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Final Report

Ecological interactions between spotted wing drosophila, African fig fly, and associated biological control agents

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Final report for project involving spotted wing drosophila, African fig fly, and associated biological control agents: Main accomplishments.

The objectives of this project were to explore the impact of African fig fly on spotted wing drosophila development, and to explore the ability of native parasitic Hymenoptera to attack both species.

Part 1 - Determine effect of presence of AFF on population growth of SWD.

1A - EFFECTS OF INTERSPECIFIC LARVAL COMPETITION ON DEVELOPMENTAL PARAMETERS IN NUTRIENT SOURCES BETWEEN *DROSOPHILA SUZUKII* (MATSUMURA) (DIPTERA: DROSOPHILIDAE) AND *ZAPRIONUS INDIANUS* GUPTA

Introduction

The insect pest ecology within Virginia vineyards has changed dramatically over the past decade with the introduction of several new invasive species. The latest introductions have been two economically significant drosophilids; spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), and the African fig fly (AFF), *Zaprionus indianus* Gupta. *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 as host plant preference, oviposition ability, overwintering capabilities and reproductive fecundity (Kansawa 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 is currently unknown.

Drosophila larvae within the same food source compete, leading to increased mortality, decreased growth and reduced fecundity as density increases (Bakker 1961). This competition, whether intraspecific or interspecific, can lead to reduced survivorship, increased developmental time and loss of body mass (Joshi and Mueller 1996, Pascual et al. 1998, Pascual et al. 2000, Takahashi and Kimura 2005). This loss of body mass is usually correlated with a reduction in female fecundity and shortened lifespan (Santos et al. 1992, Rodriguez et al. 1999, Werenkraut et al. 2008).

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). Indirect competition may also influence drosophila larval development (Budnik et al. 2001). 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).

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. 2015, 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, personnel 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* (MS unpublished data). It may be possible for *Z. indianus* to use *D. suzukii* oviposition wounds to deposit their own eggs into grapes (Appendix A) and if so, interactions between the larvae of these species within grapes may play a role in the population dynamics of *D. suzukii* in Virginia vineyards. There have been numerous studies investigating the effects of intraspecific competition on developmental parameters of *Drosophila* where one or both species are impacted developmentally (Miller 1964, Gonzalez-Candelas et al. 1990. Vineyards with both fly species present may have a lower risk of *D. suzukii* population growth due to possible interactions of *Z. indianus* on *D suzukii*. However, laboratory experiments may not be representative of actual field conditions. 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. 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. Clusters were collected, ice-cooled, and transported to Blacksburg for laboratory testing. 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 light regimen. 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 in their respective containers, 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; 2mm diameter, 15.2cm 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 (SWD: AFF) were 2:2 and 4:4. Intraspecific controls for 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 (White Cloud, Bentonville, AR) and held in a growth chamber at 23° C, 50-80% RH, and a 16:8, L:D light regimen.

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 days of collection to ensure fruit freshness. Grapes were held in a refrigerator (< 4.5 ºC) until needed. Petit Verdot grapes were randomly removed from three grape clusters and inspected under a dissecting microscope to check 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 (SWD:AFF) 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 x 15 mm) (USA Scientific, Orlando FL) that was sealed by wrapping Parafilm around the outside of the two dish halves and held in a growth chamber at 23° C, 50-80% RH and a 16:8 L:D light regimen.

Interspecific Larval Competition Utilizing Four Wine Grape Varieties 2015. Viognier, Petit Manseng, Petit Verdot and Cabernet Franc grape clusters were collected on 16 and 30 August and 9 and 16 September and all experiments were conducted within 10 days after collection. Grapes were held in a refrigerator (< 4.5 ºC) until needed. Four larval densities on each wine grape variety were compared. The larval densities evaluated for interspecific competition (SWD: AFF) 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. For each repetition of this experiment, ºBrix were measured using a handheld temperature-compensated refractometer (Zoro, Buffalo Grove, IL). A 20 g sample of grapes from each variety was pressed and the juice was

placed onto the refractometer and the ºBrix were recorded. Individual grapes were randomly selected for each wine grape variety and inspected under a dissecting microscope to check for *D. suzukii* eggs or wounds and grapes with eggs or wounds were not used. The grapes had been pulled from the cluster and the wound where the grape had been attached to the pedicle 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 (60 x 15 mm) (USA Scientific, Orlando FL) that were sealed by wrapping Parafilm around the outside of the two dish halves and held in a growth chamber at 23°C, 50-80% RH and a 16:8 L:D light regimen.

Interspecific Larval Competition In Viognier Grapes 2016. Grapes were collected on 24 August and 7 and 16 October. Grapes were used within 10 days of collection and were held in a refrigerator (< 4.5 ºC) until needed. The larval densities evaluated for interspecific competition (SWD: AFF) 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 for each of the bioassay experiments to determine if the 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 Chi-Square 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 because interpretation of a binary nominal logistic regression coefficient (β) is not as straightforward as a linear coefficient (e^{β}). 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 Chi-Square analysis. Varietal differences based upon weight (g) were analyzed via a one-way ANOVA. Data reported for larval development time, total development time, and pupal volume during all experimental years are only representative of individuals that survived to adulthood. These parameters were analyzed using a mixed-model ANOVA with egg or larval competition level and grape variety as fixed effects and dish number within experimental date as random effects (via JMP 12). A Tukey's HSD was used to separate the means and were considered

significant at $P < 0.05$. When interactions were significant ($P < 0.05$) a Slice Test was performed to look at the 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 SWD:AFF larval density had a greater likelihood of surviving to adulthood than the 4 *D. suzukii* intraspecific control (Prob > $Chi^2 = 0.0234$). The 2:2 density had 70% of the *D. suzukii* adults emerge verses only 45% from the 4 *D. suzukii* controls. The Chi^2 analysis for the *D. suzukii* eggs surviving to adulthood in the commercial medium study indicated 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 > $Chi^2 = 0.0820$). No *D. suzukii* individuals survived in the Petit Verdot grapes at the 8:8 competition level and only 2 *D. suzukii* adults emerged from the 16 *D. suzukii* alone controls, so no statistical analysis on survivorship could be performed.

Interspecific Larval Competition Utilizing Four Wine Grape Varieties 2015. Grape weight (g) differed significantly between varieties ($F= 24.3351$, $df = 3$, $P < 0.001$). Viognier (1.9g,) was significantly heavier than Cabernet Franc (1.5g). Cabernet Franc and Petit Verdot (1.31g) were similar in weight, and Petit Verdot and Petit Manseng (1.2g) were similar.

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 as indicated by the whole model test (Table 1). The percentage of *D. suzukii* larvae surviving to pupate was significantly greater in the 2 *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 binary nominal logistic regression for the 1:1 competition level and the 2 *D. suzukii* alone controls showed a significant relationship between competition level and grape variety on larvae surviving to adults as well as an interaction of competition level and grape variety (Table 1). The two main effects, competition level and grape variety, contributed significantly to the survival of *D. suzukii* larvae to adults. 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), for the 1:1 and 2 *D. suzukii* alone competition level indicated that the larvae in the 2 *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 to any other variety (Table 2). Conversely, there was increased mortality of *D. suzukii* larvae if they were reared in Petit Verdot grapes. 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 *D. suzukii* alone controls. The binary nominal logistic regression and showed the percent of *D. suzukii* larvae surviving to pupariation was significantly higher in the 4 *D. suzukii* alone control with a survival rate of 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 compared to any other grape variety (Table 2). There was an increase in mortality for *D. suzukii* reared in Petit Manseng rather than any other variety. *Drosophila suzukii* larvae also had a greater chance of survival to pupariation if reared in Cabernet Franc instead of Petit Verdot (Table 4).

The binary nominal 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 *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 *D. suzukii* alone competition level indicated that *D. suzukii* larvae had a greater chance of surviving to adulthood when reared without *Z. indianus* (Table 5). The odds ratio for the four varieties of grapes demonstrated that *D. suzukii* had a greater likelihood of surviving to adulthood when reared in Viognier grapes (Table 5). Grapes reared in Cabernet Franc had increased mortality compared to larvae reared in any other grape variety. *Drosophila suzukii* larvae reared in Petit Manseng had a greater chance of surviving to adulthood than larvae reared in Petit Verdot (Table 5).

Interspecific Larval Competition In Viognier Grapes 2016. The Chi^2 analysis showed no significant difference for the larvae surviving to either pupae or adults in the Viognier grapes at the 2:2 competition level and 4 *D. suzukii* alone controls. The Chi^2 analysis showed 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 to the 5 *D. suzukii* alone controls. However, the Chi^2 analysis for the *D. suzukii* larvae surviving to adulthood in the Viognier grapes at the 3:2 (20%) ($Prob>Chi^2 = 0.0050$) and 2:3 (15%) ($Prob>Chi^2 = 0.0077$) competition levels were significantly lower than the 5 *D. suzukii* (37%) alone controls. There was no statistical difference between the 2:3 and the 3:2 density for survivorship from larvae to pupae ($Prob>Chi^2 = 1.0$) or larvae to adults ($Prob>Chi^2 = 0.8232$).

Development:

Interspecific Larval Competition Using Commercial Medium 2014. Developmental time from egg to pupariation was not recorded for this year. The mixed model ANOVA demonstrated that total developmental time from egg to adult was only marginally affected by competition on the commercial medium cube at the 2:2 SWD:AFF density compared to the 4 *D. suzukii* alone ($P= 0.0769$). The developmental time from egg to adult at the 2:2 density was 11.1 days while the 4 *D. suzukii* density was 10.9 days. The mixed model ANOVA demonstrated that 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 to the 8 *D. suzukii* alone control (F= 37.8095, df= 1, P < 0.0001). The developmental time from egg to adult at the 4:4 competition level was 11.16 days while the 4 *D. suzukii* alone control was 10.3 days. Pupal volume was only marginally affected by larval competition level with pupal volume measuring 3.54 mm^3 at the 2:2 competition level and 3.77 mm^3 for the 4 *D. suzukii* alone control (P = 0.0917). Pupal volume was not significantly affected by larval competition level with pupal volume measuring 3.8 mm^3 at the 4:4 competition level and 3.7 mm^3 for the 8 *D. suzukii* alone control (P= 0.3068).

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). Even though no statistical analysis can be done for the larvae in Petit Verdot at the 1:1 competition level, 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 *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 6, 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 7). There was also a significant interaction between competition level and grape variety on larval developmental days (Table 7). Due to the interactions of grape variety and competition level on larval development time, a Tukey-Kramer HSD was used to separate the means for each density evaluated and a Slice Test was performed to look at the simple effects. Larval development time was longer when *D. suzukii* was in competition with *Z. indianus* at the 2:2 density. The Slice Test for larval development was significantly different for both the 2:2 density and the 4 *D. suzukii* density (Table 8). Larval developmental time was longest in Viognier at the 2:2 competition level, but was only significantly different when compared to Petit Manseng (Fig. 2A). The shortest larval developmental time was seen in the Petit Verdot grapes at the 4 *D. suzukii* alone competition level (Fig. 2A). The Slice Test for larval development

was significantly different for Petit Manseng and Petit Verdot, but not Viognier or Cabernet Franc (Table 8). The significant interaction effects for larval developmental 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 *D. suzukii* competition levels was significantly impacted by grape variety (Table 4) with no adults emerging from the Petit Verdot (Fig. 1B). There was no effect of competition level on total development, nor was there an interaction of grape variety and competition level (Table 6). Total development time from larvae to adult for the 2:2 competition level and 4 *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 *D. suzukii* density (Table 7). Due to the interactions of grape variety and competition level on total development time a Tukey-Kramer HSD was used to separate the means and a Slice Test was preformed to look at the simple effects. 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 *D. suzukii* alone controls (Fig. 2B). The Slice Test for total development was significantly different for the 2:2 density, but not at the 4 *D. suzukii* density (Table 8). 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 *D. suzukii* intraspecific control (Fig. 2B). The Slice Test for total developmental time was significantly different for Cabernet Franc and Petit Manseng, whit total development taking longer in these varieties than Petit Verdot or Viognier (Table 9). 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, with larval density effecting total development time the greatest.

Pupal volume at the 1:1 and 2 *D. suzukii* competition level was marginally affected by competition level, but not affected by grape variety (Fig. 1C), nor was there an interaction of competition level and grape variety (Table 6). Pupal volumes were affected by the grape variety, but not the competition level at the 2:2 and 4 *D. suzukii*

alone competition levels (Table 7). 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 7).

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= 0.7781) nor 3:2 (P= 0.6138) competition level relative to the 5 *D. suzukii* alone controls (Fig. 3). Larval development time was not affected at the 2:2 competition level compared to the 4 *D. suzukii* alone controls (P= 0.9423) (Fig. 4).

Total development was affected neither at the 2:3 competition level (P= 0.0844) nor 3:2 (P= 0.5167) competition level relative to the 5 *D. suzukii* alone controls (Fig. 3). Total development time was not affected at the 2:2 competition level and 4 *D. suzukii* alone controls (P= 0.4804) (Fig. 4).

Pupal volume was not significantly affected at the 2:3 competition level (P = 0.4861), or the 3:2 competition level (P = 0.7651), relative to the 5 *D. suzukii* alone controls (Fig. 3). Pupal volume was also not significantly affected by larval competition at the 2:2 competition level compared to the 4 *D. suzukii* alone controls (P = 0.2501) (Fig. 4).

Discussion

These experiments showed that interspecific larval competition between *D. suzukii* and *Z. indianus* impacted not only survivorship but also developmental parameters. Our study also demonstrated that grape varietal differences also played a role in *D. suzukii* survivorship. *D. suzukii* larval survivorship to pupariation was not affected by *Z. indianus* in commercial medium or Viognier grapes. *D. 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 to 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.

Survivorship of larvae to adults was impacted by the ratio of *D. suzukii* to *Z. indianus* with the higher competition densities experiencing greater mortality. If *D. suzukii* were outnumbered by *Z. indianus*, mortality of the *D. suzukii* was more pronounced than if the *D. suzukii* outnumbered *Z. indianus*. This study demonstrated that *D. suzukii* larvae at the 3:2 (SWD: AFF) interspecific competition level had 20% survival rate to adulthood, while the 2:3 (SWD: AFF) ratio was 15% compared to the intraspecific control treatments of 5 *D. suzukii* larvae at 37%. This further demonstrated the impact of *Z. indianus* competition pressure on *D. suzukii* survival.

Survivorship to pupariation as well as to adulthood could have been limited by the diet quality in which the larvae developed. The commercial medium study demonstrated that even at the interspecific competition level of 4:4 and intraspecific competition of 8 *D. suzukii* there was no significant difference of eggs surviving to adulthood. The medium cube weighed only 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). In contrast to the 0.38 g medium cube, the larger grapes weighed between 1.2 g and 1.9 g depending upon variety. Despite their larger size the grapes were considered a poor-quality host. Grapes have been categorized as 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. Survivorship was influenced by both interspecific competition levels and host plant variety. 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* larvae to pupae and larvae to adults reared in Viognier grapes, even while competing with *Z. indianus*, compared to 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 to 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 to 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 (Gilpin 1974).

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 day on average for *D. suzukii* at the 1:1 interspecific level and by an average increase of 2 days for *D. suzukii* larvae at the 2:2 level compared to 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. Increased development time to pupariation or adulthood have been shown to be influenced by diet quality. 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 smaller varieties had longer larval development times to pupariation. Conversely, there was no difference in development time seen in Viognier grapes across all years and interspecific larval densities. The increased development time as a result of increased competition is seen in *Drosophila 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).

Pupal volumes decreased for pupae that developed in competition with *Z. indianus* based upon grape variety for both 1:1 and 2:2 levels of interspecific competition. Pupal volumes were lower at the higher competition levels (2:2) compared to their intraspecific controls, although the decrease in size was not always statistically significant. This decrease in pupal volume is similar to previous studies in which *Drosophila* in competition at high densities produced smaller pupae. Takahashi and Kimura (2005) demonstrated that *D. suzukii* had decreased pupal volumes and decreased fecundity when reared in interspecific competition assays. Interspecific competition at high densities decreased pupal volume in *D. subobscura*, resulting in females with fewer eggs in their

ovaries (Jones et al. 1996). Pupal volume was not influenced when larvae were reared in Viognier grapes in 2016 for all competition levels. This further demonstrated the Viognier grape suitability as a host of *D. suzukii* over the other grape varieties tested.

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 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 (Appendix A). This co-infestation could decrease the survival rates of *D. suzukii* larvae as seen in our studies. Individual female *D. suzukii* lay a few eggs per fruit with a total lifetime production estimated at 380 eggs (Kansawa 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 (Appendix A). It is likely that this decrease in survivorship and decreased pupal size of individuals surviving to adulthood could cause *D. suzukii* populations in the vineyard to increase less rapidly or even decline and for those few individuals able to emerge, 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.

Viognier is a variety that is in high demand and produced nearly 1,000 tons of grapes in Virginia in 2014 (Wolf 2014). Thus, this variety should be more intensely scouted for *D. suzukii* and sprays applied regularly when grapes are ripening to keep fly populations low. Conversely, varieties that produce smaller grapes could be managed less intensely due to the higher mortality of *D. suzukii* larvae in these varieties which is compounded when co-infested with *Z. indianus*. 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.

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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, d.f.	L-R ChiSquare	Prob>ChiSq
Larval Survivorship			
Grape Variety	3,3	5.53	0.3378
Competition Level	1,1	5.24	0.0020
Grape Variety*Competition Level	3,3	5.54	0.1571
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*)	0.0180456	-1.74363
1:1 (*D. suzukii / Z. indianus*)	2 *D. suzukii* alone	55.415048	1.743628
Grape Variety			
Petit Manseng	Cabernet Franc	0.9456109	-0.02429
Petit Verdot	Cabernet Franc	4097.5935	3.612529
Viognier	Cabernet Franc	0.4970674	-0.30358
Petit Verdot	Petit Manseng	4333.2767	3.636816
Cabernet Franc	Petit Manseng	1.0575175	0.024288
Viognier	Petit Manseng	0.5256575	-0.2793
Viognier	Petit Verdot	0.0001213	-3.91614
Cabernet Franc	Petit Verdot	0.000244	-3.61261
Petit Manseng	Petit Verdot	0.0002308	-3.63676
Cabernet Franc	Viognier	2.0117995	0.303585
Petit Manseng	Viognier	1.9023795	0.279297
Petit Verdot	Viognier	8243.5366	3.916114

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, d.f.	L-R ChiSquare	Prob>ChiSq
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	0.9711
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	0.5857

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.

Variables		**Odds Ratio (e^{β})**	**β**
Competition Level			
4 *D. suzukii* alone	2:2 (*D. suzukii / Z. indianus*)	0.5998799	-0.2219357
2:2 (*D. suzukii / Z. indianus*)	4 *D. suzukii* alone	1.6670004	0.2219357
Grape Variety			
Petit Manseng	Cabernet Franc	1.7056201	0.23188231
Petit Verdot	Cabernet Franc	1.2610817	0.10074322
Viognier	Cabernet Franc	0.7375573	-0.1322042
Petit Verdot	Petit Manseng	0.7393685	-0.1311391
Cabernet Franc	Petit Manseng	0.586297	-0.2318823
Viognier	Petit Manseng	0.4324277	-0.3640865
Viognier	Petit Verdot	0.5848608	-0.2329475
Cabernet Franc	Petit Verdot	0.79297	-0.1007432
Petit Manseng	Petit Verdot	1.3525056	0.13113907
Cabernet Franc	Viognier	1.3558268	0.13220421
Petit Manseng	Viognier	2.3125255	0.36408653
Petit Verdot	Viognier	1.7098085	0.23294747

Table 5. 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 adulthood.

Variables		**Odds Ratio (e^{β})**	**β**
Competition Level			
4 *D. suzukii* alone	2:2 (*D. suzukii / Z. indianus*)	0.7645513	-0.1165934
2:2 (*D. suzukii / Z. indianus*)	4 *D. suzukii* alone	1.3079566	0.1165933
Grape Variety			
Petit Manseng	Cabernet Franc	0.6607721	-0.1799483
Petit Verdot	Cabernet Franc	0.8591821	-0.0659148
Viognier	Cabernet Franc	0.305315	-0.5152519
Petit Verdot	Petit Manseng	1.30027	0.1140335
Cabernet Franc	Petit Manseng	1.5133812	0.1799483
Viognier	Petit Manseng	0.4620579	-0.3353036
Viognier	Petit Verdot	0.3553554	-0.4493371
Cabernet Franc	Petit Verdot	1.1638976	0.0659148
Petit Manseng	Petit Verdot	0.769071	-0.1140336
Cabernet Franc	Viognier	3.2753062	0.5152519
Petit Manseng	Viognier	2.1642308	0.3353036
Petit Verdot	Viognier	2.8140844	0.4493371

Table 6. Summary outputs of full factorial mixed model ANOVA for 2 *Drosophila suzukii* and 1:1 (*Drosophila suzukii*: *Zaprionus indianus*) competition level.

Effect	d.f.	F	P
Larval Development Time*			
Grape Variety	2,66.8	0.835	0.4383
Competition Level	1,66.9	1.135	0.2904
Grape Variety*Competition Level	2,66.8	0.075	0.9280
Total Development Time*			
Grape Variety	2,43.8	3.31	0.0455
Competition Level	1,43.8	0.008	0.9288
Grape Variety*Competition Level	2,43.8	0.45	0.6402
Pupal Volume*			
Grape Variety	2,52.1	3.12	0.0526
Competition Level	1,52.1	0.007	0.9336
Grape Variety*Competition Level	2,52.1	1.77	0.1799

*Statistical analysis conducted without Petit Verdot due to lack of data

Table 7. Summary outputs of full factorial mixed model ANOVA for 4 *Drosophila suzukii* and 2:2 (*Drosophila suzukii*: *Zaprionus indianus*) competition level.

Effect	d.f.	F	P
Larval Development Time			
Grape Variety	3, 132.2	4.03	0.0088
Competition Level	1, 132.1	9.63	0.0023
Grape Variety*Competition Level	3, 132.1	3.31	0.0222
Total Development Time			
Grape Variety	3, 63.1	6.867	0.004
Competition Level	1, 63.7	37.497	< 0.0001
Grape Variety*Competition Level	3, 63.7	7.599	0.0002
Pupal Volume			
Grape Variety	3, 74.1	5.4	0.002
Competition Level	1, 74.4	2.41	0.1251
Grape Variety*Competition Level	3, 74.4	0.358	0.783

Table 8. Slice Test analysis for simple effects on mean larval 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
F	7.3569	0.6813	3.0988	11.7951	5.5724	4.3603
P	0.0072	0.4100	0.0798	0.0007	0.001	0.0053
df	1, 216	1, 216	1, 216	1, 216	3, 216	3, 216

Table 9. Slice Test analysis for simple effects on mean total 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
F	60.2386	3.8238	8.0129	0.9344	2.0133	11.4206
P	< 0.0001	0.0536	0.0057	0.3363	0.1176	< 0.0001
df	1, 216	1, 216	1, 216	1, 216	3, 216	3, 216

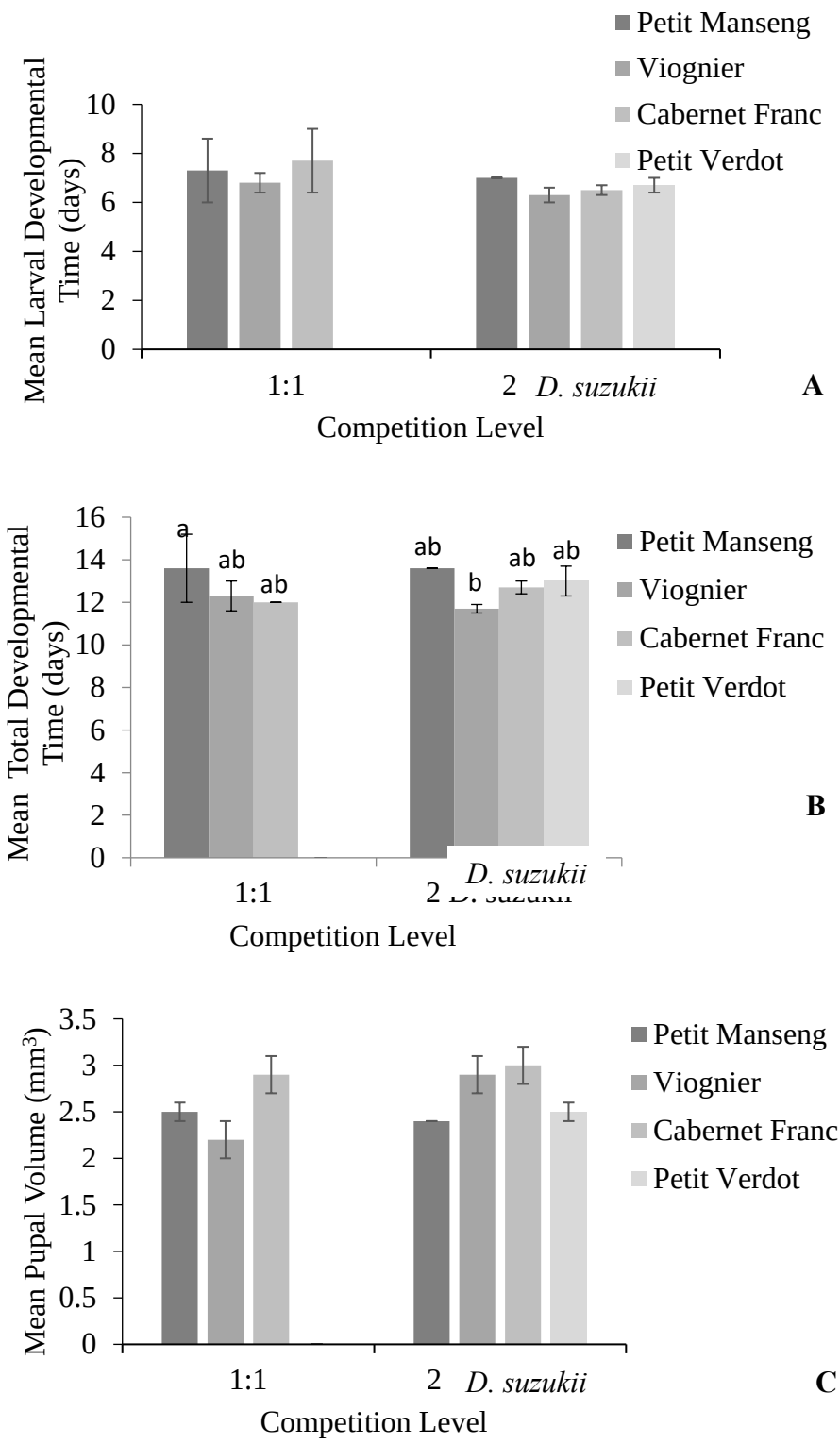

Figure 1. Mean (± SE) (A) larval development time, (B) total development time and (C) pupal volume (mm^3) of *Drosophila suzukii* on four wine grape varieties. Means sharing the same letter are not significantly different. The number of larvae of each species on a single grape: 1:1 = 1 *D. suzukii* / 1 Z. *indianus* larvae.

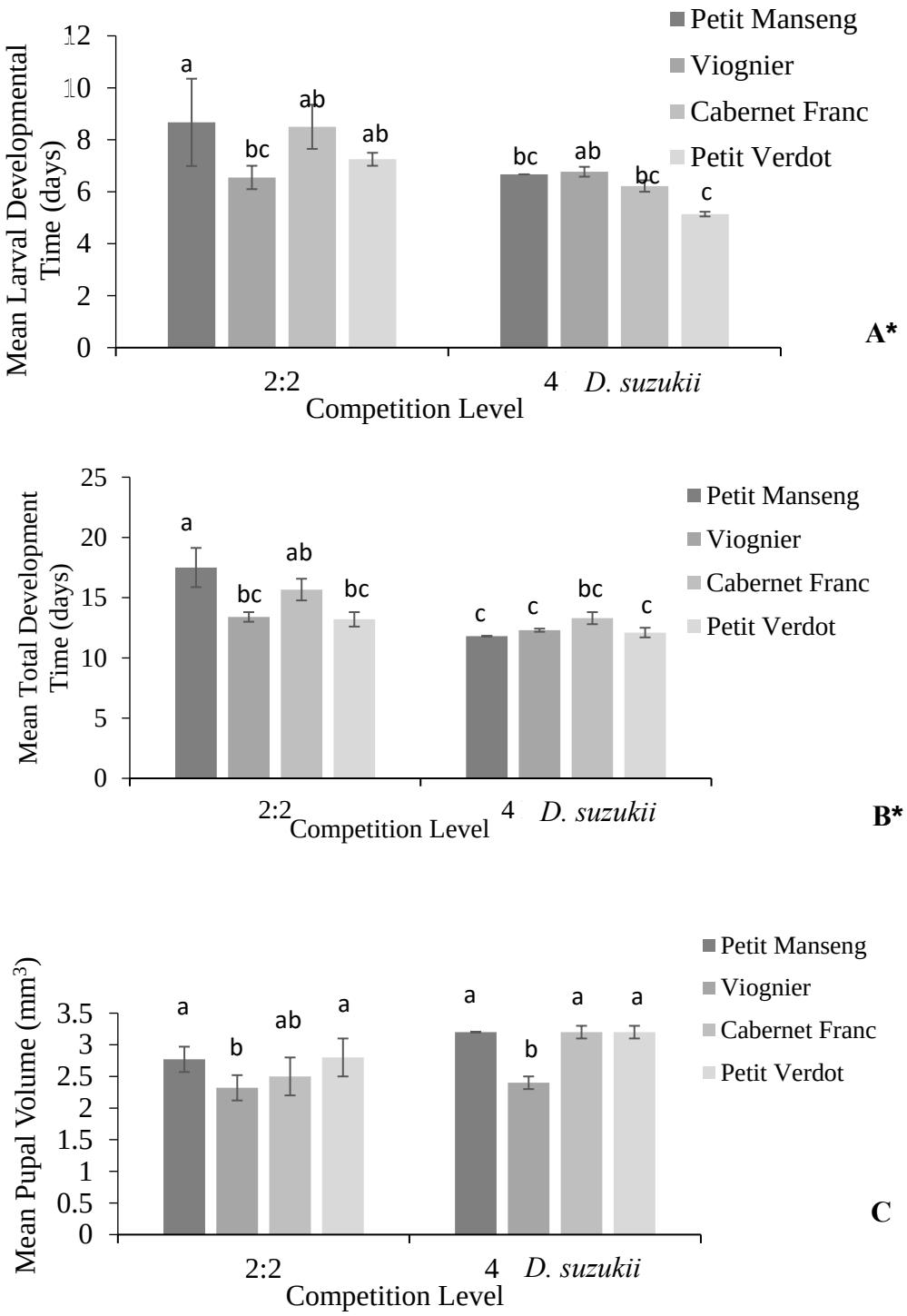

Figure 2. Mean (± SE) (A) larval development time, (B) total development time and (C) pupal volume (mm^3) of *Drosophila suzukii* on four wine grape varieties. Means sharing the same letter are not significantly different. *Indicates interactions of competition level and grape variety. The number of larvae of each species on a single grape: 2:2 = 2 *Drosophila suzukii* / 2 *Zaprionus indianus* larvae.

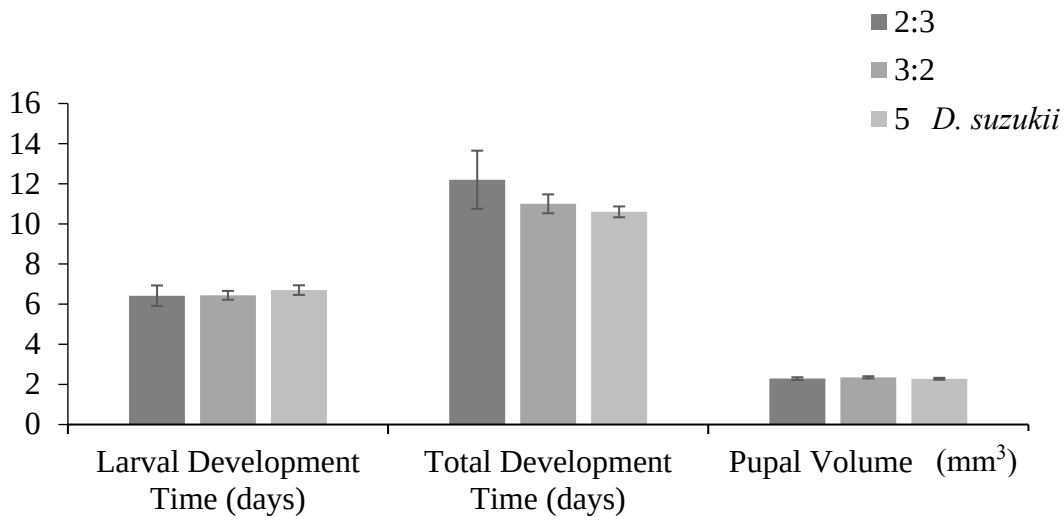

Figure 3. Mean (± SE) larval development time, total development time and pupal volume (mm^3) of *Drosophila suzukii* on Viognier grapes. Means sharing the same letter are not significantly different. Indicates the number of larvae of each species on a single Viognier grape: 2:3 = 2 *Drosophila suzukii* / 3 *Zaprionus indianus* larvae, 3:2 = 3 *Drosophila suzukii* / 2 *Zaprionus indianus* larvae.

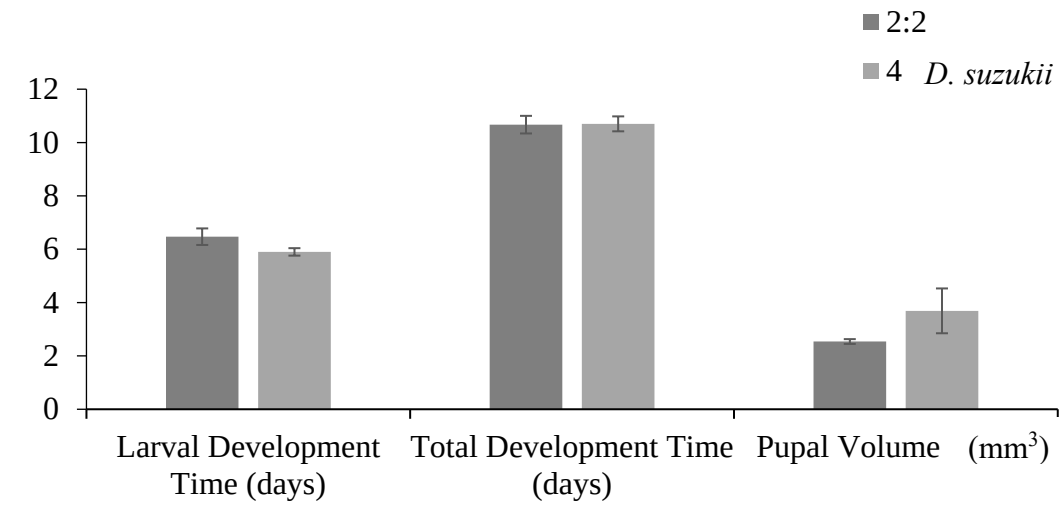

Figure 4. Mean (± SE) larval development time, total development time and pupal volume (mm^3) of *Drosophila suzukii* on Viognier grapes. Means sharing the same letter are not significantly different. Indicates the number of larvae of each species on a single Viognier grape: 2:2 = 2 *Drosophila suzukii* / 2 *Zaprionus indianus*.

1B - OVIPOSITIONAL INTERACTIONS BETWEEN *ZAPRIONUS INDIANUS* AND *DROSOPHILA SUZUKII*

Introduction

This study was performed to complement the results from Chapter 4 and to demonstrate the probability of co-infestations of both *Z. indianus* and *D. suzukii* larvae within wine grapes in Virginia vineyards. In 2012, a wine grower observed *D. suzukii* adults in a Petit Verdot block in Albemarle Co., Virginia (Carrington King personal communication, 2012). *D. suzukii* adults were present in the field, however the majority of adult flies observed were *Z. indianus*. Petit Verdot grapes infested with fly larvae were brought back to the lab and over 80% of the flies reared from those grapes were *Z. indianus*. Due to the inability of *Z. indianus* to oviposit directly into intact grapes it was reasonable to speculate that *Z. indianus* was using *D. suzukii* oviposition punctures to deposit their own eggs into the grapes. It was also reasonable to assume that *Z. indianus* larvae were impacting larval mortality of *D. suzukii* through interspecific competition within the grapes and that was why so few *D. suzukii* adults emerged from the Petit Verdot. To determine if *Z. indianus* can utilize *D. suzukii* ovipositional sites and wounds as a means to deposit their own eggs into grapes a laboratory ovipositional bioassay was conducted.

Material and Methods

Viognier Grape Oviposition 2016. *Drosophila suzukii* oviposition. Viognier grapes were collected from a single vineyard in the Piedmont region of Virginia (Orange Co.). Clusters were collected from the vineyard (22 August) using methodology described in Chapter 3. Grapes were used within a week of collection and were susceptible to *D. suzukii* oviposition based upon penetration force measurements (< 10 cN), skin thickness and titratable soluble sugars were not measured (ºBrix). Three replicates of this experiment were conducted. Three Viognier grapes were cut from a single grape cluster and scrutinized under a dissecting microscope for *D. suzukii* eggs or wounds. If wounds or eggs were present a new grape was selected. Three intact grapes for each replicate were placed into a 355 ml clear plastic rearing cup (Solo, Urbana, IL). Fifteen male and fifteen female *D. suzukii* (0 - 14 days old) were added to the cup. The cups were covered

with plastic (Saran Wrap, Oakland, CA) and placed into a (16:8, L:D) at 23°C, 50 - 80% RH for 48 h. Once the 48 h period was over all *D. suzukii* adults were removed from the container and the grapes were observed under a dissecting microscope to look for oviposition sites and eggs. *Drosophila suzukii* eggs were not counted, but direct oviposition into the grape flesh was observed.

Zaprionus indianus oviposition. Once *D. suzukii* ovipositional sites and eggs had been observed within the grapes, the grapes were placed back in the cups and fifteen male and fifteen female *Z. indianus* (0 - 14 days old) were added. The cups were re-covered with plastic wrap and placed into the growth chamber for 48 h. After the 48 h exposure period the grapes were removed and examined under a dissecting microscope for *Z. indianus* eggs.

Table Grape Oviposition 2017. The same methodology was performed for this experiment as above however, red grapes bought from a grocery store (10 March) were used instead of Viognier grapes. *Drosophila suzukii* are capable of wounding red grapes with their ovipositor, so penetration force was not recorded (Atalla et al. 2014). Grapes were only exposed to *Z. indianus* for 24 h instead of 48 h in order to attempt to observe eggs singularly instead of a large mass on the grapes as seen the previous year. A single replicate containing 3 red grapes were used for this experiment.

Results

In 2016, *D. suzukii* eggs and punctures were seen in the Viognier grapes and on the surface after the 48 h ovipositional period (Fig. 5). *Zaprionus indianus* eggs were observed on all nine Viognier grapes that had *D. suzukii* ovipositional sites or wounds resulting from attempted oviposition. *Zaprionus indianus* eggs were observed as a large mass on Viognier grapes (Fig. 6) and as individual eggs on the grapes. The first replicate had no flies emerge. The second replicate had three *Z. indianus* emerge while the third replicate had 11 *Z. indianus* and two male *D. suzukii* emerge from the three grapes.

In 2017, *D. suzukii* eggs and puncture wounds were observed in the red grapes as well as on the surface of the grape (Fig. 7). *Zaprionus indianus* eggs were also observed sharing the same ovipositional punctures in red grapes as *D. suzukii* eggs (Fig. 8). Six egg filaments can be seen radiating from a single ovipositional hole in the grape.

Drosophila suzukii eggs possess two filaments and *Z. indianus* possess four filaments. When the eggs were dissected from the ovipositional wound two eggs were observed, one from each of *D. suzukii* and *Z. indianus* (Fig. 9). Upon rearing the larvae to adults, a total of eight *Z. indianus* and four *D. suzukii* were present in the rearing cup (Fig. 10).

Discussion

Our study demonstrated that *Z. indianus* can use *D. suzukii* oviposition sites to oviposit their own eggs into grapes that they would not normally be able to penetrate. Drosophilid larval competition may increase or decrease survivorship, developmental time and body mass for one or both species within the nutrient source (Joshi and Mueller 1996, Pascual et al. 1998, Pascual et al. 2000, Budnik et al. 2001, Takahashi and Kimura 2005). The resulting interspecific co-infestation of larvae within a grape, demonstrated in Chapter 4, increased the larval mortality and developmental time of the *D. suzukii* larvae within those grapes in the laboratory. This interspecific larval competition may influence population dynamics of *D. suzukii* in vineyards. *Zaprionus indianus* may be able to impact population growth rates of *D. suzukii* in vineyards that have both species present.

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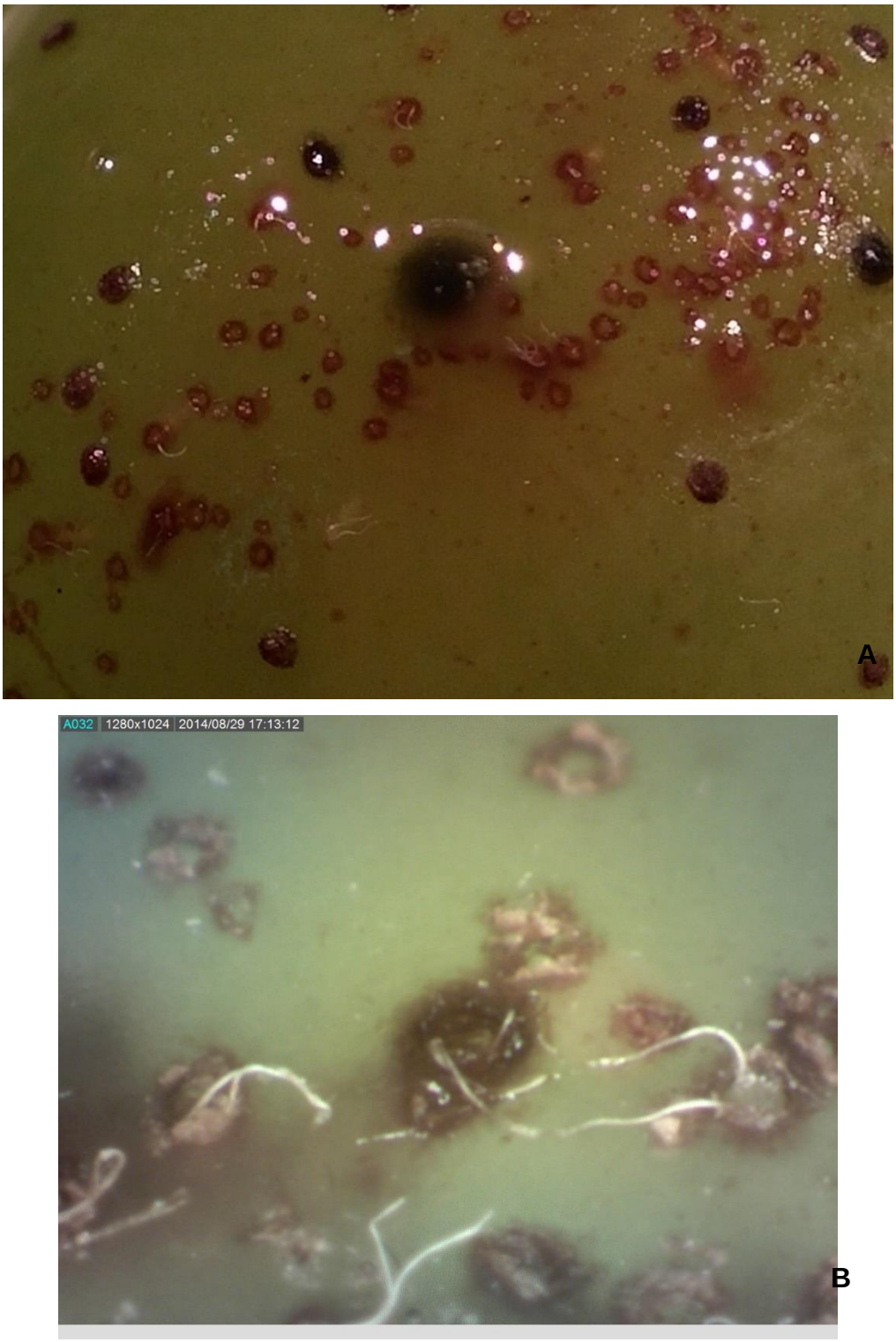

Figure 5. (A) *Drosophila suzukii* eggs and ovipositional punctures on Viognier grapes. (B) *Drosophila suzukii* egg filaments extending from a Viognier grape.

Figure 6. *Zaprionus indianus* eggs laid en masse over *Drosophila suzukii* oviposition punctures with eggs in a Viognier grape.

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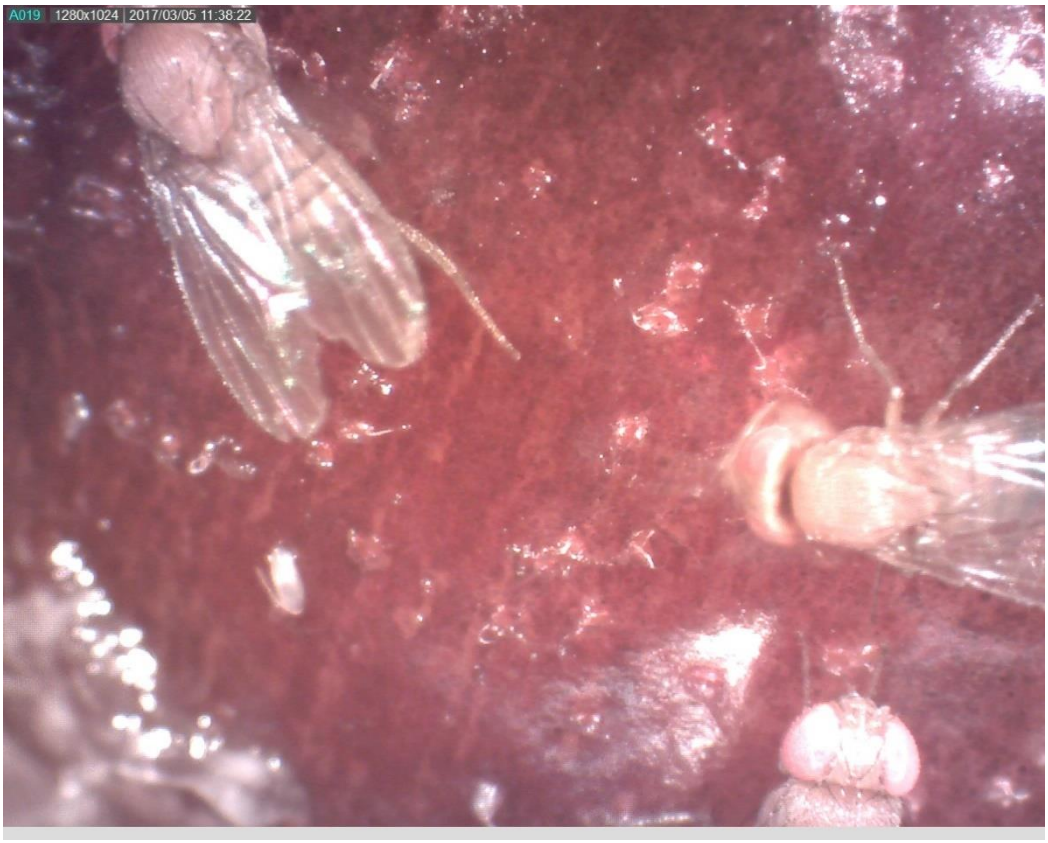

Figure 7. *Drosophila suzukii* females, oviposition punctures and eggs in a red grape.

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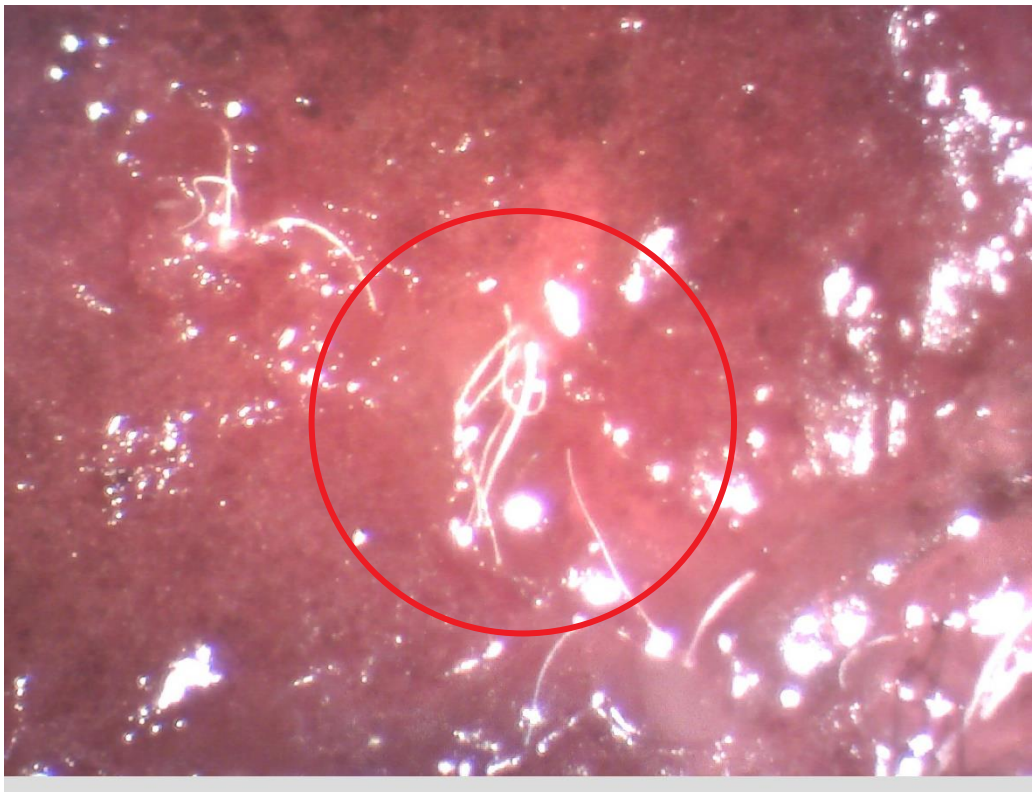

Figure 8. Combined respiratory filaments of a *Zaprionus indianus* and *Drosophila suzukii* egg in a common oviposition puncture in a red grape.

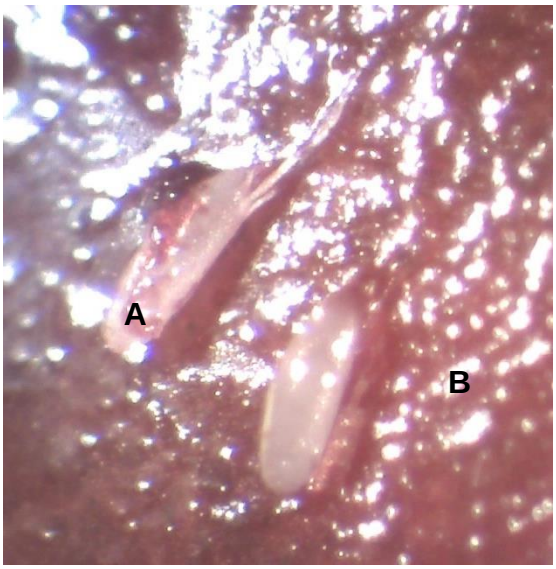

Figure 9. (A) *Zaprionus indianus* and (B) *Drosophila suzukii* eggs dissected out of the single ovipositional wound in red grape.

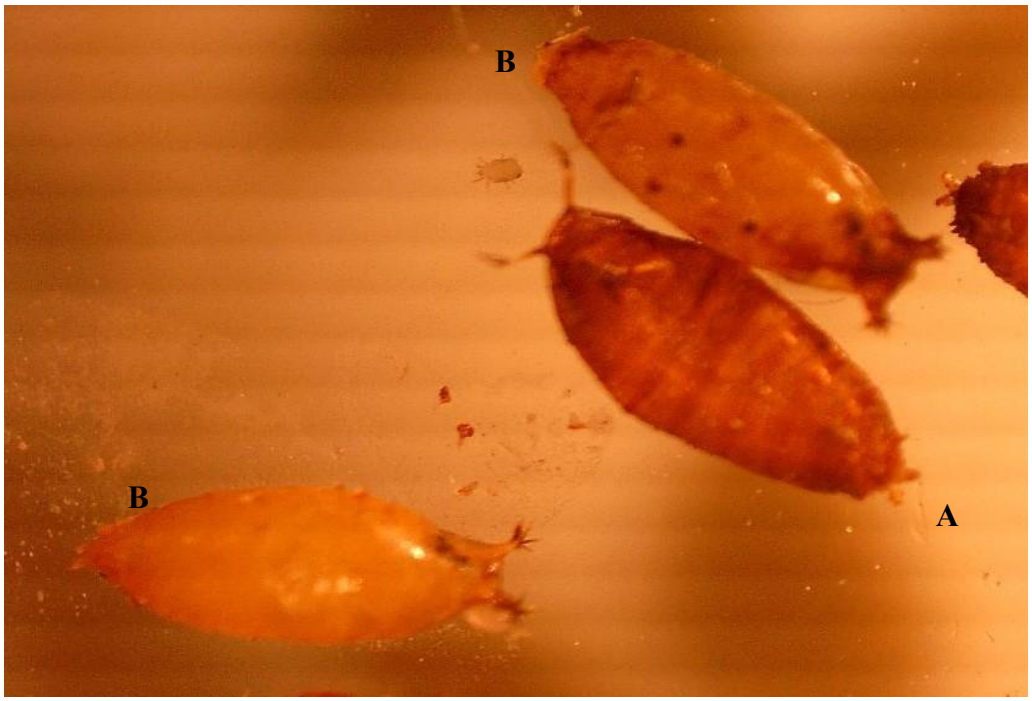

Figure 10. (A) *Zaprionus indianus* and (B) *Drosophila suzukii* pupae reared from red grapes in 2017.

Part 2 - Clarify situation of native natural enemies to attack SWD and AFF.

2A – SENTINEL TRAPPING FOR PARASITOIDS (HYMENOPTERA) OF EXOTIC DROSOPHILIDS IN VIRGINIA FRUIT CROPPING SYSTEMS

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a globally invasive pest of soft-skinned fruits, originating from Southeast Asia (Bolda et al. 2010, Hauser 2011, Cini et al. 2012, Deprá et al. 2014, Asplen et al. 2015). In 2008, spotted wing drosophila, henceforth SWD, first appeared in both N. America (CA) and Europe (Bolda et al. 2010, Hauser 2011, Calabria et al. 2012, Cini et al. 2012). In 2009, SWD spread up the west coast of the USA and was also detected in Florida (Hauser 2011). By 2011, SWD had spread to Virginia (Pfeiffer 2012, Pfeiffer et al. 2012), and is now widespread throughout the continental USA and temperate parts of Canada (Asplen et al. 2012). SWD was also discovered in Brazil in 2014 (Deprá et al. 2014). With a wide host range, high fecundity, short life cycle, and multivoltine life history, SWD is a major economic pest of many fruit cropping systems throughout the growing season (Bolda et al. 2010, Goodhue et al. 2011, Ioratti et al. 2015). SWD females use large, hardened, serrated ovipositors to cut into intact, ripe fruit where they deposit eggs, and the larvae then consume the flesh of the fruit (Hauser 2011). Affected crops include mainly cherries, strawberries, caneberries, blueberries, and grapes (Bolda et al. 2010, Goodhue et al. 2011, Asplen et al. 2015, Ioratti et al. 2015).

Due to the damage potential of SWD, it is necessary to formulate an effective integrated pest management (IPM) program for this pest. As a component of IPM, the use of biological control should be explored. Investigations of SWD parasitoids in invaded regions have discovered two species that can successfully parasitize SWD in the field: the generalist pupal parasitoid and hyperparasitoid *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), and the drosophilid-specific pupal parasitoid *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae) (Chabert et al. 2012, Rossi Stacconi et al. 2013). Both species are cosmopolitan, and have been discovered attacking SWD in western N. America, Europe, and South Korea, but estimated parasitization rates in the

field have been too low for adequate population control (Chabert et al. 2012, Rossi Stacconi et al. 2013, Daane et al. 2016, Wang et al. 2016b). No information on host-parasitoid interactions of SWD in eastern N. America has yet been published.

Another drosophilid that has recently invaded the Americas is the African fig fly, *Zaprionus indianus* Gupta, henceforth AFF. AFF originates from Africa and Eurasia, and has been invasive in S. America since 1999 (Vilela 1999, Santos et al. 2003, van der Linde et al. 2006). The species has a wide host range, but is known for being an economic pest of figs (Raga et al. 2003, van der Linde et al. 2006, Oliveira et al. 2013). AFF was first detected in N. America (FL) in 2005 (Steck 2005, van der Linde et al. 2006), and it was recorded in Virginia in 2012 (Pfeiffer 2012, Pfeiffer et al. 2012). AFF is reported to be intolerant of cold temperatures (Araripe et al. 2004, David et al. 2006), so it likely only survives year-round in the more sub-tropical regions of N. America (e.g. FL, TX, Mex.), then re-invades the more temperate regions every growing season. In support of this, AFF only appears in VA later in the growing season (Pfeiffer 2012, Pfeiffer et al. 2012). While AFF has not emerged as a significant pest in N. America, it is reported to be very adaptable and very competitive (Tidon 2003, da Silva et al. 2005, Ferreira and Tidon 2005, Galego et al. 2005, da Mata et al. 2010), and therefore threatens native drosophilid communities. Still, little is known about the ecology and impacts of AFF in N. America. In Virginia, AFF often appears in tandem with late-season SWD infestations of fruit crops (Pfeiffer 2012, Pfeiffer et al. 2012). This co-occurrence, combined with a lack of information and potential ecological threat, warranted the inclusion of AFF in this study.

The main objectives of this study were to determine which parasitoids of drosophilids are present in Virginia fruit cropping systems, and if parasitoids are successfully parasitizing *D. suzukii* or *Z. indianus* in the field. As secondary objectives, we aimed to determine if trap placement (edge vs. interior) and type of fruit bait would affect the number of parasitoids reared from traps, or which species of parasitoids were reared from traps.

Materials and Methods

Sentinel Traps. *Insects*. Species used in these experiments included *Drosophila melanogaster*, *D. suzukii* (SWD), and *Z. indianus* (AFF). The laboratory colony of *D.*

melanogaster was acquired from stock colonies maintained in the Departments of Entomology and Biological Sciences at Virginia Tech. Colonies of SWD and AFF were raised from individuals collected in southwestern Virginia. Flies were maintained on a molasses-based diet formula (Nutri-Fly™ MF, Genesee Scientific Corp., San Diego, CA) in 178-ml, square-bottom polypropylene drosophila stock bottles (Genesee Scientific Corp., San Diego, CA). Colonies were reared in an environmental chamber at 23.3º C and 14 h day length (18 W "cool white" fluorescent bulbs).

Trap Design. Sentinel traps (Fig. 11) were created using 1.4 L plastic deli containers. An opening of about 5 × 4 cm was cut into both the front and back of each container for odor dispersal and insect access. Fifty-two smaller access holes of 0.5 cm were also cut into all sides of each container, and were placed symmetrically so that opposite sides had the same number and distribution. Each container was inlaid with ~2 mm mesh aluminum screening to exclude larger insects. To minimize desiccation within the traps, the container lids were painted with an undercoat of black for shade and a topcoat of white for sunlight reflection. Each trap was also outfitted with a string for hanging in the field. Placed within each trap was a 9-cm Petri dish, which would hold the bait. The bait for each trap was ~50 g of fruit infested with larvae of one of the three fly species. Fruit used in the bait was either the same crop as produced by the cropping system (see "Experimental Design") or banana. Banana was used as the alternate fruit type because banana is common bait used for drosophilids and their parasitoids (Carson 1951, Carson and Stalker 1951, McKenzie 1974, Allemand et al. 2002, Mitsui et al. 2007, Rossi Stacconi et al. 2013).

Bait preparation. Five to seven days prior to setting traps, adult flies of the species to be used for bait were transferred into fresh rearing bottles with new food media. The bottles were placed in the environmental chamber to allow flies to reproduce. On the day of trap placement, fresh fruit to be used in the bait was purchased from the local supermarket. Fruit was rinsed with water before use. For each trap to be set, ~50 g of fruit was measured out and placed in a Petri dish, then sliced and macerated so that it fit into the dish with the lid on (the lid had to be on during transportation). Once the fruit was allocated to the dishes, larvae were harvested from the aforementioned rearing bottles. Larvae were collected from a bottle by filling it with ~3 cm of lukewarm water to

encourage larvae to come to the top of the food media, swirling the bottle to get the larvae up in the water column, and then dumping it over a fine mesh net to strain out the larvae. Larvae were then scooped from the net and placed into one of the Petri dishes with fruit, so that each bait dish ended up with an estimated 100-200 larvae, ranging from 1^{st}–3^{rd} instar. For *D. melanogaster* and AFF, 1 bottle usually sufficed for 4 dishes. For SWD, 1 bottle was usually enough for 2 dishes. Once the baits were completed, the dishes were capped, labeled, and transported to the field where they were placed in a trap.

Experimental Design. During the 2015 field season, sentinel traps were placed in four different fruit cropping systems: cherry, caneberry, blueberry, and grape (Table 10). These systems were chosen because they are the most affected by SWD in the local region (Pfeiffer *unpublished data*). The cherry orchard was located in Patrick Co., the caneberry field was in Montgomery Co., the blueberry plantation was in Giles Co., and the two vineyards were in Montgomery Co. and Amherst Co. At the beginning of trapping, only *D. melanogaster* and SWD were used for baits. AFF is only naturally present in Virginia during the late harvest season (Pfeiffer 2012, Pfeiffer et al. 2012), so baits containing AFF larvae were not deployed until AFF had appeared in the area. *Drosophila melanogaster* was chosen as an alternate host species because it is naturally occurring in southwestern Virginia, is closely related to SWD, and is known to be more susceptible to parasitization (Kopp and True 2002, Kacsoh and Schlenke 2012). Therefore, if local parasitoids are unsuccessfully attacking SWD, the same species might successfully attack *D. melanogaster* and still develop from the sentinel traps.

Trapping surveys in each cropping system were considered separate experiments. As such, each survey was a 3 × 2 × 2 factorial experiment. Infesting fly species was one factor, with 3 levels: *D. melanogaster*, SWD, and a control with no flies. Fruit type was the second factor, with 2 levels: banana and corresponding fruit crop (e.g. sweet cherry for cherry orchard, mix of raspberries and blackberries for caneberry field, blueberries for blueberry plantation, or black table grapes for vineyard). Trap placement was the last factor, where the 2 levels were field edge and interior. Therefore, 12 traps were placed in each cropping system, with 6 traps on the field edge and 6 on the field interior. Each group of six traps contained every possible fruit/fly combination. Traps were placed ≥ 20 m apart in random order. Each trapping session lasted 3-4 d, and 6-7 trapping sessions

were completed in each cropping system, so that 21-24 trapping days were accumulated for each experiment.

For the last two trapping sessions in caneberry, blueberry, and grape cropping systems, four additional traps containing baits with AFF were included, and were distributed to account for the experimental factors described above. These traps were not included in the cherry orchard because cherry is an early season crop, while AFF only occurs during the late season in Virginia. Additionally, because traps infested with AFF were only out for two trapping sessions, results from those traps were analyzed separately from the traps containing SWD and *D. melanogaster*.

At the end of each trapping session, Petri dishes were collected from traps and returned to the laboratory, where they were individually enclosed within rearing containers, and insects were allowed to complete development. Rearing containers were created from 1 L plastic deli cups, and the lids were modified with a hole covered in cloth to allow for airflow but prevent escapes. Rearing containers were monitored for fly and parasitoid emergence 2-3 times per week, for 1 month after collection. All flies and parasitoids emerged within 1 month. Emerged insects were collected, preserved in 70% ethanol, and counted. Samples of parasitoid specimens were sent away for professional identification. Data were analyzed using descriptive statistics.

Results

Cherry Orchard. Of the six sentinel-trapping sessions in the cherry orchard, the last three sessions (date range 6/8–6/26) produced parasitoids. Two parasitoid species were reared: the larval endoparasitoid *Leptopilina boulardi* (Barbotin, Carton and Kelner-Pillault) (Hymenoptera: Figitidae), and the pupal ectoparasitoid *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae), which only emerged from the 4th trapping session. Additionally, it was not unusual for traps, including control traps, to produce adult drosophilids that were not initially infesting the bait, indicating that wild flies were contaminating the traps. Therefore, the host on which the parasitoids developed was sometimes difficult to distinguish.

A total of 674 *L. boulardi* and 62 *P. vindemiae* were reared from sentinel traps. All individuals of *L. boulardi* were reared from either *D. melanogaster* or ‘other’

drosophilids that contaminated the traps. 'Other' drosophilids were defined as any drosophilids other than SWD, AFF, or *D. melanogaster*. One *P. vindemiae* was reared from SWD and all other *P. vindemiae* were reared from *D. melanogaster*. Most *L. boulardi* and all *P. vindemiae* were reared from traps baited with banana (Fig. 12), and most parasitoids of both species were reared from traps placed on the edge of the orchard (Fig. 13). However, because a large proportion of traps produced no parasitoids, only descriptive statistics could be used to interpret the data.

Caneberry Field. Only the first three trapping sessions (date range 7/6–7/26) in the caneberry field yielded parasitoids, and only one species emerged: the larval parasitoid *Leptopilina clavipes* (Hartig). A total of 207 parasitoids emerged, and only from traps baited with caneberry (Fig. 12). Most *L. clavipes* were reared from traps placed on the edge of the field (Fig. 13). Additionally, these parasitoids only emerged from traps that were contaminated with 'other' drosophilids, and mainly from control traps, i.e. traps that had no host larvae supplied, but were apparently colonized by wild drosophilids. Again, only descriptive statistics could be used to interpret the data, because most of the traps did not yield any parasitoids.

Blueberry Plantation and Vineyard. Three *Leptopilina* individuals were collected from a trap pre-infested with SWD in the blueberry plantation, which was active during the third trapping session (8/20–8/23). However, the specimens were heavily damaged and stuck within dried blueberries when they were discovered, so the species could not be identified. No other parasitoids were reared from traps placed in the blueberry plantation. Additionally, no parasitoids were reared from traps placed in vineyards.

Discussion

Parasitoid Species. Although the sentinel traps yielded three parasitoid species, the results are not promising for biological control of SWD. *Leptopilina boulardi* is a known parasitoid of frugivorous *Drosophila* (Carton et al. 1986, Dubuffet et al. 2009, Kacsoh and Schlenke 2012), but Kacsoh and Schlenke (2012) demonstrated that SWD is resistant to parasitization by *L. boulardi*, as well as several other parasitoid species, due to a high hemocyte load. Mazzetto et al. (2016) also demonstrated that *L. boulardi* in Italy could

not develop on SWD. Therefore, it makes sense that *L. boulardi* was not reared from SWD in the sentinel traps. Follow-up laboratory studies will confirm whether this strain of *L. boulardi* is capable of parasitizing SWD.

Leptopilina clavipes was only reared from traps producing 'other' drosophilids, especially control traps. Therefore, it likely preferred the other drosophilids to *D. melanogaster* or SWD. This is supported by the literature, which indicates *L. clavipes* is more associated with fungivorous drosophilids, rather than frugivorous drosophilids (Vet 1983, Carton et al. 1986, Driessen and Hemerik 1991, Pannebakker et al. 2008). Indeed, the raspberries used in the trap baits often became moldy, especially the control traps, so perhaps the 'other' drosophilids were fungal-feeding species (many of the 'other' drosophilids that emerged resembled known fungal-feeders *Drosophila phalerata* Hartig and *Drosophila subobscura* Collin (Driessen and Hemerik 1991, Pannebakker et al. 2008), but identification has not been confirmed). As a natural parasitoid of fungivorous species, *L. clavipes* would not be appropriate for biological control of SWD or AFF.

The presence of pupal parasitoid *P. vindemiae* was to be expected, because *P. vindemiae* is a cosmopolitan species, and a generalist of several schizophoran families including Drosophilidae (Nøstvik 1954, Carton et al. 1986, Goubalt et al. 2004, Marchiori et al. 2013). Furthermore, Rossi Stacconi et al. (2013) reported *P. vindemiae* as a parasitoid of SWD in Europe and Oregon, and Daane et al. (2016) reported the same in South Korea. It was somewhat surprising that only one *P. vindemiae* was reared from SWD throughout this study, and that *P. vindemiae* was only reared from one trapping session. However, the sentinel traps were only seeded with fly larvae, not pupae, so pupae would have been present for a shorter length of time. Because of that, the traps may have been attractive to *P. vindemiae* for a more limited time. Conversely, perhaps *P. vindemiae* is simply less abundant in Virginia. Regardless, because *P. vindemiae* can successfully attack SWD, it seems somewhat more promising as a potential candidate for biological control of SWD. In Costa Rica, *P. vindemiae* has been used as an augmentative biological control agent in an IPM program against *Ceratitis capitata* Weidemann (Diptera: Tephritidae), and with marked success (Camacho 1998). However, there is a valid concern about non-target impacts because of the generalist and hyperparasitic behavior of *P. vindemiae* (Guillén et al. 2002, Wang and Messing 2004,

Wang et al. 2016a). The use of *P. vindemiae* as a biocontrol agent for SWD needs to be further explored. Still, several studies have identified potential candidates for classical biological control of SWD that might prove more beneficial (Kasuya et al. 2013, Nomano et al. 2014, Asplen et al. 2015, Daane et al. 2016).

No parasitoids were reared during the first three weeks of trapping in the cherry orchard. That early in the season, insect populations may have still been recovering from winter, so fly hosts and therefore parasitoids may have been less abundant. Additionally, fruit were unripe, so the cherry orchard may have been less of a beacon to drosophilids and their parasitoids.

No parasitoids were reared from AFF in the sentinel traps, but that does not mean AFF escapes parasitization completely. Overall, the AFF-seeded traps were out for a much more limited time than the other traps, so there were fewer opportunities for parasitoids to find AFF larvae or pupae. It must also be noted that parasitoids were not reared from any of the sentinel traps during the time in which AFF-seeded traps were active (Aug.–Oct.). Perhaps the trapping sessions did not coincide with the seasonal phenology of the parasitoids, or parasitoid abundance was low, or alternate host sources were more attractive to parasitoids. In addition, the blueberry and grape growers had been using insecticides to combat SWD, which might have reduced any parasitoid presence in the area. Follow-up laboratory experiments will determine if *P. vindemiae* or *L. boulardi* will parasitize AFF under controlled conditions (Chapter 3).

Data Trends. While there were not enough overall data for an accurate and meaningful statistical analysis, interesting trends were still observed in the cherry orchard and caneberry field. In the cherry orchard, considerably more parasitoids were reared from banana-baited traps than cherry-baited traps (Fig. 2), suggesting the type of fruit containing the host may be an important factor in parasitoid host-finding behavior. Plant odors released by host feeding activity are known to be important olfactory cues for parasitoid host-finding ability (Price et al. 1980, Geervliet et al. 1994, Du et al. 1996). Furthermore, it has been shown that some parasitoids are selective about which type of plant their host feeds on (Johnson and Hara 1987, van den Berg et al. 1990, Hoballah et al. 2002). Perhaps this is the case with parasitoids of frugivorous drosophilids, and such behavior could be important for biological control efforts of SWD or AFF. An

olfactometry study investigating the relative attractiveness of different fruit odors to *Drosophila* parasitoids, such as *L. boulardi* and *P. vindemiae*, would be enlightening.

Another apparent trend in the data is that parasitoids were reared more from traps placed on the edge than on the interior (Fig. 13). Several factors could contribute to such an effect. Assuming the parasitoids enter the fruit production area from surrounding habitat, individuals would not have to venture further into the area if the edge already supplies their needs. There could also be a similar effect occurring with the host insects, with more hosts available on the edge than on the interior. If there is a higher host population on the edge, the edge might be a more attractive location for parasitoids than the interior. In addition, microclimatic conditions could have been more conducive to parasitoid presence on the edge than on the interior. The cherry orchard and caneberry field were situated within or directly adjacent to woods, so that the edges where traps were placed were less exposed to direct sunlight than the interiors. To examine this theory, temperature data were recorded throughout July 2016 in the caneberry field, in the same general locations that traps were placed in 2015. Average daytime high temperatures were consistently higher in the interior of the field than the edge (Fig. 14), indicating that microclimates were indeed different between the two areas. Assuming this difference occurs every year, and that parasitoids of frugivorous drosophilids prefer the cooler edge habitat to the warmer interior, it could help explain the observed difference in parasitoid emergence.

Design Limitations. Although some data trends were observed, the high zero count for parasitoid emergence should be addressed. While such results may be attributed to pesticide usage at field sites, parasitoid phenology, trap placement and microclimates, one cannot rule out potential design flaws of the traps themselves. Rossi Stacconi et al. (2013) used red delta traps for their sentinel traps, and reared more parasitoids over the season, especially *P. vindemiae*. Color can be an important attractive component of insect traps (Hoback et al. 1999, Campbell and Hanula 2007), so perhaps the color red is more attractive to parasitoids of *Drosophila* than white, the color of our traps. Furthermore, another factor could be the manner in which the bait was “infested” with larvae before placement in the field. The larvae were simply dumped directly onto fresh fruit immediately before trap placement, so the bait would not have had the same odors as if

the larvae had developed within the fruit for a few days prior. Specifically, the parasitoids could be attracted to the yeast and vinegar odors associated with *Drosophila* larvae, and those odors might have taken a while to develop within the bait. Consequently, the baits may not have been attractive to parasitoids for as long as they should have. It may have been better to directly expose the fruit bait to adult flies for several days before placement, so that larvae would develop within the fruit, and the proper odors would be present at the time of placement.

Conclusions and Next Steps. The results indicate that parasitization of SWD and AFF in southwestern Virginia is negligible, and that none of the reared parasitoid species would be effective biological control agents for SWD or AFF. However, *L. boulardi* and *P. vindemiae*, the two species reared that parasitize frugivorous drosophilids, must still be assessed in the laboratory to determine if they can parasitize SWD or AFF under controlled conditions. The results also raise further questions: How does AFF compare with SWD and *D. melanogaster* with respect to parasitization resistance? Are parasitoids of frugivorous drosophilids selective about what type of fruit their host feeds in? These inquiries will be pursued.

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Tables and Figures

Table 10. Date ranges (2015) of sentinel trapping sessions for each fruit cropping system in this study.

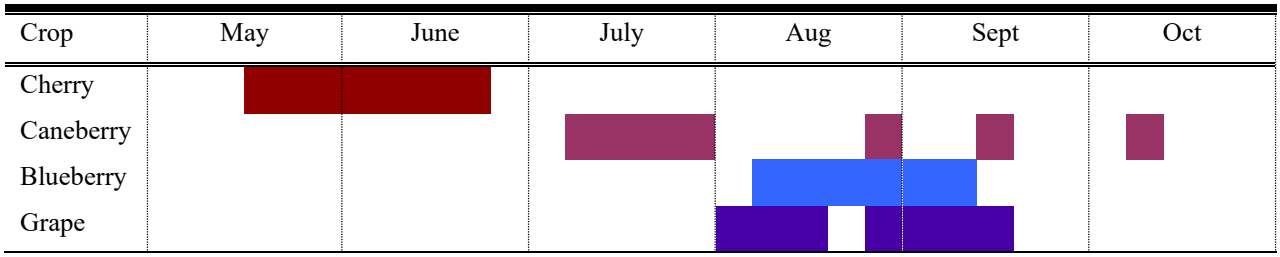

Fig. 11. An example of the sentinel traps used in this study.

Legend for Figs. 12 and 13:

Leptopilina

P. vindemiae

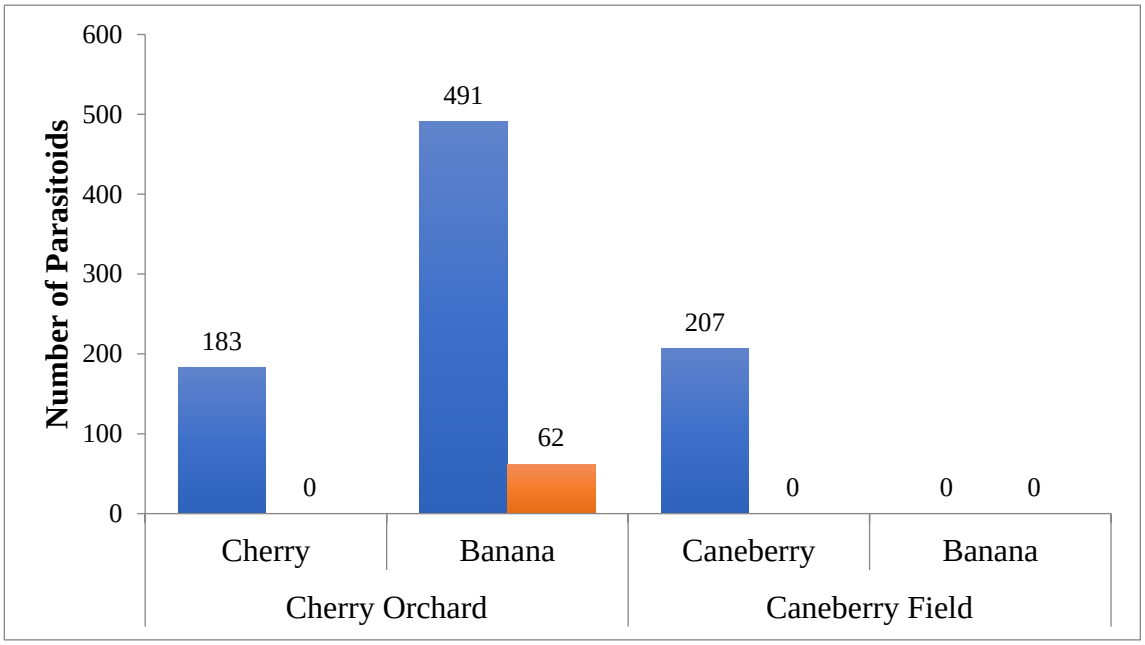

Fig. 12. Comparison of parasitoid emergence from sentinel traps, with respect to the type of fruit used to bait the trap. *Leptopilina* bars represent *L. boulardi* for the cherry orchard, and *L. clavipes* for the caneberry field.

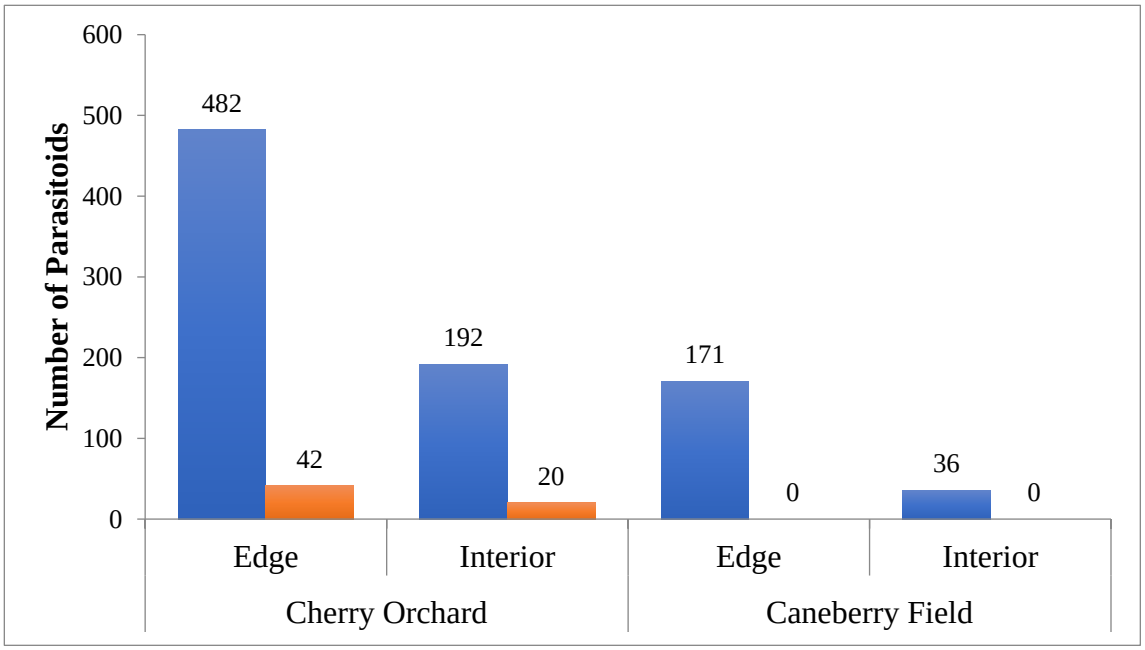

Fig. 13. Comparison of parasitoid emergence from sentinel traps, with respect to trap placement. *Leptopilina* bars represent *L. boulardi* for the cherry orchard, and *L. clavipes* for the caneberry field.

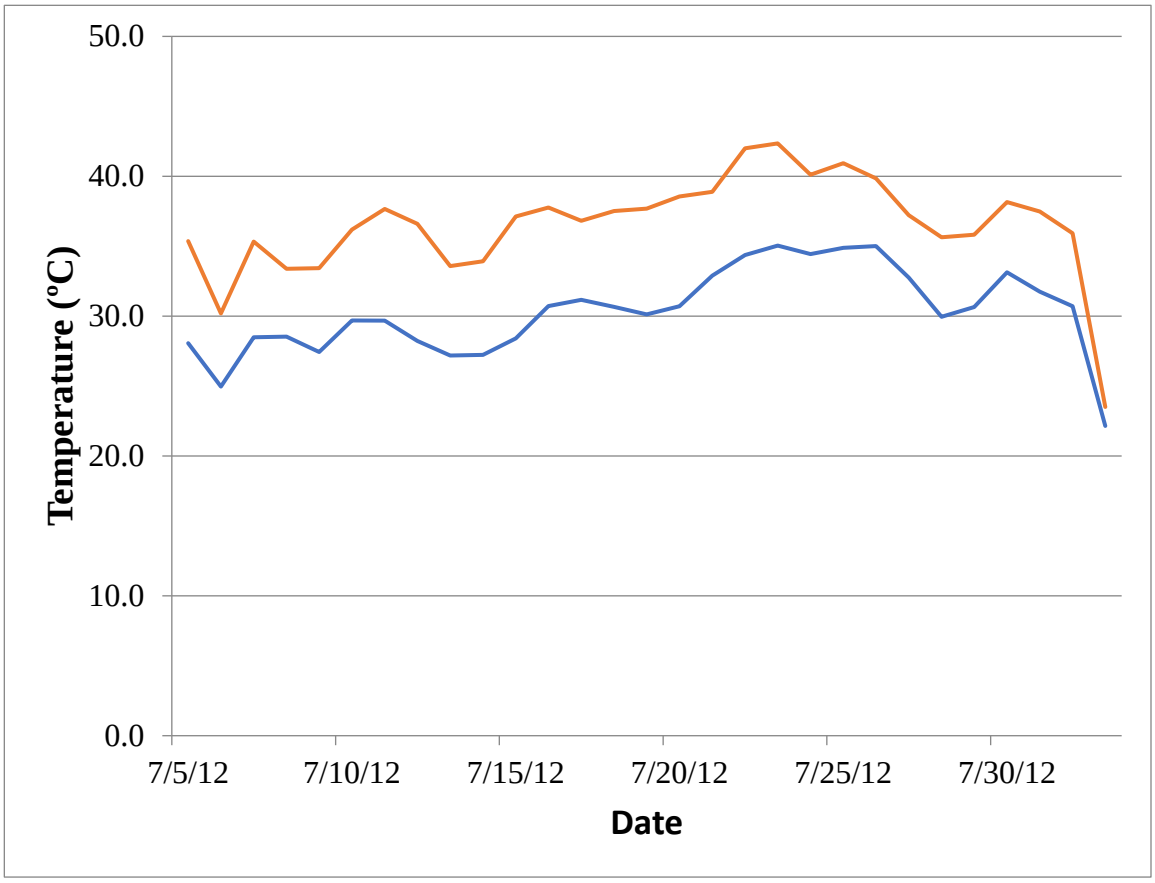

Fig. 14. Average daily high temperatures during summer 2016 for edge and interior of caneberry field. Temperature data were recorded by HOBO® Pro v2 data loggers – 4 on the edge and 3 on the interior.

2B – ABILITY OF TWO PARASITOIDS IN SOUTHWESTERN VIRGINIA TO ATTACK INVASIVE VINEGAR FLIES, *DROSOPHILA SUZUKII* (MATSUMURA) AND ZAPRIONUS *INDIANUS GUPTA* (DIPTERA: DROSOPHILIDAE)

Two exotic species of vinegar fly have recently invaded North America: the spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) of southeast Asia, and the African fig fly, *Zaprionus indianus* (Gupta) (Drosophilidae) (Steck 2005, van der Linde et al. 2006, Bolda et al. 2010, Walsh et al. 2011). Since the initial detections of *D. suzukii* in California and Europe in 2008, the species has spread rapidly and become a global economic pest of small fruits, due to its ability to cut into

ripe, intact fruit with a serrated ovipositor (Bolda et al. 2010, Cini et al. 2012, Asplen et al. 2015). In Virginia, *D. suzukii* has been an important pest of small fruit production since 2011 (Pfeiffer 2012, Pfeiffer et al. 2012).

Zaprionus indianus has been invasive in South America since the late 1990s, where it became a pest of fig production (Vilela 1999, Raga et al. 2003, Santos et al. 2003, Oliveira et al. 2013). In 2005, *Z. indianus* was discovered in Florida (Steck 2005, van der Linde et al. 2006), and was first detected in Virginia in 2012, where it has often been observed concurrently with late-season *D. suzukii* infestations (Pfeiffer 2012, Pfeiffer et al. 2012). Unlike *D. suzukii*, *Z. indianus* does not have a large, serrate ovipositor, and so cannot puncture intact fruit during oviposition (Fig. 15). Only previously damaged or overripe fruit would be susceptible to *Z. indianus* infestation (Shrader *unpublished data*). Additionally, cooler climates and winter temperatures may limit the spread of *Z. indianus* (Araripe et al. 2004, David et al. 2006). Therefore, the chance of *Z. indianus* becoming a major agricultural pest in North America is low, with the exception of fig-producing areas such as California. While there may be geographic limitations to this pest, *Z. indianus* has a wide host range (Vilela 1999, Santos et al. 2003, van der Linde et al. 2006), and is reported to be highly adaptable and highly competitive (Tidon et al. 2003, da Silva et al. 2005, Ferreira and Tidon 2005, Galego and Carareto 2005, da Mata et al. 2010), suggesting it has potential to have a major impact on drosophilid communities in invaded regions.

In the search for an effective integrated pest management program for spotted wing drosophila, biological control research has been gaining ground, particularly with hymenopteran parasitoids. Kacsoh and Schlenke (2012) examined the immune responses of *D. suzukii* and its relative *Drosophila melanogaster* (Meigen), after being attacked by 24 different strains of parasitoid wasps, which represented four hymenopteran families, and at least 14 species. The study demonstrated that *D. suzukii* is far more effective than *D. melanogaster* at neutralizing wasp eggs via melanotic encapsulation, owing to a much higher hemocyte load than *D. melanogaster* (Kacsoh and Schlenke 2012). This augmented resistance of *D. suzukii* to parasitization was corroborated by Poyet et al. (2013).

In spite of the remarkable immune system of *D. suzukii*, several potential candidates for classical biological control have been discovered in Asia, which include species in the genera *Asobara* (Hymenoptera: Braconidae), *Ganaspis* (Hym.: Figitidae), and *Leptopilina* (Hym.: Figitidae) (Kasuya et al. 2013, Nomano et al. 2014, Asplen et al. 2015, Daane et al. 2016). Yet, as a prerequisite for classical biological control, the ability of parasitoids in invaded regions to attack *D. suzukii* must be investigated. Such research has already been performed in Europe and the west coast of North America, where only the pupal parasitoids *Pachycrepoideus vindemiae* (Rondani) (Hym.: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hym.: Diapriidae) are able to parasitize *D. suzukii* with some success (Chabert et al. 2012, Rossi Stacconi et al. 2013, Gabarra et al. 2015, Rossi Stacconi et al. 2015, Mazzetto et al. 2016, Wang et al. 2016a, 2016b). No such research has yet been published from eastern North America.

In the case if *Z. indianus*, there is currently no information on its relationship with natural enemies in North America. Research in Brazil, though, has documented pupal parasitoids *P. vindemiae* and *Spalangia endius* (Walker) (Hym.: Pteromalidae), and larval parasitoid *Leptopilina boulardi* (Barbotin, Carton & and Kelner-Pillault) developing on *Z. indianus* (Marchiori et al. 2003, Marchiori and Silva 2003, Silva et al. 2004).

Sentinel trapping surveys in 2015 identified larval endoparasitoids *Leptopilina boulardi* and *L. clavipes* (Hartig), and pupal ectoparasitoid *Pachycrepoideus vindemiae* as parasitoids of frugivorous drosophilids in southwestern Virginia (Wahls *unpublished data*). Laboratory colonies of *L. boulardi* and *P. vindemiae* were successfully developed from individuals reared from sentinel traps, using host *D. melanogaster*. The primary objective of this study was to investigate whether these parasitoids could successfully develop on *D. suzukii* or *Z. indianus* in the laboratory. A second objective was to examine and compare the larval encapsulation responses of *D. melanogaster*, *D. suzukii*, and *Z. indianus* after exposure to the Virginia strain of *L. boulardi*. The purpose of these objectives is to determine if the Virginia strains of *P. vindemiae* and *L. boulardi* could be useful for augmentative or conservation biological control of *D. suzukii* or *Z. indianus*.

Materials and Methods

Insects. This study involved three species of vinegar flies, *Drosophila melanogaster*, *D. suzukii*, and *Zaprionus indianus*, and two parasitoid species, *Leptopilina boulardi* and

Pachycrepoideus vindemiae. The laboratory colony of *D. melanogaster* was developed from existing stock colonies in the Virginia Tech Departments of Biological Sciences and Entomology. Colonies of *D. suzukii* and *Z. indianus* were developed in laboratory from individuals wild-caught in southwestern Virginia. Fly colonies were maintained on molasses-based food media (Nutri-Fly™ MF, Genesee Scientific Corp., San Diego, CA) in 178-ml, square-bottom polypropylene drosophila stock bottles (Genesee Scientific Corp, San Diego, CA), and kept in an environmental chamber with 14 h daylength (18 W "cool white" fluorescent bulbs) and temperature at a constant 23.3ºC. Both laboratory colonies of parasitoids were developed from individuals collected in small fruit cropping systems in southwestern Virginia. Parasitoids were maintained on host *D. melanogaster* from the aforementioned laboratory colony, and kept in an environmental chamber with 14 h daylength, day temperature at 26ºC and night temperature at 23ºC.

Experimental Design. *Larval parasitoids*. Three days prior to experimentation, newly eclosed *L. boulardi* were collected from the laboratory colony and placed in a stock bottle with fresh food medium but no fly larvae, returned to the environmental chamber, and left to mate during that time. On the day of experimentation, 50 1st- and 2nd-instar larvae of *D. melanogaster* were placed in a 35 mm Petri dish with ~1 mm depth of food media. The Petri dish was then enclosed in a rearing bottle with three mated females and one male of *L. boulardi* (females have short antennae and males have long antennae). For the control experiment, another Petri dish was prepared the same way and enclosed in a bottle with no parasitoids. The bottles were then placed in an environmental chamber with 14 h daylength (18 W "cool white" fluorescent bulbs), 26ºC day temp, 23ºC night temp, and left for 72 h. After 72 h, the parasitoids were removed, and 10 larvae were collected from the dish that had been exposed to parasitoids. These larvae were placed in 70% ethanol and observed under a microscope. When placed in ethanol, the integument of the larvae becomes nearly transparent and internal structures can be observed, especially encapsulated parasitoid eggs/larvae (Fig. 16). For each larva, the number of eggs laid and number of encapsulated eggs was recorded, in order to determine encapsulation rate. Encapsulation rate was calculated as the number of encapsulated eggs divided by the number of eggs laid. Attack rate by *L. boulardi* was also determined based on the number of larvae that contained at least 1 wasp egg. The remaining larvae were

allowed to complete development in the environmental chamber, and the number of emerged flies and parasitoids was recorded to determine level of survival, parasitization, and overall mortality. Mortality was measured as the number of insects that did not complete development. Emerged flies were also observed for signs of attempted parasitization, i.e. encapsulated parasitoid eggs/larvae, which were still quite visible in adult flies (Fig. 17). This experiment had six replicates, and was repeated once with host *D. suzukii*, and once with host *Z. indianus*. Methods were adapted from Kacsoh and Schlenke (2012).

Pupal Parasitoids. Three days prior to experimentation, newly eclosed *P. vindemiae* were collected from the laboratory colony and placed in a stock bottle with fresh food medium but no flies, returned to the environmental chamber, and left to mate during that time. On the day of experimentation, 50 late 3rd-instar larvae and newly-formed puparia of *D. melanogaster* were placed in a 25 × 95 mm polystyrene drosophila rearing vial (Genesee Scientific Corp., San Diego, CA) with ~2 mm depth of food medium, and a paper strip for a pupariation surface. Next, three mated female and one male *P. vindemiae* were placed in the vial (females have pointed abdomens, males have rounded abdomens). A second vial was prepared with no parasitoids as a control. The vials were then placed in the environmental chamber for 72 h. After 72 h, the parasitoids were removed, and the larvae were allowed to complete development. The number of emerged flies and parasitoids were recorded to determine rates of survival, parasitization, and overall mortality. This experiment was replicated six times, and repeated using hosts *D. suzukii* and *Z. indianus*. Methods were adapted from Kacsoh and Schlenke (2012).

Statistical Analyses. The *L. boulardi* encapsulation experiments were analyzed using ANOVA and Tukey's multiple comparison, comparing differences in attack rate and encapsulation rate among the three fly species. For parasitization experiments with *L. boulardi* and *P. vindemiae*, ANOVA and Tukey's multiple comparison were used to compare survival/mortality, and parasitization rates among the three fly species. Additionally, in the *L. boulardi* parasitization experiments, the same analysis was used to compare the amount of emerged adult flies containing encapsulated wasp eggs/larvae. To determine if the presence of parasitoids influenced mortality, a Student's t-test was used

to compare the difference between mean control mortality and mean experimental mortality for each fly species in each experiment.

Results

Encapsulation response to *Leptopilina boulardi* attacks. After 72 h exposure to females of *L. boulardi*, an average of 4.8 out of 10 *D. melanogaster* larvae showed signs of attempted parasitization, for an attack rate of ~48%. Based on the number of eggs laid, and the number of eggs/larvae encapsulated, encapsulation rate was calculated at 80% (Fig. 18). *Leptopilina boulardi* attacked an average of 5.7 out of 10 *D. suzukii* larvae, for an attack rate of ~57%. The observed encapsulation rate of *D. suzukii* was ~64%. Also, an average of 1.2 out of 10 *Z. indianus* larvae were attacked, for an attack rate of ~12%, and the observed encapsulation rate of *Z. indianus* was 90%. The attack rate on *Z. indianus* was significantly less than the attack rates on *D. melanogaster* and *D. suzukii*, but attack rates on *D. melanogaster* and *D. suzukii* were not significantly different from one another (ANOVA and Tukey's multiple comparison: D.f. = 2, 15, F = 17.787, $p < 0.05$) (Fig. 18). No significant difference was observed among encapsulation rates of the three species (D.f. = 2, 14, F = 2.588, $p > 0.05$).

***Leptopilina boulardi* Parasitization Trials.** After fly larvae exposed to *L. boulardi* completed development, significant differences in emergence and mortality were observed among the three fly species (Fig. 19). Following exposure to *L. boulardi*, the mean numbers of flies emerging for each species were significantly different from the other two, with *D. melanogaster* emergence the lowest, and *Z. indianus* emergence the highest (D.f. = 2, 15, F = 103.46, $p < 0.05$). An average of 26 wasps emerged from *D. melanogaster* for a 65% parasitization rate. No wasps emerged from *D. suzukii* and *Z. indianus*. Additionally, the number of emerged flies containing at least 1 encapsulated wasp egg or larva was significantly higher in *D. suzukii* than in *D. melanogaster* and *Z. indianus* (D.f. = 2, 15, F = 75.067, $p < 0.05$). Mortality was also significantly higher in *D. suzukii* than in the other two species (D.f. = 2, 15, F = 13.858, $p < 0.05$), but the same was observed in control mortality (D.f. = 2, 15, F = 10.101, $p < 0.05$). No significant difference was observed between mortality and control mortality for each species (Table 1).

***Pachycrepoideus vindemiae* Parasitization Trials.** After fly pupae exposed to *P. vindemiae* completed development, *Z. indianus* again had the highest mean number of adult flies emerge and was significantly higher than that of *D. melanogaster* (D.f. = 2, 15, F = 3.429, $p < 0.05$) (Fig. 20). The number of adult *D. suzukii* flies emerging was between that of *D. melanogaster* and *Z. indianus*, and was not significantly different from either. *Pachycrepoideus vindemiae* was able to parasitize each fly species, but no significant difference was observed among the number of adult wasps emerged from each fly species. Also, no significant difference was observed among experimental mortality of each species. However, for each species, experimental mortality was significantly greater than control mortality (Table 11).

Discussion

The attack rates of *L. boulardi* on *D. melanogaster* and *D. suzukii* were quite similar, while *Z. indianus* seemed much less appealing to *L. boulardi.* The two *Drosophila* species are both within the *melanogaster* species group (Kopp and True 2002), likely making them similar with respect to physiology and olfactory cues. *Z. indianus* is more distantly related to the two *Drosophila* species (DeSalle 1992, Remsen and O'Grady 2002, van der Linde 2010), so the differences in physiology and scent might make it a less suitable and less attractive host to *L. boulardi*.

The observed encapsulation rates of *D. melanogaster* and *D. suzukii* were not significantly different, which initially seems surprising due to what we know about the high hemocyte load of *D. suzukii* (Kacsoh and Schlenke 2012, Poyet et al. 2013). However, Kacsoh and Schlenke (2012) reported a similar situation with another strain of *L. boulardi*, specifically LbG486, where both *D. melanogaster* and *D. suzukii* showed a comparably high level of encapsulation. The observed encapsulation rate of *Z. indianus* in this study was also not significantly different from either *Drosophila* species, but one must keep in mind that the sample size for *Z. indianus* was quite low, due to the low number of larvae that were actually attacked. Still, it can be said that the encapsulation response of *Z. indianus* is certainly not lacking, and a comparative analysis of its hemocyte load with that of *D. suzukii* and *D. melanogaster* would be of interest.

When the adult emergence results are compared with the larval encapsulation results, some inconsistencies become apparent. For example, although a high encapsulation rate was observed in larvae of all three fly species, *L. boulardi* was still able to complete development on *D. melanogaster*. Kacsoh and Schlenke (2012) reported a similar lack of correlation between encapsulation and emergence, and gave explanations that apply here. They rationalized that even if a larva had encapsulated a wasp egg, the larva still may have been super-parasitized and perhaps not all infesting wasp eggs were killed (Kacsoh and Schlenke 2012). Blumberg (1997) also explains that if parasitoid eggs/larvae are only partially encapsulated, they can still complete development. Such may have been the case here. Additionally, upon performing larval dissections, we discovered that non-encapsulated wasp eggs within a fly larva are naturally more difficult to identify than encapsulated eggs, due to similarities in coloration with internal structures. Therefore, it is possible that some non-encapsulated eggs were missed during larval dissections.

Another inconsistency was that zero parasitoids emerged from *D. suzukii* and *Z. indianus*, even though the observed encapsulation rates did not reach 100%. Again, Kacsoh and Schlenke (2012) reported similar results with *D. suzukii*, and explained that even though some wasp eggs may not have been encapsulated by the time larval dissections occurred, the eggs might have been encapsulated and killed at a later point. This is why it is also important to observe the number of emerged adult flies that contained encapsulated wasp eggs.

By examining the number of emerged flies containing encapsulated wasp eggs, one can gain a better understanding of the wasp's ability to parasitize the flies, and their ability to resist parasitization. For *D. melanogaster*, a small number of adult flies emerged compared to the number of wasps that emerged, and most flies that emerged did not have encapsulated wasp eggs, or "capsules". This, combined with the larval encapsulation results, shows that the Virginia strain of *L. boulardi* is able to somehow overcome the encapsulation response of *D. melanogaster*, and that most of the emerged flies probably avoided attack. For *D. suzukii*, a comparatively larger number of adult flies emerged with zero wasps, and a majority of the emerged flies contained capsules, showing that the Virginia strain of *L. boulardi*, like other strains, cannot overcome the encapsulation response of *D. suzukii* (Chabert et al. 2012, Kacsoh and Schlenke 2012,

Mazzetto et al. 2016). Because *Z. indianus* had such a high level of fly survival, no wasp emergence, and very few adult flies with capsules, these results are consistent with the larval encapsulation results, and indicate that *Z. indianus* is simply not an attractive host for this strain of *L. boulardi*. However, *L. boulardi* has been reported to attack *Z. indianus* in Brazil (Marchiori et al. 2003), suggesting that the susceptibility of *Z. indianus* to parasitization may vary depending on the strain of *L. boulardi*.

Based on the lack of difference between experimental and control mortality levels, one can conclude that *L. boulardi* did not affect mortality for each fly species tested. However, *D. suzukii* showed higher levels of mortality in both the experimental and control assays, indicating that the environmental conditions may have been less conducive for *D. suzukii* survival. Interestingly, evidence of cannibalism was observed in some of the *D. suzukii* puparia (Fig. 21), so perhaps cannibalism also played a role in the higher mortality levels.

Emergence results from the *P. vindemiae* trials showed that the Virginia strain of *P. vindemiae* could successfully develop on each fly species tested in the laboratory. Moreover, attack by *P. vindemiae* appeared to cause a significant increase in total mortality for the three fly species. These results are consistent with reports of other *P. vindemiae* strains attacking *D. melanogaster* and *D. suzukii* (Chabert et al. 2012, Rossi Stacconi et al. 2013, Rossi Stacconi et al. 2015, Mazzetto et al. 2016, Wang et al. 2016a), so it is not surprising that a similar result was seen with host *Z. indianus*. While *P. vindemiae* has repeatedly been reported as a natural parasitoid of *D. suzukii* (Chabert et al. 2012, Rossi Stacconi et al. 2013, Daane et al. 2016), and once as a parasitoid of *Z. indianus* (Silva et al. 2004), parasitization rates by *P. vindemiae* on these pests in the field were so low that it would make an insignificant impact on population levels. For example, Silva et al. (2004) reported a 3.5% parasitization rate of *P. vindemiae* on *Z. indianus* in Brazil, and Rossi Stacconi et al. (2013) estimated only a 1% seasonal parasitization rate on *D. suzukii* in Oregon, and even less in Italy. In Virginia, parasitization of *P. vindemiae* on *D. suzukii* in the field has also been observed as negligible, and it has yet to be observed at all on *Z. indianus* (Wahls *unpublished data*). Such low parasitization rates make sense, because *P. vindemiae* is known to be a generalist that hosts on many species within many different schizophoran families

(Nøstvik 1954, Carton et al. 1986, Goubalt et al. 2004, Marchiori et al. 2013). Therefore, one should not expect *P. vindemiae* to seek out drosophilids over other fly hosts.

Due to the generalist behavior of *P. vindemiae*, and the low parasitization rates observed in the field, it is clear that *P. vindemiae* will not be an effective conservation biological control agent. However, should it be used for augmentative biological control? *P. vindemiae* has been mass released in Costa Rica to control Mediterranean fruit fly (*Ceratitis capitata* Weidemann, Diptera: Tephritidae) in oranges, and, in conjunction with the release of sterile fruit flies, was purportedly very successful (Camacho 1998). Yet, serious problems arise when considering this strategy to control *D. suzukii*. Mainly, the invasion of *D. suzukii* is on a far greater scale, affecting at least three different continents (Asplen et al. 2015). Even if enough wasps could be mass reared in captivity, one has to consider the environmental consequences for such large-scale releases. Not only is *P. vindemiae* a generalist, it is also a hyperparasitoid (van Alphen and Thunnissen 1983), and researchers have expressed concern about the non-target impacts of mass-releasing this species, with respect to native dipteran species as well as parasitoid species (Guillén et al. 2002, Wang and Messing 2004). Therefore, the authors do not recommend using *P. vindemiae* for augmentative biological control of *D. suzukii* or *Z. indianus*, at least on a large scale, because the risk to non-targets is too high.

Conclusions. Overall, the results of this study are consistent with previous published research concerning the ability of *L. boulardi* and *P. vindemiae* to parasitize *D. suzukii*. Under the laboratory conditions of this study, the Virginia strain of *L. boulardi* cannot successfully attack pest *D. suzukii* due to its enhanced encapsulation response, and is not attracted to *Z. indianus* as a host. Consequently, the Virginia strain of *L. boulardi* is not a viable candidate for conservation or augmentative biological control of these pests. While *P. vindemiae* can parasitize both pest species, its generalist and hyperparasitic nature will likely make *P. vindemiae* ineffectual for conservation biological control. The authors also recommend extreme caution if considering *P. vindemiae* for augmentative biological control.

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Tables and Figures

Table 11. Student's t-test results comparing experimental mortality in *Drosophila melanogaster* (*D.m.*), *D. suzukii* (*D.s.*), and *Zaprionus indianus* (*Z.i.*) exposed to parasitoid females, versus control mortality in unexposed flies.

T-test Results	*D.m.*	*D.s.*	*Z.i.*
L. boulardi Trials			
Mean Experimental Mortality	7	14.5	5.5
Mean Control Mortality	8.6667	17.5	4
t	-0.6817	-0.8321	0.6311
D.f.	5	5	5
p	0.5257	0.4433	0.5557
P. vindemiae Trials			
Mean Experimental Mortality	20	17.3333	17.5
Mean Control Mortality	4	2.1667	8
t	-8.7636	-4.8711	-3.3075
D.f.	5	5	5
p	0.0003*	0.0046*	0.0213*

Mortality was measured as the number of individuals that did not complete development. Asterisks indicate a significant *p* value.

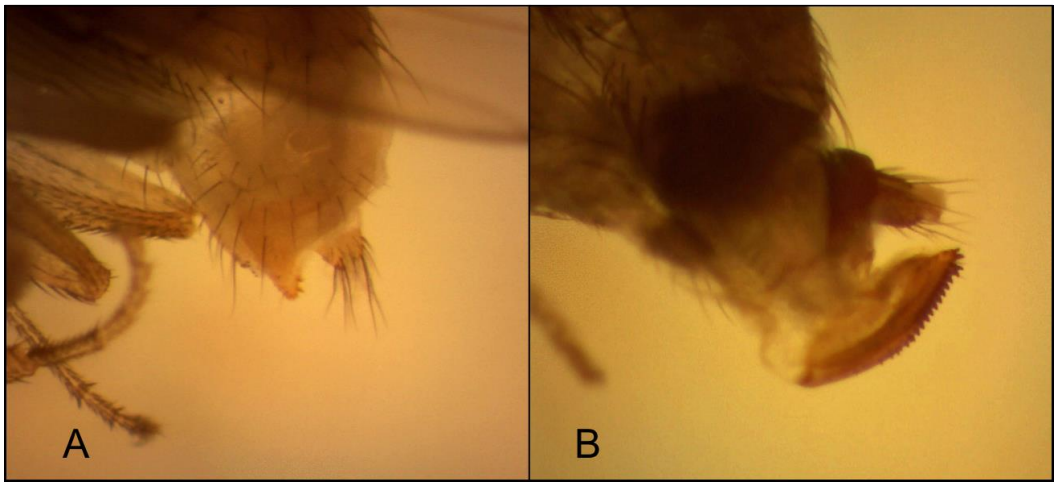

Figure 15. Comparison of ovipositors of *Zaprionus indianus* (A) and *Drosophila suzukii* (B). The ovipositor of *Z. indianus* lacks the size, sclerotization, and serration necessary to puncture the skin of intact fruit, contrary to *D. suzukii*. *Images: D. G. Pfeiffer*

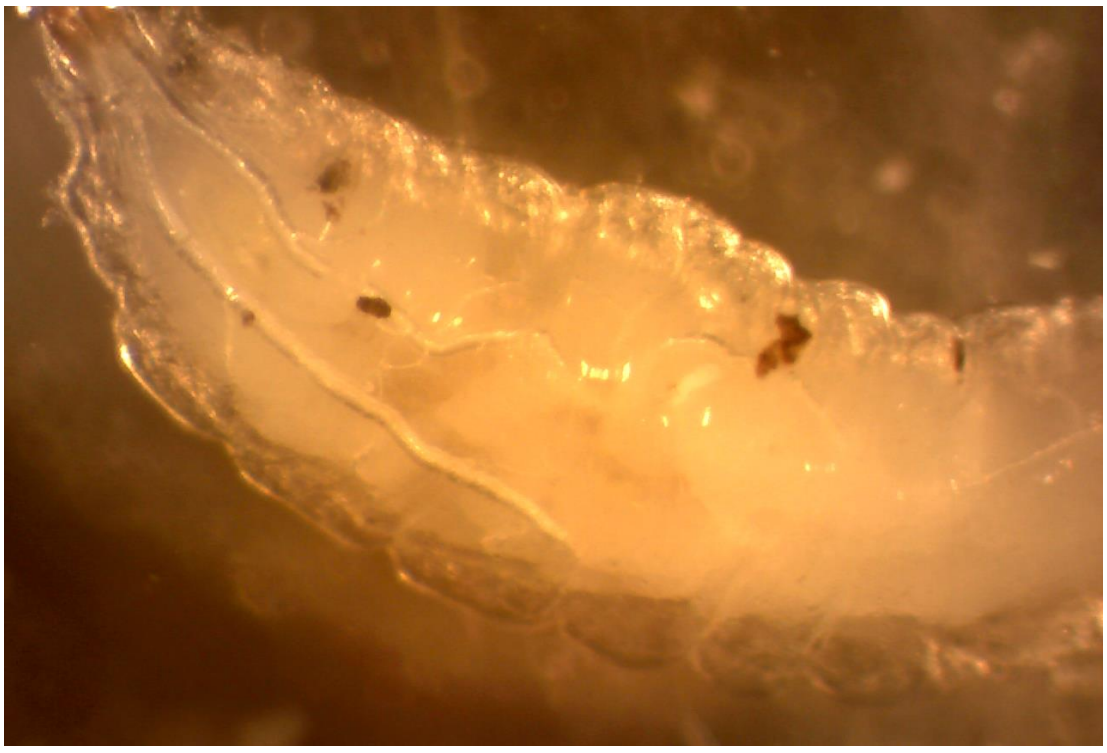

Fig. 16. *Drosophila suzukii* larva in 70% ethanol. Dark spots within fly larva demonstrate melanotic encapsulation of eggs of *Leptopilina boulardi*.

Fig. 17. Adult *Drosophila suzukii* exhibiting melanized parasitoid eggs/larvae within abdomen.

Legend for Figs. 18, 19, and 20

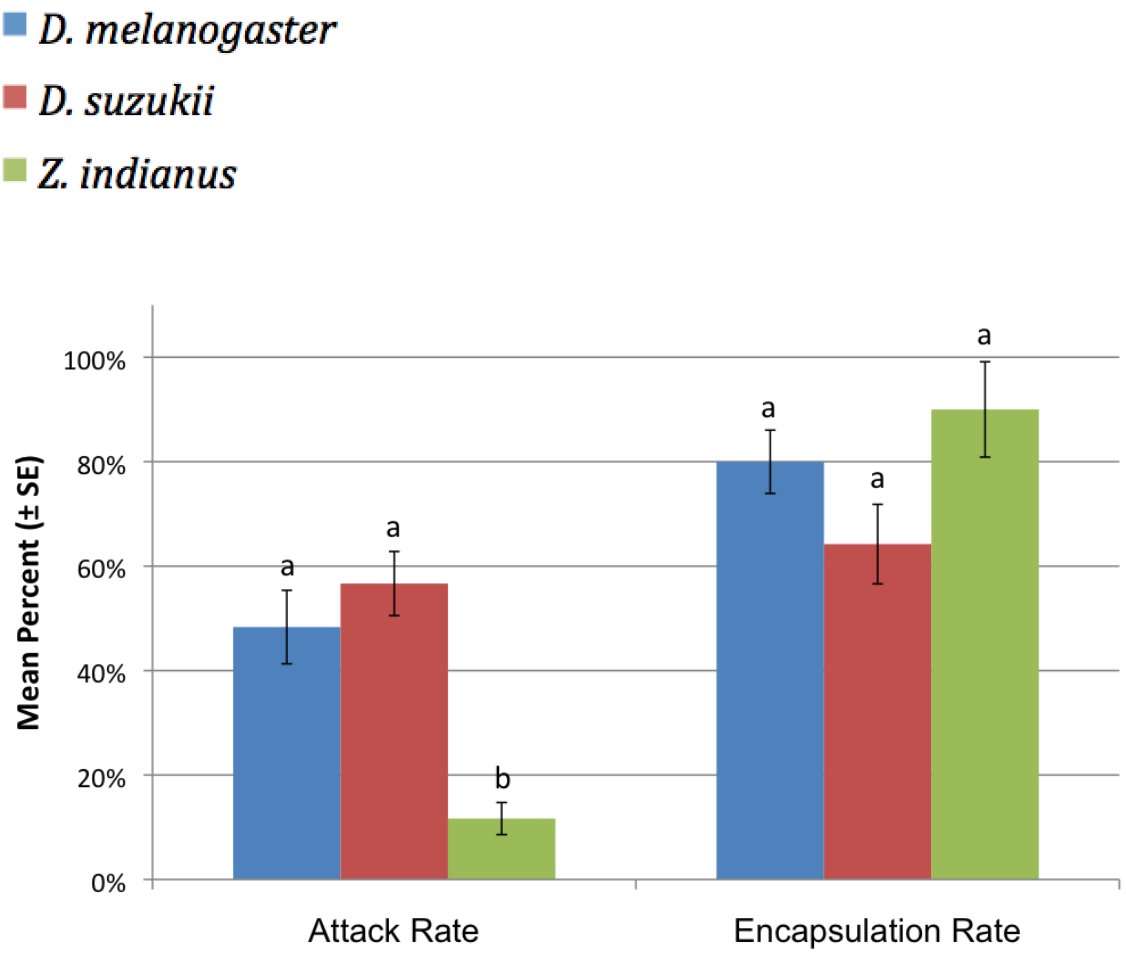

Fig. 18. Mean percent (± SE) attack rates of *Leptopilina boulardi* on larvae of *Drosophila melanogaster*, *D. suzukii*, and *Zaprionus indianus*, and encapsulation rates of wasp eggs by fly larvae. Attack rate was measured as (mean number of larvae attacked) / *N*, where *N* = 10 larvae. Encapsulation rate was measured as the mean of (no. encapsulated wasp eggs) / (no. wasp eggs laid). Within each cluster, columns with different letters are significantly different, based on Tukey's multiple comparison ($p < 0.05$).

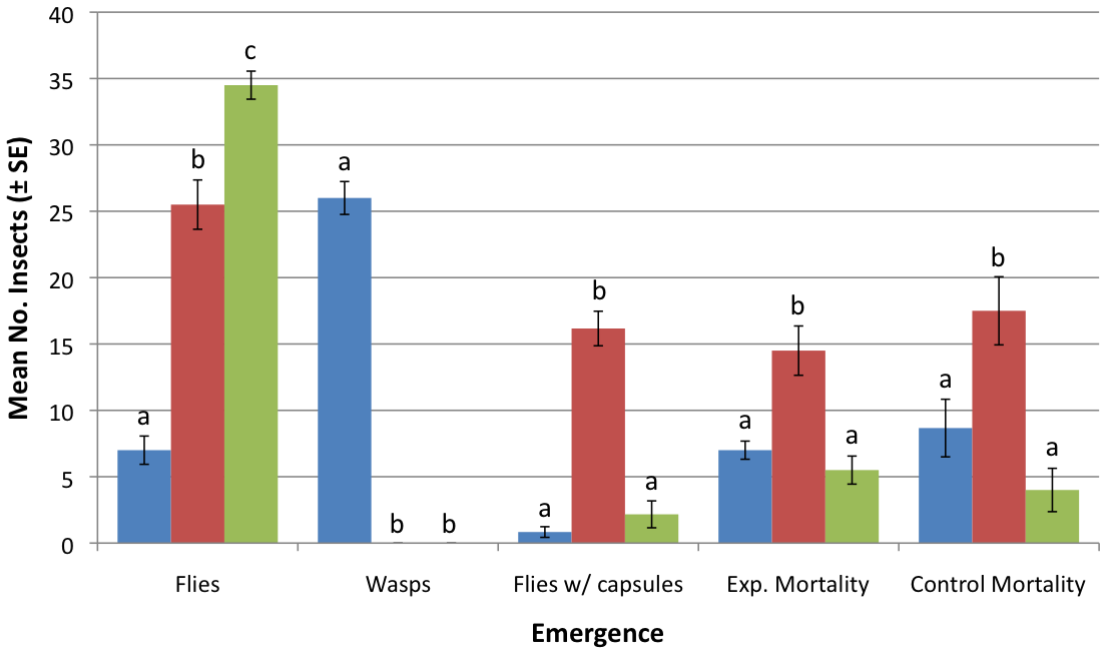

Fig. 19. Emergence and mortality results after *Drosophila melanogaster*, *D. suzukii*, and *Zaprionus indianus* larvae were exposed to *Leptopilina boulardi* females for 72 h. "Flies w/ capsules" refer to adult flies containing encapsulated wasp eggs/larvae (Fig. 3.3). "Experimental mortality" refers to individuals that did not complete development after exposure to parasitoids. "Control mortality" refers to individuals that did not complete development and were not exposed to parasitoids. Within each cluster, columns with different letters are significantly different, based on Tukey's multiple comparison ($p < 0.05$).

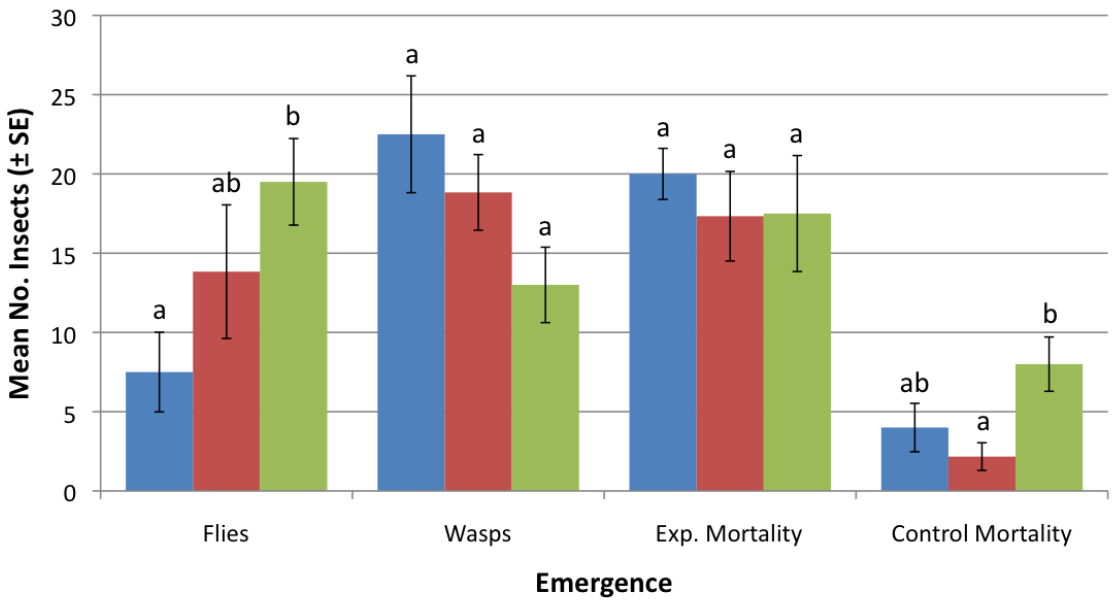

Fig. 20. Mean (± SE) emergence and mortality results after 3rd instar larvae or pupae of *Drosophila melanogaster*, *D. suzukii*, and *Zaprionus indianus* were exposed to *Pachycrepoideus vindemiae* females for 72 h. "Experimental mortality" refers to individuals that did not complete development after exposure to parasitoids. "Control mortality" refers to individuals that did not complete development and were not exposed to parasitoids. Within each cluster, columns with different letters are significantly different, based on Tukey's multiple comparison ($p < 0.05$).

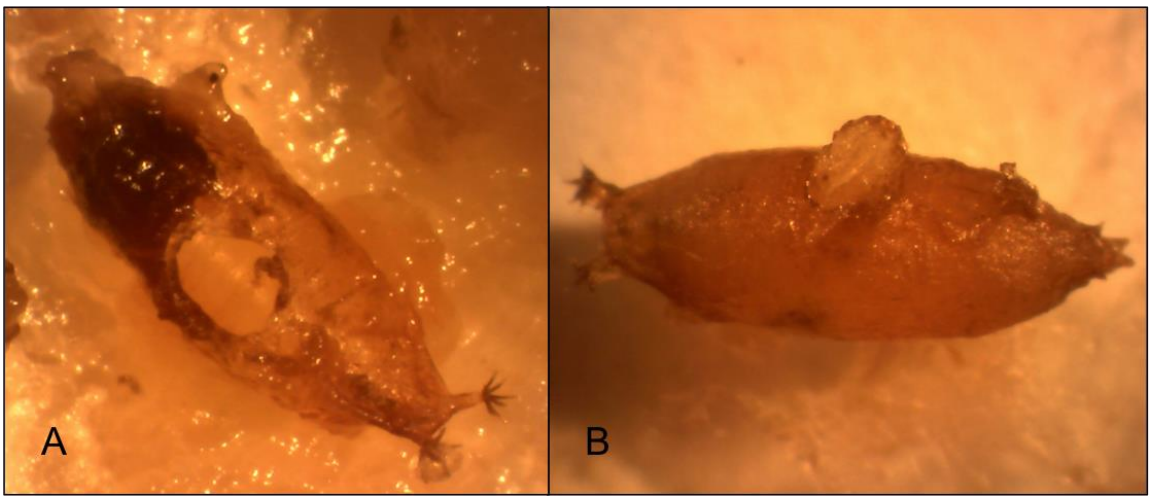

Fig. 21. A.) A cannibalized *D. suzukii* puparium from the *Leptopilina boulardi* parasitization experiment. The large hole in the side of the puparium clearly shows where another larva cut through in order to eat the pupa within. B.) Another instance of cannibalism in *D. suzukii*. Here, the antagonist larva is still feeding with its posterior visibly protruding from the hole in the puparium. These individuals were taken directly from the laboratory colony, and were never exposed to parasitic wasps, so the hole in the side cannot be attributed to predation by a wasp.

Summary:

We showed that African fig fly, though not able to penetrate intact grape berries, will oviposit at egg-laying sites of spotted wing drosophila, where they will gain access to the interior of the berry. When in the presence of AFF, development and survival may be adversely affected. Therefore, AFF is not considered a primary pest of grapes, but may slow the growth of SWD populations.

We collected to parasitoids of drosophilids most commonly, a larval parasitoid, and a pupal parasitoid. Neither of these are likely to provide effective biological control, mainly because of the ability of SWD to encapsulate and kill parasitoid eggs. Details follow:

Part 1A. The interspecific larval competition assay demonstrated the potential ecological impact that *Z. indianus* may have on *D. suzukii* populations in cultivated fruits such as grapes. *Drosophila suzukii* larval and total development time increased as did mortality when in competition with *Z. indianus*. Mortality and developmental time often increased as the density of the larvae on the grapes increased. These developmental impacts were exacerbated by the grape variety in which the two species resided (Chapter 5). The smaller grapes had increased mortality compared to the larger Viognier grapes even at the lower competition levels. Pupal volume was only marginally affected at the highest larval interspecific competition densities in grapes which suggested that the fecundity of any females emerging will not be negatively affected. This study demonstrated that Viognier grapes were a more suitable grape variety for *D. suzukii* survival and should be managed more closely than other grape varieties tested. Vineyards in which these fly species are present may have a decreased risk of *D. suzukii* populations expanding based upon the mortality of this pest when reared in competition with *Z. indianus* in the laboratory. However, those vineyards in which Viognier is grown may be at higher risk of *D. suzukii* population grown due to the survival of *D. suzukii* larvae within the fruit even when in competition with *Z. indianus* within this variety of grape.

Part 1B. Lastly, *Z. indianus* was observed laying eggs on grapes in which *D. suzukii* eggs and ovipositional wounds were observed. *Zaprionus indianus* eggs were also found in the same oviposition holes as *D. suzukii* eggs. I also documented the emergence of *Z. indianus* adults from these grapes. This confirmed my speculations that *Z. indianus* can gain ovipositional access to grapes via *D. suzukii* oviposition wounds. These experimental results will allow Virginia wine grape growers to determine the overall risk for *D. suzukii* infestation within their vineyards and help them form management strategies for this pest based on these scientific findings.

Part 2A. Sentinel trapping surveys discovered three parasitic wasp species attacking drosophilids in Virginia cherry and caneberry cropping systems, including larval parasitoids *Leptopilina boulardi* and *L. clavipes* (Hym.: Figitidae), and generalist pupal parasitoid *Pachycrepoideus vindemiae* (Hym.: Pteromalidae). However, *L. clavipes* appears to be more associated with fungivorous drosophilids versus frugivorous drosophilids, so is not relevant to biological control of *Drosophila suzukii* (spotted wing drosophila, henceforth SWD) or *Zaprionus indianus* (African fig fly, henceforth AFF). The other larval parasitoid, *L. boulardi*, did not develop on SWD or AFF in the sentinel traps, just *D. melanogaster* or other contaminating drosophilids. That *L. boulardi* did not develop on SWD was consistent with previous findings in the literature. The pupal parasitoid, *P. vindemiae*, did successfully develop on SWD on one occasion, so *P. vindemiae* can and will develop on SWD in Virginia. This result was also consistent with previous findings in the literature, except that other studies reared more *P. vindemiae* from SWD in the field. Because only one parasitoid was reared from SWD and none were reared from AFF, conservation biological control for these species is unlikely to be effective in Virginia.

Part 2B. To follow up the sentinel trapping results, the ability of *L. boulardi* and *P. vindemiae* to parasitize *D. melanogaster*, SWD, and AFF was examined in laboratory. Under controlled conditions, results were consistent with our sentinel trapping results and previous studies from the literature. Specifically, *L. boulardi* could successfully parasitize *D. melanogaster* but not SWD. Results also suggest that AFF is not an attractive host for

this strain of *L. boulardi*. Therefore, *L. boulardi* should not be considered for biological control of SWD or AFF. Conversely, *P. vindemiae* was able to successfully parasitize each of the tested fly species, demonstrating its generalist behavior. Because *P. vindemiae* can overcome the defenses of SWD and can also parasitize AFF, *P. vindemiae* might be considered as an augmentative biological control agent. However, its generalist and hyperparasitic nature described in the scientific literature raise concerns about reduced control efficiency and non-target effects.

This grant had 12 months of support for a graduate student. This was allocated to a half year of support for each of two graduate students: Meredith Shrader (PhD student) and Jamie Wahls (M.S. student). Both students successfully completed their work, and obtainied their degrees. The dissertation by Shrader and thesis by Wahls are attached as appendices. The support of the Virginia Wine Board is gratefully appreciated.

Appendix I:

Shrader, M. E. 2017. *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae): Risk assessment for an invasive vinegar fly in Virginia vineyards. PhD dissertation, Virginia Tech, Blacksburg. 141 p.

Appendix II:

Wahls, J. C. E. 2016. Host-parasitoid interactions of two invasive drosophilid species in Virginia fruit crops. Virginia Tech, Blacksburg. 98 p.