Risk factors of *Drosophila suzukii* infestation

in Upper Midwest fruit crops

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CHAPTER 1

Drosophila suzukii arrives earlier at farms in woodland landscapes but population size is unaffected in the Upper Midwest

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Abstract

Natural habitat in the landscape can affect the population dynamics of mobile insect species. The spatial and temporal effects on agricultural pest species may be especially relevant to tailor management strategies. Recent work on *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), known commonly as spotted wing drosophila, has identified the fly's host range to include wild plant species such as dogwood (*Cornus* spp.) as well as cultivated fruits such as strawberry (*Fragaria ananassa*) and raspberry (*Rubus* spp.). Many of the wild hosts grow in woodland habitat in the Upper Midwest (USA), raising the possibility that nearby woodland may increase the risk of infestation of adjacent fruit crops. A two-year field study in the Upper Midwest examined if woodland in the landscape affects the timing and abundance of *D. suzukii* populations in raspberry. In 2013, monitoring was conducted at 18 farms in Wisconsin in both the raspberry crop and the surrounding woodland; in 2014, six farms in Wisconsin, six farms in Minnesota, and five farms in Michigan were part of the study. Landscape composition was analyzed using spatial analysis for relative woodland area in the surrounding 1.5 km around farms, and farms were selected to span a gradient from low to high woodland. Woodland area was negatively correlated with *D. suzukii* timing, suggesting that flies appear earlier at farms in landscapes with high amounts of woodland. There were also significant differences between states, with flies appearing earlier and population growth rates increasing more slowly in Michigan. Woodland area was not correlated with metrics of population abundance (growth rate, peak trap catch, or total trap catch in raspberry). This study suggests the amount of woodland in the landscape affects the timing of *D. suzukii*, possibly due to the presence of alternate hosts in these non-crop habitats, but

does not increase populations in raspberry in the Upper Midwest. These results suggest early ripening summer bearing raspberries and farms in landscapes with low amounts of woodland may be less susceptible to infestation early in the season.

Keywords: landscape composition, alternate host, invasive

Introduction

Landscape composition has the potential to affect the population dynamics of mobile insect species (Gardiner et al. 2009, Tscharntke et al. 2005). The amount of natural habitat in the landscape can be an important driver if it provides resources such as alternate hosts, nesting, or overwintering sites. In agroecosystems, these resources may be otherwise limited by management practices spatially or temporally. The positive correlation between an increasing proportion of natural habitat and insect populations has been well documented for beneficial guilds such as native bees, which provide critical pollination services in apple orchards (Watson et al. 2011) and in predator species such as native ladybird beetles, which provide biocontrol services to surrounding soybean fields (Gardiner et al. 2009). However, natural habitat can also be a source of field pest populations (Rusch et al. 2013). This study aimed to examine the influence of natural habitat on the newly arrived invasive species *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), known commonly as spotted wing drosophila. Recent studies have identified the fly's host range to include wild alternate hosts (e.g., dogwood (*Cornus* spp.), honeysuckle (*Loincera* spp.); Lee et al. 2015) as well as its cultivated hosts such as strawberry (*Fragaria ananassa*) and blueberry (*Vaccinum* spp.) (Lee et al. 2011). Many of these wild alternate hosts grow in woodland habitat in the Upper Midwest raising the potential that woodland in the surrounding landscape may increase the risk of infestation of fruit crops grown in areas with a significant amount of woodland.

While the potential for many wild plants to be hosts of *D. suzukii* has been established (Lee et al. 2015), their relative contribution to the seasonal arrival and severity of crop risk remains unknown. *D. suzukii* is an emerging pest in the Upper

Midwest (initial detections in 2010-2012) and thus early season timing is still poorly understood. The status of *D. suzukii* populations during winter and early spring is unknown, but statewide monitoring has not detected adults until June or July in 2013 and 2014 (E. Pelton and C. Guédot, unpublished). If alternate hosts fruit in the spring, they may provide additional resources to build-up early season local populations which can then move into susceptible fruit crops. If alternate hosts fruit in the fall, they may provide late-season resources. Higher fall populations which overwinter successfully would provide *D. suzukii* populations earlier in the spring. The movement of adults temporally between fruit crops with varying ripening phenologies has been documented in a multi-fruit crop farm in California (Harris et al. 2014).

The amount of woodland in the surrounding landscape may influence not only timing, but also *D. suzukii* population abundance. When both alternate hosts and fruit crops are susceptible, the total amount of resources available to local population also increases. The use of both alternate hosts and fruit crops in the same area by *D. suzukii* has been documented in Florida with wild blackberry (*Rubus* spp.) and adjacent blueberry crops (Iglesias et al. 2014) and in Oregon with wild blackberry and cultivated raspberry (*Rubus* spp.; Ohrn and Dreves 2012). As *D. suzukii* is multivoltine in temperate climates, these additional resources of alternate hosts may lead to higher overall populations.

By understanding the drivers of *D. suzukii* timing and population abundance, growers may be better able to make effective management decisions. *D. suzukii* is a major pest in North America and significant economic losses in berry and tree fruit crops have been sustained since its arrival in California in 2008 (Bolda et al. 2010, Goodhue et

al. 2011). Current insecticide management recommendations for *D. suzukii* are problematic for use in integrated pest management programs because they consist of mainly broad-spectrum insecticides (Van Timmeren and Isaacs 2013, Bruck et al. 2011). These pesticides are non-selective with negative implications for natural enemies (Musser and Shelton 2003) and pollinators, which many fruit crops rely on to produce a marketable yield. Effective insecticide regimes require frequent sprays (Van Timmeren and Isaacs 2013) and increased labor and chemical costs are estimated at 6-8% of farmgate value (Bolda et al. 2010). Management options for organic growers including organically approved insecticides are generally more costly and less effective (Van Timmeren and Isaacs 2013) and cultural methods such as field sanitation are labor-intensive. By understanding the phenology and abundance of *D. suzukii* in different landscapes, growers will be better able to tailor management spatially and temporally to minimize crop risk.

The main question addressed in this study was whether increasing amounts of woodland in the landscape affects the timing and population abundance of *D. suzukii* in raspberry. To examine this question, we conducted a two-year, season-long monitoring study of *D. suzukii* at raspberry farms in three states in the Upper Midwest (USA) in landscapes spanning a low-to-high woodland gradient at the 1.5 km scale. We predicted *D. suzukii* would appear earlier and populations would increase more rapidly in landscapes with higher amounts of woodland area. Over the entire season, we predicted higher peak populations and total populations of adults in raspberry. We also conducted larval sampling of raspberry to determine if adult abundance was associated with the risk of damage to raspberry.

Materials and methods

Site Selection

To test the hypothesis of whether the amount of woodland in the landscape affects the timing and population abundance of *D. suzukii*, we conducted adult and larval monitoring at raspberry farms selected to fall over a low-to-high woodland gradient in the Upper Midwest (USA). Raspberry was used as the focal crop because it is widely grown and one of the most susceptible crops to *D. suzukii* (Bellamy et al. 2013, Lee et al. 2011). In 2013, we monitored *D. suzukii* adults and larvae at 18 farms in Southern Wisconsin. In 2014, we repeated adult monitoring at 17 farms over a wider geographic range (six farms in Southern Wisconsin, six farms in Southeast Minnesota, and five farms in Southwest Michigan). The amount of woodland in the landscape was estimated at the 1.5 km radius scale around raspberry farms. Although the dispersal capacity of *D. suzukii* is currently not known, this scale was chosen because landscape composition is typically found to have the greatest effect on moderately mobile insects at scales between 1 and 2 km (e.g., Gardiner et al. 2009).

Landscape composition surrounding raspberry field sites were evaluated using satellite-derived land cover imagery from the Cropland Data Layer (USDA NASS 2013). Land cover maps were imported into ArcGIS version 10.1 software (ESRI) and the composition of landscape around each field site was calculated for a circular buffer with a 1.5 km radius. Percent woodland area was calculated by combining all woodland classifications and dividing by total area of all landscape classifications. Cropland Data Layer classifications included as woodland consisted of forest, deciduous forest, evergreen forest, mixed forest, and woody wetland. In 2013, we selected study sites

ranging from 5 to 60 % woodland area; in 2014, sites ranging from 5 to 67 %, with five

farms repeated both years in Wisconsin. This range of low-to-high woodland composition

approximates the naturally occurring range of relative woodland area in the main

agricultural regions of Minnesota, Michigan, and Wisconsin.

Raspberry fields ranged in size from a single 75 m row to 8 hectares and pest

management strategies including certified organic, no-spray, and conventional were

represented. Self-reporting of management and spray dates were collected from

cooperating growers. The majority of farms did not report any insecticide use and farms

that did spray reported a maximum of four insecticide applications per year including

spinosyns, pyrethroids, organophosphates, and neonicotinoids to target *D. suzukii* and

other insect pests.

Habitat sampling

To determine the quality of the surrounding woodlands as potential sources of

alternate hosts, habitat sampling was conducted at farms each year of the study in June or

July. Five meters in from the woodland edge, we established a 100 m long transect

parallel to the woodland edge closest to the raspberry crop. Every 5 m, a 1x1 m quadrat

was randomly placed on the ground for a total of 20 quadrats per transect. The presence

of all plants which bear soft-skinned fruit making them a potential host for *D. suzukii*

were recorded and identified to genus or species.

Adult monitoring

To understand how *D. suzukii* populations varied between landscapes, we

conducted monitoring of adults using yeast-sugar traps in both the woodlands and

raspberry crop at each farm. Traps consisted of a clear 946 ml plastic cup (Webstaurant

Store, Lancaster, Pennsylvania, USA) and lid with ten 5 mm holes placed along the top rim of the cup. Bait consisted of 3.5 g dry active baker's yeast (Red Star, Milwaukee, Wisconsin, USA), 14 g granulated white cane sugar, and 177 ml water per trap. A drop of unscented dish soap (Seventh Generation, Burlington, Vermont, USA) was added to each trap to break surface tension and increase adult capture. Bait and trap design were chosen based on capture reliability in multiple fruit crops and regions of the USA (Burrack et al. 2015).

As early season populations were hypothesized to be present in the woodlands, three traps were placed in woodland habitat, 1 to 2 m into the woods from the edge and within 300 m of the raspberry crop. Three traps were placed in the raspberry crop in the fruiting zone on canes or trellising. The traps were placed at least 2 m apart within the crop and at least 10 m from any woodland trap. Monitoring was conducted weekly from mid-June through October in 2013 and from late-May through October or early November (depending on the state) in 2014. Each week, the yeast-sugar bait was replaced and the contents of the three traps in each habitat type (woods or raspberry) pooled and transferred to 70% ethanol. Samples were assessed for the total number of female and male *D. suzukii* adults in the laboratory at 10x to 40x magnification under a stereomicroscope. Spotless males were rare and excluded from the total. Due to high numbers of adults in trap catches, samples were subsampled if the number of *D. suzukii* were > 400 (in 2013) or > 200 (in 2014) by counting 20% of cells in a gridded tray and calculating a sample total. In 2013, samples were counted for the first four weeks and the final week of the study. Between late-July and mid-October, the high number of samples necessitated that only a subset of samples (every-other week) were counted, for a total of

60 to 95% of samples counted over the course of the season. In 2014, all samples were counted. To standardize sampling effort, all sample counts were divided by the number of traps and number of days the sample represented, then multiplied by seven for an average weekly total per trap.

From the trap catch data, we calculated four metrics of *D. suzukii* populations which represent risk to the raspberry crop: 1) week of first detection, defined as the first week an adult was trapped in either the woodland or raspberry trap type at a farm; 2) population growth rate, defined as the change in abundance from the second week of non-zero trap catches through peak trap catch (transformed by $\log_{10}$) for the raspberry trap type divided by the number of weeks between the two points; 3) peak population, defined as the highest trap catch recorded in raspberry; and 4) total population, defined as the sum across all weeks' trap catches from mid-June through October in raspberry. For weeks in 2013 without trap counts, an average of the preceding and following week was used.

Larval monitoring

To confirm adult presence and abundance was correlated to crop damage, the raspberry crop was sampled throughout the season to determine larval abundance. In 2013, fruit was sampled for larvae six times between late July and early October in order to assess infestation in both summer and fall bearing raspberry varieties. All farms were sampled at least twice, with each sample consisting of 65 to 100 g of ripe, marketable fruit (~30 to 50 berries) collected from both the edges and center of rows. Fruit was placed in a plastic bag in a cooler in the field before being transferred to a refrigerator in the lab. Within 48 hours, all fruit was subject to a fruit dunk-test consisting of 72 g salt

dissolved in 946 ml warm water per sample. Fruit was lightly crushed to break the skin

and then left in the salt water solution for a minimum of one hour before examination.

Fruit samples were placed in a shallow glass tray with white paper underneath and a

bright light shined on top in order to maximize visibility of larvae which floated to the

surface. Larvae were confirmed as *Drosophila* larvae based on the presence of spiracles

and larval form characteristics.

While *D. suzukii* are the only drosophilids able to oviposit in ripening fruit, we

reared out additional fruit samples to adulthood to confirm that larvae present in fruit

were *D. suzukii*. Samples were taken twice in 2013 (August and September) at all farms

(n = 18) and once in 2014 (August) at a subset of farms (n = 9). Fruit samples were

placed in plastic containers with a screen mesh bottom and slightly elevated so excess

liquid could drain and minimize larval drowning. After 7 to 9 days, pupae (up to 40 per

sample) were removed and placed on lightly moistened filter paper in a sealed petri dish.

After two weeks, all emerged adults were identified as *D. suzukii* or another drosophilid.

Statistical methods

To assess the hypotheses that the amount of woodland area affects *D. suzukii*

timing and population abundance, we fitted four multiple linear regressions, one for each

of the metrics: 1) week of first detection at the farm, 2) population growth rate in

raspberry, 3) peak abundance in raspberry, and 4) total population in raspberry. In each

multiple linear regression, we modeled each metric separately with percent woodland

area, year and state (to account for variation between seasons and spatial differences

between states) as fixed effects. Statistical models were also run separately for each year

and each state. To determine if adult trap catch in raspberry was a reliable proxy for fruit

infestation, larval abundance from the larval monitoring in raspberry was correlated to adult trap catch (from both the concurrent week's trap catch and the previous week's) using linear regression. All statistical analyses were performed in R (R Development Core Team 2012).

Results

Habitat sampling

At all farms, two to six alternate host plant genera were present in the woodlands of all study farms. In 2013, vegetation surveys were conducted at 13 of the 18 farms; in 2014, at 15 of the 17 farms. At five farms, there was no woodland within 300 m of the raspberry crop or the adjacent woodland was not owned by the participating grower and therefore was not accessible, and no vegetation assessment was conducted. Percent cover of alternate hosts (i.e., percent of quadrats examined with at least one host with soft-skinned fruits) ranged from 10-95% between farms, with a mean of $60 \pm 5\%$. A total of ten genera or species of potential alternate host plants were identified across all plots. The most common potential alternate hosts plants for *D. suzukii* were wild caneberries (*Rubus* spp.), gooseberries (*Ribes* spp.), and wild grape (*Vitis riparia*). Common shrubs included European buckthorn (*Rhamnus* spp.), honeysuckle (*Lonicera* spp.), and dogwood (*Cornus* spp.). Other plants found rarely included elderberry (*Sambucus racemosa*), wild blueberry (*Vaccinium corymbosum*), bittersweet nightshade (*Solanum dulcamara*), and aronia (*Aronia melanocarpa*). Trees noted included wild cherry (*Prunus serotina*) and mulberry (*Morus rubra*).

Adult monitoring

D. suzukii was detected at all farms within five weeks of one another from mid-June through mid-July in both 2013 and 2014. The week of first detection of *D. suzukii* in Wisconsin in 2013 was negatively associated with woodland area (parameter estimate: -0.028 ± 0.011, p = 0.02) and, across both years and all states, had a similar negative relationship that was significant (p = 0.05, Table 1). Between states, an ANOVA showed there were significant differences ($F_{2, 14}$ = 5.6, p = 0.02) and Tukey's HSD found *D. suzukii* was detected in Michigan earlier than Wisconsin (WI-MI: p = 0.01).

The amount of woodland surrounding sampling areas was not associated with any other indices of *D. suzukii* population including growth rate, peak population, or total population in raspberry (Table 1). Population growth rate ranged from 0.364 to 1.145 (log *D. suzukii* change/week) and varied significantly between states, with populations increasing more slowly in Michigan than Wisconsin or Minnesota (Table 1, Figures 1 & 2). Peak population in raspberry ranged from 103 to 2,637 adults. For the farms that were sampled in both years of the study in Wisconsin, peak numbers sometimes varied by up to an order of magnitude, despite a lack of differences in landscape composition or management. *D. suzukii* populations in raspberry reached peak between mid-August and late September (Fig. 1 and 2) and total population in raspberry ranged from 92 to 15,980 adults over the season.

Larval monitoring

Larvae were present in 79% of the 65 fruit samples assessed and at all sites at least once during 2013. Densities ranged from 0-7.5 larvae per raspberry with a mean of 0.01 (± 0.0002) larvae per raspberry over the fruiting season. Between both 2013 and 2014, 99% of *Drosophila* larvae reared from raspberry samples (n = 40) were confirmed

to be *D. suzukii.* Larval abundance was positively correlated to concurrent week adult trap catch within the raspberry crop ($r^2 = 0.246$, p = 0.048). There was no correlation of larval abundance with the previous week's adult trap catch ($r^2 = -0.121$, p = 0.33).

Discussion

We expected that as the amount of woodland in the landscape increased, *D. suzukii* would appear earlier and reach greater populations. In support of this hypothesis, we found that *D. suzukii* adults appear earlier in the season at farms in landscapes with higher amounts of woodland. This pattern was particularly pronounced in Wisconsin in 2013, but was also observed when combining results across Wisconsin, Michigan, and Minnesota in the second year of the study. Farms that were surrounded by relatively high amounts of woodland (50% of area within 1.5 km of a farm) had flies first collected in traps one week earlier on average than farms surrounded by low amounts of woodland (5% woodland). However, whether the pattern of earlier arrival is consistent across larger geographic areas needs to be examined in more detail since there was spatial variation in this study, with stronger patterns in Wisconsin. All other aspects of *D. suzukii* population indices, including population growth rate, peak population, and total population size did not appear to be associated with the amount of woodland in the landscape.

The early season activity of *D. suzukii* may be driven by the contribution of woodland as a source of the previous fall's alternate hosts. The presence and abundance of alternate hosts was similar across sites, with each site containing at least two different genera of hosts. Most of these hosts are fall-bearing, so they may be contributing to

populations of *D. suzukii* in the woodlands after cultivated crops have stopped bearing. Flies were still present in woodland trap catches into November (Figures 1 & 2), suggesting they are utilizing resources, whether alternate hosts or overwintering sites. Little is known about *D. suzukii* overwintering, however the Upper Midwest has cold and relatively long winters with average temperatures below 0˚C for four to five months. This likely results in high mortality of *D. suzukii* as laboratory simulations (Dalton et al. 2011) and the lack of adults found in winter trapping (E. Pelton and C. Guédot, unpublished) have suggested. So any increase of fly populations in woodlands during the fall may be reduced during the winter into low spring population levels. However, if some flies survive, they may be the first to start recolonizing and cause earlier arrival on farms in landscapes with high amount of woodland. Overall, the timing of arrival of *D. suzukii* may be important for growers to focus monitoring and could lead to cultivar selection for varieties which ripen during periods of low *D. suzukii* populations, such as early-ripening blueberries in Rhode Island (Hampton et al. 2014). Additional management implications of this study include the continued value of sampling of fruit for larvae rather than relying on adult trap catches to assess risk. While larval infestation and adult abundance were positively correlated, this relationship was weak. Unfortunately, once larvae are found in fruit, management options are already limited as current insecticides target adults only and culling fruit may be one of the few tools available.

The patterns of *D. suzukii* arrival and population growth rate varied across the three states. In particular, the dynamics of *D. suzukii* in Michigan appeared to be different than Wisconsin and Minnesota. In Michigan, flies were detected earlier than in Wisconsin and increased more slowly than Minnesota or Wisconsin. It is unclear whether

this pattern is broadly representative of the state due to the limited sample size of each state in 2014 (n ≤ 6). However, there are well known differences in abiotic factors such as temperature that may explain the differences between states on either side of Lake Michigan. Michigan farms were located in Southwest and Southcentral parts of the Lower Peninsula, which experiences a relatively warmer spring temperatures and could create earlier spring populations. *D. suzukii* activity and reproduction slows at high summer temperatures (Tochen et al. 2014), but whether or not this explains the slower growth rate recorded in Michigan was not assessed. The degree to which abiotic factors influence *D. suzukii* population trends can be further refined to better understand crop risk or to highlight the effects of landscape factors on fly populations.

Interestingly, increasing amounts of woodland area surrounding raspberry farms was not associated with variation in population abundance metrics (growth rate, population peak, and total population). It is possible that the abundance of alternate hosts could influence *D. suzukii* populations at a finer scale than this study examined, as the flies have been shown to utilize both alternate hosts and crops at field edges (Iglesias et al. 2014, Ohrn and Dreves 2012). Therefore using total woodland area within a 1.5 km area as an index of alternate resource abundance may be too coarse of a metric to find patterns in fly abundance. In the Upper Midwest study sites, most forest was deciduous and the vegetation surveys showed relatively uniform resources across sites. However, surveying was only completed 5 m into the woodland, so woodland quality may vary more in abundance and diversity of alternate hosts when moving from the edge to interior of woodlands. The distance *D. suzukii* adults forage is unknown, but if local resources are

abundant, foraging distance may be quite short and fine scale effects of alternate hosts may be most noticeable in crops within meters of woodland, not kilometers.

The phenology of common alternate hosts may also influence their contribution to local *D. suzukii* populations. In the Upper Midwest, most species confirmed as alternate hosts (e.g., wild caneberries, honeysuckle, dogwood) bear fruit in mid-to-late summer. For example, honeysuckles typically fruit in late June through the end of September. This phenology suggests alternate hosts in the Upper Midwest provide resources during the same period as raspberries and other commonly grown fruit crops (e.g., blueberry) susceptible to *D. suzukii*. Given a choice, raspberries are likely the preferred host as it has been identified as a highly preferred cultivated fruit (Belmay et al. 2013) and laboratory no-choice tests find that wild alternate hosts are less preferred than highly susceptible cultivated fruits (Lee et al. 2015). A synchronized phenology of alternate and more preferred, cultivated fruits may explain the lack of relationship between *D. suzukii* populations in raspberry and amount of woodland in the landscape.

In other geographic regions, alternate hosts may provide resources that are temporally complementary to cultivated crops. In the field, preliminary trapping in a no-spray blueberry crop and adjacent woodland in Oregon found trap catches were highest in woodland early and late in the season, with more *D. suzukii* in the crop during fruit set (Ohrn and Dreves 2012). Thus, resource-rich woodland landscapes may be more important to *D. suzukii* populations, and therefore crop risk, in warmer parts of the USA. If fly populations are not reduced by high winter mortality, alternate hosts may create temporal resource continuity (e.g., citrus in the Southern USA). *D. suzukii* has been detected year-round in other states such as California (Harris et al. 2014) and North

Carolina (Burrack et al. 2012) and higher fall woodland populations may affect the abundance of spring populations more acutely. Future studies should focus on how alternate hosts influence fruit crop risk, including the distance adults travel and temporal connectivity of resources.

Acknowledgements

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Tables

Table 1. Parameter estimates (± S.E.) of four multiple linear regressions over both 2013 and 2014 and all three states: *D. suzukii* adult trap catch metric ~ Woodland Area + State + Year

Metric	Woodland Area	State	Year	R^2	$F_{3, 31}$	p
Date of 1st Detection	-0.02 (0.01)	-0.79 (0.27)	0.37 (0.38)	0.379	6.32	< 0.01
	p = 0.05	p < 0.01	p = 0.34			
Growth Rate	-0.002 (0.002)	-0.23 (0.07)	0.15 (0.09)	0.354	5.65	< 0.01
	p = 0.29	p < 0.01	p = 0.11			
Peak Population	3 (11)	-488 (332)	87 (474)	0.105	1.21	0.32
	p = 0.76	p = 0.15	p = 0.07			
Total Population	-11 (32)	-897 (949)	421 (1355)	0.045	0.48	0.70
	p = 0.73	p = 0.35	p = 0.76			

Figures

Figure 1. Phenology of mean *D. suzukii* adults (± S.E.) per trap in the raspberry crop and in the adjacent woodlands from mid-June through October 2013 at 18 Wisconsin farms.
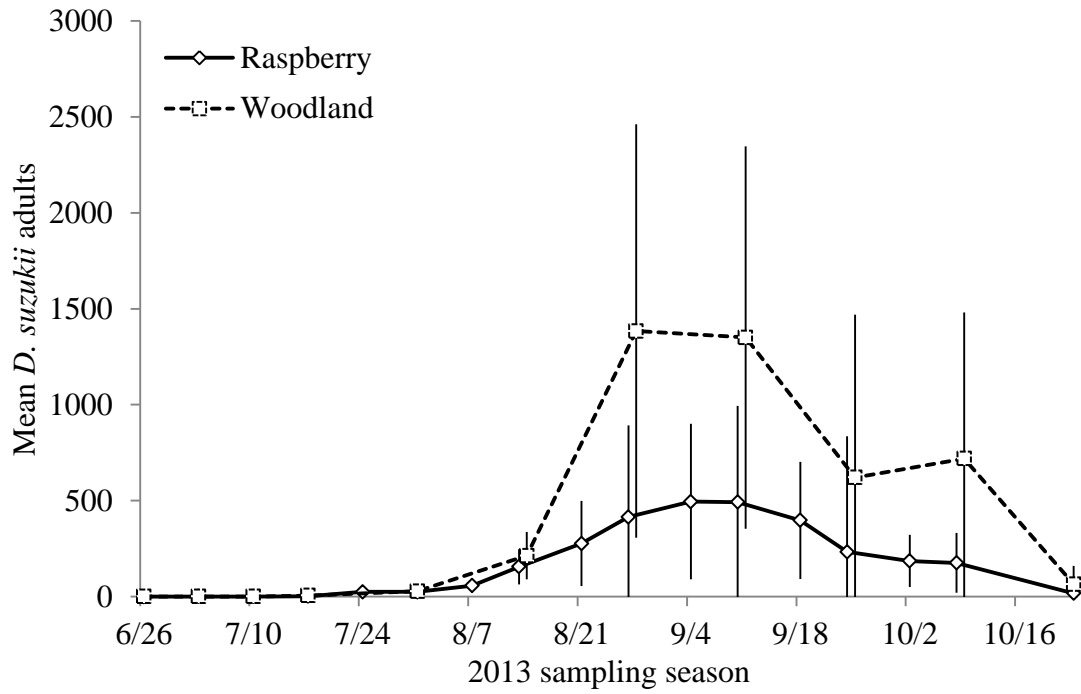
Figure 2. Phenology of mean *D. suzukii* adults (± S.E.) per trap in the raspberry crop and in the adjacent woodlands June through October or early November 2014. Trapping consisted of six farms in Wisconsin, six farms in Minnesota, and five farms in Michigan.
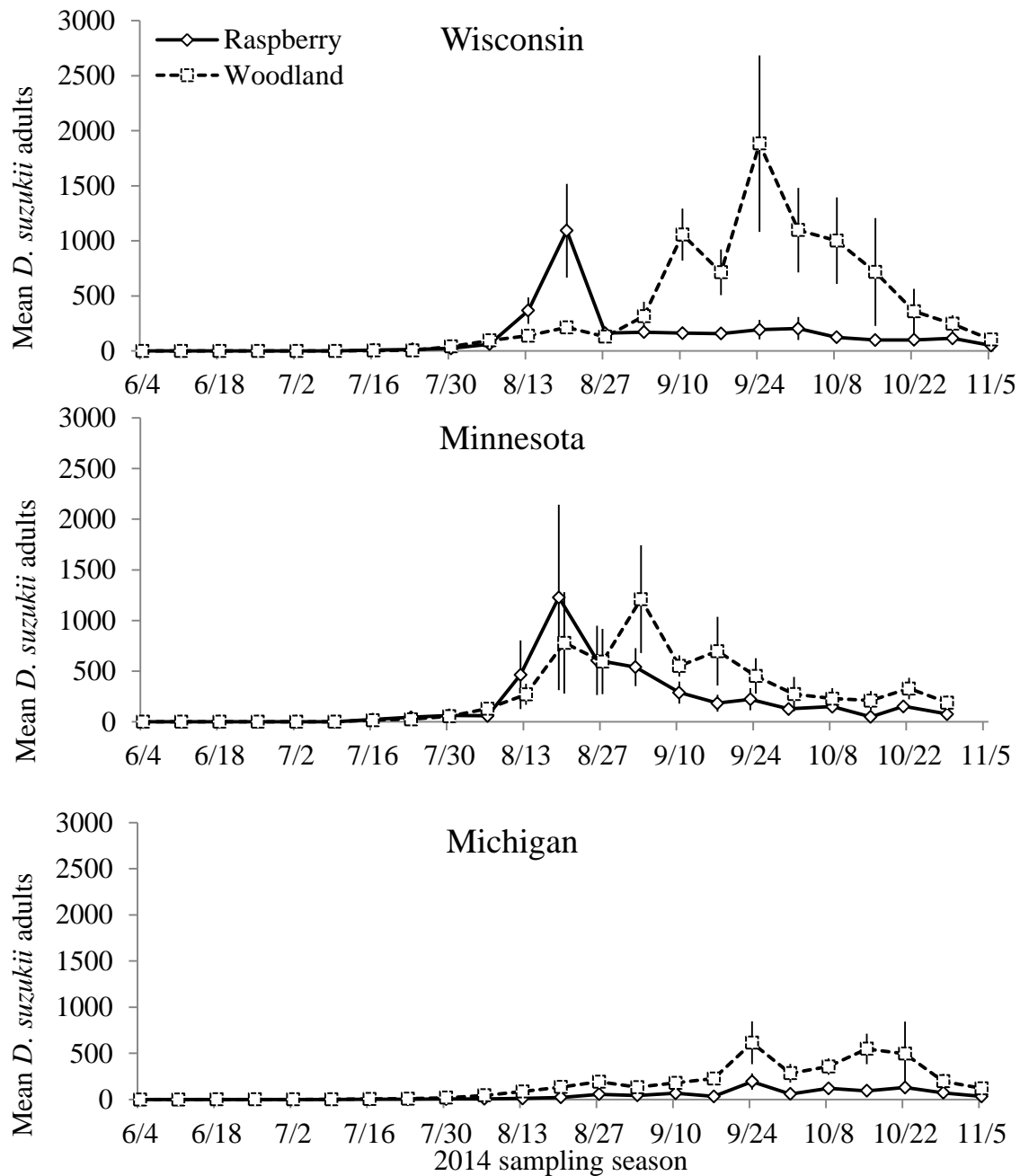
CHAPTER 2

Host susceptibility of cold hardy grapes to *Drosophila suzukii*

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Abstract

Drosophila suzukii Matsumura (Diptera: Drosophilidae), known commonly as spotted wing drosophila, is a vinegar fly originating from Southeast Asia and a major pest to many soft-skinned fruits. Due to the species recent arrival in North America in 2008, many fruit varieties are yet untested for susceptibility to infestation. While previous work has focused on *Vitis vinifera*, this study aimed to determine grape susceptibility of cold hardy varieties based on species *V. labrusca, V. riparia,* and *V. vinifera* hybrids. Field sampling was conducted in Southern Wisconsin (USA) vineyards to determine adult and larval abundance and determine if the number of adults caught in traps correlates with fruit infestation. Host susceptibility was further assessed through no-choice bioassays of both intact and damaged fruit. The field study found *D. suzukii* adults present in all varieties, low larval abundance, and no correlation between adult abundance and larval presence. Peak adult abundance occurred in the midpoint between full veraison and harvest, while larval infestation rates increased over time. In the no-choice tests, significantly more eggs, larvae, and adults occurred in damaged than undamaged grapes. In damaged grapes, larva and adult numbers were comparable between varieties and to the highly susceptible control of undamaged raspberry; however *D. suzukii* developed significantly faster in raspberry than grapes. Fruit characteristics (Brix, titratable acidity, pH) in grapes were uncorrelated to *D. suzukii* performance. Together, these experiments suggest that cold hardy grapes are overall resistant to *D. suzukii* if intact, and highly susceptible if damaged.

Keywords: spotted wing drosophila, *Vitis vinifera*, trapping, integrated pest management, no-choice

Introduction

        Drosophila suzukii Matsumura (Diptera: Drosophilidae), known commonly as spotted wing drosophila, is a major pest to many soft-skinned fruits in North America (Bolda et al. 2010), Europe (Calabria et al. 2012), and was recently discovered in South America (Deprá et al. 2014). The host crop range of *D. suzukii* is extensive and includes many fruit crops, such as raspberry (*Rubus* spp.) and strawberry (*Fragaria ananassa*; Lee et al. 2011) as well as wild non-crop hosts, such as dogwood (*Cornus* spp.) and honeysuckle (*Loincera* spp.; Lee et al. 2015). Since its arrival in California in 2008, significant economic losses have occurred in berry and tree fruit crops across North America (Bolda et al. 2010, Goodhue et al. 2011). The species was first detected in the Upper Midwest in Wisconsin and Michigan in 2010, where it is now a major pest of blueberries (*Vaccinium* spp.) and cane fruit (*Rubus* spp.; Isaacs 2011). However, the implications for the region's cold hardy grape varieties (*V. labrusca, V. riparia,* and *V. vinifera* hybrids), which are important and emerging specialty crops in this region, is still unclear.

        Previous work on the susceptibility of grapes to *D. suzukii* has focused on the more widely grown *V. vinifera* species common to temperate regions of the world. *D. suzukii* has been detected in *V. vinifera* vineyards in the USA (Harris et al. 2014, Van Timmeren and Isaacs 2014), Europe (Rouzes et al. 2012, Grassi and Pallaoro 2012), and Japan (Kanzawa 1939). A preliminary study by Maiguashca et al. (2010) found that *D. suzukii* can oviposit and develop on intact *V. vinifera* Flame variety seedless table grapes. When skin was damaged, flies also oviposited on *V. vinifera* variety Merlot, *V. vinifera* variety Riesling, and *V. labrusca* hybrid variety Concord, but fly development to pupal

stage was rare. A study on intact *V. vinifera* wine grape varieties found oviposition rates were low (< 0.8 eggs/grape) and of eggs laid, only 0-9% developed past the egg stage (Lee et al. 2011). *V. vinifera* wine grapes were also less preferred than berry crops in choice tests (Lee et al. 2011) and an index of host potential ranked grapes as having the lowest host potential compared with other susceptible, cultivated hosts (Bellamy et al. 2013).

As *D. suzukii* continues to spread to new regions, local fruit species and varieties need to be assessed for their potential as hosts. Currently, little information is available on the severity of infestations, varietal susceptibility, and the implications of *D. suzukii* for cold hardy grapes which are based on the species *V. labrusca*, *V. riparia* and *V. vinifera* hybrids (USDA NASS 2013). Adult *D. suzukii* has been detected in Michigan vineyards (which include both *V. vinifera* and cold hardy varieties), but larval infestation levels are low and constrained to southern regions of the state (Van Timmeren and Isaacs 2014). Adults have also been trapped in cold hardy variety vineyards of Québec with no larvae detected in white varieties and low infestation rates in red varieties (Saguez et al. 2013). Volunteer monitoring by Wisconsin grape growers in 2013 and 2014 detected adults in traps but few larval infestations and yield losses were reported through a statewide grower survey (Pelton and Guédot 2015). However, the relatively recent arrival of *D. suzukii* means growers may not be monitoring for larvae or may be inadvertently controlling infestation rates when using insecticides targeting other pest species.

Without an established knowledge of susceptibility, growers may detect *D. suzukii* adults while actual infestation risk remains unknown. In the Upper Midwest, vineyards are often part of diversified fruit farms and located in wooded landscapes

which contain known wild non-crop hosts (Lee et al. 2015). If growers monitor for *D. suzukii* in their vineyards and detect adults, it may be due to the proximity of other more preferred fruit crops such as caneberries and blueberries or wild non-crop hosts, possibly leading to unnecessary management. Current insecticide management recommendations are problematic for use in integrated pest management programs because they are mainly based on broad-spectrum organophosphates and synthetic pyrethroids (Van Timmeren and Isaacs 2013) which are non-selective and have a negative impact on natural enemies (Musser and Shelton 2003) and pollinators. Effective insecticide regimes require frequent sprays (Van Timmeren and Isaacs 2013) and increased labor and chemical costs are estimated at 6-8% of farmgate value (Bolda et al. 2010). Management options for organic growers including approved insecticides are generally more costly and less effective (Van Timmeren and Isaacs 2013) and cultural methods such as field sanitation are labor-intensive.

Susceptibility to *D. suzukii* may also depend on varietal differences in grapes. Varietal differences have been identified in other fruit crops such as blackberry (*Rubus* spp.) where oviposition varied by 2.5 to 2.8 fold between varieties (Lee et al. 2011). Susceptibility differences between varieties may be due to fruit characteristics, such as physical characteristics and chemical characteristics. One important chemical characteristic, which has been posited as a determinant in *D. suzukii* preference in cane fruits, is sugar content (Lee et al. 2011, Burrack et al. 2013). A weak relationship has also been noted between pH and infestation in blueberries and cherries (*Prunus* spp.; Lee et al. 2011). Physical characteristics of different varieties could also impact *D. suzukii* preference, especially skin thickness, which may be a deterrent to oviposition. In a

preliminary laboratory study, skin damage increased oviposition in *V. vinifera* wine grapes and cold hardy Concord grapes (Maiguashca et al. 2010). In other fruits, peaches with indumenta (peach fuzz) deter female oviposition, while damaged areas can have high rates of oviposition (Stewart et al. 2014) and cranberries are susceptible only if damaged (Steffan et al. 2013).

The objective of this study was to determine the susceptibility of commonly grown cold hardy grape varieties to *D. suzukii*. To establish adult varietal preference, larval infestation rates, and determine if a correlation exists between adult abundance and larval presence, we conducted a field study of six cold hardy wine grape varieties in Southern Wisconsin vineyards during 2014. To determine if susceptibility varies by varietal characteristics and skin damage, we conducted laboratory no-choice tests on eight cold hardy grape varieties (seven wine grapes, one juice/jam grape).

Materials and Methods

Field Sampling

To assess adult varietal preference, larval infestation, and establish a correlation between adults and larvae, sampling was conducted in six cold hardy grape varieties in Wisconsin vineyards during 2014. Adult monitoring consisted of trapping with yeast-sugar bait to determine presence and abundance of flies in different grape varieties. Larval sampling was conducted using fruit-dunk tests to establish larval presence and abundance.

Vineyards were located in Southern Wisconsin (USA) and traps were placed in blocks of at least five continuous rows or > 0.135 hectares of a single grape variety. Varieties studied included varieties based on the species *V. labrusca*, *V. riparia*, and *V.*

vinifera including four reds (St. Croix, Marquette, Frontenac, and Marechal Foch) and two whites (La Crescent and Edelweiss). Replicates were established using seven vineyards, which were conventionally managed and spray records were self-reported, for a total of 3 to 4 replicates per variety (Table 1).

Starting in mid-July 2014 before veraison, a single yeast-sugar trap was placed centrally in each variety. Traps were located at least ten vines in from the edge and two rows from another variety to minimize the influence of neighboring varieties. Traps consisted of a clear 946 ml plastic cup (Webstaurant Store, Lancaster, Pennsylvania, USA) and lid with ten 5 mm holes placed along the top rim of the cup. Bait consisted of 3.5 g dry active baker's yeast (Red Star, Milwaukee, Wisconsin, USA), 14 g granulated white cane sugar, and 177 ml water per trap. A drop of unscented dish soap (Seventh Generation, Burlington, Vermont, USA) was added to each trap to break surface tension and increase adult capture. Bait and trap design were chosen based on capture reliability in multiple fruit crops and regions of the USA (Burrack et al. 2015). Traps were monitored weekly at which time the bait was replaced and insect samples transferred to 70% ethanol and brought back to the laboratory where *D. suzukii* adults identified and males and females counted. Samples were subsampled if the number of *D. suzukii* > 200 by counting 20% of cells in a gridded tray and calculating the total per sample.

Marketable fruit samples were assessed for larval presence and abundance throughout the season at the seven participating vineyards. Fruit samples were collected biweekly on bait-changing days from vines adjacent to monitoring traps until the first larva was found, then samples were collected weekly until harvest. An additional sample of each Frontenac and La Crescent was taken at participating vineyards which did not

meet the minimum acreage requirement for trapping adults in these varieties, for a total of four replications of each variety (Table 1). Fruit which was visibly cracked or damaged was removed from the samples. For each sample, 100 g of sound fruit was subject to a fruit dunk-test consisting of 30 g table salt dissolved in 473 ml warm water. Fruit was lightly crushed to break the skin and then left in the salt-water solution for one hour before examination. Fruit samples were placed in a shallow glass tray with white paper underneath and a bright light shined on top in order to maximize visibility of larvae. Larvae floated to the top of the solution and were confirmed as drosophilid larvae under a microscope. If larvae were found, a paired sample of 100 g sound fruit was reared out in a 355 ml mesh-topped clear plastic cup (Solo Cup, Lake Forest, Illinois, USA) placed at room temperature for 14 days to allow any Drosophilid eggs or neonates present to complete development and determine species from the emerged adults.

No-choice assays

To assess the susceptibility of cold hardy grape varieties and the differences between undamaged and damaged fruit, no-choice tests were conducted using eight cold hardy grape varieties and raspberry as a control. Both undamaged and damaged grapes were exposed to *D. suzukii* adults and performance was measured as the number of eggs laid, number of larvae, number of adults emerged, and time to adult emergence. Fruit characteristics (Brix, pH, and titratable acidity) were assessed for each variety and correlated to preference and performance metrics.

Grape varieties Frontenac, Marechal Foch, Marquette, La Crescent, and Leon Millot were sourced from the University of Wisconsin—West Madison Agricultural Research Station (Madison, Wisconsin, USA). To minimize any effects of pesticide

residues, fruit clusters were bagged using waterproof pollination bags starting in mid-July. Fruit was bagged the day prior to any fungicide or insecticide sprays and bags were removed after the re-entry interval of the pesticide, one to three days after sprays. Grape varieties Concord, Edelweiss, St. Pepin, and St. Croix were sourced from a Southern Wisconsin vineyard (Dane County, Wisconsin, USA), which practices no-spray after fruit has reached veraison. Grapes were harvested at typical harvest Brix and acid levels and placed in plastic bags in a refrigerator until use within one week of collection to ensure peak fruit quality.

Chemical characteristics of grape varieties including sugar content (as measured by Brix values), pH, and titratable acidity (TA) were measured to determine if a correlation exists with metrics of *D. suzukii* preference and performance. A 50 ml sample of juice was taken within 24 hours of grape harvest and frozen until Brix, pH, and titratable acidity could be measured. Brix values were determined using a Hanna Instruments handheld digital refractometer (Woonsocket, Rhode Island, USA) and titratable acidity and pH were determined using the TA Wine 8.2 pH 10.00 ml method on a Hanna Instruments Titrator System HI 900 Series (Woonsocket, Rhode Island, USA).

Flies used in the no-choice tests were sourced from a colony at the University of Wisconsin-Madison (Madison, Wisconsin, USA) established in 2013 from infested raspberries. Flies are reared on a standard *Drosophila* 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).

No-choice tests were conducted in fall 2014 with eight cold hardy grape varieties. Grapes were either undamaged (8-10 reps) or damaged (8-10 reps) with each replicate containing eight individual fruits. Stems were left on the grapes to prevent access of *D. suzukii* adults to any damaged area created by removing the stem. Damage was created by cutting just below skin surface for a 10 mm long cut using a utility knife starting from the stem toward the grape's base. Ten additional replicates of eight red, store-bought organic raspberries were established as positive controls as they are known to be preferred hosts (Lee et al. 2011). Fruit was then exposed to adult *D. suzukii* with five *D. suzukii* females (0-7 days old) and three males (0-7 days old) added to each of the cups. To ensure fruit samples were not previously infested, three additional cups of each grape variety and the raspberry control were established without the addition of flies. The varieties ripened at different times so the assay was split into two sets spaced one week apart with separate control replicates. Rearing containers consisted of 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 ensure and record temperature and humidity conditions.

After 48 hours, adults were removed from rearing cups using a vacuum aspirator. Five cups from each variety of grapes were assessed the same day under the microscope to count the number of eggs laid on each of the eight grapes. An egg was recorded if one or two breathing tube filaments were visible. Raspberries were not assessed because breathing tubes were not reliably visible, as noted in Lee et al. (2011). Six days after

experiment initiation, half the fruits from each cup were dissected to determine the presence of larvae. Fruit was removed from cups and destructively sampled in order to count all present larvae (1st-3rd instar). The remaining four fruits were checked daily for emerged adults which were removed until the experiment was terminated at 21 days after initial adult removal. The following preference and performance metrics were taken during the experiment: number of eggs laid, number of larvae present, number of adults emerged, and development time (days from experiment initiation to adult emergence).

Statistical methods

Results were analyzed using the statistical software R (R Core Development Team 2012). For the field study, adult trap catches and larval abundance were pooled across the study duration, starting the first week in mid-July before veraison until the week of harvest and an analysis of variance was performed to determine varietal differences. Probability of larval presence was analyzed using a linear mixed effects model with variety, time, and adult trap catch as fixed effects and vineyard as a random effect. AIC model selection was performed using the nlme package to determine the best model.

No-choice test performance metric results were weighted per gram of fruit for use in statistical analyses to control for varying size between grape varieties and raspberry. Mean berry weight was calculated from the fresh weight of all eight berries in a cup divided by the number of berries used in each metric. Differences between the two replicate sets were assessed by comparing performance metrics (number of larvae, number of adults, and development time) of the raspberry controls. There were no significant differences so replicate sets were analyzed together. Performance metric data

did not conform to assumptions of normality, so non-parametric tests were used to compare within and between varieties: Kruskal-Wallis and Multiple Comparisons Kruskal-Wallis using the pgirmess package. Linear regressions were used to test Brix, pH, and TA in relation to each performance metric.

Results

Field monitoring

   D. suzukii adults were present at all vineyards and in all varieties during the experiment which lasted for 7 to 12 weeks (mid-July through September depending on farm and variety). *D. suzukii* were trapped in the first week at five of the seven vineyards and in the second week at all vineyards. Adult trap catches ranged from 0 to 557 adults per week. There was high variation over time as well as between varieties and between vineyards, with the overall population trend forming a curve with peak adult abundance occurring in late August through early September (Figure 1). Combining trap catches over the sampling period, Edelweiss had significantly higher adult trap catches than all other varieties except Frontenac (Table 1). For all varieties, peak occurred midway between veraison and harvest and ranged from 61 to 557 adults (Figure 1).

   Larvae were present in all varieties in at least one fruit sample, but presence and abundance was low, ranging from 0 to 4 larvae per kg of fruit on average (Table 1) out of a total of 151 samples. Larvae were found in one fruit sample of Edelweiss, four of Foch, five of Frontenac, three in La Crescent, six in Marquette, and three in St. Croix. Infested samples were present at all seven vineyards at least once during sampling. In fruit samples with larvae (22 of 151 samples) a paired fruit sample was used to rear larvae to

adults and 100% of flies reared from fruit samples (13 of 22 samples) were confirmed as

D. suzukii. Using AIC model selection (Table 2), the best fit model showed an increase in

the abundance of larvae over time but with no difference between varieties and no

association with adult trap catch.

No-choice assays

For six varieties (Concord, Frontenac, La Crescent, Marechal Foch, Marquette, St.

Pepin) of the eight varieties tested, damaged grapes had significantly higher number of

eggs, larvae, and adults than undamaged grapes. The only exceptions were Leon Millot

and St. Croix where the number of eggs laid in damaged grapes was not significantly

different than in undamaged grapes (Table 4).

Amongst undamaged varieties, more eggs were laid on Marquette than St. Pepin

and Concord. There were no statistical differences between varieties of undamaged

grapes in the number of larvae or adults present. In damaged grapes, there were

significantly more larvae in Marquette (3.03 ± 0.60) than St. Croix (0.60 ± 0.16) with

other varieties having intermediate number of larvae/gram. There were no differences in

number of emerged adults between grape varieties with more adults emerging from

raspberry compared to La Crescent and Marechal Foch. Adults developed significantly

faster in raspberries (12.88 ± 0.04 days) compared to all grape varieties. Adults

developed faster in St. Croix (14.43 ± 0.23 days) than either Marquette (16.61 ± 0.22

days) or Concord (16.48 ± 0.16 days) (Table 3).

Performance metrics were not correlated to Brix, pH, or TA. Each metric of *D.*

suzukii performance (number of eggs, number of larvae, number of adults, and

development time) in the damaged treatments were assessed using a multiple linear

regression of fruit characteristics. Undamaged treatments were not included due to very

low numbers of larvae and number of adults. In grapes, Brix ranged from 14.5-19.7, pH

ranged from 2.98-3.41, and TA ranged from 2.77-18.61. Titratable acidity and pH were

highly correlated to each other ($R^2 = 0.8259$) so only pH was included in the model with

Brix. None of the metrics were significantly correlated with any of the fruit

characteristics: number of eggs ($R^2 = 0.395$, p = 0.284), number of larvae ($R^2 = 0.229$, p =

0.522), number of adults ($R^2 = 0.023$, p = 0.944), or development time ($R^2 = 0.152$, p =

0.662). Each performance metric was also tested against a single fruit characteristic

independently and none were significant. In raspberries, Brix was 7.2, pH was 3.94, and a

measurement of TA was not taken as the method used was calibrated for grape acids.

Discussion

    In the field, adult *D. suzukii* were present in all vineyards and varieties and did not

correlate to larval presence. Overall larval abundance was relatively low (mean = 2

larvae/kg fruit) compared to Southern Wisconsin raspberries (480 larvae/kg fruit) over

the same time period (E. Pelton, unpublished). The rates of field infestation was also

slightly lower (n= 22/151) than the findings of Van Timmeren and Isaacs (2014) in

southern Michigan *V. vinifera* and cold hardy vineyards (n= 48/181) from 2010-2012. If

grape crops are slated for wine or juice making, low larval presence may make

management (e.g., field sanitation, insecticide applications) unnecessary. Using model

selection, we found the best model included the factor time, with a positive correlation

between time and probability of larval infestation. This phenomenon in the field may be

due to prolonged exposure of grapes to biotic factors (e.g., birds) and abiotic factors (e.g.,

hail, splitting due to swelling after a rain event) which may increase the likelihood that fruits skin becomes damaged allowing access to ovipositing females. While all efforts were made to select undamaged grape clusters for larval sampling, minor berry damage may have existed, especially at the site of the pedicle which was difficult to examine in tight clusters.

As adult presence does not predict larval infestation, these results suggest monitoring for adults is of limited value in integrated pest management of these cold hardy grape varieties. Trap catches of *D. suzukii* may be common in vineyards due to high populations across the landscape as other cultivated fruits such as raspberry and blueberry and non-crop wild hosts are ripening. In 2013 and 2014, trap counts were highest in raspberry crops during August and early September (up to 2,637 adults/trap) and in woodlands (where wild non-crop hosts are located) in September (E. Pelton, unpublished). More worthwhile monitoring in vineyards may consist of sampling marketable fruit for larvae; however, chemical management options are limited once fruit is infested.

While Edelweiss had higher adult abundance overall, there was no correlation with larval infestation. Higher adult counts may be because Edelweiss was harvested 1-2 weeks earlier on average than the other varieties. Peak adult populations occurred in midway between peak and harvest at all vineyards, so by being harvested earlier, Edelweiss' mean trap catch may not be impacted by landscape-level declines in *D. suzukii* populations seen in late September and October in Wisconsin (E. Pelton, unpublished).

Vineyards included in this study were conventionally managed, but results suggest insecticide use for non-*D. suzukii* insect pests does not affect the species ubiquitous presence in Wisconsin vineyards. Farms who reported spray records averaged 2-3 insecticide applications during 2014 and no sprays were reported as targeted for *D. suzukii*. Comparing reported spray events with weekly adult trap catch and larval sampling showed no clear effect on abundance (e.g., trap catches did not decrease the week following a spray). The no-choice assays confirmed low infestation rates in unsprayed grapes and these findings are corroborated by a field study in Michigan which found low infestation rates in unsprayed vineyards (Van Timmeren and Isaacs 2014).

As observed in the field, varietal differences were minimal and inconsistent in the laboratory no-choice tests. Marquette had higher numbers of eggs than two varieties in the undamaged treatments and more larvae than one variety in the damaged treatments but this did not lead to greater numbers of adults. While varietal differences have been noted for cherry and blueberry, choice tests of *V. vinifera* varieties Chardonnay, Merlot, Pinot gris, and Pinot noir by Lee et al. (2011) found no varietal differences. Lack of varietal differences was also confirmed by the non-significant results of linear regressions of performance metrics to Brix, pH, or TA.

D. suzukii females in no-choice scenarios attempted to lay eggs on undamaged grapes, despite the unviability of the eggs as suggested by low larvae and adult numbers. During the egg assessment, it was noted that the majority of eggs on undamaged grapes were laid near the stem where skin may have had small cracks at the juncture, an oviposition preference also noted in Lee et al. (2011) in *V. vinifera* varieties. In damaged grapes, most eggs were laid at the site of the damage, which were likely more suitable

sites as damaged grapes had higher rates of larvae and adults survived past the egg stage. However, failed attempts to oviposit on intact skin may have implications for susceptibility in the field. *D. suzukii* females have been shown to create small punctures when attempting to oviposit even if they did not successfully lay an egg in *V. vinifera* Thompson seedless grapes (Atallah et al. 2014). This skin injury could have implications for secondary insect pests including other *Drosophila* species and the spread of pathogens. For example, other *Drosophila* species have recently been shown to be associated with sour rot in grapes (Barata et al. 2012). Both skin thickness and fruit firmness have been posited as a deterrent to successful oviposition in fruit crops (Lee et al. 2011, Burrack et al. 2013) and in this experiment, damage by cutting affected both properties by allowing direct access to inner flesh. Further work is needed to understand how fruit firmness and skin thickness affect grape susceptibility to *D. suzukii*.

Successful development of *D. suzukii* was significantly slower in all grape varieties compared to raspberry. Flies reared on raspberries took 12.9 days $\pm$ 0.02 from egg to adulthood which is comparable to development time on artificial diet under ideal laboratory conditions (12.8 $\pm$ 0.2 days; Emiljanowicz et al. 2014). These results suggest raspberry is an ideal host for *D. suzukii* larvae despite relatively low Brix value (7.2) compared to grapes (14.5-19.7). Thus, while providing sufficient sugar, cold hardy grapes may be less suitable than raspberry due to higher acid levels (Table 2). The slower development in grapes may have interesting implications for local population source-sink dynamics.

This study focused on cold hardy wine grapes, but the susceptibility of fresh market cold hardy grapes (e.g., seedless varieties) is still unclear. Seedless varieties were

not included in this study due to a lack of acreage, but are commonly grown in small planting across the Upper Midwest. They may be more susceptible than wine grapes as they typically have thinner skins which may be susceptible to oviposition. A preliminary no-choice study in Washington found undamaged Flame variety *V. vinifera* table grapes were susceptible to egg-laying and developed larvae (Maiguashca et al. 2010). Additionally, customers may reject fresh market fruit with lower levels of larvae than processors would.

Combining these experiments, we found cold hardy wine grapes are largely resistant to *D. suzukii* if intact, but susceptible if the skin is damaged. This suggests there are minimal concerns of *D. suzukii* as a primary pest for growers of wine grapes that are undamaged. Varieties which are more prone to skin damage or easily shatter near harvest may be more susceptible.

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Table 1. Mean *D. suzukii* adults and larvae (± S.E.) per week from mid-July through September 2014 in Southern Wisconsin vineyards.

Variety	Adult sampling		Larval sampling	
	Number of Vineyards	Mean (± S.E.) Adults	Number of Vineyards	Mean (± S.E.) Larvae/kg Fruit
Frontenac	3	81.88 (17.25) ab	4	1.92 (0.79) a
Marechal Foch	4	76.13 (12.65) a	4	3.60 (1.99) a
Marquette	4	41.77 (5.22) a	4	2.94 (1.26) a
La Crescent	3	58.21 (12.12) a	4	2.40 (1.66) a
St. Croix	4	52.06 (11.27) a	4	1.85 (1.07) a
Edelweiss	4	142.10 (25.43) b	4	0.56 (0.53) a
ANOVA		$F_{5, 173} = 6.005$		$F_{5, 144} = 0.515$
		$p < 0.0001$		$p = 0.765$

Different letters within a column indicated a significant difference between varieties at $p < 0.05$ (Tukey's HSD).

Table 2. AIC values for predictor models of larval presence $\ln(\pi/(1-\pi))$.

Predictors	AIC
variety + time + adult count	107
time + adult count	99
adult count	111.1
variety + time	105.5
time	97.6

Note: larval counts without corresponding adult trap data were excluded from analysis

Table 3. Mean number of eggs, larvae, adults, and development time from egg to adult emergence in days (± S.E.) of *D. suzukii* per gram of fruit for no-choice tests.

Treatment	Variety	Eggs	Larvae	Adults	Development time in days
Undamaged	La Crescent	0.17 (0.12) ab	0 (0) a	0 (0) a	--
	Marechal Foch	0.30 (0.05) ab	0 (0) a	0 (0) a	--
	Leon Millot	1.28 (0.81) ab	0 (0) a	0.03 (0.03) a	16.00 (0)
	St. Croix	0.16 (0.05) ab	0 (0) a	0.02 (0.02) a	14.00 (0)
	Marquette	1.07 (0.08) a	0 (0) a	0 (0) a	--
	Frontenac	0.45 (0.41) ab	0.02 (0.02) a	0 (0) a	--
	St. Pepin	0.01 (0.01) b	0 (0) a	0 (0) a	--
	Concord	0.02 (0.01) b	0 (0) a	0 (0) a	--
Control	Raspberry	--[1]	1.57 (0.32) b	1.35 (0.24) b	12.88 (0.04)
	Kruskal-Wallis	$\chi^2_7 = 23.54$	$\chi^2_8 = 77.70$	$\chi^2_8 = 80.35$	--
		p = 0.0014	p < 0.0001	p < 0.0001	--
Damaged	La Crescent	3.85 (1.41) a	1.65 (0.49) ab	0.17 (0.13) a	16.71 (0.71) bc
	Marechal Foch	1.86 (0.67) a	1.15 (0.35) ab	0.32 (0.13) a	14.40 (0.21) bc
	Leon Millot	2.71 (0.70) a	2.08 (0.56) ab	0.47 (0.13) ab	14.89 (0.37) bc
	St. Croix	0.64 (0.30) a	0.60 (0.16) a	0.42 (0.16) ab	14.43 (0.23) c
	Marquette	2.67 (0.25) a	3.03 (0.60) b	0.77 (0.13) ab	16.61 (0.22) b
	Frontenac	2.28 (0.69) a	1.40 (0.55) ab	1.04 (0.28) ab	15.98 (0.21) bc
	St. Pepin	1.23 (0.61) a	1.53 (0.29) ab	0.80 (0.27) ab	15.63 (0.16) bc
	Concord	1.07 (0.21) a	1.37 (0.34) ab	0.81 (0.19) ab	16.48 (0.16) b
Control	Raspberry	--	1.57 (0.32) ab	1.35 (0.24) b	12.88 (0.04) a
	Kruskal-Wallis	$\chi^2_7 = 13.76$	$\chi^2_8 = 14.72$	$\chi^2_8 = 25.66$	$\chi^2_8 = 529.93$
		p = 0.0555	p = 0.0649	p = 0.0012	p < 0.0001

Different letters within a column indicated a significant difference between varieties at p < 0.05 (Tukey's HSD). Damage refers to a 10 mm long cut from stem towards base of fruit made by a utility knife just below skin surface. [1] Raspberries were not assessed for eggs because breathing tubes were not reliably visible.

Table 4. Results of Kruskal-Wallis tests between undamaged and damaged treatments.

Variety	Eggs		Larvae		Adults	
	χ^2_1	p	χ^2_1	p	χ^2_1	p
La Crescent	6.99	0.0082	12.51	0.0004	4.21	0.0402
Marechal Foch	6.82	0.0090	13.86	0.0002	6.17	0.0130
Leon Millot	1.84	0.17	15.78	< 0.0001	6.25	0.0124
St. Croix	2.96	0.09	11.52	0.0007	5.59	0.0181
Marquette	6.82	0.0090	13.83	0.0002	13.83	0.0002
Frontenac	3.97	0.0463	9.55	0.0020	10.90	0.0010
St. Pepin	7.26	0.0071	13.83	0.0002	11.68	0.0006
Concord	6.99	0.0082	16.31	< 0.0001	16.31	< 0.0001

Damage refers to a 10 mm long cut from stem towards base of fruit made by a utility knife just below skin surface.

Figures

Figure 1. Mean *D. suzukii* adults caught (± S.E.) per trap and mean number of larvae/kg fruit (± S.E.) phenology in six cold hardy grape varieties (Edelweiss, Frontenac, La Crescent, Marechal Foch, Marquette, and St. Croix) from mid-July through September 2014 in Southern Wisconsin vineyards.
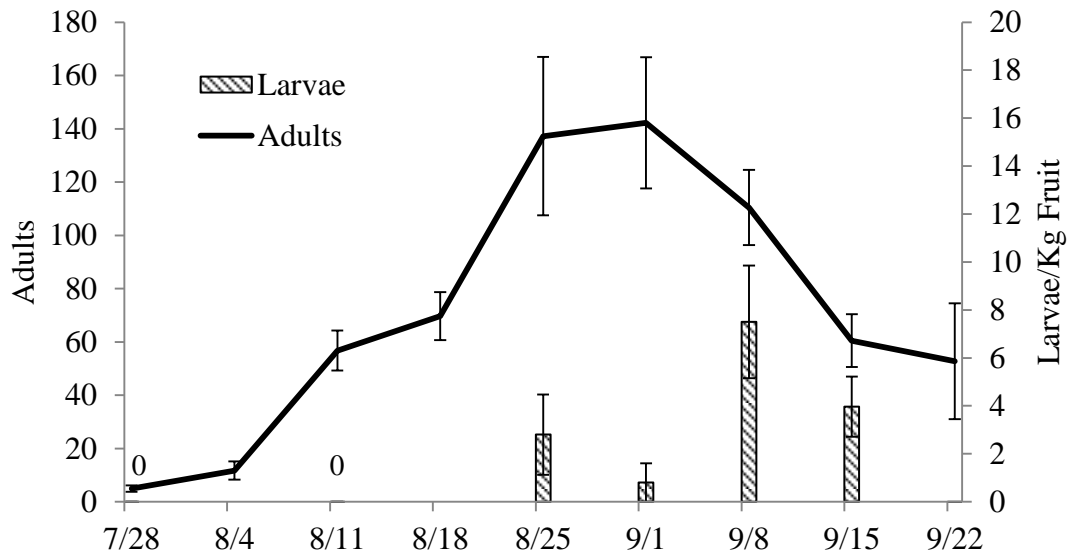
APPENDICES

Appendix A. Additional trap catch data

        In addition to trapping for adult *D. suzukii* in woodland and raspberry habitats in the study outlined in Chapter 1, a third set of traps were placed at each farm in 2013 & 2014. These traps were located in areas with presumably low resources for flies (i.e. low shade, low fruit availability) and referred to as 'open' traps. Typically, placement was in open grassland or mowed lawn on a fence post, stake, or small, non-fruit bearing shrub or tree. These traps were deployed to determine if the flies were present in this habitat type. Our results found that while *D. suzukii* are commonly present in this habitat, they were consistently found at very low numbers compared to the woodland and raspberry traps. This may suggest two interesting points: 1) the yeast-sugar traps are not attracting adults in high numbers compared to the raspberry and woodland habitats and 2) the adults are not abundant in areas of low resources. However, we chose to exclude these results from analyses in Chapter 1 given the limited value the data has in explaining the comparison between woodland and raspberry adult trap catches.

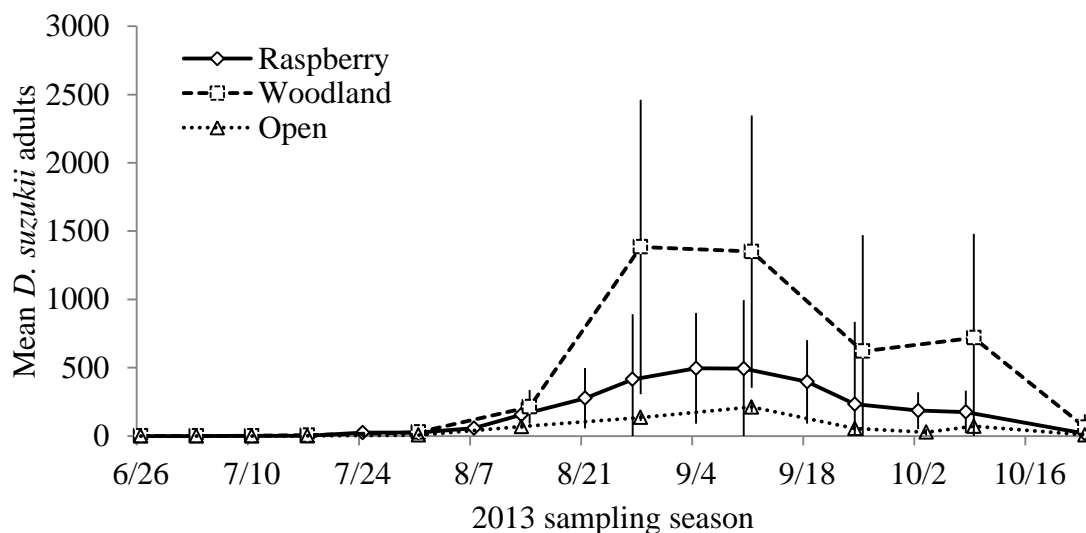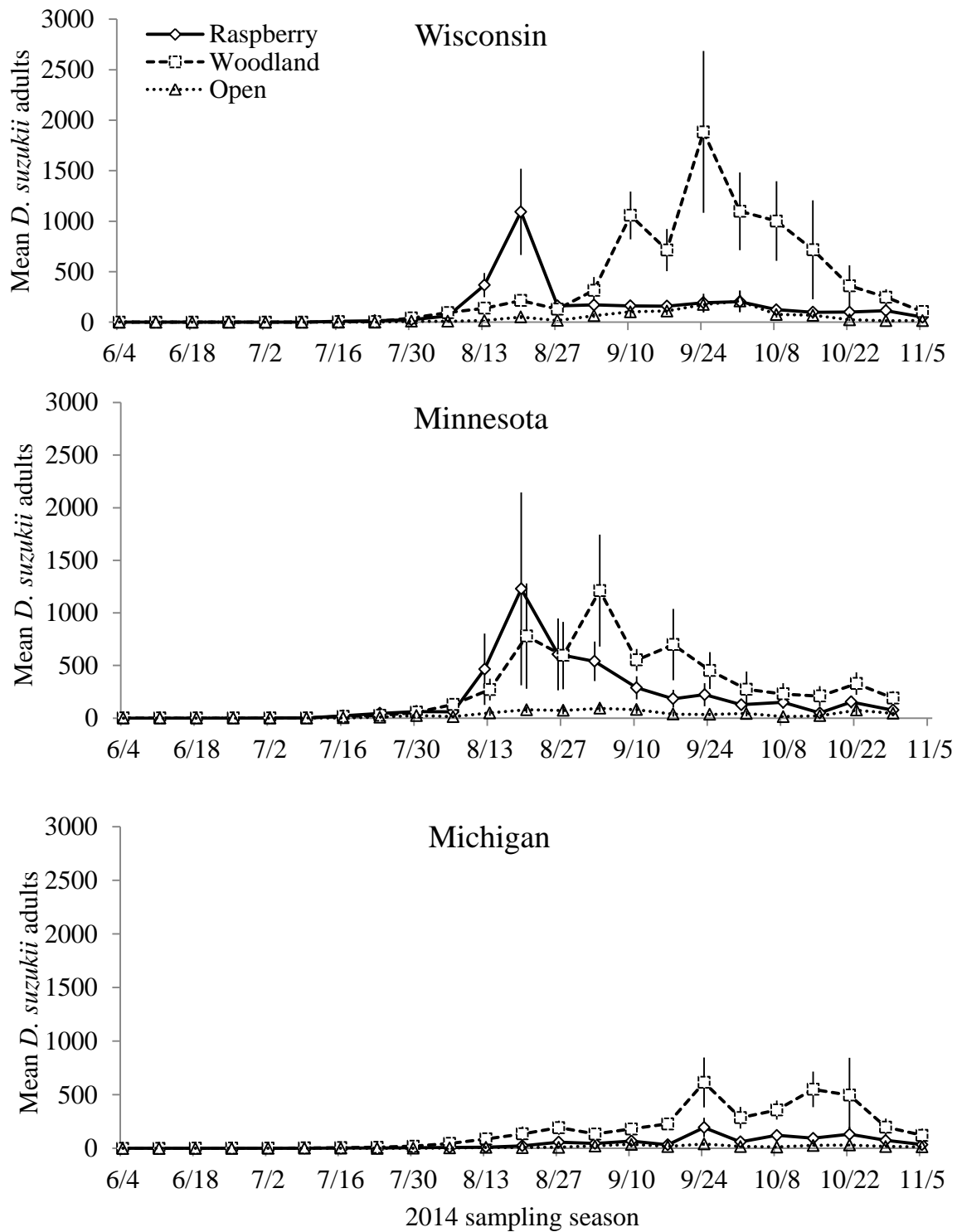Figure 1. Phenology of mean *D. suzukii* adults (± S.E.) per trap in 2013.

Figure 2. Phenology of mean *D. suzukii* adults (± S.E.) per trap in 2014.

Appendix B. Site locations

Sites included in the Chapter 1 study:

Latitude	Longitude
43.0407	-89.6148
42.9958	-89.9580
43.3868	-90.9173
43.6231	-90.7555
42.9687	-89.6824
42.7399	-89.2912
43.0408	-89.6144
42.9959	-89.9578
42.9113	-89.4396
43.0247	-89.2240
43.8007	-90.8612
43.1729	-89.3797
43.3164	-89.0769
43.6206	-90.3325
43.6170	-90.0734
43.0477	-88.6705
43.3265	-90.7942
42.9728	-88.8617
42.9429	-89.0669
43.3510	-89.0882
43.6230	-90.7556
42.9684	-89.6827
42.7398	-89.2912
43.3810	-90.8958
42.1760	-86.3675
42.6715	-84.4842
43.1416	-85.4872
43.1856	-85.3845
42.5363	-86.2073
45.2204	-93.8431
44.8908	-92.8062
44.8083	-92.8080
45.6249	-93.3596
45.2680	-93.3527
45.2248	-92.8896

Locations denote raspberry fields used in adult *D. suzukii* trapping and larval infestation assessments.

Sites included in the Chapter 2 study:

Latitude	Longitude
43.5220	-90.8277
43.6073	-90.8860
43.2879	-89.7073
43.5992	-90.9242
43.6058	-91.0041
43.6041	-90.9788
43.2951	-89.6457

Locations denote vineyards used in adult *D. suzukii* trapping and larval infestation assessments.