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Susceptibility of Aronia (*Aronia melanocarpa*) to *Drosophila suzukii* (Diptera: Drosophilidae)

Katie Hietala-Henschell,[1, 2] Emma Pelton,[1, 2] and Christelle Guédot[1, *]

ABSTRACT: *Drosophila suzukii* is an invasive pest of cultivated fruit crops in Europe, Asia, and the Americas. However, more information is needed to understand the extent of *D. suzukii* utilization of wild fruit and specialty crops as suitable hosts, such as aronia (*Aronia melanocarpa*), for which risk assessment has not yet been established. Both laboratory bioassays and field monitoring were conducted to assess the susceptibly of aronia to *D. suzukii*. No-choice bioassays were conducted on damaged, destemmed, and undamaged aronia fruit. Field infestation was assessed using yeast-sugar traps for adults and fruit samples for larvae during the 2015 growing season at three farms in south-central Wisconsin. In bioassays, *D. suzukii* successfully completed its life cycle in damaged and destemmed aronia, while undamaged aronia did not support larval or adult development. Adult flies which emerged from damaged aronia took longer to develop and weighed less compared to adults emerging from raspberry. In the field, adults were abundant throughout the growing season (late June–late September) and larvae were detected in low numbers in ripe fruit samples collected from late August through late September. After harvest, fruit sampled from the processing and packing line revealed low numbers of drosophila larvae. Overall, these findings suggest that damaged or destemmed aronia is susceptible to *D. suzukii* infestation, while intact fruit is resistant to *D. suzukii*. In addition, the bioassays suggest that aronia may serve as a suboptimal host compared to raspberry. These findings suggest the importance of preventing fruit damage before harvest and add to a growing understanding of how wild and specialty crops, such as aronia, may affect population dynamics of this invasive fly.

KEY WORDS: Spotted wing drosophila, no-choice, trapping, non-crop, post-harvest

Drosophila suzukii, also known as spotted wing drosophila, is an invasive vinegar fly that was first detected in the U.S. in 2008 and is now reported in 48 US states (Burrack 2015). *Drosophila suzukii* females have a serrated ovipositor that allows them to cut the skin of ripening and ripe fruit to lay their eggs under the skin of the fruit, as opposed to other *Drosophila* flies that can only infest rotting or damaged fruit. Estimates of yield losses vary greatly depending on crop and location (Bolda *et al.*, 2010; Walsh *et al.*, 2010; Loeb *et al.*, 2012). No economic threshold has yet been established for *D. suzukii,* thus once detected in a crop, fruit growers are advised to apply insecticides every 4–7 days until harvest. These intense control measures can be environmentally and economically costly while not completely preventing crop loss, as insecticides mainly target adult flies, while larvae inside the fruit remain mostly unaffected. However, a recent study found select insecticides (e.g., neonicotinoids, phosmet, and spinetoram) penetrated the skin and flesh of berries resulting in toxicity to eggs and larvae (Wise *et al.* 2015).

Drosophila suzukii has been reported to infest many cultivated fruit crops (e.g., blackberry, raspberry, blueberry, cherry, peach, grape and strawberry) (Lee *et al.*, 2012) and many non-crop wild hosts (e.g., buckthorn, currant, elderberry, honeysuckle, mulberry, bittersweet nightshade and autumn olive; Lee *et al.*, 2015a; Poyet *et al.,* 2015; Kenis *et al.* 2016). Since *D. suzukii* is a generalist of soft skinned fruit there is a need to identify

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susceptible hosts of both crop and non-crops to better understand the impact on farms and natural ecosystems. Recent studies suggest that *D. suzukii* utilize non-crop hosts near field edges which may be a source for crop infestation (Iglesias *et al.*, 2014; Klick *et al.*, 2015; Diepenbrock *et al.*, 2016; Pelton *et al.*, 2016). The linkages between crops and non-crops is of particular concern for growers of *Aronia melanocarpa* (Michx) Elliot (Rosaceae), which is a native cultivated crop also found growing wild in landscapes surrounding farms, and whose susceptibility to *D. suzukii* remains unassessed.

Aronia, also commonly known as chokeberry, is a perennial woody shrub native to North America that produces small clusters of dark purple, tart fruit. Cultivated varieties are growing in popularity as a specialty crop in the Midwest of the U.S. due to aronia's potential as a high value health food crop with gross income per acre around $16,000–$18,000 (Chase 2012), low pest pressure (Hannan and Jauron 2015), and low cultivation requirements (Ochmian *et al.*, 2012). Aronia is also used in landscaping due to its cold hardiness (Ochmian *et al.*, 2012; Hannan and Jauron 2015) and has been cultivated in Europe for over 70 yr (Jeppson 2000).

In Wisconsin, aronia is often present alongside other fruit crops, such as raspberry, grape, and strawberry, potentially contributing to habitats rich in *D. suzukii* hosts. Mixed-crop production systems may increase *D. suzukii* populations by providing an agroecosystem with ample resources throughout the entire season as these crops have staggered fruiting periods (Harris *et al.*, 2014; Joshi *et al.*, 2016). However, the presence of adult *D. suzukii* on a farm may not be indicative of infestation risk and growers could be implemented management practices for a crop that is in fact not susceptible to *D. suzukii* (Pelton *et al.*, 2017).

The objectives of this study were to determine the susceptibility of aronia to *D. suzukii* using no-choice bioassays and to monitor adult and larval infestation in cultivated crops. Given the growing importance of aronia as a cash crop and its presence in urban, farm, and natural ecosystems, the susceptibility of the fruit to *D. suzukii* will have direct implications for fruit growers and may help explain this pest's population dynamics.

Materials and Methods

D. suzukii colony and bioassay materials

Adult flies used in the no-choice bioassays were sourced from a colony at the University of Wisconsin-Madison (Madison, Wisconsin, USA) established in 2013 from infested raspberries and supplemented yearly with wild flies. Flies were reared on a standard *Drosophila* cornmeal and molasses-based diet containing: 4500 cc water, 500 cc cornmeal, 500 cc molasses, 200 cc yeast, 54 gm agar, 20 cc 100% propionic acid, and 45 cc 20% tegosept in 95% ethanol (University of Wisconsin-Madison Department of Genetics). Bioassays were conducted in 355 ml clear plastic cups (Solo Cup, Lake Forest, Illinois, USA) with a fine mesh lid. All cups were placed in Percival I-36LLVLC8 growth chambers (Perry, Iowa, USA) with a 16:8 (L:D) photoperiod at 22°C. Each chamber was provided with a HOBO U12 Temp/RH/Light data logger (Bourne, Massachusetts, USA) to monitor temperature and humidity conditions.

Damaged and destemmed aronia no-choice assays

To compare the susceptibility of damaged and destemmed aronia to *D. suzukii*, no-choice assays were conducted using mechanically damaged aronia, store-bought destemmed aronia, and organic raspberries (Driscoll's Organic Raspberries, USDA organic) as a control.

Aronia fruit of the 'Viking' cultivar was sourced from a farm in Dane County, Wisconsin, USA which does not apply insecticides in their pest management approach. For the mechanically damaged aronia treatment, ripe fruit was collected from the field with the stems intact and damaged by cutting a 10 mm long cut just below skin surface using a utility knife starting from the stem toward the base of the berry (Pelton *et al.*, 2017). For the destemmed aronia treatment, intact berries were obtained from a local grocery store sourced from the same farm. Red, store-bought organic raspberries (Driscoll's Organic Raspberries, USDA organic) were established as positive controls as they are known to be preferred hosts (Lee *et al.*, 2011). Each replicate consisted of eight berries per cup and a total of ten replicates per treatment. Each replicate was exposed to five *D. suzukii* females (0–7 days old) and three males (0–7 days old). To ensure fruit samples were not previously infested, three additional cups of each treatment were established without the addition of flies. After 48 hr, adults were removed from rearing cups using a vacuum aspirator. Five replicates from each aronia treatment were assessed the same day under the microscope to count the number of eggs laid on all eight fruit. An egg was recorded if one or two breathing tube filaments were visible (Pelton *et al.*, 2017). Raspberries were not assessed because breathing tubes were not reliably visible, as noted in Lee *et al.* (2011). Six days after experiment initiation, four of the berries from each replicate were destructively sampled to determine the presence of larvae and count all present larvae (1^{st}–3^{rd} instar). The remaining four berries were checked daily for emerged adults, which were removed until the experiment was terminated after 23 days.

Damaged and undamaged aronia no-choice assays

To compare the susceptibility of mechanically damaged aronia and undamaged aronia, no-choice bioassays were conducted using intact undamaged aronia, damaged aronia, and raspberry as a control. Aronia fruit of the 'Viking' cultivar were sourced from the same farm as in the previous experiment. Aronia treatments included ten replicates of undamaged fruit and ten replicates of damaged fruit with eight berries per replicate. Damage was created as previously described above. Five replicates of three red, store-bought organic raspberries (Driscoll's Organic Raspberries, USDA organic) were established as controls. Replicates were then exposed to five *D. suzukii* females (0–7 days old) and five males (0–7 days old) for 48 hr. Similar to the first assay, five additional replicates of aronia were established without flies to ensure no prior infestation. Adults were removed after 48 hr from rearing cups using a vacuum aspirator. Six days after experiment initiation, two of the aronia berries and one of the raspberries from each replicate were dissected to determine larval presence and abundance. Remaining fruit were checked daily for emerged adults, which were removed until the experiment was terminated after 32 days. Emerged adults were dried at 45–55°C for 24 hr to compare dry mass. Adult weights were summed per replicate and then divided by the total number of flies emerged to get an average weight per fly that represents a single replicate (damaged aronia $n = 10$; undamaged aronia $n = 10$; raspberry $n = 5$). Average weight per fly, representing the average weight of a fly from a single replicate, was used to compare dry mass among treatments.

Field monitoring

Drosophila suzukii adults were monitored using yeast-sugar baited traps in aronia 'Viking' cultivar at three farms in south-central Wisconsin during June–September 2015.

The trapping containers were 32 oz. clear plastic cups and lids (Webstaurant Store, Lancaster, Pennsylvania, USA). Each trap was attached to branches in the fruiting zone of aronia plants, about 60–100 cm above ground depending on shrub height. Ten 5 mm holes were drilled in the top of the cup and the yeast-sugar bait solution was chosen based on previous *D. suzukii* trapping efficacy in multiple crops (Burrack *et al.*, 2015). The bait for each trap was made of 3.5 g of dry active baker's yeast (Red Star, Milwaukee, Wisconsin, USA), 14 g of white cane sugar, and approximately 150 ml of water, and a drop of unscented dish soap (Seventh Generation, Burlington, Vermont, USA).

Three traps were set at each farm; the minimum distance between any two traps was 30 m. Trapping began at fruit set and was terminated two weeks post-harvest. Samples were collected weekly and the bait replaced. Insects were stored in 70% ethanol until further identification in the laboratory. All *D. suzukii* male and female adults were counted and samples with more than 100 *D. suzukii* adults were sub-sampled, using a 4 x 6 gridded tray and counting 20% of the cells (5 of 24 cells) in the gridded tray and calculating a sample total (Pelton *et al.*, 2017).

A total of eight samples (2–3 samples per farm) of 65–100 g of ripe aronia fruit were collected to assess larval infestations at the same three farms in 2015 on three sampling dates, August 31st, September 6th, and September 21st, during the typical ripening period for aronia in southern Wisconsin. Harvestable fruit was collected from all parts of the canopy for each sample. Fruit samples were subjected to a fruit dunk assay consisting of 72 g of salt dissolved in 946 ml of warm water per sample. Fruit was crushed and submerged in the salt solution for at least one hour and sorted under a stereomicroscope to determine presence and abundance of *Drosophila* larvae using the methods from Isaacs *et al*. (2013).

Packing line monitoring for larvae

Due to growers' concerns of *D. suzukii* infestation during the packing process, aronia fruit was collected from one farm at five stages of the fruit packing line upon harvest. The packing line starts by destemming fruit, which then pass through a float tank before being hand sorted into pint containers. The first samples were collected from the beginning of the packing line which will be referred to as "unsorted", the second stage were "floaters" defined as berries that were floating near the surface of the float tank, the third samples were "mid-floaters", the fourth stage were berries that sunk in the float tank and will be referred to as "sinkers", and the final product and the last stage were the "sorted" berries. Each sample consisted of 100 g of aronia fruit and these were subjected to the same fruit dunk assay method described above.

Statistical methods

Results were analyzed using the statistical software R (R Core Development Team 2012) and associated pgrimess package. The no-choice test performance metric results were weighted per fruit for use in statistical analyses. Non-parametric tests were used when data did not meet assumptions for normality. For two-sample comparison, Wilcoxon - Mann-Whitney tests were used. For three-sample comparison, Kruskal-Wallis and multiple comparison Kruskal-Wallis tests were used. An independent *t*-test was used for data that met normality assumptions.

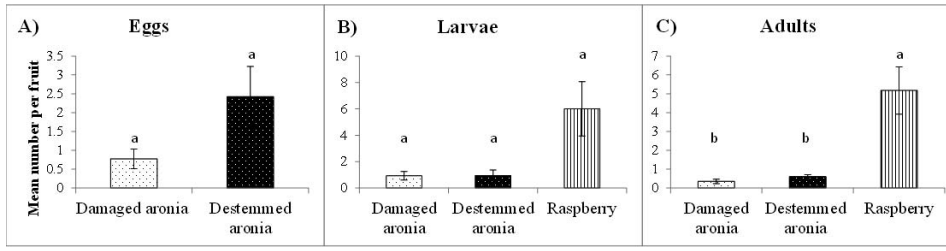

Fig. 1. Number (mean ± SEM) of *D. suzukii* A) eggs; B) larvae; and C) adults in damaged aronia, destemmed aronia, and raspberry in no-choice lab assays. Non-parametric tests were used to analyze the data. A Wilcoxon-Mann-Whitney test was used to test the number of eggs between damaged and destemmed aronia treatments (A) and Kruskal-Wallis was used to test the number of larvae (B) and adults (C) between the three treatments.

Results

Damaged and destemmed aronia no-choice assays

There was no significant difference in the number of eggs per fruit (W = 5.5, P = 0.17) when comparing mechanically damaged aronia (0.78 ± 0.26) and destemmed aronia (2.42 ± 0.80) (Fig. 1). There were also no significant differences (chi-squared = 5.3, d.f. = 2, P = 0.07) in the number of larvae present among damaged aronia (0.93 ± 0.33), destemmed aronia (0.95 ± 0.41), and raspberry (6.00 ± 2.05) (Fig. 1). Statistically significant differences were observed in adult emergence among treatments (chi-squared = 12.9, d.f. = 2, $P < 0.01$), with more adults emerged per fruit from raspberry (5.18 ± 1.25) than either damaged aronia (0.35 ± 0.12) or destemmed aronia (0.60 ± 0.11), and no significant difference between aronia treatments (Fig. 1). *Drosophila suzukii* adults also developed faster (chi-squared = 105.3, d.f. = 2, $P < 0.00001$) on raspberry (12.98 days ± 0.06) than either damaged aronia (18.21 days ± 0.39) or destemmed aronia (18.68 days ± 0.36) with no significant difference between aronia treatments.

Damaged and undamaged aronia no-choice assays

Larvae were present in the mechanically damaged aronia and the raspberry while no larvae were present in the undamaged aronia. Due to an abnormally high unexpected mortality rate of adult *D. suzukii* within the 48 hr exposure period in the damaged aronia cups, this treatment was excluded from analyses as the mortality likely had an effect on the number of eggs laid. A significant difference was seen in the number of larvae per fruit in the undamaged aronia when compared to raspberry (W = 50, $P < 0.05$). No larvae were found in the undamaged aronia whereas 48.8 (± 17.09) larvae were found per fruit in the raspberry. Emerged adults from raspberry (total 42 flies; n = 5) had an average weight of 0.42 mg (± 0.01) per fly, the emerged adults from damaged aronia (16 flies; n = 10) had an average weight of 0.12 mg (± 0.03) and the undamaged aronia had zero adults emerge (0 flies; n = 10). Emerged adults from raspberry weighed significantly more than adults emerged from damaged aronia (t = 9.85, d.f. = 12.10, $P < 0.001$).

Field monitoring

In the field, adult *D. suzukii* were first detected in aronia crops on July 6th and continued to be present at all farms until monitoring ceased on September 21st, 2015. More than 100 flies per trap were caught the week of harvest in late August, whereas peak trap catch was

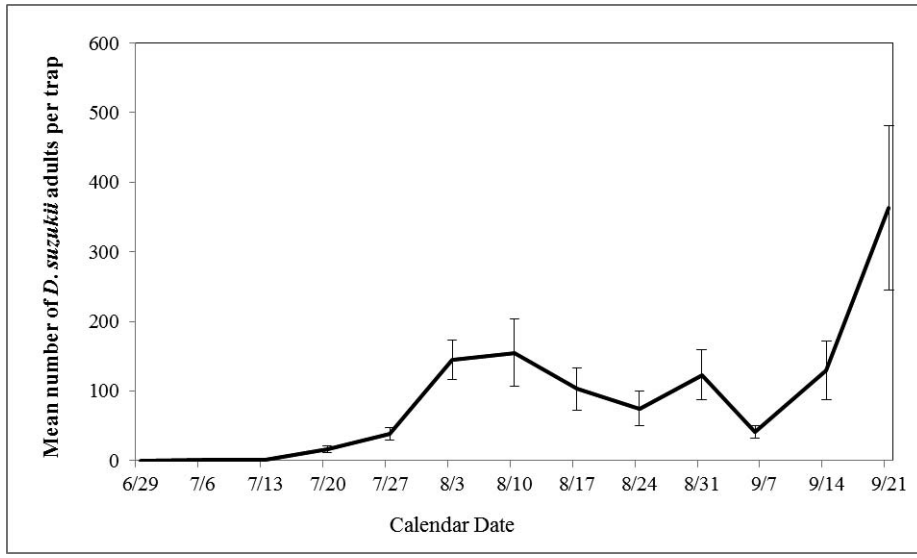

Fig. 2. Number (mean ± SEM) *D. suzukii* adults caught per trap per week from June through September 2015 in aronia at three different farms in Southern Wisconsin.

362.88 (± 118.52) adults per trap in late September, the last week of the study (Fig. 2). Field monitoring for larvae detected three drosophila larvae over the three weeks of fruit sampling. All three larvae were found in one of the eight fruit samples, the other seven samples did not recover any drosophila larvae. Due to the low numbers of larvae recovered from fruit, Drosophila larvae were not reared to adulthood to confirm species.

Packing line monitoring for larvae

Drosophila larvae were detected at low incidences at the beginning of the packing line before sorting (one larva) and during the sorting process (three larvae in floater sample; one larva in mid-floater sample). The low numbers of larvae recovered from fruit did not allow for species confirmation of Drosophila larvae or statistical analysis. No larva was detected in the final stages of the packing line, including both the sinker sample and the sorted sample. Lepidoptera larvae were also detected in the early packing line samples.

Discussion

Overall, the results presented herein suggest that *D. suzukii* can utilize aronia as a host, if the berry is either damaged or destemmed. Intact berries appear to be resistant to *D. suzukii* infestation. A similar pattern of susceptibility has been documented in cranberry fruit (Steffan *et al.*, 2013) and cold climate grape varieties (Pelton *et al.*, 2017). The resistance of undamaged aronia to *D. suzukii* may be due to physical characteristics, such as firmness and skin toughness, which may prevent successful oviposition. In one study, aronia fruit (498 G/mm) were the firmest when compared to five other small black fruits including blueberry (351 G/mm), elderberry (289 G/mm), blackberry (223 G/mm), honeysuckle (116 G/mm), and bilberry (86 G/mm) (Ochmian *et al.*, 2009). Lower *D. suzukii* oviposition rates are also associated with higher fruit penetration force, a measure of fruit firmness and skin

toughness (Lee *et al.*, 2015b; Ioriatti *et al.*, 2015). However, if the aronia fruit is damaged, physical characteristics, such as firmness and skin toughness, may also be altered and be less of a barrier to oviposition. Fruit damage from other pests or cracking of skin associated with abiotic factors (e.g., hail events) associated with field conditions could increase *D. suzukii* infestations (Ioriatti *et al.*, 2015) and aronia is known to be susceptible to cracking damage, similar to cherry crops (Jeppson 2000).

While *D. suzukii* completed its development in aronia, aronia-reared adults took significantly longer to emerge and weighed significantly less than those reared on raspberry, suggesting that aronia is a suboptimal host. During the larval assessments of the bioassays, we noted that larvae in raspberry were predominately in their third instar, while larvae in the aronia were predominately in their first instar; this pattern continued with a longer emergence time to adulthood. Because aronia has relatively high sugar content (between 12–20% SSC; Ochmian *et al.*, 2012), aronia may have other properties (e.g., plant defense compounds, acidity) which compromise growth. While beyond the scope of this study, slower development rates and smaller bodies may have negative effects on the fitness of *D. suzukii* in aronia and subsequent impacts on local population dynamics (Diepenbrock *et al.*, 2016).

Drosophila suzukii adults were trapped in aronia crops throughout the growing season, and the highest numbers occurred during the last week of trapping which was after aronia harvest. Aronia fruit is typically machine-harvested which can lead to a significant number of aronia berries left on shrubs. The remaining berries may be damaged or fall to the ground, providing oviposition and feeding opportunities for *D. suzukii* and other insects. This phenomenon has been observed in citrus crops with *D. suzukii* utilizing fallen, split fruit (Harris *et al.*, 2014). Alternately, or in addition to increased oviposition site availability, high counts of *D. suzukii* in traps in late September may also be a product of peak adult populations (typically mid-August to late September) observed in raspberry crops and surrounding woodlands in southern Wisconsin (Pelton *et al.*, 2016). Despite adult presence in all three aronia fields sampled, larval infestation rates were low in field-collected fruit samples and on the packing line. This mismatch between adult and larval numbers has also been observed in other crops that have limited susceptibility, such as cold hardy grape crops (Pelton *et al.*, 2017), and may be due to the fruits' resistance to oviposition if undamaged. Together, these findings suggest that monitoring aronia crops for berry skin damage, from biotic and/or abiotic factors, and larval infestation in berries may be a more useful monitoring approach than monitoring solely for adults. This multi-pronged approach to monitoring will allow growers to assess infestation risk and apply management strategies if warranted.

Because *D. suzukii* can utilize damaged aronia to complete its development, the shrub (cultivated and uncultivated) may be contributing to local, late season population build ups and/or serve as an alternative host until more preferable hosts become available. The effects of spill-over from susceptible non-crop hosts to adjacent crops, while still poorly understood, is an active area of research (e.g., Iglesias *et al.*, 2014; Klick *et al.*, 2015; Diepenbrock *et al.*, 2016; Pelton *et al.*, 2016). These questions are of particular importance as *D. suzukii* has become a major invasive pest of soft skinned fruit worldwide in less than 10 yr and recent models suggest the species will continue to spread (reviewed in Asplen *et al.*, 2015). Identifying more crop and non-crop hosts which are susceptible to *D. suzukii* and further understanding of source-sink dynamics will help us better assess risk to fruit growers, improve management practices, and better assess the impact of this devastating invasive pest on fruit in natural ecosystems.

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