

Research Note

Risk of Spotted-Wing *Drosophila* Injury and Associated Increases in Acetic Acid in Minnesota Winegrapes

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Abstract: Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is an invasive species to Minnesota that was first recorded in 2012. Since its arrival it has become a major pest of stone fruit and berry crops, including winegrapes. High fecundity and short generation times have allowed *D. suzukii* to flourish and spread throughout North America and Europe in a relatively short period of time. Laboratory and field trials were conducted between 2017 and 2019 to determine the risk of injury from *D. suzukii* in Minnesota winegrape varieties and to assess acetic acid (AA) levels in wine and juice samples from cold-hardy winegrape varieties in Minnesota. Results from risk of injury studies in 2017 and 2018 demonstrated a low risk of direct injury to intact grape berries. Winemakers, however, are concerned about the potential risk of *D. suzukii* infestations increasing AA-producing bacteria (e.g., *Acetobacter* spp.), known to expedite the development of sour rot in grapes. AA trials in 2017 and 2019 demonstrated significant increases in AA for select grape varieties as fly density increased. However, the 2018 AA trials with modified infestation protocols did not result in significant differences in AA. Our results are discussed within the context of improving integrated pest management programs for *D. suzukii*.

Key words: acetic acid bacteria, *Drosophila suzukii*, integrated pest management, risk of injury

Drosophila suzukii (Matsumura), commonly known as spotted-wing drosophila, is an invasive species native to East Asia (Walsh et al. 2011, Daane et al. 2016) and has become a major pest of berry crops in all new countries where it has invaded. *D. suzukii* was first recorded in North America in 2008 (Hauser 2011). Soon after, *D. suzukii* was initially detected in Minnesota in 2012, with severe economic damage observed there every year since then (Asplen et al. 2015, Digiorgio et al. 2019). *D. suzukii* prefers oviposition in healthy, maturing

fruit, which is the leading cause for the excessive economic losses associated with this pest (Asplen et al. 2015). The high level of fruit damage is facilitated by the female's serrated ovipositor, which allows for penetration of healthy fruit skin and deposition of eggs just under the fruit's skin (Lee et al. 2011a, Atallah et al. 2014). The most damaging periods of the *D. suzukii* lifecycle are female oviposition, egg hatch, and the development of larvae within berries. Once eggs hatch, the larvae begin to consume the flesh of the fruit as they undergo three larval instars, eventually making the fruit soft and unmarketable (Asplen et al. 2015).

D. suzukii has a wide host range among numerous stone fruit and berry crops (Bellamy et al. 2013, Asplen et al. 2015). *D. suzukii* prefers hosts such as raspberries and strawberries, and winegrapes are less preferred (Lee et al. 2011b). The lack of preference or suitability has been determined from studies demonstrating that *D. suzukii* females have difficulty ovipositing in grapes unless previous injury has occurred (Ioriatti et al. 2015, Holle et al. 2017, Pelton et al. 2017). However, berry injury can be common in winegrapes where growing conditions support the rapid uptake of water, resulting in a physiological condition known as splitting of the fruit skin (Opara et al. 1997, Galvan et al. 2006). Splitting, along with other forms of injury from birds, yellowjackets, and pathogens, may also facilitate injury and compromise the integrity of the berry skin (Galvan et al. 2006, 2007), which in turn allows *D. suzukii* to oviposit successfully in grapes (Holle et al. 2017). However, even when given the opportunity to infest winegrapes via previous injury, it has been found that *D. suzukii* eggs and/or larvae often have a low survival rate within the berry compared to other fruit species (Lee et al. 2011b, Holle et al. 2017, Pelton et al. 2017, Shrader et al. 2020).

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Despite these results, and because splitting and other forms of skin injury are common in the Midwest United States, a more recent, critical concern for winegrape growers is the degree to which *D. suzukii* may vector various microorganisms, as has been shown with many other *Drosophila* spp. (Barata et al. 2012). Specifically, *D. suzukii* has the potential to vector *Acetobacter* spp. from one grape to another (Ioriatti et al. 2018). The introduction of *Gluconobacter* and *Acetobacter* spp., also known as acetic acid bacteria (AAB), on grapes can lead to high levels of AAB in the berry crop and eventually increased concentrations of AAB in grape juice or wine (Ioriatti et al. 2018). If grapes contain high levels of AAB, this can increase the spoilage rate and also cause a disease known as sour rot (Barata et al. 2012, Ioriatti et al. 2018). Sour rot is a disease that occurs when grapes convert sugars into ethanol and eventually convert the ethanol into acetic acid (AA) (Hall et al. 2018). In vineyards where sour rot is abundant, the resulting juice becomes contaminated and may result in unacceptable levels of AA and unmarketable wine (Zoecklein et al. 1995).

The Minnesota winegrape industry is now estimated to have >70 wineries and an approximate \$80 million annual impact to the state's economy (Tuck and Gartner 2016). It is therefore imperative that timely research be conducted on both endemic and invasive pest species that pose a threat to the industry. Due to the limited amount of research conducted thus far with *D. suzukii* on winegrapes under Minnesota climatic conditions, new studies were initiated to assess the overall risk of direct injury to intact berries of popular commercial, cold-hardy grape varieties. An additional aim of this study was to assess the potential for increased volatile acidity, or more specifically AA concentrations, in the juice and wine when *D. suzukii* are present on berries.

Materials and Methods

Risk of injury: Laboratory studies. All varietal susceptibility studies during 2017 to 2018 were conducted using winegrapes produced at the Horticultural Research Center (HRC) in Excelsior, MN (44°52'N; 93°38'W). All varieties were produced using standard Midwest region practices for fertility and vine management. Thirty-four varieties and breeding selections were screened to assess the risk of injury to obtain a preliminary assessment of varietal susceptibility to *D. suzukii*. For each variety, 10 intact berries with attached pedicel were collected at harvest maturity and placed in individual 30-mL plastic cups with lids (Dart Container Corp.). Harvest maturity was determined by the viticulturist on-site at the HRC, where the desired total soluble solids (TSS) level for each variety was monitored; when grapes reached their desired TSS (range: 17 to 25 Brix, depending on variety), we initiated harvest within one to two days. Following berry collection, samples were placed in a cooler and transferred to the lab, where each undamaged berry was placed in an individual 25 × 95 mm polystyrene vial (Genesee Science). Prior to placing berries in vials, they were examined for any breaks in the berry skin under a dissecting microscope (Leica EZ4W, Leica Microsystems) and checked to be sure the pedicel was intact.

D. suzukii flies were obtained from a University of Minnesota laboratory colony maintained at 23°C, 40% humidity, and photoperiod of 16L:8D (Stephens et al. 2015). For each berry, three male and three female *D. suzukii* adults were placed in each vial; the vials were then sealed with a foam stopper (Genesee Science). Flies and berry samples were maintained under the same temperature, humidity, and photoperiod conditions described previously for colony rearing; berries were held for one week to allow ample time for mating and oviposition (Holle et al. 2017). By the end of the week, the majority of flies had died; any remaining flies were removed using gloves and a small camel hair brush. Vials were then held for an additional week in the same chamber until they were examined for risk of injury using a dissecting microscope to identify and count the total number of *D. suzukii* larvae, pupae, and adults present. Risk of injury was characterized by *D. suzukii* females' ability to infest intact berries. Data were recorded for total infestation per berry.

The 2018 studies were modified to screen fewer varieties but evaluate injury risk over time as berry skin aged, via multiple harvest dates, versus taking measurements only at harvest maturity. Varietal selection was based on the 2017 results, from which we intentionally selected four varieties with low risk of injury and four varieties with a higher risk of injury. The four low-risk varieties selected were Frontenac, Itasca, Marquette, and La Crescent. The four high-risk varieties selected were MN1259, MN1280, Swenson Red, and Vanessa. Berries were collected weekly starting at veraison and continued until harvest maturity, as previously described. Each week, 30 intact berries with pedicel attached were collected from each variety and placed in individual 30-mL plastic cups. In the laboratory, each berry was placed in a 25 × 95 mm polystyrene vial. Twenty-seven berries were each infested with three male and three female *D. suzukii* adults from the laboratory colony. The remaining three berries were not infested, to use as a negative control. Vials were sealed with a foam stopper. Flies were held on the berries for one week and then removed. Vials were then held for one more week and afterward were examined for infestation using a dissecting microscope to identify and count the total number of *D. suzukii* larvae, pupae, or adults; data were recorded on a per-berry basis.

AA levels in juice: Field studies. To assess how much AA may accumulate in fruit in the field, vineyard trials were conducted in 2017 and 2019 at the HRC, University of Minnesota, in Excelsior, MN. Varieties studied in 2017 and 2019 were Marquette, Frontenac, La Crescent, and Itasca. Trials were set up ~2 weeks prior to the projected harvest maturity date. Trials consisted of three treatments with four replications in each variety. Treatment 1: control with zero flies; Treatment 2: five pairs of male and female adult *D. suzukii*; Treatment 3: 10 pairs of male and female adult *D. suzukii*. An in-situ vineyard experiment was conducted to enclose *D. suzukii* with a single cluster of grapes using handmade fine-mesh bags with hole sizes of 0.60 mm × 1.05 mm. The handmade bags also excluded other vertebrate and invertebrate pests from the clusters. Before infesting, small 5 to 10 mm

incisions were made on the exterior of 15 to 20 berries to imitate berry injury and to ensure *D. suzukii* adults could successfully feed on and oviposit onto the berries. Clusters that were not infested with *D. suzukii* also received small incisions in ~15 to 20 berries to ensure fair comparison across treatments. Incisions on the berries were made using a small scalpel and meant only to cut the exocarp of the berry, exposing the flesh but not cutting into the flesh. Mesh bags were tied up with the drawstring sewn into the top, and clusters were left on the vine for 2 weeks. After the berries had been harvested, the clusters were crushed and juiced individually. The juicing process consisted of placing each individual cluster in a 1-gallon Ziploc bag (S.C. Johnson & Son, Inc.) to be crushed by hand. Once crushed in the Ziploc bag, the contents were poured and pressed through a stainless steel china cap strainer (New Star Foodservice). The resulting juice was collected in Falcon 50-mL conical centrifuge tubes (Fisher Scientific) in amounts no less than 25 mL and stored in a freezer at -62°C until further analysis. Since studies were conducted on a single cluster of grapes per variety, only a small amount of juice could be used for testing.

Juice samples were delivered via overnight express shipments to the Iowa State University, Midwest Grape and Wine Industry Lab, Ames, IA, to conduct the enzymatic assays to obtain the AA concentrations.

Temperature data. For the 2017 and 2019 trials conducted in the field, ambient temperature data (daily) were collected at the HRC, from the automated weather station (RainWise Co.) “Chaska (Univ. of MN-HRC)” via <http://newa.cornell.edu> to assess potential impacts on *D. suzukii* activity. Date ranges for 2017 were 13 to 28 Sept, and 3 to 20 Oct; for 2019 the dates were 18 Sept to 1 Oct and 1 to 15 Oct. Average daily maximum and minimum temperatures were calculated for each of the four trials.

AA levels in wine and juice: Laboratory study. To evaluate how much AA could accumulate in both wine and juice, a laboratory study was conducted in 2018. Methods were modified by decreasing the number of varieties screened to increase the number of grapes per variety, to provide ample juice and wine for AA measurements, and to conduct infestations indoors versus the field. For the wine studies we did not collect information on pH, titratable acidity, ethanol, or residual sugars at harvest, nor in the resulting wine samples. Frontenac and Itasca berry samples were covered with 80-g mesh netting (ExcludeNet, Tek-knit Industries) just before veraison occurred to exclude any potential pests from infesting the fruit prior to harvest. Once grapes reached harvest maturity (as defined previously), they were collected in tubs and transferred to the winery for infestation. Grapes placed in the tubs were indirectly injured during the harvest process as normal, less cautious harvesting will remove pedicels and damage the exocarp of berries, to ensure *D. suzukii* would feed on and oviposit in the grapes. Next, 2 kg (~28.5 clusters) of grapes of each variety were placed in individual tubs. Once this was done, infestations occurred. The two treatments included a control with no flies and a treatment with 2000 flies (40 vials with an average of 50 flies per vial) per 28.5 clus-

ters. Treatments were replicated three times. Once infestations were completed, bins were secured with 80-g mesh over the top and placed in a large walk-in cooler for four days at 13°C. Once bins were removed, the berries were juiced. All 28.5 clusters, of each treatment and replicate, were placed in a 1-gallon Ziploc bag, where they were crushed by hand. Once crushed in the Ziploc bag, they were poured and pressed through a stainless steel china cap strainer. The resulting juice was collected in 500-mL borosilicate glass Erlenmeyer flasks. Before preparing for fermentation, a Falcon 50-mL sample was collected for each replicate of each treatment. Juice samples were stored in a freezer at -62°C until they were processed to obtain AA concentrations. The remaining juice was then fermented into wine. Yeast used on all batches was DV-10 (Lallemand, Inc.), with a dose rate of 0.25 g/L of juice. Yeast hydration nutrient (GoFerm, Lallemand, Inc.) was also added when the yeast was mixed with water at the rate of 0.35 g/L. Juice was treated with a 29.97 mg/L addition of potassium metabisulfite (KMBS) and cold-settled overnight at 7.2°C, then racked off of juice prior to yeast addition. Proper nutrient levels were not established after nutrient additions had been made. Fermentations lasted 12 days at ~21°C. The clear/settled wine was racked into 250-mL flasks along with a 49.94 mg/L addition of KMBS and was held at -2°C for 14 days until cold stabilization was assumed to be achieved. Once the wine had finished fermentation and no stuck or sluggish fermentations were observed, wine was bottled and stored at 7.2°C, and 50-mL samples of the wine were collected at bottling to send for AA testing.

Juice and wine samples were shipped overnight to the Iowa State University Midwest Grape and Wine Industry Lab in Ames, IA for completion of the enzymatic assays to obtain the AA concentrations.

Statistical analysis. Survey data to assess the risk of injury among multiple winegrape varieties in 2017 were summarized by examining the means and standard errors for each variety. Individual berry infestations were calculated across the 10-berry sample per variety. In 2018, data were summarized as percentage infestation. For AA levels of juice and wine, data were analyzed using an analysis of variance (ANOVA) with R statistical software (R Core Team 2017). A means separation test was conducted using Tukey’s honest significant difference test (Agricolae, *HSD.test*, [Mendiburu 2019]). The analysis was conducted on each individual variety. Analytical assumptions were met prior to analysis.

Results

Risk of injury: Laboratory studies. Infestation results in 2017 with intact berries indicated that among the 34 varieties screened, only four were observed to have a risk of injury based on larval infestations (Table 1). The varieties infested with *D. suzukii* larvae included Swenson Red and Vanessa and breeding selections MN1259 and MN1280. Breeding line MN1259 incurred the highest infestation, with 60% of the berries infested, with an average of 3.9 ± 2.27 (+/-SEM) larvae per berry. Vanessa was 40% infested with an average of 0.4 ± 0.21 larvae per berry, and Swenson Red incurred a

Table 1 Infestation levels of Minnesota winegrapes by *Drosophila suzukii* in laboratory assays in 2017, summarized as mean (\pm SEM) and percent *D. suzukii* infestations of larvae and/or pupae per berry, for 34 varieties collected at harvest maturity. TSS, total soluble solids.

Grape variety ^a	Date harvested	TSS (Brix)	Total berries screened	Mean larvae per berry	% Infested
Brianna	31 Aug 2017	17.7	10	0.0 \pm 0.0	0
MN1259	31 Aug 2017	23.3	10	3.9 \pm 2.27	60
Edelweiss	31 Aug 2017		10	0.0 \pm 0.0	0
Saint Croix	12 Sept 2017	19.6	10	0.0 \pm 0.0	0
MN1369	12 Sept 2017		10	0.0 \pm 0.0	0
Swenson Red	15 Sept 2017		10	0.2 \pm 0.19	10
Jupiter	15 Sept 2017		10	0.0 \pm 0.0	0
Vanessa	15 Sept 2017		10	0.4 \pm 0.21	40
MN1213	15 Sept 2017		10	0.0 \pm 0.0	0
Leon Millot	21 Sept 2017	21.1	10	0.0 \pm 0.0	0
Aromella	21 Sept 2017	19.6	10	0.0 \pm 0.0	0
Louise Swenson	27 Sept 2017	20.3	10	0.0 \pm 0.0	0
Itasca	28 Sept 2017	23.9	10	0.0 \pm 0.0	0
Marquette	4 Oct 2017	25.0	10	0.0 \pm 0.0	0
Petite Pearl	4 Oct 2017	20.9	10	0.0 \pm 0.0	0
MN1326	5 Oct 2017	19.9	10	0.0 \pm 0.0	0
Seyval blanc	5 Oct 2017	19.6	10	0.0 \pm 0.0	0
Kay Gray	10 Oct 2017		10	0.0 \pm 0.0	0
Marechal Foch	10 Oct 2017	24.3	10	0.0 \pm 0.0	0
La Crescent	10 Oct 2017	22.1	10	0.0 \pm 0.0	0
Prairie Star	10 Oct 2017	19.1	10	0.0 \pm 0.0	0
Blue Jay	10 Oct 2017		10	0.0 \pm 0.0	0
MN1277	10 Oct 2017		10	0.0 \pm 0.0	0
Frontenac blanc	10 Oct 2017	24.0	10	0.0 \pm 0.0	0
Frontenac gris	10 Oct 2017	24.5	10	0.0 \pm 0.0	0
Pinot noir	13 Oct 2017	21.3	10	0.0 \pm 0.0	0
Frontenac	16 Oct 2017	24.8	10	0.0 \pm 0.0	0
Marechal Foch	18 Oct 2017	25.0	10	0.0 \pm 0.0	0
Chardonnay	19 Oct 2017	21.9	10	0.0 \pm 0.0	0
MN1280	24 Oct 2017		10	0.2 \pm 0.19	10
Valde Penas	25 Oct 2017		10	0.0 \pm 0.0	0
Malbec	25 Oct 2017		10	0.0 \pm 0.0	0
MN1307	31 Oct 2017		10	0.0 \pm 0.0	0
Riesling	3 Nov 2017		10	0.0 \pm 0.0	0

^aVariety sequence listed from early to late maturity, which in turn resulted in early to late harvest times, respectively.

10% infestation with an average of 0.2 ± 0.19 larvae per berry. Breeding line MN1280 also experienced a 10% infestation with an average infestation of 0.2 ± 0.19 larvae per berry. All other varieties experienced zero infestation.

Infestation results for *D. suzukii* larvae in 2018 demonstrated a low level of infestation never exceeding 1%. Among the eight varieties, only four exhibited any risk of infestation (Table 2). However, the pattern of varietal infestation in 2018 did not fully align with the 2017 results. Varieties infested in 2018 included Itasca, Vanessa, La Crescent, and MN1280. Despite a high sample size ranging from 162 to 216 berries for these varieties, only one to two berries were found to be infested.

AA levels in juice: Field studies. Field studies in 2017 indicated significant differences in AA production between varieties as a result of spotted-wing drosophila infestation. Results indicated that as the *D. suzukii* infestation or expo-

sure level increased on Frontenac and La Crescent clusters, a significant increase in AA levels was detected in the juice (Frontenac, $F = 6.264$, $p = 0.009$, $df = 2$; La Crescent, $F = 10.051$, $p = 0.001$, $df = 2$) (Figure 1). Marquette and Itasca, however, did not exhibit significant differences (Marquette, $F = 3.291$, $p = 0.061$, $df = 2$; Itasca, $F = 1.087$, $p = 0.359$, $df = 2$) in AA between 0, 5, and 10 pairs of flies.

In 2019, Marquette and La Crescent showed significant increases in AA levels (Marquette, $F = 6.984$, $p = 0.006$, $df = 2$; La Crescent, $F = 43.284$, $p < 0.001$, $df = 2$) compared to the uninfested control treatment (Figure 2). The varieties Frontenac and Itasca did not differ in their AA levels across different levels of *D. suzukii* infestations (Frontenac, $F = 0.065$, $p = 0.937$, $df = 2$; Itasca, $F = 2.1713$, $p = 0.143$, $df = 2$) across treatments.

Temperature data: Field studies. Weather data indicated that in 2017, average daily maximum temperatures for the

early and late trials were 24°C and 17°C, respectively. 2017 average daily minimum temperatures for early and late trials were 14°C and 7°C, respectively. For trials conducted in 2019, average maximum temperatures for early and late trials were 22°C and 12°C, respectively. Average minimum temperatures for 2019 for early and late trials were 12°C and 5°C, respectively.

AA levels in wine and juice: Laboratory study. In the 2018 lab studies, Frontenac and Itasca juice and wine AA levels did not differ across treatments (Frontenac juice, $F = 4.0$, $p = 0.1161$, $df = 1$; Frontenac wine, $F = 1.2047$, $p = 0.3340$, $df = 1$; Itasca juice, $F = 0.4717$, $p = 0.5300$, $df = 1$; Itasca wine, $F = 0.0901$, $p = 0.7790$, $df = 1$). Frontenac juice averaged 0.005 ± 0.00017 and 0.008 ± 0.00128 AA g/L for uninfested and infested *D. suzukii* treatments, respectively. Frontenac wine averaged 0.177 ± 0.034 and 0.213 ± 0.0038 AA g/L for uninfested and infested treatments, respectively. Itasca juice averaged 0.010 ± 0.0011 and 0.009 ± 0.0003 AA g/L for uninfested and infested treatments, respectively. Itasca wine samples averaged 0.180 ± 0.071 and 0.124 ± 0.0818 AA g/L for uninfested and infested treatments, respectively.

Discussion

Our study to assess the possibility of direct injury by *D. suzukii* in cold-hardy winegrapes in both 2017 and 2018 demonstrated that there is a low risk of injury, with numerous winegrape varieties having zero or very low larval/pupal infestations when intact berries are exposed to *D. suzukii* (Tables 1 and 2). These results are in agreement with a previous Minnesota study in which both intact and previously damaged table grape berries were exposed to *D. suzukii* (Holle et al. 2017). These authors found that only previously damaged berries harbored larval infestations, and averaged 3.57 larvae per berry. For our 2017 study, designed to assess berry susceptibility at harvest, only four of the 34 varieties were found to be at risk for *D. suzukii* injury (Table 1). Our results are also in agreement with Bellamy et al. (2013), who found that California winegrape varieties were much less susceptible to *D. suzukii* compared to other fruit species.

Previous studies have demonstrated that once grapes reach veraison, the outer skin (exocarp) begins to weaken as sugars increase (Ioriatti et al. 2015, Shrader et al. 2019). This characteristic in grapes is what leads researchers to believe that as

Table 2 Infestation levels of Minnesota winegrapes by *Drosophila suzukii* in 2018 laboratory assays, summarized as percent *D. suzukii* infestations of larvae and/or pupae across the total berries screened from veraison to harvest, and mean total soluble solids (TSS) levels at harvest.

Variety ^a	Berry collection start date	TSS (Brix)	Weekly collections	Total berries screened	Total berries infested	Infested (%)
MN1259	30 July 2018	24.53 ± 0.68 ^b	4	108	0	0.0
Itasca	6 Aug 2018	24.70 ± 0.30	6	162	1	0.6
Vanessa	6 Aug 2018	18.83 ± 0.22	7	189	1	0.5
Swenson Red	6 Aug 2018	18.23 ± 0.12	7	189	0	0.0
La Crescent	6 Aug 2018	22.17 ± 0.12	7	189	1	0.5
Marquette	6 Aug 2018	25.23 ± 0.50	7	189	0	0.0
MN1280	6 Aug 2018	23.50 ± 0.25	8	216	2	0.9
Frontenac	6 Aug 2018	26.00 ± 0.58	8	216	0	0.0

^aVarietal sequence listed from early to late maturity, which in turn determined the number of weeks of berry exposure to *D. suzukii*. Because of the near-zero infestation rates, data were pooled for all sample dates.

^bMean ± standard error of the mean.

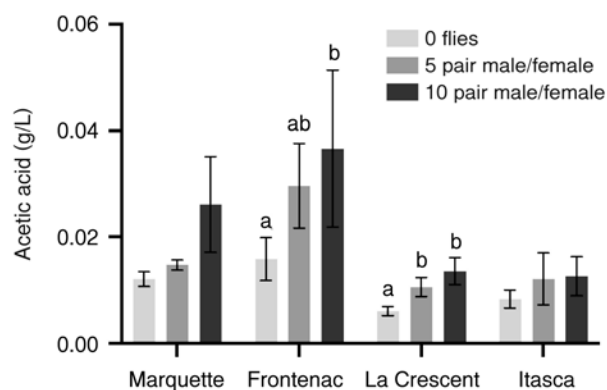


Figure 1 Mean acetic acid levels for four winegrape varieties infested with *Drosophila suzukii* and left on the vine ~2 weeks prior to harvest at the Horticultural Research Center, Excelsior, MN, 2017. Harvest dates were Itasca and Marquette 28 Sept 2017, and Frontenac and La Crescent 20 Oct 2017. Tukey's honest significant difference test, where different letters indicate significance ($p < 0.05$), are exclusive to each variety.

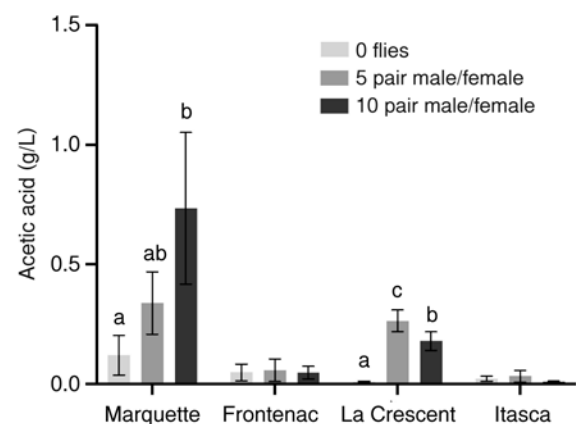


Figure 2 Mean acetic acid levels for four winegrape varieties infested with *Drosophila suzukii*, and left on the vine 2 weeks prior to harvest at the Horticultural Research Center, Excelsior, MN, 2019. Harvest dates were La Crescent and Marquette 1 Oct 2019, and Frontenac and Itasca 15 Oct 2019. Tukey's honest significant difference test, where different letters indicate significance ($p < 0.05$), are exclusive to each variety.

the grape skin matures and weakens, there could also be an increase in berry susceptibility to *D. suzukii*. Data presented in Table 2 do not show an increased infestation over time, but they do demonstrate the ability of *D. suzukii* to oviposit into intact berries of selected varieties at harvest when grape berries are assumed to be most vulnerable to *D. suzukii* infestations. In the 2018 risk of injury study, the results indicated that even when conducting the study over multiple weeks as grapes reach maturity, when berries should be at higher risk, we continued to observe only minimal infestations (Table 2). This trend also occurred despite the higher TSS levels observed for the four late-season varieties (La Crescent, Marquette, MN1280, Frontenac), where TSS ranged from 23 to 25 Brix (Table 2). Another study in the Midwest region, conducted by Pelton et al. (2017) on cold-hardy grape varieties, also found a high level of inherent resistance to *D. suzukii* oviposition on intact grapes. In Virginia, a study conducted for six winegrape varieties demonstrated results similar to those found in our study, where minimal infestation levels were recorded when *D. suzukii* had the option to oviposit only on intact berries (Shrader et al. 2019).

Overall, few studies have been conducted in other states or countries to assess *D. suzukii* oviposition on intact versus previously damaged grape berries (Ioriatti et al. 2015, Holle et al. 2017, Pelton et al. 2017, Shrader et al. 2019). It has been hypothesized that one factor in *D. suzukii*'s ability to oviposit in grape berries arises from the amount of force required to penetrate the skin. Ioriatti et al. (2015) confirmed that as the penetration force needed to penetrate the grape skin decreased with aging grapes, there was an increase in *D. suzukii* oviposition, indicating that this physical characteristic is an important factor in determining the risk of *D. suzukii* injury (Entling et al. 2019). Shrader et al. (2019), with U.S. varieties, also found that penetration force was a reliable indication of susceptibility; grape berries with low penetration force experienced higher *D. suzukii* infestations. One concern regarding differences in varietal injury would be the potential for some berries to be injured prior to infestation with *D. suzukii*. In the present study, all berries were thoroughly evaluated for injury prior to collection, but there is a small possibility that a small skin split could have been missed, which would allow egg-lay to occur. However, the overall low risk of injury to *D. suzukii* infestation for intact grapes is encouraging news for the winegrape industry, yet also demonstrates the need for production practices that minimize other causes of injury to the fruit (Galvan et al. 2006), and for development of new varieties with more pest-resistant characteristics (Clark et al. 2018). For example, Ebbenga et al. (2019) recently demonstrated that the use of exclusion netting in vineyards decreases season-long berry infestations of *D. suzukii* and subsequent berry injury.

Our studies designed to assess the influence of *D. suzukii* on AA indicated similar results in 2017 and 2019, when the same fly exposure protocol was used (Figures 1 and 2). In the 2017 AA field study, we found a significant increase in AA as fly exposure increased for the varieties Frontenac and La Crescent. In the 2019 AA field study, a significant AA

increase was observed for Marquette and La Crescent. These three varieties represent some of the most popular cold-hardy winegrapes grown in the Midwest region. While none of the reported AA levels were found to exceed the legal sensory threshold limits (1.2 g/L for white wines; 1.4 g/L for red wines) as defined by the Standards of Identity in the Code of Federal Regulations (27 CFR), it is important to note that the presence of *D. suzukii* can cause statistically significant increases in AA levels if berry injury is present. Similarly, Ioriatti et al. (2018) demonstrated that with increased numbers of *D. suzukii* adults, there were increases in AAB on grapes that led to increases in sour rot development.

Our studies attempted to take this one step further and measure whether the increase in AA would affect the juice and wine quality. Because the field study in 2017 did not allow enough grapes to make wine, methods were adjusted in 2018 to create a lab study that included more grapes for the wine-making process. But results in the 2018 AA lab study did not demonstrate a significant difference in AA levels between the control and infested grapes for either juice or wine samples. However, observations made while preparing to process the grapes revealed an obvious difference in the condition of the grapes. Grapes that were not exposed to *D. suzukii* appeared clean and unaffected by filamentous fungi or bacteria, whereas grapes that had *D. suzukii* introduced into the containers showed signs of mold and bacterial growth. While this difference did not translate into significant elevated AA levels, the study indeed provided insight into *D. suzukii*'s effect on the overall grape health. Improved methods for obtaining large samples of infested grapes to produce wine and further studies testing *D. suzukii*'s effect on wine quality could give more insight to growers and stakeholders on how the grapes are being affected.

For the 2017 and 2019 AA field studies, trials were conducted at a similar time with similar methods. However, temperatures during the time interval of each trial varied tremendously and may have had an impact on the results by limiting *D. suzukii* activity. Previous studies have indicated temperatures below 10°C will arrest movement and development of *D. suzukii* (Ryan et al. 2016, Leach et al. 2019). In 2019, the late-season trials experienced an average temperature of 8°C, which falls below the 10°C threshold for *D. suzukii*. For both the 2017 infestation and early infestations of 2019, the vineyard temperatures were much higher and would have been more conducive to *D. suzukii* movement and activity (Ryan et al. 2016, Leach et al. 2019). Future studies would benefit in making sure temperature is accounted for when assessing the impact of late-season *D. suzukii* infestations on winegrapes.

Conclusions

In summary, the results for several Minnesota cold-hardy varieties evaluated in this study indicate a low risk of injury from *D. suzukii* when grapes are managed well to minimize splitting and damage by birds or insects that may compromise the berry exocarp. To date, the primary mechanism impacting the risk of infestation and subsequent injury appears to be the physical strength and integrity of the berry skin, particularly

as grapes mature from veraison to harvest. Our results regarding inherent resistance to *D. suzukii*, or lack of direct damage to winegrapes, are similar to previous, yet limited, studies conducted in the U.S. The greater concern for winegrapes is the degree to which AA is produced, particularly late season, near harvest. It is important to conduct more extensive AA studies for additional varieties to better characterize this risk from *D. suzukii*. To better understand the role of specific resistance mechanisms in Minnesota winegrapes, detailed penetrometer studies should be conducted on berries from veraison to harvest for selected commercial varieties and early germplasm sources being used for new variety development. Additional TSS and sugar/acidity ratios should also be examined in more detail in the future. Comprehensive research on resistance mechanisms will help contribute to the development of varieties with multiple pest resistance. Expanding our knowledge of the risk of injury among grape varieties from *D. suzukii* will also help pest managers better understand the extent to which growers can rely on host plant resistance as part of an effective integrated pest management strategy.

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