

ABSTRACT

SWOBODA BHATTARAI, KATHARINE ANNE. Determining Factors that Affect Host Use by the Invasive Vinegar Fly *Drosophila suzukii* (Diptera: Drosophilidae). (Under the direction of Dr. Hannah J. Burrack).

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is an invasive species that has devastated integrated pest management programs in North America and Europe in recent years. Females use their saw-like ovipositor to lay eggs in ripe and ripening fruits and threaten the viability of berry and cherry production in many areas.

Non-crop habitat may serve as a source of infesting populations and provide *D. suzukii* with refuge from adverse conditions. To determine if non-crop habitat positively affects adult abundance in crop fields, we tested the hypothesis that adult trap captures would decrease as distance from non-crop habitat increased. We deployed monitoring traps with a fermentation-based bait weekly at equidistant points along transects running from 1) a standing water source and 2) a wooded edge into crop fields at two commercial blackberry farms in western North Carolina. Overall, more flies were caught in traps placed outside of crop fields than within crop fields. The hypothesis that *D. suzukii* trap captures would decrease as distance from non-crop habitat increased was only supported when fly populations were high towards the end of the season. We also hypothesized that *D. suzukii* and other sympatric drosophilids may exhibit patterns associated with 1) diurnal movement into crop fields, 2) diurnal attraction to monitoring traps with fermentation-based baits, and 3) seasonal changes to these patterns. Flight interception traps and monitoring traps were deployed biweekly between crop fields and wooded edges at two blackberry farms; traps were checked hourly for 24 hours, except during darkness. Both *D. suzukii* and other drosophilids moved between crop fields and wooded edges and were attracted to monitoring

traps primarily during the morning and evening hours. Mating behavior was also observed on the exterior of monitoring traps, suggesting that *D. suzukii* may visit such traps to interact with conspecifics.

Because laboratory studies may underestimate the susceptibility of unripe fruits to infestation when the natural ripening process is interrupted in prematurely-harvested fruit, we hypothesized that eggs laid in unripe fruit in the field would be more likely to complete their development as the fruit ripens naturally on the plant. *D. suzukii* infestation rates in ripe blackberries and unripe blackberries that were bagged and allowed to ripen naturally were compared in two contrasting field settings: a small, unmanaged research planting and a large, managed commercial blackberry field with high and low numbers of *D. suzukii* adults, respectively. *D. suzukii* were able to infest and survive in unripe blackberries at all stages tested, but had the most success in berries at later stages of ripening. *D. suzukii* emerged from unripe berries bagged at all stages in the research planting, whereas very few emerged from unripe berries bagged at any stage in the commercial field. This discrepancy, coupled with results from a study in which infestation rates in ripe and unripe blackberries were measured weekly as fly numbers increased over the course of a growing season, suggested that unripe berries were more likely to be infested when *D. suzukii* population densities were high.

Finally, we hypothesized that *D. suzukii* females at different developmental stages would be differentially attracted to monitoring traps with fermentation-based baits and ripe fruits. Females were collected on the surface of traps, within traps, and/or on ripe fruits at three field locations in North Carolina. Females collected on ripe fruits were more likely to have mature eggs present in their ovaries and had higher numbers of mature eggs than females collected on the surface of or within monitoring traps.

The results of this research can be used to inform monitoring recommendations for *D. suzukii* detection programs, integrated pest management decisions, and non-crop habitat management strategies.

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Determining Factors that Affect Host Use by the Invasive Vinegar Fly
Drosophila suzukii (Diptera: Drosophilidae)

by
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DEDICATION

I dedicate this dissertation to my husband Kishor and our daughter Kiran with all my heart.

BIOGRAPHY

Katharine Anne Swoboda Bhattarai was born and raised in Lincoln, Nebraska. She earned a Bachelor's degree at the University of Nebraska-Lincoln before heading west to pursue a Master's degree at Utah State University studying native bees as managed crop pollinators. She joined specialty crop integrated pest management laboratory led by Dr. Hannah Burrack at NC State in 2012. Ultimately, Katharine would like to combine her experience working with managed pollinators and integrated pest management to help growers both produce and protect their crops.

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Finally, I would not be where I am today without the love and support of my family and friends. Thank you all from the bottom of my heart.

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CHAPTER 1: Effects of Non-Crop Habitat on *Drosophila suzukii*

Abundance in Crop Fields

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Abstract

Drosophila suzukii (Matsumura), the spotted wing drosophila, is a highly invasive vinegar fly that was first detected in the continental United States in 2008. Females use their saw-like ovipositor to lay eggs in soft-skinned fruits and threaten the viability of raspberry, blackberry, blueberry, cherry, and strawberry production. Understanding the ecology and population dynamics of *D. suzukii* is essential for the development of effective management programs. Non-crop habitat may provide *D. suzukii* with a refuge from pesticide treatments and serve as a source of infesting populations, and the objective of this research was to determine if proximity to different types of non-crop habitat affected adult abundance in crop fields. The hypothesis that abundance, as measured by captures of adult flies in monitoring traps, will decrease as distance from non-crop habitat increases was tested during 2013 and 2014 at two commercial blackberry farms in western North Carolina. At each farm, *D. suzukii* were trapped weekly using a fermentation-based bait along transects that ran perpendicular from 1) a water source into a crop field and 2) a wooded edge into a crop field. Overall, more flies were caught in traps placed outside of crop fields than within crop fields. The hypothesis that the number of adult *D. suzukii* caught in traps would decrease as distance from non-crop habitat increased was only supported when fly populations were high towards the end of the season. Results of this study can be used to inform monitoring recommendations for *D. suzukii* detection programs, integrated pest management decisions, and non-crop habitat management strategies.

Introduction

Invasions of agriculture pest insects are increasingly common and of great environmental and economic significance. It has been estimated that the approximately 50,000 foreign species of plants, animals, arthropods, mollusks, and microbes that have been introduced into the United States cause environmental damage and losses totaling US \$120 billion per year (Pimentel *et al.*, 2005). The establishment of invasive crop pests disrupts established management programs because pesticides are generally the first line of defense against economic loss. It is important to understand patterns of host use and local movement by invasive species because these directly impact growth potential and ultimately determine the success of a biological invasion.

Drosophila suzukii, the spotted wing drosophila, is an invasive vinegar fly that has caused extensive economic damage to small and stone fruit crops throughout its introduced range. It is native to parts of Southeast Asia and was first described as a pest of cherries in Japan in the 1930s (Kanzawa 1935, 1939). It was detected in Hawaii in the early 1980s (Kaneshiro 1983), but was not found in the continental United States until 2008 when it was identified from samples collected in a commercial raspberry field in California (Hauser 2011). It has since spread rapidly throughout fruit growing regions in the United States, Mexico, Canada, Europe, and South America (Asplen *et al.* 2014, Cini *et al.* 2014, Depra *et al.* 2014). *D. suzukii* is highly mobile (Mitsui *et al.* 2010) and widely polyphagous (Lee *et al.* 2015, Poyet *et al.* 2015). Females use their saw-like ovipositor to lay eggs in soft-skinned fruits and attack a wide variety of crop hosts including blackberries, raspberries, blueberries, cherries, and strawberries (Lee *et al.* 2011, Burrack *et al.* 2013). It has been estimated that *D.*

suzukii, assuming a 30% damage level, may cause more than US \$500 million in annual losses in western US production areas alone (Bolda et al. 2010, Goodhue et al. 2011). Unfortunately, monitoring programs that reliably predict *D. suzukii* infestation risk are not currently available (Wiman et al. 2014, Asplen et al. 2015).

The landscape surrounding crop fields influences pest populations. Pest insect densities may be higher on field edges, but nearby non-crop habitat may also enhance conservation biological control (Bianchi et al. 2006, Rand et al. 2006, Sivakoff et al. 2013, Thomson and Hoffmann 2013). Similarly, it has been suggested that non-crop habitat may serve as either a source or sink for *D. suzukii* populations in agricultural fields. Flies have been shown to use both wild hosts and crops at field edges (Iglesias et al. 2014, Klick et al. 2015), and non-crop habitat can be a source of infesting populations when crop and non-crop hosts are used concurrently or in sequence (Diepenbrock et al. 2016). Non-crop habitat may also provide *D. suzukii* with refuge from management efforts such as insecticide applications (Van Timmeren and Isaacs 2013) or other adverse conditions within crop fields such as elevated temperatures or low relative humidity (Kimura 2004, Tochen et al. 2014, 2015). Recent research explored the contribution of non-crop habitat to in-field populations of *D. suzukii*, but was conducted at a broad landscape scale (Pelton et al. 2016). Because fine-scale spatial analysis is required to determine if the costs or benefits associated with non-crop habitat extend into crop fields (Thomson and Hoffmann 2013), our objective was to take a close look at the interface between non-crop habitat and crop fields and determine if the presence of these non-crop habitats increased *D. suzukii* abundance in nearby fields.

Cultivated blackberries (*Rubus fruticosus* L. and other *Rubus* spp.) are highly-preferred *D. suzukii* hosts (Lee et al. 2011, Bellamy et al. 2013), and the blackberry industry in the United States has been severely impacted by its presence (Diepenbrock et al. 2017). We selected two types of non-crop habitat that are common on many blackberry farms in the southeastern United States, surface water sources, such as irrigation ponds, and wooded edges. We conducted a two-part analysis. In Part I, we focused on adult fly populations as measured by trap captures along transects running from non-crop habitat in to crop fields over the course of the growing season. We simultaneously assessed infestation within pooled berry samples and the likelihood that a single berry would be infested at the same locations, which will be presented in a separate analysis. We hypothesized that the number of adult *D. suzukii* caught in traps would decrease as distance from non-crop habitat increased and that flies would be more likely to be caught in traps located closer to non-crop habitat. We also hypothesized that trap captures might be influenced by the type of non-crop habitat present and abiotic conditions within crop fields and the larger agroecosystem.

It is important to understand how, over space and time, *D. suzukii* populations are distributed within crop fields and the surrounding landscape. This knowledge can inform monitoring recommendations for detection programs, integrated pest management decisions, and non-crop habitat management strategies.

Materials and Methods

Experimental design

We conducted experiments at three commercial blackberry farms in Cleveland and Lincoln Counties in western North Carolina during 2013 and 2014. Standard conventional pest management practices, including insecticide applications, were employed at all locations (SRSFC 2017). Experiments were replicated at two farms each year. We deployed monitoring traps and collected fruit samples along transects that ran into crop fields planted with ‘Ouachita’ and ‘Navaho’ blackberry cultivars. Transects were perpendicular to non-crop habitat consisting of either surface water sources or wooded edges that were selected based on their proximity to a crop field. Water sources consisted of streams (Site 2 in 2013 only; Table 1.1) or irrigation ponds that ran alongside or were located within 30 m of a crop field. Wooded edges ran along the length of a crop field and were part of larger wooded areas that had been partially removed and replaced with crop fields. Wild brambles, black cherry (*Prunus serotina*), and American pokeweed (*Phytolacca americana*) plants were present along the wooded edges at the farms.

Experimental layouts varied somewhat between non-crop habitat types and years (Table 1.1). During 2013, three transects spaced at least 20 m apart were replicated per wooded edge experiment, with traps and fruit samples collected at 20 m intervals along each transect. Fields near water sources were smaller, and we sampled at 10 m intervals along three replicated water source transects spaced greater than 20 m apart at each farm. We altered the experimental design slightly in 2014 and sampled at 30 m intervals along wooded edge transects and 15 m intervals along water source transects, both of which were spaced 30

m apart. We also added transects to some fields at both farms in 2014 to increase replication where space allowed. At Site 3 during 2014, we established three water source transects and two separate wooded edge experiments, each with four or five transects, in fields that were located over 500 m apart and separated by woods.

Transects were established and adult trapping began on 5-6 June in 2013 and on 27-28 May 2014 and ended in late July or August in both years, when fruit were no longer present on plants (Table 1.1).

Adult fly monitoring

We deployed *D. suzukii* monitoring traps at regular intervals along each transect (Table 1.1). Traps consisted of 946 ml clear plastic cups and lids with ten 5 mm holes drilled near the top (DeliPRO brand, Tri-pack Industrial USA, White Plains, NY) and were baited with 1.69 g of dry active yeast, 8.45 g of sugar, and 150 ml of water (Burrack et al. 2015). In both years, we collected and replaced the trap bait weekly. Trap contents were filtered in the laboratory using fine-mesh sieves, and female *D. suzukii*, male *D. suzukii*, and non-*D. suzukii* drosophilids caught in each trap weekly were enumerated.

Data analysis

We conducted a four-part analysis to determine if there were patterns associated with female and total *D. suzukii* and total non-*D. suzukii* trap captures along water source and wooded edge transects during the two years of study. Analyses addressed the following questions: 1) Did trap capture patterns within habitat types differ between years; 2) Did trap capture

patterns differ between habitat types within individual years; 3) Did finer scale trap capture patterns differ within a given year; and 4) Where along transects and when during the season were trap captures more likely to occur?

For questions 1 and 2, only data from distances and weeks of the year in common between data sets were analyzed. To address question 3, weekly trap captures at all distances observed within an experiment were analyzed. Finally, to address question 4, weekly trap captures were converted to binomial (presence/absence) data as follows: individual traps were scored as “1” if at least one female *D. suzukii*, one male *D. suzukii*, or one male or female *D. suzukii* (for total *D. suzukii*) was present and scored as “0” if no *D. suzukii* were present. Individual distances or weeks of the year were removed from an analysis if no flies were present (e.g., no male *D. suzukii* were caught in traps located 160 m from a wooded edge in 2014).

Analyses were conducted using generalized linear mixed models in PROC GLIMMIX (SAS v. 9.4). The data set, response variable, fixed and random effects, response distribution and link function, and post hoc analyses for each question are presented in Table 1.2. When significant interactions occurred between fixed effects in a model, we used the SLICE and SLICEDIFF options in the lsmeans statement of PROC GLIMMIX to obtain the overall simple effect *F*-tests and pair-wise simple effect differences (Stroup 2013) and applied the Tukey-Kramer adjustment for multiple comparisons using the ADJUST=Tukey option.

Results

Question 1. Did trap capture patterns for female D. sukukii, total D. sukukii, and other drosophilids within habitat types differ between years?

Distance from water sources in 2013 and 2014. First, we ran a 3-way analysis to determine if patterns of female *D. sukukii* trap captures at 0, 30, and 60 m from a water source during weeks 25-34 of the year were different in 2013 and 2014, and they were not (distance*year*week of year: $F_{18,242} = 1.45$, $P = 0.11$). Next, we compared the two-way effects of distance and week between years. Numbers of female *D. sukukii* captured in traps differed significantly between weeks 25 and 29-32 of the year in 2013 and 2014, with peak trap captures occurring one week earlier in 2013 (week 31: 31 July) than in 2014 (week 32: 6 August) (year*week of year: $F_{9,242} = 8.30$, $P < 0.0001$) (Figure 1.1A). When trap captures were high toward the end of the growing season, numbers of female *D. sukukii* captured in traps located 0, 30, and 60 m from a water source differed. During weeks 30-32 of the year, more females were caught in traps located 0 m from a water source than in traps located 30 or 60 m from a water source. More females were also captured 0 m from a water source than at 30 during week 33, while more females were captured at 0 m than at 60 m during week 34 (distance*week of year: $F_{18,242} = 2.96$, $P < 0.0001$) (Figure 1.1D). The overall effect of distance from a water source on female *D. sukukii* trap captures was the same in 2013 and 2014 (distance*year: $F_{2,242} = 0.88$, $P = 0.42$).

Trap capture results for total *D. sukukii* were very similar to those of female *D. sukukii*. Patterns of total *D. sukukii* trap captures at 0, 30, and 60 m from a water source during weeks 25-34 of the year were not different in 2013 and 2014 (distance*year*week of

year: $F_{18,242} = 1.12$, $P = 0.33$). Numbers of total *D. suzukii* captured in traps differed significantly between weeks 25-27 and 29-32 of the year, with peak trap captures again occurring one week earlier in 2013 (week 31: 31 July) than in 2014 (week 32: 6 August) (year*week of year: $F_{9,242} = 8.26$, $P < 0.0001$) (Figure 1.1B). More total *D. suzukii* were caught in traps located 0 m from a water source than in traps located 30 or 60 m from a water source during weeks 30-32 and 34 of the year, while more flies were captured 0 m from a water source than at 30 m in week 33 (distance*week of year: $F_{18,242} = 2.72$, $P = 0.0003$) (Figure 1.1E). Distance effects were not different between the two years (distance*year: $F_{2,242} = 0.68$, $P = 0.51$).

Trap capture patterns of total non-*D. suzukii* drosophilids at 0, 30, and 60 m from a water source in 2013 and 2014 were different than those of *D. suzukii*. As was true for *D. suzukii*, the overall trap capture patterns of other drosophilids did not differ between years (distance*year*week of year: $F_{18,242} = 0.53$, $P = 0.94$). Although more non-*D. suzukii* drosophilids were captured in traps in 2013 than in 2014 during weeks 27, 30, 31, and 34 of the year (year*week of year: $F_{9,242} = 4.80$, $P < 0.0001$) (Figure 1.1C), the number of other drosophilids caught in traps located 0, 30, and 60 m from a water source did not differ among weeks of the year as they did for *D. suzukii* (distance*week of year: $F_{18,242} = 0.84$, $P = 0.65$) (Figure 1.1F). Instead, more other drosophilids were caught 0 m from a water source than at 30 or 60 m from a water source in 2013, whereas more flies were captured in traps located 0 and 30 m from a water source in 2014 than in traps placed 60 m away in 2014 (distance*year: $F_{2,242} = 4.54$, $P = 0.0116$).

Distance from wooded edges in 2013 and 2014. We ran a 3-way analysis to determine if patterns of female *D. suzukii* trap captures at 0, 60, and 120 m from a wooded edge during weeks 25-34 of the year were different in 2013 and 2014, and they were (year*week of year*distance: $F_{18,440} = 2.20$, $P = 0.0032$). During weeks 29-32 and 34 of the year in 2013, more female *D. suzukii* were captured in traps located 0 m from a wooded edge than in traps located 60 or 120 m from a wooded edge (Figure 1.2A). The same trap capture pattern was only observed during week 32 of the year in 2014; otherwise, more female *D. suzukii* were captured at 0 m than at 60 m, but not 120 m, in weeks 28 and 33 of the year in 2014 (Figure 1.2B).

As was true for female *D. suzukii*, patterns of total *D. suzukii* trap captures at 0, 60, and 120 m from a wooded edge during weeks 25-34 of the year were different in 2013 and 2014 (year*week of year*distance: $F_{18,440} = 2.27$, $P = 0.0022$). During weeks 29-32 and 34 of the year in 2013, more total *D. suzukii* were captured in traps located 0 m from a wooded edge than in traps located 60 or 120 m from a wooded edge (Figure 1.2C). The same trap capture pattern was observed during weeks 32-34 of the year in 2014, whereas this pattern was only observed for female *D. suzukii* during week 32 of the year. Otherwise, more total *D. suzukii* were captured at 0 m than at 60 m in weeks 28 and 31 of the year in 2014 (Figure 1.2D).

Patterns of total non-*D. suzukii* trap captures at 0, 60, 120 m from a wooded edge during weeks 25-34 of the year were not different in 2013 and 2014 (year*week of year*distance: $F_{18,440} = 0.66$, $P = 0.85$). Numbers of total non-*D. suzukii* captured in traps differed significantly among weeks of the year and more flies were caught in 2013 than 2014

during weeks 30, 31, and 34 (year*week of year: $F_{9,440} = 4.52$, $P < 0.0001$) (Figure 1.2E). Numbers of total non-*D. suzukii* caught in traps located 0, 60, and 120 m from a wooded edge did not differ among weeks of the year (distance*week of year: $F_{18,440} = 1.24$, $P = 0.22$) (Figure 1.2F), but did differ between years (year*distance: $F_{2,440} = 11.77$, $P < 0.0001$). In both years, more flies were captured at 0 m from a wooded edge than at 60 and 120 m from a wooded edge.

Question 2. Did habitat type affect trap capture patterns for female D. suzukii, total D. suzukii, and other drosophilids within individual years?

Distance from non-crop habitat in 2013. First, we ran a 3-way analysis to determine if patterns of female *D. suzukii* trap captures at 0, 20, 40, and 60 m from a water source or wooded edge were different during weeks 25- 34 of 2013, and they were not (distance*habitat*week of year: $F_{27,257} = 0.95$, $P = 0.54$). Overall, type of habitat did not affect female *D. suzukii* trap capture patterns in 2013 (distance*habitat: $F_{3,257} = 0.89$, $P = 0.44$; habitat*week of year: $F_{9,257} = 1.59$, $P = 0.12$; habitat: $F_{1,257} = 2.6$, $P = 0.11$). The numbers of female *D. suzukii* captured in traps located 0, 20, 40, and 60 m from either non-crop habitat did differ between weeks of the year (distance*week of year: $F_{27,257} = 8.75$, $P < 0.0001$). During week 25, more females were caught at 20 and 40 m from non-crop habitat than at 0 m. During week 29, more flies were caught at 0 m than at 20, 40, and 60 m from non-crop habitat, while more flies were caught at 20 m than at 40 m. During weeks 30 and 31, more flies were caught at 0 m than at 20, 40, and 60 m from non-crop habitat, while more

flies were caught at 20 m than at 40 and 60 m. Finally, during weeks 32 and 34, more flies were caught at 0 m than at 20, 40, and 60 m from non-crop habitat (Figure 1.3A).

Results for total *D. sukuzii* trap captures at 0, 20, 40, and 60 m from non-crop habitat were very similar to those of female *D. sukuzii* in 2013. Overall, type of habitat did not affect total *D. sukuzii* trap capture patterns in 2013 (distance*habitat*week of year: $F_{27,257} = 0.67$, $P = 0.89$; distance*habitat: $F_{3,257} = 1.06$, $P = 0.37$; habitat*week of year: $F_{9,257} = 1.23$, $P = 0.28$; habitat: $F_{1,257} = 3.72$, $P = 0.06$). Again, total *D. sukuzii* trap captures at 0, 20, 40, and 60 m from either non-crop habitat differed between weeks of the year (distance*week of year: $F_{27,257} = 8.01$, $P < 0.0001$) and significant differences between trap captures at 0, 20, 40, and 60 m from non-crop habitat were the same as for female *D. sukuzii* during weeks 25, 29-32, and 34 of the year (Figure 1.3B).

Type of habitat also did not have a significant effect on trap captures of other drosophilids species at 0, 20, 40, and 60 m during weeks 25-34 of the year in 2013 (Figure 1.3C). Overall, more flies were captured 0 m from non-crop habitat in 2013 than at 20, 40, and 60 m, while more flies were captured at 20 m than at 40m (distance: $F_{3,257} = 34.39$, $P < 0.0001$). Trap captures also varied between weeks of the year; the highest numbers of flies were captured during weeks 26, 27, 31, and 34 and the lowest numbers of flies during weeks 28, 29, 32, and 33 of the year (week of year: $F_{9,257} = 20.65$, $P < 0.0001$).

Distance from non-crop habitat in 2014. As was true in 2013, patterns of female *D. sukuzii* trap captures between 0 and 60 m from a water source or wooded edge were not different during weeks 25-34 of the year in 2014 (distance*habitat*week of year: $F_{18,511} = 0.37$, $P =$

0.99). Overall, type of habitat did not affect female *D. sukukii* trap capture patterns (distance*habitat: $F_{2,511} = 1.75$, $P = 0.17$; habitat*week of year: $F_{9,511} = 1.87$, $P = 0.05$; habitat: $F_{1,511} = 3.08$, $P = 0.08$) (Figure 1.3D). As was also true in 2013, female *D. sukukii* trap captures at distances within 60 m from non-crop habitat differed between weeks of the year in 2014 (distance*week of year: $F_{18,511} = 3.79$, $P < 0.0001$). During weeks 30 and 33, more females were captured at 0 m than at 30 and 60 m from non-crop habitat, while more flies were captured at 0 m than at 60 m during week 31. During week 32, when the most female *D. sukukii* were captured overall, more flies were captured at 0 m than at 30 and 60 m and at 30 m than at 60 m. Finally, during week 34, more females were captured at 0 and 30 m than at 60 m from non-crop habitat (Figure 1.3G).

However, the numbers of total *D. sukukii* captured in traps placed near water sources traps and traps near wooded edges differed between weeks of the year in 2014 (habitat*week of the year: $F_{9,511} = 1.99$, $P = 0.0388$). More flies were generally captured along water source transects than wooded edge transects during the second half of the season in 2014, but more flies were captured along wooded edge transects during week 33 (13 August) (Figure 1.3E). Total *D. sukukii* trap captures at distances within 60 m from non-crop habitat differed between weeks of the year in 2014 (distance*week of year: $F_{18,511} = 4.64$, $P < 0.0001$). During weeks 30 and 33 of the year, more total *D. sukukii* were captured at 0 m than at 30 and 60 m from non-crop habitat. More flies were also captured at 0 m than at 60 m during week 31, while more flies were captured at 0 m than at 30 and 60 m and at 30 m than at 60 m during week 32, when the most flies were captured overall. Finally, during week 34, more flies were captured at 0 and 30 m than at 60 m from non-crop habitat (Figure 1.3H).

The numbers of other drosophilids captured in water source traps and in wooded edge traps differed between weeks of the year in 2014 (habitat*week of the year: $F_{9,511} = 2.18$, $P = 0.0224$). More flies were captured in wooded edge traps during weeks 27 and 28 of the year, while more flies were captured in water source traps during weeks 33 and 34 (Figure 1.3F). Overall, more flies were captured in traps placed 0 m from non-crop habitat than at 30 or 60 m, while more flies were captured at 30 m than at 60 m (distance: $F_{2,511} = 20.77$, $P < 0.0001$) (Figure 1.3I).

Question 3. Did trap capture patterns for female D. sukukii, total D. sukukii, and other drosophilids differ at a finer scale along transects within habitat types each year?

Distance from water sources in 2013. More female *D. sukukii* were caught in traps placed 0-20 m than in traps placed 50 m from a water source during week 29 of the year in 2013, while more females were also caught in traps at 10 m than at 40 m. During week 30, more females were caught at 0 m and 10 m than at 20-60 m and at 20 m than at 40-60 m. During week 31, when trap captures peaked, more females were caught at 0 m than at 40-70 m, at 10 m than at 30-70 m, and at 20-30 m than at 70 m. Trap captures increased again during week 34, and more females were caught at 0 m than at 30-70 m, at 10 m than at 40-50 m, and at 20 m than at 50 m (distance*week of the year: $F_{63,235} = 3.24$, $P < 0.0001$) (Figure 1.4A).

Trap capture patterns for total *D. sukukii* were very similar to those of female *D. sukukii*. During week 29 of the year, more total *D. sukukii* were caught in traps placed 0-20 m than in traps placed 50 m from a water source. During week 30, more flies were caught at 0 m and 10 m than at 20-60 m and at 20 m than at 40-60 m. During week 31, when trap

captures peaked during the season, more flies were caught at 0 m and 10 m than at 30-70 m and at 20-30 m than at 70 m. Trap captures increased again during week 34, and more flies were caught at 0 and 10 m than at 30-50 m and 70 m (distance*week of the year: $F_{63,235} = 2.88$, $P < 0.0001$) (Figure 1.4B).

For other drosophilids, there was not a significant interaction between distance from a water source and week of the year in 2013 (distance*week of the year: $F_{63,235} = 0.84$, $P = 0.78$) (Figure 1.4C). Overall, more flies were captured at 0 m from a water source than at 20-70 m, at 10 m than at 30-50 and 70 m, at 20 and 30 m than at 50 and 70 m, and at 60 m than at 70 m (distance: $F_{7,235} = 17.45$, $P < 0.0001$). More flies were captured during weeks 26, 27, and 34 than the others, while the fewest flies were captured during week 28 of the year (week of the year: $F_{9,235} = 20.50$, $P < 0.0001$).

Distance from wooded edges in 2013. During weeks 29 and 32 of the year, more female *D. suzukii* were caught in traps placed along a wooded edge (0 m) than in traps placed at 20-160 m from the wooded edge. The same pattern occurred during weeks 30 and 31; however, more females were also caught at 20 m than at 40-60 m during week 30 and at 20 m than at 40-160 m during week 31. Finally, more females were caught at 0 m than at 40-140 m during week 34 (distance*week of the year: $F_{72,232} = 3.34$, $P < 0.0001$) (Figure 1.4D).

The trap capture patterns for total *D. suzukii* at different distances from a wooded edge during weeks 25-34 of the year in 2014 were identical to those of female *D. suzukii* (distance*week of the year: $F_{72,232} = 3.58$, $P < 0.0001$) (Figure 1.4E).

For other drosophilids, there was not a significant interaction between distance from a wooded edge and week of the year in 2013 (distance*week of the year: $F_{72,232} = 0.74$, $P = 0.93$) (Figure 1.4F). Overall, more flies were captured along the wooded edge (0 m) than at any distance within the crop field (20-160 m), while more flies were captured at 20, 40, 80, and 100 m than at 140 m within the crop field (distance: $F_{8,232} = 14.83$, $P < 0.0001$). More flies were captured during week 26 and fewer flies during week 28 than the other weeks of the year (week of the year: $F_{9,232} = 14.79$, $P < 0.0001$).

Distance from water sources in 2014. In 2014, more female *D. suzukii* were caught in traps placed along a water source (0 m) than in traps placed 30-75 m from a water source during week 32 of the year, while more females were also caught at 15 m than at 45 and 60 m and at 30 m than at 45 m. During week 33, more females were caught at 0 m than at 45 and 60 m, while more females were caught at 0 and 30 m than at 60 m during week 34. Finally, more females were caught at 0 and 15 m than at 60 m during week 35 (distance*week of the year: $F_{60,355} = 1.56$, $P = 0.0082$) (Figure 1.5A).

The trap capture patterns for total *D. suzukii* at different distances from a water source during weeks 25-34 of the year in 2014 were similar to those of female *D. suzukii*. During week 32 of the year, more total *D. suzukii* were caught in traps placed along a water source (0 m) than in traps placed 30-75 m from a water source, while more flies were also caught at 15 m than at 45 and 60 m. During week 33, more flies were caught at 0 m than at 30-60 m, while more flies were caught at 0 and 30 m than at 60 m during week 34. Finally, more flies

were caught at 0, 15, and 30 m than at 60 m during week 35 (distance*week of the year: $F_{60,355} = 1.62$, $P = 0.0045$) (Figure 1.5B).

For other drosophilids captured along water source transects in 2014, there was not a significant interaction between distance from a water source and week of the year (distance*week of the year: $F_{60,355} = 0.68$, $P = 0.97$) (Figure 1.5C). More flies were captured in traps placed 0 and 15 m from a water source than at 30-75 m away (distance: $F_{5,355} = 10.96$, $P < 0.0001$). Trap captures fluctuated throughout the season, with the most flies caught in traps during weeks 25 and 26 and the lowest numbers caught during week 31 (week of the year: $F_{12,355} = 7.81$, $P < 0.0001$).

Distance from wooded edges in 2014. During week 32 of the year in 2014, more female *D. suzukii* were captured in traps placed along a wooded edge (0 m) than in traps placed within a crop field (30-120 m). During week 33, more females were captured at 0 m than at 60 and 90 m, while more females were captured at 0 and 30 m than at 120 m during week 35 (distance*week of the year: $F_{48,766} = 1.51$, $P = 0.0159$) (Figure 1.5D).

Trap capture patterns for total *D. suzukii* were similar to those of female *D. suzukii*. During week 32, more total *D. suzukii* were captured in traps placed along a wooded edge (0 m) than in traps placed within a crop field (30-120 m from a wooded edge). During week 33, more flies were captured at 0 m than at 60, 90, and 120 m from a wooded edge, while more flies were captured at 0 and 30 m than at 120 m during week 35 (distance*week of the year: $F_{48,766} = 2.03$, $P < 0.0001$) (Figure 1.5E).

There was not a significant interaction between distance from a water source and week of the year for other drosophilids captured along wooded edge transects in 2014 (distance*week of the year: $F_{48,766} = 0.86$, $P = 0.74$) (Figure 1.5F). More flies were captured in traps placed 0 m from a wooded edge than 60, 90, and 120 m away, while more flies were captured at 30 m than at 60 and 90 m (distance: $F_{4,766} = 12.33$, $P < 0.0001$). Trap captures varied throughout the season, with the most flies caught in traps during week 26 and the lowest numbers caught during week 31 (week of the year: $F_{12,766} = 23.21$, $P < 0.0001$).

Question 4: Where along transects and when during the season were females, males, and total D. sukuzii more likely to be caught in traps?

Distance from water sources in 2013. Female *D. sukuzii* were not equally likely to be caught in traps located 0-70 m from a water source during weeks 25-34 of the year in 2013 (distance: $F_{7,301} = 3.45$, $P = 0.0014$). Females were more likely to be caught in traps placed 20 m from a water source than 40 and 50 m away and at 10 m than at 50 m (Figure 1.6A). Female *D. sukuzii* were also not equally likely to be caught in traps during weeks 25-34 of the year (week of year: $F_{9,301} = 6.50$, $P < 0.0001$) and were more likely to be caught during weeks 25-27, 29-32 and 34 of the year than week 28 (Figure 1.6D).

As was true for *D. sukuzii* females, total *D. sukuzii* were more likely to be caught in traps placed 20 m from a water source than 40 and 50 m away and in traps at 10 m than at 50 m (distance: $F_{7,301} = 3.63$, $P = 0.0009$) (Figure 1.6B). When males and females were combined, flies were less likely to be caught in traps during week 28 of the year than weeks 25-27 and 29-34 (week of year: $F_{9,301} = 6.48$, $P < 0.0001$) (Figure 1.6E).

No *D. sukuzii* males were caught during week 28 of the year, which was not included in the analysis. Male *D. sukuzii* were not equally likely to be caught in traps located 0-70 m from a water source in 2013 and were more likely to be caught at 0 m than at 30, 40, and 50 m and at 10 m than at 40 or 50 m (distance: $F_{7,261} = 4.93$, $P < 0.0001$) (Figure 1.6C). Male were also more likely to be caught during weeks 26 and 31 of the year than during weeks 29, 32, and 33 (week of year: $F_{8,261} = 4.83$, $P < 0.0001$) (Figure 1.6F).

Distance from wooded edges in 2013. Female *D. sukuzii* were equally likely to be caught in traps located 0-160 m from a wooded edge during weeks 25-34 of the year in 2013 (distance: $F_{8,307} = 1.86$, $P = 0.0661$) (Figure 1.7A). In contrast, females were not equally likely to be caught in traps during weeks 25-34 of the year (week of year: $F_{9,307} = 7.73$, $P < 0.0001$). Females were more likely to be caught during weeks 25, 26, and 31 than weeks 27, 28, and 32 of the year, during weeks 29 and 34 than weeks 28 and 32, and during weeks 30 and 33 than week 28 (Figure 1.7D).

As was true for females, total *D. sukuzii* were captured with equal likelihood in traps placed 0-160 m from a wooded edge (distance: $F_{8,307} = 1.91$, $P = 0.0587$) (Figure 1.7B) but were not equally likely found in traps during weeks 25-34 of the year (week of year: $F_{9,307} = 7.74$, $P < 0.0001$). Males and females were more likely to be caught during weeks 25, 26, and 31 than weeks 27, 28, and 32 of the year, and during weeks 29, 30, 33 and 34 than week 28 (Figure 1.7E).

No *D. sukuzii* males were caught in traps placed 160 m from a wooded edge, which was not included in the analysis. Male *D. sukuzii* were not equally likely to be caught in traps

placed 0-140 m from a wooded edge (distance: $F_{7,289} = 2.60$, $P = 0.0128$), nor during weeks 25-34 of the year in 2013 (week of year: $F_{9,289} = 7.52$, $P < 0.0001$). Males were more likely to be caught in traps placed along a wooded edge (0 m) than at 60 and 100 m away (Figure 1.7C). Males were more likely to be caught during weeks 25 and 26 of the year than weeks 27-29 and 32-33, during week 26 than week 30, and during week 31 than weeks 27-29 and 33 (Figure 1.7F).

Distance from water sources in 2014. No *D. suzukii* females or males were caught during week 23 of the year in 2014, which was not included in the following analyses. Females *D. suzukii* were not equally likely to be caught in traps located 0-75 m from a water source (distance: $F_{5,391} = 2.86$, $P = 0.0149$), although Tukey's test failed to detect pairwise differences between distances. According to Fisher's LSD, female *D. suzukii* were more likely to be caught in traps placed 0 and 15 m from a water source than at 45, 60, and 75 m (Figure 1.8A). Females were also not equally likely to be caught in traps during weeks 25-34 of the year in 2014 (week of year: $F_{11,391} = 7.17$, $P < 0.0001$). Females were more likely to be caught during weeks 32 and 34 than weeks 24-29 and 31, during week 33 than weeks 24, 25, 27-29, and 31, and during weeks 30 and 35 than week 27 (Figure 1.8D).

As was true for females, total *D. suzukii* were not equally likely to be caught in traps placed 0-75 m from a water source in 2014 (distance: $F_{5,391} = 2.31$, $P = 0.0433$) (Figure 1.8B). Tukey's test again failed to detect pairwise differences in trap captures. According to Fisher's LSD, male and female *D. suzukii* were more likely to be caught in traps placed 0 m from a water source than at 75 m and in traps at 15 m than at 60 and 75 m. Total *D. suzukii*

were also not equally likely to be caught in traps during weeks 25-34 of the year (week of year: $F_{11,391} = 7.02$, $P < 0.0001$). Males and females were more likely to be caught during weeks 32 and 34 than weeks 24-29 and 31, during weeks 33 and 35 than weeks 25 and 27-29, and during week 30 than week 27 (Figure 1.8E).

Male *D. suzukii* were not equally likely to be caught in traps placed 0-75 m from a water source in 2014 (distance: $F_{5,391} = 4.89$, $P = 0.0002$). According to Fisher's LSD, male *D. suzukii* were more likely to be caught in traps placed 0 and 15 m from a water source than at 30, 45, 60, and 75 m away (Figure 1.8C). Male *D. suzukii* were also not equally likely to be caught in traps during weeks 25-34 of the year (week of year: $F_{11,391} = 8.12$, $P < 0.0001$). Males were more likely to be caught during week 32 and 34 than weeks 24-30, and during week 35 than weeks 24-29 (Figure 1.8F).

Distance from wooded edges in 2014. No *D. suzukii* females were caught during week 23 of the year in 2014, which was not included in the analysis. Female *D. suzukii* were equally likely to be caught in traps located 0-120 m from a wooded edge (distance: $F_{4,756} = 1.64$, $P = 0.16$) (Figure 1.9A). Females were more likely to be caught in traps during weeks 32-35 than weeks 24-31, and during weeks 24, 26, and 29-31 than week 27 (week of year: $F_{11,756} = 13.96$, $P < 0.0001$) (Figure 1.9D).

As was true for females, total *D. suzukii* were equally likely to be caught in traps located 0-120 m from a wooded edge (distance: $F_{4,820} = 1.87$, $P = 0.11$) (Figure 1.9B). Males and females were not equally likely to be caught in traps during weeks 23-35 of the year (week of year: $F_{12,820} = 14.05$, $P < 0.0001$) (Figure 1.9E). Flies were more likely to be caught

in traps during weeks 32-35 than weeks 24-31, during weeks 24-26 and 29-31 than weeks 23 and 27, and during week 28 than week 23.

Male *D. suzukii* were not equally likely to be caught in traps located 0-120 m from a wooded edge in 2014 (distance: $F_{4,820} = 4.81$, $P = 0.0008$), nor during weeks 23-35 of the year (week of year: $F_{12,820} = 16.07$, $P < 0.0001$). Males were more likely to be caught along a wooded edge (0 m) than at 60 or 90 m away (Figure 1.9C) and during weeks 32-35 than weeks 23-31 (Figure 1.9F).

Discussion

Overall trap capture patterns for female and total *D. suzukii* were similar along water source and wooded edge transects during 2013 and 2014. The numbers of females and total *D. suzukii* caught in traps were low early in the season and started to increase around week 29 (15-16 July) in both years. Peak trap captures occurred one week earlier along both water source and wooded edge transects in 2013 (week 31: 31 July) than in 2014 (week 32: 6 August). Similar trap capture patterns have been observed before for *D. suzukii* in multiple cropping systems; the number of *D. suzukii* caught in traps tends to remain low before peaking, sometimes several times, during the harvest period (Hamby et al. 2014, Harris et al. 2014, Bahder et al. 2016, Haviland et al. 2016, Joshi et al. 2016, Pelton et al. 2016). For example, adult trap captures were the highest near harvest in cherry orchards and then declined as fly populations moved elsewhere (Wang et al. 2016).

In both years, fly captures in traps located at increasing distances from non-crop habitat differed only when fly numbers were high at the end of the growing season. Trap

capture patterns for female and total *D. suzukii* did not differ between years along water source transects but did differ along wooded edge transects. Along water source transects, more flies were caught in traps placed outside of crop fields (0 m) than within crop fields (30 and 60 m) when differences were observed in both years. Along wooded edge transects, more flies were caught outside of crop fields (0 m) than within crop fields (60 and 120 m) during the second half of the season (weeks 29-32 and 34) in 2013, but only during one week in 2014. This discrepancy could be explained, at least in part, by the fact that more *D. suzukii* were caught in 2013 than in 2014; higher fly numbers may have provided more power to detect differences in trap captures at increasing distances from non-crop habitat in 2013.

Differences in trap capture patterns along water source and wooded edge transects could have been affected by weather patterns during the two years of the study. The growth of *D. suzukii* populations is affected by temperature and relative humidity (Tochen et al. 2013, 2014, Wiman et al. 2014, 2016). Cold temperatures during Spring 2014, coupled with elevated temperatures and lower relative humidity during the first half of the sampling period in 2014 (Figure 1.10), could have suppressed *D. suzukii* populations. This could also explain why trap captures peaked one week later in 2014 than in 2013.

Trap capture patterns of other drosophilids were different than those of *D. suzukii*. Whereas *D. suzukii* trap captures showed definite seasonality, with low numbers followed by large peaks in both trap captures and fruit infestation (data not shown), other drosophilids were caught in traps throughout the season in both years. Other drosophilids exhibited an edge effect throughout the 2013 and 2014 seasons along both water source and wooded edge

transects (i.e., distance*week of year interactions were not significant), unlike *D. suzukii* which was more abundant on field edges only when overall abundance appeared high.

Species present among the non-*D. suzukii* drosophilids caught in traps represent different life histories and ecologies (i.e., some overripe fruit feeders, fungi feeders, and some woodland species were present). Past collections of the *Drosophila* species present in western North Carolina (Macon County) documented the presence of at least 25 species (Miller and Weeks 1964). Despite the potentially large number of congeneric species present, *D. suzukii* was the dominant *Drosophila* species present in blackberry fields in both years of our experiment. In contrast, other *Drosophila* species were more abundant than *D. suzukii* in all but the final year of a 3-year study conducted in the Pacific Northwest (Bahder et al. 2016).

D. suzukii trap captures were, in general, higher on the edges of fields, regardless of the type of habitat on the edge. More flies were caught outside of crop fields than within crop fields located near both wooded edges and water sources. *D. suzukii* is often found in wooded areas and has adapted to utilize resources present in such habitats (Ometto et al. 2013). Wooded areas may be attractive to *D. suzukii* because non-crop hosts are often present (Lee et al. 2015, Poyet et al. 2016). Klick et al. (2016) caught more *D. suzukii* in field margins with Himalayan blackberry, a known *D. suzukii* host, than in field margins without an alternative host. Pelton et al. (2016) caught high numbers of *D. suzukii* in wooded areas and suggested that they may be providing flies with resources such as wild hosts and overwintering sites. Wild *Rubus* spp., black cherry, and American pokeweed were present along wooded edges at the three sites used in this study. In fact, during these experiments, we

documented that flies developing in American pokeweed were more likely to infest blackberries than their natal host (Diepenbrock et al. 2016). Although non-crop hosts were not present near water sources, this habitat can provide other resources and/or conditions that may be attractive to *D. suzukii*. Individual trees provided shade near each of the water sources tested in this study, while proximity to water likely provided levels of relative humidity that were higher than those within crop fields.

Trap capture patterns for *D. suzukii* at a finer scale indicated that fly captures near non-crop habitat were higher up to 15-30 m into crop fields, but no further, so the effect of distance from non-crop habitat within habitat types does not extend very far into crop fields. The commercial fields used in this study were treated with insecticides at least once a week for *D. suzukii* control; therefore, all traps within crop fields were also “treated” with insecticide. Assuming that all traps within a crop field received a comparable amount of insecticide during each application, the fact that traps close to non-crop habitat had higher trap captures suggests that more flies may have been present and encountered traps in these areas.

The likelihood of flies getting caught in traps along water source and wooded edge transects in the two years differed between male and female *D. suzukii*. Males were more likely to be caught in traps located outside of crop fields than within crop fields along both water source and wooded edge transects. Conversely, females were more likely to be caught outside of crop fields near water sources in both years, but equally likely to be caught in traps placed within and outside of crop fields near wooded areas in both years. This discrepancy may reflect that female and male flies may be seeking different resources within a system;

for example, females may spend most of their time searching for oviposition hosts, while males are searching for females to mate with or food resources. Fermentation-based baits do not accurately predict fruit infestation, and there is mounting evidence that they attract female flies that are physiologically different from females that are attracted to ripe fruits (Burrack et al. 2015, Wallingford et al. 2016, Swoboda-Bhattarai et al. 2017). Therefore, trap captures are not necessarily representative of the egg-laying population in an area and may over sample reproductively immature female flies. If so, trap capture patterns within crop fields may reflect infestation rates in nearby fruits and may be less affected by the presence of non-crop habitat near crop fields.

Management Implications

Management activities for *D. suzukii* in the United States are primarily based on adult trap captures and fruit ripeness levels, as there are currently no reliable metrics available that reliably link adult *D. suzukii* presence with infestation in crop fields (Asplen et al. 2015). The results presented in this study move us closer to understanding how *D. suzukii* adults are distributed within crop fields on both spatial and temporal scales and may lead to improved monitoring tools and recommendations that directly impact the growth potential of *D. suzukii* populations in fruit crops.

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TABLES

Table 1.1. Description of transect experiments conducted at commercial blackberry farms in North Carolina during 2013 and 2014.

Year	Site	County	Edge Habitat	Field	Crop Cultivar	Number of transects	Transect length (m)	Distance between transects (m)	Distance between sample points within transects (m)	Weeks of year (dates) trapped	Weeks of year (dates) fruit samples collected
2013	1	Cleveland	Water	1	'Navaho'	3	≤70	≥20	10	25 (18-Jun) to 34 (20-Aug)	26 (25-Jun) to 33 (13-Aug)
			Woods	1	'Ouachita' and 'Navaho'	3	≤160	≥20	20	25 (18-Jun) to 34 (20-Aug)	26 (25-Jun) to 33 (13-Aug)
	2	Lincoln	Water	1	'Navaho'	3	≤70	≥20	10	25 (18-Jun) to 30 (23-Jul)	26 (25-Jun) to 30 (23-Jul)
			Woods	1	'Navaho'	3	≤140	≥20	20	25 (18-Jun) to 30 (23-Jul)	26 (25-Jun) to 30 (23-Jul)
2014	1	Cleveland	Water	1	'Navaho'	4	≤75	30	15	23 (3-Jun) to 35 (27-Aug)	25 (17-Jun) to 34 (20-Aug)
			Woods	1	'Ouachita' and 'Navaho'	4	120	30	30	23 (3-Jun) to 35 (27-Aug)	25 (17-Jun) to 34 (20-Aug)
	3	Cleveland	Water	1	'Navaho'	3	≤60	30	15	23 (3-Jun) to 35 (27-Aug)	25 (17-Jun) to 34 (20-Aug)
			Woods	1	'Ouachita' and 'Navaho'	5	120	30	30	23 (3-Jun) to 35 (27-Aug)	25 (17-Jun) to 34 (20-Aug)
				2	'Ouachita'	4	120	30	30	23 (3-Jun) to 35 (27-Aug)	25 (17-Jun) to 34 (20-Aug)

Table 1.2. Statistical analyses conducted.

Data set	Response variables	Fixed effects	Random effects	Distribution/link	Post hoc analysis
Question 1: Did trap capture patterns within habitat types differ between years?					
1. Water transects in 2013 and 2014	SWDf, SWDtot, NONtot	3-way interaction: Distance (0, 30, 60 m) Year (2013, 2014) Week of year (25 – 34)	Site (1, 2, 3) – nested within year Transect (1 – 4)	Lognormal/identity	<i>SWDf</i> : 1. Year*week of year, sliced by week of year; 2. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment for both analyses <i>SWDtot</i> : Same as SWDf <i>NONtot</i> : 1. Year*week of year, sliced by week of year; 2. Distance*year, sliced by year; Tukey-Kramer adjustment for both analyses
2. Woods transects in 2013 and 2014	SWDf, SWDtot, NONtot	3-way interaction: Distance (0, 60, 120 m) Year (2013, 2014) Week of year (25 – 34)	Site (1, 2, 3) – nested within year Transect (1 – 5)	Lognormal/identity	<i>SWDf</i> : 1. Distance*year*week of year, sliced by year*week of year; Tukey-Kramer adjustment <i>SWDtot</i> : Same as SWDf <i>NONtot</i> : 1. Year*week of year, sliced by week of year; 2. Distance*year, sliced by year; Tukey-Kramer adjustment for both analyses
Question 2: Did trap capture patterns within individual years differ between habitat types?					
1. Water and woods transects in 2013	SWDf, SWDtot, NONtot	3-way interaction: Distance (0, 20, 40, 60 m) Habitat (water, woods) Week of year (25 – 34)	Site (1, 2) Transect (1 – 3)	Lognormal/identity	<i>SWDf</i> : 1. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment <i>SWDtot</i> : Same as SWDf <i>NONtot</i> : Analysis of main effects with Tukey-Kramer adjustment
2. Water and woods transects in 2014	SWDf, SWDtot, NONtot	3-way interaction: Distance (0, 30, 60 m) Habitat (water, woods) Week of year (25 – 34)	Site (1, 3) Transect (1 – 5)	Lognormal/identity	<i>SWDf</i> : 1. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment <i>SWDtot</i> : 1. Habitat*week of year, sliced by week of year; 2. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment for both analyses <i>NONtot</i> : 1. Habitat*week of year, sliced by week of year; 2. Analysis of main effects with Tukey-Kramer adjustment
Question 3: Did finer scale trap capture patterns differ within a given year?					
1. Water transects in 2013	SWDf, SWDtot, NONtot	2-way interaction: Distance (0, 10, 20, 30, 40, 50, 60, 70 m) Week of year (25 – 34)	Site (1, 2) Transect (1 – 3)	Lognormal/identity	<i>SWDf</i> : 1. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment <i>SWDtot</i> : Same as SWDf <i>NONtot</i> : Analysis of main effects with Tukey-Kramer adjustment

Table 1.2. Continued.

Data set	Response variables	Fixed effects	Random effects	Distribution/link	Post hoc analysis
Question 3: Did finer scale trap capture patterns differ within a given year?					
2. Woods transects in 2013	SWDf, SWDtot, NONtot	2-way interaction: Distance (0, 20, 40, 60, 80, 100, 120, 140, 160 m) Week of year (25 – 34)	Site (1, 2) Transect (1 – 3)	Lognormal/identity	<i>SWDf</i> : 1. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment <i>SWDtot</i> : Same as <i>SWDf</i> <i>NONtot</i> : Analysis of main effects with Tukey-Kramer adjustment
3. Water transects in 2014	SWDf, SWDtot, NONtot	2-way interaction: Distance (0, 15, 30, 45, 60, 75 m) Week of year (23 – 35)	Site (1, 3) Transect (1 – 4)	Lognormal/identity	<i>SWDf</i> : 1. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment <i>SWDtot</i> : Same as <i>SWDf</i> <i>NONtot</i> : Analysis of main effects with Tukey-Kramer adjustment
4. Woods transects in 2014	SWDf, SWDtot, NONtot	2-way interaction: Distance (0, 30, 60, 90, 120 m) Week of year (23 – 35)	Site (1, 3) Field (1 – 2) Transect (1 – 5)	Lognormal/identity	<i>SWDf</i> : 1. Distance*week of year, sliced by week of year; Tukey-Kramer adjustment <i>SWDtot</i> : Same as <i>SWDf</i> <i>NONtot</i> : Analysis of main effects with Tukey-Kramer adjustment
Question 4: Where along transects and when during the season were trap captures more likely to occur?					
1. Water transects in 2013	SWDf, SWDm, SWDtot	Distance (0, 10, 20, 30, 40, 50, 60, 70 m) Week of year (25 – 34)		Binomial/logit	<i>All</i> : Analysis of main effects with Tukey-Kramer adjustment
2. Woods transects in 2013	SWDf, SWDm, SWDtot	Distance (0, 20, 40, 60, 80, 100, 120, 140, 160 m) Week of year (25 – 34)		Binomial/logit	<i>All</i> : Analysis of main effects with Tukey-Kramer adjustment
3. Water transects in 2014	SWDf, SWDm, SWDtot	Distance (0, 15, 30, 45, 60, 75 m) Week of year (23 – 35)		Binomial/logit	<i>All</i> : Analysis of main effects with Tukey-Kramer adjustment
4. Woods transects in 2014	SWDf, SWDm, SWDtot	Distance (0, 30, 60, 90, 120 m) Week of year (23 – 35)		Binomial/logit	<i>All</i> : Analysis of main effects with Tukey-Kramer adjustment

^aResponse variable abbreviations: SWDf = female *D. suzukii*; SWDm = male *D. suzukii*; SWDtot = total *D. suzukii*; NONtot = total other drosophilids.

FIGURES

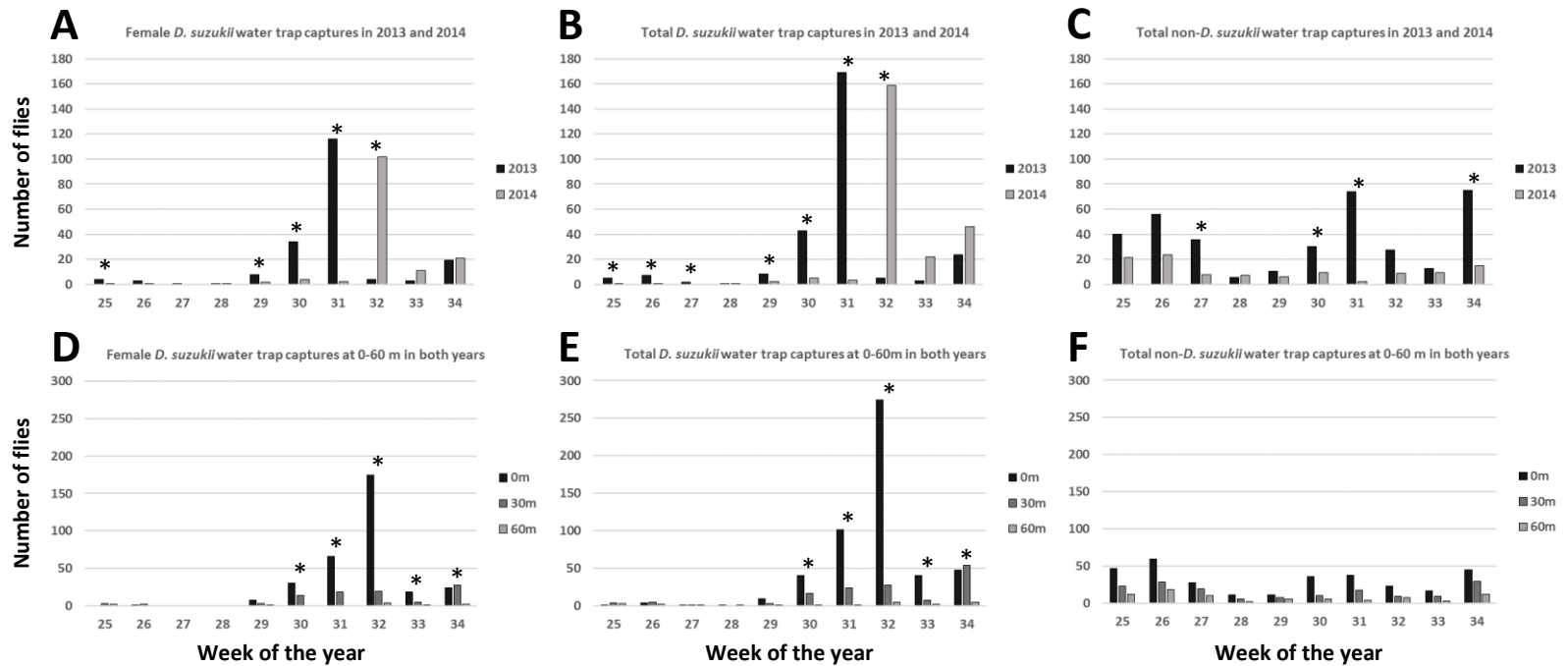


Figure 1.1. Trap captures for female *D. sukukii*, total *D. sukukii*, and total non-*D. sukukii* drosophilids along water source transects in 2013 and 2014 (A-C) and at 0, 30, and 60 m away from a water source during weeks 25-34 of the year in both years (D-F). Significant differences among treatment means within weeks of the year are denoted by an asterisk (*); significant differences between treatment means are described in the text.

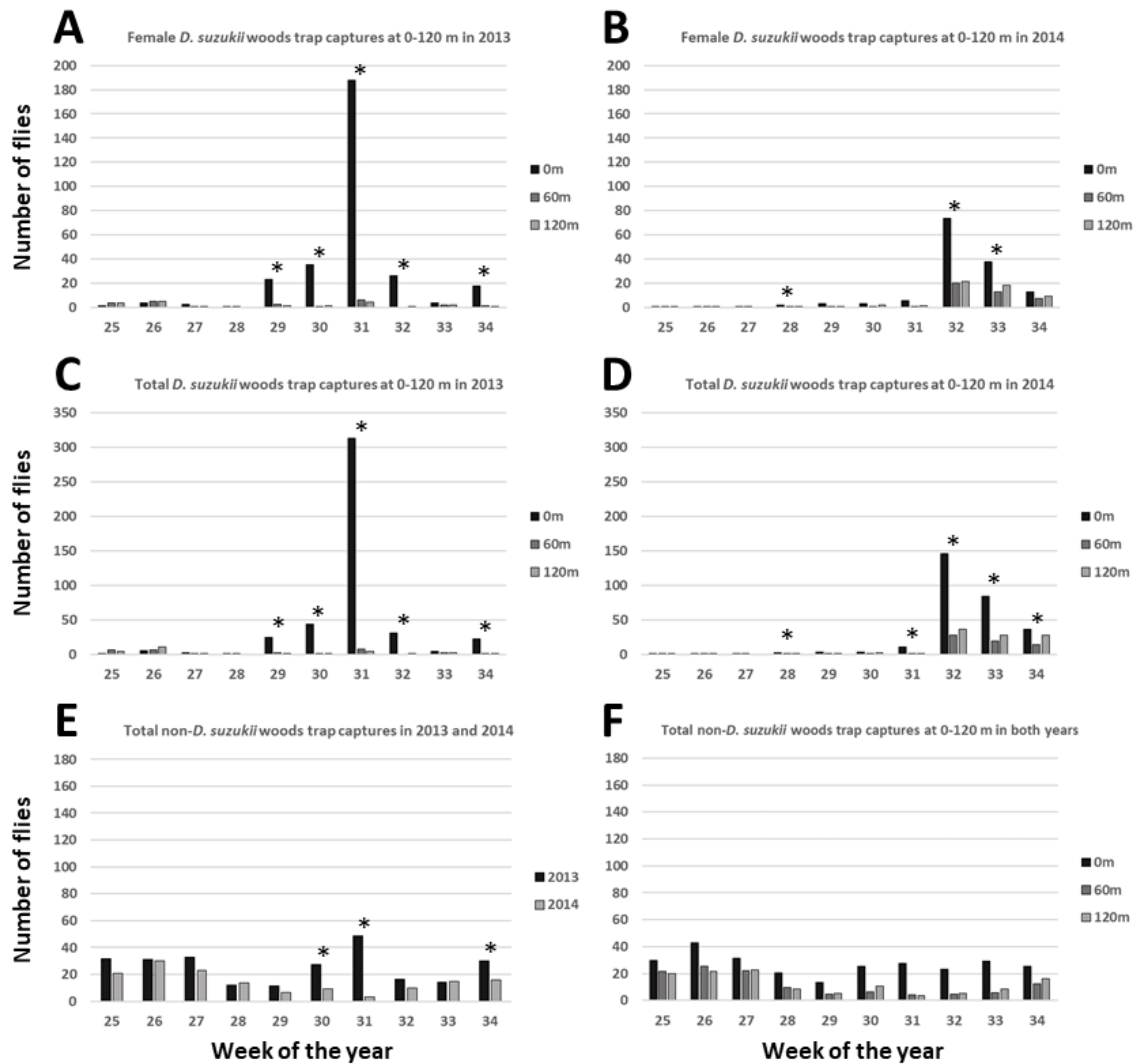


Figure 1.2. Trap captures for female *D. sukukii* (A-B), total *D. sukukii* (C-D), and total non-*D. sukukii* drosophilids (E-F) at 0, 60, and 120 m away from a wooded edge during weeks 25-34 of the year in 2013 and 2014. Significant differences among treatment means within weeks of the year are denoted by an asterisk (*); significant differences between treatment means are described in the text.

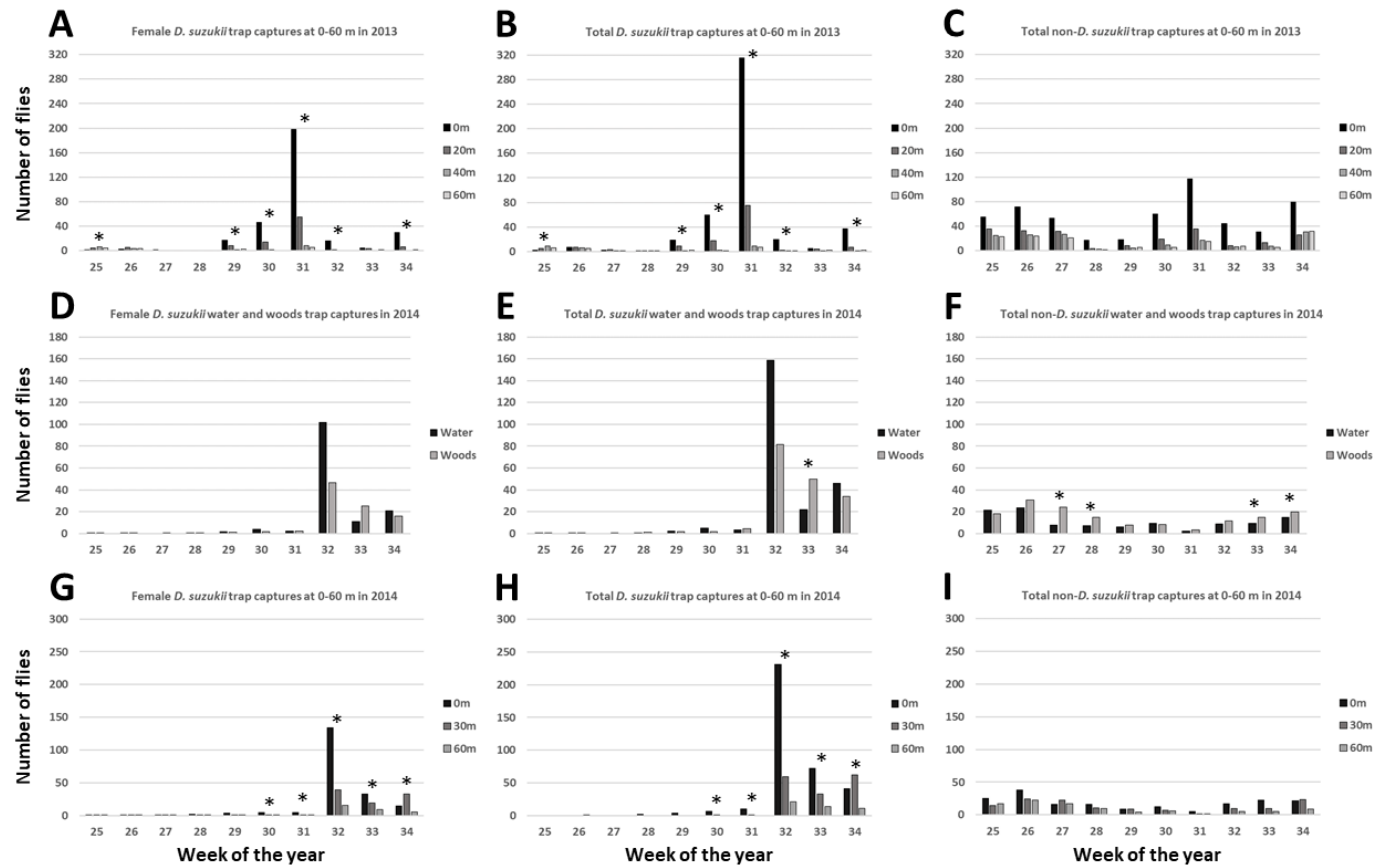


Figure 1.3. Trap captures for female *D. sukikii*, total *D. sukikii*, and total non-*D. sukikii* drosophilids at 0-60 m away from a water source or a wooded edge during weeks 25-34 of the year in 2013 (A-C) or 2014 (D-I). Significant differences among treatment means within weeks of the year are denoted by an asterisk (*); significant differences between treatment means are described in the text.

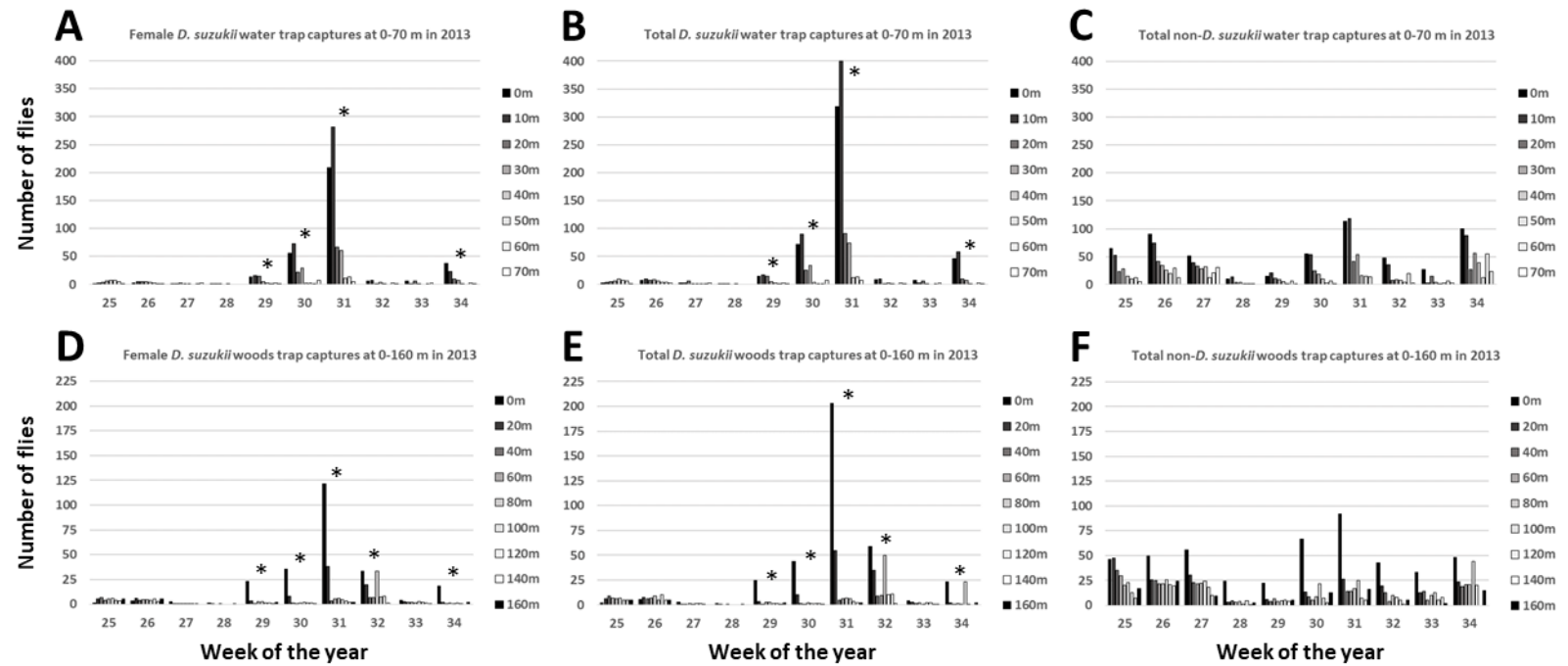


Figure 1.4. Fine scale trap captures for female *D. sukuzii*, total *D. sukuzii* and total non-*D. sukuzii* drosophilids along water source (A-C) or wooded edge (D-F) transects during weeks 25-34 of the year in 2013. Significant differences among treatment means within weeks of the year are denoted by an asterisk (*); significant differences between treatment means are described in the text.

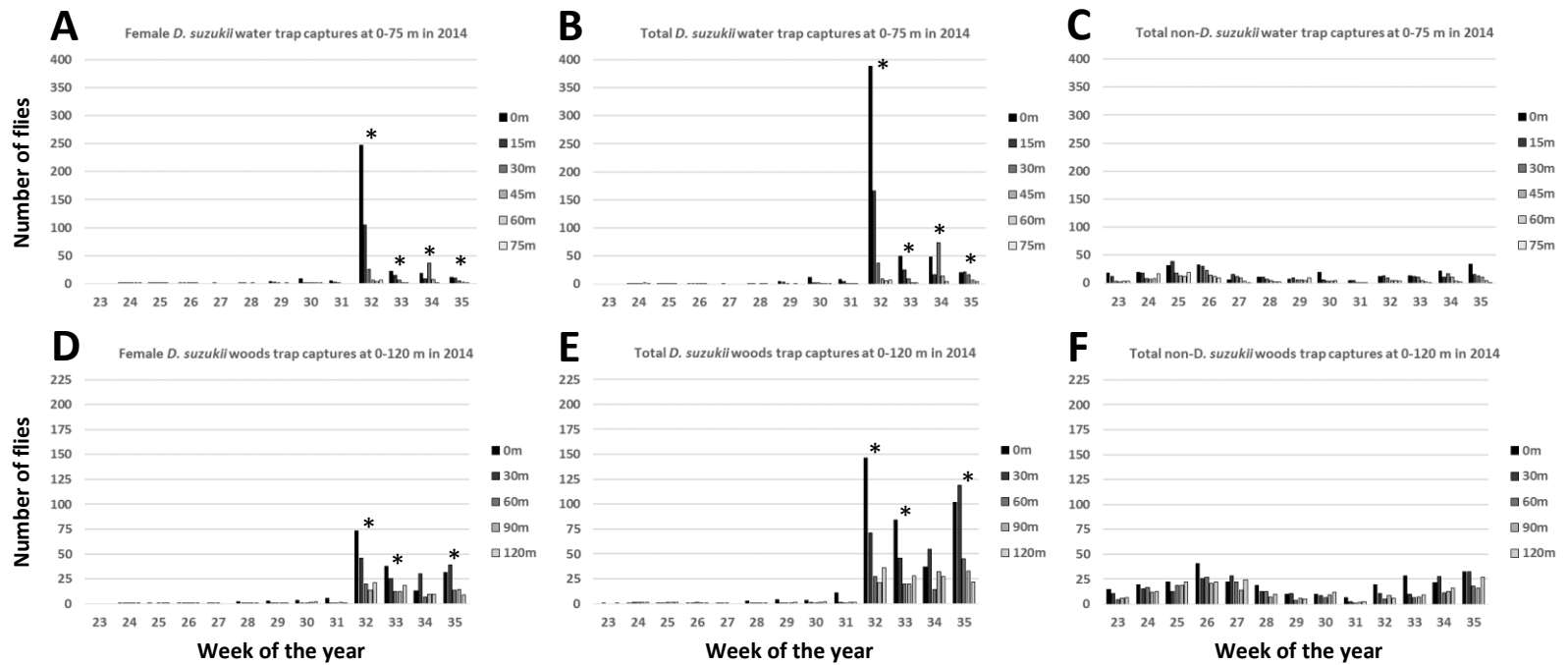


Figure 1.5. Fine scale trap captures for female *D. sukukii*, total *D. sukukii* and total non-*D. sukukii* drosophilids along water source (A-C) or wooded edge (D-F) transects during weeks 25-34 of the year in 2014. Significant differences among treatment means within weeks of the year are denoted by an asterisk (*); significant differences between treatment means are described in the text.

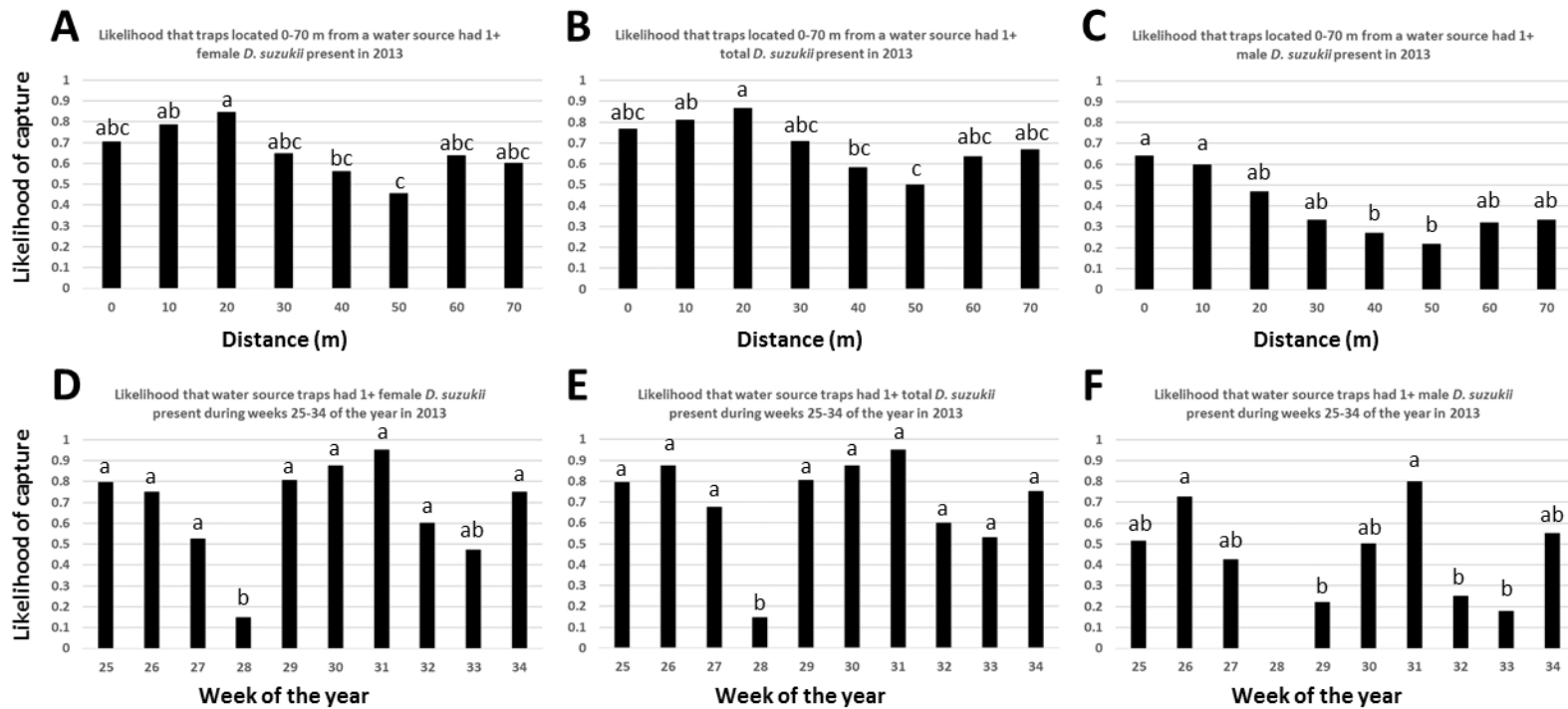


Figure 1.6. Likelihood that female, male, and total *D. sukuzii* were caught in traps located 0-70 m along transects running from water sources into crop fields (A-C) and during weeks 25-34 of the year (D-F) in 2013. Bars that share a letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer test).

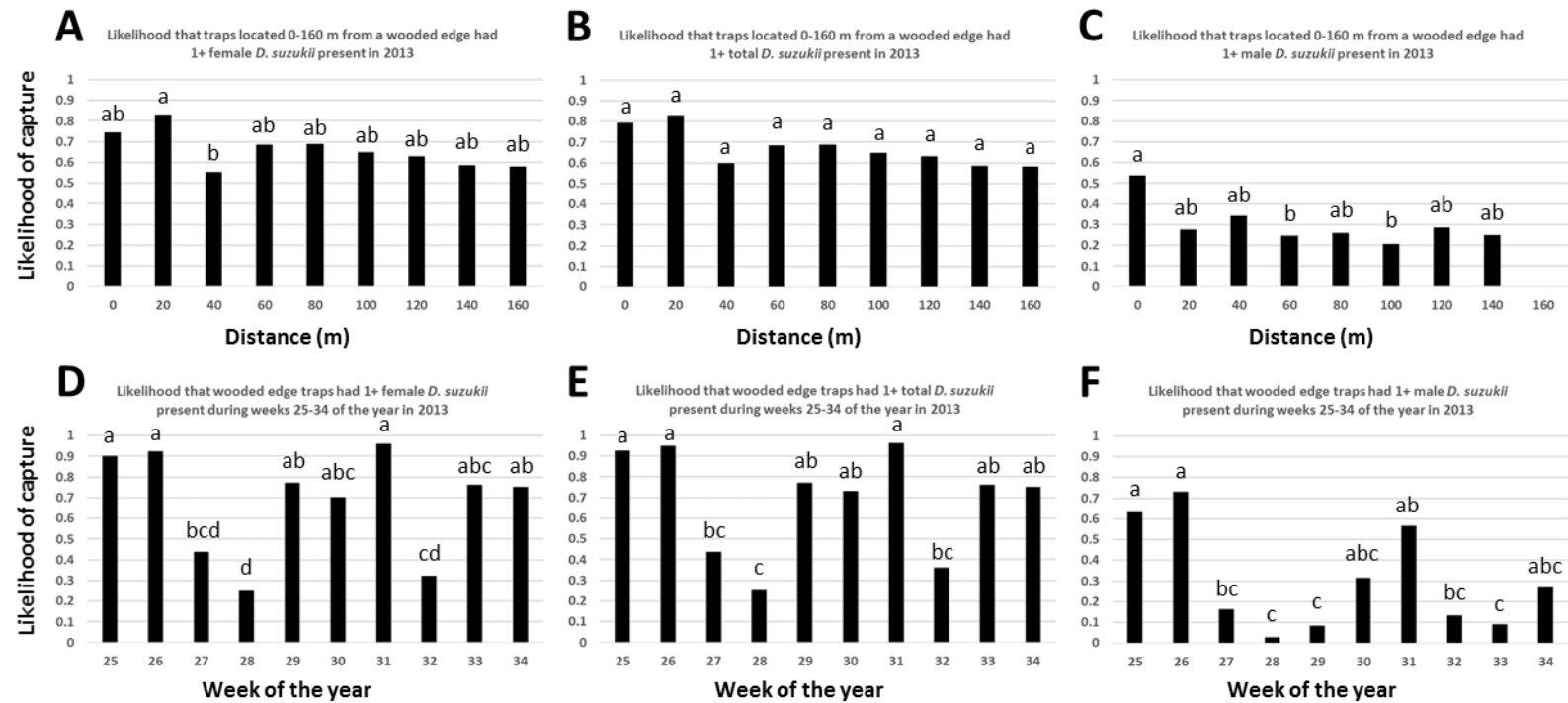


Figure 1.7. Likelihood that female, male, and total *D. sukuzii* were caught in traps located 0-160 m along transects running from wooded edges into crop fields (A-C) and during weeks 25-34 of the year (D-F) in 2013. Bars that share a letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer test).

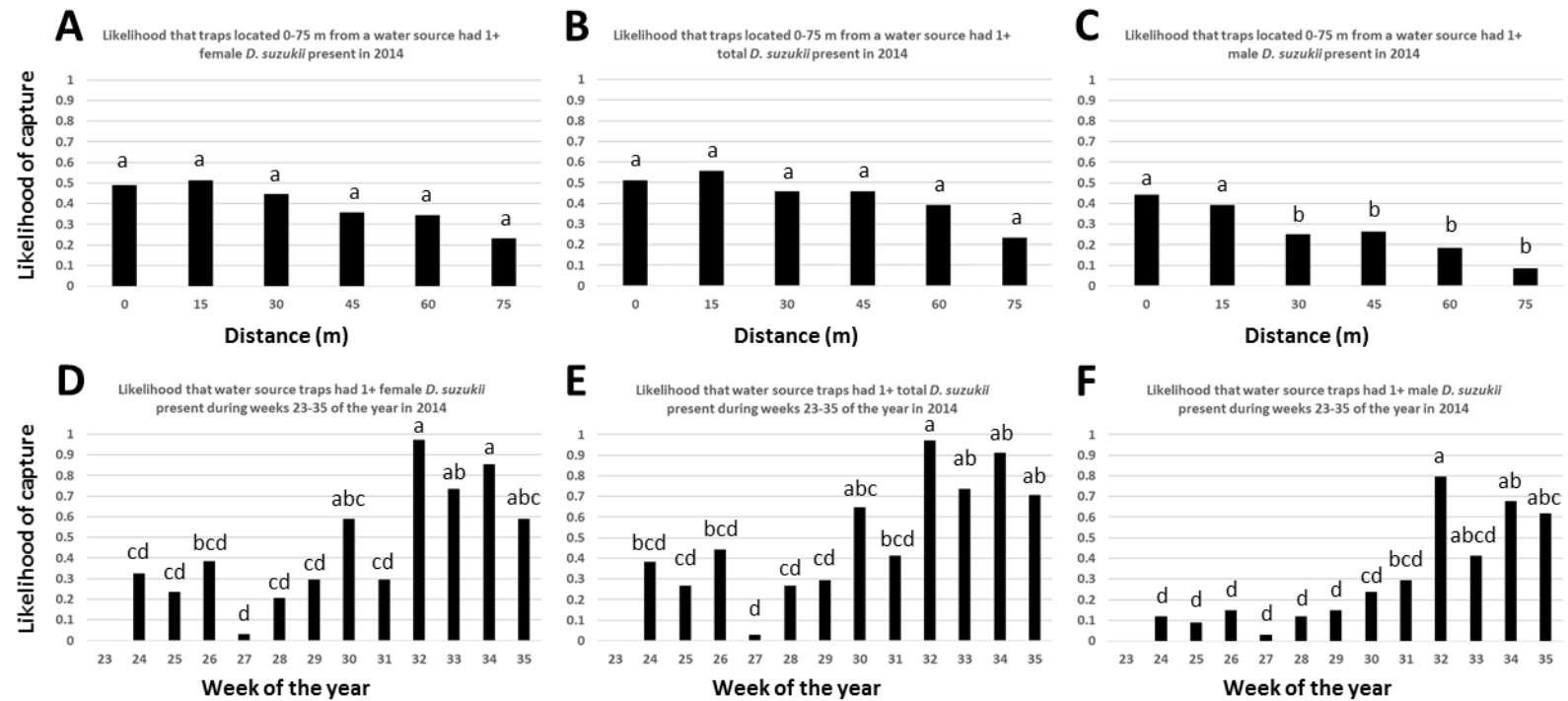


Figure 1.8. Likelihood that female, male, and total *D. sukuzii* were caught in traps located 0-75 m along transects running from water sources into crop fields (A-C) and during weeks 23-35 of the year (D-F) in 2014. Bars that share a letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer test).

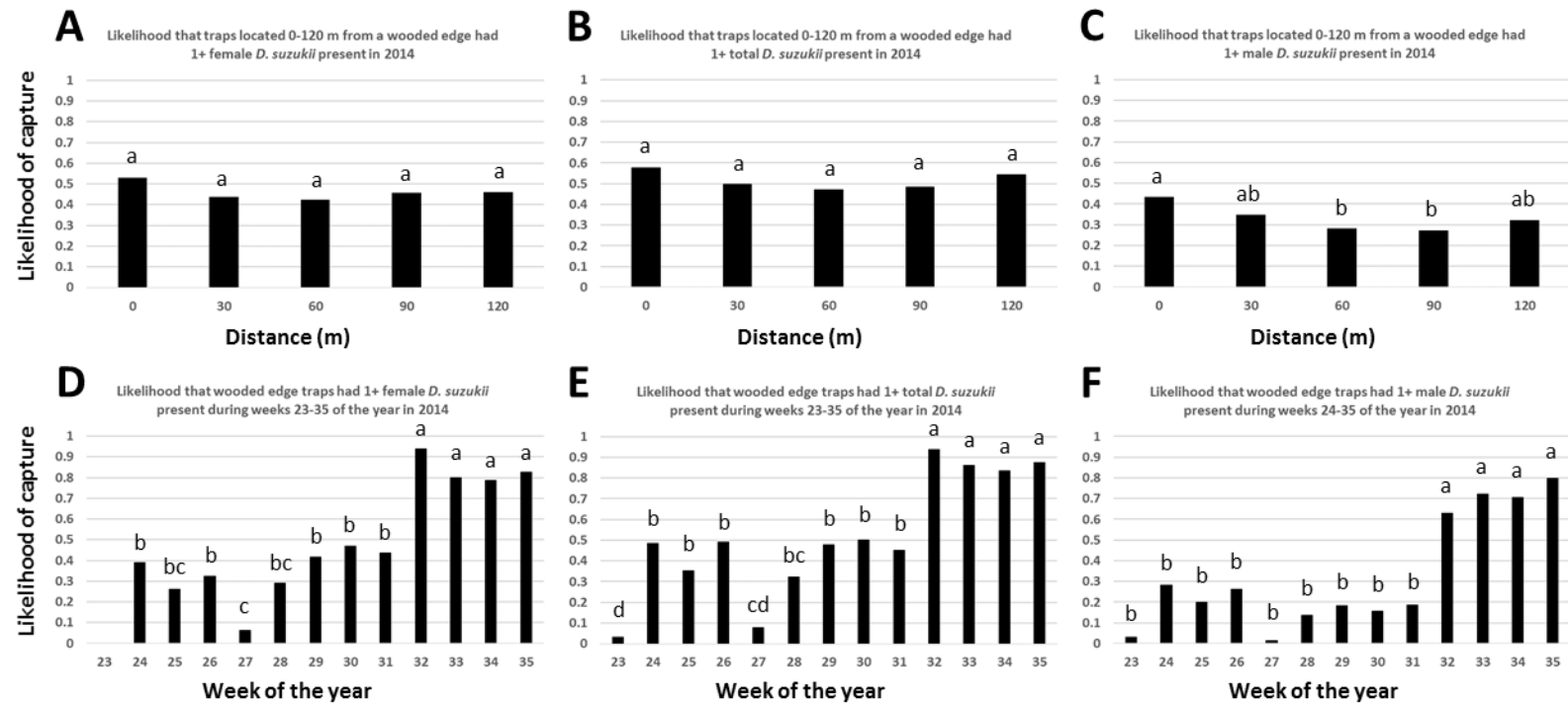


Figure 1.9. Likelihood that female, male, and total *D. suzukii* were caught in traps located 0-120 m along transects running from wooded edges into crop fields (A-C) and during weeks 23-35 of the year (D-F) in 2014. Bars that share a letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer test).

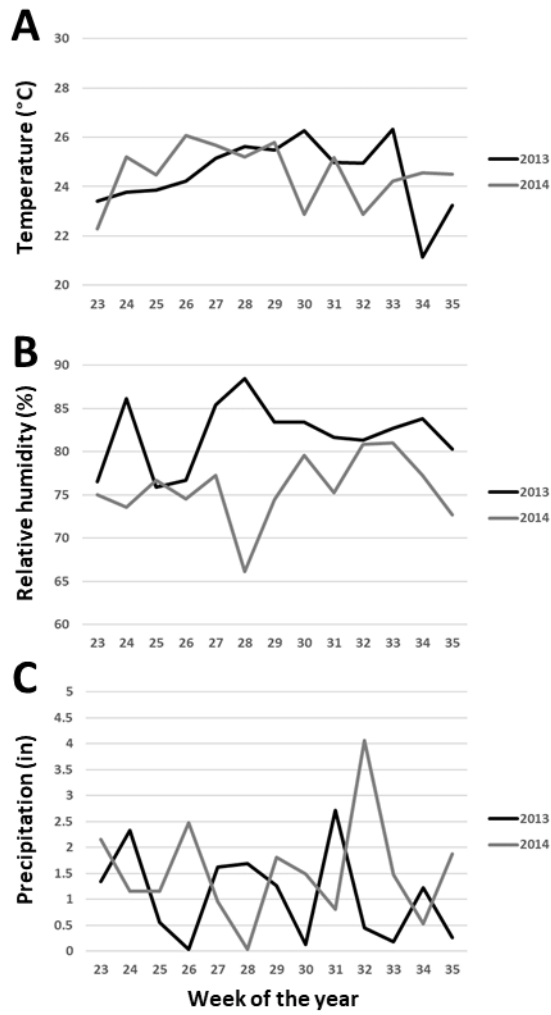


Figure 1.10. Average daily temperatures (A), average relative humidity (B), and sum of hourly precipitation (C) from a weather station located near Site 1 in Cleveland County, NC. Data from 2013 and 2014 were obtained from the State Climate Office of North Carolina and averaged over the seven days prior to each collection date.

**CHAPTER 2: A Comparison of the Diurnal and Seasonal Activity Patterns of
Drosophila suzukii and Other Drosophilid Species (Diptera:
Drosophilidae) Present in Blackberry Agroecosystems**

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Abstract

Drosophila species have provided scientists with an invaluable tool with which to study behavioral innovation. *Drosophila suzukii* (Matsumura) is an invasive species that differs from other frugivorous drosophilids in that females lay eggs in ripe and ripening fruits instead of overripe or rotten fruits. We hypothesized that there may be diurnal or seasonal patterns associated with the movement of *D. suzukii* and other drosophilid species into and out of crop fields and attraction to monitoring traps with a fermentation-based bait, and that *D. suzukii* may exhibit different behaviors than other drosophilid species based on its unique ecology. To test these hypotheses, we deployed 2-headed Malaise traps between crop fields and wooded edges to simultaneously catch flies moving into and out of crop fields. We also deployed monitoring traps with a fermentation-based bait between crop fields and wooded edges and within crop rows. Traps were deployed weekly in June-August in 2014 and 2015 at two commercial blackberry fields in Cleveland County, NC, and were checked hourly for 24 hours, except during darkness. Both *D. suzukii* and other drosophilids moved between crop fields and wooded edges and were attracted to monitoring traps with a fermentation-based bait primarily during the morning and evening hours. Whereas other drosophilids were captured in traps throughout the season, few *D. suzukii* were caught in traps until early to mid-July in both years and increased as the season progressed. Understanding *D. suzukii* movement and diurnal activity patterns will help growers to effectively time insecticide applications and is essential for the development of future management strategies, including attract and kill and mass trapping.

Introduction

Drosophila species have provided scientists with an invaluable tool with which to study behavioral innovation both in the laboratory and in natural settings. Changes in behavior, along with a combination of reproductive and ecological traits including high fecundity, short generation times, and adaptation to a wide range of niches have allowed several *Drosophila* species to expand beyond their ancestral ranges (Ometto *et al.*, 2013). *D. melanogaster* is a classic example, whose current worldwide distribution is the result of an out-of-Africa expansion that occurred 15,000 years ago (David & Capy 1988). Similarly, *D. subobscura* is a Palearctic species that rapidly invaded broad latitudinal ranges in North and South America beginning in the early 1980s (Pascual *et al.*, 2007). Recently, another drosophilid species has undergone a dramatic range expansion, but differs from previous invaders in that it is a crop pest of grave concern. *Drosophila suzukii* (Matsumura) differs from many other frugivorous drosophilids in that females lay eggs in ripe and ripening fruits instead of overripe or rotten fruits. This difference in ecology is reflected in morphological adaptations such as the large sclerotized ovipositor that *D. suzukii* females use to lay eggs in sound fruit (Atallah *et al.*, 2014), but is it also reflected in behavioral adaptations that set *D. suzukii* apart from other drosophilids present in the same systems?

D. suzukii is endemic to Southeast Asia and is present in several countries with temperate climates from Pakistan to Japan (Kanzawa 1936, Cini *et al.* 2014). *D. suzukii* has demonstrated a propensity for invasion and quickly became one of the most abundant drosophilid species in some Hawaiian ecosystems after its detection in the early 1980s (Kaneshiro, 1983; Asquith & Messing, 1992; Leblanc *et al.*, 2009). It was first detected in the

continental United States in California in 2008 and again in Florida in 2009, and has since been detected throughout much of North America (Hauser, 2011) and Europe (Cini *et al.*, 2012), and in South America (Depra *et al.*, 2014). Assuming 20% yield loss, revenue losses to *D. suzukii* in strawberries, blueberries, raspberries, blackberries and cherries in California, Oregon, and Washington alone have been estimated at \$511 million annually (Bolda *et al.*, 2010; Walsh *et al.*, 2012).

Recent research has illuminated aspects of *D. suzukii* resource use. Females prefer some crop hosts over others for oviposition, and offspring perform better in preferred female oviposition substrates (Bellamy *et al.*, 2013; Burrack *et al.*, 2013; Diepenbrock *et al.*, 2016). Host phenology drives infestation timing, with flies preferring soft ripe fruit over firm unripe fruit (Burrack *et al.*, 2013; Lee *et al.*, 2016; Swoboda-Bhattarai & Burrack, 2016). The presence of non-crop habitat, which can serve as a source of infesting populations or provide *D. suzukii* with refuge from management treatments within crop fields, might also affect *D. suzukii* activity patterns related to host use (Klick *et al.*, 2016). Despite recent advances, little is known about the ecology of *D. suzukii* in agroecosystems, which has made it difficult to develop management strategies beyond preventative insecticide use.

Drosophilid species with very different life histories have been shown to exhibit similar behavioral patterns related to locating and utilizing resources. It may be possible to illuminate some aspects of *D. suzukii* ecology by looking at the movement and behavioral patterns of other co-occurring drosophilid species. For example, adults of a wide range of *Drosophila* species are attracted to simple banana baits fermented with *Saccharomyces cerevisiae*, even though their natural feeding and breeding sites are very different (Markow &

O’Grady, 2008). Several *Drosophila* species have been shown to be attracted to such baits and other food sources during the morning and late afternoon/evening hours (Dobzhansky & Epling, 1944; Pavan *et al.*, 1950, Mitchell & Epling, 1951; Miller & Weeks, 1964; Noor, 1991). Under simulated “summer” conditions in the laboratory, *D. suzukii* exhibited a bimodal locomotor activity pattern with maximum activity occurring at dawn and dusk, while ‘winter’ activity was unimodal and restricted to the warmest part of the day (Hamby *et al.*, 2013). Similarly, *D. suzukii* males and virgin females displayed nighttime inactivity followed by weak activity in the morning, another inactive period in the afternoon, and a prominent evening peak of activity; however, the afternoon lull and evening peak of activity were reduced in mated females (Ferguson *et al.*, 2015). Understanding the timing of *D. suzukii* activity in natural settings, and how it compares to that of co-occurring drosophilid species, provides us with the opportunity to further understand the mechanisms of its invasion success and provide information necessary to develop new management practices.

Experiments were conducted in 2014 to track the temporal and spatial movement of *D. suzukii* and other drosophilids in blackberry agroecosystems. Blackberry is a preferred crop host and insecticide-treated farms still support high *D. suzukii* populations (Lee *et al.*, 2011; Bellamy *et al.*, 2013; Burrack *et al.*, 2013). Our objectives were to determine if there were diurnal patterns associated with drosophilid 1) movement between crop fields and adjacent wooded areas, 2) attraction to monitoring traps baited with a yeast and sugar solution (a food bait that releases fermentation-based volatiles attractive to both sexes that is used widely for *D. suzukii* monitoring), 3) seasonal changes to these patterns, and 4) to relate these patterns to *D. suzukii* oviposition behavior. We focused on wooded areas because

previous observations suggested that adult *D. suzukii* were more abundant on field edges than within crop fields (Pelton *et al.*, 2016). We expected that *D. suzukii* would exhibit both diurnal and seasonal patterns and that these patterns would be similar to those of other drosophilid species in the system. Because *D. suzukii* is unusual among *Drosophila* species in regard to where it lays its eggs, we also predicted that behaviors related to finding oviposition hosts might differ between *D. suzukii* and other drosophilids more than behaviors related to feeding.

Materials and Methods

Sampling period

During 2014 and 2015, we collected samples at two commercial blackberry farms in Cleveland County, North Carolina. We sampled at each farm every two weeks, weather permitting, starting before ripe berries were present and continuing through the postharvest period. In both years, we sampled at each farm at least once i) before the host crop ripened, ii) during fruit harvest, and iii) postharvest, except at Farm 2 in 2014 when no postharvest sample was collected. We collected samples on four dates at Farm 1 and three dates at Farm 2 in 2014, and on seven dates at Farm 1 and four dates at Farm 2 in 2015 (Table 2.1). The number of sample dates differed between farms and years due to inclement weather and on-farm activities that precluded sampling. Sample dates were timed to crop phenology instead of week of the year, and we started collecting samples at Farm 1 earlier in 2015 than during 2014.

Movement between crop fields and non-crop habitat, 2014-2015

We used ez-Migration traps (BugDorm, Taiwan), which are 2-headed, unbaited Malaise traps designed to simultaneously capture insects moving in two opposite directions by funneling them into separate collection canisters, which we filled with 70% ethanol. On each sampling date, we set up four Malaise traps in the area between the crop field and the wooded edge (Fig. 2.1). Traps were set up perpendicular to and located ~3 m away from the end of the crop rows. During each sampling period, we checked traps hourly, on the hour, from approximately 5 am to 10 pm (daylight hours), once after it was completely dark, and once before sunrise. The contents of each canister were removed whenever a *Drosophila*-like insect was captured or after every four hours. Canister contents were removed by pouring them through a handheld kitchen strainer; all insects present were collected using soft forceps and placed into a 20 ml scintillation vial with 70% ethanol. Samples were examined under a stereomicroscope. All *D. suzukii* and non-*D. suzukii* Drosophilidae were identified, sexed, and counted, and preserved in 70% ethanol for future use. Because this experiment was conducted in commercial fields, the large Malaise traps could not be left out for longer than 24 hours and were removed between sampling dates.

Attraction to monitoring traps with a fermentation-based bait, 2014-2015

Monitoring traps consisted of 32 fl oz. clear plastic cups and lids with 10 equidistant holes drilled near the top (DeliPRO brand, Tri-pack Industrial USA, White Plains, NY) baited with 1.69 g of dry active yeast, 8.45 g of sugar, and 150 ml of water (Burrack *et al.*, 2015). In concert with the Malaise trap experiment described above, we set up four traps with

yeast/sugar bait between the crop field and wooded edge and four traps within the crop field. Monitoring traps at Farm 1 were located ~30 m away from the wooded edge (Fig. 2.1). Because the wooded edge was located farther away from the crop field at Farm 2 than at Farm 1, monitoring traps were set up 17.7 m from the end of crop rows to keep the spacing consistent between the two farms.

In 2014, we deployed monitoring traps next to the Malaise traps because another experiment was being conducted in the same field. However, because the monitoring traps proved to be very attractive to *D. suzukii* in 2014, we placed the Malaise and monitoring traps farther apart in 2015 to reduce the likelihood of interference occurring between the two types of traps. We also added an extra pair of monitoring traps in 2015 so that each Malaise trap had an equidistant monitoring trap set up on either side. In both years, monitoring traps in line with the Malaise traps were set up ~3 m away from the end of the crop row.

Monitoring traps were also checked hourly as described above for the Malaise traps. Flies were collected from the surface of the bait using soft forceps and placed into vials with 70% ethanol; the contents of each monitoring trap were then poured through a handheld kitchen strainer to look for flies that may have drowned. During 2014 collections, we observed that many flies were present on the outside of the monitoring traps but did not necessarily enter them. Therefore, starting in late July 2014 and during the entire 2015 season, flies were aspirated off the surface of monitoring traps using a handheld aspirator (BioQuip, Rancho Dominguez, CA) for one minute before flies were collected from within traps. Flies collected off the surface of monitoring traps were transferred to a separate vial

with 70% ethanol. All Drosophilidae captured were separated by *D. suzukii* and non-*D. suzukii*, sexed, counted, and preserved for later identification to species.

Two WatchDog® data loggers (Model A150, Spectrum Technologies, Inc., Aurora, IL) housed within radiation shields (WatchDog® Item Number 3663A) were used to record hourly ambient temperatures during each sampling period in 2015. One logger was placed within the crop field ~60 m away from the wooded edge, while another was placed along the wooded edge in line with the other logger. Both loggers were hung ~1.5 m off the ground, on a trellis post within the crop field and on a tree branch along the wooded edge.

Oviposition behavior, 2015

To determine when *D. suzukii* females lay eggs in fruit throughout the day, infestation rates were compared in blackberries that were exposed to wild flies from 6-10am, 10am-2pm, 2-6pm, 6-10pm, or overnight from 10pm-6am. On 20 July at Farm 1, small 5-7" mesh bags were placed over clusters of unripe blackberries to prevent oviposition. Six clusters were bagged in each of the 10 rows (60 clusters total) between the two northernmost Malaise traps. Clusters were bagged in the first trellis section of each row, closest to the wooded edge, and were left to ripen in the bags. During the next sampling period (3-4 August), one bag was removed in each row during each time period. At the end of each time period, all of the ripe berries on each cluster were collected, brought back to the lab and weighed, and were individually suspended in an organza sling inside a 2 oz. plastic portion cup with holes poked in the bottom to promote juice drainage. Berries were held in a growth chamber at 20°C for

10 days, at which time they were dissected and all pupae moved to a small Petri dish with a moistened paper towel square until adults emerged and were confirmed to be *D. suzukii*.

Data analyses

Movement between crop fields and non-crop habitat, 2014-2015

D. suzukii captures in Malaise traps were too low to allow formal statistical analysis.

However, sufficient numbers of other drosophilid species were captured in Malaise traps for statistical analysis. To determine if there are diurnal and/or seasonal patterns associated with the movement of other drosophilid species between crop fields and non-crop habitat, we compared the number of female, male, and total (females and males combined) non-*D. suzukii* drosophilids caught moving into or out of the crop field during five diurnal time periods and during preharvest, harvest, and postharvest periods. Data were analyzed using a generalized linear mixed model with a lognormal distribution and an identity link in PROC GLIMMIX in SAS v. 9.4. Direction of movement (into the field and out of the field), time period (6-10am, 10am-2pm, 2-6pm, 6-10pm, and 10pm-6am), and season (preharvest, harvest, and postharvest) were included in the model as fixed effects, while year, site, and trap were included as random effects. Post hoc means comparisons were conducted using the Tukey-Kramer test in this and all of the following analyses.

Attraction to monitoring traps with a fermentation-based bait, 2014-2015

Because large numbers of flies were observed on the outside of traps, we first compared the number of flies aspirated off the surface of traps to the number of flies collected within traps.

We calculated the total numbers of *D. suzukii* and of other drosophilid species captured at individual monitoring traps using each collection method during each 24-hour sampling period when both collection methods were used (final two sampling dates in 2014 and throughout the 2015 season). We analyzed data for *D. suzukii* (females, males, and total) and other drosophilid species (females, males, and total) separately using generalized linear mixed models with a lognormal distribution and an identity link in PROC GLIMMIX in SAS v. 9.4. Collection method (aspirated off the surface of traps vs. collected within traps) and trap placement (within the crop field vs. between the crop field and wooded edge) were included in the model as fixed effects, while year, season, site, transect, and trap were included as random effects. We also compared the numbers of males and females aspirated off the surface of traps to those collected within traps for both *D. suzukii* and other drosophilids in separate two-way analyses with sex and collection methods as fixed effects.

For the remaining analyses, the number of flies aspirated off the surface of a trap were combined with the number of flies captured within the trap to calculate a trap total. For the monitoring traps, we conducted a two-part analysis to determine if there are diurnal and seasonal patterns associated with trap captures of *D. suzukii* and other drosophilid species in monitoring traps with a fermentation-based bait. First, we wanted to determine if there are daily activity patterns associated with trap attraction and whether these patterns are affected by where traps were placed within the system (in the crop field vs. between the crop field and wooded edge). We calculated the total numbers of *D. suzukii* and other drosophilid species captured at individual traps during each daily time period and analyzed the data using a generalized linear mixed model with a lognormal distribution and an identity link in PROC

GLIMMIX in SAS v. 9.4. Time period (6-10am, 10am-2pm, 2-6pm, 6-10pm, and 10pm-6am) and trap placement (within the crop field or between the crop field and the wooded edge) were included in the model as fixed effects, while season, year, site, transect, and trap were included in the model as random effects. Next, we wanted to determine if daily patterns of trap attraction changed over the course of the season and compared the numbers of *D. suzukii* and other drosophilid species captured during five daily time periods and during preharvest, harvest, and postharvest periods. Data were analyzed using a generalized linear mixed model with a lognormal distribution and an identity link in PROC GLIMMIX in SAS v. 9.4. Time period (6-10am, 10am-2pm, 2-6pm, 6-10pm, and 10pm-6am) and season (preharvest, harvest, and postharvest) were included in the model as fixed effects, while trap placement, year, site, transect, and trap were included in the model as random effects.

Oviposition behavior, 2015

To determine if there are diurnal patterns associated with the oviposition behavior of *D. suzukii* females, we conducted a two-part analysis. First, to determine if the likelihood of a berry becoming infested differs throughout the day, the ratio of infested berries (number of events) over the number of exposed berries (number of trials) for clusters exposed during the five time periods were compared. Data were analyzed using a generalized linear mixed model with a binomial distribution and a logit link in PROC GLIMMIX in SAS v. 9.4, with time period (6-10am, 10am-2pm, 2-6pm, 6-10pm, and 10pm-6am) and row (1-10) included in the model as fixed effects. Next, we determined if infestation rate varied among berries exposed during the five time periods using a generalized linear mixed model with a

lognormal distribution and an identity link in PROC GLIMMIX in SAS v. 9.4. Time period was included in the model as a fixed effect, while row and cluster were included as random effects.

Results

Preliminary analyses suggest that at least 17 naturally occurring drosophilid species in 9 genera were present in our samples. Ambient temperatures were a few degrees higher in crop fields during daylight hours than along wooded edges in 2015 and vice versa during the nighttime (Fig 2.2).

Movement between crop fields and non-crop habitat, 2014-2015

Diurnal patterns

A total of 595 non-*D. suzukii* drosophilids were caught in the Malaise traps over the course of the study. Overall, the movement of other drosophilid species into and out of the crop field followed a U-shaped pattern. Peaks of movement in both directions occurred during the morning (6-10am) and evening (6-10pm) hours, with less movement observed during the midday (10am-2pm), afternoon (2-6pm), and overnight (10pm-6am) hours (Fig. 2.3). More females were caught in the Malaise traps during the evening hours than during the other four time periods ($F_{4,667} = 28.14$, $P < 0.0001$). Females were also caught more often during the morning hours than during the midday and afternoon hours, while more females were caught overnight than during the afternoon hours. More males of other drosophilid species were also caught in the Malaise traps during the evening hours than during the other four time periods

($F_{4,667} = 22.36$, $P < 0.0001$). When females and males were combined, more flies were caught during the evening hours than during the other four time periods, while more flies were also caught during the morning and overnight hours than during the midday and afternoon hours ($F_{4,667} = 38.30$, $P < 0.0001$).

A total of 31 *D. sukukii* were caught in the Malaise traps over the course of the study. As was true for other drosophilid species, *D. sukukii* movement into and out of the crop field followed a U-shaped pattern with peaks of movement in both directions during the morning and evening hours (Fig. 2.3). Very few *D. sukukii* were caught in the Malaise traps from 10am-2pm (midday), and no *D. sukukii* were caught during the afternoon hours from 2-6pm (Fig. 2.3).

Flies were not caught moving into and out of crop fields with equal frequency. More non-*D. sukukii* drosophilids were caught moving into crop fields than were caught moving out of crop fields overall (female: $F_{1,667} = 17.10$, $P < 0.0001$; male: $F_{1,667} = 22.80$, $P < 0.0001$; total: $F_{1,667} = 28.70$, $P < 0.0001$; Fig. 2.3). Patterns of movement into and out of crop fields differed among time periods for female, male, and total non-*D. sukukii* drosophilids (Table 2.2). More flies were caught moving into the crop field than out of the crop field during the evening hours; such differences in the numbers of flies moving into and out of the crop field were not observed during the other four time periods.

All *D. sukukii* males that were caught in the Malaise traps were caught moving into the field during the evening hours and moving out of the field during the morning hours. Female *D. sukukii* were caught moving in both directions during the evening and morning hours.

Seasonal patterns

Individuals of non-*D. suzukii* drosophilid species were captured in the Malaise traps on all the sampling dates at both sites in both years. Similar numbers of female, male, and total drosophilids were caught in the Malaise traps during the preharvest, harvest, and postharvest periods (female: $F_{2,667} = 0.57$, $P = 0.5672$; male: $F_{2,667} = 1.13$, $P = 0.3243$; total: $F_{2,667} = 0.83$, $P = 0.4345$). However, patterns associated with the direction of movement did change over the course of the season for female and total non-*D. suzukii* drosophilids. There was a significant interaction between direction of movement and season (female: $F_{2,667} = 3.45$, $P = 0.0324$; total: $F_{2,667} = 3.59$, $P = 0.0283$). There were always a lot of flies moving into the field regardless of the season, but more flies were caught moving out of the field during the postharvest period than during the preharvest period (Table 2.3).

More *D. suzukii* were caught in the Malaise traps in 2015 ($n = 28$) than in 2014 ($n = 3$). Although individuals of other drosophilid species were captured in the Malaise traps on all the sampling dates at both sites in both years, we did not catch *D. suzukii* in the Malaise traps until mid-July in 2014 and 2015 and on some subsequent sampling dates (Table 2.3).

Attraction to monitoring traps with a fermentation-based bait, 2014-2015

We observed *D. suzukii* male courting behavior, male-male aggression, and mating pairs on the surface of some monitoring traps, as well as on nearby fruits and leaves. Males of other drosophilid species were also observed to court other flies on the surface of monitoring traps.

Diurnal patterns

Non-*D. suzukii* drosophilid species were captured inside of monitoring traps more often than they were aspirated off the surface of traps (Table 2.4). These patterns were not affected by trap placement; results were similar for traps placed within the crop field and for traps placed between the crop field and wooded edge (collection method x trap placement: other female drosophilids: $F_{1,220} = 0.09$, $P = 0.7622$; other male drosophilids: $F_{1,220} = 0.16$, $P = 0.6937$; other drosophilids in total: $F_{1,220} = 0.00$, $P = 0.9517$). Females and males of other drosophilid species were equally likely to be caught within traps or on the surface of traps (sex x collection method: $F_{1,471} = 2.43$, $P = 0.12$).

Unlike other drosophilid species, *D. suzukii* were caught within traps as often as they were aspirated off the surface of traps (Table 2.4). These results were similar for traps placed within the crop field and for those placed between the crop field and wooded edge (collection method x trap placement: female *D. suzukii*: $F_{1,220} = 0.05$, $P = 0.8219$; male *D. suzukii*: $F_{1,220} = 0.57$, $P = 0.4527$; total *D. suzukii*: $F_{1,220} = 0.12$, $P = 0.7286$). As was true for other drosophilid species, *D. suzukii* females and males did not differ in the frequency with which they were aspirated off the surface of traps or were collected within them (sex x collection method: $F_{1,471} = 3.77$, $P = 0.053$).

The capture of non-*D. suzukii* drosophilids at monitoring traps followed a U-shaped pattern, with peaks during the morning (6-10am) and evening (6-10pm) hours and very little capture observed during the midday (10am-2pm), afternoon (2-6pm), and overnight (10pm-6am) hours (Fig. 2.3). Overall, more flies were caught at monitoring traps during the evening and morning hours than during the midday, afternoon, and overnight hours. However, the

number of non-*D. suzukii* drosophilids caught at monitoring traps during the five time periods was affected by where traps were placed within the system (time period x trap placement: females: $F_{4,724} = 6.18$, $P < 0.0001$; males: $F_{4,724} = 2.86$, $P = 0.0227$; total: $F_{4,724} = 5.97$, $P < 0.0001$) (Table 2.5). During the midday, afternoon, evening, and overnight hours, flies were captured equally often at traps located within the crop field and between the crop field and wooded edge. However, during the morning hours, significantly more flies were caught at monitoring traps placed between the crop field and wooded edge than at traps placed within the crop field.

The diurnal patterns of attraction to monitoring traps exhibited by *D. suzukii* were similar to those of other drosophilid species (Fig 2.3). Nearly twice as many *D. suzukii* females were caught at monitoring traps during the evening hours than during the morning hours, while very few females were caught during the midday, afternoon, and overnight hours (Table 2.5). Similarly, more male and total *D. suzukii* were caught at monitoring traps during the evening and morning hours than during the midday, afternoon, and overnight hours. Unlike other drosophilids, the number of *D. suzukii* caught at monitoring traps during the five time periods was not affected by where traps were placed within the system. More female, male, and total *D. suzukii* were caught at traps placed between the crop field and wooded edge than in traps placed within the crop field (Table 2.5; Fig. 2.3).

Seasonal patterns

Females and males of non-*D. suzukii* drosophilid species were captured at monitoring traps on all sampling dates in 2014 and 2015. In contrast, *D. suzukii* females and males were not

caught until 11 July and 25 July in 2014, respectively. Although several *D. suzukii* females and males were caught at monitoring traps in early June in 2015, very few additional flies were caught until mid to late July.

Activity at monitoring traps increased as the season progressed. More non-*D. suzukii* drosophilid flies were caught during the harvest period than during the preharvest period, and more were caught during the postharvest period than either during the preharvest and harvest periods (females: $F_{2,720} = 25.21$, $P < 0.0001$; males: $F_{2,720} = 19.99$, $P < 0.0001$; total: $F_{2,720} = 27.86$, $P < 0.0001$). The diurnal patterns of attraction to monitoring traps exhibited by other drosophilid species changed over the course of the season. Preharvest, the numbers of other drosophilids caught at monitoring traps did not differ among the five daily time periods. During both the harvest and postharvest periods, however, more other drosophilids were caught during the evening and morning hours than were caught during the midday, afternoon, and overnight hours (Table 2.6).

As was true for other drosophilid species, the number of *D. suzukii* flies caught at monitoring traps also increased over the course of the season. More female and total *D. suzukii* were caught during the postharvest period than during both the preharvest and harvest periods, while more *D. suzukii* males were caught during the postharvest period than during the preharvest period (females: $F_{2,720} = 11.91$, $P < 0.0001$; males: $F_{2,720} = 3.68$, $P = 0.0256$; total: $F_{2,720} = 11.08$, $P < 0.0001$). Very few *D. suzukii* were caught preharvest and were caught in monitoring traps equally often during the five daily time periods. During the harvest period, however, more female, male, and total *D. suzukii* were caught during the evening hours than during the midday, afternoon, and overnight hours. In addition, more

male and total *D. suzukii* were also caught during the morning hours than during the midday, afternoon, and overnight hours. Postharvest, more female, male, and total *D. suzukii* were caught during the evening and morning hours than during the midday and afternoon hours. More female and total *D. suzukii* were also caught during the evening hours than during the morning and overnight hours, while more males were caught during the evening hours than during the morning hours (Table 2.6).

***D. suzukii* oviposition behavior, 2015**

Clusters of previously-bagged berries were exposed during five time periods on 3-4 August 2015 at Farm 1. No *D. suzukii* were reared from berries exposed from 10am-2pm and overnight from 10pm-6am (Table 2.7); these two periods correspond with times of the day when little to no *D. suzukii* activity was observed at the Malaise and monitoring traps. Conversely, at least one *D. suzukii* was reared from berries exposed during the three remaining time periods. Infestation rates were higher in berries exposed during the evening hours, although the likelihood that a berry would become infested did not differ between time periods ($F_{4,27} = 1.62, P = 0.1987$) or among the crop rows where berries were exposed ($F_{9,27} = 0.46, P = 0.8871$) (Table 2.7).

Discussion

We found that *D. suzukii* detection was related to both time of day and time of year, and that the diurnal and seasonal behaviors exhibited by *D. suzukii* were similar to those of other drosophilid species present in the system – but also different in some important ways.

Other drosophilid species were captured in Malaise traps and at monitoring traps on all sampling dates during the two years of study, whereas *D. suzukii* were not captured until mid-July in both years. This difference likely reflects the fact that multiple species of other non-*D. suzukii* drosophilids were present in our samples, many of which have very different life histories than *D. suzukii*. *Drosophila affinis*, one of the most abundant species captured in traps during both years, was previously reared from decomposing blackberries collected from under wild blackberry bushes in western NC (Miller and Weeks 1964). Females of other species may have been attracted to different oviposition or food resources within the system, such as fungi, tree sap, or plant leaves, that were available at throughout or at various times of the season. Because *D. suzukii* uses ripening and ripe fruits for oviposition, it is unsurprising that they were not caught in large numbers in either the Malaise traps or monitoring traps until mid-July in both years, which is during the main part of the blackberry harvest season.

D. suzukii appears to have similar daily activity patterns in the wild as other drosophilids (Miller & Weeks, 1964; Mitchell & Epling, 1951) with most activity occurring during two distinct periods of the day, between 6pm and sunset and between sunrise and 10am. Interestingly, more females and males of other drosophilid species were caught within monitoring traps, whereas equal numbers of *D. suzukii* females and males were caught on the surface of and within traps. *D. suzukii* may spend more time on the surface of monitoring traps than other species and may be oriented to the fermenting attractant in these traps for different reasons. Our observations of courting and mating behavior on the surface on monitoring traps suggest that *D. suzukii* may be attracted to traps not only because they are

seeking food, but also to interact with conspecifics. Monitoring traps may provide male *D. suzukii* with a suitable substrate on which to perform their courtship dance, which involves both a visual display and substrate-borne vibrations (Fuyama, 1979; Mazzoni *et al.*, 2013; Revadi *et al.*, 2015).

More non-*D. suzukii* drosophilids were caught at monitoring traps placed within crop fields than in traps placed between crop fields and wooded edges, while the opposite pattern was true for *D. suzukii*. *D. suzukii* has been shown to be more sensitive than *D. melanogaster* to volatiles associated with the fruit-ripening process (Abraham *et al.*, 2015) and to leaf odors (Keeseey *et al.*, 2015). As such, the presence of fruit or the plants themselves may have interfered with *D. suzukii* attraction to traps within crop fields. Other species that are primarily attracted to rotting substrates for oviposition or food, such as *D. affinis*, may have been more likely to detect and get caught in monitoring traps within crop fields. In addition, it is possible that monitoring traps were not equally attractive to *D. suzukii* females at different reproductive stages and that older egg-laying females were more attracted to ripe fruits than to monitoring traps within crop fields (Swoboda-Bhattarai *et al.*, 2017).

Fruit infestation appears to occur during the same time periods when flies were captured in monitoring traps. Some small degree of egg laying appears to occur during the day despite the lack of trap captures, but the highest rates of infestation were observed during the evening hours preceding sunset. While there was not a significant difference in the probability of infestation during the day, significantly higher infestation rates were observed during the evening hours preceding sunset. Although few studies have looked at the diurnal timing of oviposition behavior in *D. suzukii*, a peak of oviposition activity in cut grapes was

observed during the 8pm-midnight hours of a 16:8 light:dark cycle (lights on from 4:30am to 8:30pm), although some eggs were laid throughout the 24-hr test period (Lin *et al.*, 2014).

A number of different factors likely drive our observations. Some clearly relate to abiotic conditions. *Drosophila* species observed in California showed two peaks of daily activity in their natural habitats, one in the morning and another before sunset (Dobzhansky & Epling, 1944). In another early study, it was suggested that temperature, humidity, and light might be factors that limit the periods during which *Drosophila* visit food sources (Michell & Epling, 1951). In a more recent study, it was suggested that the high levels of activity exhibited by *D. subobscura* and *D. pseudoobscura* near sunrise and sunset could be explained by decreasing sun angles (Noor, 1998). At our sampling locations, inflection points in ambient temperature occurred between 7-8pm and 7-8am; temps were higher within the crop field than along the wooded edge during the day, but switched at night. Because *D. suzukii* has a limited tolerance for high temperatures and low relative humidity (Tochen *et al.* 2014, 2015), these observations could explain why we saw peaks of movement into crop fields during the evening hours. Weather conditions have also been shown to play a role in determining activity patterns. Neotropical species of *Drosophila* were active in the morning and before sunset on clear, warm, and dry days, but were mostly quiescent during the middle part of the day; on rainy days, however, the flies were active throughout the day (Pavan *et al.*, 1950). We sampled on days with predominately clear conditions, which likely influenced the daily activity patterns we observed.

Crop phenology, or more broadly resource availability, may be the main driver of some of our observations. *D. suzukii* are not common in fields until host crop fruit ripen, but

naturally occurring non-*D. suzukii* drosophilids were captured at all sample dates. The other drosophilid species captured utilize a wide range of natural materials for oviposition, including tree sap, various types of fungi, rotting fruit, and even spittle masses created by spittlebug nymphs (*Cladochaeta* spp.). Due to their highly polyphagous nature, when resources become scarce and/or population densities exceed optimal levels within an available resource, it is thought that *D. suzukii* migrate to more favorable habitats (Mitsui *et al.*, 2010). Our data also suggest that *D. suzukii* might also move between habitats that vary in favorability or resource availability on a much finer geographic scale. *D. suzukii* is known to utilize both blackberry crops and non-crop hosts present along wooded edges (Diepenbrock *et al.*, 2016). In another study, following weekly treatments of field margin vegetation with a 10% chicken egg white mark solution, Klick *et al.* (2016) caught more marked flies and total flies in field margins containing Himalayan blackberry, a known non-crop host, than in field margins without a non-crop host present. Similarly, more flies were caught in crop fields near patches of Himalayan blackberry than near areas without non-crop hosts, suggesting that field margins containing alternative hosts may result in increased pest pressure within crop fields if *D. suzukii* move from such areas into crop fields, which Malaise trap captures suggest that they do.

Finally, some of our observations are likely driven primarily by human activity. We conducted our research at commercially managed farms, where insecticides were applied at least weekly in an attempt to prevent *D. suzukii* infestation. These insecticide applications may prevent flies from exiting the field, which is a limitation of our experimental design. Insecticide applications may also impact trap captures within the field, which was observed

in both conventional and organic raspberry production systems in California (Hamby *et al.*, 2014). Similar to previous research (Klick *et al.*, 2016; Pelton *et al.*, 2016), our results also suggest that growers interested in using traps to monitor for *D. suzukii* should place traps in the area between crop fields and wooded edges or other types of non-crop habitat to maximize the number of flies caught.

Our results present a picture of *D. suzukii* movement in the context of naturally co-occurring congeneric species and also have significant implications for management recommendations. When pesticide treatments are needed, growers should apply insecticides during the evening to increase the likelihood of adult *D. suzukii* exposure during times when they are laying eggs. This recommendation has benefits for species other than *D. suzukii*, however. Because pollinators are often most active during the midday hours, the adoption of an evening spray schedule for *D. suzukii*, whenever possible, could have additional benefits such as increased pollination for caneberries and other indeterminately-fruiting crops attacked by *D. suzukii*. As we develop a more complete understanding of what drives *D. suzukii* activity, this information should be incorporated into predictive tools to model risk and suggest mitigation.

In conclusion, some important insights into the behavior and activity periods of *D. suzukii* were gained during this study that have important implications for its management in fruit crops. Understanding the timing and direction of movement between hosts will allow for the improved timing of insecticide applications. Based on our results, it might be most effective for growers to apply insecticides during periods of high *D. suzukii* activity, i.e., late in the day or early in the morning, to increase the probability of *D. suzukii* adults coming into

contact with a lethal dose of insecticide. Understanding the directionality of *D. suzukii* movement will also be useful for potential future management strategies, including attract and kill, mass trapping, and augmentative biological control. Based on our preliminary results, it might be effective to deploy attract and kill traps with a fermentation-based bait in the area between a wooded edge or other type of non-crop habitat and the crop field to be protected. Ultimately, such tactics would reduce the non-target effects of pesticides and reduce pesticide residues on fruit and in the environment, and would help to sustain the small fruits industry in the United States.

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TABLES

Table 2.1. Dates when traps were deployed at two blackberry farms during 2014 and 2015, sampling period, crop phenology, and the sunset and sunrise times for Cleveland County, NC.

Farm	Week	Date	Start time	Fruiting period	Sunset	Sunrise
2014						
1	2	14-15 June	12 PM	Pre-fruiting	8:41 PM	6:10 AM
	6	11-12 July	5 PM	Full fruiting, harvest period	8:42 PM	6:20 AM
	8	25-26 July	12 PM	Full fruiting, harvest period	8:34 PM	6:30 AM
	13	30-31 August	2 PM	Postharvest	7:55 PM	6:57 AM
2	1	6-7 June	7 PM	Pre-fruiting	8:39 PM	6:11 AM
	3	21-22 June	2 PM	Some ripe fruit present	8:44 PM	6:11 AM
	5	5-6 July	2 PM	Full fruiting, harvest period	8:45 PM	6:17 AM
2015						
1	1	6-7 June	2 PM	Pre-fruiting	8:37 PM	6:11 AM
	3	20-21 June	1 PM	Some ripe fruit present	8:43 PM	6:11 AM
	5	6-7 July	11 AM	Full fruiting, harvest period	8:43 PM	6:17 AM
	7	19-20 July	10 AM	Full fruiting, harvest period	8:38 PM	6:25 AM
	9	3-4 August	10 AM	Full fruiting, harvest period	8:27 PM	6:36 AM
	11	17-18 August	11AM	Some ripe fruit remaining	8:12 PM	6:47 AM
	13	29-30 August	11 AM	Postharvest	7:57 PM	6:56 AM
2	2	12-13 June	1 PM	Some ripe fruit present	8:42 PM	6:10 AM
	4	28-29 June	3 PM	Full fruiting, harvest period	8:45 PM	6:14 AM
	6	--	--			
	8	24-25 July	2 PM	Full fruiting, harvest period	8:36 PM	6:29 AM
	10	--	--			
12	24-25 August	11AM	Postharvest	8:04 PM	6:53 AM	

Table 2.2. Mean (\pm SE) number of females, males, and females and males combined (total) of non-*D. suzukii* drosophilids captured moving into or out of the crop field during five diurnal time periods at two commercial blackberry farms in Cleveland County, NC, in 2014 and 2015.

Direction of movement	Time period*					
	6-10am	10am-2pm	2-6pm	6-10pm	10pm-6am	
Females						
Into field	0.5 \pm 0.10b	0.1 \pm 0.03cd	0.1 \pm 0.03cd	2.6 \pm 0.59a	0.5 \pm 0.13bcd	$F_{4,667} = 11.79$
Out of field	0.4 \pm 0.11bcd	0.2 \pm 0.06bcd	0.0 \pm 0.00d	0.5 \pm 0.12bc	0.3 \pm 0.09bcd	$P < 0.0001$
Males						
Into field	0.4 \pm 0.08b	0.0 \pm 0.02b	0.1 \pm 0.03b	1.7 \pm 0.37a	0.3 \pm 0.07b	$F_{4,667} = 12.00$
Out of field	0.2 \pm 0.06b	0.1 \pm 0.04b	0.0 \pm 0.01b	0.3 \pm 0.08b	0.2 \pm 0.07b	$P < 0.0001$
Total						
Into field	0.9 \pm 0.13b	0.1 \pm 0.03d	0.1 \pm 0.05cd	4.3 \pm 0.93a	0.8 \pm 0.15bc	$F_{4,667} = 15.32$
Out of field	0.6 \pm 0.14bcd	0.3 \pm 0.08cd	0.0 \pm 0.01d	0.8 \pm 0.16bc	0.5 \pm 0.13bcd	$P < 0.0001$

*Means that share a letter within rows and columns for each separate analysis (female, male, and total) are not significantly different at $\alpha = 5\%$.

Table 2.3. Dates of first capture and mean (\pm SE) number of females, males, and females and males combined (total) of *D. suzukii* and non-*D. suzukii* drosophilids captured moving into or out of the crop field during the preharvest, harvest, and postharvest periods at two commercial blackberry farms in Cleveland County, NC, in 2014 and 2015.

Species	Sex	Captures	Direction of movement*	
			Into field	Out of field
<i>D. suzukii</i>	Females	First of year	12 Jul 2014 & 3 Aug 2015	25 Jul 2014 & 19 Jul 2015
	Males	First of year	19 Jul 2015	26 Jul 2014 & 20 Jul 2015
Other drosophilids	Females	First of year	6 Jun 2014 & 6 Jun 2015	6 Jun 2014 & 7 Jun 2015
		Preharvest	0.7 \pm 0.16a	0.1 \pm 0.05c
		Harvest	0.8 \pm 0.22ab	0.3 \pm 0.06bc
		Postharvest	0.5 \pm 0.11ab	0.4 \pm 0.10abc
			$F_{2,667} = 3.45$	
			$P = 0.0324$	
	Males	First of year	6 Jun 2014 & 6 Jun 2015	6 Jun 2014 & 7 Jun 2015
		Preharvest	0.5 \pm 0.12a	0.1 \pm 0.03a
		Harvest	0.6 \pm 0.14a	0.2 \pm 0.04a
		Postharvest	0.3 \pm 0.08a	0.2 \pm 0.05a
			$F_{2,667} = 0.70$	
			$P = 0.4976$	
Total	First of year	6 Jun 2014 & 6 Jun 2015	6 Jun 2014 & 7 Jun 2015	
	Preharvest	1.2 \pm 0.25a	0.2 \pm 0.05c	
	Harvest	1.4 \pm 0.35a	0.5 \pm 0.08bc	
	Postharvest	0.9 \pm 0.17ab	0.6 \pm 0.12abc	
		$F_{2,667} = 3.59$		
		$P = 0.0283$		

*Means that share a letter within rows and columns for each separate analysis (female, male, and total non-*D. suzukii* drosophilids) are not significantly different at $\alpha = 5\%$

Table 2.4. Mean (\pm SE) number of females, males, and total *D. suzukii* and other drosophilids aspirated off the surface of or collected within monitoring traps during 24-hour collection periods at two commercial blackberry farms in Cleveland County, NC, in 2014 and 2015.

Collection method*	Female	Male	Total
<i>D. suzukii</i>			
Aspirated off trap surface	1.7 \pm 0.28a	0.9 \pm 0.18a	2.6 \pm 0.42a
Collected within trap	4.0 \pm 0.85a	0.8 \pm 0.16a	4.8 \pm 0.99a
	$F_{1,220} = 3.53$	$F_{1,220} = 0.55$	$F_{1,220} = 0.67$
	$P = 0.0616$	$P = 0.4597$	$P = 0.4141$
Other drosophilids			
Aspirated off trap surface	3.9 \pm 0.48b	3.0 \pm 0.39b	6.9 \pm 0.81b
Collected within trap	7.6 \pm 0.97a	4.6 \pm 0.62a	12.2 \pm 1.55a
	$F_{1,220} = 19.15$	$F_{1,220} = 6.85$	$F_{1,220} = 17.28$
	$P < 0.0001$	$P = 0.0095$	$P < 0.0001$

*Means that share a letter within a column for each separate analysis (female, male, and total *D. suzukii* and other drosophilids) are not significantly different at $\alpha = 5\%$.

Table 2.5. Mean (\pm SE) number of *D. suzukii* and other drosophilids (females, males, and total) captured at monitoring traps with a fermentation-based bait during five diurnal time periods, and at traps placed within the crop field and those placed between the crop field and wooded edge, at two commercial blackberry farms in Cleveland County, NC, in 2014 and 2015.

Variable*	<i>D. suzukii</i>			Other drosophilid species			
	Females	Males	Total	Females	Males	Total	
Time period x trap placement							
6-10am	Field	0.9 \pm 0.29a	0.3 \pm 0.09a	1.29 \pm 0.33a	2.3 \pm 0.43b	1.8 \pm 0.36b	4.1 \pm 0.74b
	Edge	1.9 \pm 0.53a	0.7 \pm 0.19a	2.64 \pm 0.64a	4.7 \pm 0.66a	2.8 \pm 0.44a	7.5 \pm 1.05a
10am-2pm	Field	0.0 \pm 0.03a	0.0 \pm 0.00a	0.0 \pm 0.03a	0.2 \pm 0.06c	0.0 \pm 0.02c	0.2 \pm 0.07c
	Edge	0.0 \pm 0.01a	0.0 \pm 0.00a	0.0 \pm 0.01a	0.4 \pm 0.02c	0.0 \pm 0.02c	0.1 \pm 0.03c
2-6pm	Field	0.0 \pm 0.01a	0.0 \pm 0.00a	0.0 \pm 0.01a	0.2 \pm 0.08c	0.0 \pm 0.02c	0.3 \pm 0.09c
	Edge	0.0 \pm 0.01a	0.0 \pm 0.00a	0.0 \pm 0.01a	0.1 \pm 0.03c	0.0 \pm 0.00c	0.1 \pm 0.03c
6-10pm	Field	2.5 \pm 0.72a	0.5 \pm 0.17a	3.0 \pm 0.88a	6.1 \pm 1.33a	4.4 \pm 1.07a	10.4 \pm 2.36a
	Edge	3.4 \pm 0.85a	1.0 \pm 0.24a	4.4 \pm 1.05a	5.1 \pm 0.86a	3.3 \pm 0.46a	8.4 \pm 1.27a
10pm-6am	Field	0.0 \pm 0.02a	0.0 \pm 0.00a	0.0 \pm 0.02a	0.2 \pm 0.07c	0.3 \pm 0.10bc	0.5 \pm 0.17c
	Edge	0.0 \pm 0.04a	0.0 \pm 0.02a	0.0 \pm 0.05a	0.1 \pm 0.03c	0.0 \pm 0.03c	0.1 \pm 0.05c
		$F_{4,724} = 1.61$ $P = 0.1692$	$F_{4,724} = 1.94$ $P = 0.1021$	$F_{4,724} = 1.85$ $P = 0.1178$	$F_{4,724} = 6.18$ $P < 0.0001$	$F_{4,724} = 2.86$ $P < 0.0001$	$F_{4,724} = 5.97$ $P < 0.0001$
Time period							
	6-10am	1.4 \pm 0.31b	0.5 \pm 0.11a	2.0 \pm 0.37a	-	-	-
	10am-2pm	0.0 \pm 0.02c	0.0 \pm 0.00b	0.0 \pm 0.02b	-	-	-
	2-6pm	0.0 \pm 0.01c	0.0 \pm 0.00b	0.0 \pm 0.01b	-	-	-
	6-10pm	2.9 \pm 0.56a	0.8 \pm 0.15a	3.7 \pm 0.69a	-	-	-
	10pm-6am	0.0 \pm 0.02c	0.0 \pm 0.01b	0.0 \pm 0.03b	-	-	-
		$F_{4,724} = 40.03$ $P < 0.0001$	$F_{4,724} = 26.15$ $P < 0.0001$	$F_{4,724} = 44.76$ $P < 0.0001$			
Trap placement							
	Within field	0.7 \pm 0.17b	0.2 \pm 0.04b	0.9 \pm 0.21b	-	-	-
	Near woods	1.1 \pm 0.22a	0.4 \pm 0.07a	1.5 \pm 0.27a	-	-	-
		$F_{1,724} = 5.01$ $P = 0.0255$	$F_{1,724} = 6.44$ $P = 0.0114$	$F_{1,724} = 6.31$ $P = 0.0122$			

*Means that share a letter within columns for each separate analysis (time period x trap placement, time period, and trap placement) are not significantly different at $\alpha = 5\%$.

Table 2.6. Mean (\pm SE) number of female, male, and total *D. suzukii* and other drosophilids captured during five diurnal time periods, and at traps placed within the crop field and those placed between the crop field and wooded edge, at two commercial blackberry farms in Cleveland County, NC, in 2014 and 2015.

Time period*	<i>D. suzukii</i>			Other drosophilids		
	Preharvest	Harvest	Postharvest	Preharvest	Harvest	Postharvest
Females						
6-10am 5	0.3 \pm 0.13a	0.6 \pm 0.18ab	4.3 \pm 1.12b	1.3 \pm 0.48a	2.5 \pm 0.45a	8.0 \pm 1.01a
10am-2pm 1	0.1 \pm 0.06a	0.0 \pm 0.02b	0.0 \pm 0.02c	0.0 \pm 0.00a	0.0 \pm 0.02b	0.3 \pm 0.12b
2-6pm 2	0.0 \pm 0.00a	0.0 \pm 0.02b	0.0 \pm 0.00c	0.0 \pm 0.03a	0.2 \pm 0.07b	0.2 \pm 0.07b
6-10pm 3	0.0 \pm 0.00a	1.9 \pm 0.57a	7.9 \pm 1.71a	0.3 \pm 0.15a	5.2 \pm 1.12a	11.1 \pm 1.6a
10pm-6am 4	0.0 \pm 0.00a	0.0 \pm 0.03b	0.0 \pm 0.00c	0.0 \pm 0.00a	0.2 \pm 0.05b	0.0 \pm 0.00b
	$F_{8,720} = 19.63$ $P < 0.0001$			$F_{8,720} = 19.02$ $P < 0.0001$		
Males						
6-10am	0.4 \pm 0.16a	0.5 \pm 0.16a	0.8 \pm 0.19b	0.6 \pm 0.23a	1.6 \pm 0.31a	5.4 \pm 0.72a
10am-2pm	0.0 \pm 0.00a	0.0 \pm 0.00b	0.0 \pm 0.00c	0.0 \pm 0.00a	0.0 \pm 0.00b	0.1 \pm 0.06b
2-6pm	0.0 \pm 0.00a	0.0 \pm 0.00b	0.0 \pm 0.00c	0.0 \pm 0.00a	0.0 \pm 0.01b	0.0 \pm 0.03b
6-10pm	0.0 \pm 0.00a	0.6 \pm 0.18a	1.9 \pm 0.41a	0.5 \pm 0.15a	3.2 \pm 0.72a	8.0 \pm 1.48a
10pm-6am	0.0 \pm 0.04a	0.0 \pm 0.01b	0.0 \pm 0.00c	0.1 \pm 0.05a	0.2 \pm 0.07b	0.0 \pm 0.00b
	$F_{8,720} = 8.82$ $P < 0.0001$			$F_{8,720} = 18.00$ $P < 0.0001$		
Total						
6-10am	0.8 \pm 0.28a	1.1 \pm 0.31a	5.1 \pm 1.25b	1.9 \pm 0.68a	4.1 \pm 0.74a	13.4 \pm 1.55a
10am-2pm	0.1 \pm 0.06a	0.0 \pm 0.02b	0.0 \pm 0.03c	0.0 \pm 0.00a	0.1 \pm 0.02b	0.4 \pm 0.15b
2-6pm	0.0 \pm 0.00a	0.0 \pm 0.02b	0.0 \pm 0.00c	0.0 \pm 0.03a	0.2 \pm 0.07b	0.2 \pm 0.07b
6-10pm	0.0 \pm 0.00a	2.4 \pm 0.72a	9.8 \pm 2.06a	0.8 \pm 0.25a	8.4 \pm 1.81a	19.1 \pm 2.90a
10pm-6am	0.0 \pm 0.04a	0.1 \pm 0.03b	0.0 \pm 0.00c	0.1 \pm 0.05a	0.4 \pm 0.12b	0.0 \pm 0.00b
	$F_{8,720} = 19.48$ $P < 0.0001$			$F_{8,720} = 20.25$ $P < 0.0001$		

*Means that share a letter *within columns* for each separate analysis (female, male, and total *D. suzukii* and other drosophilids) are not significantly different at $\alpha = 5\%$.

Table 2.7. Proportions of infested berries and mean infestation rates in blackberries exposed during five time periods on 3-4 August 2015 at a commercial blackberry farm in Cleveland, County, NC.

Time period*	Proportion of berries infested (# exposed)	Mean <i>D. suzukii</i> per berry
6-10am	0.18 (22)	0.3 ± 0.12ab
10am-2pm	0.00 (20)	0.0 ± 0.00b
2-6pm	0.05 (22)	0.0 ± 0.05b
6-10pm	0.33 (18)	0.5 ± 0.20a
10pm-6am	0.00 (17)	0.0 ± 0.00b
	$F_{4,27} = 1.62$	$F_{4,72} = 4.66$
	$P = 0.1987$	$P = 0.0021$

*Means that share a letter within columns are not significantly different at $\alpha = 5\%$.

FIGURES

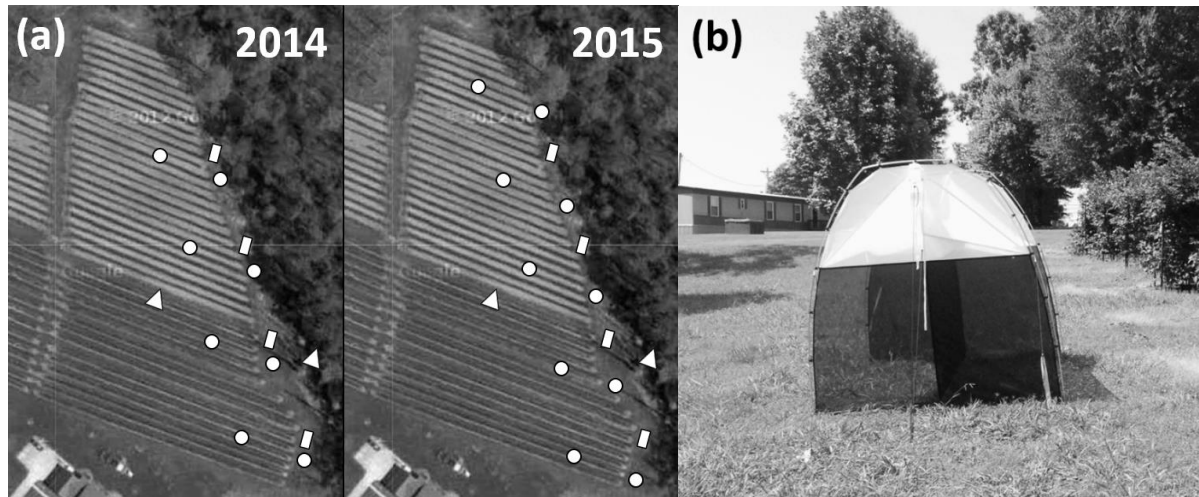


Fig. 2.1. Plot maps showing the placement and orientation of Malaise traps (rectangles) and monitoring traps with a fermentation-based bait (circles) used to determine the movement and activity patterns of *D. suzukii* at Farm 1 in North Carolina in 2014 and 2015. Data loggers (triangles) were placed within the field and along the wooded edge to record abiotic conditions (a). Side view of a Malaise trap (b).

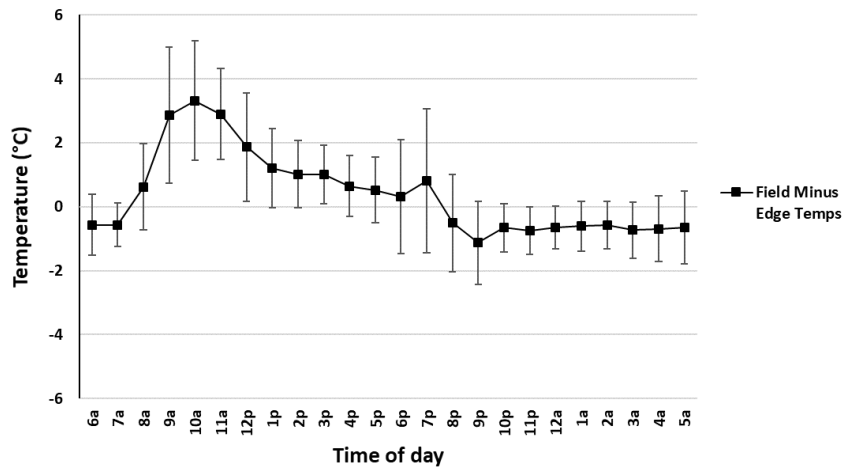


Fig. 2.2. Differences in hourly temperature measurements between crop fields and wooded edges averaged across sampling dates in 2015.

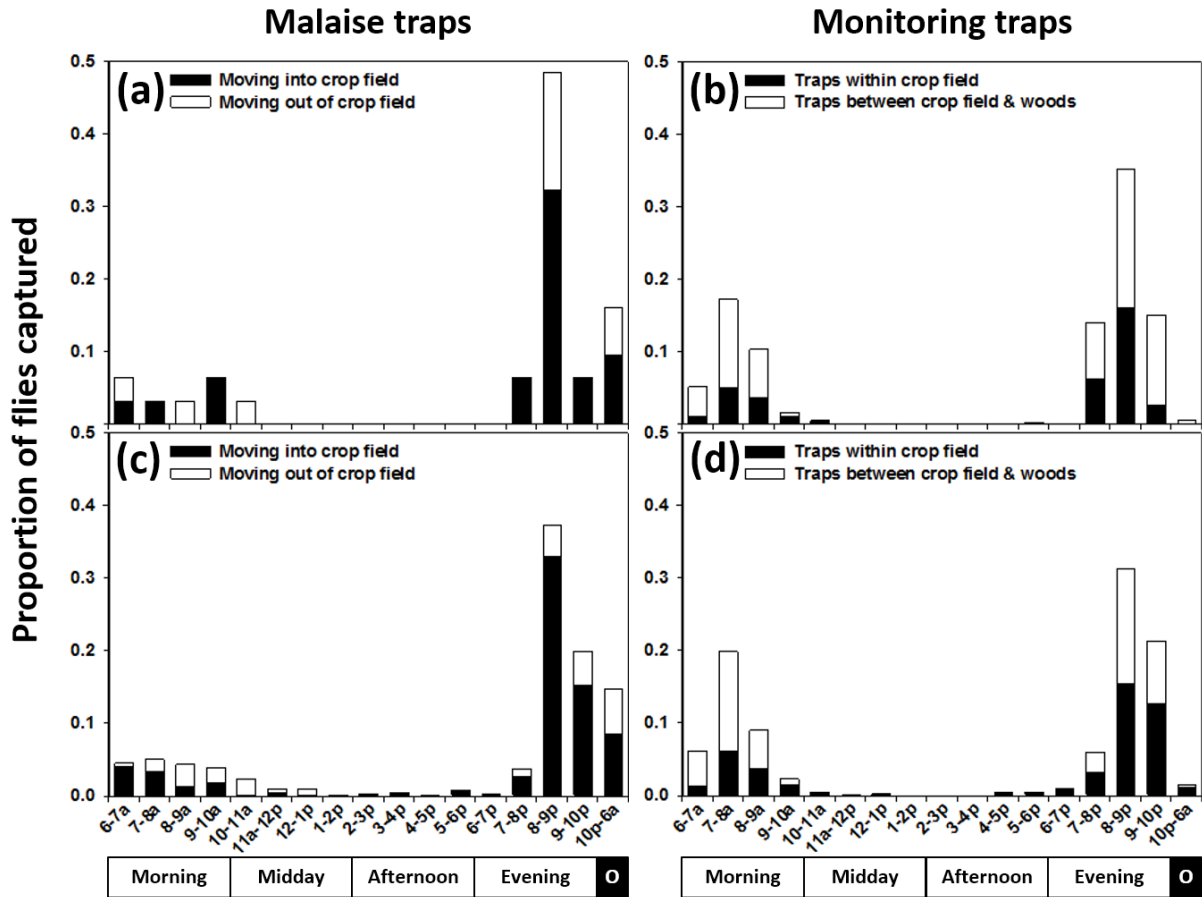


Fig. 2.3. *D. sukuzii* (a,b) and other drosophilid species (c,d) caught in Malaise traps while moving into or out of crop fields and in monitoring traps placed within crop fields or between crop fields and adjacent wooded edge at each hour of the day, expressed as proportions of the total number of individuals intercepted at the two farms during the two years of study. Hours of the day are grouped into five daily time periods including morning (6-10am), midday (10a-2p), afternoon (2-6p), evening (6-10p), and overnight (10p-6a; represented by dark square with “O”). Sample sizes: Malaise traps = 31 *D. sukuzii* and 595 other drosophilids; monitoring traps = 933 *D. sukuzii* and 2552 other drosophilids.

CHAPTER 3: *Drosophila suzukii* Infestation in Ripe and Ripening Caneberries

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Abstract

Drosophila suzukii (Matsumura) is a highly invasive vinegar fly that was first detected in the continental United States in 2008. Females use their saw-like ovipositor to lay eggs in soft-skinned fruits and severely threaten the viability of raspberry, blackberry, blueberry, cherry, and strawberry production. Females observed in no-choice laboratory bioassays laid eggs in unripe blueberries and blackberries, but most of the eggs failed to develop, perhaps because the ripening process was interrupted in the prematurely-harvested fruit. We hypothesized that eggs laid in unripe fruit in a field may be able to complete development as the fruit continues to ripen naturally. To test this hypothesis, we used fine mesh cages to prevent later egg laying by *D. suzukii* in caneberries at several ripeness stages: green-pink, pink, and ripe raspberries and in green-pink, red, purple, and ripe blackberries. We collected the fruit once ripe, and reared out and counted all *D. suzukii* present. This experiment was conducted in two contrasting field settings in 2013 and 2014: a small, unmanaged research planting with high numbers of *D. suzukii* adults and a large, managed commercial blackberry field with comparatively low numbers of *D. suzukii* adults. In the research planting, very few flies emerged from blackberries that were bagged at the green-pink stage. In general, more flies emerged from berries bagged at later stages of ripening (pink raspberries and red and purple blackberries) than from green-pink berries. In the commercial blackberry field, very few flies emerged from unripe berries bagged at any stage. Knowing which ripeness stages are susceptible to *D. suzukii* infestation under what conditions will help growers to decide when to begin protecting fruit from this damaging pest and to identify high-risk areas, or times of

the season, where management tactics could be focused to reduce *D. suzukii* infestation in fruit crops.

Keywords: *Rubus*, integrated pest management, fruit susceptibility, frugivore

INTRODUCTION

Drosophila suzukii (Matsumura), referred to as the spotted wing drosophila, is a highly invasive vinegar fly first discovered in the United States in California in 2008 and again in Florida in 2009 (Hauser, 2011). It has since been detected throughout the United States (Walsh et al., 2011; Burrack et al., 2012), and represents one of the most severe ongoing biological invasions in the Western Hemisphere (Atallah et al., 2014; Depra et al., 2014) and Europe (Cini et al., 2012). *D. suzukii* is a devastating pest of soft-skinned fruit crops and severely threatens the viability of berry production, most significantly impacting raspberries, blackberries, blueberries, cherries, and strawberries. Females use their large, serrated ovipositor to lay eggs in ripe and ripening fruit, instead of in wounded or overripe fruit like other drosophilids. Larval feeding damages fruit and decreases shelf life, while oviposition can facilitate secondary infection by bacteria, fungi, and yeast pathogens. As such, there is currently zero tolerance for *D. suzukii* larvae in fresh market fruit and a single infested fruit can result in the rejection of an entire shipment.

It is essential for growers to protect their fruit throughout its susceptible phase until harvest. Insecticides are the primary tool currently used to manage *D. suzukii*, but have limitations. Very few insecticides are both efficacious against *D. suzukii* and have short enough preharvest intervals that they can be applied to fruit that will go to market within a few days (Beers et al., 2011). The list of materials that are currently recommended for use against *D. suzukii* in caneberries in North Carolina includes a handful of materials, representing three modes of action, including organophosphates, pyrethroids, and spinosyns (SRSFC, 2015). In addition, rainfall, which is common in the southeastern United States

during the summer months, can greatly reduce the level of control achieved by some insecticides (Van Timmeren and Isaacs, 2013). With such limited tools, growers must carefully craft a season-long management plan for *D. suzukii* with enough flexibility to adjust for inclement weather and other unexpected events.

Knowing which ripeness stages are susceptible to infestation, and can support *D. suzukii* development, would allow growers to pinpoint when to initiate management. In turn, this would allow them to conserve insecticide applications for the end of the season when *D. suzukii* pressure is often highest. In previous studies of fruit susceptibility, female *D. suzukii* laid eggs in green and ripening fruit in no-choice laboratory assays (Kanzawa, 1935, 1939; Lee et al., 2011). Many of the eggs failed to develop, however, perhaps because the natural ripening process was interrupted when the fruit were harvested early and brought into the laboratory for bioassays. Therefore, the objectives of our study were twofold: 1) determine which stages of unripe caneberries are susceptible to *D. suzukii* infestation in the field; 2) determine if *D. suzukii* are able to develop in unripe fruit that are allowed to complete the natural ripening process on the plant. Because infestation in field settings can depend on characteristics of the host plant and the larger agroecosystem (Lee et al., 2015), the susceptibility of caneberry ripeness stages was tested in two contrasting field settings: a small, unmanaged research plot and a large, managed commercial field. Understanding host use and susceptibility could help growers to identify high-risk areas, or times of the season, where management tactics could be focused to reduce *D. suzukii* infestation in fruit crops.

METHODS

Varieties

Patterns of infestation in ‘Nantahala’ raspberries and NC537 blackberries were measured at Upper Mountain Research Station (UMRS) in Ashe County, North Carolina, in October 2013. Plantings were part of a replicated field trial established in 2009 with multiple varieties of both floricanefruiting and primocanefruiting raspberries and blackberries.

‘Nantahala’ is a primocanefruiting red raspberry (*Rubus idaeus* L.) released by the NCSU breeding program in 2009, which is grown commercially in North Carolina. Its large berry size and late harvest season, from late August until the first hard freeze, are the primary characteristics that distinguish ‘Nantahala’ from other cultivars of red raspberry (Fernandez et al., 2009). NC537 is an experimental cultivar of primocanefruiting blackberry in the NCSU breeding program that had high marketable yields compared to several other experimental blackberry cultivars during a performance trial at UMRS in 2007 (Fernandez and Ballington, 2010).

Infestation patterns in a commercial field of Prime-Ark® 45 blackberries located in Cleveland County, North Carolina, were measured in September-October 2014. Prime-Ark® 45 was released by the University of Arkansas breeding program in 2011 and is the first primocanefruiting cultivar with commercial quality fruit and postharvest shipping potential (Clark and Perkins-Veazie 2011). In general, primocanefruiting blackberry varieties fruit in the fall, when *D. suzukii* populations are often largest.

Approach

Small mesh bags with drawstring closures, measuring approximately 13 x 18 cm, were placed over unripe berries at various ripeness stages to prevent *D. suzukii* females from ovipositing further. Bags were secured with a strip of foam placed around the stem beneath the drawstring to prevent any insects from entering or leaving the bag. Bags were marked with colored flagging to indicate the ripeness stage of the berries within, while the number of berries within each bag was recorded. Bagged berries were left to ripen naturally on the plant and were collected once ripe.

On each sampling date, ripe berries were collected and placed individually in paper lunch bags for transport back to the laboratory. After collection, all berries were held in individual rearing containers in the laboratory until *D. suzukii* completed their development. Berries were housed in 1 or 2 oz. clear polystyrene portion cups (Dart Conex, Mason, MI) depending on their size. Each berry was suspended within the cup in a sling made from a 10 x 10 cm square of organza to promote juice drainage. Holes were poked in the bottom of each cup, which were then placed in layers on top of paper towels to wick any juice out of the bottom of the cups.

Cups were held in a growth chamber at 20°C, 16L:8D to allow flies to develop. Most berries were dissected within two weeks of collection. For each berry, dead larvae were counted during the dissection process, while all pupae were removed and placed on a moistened paper towel square in a 60 mm diameter Petri dish. Adults were sexed and counted after they emerged within the Petri dish, and any non-*D. suzukii* flies were noted. If adults

emerged inside the rearing containers, they were removed using an aspirator, frozen, sexed, and counted.

Infestation in ripe and unripe caneberries

Three ripeness stages of 'Nantahala' raspberries were tested. Green-pink berries were almost full-sized and had a slight hint of pink, but were difficult to remove from the receptacle and were collected by cutting the stem behind the berry. Pink berries were full-sized, coral-colored berries that could be pulled from the receptacle with ease. Ripe berries were blemish free berries that could have been picked for market. On 4 October 2013, 41 green-pink berries on 20 clusters and 28 pink berries on 20 clusters were bagged, while 24 ripe berries from 20 clusters were collected.

All green-pink and pink berries were ripe on 12 October and were collected and placed into rearing containers on the same day. Clusters, with the mesh bag still attached, were cut using clippers and placed into separate paper bags. Some of the raspberries became dislodged from the receptacle during transport to the laboratory; as a result of the disturbance, some larvae exited the berries but remained within the mesh bags. For these clusters, all berries and larvae were put into single rearing containers; multiple berries were placed into single containers for 6 out of 20 green-pink clusters and 4 out of 20 pink clusters.

Four ripeness stages of NC537 blackberries were tested. Green-pink berries were mostly green but had a slight hint of pink. Red berries were bright red in color, but not yet full-sized. Purple berries were nearly full-sized and dark red to purple in color, and were often mottled in appearance indicating that some drupelets were ripening faster than others.

Ripe berries were sound, blemish free berries that could have been picked for market. On 4 October 2013, 44 green-pink berries on 20 clusters, 26 red berries on 20 clusters, and 24 purple berries on 20 clusters were bagged, while 28 ripe berries from 20 clusters were collected. Berries from clusters with more than one ripe berry ($n = 7$) were placed into single rearing containers.

All purple berries were ripe on 12 October and were collected and placed into rearing containers on the same day. All red berries were ripe on 12 October except for two, which were left to ripen until 18 October. Similarly, six green-pink berries were ripe on 12 October, while the remaining berries were left to ripen until 18 October. In contrast to raspberries, no larvae exited blackberries during transport to the laboratory, so all green-pink, red, and purple berries were placed into separate rearing containers.

Infestation patterns in Prime-Ark® 45 blackberries were measured twice in 2014. On 28 September, ripe berries were collected, while unripe berries were bagged at the stages described above for NC537, with the addition of the green stage. Green berries were completely green in color and had started to swell, but had to be removed from the stem using clippers. In addition, the red stage of Prime-Ark® 45 berries often appeared more pink in color than red. The experiment was repeated two weeks later on 12 October, although only ripe berries were collected and purple and red berries bagged due to the later date.

On 28 September, 22 ripe berries from 22 clusters were collected, while 25 purple berries on 23 clusters, 40 red berries on 22 clusters, 52 green-pink berries on 22 clusters, and 54 green berries on 23 clusters were bagged. On 12 October, 20 ripe berries from 20 clusters were collected, while 23 purple berries on 20 clusters, and 23 red berries on 20 clusters were

bagged. Because the commercial Prime-Ark® 45 field was visited approximately every 3 days from 28 September to 28 October (every Wednesday and Sunday, except 26 October), detailed data were collected regarding the number of days required for berries at different initial ripeness stages to fully ripen. Once ripe, all berries were collected, transported to the laboratory in individual paper lunch sacks, weighed, and placed into individual rearing containers.

Adult fly populations

Traps were deployed during experiments in 2013 and 2014 to monitor populations of adult *D. suzukii*. Traps consisted of 32 fl oz. plastic deli containers with lids (Tripak Industrial USA, LLC, White Plains, NY) with ten 5 cm diameter entrance holes made with a soldering iron approximately 2.5 cm below the lid. Traps were hung over trellis poles using synthetic tie-down cord. Yeast and sugar bait, made from 1.69 g dry active yeast, 8.45 g sugar, and 150 ml water, was added to each trap.

Two traps, one placed in a row with raspberry cultivars and another in a blackberry row, were deployed for three weeks at UMRS in 2013, starting on 27 September. Traps were serviced and the yeast and sugar bait replaced weekly until 18 October. Two traps were deployed within blackberry rows in the commercial Prime-Ark® 45 field on 28 September 2014. Traps were serviced and the yeast and sugar bait replaced weekly until 28 October.

Data analysis

Total infestation was measured as the sum of dead larvae, dead pupae, and adult *D. suzukii* (i.e., the total number of individuals present) because fairly high mortality was observed in some of the rearing containers, likely due to poor drainage. For clusters from UMRS in which multiple fruits were placed in a single container, the total number of *D. suzukii* present was divided by the number of berries in the container, rendering an average value for the berries in the cluster. Therefore, average infestation rates in clusters of berries at different ripeness stages were compared, with ripeness as a fixed effect and cluster as a random effect in PROC MIXED in SAS 9.4. The response variable was calculated by averaging total infestation across all of the berries on a cluster, regardless of whether they were housed individually or together following collection. NC537 data were normalized using a square root transformation, while 'Nantahala' data did not require transformation. Post hoc means comparisons were performed using Tukey-Kramer's HSD. Infestation rates were low in unripe berries in the commercial Prime-Ark® 45 field in 2014. Therefore, data were analyzed using binomial regression in PROC GLIMMIX in SAS 9.4, with ripeness stage and date as fixed effects. Only those ripeness stages in which infestation was observed on either date were included in the analysis (i.e., red, purple, and ripe berries). Berries were scored as being 1) infested or 2) not infested; infested berries were those from which at least one *D. suzukii* adult emerged.

All berries from the commercial Prime-Ark® 45 field were weighed before they were placed into individual rearing containers to determine if bagging had a negative effect on berry development. The weights of berries bagged at the green, green-pink, red, and purple

stages on 28 September were determined once berries were ripe and were compared with the weights of ripe berries collected on 28 September. Data were analyzed using PROC MIXED in SAS 9.4 with ripeness stage as a fixed effect. Post hoc means comparisons were performed using Tukey-Kramer's HSD.

RESULTS

Infestation patterns in ripe and unripe caneberries

D. suzukii were able to complete development in green-pink and pink 'Nantahala' raspberries that were bagged and left to ripen on the plant at UMRS in 2013 (Fig. 3.1). On average, more *D. suzukii* emerged from ripe berries than from berries bagged at the pink or green-pink stages. Similarly, more flies emerged from berries bagged at the pink stage than from berries bagged at the green-pink stage ($F = 23.90$, $df = 2, 57$, $P < 0.0001$). *D. suzukii* were also able to complete development in green-pink, red, and purple NC537 blackberries at UMRS in 2013 (Fig. 3.2). On average, more *D. suzukii* emerged from ripe berries than from berries bagged at the purple, red, or green-pink stages. Similarly, more flies emerged from berries bagged at the purple stage than from bagged red or green-pink berries, while more flies emerged from bagged red berries than from berries bagged at the green-pink stage ($F = 32.11$, $df = 3, 75$, $P < 0.0001$).

In 2014, very few *D. suzukii* emerged from unripe Prime-Ark® 45 blackberries bagged in the commercial field on 28 September and 12 October. No infestation was detected in berries bagged at the green, green-pink, or purple stages on 28 September, while only one

of 40 red berries (2.5%) and 14 of 22 ripe berries (63.6%) were infested with *D. suzukii* (Fig 3.3). No infestation was detected in red berries bagged on 12 October, while 3 of 23 purple berries (13.0%) and 10 of 20 ripe berries (50.0%) were infested (Fig 3.3). The three ripeness stages in which infestation was observed were not equally susceptible to infestation and more *D. suzukii* emerged from ripe berries than from berries bagged at the red or purple stages ($F = 16.41$, $df = 2$, 149 , $P < 0.001$). However, differences in the susceptibility of red, purple, and ripe berries to infestation did not differ between the two sampling dates ($F = 0.02$, $df = 1$, 149 , $P = 0.90$).

Berry ripening times and weight at ripeness

Purple berries bagged in the commercial field on 28 September took 3.0 days to ripen, on average, while bagged red, green-pink, and green berries took 6.7, 16.0, and 28.2 days to ripen, respectively. Approximately 41% of berries bagged at the green stage on 28 September failed to ripen completely by 28 October, while 14.8% failed to develop altogether. Similarly, 3.8% of berries bagged at the green-pink stage on 28 September failed to ripen by 28 October, while 3.8% failed to develop. In contrast, all of the berries bagged at the red and purple stages on 28 September ripened completely by 28 October. Bagged berries that did ripen completely did not weigh less at ripeness than the ripe berries that were collected on 28 September ($F = 3.17$, $df = 4$, 154 , $P = 0.0154$) (Fig. 3.4).

Adult fly populations

At UMRS in 2013, an average of 17.1, 26.6, and 36.8 adult *D. suzukii* were caught daily in a trap placed among raspberry cultivars during the first, second, and third week of the experiment, respectively. Fewer *D. suzukii* adults were caught in a trap placed among blackberry cultivars at UMRS in 2013; an average of 1.1, 2.8, and 0.8 adults were caught daily during the first, second, and third week of the experiment, respectively. Even fewer *D. suzukii* adults were caught in the commercial field of Prime-Ark® 45 blackberries in 2014, where an average of 0.4, 0.6, 0.1, and 0.4 adults were caught daily in two traps during the first through fourth week of the experiment, respectively.

DISCUSSION

Although infestation was detected in all caneberry ripeness stages tested, in general, more flies emerged from ripe berries and berries bagged at later stages of ripening. However, 'Nantahala' raspberries that had started to turn color were highly susceptible to infestation at UMRS in 2013 and approximately 10 flies, on average, emerged from raspberries bagged at the green-pink stage. In contrast, very few *D. suzukii* emerged from NC537 blackberries bagged at the green-pink stage, suggesting that green-pink blackberries were less susceptible to infestation than green-pink raspberries grown in the same area. Nonetheless, these results add additional support to the idea that fruit are generally susceptible to infestation by *D. suzukii* once they start to turn color (Lee et al., 2012).

Infestation rates were highest in ripe berries in both the research planting and the commercial field. The physical characteristics of individual fruits may affect how susceptible

they are to infestation by *D. suzukii*. Because fruits tend to become softer as they ripen, ripe fruits and fruits at later stages of ripening may be more susceptible to infestation because they are easier for females to puncture with their ovipositor. In fact, several studies have shown a negative relationship between fruit firmness and oviposition (Lee et al., 2012; Kinjo et al., 2013; Poyet et al., 2014). Burrack et al. (2013) observed that softer fruit and artificial substrates were more heavily infested than firmer substrates in the laboratory, and that no eggs were laid in artificial substrates exceeding 52 centinewtons surface penetration force. These results suggest that some fruit, such as those at early stages of ripening, may be too firm for *D. suzukii* to lay eggs in and that *D. suzukii* may have a preferred firmness range for oviposition above which they cannot or will not lay eggs. Therefore, it is possible that the firmer nature of some berries, such as green-pink raspberries and green-pink and red blackberries, may have deterred some oviposition attempts and led to higher rates of infestation in riper berries. As such, female preference for riper fruit is one mechanism that could explain the observed patterns of infestation in both raspberries at UMRS and in blackberries at both sites. An intriguing alternative, which cannot be ruled out with the present data, is that female *D. suzukii* show no preference and lay equal numbers of eggs in all ripeness stages, but fewer individuals are able to develop in unripe berries.

It may be easier females to locate suitable oviposition sites in complex fruits, which may render them more susceptible to infestation than comparatively simple fruits. Although firmness readings are often taken for individual fruits as a whole, firmness may vary across the surface of a fruit and may not be independent of fruit size, texture, or overall structure. Firmness may be more variable across the surface of complex fruits like caneberries, with

their many individual drupelets, than across the surface of blueberries, cherries, or other fruits that are relatively simple and uniform in structure. Female *D. suzukii* may prefer to lay eggs in riper fruit, the fact that some *D. suzukii* emerged from raspberries and blackberries bagged at the green-pink stage suggests that females were able to locate suitable oviposition sites within the firmer berries. Interestingly, overall patterns of infestation in raspberries and blackberries were very similar at UMRS in 2013. Infestation rates were lowest in green-pink berries and increased in a nearly stepwise fashion in more and more ripe berries (i.e., pink, then ripe raspberries, Fig. 3.1; red, then purple, then ripe blackberries, Fig. 3.2). As such, the accumulation of eggs in unripe fruit is another mechanism that could explain the observed patterns of infestation in both raspberries and blackberries at UMRS. Such accumulation could occur if females begin to lay eggs in fruit once they are physically able to make an incision and insert an egg, and then more and more eggs are laid as fruits ripen. It remains to be seen whether individual *D. suzukii* females indiscriminately lay eggs in fruit at various ripeness stages or whether some females specialize on particular stages.

The idea that female *D. suzukii* search for suitable oviposition sites has been supported by other studies of fruit susceptibility. Females laid more eggs in intentionally damaged wine grapes than in intact grapes (Ioriatti et al., 2015). Similarly, *D. suzukii* laid eggs in intentionally damaged cranberries but not in intact cranberries at any developmental stage (Steffan et al., 2013). In another study, female *D. suzukii* did not oviposit in intact, fuzzy peach sections or in small punctures, but readily laid eggs in sections without fuzz, in those with insect damage, and in large punctures (Stewart et al., 2014). Finally, edible fruit coatings did not prevent oviposition in raspberries and blueberries, as females were able to

locate suitable oviposition sites despite the presence of the coating (Swoboda-Bhattarai and Burrack, 2014).

Female *D. suzukii* may be more likely to accept suboptimal oviposition sites when population densities are high. For example, although *D. suzukii* females rarely lay eggs in intact peaches, in cases of extreme pest pressure they will occasionally lay eggs in intact peaches near the petiole where there is less obstructive fuzz (Stewart et al., 2014). This observation may help to explain differences in infestation patterns between the research planting and commercial field, namely that *D. suzukii* emerged from berries at all ripeness stages tested at UMRS, whereas very few flies emerged from unripe blackberries in the commercial field. The two sites differed in several respects, in particular that *D. suzukii* population densities were much lower in the commercial field than at UMRS. The commercial field was treated and picked approximately every seven and two days, respectively, in 2014. Active management, in combination with the larger size of the commercial field, likely helped to keep *D. suzukii* population densities comparatively low and contributed to lower levels of infestation in ripe fruit and very low levels of infestation in unripe berries. Conversely, the plot at UMRS was untreated and unpicked in 2013, resulting in very high *D. suzukii* population densities. Under such conditions, females may have been forced to lay eggs in increasingly less ripe berries as population densities and intraspecific competition for resources increased at UMRS. This conclusion is supported by the fact that infestation was observed, but not quantified, in red berries collected in commercial fields under two higher density scenarios: 1) earlier in the season in the same commercial Prime-Ark® 45 field, and 2) at another commercial Prime-Ark® 45 field in Cleveland County with

high numbers of *D. suzukii*. Together, these results suggest that infestation in unripe berries was higher when *D. suzukii* pressure was higher.

When population densities are high, laying eggs in unripe fruit would be a good strategy if the resulting larvae have a competitive advantage over larvae that develop from eggs laid later on. Individual fruits represent a finite resource, and competition for food may occur among developing progeny and reduce their survivorship, size, or overall fitness (Skinner 1985). Therefore, eggs laid in fruit at earlier ripeness stages may be able to eclose sooner, develop faster, and use more resources as larvae because they are larger than their competitors. As such, high levels of intraspecific competition have been shown to increase resource use diversity within natural populations (Svanback and Bolnick, 2007) and may favor individuals who are less selective and willing to accept a wider range of host quality (Davis et al., 2011). It is possible that intraspecific competition may have contributed to the highly polyphagous nature of *D. suzukii* (Lee et al., 2015), but may also push *D. suzukii* females to maximize their reproductive potential within individual hosts by accepting less preferred ripeness stages when population densities are high.

Laying eggs in unripe fruit would not be a good strategy, if larvae cannot complete their development due to host quality, larval development time, or a combination of these factors. We observed *D. suzukii* infesting and surviving in all stages of unripe raspberries and blackberries tested. In contrast to previous laboratory studies in which high mortality was observed in green and other unripe fruit (Lee et al. 2011), our observations show that eggs laid in unripe fruit in the field are not necessarily dead ends because fruits become more suitable for larval development as they ripen. Unripe fruit may initially be less suitable

substrates for larval development, but they undergo a series of interrelated physiological changes in soluble sugar content, pH, and firmness as they ripen (Vicente et al., 2007), which render them softer, sweeter, and more suitable for *D. suzukii* larval development as a result.

Several factors can affect the larval development time of *D. suzukii*, including temperature (Tochen et al., 2014), substrate quality, and larval density (Hardin et al., 2015). A catastrophic mismatch could occur if females lay eggs in fruit that ripen too fast to support larval development. For example, Poyet et al. (2014) concluded that female *D. suzukii* laid more eggs in red, unripe cherries than in ripe cherries to give larvae enough time to fully develop before the fruit mesocarp decayed. A mismatch could also occur if fruit ripen too slowly to provide adequate nutrition for larval development. It took approximately 9 days for *D. suzukii* to develop from egg to pupa in low carbohydrate diets, akin to unripe fruit (Hardin et al., 2015), but took bagged green-pink blackberries 12.9 and 16.0 days, on average, to ripen at UMRS and in the commercial field, respectively. This discrepancy in ripening times could explain why some *D. suzukii* larvae developed in green-pink blackberries at UMRS, but not in green-pink berries in the commercial field. This discrepancy in ripening times could be due to the different varieties used in the study; Prime-Ark 45 berries were larger than NC537 berries on average and may have taken longer to ripen. The fact that unripe berries bagged on 28 September did not weigh less at ripeness than the ripe berries collected on the same day suggests that most berries bagged at early stages of ripening were able to reach their full developmental potential in spite of being bagged for an extended period of time.

Allowing fruit to ripen naturally in the field appears to support greater larval development than is possible in fruit removed from plants, which helps explain differences between our results and those obtained by others (Lee et al. 2011), in which few *D. suzukii* survived in green and other unripe fruit. Further studies (Lee et al. 2015) observed that few or no *D. suzukii* larvae developed in fruits collected from three wild host species in laboratory bioassays, despite the fact that field-collected fruits from all three species were infested with developing *D. suzukii*. The authors suggested that the observed discrepancies could have resulted from differences in suitability between picked and unpicked fruits. For example, some of the picked fruits dried out and probably prevented development of *D. suzukii*, whereas unpicked fruits that remained hanging on the shrub for some time after oviposition were suitable for development (Lee et al., 2015). Therefore, our approach can provide a powerful method to assess the susceptibility, and suitability for larval development, of various ripeness stages of a particular host species, or of several host species that fruit concurrently and in close proximity.

CONCLUSIONS

D. suzukii were able to infest and survive in unripe raspberries and blackberries, but had the most success in berries at later stages of ripening. Berries at earlier stages of ripening may be more susceptible to infestation in areas where, or at times of the season when, pressure from *D. suzukii* is higher. This information may help growers to optimally time management treatments and to maintain season-long control of *D. suzukii*. These data also

suggest that unless *D. suzukii* populations are large, growers may not need to apply control measures until their fruit is transitioning from unripe to ripe developmental stages.

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FIGURES

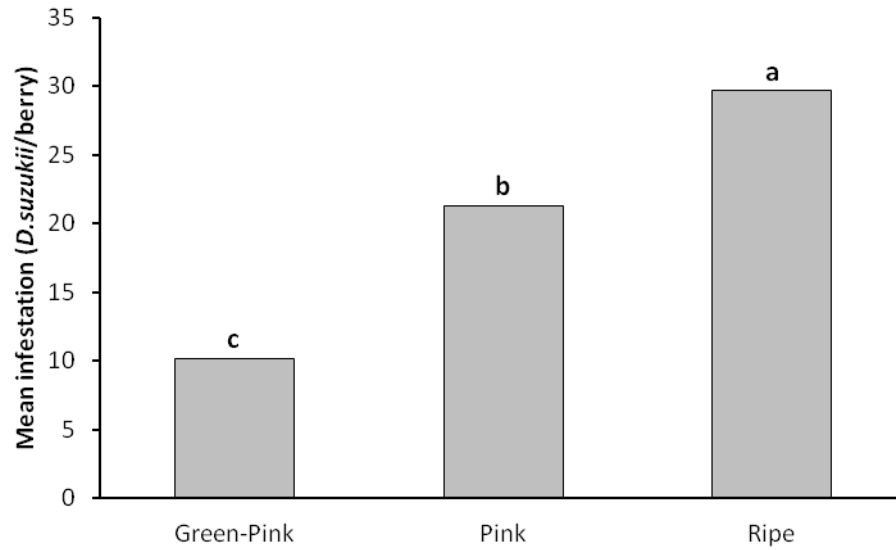


Fig. 3.1. *D. suzukii* infestation in unripe (green-pink and pink) and ripe 'Nantahala' raspberries at Upper Mountain Research Station, Ashe County, North Carolina, in October 2013. Infestation was calculated by averaging total infestation across all berries on a cluster (i.e., cluster averages). Bars that share a letter are not different at $\alpha = 5\%$. $n = 20$ cluster averages for each ripeness stage.

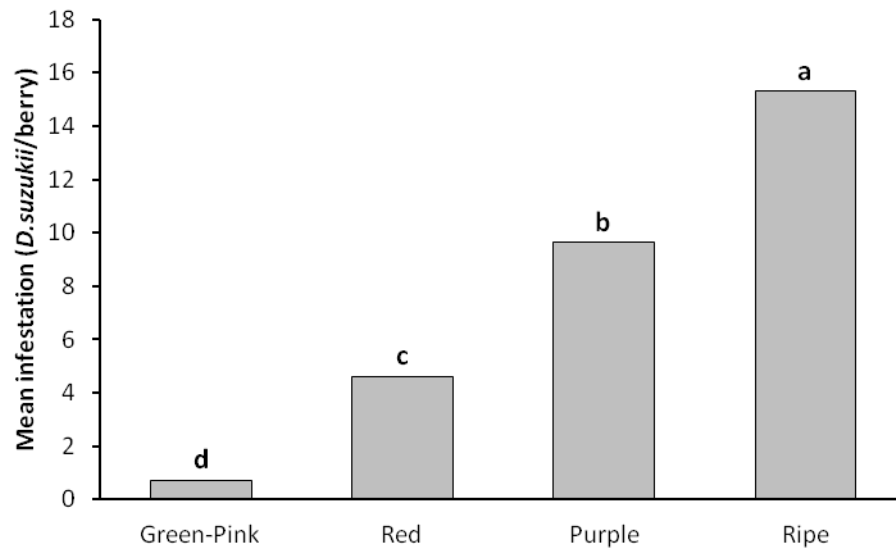


Fig. 3.2. *D. suzukii* infestation in unripe (green-pink, red, and purple) and ripe NC537 blackberries at Upper Mountain Research Station, Ashe County, North Carolina, in October 2013. Infestation was calculated by averaging total infestation across all berries on a cluster (i.e., cluster averages). Bars that share a letter are not different at $\alpha = 5\%$. $n = 20$ cluster averages for each ripeness stage, except $n = 19$ for red clusters.

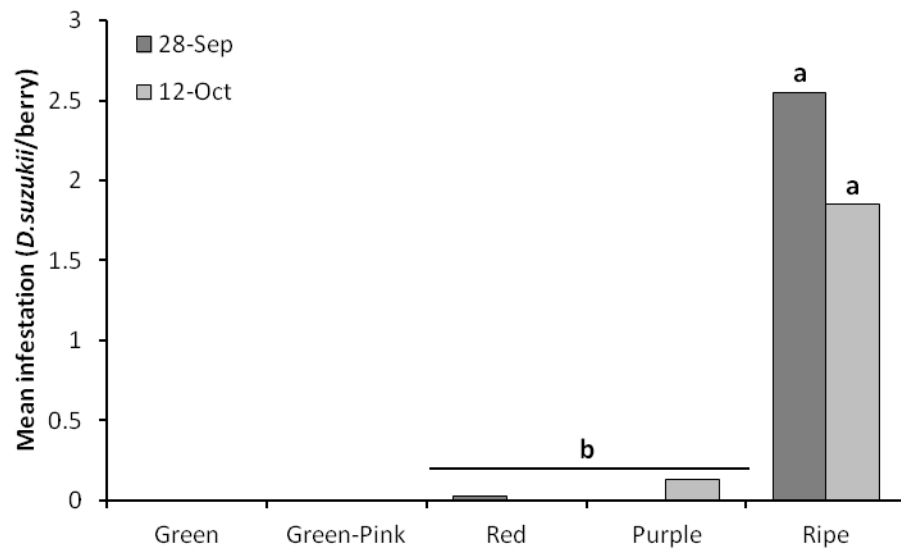


Fig. 3.3. *D. suzukii* infestation in unripe (green, green-pink, red, and purple) and ripe Prime-Ark® 45 blackberries at a commercial farm in Cleveland County, North Carolina, on 28 September and 12 October 2014. Green and pink-green berries were not bagged on 12 October. Bars that share a letter are not different at $\alpha = 5\%$. 28 September: $n = 24$ green, 48 green-pink, 40 red, 25 purple, and 22 ripe berries; 12 October: $n = 23$ red, 23 purple, and 20 ripe berries.

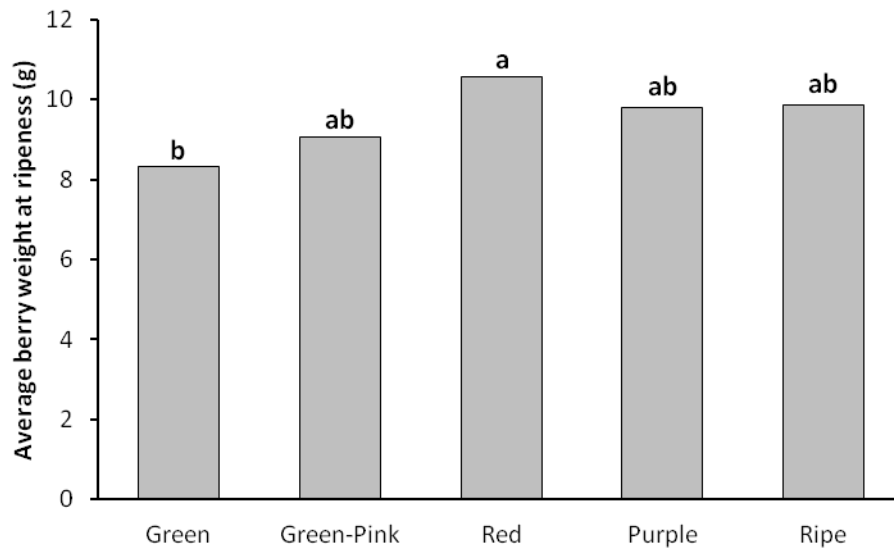


Fig. 3.4. Average weight at ripeness of unripe (green, green-pink, red, and purple) and ripe Prime-Ark® 45 blackberries that were bagged or collected 28 September 2014 at a commercial farm in Cleveland County, North Carolina. Bars that share a letter are not different at $\alpha = 5\%$. $n = 24$ green, 48 green-pink, 40 red, 25 purple, and 22 ripe berries.

CHAPTER 4: Resource Use Diversity in *Drosophila suzukii* (Diptera: Drosophilidae)

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Abstract

Efficient resource use by invasive species in their introduced environment is essential to their success. Intraspecific competition can increase resource use diversity within natural populations and may favor individuals who are willing to accept a wider range of hosts and/or host qualities. *Drosophila suzukii* (Matsumura) is a highly polyphagous, invasive vinegar fly that severely threatens berry and cherry production in North America, Europe, and South America. To determine if intraspecific competition affects *D. suzukii* host use and population growth, we compared oviposition and infestation patterns in ripe and unripe blackberries, a highly-preferred host, as fly numbers increased in an unmanaged research planting with a history of high *D. suzukii* infestation. We hypothesized that females would oviposit in unripe berries, which are suboptimal hosts compared to ripe berries, as levels of intraspecific competition increased. We collected ripe berries; secured drawstring mesh bags over purple, red, and green-pink berries; and deployed traps to catch adults, weekly in June-August 2015. Infestation occurred first in ripe berries, while unripe berries were infested only after the number of female *D. suzukii* caught in traps increased sharply. These results suggest that intraspecific competition affects *D. suzukii* host use and population growth. These data may help growers better time management treatments and more effectively manage *D. suzukii* populations.

Introduction

The ecological and economic costs associated with invasive species have been estimated to exceed \$120 billion annually (Pimentel et al. 2005) and the threat of continued global species exchange is high due to increased trade and climate change. Understanding the factors that regulate invasive species populations is a major goal for basic and applied ecologists (Bulleri et al. 2008). Characteristics common to successful colonists across many taxa include r-selected life histories (MacArthur and Wilson 1967), which involve the use of pioneer habit, short generation time, high fecundity, high growth rates, and the ability to disperse offspring widely. When invasive species fail to establish, it may be due to lack of pre-adaptation to the new climate, disturbance, predation, competition, or diseases in their new environment (Sakai et al. 2001). However, the innate characteristics of individual species also play a role, and determining how behavioral mechanisms contribute to invasion success is an important but understudied area (Holway and Suarez 1999) that may provide insight into why some invasive species are particularly successful (Tsutsui et al. 2000).

Drosophila suzukii (Matsumura), the spotted wing drosophila, is an invasive species originally from southeast Asia that has been detected in many parts of North America, Europe (Hauser 2011, Cini et al. 2012), and South America (Depra et al. 2014) since 2008. Females possess a large serrated ovipositor and preferentially oviposit in undamaged, ripening fruits instead of overripe or rotting fruits like most other *Drosophila* species. They attack a wide variety of soft-skinned fruit crops, including berries and cherries, and non-crop hosts and have the potential to cause great economic harm to fruit industries across their introduced range (Bolda et al. 2010).

Resource competition between species can play a major role in driving ecological and evolutionary diversification, and interspecific competition for oviposition sites has clearly contributed to the ecological diversity of *Drosophila* (Markow and O'Grady 2008). Many species aggregate, feed, and breed on a variety of patchy resources (Wertheim 2002), which can result in negative fitness consequences for progeny if patches are overused (Skinner 1985). Thus, many *Drosophila* species avoid laying eggs within the same patches or resort to resource partitioning if avoidance is not possible (Chess et al. 1990, Markow and O'Grady 2008). *D. suzukii* is unusual among *Drosophila* species because the niche it occupies is relatively free of interspecific competition. The evolution of its serrated ovipositor proved advantageous by allowing females to exploit a resource inaccessible to other species (Atallah et al. 2014), and it is possible that *D. suzukii* evolved to occupy its unusual niche in response to strong competition for resources among *Drosophila* species in its native range.

While the niche occupied by *D. suzukii* is relatively free of interspecific competition, intraspecific competition is likely an important force influencing *D. suzukii* host use patterns. In general, conspecifics can serve as sources of information about resource quality, either by indicating the presence of a high-quality resource or by indicating the presence of potential competitors for a particular resource. High levels of competition may favor individuals who are less selective and willing to accept a wider range of host quality (Davis et al. 2011). As such, intraspecific competition has been shown to increase resource use diversity within natural populations (Svanback and Bolnick 2007) and to drive niche expansion onto resources where intraspecific competition is less severe (Bolnick 2001). Intraspecific competition may have contributed to the highly polyphagous nature of *D. suzukii* (Lee et al.

2015), but may also push *D. suzukii* females to maximize their reproductive potential within a given host.

Resource quality influences *D. suzukii* host choice. Females prefer to lay eggs in ripe fruit, as riper fruit are generally more suitable for larval development than less ripe fruit (Lee et al. 2011). However, intraspecific competition can reduce *D. suzukii* survivorship at high larval densities even in high quality diets that are akin to ripe fruit (Hardin et al. 2015). Previously, different patterns of infestation in ripe and unripe blackberries, a highly-preferred host, were observed in two contrasting environments: a small, unmanaged research planting with high numbers of *D. suzukii* and a large, managed commercial field with comparatively low numbers of *D. suzukii*. *D. suzukii* eggs were able to develop to adults when laid in green-pink, red, and purple blackberries that ripened naturally on the plant. In the research planting, infestation rates were highest in ripe berries, although all stages of unripe berries (green-pink, red, and purple berries) were infested at some level. Infestation rates in the commercial field were much lower than in the research planting, and very little infestation was observed in unripe berries (Swoboda-Bhattarai and Burrack 2016). These results suggest that both host quality and population density are factors that may influence *D. suzukii* infestation patterns, and raise the questions of whether population density also affects *D. suzukii* host choice and how females choose among hosts of varying quality as population densities and the potential negative effects of intraspecific competition increase (Bolnick 2001, Svanback and Bolnick 2007).

The objective of this study was to determine if *D. suzukii* host use patterns changed as population densities increased by obtaining weekly "snapshots" of infestation patterns 1)

across ripe and unripe berries and 2) within individual ripe and unripe berries. We predicted that infestation would occur first in ripe berries and that no infestation would occur in unripe berries early in the season when population densities are low, and that infestation rates in ripening berries (starting with purple berries, then red, then green-pink; Fig. 1) would increase as fly populations increased throughout the season. We also predicted that the proportions of immature *D. suzukii* life stages (eggs and first, second, and third instar larvae) within individual ripe and unripe berries would change over time as females modify their oviposition behavior in response to increased resource competition. Specifically, we predicted that higher proportions of developing larvae would be found in unripe berries as fly populations increased throughout the season. If these predictions are correct, this experiment will provide evidence that *D. suzukii* modifies its oviposition behavior in response to increased intraspecific competition for individual hosts, which may be a behavioral mechanism that has contributed to its invasion success.

Materials and Methods

Study site. Experiments were conducted in a planting of ‘Von’ blackberries at the NCDA&CS Piedmont Research Station in Rowan County, NC, which received no pesticide applications, from June to August 2015. ‘Von’ is a thornless, floricanne-fruiting blackberry cultivar suitable for cultivation in the southeastern United States released from the North Carolina State University *Rubus* breeding program in 2013 (Fernandez et al. 2013). All berries in this study were collected and bagged along the northern, shady side of one 36m

long row. Previous research at this location has demonstrated that it fosters a large naturally occurring population of *D. suzukii* that is stable from year to year.

As it is not feasible to measure the abundance of adult *D. suzukii* by counting individual flies on fruits, the numbers of female and male *D. suzukii* captured in three monitoring traps each week were used to approximate the adult population present in the planting. Traps consisted of 32 fl oz. clear plastic cups and lids with 10 equidistant holes drilled near the top (DeliPRO brand, Tri-pack Industrial USA, White Plains, NY). Traps were deployed 12m apart from each other along the same row where berries were collected and were baited with 1.69 g of dry active yeast, 8.45 g of sugar, and 150 ml of water (Burrack et al. 2015). Bait was collected from each trap and replaced weekly over the course of the experiment.

Fruiting phenology and characteristics of ripe and unripe berries. To document the progression of fruit ripening within the plot, weekly counts of green, green-pink, red, purple, and ripe berries were made on 10 tagged branches that were spaced equally along the row. Counts began on 17 June when all berries were at the green stage and continued through 5 August when very few berries remained in the planting. To determine how the characteristics of ripe and unripe berries differ, 20 berries each at the green-pink, red, purple, and ripe stages were collected throughout the plot on 22 July. Each berry was individually weighed in the laboratory, after which its length and two measurements of its width were made using digital calipers (Mitutoyo Corporation, Kawasaki, Japan). The volume of each berry was then estimated by averaging the two width measurements to determine the diameter and radius

and then taking the berry length times the radius squared times pi. A Wagner gram force (gf) gage (Wagner Instruments, Greenwich, CT) fitted with a blunted No. 3 insect pin (Elephant Brand, Austria) was used to measure the surface penetration force in centinewtons (cN) required to puncture the skin of three drupelets located around the center of each berry; measurements were then averaged to create a single measurement of firmness for each berry. Finally, a handheld refractometer (QA Supplies, Norfolk, VA) was used to measure the soluble sugar concentration (i.e., Brix) of each berry. It was not possible to obtain enough juice from some green-pink berries to obtain a reliable Brix measurement.

Weekly snapshots of infestation patterns across ripe and unripe berries. Each week from 12 June to 22 July, 20 ripe berries were collected and 20 purple, 20 red, and 20 green-pink berries were covered with 13 x 18 cm fine mesh bags with drawstring closures to prevent additional oviposition by *D. suzukii* females. On 29 July and 5 August, fewer ripe and unripe berries were available throughout the planting and only 10 berries at each ripeness stage were collected or bagged. During all weeks, unripe bagged berries were labelled and marked with colored flagging tape to indicate ripeness stage, left to ripen naturally on the plant, and collected once ripe. After collection, berries were held individually in rearing containers composed of 1 or 2 oz. clear polystyrene portion cups (Dart Conex, Mason, MI); each berry was suspended within the cup in a 10 × 10 cm square of organza fabric to allow juice drainage. Three holes were poked in the bottom of each cup, which were then placed in layers on top of paper towels to allow further drainage and reduce larval mortality.

Rearing containers were held in a growth chamber at 20°C, 16L:8D to allow flies to develop. Berries were dissected between 10 and 14 days after collection. For each berry, third instar larvae were counted during the dissection process, and all pupae were removed and placed on a moistened paper towel square in a 60 mm diameter Petri dish (Burrack et al. 2013). Dishes were monitored for adult emergence; all *D. suzukii* adults were sexed and counted after emergence using a stereomicroscope (Olympus SZX10, Center Valley, PA). If adults emerged inside the rearing containers, they were removed using an aspirator, frozen, sexed, and counted. All pupal cases in each dish were then examined under a stereomicroscope and any dead pupae that failed to emerge were counted. *D. suzukii* infestation rates were calculated by adding the number of third instar larvae, dead pupae, and emerged adults for each berry.

Most flies observed were *D. suzukii* and the few non-*D. suzukii* adults and dead pupae present were excluded from infestation calculations. Of a total of 1571 adults reared from berries, 145 (9.23%) were confirmed as non-*D. suzukii* adults, while 18 of 211 (8.53%) dead pupae were non-*D. suzukii*. Nearly four-fifths (78.62%) of non-*D. suzukii* adults present were reared from ripe berries collected on 8 July, 15 July, and 22 July.

Weekly snapshots of infestation patterns within individual ripe and unripe berries. Starting on 8 July, the week after *D. suzukii* infestation was first detected, 20 berries at each ripeness stage except green were collected along the length of the row. Berry samples were collected again on 15 and 22 July. Each week, berries were stored in a refrigerator at 4°C to suspend the development of any immature stages present (Aly et al. 2017). A stereomicroscope was

used to count *D. suzukii* eggs as soon as possible after berries were collected. Berries were then preserved individually in 1 or 2 oz. portion cups filled with a saltwater solution, which killed any immature flies present and preserved them along with the berry tissue. Preserved berries were dissected under a stereomicroscope, one drupelet at a time, and the numbers of first, second, and third instar larvae present were counted.

Data analysis. To document fruiting phenology within the plot, the overall proportions of ripe, purple, red, green-pink, and green berries were calculated by summing the total number of berries at each ripeness stage that were present on all 10 tagged branches each week. The average proportions of berries at each ripeness stage on each branch were then compared across weeks using a generalized linear mixed model with a normal distribution and identity link (Proc GLIMMIX; SAS v. 9.4) with ripeness stage and date as fixed effects and branch as a random effect. Data from 5 August were not included in the analysis due to low sample sizes. Data were arcsine square root transformed prior to analysis. The SLICE and SLICEDIFF options in the lsmeans statement of PROC GLIMMIX were used to obtain the overall simple effect *F*-tests and pair-wise simple effect differences (Stroup 2013) for the significant interaction between date and ripeness stage. Finally, the Tukey-Kramer adjustment for multiple comparisons was applied using the ADJUST=Tukey option.

Weight, volume, firmness, and Brix of ripe, purple, red, and green-pink berries were compared in separate analyses using mixed model ANOVA (Proc Mixed; SAS v. 9.4) with ripeness stage as a fixed effect and berry as a random effect. Berry volume was initially included as a covariate in the analyses of firmness and Brix fruit traits, but was removed

from the both models due to non-significance. Weight, volume, and firmness data were transformed to meet the assumption of normality using a square root transformation, while Brix data were transformed using a fourth root transformation.

To assess weekly infestation patterns across ripe and unripe berries, infestation rates in ripe, purple, red, and green-pink berries were compared across weeks using a generalized linear mixed model with a lognormal distribution and an identity link (Proc GLIMMIX; SAS v. 9.4) with ripeness stage and week as fixed effects and berry as a random effect. Data from weeks prior to 1 July and after 5 August were excluded from the analysis because no infestation occurred prior to 1 July and very few unripe berries remained in the planting on 5 August. The numbers of female and male *D. suzukii* captured in monitoring traps across weeks were compared separately using mixed model ANOVA (Proc MIXED; SAS v. 9.4) with date as a fixed effect and trap as a random effect.

To assess weekly infestation patterns within individual ripe and unripe berries, the proportions of *D. suzukii* eggs and first, second, and third instar larvae present in ripe, purple, red, and green-pink berries were compared across weeks using a generalized linear mixed model with a normal distribution and identity link (Proc GLIMMIX; SAS v. 9.4). Date, ripeness stage, and immature life stage were included in the model as fixed effects and berry as a random effect. Data were arcsine square root transformed prior to analysis. The SLICE and SLICEDIFF options in the lsmeans statement of PROC GLIMMIX were used to obtain the overall simple effect *F*-tests and pair-wise simple effect differences (Stroup 2013) for significant interactions between fixed effects in the model. In addition, the Tukey-Kramer adjustment for multiple comparisons was applied using the ADJUST=Tukey option.

Post hoc means comparisons were conducted for all analyses described above using the Tukey-Kramer test.

Results

Fruiting phenology and characteristics of ripe and unripe berries. Overall proportions of ripe and unripe berries present on the 10 tagged branches changed over the course of the season. The proportion of green berries present declined steadily over the season as berries underwent the ripening process. The proportion of green-pink berries present started to increase on 24 June and peaked on 22 July. Red and purple berries each accounted for less than 10% of the berries present throughout the season, except for red berries on 5 August. Ripe berries became more prevalent in the plot after 15 July, at which point they accounted for over 20% of the berries present and remained that way for the rest of the season.

Average proportions of ripe and unripe berries on the 10 branches selected to estimate fruiting phenology changed over the course of the season (date*ripeness stage: $F_{[24,285]} = 38.78, P < 0.0001$). On 17 June, 24 June, and 1 July, there were more green berries on average than ripe, purple, red, and green-pink berries (Table 4.1). The same pattern was observed on 8 and 15 July as well; however, more green-pink berries were also present on 8 July than ripe, purple, and red berries, while more ripe berries were also present on 15 July than purple and red berries and more green-pink berries than purple. For the first time during the season, there were as many ripe berries present on 22 July as green and green-pink berries, while all three stages were more abundant than red and purple berries. Finally, there

were more ripe berries present than green, green-pink, red, and purple berries on 29 July. Only one ripe and one red berry remained on the branches on 5 August.

Unripe berries generally took less than one week to ripen, and most berries bagged at the green-pink stage were ripe by the following week. Berry weight, volume, firmness, and soluble sugars (i.e., sweetness) changed significantly during the ripening process (Table 4.2). Berries increased in both weight and volume as they ripened. Ripe berries weighed more and were larger than purple, red, and green-pink berries, while purple berries in turn weighed more and were larger than both red and green-pink berries. Berries also became softer and sweeter as they ripened. Green-pink berries were the firmest and least sweet of the four stages, while red berries were softer and sweeter than green-pink berries, purple berries were softer and sweeter than red berries, and ripe berries were the softest and sweetest of the four stages.

Adult fly trap captures. The average number of female *D. suzukii* captured in traps stayed consistently low, below 5 individuals on average, on and prior to 8 July. The number of females caught in traps increased sharply on 15 July and then peaked the following week on 22 July. Compared to females, male trap captures remained comparatively low throughout the season (Table 4.3).

Weekly infestation patterns across ripe and unripe berries. Infestation was first detected on 1 July and was detected in ripe berries before unripe berries (Table 4.3). Ripe berries were infested at higher rates than all unripe stages of berries on 1, 8, 15, 22, and 29 July, while

infestation rates in purple berries were higher than in red and green-pink berries on 15 July only (stage*week: $F_{[12,339]} = 9.15$, $P < 0.0001$). Infestation rates in unripe berries were highest on 15 July, when the number of female *D. suzukii* caught in traps increased sharply.

Weekly infestation patterns within individual ripe and unripe berries. Overall, the total number of immature *D. suzukii* present in berries increased as they ripened (Table 4.4). Within each week, green-pink berries had the smallest number of immature *D. suzukii* present, while ripe berries had the highest number of immature *D. suzukii* present. Only eggs were found in green-pink berries, and red berries contained only eggs and first instar larvae. Purple berries contained eggs, first instar, and second instar larvae, although second instars were only found in berries collected on 22 July. Eggs and first, second, and third instar larvae were found in ripe berries collected each week.

The relative proportions of *D. suzukii* immature stages present in ripe, purple, red, and green-pink berries did not differ when compared across weeks (date*ripeness stage*immature stage: $F_{[18,911]} = 0.97$, $P = 0.49$). However, there were differences in the proportions of eggs and first, second, and third instars present in ripe and unripe berries overall (ripeness stage*immature stage: $F_{[9,911]} = 42.09$, $P < 0.0001$) (Table 4.4). Green-pink and red berries had more eggs present than first instar larvae. Purple and ripe berries had more eggs present than first, second, and third instar larvae, while more first instars were present than second and third instars. In addition, the relative proportions of *D. suzukii* immature stages present in ripe and unripe berries differed across weeks (date*immature stage: $F_{[6,911]} = 2.34$, $P = 0.0301$). On both 8 July and 15 July, more eggs were present overall

than first, second, and third instar larvae, while more first instars were present than second and third instars. On 22 July, more eggs were present than first, second, and third instar larvae. However, higher proportions of second instar larvae were observed on 22 July; more first instar larvae were present than third instars, but not second instars.

Discussion

The experiments conducted in this study were designed to address whether population density affects *D. suzukii* host choice and how *D. suzukii* females choose among hosts of varying quality as population densities, and the potential negative effects of intraspecific competition, increase. The host use patterns of *D. suzukii* females did change over time, and this was correlated with increased population density. As was predicted, infestation occurred in ripe berries first and increased in unripe berries as fly populations increased through the end of fruit availability.

D. suzukii females have been shown to prefer ripe blackberries over unripe blackberries for oviposition (Lee et al. 2011, Swoboda-Bhattarai and Burrack 2016), perhaps because they are more suitable for larval development than unripe berries or artificial substrates that approximate unripe berries (Lee et al. 2011, Hardin et al. 2015). Once established, patterns of infestation in unripe berries progressed as was predicted (Figure 4.1). Infestation in unripe berries first occurred in purple berries, followed by infestation in red and green-pink berries one week later. However, infestation rates in purple berries were not significantly higher than in red and green-pink berries until 15 July, at which time patterns of infestation in ripe and unripe berries resembled those observed previously in an unmanaged

research planting with high population densities of *D. suzukii* (Swoboda-Bhattarai and Burrack 2016).

Our observations suggest that female flies may have altered their host choice behavior in response to increasing levels of adult competition. The amount of competition experienced by individual females is likely a function of both the relative abundance of resources and the number of other flies, i.e., potential competitors, encountered. Insects can potentially detect competitors both visually and chemically, and may be able to form some sense of the level of competition from both methods (Nufio and Papaj 2004a). It is unclear if *D. suzukii* detects infestation in potential host fruit. Many true fruit flies and parasitoid species leave chemical markers behind following oviposition, but this seems unlikely in the case of *D. suzukii* as most *Drosophila* do not utilize such host marking pheromones. In fact, some species aggregate instead using aggregation pheromones. Alternatively, the breathing filaments that remain outside the fruit skin following oviposition by *D. suzukii* may serve as a kind of host-marking device that deters other females from laying additional eggs within a single fruit or a single drupelet.

Patterns of insect host choice observed at the population level result from choices made by individuals in response to local conditions, and individual variation is a basic feature of insect-plant relationships that can help explain different resource use strategies (Janz et al. 1994, Schoonhoven et al. 1998). There are many reasons why female insects may choose to oviposit on a particular host. The oviposition preference-offspring performance (P-P) hypothesis predicts that when insects utilize discrete hosts for oviposition, from which juveniles have limited ability to disperse, females should evolve oviposition behaviors that

maximize larval fitness (Jaenike 1978). While many studies support this hypothesis, the relationship between female preference and offspring performance is often stronger in specialist versus generalist species (Gripenberg et al. 2010, Liu et al. 2012). Conversely, some phytophagous species regularly oviposit on plant species that maximize adult, not offspring, performance (Scheirs 2002). Physiological state and egg load can also influence how persistently some females forage for oviposition sites, their probability of accepting a host, and the number of eggs they lay (Courtney et al. 1989, Minkenberg et al. 1992, Aluja et al. 2001). This may lead some females to make oviposition decisions that optimize their own fitness, perhaps by increasing the number of offspring they produce over a lifetime, even if this strategy decreases the performance of individual offspring (Nufio and Papaj 2004b).

In this study, in the absence of intraspecific competition, *D. suzukii* egg dispersion appeared to conform to the P-P hypothesis, but when adult competition for oviposition resources was higher, flies accepted lower quality hosts in the form of unripe berries. To more fully test this hypothesis, future studies should explore whether larvae that develop from eggs laid first (i.e., at low densities in less ripe fruit) are more fit with respect to development rate, pupal weight, and lifetime fecundity than larvae that develop in competition with large numbers of conspecifics in riper fruit (Averill and Prokopy 1987). If they are, this would provide evidence for why some females might choose to lay eggs in less ripe fruits under conditions of high resource competition.

Population structure within a variable host crop also changed as population densities increased, as was evident from changes in infestation patterns within individual ripe and unripe berries. Previously, we confirmed that *D. suzukii* could lay eggs and complete their

development in green-pink, red, and purple blackberries that were left to ripen on the plant (Swoboda-Bhattarai and Burrack 2016), but were unable to confirm at what ripeness stage eggs hatched. In the current study, only eggs were found in green-pink berries, while eggs and developing larvae were found in all other ripeness stages. First instar larvae were found in red berries, indicating that *D. suzukii* eggs were able to hatch and larvae were able to begin developing in blackberries at this red stage. Unripe berries generally took less than one week to ripen and most berries bagged at the green-pink stage were ripe or close to ripe by the following week. The length of time that it takes *D. suzukii* to develop from the egg to the pupal or adult stage depends both on the temperature and the nature of the substrate (Tochen et al. 2014, Hardin et al. 2015), but none of the ripeness stages observed in this study took so long to ripen that *D. suzukii* eggs would not have been able to develop in them.

Not all females may make the same oviposition decisions when responding to increasing levels of resource competition. Because more second instar larvae were found in purple and ripe berries later in the season on 22 July than earlier in the season, females may have started to lay eggs in berries at earlier stages of the ripening process later in the season as fly populations increased. Therefore, unripe berries may be more susceptible to infestation in areas or at times of the season when pressure from *D. suzukii* is higher. However, the fact that eggs and first instar larvae were also present in purple and ripe berries on 22 July suggests that some females may have continued to lay eggs in ripe fruit while others selected suboptimal, less ripe host stages for oviposition under conditions of increased population pressure. Therefore, females might adopt multiple oviposition strategies in response to local conditions.

Our results suggest that population-level patterns of host use can be influenced by multiple factors including host quality and population density. When population densities and intraspecific competition are low, female *D. suzukii* lay eggs in ripe fruit, which is a good choice for their offspring in terms of growth and development. However, when population densities increase and result in increased levels of competition, female *D. suzukii* appear to make use of less suitable substrates. Intraspecific competition likely contributed to the highly polyphagous nature of *D. suzukii* and may also push females to maximize their reproductive potential within individual hosts by accepting a wider range of host qualities. If so, this tendency could help explain why *D. suzukii* has become established in a wide variety of environments across North America, Europe, and South America. Our findings are important for the development of population models such as that of Wiman et al. (2014) that integrates temperature-dependent life table data into a model that projects *D. suzukii* population growth. In addition, crop risk may be related to resource availability, as the hosts that *D. suzukii* chooses to attack may depend on what other hosts are available.

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TABLES

Table 4.1. Mean^a (\pm SE) proportions of ripe and unripe (purple, red, green-pink, and green) ‘Von’ blackberries on 10 tagged branches over the course of the 2015 growing season.

Ripeness stage	Date						
	17-Jun	24-Jun	1-Jul	8-Jul	15-Jul	22-Jul	29-Jul
Ripe	0.00 \pm 0.00b	0.01 \pm 0.01b	0.01 \pm 0.01b	0.03 \pm 0.01c	0.22 \pm 0.05b	0.23 \pm 0.03a	0.75 \pm 0.10b
Purple	0.00 \pm 0.00b	0.01 \pm 0.01b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.02 \pm 0.02d	0.07 \pm 0.03b	0.08 \pm 0.04b
Red	0.00 \pm 0.00b	0.00 \pm 0.00b	0.01 \pm 0.01b	0.04 \pm 0.02c	0.06 \pm 0.03c	0.08 \pm 0.03b	0.09 \pm 0.04b
Green-pink	0.00 \pm 0.00b	0.00 \pm 0.00b	0.04 \pm 0.01b	0.14 \pm 0.04b	0.13 \pm 0.02b	0.30 \pm 0.06a	0.02 \pm 0.02b
Green	1.00 \pm 0.00a	0.98 \pm 0.02a	0.93 \pm 0.02a	0.79 \pm 0.05a	0.56 \pm 0.06a	0.32 \pm 0.06a	0.06 \pm 0.06a

^aValues followed by the same letter within a column are not significantly different ($\alpha = 0.05$, Tukey-Kramer).

Table 4.2. Mean^a (\pm SE) measurements of four fruit characteristics of ripe and unripe (purple, red, and green-pink) ‘Von’ blackberries.

Ripeness stage	Weight (g)	Volume (mm ³)	Firmness (cN)	Brix (%)
Ripe	6.6 \pm 0.4a	10,167.8 \pm 788.4a	22.2 \pm 0.8d	10.8 \pm 0.4a
Purple	4.4 \pm 0.2b	6943.0 \pm 452.7b	26.1 \pm 0.8c	8.3 \pm 0.2b
Red	3.0 \pm 0.2c	4569.3 \pm 258.6c	46.2 \pm 1.7b	7.0 \pm 0.1c
Green-pink	2.7 \pm 0.2c	4305.2 \pm 235.8c	87.6 \pm 2.3a	5.9 \pm 0.1d
<i>F</i>	44.23	36.82	471.83	112.54
<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001
df	3,76	3,57	3,57	3,57

^aValues within a column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer adjustment).

Table 4.3. Mean^a (\pm SE) *D. suzukii* infestation per berry in ripe and unripe (purple, red, and green-pink) ‘Von’ blackberries and adult *D. suzukii* trap captures over the course of the 2015 growing season.

	Date						
	24-Jun ^b	1-Jul	8-Jul	15-Jul	22-Jul	29-Jul	5-Aug ^b
Ripeness stage							
Ripe	0.0 \pm 0.00	2.4 \pm 0.76a	9.3 \pm 1.97a	16.4 \pm 1.88a	22.9 \pm 2.71a	2.9 \pm 1.17a	3.0 \pm 0.84
Purple	0.0 \pm 0.00	0.1 \pm 0.05b	1.2 \pm 0.36b	8.3 \pm 2.84b	0.9 \pm 0.28b	0.2 \pm 0.13b	--
Red	0.0 \pm 0.00	0.0 \pm 0.00b	0.9 \pm 0.48b	2.3 \pm 0.46c	0.3 \pm 0.15b	0.1 \pm 0.10b	--
Green-pink	0.0 \pm 0.00	0.0 \pm 0.00b	0.9 \pm 0.75b	1.5 \pm 0.32c	0.1 \pm 0.05b	0.0 \pm 0.00b	--
Trap captures							
Female	4.0 \pm 0.00B	2.0 \pm 1.15B	0.7 \pm 0.33B	18.0 \pm 7.00AB	41.0 \pm 8.74A	23.3 \pm 11.92AB	29.3 \pm 7.22AB
Male	2.5 \pm 1.50A	0.3 \pm 0.33A	0.3 \pm 0.33A	1.0 \pm 0.00A	4.3 \pm 1.76A	9.0 \pm 4.58A	6.3 \pm 2.67A

^aRipeness stage values within a column (lower case) and trap capture values within a row (upper case) followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer adjustment).

^bData from 24 June and 5 August were not included in the analysis.

Table 4.4. Mean^a (\pm SE) proportions of eggs, first, second, and third instars present in unripe (green-pink, red, and purple) and ripe blackberries collected on 8 July, 15 July, and 22 July 2015, and the total number of immature *D. suzukii* present in 20 berries.

Immature stage	Ripeness stage			
	Green-pink	Red	Purple	Ripe
8 July				
Egg	0.05 \pm 0.05a	0.47 \pm 0.11a	0.79 \pm 0.08a	0.66 \pm 0.05a
First instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.11 \pm 0.05b	0.27 \pm 0.05b
Second instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.03 \pm 0.02c
Third instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.03 \pm 0.01c
Total number	1	44	196	308
15 July				
Egg	0.05 \pm 0.05a	0.68 \pm 0.10a	0.90 \pm 0.03a	0.71 \pm 0.02a
First instar	0.00 \pm 0.00b	0.02 \pm 0.01b	0.11 \pm 0.03b	0.25 \pm 0.02b
Second instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.02 \pm 0.01c
Third instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.02 \pm 0.01c
Total number	4	107	542	625
22 July				
Egg	0.20 \pm 0.09a	0.66 \pm 0.10a	0.85 \pm 0.07a	0.69 \pm 0.04a
First instar	0.00 \pm 0.00b	0.04 \pm 0.03b	0.05 \pm 0.02b	0.19 \pm 0.03b
Second instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.08 \pm 0.02c
Third instar	0.00 \pm 0.00b	0.00 \pm 0.00b	0.00 \pm 0.00c	0.04 \pm 0.01c
Total number	4	82	430	877

^aWithin each date, values within a column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer adjustment).

FIGURES

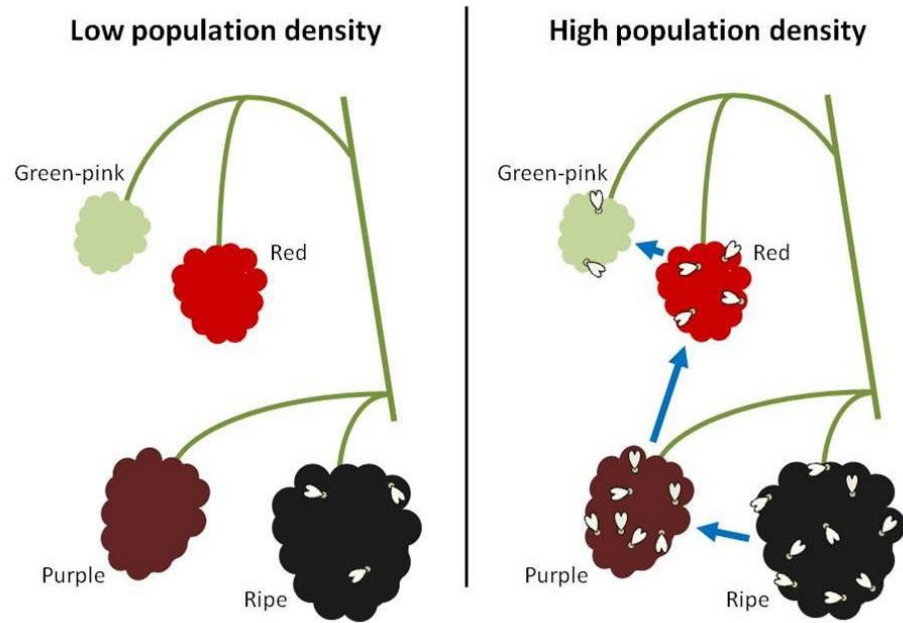


Figure 4.1. Model of female *D. suzukii* host choice under low and high population density scenarios.

CHAPTER 5: Reproductive Status of *Drosophila suzukii* (Diptera: Drosophilidae)

Females Influences Attraction to Fermentation-Based Baits and Ripe Fruits

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Abstract

Drosophila suzukii (Matsumura), the spotted winged drosophila, is an invasive species that is a devastating pest of soft-skinned fruit crops. Although much effort has been directed towards developing traps and attractants to monitor for *D. suzukii*, current monitoring tools do not reliably predict fruit infestation. The objective of this study was to determine if *D. suzukii* females at different developmental stages are differentially attracted to monitoring traps with fermentation-based baits and ripe fruits. Females were collected on the surface of traps, within traps, and/or on ripe fruits during three experiments at field locations in North Carolina, USA, and were dissected to determine their reproductive status. In general, females collected on ripe fruits were more likely to have mature eggs present in their ovaries and had higher numbers of mature eggs than females collected on the surface of or within monitoring traps. The results of this study have implications for *D. suzukii* monitoring and the development of effective baits for use in integrated pest management programs.

Key words: Monitoring, raspberry, blackberry, invasive species

Introduction

The invasive vinegar fly *Drosophila suzukii* (Matsumura), the spotted wing drosophila, attacks and can cause substantial economic damage to soft-skinned fruit crops such as raspberries, blackberries, blueberries, cherries, and strawberries (Bolda et al. 2010, Lee et al. 2011). Much effort has been directed to developing traps (Lee et al. 2012, Lee et al. 2013, Iglesias et al. 2014) and attractants (Cha et al. 2012, Landolt et al. 2012, Basoalto et al. 2013, Cha et al. 2013) to monitor for *D. suzukii*, but captures in current traps are poorly correlated to fruit infestation. When six fermentation-based attractants were compared within four host crops across 10 states (Burrack et al. 2015), five of six attractants detected the presence of *D. suzukii* before the development of fruit infestation. However, once ripe fruits were available, captured flies had fewer mature eggs within their ovaries. One explanation for this result is that females with mature eggs may be more attracted to ripe fruits than to traps with fermentation-based baits. If so, such baits may underestimate the presence of egg-laying *D. suzukii* females when ripe fruits are available and may help explain why captures in currently used monitoring traps do not effectively predict fruit infestation.

Female *Drosophila* must feed to mature eggs (Markow and O'Grady 2008). Yeasts, a common component of fermentation-based baits, are an important source of nutrients for *Drosophila* species that can dramatically impact their ability to produce eggs (Chippindale et al. 1993, Chippindale et al. 1997, Simmons and Bradley 1997, Ganter 2006). During previous experiments, we observed male courting behavior, male-male aggression, and mating pairs on the surface of traps containing a yeast, sugar, and water mixture, which suggested to us that flies may be using traps to locate mates.

Based on these observations, we developed a series of hypotheses. First, young, reproductively immature females will be more attracted to fermentation-based baits than older, reproductively mature, egg-laying females. Next, older flies will be more attracted to ripe fruits than to baits. Finally, flies orienting to traps will spatially partition this resource. Specifically, flies within traps will more likely be young females seeking food to mature eggs, while females on the surface of traps will be seeking mates and be more likely to already have mature eggs.

Our objective was to determine if reproductive status affects female *D. suzukii* attraction to and capture in monitoring traps containing fermentation-based baits, and whether flies collected directly from ripe fruits differed in reproductive status from those collected on and within traps. Determining if there are patterns associated with female attraction to fermentation-based baits and ripe fruits will lead to improved monitoring tools and a better understanding of how to interpret fermentation-based trap captures and their use in IPM programs.

Materials and Methods

Sample collection

We conducted three experiments. The first compared flies collected within traps to those collected on ripe fruits. Three traps (fermenting cup bait; Burrack et al. 2015) were deployed at least 3 m apart within a mixed planting of raspberry and blackberry cultivars at the Upper Mountain Research Station (UMRS) in Ashe County, NC, from 11-12 October 2013. Three traps were also deployed along a brush-lined creek that ran parallel to the planting; each trap

was located 10m away from a trap within the planting. Flies were collected from within traps 24h after they were deployed, while flies were periodically aspirated off ripe raspberries and blackberries located near traps.

Next, we compared flies collected on the surface of traps to those collected within traps. Four traps (yeast and sugar bait; Burrack et al. 2015) were deployed within a commercial blackberry field in Cleveland County (CC), NC, on 25-26 July and 30-31 August 2014. Four traps were also deployed between the field and an adjacent wooded edge, 20m away from traps within the field. Many *D. suzukii* and other drosophilids were observed on the surface of traps but did not necessarily enter them, and these flies were aspirated from the surface of traps for one minute before flies were collected within traps. Flies were collected hourly for 24h, except during darkness.

Finally, we compared flies collected in all three locations: on the surface of traps, within traps, and on ripe fruits. Eight traps (yeast and sugar bait; Burrack et al. 2015) were deployed within a mixed planting of blackberry cultivars at the Piedmont Research Station (PRS) in Rowan County, NC, on 20-21 July 2016. Traps were placed 12m apart within three rows, and were checked for fly activity every 30m from 5:30pm until dark and again from 6:45-9am. Flies were aspirated from the surface of each trap for one minute before flies were collected within. Flies were then collected from ripe berries surrounding the trap by blowing them into small 10x18 cm mesh bags with drawstring closures.

Flies collected in all three experiments were preserved in 70% ethanol and dissected under a stereomicroscope (Olympus SZX10, Center Valley, PA). The total number of mature

eggs in both ovaries were counted; eggs were considered mature when they possessed fully formed respiratory filaments (Fig. 5.1).

Data analysis

Females were compared according to 1) the likelihood that they had one or more mature egg(s) and 2) the total number of mature eggs present in their ovaries. Trap placement (CC, UMRS) and time of year (CC) were also tested to determine if they affected mature egg counts. Analyses were conducted using generalized linear mixed models via PROC GLIMMIX in SAS v. 9.4 (Table 5.1), and post hoc means comparisons made using the Tukey-Kramer test.

Results

The likelihood of having one or more mature eggs(s) present differed among females. Those collected on ripe berries at UMRS were more likely to have one or more mature eggs (range = 0-14 per female) than females within traps (range = 0-7 per female) ($F_{1,50} = 16.19, P = 0.0002$). Similarly, females collected on ripe berries (range = 0-19 per female) and on the surface of traps (range = 0-10 per female) at PRS were more likely to have one or more mature eggs than females collected within traps (range = 0-7 per female) ($F_{2,227} = 8.64, P = 0.0002$). However, females collected on the surface of (range = 0-10 per female) and within traps (range = 0-2 per female) at CC were equally likely to have one or more mature eggs ($F_{1,67} = 0.05, P = 0.83$).

Females collected on ripe berries generally had more mature eggs than females on the surface of and within traps, while females on the surface of traps had more mature eggs than females within traps; however, the magnitude and significance of these relationships differed between sites (Fig. 5.2). Females collected on ripe berries at UMRS had over five times more mature eggs on average than females within traps ($F_{1,50} = 27.09$, $P < 0.0001$). Although females collected on ripe berries and on the surface of traps at PRS had similar numbers of mature eggs, females collected in both locations had over twice as many mature eggs as females within traps ($F_{2,198} = 10.98$, $P < 0.0001$). However, females collected on the surface of traps at CC did not have more mature eggs than females within traps ($F_{1,59} = 1.05$, $P = 0.31$).

When ripe fruits were readily available, monitoring traps attracted females with low mature egg counts regardless of whether they were located within or outside of the crop. Females collected within traps placed inside the crop planting and within traps placed along the creek at UMRS had similarly low numbers of mature eggs ($F_{1,55} = 0.57$, $P = 0.45$), as did females collected at traps placed within the crop field and between the crop field and wooded edge during the harvest period at CC ($F_{1,6} = 0.04$, $P = 0.84$) (Table 5.2). During both the harvest and postharvest periods at CC, females collected on the surface and within traps had similar numbers of mature eggs ($F_{1,69} = 1.46$, $P = 0.23$), although females collected at traps during the postharvest period had nearly three times as many mature eggs as females collected during harvest ($F_{1,10} = 5.51$, $P = 0.041$) (Table 5.2).

Discussion

Our results support the idea that reproductively mature females with higher numbers of mature eggs are more attracted to ripe fruits than to fermentation-based baits, although the olfactory responses of individual females was likely affected by their physiological state. Female *D. suzukii* raised on diet in the laboratory were sensitive to fruit volatiles in two recent studies (Keeseey et al. 2015, Revadi et al. 2015a). However, headspace volatiles from vinegar were attractive to *D. melanogaster* regardless of age, sex, and mating status, provided the flies had been starved (Becher et al. 2010). Reproductively mature *D. suzukii* females may therefore be more attracted to ripe fruits for oviposition if they are well-fed, but more attracted to fermentation odors from rotting fruits or monitoring traps when hungry or unable to find sufficient nutrients to maintain egg maturation. In fact, *D. suzukii* females became more attracted to both fruit and yeast odors following mating (Mori et al. 2017), suggesting the importance of being able to locate both resources.

Wild *D. suzukii* females had a low degree of egg maturation compared to females reared under controlled laboratory conditions (Plantamp et al. 2016), which suggests that wild flies may not necessarily achieve full reproductive potential. In our study, *D. suzukii* females collected on ripe fruits at UMRS had twice as many mature eggs as females collected on ripe fruits at PRS. Ripe fruits at UMRS were not harvested and fermented and rotted in the field, which may have provided food for flies, as we observed several *D. suzukii* females collected on ripe fruits had red and purple midguts when dissected. Conversely, ripe fruits at PRS were removed for use in other experiments and all females collected on ripe fruits had clear midguts. Our results support the hypothesis that females with higher numbers

of mature eggs were attracted to monitoring traps once oviposition and food resources were depleted during the postharvest period at CC (Table 5.2). It is therefore likely that both resource quality and abundance can affect the responses of reproductively mature *D. suzukii* females to olfactory cues in the field.

Our results also support the idea that reproductively immature females with low numbers of mature eggs are more attracted to fermentation-based baits than to ripe fruits. Regardless of whether such females are looking for nutrients to mature eggs or potential mates, the fermentation-based attractants used in monitoring traps may be useful in removing reproductively immature females from the field before they mate and commence egg-laying. Laboratory studies suggest that *D. suzukii* females can become inseminated within 24 h of emergence, but do not produce offspring until 2.5 d after emergence (Revadi et al. 2015b), which agrees with an earlier estimate of 1-4 d following emergence for the onset of offspring production (Kanzawa 1935). If wild *D. suzukii* behave similarly, under optimal conditions females have approximately 2-4 d to mate, find a protein-rich food source to start egg maturation, and find a suitable oviposition substrate. Therefore, there is potentially a 4 d window in which mass trapping with fermentation-based baits could remove reproductively immature females before they start to lay eggs in crop fields, perhaps using a yeast species or strain attractive to *D. suzukii* (Hamby et al. 2012, Scheidler et al. 2015).

Our results point to differing levels of attraction to ripe fruits and monitoring traps with fermentation-based baits by *D. suzukii* females at different developmental stages, and provide needed context to interpret results of previous studies in which traps with fermentation-based baits were used to monitor for *D. suzukii*. Future research should address

whether the mating status of females on the surface of monitoring traps differs from those within traps. Controlled laboratory studies that compare newly emerged, virgin females and mated, reproductively mature females for differences in sensitivity to fermentation volatiles and volatiles associated with ripe fruits would also be helpful in further defining differential attraction.

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TABLES

Table 5.1. Response and explanatory variables, including fixed and random effects, for all generalized linear mixed models and the distribution of response variables.

Site(s)	Fixed effects	Random effects	Distribution/link
<i>Response variable: Likelihood of having one of more mature egg(s) present in ovaries</i>			
All	Collection location (on trap surface/within trap/on ripe fruit)		Binomial/logit
<i>Response variable: Number of mature eggs present in ovaries</i>			
UMRS	1. Collection location (within trap/on ripe fruit) 2. Trap placement (within planting/along creek)	Trap	Lognormal/identity
CC	1. Collection location (on trap surface/within trap) trap placement (within crop field/between field & edge) 2. Collection location (on trap surface/within trap) collection period (harvest/postharvest)	Trap Trap placement + trap	Lognormal/identity Lognormal/identity
PRS	1. Collection location (on trap surface/within trap/on ripe fruit)	Time of day (30-m interval) + trap	Lognormal/identity

Table 5.2. Effects of trap placement and harvest period on the mean number of mature eggs per fly collected on the surface of and/or within monitoring traps with a fermentation-based bait at Upper Mountain Research Station (UMRS) in Ashe County, NC, in 2013 and a commercial blackberry farm (CC) in Cleveland County, NC, in 2014.

<i>A. Effect of trap placement on mature egg counts</i>				
<u>Period</u>	<u>Trap placement at UMRS</u>	<u>Mature eggs ± SE</u>	<u>Trap placement at CC</u>	<u>Mature eggs ± SE</u>
Harvest	Within crop (n=29)	0.793±0.334a	Within crop (n=16)	1.687±0.746a
	Outside crop (n=30)	1.500±0.596a	Outside crop (n=53)	0.528±0.194a
<i>B. Effect of harvest season on mature egg counts</i>				
<u>Period^a</u>	<u>Collection location at CC</u>	<u>Mature eggs ± SE</u>	<u>Collection location at CC</u>	<u>Mature eggs ± SE</u>
Harvest	Trap surface (n=58)	0.862±0.272a	Combined ^b (n=69)	0.797±0.232b
	Within trap (n=11)	0.454±0.247a		
Postharvest	Trap surface (n=6)	1.333±0.715a	Combined (n=15)	2.267±0.720a
	Within trap (n=9)	2.889±1.086a		

Within sections A and B, values followed by the same letter within a column are not significantly different ($\alpha=0.05$, Tukey-Kramer adjustment).

^aHarvest and postharvest periods at CC were 25-26 July 2014 and 30-31 August 2014, respectively.

^bIncludes flies collected on the surface of and within traps.

FIGURES

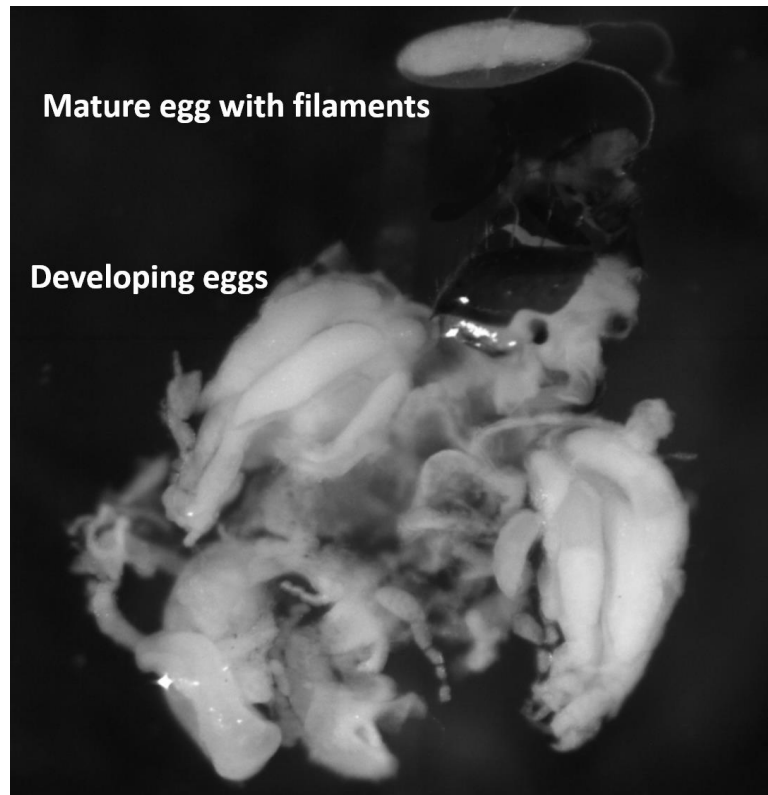


Fig. 5.1. *D. sukuzii* reproductive tract containing developing and mature eggs.

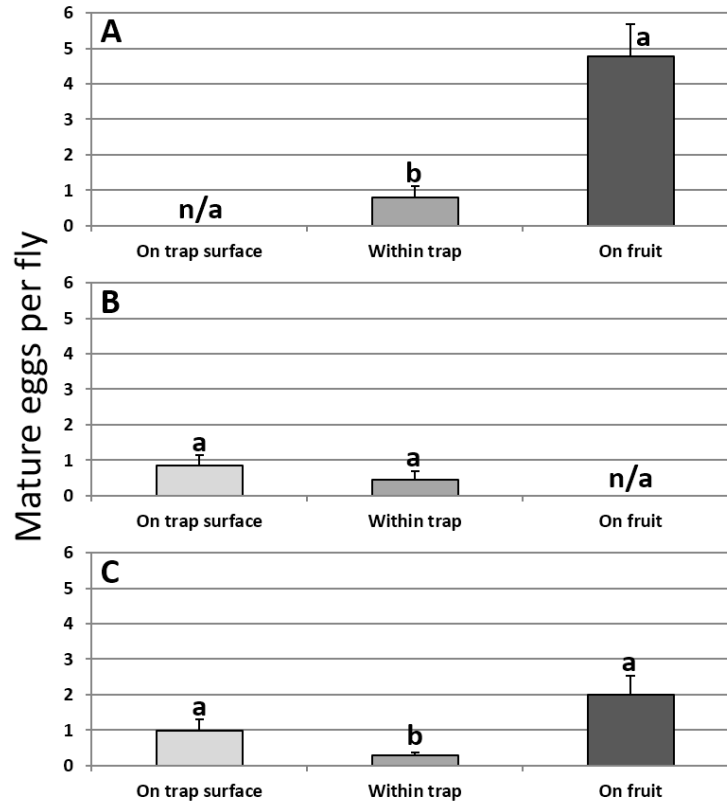


Fig. 5.2. Mean number of mature eggs present in *D. suzukii* females collected on the surface or within monitoring traps and on ripe fruit at A) Upper Mountain Research Station (UMRS) in 2013, B) a commercial blackberry farm in Cleveland County (CC) in 2014, and C) Piedmont Research Station (PRS) in 2016. Bars that share a letter within each location are not significantly different at $\alpha=5\%$.