects that exclusion may have on temperature and fruit quality. The objectives of this study were to determine the efficacy of different types of exclusion (high tunnel, netted and plastic low tunnels) and compare the differences in temperature and fruit quality (titratable acidity, Brix and firmness). In 2016, the tunnels were successful in excluding SWD. However, infestations were delayed in 2017. Overall, the temperatures among the tunnels were relatively similar and only slightly warmer than the open plot. Infestations lasted longer in the netted low tunnels compared to the plastic, which could be due to the shorter amount of time that temperatures were above 30°C (lethal temperature for SWD). For the fruit quality component, the netted low tunnels were on the lower spectrum of the acceptable range of firmness which was significantly different from the other treatments. The open plot and netted low tunnels had a markedly higher titratable, which is a sign of good quality. Despite the lack of significant differences, the netted high and plastic low tunnels had higher Brix that is within the recommended range of 10-12%. It appears that tunnel type may affect fruit quality. However,

other abiotic factors could also contribute to the differences. There is potential for exclusion to be an effective control method against SWD, but more studies should be conducted to address modifications as well as the economics of building and implementing exclusion netting into a management program.

**Key words:** spotted wing drosophila, insect netting, exclusion, low tunnels, fruit quality

## Introduction

*Drosophila suzukii* Matsumura, commonly known as spotted wing drosophila (SWD), is an invasive fruit pest native to Southeast Asia. From 2008 to 2013, first detections began to surface across the North American mainland and into Europe (Hauser 2011, Lee et al. 2011, Asplen et al. 2015). Unlike many native drosophilids that feed on overripe or rotting material, *D. suzukii* is unique by feeding on ripening and ripe fruit. The female has a serrated ovipositor, enabling oviposition underneath the skin of soft-bodied fruit (Goodhue et al. 2011, Diepenbrock et al. 2016). The larvae feed inside the fruit which causes the most damage. In addition, a female may lay up to 300 eggs which has allowed SWD to become a severe economic pest (Cini et al. 2012). Infestations have resulted in over \$700 million in losses of caneberries, blueberries, cherries, grapes, and strawberries. Weekly insecticide sprays during ripening and harvest have significantly added to the cost of fruit production (Goodhue et al. 2011, Diepenbrock et al. 2016). Damage caused by SWD can reach 80% loss of susceptible fruit crops (Dreves et al. 2009, Lee, Bruck, Curry, et al. 2011a, Walsh et al. 2011) with up to 100% fruit infestation in caneberries in Arkansas (Johnson et al. 2016).

Blackberry (*Rubus spp.*) is one susceptible host crop in the Southeastern United States and as such requires continuous protection once the fruit begins to ripen through harvest (eFly Working Group 2014, Johnson et al. 2016, Swoboda-Bhattarai and Burrack 2016). As a result, insecticides are the primary control method, with up to a 90% increase compared to prearrival of *D. suzukii*. The frequent application of conventional pesticides poses a challenge for conventional growers financially and the growing demand for organic products. Currently, there is only one OMRI approved formulation, spinosad (Entrust™) reported as an effective organic insecticide.

Alternatives to chemical treatments such as insect netting have shown to be useful in excluding SWD and successfully implemented in IPM programs for a range of insect pests (Dib et al. 2010, Sauphanor et al. 2012, Leach, Van Timmeren, et al. 2016, Alnajjar et al. 2017). The effectiveness of exclusion provides organic growers with alternatives for control in a variety of agricultural systems (Link 2014, Cormier et al. 2015, Schattman et al. 2015). Exclusion netting can be beneficial in managing additional pests as well as enhancing the harvest season, yield, fruit quality (Lloyd et al. 2005, Hanson et al. 2011). Striving for good fruit quality is imperative to produce marketable fruit, especially in fresh markets. Each aspect of fruit quality has a range that is deemed suitable for customer acceptance. Brix (total soluble solids) is a measurement of the sugar concentration of liquids, in this case, fruit juice and is essential to ensure high fruit quality. Similar to Brix, titratable acidity is the total amount of acid in a solution, typically as a weight or volume, acidity is especially important in the flavor of blackberry (Cahn et al. 1992, Badenes and Byrne 2012). According to Sebesta 2014, higher amounts of total soluble solids and acidity improve flavor perception for the consumer. Relatively low firmness, high acidity, and sugar content are what would classify a fruit as being of good quality (Cahn et al. 1992).

There is limited information on the effectiveness of different types of exclusion as a control method for spotted wing drosophila in blackberry, and the effect of increased temperature on fruit quality and SWD survivability is unknown. A study was conducted over two seasons comparing SWD adults collected in baited traps, immatures found in fruit, temperatures and fruit quality (Brix, acidity, firmness) of each treatment in open blackberry plantings versus tunnels which differed in proportion covered with insect exclusion netting: 1) attached along sides and ends of a plastic covered high tunnel; 2) low tunnels fully enclosed in insect exclusion netting; and 3) plastic covered low tunnels with ends covered with insect exclusion netting.



## Materials and Methods

### *Locations*

There were three organically managed sites used in this study, varying from blackberry to blueberry plantings located in the Northwest Arkansas region (**Table 1**). Due to the lack of SWD activity within the blueberries at Farm 2 in 2016, the second site for 2017 was relocated to Farm 3. The timing of tunnel installment in all locations for this study occurred after petal fall and development of mostly unripe green fruit to ensure the tunnels would not prevent pollination or reduce fruit set.

### *Plantings and Tunnel Descriptions*

#### Farm 1:

There were two identical, 55 m long blackberry rows planted 15 m apart. Both rows were subdivided into three consecutive 18 m plots of each cultivar with the blackberry plants spaced 0.5 m apart. The east row served as the uncovered control. The west row was inside a high tunnel (60 m x 7.3 m x 3.6 m) with greenhouse grade poly film roof covering. This tunnel had both sides (1.5 m) and ends covered with 80 gram Tek-Knit netting (Tek-Knit Industries, Quebec, Canada) (**Fig. 1A**). Each year, a colony of eastern bumble bees, *Bombus impatiens* Cresson (Koppert Co., Howell, MI) was placed at fruit level on cinder blocks inside the high tunnel to ensure adequate pollination of the plants. Outside rows were pollinated naturally. Each row was fertilized in spring and trickle irrigation was applied as needed. Planted 78 m to east were six rows (18.3 m ) of blackberry plants (same three cultivars and spacing per row as above).

On June 1<sup>st</sup> and 3<sup>rd</sup> 2016, portions of these rows remained uncovered or were covered with either an all netted low tunnel or plastic low tunnel with netted ends. Three netted low tunnels (3.7 m x 2 m x 1.2 m) had roof, sides, and ends covered in insect exclusion netting (**Fig.**

**1B).** Two low plastic tunnels had sides and top covered with greenhouse plastic and ends covered in insect exclusion netting (**Fig. 1B**).

On May 23<sup>rd</sup> or 24<sup>th</sup> 2017, three netted low tunnels were set up on part of the 55 m row of blackberry plants 15 m to east of the high tunnel after removal of the trellis wire. The ribs of the low plastic tunnels were redesigned to withstand wind and rain (**Fig. 2A-B**). The new tunnels had dimensions of 2 m x 1.2 m x 6 m and made of 1-inch PVC pipe to obtain an arched shape, but still had netted ends. Black PVC pipes were cut in half to provide a securing mechanism for the material to the frame in which half of one end of the low tunnels had to be unclipped to allow access inside the low tunnels (**Fig. 2C-D**).

#### Farm 2:

On June 2<sup>nd</sup> 2016, three netted low tunnels (3.7 m x 2 m x 1.2 m) were installed 3.7 m apart over portions of one of two 60 m rows of blueberries. The other blueberry row served as the uncovered control (**Fig. 3A**).

#### Farm 3:

A 76 m row of blackberry plants were trained to a trellis with two vertical wires (**Fig. 3B**). On May 31<sup>st</sup> 2017, three netted low tunnels (3.7 m x 2 m x 1.2 m) each had the netting on both ends partially cut and secured with large binder clips to seal the tunnels around the trellis wires (**Fig. 4A-B**). The other plants served as the uncovered control.

#### *Tunnel Construction*

At Farm 1, the high tunnel had the Tek-Knit insect exclusion netting secured to each side and both ends along with screened doors at both ends (**Fig. 1A**). The netting (60 m x 1.5 m) attached by large binder clips (41 mm) to both the baseboard and to the board at 1.5 m high with aluminum lock channel and Wiggle Wire® (WW) which secured the poly film roof covering.

Both curved ends (3.7m x 7.3 m) had netting held by WW in aluminum lock channels to end wall framing or attached directly to tube framing by an aluminum fabric clip. In 2016, the low tunnels used three galvanized tube frames (1.2 m x 1.8 m x 2.5 cm diameter), one at each end and one in the center. These three structures were held together at the two upper curved corners with horizontal galvanized tube purlins (1.8 m x 2.5 cm diameter). An aluminum lock channel bolted along these frames facing outward on both sides and the top of the two end frames. Netting or plastic was thrown over and held to frames with WW inserted into the lock channels. Each of the six tube frame bases was placed onto one of six rebars (0.3 m x 1 cm diameter) driven vertically into the ground (**Fig. 5A-D**).

Mesh size is imperative to the success of excluding SWD, which are 2 to 3.5 mm in length and have a 5 to 6.5 mm in wingspan. Mesh openings of 1.0 mm or smaller will prevent SWD from entering crop areas (Kawase and Uchino 2005). The amount of netting or plastic needed to cover and secure to the body of the tunnel was 5.8 m x 4 m.

This material coiled around a long piece(s) of rebar (1 cm diameter) and fastened to the ground with multiple landscape staples. The end frames were covered using WW to set the material for the sides and top in place as well as closing off one end of the tunnel. To allow easy access a door with magnetic stripping was made. Adhesive magnetic tape (.75in-W Horizon Group Adhesive Magnetic Tape, Walmart) was hot glued on the edge of two 1.8 m x 0.6 m netting pieces with a 1” ribbon glued on top of the exposed netting to strengthen the bond. The top and side of each half of the door was attached with WW, and one-half of the door was secured at the bottom with a piece of rebar and landscape pins (**Fig. 6A-B**).

### *Monitoring*

Each site was monitored weekly for SWD presence at multiple stages with the

combination of SWD traps for adults and fruit sampling for immature stages (**Table 1**). The trap is designed to capture adult SWD and consists of a 1-quart clear plastic deli cup with ~twenty (4.8 mm diameter) holes punctured in the upper half, two-thirds of the way around the container (**Fig. 7A**). Too many holes clustered around the whole cup will cause flies to be lost during the sampling process and cause the trap to crack. A 2.5 cm wide red and black duct tape strips were wrapped horizontally on the trap on either side of holes to increase visual attractiveness to SWD (Lee et al. 2013). One Scentry SWD bag lure was attached to the underside of the lid to act as an odor attractant. Each trap had 300 ml of apple cider vinegar serving as both a fly drowning solution and attractant. Samples were processed weekly. The vinegar solution containing flies was poured through a kitchen sieve and flies were transferred with a fine paint brush into vials and later counted in the laboratory (**Fig. 7B**). Fresh apple cider vinegar was added to the trap and placed back in the field. The Scentry SWD lures were replaced monthly to maintain attractiveness of the SWD traps.

Weekly, a 30-fruit sample was collected from each treatment (uncovered and tunnels). Each fruit was scanned under a stereomicroscope and the numbers of eggs, larvae or pupae per fruit was recorded. The percentage infestation was calculated by the proportion of infested fruit out of the total number of fruit for each treatment.

### *Weather*

The increased temperature inside the tunnels was hypothesized to alter fruit quality. Temperature and relative humidity were recorded inside each tunnel and in the open planting (ambient). WatchDog loggers (Spectrum Technologies, Aurora, IL) were installed at fruit height in all locations to record at half hour intervals the temperature, percentage relative humidity, and soil temperature. On June 1st-3rd 2016 and May 23<sup>rd</sup>- May 24<sup>th</sup> 2017 at Farm 1, a logger was

installed: outside in the uncovered row (referred to as ambient), inside one high tunnel, and in each of two netted and two plastic low tunnels. In 2016 at Farm 2, loggers recorded the ambient outside temperatures and inside netted low tunnels installed on June 1st-3rd of 2016 (**Fig. 8A**). For the 2017 season, the loggers were installed on May 23<sup>rd</sup> - May 24<sup>th</sup> at Farm 1 and May 31<sup>st</sup> at Farm 3 (**Fig. 8B**). Data from each logger was manually downloaded weekly, and the WeatherTracker data was accessed via satellite. The temperature was recorded every half hour in and outside each type of tunnel. The cumulative number of hours per week that temperatures exceeded 30°C was calculated to compare the presence of spotted wing drosophila during times above lethal temperatures. Kanzawa (1939) observed a decrease in SWD activity at this temperature and Kimura 2004 estimated 50% lethality at 35°C.

A side experiment was conducted during this study in which the low tunnels were opened and exposed to infestations for one week (July 4<sup>th</sup>-July 10<sup>th</sup>) as temperatures began to reach the lethal limit for SWD survival. This study was performed to see if temperature could play a role in SWD's ability to survive inside the different types of low tunnels.

### *Fruit Quality*

In 2017 at Farm 1, five samples of up to 30 ripe fruit (when available) were collected weekly during the harvest period from each cultivar inside the high tunnel. In the adjacent row, all ripe fruit were collected weekly from each tunnel and the uncovered blackberries.

The following components of fruit quality were measured in this study: 1) color and appearance; 2) texture; and 3) flavor. Each ripe fruit sample was categorized and recorded as either marketable (#1) or culled (#2) based on the grading system by USDA standards (USDA Specialty Crops Inspection Division 2016) (**Fig. 9A**). The culled fruit were further segregated into six categories: 1) drupe damage (feeding, disease, etc.); 2) discoloration (color <100%

black); 3) size ( $> 0.5''$ ); 4) shape (irregular drupelets); 5) overripe (leaky, soft etc.); and 6) under-ripe (red, hard, etc.) (**Fig. 9B-D**).

Ten marketable fruit (if available) were randomly selected from each sample. A stereomicroscope was used to scan each fruit and note presence/absence of spotted wing drosophila. Then each fruit was placed under the compression plate attachment in the TA.XT *plus* Texture Analyser (Texture Technologies Corp., South Hamilton, MA) to determine firmness calculated as the amount of force in pounds to crush each fruit (**Fig. 10A-B**).

Two components of flavor that were measured included: percentage sugar (Brix) as a measure of total soluble solids (TSS); and acid (titratable acidity) content of each fruit in a sample (10 replicates). The 10-fruit sample previously used for the texture analysis was ground up to produce juice needed for flavor analysis or remaining marketable fruit were used if texture samples did not provide substantial amounts of juice. Total soluble solids or percentage sugar (Brix) were measured with an Atago® PR-32 $\alpha$  digital refractometer (Pulse Instruments®). A 5-mL syringe was used to obtain juice from each ground up fruit sample and then a small amount was placed onto the refractometer prism to determine the percentage of sugar present. After each 10-fruit sample, a drop of deionized water was applied to the refractometer to ensure 0% calibration (**Fig. 11A-B**). A quality rating was recorded for each fruit sample as follows: 6 = poor; 8 = average; 12 = good; and 16+ = excellent (Harrill 1998).

A 6 g sample of blackberry juice was measured out using an Ohaus® SP-2001 Scout™ Pro Balance (Ohaus, Parsippany, NJ) and diluted with 50 g of deionized, degassed water by titration with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2; results were expressed as a percentage of citric acid (g/100g). (**Fig. 12 A-C**) (Garner et. al 2013).

### *Statistical Analyses*

In June and July 2016 and 2017, 78% of trap count data were zero. A Chi Square test found an association between zero counts and treatments (open field versus three tunnel types) showing an extremely skewed distribution with zero-inflation. Logistic regression, PROC Logistic procedure, was applied to analyze the number of zeros as a binomial distribution which resulted in estimating the adjusted odds ratio (aOR), i.e., the odds of a zero in each tunnel type compared to Ambient after adjustment for year, site and week. The 95% confidence intervals (CI) of aOR are reported accordingly. Means were computed by PROC GLM LSMEANS procedure, giving pairwise-comparisons at 0.05 significance level. Goodness of fit (Deviance test) found  $P\text{-value} > 0.05$  indicating the binomial model fits well (SAS Institute Inc. 2013).

In 2017, each component of fruit quality data (titratable acidity, Brix and firmness) by treatment (open field versus three tunnel types) was analyzed by analysis of covariance (ANCOVA) taking the effect of the weekly cumulative number of hours <65% relative humidity into account. The titratable acidity and firmness variables were transformed with natural log and square root, respectively, to meet the normality conditions in ANCOVA. Respective treatment means were back-transformed with square (**Table 8**) and exponential natural log (**Table 9**). Multiple comparison by treatment was conducted by Tukey's method at 0.05 significance level (SAS Institute Inc. 2013).

## **Results**

### *Monitoring*

For the 2016 season, all netted tunnels at Farms 1 and 2 successfully excluded SWD flies and prevented SWD infestation of fruit (**Table 2 and 3**). In 2017, exclusion netting used on tunnels at Farms 1 delayed SWD infestation of blackberry fruit until 12 June in the netted low

tunnel, 19 June in the netted plastic tunnel and 3 July in the netted high tunnel. The uncovered plants had the highest total number of SWD flies per trap (11.36) compared to the low netted tunnel (5.21), netted high tunnel (0.38) and low plastic netted tunnel (0.13) (**Table 4**). In 2017 in Farm 3, the uncovered plants had a higher total number of SWD flies per trap (5.75) compared to the netted low tunnel (0.16) and had season average of 2.38% of fruit infested by SWD compared to 1.04 in the netted low tunnel with first fruit infestation on 26 June (**Table 5**). Overall, the odds ratio of having zero SWD present was significantly higher in the netted high tunnel (237.1) compared to the screened and plastic low tunnels (30.6 and 82.5) and uncovered (1) (**Table 6**).

#### *Weather*

In 2016 at Farm 1, the seasonal average cumulative number of lethal hours per week (LH/wk) greater than or equal to 30°C was consistently higher at 69.4 LH/wk in the netted high tunnel than all the other treatments which were similar: plastic low tunnels (58.6 LH/wk), netted low tunnels (54.6 LH/wk) and uncovered plots (53.4 LH/wk) (**Fig. 13**). At Farm 2, the netted low tunnels had 56.8 LH/wk compared to 42.5 LH/wk in the uncovered plots (**Fig. 14**).

In 2017 at Farm 1, the 68.0 LH/wk in plastic low tunnels was consistently higher than the netted high tunnel (50.8 LH/wk) tunnel and netted low tunnels (41.7 LH/wk). In 2017, these plastic tunnel was slightly longer (6 m) than in 2016 (4 m) that may have caused more heat build than in 2016. These values in both netted tunnels were higher than the uncovered plots (27.7 LH/wk) (**Fig. 15**). At Farm 3, the 50.5 LH/wk in the netted low tunnels was higher than the uncovered plots (32.6 LH/wk). The warmer tunnels in 2017 had relatively low numbers of SWD flies trap that did not directly correspond to the percentage of SWD-infested fruit.



### *Fruit Quality*

The interaction of treatment and number of hours of exposure to air  $\leq 65\%$  RH significantly affected Brix levels in harvested fruit ( $F_{3,190} = 4.0$ ,  $P < 0.009$ ). The cumulative number of hours exposure to air  $\leq 65\%$  RH alone explained more ( $R^2 = 0.4$ ) of the observed variation in Brix levels across treatments ( $F_{1,190} = 140.0$ ,  $P < 0.0001$ ) compared to that for treatment effects alone ( $F_{3,190} = 11.7$ ,  $P < 0.0001$ ;  $R^2 = 0.09$ ) (**Table 6, Fig. 17**). After adjusting Brix to 67% RH, the fruit collected from the uncovered blackberry treatment were significantly different than all the netted tunnels which were dryer with more hours of exposure to air  $\leq 65\%$  RH than did the uncovered blackberry plants (**Table 7**).

The treatment of uncovered blackberry fruit had titratable acidity of 1.5% which was significantly higher ( $F_{1,137} = 14.6$ ,  $P < 0.0001$ ;  $R^2 = 0.3$ ) than all netted tunnel treatments. Similar values for titratable acidity were noted for both the netted plastic low tunnels (1.1%) and netted low tunnels (0.9%). The netted low tunnel had similar titratable acidity as the netted high tunnel (0.6%) (**Table 8**).

There was not as much variability in firmness with treatment effects ( $F_{1,1631} = 5.9$ ,  $P < 0.0005$ ;  $R^2 = 0.03$ ). The netted high tunnel blackberries had a significantly higher firmness (8.3 Newtons) compared to blackberries in the netted low tunnel (6.9 N), which were similar to firmness of blackberries in the other tunnels treatments (**Table 9**).

### **Discussion**

Based on observation it is evident that SWD was able to be excluded during the first year. However, infestations were delayed a few weeks in 2017. There were many factors that most likely contributed to the outbreak of SWD within the tunnels, such as tunnel exposure during harvest or SWD entry through holes in the netting (Rogers et al. 2016). The tunnel design also

played a role in SWD introduction into the low plastic tunnels due to the inability to withstand intense weather, which occurred in both years on multiple occasions. While the insect netting has a lifespan of seven years, the netted tunnels required frequent maintenance in repairing torn holes, which may not have been corrected in time and allowed SWD entry. According to Link 2014, insect netting wears down the more the material is handled and should be replaced yearly. The netting material from 2017 was the same used in 2016, which could have contributed to degradation. Some concern growers had in Link 2014 was in regard to the tunnels inhibiting equipment to enter or maneuver around the tunnels. Weed management proved challenging to control inside the low tunnels due to the narrow dimensions of the structure. Studies have shown that plant canopies promote SWD populations through a decrease in temperature and increase in relative humidity. Poor weed control can contribute to an environment more suitable for SWD (Diepenbrock and Burrack 2017).

While the low netted and plastic tunnels were based on similar structures, SWD infestations lasted longer inside the netted low tunnels compared to the plastic. The reason could be due to constant high numbers of lethal hours per week within the plastic low tunnels. However, the reproductive rate and developmental periods decrease as temperatures began to reach 28°C (Tochen et al. 2014). Overall, the temperatures among the tunnels were relatively similar and only slightly warmer than the open plots.

A few studies have shown the effect tunnels have on fruit quality. Some state that exclusion netting doesn't significantly impact aspects such as acidity or sugar content, while others noticed a slight increase in fruit size and yield (Thompson et al. 2009, Daniel Cormier et al. 2015, Leach, Van Timmeren, et al. 2016). The ranges of these quality components vary depending on the blackberry cultivar: Brix (8.2-16.6%), titratable acidity (0.83-1.76%) and

firmness (7.13-13.26N).

Low percentage of relative humidity (<65%RH) seems to have an effect on fruit quality when compared among the treatments. All of the blackberry treatments except for the netted low tunnel blackberries were in the acceptable range for firmness. The open plot and netted low tunnel blackberries had the highest amount of acidity, which is a sign of good blackberry quality. All of the blackberry treatments except for the netted high tunnel blackberries were within the recommended quality range of 10-12%. It appears that tunnel type may have an affect on fruit quality. However, other abiotic factors such as relative humidity appear to play a larger role within each tunnel type and its effect on fruit quality.

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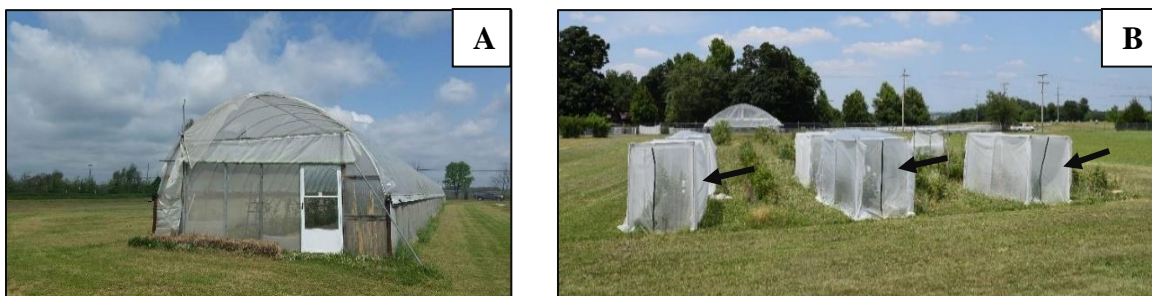


Figure 1. Tunnel designs in Fayetteville, AR (2016): A) 60 m long high tunnel with insect exclusion netting secured to sides and ends and with wire screened entry door on both ends. B) The layout of three netted low tunnels and three low plastic tunnels each with secured entry doors using magnetic strips (arrows) hot glued to insect exclusion netting.

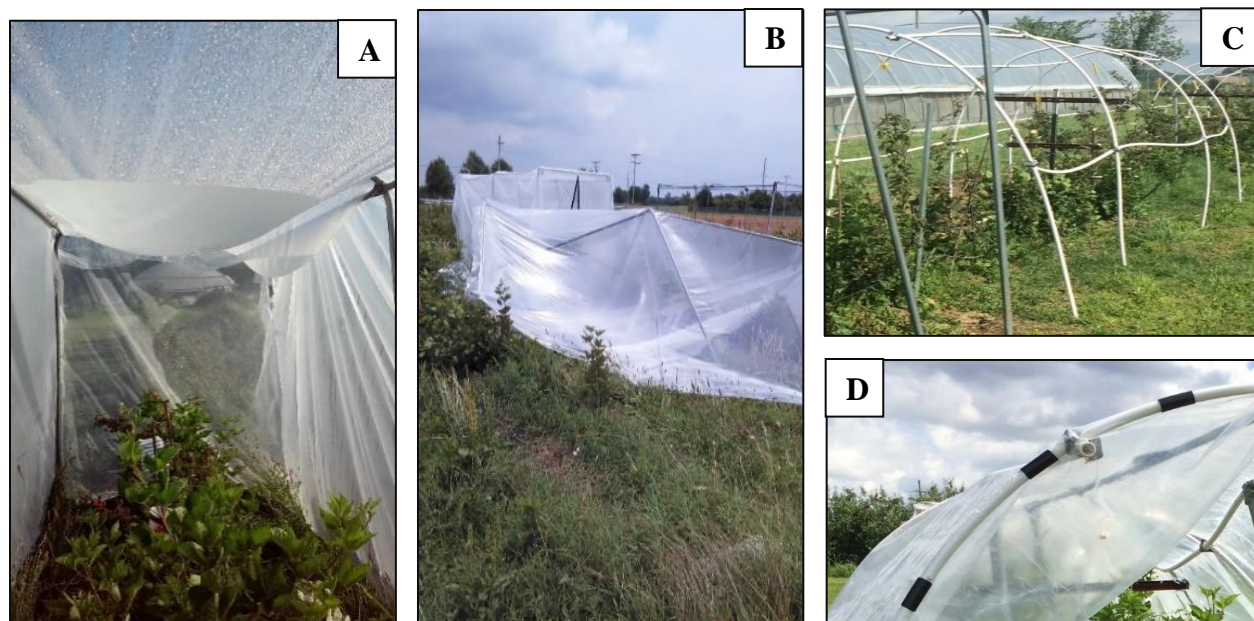


Figure 2. Changes in plastic low tunnel designs: in 2016, the tunnel had A) insect exclusion netting on both ends and plastic on both sides and the roof. B) The combination of the roof accumulating rain and strong winds caused plastic tunnels to collapse. In 2017, plastic low tunnels were redesigned to include a C) curved frame of 2.5 cm diameter PVC tubing to shed water and D) 6 cm long pieces of black PVC pipe cut in half to snap over and secure plastic to the frame.



Figure 3. Layout of three netted low tunnels at A) Farm 2 (2016) and B) Farm 3 (2017).

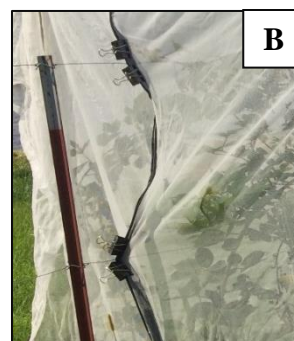


Figure 4. Insect exclusion netting on ends A-B) clipped to hold it closed around trellis wire in an organic farm in West Fork, AR (2017).



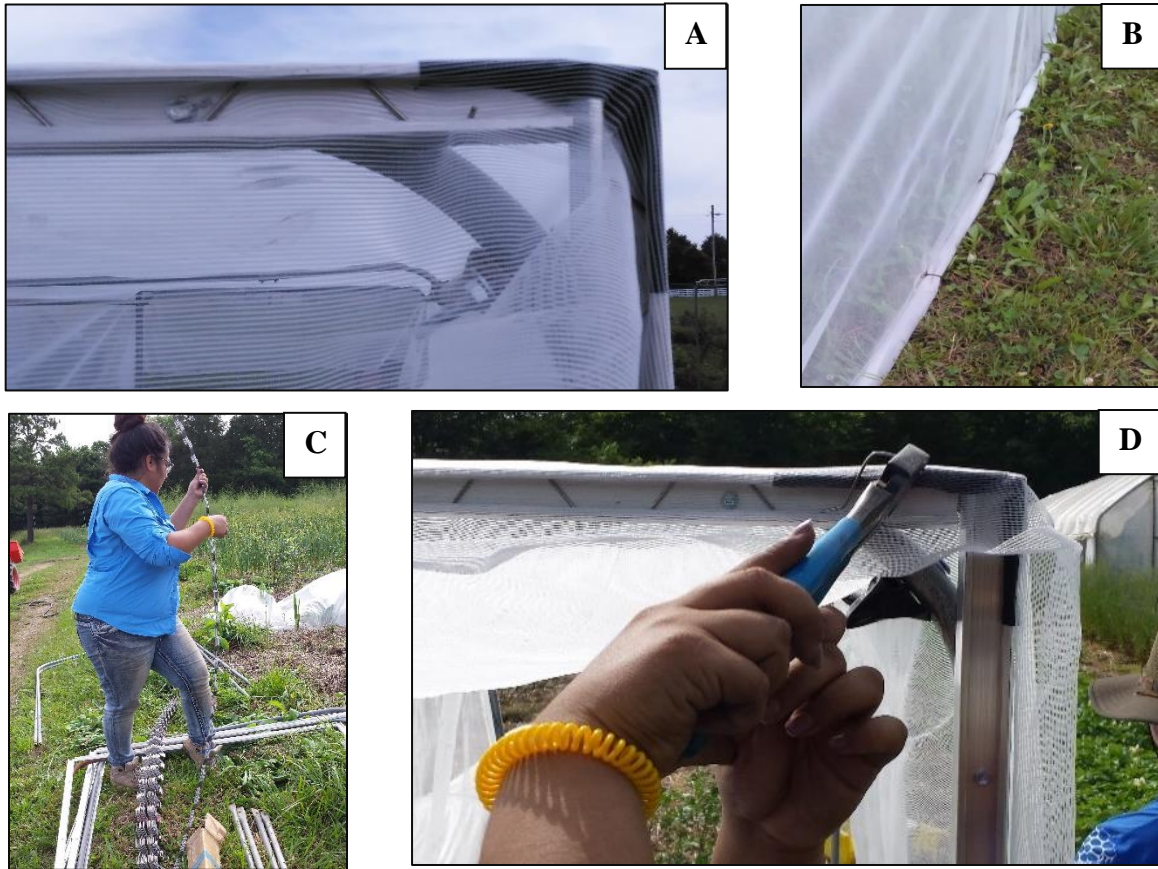


Figure 5. Low tunnel construction: A) attached to frame of aluminum lock channel with WW; B) bottom of netting on all sides was rolled around rebar and secured to the ground with galvanized landscape fabric staples; C) Array of frames and WW supplies and D) using pliers to insert WW into the aluminum lock channel to hold the netting in place.

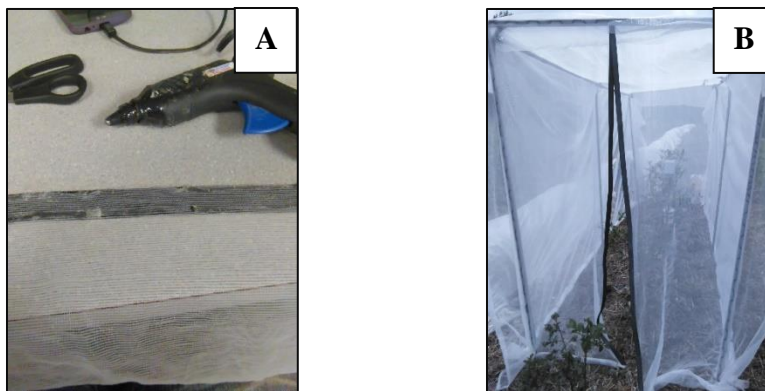


Figure 6. Assembly and installation of easy closing netted end door for low tunnels: A) hot glue magnetic strips at edges of end door netting and B) netted door installed.



Figure 7. A) Spotted wing drosophila trap with apple cider vinegar; B) Tools used to transfer spotted wing drosophila flies to sample vial.

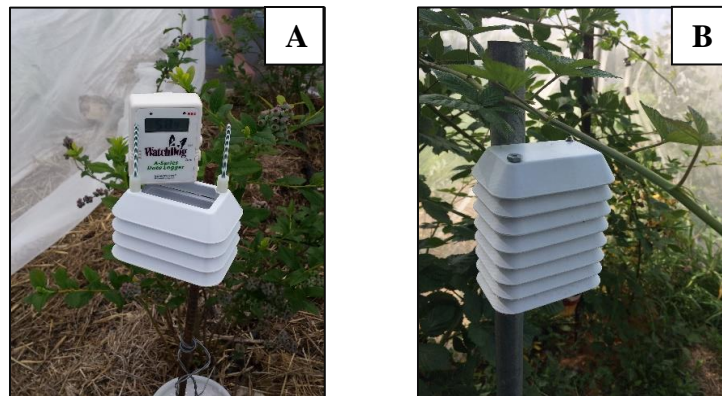


Figure 8. Watchdog weather logger placement: A) Berryville, AR (2016) and B) West Fork, AR (2017).

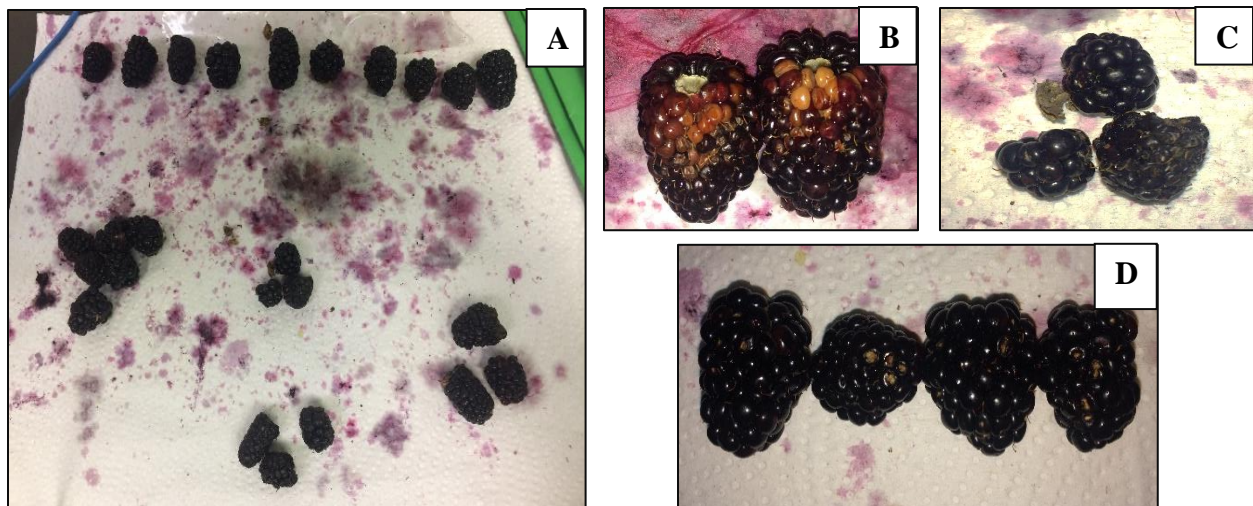


Figure 9. A) Sorting samples based on marketability; B) Example of fruit discoloration; C) example of small fruit size; D) example of drupe damage (2017).





Figure 10. A) Sample of ten blackberry fruit numbered before scanning for the presence of spotted wing drosophila eggs followed by analysis for firmness in the B) TA.XT *plus* Texture Analyser used to measure fruit firmness through compression.

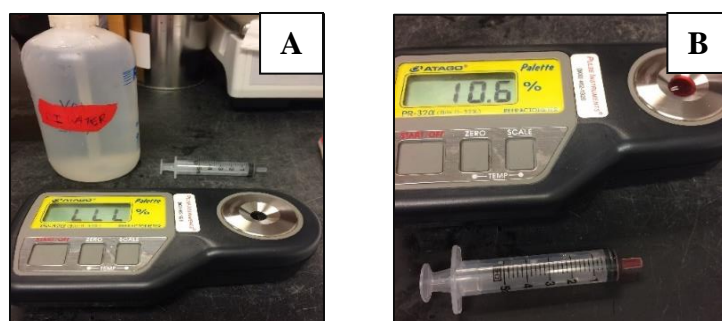


Figure 11. A) Atago® PR32α refractometer reading with deionized water. B) Refractometer reading as percentage sugar (Brix) as a measure of total percentage soluble solids for blackberry juice sample.

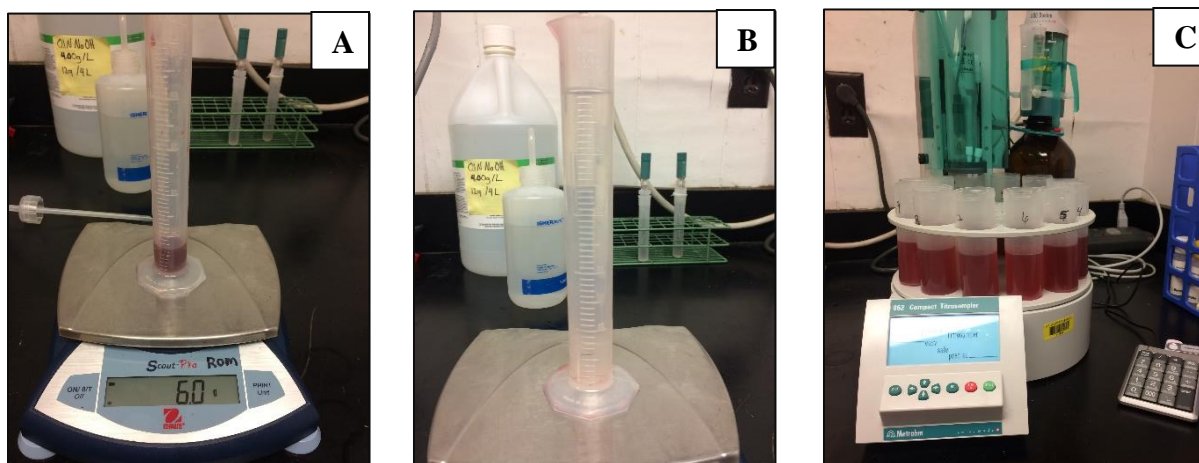


Figure 12. Measuring out A) 6.0 g of blackberry juice to be added to B) 50 ml of deionized water. C) A sample of 6 g blackberry juice in tubes analyzed for pH and acidity in a Metrohm 862 compact titrator (Mettler-Toledo, LLC, Columbus, OH).

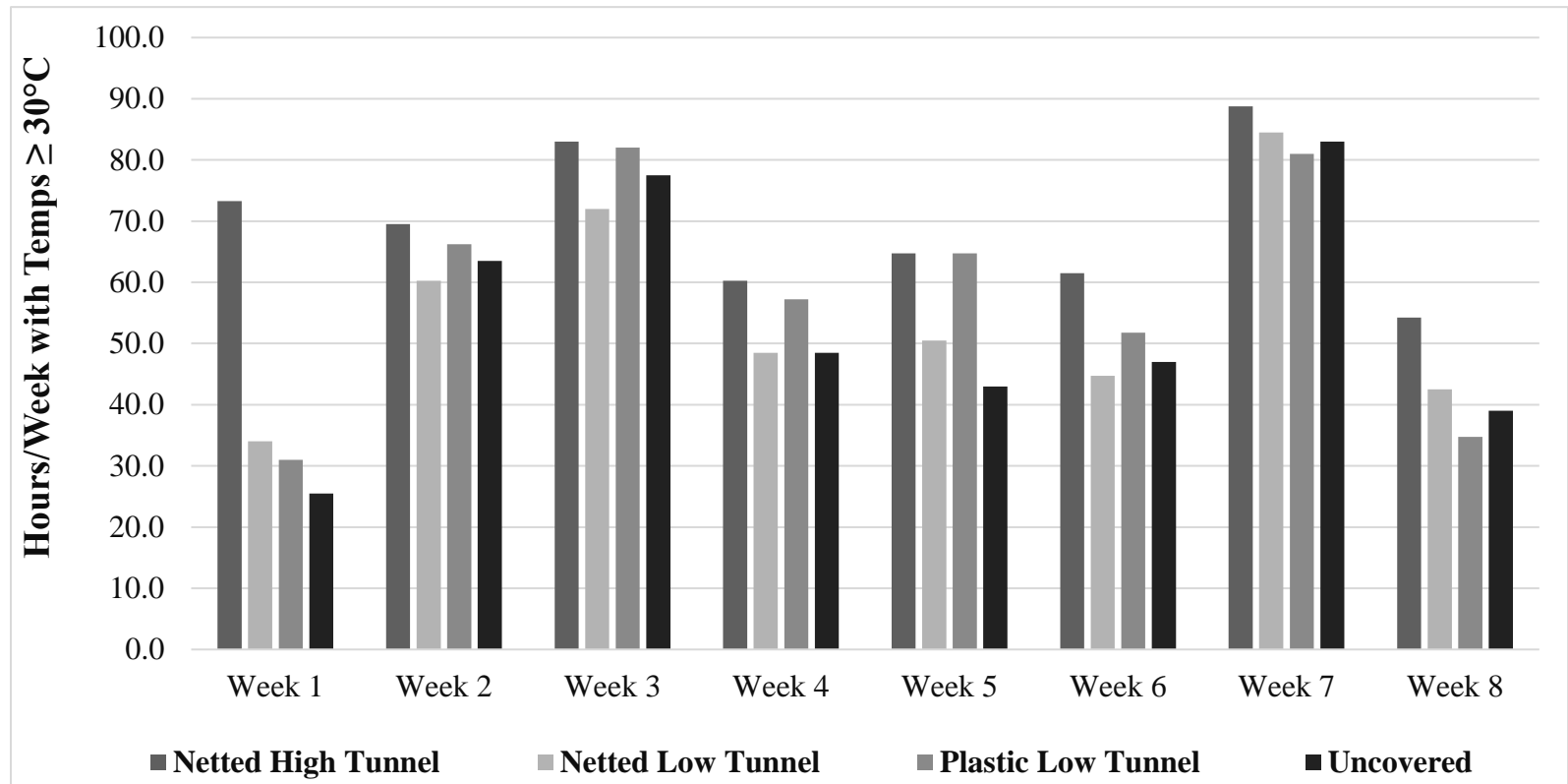


Figure 13. Hours per week in which the temperature was  $\geq 30^{\circ}\text{C}$  within each treatment and uncovered plots (Farm 1, 2016)

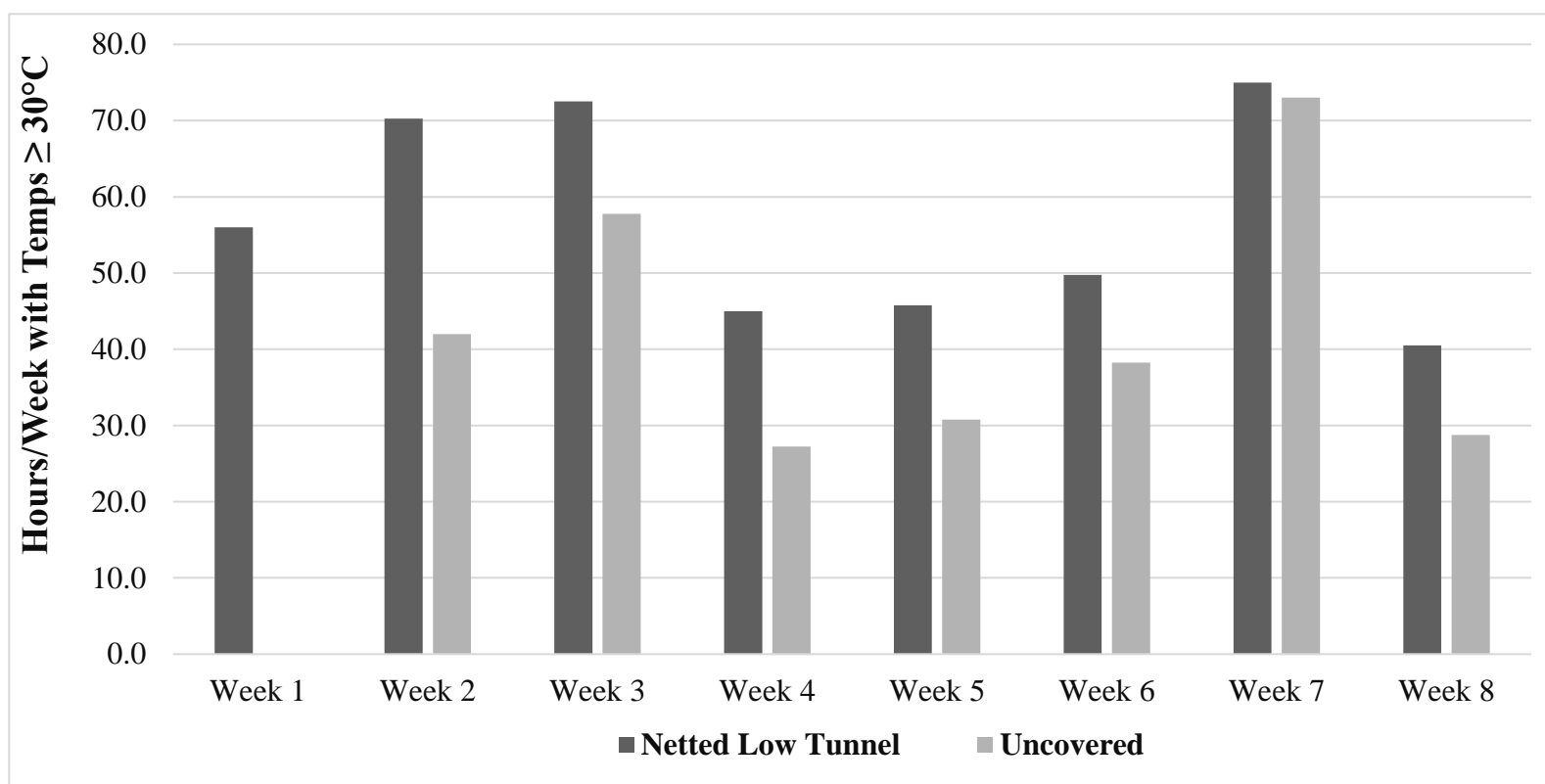


Figure 14. Hours per week in which the temperature was  $\geq 30^{\circ}\text{C}$  within netted low tunnel and uncovered plots (Farm 2, 2016).

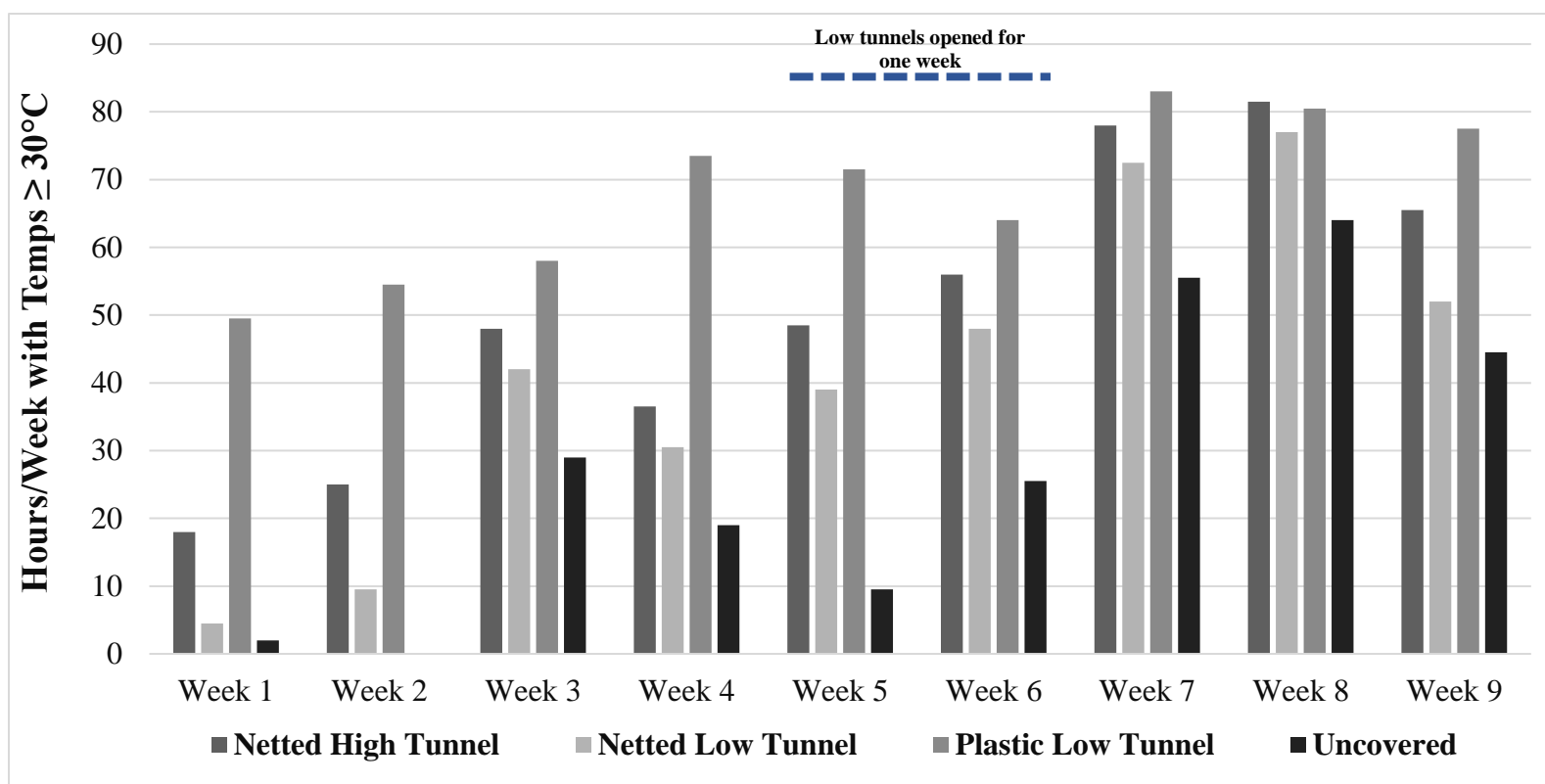


Figure 15. Hours per week in which the temperature was  $\geq 30^{\circ}\text{C}$  within each treatment and uncovered plots (Farm 1, 2017)

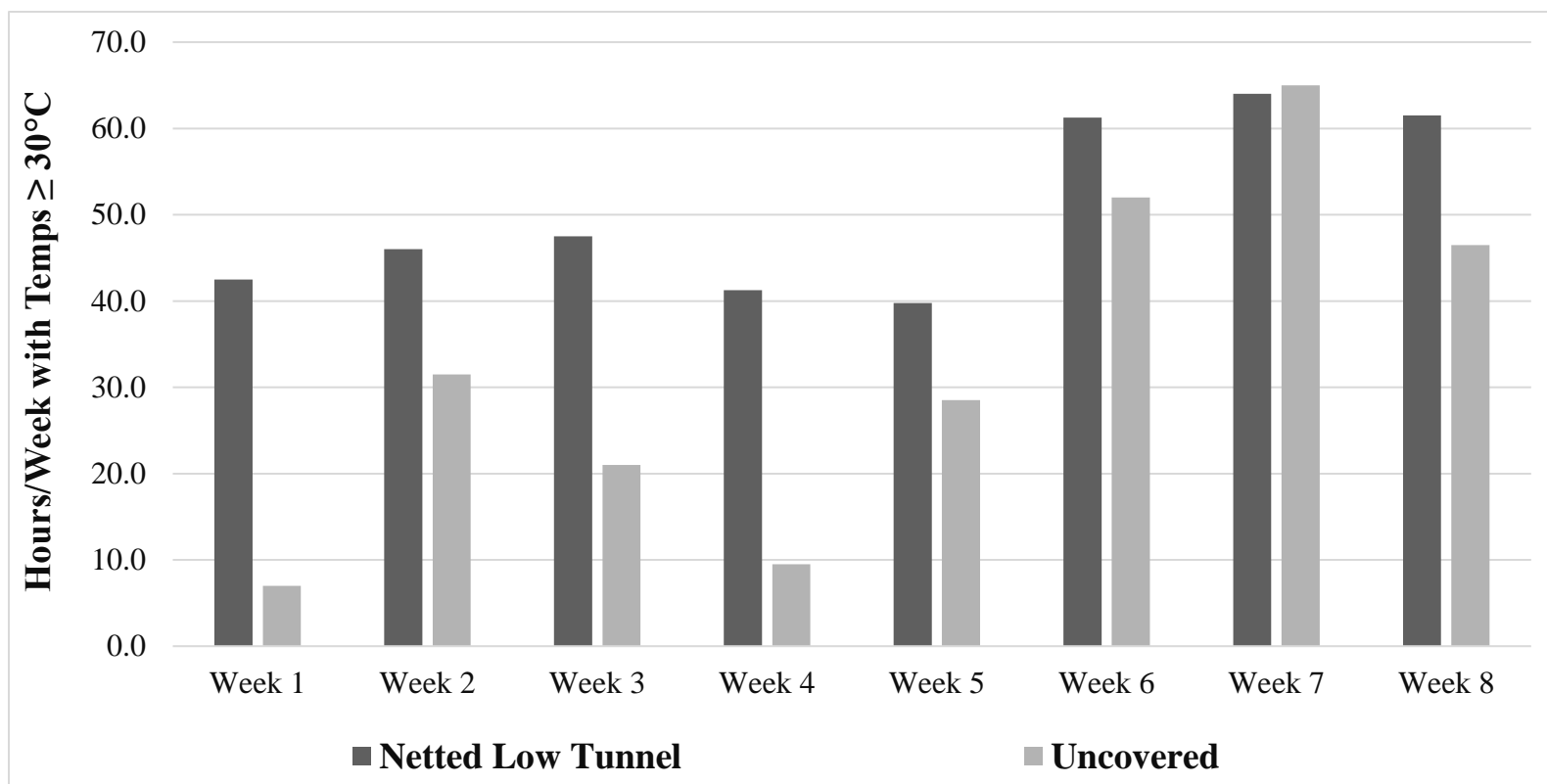


Figure 16. Hours per week in which the temperature was  $\geq 30^{\circ}\text{C}$  within netted low tunnels and uncovered plots (Farm 3, 2017)

Table 1. Site descriptions that include farm name, location, crop, cultivars, number of netted high tunnels (NHT), netted low tunnels (NLT) and plastic low tunnels (PLT) and number of SWD traps per treatment for 2016 and 2017.

Year	Name	Farm	Location	Crop	Cultivars	NHT	NLT	PLT	NHT Traps	NLT Traps	PLT Traps	Uncovered Traps
2016-2017	Farm 1	AAREC	Fayetteville, AR	Blackberry	Prime-Ark®45 Prime-Ark®Freedom Prime-Ark®Traveler	1	3	2	2-4	3	2	2-1
2016	Farm 2	Dripping Springs Garden	Berryville, AR	Blueberry	Blueray Bluecrop Bluejay	0	3	0	-----	3	----	3
2017	Farm 3	Henderson Farm	West Fork, AR	Blackberry	-----	0	3	0	-----	3	----	1



Table 2. Mean numbers of spotted wing drosophila (SWD) flies per trap and eggs per fruit ( $\pm$ SE) by treatment and sample date (Farm 1, 2016).

Sample Date	High Tunnel				Low Tunnel			
	(sides and ends netted, N=2)		Low Tunnel (all netted, N=3)		(plastic sides/top, netted ends, N=2)		Outside Ambient (uncovered, N=2)	
	Flies	Eggs	Flies	Eggs	Flies	Eggs	Flies	Eggs
	per trap	per fruit	per trap	per fruit	per trap	per fruit	per trap	per fruit
6-Jun	0.0	0.0	0.0	0.0	0.0	----	0.0 $\pm$ 0.0	----
13-Jun	0.0	0.0	0.0	0.0	0.0	----	0.0 $\pm$ 0.0	0.1 $\pm$ 0.28
20-Jun	0.0	0.0	0.0	0.0	0.0	0.0	0.5 $\pm$ 0.5	----
27-Jun	0.0	0.0	0.0	0.0	0.0	0.0	2.0 $\pm$ 2.0	0.03 $\pm$ 0.00
4-Jul	0.0	0.0	0.0	0.0	0.0	0.0	0.0 $\pm$ 0.0	0.03 $\pm$ 0.00
11-Jul	0.0	0.0	0.0	0.0	0.0	0.0	0.0 $\pm$ 0.0	0.7 $\pm$ 0.20
18-Jul	0.0	0.0	0.0	0.0	0.0	----	17.5 $\pm$ 4.50	1.1 $\pm$ 0.29
25-Jul	0.0	0.0	0.0	0.0	0.0	----	31.5 $\pm$ 10.50	0.2 $\pm$ 0.10

Table 3. By treatment and sample date mean number of spotted wing drosophila (SWD) flies per trap ( $\pm$ SE), and eggs per fruit (Farm 2, 2016).

Sample Date	Low Tunnel (netted, N=3)		Open Crop (uncovered, N=3)	
	Flies per trap	Eggs per fruit	Flies per trap	Eggs per fruit
6-Jun	0.0	0.0	0.0	0.0
13-Jun	0.0	----	0.0	----
20-Jun	0.0	0.0	0.0	0.0
27-Jun	0.0	0.0	0.0	0.0
4-Jul	0.0	----	0.0	----
18-Jul	0.0	----	0.7 $\pm$ 0.3	----
25-Jul	0.0	----	7.0 $\pm$ 0.0	----

Table 4. By treatment and sample date mean number of spotted wing drosophila (SWD) flies per trap ( $\pm$ SE) and eggs per fruit ( $\pm$  SE) (Farm 1, 2017).

Sample Date	High Tunnel (sides and ends netted, N=4)		Low Tunnel (netted, N=3)		Low Tunnel (plastic, N=2)		Outside Ambient (uncovered, N=1)	
	Flies	Eggs	Flies	Eggs	Flies	Eggs	Flies	Eggs
	per	per	per	per	per	per	per	per
	trap	fruit	trap	fruit	trap	fruit	trap	fruit
5-Jun	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9 $\pm$ 1.10
12-Jun	0.0	0.0	0.0	0.04 $\pm$ 0.0	0.0	0.0	3.0	0.8 $\pm$ 0.29
19-Jun	0.0	0.0	0.7 $\pm$ 0.33	0.4 $\pm$ 0.10	0.0	0.1 $\pm$ 0.13	0.0	1.3 $\pm$ 0.24
26-Jun	0.0	0.0	2.3 $\pm$ 0.88	0.3 $\pm$ 0.10	0.0	0.05 $\pm$ 0.04	6.0	7.6 $\pm$ 0.87
3-Jul	0.3 $\pm$ 0.25	0.03 $\pm$ 0.0	9.3 $\pm$ 6.84	1.4 $\pm$ 0.17	0.5 $\pm$ 0.50	0.9 $\pm$ 0.35	1.0	8.9 $\pm$ 1.08
10-Jul	0.8 $\pm$ 0.75	0.1 $\pm$ 0.10	24.0 $\pm$ 6.35	1.4 $\pm$ 0.21	1.5 0.50	1.6	8.2 $\pm$ 1.73	8.2 $\pm$ 1.73
17-Jul	1.3 $\pm$ 0.75	0.8 $\pm$ 0.18	7.3 $\pm$ 4.48	3.3 $\pm$ 1.21	0.5 $\pm$ 0.50	1.5 $\pm$ 1.00	49.0	1.0 $\pm$ 0.34
24-Jul	0.8 $\pm$ 0.48	0.3 $\pm$ 0.10	3.3 $\pm$ 3.33	1.4 $\pm$ 0.79	0.0	0.1 $\pm$ 0.10	33.0	1.3 $\pm$ 0.64
31-Jul	0.2 $\pm$ 0.10	0.2 $\pm$ 0.10	0.00	0.8 $\pm$ 0.22	0.0	0.0	2.0	0.4 $\pm$ 0.17
Average	0.38	0.16	5.21	1.00	0.13	0.47	11.36	3.60

Table 5. By treatment and sample date, mean number of spotted wing drosophila (SWD) flies per trap ( $\pm$ SE) and mean eggs per fruit ( $\pm$ SE) (Farm 3, 2017).

Sample Date	Low Tunnel (all netted, N=3)		Outside Ambient (uncovered, N=1)	
	Flies	Eggs	Flies	Eggs
	per	per	per	per
	trap	fruit	trap	fruit
5-Jun	0.0	----	17.0	----
12-Jun	0.0	----	24.0	----
19-Jun	0.0	----	1.0	----
26-Jun	0.0	0.1 $\pm$ 0.10	0.0	1.4 $\pm$ 0.27
3-Jul	0.0	1.2 $\pm$ 0.37	1.0	----
10-Jul	0.0	0.6 $\pm$ 0.21	1.0	0.9 $\pm$ 0.28
17-Jul	0.0	1.0 $\pm$ 0.33	2.0	0.2 $\pm$ 0.14
24-Jul	1.3 $\pm$ 1.33	2.3 $\pm$ 1.10	0.0	7.0 $\pm$ 2.16
Average	0.16	1.04	5.75	2.38

Table 6. Effects of cumulative hours  $\geq 30^{\circ}\text{C}$ , and hours  $\leq 65\%$  RH and  $> 95\%$  RH on treatment means of Brix of harvested blackberry fruit (Farm 1, 2017).

Treatment	Brix (%)	Cum. Hrs. $\geq 30^{\circ}$	Cum. Hrs. $\leq 65\%$ RH	Cum. Hrs. $\geq 95\%$ RH
Netted High Tunnel	11.1a	51.9	76.7	19.9
Plastic Low Tunnel	10.1ab	68.0	60.7	68.8
Netted Low Tunnel	9.4b	40.8	46.8	59.0
Uncovered	9.6b	27.7	44.1	38.7

Means in same column followed by the same letter are not significantly different (one-way ANOVA, Tukey's HSD,  $P>0.05$ )

Table 7. Differences in Brix based on treatment and percent relative humidity ( $<65\%$ ) of harvested blackberry fruit (Farm 1, 2017).

Treatment	LSMean (df = 190)	95% CI
Netted High Tunnel	9.7 $\pm$ 0.20b	9.31, 10.11
Plastic Low Tunnel	10.6 $\pm$ 0.46b	9.68, 11.50
Netted Low Tunnel	10.6 $\pm$ 0.54b	9.55, 11.67
Uncovered	12.7 $\pm$ 0.59a	11.52, 13.83

LSMeans in same column followed by the same letters are not significantly different (Tukey's HSD,  $P>0.05$ )

Table 8. Effects of cumulative hours <65% RH on treatment mean titratable acidity of harvested blackberry fruit (Farm 1, 2017)

Treatment	LSMean (df =137)*	95% CI	Titratable acidity (%)*
Netted High Tunnel	-0.5	-0.57, -0.40	0.62±0.004c
Plastic Low Tunnel	-0.1	-0.35, 0.06	0.86±0.10bc
Netted Low Tunnel	0.1	-0.09, 0.30	1.11±0.1b
Uncovered	0.4	0.19, 0.61	1.49±0.11a

\* Back transformed from LSMeans in column with the same letters are not significantly different ( $X^2$ , Tukey's HSD,  $P>0.05$ )

Table 9. Back transformation of the LSMeans of firmness using exponential natural log to determine differences in firmness based on blackberry treatments and percent relative humidity (<65%RH) (Farm 1, 2017).

Treatment	LSMean (df = 1628)*	95% CI	Firmness (N)
Netted Low Tunnel	2.6	2.54, 2.74	6.9±0.06b
Uncovered	2.7	2.59, 2.85	7.4±0.07ab
Plastic Low Tunnel	2.8	2.68, 2.87	7.7±0.05ab
Netted High Tunnel	2.9	2.82, 2.95	8.3±0.03a

\*Back transformed from LSMeans in column with the same letters are not significantly different ( $\log(x)$ , Tukey's HSD,  $P>0.05$ )

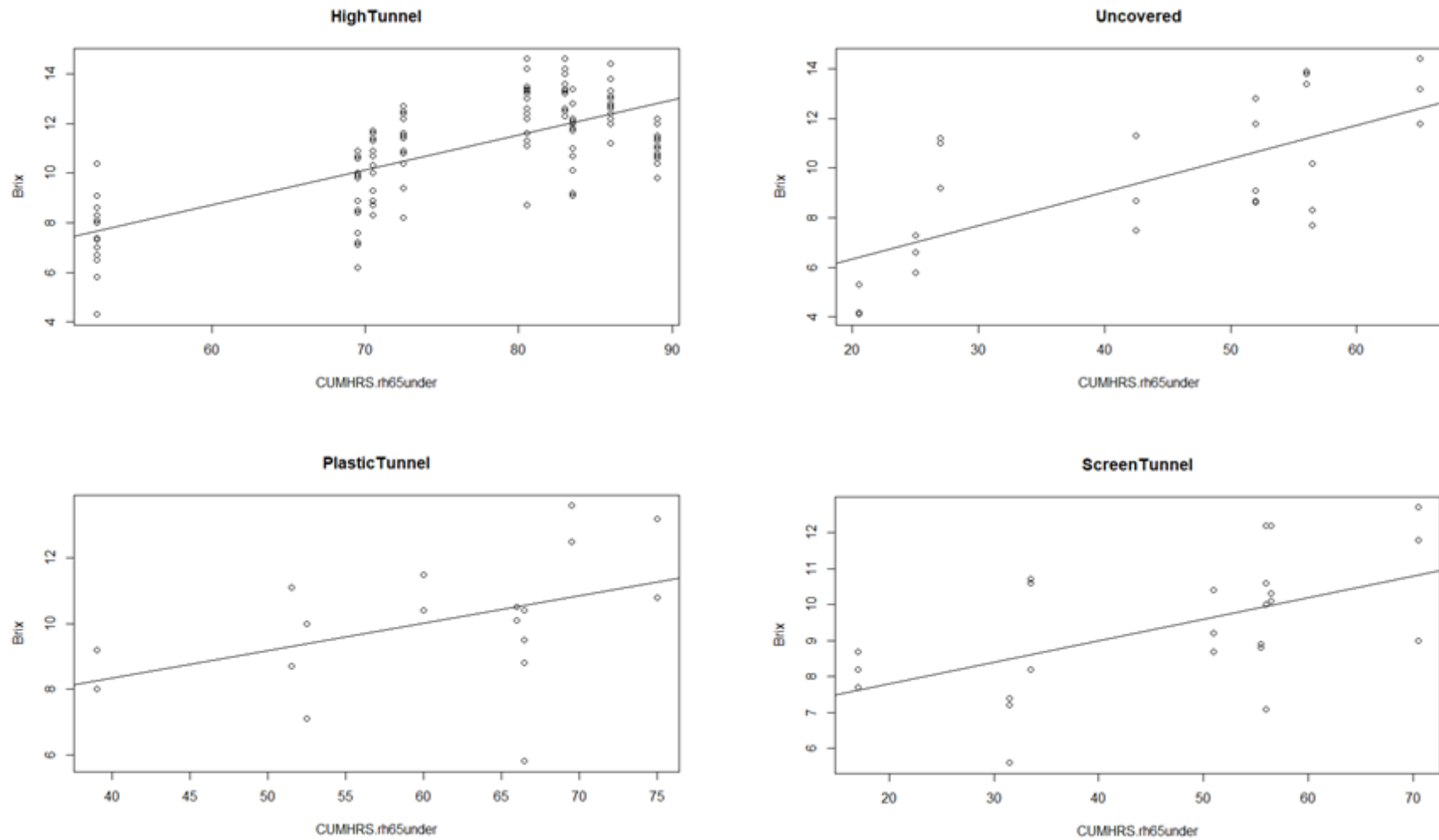


Figure 17. Brix values of blackberry fruit for the interaction of treatments (uncovered or 3 tunnel types with insect exclusion netting) by cumulative number of hours exposure to air  $\leq$  65% relative humidity (RH)

## CHAPTER 5: Conclusion

Spotted wing drosophila (SWD) is an invasive pest of ripening and ripe thinned skinned fruits. Global trade, initial lack of regulation of infested berry fruits, high reproductive potential and a wide range of wild hosts have contributed to the recent (after 2008) expansion of SWD range from eastern Asia to Europe and the Americas (Ioriatti et al. 2013). The current study was conducted in Arkansas. It included monitoring of SWD populations in different crop systems and adjacent landscape habitats, identifying wild hosts of SWD, evaluate is effectiveness of insect exclusion netting in tunnels to prevent SWD fruit infestations, and comparing effects of netted tunnels on temperature and fruit quality. Hopefully, the following findings will lead to improved pest management practices against SWD.

The season total numbers of SWD were lower in the fruit crop plots than in the perimeter traps located next to a refuse pile of culled fruit and mulch and in the shaded perimeter tree canopy. In Arkansas, these perimeter sites had 12 wild host plants where there were SWD infested fruit from early June into October. The Arkansas perimeter landscape habitats with wild SWD host plants appeared to be an important refuge for SWD dispersal to ripening fruits in adjacent commercial blocks. This agreed with the findings reported by Lee et al. (2015). Many other pests are also dependent on both the crop and landscape habitat to sustain populations (Ricci et al. 2009, Mitsui et al. 2010, Klick et al. 2016). Furthering our understanding of the dispersal between adjacent wild host plants and fruit crops could be beneficial in the design and implementation of long-term integrated pest management strategies. With this information, growers may be able to monitor the perimeter habitat and apply attract-and-kill tactic near early maturing crops (Hampton et al. 2014) before SWD densities get too high or apply insecticides when SWD are at higher densities.



In tunnel production systems, insect exclusion netting is proving to be effective against higher SWD densities (Link 2014, Cormier et al. 2015, Schattman et al. 2015, Chouinard et al. 2016, Leach et al. 2016, Rogers et al. 2016, Alnajjar et al. 2017). In the 2016 study, netting on most of the low (1.8 m) and high (3.7 m) tunnels prevented SWD fruit infestations but one tunnel had SWD infested fruit late in the season. In 2017, SWD infestation were delayed for a few weeks in these netted tunnels. Exclusion tunnels could broaden the distribution of small fruit production especially in areas where SWD densities are high. However, more should be conducted to improve timing of placement of exclusion netting as well as the economics of building and implementing exclusion netting into a management program (Link 2014). Monitoring SWD fly densities and oviposition on fruit could aid in integrating control tactics and contribute to reducing the dependency on one control and extend SWD management over an extended harvest period from early summer florican blackberries and raspberries through the fall harvest of primocane-fruiting blackberries and raspberries.

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