

**INTEGRATED PEST MANAGEMENT OF SPOTTED WING DROSOPHILA
(*DROSOPHILA SUZUKII*) IN MICHIGAN HIGH TUNNEL RASPBERRIES**

By

Heather Leach

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ABSTRACT

INTEGRATED PEST MANAGEMENT OF SPOTTED WING DROSOPHILA (*DROSOPHILA SUZUKII*) IN MICHIGAN HIGH TUNNEL RASPBERRIES

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Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a global pest of economic importance in raspberry and other berry crops. High fecundity, short generation time, and limited understanding of this invasive pest contribute to the challenges in managing this fly. Current management is largely dependent on frequent applications of broad-spectrum insecticides and this thesis explores new management options for control of this pest in high tunnel raspberries, an increasingly common popular production method for this crop. During 2015 and 2016, surveys were conducted to characterize the arthropod community in high tunnel raspberry plantings in Central and Southwest Michigan. Methods to control the insect pests of raspberries, including *D. suzukii*, were evaluated over these two years. This includes the use of specialty plastics to reduce ultra-violet (UV) light transmission in the growing environment, which may disorient insects and reduce pesticide degradation, and the use of exclusion netting. While UV blocking plastics did not strongly affect the insect community, they reduced the degradation of insecticides up to 50% compared to plastics that transmitted UV. Moreover, exclusion netting reduced *D. suzukii* adult and larval populations by up to 75% and delayed their arrival by 4 weeks. Additionally, netting used with insecticides contributed to a greater reduction of *D. suzukii* than either netting or insecticides alone. This research demonstrates effective new knowledge that can be used to increase sustainability in raspberry production and help rebuild integrated pest management after invasion by *D. suzukii*.

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CHAPTER 1. MANAGEMENT OF SPOTTED WING DROSOPHILA AND OTHER PESTS IN HIGH TUNNEL RASPBERRY PRODUCTION SYSTEMS

INTRODUCTION

Identification and biology

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a polyphagous frugivore commonly referred to as spotted wing *Drosophila*. This pest is closely related to other vinegar flies, including the well-known and studied model organism *Drosophila melanogaster* (Meigen). Both species are within the subgenus *Sophophora* along with *Drosophila biarmipes*, which is closely related and considered a sister group to *D. suzukii* (Yang et al. 2011, Chiu et al. 2013). Female *D. suzukii* are unlike most other *Drosophila* flies in that they have a large doubly-serrate ovipositor which allows them to expand their host range to ripening and ripe fruits, compared to other common vinegar flies which infest rotten or damaged fruits (Walsh et al. 2011). *Drosophila subpulchrella* also has a serrated ovipositor which allows it to deviate from rotten or damaged fruits as a host, but the shape and overall morphology allow these two species to be distinguished (Atallah et al. 2014). Adult *D. suzukii* are 2-3 mm in length, with females being slightly larger than their male counterparts (Walsh et al. 2011). They are golden brown in body color and have red eyes (Figure 1.1). Male *D. suzukii* have one dark spot on the leading edge of each wing, from which this species gets its common name. The eggs are white, shiny, and cylindrical with two spiracles that remain on the outside of the host (Kanzawa 1939; Walsh et al. 2011). Larvae are elongated and cream-colored, with respiratory structures, creeping welts, and mouthparts visible (Kanzawa 1939; Walsh et al. 2011; Van Timmeren et al. 2017). The pupae are oval and

cylindrical with two distinctive spiracles that protrude from the front and are golden brown, darkening as they harden (Kanzawa 1939; Walsh et al. 2011).

Life cycle

Optimal environmental conditions for *D. suzukii* are largely determined by temperature and humidity. Optimal temperature estimates vary dependent on the study and the diet substrate, but general trends are consistent. Based on parameters developed by Tochen et al. (2014) from cherries, their reproductive rate is highest at 22 °C and optimal developmental temperatures are 28.1 °C. Minimal. Minimum and maximum temperatures for development were found to be 7.2 °C and 42.1 °C, respectively, which were determined through modeling the temperature-dependent survivorship curve (Tochen et al. 2014). Using parameters from Kinjo et al. (2014), female *D. suzukii* lay the greatest number of eggs at 25 °C and this declines with increasing temperatures. *Drosophila suzukii* are also sensitive to high temperatures with an upper temperature threshold of 31°C for the egg and larval stage (Kinjo et al. 2014). Some studies suggest that *D. suzukii* will live up to 30 days and lay about 300 eggs in their lifetime (Kinjo et al. 2014). Emiljanowicz et al. (2014) found that the average lifespan of *D. suzukii* was about 86 days at 22 °C with a maximum of up to 153 days. At this temperature, they were able to generate an average lifetime production of 536 eggs (Emiljanowicz et al. 2014). Early studies on *D. suzukii* from Kanzawa (1939) found that females will produce between 219 and 563 eggs in their lifetime. *Drosophila suzukii* also desiccate easily and require moisture within their environment to survive (Shearer et al. 2016; Wiman et al. 2016).

Adult female *D. suzukii* lay eggs after they are 1-3 days old, and fecundity per day increases after this period until they are about 20 days old (Kanzawa 1939; Kinjo et al. 2014; Tochen et al. 2014). Eggs are deposited just below the surface of their hosts and the two spiracles remain on the outside of the host, presumably to aid in oxygen uptake (Hinton 1960; Demerec 1994). The egg-laying process can take anywhere from seconds to hours, depending on the penetration force required to break through the skin of the host fruit (Lee et al. 2011). It takes about 24 hours for the egg to hatch into a first instar larva. The larvae go through three larval instars before pupating. Pupation occurs both within and outside of the fruit, but more commonly larvae are seen on the outside of the fruit (Woltz and Lee 2017). At 26 °C, it takes only about 10 days to go from egg to adult (Figure 1.1) (Lee et al. 2011; Tochen et al. 2014). Adults will feed on a variety of substrates, including fruits, mushrooms, bird feces, and flowers (Lee et al. 2015; Wallingford et al., unpublished). Both the high fecundity and rapid life cycle of this insect allows it to have up to 13 generations per growing season, dependent on environmental conditions (Asplen et al. 2015; Burrack et al. 2015). Depending on climate and region, *D. suzukii* may be active all year or have a period of quiescence during cold or hot temperatures.

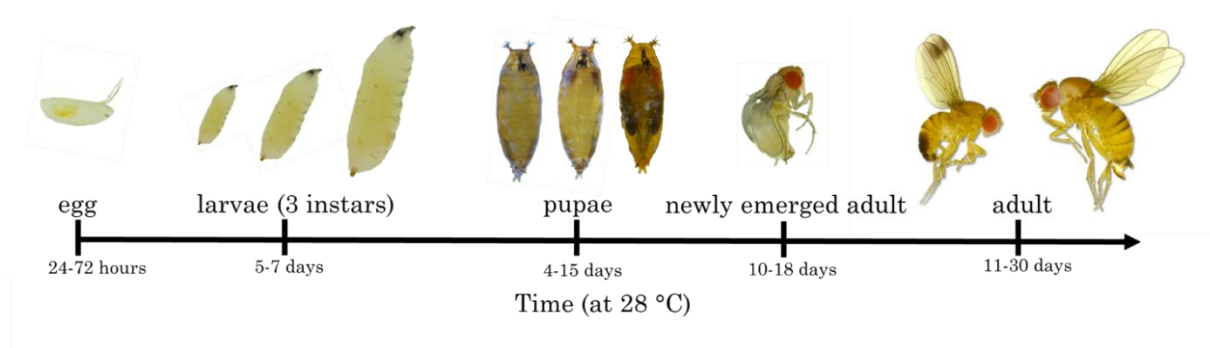


Figure 1.1. The life cycle of *Drosophila suzukii* at 28 °C, where one generation takes approximately 10-12 days to go from egg to adult.

Little is known about the overwintering capabilities of *D. suzukii*, except that it has a winter morph that occurs when eggs and young instar larvae are exposed to colder temperatures. These adults have larger wings and a darker and smaller body. In this stage, they have both down-regulated and up-regulated genes which allow them to be longer-lived and to survive at cooler temperatures (Shearer et al. 2016). Despite these defenses against the cold, harsh winters such as those in Michigan are still thought to limit populations (Wallingford et al., unpublished). This could be a vulnerable bottleneck period, with implications for how their genes flow to the following season. However, the overwintering behavior and habitat of *D. suzukii* in Michigan and elsewhere is still poorly understood.

Modeling *D. suzukii* population development has been done primarily using degree-day Leslie-matrix models (Wiman et al. 2014; Tochen et al. 2014; Wiman et al. 2016). Some models, including those from Wiman et al. (2016) have been run with control options, such as insecticides and varying levels of biological control, to better understand when populations of *D. suzukii* are most vulnerable. These models may also explain the failure of some control methods, including insecticide applications that target the adult, since adults are a small proportion of the population during the growing season (Wiman et al. 2014; Emiljanowicz et al. 2014). Some online modeling tools already exist for *D. suzukii* (e.g. www.uspest.org) that are based on region and degree days using models provided by Oregon State University (Coop 2015) that predict when first egg-laying and population peaks may occur. However, it has also been recognized that degree day models for *D. suzukii* are not an ideal approach to population prediction (Wiman et al. 2014), partly due to the generational overlap of this species (Asplen et al. 2015). Better results are found using a stage-structured model combined with degree day modeling (Wiman et al. 2016). As knowledge on the overwintering behavior and biology of *D. suzukii* continue to

develop, and potential new control measures are put in place, these models will continue to get more specific. Ideally, these models can become more relevant to crops and regions so that growers may use it them as tools for applying their control measures. dates of

Spread and distribution

While this pest is native to East Asia (China, Japan, North, South Korea), it is now present in North America, Europe, Central America, South America, and continued expansion is expected (Asplen et al. 2015). The first detection outside of East Asia was in 1980 in Hawaii (Kaneshiro 1983), but it has not been classified as a pest in this area (Beardsley et al. 1999; O’Grady et al. 2002; Hauser 2011). In 2008, *D. suzukii* was detected in California and in the five years following, it had spread to all major fruit producing regions of the U.S. and is now considered a critical pest to manage in the affected crops (Goodhue et al. 2011; Asplen et al. 2015). In Europe, *D. suzukii* was first detected in Italy and Spain in 2009 (Grassi et al. 2009; Calabria et al. 2012). It has since spread to most of Europe, including Slovenia, Germany, Hungary, and Poland (Seljak 2011; Vogt et al. 2012; Kiss et al. 2013; Łabanowska and Piotrowski 2015). *Drosophila suzukii* has been documented in Costa Rica and Ecuador (Hauser 2011) and continues to spread south into Brazil (Depra’ et al. 2014; Vileia and Mori 2014), Argentina (Santadino et al. 2015), and Chile (Medina-Munoz et al. 2015).

Phenology and trapping

The phenology of *D. suzukii* is highly dependent on weather conditions. In the Midwest with cold winters, *D. suzukii* are active from approximately June through October and populations build as the season continues (Hamby et al. 2014; Van Timmeren et al., unpublished). In southern California, *D. suzukii* are active season-long, except in the hottest periods of the year where temperatures exceed their upper threshold (Dalton et al. 2011; Tochen et al. 2014). In other regions, including Europe, populations follow similar phenology trends dependent on the climate.



Figure 1.2. A 32 oz. adult *Drosophila suzukii* trap made with a mixture of yeast, sugar, and water, with a yellow-sticky trap hung in the center. After being hung in a blueberry bush for one week, it shows the many Drosophilids and non-target insects caught.

Adult activity is generally monitored with traps, which differ depending on region and preference. Several commercial lures have been developed for *D. suzukii*, and homemade traps baited with a solution of yeast and sugar (Figure 1.2) or apple cider vinegar are common (Landolt et al. 2010; Cha et al. 2012; Lee et al. 2012). The commercial lures consist of four principal components: acetic acid, ethanol, acetoin, and methionol. Generally, traps are deployed and checked once a week. In Michigan, captures of *D. suzukii* during high population periods can easily exceed 1,000 adults per trap per week. Early season captures tend to have a 50:50 sex ratio, whereas late season fly capture tends to be more dominated by females (Hamby et al. 2014; Van Timmeren et al., unpublished). Larval population activity can be monitored by sampling the fruit in the crop of concern. This can include visual scouting for damage, which may include leaky deflated fruit or fruit with emergence holes from the third instar larvae (Figure 1.3). For precise counts of infestation, lightly crushed fruit can also be immersed in a strong salt or sugar solution (Leach et al. 2016). This causes the larvae to emerge from the fruit, which can then be counted using a stereomicroscope.



Figure 1.3. Characteristic emergence holes and deflation of a blueberry from the third instar *Drosophila suzukii* larvae leaving the fruit to pupate.

Host range

A key factor limiting the host range of *D. suzukii* is their ability to penetrate the skin for laying eggs (Bellamy et al. 2013). The most important crop hosts are raspberries, cherries, blueberries, and blackberries. Based on host odors, to the most attractive fruits are raspberries, followed by strawberry, blackberry, and blueberry (Bellamy et al. 2013; Burrack et al. 2013). The number of eggs laid per gram is highest in strawberry, followed by raspberry and blackberry (Lee et al. 2011). Overall, the most preferred and successful host of cultivated crops in the field appears to be raspberry, closely followed by blackberry. In some fruits with tougher skins, such as plums and peaches, they appear to be opportunistic, similar to other *Drosophila* species. Pre-existing damage, such as insect feeding or splitting, allows *D. suzukii* to lay eggs in these crops. As with these stone fruits, grapes are not preferred since oviposition decreases with the increased penetration force needed to penetrate grape skins (Ioriatti et al. 2015). However, damaged grapes during harvest time have been shown to experience increased *D. suzukii* feeding and oviposition, and they also spread sour rot bacteria that decrease the marketability of the grapes (Ioriatti et al. 2015). *Drosophila suzukii* also infest many wild fruits, which provide opportunities to build populations in the early part of the growing season, before cultivated fruit ripen (Kimura et al. 1977; Nishiharu 1980; Mitsui et al. 2010; Lee et al. 2015; Briem et al. 2015; Kenis et al. 2016).

CURRENT STATUS OF *D. SUZUKII*

Economic losses

In both its native and invaded regions, extensive economic damage has been documented from *D. suzukii*. Economic impact within the invaded region is much more significant due to the lack of natural enemies or competitors (Yamakawa and Watanabe 1991; Sasaki and Sato 1995; Kawase et al. 2008). In the western U.S., damage to cherries, raspberries, blueberries, strawberries, and blackberries equated to \$511 million in annual losses, assuming a 20% crop loss across each of these crops (Walsh et al. 2011). Bolda et al. (2010) used maximum crop loss values of 40% in blueberries, 50% for caneberries, 33% for cherries, and 20% for processing strawberries and found that revenue losses could be as large as \$421 million in the same region. In one province in Italy, economic losses from *D. suzukii* in one year were \$2.5 million (De Ros et al. 2013). In the U.S. in 2014, raspberry production had reportedly lost \$150 million due to this pest (Burrack et al., unpublished data). However, conventional raspberry production in California in the past few years have been only 3% or less as a result of adequate chemical management schedules (Farnsworth et al. 2016). However, losses remain significant in organic systems (Farnsworth et al. 2016), and the higher costs of production to manage this pest challenges the economic viability of berry production.

Estimated economic losses from *D. suzukii* in Michigan were \$20 million in 2012 (R. Isaacs, unpublished). *Drosophila suzukii* has caused a dramatic increase in insecticide applications and a reduction in saleable fruit, resulting in lost business for Michigan growers. Furthermore, environmental and landscape factors in Michigan are highly favorable for the

success of *D. suzukii* (Pelton et al. 2016). Recent modeling suggests that economic impact is minimized by using high efficiency traps to guide spray application timing (Fan et al. 2016).

Current management and thresholds

The primary means of control of *D. suzukii*, has been the use of broad-spectrum insecticides (Bruck et al. 2011; Van Timmeren and Isaacs 2013). In most crops, both direct sale and processing markets have zero tolerance for *D. suzukii* infestation, so the pressure to keep fruit free of larvae is high (Asplen et al. 2015; Diepenbrock et al. 2016; Farnsworth et al. 2016). Sufficient control has been obtained from applications of insecticide every 5 to 7 days during the crop ripening and harvest season. In the nine years that *D. suzukii* has been a pest in the U.S., few successful control tactics have been developed beyond chemical control, especially in large-scale cropping systems. Chemical control in conventional farms includes using rotation of organophosphates, pyrethroids, carbamates, spinosyns and some other chemical classes. In organic production, there are fewer options and control has largely relied on the use of one effective spinosyn chemical, i.e. spinosad. There is no formal threshold for *D. suzukii*, but most growers use control measures after the first fly is found in monitoring traps in or near their fields and fruit is susceptible to damage (Diepenbrock et al. 2016).

While this chemical-based management approach is currently working in some regions, other regions such as the Midwest have greater challenges with insecticide-based management, largely due to rainfall and pesticide wash-off (VanTimmeren and Isaacs 2013). Furthermore, repeated chemical applications are not sustainable for the environment or the agricultural production system. Insecticide resistance is a major concern in management of *D. suzukii* due to

the insect's high fecundity and fast generation turnover (Asplen et al. 2015; Lee et al. 2015).

While rotation of chemical classes is strongly suggested, many growers repeatedly spray the least expensive and most effective insecticides. Frequent applications of insecticides have also led to problems exporting fruit with excessive pesticide residue. Some countries, particularly those in the European Union, have very low tolerances for insecticide residues than does the United States (Haviland and Beers 2012).

Insecticides that are active on *D. suzukii* can also damage natural enemy complexes in fruit production systems (Roubos et al. 2014). The adverse consequences of insecticide applications have been widely documented and include reducing populations of beneficial arthropods (Smith and Stratton 1986; Biondi et al. 2012), killing non-target mammals, fish, and birds (Devine and Furlong 2007; Goulson et al. 2013), leaching into the soil and water in the surrounding landscape (Devine and Furlong 2007; Goulson 2013), and potential effects on public health (Pimentel 1995; Devine and Furlong 2007). It is well understood that frequent applications of insecticides can trigger resurgence or growth in secondary pests that result from reduced populations of the natural enemies which would normally keep these pests in check (Heinrichs and Mochida 1984; Beers et al. 2016; Shearer et al. 2016).

While all of these concerns are important, insecticide-based management of *D. suzukii* will likely be necessary in production systems of susceptible crops that have a zero-tolerance for this pest. This insect has proven to be highly challenging for fruit growers in every invaded region where susceptible crops are grown. However, the need to integrate other control strategies with chemical management is clear and should be the primary focus of future *D. suzukii* management research.

RASPBERRY PRODUCTION

The United States is the third largest producer of fresh market raspberries in the world after Russia and Poland. The majority of US production comes from the western U.S. (USDA-NASS 2014), with California as the largest producer in the U.S., with a market value of \$267 million for fresh-market production (Agricultural Marketing Resource Center, USDA 2015). The U.S. has over 8,000 raspberry farms totaling a little over 23,000 acres (USDA-NASS 2012).

California, Oregon, and Washington make up about 20,320 acres of raspberries (USDA-NASS, 2014). Michigan produces this crop on about 589 acres (USDA-NASS, 2012). California, Oregon, and Washington together produced 173.86 million pounds of red raspberries with 48% going to fresh-market production (USDA-NASS 2014). The U.S. exports some of this crop with about 57.6 million pounds exported in 2014, valued at \$175 million (USDA 2014). The majority of our exports go to Canada (ERS-USDA 2016). The U.S. also imports raspberries, primarily from Chile, Mexico, and Canada (USDA 2014). Historically, Michigan produced about 14,000 acres in the 1950's (Ramsdell and Perry 1994). However, California and other western states largely outcompeted growers in Michigan, primarily due to disease pressure from our humid region, viruses, winter damage, and a short production season (Ramsdell and Perry 1994).

The majority of the raspberry crop is from primocane harvest in the fall, but raspberries can also be double-cropped and produce a crop in the late spring and summer (floricane harvest). Production of caneberries (raspberries and blackberries) is fairly different from most other fruit crops because the harvest is continuous. This provides several advantages for the grower, including season-long production of the fruit, but also requires labor for hand harvesting and pruning. There are many cultivars of raspberry and new types continue to be developed (Gambardella et al. 2014; Suster 2016). New cultivars are mostly selected for flavor, size, color,

increased shelf-life, and vigor of the plant, as well as susceptibility to diseases and some arthropod pests, like mites (Molina-Bravo et al. 2014; Bradish et al. 2015; Kula et al. 2016). Most raspberries are red (*Rubus idaeus*), but popularity of black (*R. occidentalis*), yellow, and purple raspberries (crosses of both species) is also growing.

Prior to *D. suzukii*, the main insect pests in raspberry were primarily controlled through occasional applications of insecticides and cultural management (Bushway et al. 2008). The primary pests of raspberries in Michigan are small sucking pests, including the western flower thrips (*Frankliniella occidentalis*), the small and large raspberry aphid (*Aphis idaei*, and *Amphorophora idaei*, respectively), potato leafhoppers (*Empoasca fabae*), sap beetles (*Glischrochilus quadrisignatus* and *G. fasciatus*), the tarnished plant bug (*Lygus lineolaris*) and two-spotted spider mites (*Tetranychus urticae*) (Bushway et al. 2008; Heidenreich et al. 2012). Other common pests include the raspberry sawfly (*Monophadnoides geniculatus*), the strawberry root weevil (*Otiorhynchus ovatus*), the raspberry cane borer (*Oberea bimaculate*), raspberry crown borer (*Pennisetia marginata*), the Eastern raspberry fruitworm (*Byturus rubi*) and a few others (Bushway et al. 2008). Both rose chafers (*Macrodactylus subspinosus*) and Japanese beetles (*Popillia japonica*) are common leaf-feeders of raspberries (Bushway et al. 2008; Heidenreich et al. 2012). These pests are easy to manage with infrequent applications of insecticides or miticides as the pest populations grow. For some pests, like the raspberry stem borer, removal of canes with oviposition stings can be an effective management technique (Bushway et al. 2008). *Drosophila suzukii* has changed the way that raspberries are managed and the intensity of insecticide applications, making it more costly to produce raspberries in most regions (Bolda et al. 2010; Goodhue et al. 2011). This pest's strong affinity for raspberries and

the continuous ripening and harvest of fruits make management in this crop particularly challenging.

ESTABLISHING INTEGRATED PEST MANAGEMENT PROGRAMS FOR *D. SUZUKII*

Biological control

Integrated pest management relies on the combination of chemical, biological, cultural, and physical tactics (Elliott et al. 1995; Norris et al. 2003; Flint 2012; Pedigo and Rice 2014). The goal of integrated pest management is to provide a sustainable framework that keeps pests below economic thresholds (Higley and Pedigo 1996; Pedigo and Rice 2014), and biological control agents can play an important role in maintaining pest populations below their carrying capacity (Higley and Pedigo 1996). Native biological control options for *D. suzukii* have been explored in Europe and the United States and several species of wasps from both areas have been found to parasitize *D. suzukii*, but at low levels (<5%) (Poyet et al. 2013; Rossi Stacconi et al. 2013; Gabarra et al. 2015; Wang et al. 2016). In addition to low parasitism rates, *D. suzukii* larvae can encapsulate the eggs of some parasitoid wasps, further reducing their success (Kacsoh and Schlenke 2012; Chabert et al. 2012; Poyet et al. 2013). Additionally, commercially available fungal pathogens, entomopathogenic nematodes, and predators have been evaluated for their ability to attack *D. suzukii* with low success rates (Woltz et al. 2015). Some native predators, such as ants, have been found to attack *D. suzukii* pupae, but only when *D. suzukii* populations are high (Woltz and Lee 2017). These findings emphasize the need for classical biological control.

Classical biological control involves searching for native parasitoids in the pest's home range, testing for non-target effects, then releasing promising species to reduce populations of the pest (Huffaker 2012; Hajek et al. 2016). This has been done successfully for many pests (Cameron et al. 1989; Van Driesche et al. 2010; Mason and Gillespie 2013). However, some introductions have had unintended consequences. First introductions of the multicolored Asian lady beetle (*Harmonia axyridis*) in the U.S. began in 1916 and continued with multiple releases in years following (Koch 2003). While it was a voracious predator on aphids and many other small pests, it has displaced many other Coccinellid species (Bahlai et al. 2015) and has become a nuisance pest, entering households in the winter months (Koch 2003). Despite examples of unintended consequences of some classical biological control agents, regulations for introducing a new biological control agent into the U.S. have grown and as a result, fewer non-target impacts and unsuccessful releases have been noted (Hajek et al. 2016). *Drosophila suzukii* is recognized as a particularly challenging pest to manage with our current understanding of its behavior and biology, and additional control measures, including biological control, are needed to suppress its populations and the damage it causes.

Since *D. suzukii* was detected in California, research teams from the U.S. have been working with colleagues in Asia to seek candidate natural enemies for classical biological control release programs. Five species of parasitoid wasps from China and South Korea were commonly reared from *D. suzukii* pupae and were brought to the U.S. in a quarantine facility to complete host-specificity testing (Daane et al. 2016). Two larval parasitoids from this group, *Ganaspis brasiliensis* and *Leptopilina japonica* have been found to be fairly host specific on *D. suzukii* and in November 2016, a USDA-APHIS permit was filed for their release into the U.S. (K. Daane, personal communication).

These classical biological control agents show promise, but represent a long-term management scheme that may take years to become successfully established. Their release is expected to be focused in non-crop refugia so that the heavy reliance on chemical management in the crop will not disrupt the establishment of these species (K. Daane, personal communication). Ideally, the parasitoids would be able to reduce the carrying capacity of the natural areas and chemical control would drive populations down within the crop, thereby suppressing the whole population. Furthermore, driving the population lower in late season would result in fewer *D. suzukii* overwintering, potentially reducing the emergent spring population. Other control efforts during crop harvest are likely to remain necessary, and cultural and physical control of this pest both show promise for adoption in a shorter timeframe. Their potential to be integrated into current management schemes is discussed below.

Cultural control

Cultural control tactics change growing practices or the growing environment to control a pest or disease, or to enhance services from beneficial organisms (Flint 2012; Pedigo and Rice 2014). They are considered one of the four main tactics for management within the integrated pest management framework. Cultural control has been used in many different situations through our agricultural history, including crop rotation to decrease disease or nematode prevalence or host plant resistance. Cultural control is generally highly compatible with other management techniques, including chemical management. For this reason, it should be considered as a bridge to move from our current chemical-dominated system to an integrated pest management scheme. Once these tactics are adopted, fewer applications of insecticides should be needed, thereby facilitating the introduction or expansion of biological control. For *D. suzukii* management,

potential cultural control tactics have primarily been through the utilization of habitat modification, including pruning, tilling, and mulching (Lee et al. 2015; Tochen et al. 2014; Iglesias and Liburd 2017). The goal of most of these cultural controls is to modify the temperature and humidity to put *D. suzukii* under stress. Dark mulches, for example, have the potential to heat *D. suzukii* adults and pupae on the ground to a temperature that is lethal. Pruning may increase airflow and decrease relative humidity, promoting desiccation of the fly within the crop area. This area of research is still growing and in many cases, these controls methods remain regionally specific. Additional cultural control strategies for *D. suzukii* include increasing the harvest frequency and the use of specialty plastics in high tunnel production to deter host finding.

Unlike some other susceptible berry crops, raspberries and blackberries ripen quickly and need to be harvested on a regular schedule. Mechanical harvesting of these crops is rare, and is generally not done for fresh market production. This limits the chemical options for growers to meet their pre-harvest intervals (PHIs) and still be able to harvest their fruit. However, increasing the harvest interval could be a potential way to decrease the number of *D. suzukii* eggs and larvae inside the fruit. Rapid harvest schedules would result in fewer ripe fruit on the cane for female *D. suzukii* to oviposit on, and may also prevent eggs and small larvae from reaching their last instar, which is the largest and most detectable.

Tunnel production is an increasingly common way to produce berries and vegetables, and involves seasonally covering the plants with plastic to expand the growing season in the spring and/or fall. High tunnels, or hoop houses, are fixed structures that resemble a temporary greenhouse, covered with plastic on the top and open on the sides and ends of the tunnel (Lamont 2009; Giacomelli 2009; Hanson et al. 2011). The sides and ends of the tunnel can also be

covered for season-long production in some regions, greatly extending the growing season. Low tunnel production is a small-scale version of high tunnels, usually used for shorter crops, such as strawberries. For temperate climates like those found in Michigan, tunnels provide a potential solution for addressing late and early frosts that can severely affect production of some fruit crops (Demchak 2009). In Michigan, adoption of high tunnels is relatively new and on limited acreage, but adoption is growing and growers that have taken advantage of these structures to grow high-value crops including raspberries, strawberries, tomatoes, and grapes are experiencing higher yields and much better fruit quality. Often, disease and insect pest pressure under plasticulture is reduced and the pest complex tends to be dominated by pests found in greenhouses due to similar temperature and humidity conditions (Pottorff and Panter 2009).

Recent research in greenhouses has focused on manipulating the wavelengths of light that reach the plants through modified plastic covering types. This modification of light transmittance by plastic began as a way to increase photosynthetically active radiation (PAR) but has since grown to manipulate the light in other ways. This includes changing light scatter, diffusion, intensity, and ultraviolet (UV) light. While light modification was initially done to increase plant health and productivity, changing these light parameters, particularly UV light, has also been found to affect insect and pathogen populations (Antignus et al. 1996; Costa et al. 2002; Mutwiwa et al. 2005; Diaz et al. 2006). For example, the spores of many fungi require UV light to sporulate and the absence of UV light can thereby reduce disease pressure (Reuveni et al. 1989; Nicot 1996; Raviv and Antignus 2004; Paul et al. 2005; Ben-Yakir and Fereres 2016). Disease pressure can also be reduced by mitigating insect vector populations through a reduction in UV light (Goldsmith and Bernard 1974; Goldsmith 1993; Doring and Chittka 2007; Johansen et al. 2011).

Insects have two compound eyes that consist of ommatidia which can detect light at different wavelengths and together these ommatidia create one larger image. Insects vary significantly in the wavelengths their ommatidia can detect. Bees, for example, are highly sensitive to UV light which allows them to forage for floral resources (Foster et al. 2014; Koski and Ashman 2014). Many other insects, including aphids, are less sensitive to UV and use spectral differences to distinguish between bare ground, sky, and plants (Costa and Robb 1999). Manipulation of the light transmittance under tunnels? has the potential to modify host finding and selection, and many other behavioral cues associated with vision. Whiteflies, a common greenhouse pest, have lower flight capacity and mate finding success under reduced UV light conditions (Costa and Robb 1999; Ben-Yakir et al. 2008). Aphids also cause less damage to plants covered in UV reducing plastics (Summers et al. 2004; Shahak et al. 2008). While high tunnels vary in their structure shapes compared to greenhouses, similar changes in the behavior of these insects may be seen in high tunnels. Selecting plastics with pest reducing properties could be a new cultural method to control some of these pests.

For *D. suzukii*, both vision and odor are important factors in host selection. However, there is still little knowledge of both the small and large scale host searching behaviors of this pest. Much more information on odor stimuli and repellents for *D. suzukii* is available because of interest in developing an effective and selective lure for monitoring and trapping (Keesey et al. 2015; Revadi et al. 2015). However, we know that *Drosophila melanogaster* have ommatidia that can detect UV light (Menne and Spatz 1977; Schümperli 1973; Paulk et al. 2013) and it is likely that *D. suzukii* share this trait. Therefore, UV light manipulation under plasticulture offers an opportunity for disruption of the damage that this and other pests cause. The behavior of *D.*

suzukii under reduced UV light conditions has not previously been studied, but I expect these changes may interfere with host finding, feeding, or mating.

Reducing UV light is also expected to affect pesticide degradation over time. Pesticides degrade under UV light (Matsumura 1982; Katagi 2004), so reducing UV light may decrease their rate of breakdown on plant surfaces. This could have added benefits for longer control of insects and pathogens, resulting in fewer sprays and lower pest management expenses. However, it may also have other effects, such as increasing residues found on fruits. This has the potential to decrease the ability to market those fruits to areas with lower pesticide residue limits. It is not well understood if reducing UV light can effectively control pests in high tunnel production or how it may change pesticide degradation, but it will be important to understand this to support widespread adoption of tunnel production systems.

Physical control

Physical control is often done to exclude pests or diseases from a crop. This is common in greenhouses, but is generally only seen in high-value field-grown crops or in small-scale plantings due to cost restrictions. However, the devastation caused by *D. suzukii* has driven many growers to become much more interested in physical barriers to prevent infestation. This mostly exists in the form of exclusion netting draping over the crops to prevent access. Different structures to hold the netting have been experimented with, mostly in small-scale systems, but with good success (Caprile et al. 2013; Cormier et al. 2015; Link 2014; Rogers et al. 2016; Leach et al. 2016). In many cases, the netting not only reduces *D. suzukii* infestation but also many other secondary pests. The netting also provides an opportunity to reduce or even eliminate

chemical control programs. Like cultural control, this physical control tactic can work well in concert with chemical management and can provide a new opportunity to restructure current management programs for *D. suzukii* (Leach et al. 2016).

SUMMARY

Drosophila suzukii is an invasive frugivorous pest present throughout all major fruit producing regions of North America and abroad that is causing significant crop loss and higher production costs. The current control strategies rely heavily on insecticides but it is widely recognized that this approach alone is unsustainable (Asplen et al. 2015), so diversifying pest management programs is very important for the continued viability of berry production. New management strategies need to be evaluated for their ability to cost-effectively control *D. suzukii* populations that are compatible with current management techniques. Raspberries are of particular importance because they are highly susceptible to *D. suzukii* damage. Both cultural and physical control tactics show promise for reducing populations of *D. suzukii* in raspberry. This includes producing raspberries in high tunnels, which can manipulate light for disruption of visual cues while also providing a structure for pest exclusion. Information gained from the studies presented in this thesis will also be relevant for other crops and regions where *D. suzukii* is problematic.

OBJECTIVES

The overall scope of this research project is to expand control tactics for management of *D. suzukii* in high tunnel raspberries. The first objective was to better understand the insect community present in Michigan raspberries and to determine whether modifying the visual

landscape, specifically reducing UV light, interferes with the abundance of these insects and their damage, with a particular focus on *D. suzukii*. The second objective was to determine how insecticides degrade under different plastic types with varying levels of UV transmittance. The final objective was to determine how exclusion netting may be utilized for *D. suzukii* control in combination with chemical control and in a commercial high tunnel raspberry setting.

CHAPTER 2. ABUNDANCE AND TIMING OF ARTHROPODS IN MICHIGAN HIGH TUNNEL RASPBERRIES

ABSTRACT

Global raspberry production has almost doubled in the past 20 years as the demand for nutritious and locally produced food increases. This has been made possible in part by the use of high tunnels, which are cost-effective structures that protect crops from adverse weather and lengthen the growing season. To best optimize the growing environment and pest and disease management of these crops, the plastics that cover these structures can be used to modify light conditions, including ultraviolet (UV) light. We evaluated two commercially available films, Luminance® and Lumisol®, and two research-grade films, that differ in UV transmission properties. We also characterized the phenology of arthropod pests, beneficials, and pollinators under these protected structures p using yellow sticky traps and yeast-sugar traps as well as direct observations in 2015 and 2016 at three sites in southern Michigan. We found 36 pest groups and 31 beneficial insect groups across the two years of this study. Eight pest groups were identified to be the most damaging on raspberry, including spotted wing *Drosophila*, aphids, leafhoppers, and thrips. The six most abundant natural enemy groups included lady beetles, lacewings and parasitoid wasps. We found that these arthropod communities are highly dependent on location and time of year, and different levels of UV transmission under the tunnels do not strongly affect their activity or abundance. We saw no effect of the different plastic treatments on pests or natural enemies or on feeding damage. In one of the year, the abundance of thrips and aphids was reduced and leafhopper populations were increased under the UV blocking plastic, though this trend was not consistent among locations. There was no

significant effect of plastics on most pollinators, except that honey bees were significantly less active under tunnels with UV blocking plastic. This research informs our understanding of the arthropod community in Michigan high tunnel raspberries and suggests that varying levels of UV transmission under these growing environments is unlikely to strongly affect pest incidence or damage.

INTRODUCTION

Fruit production and consumption have increased globally as the demand for nutritious and fresh food increases (Inwood et al. 2009; Clark and Inwood 2016). This includes berries such as raspberries, which have been cultivated by humans as early as the Greeks and Romans (Gunther 1934; Kempler and Hall 2013). Top producers of raspberries include Russia, Poland, and the U.S., though production occurs globally. Within the U.S., most raspberries are produced in the west coast states, due to ideal weather, limited pest issues, long growing seasons, and regional marketing and export opportunities. Raspberries in other regions, including the Midwest, tend to be grown on smaller farms with more direct market sales (Hanson et al. 2011). However, as the demand for locally produced food increases (Coit 2008; Schupp 2016), raspberry growers in the Midwest and other regions are utilizing innovative new production methods to increase production. This has primarily been done through the use of high tunnels, which protect crops from rain, frost, and other adverse weather conditions. They also allow for season extension, producing florican berries earlier in the spring and primocane berries later into the fall (Lamont et al. 2009; Hanson et al. 2011; Xu et al. 2014) when prices are higher. Moreover, raspberry yields under tunnels can be doubled compared to field conditions in northern latitudes, increasing potential profitability in these regions (Fernandez and Perkins-Veazie 2011; Palonen et al. 2017).

The U.S. ranks 10th in the global acreage of crops that are covered with high tunnels or greenhouses with primary production in tomatoes, cucumbers, and lettuce (Lamont et al. 2009). The projected growth rate of high tunnel construction in the U.S. is 10-15% per year (Orzolek 2013), so it will also be important to understand how this change in production practices will affect pest management.

While some predict lower pressure of pests under high tunnels because of the earlier crop phenology (Rom et al. 2010), others have found that arthropod pests of caneberries, particularly traditional greenhouse pests like two-spotted spider mites, aphids, and thrips are more abundant under high tunnels compared to field production (Demchack 2009, Yao and Rosen 2011). Similarly, Lang (2009) found that Japanese beetle and cherry fruit fly were reduced under high tunnel tree fruit production, whereas aphid and spider mite populations were much higher. High tunnels create a protected environment that remains dry and hot (Wien 2009; Yao and Rosen 2011) similar to greenhouse conditions, that are ideal for some pests.

Recent developments in the types of plastic coverings for protected culture systems have aimed to optimize plant growth and to deter pests and diseases. These plastics can be specialized in a variety of ways, including reducing the amount of ambient ultraviolet (UV) light transmitted into the tunnel. Decreasing ultraviolet light can help to mitigate disease pressure, especially among fungal pathogens which require UV light to sporulate (Nicot et al. 1996; Paul et al. 2005). Reducing UV light in these growing structures is also now being considered as a pest management strategy in greenhouses. Ultraviolet light has a wavelength of 290-400nm on the electromagnetic spectrum, and insects are capable of detecting light at these wavelengths. While there are variations in the exact amount of UV light that different insects can see, most insects have peaks in UV light sensitivity from 340-400nm (Coombe 1982; Peitsch et al. 1992; Matteson

and Terry 1992). Because UV light is part of the visible spectrum for insects, reducing the amount of available visible light may have consequences for their feeding, mating, or dispersion. Most research on these phenomena has been focused in greenhouse production, where decreased abundance, feeding, and diseases vectored from whiteflies, aphids, and thrips have been observed under reduced UV light (Antignus 2000; Diaz et al. 2006; Kigathi and Poehling 2012; Lamnatou and Chemisana 2013). Antignus et al. (2001) found that whiteflies have decreased dispersal when released from a central location under UV-reducing materials in a closed growing system. In protected high tunnel cucumber production, Doukas and Payne (2007) found that there were fewer thrips and beetles under the UV-blocking plastic compared to the UV-transparent plastic.

Far less research has focused on the effect of natural enemies when UV light is reduced, and there are variable results depending on the species. Chiel et al. (2006) found that two out of three whitefly parasitoids were able to navigate to their host regardless of a modification of the light spectrum, whereas one parasitoid species failed to locate its host under reduced UV light conditions. Likewise, fewer parasitoid wasps and syrphids have been observed under reduced UV light conditions, but this may be in response to lower numbers of prey in these environments (Doukas and Payne 2007). Because most research on insect behavior with plastics has been focused on greenhouse production, little research has been conducted on pollinators. However, most bees are trichromatic in UV, blue, and green, and disrupting this wavelength may affect their pollination efficiency (Skorupski et al. 2007). Most research on pollinators in these reduced UV environments have been focused on bumble bees and are inconclusive, with some studies showing a reduction in activity (Morandin et al. 2001), some with no effect (Morandin et al.

2002), and some evidence of learning and adjustment in their behavior when to less UV light is reduced (Dyer and Chittka 2004).

The pest management implications of specialty plastics used for high tunnel raspberry production is still unknown, and this work seeks to clarify the effect that reduced UV light may have on insect communities in high tunnels. This research also addresses the need to update our understanding of the raspberry pests, pollinators, and beneficial arthropods as raspberry production under tunnels increases in this area. The objectives of this study were to (1) characterize the pest and beneficial insect community in raspberries grown in Michigan, and to (2) determine the response of these insects in high tunnel raspberries when exposed to plastics that vary in UV transmittance. This includes focus on pests such as spotted wing *Drosophila* (*Drosophila suzukii*), as well as natural enemies and pollinators. This research will help to predict arthropod communities and optimize plastic selection for insect management in high tunnel production for raspberries and other berry crops.

MATERIALS AND METHODS

Site selection

In 2015, this research took place at the Horticultural Teaching and Research Center (HTRC) in East Lansing, MI and at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, MI. In 2016, this research took place at HTRC and at a commercial farm, “Farm 1” in Coloma, MI. At HTRC, the south half of the three 7.6 × 60 m Haygrove high tunnels (Haygrove Ltd, Herefordshire, UK) were covered with diffuse Luminance® plastic (Visqueen, Stevenston, UK) and the north half were covered with diffuse Lumisol® plastic (Visqueen,

Stevenston, UK). Each tunnel contained a row of the primocane fruiting red raspberry cultivars Polka, Joan J, and Himbo Top. Floricanes were retained to produce summer floricanes fruit and fall primocane fruit. Two applications of organic insecticides were made in 2015 and 6 in 2016 for control of whiteflies, sawflies, and *D. suzukii*. At SWMREC, the north half of one 7.6 x 60 m Haygrove high tunnel (Haygrove Ltd, Herefordshire, UK) was covered with clear research grade UV-blocking plastic (Visqueen, Stevenston, UK) and the south half was covered with clear research grade UV-transparent plastic (Visqueen, Stevenston, UK). The tunnel contained potted raspberries, planted in spring 2015, with a mix of many cultivars. Maintenance pesticide sprays were applied throughout the season to keep pests low with a total of 5 insecticide applications in 2015. At Farm 1, three 7.6 × 122 m Haygrove high tunnels were covered in Luminance® and three were covered in Lumisol® plastic. Summer and fall-bearing red raspberries were grown, one tunnel per variety (cv. ‘Prelude’, ‘Joan J’, and ‘Himbo Top’). Farm 1 was conventionally managed in 2015 and 2016, with a total of 16 insecticide applications made in 2015 and 14 in 2016 (primarily for *D. suzukii* management). In 2015, there was one replicate of each plastic type at SWMREC and three replicates at HTRC. In 2016, there were three replicates of each plastic type at both sites. UV light was measured once weekly on the inside and the outside of each tunnel with a UV light meter, sensitive from 250-400 nm (MU-200, Apogee Instruments Inc., Logan, UT) in 2016 to determine the % reduction in UV by each plastic type.

Pests and natural enemies

To sample insects attracted to plant canopies, one 14 × 23.5 cm yellow sticky trap (Scentry MultiGuard, Great Lakes IPM, Vestaburg, MI) was placed just above the crop canopy at each trap location, facing into the tunnel. Yellow sticky traps were replaced weekly from June-

September of each year, and the number and type of insects on each trap were identified to family. As a companion method for sampling insects on the plant canopy, visual scouting of the raspberry plants for insect and disease damage was done weekly from June through September in 2015 and 2016. Under each plastic treatment, 25 leaves were randomly selected and observed for percent damage (either sucking or chewing pest damage, presence of molds, etc.). Insects present on the leaves were identified to at least family level and counted.

Adult *D. suzukii* were sampled using yeast-sugar traps with two traps placed in the middle row, 10 m from the edge on either side of each treatment. Traps were made from 0.95 liter deli-cups filled with 150 ml of solution and a yellow sticky insert was hung from the top (Van Timmeren and Isaacs 2013), and they were checked weekly. Each treatment had a total of three traps per site in 2015 and six traps per site in 2016. During harvest, larval *D. suzukii* were sampled by harvesting 25 ripe fruit within 5 m of each trap location and immersing them in a strong salt solution, which was left for one hour before sifting the solution through a reusable coffee filter and counting the eggs and larvae using a stereomicroscope (Van Timmeren et al. 2017).

Pollinators

Pollinators were sampled during peak bloom in 2015 and 2016 (when bloom was estimated to be at or above 20% for all plants in a tunnel) using visual observations. For a total of 10 min, trained observers would walk through each treatment and count the number and type of bees pollinating raspberry flowers. Each transect was inside the treatment by at least 10 m, to avoid any edge effect of the tunnels. Pollinators were categorized by small dark bee, large dark bee, bumble bee, honeybee, or non-bee pollinators. Small and large dark bees were combined for statistical analysis and were re-categorized as wild bees. In 2015, there were a total of three

observations per site. Pollinator observations were not carried out at SWMREC, since many of these plants were in early growth stages and had little flower and fruit production. In 2016, I conducted two observations per site.

Statistical analysis

The light data satisfied normality assumptions using Levene's test and were analyzed using analysis of variance. The *D. sukikii* adult and larval data were analyzed using a linear mixed-effect model using the "nlme" package in R with repeated measures using a first-order continuous autoregressive correlation structure (Pinheiro et al. 2014). The direct leaf observations and trap data were analyzed using a linear mixed-effect model, with repeated measures and a zero-inflated negative binomial distribution, due to large amounts of zeros in the count data (R Package "glmmadmb", Fournier et al. (2012)). Pollinator counts were analyzed using a linear mixed-effect model. Tukey's Honest Significant Difference was used for post-hoc comparisons. All data analyses were conducted using the R program (3.3.3., R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Plastics

In 2016, there was a significant season-long reduction in UV light under the blocking plastics at HTRC and Farm 1 (Figure 2.1). At Farm 1, the Luminance® UV blocking plastic reduced 17.8% of the UV light on average compared to the Lumisol® UV transparent plastic. Compared to outside conditions, the Luminance® UV blocking plastic allowed $37.81 \pm 1.34\%$ of UV light through where the Lumisol® UV transparent plastic allowed $55.64 \pm 1.19\%$ of UV light through

on average. At HTRC, the Luminance® partial UV blocking plastic reduced the UV light on average 19% compared to the Lumisol® UV transparent plastic. Compared to outside conditions, the Luminance® UV blocking plastic allowed an average of $39.23 \pm 1.03\%$ of UV light through where the Lumisol® UV transparent plastic allowed $58.34 \pm 1.89\%$ of UV light through on average. The plastics that were placed at SWMREC allowed only 0.6% of the UV light through under the blocking plastic, and 74.2% of the UV light through under the transparent plastic (Figure 2.1). This reduction in UV light was consistent across different cloud cover conditions throughout the season.

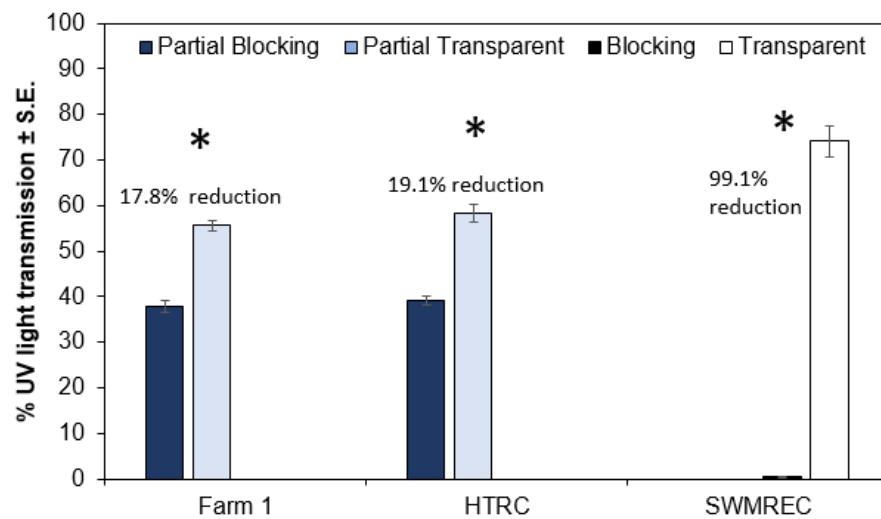


Figure 2.1. The average season-long UV light transmission under the tunnels in 2015 and 2016, measured using an Apogee MU-200 light meter. Bars marked with asterisk denote a significant difference between treatments at $\alpha = 0.05$.

Pests

Common pests varied between sites, but generally included small sucking pests, like aphids (*Amphorophora agathonica* Hottes and *Aphis rubicola* Patch), thrips (*Frankliniella occidentalis*

Pergande and *Thrips tabaci* Linderman), leafhoppers (*Empoasca* spp. Walsh, *Typhlocyba pomaria* McAtee, and *Edwardsiana rosae* Linnaeus), whiteflies (*Trialeurodes abutiloneus* Haldeman and *Bemisia tabaci* Gennadius), and spider mites (*Tetranychus urticae* Koch). Raspberry beetles (*Byturus rubi* Barber) and *D. suzukii* were also common across all sites in both years. Populations of most pests were low in the spring and became more abundant later when there were warmer temperatures and increasing fruit production (Figure 2.2). Thrips and raspberry beetles were most abundant in the spring and they decreased as the season progressed.

In 2015, there were no significant differences in *D. suzukii* captured season-long between the two plastic types at both sites (Figure 2.3) ($F_{1, 118} = 0.3$, $p = 0.6$). In 2016, however, significantly more *D. suzukii* were captured in traps under the UV-blocking plastic at HTRC ($F_{1, 317} = 3.3$, $p = 0.006$). At the commercial farm in 2016, there were no statistical differences between adult *D. suzukii* captured under the two treatments ($F_{1, 317} = 1.2$, $p = 0.6$). In 2015, no differences were found among the cumulative *D. suzukii* infestation in the sampled fruit (Figure 4B). This was also true for Farm 1 in 2016 ($F_{1, 228} = 0.2$, $p = 0.9$). There were significantly more cumulative *D. suzukii* larvae found under the transparent plastic compared to the blocking plastic at HTRC in 2016 ($F_{1, 228} = 3.2$, $p = 0.009$), which is opposite of where adults were trapped most at this site (Figure 2.3).

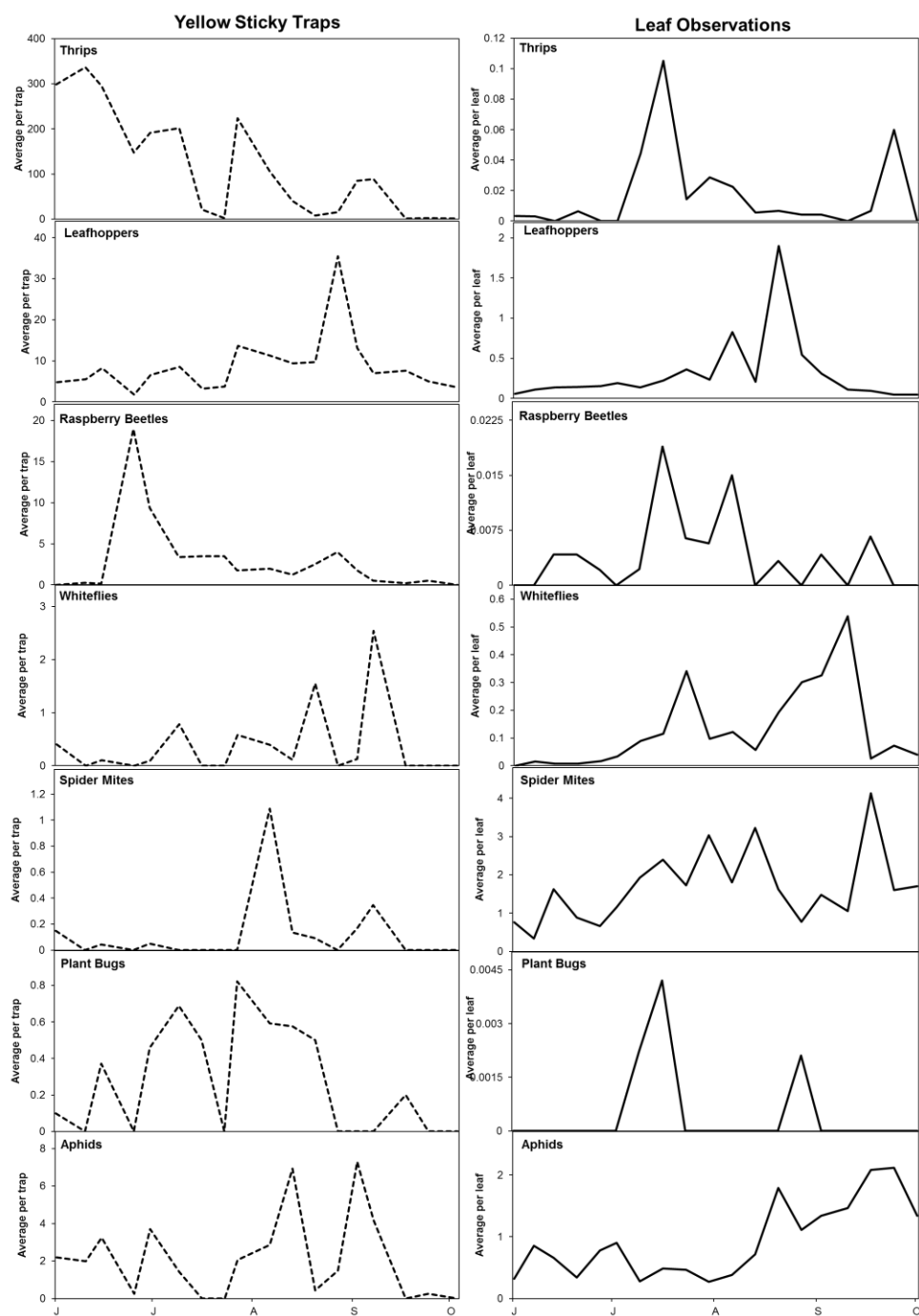


Figure 2.2. Average season-long abundance of the most common insect and mite pests on yellow sticky traps (dotted) and on raspberry leaves (solid) across both Farm 1 and HTRC in 2015 and 2016.

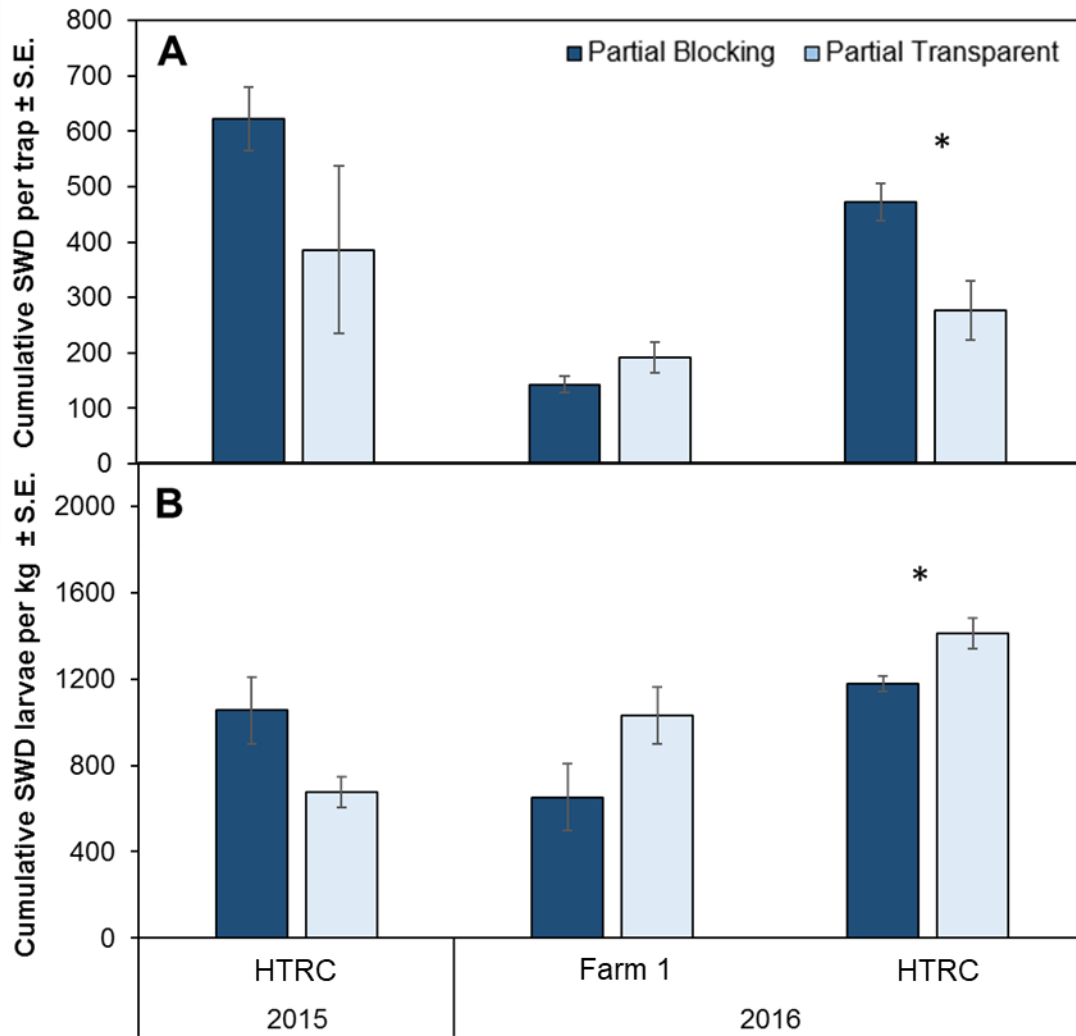


Figure 2.3. (A) Average cumulative number of SWD adults found per trap ($\pm$ S.E.) and **(B)** SWD larvae per kilogram ($\pm$ S.E.) of fruit under UV-blocking or transparent plastics. Bars marked with an asterisk denote treatment differences at each site at $\alpha = 0.05$.

In 2015, there were statistically similar numbers of pests observed from the direct leaf observations between the plastic treatments ($F_{1, 1725} = 0.6$; $p = 0.5$) (Figure 2.4). The sites were statistically different from each other ($F_{1, 1725} = 23.2$; $p < 0.001$) and the date of the observation

was also a significant factor contributing to the model ($F_{1, 1725} = 5.6$; $p < 0.001$). The percent of leaf damage was similar across all locations and plastic treatments in 2015 ($F_{1, 1725} = 0.24$; $p = 0.8$). Likewise in 2016, there were statistically similar numbers of pests observed from the direct leaf observations among plastic types ($F_{1, 4023} = 0.04$; $p = 0.97$) (Figure 2.4, Table 2.1). This year, the sites were statistically different from each other ($F_{1, 4023} = 9.6$; $p < 0.001$) and the date of the observation was also significant ($F_{1, 4023} = 2.7$; $p = 0.007$). The percent of leaf damage in 2016 was also similar across all locations and plastic treatments ($F_{1, 4023} = 0.03$; $p = 0.9$).

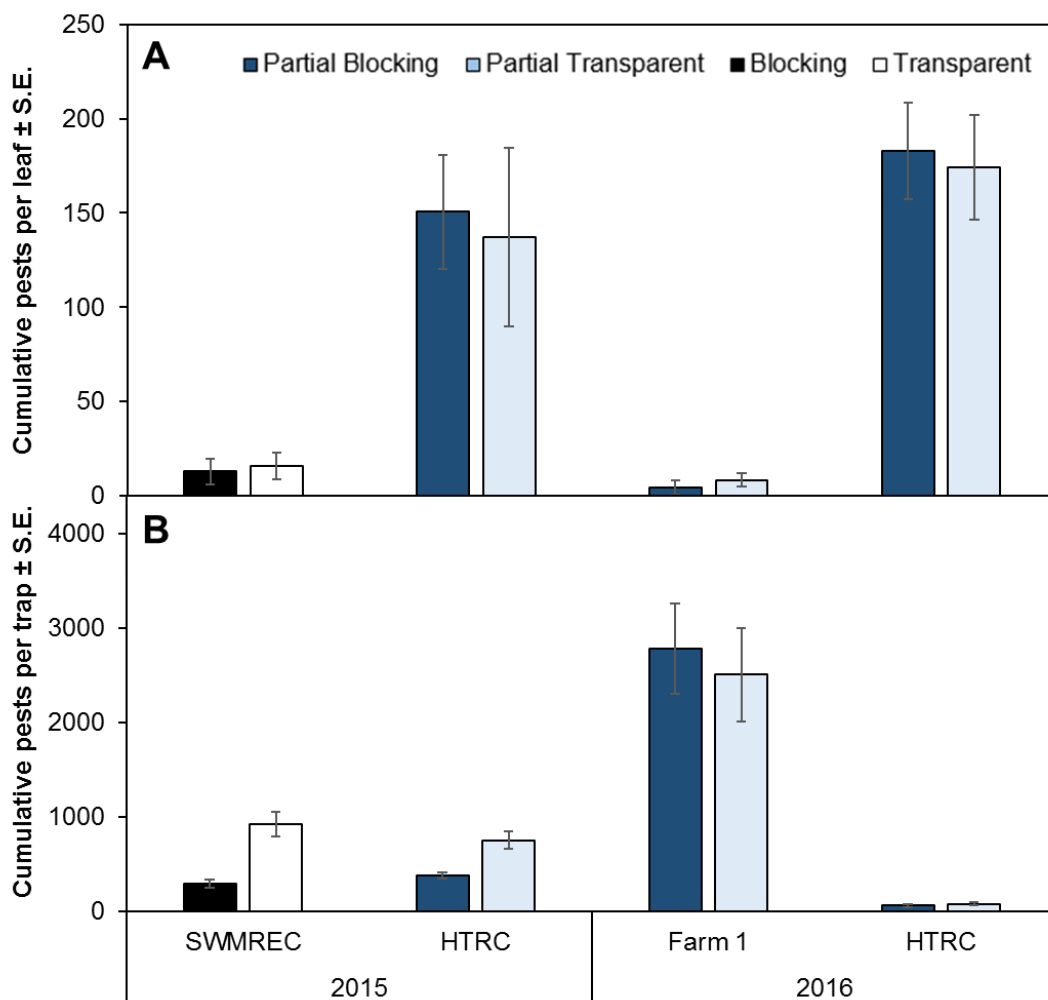


Figure 2.4. (A) Average number of arthropod pests per leaf ($\pm$ S.E.) at each location and **(B)** the average cumulative number of arthropod pests found on yellow sticky traps ($\pm$ S.E.) season-long.

Table 2.1. Average cumulative arthropod pests and natural enemies per yellow sticky trap ($\pm$ S.E.) captured throughout the raspberry growing season in 2015 and 2016. Values are followed by their F and p-values (2015: $F_{1,66}$; 2016: $F_{1,98}$). P-values given in bold denote significant differences between UV blocking treatments.

	2015					2016				
	SWMREC		HTRC		F-value p-value	Farm 1		HTRC		F-value p-value
	Block	No Block	Block	No Block		Block	No Block	Block	No Block	
All pests	292.5 $\pm$ 41.6	925.5 $\pm$ 127.9	378.0 $\pm$ 36.4	752.3 $\pm$ 90.6	F = 1.4, p = 0.2	2791.7 $\pm$ 52.7	3114.5 $\pm$ 23.9	84.0 $\pm$ 1.7	79.8 $\pm$ 3.9	F = 0.1, p = 0.9
Leafhoppers	68.3 $\pm$ 4.2	62.3 $\pm$ 1.9	242.7 $\pm$ 9.1	134.5 $\pm$ 5.7	F = 2.0, p = 0.04	41.5 $\pm$ 6.3	30.3 $\pm$ 0.7	26.3 $\pm$ 4.2	26.0 $\pm$ 4.2	F = 0.5, p = 0.6
Thrips	382.2 $\pm$ 39.9	1622.1 $\pm$ 125.9	420.8 $\pm$ 36.6	1300.6 $\pm$ 91.6	F = 4.1, p < 0.001	2639.3 $\pm$ 460.2	2936.3 $\pm$ 483.4	17.3 $\pm$ 4.7	34.7 $\pm$ 8.1	F = 0.4, p = 0.7
Aphids	5.3 $\pm$ 0.7	9.7 $\pm$ 1.1	9.6 $\pm$ 0.6	4.2 $\pm$ 0.3	F = 0.9, p = 0.3	22.8 $\pm$ 0.5	65.8 $\pm$ 3.0	1.8 $\pm$ 0.1	1 $\pm$ 0.4	F = 2.7, p < 0.01
Plant bugs	9.3 $\pm$ 0.8	8.4 $\pm$ 0.7	0.0 $\pm$ 0.0	6.1 $\pm$ 1.1	F = 0.6, p = 0.5	4.5 $\pm$ 0.5	4.3 $\pm$ 0.3	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1	F = 0.2, p = 0.9
Raspberry beetles	87.9 $\pm$ 7.5	126.2 $\pm$ 10.1	48.5 $\pm$ 1.9	34.7 $\pm$ 2.2	F = 1.3, p = 0.2	9.2 $\pm$ 0.5	6.3 $\pm$ 0.8	2.8 $\pm$ 0.3	0.7 $\pm$ 0.2	F = 0.6, p = 0.6
All natural enemies	72.3 $\pm$ 3.5	54.8 $\pm$ 1.6	79.3 $\pm$ 2.1	72.9 $\pm$ 2.5	F = 0.2, p = 0.9	29.2 $\pm$ 0.4	36.5 $\pm$ 0.5	29.0 $\pm$ 3.1	20.8 $\pm$ 2.8	F = 0.9, p = 0.4
Spiders	5.3 $\pm$ 0.4	9.1 $\pm$ 0.7	6.3 $\pm$ 0.4	12.4 $\pm$ 0.7	F = 0.2, p = 0.9	5.8 $\pm$ 0.5	3.8 $\pm$ 0.1	1.5 $\pm$ 0.2	0.5 $\pm$ 0.2	F = 1.0, p = 0.3
Syrphids	6.8 $\pm$ 0.7	10.2 $\pm$ 0.7	2.7 $\pm$ 0.2	7.2 $\pm$ 0.6	F = 0.01, p = 0.9	1.3 $\pm$ 0.2	0.5 $\pm$ 0.1	2.8 $\pm$ 1.2	0.3 $\pm$ 0.2	F = 0.9, p = 0.3
Parasitoid wasps	47.0 $\pm$ 2.9	31.3 $\pm$ 1.6	53.5 $\pm$ 1.8	41.3 $\pm$ 1.8	F = 0.01, p = 0.9	9.8 $\pm$ 0.2	14.8 $\pm$ 0.1	14.0 $\pm$ 1.4	14.7 $\pm$ 2.1	F = 1.1, p = 0.3
Lady beetles	7.6 $\pm$ 0.4	1.4 $\pm$ 0.2	16.4 $\pm$ 0.7	5.7 $\pm$ 0.4	F = 0.8, p = 0.4	1.3 $\pm$ 0.1	3.0 $\pm$ 0.2	2.0 $\pm$ 0.5	0.8 $\pm$ 0.3	F = 1.0, p = 0.3
Lacewings	2.2 $\pm$ 0.2	1.0 $\pm$ 0.2	2.2 $\pm$ 0.2	7.3 $\pm$ 0.2	F = 2.4, p = 0.07	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.0 $\pm$ 0.0	0.5 $\pm$ 0.2	F = 0.7, p = 0.4

The number of pests on the yellow sticky traps in 2015 were not significantly different between the plastic types across the two sites ($F_{1,66} = 0.2$; $p = 0.8$). As with the direct leaf observations in 2015, there was significant variation in pest populations among the sites ($F_{1,66} = 2.1$; $p = 0.04$) and time of year ($F_{1,66} = 8.4$; $p < 0.001$). The abundance of thrips found on the yellow sticky traps in 2015 was significantly related to the plastic treatment, with lower populations under the UV-blocking treatment ($F_{1,66} = 3.8$; $p < 0.001$). I found higher abundance of leafhoppers under the UV-blocking treatment ($F_{1,66} = 8.4$; $p < 0.001$).

The same pattern also held true for the yellow sticky traps in 2016, where the plastic covering was not a significant factor ($F_{1,98} = 0.1$; $p = 0.9$), but site ($F_{1,98} = 10.3$; $p < 0.001$) and date ($F_{1,98} = 3.5$; $p < 0.001$), were significant in determining pest abundance. Aphid populations were significantly lower under the UV-blocking material compared to the UV-transparent ($F_{1,98} = 2.7$; $p = 0.006$), but this trend was only apparent at Farm 1.

Natural enemies

The majority of the natural enemies found on the traps and observed on plants were parasitoid wasps (many families, primarily from Chalcidoidea, including Aphelinidae, Pteromalidae, etc.). I also found lacewings (Chrysopidae and Hemerobiidae), spiders (all spiders), syrphids (Syrphidae), lady beetles (Coccinellidae), and predatory mites (Phytoseiidae). The populations of all natural enemies remained fairly constant through the middle part of the season, with population increases throughout harvest, when pest populations were also higher (Figure 2.5).

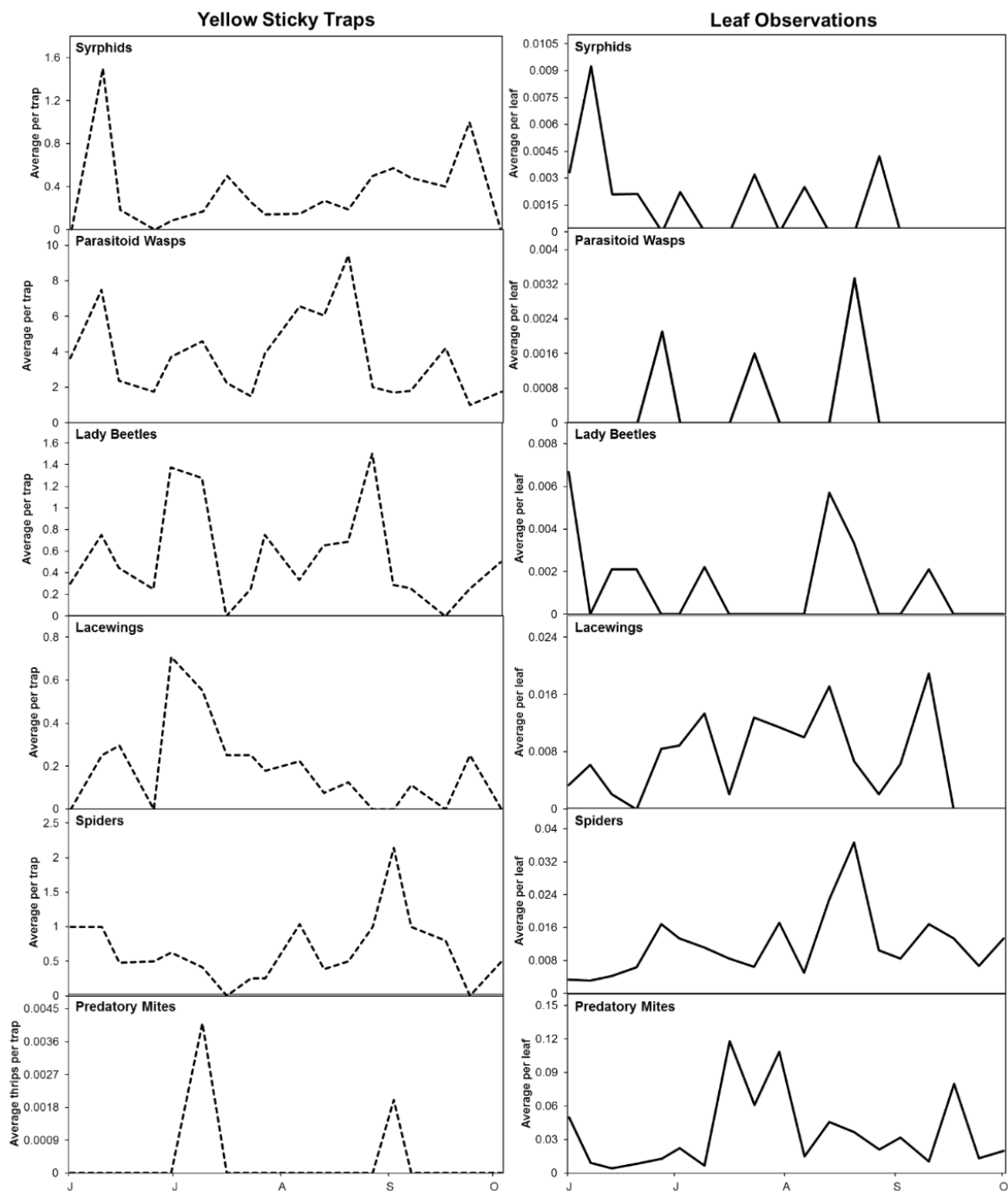


Figure 2.5. Average season-long abundance of the most common natural enemies on yellow sticky traps (dotted) and leaf observations (solid) across both Farm 1 and HTRC in 2015 and 2016.

In 2015 there were similar numbers of natural enemies observed in each plastic treatment ($F_{1, 1725} = 0.07$; $p = 0.9$) (Figure 2.6, Table 2.1, 2.2). The sites were different from each other ($F_{1, 1725} = 8.04$; $p < 0.001$,) but the date of the observation was not a significant factor in the abundance of natural enemies ($F_{1, 1725} = 0.2$; $p = 0.8$). The leaf observations for natural enemies in 2016 also had similar results with no significant effect of treatment ($F_{1, 4023} = 1.1$; $p = 0.3$), but significant variation among locations ($F_{1, 4023} = 2.0$; $p = 0.04$). For insects on the yellow sticky traps in 2015, there was no significant variation in natural enemies based on plastic treatment, site, or date ($F_{1, 66} = 0.9$; $p > 0.4$) (Figure 2.6). For the yellow sticky traps in 2016, plastic treatment was also not a significant factor ($F_{1, 98} = 0.6$; $p = 0.9$) in determining natural enemy abundance. There was significant variation between the sites ($F_{1, 98} = 5.2$; $p < 0.001$), but date was not a significant determinant of the natural enemy populations ($F_{1, 98} = 0.9$; $p = 0.4$). For both methods in 2015 and 2016, none of the six most abundant natural enemies significantly differed between plastic types (Table 2.1, 2.2).

Table 2.2. Average cumulative arthropod pests and natural enemies per raspberry leaf ($\pm$ S.E.) throughout the raspberry growing season in 2015 and 2016, followed by their F and p-values (2015: $F_{1, 1725}$; 2016: $F_{1, 4023}$).

	SWMREC		2015 HTRC		F-value p-value	Farm 1		2016 HTRC		F-value p-value
	Block	No Block	Block	No Block		Block	No Block	Block	No Block	
All pests	12.9 $\pm$ 7.0	15.8 $\pm$ 7.2	150.6 $\pm$ 30.2	137.3 $\pm$ 47.5	F= 0.4, p = 0.7	4.2 $\pm$ 4.0	8.2 $\pm$ 3.5	182.7 $\pm$ 25.6	174.1 $\pm$ 27.9	F = 0.4, p = 0.7
Leafhoppers	2.2 $\pm$ 1.6	3.6 $\pm$ 2.5	4.4 $\pm$ 2.1	4.6 $\pm$ 2.3	F= 0.8, p = 0.4	0.08 $\pm$ 0.2	0.08 $\pm$ 0.2	35.0 $\pm$ 6.7	36.7 $\pm$ 7.0	F = 1.6, p = 0.1
Thrips	0.1 $\pm$ 0.2	0.2 $\pm$ 0.6	0.04 $\pm$ 0.1	0.0 $\pm$ 0.0	F= 0.08, p = 0.9	0.3 $\pm$ 0.4	2.9 $\pm$ 2.5	0.3 $\pm$ 0.4	0.1 $\pm$ 0.2	F = 1.1, p = 0.3
Aphids	0.7 $\pm$ 1.0	1.3 $\pm$ 0.8	28.6 $\pm$ 12.6	53.3 $\pm$ 37.2	F= 0.1, p = 0.9	0.4 $\pm$ 0.6	1.8 $\pm$ 1.5	73.3 $\pm$ 13.6	70.9 $\pm$ 18.9	F = 0.3, p = 0.7
Whiteflies	0.2 $\pm$ 0.3	0.4 $\pm$ 0.7	1.8 $\pm$ 0.9	3.0 $\pm$ 1.4	F= 0.9, p = 0.3	0.0 $\pm$ 0.0	0.2 $\pm$ 0.4	15.5 $\pm$ 3.8	21.1 $\pm$ 3.8	F = 1.8, p = 0.06
Spider mites	9.8 $\pm$ 5.3	10.1 $\pm$ 6.3	124.3 $\pm$ 23.3	86.1 $\pm$ 19.6	F= 0.1, p = 0.9	3.1 $\pm$ 3.8	2.8 $\pm$ 2.2	58.0 $\pm$ 11.8	44.4 $\pm$ 12.8	F = 1.1, p = 0.3
Raspberry beetles	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	F= 0.05, p = 0.9	0.2 $\pm$ 0.3	0.3 $\pm$ 0.3	0.04 $\pm$ 0.1	0.08 $\pm$ 0.2	F = 0.4, p = 0.7
All natural enemies	0.9 $\pm$ 0.7	0.8 $\pm$ 0.6	4.3 $\pm$ 2.2	4.1 $\pm$ 1.8	F= 1.5, p = 0.1	0.4 $\pm$ 0.5	0.2 $\pm$ 0.2	1.5 $\pm$ 0.9	1.9 $\pm$ 0.9	F = 1.1, p = 0.3
Lady beetles	0.04 $\pm$ 0.1	0.1 $\pm$ 0.2	0.04 $\pm$ 0.2	0.04 $\pm$ 0.2	F= 0.3, p = 0.8	0.08 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	F = 0.4, p = 0.7
Predatory mites	0.3 $\pm$ 0.6	0.1 $\pm$ 0.2	3.7 $\pm$ 1.9	3.6 $\pm$ 1.8	F= 1.5, p = 0.1	0.2 $\pm$ 0.4	0.0 $\pm$ 0.0	0.5 $\pm$ 0.4	0.4 $\pm$ 0.3	F = 1.1, p = 0.3
Lacewings	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.04 $\pm$ 0.1	0.04 $\pm$ 0.1	F= 0.09, p = 0.9	0.08 $\pm$ 0.2	0.08 $\pm$ 0.2	0.2 $\pm$ 0.3	0.9 $\pm$ 0.6	F= 0.9, p = 0.4

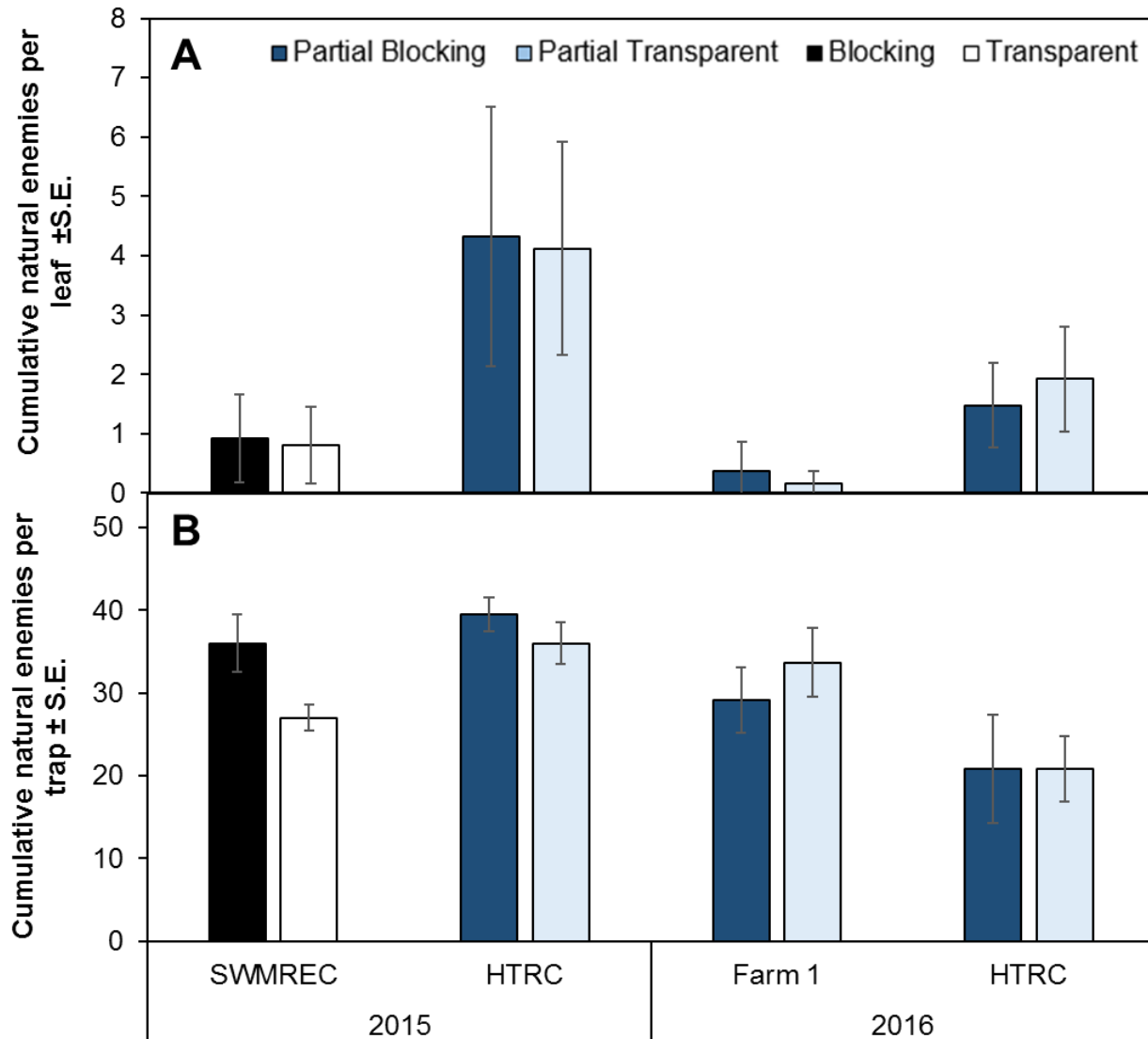


Figure 2.6. (A) Average cumulative number of arthropod natural enemies counted per leaf ($\pm$ S.E.) at each location season long and **(B)** the average cumulative number of arthropod natural enemies found on yellow sticky traps ($\pm$ S.E.) season-long.

Pollinators

Across both years, there was no difference in the number of bumble bees, wild bees, or the total number of flower visits between the two plastic types at both locations (bumble bees: $F_{1,8} = 0.002$, $p = 0.97$; wild bees: $F_{1,8} = 0.1$, $p = 0.77$; total bees: $F_{1,8} = 0.28$, $p = 0.61$). However, there were 74.6 % fewer honey bees observed making floral visits under the UV blocking treatment compared to the transparent treatment in the ten-minute observations ($F_{1,8} = 8.19$; $p = 0.02$) (Figure 2.7). The overall number of wild bees counted was statistically similar between treatments, but with a trend for fewer under the blocking treatment (Figure 2.7).

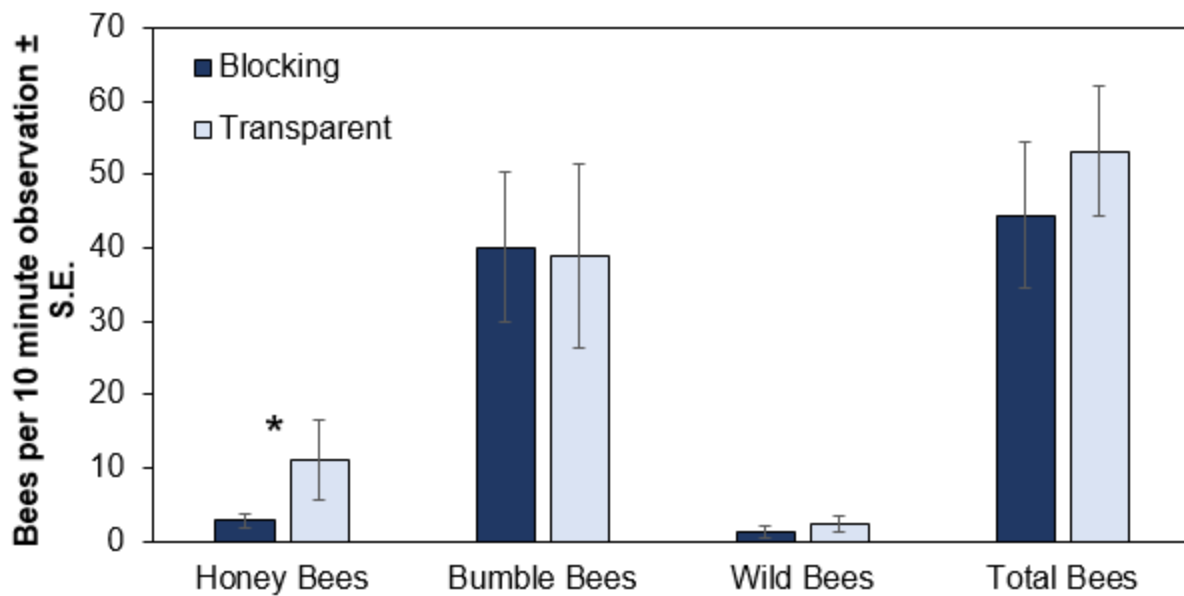


Figure 2.7. Average number of bees per ten-minute observation ($\pm$ S.E.) under tunnels covered in UV-blocking or UV-transparent plastics between both sites and years. Bars marked with an asterisk denote treatment differences at $\alpha = 0.05$.

DISCUSSION

Throughout both years of the study, eight key pests were responsible for most of the plant or fruit damage and six abundant natural enemy groups under the high tunnels (Figure 2.2, 2.3, 2.4).

While some insects, particularly pests including thrips and aphids, had outbreaks at some sites and not others (Table 2.1), they were abundant at all of the raspberry plantings sampled in this study.

Overall, there was no consistent effect of plastic type on natural enemy or pest populations. In 2016 at HTRC, significantly more adult *D. suzukii* were found under the UV-blocking treatment compared to the UV-transparent treatment (Figure 2.3A), but there were significantly more larval *D. suzukii* found under the UV-transparent treatment (Figure 2.3B). While it is difficult to elucidate the effect of the plastic, these treatments were not randomized to account for directionality and previous data have shown higher pressure of *D. suzukii* from the south compared to the north. It is possible that while adult fly presence may be higher under the UV-blocking plastics, egg-laying or larval survivorship is lower under reduced UV conditions.

More research is needed on how *D. suzukii* egg-laying, mating, and dispersal behavior may be modified by these plastics. However, general trends in our data suggest that plastic type is not a primary cause driving the population of *D. suzukii* in this study (Figure 2.3). Similarly, differences were documented in thrips and leafhopper populations in 2015 and aphid populations in 2016 under the plastic types (Table 2.1). Other studies which have focused on these insects have found that ambient UV light can impact their behavior and ability to cause plant damage. However, these trends are not consistent from year to year and these insects are still found in abundant numbers under both treatments, indicating that any effects are subtle and may not have biological or agricultural significance. In this study, most arthropod populations don't appear to

be driven by the UV light conditions in these tunnels, possibly because their primary way of finding hosts for feeding and oviposition are done using olfaction cues, and also because they spend much of their time under the leaves which would block UV naturally. While visual and olfactory cues have been shown to be complementary in insects (Prokopy and Owens 1983; Dobson 1994; Terry 1997), odor cues may predominate when the insects are close to a potential host (Terry 1997; Antignus 2000).

Additionally, most studies conducted on this topic have been focused in closed environments, like greenhouses (Antignus et al. 1996, Costa and Robb 1999). These findings could vary if the environment was open for insects to go in and out, such as with a high tunnel. Costa et al. (2002) compared plastics that varied in UV transmission and found reduced populations of thrips and whiteflies under the UV blocking plastic in closed greenhouse experiments, but this treatment response was not found when the study was conducted in high tunnels. It's possible that unfiltered sunlight at the ends and sides of the high tunnels can correct for the modified light from the plastics (Diaz and Fereres 2007). Moreover, the UV blocking plastic used at two of the three sites in this study was not 100% UV blocking, and allowed about 40% of the UV light through (Figure 2.1). Complete UV reduction may be necessary for these open systems to see a similar response as with greenhouses, though similar trends in insect abundance between plastics also occurred at SWMREC in 2015, which had a 99.1% UV reducing plastic.

I found that honey bees were less active under reduced UV light conditions, and this trend was also present for wild bees, though not significantly so. However, the number of pollinator observations at these sites were relatively low, and more effort to observe bee activity may better reveal wild bee sensitivity to UV light conditions. Bumble bee activity under these

tunnels was similar regardless of the UV light conditions and these findings are consistent with Morandin et al. (2002). However, we know that some bees are sensitive to reduced UV light conditions (Skorupski et al. 2007) and more research is needed on whether plastic choice affects pollination services in this or other crops, especially in areas where bumble bees are not naturally abundant or cannot be stocked as commercial colonies.

The phenology and abundance of arthropod pests and natural enemies highlighted in this research can be provided to growers, scouts, and others involved in raspberry production to better guide integrated pest management decisions in this crop. This study did not investigate fruit quality parameters, including fruit size, brix, shelf-life, and more research should be done to expand on these factors. Moreover, regions with different light intensity, such as locations closer to the equator, may have different arthropod communities and sensitivity to UV conditions. Throughout Michigan and at similar latitudes, however, similar results can be expected for the insects in this crop. For most greenhouse and high tunnel raspberry pests, including thrips, whiteflies, and aphids, behavioral responses are not expected under the plastics presented in this study. This study focused on UV transmission, and plastics can be engineered in other ways to modify the light, including diffusion and PAR, and it is unknown how modifying these factors might change arthropod communities and abundance. The results of this study suggest that plastic choice in terms of UV transmission is not likely to affect insect populations in high tunnel raspberries given the UV characteristics of the plastics presented in this study. This information allows high tunnel growers to make their plastic choice based on other parameters, including cost and effect on fruit quality.

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CHAPTER 3. REDUCED ULTRAVIOLET LIGHT TRANSMISSION INCREASES INSECTICIDE LONGEVITY IN PROTECTED CULTURE RASPBERRY PRODUCTION

Heather Leach, John C. Wise, Rufus Isaacs

Department of Entomology, Michigan State University, 202 Center for Integrated Plant Systems,
578 Wilson Road, East Lansing, MI 48824

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ABSTRACT

High tunnels are large protective structures used for season extension of many crops, including raspberries. These structures are often covered in plastic films to reduce and diffuse ultraviolet light transmission for pest and disease control, but this may also affect the photodegradation and efficacy of pesticides applied under these tunnels. We compared the residue levels of ten insecticides under three tunnel plastics with varying levels of UV transmission and open field conditions. Raspberry plants placed in research-scale tunnels were treated with insecticides and residues on fruit and foliage were monitored for one or two weeks in early 2015 and early and late 2016. Plastics that reduce UV transmission resulted in 50% greater residues of some insecticides compared to transparent plastics, and 60% compared to uncovered tunnels. This increased persistence of residues was evident within 1 day and remained consistently higher for up to 14 days. This pattern was demonstrated for multiple insecticides, including bifenthrin, esfenvalerate, imidacloprid, thiamethoxam, and spinosad. In contrast, the insecticide malathion degraded rapidly regardless of the plastic treatment, indicating less sensitivity to photodegradation. These results suggest that the activity of pesticides under high tunnels covered in UV-reducing plastics may be prolonged, potentially allowing for fewer insecticide

applications and longer intervals between sprays, and this information can be used to optimize pest control in protected culture berry production.

INTRODUCTION

Raspberries are an economically important crop that enhance human diets throughout the world (Manganaris et al. 2014; Yang and Kortessniemi 2015). Raspberry growers are increasingly implementing high tunnels to better control their climatic variability and expand the regions where berry production can be profitable (Kadir et al. 2006; Thompson et al. 2009; Lamont 2009; Demchak 2009; Neri et al. 2012). High tunnels are steel structures covered with plastic which enable modification and greater control of the crop environment, extending the growing season into both the spring and the fall (Lamont 2009; Giacomelli 2009; Hanson et al. 2011). This approach also protects the plants from rain, which is a frequent concern for producers in many temperate production regions, thereby reducing disease incidence and preventing wash-off of pesticide residues (Demchak 2009; Hanson et al. 2011; Neri et al. 2012).

Increasingly, production under these tunnels is being optimized through the manipulation of the plastic covering type. Various plastics can be selected for their specialized features, based on the needs of the crop and region, including light diffusion, manipulation of infrared radiation, and decreased condensation (Espí et al. 2006; Heidenreich et al. 2008; Lamont 2009). Plastics manufacturers are also developing ways to reduce the transmission of ultraviolet (UV) light. This began primarily for improved plant growth and yield (Kataoka et al. 2003; Dufault and Ward 2009), but blocking UV light has also been found to reduce disease and pest incidence in tunnel-grown crops (Espí et al. 2006; Heidenreich et al. 2008). Ultraviolet light has a wavelength from 10 to 400 nm, slightly shorter than the visible light spectrum for humans. The visible spectrum of

light for insects, however, includes UV light, and disrupting this has been shown to have behavioral effects on dispersion, feeding, and mating of whiteflies, aphids, and some other pests (Antignus et al. 2001; Costa et al. 2002; Diaz and Fereres 2007; Doukas and Payne 2007; Johansen et al. 2011; Ben-Yakir and Fereres 2016). Blocking UV light transmission also reduces disease incidence, since it is required for sporulation by many common fungal pathogens (Reuveni et al. 1989; Nicot 1996; Raviv and Antignus 2004; Paul et al. 2005; Ben-Yakir and Fereres 2016).

While these plastics may be promising for reducing disease and insect pest pressure in raspberry production, occasional pesticide applications are still required for controlling pest outbreaks, and these plastics have the potential to affect pesticide degradation. This is especially important for managing the invasive insect, spotted wing *Drosophila* (*Drosophila suzukii*), a devastating new pest of this and other berry crops (Asplen et al. 2015). Management of this insect can include cultural (Leach et al. 2016, 2017) and biological (Daane et al. 2016; Woltz and Lee 2017) approaches, but in larger commercial production settings, frequent insecticide applications are commonly used to protect berries from infestation by its larvae (VanTimmeren and Isaacs 2013; Diepenbrock et al. 2016). It is important to maintain control of *D. suzukii* through the long ripening period of raspberries, and high tunnel coverings that reduce pesticide degradation may allow for less frequent applications and/or improved control.

The rates of degradation of pesticides are influenced by light, plant metabolism, temperature, and microorganisms (Baskaran et al. 1999; Burrows et al. 2002; Sinderhauf and Schwack 2003). Photodegradation by sunlight is a major abiotic degradation pathway of chemicals largely caused by light in the ultraviolet spectrum (Schwarzenbach et al. 2003). Photodegradation of pesticides tends to happen within the first few hours after the application, so

reducing UV light is expected to change the initial degradation curve of these chemicals (Burrows et al. 2002; de Urzedo et al. 2007; Weber et al. 2009). Reduced degradation of pesticides has previously been reported on crops grown under protective structures compared to open fields (Garau et al. 2002; Weber et al. 2009; Allen et al. 2015; Sun et al. 2015). Van Emden and Hadley (2011) found that the insecticide cypermethrin can provide sustained control on the confused flour beetle (*Tribolium confusum*) for up to 6 months longer in bioassays when exposed to a UV reducing plastic compared to a UV transparent plastic. However, the dissipation of commonly used insecticides under protected culture with UV reducing plastics has not been studied in raspberries or other berry crops, despite the widespread use of these plastics across this industry. Understanding how insecticides may degrade differently under specialty plastics is important for developing appropriate intervals between applications, and for exploring potential prolonged efficacy on pests and also longer periods of risk to beneficial arthropods.

The objectives of this study were (1) to determine how different agricultural plastics alter UV transmittance under small-scale tunnels compared to field conditions, (2) to determine how ten different insecticides degrade on raspberry foliage across these treatments in early and late summer, (3) to determine how these insecticides degrade differently on raspberry fruit under these treatments in late summer, and finally (4) to observe the degradation of these chemicals over time across the different plastic treatments, compared to uncovered tunnels.

MATERIALS AND METHODS

Experimental setup

This research was conducted in small research-scale tunnels covered with different plastic types at the Horticultural Teaching and Research Center in East Lansing, Michigan, USA. The tunnels

were 1.2 m × 3 m and each tunnel covered eight potted raspberry plants (cv. “Polka”). Plants were irrigated daily using 3.2 GPH Netafim spray stakes (Trickl-Eez Irrigation Inc., St. Joseph, MI) inserted into the base of each 11.4 L pot. Each tunnel was constructed from five hoops of 1.9 cm diameter metal conduit, shaped using a hoop bender (QuickHoops™, Johnny’s Selected Seeds, Fairfield, ME) (Figure 3.1). The hoops were anchored by sliding them over 1.3 cm diameter conduit stakes that were pounded into the ground, 0.75 m apart and leaving 0.6 m above the ground. Plastic was secured on the sides of the tunnel using 3.8 cm x 8.9 cm × 3 m wood on each side, raised 0.3 m above the ground to allow for airflow (Figure 3.1). In 2015, we constructed 12 tunnels, with two plastic types covering each of four replicates and uncovered controls arranged in a randomized complete block design. Tunnels were 2 m apart from each other. The following plastic types were compared: diffuse Luminance® plastic (Visqueen, Stevenston, UK) and diffuse Lumisol® plastic (Visqueen, Stevenston, UK). In 2016, we constructed 16 tunnels with 4 replicates covered in three plastic types and uncovered controls arranged in a randomized complete block design. The three plastic types were Luminance®, research-grade clear UV-blocking (Visqueen, Stevenston, UK), and research-grade clear UV-transparent (Visqueen, Stevenston, UK). The uncovered control had the same metal frame, but no plastic unless rain was predicted, in which case they were temporarily covered in plastic to keep the plants dry. Plants in this treatment were covered for no more than 12 hours, once in the first trial, three times in the second trial, four times in the third trial, and once in the final trial, and all rain events occurred overnight so there was little effect on UV exposure.



Figure 3.1. Research tunnels containing eight potted raspberry plants and covered with different plastic types, replicated in a randomized complete block design. Tunnels measured 0.6 m x 3 m and were raised 0.3 m above the ground to allow for airflow.

During each trial in 2016, the UV light under each tunnel was measured at canopy height using a UV sensor sensitive to 250-400 nm (MU-200, Apogee Instruments Inc, Logan, UT) and compared to the UV light conditions immediately outside of the tunnel between 12 and 2pm. UV light data for the periods of the experiments were also gathered from the USDA's (Natural Resource Ecology Laboratory, Colorado State University, Fort Collins) UV-B monitoring research program at the closest recording station with a similar latitude in Geneva, NY. One temperature probe (HoboWare Data Logger, Onset Computer Corp., Bourne, MA) was hung in the center of each tunnel at canopy height to record temperature once every hour for June-October in 2016. Additional temperature and humidity values were taken from a MSU Enviroweather weather station 3.9 km from the site for dew point information (www.enviroweather.msu.edu).

Insecticide applications and plant samples

In 2015, one application of insecticides was made to the raspberry plants on 9 September. The insecticides were combined as a tank mix and were applied to the plants at the equivalent of 468 liters of water per hectare using a CO₂ powered backpack sprayer operating at 50 psi with a single head boom and TeeJet 8003VS spray nozzle. Insecticides were applied at their maximum labeled rate for raspberries: thiamethoxam (Actara 25WDG, 70.61 g AI ha⁻¹, Syngenta Crop Protection, LLC, Greensboro, NC); imidacloprid (Admire Pro 2F, 347.5 g AI ha⁻¹, Bayer Crop Science LP, Research Triangle Park, NC); esfenvalerate (Asana XL 0.66EC, 56.04 g AI ha⁻¹, DuPont de Nemours and Company, Wilmington, DE); acetamiprid (Assail 30SG, 112.09 g AI ha⁻¹, United Phosphorus, Inc., King of Prussia, PA); bifenthrin (Brigade 2EC, 112.09 g AI ha⁻¹, FMC Corp., Philadelphia, PA); spinetoram (Delegate 25WG, 105.1 g AI ha⁻¹, Dow AgroSciences LLC, Indianapolis, IN); spinosad (Entrust 2SC, 94.6 g AI ha⁻¹, Dow AgroSciences LLC, Indianapolis, IN); cyantraniliprole (Exirel 10SE, 149.07 g AI ha⁻¹, E. I. du Pont de Nemours and Company, Wilmington, DE); malathion (Malathion 8F, 1,782.17 g AI ha⁻¹, Gowan Company LLC, Yuma, AZ); and zeta-cypermethrin (Mustang Maxx 0.8EC, 28.0 g AI ha⁻¹, FMC Corp., Philadelphia, PA). No adjuvants were included in the tank mix.

Twenty-five leaves (approx. 12 g) were sampled from one replicate of each treatment before the application (0 days after treatment (DAT)) to make sure no insecticides were found on the leaves prior to the application. Immediately after the application dried (0.5 DAT), and at 1, 3, 5, and 7 DAT, twenty-five leaves were sampled from under each tunnel. The leaves sampled were fully expanded leaves on the upper part of the canopy, so that they were exposed to the

light conditions within each tunnel. Samples were placed immediately in 0 °C conditions in a cooler with ice and then shipped overnight in a freezer box for residue analysis.

In 2016, the same insecticides were studied, and all except thiamethoxam, spinetoram, and spinosad were applied at a reduced rate of 50% from the previous year. Thiamethoxam, spinetoram, and spinosad were applied at 100% of the suggested field rate as described above. These three materials degraded more quickly than the others in our 2015 results, and this reduced rate allowed all chemicals to be brought to a similar scale for the residue analyses. Applications were made on the raspberry plants on 8 July (early summer) and 6 September (late summer), and leaf samples were subsequently taken after each application at 1, 3, 5, 7, and 14 DAT. A longer sampling period of 14 days was added to better quantify the end of the residue decline. Sampling was also done at 0 DAT as described above.

In 2016, an additional application was made on 7 October to measure residues on raspberry fruit. Raspberry plants grown under the small tunnels did not produce enough fruit for this, so unsprayed canes producing raspberries from the MSU Clarksville Research Center in Clarksville, MI were cut and placed in buckets with water and floral foam. The fruits and stems were then sprayed with the tank mixture as described above. Fifty ripe fruits (approx. 100 g) from each tunnel were harvested on 1, 3, and 5 DAT. Fruits located in the upper part of the canopy were selected so they were exposed to the light conditions of each tunnel. Sampling was done at 0 DAT as described above with fifty fruit for each sample. They were frozen immediately after collection and sent overnight for residue analysis.

Residue analysis

To analyze the residues of the active ingredients of the insecticides on the leaves, 1 g of frozen homogenized leaves were combined with 10 mL of acetonitrile and 5 mL of deionized water. Extracting salts (Supel QuE Citrate, Sigma-Aldrich Corporation, St. Louis, MO) (AOAC Official Method 2007.01; Anastassiades et al. 2003) were added, and the sample was centrifuged. Extracts were then put into the dispersive Solid Phase Extraction (dSPE, Supel QuE PS, Sigma-Aldrich Corporation, St. Louis, MO) and centrifuged again. Samples were then analyzed using gas chromatography triple-quadrupole mass spectrometry (GC-MS/MS) and liquid chromatography triple-quadrupole mass spectrometry (LC-MS/MS). GC-MS/MS analysis was performed using a Varian 4000 GC/MS Ion Trap (Varian Medical Systems Inc, Palo Alto, CA) for bifenthrin, cypermethrin and esfenvalerate. Injections were made in splitless mode at 250°C onto a VF-XMS column (30m x 0.25mm ID). The column started at 50°C and increased to 260°C at a rate of 45°C/minute, then increased to 310°C at a rate of 15°C/minute for a total run time of 12 minutes.

LC-MS/MS analysis was performed using a Thermo TSQ Endura MS and Vanquish HPLC for all other analytes (Thermo Fisher Scientific Inc., Waltham, MA). An Accucore Phenyl-X column (2.6µm, 100 × 2.1mm, Thermo Fisher Scientific Inc., Waltham, MA) was used with a flow rate of 0.3 mL/min. The gradient program began with 100% of an aqueous solution containing 0.05% acetic acid, 10mM ammonium acetate, and 2% ACN. This mobile-phase composition was held for 0.5 min and ramped to 100% of an ACN solution containing 0.05% acetic acid, 10mM ammonium acetate, 5% H₂O and held from 5.5 to 9.0 min. The column was re-equilibrated at the initial mobile-phase conditions for a minute resulting in a 10 min run time. A separate method was run for cyantraniliprole using the same mobile-phases with a different

pump profile. This gradient program began with 50% of aqueous solution and 50% of ACN solution, ramped to 100% at 3.5 min and held until 5.5 min. The column was re-equilibrated at the initial mobile-phase condition for a 0.5 min resulting in a 6 min run time. Both LC methods used Atmospheric Pressure Chemical Ionization (APCI) as the mass spectrometer ion source.

To analyze the residues of the active ingredients of the insecticides on the fruit, 10 g of frozen homogenized fruit were combined with 10 mL of acetonitrile and 1.5mL of deionized water. A packet of extracting salts QuEChERS (Supel QuE Citrate, Sigma-Aldrich Corporation, St. Louis, MO) (AOAC Official Method 2007.01; Anastassiades et al. 2003) was added. The sample was shaken for 15 minutes in a mixer (MIX-003-001), then centrifuged for 5 minutes. The supernatant was added to a tube containing the dispersive Solid Phase salts (dSPE, Supel QuE PS, Sigma-Aldrich Corporation, St. Louis, MO), shaken, and centrifuged for two minutes. The extract was then analyzed using gas and liquid chromatography coupled with mass spectrometry detectors triple-quadrupole (GC-MS/MS) and (LC-MS/MS). The GC-MS/MS analysis was performed using a 436-GC and EVOQ-TQ (Bruker Corp., Billerica, MA) for bifenthrin, cypermethrin and esfenvalerate. Injections were made onto a 5% phenyl-methyl siloxano column (20m x 0.18mm ID and 0.18µm of film) in split mode with the injector at 70 °C and held for 0.50 minutes, then heated at 200°C/min to 300°C. The column started at 60°C and increased to 180°C at 45°C/minute, then increased to 300 °C at 25°C/minute, then to 330°C at 50°C/minute for a total run time of 16.07 minutes.

LC/MS/MS analysis was performed using a UHPLC-Advance and EVOQ-Elite-TQ (Bruker Corp., Billerica, MA) for all other analytes. A built-in oven and a HPLC column (Intensity Solo 2 C18, 2µm, 100 x 2.1mm, Bruker Corp., Billerica, MA) was used with a flow rate of 0.4 mL/min. The gradient program began with 98% of mobile phase A containing 0.05%

formic acid and 2mM ammonium formate in water and 2% mobile phase B containing 0.05% formic acid in methanol. This mobile-phase composition was held for 0.10 min and ramped to 65% mobile phase A and held for 7 minutes then 2% mobile phase A and held for 3 minutes. The column was re equilibrated at the initial mobile-phase conditions for 3 minutes resulting in a 13 min run time. For the LC- MS/MS technique, an Electrospray Ionization (ESI) source was used. For the GC-MS/MS analysis an Electron Ionization (EI) source was used.

For leaf and fruit analysis, one quantitative transition and at least one qualifier transition were monitored for each analyte. Retention times and ion ratios of quantitative and qualitative ions were determined from analytical standards. All analyses were calibrated with a minimum of a 5 point curve and samples were bracketed with Continuing Calibration Verifications (CCVs). Both the limit of detection (LOD) and the limit of quantification (LOQ) for leaf and fruit residues was 0.01 ppm.

Statistical analyses

Residue samples from each of the four experiments were analyzed using a linear mixed-effect model with repeated measures. Data were tested for normality using the Levene's and Shapiro-Wilks tests. Residues from plants under the different plastic treatments were compared along with the uncovered control plants using analysis of variance with block as a random factor using the "nlme" package in R (Pinheiro et al. 2017). Tukey's Honestly Significant Difference test was used to conduct post-hoc comparisons among treatments using the "lsmeans" package in R (Lenth 2016). All data were analyzed using R (3.3.3., R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The amount of UV light allowed through the plastic treatments was significantly reduced compared to the uncovered tunnel, with the greatest reduction occurring from the complete UV blocking plastic, which allowed only 0.6% of UV light through, followed by 33.1% of UV light penetration under the partial blocking treatment, 74.2% of UV light penetration under the transparent plastic treatment, while the open treatment allowed an average of 99.4% of the UV light through (Figure 3.2) ($F[3,12] = 571.3$; $p < 0.001$). Average temperature in the plant canopy during the daytime was 24.3 ± 0.3 °C in the open treatment, 25.3 ± 0.2 °C in the transparent treatment, 25.2 ± 0.4 °C in the partial blocking treatment, and 25.4 ± 0.2 °C in the blocking treatment. The temperature within the tunnels was not significantly different between any of the treatments in July ($F [3,12] = 1.2$; $p = 0.3$) or throughout the entire season ($F [3,12] = 2.9$; $p = 0.1$). In September, temperatures recorded in the uncovered tunnels were significantly lower than those in all other treatments ($F [3,12] = 8.7$; $p = 0.005$). However, the control treatments were within 1.1 ± 0.1 °C of the other treatments on average in September. Readings for UV light in Geneva, NY in September 2015 on average were 18.3 ± 1.2 kJ/m². In July 2016, total UV light was 31.1 ± 1.3 kJ/m², 21.7 ± 1.1 kJ/m² in September, and 9.6 ± 1.0 kJ/m² in October.

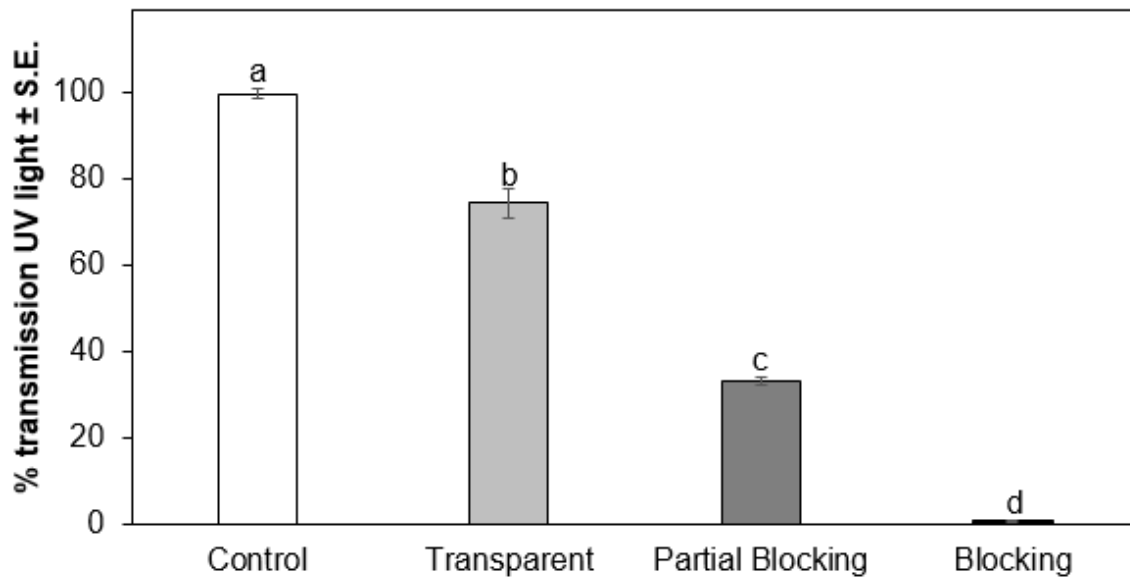


Figure 3.2. Average percent transmission of UV light ($\pm$ S.E.) under each of the plastic types covering tunnels in 2016. UV light was measured using a handheld UV meter (Apogee MU-200). Bars marked with different letters denote treatment differences at $\alpha = 0.05$.

In 2015, the partial blocking treatment had significantly greater insecticide residues than the open uncovered treatment for nine of the ten insecticides tested over the entire experiment (Figure 3.3). The leaf samples taken at 0 DAT were all found to be below detectable levels for the insecticides evaluated. Spinosad was the only insecticide found to not degrade differently among the treatments ($F [2,53] = 2.74$; $p = 0.07$), but the levels of this insecticide detected were very low by 1 DAT across all treatments. For most insecticides, the amount of residue on leaves of raspberry plants growing under the partial transparent treatment did not differ from those on plants growing under the two other plastics. In some cases, as with acetamiprid, the partial transparent plastic was similar to partial blocking plastic, and had 26% and 35.5% higher residues, respectively, compared to the open control ($F [2,53] = 8.74$; $p < 0.001$). For

imidacloprid, the blocking treatment had 30% and 49% greater residues compared to the partial transparent and open treatment, respectively ($F [2,53] = 19.09$; $p < 0.001$).

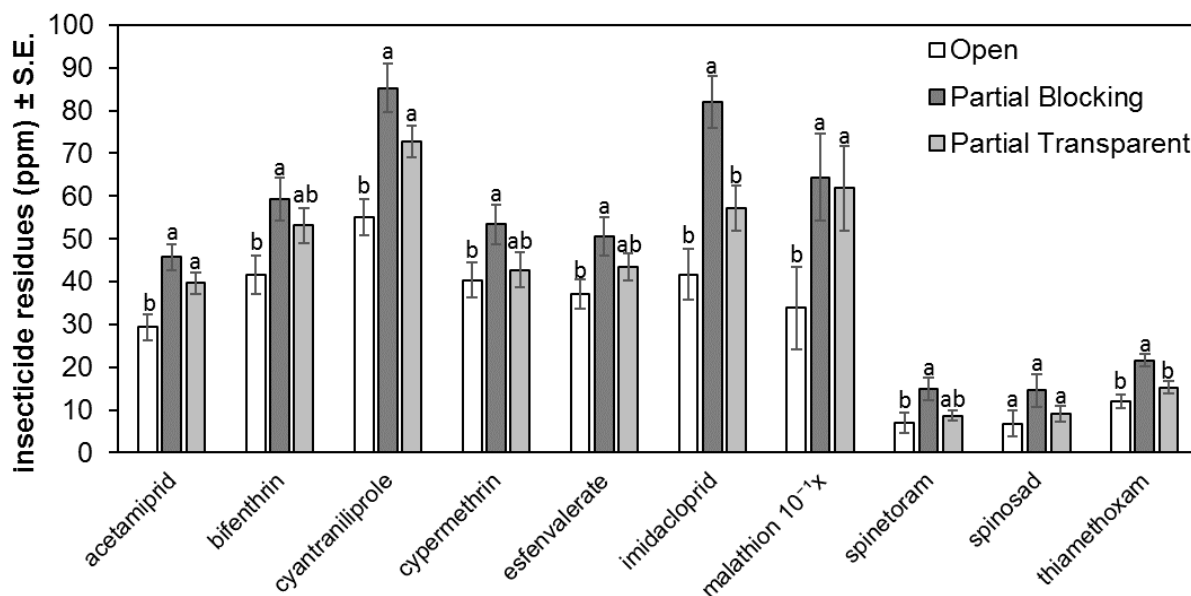


Figure 3.3. Average parts per million of insecticide residues on leaves ($\pm$ S.E.) under two plastic treatments and an uncovered control across 0.5, 1, 3, 5, and 7 days after the insecticides were applied in 2015. Bars within a group marked with different letters denote treatment differences at $\alpha = 0.05$.

In the July 2016 trial with leaf samples, the fully or partially blocking plastics resulted in significantly higher insecticide residues compared to the transparent plastic or open control treatments for nine out of the ten insecticides tested (Figure 3.4). In contrast, malathion residues did not differ among any the treatments ($F [3,71] = 0.25$; $p = 0.8$), partly because it degraded very quickly (Figure 3.4). The leaf samples taken at 0 DAT in this trial were all found to be below detectable levels for the insecticides evaluated. For all other insecticides except

imidacloprid, the blocking plastic treatment was statistically similar to the partial blocking plastic treatment. For imidacloprid, residues under the blocking treatment were 11.5% higher than the partially blocking treatment ($F [3,75] = 221.51; p < 0.001$). For all insecticides except acetamiprid, the transparent treatment resulted in similar residue levels to the uncovered control. Acetamiprid residues were 22.5% higher under the transparent treatment compared to the uncovered tunnels ($F [3,75] = 65.46; p < 0.001$).

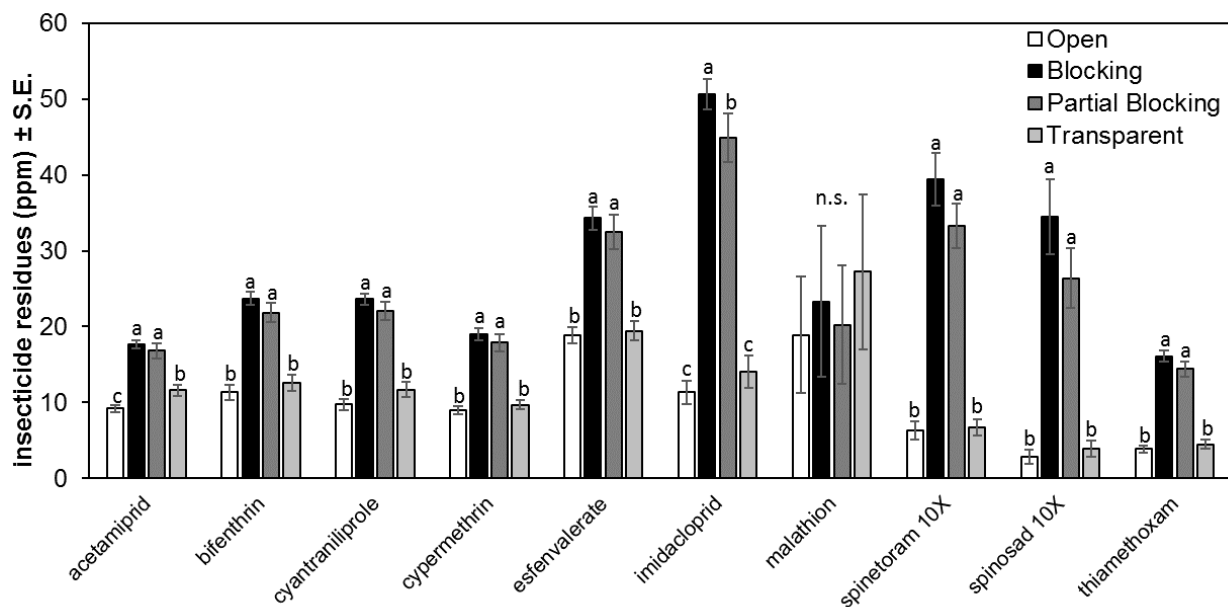


Figure 3.4. Average parts per million of insecticide residues on leaves ($\pm$ S.E.) under three plastic treatments and an uncovered control across 1, 3, 5, 7, and 14 days after the insecticides were applied in July 2016. Bars within a group marked with different letters denote treatment differences at $\alpha = 0.05$.

The pattern of insecticide residues measured from the September 2016 trial were similar to those of the July 2016 trial (Figure 3.5). Leaf samples taken at 0 DAT in this trial had variable levels of residues for each of the chemicals except malathion. All values were below 10 ppm

with 72.5% of the samples below 4 ppm. Malathion again degraded quickly across the treatments and had high variability with no significant differences among the treatments ($F [3,75] = 1.20$; $p = 0.3$). For all other insecticides tested, the complete and partial UV blocking materials had significantly higher residues averaged across all sample dates compared to the transparent plastic or the uncovered control (Figure 3.5). In some cases, the complete UV blocking material had higher residues than the partial UV blocking plastic, as with imidacloprid, spinetoram, and spinosad ($F [3, 75] = 58.85$; $p < 0.001$, $F [3, 75] = 49.59$; $p < 0.001$, and $F [3, 75] = 30.21$; $p < 0.001$, respectively). For spinosad, the blocking treatment had an average of 481.6% higher residue levels compared to the uncovered tunnel, and 30.8% higher residues than the partial blocking treatment.

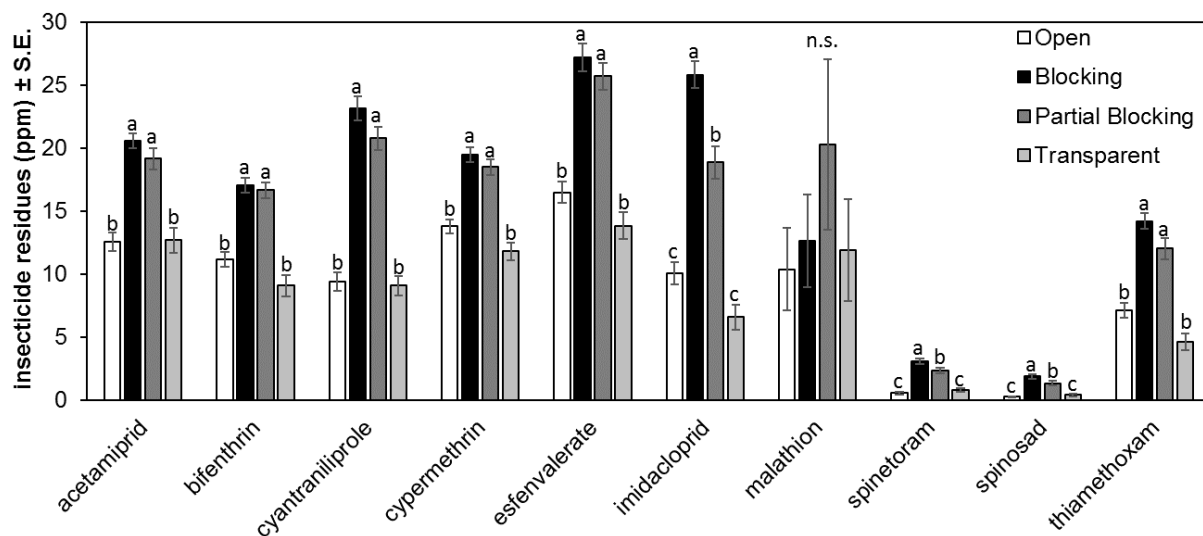


Figure 3.5. Average parts per million of insecticide residues on leaves ($\pm$ S.E.) under three plastic treatments and an uncovered control across 1, 3, 5, 7, and 14 days after the insecticides were applied in September 2016. Bars within a group marked with different letters denote treatment differences at $\alpha = 0.05$.

For the residues on raspberry fruit sampled in 2016, there were fewer differences among the plastic treatments than for the leaf analyses (Figure 3.6). The fruit samples taken at 0 DAT in this trial were all found to be below detectable levels for the insecticides evaluated. Most insecticides had higher residues under plastic treatments compared to the uncovered controls. Some insecticides, such as acetamiprid and malathion, had no significant differences among the plastic treatments ($F [3,39] = 1.95$; $p = 0.1$, and $F [3, 39] = 1.06$; $p = 0.4$, respectively). In some cases, residues from the blocking treatments were lower than the residues from the transparent plastics, as seen for bifenthrin which had an average of 33.7% higher residues under the plastics, as seen for bifenthrin which had an average of 33.7% higher residues under the transparent treatment compared to the blocking treatment ($F [3, 39] = 6.28$; $p = 0.001$).

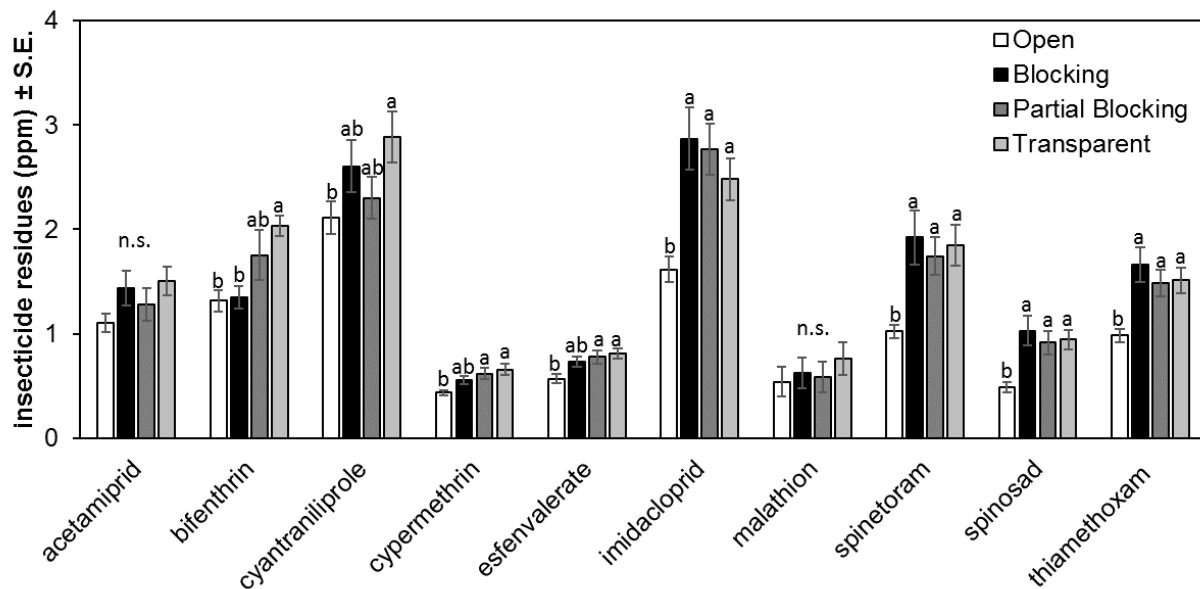


Figure 3.6. Average parts per million of residues of insecticides on raspberry fruits ($\pm$ S.E.) under three plastic treatments and an uncovered control across 1, 3, and 5 days after the insecticides were applied in October 2016. Bars within a group marked with different letters denote treatment differences at $\alpha = 0.05$.

For most insecticides under the transparent and open treatments, residues declined rapidly within 1 d after the application, as seen with imidacloprid and cyantraniliprole (Figure 3.7). This was also true with cypermethrin and spinosad (Figure 3.8). The blocking or partially blocking plastics, however, resulted in greater retention of the insecticides, and this remained consistent through time in most trials. In some cases, residues returned to statistically equivalent levels near the end of the trial, as seen with both imidacloprid and cyantraniliprole in the September 2015 trial (7 DAT: $F [2, 8] = 1.62$; $p = 0.2$, and $F [2, 8] = 0.32$; $p = 0.7$, respectively). For many of the trials, both the partial blocking and the blocking treatment had similar residues through time, as seen with the July 2016 trial. In the fruit residue trial, these treatment differences were less evident, except that the open treatment had overall reduced residues compared to the covered treatments on 1 and 3 DAT.

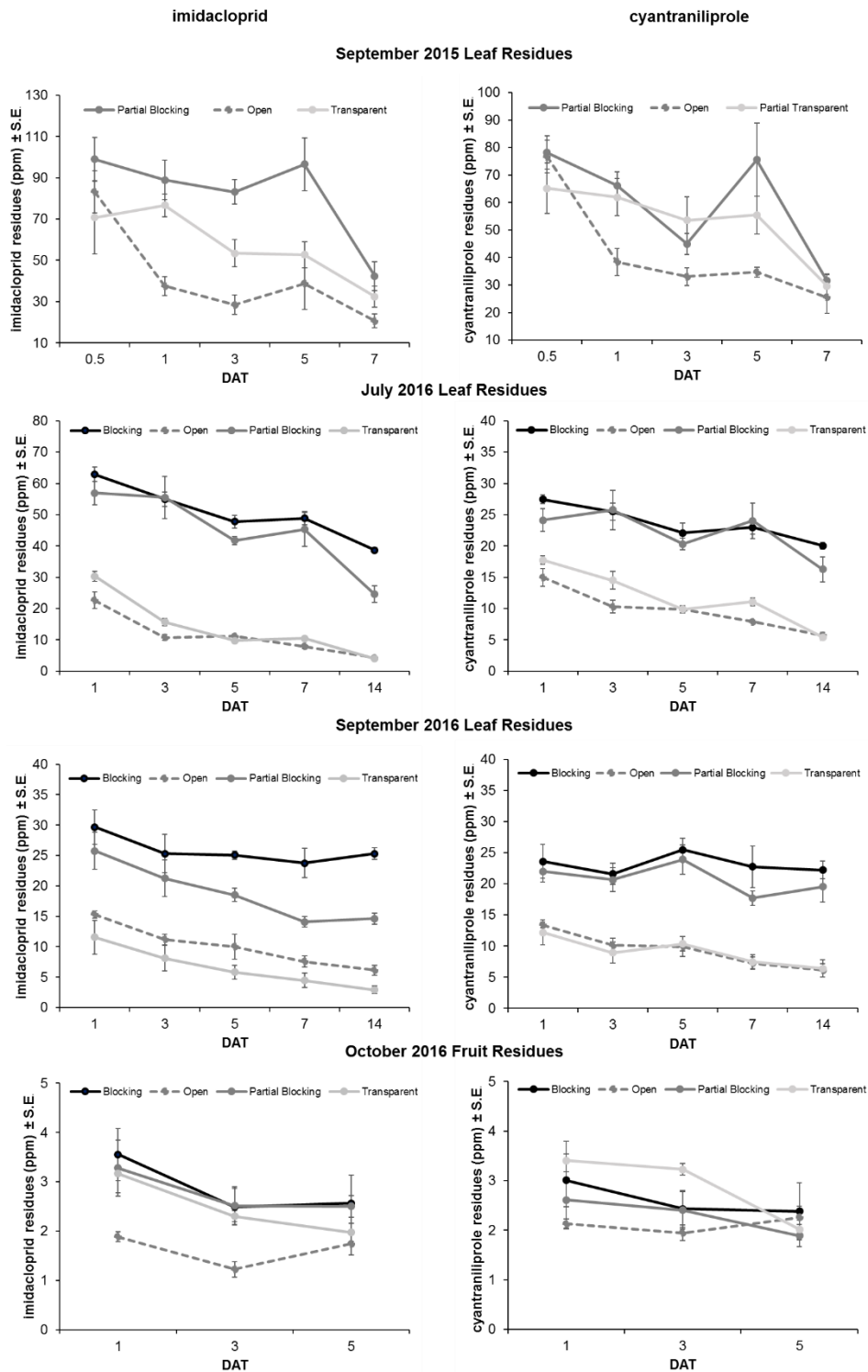


Figure 3.7. Residue levels of imidacloprid (left) and cyantraniliprole (right) ($\pm$ S.E.) found on raspberry foliage and fruit across the four residue decline trials conducted in 2015 and 2016.

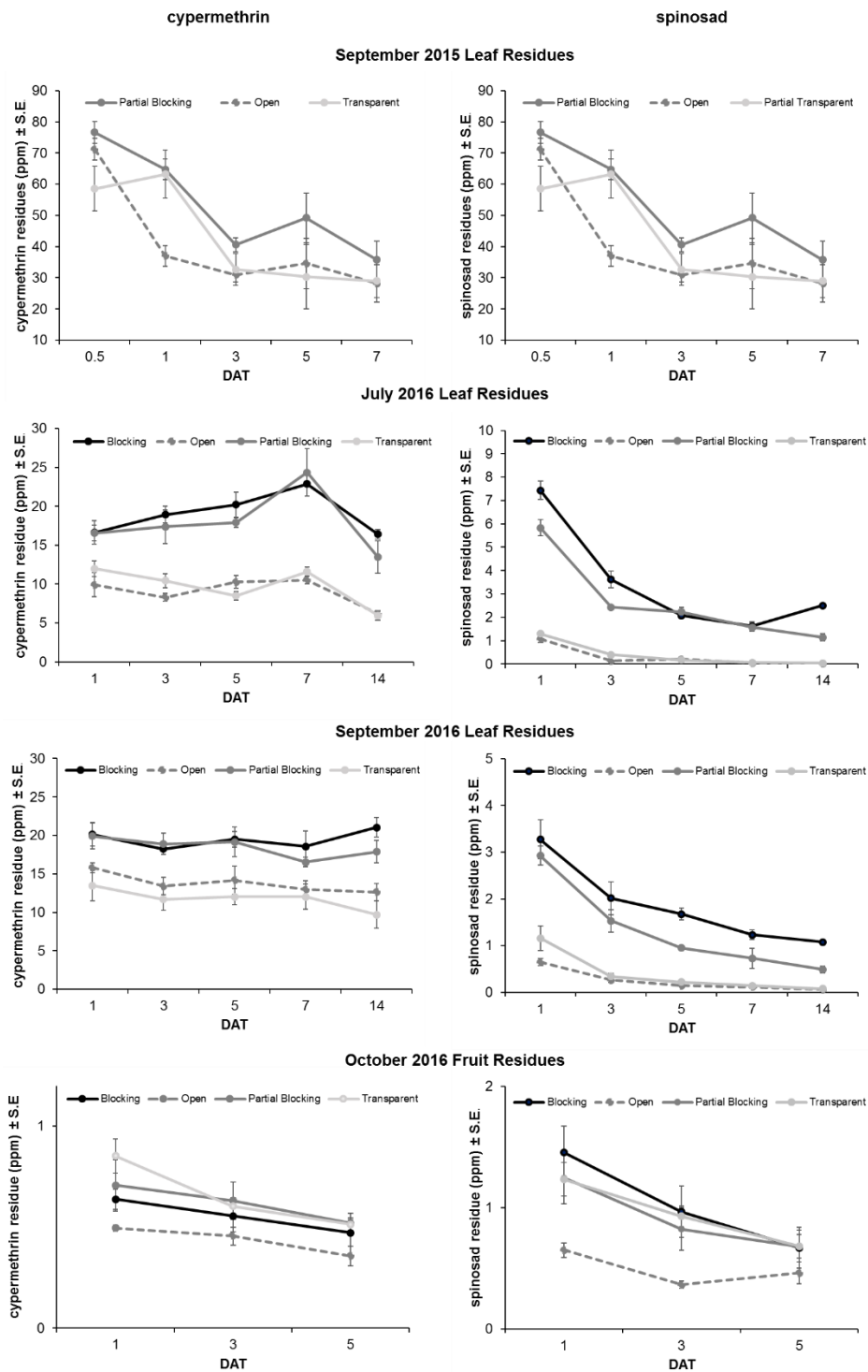


Figure 3.8. Residue levels of cypermethrin (left) and spinosad (right) ($\pm$ S.E.) found on raspberry foliage and fruit across the four residue decline trials conducted in 2015 and 2016.

DISCUSSION

This study highlights the influence of plastic coverings used in high tunnels on the degradation of insecticides after they are applied. We found that the persistence of most insecticides is influenced by the level of UV exposure, with imidacloprid, cyantraniliprole, cypermethrin, and spinosad frequently declining more slowly when UV light was blocked from interacting with residues on the plant canopy and on the fruit. This longer period of activity is expected to improve control of insect pests under tunnels using UV blocking plastic, compared to field-grown settings, particularly when combined with the disruption of insect behavior reported under these lower UV levels (Antignus 2000; Heidenreich et al. 2008; Kigathi and Poehling 2012; Lamnatou and Chemisana 2013). The consistent pattern of how UV-blocking plastics affected residues between years and times of year in our experiments suggests that this is a general pattern that can be expected in other regions. Given that high tunnels are used globally for berry, tree fruit, and vegetable production (Lamont 2009), it would be valuable to better understand how local environmental conditions affect the degree to which insecticide residue decline is delayed by selective plastics. A combination of laboratory studies to recreate the UV and temperature conditions experienced in different regions could then be validated using a network of the research-scale tunnels described in this study.

Temperature and humidity are additional environmental factors that can affect residue decline of pesticides (Wu and Nofziger 1999; Schwarzenbach et al. 2003). In some cases, we observed that the residues increased on certain sampling dates, as with imidacloprid on the 5 DAT sample in the September 2015 trial (Figure 3.7), and it is possible that leaf wetness from dew can play a role in redistributing the chemicals in the plant canopy (Mota-Sanchez et al. 2012). In each trial, there were days where the minimum recorded temperature at the site was

below the calculated dew point, causing the potential for leaf wetness. In the 2015 leaf residue trial, this occurred on 5 DAT, aligning with the observed residue increase. During the July 2016 leaf residue trial, these conditions also occurred on 5, 10, and 12-13 DAT, and on 1-3, 6-7, and 9-13 DAT during the September 2016 leaf residue trial. Throughout the 2016 fruit residue trial, this occurred on 2-5 DAT of the trial. While leaf wetness may be a factor influencing the residues, in general the relative levels of insecticide remained consistent through the period of these trials. We also found higher temperatures under the covered tunnels compared to the uncovered tunnels in the September 2016 trial, which could influence insecticide degradation (Wu and Nofziger 1999; Schwarzenbach et al. 2003). For both imidacloprid and cypermethrin, residues under the transparent treatment were lower than those under the uncovered tunnels in the September 2016 trial, suggesting that heat could be the cause of the faster decline.

Increased persistence of insecticide residues can translate into improved efficacy for longer periods (Borchert et al. 2004; Wise et al. 2006). This could result in fewer insecticide applications needing to be applied to maintain pest control, which would be significant for berry producers that are rebuilding integrated pest management systems after the arrival of the invasive fly, *D. suzukii*. It could also translate into longer spray intervals, giving growers some relief from the time and money needed for repeated spraying to protect fruit from this pest. Slowing the degradation of insecticides may also provide an opportunity to increase sustainability on farms using protected culture. This could be particularly beneficial for organic growers struggling to control problematic insect pests, including *D. suzukii* and *Halyomorpha halys* Stål (Van Timmeren and Isaacs 2013; Lee et al. 2014). Spinosad is an organically certified chemical that has good efficacy against *D. suzukii*, but has a short period of residual activity and it needs to be rotated frequently to reduce the risk of resistance (Van Timmeren and Isaacs 2013). As shown in

this study, reducing the UV light penetration into the growing environment can increase the retention of spinosad residues by up to 85% one day after the application. Residues of spinosad can also be retained up to 14 days after application under UV blocking plastics, unlike the transparent or uncovered treatments, which fall to undetectable levels at 1-3 days after the application (Figure 3.7). This could offer greater initial control as well as a longer duration of control, allowing organic growers to be more successful and economical in their production of berry crops.

Reduced degradation of pesticides has been observed for tomatoes and lettuce, which are commonly produced under protected culture (Garau et al. 2002; Cengiz et al. 2007; Chuanjiang et al. 2010; Allen et al. 2015). Allen et al. (2015) tested pesticide residues of multiple crop types grown under protected culture or in open field settings, and found significantly more types of pesticides and greater residues on the protected crops. Additionally, Garua et al. (2002) found that on greenhouse-grown tomatoes, some fungicides have slower degradation, particularly cyprodinil, and they discussed how this may have implications for meeting the maximum residue limit or for the pre-harvest intervals (PHI) set for this pesticide. While the residual activity of fungicides, miticides, and other pesticides were not evaluated in our study, similar results are expected based on these and other chemical degradation studies (Burrows et al. 2002; Sun et al. 2015).

The time of year and corresponding sun intensity may change the degree of benefit provided by UV blocking plastics, which could explain the different results between our fruit and leaf residue trials. The fruit trial was conducted in early October, and based on light data from Geneva, NY, there is an average of 12.1 kJ/m² less UV light compared to September, and 21.5 kJ/m² less UV light compared to July. Raspberry production in high tunnels in northern

temperate regions tends to begin in late June and can last through October, depending on the specific region and the cropping system used (Hanson et al. 2011). However, given that our results for both the early summer and late summer applications in 2016 are very similar, we do not expect time of year to have a profound impact on how plastic coverings affect insecticide residues.

While slowing insecticide residue decline has potential benefits for growers, it may also be a concern for meeting harvest tolerances set for insecticides, safety for the growers/harvesters, as well as the risks to beneficial insects. Increased insecticide use has been shown to harm pollinators and natural enemies in multiple systems (Desneux et al. 2007; Biondi et al. 2012; Gill and Raine 2014; Roubos et al. 2014; Chagnon et al. 2015), and the potential for increased longevity of insecticide residues affecting beneficial insects under tunnel production should be explored further. Decreased populations of natural enemies may result in surges of secondary pests (Beers et al. 2016; Yang et al. 2016), and research is needed to fully understand the long-term effect of prolonged insecticide residues on pest and beneficial insect populations. Developing new label requirements for pesticides in protected culture berry production, including PHI, retreatment intervals, and reentry intervals (REI), could be a way to manage the potential concerns about increased persistence of residues although the variation in types of plastics used on tunnels may make it challenging to provide a broadly applicable standard approach. While plastic type and UV transmittance of agricultural plastics are not standardized among plastic producers, they could fit into generalized UV blocking categories, such as those presented in this study, that would help to clarify pesticide labeling. The US EPA currently sets pesticide restrictions based on Good Laboratory Practice field residue data from relevant production systems (U.S. EPA, 2011), thus standards for protective culture are different than

open-field practices. However, if the protective culture industry moved predominantly to UV-blocking plastics, bridging data from one crop setting could potentially be used to amend labels for a broader range of crops. The effect of other plastic parameters, including light diffusion and manipulation of wavelengths beyond the UV spectrum were not evaluated in this study. While these factors may also affect the degradation of insecticides (Burrows et al. 2002; Katagi 2004; Remucal 2014), we expect the strongest effect to result from manipulation of UV light, since this is the primary way that insecticides are initially degraded (Burrows et al. 2002).

The information presented in this study highlights that UV degradation is an important breakdown pathway for the active ingredients of most of the insecticides studied, particularly the pyrethroids, neonicotinoids, and spinosyns, where the blocking plastics consistently reduced residue decline compared to the transparent plastic and the open control. Interestingly, this pattern was not observed for the one organophosphate studied, which could be used strategically before harvesting since its degradation is similar regardless of plastic covering. To our knowledge, this is the first time that the degradation of insecticides under protected culture plastics that modify UV transmittance has been studied on berry crops. Reducing UV exposure after insecticide applications through specialty plastics presents a new way to prolong the retention of the active ingredient and the efficacy of these compounds, in addition to the benefits of keeping the plants dry. These plastics should be considered as a component of integrated insect and disease management approaches for high tunnels, with the potential for reducing the frequency of pesticide applications.

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CHAPTER 4. EXCLUSION NETTING DELAYS AND REDUCES *DROSOPHILA SUZUKII* INFESTATION IN RASPBERRIES

Heather Leach, Steven Van Timmeren, and Rufus Isaacs

Department of Entomology, Michigan State University, 202 Center for Integrated Plant Systems,
Michigan State University, East Lansing, MI 48824, USA.

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ABSTRACT

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a new frugivorous pest of raspberries and other soft fruits in North America, causing infestation of fruit at harvest time. Control of this pest has primarily been through the application of broad-spectrum insecticides to prevent oviposition and larval development, and there is an urgent need for alternative approaches. Over two growing seasons, we compared *D. suzukii* control in a research planting with insecticide and exclusion treatments in a factorial design, monitoring first, second, and third instar *Drosophila* larvae in ripening, ripe, and overripe berries. Each of the two control approaches provided significant reduction of infestation in raspberry fruit, but the combination treatment had the lowest overall abundance of larvae in fruit. This pattern was seen for all larval instars in both years. The combination treatment also delayed the first detected larval infestation by ten days compared to the untreated plots. Exclusion netting applied to commercial size high tunnels resulted in a significant reduction in overall *D. suzukii* infestation in raspberries, as well as a three week delay in the average first detectable fruit infestation. Raspberry size and quality were not affected by the exclusion treatments, indicating that this approach can be an important component of growers' response to invasion by *D. suzukii* in temperate climates. We discuss the opportunities and limitations for implementing exclusion netting in raspberry production.

INTRODUCTION

Spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), was first detected in the United States in 2008 and has since become an important economic pest of soft fruit production (Bolda et al. 2010, Hauser 2011). *Drosophila suzukii* is equipped with a doubly-serrated ovipositor, allowing it to lay eggs in fresh, undamaged fruit (Lee et al. 2011b). The resulting larvae degrade fruit quality, causing a risk of detectable contamination that can reduce marketability (Goodhue et al 2011, Walsh et al. 2011). Puncture wounds from oviposition also increase the ability of pathogens to colonize the fruit (Walsh et al. 2011), further reducing marketable yields. Female flies can lay up to 25 eggs per day, depending on host and environmental conditions (Kinjo et al. 2014), making continued and efficacious control important. While *D. suzukii* has a broad host range, the most impacted crops include raspberry, blackberry, blueberry, and cherry (Lee et al. 2011a, Asplen et al. 2015). Raspberry is particularly at risk due to its highly attractive odors and soft epicarp, making oviposition relatively easy for *D. suzukii* (Lee et al. 2011b, Bellamy et al. 2013, Burrack et al. 2014, Abraham et al. 2015). Growers use baited traps to monitor for the presence of *D. suzukii*, with insecticidal protection of crops beginning when the fruit start to ripen and when *D. suzukii* flies have been trapped in the vicinity (Diepenbrock et al. 2016). Since the invasion by *D. suzukii*, insecticide applications in these systems have increased dramatically (Bruck et al. 2011, Van Timmeren and Isaacs 2013, Diepenbrock et al. 2016). Without repeated treatment of fruit, the high fecundity and short life cycle of *D. suzukii* allow it to rapidly increase in abundance (Wiman et al. 2014). *Drosophila suzukii* is known to use non-crop hosts, often at the borders of crop fields, so immigration into fields is a major source of ovipositing flies during the growing season (Klick et al. 2015, Lee et

al. 2015, Pelton et al. 2016). This makes complete control of this pest with insecticides highly challenging and very expensive (Bruck et al. 2011). Few alternative controls exist, limiting the options for organic and sustainable production of these fruit (Bruck et al. 2011).

The invasion of *D. suzukii* into fruit production regions has disrupted previously reliable IPM systems, and long-term restructuring of those programs should include biological, physical, cultural, and chemical control methods (Cini et al. 2012, Asplen et al. 2015, Haye et al. 2016). Physical exclusion has significant potential for use under protected culture such as high tunnels (Lee et al. 2011b). Exclusion netting has shown promise for reducing *D. suzukii* infestation in small-scale plantings of blueberries and raspberries in North America (Link et al. 2014, Cormier et al. 2015, Rogers et al. 2016) and for blueberries in Europe (Kawase et al. 2007, Grassi and Pallaoro 2012). Rogers et al. (2016) found that exclusion netting significantly lowered the number of infested raspberries when compared to either untreated or insecticide-treated field plots. However, effects on the timing of *D. suzukii* arrival and subsequent population growth in these protected areas will also have important implications for management of this pest. Mini-tunnels covered with netting or plastic reduced infestation by *D. suzukii* (Rogers et al. 2016), but the high levels of control reported in the plastic exclusion treatment was thought to be caused by extreme high temperatures that may not be as likely in a commercial high tunnel. There is limited information on the performance and feasibility of the exclusion approach in commercial production settings, and growers are also interested in combining control approaches to increase the proportion of fruit that meet the marketable standard. To explore the efficacy, feasibility, and limitations of using exclusion combined with insecticides for control of *D. suzukii* in raspberries, we tested these approaches separately and together over two seasons. Fruit were sampled using a method that revealed larval stage, allowing us to compare treatment effects on recent infestations

and on larvae most likely to be detected. We also tested the efficacy of exclusion netting for controlling *D. suzukii* adults and larvae in commercial production of high tunnel grown red raspberry and measured its effect on temperature, fruit quality, and the abundance of pest and beneficial arthropods.

MATERIALS AND METHODS

Exclusion netting combined with insecticides

A trial was conducted in a raspberry planting (cv. ‘Caroline’) in 2014 and 2015 at the Trevor Nichols Research Center in Fennville, Michigan. Plots were established in the planting (1.8 m by 1.8 m) and were either covered with 32 x 32 Lumite mesh-covered cage frames (BioQuip Products, Rancho Dominguez, CA) or left uncovered. These plots were either sprayed with insecticides or not, creating a factorial design with four replicates of each treatment in a randomized complete block design. Temperature probes (Hobo Pendant[®] Temperature Data Logger, Onset Computer Corporation, Bourne, MA) were placed inside radiation shields (Spectrum[®] Technologies, Inc., Aurora, IL) and attached to a PVC pole (3.8 cm diameter) in the center of each plot to determine the effect of netting on temperature in the plant canopy. In 2014, netting was applied to the fall raspberry crop in late August, and to reduce the starting infestation level all ripening or ripe fruit were removed from the plants immediately prior to the start of the experiment. In 2015, netting was applied to the summer crop in early July as soon as the fruit began to ripen. Insecticides were applied using a CO₂-powered backpack sprayer operating at 3.5 kgf/cm² in a volume of water equivalent to 1,496 liters per hectare and equipped with a single head boom and a TeeJet 8003VS spray nozzle (TeeJet Technologies, Wheaton, IL). Insecticide-treated plots received 4 applications of insecticide on 7d intervals rotating between zeta-

cypermethrin (Mustang Maxx, 292 ml/Ha), spinetoram (Delegate WG, 438 ml/Ha), malathion (Malathion 8F, 2,338 ml/Ha), and zeta-cypermethrin (Mustang Maxx, 292 ml/Ha). Fruit were collected immediately before the next application was applied, to provide three (2014) and four (2015) samples during the crop ripening period.

At each sample date, five ripening, ripe, and overripe berries were collected from each plot and assessed for presence of immature *Drosophila* using a modified salt test. This consisted of placing the berries in a one gallon resealable plastic bag and lightly crushing the fruit before adding salt water (237 ml of table salt added to 3.78 L of tap water). After 30 minutes, the fruit and liquid were poured over a coarse screen to remove the berries and then into a reusable coffee filter (Medelco 4-Cup Universal Coffee Filter, Medelco Incorporated, Bridgeport, CT) and the retained solids were examined under a stereomicroscope (Olympus SZX10 set at 20X magnification [10X eyepiece lens, 0.5X objective lens], Olympus America, Inc., Center Valley, PA) to facilitate accurate counting of *Drosophila* eggs and larvae. Larvae were classified as small, medium, and large which correlates approximately with the first, second, and third instar stages.

High tunnel exclusion netting

Fly-proof netting was installed on two high tunnels over raspberry plantings at a commercial, conventionally managed farm in Coloma, MI. Netting was also installed on one high tunnel over raspberry plants at an organic research farm in East Lansing, MI. All three netted tunnels were adjacent to a paired control tunnel that was open at the ends, and all six tunnels were covered by UV-blocking Lumite plastic coverings. To exclude *D. suzukii* from the three tunnels, 80 gram Tek-Knit netting (Berry Protection Solutions Stephentown, New York) was applied to the sides

of the tunnels by suspending it using 16-gauge galvanized steel wire attached along its length using zip ties along the interior of the curved roof struts, and with shade clips (FarmTek, Dyersville, IA) that held the netting to the wire. To allow movement of pickers to the outside rows of raspberry, the netting was also secured to the sides of the tunnel using 40mm metal clips (Haygrove Tunnels, Mount Joy, PA) and to the ground using landscaping fabric staples. Netting was applied to the outside frame on the two ends of each tunnel, using a different door design to accommodate different needs for access. The research farm site had two 7.6 x 60 m Haygrove tunnels oriented north-south, with each containing organic summer and fall red raspberries (cv. 'Polka', 'Himbo Top', and 'Joan J'). In the netted tunnel, an access point was installed in the south end using a small door frame with a magnetic closure. At the commercial farm, four 7.6 x 122 m Haygrove tunnels were oriented east-west with three rows of summer and fall red raspberries (cv. 'Prelude') in each. Two of these were netted, and both ends of the tunnels were fitted with two 2.4 x 2.1 m barn style wooden doors covered in netting, which allowed for access by a sprayer (Figure 4.1). Netting was installed on the tunnels in early June prior to activity of *D. suzukii*. Bumble bee colonies (Koppert Biological Systems, Howell, MI) were introduced for pollination, using three hives per tunnel at the commercial farm and two hives per tunnel at the research farm. Summer berry harvest occurred in late June through July and fall harvest occurred from the middle of August through early October.



Figure 4.1. High tunnel raspberry planting with netting added to exclude *Drosophila suzukii*.

The barn style wooden doors were installed to allow access for a tractor-pulled sprayer.

Temperature probes (Hobo Pendant[®] Temperature Data Logger, Onset Computer Corporation, Bourne, MA) inside radiation shields were hung in the center of each tunnel, taking readings every hour. Two monitoring traps baited with a yeast and sugar mix (Van Timmeren and Isaacs 2013) were placed 9 m from the end of each tunnel at canopy height and were checked weekly. Traps were made from 32 oz. deli cups filled with 150ml of solution and a yellow sticky insert hung from the top. When ripe fruit were available to harvest, 25 ripe raspberries were sampled every week within 5 meters of each yeast-sugar trap and in the center of the tunnel. The weight and diameter of these berries was recorded, and the degrees brix was recorded using a portable refractometer (Model RHB-32ATC, Westover Scientific Inc., Bothell, WA). These fruit were then immersed in a salt solution as described above and the number of

Drosophila eggs were counted, along with the number of larvae of each stage as described above. At regular intervals during summer and fall harvest, additional ripe fruit were sampled and the flies were reared to confirm the infestation as *D. suzukii*. All flies emerging from the fruit were identified as *D. suzukii*.

To monitor activity of other arthropods in the tunnels, 14 cm by 23.5cm yellow sticky traps (Scentry MultiGuard; Great Lakes IPM, Vestaburg, MI) were suspended above the plant canopy in the middle of each tunnel and replaced weekly. Arthropods captured were identified at least to family and later sorted by functional group. Direct leaf observations were also conducted weekly on 25 randomly selected raspberry leaves in each tunnel. Leaves were evaluated for percent leaf damage and suspected causes of the damage. The numbers and types of arthropods found on the leaves were also recorded.

Statistical analyses

In the factorial experiment with netting and insecticide treatments, the numbers of first, second, and third instar, and total larvae per gram of fruit were analyzed using a Kruskal-Wallis test followed by a Conover-Inman test for post-hoc comparisons. For the high tunnel experiment, fruit quality, temperature, and immature and adult *D. suzukii* data were analyzed using analysis of variance to compare netted and open tunnels, followed by Tukey's Honest Significant Difference for post-hoc comparisons. A Student's t-test was used to analyze data from the direct leaf observations and yellow sticky traps. Data were analyzed using R (3.2.2., R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Exclusion netting combined with insecticides

In two growing seasons, there were significantly fewer *Drosophila* larvae present in netted raspberries than in raspberries grown in the open (Table 4.1). This was most apparent and was statistically significant in the overripe fruit, though the same trend was also found in the ripening and ripe fruit. For all ripeness stages across 2014 and 2015, the open unsprayed plots had the highest number of *D. suzukii* larvae, the plots receiving exclusion netting or insecticide applications were intermediate, and the fewest larvae were found in plots with the combination of insecticide applications and netting. The differences among treatments varied depending on the sampling date, in part because the earlier sampling dates, especially those in early July 2015, had fewer larvae overall (Table 4.1).

In 2015, netting was installed on summer red raspberry plants before *D. suzukii* activity increased, allowing us to detect the first infestation in each plot. Average first infestation in berries on the open control plants July 10 (± 1.0 day) was ten days earlier than the combination netting and insecticide treatment July 20 (± 1.7 days). The other treatments were intermediate, with average first larval detection for the open insecticide treatment on July 15 (± 1.1 days) and the netted non-insecticide treatment on July 16 (± 1.0 day).

The combination of netting and insecticides resulted in significantly lower abundance of first, second, and third instar *D. suzukii* larvae in berries in 2014 and 2015 compared to the untreated control (Table 4.2). The insecticide treatment and exclusion treatment alone had intermediate levels of infestation for all instars in both years. While the presence of *Drosophila* larvae was

lower in the netted treatments, it never remained at zero. As pest pressure continued to build throughout the 2014 and 2015 growing seasons, we found that netting alone was not sufficient to control *D. suzukii* (Table 4.1). In contrast, combining netting with insecticide applications resulted in significantly lower infestation of the overripe fruit with *D. suzukii* than with netting alone (Table 4.1). The trends were similar in ripening and ripe fruit, with lower abundance of larvae in the combined treatment compared with the netting treatment on nine of the ten assessment dates when larvae were detected in the berries.

The average temperature inside the netted cages was very similar to the outside temperature over the course of the experiment in 2014 (Netted: 21.7 ± 0.5 °C, Open: 21.8 ± 0.5 °C) and in 2015 (Netted: 18.0 ± 0.4 °C, Open: 18.1 ± 0.4 °C). The average maximum temperature was slightly higher inside the netted cages over the course of the experiment in 2014 (Netted: 30.9 ± 0.6 °C, Open: 29.7 ± 0.4 °C), though this same trend was not found for 2015 (Netted: 25.9 ± 0.5 °C, Open: 25.4 ± 0.5 °C).

Table 4.1 Average number of *Drosophila* larvae per gram of raspberries collected from plots receiving insecticide and netting treatments. Five ripening, ripe, and overripe berries were collected from each plot weekly and assessed using a modified salt test. Averages with the same letter within each column are not significantly different at $\alpha=0.05$.

Ripeness	Netting Treatment	Insecticide Treatment	2014			2015				Overall 2014 & 2015
			27 Aug	4 Sept	10 Sept	9 July	13 July	17 July	23 July	
Ripening	No Netting	No Insecticide	0.8 ± 0.4a	1.2 ± 0.2a	0.3 ± 0.1a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.4 ± 0.1a	0.3 ± 0.09a
	No Netting	Insecticide	0.09 ± 0.03b	0.7 ± 0.3ab	0.3 ± 0.1a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.07 ± 0.07ab	0.1 ± 0.03ab
	Netting	No Insecticide	0.05 ± 0.05c	0.2 ± 0.1bc	0.8 ± 0.5a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.09 ± 0.09ab	0.1 ± 0.08b
	Netting	Insecticide	0.03 ± 0.03d	0.08 ± 0.08c	0.1 ± 0.01a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.03 ± 0.03b	0.03 ± 0.01b
	Statistics (Kruskal-Wallis):		H = 11.3, df = 3,10, P = 0.01	H=10.2, df=3,12, P = 0.02	H=2.6, df=3,12, P = 0.47	H= n/a, df= 3,12, P = n/a	H= n/a, df= 3,12, P = n/a	H= n/a, df= 3,12, P = n/a	H=8.3, df=3,12, P = 0.03	H= 13.7, df= 3,12, P = 0.003
Ripe	No Netting	No Insecticide	3.3 ± 0.6a	2.85 ± 0.6a	3.7 ± 1.1a	0.0 ± 0.0a	0.01 ± 0.01a	0.03 ± 0.03a	0.1 ± 0.08a	1.2 ± 0.2a
	No Netting	Insecticide	0.5 ± 0.2b	1.0 ± 0.2b	1.9 ± 0.4a	0.0 ± 0.0a	0.01 ± 0.01 a	0.02 ± 0.02a	0.2 ± 0.1a	0.5 ± 0.1ab
	Netting	No Insecticide	1.0 ± 0.6b	0.6 ± 0.2bc	0.7 ± 0.5a	0.0 ± 0.0a	0.02 ± 0.02a	0.06 ± 0.03a	0.2 ± 0.1a	0.4 ± 0.1ab
	Netting	Insecticide	0.08 ± 0.01c	0.2 ± 0.2c	1.0 ± 0.8a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.01 ± 0.01a	0.1 ± 0.09b
	Statistics (Kruskal-Wallis):		H=11.8, df=3,12, P = 0.008	H=11.6, df=3,12, P = 0.009	H=5.9, df=3,12, P = 0.12	H= n/a, df= 3,12, P = n/a	H=1.1, df=3,12, P = 0.75	H=3.1, df=3,12, P=0.36	H=4.3, df=3, 12, P=0.20	H= 7.7, df= 3,12, P = 0.05
Overripe	No Netting	No Insecticide	5.8 ± 1.5a	6.9 ± 0.8a	5.2 ± 1.3a	0.1 ± 0.07a	0.2 ± 0.07a	1.2 ± 0.1a	7.6 ± 1.8a	3.7 ± 0.6a
	No Netting	Insecticide	1.9 ± 0.4a	2.3 ± 0.5b	1.7 ± 0.3b	0.0 ± 0.0a	0.08 ± 0.04ab	0.6 ± 0.2ab	1.9 ± 1.9ab	1.1 ± 0.1b
	Netting	No Insecticide	0.8 ± 0.1b	1.6 ± 0.5b	3.3 ± 1.8ab	0.0 ± 0.0a	0.02 ± 0.02ab	0.4 ± 0.2ab	3.6 ± 1.9a	1.3 ± 0.4b
	Netting	Insecticide	0.2 ± 0.05b	0.3 ± 0.2c	0.6 ± 0.2c	0.0 ± 0.0a	0.0 ± 0.0b	0.09 ± 0.06b	0.02 ± 0.02b	0.1 ± 0.04c
	Statistics (Kruskal-Wallis):		H=12.1, df=3,12, P = 0.007	H=12.3, df=3,12, P=0.006	H=9.1, df=3,12, P=0.03	H=5.8, df=3,12, P=0.11	H=7.7, df=3,12, P=0.05	H=8.2, df=3,12, P=0.04	H=10.8, df=3,12, P=0.01	H=29.4, df=3,12, P<0.0001

Table 4.2 Average number of 1st, 2nd, and 3rd instar *Drosophila* larvae per gram of raspberries in fruit of all ripening stages collected from plots receiving insecticide and netting treatments. Five ripening, ripe, and overripe berries were collected from each plot and assessed using a modified salt test. Averages with the same letter within each column are not significantly different at $\alpha=0.05$.

Year	Netting Treatment	Insecticide Treatment	1 st Instar	2 nd Instar	3 rd Instar
2014	No Netting	No Insecticide	1.8 ± 0.2a	1.1 ± 0.2a	0.3 ± 0.07a
	No Netting	Insecticide	0.7 ± 0.1b	0.3 ± 0.05b	0.04 ± 0.01b
	Netting	No Insecticide	0.4 ± 0.07c	0.3 ± 0.1bc	0.2 ± 0.1ab
	Netting	Insecticide	0.1 ± 0.02d	0.08 ± 0.03c	0.07 ± 0.04b
	Statistics (Kruskal-Wallis):		H = 60.75	H=29.01	H=18.97,
			df = 3,12, P < 0.0001	df=3,12, P < 0.0001	df=3,12, P = 0.0002
2015	No Netting	No Insecticide	0.5 ± 0.1a	0.2 ± 0.1a	0.07 ± 0.03a
	No Netting	Insecticide	0.2 ± 0.07ab	0.07 ± 0.02ab	0.03 ± 0.01ab
	Netting	No Insecticide	0.3 ± 0.1ab	0.06 ± 0.03ab	0.09 ± 0.04ab
	Netting	Insecticide	0.01 ± 0.006b	0.007 ± 0.005b	0.0 ± 0.0b
	Statistics (Kruskal-Wallis):		H=13.89,	H=13.32,	H= 9.07,
			df=3,12, P = 0.003	df=3,12, P = 0.003	df=3,12, P = 0.02

High tunnel exclusion netting

There were significantly fewer *Drosophila* eggs, larvae, and adults in the netted tunnels than the open tunnels at both sites (Figure 4.2). Over the entire season, there was an 82% reduction in *Drosophila* eggs ($F=18.5$; $df=1,16$; $p=0.0002$), a 74% reduction in *Drosophila* larvae ($F=4.7$; $df=1,16$; $p=0.02$), and a 65% reduction in *D. suzukii* adults ($F=30.0$; $df=1,10$; $p=0.0003$) in the netted tunnels (Figure 2). Over the entire season, there were significantly fewer first instar larvae found in fruit in the netted tunnels (81.4 ± 67.8 larvae/kg) than the open tunnels (361.6 ± 206.6 larvae/kg) ($F=4.8$; $df=1,16$; $p=0.02$). There were also significantly fewer second instars from fruit in the netted tunnels (45.2 ± 45.2 larvae/kg) compared to the open tunnels (162.7 ± 62.6 larvae/kg) ($F=3.6$; $df=1,16$; $p=0.03$). Third instar larvae from fruit in the netted tunnels (9.0 ± 9.0 larvae/kg) was reduced compared to the open tunnels (22.6 ± 4.5 larvae/kg), but not significantly so ($F=0.8$; $df=1,16$; $p=0.18$). Furthermore, the netted treatments delayed the arrival of *D. suzukii* adults by twenty-three days (Figure 3). The average first catch in the open tunnels was on July 8 (± 12.5 days) and the netted tunnels on July 31 (± 18.7 days). Larval infestation was delayed by twenty-four days with the average first larval detection in the open tunnels on August 16 (± 17.6 days) and the netted tunnels on September 3 (± 14.1 days). However, none of the netted treatments maintained zero adult *D. suzukii* captures in the traps or zero larval infestation in the raspberries, and later in the season the infestation

built up inside the netted tunnels (Figure 4.3). Despite this, the overall level of infestation remained lower in the netted tunnels than in the open tunnels.

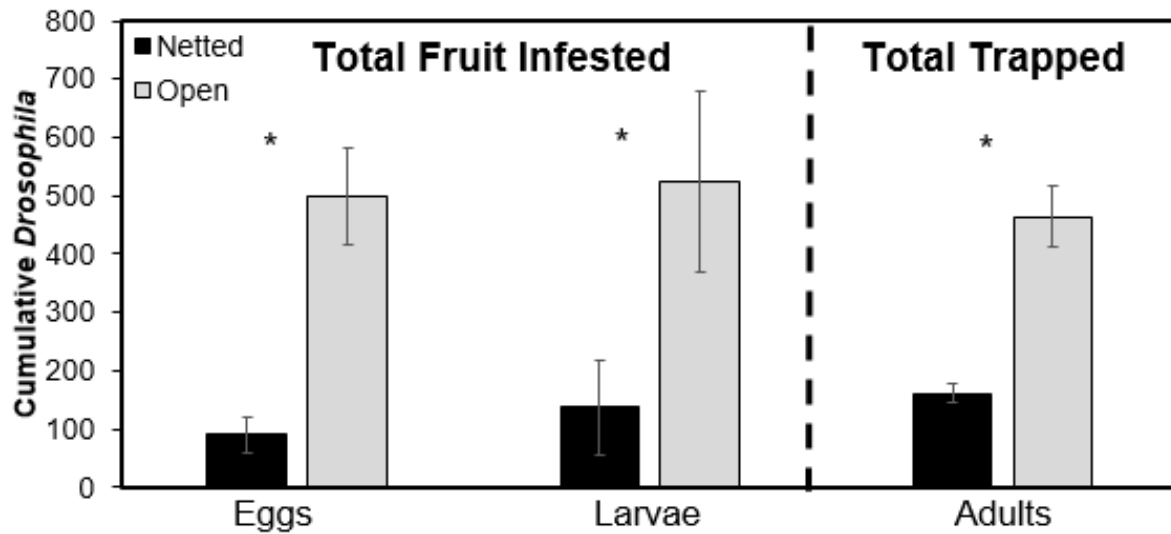


Figure 4.2. Cumulative number of *Drosophila* eggs and larvae per kilogram of raspberries ($\pm$ S.E.) and the cumulative number of *D. suzukii* adults caught each week per trap ($\pm$ S.E.) in open and netted high tunnels throughout the entire raspberry season. Bars marked with an asterisk denote life stages where treatments were significantly different at $\alpha=0.05$.

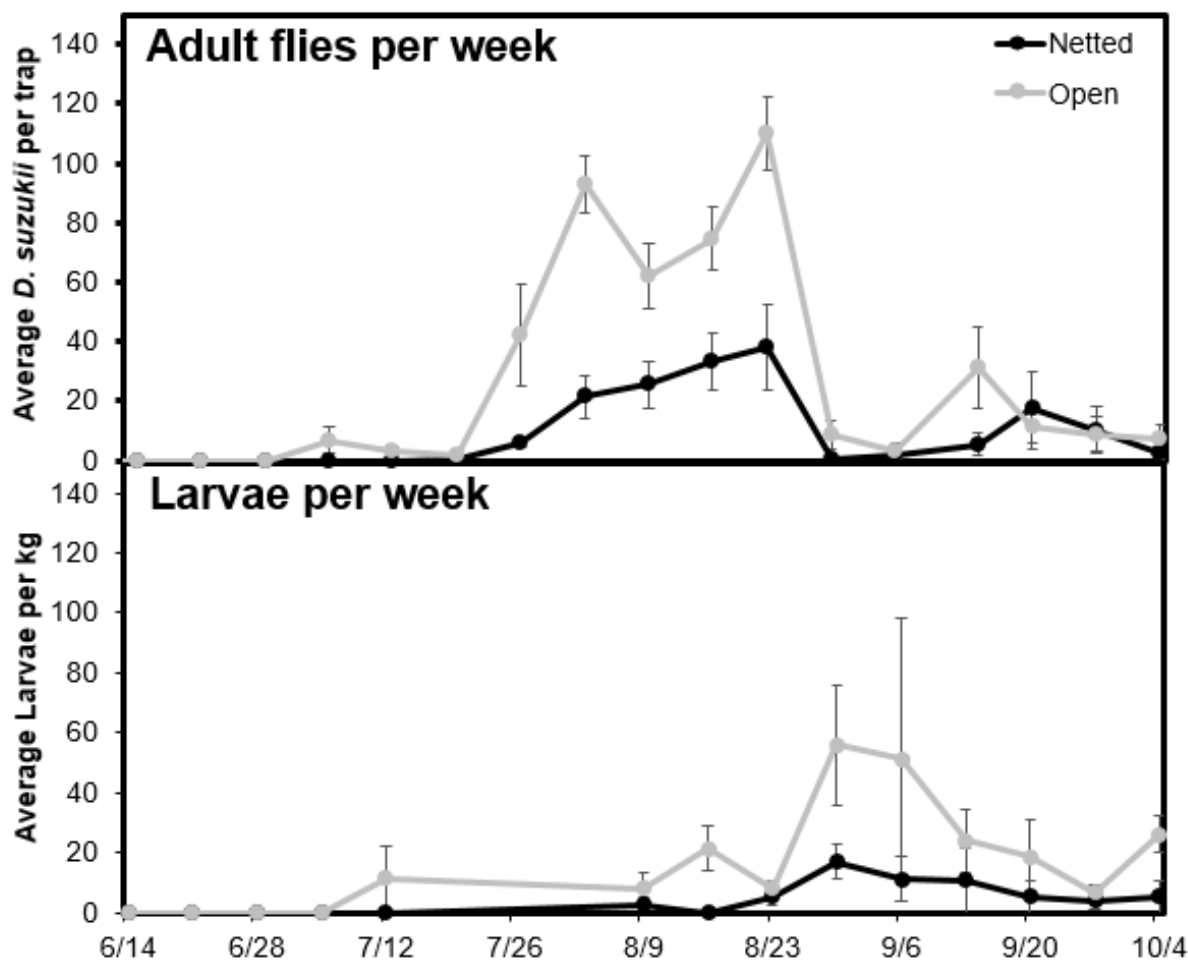


Figure 4.3. The number of *D. suzukii* adults caught per trap ($\pm$ S.E.) per week in open and netted tunnels (top) and the number of *Drosophila* larvae per kilogram of fruit ($\pm$ S.E.) per week in open and netted tunnels (bottom) throughout the raspberry growing season. Summer berry harvest started in mid-June and ran through mid-July, whereas fall raspberry harvest began in August and ended in late September.

Netting of the high tunnels affected other arthropods present in this system, though the composition of the arthropods remained similar between the two treatments. The most abundant pests and natural enemies from the combined sampling using yellow sticky traps and direct leaf

observations within the open and netted tunnels are shown in Figure 4.4. Examination of the yellow sticky traps revealed that the abundance of pests, natural enemies, and pollinators was reduced in the netted tunnels compared to open tunnels. Pest insects and mites at the commercial farm were reduced by 44% ($t = -7.58$, $p = 0.008$). Natural enemies were reduced at both sites by 48% ($t = -2.88$, $p = 0.02$). Pollinators, excluding the supplemented bumble bees, declined by 77% although this reduction wasn't statistically significant due to variability among sites ($t = -1.78$, $p = 0.07$). From the direct leaf observations, we observed similar reductions of pests (42% decline) and natural enemies (32% decline) but these were highly variable between sites and there was no significant difference between netted and open tunnels ($t = -0.47$, $p > 0.32$; $t = -0.30$, $p > 0.38$, respectively). We found no instances of insect abundance increasing in the netted tunnels, though some insects were more affected by the netting than others. Thrips, mites, and leafhoppers were reduced by greater than 50%, whereas aphids and raspberry beetles were reduced by fewer than 20% compared to populations found in the open tunnels, from both yellow sticky trap and direct leaf observation data. Spiders were the only natural enemy that appeared to be less affected by the netting with an average reduction of 21%. Average percent leaf damage throughout the entire season in the open tunnels ($2.3 \pm 1.3\%$) was slightly higher than the netted tunnels ($2.2 \pm 1.8\%$), but not significantly so ($t = -0.05$, $p = 0.47$).

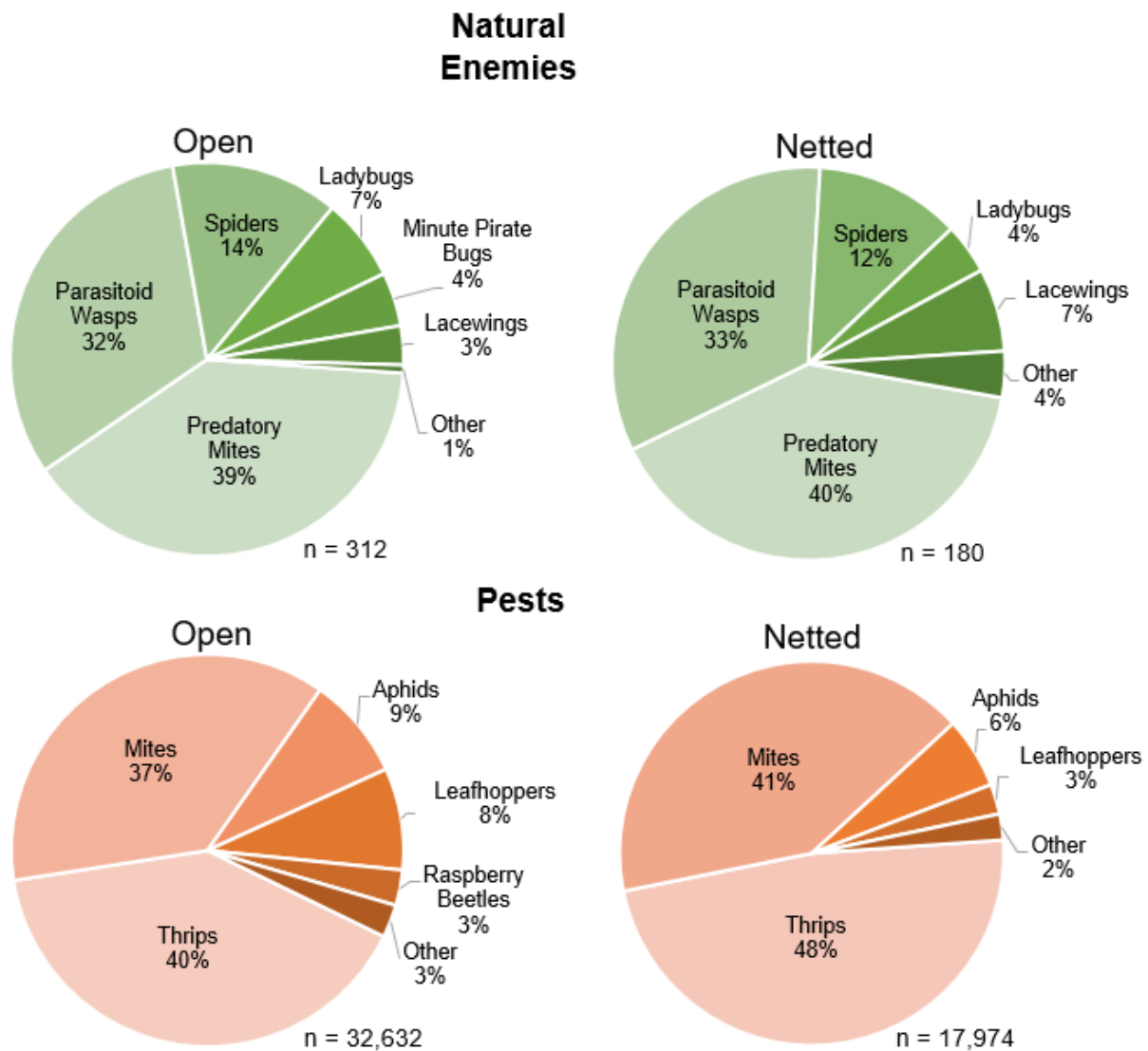


Figure 4.4. The composition of the most abundant natural enemies (top) and raspberry pests (bottom) caught on yellow sticky traps and observed on raspberry leaves throughout the season in the open (left) and netted (right) high tunnels. *N* represents the total number of insects observed throughout the season.

There was little effect on fruit quality characteristics of raspberries grown under the netted high tunnels. The average weight (Netted: $2.7 \pm 0.1\text{g}$; Open: $2.5 \pm 0.1\text{g}$), diameter (Netted: $16.1 \pm 0.2\text{mm}$; Open: $15.7 \pm 0.2\text{mm}$), and brix (Netted: $7.5 \pm 0.6 \text{ }^\circ\text{Bx}$; Open: $7.1 \pm 0.2 \text{ }^\circ\text{Bx}$), of the fruit collected from the netted tunnels was not significantly different from the fruit collected from the open tunnels ($F = 2.91$, $df = 1,4$, $p > 0.16$). The temperature in the netted tunnels was not significantly different from the open tunnels (Netted: $21.7 \pm 0.2 \text{ }^\circ\text{C}$, Open: $21.6 \pm 0.3 \text{ }^\circ\text{C}$) ($F = 0.04$; $df = 1,4$; $p = 0.83$). Likewise, the average maximum temperature was similar in the netted tunnels (Netted: $42.9 \pm 0.8 \text{ }^\circ\text{C}$, Open: $42.2 \pm 0.8 \text{ }^\circ\text{C}$) ($F = 0.39$; $df = 1,4$; $p = 0.56$).

DISCUSSION

Exclusion netting is a non-chemical approach to preventing insect infestation of crops, and in this study we found that netting can significantly reduce and delay *D. suzukii* infestation in red raspberries. This delay may be sufficient to eliminate the threat of *D. suzukii* from the summer crop of raspberries altogether and facilitate the production of insecticide free or organic-certified berries. Delayed fly activity could also prevent up to three weeks of insecticide sprays, lowering pesticide risks to pollinators, reducing the risk of insecticide resistance for *D. suzukii*, and saving growers both time and money. The delay of infestation that we observed was greater in the high tunnels than the smaller netted plots. Previous research has shown that high tunnels without exclusion netting can offer protection from pests compared field-grown raspberries (Demchak 2009, Hanson et al. 2013), so the combination of netting and high tunnels may provide even greater delay. The combined netting and insecticide treatments evaluated in this study also reduced all sizes of *D. suzukii* larvae detected in berries. This is particularly important for third

instar larvae, because these are the largest and are most visible to consumers and processors. Larvae that survive to that terminal instar have also usually caused collapse of fruit structure and leaking of juices that can also be apparent. Preventing *D. suzukii* from reaching third instar stages is important for growers and knowing that combinations of netting and insecticides will complement each other to achieve this provides insights into how best to ensure marketable fruit in regions with *D. suzukii*. These measures could be further complemented by increased raspberry harvest frequency, which has been found to reduce *D. suzukii* infestation in the fruit (Leach et al., unpublished data).

Importantly, exclusion netting did not have a negative impact on the quality of raspberries harvested from netted tunnels, and the temperature differences caused by exclusion netting were minimal. The trends for small increases in fruit weight, diameter, and sugar content among berries from the netted treatments could be due to a number of factors, such as increased vigor from loss of other pests or the slight increase in temperature. Cormier et al. (2015) observed similar trends in the weight of blueberries under netted field plots. While the fine mesh netting would block air flow, it also provides shading, which may be responsible for the similarity in temperature despite the enclosure. However, the presence of the netting has the potential to increase the ambient temperature, especially in the later parts of the growing season or in warmer production regions. Extreme temperatures in netted high tunnels is a concern that should be kept in mind for fruit production in regions with different climates. However, there are fan systems and venting options that can be used to minimize the risk of extreme temperatures in high tunnels. The reproductive rate of *D. suzukii* declines as temperatures surpass 28 °C (Tochen et al. 2014), so hotter conditions may also reduce the potential for this pest to cause fruit infestation. Indeed, a recent study using low tunnels found that raspberries grown under plastic covering had

much lower infestation rates than those covered with netting, presumably due to the hot microclimate created by the plastic that exceeded the thermal threshold for population growth by *D. suzukii* (Rogers et al. 2016). However, optimum flowering and growth in primocane raspberries occurs at 24-27°C, dependent on variety (Carew et al. 2003, Sønsteby and Heide 2009), so temperatures higher than this range may have negative implications for plant health and berry yield.

Exclusion netting and screening can have additional pest management benefits by acting as a barrier against other pests including insects and birds (Blua et al. 2005, Dellamano 2006, Qureshi et al. 2007, Simon 2008), thereby providing economic benefits in addition to the reductions of *D. suzukii* noted here. We found that raspberry aphids and raspberry beetles were relatively unaffected by the netting, perhaps because they were already established in the plantings, so these will still require active management in a netted tunnel setting. It is also possible that netting high tunnel plantings from the first year of growth could also prevent these pests from becoming established. Still, mobile insects and those with alternative hosts or different overwintering sites may be more affected by the netting than permanent residents that overwinter and complete their life cycle on or near the crop. We therefore recommend that monitoring for pests, including *D. suzukii*, should continue with the implementation of exclusion netting.

We have found that the exclusion netting is an effective way to delay the start of insecticide inputs for *D. suzukii* management, but this does not address grower concerns about netting, including the cost and potential for intensive labor for installation and maintenance (Link 2014). Installation of netting requires a structure for its support, and some producers have adapted a less expensive modified bird netting support system for excluding *D. suzukii* (Pullano 2015). If a structure is already in place, such as a high tunnel or bird netting support, exclusion netting can

be a less expensive addition to production costs. In the approach tested here, we calculated that netting the sides and ends of one acre of 122 meter long tunnels would cost about \$6,100. This estimate includes the cost of the netting plus its shipping, accessories to secure the netting, bumble bee colonies for supplemental pollination, and labor costs for installation of the netting. This cost could be amortized across the lifespan of the netting, which is projected to be seven years (Tek-Knit Industries). Supplemental pollinators are necessary for raspberries which produce in both the summer and fall. For other crops such as blueberries that bloom before *D. suzukii* is active, netting could be installed after pollination. Furthermore, we expect that labor costs would be reduced with practice in its installation. Further experience is needed with this approach to determine whether reduced insecticide applications or the potential increase in fruit quality and sale price would cover the cost of netting. Nevertheless, netting provides additional insurance for growers to have a marketable crop in years or regions where *D. suzukii* is a pest of concern.

Future research should focus on economic analyses of netting application and the possibility of insecticide-treated netting. While *D. suzukii* adults were not observed resting on the netting in this study, *D. suzukii* attraction is primarily driven by odor (Keesey et al. 2015, Revadi et al. 2015), making encounters with netting likely. Repellents are also being developed for *D. suzukii* (Pham and Ray 2015, Wallingford et al. 2015, Renkema et al. 2016) and their application in combination with netting could be explored.

This pest has rapidly disrupted established IPM programs in small fruit and berry production around the world. To rebuild these programs, multiple non-chemical management approaches must be explored including the search for classical biological control agents (Guerrieri et al. 2016, Daane et al. 2016), pest monitoring (Burrack et al. 2015), removal of non-crop hosts

(Klick et al. 2015, Lee et al. 2015; Briem et al. 2016, Pelton et al. 2016), and use of exclusion netting. This will provide growers across a range of production systems with diverse options to manage *D. suzukii*, and it can also serve as an example for invasive pest challenges that we may face in the future.

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CHAPTER 5. CONCLUSIONS

Drosophila suzukii is a critical pest of raspberry and other soft fruits and current management is heavily dependent on the use of frequent broad-spectrum insecticide applications (Asplen et al. 2015). This creates a demand for new management tools so that dependence on chemical control can be lessened. As the acreage of high tunnel berry production increases (Hanson et al. 2011), this presents an opportunity to utilize innovative new strategies for pest control, including for *D. suzukii*. In the above work, we have determined new management techniques to improve our control and rebuild integrated pest management schemes in high-tunnel grown systems.

From this research, there are seven key arthropod pests in high tunnels: *D. suzukii*, aphids, thrips, leafhoppers, spider mites, raspberry beetles, and plant bugs. There are also six common natural enemies in these systems: lady beetles, lacewings, parasitoids wasps, syrphids, spiders, and predatory mites. The type of plastic covering these structures in terms of UV transmittance does not strongly effect *D. suzukii* or other insect populations except for some pollinators. This research suggests that bee activity may be reduced in these reduced UV environments, particularly for honey bees and wild bees. Additional studies should focus on the effect of pollination and bee behavior under UV blocking plastics, especially with crops that are highly dependent on pollination and some crops that utilize host-specific pollinators. However, this could be dependent on other factors and more research is needed to fully elucidate the effect of plastics on pollinators.

While UV transmittance under these tunnel environments did not strongly effect arthropod communities, we did see a strong effect on pesticide degradation. UV blocking materials were able to reduce and delay the degradation of nearly all insecticides evaluated in our

study, including the organic insecticide tested. This work is also supported by other studies using plastics with modified spectral properties in different crops (Garau et al. 2002; Cengiz et al. 2007; Chuanjiang et al. 2010; Allen et al. 2015). To our knowledge, this is the first time that these plastics have been investigated for their ability to modify insecticide degradation on raspberries, and this information can be used to support an integrated pest management system under these tunnels. This indicates that while plastic choice may not be important for growers in terms of direct effect on insect behavior, increased residues of insecticides may increase our pesticide-use efficiency and thereby allow us to increase possible tools for management of raspberry pests. Increased active ingredient could lead to increased sustainability, with fewer needed applications and longer intervals in between applications. These results have implications beyond raspberry high tunnel growers and can be useful information for guiding all high tunnel producers on spray programs and schedules. The effect of UV blocking plastics on fungicides, miticides, and other pesticides should be evaluated for their ability to control pests and diseases that were not studied here. Understanding how these increased residues translate to efficacy on *D. suzukii* and other raspberry pests would also be valuable information.

Utilizing exclusion netting is a significant way to reduce *D. suzukii* infestation under high tunnels, another method for management that decreases our reliance on chemical control. While netting is an effective control measures for reduced-spray or organic operations, we also found that insecticides can complement netting for better control of *D. suzukii*. Netting also requires an existing structure, annual labor, and high upfront costs, which may not be feasible for some growers. Additional research should focus on the economics of netting and developing new strategies for the deployment of netting. Moreover, the use of insecticide or repellent impregnated netting may further deter and reduce *D. suzukii* populations and other important

raspberry pests, and this should be evaluated. While some other studies have focused on netting (Link 2015; Cormier 2015; Rogers et al. 2016), the use of netting in warmer climates is not well-understood and should be evaluated.

The combination of reduced UV light conditions, exclusion netting, and insecticide applications may provide the best sustainable control for *D. suzukii* in high tunnel raspberry. Other cultural control techniques, including harvest frequency, sanitation, mulching, and pruning have been evaluated in raspberries and other fruit crops (Lee et al. 2015; Tochen et al. 2014; Leach et al. 2017) and should also be incorporated into these high tunnel systems. Utilizing these strategies can help us bridge to other integrated pest management tactics, namely biological control (Daane et al. 2016). *Drosophila suzukii* is an extremely challenging pest, particularly for raspberries (Lee et al. 2011b) and will require multiple management tactics in order to achieve sustainable control. The control measures developed in this thesis will help to bring growers closer to integrated pest management in high tunnel raspberries.

APPENDIX

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

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

Voucher Number: 2017-08

Author and Title of thesis: Heather Leach. “Integrated pest management of spotted wing *Drosophila* (*Drosophila suzukii*) in Michigan high tunnel raspberries”.

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

Specimens:

Table A1. List of voucher specimens

Family	Genus-Species	Life Stage	Sex	Quantity	Preservation
Diptera	<i>Drosophila suzukii</i>	Adult	Male	5	Pinned
Diptera	<i>Drosophila suzukii</i>	Adult	Female	5	Pinned
Diptera	<i>Drosophila suzukii</i>	Adult	Male	5	75% ETOH
Diptera	<i>Drosophila suzukii</i>	Adult	Female	5	75% ETOH

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