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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, and since its arrival 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 during 2017-2019 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

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berries. Winemakers, however, are concerned about the potential risk of *D. suzukii* infestations increasing AA producing bacteria (e.g., *Acetobactor spp*.), known to expedite the development of sour rot in grapes. Acetic acid 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 (IPM) for *D. suzukii*.

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

Introduction

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. *Drosophila 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 every year since then (Asplen et al. 2015, Digiacomo et al. 2019). *Drosophila suzukii* prefers oviposition in healthy, maturing fruit, which is the leading cause for the excessive economic losses exhibited by this pest (Asplen et al. 2015). The high level of fruit damage is facilitated via the female's serrated ovipositor that 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 period of the *D. suzukii* lifecycle is female oviposition, egg hatch and the development of larvae within berries. Once eggs hatch, the larvae

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53 begin to consume the flesh of the fruit as they undergo three larval instars, eventually making
54 the fruit soft and unmarketable (Asplen et al. 2015).

55 *Drosophila suzukii* has a wide host range among numerous stone fruit and berry crops
56 (Bellamy et al. 2013, Asplen et al. 2015). Currently, it is known that *D. suzukii* prefers hosts such
57 as raspberries and strawberries, and that winegrapes are less preferred (Lee et al. 2011b). The
58 lack of preference or suitability has been determined from studies demonstrating that *D. suzukii*
59 females have difficulty ovipositing in grapes unless previous injury has occurred (Ioriatti et al.,
60 2015, Holle et al. 2017, Pelton et al. 2017). However, berry injury can be common in
61 winegrapes, where growing conditions support the rapid uptake of water, resulting in a
62 physiological condition known as splitting of the fruit skin (Opara et al. 1997, Galvan et al.
63 2006). Splitting, along with other forms of injury from birds, yellowjackets, and pathogens may
64 also facilitate injury and compromise the integrity of the berry skin (Galvan et al. 2006, Galvan
65 et al. 2007), which in turn allows *D. suzukii* to oviposit successfully in grapes (Holle et al. 2017).
66 However, even when given the opportunity to infest winegrapes via previous injury, it has been
67 found that *D. suzukii* eggs and/or larvae often have a low survival rate within the berry compared
68 to other fruit species (Lee et al. 2011b, Pelton et al. 2017, Holle et al. 2017, Shrader et al. 2019).
69 Despite these results, and because splitting and other forms of skin injury are common in the
70 Midwest U.S., a more recent, critical concern for winegrape growers is the degree to which *D.*
71 *suzukii* may vector various microorganisms, as has been shown with many other *Drosophila spp*.
72 (Barata et al. 2012). Specifically, *D. suzukii* has the potential to vector *Acetobactor spp*. from one
73 grape to another (Ioriatti et al. 2018). The introduction of *Gluconobacter* and *Acetobactor spp.*,
74 also known as acetic acid bacteria (AAB), on grapes can lead to high levels of AAB in the berry

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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 disorder 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 acetic acid 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 2017-2018 were conducted using winegrapes produced at the Horticultural Research Center (HRC) in Excelsior, Minnesota (44˚52'08.1"N, 93˚38'17.3"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

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collected at harvest maturity and placed in individual 30 ml plastic cups with lids (Dart Container Corp., Mason, MI). Harvest maturity was determined by the viticulturist onsite at the HRC, where the desired brix level for each variety was monitored; when grapes reached their desired brix (range: 17-25%, depending on variety), we initiated harvest within 1-2 days. Following berry collection, samples were placed in a cooler, transferred to the lab, where only undamaged berries were placed in individual 25 x 95 mm polystyrene vials (Genesee Science, San Diego, CA). Prior to placing berries in vials, they were examined for any breaks in the berry skin under a dissecting microscope (Leica EZ4W, Leica Microsystems, Buffalo Grove, IL), and checked to be sure the pedicel was intact. *Drosophila 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, San Diego, CA). 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's 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* female's 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

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maturity. Varietal selection was based on the 2017 results, where we intentionally selected 4 varieties with low risk of injury, and 4 varieties with a higher risk of injury. The four low risk varieties selected were: 'Frontenac', 'Itasca', 'Marquette', and 'LaCrescent'. The four high-risk varieties selected were: 'MN1259', 'MN1280', 'Swenson Red', 'Vanessa'. Berries were collected weekly starting at véraison 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, 30 berries were each placed in individual 25 x 95 mm polystyrene vials, 27 berries were 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.

Acetic acid levels in juice: Field studies. To assess how much acetic acid may accumulate in fruit in the field, vineyard trials were conducted in 2017 and 2019 at the Horticultural Research Center (HRC), University of Minnesota, in Excelsior, Minnesota. 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 3 treatments with 4 replications in each variety. Treatment 1: check with zero flies; treatment 2: 5 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 holes sizes of 0.60 mm x 1.05 mm. The

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handmade bags also excluded other vertebrate and invertebrate pests from the clusters. Before infesting, small, 5 – 10 mm, incisions were made on the exterior of 15-20 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 of approximately 15–20 berries to ensure fair comparison across treatments. Incisions on the berries were made using a small scalpel and meant to only 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 two 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., Racine, WI), to be crushed by hand. Once crushed in the Ziploc® bag, they were poured and pressed through a stainless-steel china cap strainer (New Star Foodservice, Chino, CA). The resulting juice was collected in Falcon 50 ml Conical Centrifuge Tubes (Fisher Scientific, Hampton, NH) of 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, Iowa to conduct the enzymatic assays to obtain the acetic acid concentrations.

Temperature data. For the 2017 and 2019 trials conducted in the field, ambient temperature data (daily) were collected at the Horticultural Research Center, from the automated weather station (RainWise Co., Trenton, ME) “Chaska (Univ. of MN-HRC)” via http://newa.cornell.edu, to assess potential impacts on *D. suzukii* activity. Date ranges for 2017

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were 9/13 -9/28, and 10/3-10/20; for 2019 the dates were 9/18-10/1 and 10/1-10/15. Average daily maximum and minimum temperatures were calculated for each of the four trials.

Acetic acid levels in wine and juice: Laboratory study. To evaluate how much acetic acid 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, TA, ethanol, or residual sugars at harvest, nor in the resulting wine samples. 'Frontenac' and 'Itasca' berry samples were covered with 80-gram mesh netting (ExcludeNet, Tek-knit Industries, Quebec, CA) just before véraison 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 pedicles and damage the exocarp of berries, to ensure *D. suzukii* would feed on and oviposit in the grapes. Next, two kilograms (~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 clusters. Treatments were replicated 3 times. Once infestations were completed, bins were secured with 80-gram mesh over the top, and placed in a large walk-in cooler for 4 days at 13°C. Once bins were removed, they 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

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strainer. The resulting juice was collected in 500mL 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., Rhinelander, WI), with a dose rate of 0.25 gram per liter of juice. Yeast hydration nutrient was also added when the yeast was mixed with water, (GoFerm, Lallemand inc. Rhinelander, WI) at the rate of 0.35 gram per liter. Juice was treated with a 30ppm addition of KMBS (potassium metabisulfite) 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 approximately 21°C. The clear/settled wine was racked into 250mL flasks along with a 50ppm addition of KMBS and were 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 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, Iowa to conduct the enzymatic assays to obtain the acetic acid concentrations.

Statistical analysis. Survey data to assess the risk of injury among multiple winegrape varieties in 2017 was 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 acetic acid levels of

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juice and wine, data were analyzed using an analysis of variance (ANOVA) with R statistical software (R Core Team 2017). A mean 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: Lab study. 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 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'. For 'Itasca', with 162 berries screened, only one berry was infested. For 'Vanessa', with 189 berries screened, only one berry was infested. For 'La Crescent', with 189 berries screened, only one

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226 berry was infested. Finally, for the breeding selection 'MN1280', with 216 berries screened, two
227 berries were infested.

228 **Acetic acid levels in juice: Field studies.** Field studies in 2017 indicated significant
229 differences in acetic acid production between varieties as a result of SWD infestation. Results
230 indicated that as the *D. suzukii* infestation or exposure level increased on 'Frontenac' and 'La
231 Crescent' clusters, there was a significant increase in AA levels detected in the juice (Frontenac,
232 $F= 6.264$, $p= 0.009$, $df= 2$; La Crescent, $F= 10.051$, $p= 0.001$, $df= 2$) (Fig. 1). 'Marquette' and
233 'Itasca', however, did not exhibit significant differences (Marquette, $F= 3.291$, $p= 0.061$, $df= 2$;
234 Itasca, $F= 1.087$, $p= 0.359$, $df= 2$) in AA, between 0, 5, and 10 pairs of flies.

235 In 2019, 'Marquette' and 'LaCrescent' showed significant increases in AA levels
236 (Marquette, $F= 6.984$, $p= 0.006$, $df= 2$; La Crescent, $F= 43.284$, $p<0.001$, $df= 2$) compared to the
237 uninfested check treatment. The varieties 'Frontenac' and 'Itasca' did not differ in their acetic
238 acid levels across different levels of *D. suzukii* infestations (Frontenac, $F= 0.065$, $p= 0.937$, $df=$
239 2; Itasca, $F= 0.001$, $p= 2.171$, $df= 2$) across treatments (Fig. 2).

240 **Temperature data: Field studies.** Weather data indicated that in 2017, average daily
241 maximum temperatures for the early and late trials were 24°C and 17°C, respectively. 2017
242 average daily minimum temperatures for early and late trials were 14°C and 7°C, respectively.
243 Trials conducted in 2019, average maximum temperatures for early and late trials were 22°C and
244 12°C, respectively. Average minimum temperatures for 2019, early and late trials were 12°C and
245 5°C, respectively.

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Acetic acid levels in wine and juice: lab 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 acetic acid g/l for uninfested and infested *D. suzukii* treatments respectively. Frontenac wine averaged 0.177±0.034 and 0.213±0.0038 acetic acid g/l for uninfested and infested treatments respectively. Itasca juice averaged 0.010±0.0011 and 0.009±0.0003 acetic acid g/l for uninfested and infested treatments respectively. Itasca wine samples averaged 0.180±0.071 and 0.124±0.0818 acetic acid 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 & 2). These results are in agreement with a previous Minnesota study, where 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), where California winegrape varieties were much less susceptible to *D. suzukii* compared to other fruit species.

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Previous studies have demonstrated that once grapes reach véraison, the outer skin (exocarp) begins to weaken as sugars increase (Ioriatti et al. 2015, Shrader et al. 2018). This characteristic in grapes is what leads researchers to believe that as the grape skin matures and weakens, there could also be an increase in berry susceptibility to *D. suzukii*. Data presented in Table 2 does not show an increased infestation over time, but it does 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 Brix levels observed for the four late season varieties (La Crescent, Marquette, MN 1280, Frontenac), where Brix values ranged from 23-25% (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* only had the option to oviposit on intact berries (Shrader et al. 2018).

Overall, few studies have been conducted in other states or countries to assess *D. suzukii* oviposition on intact vs. previously damaged grape berries (Ioriatti et al. 2015; Holle et al. 2017, Pelton et al. 2017, Shrader et al. 2018). 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 decreases with aging grapes, there was an increase in *D. suzukii* oviposition, indicating this

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289 physical characteristic is an important factor in determining the risk of *D. suzukii* injury (Entling
290 et al. 2018). Shrader et al. (2018), with U.S. varieties, also found that penetration force was a
291 reliable indication of susceptibility; grape berries with low penetration force experienced higher
292 *D. suzukii* infestations. One concern regarding differences in varietal injury would be the
293 potential of some berries having been injured prior to infestation with *D. suzukii*. In the present
294 study, all berries were thoroughly evaluated for injury prior to collection, but there is a small
295 possibility that a small skin split could have been missed, which would allow for egg-lay to
296 occur. However, the overall low risk of injury to *D. suzukii* infestation for intact grapes is
297 encouraging news for the winegrape industry, yet also demonstrates the need for production
298 practices that minimize other causes of injury to the fruit (Galvan et al. 2006), and for
299 development of new varieties with more pest resistant characteristics (Clark et al. 2018). For
300 example, Ebbenga et al. (2019) recently demonstrated that the use of exclusion netting in
301 vineyards decreases season-long berry infestations of *D. suzukii*, and subsequent berry injury.
302 Our studies designed to assess the influence of *D. suzukii* on AA indicated similar results
303 in 2017 and 2019, when the same fly exposure protocol was used (Figs. 1,2). In the 2017 AA
304 field study, we found a significant increase in AA as fly exposure increased, for the varieties,
305 Frontenac and La Crescent. In the 2019 AA field study, a significant AA increase was observed
306 for Marquette and La Crescent. These three varieties represent some of the most popular cold-
307 hardy winegrapes grown in the Midwest region. While none of the reported AA levels were
308 found to exceed the legal sensory threshold limits (1.2 g/l for white wines; 1.4 g/l for red wines),
309 as defined by the Standards of Identity in the Code of Federal Regulations (27 CFR), it is
310 important to note that the presence of *D. suzukii* can cause statistically significant increases in

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311 acetic acid levels, if berry injury is present. Similarly, Ioriatti et al. (2018) demonstrated that with
312 increased numbers of *D. suzukii* adults, there were increases in AAB on grapes that led to
313 increases in sour rot development. Our studies attempted to take this one step further and
314 measure whether the increase in AA would affect the juice and wine quality. Because the field
315 study in 2017 did not allow enough grapes to make wine, methods were adjusted in 2018 to
316 create a lab study that included more grapes for the wine making process. Results, however in
317 the 2018 AA lab study did not demonstrate a significant difference in AA levels between the
318 check and infested grapes for either juice or wine samples. However, observations made while
319 preparing to process the grapes, revealed an obvious difference in the condition of the grapes.
320 Grapes that were not exposed to *D. suzukii* appeared clean and unaffected by filamentous fungi
321 or bacteria, whereas grapes that had *D. suzukii* introduced into the containers showed signs of
322 mold and bacterial growth. While this difference did not translate into significant, elevated AA
323 levels, the study indeed provided insight into *D. suzukii*‘s effect on the overall grape health.
324 Improved methods for obtaining large samples of infested grapes to produce wine, further studies
325 testing *D. suzukii*’s effect on wine quality could give more insight to growers and stakeholders
326 on how the grapes are being affected.

327 For the 2017 and 2019 AA field studies, trials were conducted at a similar time with
328 similar methods. However, temperatures during the time interval of each trial varied
329 tremendously and may have had an impact on the results by limiting *D. suzukii* activity. Previous
330 studies have indicated temperatures below 10°C will arrest movement and development of *D.*
331 *suzukii* (Ryan et al. 2016; Leach et al. 2019). In 2019, the late season trials experienced an
332 average temperature 8°C, which falls below the 10°C threshold for *D. suzukii.* For both the 2017

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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, bird damage, or other 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 véraison 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 véraison to harvest, for selected commercial varieties and early germplasm sources being used for new variety development. Additional Brix 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

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354 managers better understand the extent to which growers can rely on host plant resistance as part
355 of an effective integrated pest management (IPM) strategy.

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

Grape variety	Date harvested	Brix at harvest	Total berries screened	Mean larvae per berry	% Infested
Brianna	8/31/17	17.7	10	0.0 ± 0.0	0
MN 1259	8/31/17	23.3	10	3.9 ± 2.27	60
Edelweiss	8/31/17		10	0.0 ± 0.0	0
Saint Croix	9/12/17	19.6	10	0.0 ± 0.0	0
MN 1369	9/12/17		10	0.0 ± 0.0	0
Swenson Red	9/15/17		10	0.2 ± 0.19	10
Jupiter	9/15/17		10	0.0 ± 0.0	0
Vanessa	9/15/17		10	0.4 ± 0.21	40
MN 1213	9/15/17		10	0.0 ± 0.0	0
Leon Millot	9/21/17	21.1	10	0.0 ± 0.0	0
Aromella	9/21/17	19.6	10	0.0 ± 0.0	0
Louise Swenson	9/27/17	20.3	10	0.0 ± 0.0	0
Itasca	9/28/17	23.9	10	0.0 ± 0.0	0
Marquette	10/4/17	25	10	0.0 ± 0.0	0
Petite Pearl	10/4/17	20.9	10	0.0 ± 0.0	0
MN 1326	10/5/17	19.9	10	0.0 ± 0.0	0
Seyval Blanc	10/5/17	19.6	10	0.0 ± 0.0	0
Kay Gray	10/10/17		10	0.0 ± 0.0	0
Marechal Foch	10/10/17	24.3	10	0.0 ± 0.0	0
La Crescent	10/10/17	22.1	10	0.0 ± 0.0	0
Prairie Star	10/10/17	19.1	10	0.0 ± 0.0	0
Blue Jay	10/10/17		10	0.0 ± 0.0	0
MN 1277	10/10/17		10	0.0 ± 0.0	0
Frontenac Blanc	10/10/17	24	10	0.0 ± 0.0	0
Frontenac Gris	10/10/17	24.5	10	0.0 ± 0.0	0
Pinot Noir	10/13/17	21.3	10	0.0 ± 0.0	0
Frontenac	10/16/17	24.8	10	0.0 ± 0.0	0
Marechal Foch	10/18/17	25	10	0.0 ± 0.0	0
Chardonnay	10/19/17	21.9	10	0.0 ± 0.0	0
MN 1280	10/24/17		10	0.2 ± 0.19	10
Valde Penas	10/25/17		10	0.0 ± 0.0	0
Malbec	10/25/17		10	0.0 ± 0.0	0
MN 1307	10/31/17		10	0.0 ± 0.0	0
Riesling	11/3/17		10	0.0 ± 0.0	0

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

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

Variety	Berry collection start date	Brix Mean ± SEM at harvest	Weekly Collections	Total Berries Screened	Total Berries Infested	% Infested
MN 1259	7/30/18	24.53±0.68	4	108	0	0.0
Itasca	8/6/18	24.70±0.30	6	162	1	0.6
Vanessa	8/6/18	18.83±0.22	7	189	1	0.5
Swenson Red	8/6/18	18.23±0.12	7	189	0	0.0
La Crescent	8/6/18	22.17±0.12	7	189	1	0.5
Marquette	8/6/18	25.23±0.50	7	189	0	0.0
MN 1280	8/6/18	23.50±0.25	8	216	2	0.9
Frontenac	8/6/18	26.00±0.58	8	216	0	0.0

Varietal 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.

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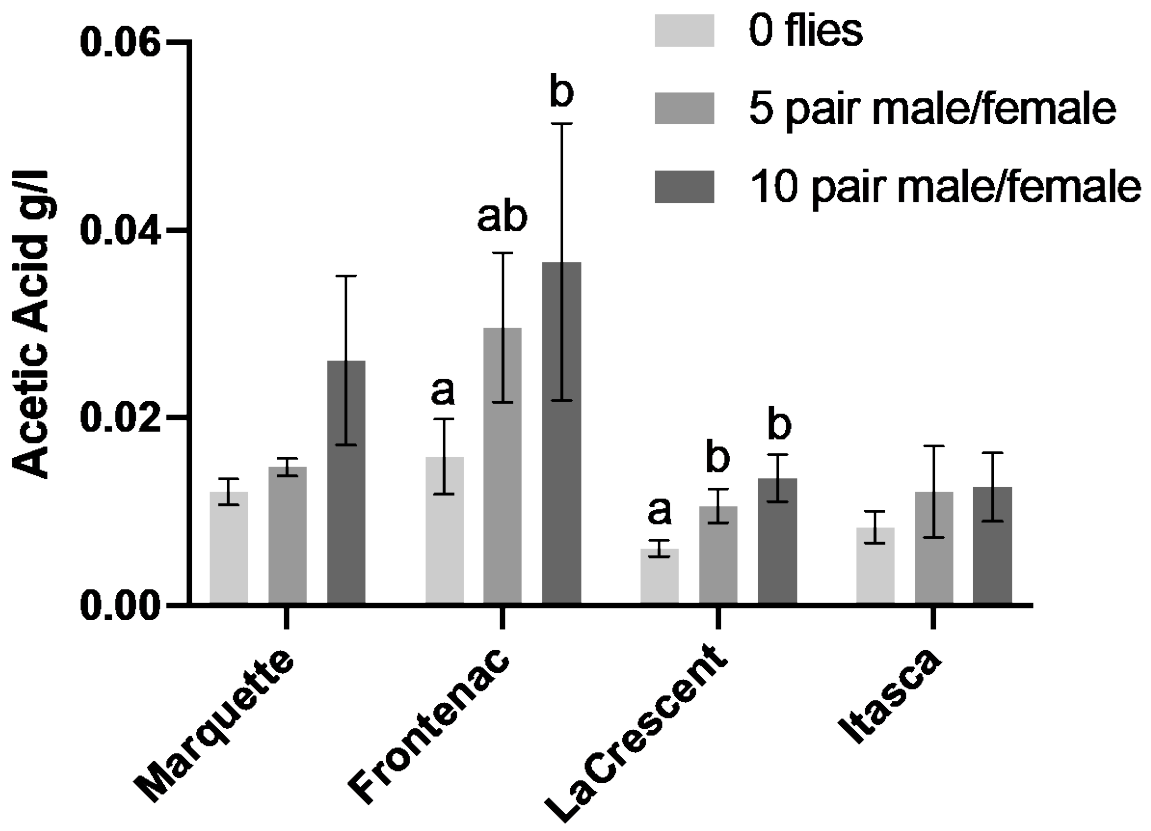

Figure 1 Mean acetic acid levels for 4 winegrape varieties infested with *D. suzukii*, and left on the vine approximately 2 weeks prior to harvest; Horticultural Research Center, Excelsior MN, 2017. Harvest dates were Itasca and Marquette 9/28/17, and Frontenac and La Crescent 10/20/17. Tukey's HSD test, where different letters indicate significance ($P<0.05$), are exclusive to each variety.

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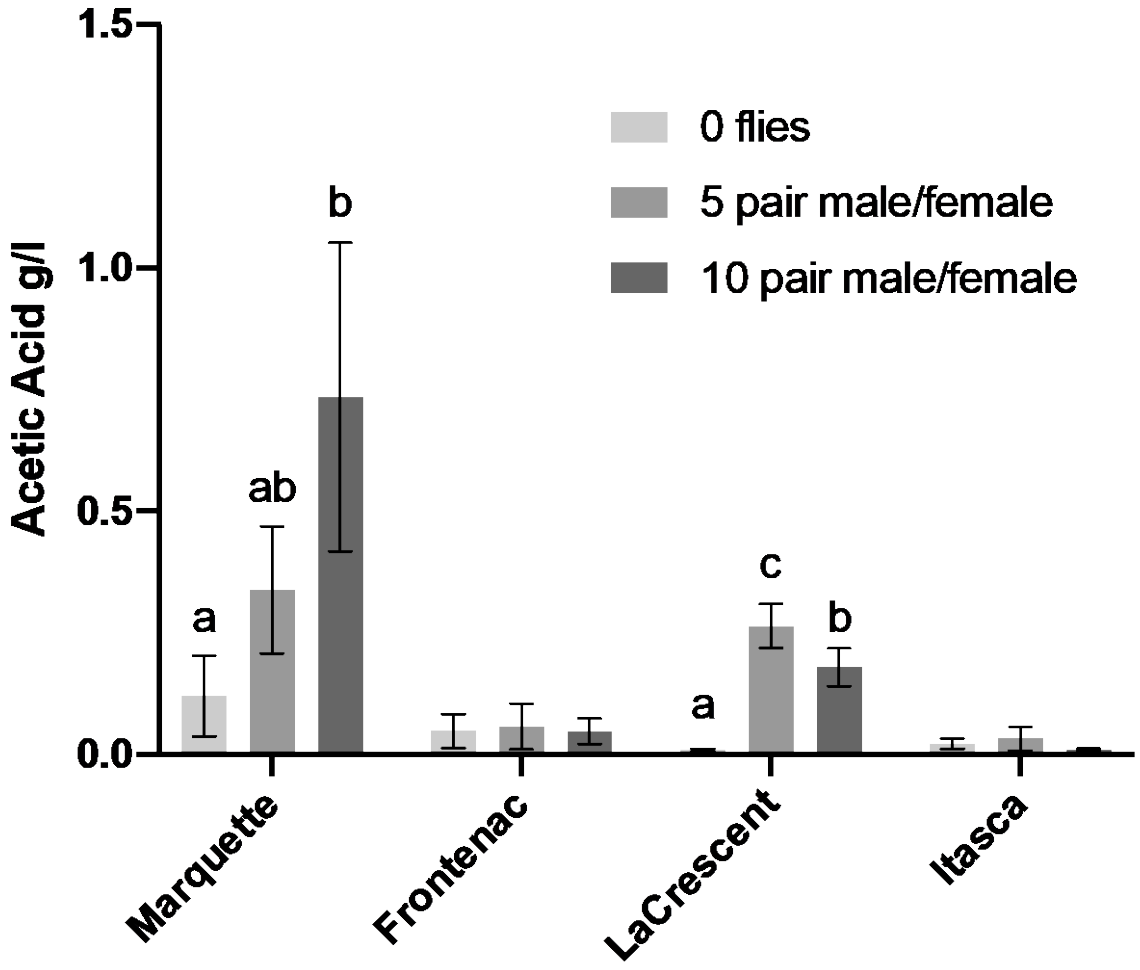

Figure 2 Mean acetic acid levels for 4 winegrape varieties infested with *D. suzukii*, and left on the vine 2 weeks prior to harvest; Horticultural Research Center, Excelsior MN, 2019. Harvest dates were La Crescent and Marquette 10/1/19, and Frontenac and Itasca 10/15/19. Tukey's HSD test, where different letters indicate significance ($P<0.05$), are exclusive to each variety.