Journal of Economic Entomology, 113(1), 2020, 230–238
doi: 10.1093/jee/toz297
Advance Access Publication Date: 19 November 2019
Research

OXFORD

Ecology and Behavior

Effects of Interspecific Larval Competition on Developmental Parameters in Nutrient Sources Between *Drosophila suzukii* (Diptera: Drosophilidae) and *Zaprionus indianus*

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Subject Editor: Jana Lee

Received 28 February 2019; Editorial decision 10 October 2019

Abstract

Two invasive drosophilids, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) and *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae) are expanding their geographic distribution and cohabiting grape production in the Mid-Atlantic. The ecological and economic impact of these two species within vineyards is currently unknown. *Zaprionus indianus* was presumably not capable of ovipositing directly into grapes because they lack a serrated ovipositor and may use *D. suzukii* oviposition punctures for depositing their own eggs. Therefore, an interspecific larval competition assay was performed at varying larval densities using commercial medium and four commonly grown wine grapes in Virginia to investigate the impact *Z. indianus* larvae may have on the mortality and developmental parameters of *D. suzukii* larvae. *Zaprionus indianus* did not affect *D. suzukii* mortality or development parameters even at high interspecific densities when reared in commercial medium, but it did cause higher *D. suzukii* mortality within grapes. Mortality was also influenced by the variety of grape in which the larvae were reared, with smaller grapes having the highest *D. suzukii* mortality. Presence of *Z. indianus* also increased development time to pupariation and adult emergence for most interspecific competition levels compared with the intraspecific *D. suzukii* controls. Pupal volume was marginally affected at the highest interspecific larval densities. This laboratory study suggests that competition from *Z. indianus* and grape variety can limit *D. suzukii* numbers, and the implications on *D. suzukii* pest management be further verified in the field.

Key words: interspecific competition, intraspecific competition, wine grapes, medium, pupal volume

The insect pest ecology within Virginia vineyards has changed dramatically over the past decade with the introduction of two economically significant drosophilids; spotted wing drosophila, *Drosophila suzukii* Matsumura, and the African fig fly (AFF), *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae). *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), spotted-wing drosophila, is economically damaging to small fruits, cherries and grapes in areas where these crops are produced (Goodhue et al. 2011, Walsh et al. 2011). *Drosophila suzukii* can develop in both wild and cultivated grapes, including both table and wine grapes (Ioriatti et al. 2015, Kim et al. 2015). The serrated ovipositor of female *D. suzukii* facilitates oviposition in ripening fruit that other drosophilid species cannot utilize (Lee et al. 2011). Larvae developing within the fruit reduce fruit quality and marketability (Walsh et al. 2011). Furthermore, female *D. suzukii* can render grapes susceptible to secondary pathogens, such as *Acetobacter* spp., *Gluconobacter* spp., and *Hanseniaspora uvarum*, and facilitate sour rot outbreaks in vineyards through piercing and wounding the fruit during oviposition (Barata et al. 2012, Hamby and Becher 2016). If sour rot is found in a few grapes within a cluster or packing crates, the whole cluster and crates may be culled or rejected by processors and wine makers.

Drosophila suzukii and *Z. indianus* are currently expanding their distribution globally and sharing new fruit hosts. While sharing some ecological attributes, *D. suzukii* and *Z. indianus* differ in several key characteristics such oviposition ability, overwintering capabilities and reproductive fecundity (Kanzawa 1939, Biddinger et al. 2012, Ramniwas et al. 2012, Asplen et al. 2015, Wallingford and Loeb 2016). The ecological and economic impacts of these two drosophilids when sharing cultivated fruit hosts are currently unknown.

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Drosophila larvae within the same food source compete, leading to increased mortality, decreased growth, and reduced fecundity as density increases (Bakker 1961). Direct interspecific completion between *Drosophila buzzatii* (Patterson and Wheeler) and *Drosophila koepferae* (Fontdevila and Wasserman) resulted in the former experiencing increased developmental times, smaller body mass, and lower viability when reared with the latter (Werenkraut et al. 2008). The egg-to-adult viability of *Drosophila willistoni* (Sturtevant) larvae were negatively affected by metabolic waste products in food medium previously used by *Drosophila pavani* (Brncic) (Budnik and Brncic 1974). The presence of *D. melanogaster* in a substrate significantly reduced *D. suzukii* emergence and the potential egg laying of the females exposed to the *D. melanogaster* preinoculated medium (Shaw et al. 2018). Furthermore, the number of *D. suzukii* offspring in both pairwise and cage experiments was dramatically reduced when in competition with *D. melanogaster* (Dancau et al. 2016).

Intraspecific competition also affects developmental performance. *Drosophila subobscura* (Collin) had a decrease in pupal volume, but not an increase of developmental time at high densities (Miller 1964, Gonzalez-Candelas et al. 1990). Among the species of interest to the present studies, *Z. indianus* reared at high larval densities (more than 30 per tube) had longer developmental times and lower survivorship and body mass (Amoudi et al. 1993).

Drosophila suzukii may attempt to avoid interspecific competition by ovipositing in intact, carbohydrate-rich, and protein-poor fruit such as blueberries or grapes (Bellamy et al. 2013, Sandra et al. 2015). *Drosophila suzukii* can develop in nutrient deficient hosts; however, other *Drosophila* species may not be able to compensate developmentally from feeding on low-protein hosts (Begon 1983, Jaramillo et al. 2015). The quality of the nutrient substrate may also impact the development and survival of *Drosophila* within the medium. Larval competition density as well as nutrient profiles of host plants may be important when considering population dynamics within specific host crops (Bellamy et al. 2013, Hardin et al. 2015, Jaramillo et al. 2015).

An observation by a Virginia wine grower in 2012 estimated 80% loss of grapes in a Petit Verdot block due to fly infestation and sour rot (Carrington King, personal communication). *Drosophila suzukii* was visually detected in the vineyard; however, most flies observed in the field and flies reared from infested grape clusters in the laboratory were *Z. indianus* (M.E.S., unpublished data). If *Z. indianus* uses *D. suzukii* oviposition wounds to deposit their own eggs into grapes then the interactions between the larvae of these species within grapes may affect the population dynamics of *D. suzukii* in Virginia vineyards (Pfeiffer et al. 2019). Vineyards with both fly species present may have a lower risk of *D. suzukii* population growth due to possible interactions of *Z. indianus*. The objective of this study was to determine the interspecific interactions of *Z. indianus* larvae and *D. suzukii* larvae within commercial medium and wine grapes commonly grown in Virginia. Our hypothesis is that *Z. indianus* larvae will out-compete *D. suzukii* larvae within a nutrient source, impacting the developmental parameters by increasing development time and decreasing survival of *D. suzukii*. Developmental impacts of interspecific larval competition were assessed using commercial food medium and four varieties of grapes as well as different densities of *D. suzukii* and *Z. indianus* eggs and larvae. Dietary medium as well as grapes were used to observe developmental parameters in *Drosophilae* when all nutritional needs were met compared with available resources in a vineyard. Different fitness components and parameters analyzed were larval development time, total development time, larval mortality, adult emergence, and pupal volume.

Materials and Methods

Grape Cluster Collection

Field-collected grape clusters came from a single vineyard located in Virginia's Piedmont region (Orange County) (coordinates: 38.234451, −78.102461). The size of vineyard blocks from which wine grapes of each variety were collected was: Petit Verdot 0.65 ha, Cabernet Franc 1.07 ha, Viognier 0.5 ha, and Petit Manseng 0.5 ha. Clusters were collected from the middle of each block (>9 m from adjacent varietal blocks) and from the middle of the selected row (>50 m from row edge). Row lengths ranged from 160 m to 170 m. Grapes were sprayed for fungus and insects, but grape clusters were collected when chemicals had passed peak residual effectiveness. Clusters were collected, ice-cooled, and transported to Blacksburg for laboratory testing. All grapes clusters collected were maturing and above 15° Brix. Petit Verdot grape clusters were collected and used in 2013, all four varieties were used in 2014, and Viognier was used in 2015.

Drosophila suzukii and *Z. indianus* Egg and Larva Collection

Drosophila suzukii and *Z. indianus* colonies have been maintained in laboratory growth chambers at Virginia Tech, Blacksburg, Virginia since 2012 from flies that were collected and reared from raspberries. Eggs of *Z. indianus* and *D. suzukii* were acquired by exposing adult flies to 50 ml of a commercial medium (Nutri-Fly MF-molasses formulation, no antimicrobials) (Genesee Scientific Corporation, San Diego, CA) in 177 ml square bottom, polypropylene flasks (Genesee Scientific Corporation, San Diego, CA) for 48 h in a growth chamber at 23°C, 50–80% RH, and a 16:8 (L:D) h photoperiod. Adult flies were removed after 48 h and the medium was checked for eggs, which were used immediately after the 48 h ovipositional period.

First instar larvae (L1) were collected by exposing adult flies to the medium and environmental conditions described above. Flies were removed after 48 h and the medium with eggs was returned to the growth chamber for an additional 24–36 h to allow for egg hatch. Once eggs or L1 larvae were observed, eggs and larvae were removed under a dissecting microscope using a homemade scoop (9 mm^2 piece of metal glued to a small wooden dowel rod; 2 mm diameter, 15.2 cm long) and placed on a medium cube or a grape for bioassay experiments.

Interspecific Larval Competition in Commercial Medium 2014

These methods were adapted from Takahashi and Kimura (2005). Nutri-Fly MF (molasses formulation) medium (Genesee Scientific Corporation, San Diego, CA) was prepared to package specifications and no additional antimicrobial agents were added. A 0.38 g medium cube was placed under a dissecting microscope and the eggs of each species were transferred to the cube. The interspecific egg densities tested (*D.s.: Z.i.*) were 2:2 and 4:4. Intraspecific controls were four and eight *D. suzukii* eggs per cube and all densities were replicated 15 times. The cubes with eggs were placed individually in 16 ml glass shell vials (Fisher Scientific, Waltham, MA) which were capped with a cotton ball and held in a growth chamber at 23°C, 50–80% RH, and a 16:8 (L:D) h photoperiod.

Interspecific Larval Competition in Petit Verdot Grapes 2014

Petit Verdot clusters were collected on 27 August and 9 September and all experiments were conducted within 10 d of collection to

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ensure fruit freshness. Grapes were held in a refrigerator (<4.5°C) until needed. Grapes were randomly removed from three grape clusters and inspected under a dissecting microscope for *D. suzukii* eggs or wounds. Grapes containing eggs or wounds were not used. L1 larvae were transferred to single Petit Verdot grapes to ensure that individual larvae were alive at the beginning of the experiment. The interspecific larval densities tested (*D.s.: Z.i.*) were 4:4 and 8:8 and intraspecific controls were 8 and 16 *D. suzukii* larvae per grape. There were 15 replicates for each larval density tested. Each grape was then placed in a polystyrene petri dish (60 × 15 mm) (USA Scientific, Orlando, FL) sealed by Parafilm and held in a growth chamber at 23°C, 50–80% RH and a 16:8 (L:D) h photoperiod. Sealing with Parafilm prevented larval escape and enough ventilation to ensure survival.

Interspecific Larval Competition Utilizing Four Wine Grape Varieties 2015

Four varieties of wine grapes were utilized to determine whether larval interactions were the same or varied based upon the type of grape in which the competition took place. Viognier, Petit Manseng, Petit Verdot, and Cabernet Franc grape clusters were collected on 16 and 30 August and 9 and 16 September, stored at <4.5°C, and all experiments were conducted within 10 d after collection. Four larval densities on each wine grape variety were compared. The larval densities evaluated for interspecific competition (*D.s.: Z.i.*) were 1:1, 2:2, with two and four *D. suzukii* alone serving as an intraspecific competition control. Twenty replicates were performed for each larval density and grape variety. Ten randomly selected grapes were removed from the clusters of each variety and weighed (g) in case grape volume became a statistically significant factor. Individual grapes were randomly selected for each wine grape variety and inspected for *D. suzukii* eggs or wounds. The grapes had been pulled from the cluster and the wound where the grape had been attached to the pedicel was the site of larval deposition. Larvae were then placed onto the grapes at the various densities for each fly species. Grapes containing larvae were placed individually in polystyrene petri dishes at 23°C, 50–80% RH, and a 16:8 (L:D) h photoperiod.

Interspecific Larval Competition in Viognier Grapes 2016

In 2016, further experiments were conducted using Viognier grapes due to the increased survivorship seen in the previous year (2015) when larval were reared on this particular variety. Grapes were collected on 24 August and 7 and 16 October. Grapes were used within 10 d of collection and were held in a refrigerator (<4.5°C) until needed. The larval densities evaluated for interspecific competition (*D.s.: Z.i.*) were 2:3, 3:2, and 2:2 with intraspecific competition densities of four or five *D. suzukii* per grape acting as controls. Twenty replicates for each competition level were performed. The same methodology used for the 2015 study was used.

Larval Developmental Performance Observations

Medium and grapes were observed daily through visual inspection for 21 d and larval mortality was recorded when dead larvae were outside the medium or grape within the container. If neither larvae nor pupae could be observed in the container, the grape or medium were dissected to look for larvae or pupae. If no individuals were found, then the individuals were marked as dead at the larval stage. If pupation occurred, the date was recorded so larval development time could be determined. Each pupa was removed from the grape or container with soft forceps and placed under a dissecting microscope for estimation of pupal volume. Pupal volume was estimated based on measurements of pupal length and width using an ocular micrometer and calculated using this following formula (Takahashi and Kimura 2005).

$$^{*}V = \frac{4}{3}\pi\left(\frac{w}{2}\right)^2\left(\frac{l}{2}\right)$$

Pupal volume has been used to determine fecundity in drosophilid females as well as overall fly vitality (Santos et al. 1992, Rodriguez et al. 1999, Takahashi and Kimura 2005). Larval development time (days) period was the period from the day the egg or L1 larva was placed on the medium or grape until pupation. Total development time (days) was the period from egg or L1 larva to adult eclosion. Larval and total development times were used as evaluation parameters based upon *D. melanogaster* extending or arrested developmental time in order to overcome competition in medium (Miller 1964, Gonzalez-Candelas et al. 1990). Larval mortality and adult emergence were also recorded to determine whether interspecific competition affected mortality more than intraspecific competition.

Statistical Analysis

Survivorship of eggs to adults in the commercial medium and Petit Verdot grape trials in 2014 at varying densities were analyzed via a χ^2 analysis. In 2015, survivorship (0 = dead, 1 = alive) of larvae to pupae and larvae to adults comparing four varieties of grapes at varying densities were analyzed using a binary nominal logistic regression. In order to identify which main effect had the greatest impact on survivorship, an odds ratio test was performed. Odds is defined as the probability of an event occurring divided by the probability of the event not occurring. The odds ratio (i.e., survival) for a unit change (negative or positive) in the predictor variable was determined after taking into account all other predictors in the model (i.e., competition level and grape variety) (King 2008, Maroof 2012, Rijal et al. 2014). In 2016, survivorship of larvae to pupae and larvae to adults in the Viognier grape trials at varying densities were analyzed via a χ^2 analysis. Varietal differences based upon weight (g) were analyzed via a one-way ANOVA. Data reported for all developmental parameters are only representative of individuals that survived to adulthood. These parameters were analyzed using a mixed-model ANOVA with egg or larval competition level, grape variety, and their interactions as fixed effects and dish number within experimental date as random effects (via JMP 12). A Tukey's HSD separated the means ($P < 0.05$). When interactions were significant ($P < 0.05$) a Slice Test compared simple effects of competition level and grape variety.

Results

Survival

Interspecific Larval Competition in Commercial Medium and Petit Verdot Grapes 2014

Eggs surviving to pupariation were not recorded for this year. *Drosophila suzukii* eggs at the 2:2 *D.s.: Z.i.* larval density had a greater likelihood of surviving to adulthood than the 4:0 *D. suzukii* intraspecific control (Prob > χ^2 = 0.0234). The 2:2 density had 70% of the *D. suzukii* adults emerge versus only 45% from the 4:0 *D. suzukii* controls. There was no significant difference in survivorship based upon the density of the eggs on the medium cube at the 4:4 versus 8 *D. suzukii* alone controls (Prob > χ^2 = 0.082). No *D. suzukii* individuals survived in the Petit Verdot grapes at the 8:8 competition level and only 2:0 *D. suzukii* adults emerged from the 16:0 *D. suzukii* alone controls, so no statistical analysis could be performed.

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Interspecific Larval Competition Utilizing Four Wine Grape Varieties 2015

Grape weight (g) differed significantly between varieties (F = 24.3, df = 3, P < 0.001). Viognier (1.9 g,) was significantly heavier than Cabernet Franc (1.5 g). Cabernet Franc and Petit Verdot (1.31 g) were similar in weight as was Petit Verdot and Petit Manseng (1.2 g).

Competition level and grape variety both significantly impacted *D. suzukii* survivorship to pupariation and adulthood, but these effects were not always independent. The binary nominal logistic regression analysis showed a statistically significant relationship between competition level (1:1 and 2 *D. suzukii*) and larvae surviving to pupariation (Table 1). The percentage of *D. suzukii* larvae surviving to pupate was significantly greater in the 2:0 *D. suzukii* (58%) alone relative to the 1:1 (38%) competition level. Survival rate was not significantly impacted by grape variety. There were no interaction effects of grape variety and competition level on larval survivorship to pupariation (Table 1).

The logistic regression for the 1:1 competition level and the 2:0 *D. suzukii* alone controls showed a significant relationship between the main effects of competition level and grape variety on larvae surviving to adults as well as an interaction of competition level and grape variety (Table 1). These main effects were separated and the individual odds ratios for larval survival were calculated for each competition level (1:1 and 2 *D. suzukii* alone) and each grape variety (Table 2). The odds ratio (e^{β}; survival) and β (positive or negative correlation), indicated that the larvae in the 2:0 *D. suzukii* alone competition level had a greater chance of surviving to adulthood than the *D. suzukii* larvae in competition with *Z. indianus*. The odds ratio for the four varieties of grapes demonstrated that *D. suzukii* larvae survivorship to adulthood was greatest when reared in Viognier grapes when compared with any other variety (Table 2). There was a greater likelihood of *D. suzukii* larvae surviving to adulthood if they were reared in Petit Manseng rather than in the Cabernet Franc (Table 2).

There was a significant relationship between competition level and grape variety on larvae surviving to pupariation based upon the nominal logistic regression analysis (Table 3) for the 2:2 and 4:0 *D. suzukii* alone controls. A significantly higher percent of *D. suzukii* larvae surviving to pupariation in the 4:0 *D. suzukii* alone control at 50% while the 2:2 competition level was 39%. The odds ratio also demonstrated that larvae surviving to pupariation was greatest when reared in the 4 *D. suzukii* alone controls (Table 4). The odds ratio for the four varieties of grapes demonstrated that *D. suzukii* survivorship to pupariation was greater when they are reared in Viognier grapes with Petit Manseng having the highest mortality (Table 4).

The logistic regression showed a significant relationship between competition level and grape variety on larvae surviving to adulthood. (Table 3). The survival rate of *D. suzukii* to adulthood at the 2:2 competition level was 18%, while the 4:0 *D. suzukii* alone controls had a significantly greater survival rate of 23%. The odds ratio for competition level of *D. suzukii* larvae at the 2:2 and 4:0 *D. suzukii* alone competition level indicated that *D. suzukii* larvae had a greater chance of surviving to adulthood when reared without *Z. indianus* (Table 4). The odds ratio showed *D. suzukii* had a greater likelihood of surviving to adulthood when reared in Viognier grapes (Table 4). Grapes reared in Cabernet Franc had increased mortality.

Table 1. Summary of the statistically significant values from the binary nominal logistic regression effects for competition level 2 *Drosophila suzukii* and 1:1 (*D. suzukii*: *Z. indianus*)

Effect	Nparm, df	L-R χ^2	Prob > χ^2
Larval survivorship			
Grape variety	3,3	5.53	NSD
Competition level	1,1	5.24	0.0020
Grape variety*competition level	3,3	5.54	NSD
Adult survivorship			
Grape variety	3,3	18.73	0.0003
Competition level	1,1	4.489	0.0341
Grape variety*competition level	3,3	8.213	0.0418

Table 2. Binary logistic regression parameters and associated statistics derived from the 1:1 (*D. suzukii*: *Z. indianus*) and 2 *Drosophila suzukii* competition levels and four wine grape varieties on *Drosophila suzukii* larval survivorship to adults

Variables		Odds ratio (e^{β})	β
Competition level			
2 *D. suzukii* alone	1:1 (*D. suzukii* / *Z. indianus*)	0.018	−1.744
1:1 (*D. suzukii* / *Z. indianus*)	2 *D. suzukii* alone	55.415	1.744
Grape variety			
Petit Manseng	Cabernet Franc	0.946	−0.024
Petit Verdot	Cabernet Franc	4097.594	3.613
Viognier	Cabernet Franc	0.497	−0.304
Petit Verdot	Petit Manseng	4333.276	3.637
Cabernet Franc	Petit Manseng	1.058	0.0243
Viognier	Petit Manseng	0.526	−0.279
Viognier	Petit Verdot	0.0001	−3.916
Cabernet Franc	Petit Verdot	0.0002	−3.613
Petit Manseng	Petit Verdot	0.0002	−3.637
Cabernet Franc	Viognier	2.012	0.304
Petit Manseng	Viognier	1.902	0.279
Petit Verdot	Viognier	8243.537	3.916

Interspecific Larval Competition in Viognier Grapes 2016

There was no significant difference for the larvae surviving to either pupae or adults in the Viognier grapes at the 2:2 competition level and 4:0 *D. suzukii* alone controls. No significant difference in survivorship for the larvae surviving to pupariation in the Viognier grapes at the 3:2 and 2:3 competition levels compared with the 5:0 *D. suzukii* alone controls. However, *D. suzukii* larvae surviving to adulthood in the Viognier grapes at the 3:2 (20%) (Prob > χ^2 = 0.0050) and 2:3 (15%) (Prob > χ^2 = 0.0077) competition levels were significantly lower than the 5:0 *D. suzukii* (37%) alone controls.

Development

Interspecific Larval Competition Using Commercial Medium 2014

Developmental time from egg to pupariation was not recorded for this year. Total developmental time from egg to adult was not statistically

Table 3. Summary of the statistically significant values from the binary nominal logistic regression effects for competition level 4 *Drosophila suzukii* and 2:2 (*Drosophila suzukii*: *Zaprionus indianus*)

Effect	Nparm, df	L-R χ^2	Prob > χ^2
Larval survivorship			
Grape variety	3,3	9.5	0.0233
Competition level	1,1	6.61	0.0101
Grape variety*Competition level	3,3	0.24	NSD
Adult survivorship			
Grape variety	3,3	14.40	0.0024
Competition level	1,1	19.25	<0.0001
Grape variety*Competition level	3,3	1.934	NSD

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significantly affected by competition on the commercial medium cube at the 2:2 *D.s.: Z.i.* density compared with the 4:0 *D. suzukii* alone. The developmental time from egg to adult at the 2:2 density was 11.1 d while the 4:0 *D. suzukii* density was 10.9 d (data not shown). Total development time from egg to adult was significantly affected by competition level on the commercial medium cube diet at the 4:4 competition level compared with the 8:0 *D. suzukii* alone control ($F = 37.8$, df = 1, $P < 0.0001$). The developmental time from egg to adult at the 4:4 competition level was 11.16 d while the 4 *D. suzukii* alone control was 10.3 d. Pupal volume was not affected by larval competition level for the 4:0 or 8:0 *D. suzukii* alone control (data not shown).

Interspecific Larval Competition Utilizing Four Wine Grape Varieties 2015

Due to no adults emerging from the Petit Verdot grapes, they were excluded from the statistical analysis performed at the 1:1 competition level (Table 4). Nevertheless, it can be stated that grape variety is important when analyzing developmental parameters for *D. suzukii* because none survived to adulthood in the Petit Verdot grapes.

The mixed model ANOVA showed that larval developmental days from at the 1:1 competition level and 2:0 *D. suzukii* alone were not significantly impacted by competition or grape variety, nor were there any significant interactions between grape variety and competition level (Table 5; Fig. 1A). Larval developmental days at the 2:2 competition level and 4 *D. suzukii* alone control were significantly impacted by both grape variety and competition level (Table 5). There was also a significant interaction between competition level and grape variety on larval developmental days (Table 5). Larval development time was longer when *D. suzukii* was in competition with *Z. indianus* at the 2:2 density and was significantly different for both the 2:2 density and the 4:0 *D. suzukii* density (Table 6). Larval developmental time was longest in Viognier at the 2:2 competition level, but was only significantly different when compared with Petit Manseng (Fig. 2A). The shortest larval developmental time was seen in the Petit Verdot grapes at the 4:0 *D. suzukii* alone competition level (Fig. 2A). Larval development was significantly different for Petit Manseng and Petit Verdot, but not Viognier or Cabernet Franc (Table 6). The significant interaction effects for larval developmental

Table 4. Binary logistic regression parameters and associated statistics derived from the 2:2 (*Drosophila suzukii*: *Zaprionus indianus*) and 4 *Drosophila suzukii* competition levels and four wine grape varieties on *Drosophila suzukii* larval survivorship to pupariation (A) and adulthood (B)

Variables		Odds ratio (e^{β})	β	Odds ratio (e^{β})	β
Competition Level		A		B	
4 *D. suzukii* alone	2:2 (*D. suzukii* / *Z. indianus*)	0.599	−0.222	0.765	−0.117
2:2 (*D. suzukii* / *Z. indianus*)	4 *D. suzukii* alone	1.667	0.222	1.308	0.117
Grape Variety					
Petit Manseng	Cabernet Franc	1.706	0.232	0.660	−0.179
Petit Verdot	Cabernet Franc	1.261	0.101	0.859	−0.066
Viognier	Cabernet Franc	0.738	−0.132	0.305	−0.515
Petit Verdot	Petit Manseng	0.739	−0.131	1.300	0.114
Cabernet Franc	Petit Manseng	0.587	−0.232	1.513	0.179
Viognier	Petit Manseng	0.432	−0.364	0.462	−0.335
Viognier	Petit Verdot	0.585	−0.233	0.355	−0.449
Cabernet Franc	Petit Verdot	0.792	−0.101	1.164	0.066
Petit Manseng	Petit Verdot	1.352	0.131	0.769	−0.114
Cabernet Franc	Viognier	1.356	0.132	3.275	0.515
Petit Manseng	Viognier	2.312	0.364	2.164	0.335
Petit Verdot	Viognier	1.709	0.233	2.814	0.449

Table 5. Summary outputs of full factorial mixed model ANOVA for 2 *Drosophila suzukii* and 1:1 (*Drosophila suzukii*: *Zaprionus indianus*) competition level (A) and summary outputs of full factorial mixed model ANOVA for 4 *Drosophila suzukii* and 2:2 (*Drosophila suzukii*: *Zaprionus indianus*) competition level (B)

	df	*F*	*P*	df	*F*	*P*
Effect		A			B	
Larval development time[a]						
Grape variety	2,66.8	0.835	NSD	3, 132.2	4.03	0.0088
Competition level	1,66.9	1.135	NSD	1, 132.1	9.63	0.0023
Grape variety*Competition level	2,66.8	0.075	NSD	3, 132.1	3.31	0.0222
Total development time[a]						
Grape variety	2,43.8	3.31	0.0455	3, 63.1	6.867	0.004
Competition level	1,43.8	0.008	NSD	1, 63.7	37.497	<0.0001
Grape variety*Competition level	2,43.8	0.45	NSD	3, 63.7	7.599	0.0002
Pupal volume[a]						
Grape variety	2,52.1	3.12	0.0526	3, 74.1	5.4	0.002
Competition level	1,52.1	0.007	NSD	1, 74.4	2.41	NSD
Grape variety*Competition level	2,52.1	1.77	NSD	3, 74.4	0.358	NSD

[a]Statistical analysis conducted without Petit Verdot due to lack of data.

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time did not affect the overall conclusions of the analysis and were due to large variation among replicates of a grape variety, with larval density effecting larval development time the greatest.

Total development time from larvae to adult for the 1:1 and 2:0 *D. suzukii* competition levels was significantly impacted by grape variety (Fig. 1B). There was no effect of competition level on total development, nor was there an interaction of grape variety and competition level (Table 5). Total development time from larvae to adult for the 2:2 competition level and 4:0 *D. suzukii* alone controls was significantly impacted by grape variety and competition level. There was also a significant interaction of both competition levels and grape varieties on the total developmental days from larvae to adult at the 2:2 and 4:0 *D. suzukii* density (Table 5). A Slice Test showed that the longest total development time was seen in the Petit Manseng at the 2:2 competition level while Cabernet Franc had the longest total development time in the 4:0 *D. suzukii* alone controls (Fig. 2B). Total development was significantly different for the 2:2 density, but not at the 4:0 *D. suzukii* density (Table 6). The grape variety contribution to the significant interaction appears to arise from greater varietal variation at the 2:2 competition level relative to the 4:0 *D. suzukii* intraspecific control (Fig. 2B). Total developmental time was significantly different for Cabernet Franc and Petit Manseng, with total development taking longer in these varieties than Petit Verdot or Viognier (Table 6). The significant interaction effects for total developmental time did not affect the overall conclusions of the analysis and were due to variations among replicates of a grape variety.

Pupal volumes were affected by the grape variety, but not the competition level at the 2:2 and 4:0 *D. suzukii* alone competition levels (Table 5). Pupal volumes were smallest when larvae were reared on the Viognier grapes, at both competition levels (Fig 2C). There were no significant interactions between competition level and grape variety on the volume of the pupae (Table 5).

Interspecific Larval Competition Within Viognier Grapes 2016

The mixed model ANOVA demonstrated that larval development time was neither affected at the 2:3 competition level (P = 0.78) nor 3:2 competition level (P = 0.61) relative to the 5:0 *D. suzukii* alone controls. Larval development time was not affected at the 2:2 competition level compared with the 4:0 *D. suzukii* alone controls (P = 0.94).

Total development was affected neither at the 2:3 competition level (P = 0.08) nor 3:2 competition level (P = 0.52) relative to the 5:0 *D. suzukii* alone controls. Total development time was not affected at the 2:2 competition level and 4:0 *D. suzukii* alone controls (P = 0.48).

Pupal volume was not significantly affected at the 2:3 competition level (P = 0.49), or the 3:2 competition level (P = 0.77), relative to the 5:0 *D. suzukii* alone controls. Pupal volume was also not

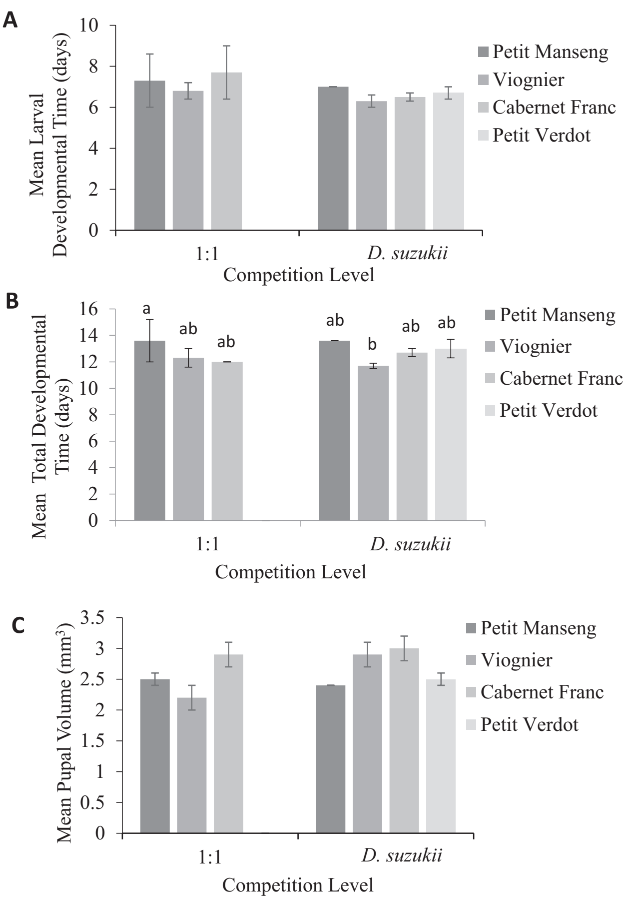

Fig. 1. Mean (± SE) (A) larval development time, (B) total development time, and (C) pupal volume (mm³) of *Drosophila suzukii* on four wine grape varieties. Means sharing the same letter are not significantly different. The number of larvae of each species on a single grape: 1:1 = 1 *D. suzukii* / 1 *Z. indianus* larvae.

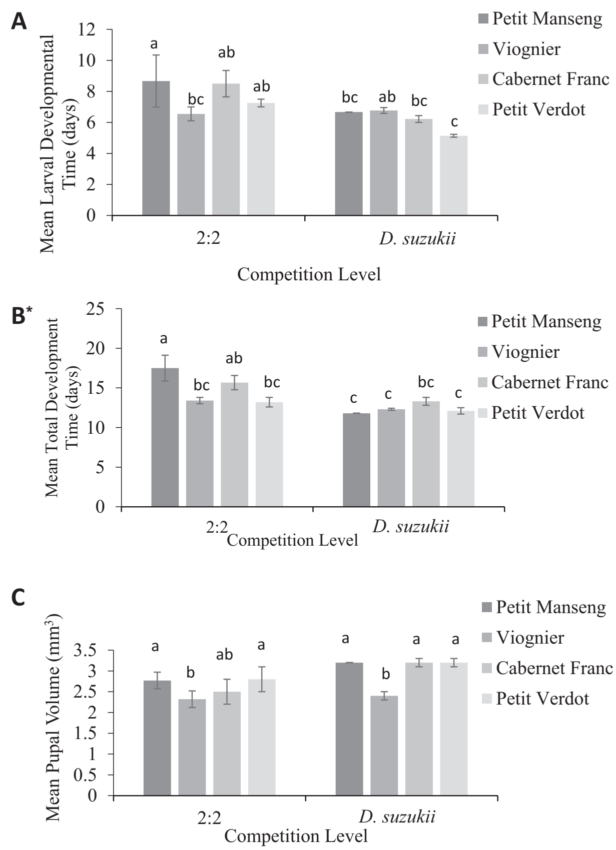

Fig. 2. Mean (± SE) (A) larval development time, (B) total development time, and (C) pupal volume (mm³) of *Drosophila suzukii* on four wine grape varieties. Means sharing the same letter are not significantly different. *Indicates interactions of competition level and grape variety. The number of larvae of each species on a single grape: 2:2 = 2 *Drosophila suzukii* / 2 *Zaprionus indianus* larvae.

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Table 6. SliceTest analysis for simple effects on mean larval (A) and total (B) developmental days for 4 *Drosophila suzukii* and 2:2 (*Drosophila suzukii*: *Zaprionus indianus*) competition level

		Grape Variety			Competition Level		
		Petit Manseng	Viognier	Cabernet Franc	Petit Verdot	4 *D. suzukii*	2:2
A	*F*	7.3569	0.6813	3.0988	11.7951	5.5724	4.3603
	P	0.0072	NSD	NSD	0.0007	0.001	0.0053
	df	1, 216	1, 216	1, 216	1, 216	3, 216	3, 216
B	*F*	60.2386	3.8238	8.0129	0.9344	2.0133	11.4206
	P	<0.0001	NSD	0.0057	NSD	NSD	<0.0001
	df	1, 216	1, 216	1, 216	1, 216	3, 216	3, 216

significantly affected by larval competition at the 2:2 competition level compared with the 4:0 *D. suzukii* alone controls ($P = 0.25$).

Discussion

These experiments showed that interspecific larval competition between *D. suzukii* and *Z. indianus* impacted not only survivorship but also developmental parameters. However, grape varietal differences may play a larger role in *D. suzukii* survivorship than competition levels. *Drosophila suzukii* larval survivorship to pupariation was not affected by *Z. indianus* in commercial medium or Viognier grapes. *Drosophila suzukii* larval survivorship to adulthood was significantly reduced in the presence of *Z. indianus* in Petit Manseng, Petit Verdot, and Cabernet Franc for all interspecific densities tested compared with the intraspecific *D. suzukii* controls. Varietal differences in survivorship could have resulted from nutritional factors, grape mass (g), or a combination of both which may have been limiting components in certain grape varieties. Physical interactions as well as metabolic wastes or allelochemicals produced by *Z. indianus* may have also played a role in *D. suzukii* larval survivorship. The interspecific competition impacts on survivorship and developmental time become more pronounced as the level of interspecific larval competition density increased. Our results of decreased adult emergence are similar to those seen when the rate of emergence of *D. suzukii* was significantly lower from medium preinoculated by *D. melanogaster* than from a fresh blank medium (Shaw et al. 2018).

This study demonstrated that *D. suzukii* larvae at the 3:2 *(D.s.: Z.i.)* interspecific competition level had 20% survival rate to adulthood, while the 2:3 *(D.s.: Z.i.)* ratio was 15% compared with the intraspecific control treatments of 5:0 *D. suzukii* larvae at 37%. This is similar to the competition results seen with *D. suzukii* reared in the presence of *D. melanogaster*, the number of *D. suzukii* offspring in both pairwise and cage experiments was dramatically decreased when the two species were together compared with cages without this competitor (Dancau et al. 2016). Survivorship of larvae to adults was impacted by the ratio of *D. suzukii* to *Z. indianus* with the higher competition densities experiencing greater mortality.

Survivorship to pupariation as well as to adulthood could have been limited by the diet quality in which larvae developed. The commercial medium study demonstrated that even at the interspecific competition level of 4:4 and intraspecific competition of 8:0 *D. suzukii* there was no significant difference of eggs surviving to adulthood. The medium cube weighed 0.38 g, but the commercial diet had been specifically formulated to maximize the development and survival of *Drosophila* larvae. *Drosophila suzukii* can overcome intraspecific competition if the dietary resource provides enough protein to support larval development (Hardin et al. 2015). Despite their larger size grapes (1.2–1.9 g), they were considered a poor-quality host. Grapes are carbohydrate rich and protein poor, a poor nutritional environment for *Drosophila* larvae (Bellamy et al. 2013). Furthermore, grape variety was a main effect when assessing survivorship of larva to pupa as well as larva to adult. The smaller-fruited grape varieties, Petit Verdot, Petit Manseng, and Cabernet Franc had significantly lower survivorship than the larger-fruited Viognier at the higher interspecific competition level. These varietal differences became very apparent when assessing the odds ratio test for survivorship to adulthood. Larvae reared in the Viognier grapes had a significantly greater chance of surviving to adulthood than in any other grape variety. However, the differences in survivorship were less pronounced when comparing larvae to pupariation and larvae to adulthood at the lower interspecific competition level. Differences in survivorship of *Drosophila* from larvae to adults in different varieties of cacti were demonstrated by Werenkraut et al. (2008), in which both interspecific densities of larvae as well as cactus variety influenced survivorship of larvae to adults.

The increased survival rate for *D. suzukii* in Viognier grapes, even while competing with *Z. indianus*, compared with larvae reared in other grape varieties tested was confirmed in the 2016 study. In our study, the survivorship of larvae to pupae and larvae to adult at the interspecific competition level of 2:2 was not statistically different from the 4 *D. suzukii* controls. Furthermore, larval survivorship to adulthood at these levels of interspecific competition did not appear to be influenced by metabolic wastes given that the food available appeared to be substantial enough to allow 4 *Drosophila* to survive to adults. Assuming, the metabolic waste of *D. suzukii* is equally detrimental as that of *Z. indianus*, mortality should have increased at the 4 *Drosophila* density. Had metabolic waste influenced survivorship, the viability of larvae to adults would have decreased even when food was in excess. The increase in density within a medium can cause a loss of nutrient quality through metabolic residue contamination (uric acid and CO_2) during larval development (Ohba 1961, Scheiring et al. 1984). This provides further proof that survivorship of larvae to pupae and larvae to adults is influenced by food availability and interspecific competition levels and not metabolic wastes produced by *Z. indianus*. The larval survival rate to pupariation was not affected at the interspecific competition levels of 2:3 and 3:2 *D. suzukii* and *Z. indianus* compared with the intraspecific control of 5 *D. suzukii*. However, larval survival to adulthood was affected at these densities and more so in the 2:3 (15%) interspecific competition level compared with the 3:2 (20%) competition level. The decreased survivorship seen when *Z. indianus* outnumbered *D. suzukii* may have been influenced by exclusion competition in which the *Z. indianus* larvae excluded *D. suzukii* larvae from feeding by physically using their bodies to push the competing larvae away from the food source. *Zaprionus* spp. has been described as being competitive in food medium by drowning other larvae in the medium.

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Larval development time to pupariation and total development time to adulthood increased as the level of interspecific competition increased. Larval development time to pupation increased 1 d on average for *D. suzukii* at the 1:1 interspecific level and by an average increase of 2 d for *D. suzukii* larvae at the 2:2 level compared with the intraspecific controls. Total development time to adults also increased based upon the level of interspecific competition. Varietal differences were also seen in larval to adult development time, with the largest increase seen in Petit Manseng. Larvae had to feed for prolonged periods to acquire enough nutrients through increased food consumption in poor nutrient environments. Hardin et al. (2015) showed that *D. suzukii* will increase development time to consume enough nutrients to reach pupariation in a poor nutrient environment and that development time was also influenced by density with the highest densities having the longest development times. Smaller grapes may contain less nutrients, which might explain why *D. suzukii* reared in competition within smaller varieties had longer larval development times to pupariation. The increased development time as a result of increased competition is seen in *D. melanogaster*. In order to overcome competition pressure, *D. melanogaster* showed prolonged or arrested larval development at high interspecific competition levels (Miller 1964). Larvae developing in the commercial medium showed an increase in development time at the highest densities, which may have been due to a decrease in diet quality (Ohba 1961).

The interactions seen between diet quality manifested by morphological variances in grape variety and the levels of interspecific drosophilid competition raise several important considerations for ecological *Drosophila* population interactions, varietal selection, and pest management in Virginia vineyards. Our study indicated that *D. suzukii* have a greater chance of surviving to the adult stage if high densities of interspecific competition can be avoided in grapes. However, *Z. indianus* could potentially use *D. suzukii* oviposition sites to lay their own eggs creating a co-infestation within the grapes (Pfeiffer et al. 2019). This co-infestation could decrease the survival rates of *D. suzukii* larvae as seen in our studies at high densities. Individual female *D. suzukii* lay a few eggs per fruit with a total lifetime production estimated at 380 eggs (Kanzawa 1939, Mitsui et al. 2006). However, *Z. indianus* is capable of laying large clutches on a single fruit which would impact *D. suzukii* development in the grape. It is likely that this decrease in survivorship and pupal size of individuals surviving to adulthood could cause *D. suzukii* populations in the vineyard to increase less rapidly or even decline. Surviving females may have lower fecundity as a result of small pupal size. This may be especially important if *Z. indianus* larvae outnumber *D. suzukii* larvae in a grape. The decrease of *D. suzukii* populations within a vineyard could reduce management costs by decreasing spray applications and cluster sorting.

Further studies evaluating the co-infestations of these two invasive drosophilids in the vineyard should be conducted. This would ascertain to what degree these co-infestations are occurring naturally in the vineyard.

Acknowledgments

We thank the Virginia Wine Board for funding this research as well as The Horton Vineyard for the use of their vines. We thank Corey Reidel who helped perform this research, and Chris Bergh, Kim van der Linde, and Jayesh Samtami, who reviewed an earlier draft of this paper. We also thank Carlyle Brewster for his help with the statistical analysis.

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