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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. In a recent study, females in no-choice laboratory bioassays laid eggs in ripening blueberries and blackberries. However, 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 ripening fruit in a field may be able to complete development as the fruit continues to ripen. To test this hypothesis, we used fine mesh cages to prevent later egg laying by *D. suzukii* in fruit at several ripeness stages: green-pink, pink, and ripe raspberries and in green-pink, red, purple, and ripe blackberries. We collected the fruit once they were ripe, and reared out and counted all *D. suzukii* present. This experiment was conducted at two locations during 2013 and 2014, one with very high fly populations and one with low populations. Very few flies emerged from blackberry clusters that were bagged at the green-pink stage, and in general, more flies emerged from clusters bagged at later stages of ripening (purple and red blackberries and pink raspberries) than from green-pink fruit. Knowing what ripeness stages are susceptible to *D. suzukii* infestation will help growers to better pinpoint when to begin applying management tools.

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

Introduction

Drosophila suzukii (Matsumura), 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 ranks among the most severe ongoing biological invasions in the Western Hemisphere (Atallah et al., 2014; Deprá 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 saw-like 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 a zero tolerance threshold for *D. suzukii* larvae in fresh market fruit and a single infested fruit can result in the rejection of an entire shipment.

Insecticides are the primary tool currently used to manage *D. suzukii*, but they have limitations. There are very few insecticides that are both efficacious against *D. suzukii* and have short enough preharvest intervals so that they can be applied to fruit that will go to market within a few days (Beers et al., 2011). For example, 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). In fact, some fields that were sprayed multiple times still contained larvae in 100% of fruit (Burrack et al., 2013), suggesting that insecticides alone may not provide effective *D. suzukii* control in the rainy and humid southeastern United States.

Season-long management of *D. suzukii* is essential. Within individual hosts, knowing which ripeness stages are susceptible to infestation and support *D. suzukii* development would allow growers to pinpoint when to begin applying management treatments. In turn, this would allow them to conserve insecticide applications for the end of the season when *D. suzukii* pressure is higher. Females have been observed laying eggs in green and ripening fruit in no-choice laboratory assays (Kanzawa, 1935, 1939; Lee et al., 2011), although many of these eggs failed to develop. This could be because the fruit used in bioassays was harvested early and brought to the lab, halting the ripening process. Therefore, the objectives of our study were twofold: 1) determine which stages of ripening caneberries are susceptible to *D. suzukii* infestation in the field; 2) determine if *D. suzukii* are able to develop in ripening fruit that are allowed to complete the natural ripening process on the plant. Because infestation in field settings can depend on several factors related to the host plant itself, the larger agroecosystem, and *D. suzukii* populations (Lee et al., 2015), the susceptibility of caneberry ripeness stages was tested in two different field scenarios, 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 could reduce *D. suzukii* infestation in crops (Lee et al., 2015).

Methods

Cultivars

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 cultivars of both floricane-fruiting and primocane-fruiting raspberries and blackberries. 'Nantahala' is a primocane-fruiting 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 in the North Carolina mountains 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 primocane-fruiting blackberry in the NCSU breeding program that had high marketable yields compared with 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 primocane-fruiting cultivar with commercial quality fruit and postharvest shipping potential (Clark and Perkins-Veazie, 2011). In general, primocane-fruiting blackberry cultivars fruit in the fall.

Approach

Mesh bags were placed over ripening berries to prevent *D. suzukii* females from ovipositing further in berries at various ripeness stages, which were left to ripen naturally on the plant and were collected once ripe. The bags measured approximately 13×18 cm and were closed using a drawstring. Bags were secured using 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, numbered, and the number of berries within the bag recorded.

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. Most berries were housed in 1 oz. clear polystyrene portion cups (Dart Conex, Mason, MI), while some of the larger blackberries were housed in 2 oz. clear polystyrene portion cups. Each berry was suspended within the cup in a sling made from a 10×10 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 third instar 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. *D. suzukii* adults were sexed and counted after they emerged within the petri dish, and any non-*D. suzukii* flies noted. If adults emerged inside the rearing containers, they were removed using an aspirator, frozen, sexed, and counted.

Infestation in ripe and ripening caneberries

Three ripeness stages of 'Nantahala' raspberries were tested. Green-pink berries were almost full-sized and had a slight hint of pink. They could not be easily removed 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 October 4, 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 October 12 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 lunch sacks. Some of the raspberries became dislodged from the receptacle during transport to the laboratory and some larvae exited the berries due to the disturbance (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 October 4, 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 and placed into rearing containers in the laboratory. Berries from clusters with more than one ripe berry (n=7) were placed into single rearing containers.

Clusters, with the mesh bag still attached, were cut using clippers and placed into individual paper lunch sacks. 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. All purple berries were ripe on October 12, and were cut and placed into rearing containers on the same day. All red berries were ripe and were collected on October 12, except for two, which were left to ripen until October 18, when they were collected and placed into rearing containers. Similarly, six green-pink berries were ripe on October 12, and were collected, while the remaining berries were left to ripen until October 18, when they were collected and placed into rearing containers.

Infestation patterns in 'Prime-Ark® 45' were measured twice in September-October 2014. On September 28, ripe berries were collected and ripening berries were bagged at the ripeness 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 pinker in color than red. The experiment was repeated two weeks later on October 12, although only ripe berries were collected and purple and red berries bagged due to the later date.

All berries were collected, transported to the laboratory in individual paper lunch sacks, weighed, and placed into individual rearing containers. On September 28, 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 October 12, 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 (every Wednesday and Sunday, except October 26) from September 28 to October 28, detailed data were collected regarding the number of days required for berries at different initial ripeness stages to fully ripen.

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 September 27. Traps were serviced and the yeast and sugar bait replaced on October 5 and 12, and were taken down on October 18. Two traps were deployed within blackberry rows in the commercial 'Prime- Ark® 45' field on September 28, 2014. Traps were serviced and the yeast and sugar bait replaced on October 5, 12 and 19, and were taken down on October 28.

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 found) 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* found 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 was lower in ripening berries in the commercial 'Prime-Ark® 45' field in 2014, and 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 September 28 were determined once berries were ripe and were compared with the weights of ripe berries collected on September 28. 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 ripening caneberries

Infestation in green-pink, pink, and ripe 'Nantahala' raspberries was compared at UMRS in October 2013. *D. suzukii* were able to complete development in green-pink and pink berries that were bagged and left to ripen on the plant (**Figure 1**). On average, more *D. suzukii* emerged from ripe berries than from berries that were 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).

Infestation in green-pink, red, purple, and ripe NC537 blackberries was compared at UMRS in October 2013. *D. suzukii* were able to complete development in green-pink, red, and purple berries that were bagged and left

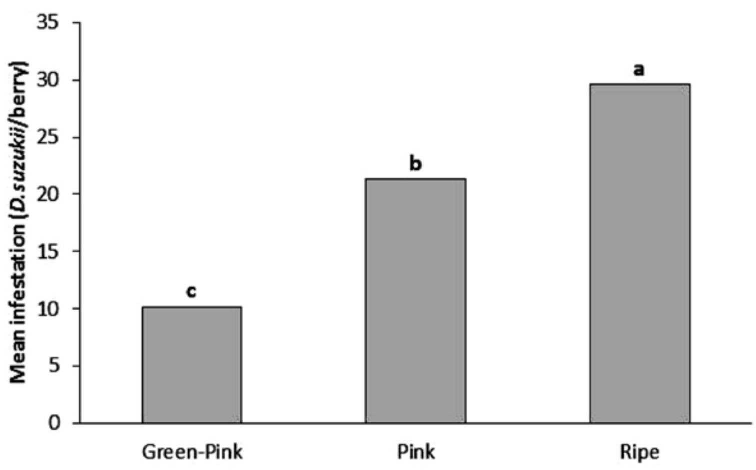

Figure 1. *D. suzukii* infestation in ripening (green-pink and pink) and ripe 'Nantahala' raspberries at Upper Mountain Research Station, Ashe County, North Carolina, in October 2013. Infestation was calculated by averaging total infestation across all berries on a cluster (i.e., cluster averages). Bars that share a letter are not different at α=5%. n=20 cluster averages for each ripeness stage.

to ripen on the plant. However, very few flies emerged from berries bagged at the green-pink stage (**Figure 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 purple berries than from red or green-pink berries. Finally, more flies emerged from red berries than from green-pink berries (F=32.11, df=3, 75, P<0.0001).

Infestation in green, green-pink, red, purple, and ripe 'Prime-Ark® 45' blackberries was compared in a commercial field on September 28, 2014, while infestation in red, purple, and ripe berries was compared on October 12. Overall, very few flies emerged from ripening berries bagged on either date. No infestation was detected in berries bagged at the green, green-pink, or purple stages on September 28. One of 40 red berries (2.5%) bagged on September 28 was infested with *D. suzukii*, while 14 of 22 ripe berries (63.6%) collected on September 28 were infested (**Figure 3**). No infestation was detected in berries bagged at the red stage on October 12, while 3 of 23 purple berries (13.0%) bagged on October 12 and 10 of 20 ripe berries (50.0%) collected on October 12 were infested with *D. suzukii* (Figure 3). The three ripeness stages in which infestation was observed, red, purple, and ripe berries, were not equally susceptible to infestation by *D. suzukii* (F=16.41, df=2, 149, P<0.001). However, differences in the susceptibility of red, purple, and ripe berries to *D. suzukii* infestation did not differ between the two sampling dates (F=0.02, df=1, 149, P=0.9018).

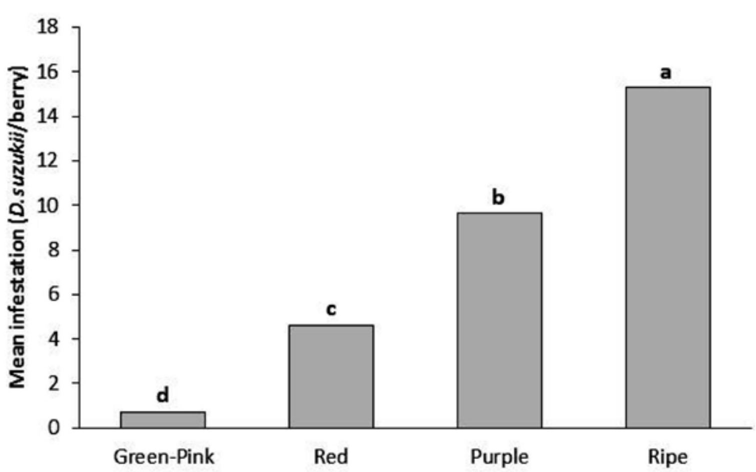

Figure 2. *D. suzukii* infestation in ripening (green-pink, red, and purple) and ripe NC537 blackberries at Upper Mountain Research Station, Ashe County, North Carolina, in October 2013. Infestation was calculated by averaging total infestation across all berries on a cluster (i.e., cluster averages). Bars that share a letter are not different at α=5%. n=20 cluster averages for each ripeness stage, except n=19 for red clusters.

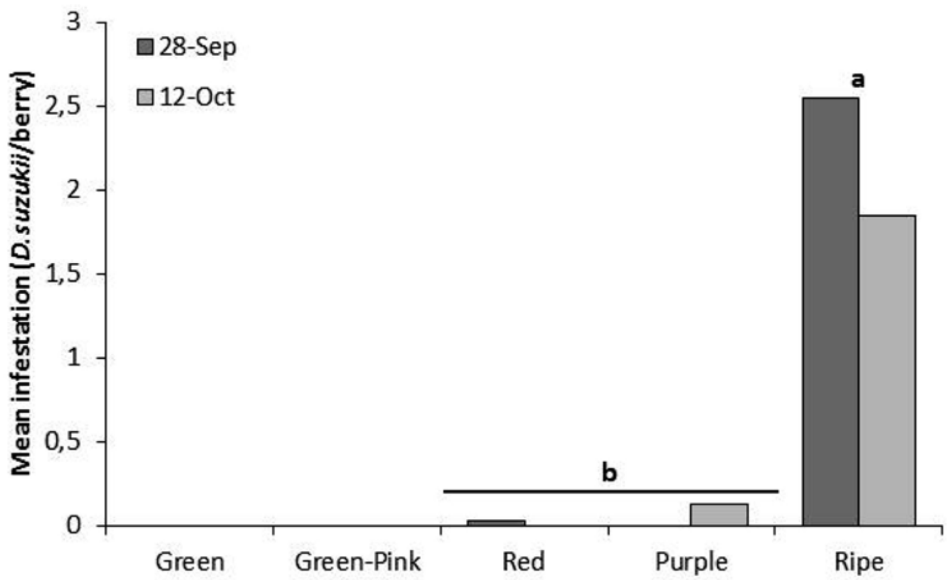

Figure 3. *D. suzukii* infestation in ripening (green, green-pink, red, and purple) and ripe 'Prime-Ark® 45' blackberries at a commercial farm in Cleveland County, North Carolina, on 28 September and 12 October 2014. Green and pink-green berries were not bagged on 12 October. Bars that share a letter are not different at α=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.

Berry ripening times and weight at ripeness

For berries bagged in the commercial field on September 28, purple berries took 3.0 days on average to ripen (n=25 berries), red berries took 6.7 days (n=40), green-pink berries took 16.0 days (n=48), while green berries took 28.2 days (n=24). Of the 54 berries bagged at the green stage on September 28, 22 failed to ripen by October 28 (40.7%), while 8 failed to develop entirely (14.8%). Similarly, 2 of 52 (3.8%) berries bagged at the green-pink stage on September 28 failed to ripen by October 28 and two failed to develop (3.8%). In contrast, all of the berries bagged at the red and purple stages on September 28 ripened completely by October 28. Although some berries failed to ripen completely by October 28, those that did ripen did not weigh less when they were ripe than the ripe berries that were collected on September 28 (F=3.17, df=4, 154, P=0.0154) (**Figure 4**). Interestingly, berries that were bagged at the red stage weighed more when ripe than berries bagged at the green stage.

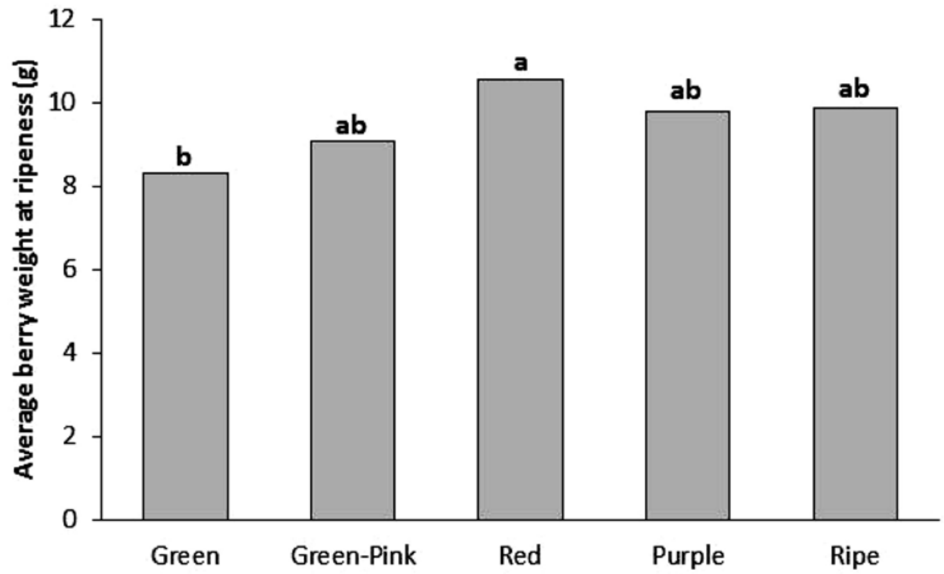

Figure 4. Average weight at ripeness of ripening (green, green-pink, red, and purple) and ripe 'Prime-Ark® 45' blackberries that were bagged or collected September 28, 2014 at a commercial farm in Cleveland County, North Carolina. Bars that share a letter are not different at α=5%. *n*=24 green, 48 green-pink, 40 red, 25 purple, and 22 ripe berries.

Adult fly populations

At UMRS in 2013, an average of 33.3 (±8.1) (mean ±SD) female and 20.4 (±12.1) male *D. suzukii* were caught each day, over the course of the three week experiment, in a trap placed among raspberry cultivars. Similarly, an average of 2.3 (±1.3) female and 0.8 (±0.9) male *D. suzukii* were caught each day, over the course of the experiment, in a trap placed among blackberry cultivars at UMRS. In the commercial field of 'Prime-Ark® 45' blackberries in 2014, an average of 0.6 (±0.6) female and 0.3 (±0.3) male *D. suzukii* were caught each day in two traps deployed over the course of the month long experiment (**Figure 5**).

Discussion

The first objective of this study was to determine which ripeness stages of caneberries are susceptible to infestation by *D. suzukii* in the field. Although infestation was detected in all caneberry ripeness stages observed, in general, more flies emerged from ripe berries and berries bagged at later stages

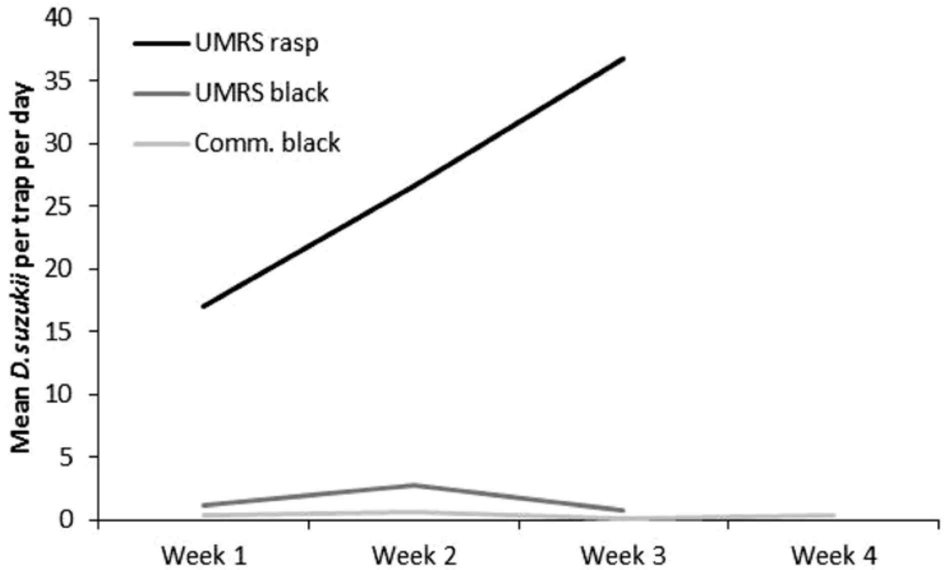

Figure 5. Average number of adult *D. suzukii* caught per trap per day in traps placed within raspberry and blackberry rows at UMRS for three weeks in October 2013 and in traps placed within in a commercial blackberry field for four weeks in October 2014.

of ripening. Infestation rates in ripe raspberries and blackberries at UMRS in October 2013 were very high overall; on average, approximately 30 and 15 flies emerged from ripe raspberries and blackberries, respectively. However, 'Nantahala' raspberries were highly susceptible to infestation once they started to turn color 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 the green-pink stage, suggesting that green-pink blackberries were less susceptible to infestation than green-pink raspberries. Nonetheless, these results add additional support to the idea that fruit are generally susceptible to infestation by *D. suzukii* once they start to color (Lee et al., 2011).

The susceptibility of different ripeness stages of a particular fruit likely depends on characteristics of the fruit at each stage. For example, because fruits tend to become softer as they ripen, ripe fruits and those at later stages of ripening may be easier for *D. suzukii* to puncture with their ovipositor. In fact, several studies have shown a negative relationship between fruit firmness and oviposition. Female *D. suzukii* preferred to lay eggs in ripe fruit versus ripening or green fruit in a series of no-choice and choice bioassays (Lee et al., 2011), while the authors of another study concluded that *D. suzukii* clearly tended to lay more eggs in softer than in firmer fruits (Kinjo et al., 2013). Firmness also influenced *D. suzukii* oviposition in the American black cherry, *Prunus serotina*, in France (Poyet et al., 2014). In fact, it has been suggested that a firmness may exist where *D. suzukii* will not lay eggs. Softer fruit and artificial substrates were more heavily infested than firmer substrates in the laboratory, while no eggs were laid in artificial substrates exceeding 52 centinewtons surface penetration force (Burrack et al., 2013).

Although more *D. suzukii* were present in ripe raspberries and blackberries at UMRS, many *D. suzukii* emerged from berries bagged at earlier stages of ripening, suggesting that females succeeded in laying eggs in some very firm berries. Although firmness readings are usually taken for individual fruits as a whole, firmness may not in fact be independent of fruit size, texture, or overall structure, and may vary across the surface of individual fruits. For example, firmness may be more variable across the comparatively complex surface of raspberries and blackberries, with their many individual drupelets, than across the surface of blueberries, cherries, or other fruits that are comparatively simple and uniform in structure. In fact, the mottled appearance of many purple blackberries suggests that some drupelets start to ripen before others and it is possible that *D. suzukii* can take advantage of this by selecting softer areas within a berry for oviposition. Female *D. suzukii* may begin to lay eggs in fruit once they are physically able to make an incision and insert an egg, and that more and more eggs are laid as individual fruits ripen. Such a tendency could explain the stepwise pattern to infestation observed in increasingly ripe raspberries and blackberries at UMRS (Figures 1 and 2). As such, the accumulation of eggs in ripening fruit is one mechanism that could explain the observed patterns of infestation in both raspberries and blackberries at UMRS.

The idea that female *D. suzukii* may seek out suitable spots for oviposition has been supported by other studies of fruit susceptibility. Incised wine grapes were more favorable for *D. suzukii* oviposition and as a nutrient substrate than intact grapes (Ioriatti et al., 2015). Intact cranberries were not suitable hosts for *D. suzukii* regardless of whether they were unripe, ripe, or overripe; in all cases, very few eggs were laid and none of the larvae survived. However, *D. suzukii* were able to oviposit and survive in cranberries that had been intentionally wounded (Steffan et al., 2013). In another study, peach fuzz appeared to be an obstacle for ovipositing females, who did not oviposit in intact fuzzy peach sections or in small punctures (0.3 or 0.5 mm), but readily laid eggs in sections without fuzz, with insect damage, and with large punctures (1 mm) (Stewart et al., 2014). In addition, fruit coatings did not prevent oviposition in coated raspberries and blueberries; females located suitable oviposition sites despite the presence of the coating (Swoboda-Bhattarai and Burrack, 2014).

The second objective of this study was to determine if *D. suzukii* are able to complete their development in ripening fruit that are allowed to ripen naturally on the plant. In this study, *D. suzukii* were able to infest and survive in all stages of ripening raspberries and blackberries that were bagged and left to ripen naturally on the plant. Previous studies have shown that ripe fruit are a more suitable substrate for *D. suzukii* larval development; more larvae developed in ripe fruit than in ripening or green fruit (Lee et al., 2011) and in artificial diets akin to ripe fruit than in lower quality diets (Hardin et al., 2015). Although green and ripening fruit may initially be less suitable substrates for larval development, 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. In fact, Lee et al. (2011) observed a positive relationship between soluble sugar content and oviposition, while oviposition in wine grape cultivars was correlated with physiological changes that occurred during grape berry development (Ioriatti et al., 2015).

In this study, immature *D. suzukii* were able to develop in green-pink and pink raspberries and green-pink, red, and purple blackberries that were bagged and left to ripen naturally on the plant. The fact that ripening berries bagged on September 28 did not weight less when they were ripe than ripe berries collected on September 28 (Figure 4) 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. This observation could help explain differences between the results obtained during this field study and those obtained by Lee et al. (2011) in the laboratory, in which few *D. suzukii* survived in green and ripening fruit. Additionally, in no-choice laboratory assays, Lee et al. (2015) observed that few or no *D. suzukii* larvae developed in fruits collected from three wild host species, despite the fact that field-collected fruit 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 during the laboratory assays, which 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, using the approach described in this study can provide a powerful method to assess the susceptibility, and suitability for larval development, of different ripeness stages of a particular host species or of several hosts fruiting concurrently and in close proximity.

In addition, the amount of time it takes a berry to ripen might influence how susceptible it is to infestation by *D. suzukii*. In other words, depending on how long berries take to ripen and how long *D. suzukii* larvae take to develop, female *D. suzukii* may or may not choose to lay an egg in a berry at a particular ripeness stage. Several factors can affect *D. suzukii* larval development time. Temperature can have significant effect on *D. suzukii* developmental period and survival. In one study, developmental periods decreased as temperatures increased to 28°C, but increased above 30°C, suggesting that the developmental extremes for *D. suzukii* were approached above this temperature (Tochen et al., 2014). Substrate quality is another factor that can also influence larval development time. For example, *D. suzukii* took 5.7 days to develop from egg to pupa in raspberries and 5.8 days in blackberries that were artificially infested in laboratory no-choice assays (Burrack et al., 2013). Substrate quality and larval density can interact to impact larval development and survival. For example, *D. suzukii* took approximately 8.0 and 8.5 days to develop from egg to pupa in raspberry-based diet, a high quality diet, at low and high densities, respectively. Similarly, *D. suzukii* took 8.5 days to develop at low densities, but 9.5 days at high densities, to develop from egg to pupa in low carbohydrate diets (Hardin et al., 2015). Based on this evidence, *D. suzukii* larvae may take longer to develop in ripening fruit, which is akin to low carbohydrate diets, because soluble sugar content increases as fruit ripen.

However, even though development time may be longer in ripening fruit, some ripening fruit may take too long to ripen to support complete larval development. In the commercial field in October 2014, bagged purple berries took 3.0 days on average to ripen, while red, green-pink, and green berries took 6.7, 16.0, and 28.2 days to ripen on average. If you take into account that *D. suzukii* larvae took approximately 8.5 and 9.5 days on average to develop in low carbohydrate diets at low and high larval densities, respectively (Hardin et al., 2015), it is possible that green and green-pink berries in the commercial field simply took too long to ripen to support the development of *D. suzukii* larvae. In comparison, green-pink berries took 12.9 days to ripen on average at UMRS in 2013, which was a few days faster than those in the commercial blackberry field in 2014. This discrepancy could be due to the different cultivars used in the study; 'Prime-Ark® 45' berries were larger than NC537 berries on average and may have taken longer to ripen. As a result, some larvae may have just made it in green-pink berries at UMRS in 2013, which could explain the comparatively low infestation rates in green-pink blackberries versus green-pink raspberries observed. However, based on the patterns of infestation observed at UMRS in 2013, and the fact that very few *D. suzukii* emerged from red and purple berries in the commercial blackberry field in 2014, it seems unlikely that many eggs were laid in green or green-pink berries in the commercial field in 2014. Even if eggs were laid in green or green-pink berries in the commercial blackberry field, it is possible that they may have failed to develop because of the comparatively long ripening times observed.

Infestation patterns in ripe and ripening blackberries were examined in, and found to differ between, two contrasting field settings that included a small research planting and a large commercial field. The overall patterns of infestation in ripe and ripening raspberries and blackberries were very similar at UMRS in 2013, with highest rates of infestation in ripe fruit and decreasing rates of infestation in less and less ripe fruit (i.e., pink, then green-pink raspberries; purple, then red, then green-pink blackberries). Infestation patterns in ripe and ripening blackberries in the commercial field in 2014 were somewhat different, however. Many of the ripe berries collected on September 28 and October 12 were infested with *D. suzukii*, whereas, very few *D. suzukii* emerged from ripening berries overall. No *D. suzukii* emerged from green and green-pink berries bagged on September 28, while very few *D. suzukii* emerged from red and purple berries bagged on either date. Overall, infestation rates were highest in ripe berries in both the research planting and the commercial field, yet patterns of infestation in ripening berries differed between the two sites.

Our two research sites differed in several respects, but one of the most obvious was that *D. suzukii* population densities were much higher at UMRS than in the commercial field, albeit differed between raspberry and blackberry sections of the planting. Over 10 times more females were caught each day in a trap placed among raspberry cultivars at UMRS than in a trap placed among blackberry cultivars: 33 vs. 2 females, respectively. In contrast, less than one female was caught each day in two traps deployed over the course of the month long experiment in the commercial blackberry field. It is probable that females seek out suitable oviposition sites in the field and may be more likely to do so in high pressure situations. For example, in cases of extreme pest pressure, *D. suzukii* females occasionally oviposit in peach near the petiole where there is less obstructive fuzz (Stewart et al., 2014). Therefore, female *D. suzukii* at UMRS may have been "pushed" to lay eggs in less ripe stages such as green-pink berries, which might not happen in commercial settings because of their larger size and lower population densities.

Fewer adult *D. suzukii* were caught in traps in the commercial blackberry field in 2014 than at UMRS in 2013. The plot at UMRS was untreated and unpicked in 2013, resulting in very high *D. suzukii* population densities, whereas the commercial blackberry 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 may have contributed to very low levels of infestation in all stages of ripening blackberries in the commercial field. All together, these results suggest that infestation in ripening berries was higher when *D. suzukii* pressure was higher. This idea is supported by the fact that infestation was observed, but not quantified, in red berries collected under two higher density scenarios: 1) earlier in the season in the commercial 'Prime-Ark® 45' field, and 2) at another commercial 'Prime-Ark® 45' field in Cleveland County with high infestation rates.

Interestingly, infestation rates in ripe fruit were lower at UMRS in 2011 and 2012 when the plot was being actively managed (i.e., treated with insecticides as needed and picked regularly) (Burrack et al., 2013).

Conclusions

D. suzukii were able to infest and survive in ripening berries. However, ripening berries may be more susceptible to infestation in areas 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*.

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