Influence of Previous Fruit Injury on Susceptibility to Spotted Wing Drosophila (Diptera: Drosophilidae) Infestation in the Midwestern United States[1]

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Abstract Spotted wing drosophila, *Drosophila suzukii* (Matsumura), is a major agricultural pest throughout most of the fruit growing regions of the United States, with high reproductive rates and short generation times resulting in exponential population growth in berry crops across the country. Fruit suitability research has been conducted for some small fruit species such as raspberries, strawberries, cherries, and blackberries; however, there is a paucity of data regarding the role of previous injury on host susceptibility and suitability for *D. suzukii*. This study examined the role of previous fruit injury on adult *D. suzukii* survival, adult production, and host susceptibility, with injury referring to physiological splitting, disease, and vertebrate feeding. Results indicate that intact raspberries, blueberries, and table grapes are susceptible to oviposition by *D. suzukii* and the presence of injury increases survival of both male and female flies. When fruit was injured, berry infestation levels increases, but this did not always translate into increased adult populations. We found that the proportions of *D. suzukii*–infested fruit were significantly higher for injured versus intact fruit. When comparing adult emergence for intact and injured fruit, only table grape and cherry tomato had significantly higher adult counts for injured berries. Overall, adult emergence per injured fruit for raspberries of 19.77 was significantly higher than all other fruit types tested, with 2.87, 3.57, and 3.43 adults for cherry tomatoes, grapes, and blueberries, respectively. Results suggest reducing previous injury in ripe fruit may facilitate more effective management of *D. suzukii* populations.

Key Words fruit splitting, raspberry, blueberry, grape, tomato

Throughout Asia, Europe, and North America, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), commonly known as spotted wing drosophila, continues to be a significant economic pest of numerous small-fruit crops (Asplen et al. 2015, Goodhue et al. 2011). As a pest with an ever expanding geographic range, *D. suzukii* has been a major agricultural pest in the contiguous United States since 2008. When first detected in California and in the absence of effective control measures, annual yield reductions for raspberry and strawberry production were estimated to range from 20 to 50%, with 20 to 37% revenue losses (Goodhue et al. 2011). In Minnesota, *D. suzukii* was first documented in 2012, after which it quickly spread throughout the state and has had considerable negative impacts in both

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production costs and reductions in marketable yield (Asplen et al. 2015). While insecticide use may provide acceptable levels of control (Bruck et al. 2011) and high tunnel production has been shown as a promising management tool for *D. suzukii* (Rogers et al. 2016), several growers in Minnesota have temporarily suspended raspberry production until new management solutions are developed (W.D.H. unpubl. data).

Drosophila suzukii differs from nearly all other *Drosophila* species, which infest overripe fruit, in that *D. suzukii* females have a serrated, sclerotized ovipositor that can penetrate the skin of ripening fruit, allowing them to lay eggs in fruit (Lee et al. 2011a, b). Previously injured fruit, resulting from physiological splitting, disease infection, or vertebrate feeding in wine grapes in Minnesota, has been associated with infestations of the multicolored Asian ladybird beetle, *Harmonia axyridis* Pallas (Galvan et al. 2006). Moreover, splitting or cracking in small fruits such as cherries (Grimm et al. 2012) and cherry tomatoes (Lichter et al. 2002) is a documented phenomenon caused by imbalances in water and nutrients. Despite the ability of *D. suzukii* to attack ripening intact fruit, the ability or likelihood of *D. suzukii* attacking overripe or previously injured fruit exists, and has not been fully examined with regard to the role this injury may play in subsequent population increase. Few studies have explored the role that previous injury of ripe fruit may play in fruit infestation by *D. suzukii,* and have primarily been limited to cranberries (Steffan et al. 2013), peaches (Stewart et al. 2014), mistletoe (Briem et al. 2016), and wine grapes (Ioriatti et al. 2015). As shown by Bellamy et al. (2013) and Ioriatti et al. (2015), there are substantial differences among small fruit species regarding host suitability and susceptibility for *D. suzukii*. While Bellamy et al. (2013) explored host potential for a range of seven small-fruit hosts, they did not explore the role of previous injury. Alternatively, while Ioriatti et al. (2015) considered previous injury in wine grapes, they viewed this injury mainly from the standpoint of increased vectoring of spoilage bacteria by *D. suzukii* rather than the influence on adult *D. suzukii* populations. Stewart et al. (2014) examined how *D. suzukii* oviposition is limited by the physical barrier of peach fuzz on intact and injured fruit, and Briem et al. (2016) examined mistletoe berries, which serve as an alternate host and are not considered a cash crop.

Therefore, the purpose of this study was to characterize the role of previous injury to ripe fruit on the level of *D. suzukii* infestation (i.e., host susceptibility), and adult survival and population density (i.e., host suitability), on commonly grown fruit crops in the Midwest. This study examined small fruit thought to be highly suitable and susceptible as hosts for *D. suzukii* (raspberry and blueberry) as well as fruit thought to have a low level of suitability and susceptibility to *D. suzukii* (table grape and cherry tomato).

Materials and Methods

To assess the impact of previous injury on *D. suzukii* infestation, no-choice assays were conducted in 2014 with organic raspberries, blueberries, table grapes, and cherry tomatoes that were purchased from local grocery stores. All samples were carefully examined before use for existing injury or skin defects before use in an assay. All fruit samples were washed twice with warm water to remove potential pesticide residues. Berries from each crop were randomly selected and assigned into one of four treatments. Treatments included intact fruit with *D. suzukii* flies added, intact fruit with no flies added, fruit injured with a scalpel and *D. suzukii* flies added, and fruit injured with a scalpel without flies added. Flies used were collected from a University of Minnesota colony, which was started with flies from Michigan State University in 2013. The colony is maintained at 25–27°C with 40–60**%** relative humidity (RH), and a 16:8-h (light:dark [L:D]) photoperiod; flies are fed a diet made with agar, cornmeal, nutritional yeast, and sugar; male and female adults were ~2–4 d old when placed in vials for the assays. Fruit injured with a scalpel consisted of a 1-cm incision in the skin of the fruit. For fruit that develops on vines, such as table grape, a small part of the stem (pedicel) was left on the berry when separated from the cluster to minimize any unintentional injury occurring from stem removal.

Individual fruit was placed into 25 × 95–mm polystyrene vials (Genesee Scientific, San Diego, CA) and sealed with a foam stopper (Genesee Scientific). To provide moisture for adult flies inside the vial, a microcentrifuge tube (Dot Scientific, Burton, MI) filled with water and plugged with cotton (Curad, Mundelein, IL) was added to each vial. In trials conducted using raspberries and to minimize the chances of larvae mortality, 5 ml of sand was placed at the bottom of each vial to absorb excess juices that may collect on the bottom of the vial as berries deteriorated from larval feeding. Treatments where *D. suzukii* flies were added to the vial were comprised of five adult male and five adult female *D. suzukii*. Prior to transfer into the treatment vials, flies were anaesthetized using a carbon dioxide gun (Genesee Scientific) to allow counting and sex determination. For each fruit type, four treatments were arranged in a randomized complete block design with each vial considered a replicate and 30 replicates for each treatment. Vials were placed in a Percival incubator (Percival Scientific; Perry, IA) at 25°C, 16:8 (L:D), and 40–60**%** RH. Flies were left in the vials for 5 d to allow for mating and oviposition. Fly mortality was recorded at 3 d after infestation (DAI) and flies were removed at 5 DAI. Vials were then left for an additional 14 d to allow for egg hatch, larval development, and adult emergence. Final counts of emerged adult *D. suzukii* flies were recorded at 19 DAI.

Values for proportion mortality of male and female *D. suzukii* at 3 DAI were compared between intact and injured fruit treatments for each fruit type using a χ^2 test. Data for the number of flies emerged from intact and injured fruit as well as the proportion of fruit infested for intact and injured fruit were tested for normality (Shapiro–Wilk test) and homoscedasticity (Levene's test). Tests showed our data did not meet the assumptions for an analysis of variance and no appropriate transformations were found. Therefore, a Kruskal–Wallis nonparametric test with a subsequent multiple comparison test was run for the number of flies emerged and the proportion of fruit infested data. R version 3.2.0 (R Development Core Team 2015) and RStudio version 0.98.1103 (RStudio Team 2015) were used for all statistical analyses. The following R packages and commands were used to compare the number of flies emerged from fruit and proportion of fruit infested: kruskal.test in *stats* (R Development Core Team 2015) and kruskalmc in *pgirmess* (Giraudoux 2016).

Results

Based on 3-d mortality assessments after initial fly infestation, both female and male flies in previously injured berry treatments experienced significantly increased survivorship compared with flies from intact berry treatments, with the exception of raspberry (Table 1). While comparisons were not made across fruit type, raspberry consistently supported the highest overall survivorship of adult flies at 3 DAI (Table 1). Male survivorship at 3 DAI tended to be lower than female survivorship regardless of fruit type.

Despite relatively low fly survivorship levels for several fruit types, *D. suzukii* adults still emerged from most treatments at 19 DAI (Table 2). With the exception of raspberry, no fly emergence was recorded from "uninfested" check treatments (i.e., intended to be uninfested) of either intact or injured fruit (data not shown). For "uninfested" raspberry treatments, mean fly emergence $\pm$ standard error of the mean (SEM) per fruit for intact and injured treatments was 3.30 $\pm$ 0.89 and 3.83 $\pm$ 0.81, respectively. This indicates the raspberries purchased from source stores were infested at the time of purchase. Adult emergence for intact and injured fruit was not significantly different for either raspberry or blueberry, but was significantly higher for injured fruit for both table grape and cherry tomato (Table 2). Significant differences in adult emergence were found for intact fruit, with raspberry producing the highest number of flies and cherry tomato not producing any flies. Among injured fruit types, fly emergence was much more uniform across treatments but raspberry still produced significantly more flies than the other treatments (Table 2).

Data for the proportion of fruit infested for intact fruit follows a trend similar to data for adult fly emergence, with raspberry and blueberry exhibiting relatively high infestation rates of 0.83 and 0.50, respectively, and both fruit types having significantly higher rates of infestation than cherry tomato (Fig. 1). No significant differences in the proportion of fruit infested were detected among fruit types for treatments with injury and infestation rates ranged from 0.73 to 1.00. When comparing the proportion of fruit infested for intact and injured treatments, all fruit types had significantly higher infestation rates in treatments with injury except for raspberry (Fig. 1).

Discussion

Our results were similar to findings of Ioraitti et al. (2015) on wine grapes, in that we found previously injured (incised) fruit provides a more favorable environment for survival of both male and female adult *D. suzukii* than intact fruit for all fruit types tested, with the exception of female flies on intact raspberry (Table 1). The presence of injury on fruit hosts likely provides a source of nutrition for adults as well as allowing easier access to oviposition sites. Increased adult survival is also likely to increase overall oviposition and subsequent adult production allowing for population increases to be magnified over time (e.g., Wiman et al. 2014). However, increased adult survival did not result in increased adult production on a per-fruit basis (Table 2). For both raspberry and blueberry, injured and intact berries produced similar numbers of adults; however, raspberries produced significantly higher numbers of adults than blueberries regardless of the presence of fruit injury.

Table 1. Mean ± SEM mortality of female and male *Drosophila suzukii* on intact and injured fruit.

Fruit type	Mean ± SEM proportion female adult fly mortality at 3 DAI*		χ^2 value	*P* value	Mean ± SEM proportion male adult fly mortality at 3 DAI		χ^2 value	*P* value
	Intact**	Injured**			Intact	Injured		
Raspberry	0.22 ± 0.04	0.14 ± 0.03	2.73	0.1000	0.26 ± 0.03	0.14 ± 0.03	6.02	0.0100
Blueberry	0.94 ± 0.02	0.57 ± 0.04	121.11	<0.0001	0.93 ± 0.02	0.62 ± 0.04	39.34	<0.0001
Grape (table)	0.86 ± 0.03	0.22 ± 0.03	176.36	<0.0001	0.89 ± 0.03	0.12 ± 0.03	18.48	<0.0001
Tomato (cherry)	0.98 ± 0.00	0.83 ± 0.03	52.79	<0.0001	1.00 ± 0.01	0.85 ± 0.03	22.79	<0.0001

* DAI = days after infestation of vials containing ripe fruit; infestation of berries is defined as the introduction of five female and five male adult *D. suzukii* into vials for 5 d, and mortality being measured after 3 d.

** Intact and injured refer to the status of fruit skin at time of infestation and describes whether the berry was intact, completely free of any known visible injury, or injured, where the berry skin received a 1-cm incision with a scalpel.

Table 2. Mean ± SEM *Drosophila suzukii* adult fly emergence from intact and injured fruit after artificial infestation.

Fruit type	Mean ± SEM number of adult flies / fruit at 19 DAI*		*t* value†	df	*P* value
	Intact**	Injured**			
Raspberry	18.70 ± 2.30 a‡	19.77 ± 2.44 a	−0.25	56.25	0.8000
Blueberry	2.13 ± 0.44 b	3.43 ± 0.33 b	−1.66	46.63	0.1000
Grape (table)	0.40 ± 0.11 bc	3.57 ± 0.29 b	−6.67	34.62	<0.0001
Tomato (cherry)	0.00 ± 0.00 c	2.87 ± 0.40 b	−6.08	29.00	<0.0001

* DAI = days after infestation of vials containing ripe fruit; infestation of berries is defined as the introduction of five female and five male adult *D. suzukii* into vials for 5 d and fruit infestation being measured after 19 d by presence of larvae, pupae, or adults.

** Intact and injured refer to the status of fruit skin at infestation and describes whether the berry was intact, completely free of any known injury, or injured, where the berry skin received a 1-cm incision with a scalpel. Intact and injured fruit was also set up with no fly infestation as a check for previous exposure to *D. suzukii*. Of the four fruit species, only uninfested raspberry fruit produced *D. suzukii* flies after 19 d with a mean of 3.30 ± 0.89 and 3.83 ± 0.81 for intact and injured treatments, respectively.

† Values of *t*, df, and *P* provided for comparison of the mean number of flies emerged from intact and injured fruit of the same fruit type.

‡ Means within columns followed by the same letter are not significantly different using a Kruskal–Wallis nonparametric test with subsequent multiple comparison test.

Conversely, both intact table grapes and cherry tomatoes produced significantly fewer adults than berries with injury (Table 2). Differences in the production of *D. suzukii* adults between intact and injured fruit highlights host susceptibility, where oviposition is prohibited by characteristics of the fruit skin that do not allow the female ovipositor to penetrate the skin. Ioriatti et al. (2015) found that over time, penetration force required to pierce grape skin decreased as wine grapes ripened and demonstrated that susceptibility to oviposition increased over time. Bellamy et al. (2013) developed a host potential index for several fruit crops which includes oviposition frequency as a factor. Oviposition frequency was found to differ among fruit types with raspberry, strawberry, and blueberry having higher oviposition frequency and grape and peach having lower oviposition frequency. Consequently, fruit with lower oviposition frequency had a lower overall probability of being a potential host. While intact fruit skin among different fruit types may present varying degrees of ovipositional barriers and eventual adult emergence (Table 2), it is clear that different fruits also differ in suitability for *D. suzukii* development to the adult stage (e.g., Diepenbrock et al. 2016). Raspberries clearly produced significantly higher numbers of *D. suzukii* flies during the course of this study for both intact and injured fruit. However, in the case of injured blueberries, table grapes, and cherry tomatoes, the potential oviposition barrier that the fruit skin may create should no longer be an issue. So, there must be other explanations for the significantly lower adult production in injured blueberry, table grape, and cherry tomato when compared with injured raspberry.

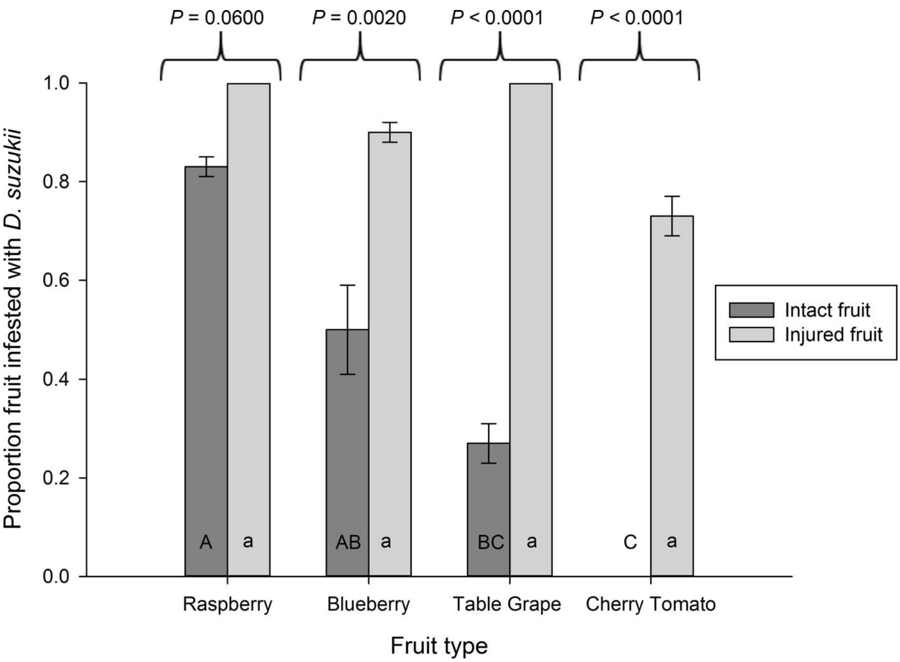

Fig. 1. Proportion of fruit infested with *Drosophila suzukii* 19 d after five male and five female *D. suzukii* flies were added to each vial. The five male and five female flies were removed from each vial after 5 d. Mean proportion of fruit infested is indicated by bars; for intact fruit with the same uppercase letter and for injured fruit with the same lowercase letter are not significantly different ($P > 0.05$). Comparisons of proportions of infested fruit were made between intact and injured fruits within each fruit type using a two-sample *t* test analysis and *P* values above each fruit type indicate significance. Analysis of proportion of fruit infested comparing different fruit types was conducted using a Kruskal–Wallis nonparametric test with subsequent multiple comparison test.

For three of the four varieties of wine grapes tested, Ioriatti et al. (2015) found that incised berries had significantly higher oviposition per berry than intact berries, suggesting that reduced oviposition would not be a limiting factor for injured berries. Moreover, Pelton et al. (2017) found that even when *D. suzukii* eggs were laid in intact wine grapes, almost no larvae were recovered, suggesting lack of egg hatch or larval development. One explanation for differential host suitability could reflect either nutritional composition of the fruit and/or the physiological composition of the environment the eggs and larvae develop under (e.g., Brix, fruit acidity), which in turn impacts adult production for each fruit type. In the case of cherry tomato, wound healing (Dean and Kolattukudy 1976, Whaley Emmons and Scott 1997) may also play a role in decreased survival of *D. suzukii* eggs and/or larvae. The issue of host suitability is further illustrated in Fig. 1, where the proportion of fruit infested differs for intact fruit, providing a similar trend to adult production values in Table 2. However, for injured fruit, despite the differences in adult production for each fruit type, the proportion of fruit infested is not statistically different.

Both host susceptibility and suitability are critical considerations when evaluating the overall risk of each crop to *D. suzukii*. To minimize host susceptibility to *D. suzukii* oviposition, management of irrigation, disease, physiological disorders, vertebrate feeding, and physical damage are all critical factors. In addition, sanitation during harvest is an important consideration; this has been particularly problematic for "pick your own" operations in Minnesota. Often imperfect or injured fruit is left in the field because the berries are considered unmarketable or undesirable. However, the leftover fruit may serve as hosts for *D. suzukii*, leading to an increase in population growth in the field. In order to remedy this issue, berry growers have been encouraged to consider harvesting all fruit and properly disposing of the unwanted fruit. Fruit crops previously thought to not be susceptible hosts when intact may become susceptible when previous injury is present. Knowing whether *D. suzukii* is causing primary injury or is taking advantage of previous injury can influence the control options used in managing *D. suzukii* populations.

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