DROSOPHILA SUZUKII (MATSUMURA) (DIPTERA: DROSPHILIDAE): RISK ASSESSMENT FOR AN INVASIVE VINEGAR FLY IN VIRGINIA VINEYARDS

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Drosophila suzukii (Matsumura) (Diptera: Drosophila): Risk Assessment For An Invasive Vinegar Fly In Virginia Vineyards

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ABSTRACT (Academic)

Drosophila suzukii (Matsumura) (Diptera: Drosophila) is an invasive frugivore and has become a significant pest of small fruit, cherry and grape throughout the United States. It may be possible to determine if a Virginia vineyard is at risk of *D. suzukii* infestation by analyzing the biotic and abiotic factors around each vineyard. This pest is known to utilize a wide range of cultivated and wild host plants. A host plant survey was conducted at four vineyards in the Piedmont Region of Virginia to identify hosts used by *D. suzukii* around vineyards. The seasonal availability of host plants and adult emergence from them were tracked. Six host plant species of *D. suzukii* were identified, some available season-long. Monitoring *D. suzukii* in cultivated crops is crucial for the timing of spray applications. Homemade and commercially available baits and traps were deployed in two vineyards to determine the efficacy and selectivity towards *D. suzukii*. The homemade and commercially available baits that contained red wine caught the most *D. suzukii*, but none were exclusively attractive to *D. suzukii*. Wine grape susceptibility was assessed in laboratory choice and no-choice ovipositional bioassays. Ovipositional susceptibility was determined by measuring the physiological and morphological parameters using six wine grape varieties. More eggs were laid in grapes as penetration force decreased. Penetration force and not skin thickness was the limiting factor for oviposition. Survivorship of eggs laid in intact grapes was analyzed and survivorship to adulthood was dependent upon variety and survivorship usually exceeded 9% survival seen in previous studies. Larval developmental parameters of *D. suzukii* were affected by grape variety and the density of *Z. indianus*. *D. suzukii* mortality was increased in most cases when in competition with *Z. indianus*, but was less pronounced when reared in Viognier grapes. My *Z. indianus* oviposition study demonstrated that they will follow injury created by *D. suzukii*, and then the Z. indianus larvae may outcompete *D. suzukii* within the berries. These studies greatly improved our understanding of *D. suzukii* biology and ecology in Virginia vineyards.

Drosophila suzukii (Matsumura) (Diptera: Drosophila): Risk Assessment For An Invasive Vinegar Fly In Virginia Vineyards

Meredith Edana Shrader

ABSTRACT (Public)

Drosophila suzukii (Matsumura) (Diptera: Drosophila), henceforth referred to as spotted wing drosophila (SWD) is an invasive pest of small fruits, cherries and grapes grown throughout the United States. This pest has a wide host range including cultivated and wild host plants. A host plant survey was conducted at four vineyards in the Piedmont Region of Virginia to identify those used by SWD. The seasonal availability of host plants and adult emergence from their fruit were tracked throughout the growing season. Six host plant species were found and these host plants were available to SWD throughout the season. Monitoring SWD in cultivated crops is crucial for the timing of spray applications. Homemade and commercially available baits and traps were deployed in two vineyards to determine the efficacy and selectivity towards SWD in the vineyard. Baits containing red wine, whether homemade or commercially available caught the most SWD, but none were exclusively attractive to SWD. Wine grape susceptibility to SWD oviposition was assessed in laboratory no-choice and choice ovipositional bioassays using six wine grape varieties; physiological and morphological parameters were considered. More eggs were laid in grapes as penetration force decreased. Penetration force and not skin thickness was the limiting factor for oviposition. Survivorship of eggs laid in intact grapes was analyzed and up to 50% of the eggs laid in larger grapes survived to adulthood. Larval interactions between SWD and *Z. indianus*, African fig fly (AFF), were also analyzed based on competition intensity and grape variety in which they were reared. SWD mortality, developmental parameters and pupal volume were impacted when in competition with AFF. SWD mortality was less pronounced, even when in competition with AFF, when reared in Viognier grapes compared to any other grape variety tested. My *Z. indianus* oviposition study demonstrated that they will flow injury created by *D. suzukii* and lay oviposit eggs into those wounds. These studies greatly improved our understanding of SWD biology and ecology in Virginia vineyards.

DEDICATION

To my loving husband, wonderful children, family, friends and mentors whose never-ending love, patience and encouragement gave me the strength to achieve anything

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CHAPTER 1: LITERATURE REVIEW AND RESEARCH JUSTIFICATION

Origin and Distribution. *Drosophila suzukii* (Matsumura), spotted wing drosophila, was first described by Matsumura in 1931 in Japan (Kanzawa 1936). Reports of *D. suzukii* date back to 1916, where it was described as a pest of cherries (*Prunus* spp. L.) (Kanzawa 1939). There has been speculation that the species had been introduced to Japan from the neighboring countries of Korea, Thailand and India (Kanzawa 1936, Hauser et al. 2009). The species was documented in the United States on the island of Oahu, Hawaii in 1980 and was reported in several other Hawaiian Islands shortly thereafter (Kaneshiro 1983). *Drosophila suzukii* was first reported in the mainland United States in 2008 in Santa Cruz, California from a fly sample collected from a raspberry (*Rubus* spp. L.) planting (Lee et al. 2011). By 2009, *D. suzukii* had been detected in more than 20 counties in California as well as into Washington, Oregon, and British Columbia (Canada). It also appeared in Florida around the same time period in 2009 (Bolda et al. 2010, NAPPO 2010). In 2010, *D. suzukii* was recorded in Louisiana, South Carolina, North Carolina, Michigan, Wisconsin, and Utah, and in the Canadian provinces of Alberta, Manitoba, Ontario and Quebec (Hauser 2011). It was detected in Virginia in a raspberry field in 2011 and has been collected in traps every year since then (Pfeiffer 2011, Burrack et al. 2012). *Drosophila suzukii* has become a global pest and was detected in Europe (Spain, Italy and France) between 2008 and 2012 (Cini et al. 2012, Lee et al. 2012). As of 2015, it has been detected on every continent except Antarctica and Australia (Asplen et al. 2015).

Morphological Descriptions and Life History. *Drosophila suzukii* eggs are translucent milky white (Walsh et al. 2011). The egg is glossy and grows increasingly transparent as the embryos develop (Kanzawa 1939). Eggs are an average 0.62 mm long and 0.18 mm wide (Kanzawa 1939), and typically hatch in ~1.4 days at 22 ºC (Emiljanowicz et al. 2014). White larvae have distinctive black mouthparts clearly visible in their head. There are three instars, which grow up to an average length of 3.94 mm and width of 0.88 mm. Larval development typically takes ~6 days at 22ºC, ending in pupariation (Emiljanowicz et al. 2014). Puparia range in color from a light brown to dark amber and have two protruding respiratory horns, which can be used to distinguish *D. suzukii* from

other co-occurring species. Adults emerge in about 6 days and may live an average of 86 days under optimal conditions in the laboratory (Emiljanowicz et al. 2014).

Adult *D. suzukii* are small (2-3 mm) flies with red eyes, have a light brown to amber colored thorax and possess a light brown abdomen with darker brown to blackish stripes (Kanzawa 1939, Walsh et al. 2011). Females and males are sexually dimorphic, with the males possessing a dark spot on the leading edge of each wing. Males are also generally smaller in size than females and possess two sets of tarsal combs which can be seen under a dissecting microscope. Females can also be distinguished from males and other drosophilids by the very large sclerotized and serrated ovipositor (Hauser 2011). Females are very fecund, producing an average of 5.7 eggs per day over a ten day period in the laboratory, with mean total lifetime production of 635 eggs (Emiljanowicz et al. 2014).

The overwintering physiology of *D. suzukii* is still being investigated; however, it is believed that males and females overwinter as adults (Hoffmann et al. 2003, Strachan et al. 2011). Adult flies disperse from cultivated crops into wooded areas in the fall and may overwinter in man-made structures and forested areas around host plants (Kanzawa 1939). It is also believed that *D. suzukii* overwinters as a winter morph, which is larger and darker than the summer form of the fly (Stephens et al. 2015, Shearer 2016). These winter-morphs have larger bodies and wing lengths as well as darker coloration when compared to summer-morphs (Vonlanthen et al. 2016, Wallingford and Loeb 2016). It has been suggested that *D. suzukii* may not survive extremely cold winters, however in its native range in Japan minimum temperatures usually range from -4º to -12º C (Kimura 2004). Emergence of adults from overwintering habitats may begin as early as April or in May, when temperatures exceed 17º C and wild host plants begin to bloom (Mitsui and Kimura 2010, Dalton et al. 2011, Lee et al. 2015). Upon emergence, adults forage among and oviposit within flowering and fruiting wild host plants (Tochen et al. 2016). This gives female *D. suzukii* time to acquire nutrients for reproduction. Females also need time upon emerging to become reproductively active. This delay in oviposition is due to their reproductive diapause from the winter aestivation (Stratchan et al. 2011). Dispersion from wild hosts to cultivated fruit crops depends upon the maturity and phenological state of cultivated fruit. Once female flies are capable of ovipositing in

fruit, growers must begin implementing management strategies to prevent economic losses from it.

Pest Status and Host Plants in the Mid-Atlantic Region. Since its invasion of the Mid-Atlantic region in 2010 and 2011, *D. suzukii* has become a significant pest of small fruits and soft-fleshed tree fruits (Walsh et al. 2011, Burrack et al. 2012). Potential economic loss due to this pest in the top five small fruit crops produced on the Pacific Coast of the U.S., assuming 20% loss, was $511 million (Bolda et al. 2010). Some wine grape producers estimated losses above 80% for some varieties in which *D. suzukii* was detected (Carrington King personal communication 2012). It is thought that this pest overwinters in areas around potential host plants and thus is seen in the same areas year to year, increasing crop management costs through additional insecticidal sprays needed to control its populations (Harris et al. 2014). These increased management costs and crop losses vary year to year, as do trapping numbers from infested production areas.

Drosophila suzukii females oviposit directly in fruit and may be capable of spreading secondary pathogens such as *Acetobacter* spp. and *Hanseniaspora uvarum* (Niehaus), which are associated with *D. suzukii* (Hamby et al. 2012, Chandler et al. 2014, Ioriatti et al. 2015, Rombaut et al. 2017). Larval feeding injury on fruit manifests as depressions or deformations on the fruit surface (Goodhue et al. 2011). Larvae then quickly liquefy infested berry crops, such as raspberries and blueberries (*Vaccinium* spp.), and cause large areas of necrotic tissue in cherries that make the fruit unmarketable. The larvae also decrease the quality of processing fruit (Walton et al. 2016). *Drosophila suzukii* oviposition attempts, even if unsuccessful in depositing eggs into grapes will wound fruit (Attellah et al. 2014). Damaged fruit flesh is at increased risk of secondary pathogen infection. If only a few grapes in a cluster are infected with secondary pathogens, such as yeasts and bacterial sour rot, the whole cluster may be culled (Sharon Horton personal communication). Fruit and grape growers are economically affected when secondary pathogens are present in the fruit production area, due to increased handling cost to cull affected fruit by hand in the field, as well as the monetary loss of the affected fruit.

Biological and ecological factors underlying the great economic impact of *D. suzukii* has on numerous fruit crops in the US include: 1) it is highly polyphagous, 2) it is a season-long, annual pest, 3) females are highly fecund, 4) it exhibits multivoltinism and 5) flies are able to utilize unripe, ripening, ripe, and even rotten fruit for oviposition, with greatest risk to ripe fruit. Lee et al. (2015) reported that *D. suzukii* utilizes > 60 cultivated and wild host plants and is not bound by the availability of cultivated hosts. This makes *D. suzukii* particularly difficult to control because it can be found in unmanaged wooded areas adjacent to intensively managed cultivated crops in which insecticidal sprays are used to control pests (Klick et al. 2016). In the spring, adult flies exploit flowering and fruiting wild host plants around Virginia vineyards such as tartarian honeysuckle (*Lonicera tatarica* L.) and bird cherry trees (*Prunus avium* L.) for food and oviposition sites (Shrader, Ch. 2). Other host plants include wild blackberries (*Rubus* spp.), mulberries (*Morus nigra* L.), and American pokeweed (*Phytolacca americana* L.), (Lee et al. 2015). Unmanaged wooded areas act as reservoirs and allow for populations of this fly to increase unchecked. This also increases the risk of season-long invasion into nearby cultivated small fruit crop hosts.

Once cultivated crops begin to ripen, flies immigrate into them. Early-maturing cultivated hosts such as cherries are utilized first, flies then move into other fruiting plants as they ripen. Late-season fruit, including grapes, are particularly at risk because by that time population levels of *D. suzukii* have increased to very high levels.

Monitoring *D. suzukii* in Small Fruit and Grape Plantings. Many baits have been suggested for use in *D. suzukii* monitoring traps, including various types of wine, vinegars, and combinations of these materials (Landolt et al. 2012, Grassi et al. 2015). The addition of sugars and other fermented materials such as yeasts and bread have also been evaluated in traps. Various trap designs (cups, sticky cards and domes) have also been assessed (Lee et al. 2012, Lee et al. 2013). None of these monitoring systems are selective for *D. suzukii*, but a four-component chemical lure for *D. suzukii* was found to reduce non-target fly catch by 37.2 – 84.7% (Cha et al. 2015), but there is still no standardized trapping and monitoring methodology for this pest, despite the availability of several commercial systems (Walton et al. 2016, Zerulla et al. 2016). An effective

monitoring tool should help growers to understand the phenology of the target pest, predict population events, and aid in timing of control actions, however that has not yet been the case for *D. suzukii* (Landolt et al. 2012, Lee et al. 2012, Lee et al. 2013, Walton et al. 2016). Trapping systems for detecting *D. suzukii* are expensive and time consuming to deploy, monitor, and service. The costs associated with trapping are high, with little to no quantitative information about infestation levels in the cultivated crop gained from the numbers of flies captured (Lee et al. 2012). Fly captures vary depending upon the bait used and the type of cultivated crop (Dalton et al. 2011). It is suggested that growers use trapping merely as a qualitative measure of pest presence, since population numbers cannot be estimated by trapping, thus no insecticidal spray management strategies can be implemented based on the number of flies captured (Walton et al. 2016). In some instances, trapping to determine the presence of *D. suzukii* in the field has not been effective at warning growers about an imminent *D. suzukii* attack because traps are not competitive with ripening fruit (Wiman et al. 2014). Additionally, direct sampling of fruit (i.e. salt or sugar flotation) for its presence has limited utility beyond determining presence because there is no established protocol for the number of fruit to be sampled to estimate population levels within the field. Also, by the time larvae are detected in fruit, the whole berry crop may be infested and thus not marketable (Walton et al 2016). Ongoing efforts are focusing on the spatial analysis of *D. suzukii* populations in the field, lure formulation, and standardizing monitoring tools that will allow their more practical use by growers and crop scouts to combat this pest.

Managing *D. suzukii* in Small Fruit and Grape Plantings. Following the introduction of *D. suzukii* in the U.S. in 2008, and in the absence of non-chemical biological or cultural management strategies, the initial response of small fruit and cherry growers to this invasive pest was to increase insecticide-based control tactics. Due to the short generation time and high value of the crops attacked, growers spray at 4- to 7-day intervals in small fruits (Beers et al. 2011, Bruck et al. 2011). This resulted in an increase in insecticide use of 4.8-fold in cherries, 3.5-fold in raspberries and 1.2-fold in strawberries from 2007 to 2012 in the Western U.S. (Steenwyk and Bolda 2015). The reliance on insecticidal sprays to control *D. suzukii* has had a substantial impact on the fruit industry; 1) growers have a limited number of effective insecticidal spray options

based on mode of action and pre harvest interval, 2) potential insecticide resistance development, 3) potential secondary pest outbreaks via disruption of biological control, 4) insecticide residues may exceed MRLs, interfering with international marketing of fruit and, 5) the increased cost of spray applications (Bruck et al. 2011, Walsh et al. 2011, Steenwyk and Bolda 2015, WHO/FAO 2016.). Insecticides targeting adult *D. suzukii* have mainly been organophosphates, carbamates, pyrethroids, and spinosyns (Beers et al. 2011, Bruck et al. 2011, Cini et al. 2012). Chemicals applied in the west coast tend to have longer residual activity (5-14 days) because there is less rainfall than in eastern states, where their residual activity is < 7 days for some chemicals (Bruck et al. 2011, Diepenbrock et al. 2016, Diepenbrock et al. 2017). Gautam et al. (2016) showed that rainfall reduced the mortality of *D. suzukii* exposed to sprayed blueberry fruit and foliage at 1 day-after-treatment. Rain fastness was improved with the addition of an adjuvant, which helped increase *D. suzukii* mortality exposed to treated blueberry fruit and foliage. There are currently few insecticides that target larvae within the fruit (Beers et al. 2011, Bruck et al. 2011), and few efficacious organic chemicals, for which the organic fruit production areas has been disproportionately affected by *D. suzukii*.

Many of the most effective insecticides for *D. suzukii* management have broad-spectrum effects on arthropods and their increased use in fruit production has significantly disrupted IPM programs (Steenwyk and Bolda 2015). Miticide applications have increased because insect and mite predators of phytophagous mites have been killed. IPM tactics for *D. suzukii*, including harvesting in a timely manner (Lee et al. 2013), physical exclusion of *D. suzukii* with fine mesh netting (Grassi and Pallaoro 2012), and removing infested fruit by hand before harvesting or spraying have been implemented in some systems but have not replaced the need for insecticide applications. All of these methods have increased management costs associated with them, which may make several of them too expensive to implement. Efforts to find a non-chemical control option for growers are on-going and may offer a more affordable approach than other control options.

In its native range in Japan, *D. suzukii* is attacked by several biological agents including both larval and pupal parasitoids (Mitsui et al. 2007). However, these parasitoids are generalists and do not specifically target *D. suzukii*. Some common larval

parasitoids in Japan include *Asobara*, *Leptopilina,* and *Ganaspis* spp., while the parasitoids *Trichopria* spp. and *Pachycrepoideus vindemiae* (Rondani) (Mitsui et al. 2007) attack pupae. Research to identify a parasitoid that would specifically target *D. suzukii* has revealed that most of the parasitoids tested did not complete development on this pest. Only seven of the 15 candidate parasitoids native to the U.S. successfully developed on *D. suzukii* (Kacsoh and Schlenke 2012). This low success rate was due to the flies' innate immune response of encapsulating hymenopteran eggs (Kacsoh and Schlenke 2012). Of the seven parasitoids that successfully developed in *D. suzukii*, *Asobara japonica* (Foerster) showed the most promise due to a high female to male egg ratio and its ability to establish in a wide range of climates (Mitsui et al. 2007). *Asobara japonica* also laid three times more eggs in *D. suzukii* larva than in *D. melanogaster* (Meigen). A study in Europe examined the parasitization abilities of three larval and two pupal parasitoids (Chabert et al. 2012). Only the two pupal parasitoids, *Trichopria* sp. and *P. vindemiae* successfully parasitized *D. suzukii* pupae. Rossi-Stacconi et al. (2013, 2015) confirmed the ability of *Trichopria* sp. and *P. vindemiae* to parasitize *D. suzukii* in Italy and also showed that *L. heterotoma* was able to complete its life cycle and emerge from the fly, but only under laboratory conditions. Wang et al. 2016, demonstrated that *Trichopria drosophilae* (Perkins) did not discriminate against young or old *D. suzukii* pupae, but developmental time increased as pupal age increased. This is important due to the overlap of generations seen with *Drosophila* field populations. However, the only wasp species that could parasitize and complete development to the adult stage within *D. suzukii* at a rate that would possibly affect the fly's population levels was an undescribed species of *Ganaspis* (sp. 1) from Florida and Hawaii (Kacsoh and Schlenke 2012). It is believed that the impact of parasitoids currently occurring in the United States on *D. suzukii* population levels will be limited, based upon sentinel trapping data (Wahls 2016).

***Zaprionus indianus* Gupta.** The African fig fly (AFF), *Zapriounus indianus* Gupta (Diptera: Drosophilidae), is native to Africa, the Middle East and southern Eurasia (Gupta 1970) and was first detected in Sao Paulo, Brazil, in 1999 where it became a pest on figs (Vilela 1999). It was first detected in the U.S. in 2005 (Florida), has spread rapidly, and has now been detected throughout much of North America (Steck 2005, van

der Linde et al. 2006, Biddinger et al. 2012, van der Linde 2013). *Zaprionus indianus* has a wide host range and has been reported on oranges, peaches, raspberries and strawberries (Santos et al. 2003, van der Linde et al. 2006, Biddinger et al. 2012). This makes *Z. indianus* a concern to the small fruit and tropical fruit crop growers; however, it is unclear if it is a primary or secondary pest. *Zaprionus indianus* has been reared from fallen fruit and ripe fruit harvested directly from trees, but it may not be capable of ovipositing in intact ripening fruit on the tree (Steck 2005).

Zaprionus indianus can readily utilize *D. suzukii* oviposition wounds, bird damaged or cracked fruit to lay eggs, develop and emerge as adults. It is highly fecund and the eggs and pupae can survive very high temperatures, which allow it to colonize several types of habitats (Ramniwas et al. 2012). Larval *Z. indianus* use physical aggression and habitat destruction to outcompete other drosophilid species within a food resource (Gilpin 1974). This interspecies competition may play a role in its ability to utilize an extensive host plant range and ecological habitats including Virginia vineyards. In 2012, *Z. indianus* was identified in Petit Verdot grapes in a Virginia vineyard in the Piedmont region and has been detected in several Virginia vineyards annually since then.

***Drosophila* Larval Competition.** *Drosophila* larvae compete within a food source, which can impact larval development since this is the most susceptible stage to resource limitation (Bakker 1961, Miller 1964, Gilpin 1974, Roper et al. 1996, Shiotsuga et al. 1997). Competition between phytophagous insects, such as *Drosophila*, has been documented at the larval stage of development (Case and Gilpin 1974, Gilpin et al. 1986). Interspecific competition may involve a direct behavioral response by one species to another. This behavioral response may negatively impact the subset of competing individuals through physical aggression or predation on juveniles. Larval developmental stage had a significant effect on survivorship and nutrient sequestration through physical competition in *Drosophila*. Gilpin (1974) demonstrated that 2-day-old larvae (L2) had a competitive advantage over newly-hatched larvae (L1); the larger larvae developed faster and showed lower mortality. The larger larvae prevented smaller larvae from feeding by using their bodies as physical barriers and pinning the smaller larvae in the food medium.

Further competition between larvae involves limiting the use of or depleting resources through indirect means such as habitat destruction, consuming of nutrient sources or metabolic poisons. Gilpin (1974) demonstrated that third instar *Drosophila* larvae caused food medium liquidation when maneuvering within the medium, which may drown smaller larvae. Habitat destruction occurred after the larger larvae consumed the food; third instar larvae defecated and deposited harmful metabolites at the surface of the medium where the first and second instars were confined. These poisonous metabolites negatively impacted the development of younger larvae. Developmental time and larval mortality were increased when larval growth took place in culture medium that was previously used by larvae of the same or differing species (Weisbrot 1966, Dawood and Strickberger 1969, Budnik 2001).

The effects of density and competition on individual fitness vary considerably among *Drosophila* species. Budnik et al. (2001) demonstrated that interspecific larval competition may increase or decrease the viability of one or both species within a nutrient source. 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 life span (Santos et al. 1992, Rodriguez et al. 1999, Werenkraut et al. 2008). Budnik and Brncic (1974) established the effects of intraspecific competition, describing the egg-to-adult viability of *Drosophila willistoni* (Sturtevant) larvae that were negatively affected by metabolites in food medium previously used by *Drosophila pavani* (Brncic). This experiment showed that metabolic waste products of the first species likely interfered with the development of the second species. Werenkraut et al. (2008) further demonstrated that *Drosophila buzzatii* (Patterson and Wheeler) and *Drosophila koepferae* (Fontdevila and Wasserman) had increased developmental times, smaller body mass and lower viability when reared with interspecific competitors. *Drosophila melanogaster*, to overcome competition pressure, showed prolonged or arrested larval development at high interspecific competition levels, while *Drosophila subobscura* (Collin) had a decrease in pupal volume, but not an increase of developmental time at high densities (Miller 1964, Gonzalalez-Candelas et al. 1990).

Intraspecific competition can also affect development; when *Z. indianus* was reared at high larval densities (30 + per tube), the developmental time increased while survivorship and body mass decreased (Amoudi et al. 1993). Takahashi and Kimura (2005) verified that per capita egg production decreased in female *D. simulans* (Sturtevant), *D. suzukii*, *D. auraria* (Peng), *D. rufa* (Kikkawa and Peng) and *D. immigrans* (Sturtevant) that were under interspecific competition as larvae at high densities. Jones et al. (1996) confirmed that intraspecific competition at high densities decreased pupal volume in *D. subobscura*, resulting in females with fewer eggs in their ovaries and also demonstrated that larval mortality was density-dependent, with higher populations resulting in increased mortality of *D. subobscura*.

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 while feeding on low-protein hosts (Begon 1983, Hardin et al. 2015). This may also allow *D. suzukii* larvae a chance to develop alone, so that *D. suzukii* larvae are larger than larvae of other species that may develop after the first act of oviposition. The quality of the nutrient substrate may also impact the development and survival of *Drosophila* within the medium. Hardin et al. (2015) demonstrated that *D. suzukii* showed increased mortality when the nutrient value of the medium decreased at high population densities. 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). Larval competition as well as nutrient profiles of host plants may be important when considering population dynamics within specific host crops. This host plant suitability and larval competition are especially important in mixed crop production, where one host may be preferred over another, based on nutrient potential. The more nutrient rich host crop may be utilized first if both plants are ripening simultaneously.

***Drosophila* Cannibalism.** Cannibalism has ecological implications by influencing population dynamics and stability, especially if it occurs in the form of interspecific

competition (Richardson et al. 2010, Crump 1986). Cannibalism within *Drosophila* has been described as being due to intense competition within a resource that is often density-dependent. Cannibalism is most often seen in intraguild competition in a medium where smaller larvae attack and feed upon older, slower-moving larvae (Bhattacharyya 2014). This cannibalistic behavior may be an adaption in response to situations where there is little nutrient value within a medium or host plant (Vijendravarma et al. 2013). Both predation and cannibalism may be important when addressing invasive species ecology in landscapes such as Virginia vineyards, where both *D. suzukii* and *Z. indianus* are spatially and temporally sympatric.

Wine Grape Production in Virginia. Wine grapes are an economically important crop in Virginia, which was the nation's fifth largest wine grape producer in 2012, with 1,366 ha in production. A 2010 economic impact study estimated that the Virginia wine industry employs more than 4,700 people and contributes almost $750 million to the Virginia economy on an annual basis (Frank et al. 2012). In 2013, Virginia ranked fifth in the number of wineries nationwide, with more than 222 in the Commonwealth (U.S. Winery Database 2013). More than 1.6 million tourists visited Virginia wineries in 2013, according to the Virginia Tourism Corporation and Virginia was in the top 10 wine tasting destinations in 2012.

Wine grapes grown in Mid-Atlantic vineyards may be at greater risk of *D. suzukii* oviposition, than small fruit and grapes grown on the west coast, because the environmental conditions in this region are more suitable for *D. suzukii* survival, population growth and dispersal (Damus 2009). Climex models compared the United States climate to *D. suzukii* home range in Asia and the map generated indicated that the eastern half of the US had "optimal" conditions for this pest to complete its life cycle (Damus 2009). The risk of *D. suzukii* fruit infestation is also higher in the Mid-Atlantic region than the west coast of the US because chemical residual activity is shorter (Bruck et al. 2011). Season-long rainfall reduces chemical success in the fields with efficacy decreasing when residues are washed off grapes by rain (Van Timmerman and Isaacs 2014).

Petit Manseng, Petit Verdot, Vidal, Cabernet Franc, and Viognier are extensively grown throughout Virginia producing 162, 384, 579, 869 and 457 metric tons, respectively in 2013 and 206, 502, 741, 964 and 366 metric tons produced, respectively, in 2014 (Wolf 2014). Pinotage is a less common variety (<12 tons per year). Petit Verdot, Cabernet Franc and Pinotage are red varieties while Petit Manseng, Vidal Blanc and Viognier are white varieties. Pinotage, Cabernet Franc and Viognier have tightly bound clusters; whereas Petit Manseng, Vidal Blanc and Petit Verdot have loosely bound clusters with spacing between each individual grape.

Pest Infestation Risk Assessment. It may be possible to determine if a crop production area is likely to experience pest infestations by analyzing the abiotic and biotic factors associated with that growing region. Probable habitat ranges for invasive insect pests have been identified through the use of climate imaging software such as CLIMAX. Habitats that have comparable climatic temperatures and precipitation of the pest's native range may be suitable for invasive pests such as *D. suzukii* in Virginia vineyards. Therefore, these areas are at higher risk for infestation than those habitats that do not fit the climatic home range data of *D. suzukii* (Alspen et al. 2015). It may be possible to determine the likelihood of pest infestation within a field based upon the amount of plant materials surrounding the field (Weber et al. 1990). The availability of host plants within a production area may also help determine risk for pest infestation (Lee et al. 2015, Kenis et al. 2016). Production areas that have several host plants present in large numbers may be at higher risk than those with few host plants. Monitoring pest populations through effective trapping protocols will allow growers to determine if *D. suzukii* is in the area (Lee et al 2012, Burrack et al. 2015). This will allow growers to determine if their field are at risk of D. suzukii infestation and make informed decision on spraying based upon the presence of absence of the pest within certain grape cultivars. Certain berry and grape cultivars may be more susceptible and at higher risk of infestation based upon the morphology of the fruit (Burrack et al. 2012, Ioratti et al. 2015). Grapes and berries with greater penetration forces may avoid *D. suzukii* oviposition, however those with low penetration forces (< 15 cN) may be at risk for *D. suzukii* infestation. Pest population levels and risk of infestations within the fruit may be influenced by other insect species

within the same habitat, since competition for the same resources may impact one species over the other (Shrader Ch. 4). By analyzing these factors, it may be possible to determine risk of *D. suzukii* infestation within Virginia vineyards.

Research Justification

When developing a pest management strategy for a new invasive species it is important to determine the host plants with which the pest is associated. With a highly polyphagous drosophilid pest, different host plants may have a broad range of effects on the insect, including developmental time, reproductive rates, and survivorship. The availability of cultivated and wild host plants may determine the geographic range and the availability of wild host plants has important implications for understanding the susceptibility of economic crops to *D. suzukii* attack. Furthermore, it may be possible to determine a risk analysis for *D. suzukii* infestations based upon the host plants available, the time of year they are utilized, and the phenology of economic crop hosts. The availability of host plants adjacent to cultivated crops may allow for harborage from sprays and facilitate season-long re-infestation. In Chapter 2, the results of experiments pertaining to the suitability of vegetative and reproductive plant tissue from several wild host plants collected from four different vineyards are reported. Once the most prevalent wild host plants are determined in these vineyards, the removal of these plants from the landscape may play a role in *D. suzukii* population dynamics. By removing these wild host plants from the area, especially those used in early spring when populations are increasing, the *D. suzukii* population around the vineyard may remain low due to the unavailability of nutrient and ovipositional resources. It may also be possible to use these wild host plants as trap crops and then spray the plants once flies are detected around these host plants.

The ability to monitor fly populations and determine when *D. suzukii* is present within a crop is the cornerstone of pest management. It is imperative to be able to capture flies in the presence of ripening fruit, which may be more attractive than trapping baits (Lee et al. 2012, Burrack et al. 2015). A selective trap for *D. suzukii* would help with the correct identification of this pest, so proper management tactics can be taken. Insecticide sprays targeting *D. suzukii* should be planned only when the fly is present in the field as determined by monitoring tools. Chapter 3 reports experiments that evaluated

the efficacy and selectivity of several trapping baits and trap designs for capturing *D. suzukii* in Virginia vineyards.

The ability of *D. suzukii* to oviposit in fruit appears to be based upon the firmness of the fruit, as documented through penetration force analysis (Burrack et al. 2013, Ioratti et al. 2015). However, the physical properties of wine grapes and *D. suzukii* ovipositional preference, particularly those grown in the eastern United States, have not been extensively studied. Wine grape cultivars have a wide range of physiological and morphological characteristics that may make oviposition in certain cultivars more difficult for *D. suzukii*. Chapter 4 reports experiments that evaluated the penetration force, skin thickness, and degrees Brix using a wine grape susceptibility bioassay. Survivorship from each of the six varieties tested was also recorded. The relative vulnerability of grapes to *D. suzukii* oviposition at varying points in the growing season may have important implications for management strategies, especially for those varieties that mature early in the growing season when *D. suzukii* are moving into the vineyard from the surrounding wild host plants.

Interspecies competition may play a role in *D. suzukii* population dynamics within Virginia vineyards if they utilize grapes co-infested with *Zaprionus indianus*. *Drosophila* competition has been known to increase developmental times and mortality of larvae within a nutrient source. Petit Verdot grape clusters infested with fly larvae were collected from the field in 2012 and 90% of the flies reared from the cluster were *Z. indianus*. In Chapter 5, competition of these two drosophilid species within grapes was quantified for four grape varieties as well as several larval densities. The developmental time, pupal volume and survivorship was recorded for *D. suzukii* to determine the impact of *Z. indianus* larvae within grapes. *D. suzukii* populations may be suppressed if *Z. indianus* negatively impacts growth or increases mortality of the larvae within the grapes.

Research Objectives

Objective 1. Identify the host plant species used for oviposition by *D. suzukii* as well as the seasonal availability of these plants around four Virginia vineyards.

Objective 2. Evaluate and compare the efficacy and selectivity of several homemade and

commercially available baits and trap designs for monitoring *D. suzukii* in Virginia vineyards.

Objective 3. Identify the morphological and physiological characteristics associated with differences in the susceptibility of wine grapes to *D. suzukii* oviposition.

Objective 4. Determine the ovipositional preference and survivorship of *D. suzukii* larvae in red and white wine grape varieties.

Objective 5. Determine the developmental impact of *Z. indianus* larvae on *D. suzukii* larvae in wine grape varieties at several larval densities.

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Chapter 2: Wild and Ornamental Host Plant Survey for *D. suzukii* Adults in Four Virginia Vineyards

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Abstract

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is an invasive, highly polyphagous pest of berries, cherries and grapes. *Drosophila suzukii* is also known to utilize a wide range of ornamental and unmanaged wild host plants. These host plants may be found adjacent to vineyards, posing a season-long threat to grapes. To determine the most common host plants for *D. suzukii* near our vineyards, I surveyed four vineyards in the Piedmont region of Virginia. In 2013, I also deployed traps to determine when *D. suzukii* was active in the vineyard. Collections of fruiting bodies as well as flowers and ovaries were made for two years with plants sampled May – October in 2013, and June – September in 2014. Plants were collected from the unmanaged woodland and grassland edges around vineyards. I sampled 48 plant species representing 24 plant families and identified six *D. suzukii* host plants. The seasonal availability of these host plants was also recorded and there were host plants available to *D. suzukii* throughout the grape ripening period. *Drosophila suzukii* were detected in wild host plants before they were detected in traps within the vineyard. The rate of occurrence analysis for *D. suzukii*-positive host plant samples allowed me to determine the prevalence and importance of these six host plants in areas surrounding the vineyards. By understanding host plant use, it may be possible to influence *D. suzukii* populations through the removal of host plants, or develop a *D. suzukii* vineyard risk assessment based upon the prevalence and abundance of these six host plants in the landscape.

Keywords: host plant survey, *D. suzukii*, wild hosts, vineyards, grapes

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is an invasive frugivore native to eastern Asia, which attacks and injures several cultivated host plants including stone fruits, small fruits and grapes (Cini et al. 2012, Lee et al. 2012). This pest causes severe economic harm through the loss of crop yield, increased management costs due to spraying, fruit culling and possible rejection of fruit by wholesale markets if larvae are discovered within the fruit (Goodhue et al. 2011, Walsh et al. 2011). In addition to cultivated crops *D. suzukii* also utilizes non-cultivated wild host plants for food and oviposition sites when preferred cultivated crops are not available. Some wild hosts include wild caneberries (*Rubus* spp.), pokeweed, wild rose hips and several other wild and ornamental fruiting plants (Lee et al. 2015, Kenis et al. 2016). Although some of the alternative host plants have been identified, the nutrient sources for *D. suzukii* in the spring and early summer have yet to be fully investigated and alternative host plants may differ by geographic location (Hauser 2011). It may be possible to determine if a vineyard is at a higher risk of *D. suzukii* infestation based upon the presence or absence of specific host plants.

Cultivated fruits that ripen in the late summer may be at a higher risk of *D. suzukii* infestation because fly populations may have increased in neighboring wild host plants and immigrated into cultivated crops. If prominent wild and non-cultivated host plants could be identified that were responsible for sustaining and amplifying the *D. suzukii* population in the spring, they could be removed from the cropping landscape which may slow fly population growth. The abundance and overall biomass of weeds and potential host plants around sweet corn (*Zea mays* L.) fields increased European corn borer (*Ostrinia nubilalis* (Hübner)) populations within the corn, thus decreasing yields, whereas weed free fields decreased European corn borer populations and increased yields (Weber et al. 1990). Habitats directly surrounding vineyards may also play a role in *D. suzukii* population dynamics especially when it comes to overwinter survival and early spring population dynamics. Adults may avoid extremely cold winter temperatures by hiding in sheltered areas such as unmanaged field edges around cultivated crops (Stephens et al. 2015, Jakobs et al. 2015). *Drosophila suzukii* acquires sugars from alternative host plants during the winter months using sap from oak trees and may use spring flowers such as

cherry blossoms as a food source when other nutrient sources are unavailable (Kansawa 1939, Tochen 2016). These early flowering spring plants would be especially important when overwintering populations are low and when flies are in search of nourishment and oviposition sites. Furthermore, host plants that are identified throughout the growing season could be removed, where practical, as an attempt to remove oviposition sites from the immediate vicinity of surrounding cultivated fruit crops (Lee et al. 2015). Alternative host plants are also important when cultivated crops are sprayed, as they may provide refugia for *D. suzukii*. Once sprays have dissipated, flies can then move back into the crop. Determining the risk potential for a vineyard based upon surrounding vegetation may impact management strategies. A vineyard with few *D. suzukii* host plants maybe at a lower risk of infestation depending upon the prevalence of wild and ornamental host plant species and the rate of occurrence of *D. suzukii* positive samples found in Virginia vineyards. Our hypothesis is that there will be no *D. suzukii*-positive host plants surrounding the four Virginian vineyards. This project was undertaken to ascertain what wild and ornamental host plants *D. suzukii* uses and the seasonal availability of these plants in woodlands and fields adjacent to four geographically diverse vineyards in Virginia.

Materials and Methods

Vineyard locations. Plants were sampled from four vineyards in the Piedmont region of Virginia; one vineyard each in Amherst, Amherst Co. (Site 1), Tyro, Nelson Co. (Site 2), Crozet, Albemarle Co. (Site 3), and in Gordonsville, Orange Co. (Site 4) during 2013 and 2014 (Table 2.1). Site 1 was a small vineyard (< 2 ha) located in a forest clearing surrounded by deciduous woods. Site 2 was a large vineyard (> 15 ha) adjacent to apple production, with deciduous woods on two sides of the vineyard and grasslands on the other two sides, Site 3 was a small vineyard with deciduous woods on one side and pasture land on the other 3 sides. Finally, Site 4 was a large vineyard with an adjacent field of soybean or corn on two sides, grassland and patchy deciduous wooded areas on the other sides. Geographic analysis of each vineyard was performed using satellite-derived land cover imagery from the ESRI; World Boundaries and Places, and World Imagery layers and were imported into ArcGIS version 10.1 software (ESRI, Redlands,

CA). The composition of the land around each field site was calculated within a 500-meter diameter circle radiating from a central point within the vineyard. The location, land type, and grape acreage as well as GPS location coordinates for each of the four vineyards were quantified (Table 2.1).

***Drosophila suzukii* monitoring**. In 2013, to determine when *D. suzukii* adults were present, apple cider vinegar baited (ACV) monitoring traps were placed in each of the four vineyards. Four traps consisting of a clear 1-liter plastic deli cup (model: TD41032-A01, Solo, Urbana, IL) with eight 7-mm diameter holes positioned equidistant around the cup at approximately 2 cm below the rim were placed in each vineyard. A translucent plastic lid was used to cover the top of the cup. The deli cups were filled with 200 ml ACV (5% acidity; Kroger, Cincinnati, OH) and a drop of clear unscented hand soap (Softsoap Advanced Clean Liquid Hand Soap, Colgate-Palmolive, New York, NY) to break the surface tension. Traps were placed on the edge of the vineyard blocks within the first panel of grape vines located immediately next to wooded areas. Traps were hung 10-20 meters from the wooded edge and at least 10m apart. Trapping began on 31 May at Sites 1 and 2 and on 5 June at Sites 3 and 4. Flies were monitored throughout the growing season at all four vineyards until they were collected on 13 September. Traps were checked biweekly and any flies captured were collected, counted and identified in a laboratory at Virginia Tech, Blacksburg, Virginia.

Non-crop plant sample collection. Plant samples around the vineyards were collected from adjacent woodlands or field edges up to 3 meters into the unmanaged boarder. Biweekly plant samples were collected from each location in 2013 and 2014. In 2013, plant samples were collect from 31 May to 18 October and from 1 June to 18 August in 2014. The survey focused on flowering, green, ripening and ripe fruits of wild and ornamental non-crop hosts in numerous habitats. If flowers were present, then 10 or more flower heads were collected per plant. If fruit were present, then at least 10 ripe or ripening fruit per plant were collected. When ovaries were present then 10 or more per plant were collected. Foliage or fruit samples were placed in a plastic quart size bag (Home Sense, Kroger, Cincinnati, OH), labeled with sample location and date and taken

back to Virginia Tech, Blacksburg, Virginia for identification and cataloging. Plant samples were identified using field guides (Dirr 1990, Uva 1997) and with assistance from the Plant Disease Clinic, Virginia Tech. After plant samples were identified the foliage or fruits were placed back into the bags and held in storage containers at room temperature (~24 ºC) in a laboratory. Plant samples were visually inspected each day for 21 days for larvae, pupae or adult flies. Sample bags were opened daily to allow fresh air into the bags. Plant foliage was manipulated within the bags to look for pupa, the bags were then resealed and replaced onto the laboratory bench. The 21-day observation period should have been long enough for pupae or adults to emerge from the plant samples (Emiljanowicz et al. 2014, Lee et al. 2015).

Statistical Analysis. The rate of occurrence indicates the probability that a *D. suzukii* host plant sample, at any of the four vineyard locations, will give rise to a *D. suzukii* adult. This was calculated as the number of sites where a plant sample gave rise to *D. suzukii* adult multiplied by the number of years' the plant was sampled from that location, divided by all sites and year combinations where the fruit was collected (adapted from Kenis et al. 2016). Only plant species that yielded emerged *D. suzukii* adults were used in this analysis.

Results

***Drosophila suzukii* monitoring**. In 2013, *D. suzukii* were caught on 8 July at site 4, 16 July at site 1, 5 August at site 3 and 15 August at site 2. Traps in all vineyards yielded *D. suzukii*, but total captures were low. Traps at site 1 captured 8 *D. suzukii*, 32 were captured at site 3, 32 captured at site 4, and a single fly was captured at site 3 for the whole plant sampling period. This suggests that the majority of *D. suzukii* appeared in the vineyard between July and August, and none were caught in early summer (June).

Non-crop plant species positive for *D. suzukii*. In total, 48 plant species representing 24 plant families were collected around four vineyard sites (Table 2.2). In 2013, over 390 plant samples were collected from May to mid-October, when frost occurred. Host plants came from three families: Rosaceae, Phytolaccaceae, and Caprifoliaceae. Specific plants

that had adult flies emerge were wild blackberry (*Rubus* spp. L.), mock strawberry (*Duchesnea indica* Theodor Wolf), pokeweed (*Phytolacca americana* L.), Japanese honey suckle (*Lonicera japonica* Thunberg), bird cherry (*Prunus avium* L.), and tatarian honeysuckle (*Lonicera tatarica* L.). This is the first report of mock strawberry (*D. indica*) as a host of *D. suzukii* in the field (Lee et al. 2015, Kenis et al. 2016).

In 2014, over 590 plant samples were collected from May to September, when grapes were harvested. Host plant were from the same three families as during 2013, Rosaceae, Phytolaccaceae, Caprifoliaceae. Species from which adult *D. suzukii* emerged were wild blackberry *(Rubus* spp.), wild cherry, pokeweed, Japanese honeysuckle, and tatarian honeysuckle (Table 2.2).

The rate of occurrence for all species from which *D. suzukii* emerged from field samples is presented in Figure 2.1. *D. suzukii* emerged from the berries of tatarian honeysuckle and wild caneberries at 100% of the sites in all years. In contrast, Japanese honeysuckle and mock strawberries were occasional host plants, with 33% and 25% rates of occurrence, respectively (Fig. 2.1).

The seasonal patterns of *D. suzukii* emergence from host plants were similar for both years. Early spring host plants included tatarian honeysuckle and caneberries (June and July). Early spring host plant samples collected 26 June in tatarian honeysuckle at site 4 and caneberries collected from site 3 had adult flies emerge, however traps at these locations did not capture *D. suzukii* within the vineyards until 8 July and 5 August, respectively (Table 2.3 and 2.4). Late season host plants (August – October) included pokeweed, Japanese honeysuckle, mock strawberry and wild bird cherry. These host plants yielded *D. suzukii* positive samples during and after August

Discussion

Although *D. suzukii* is highly polyphagous, landscapes immediately surrounding vineyards in this experiment were not diverse and had a limited number of host plants. Of the 48 plant species sampled, only six produced *D. suzukii*. Our field survey identified a newly reported host of *D. suzukii* in the field, *D. indica* (Rosaceae). This species had previously been described as a host plant in laboratory assays but not in the field (Lee et al. 2015, Kenis et al. 2016). Of the six host plants identified around the

vineyards the rate of occurrence in these samples was high with the exception of *D. indica*, which had a rate of 25%. This indicated that if these plants exist in the landscape around vineyards it is probable that *D. suzukii* will use them as oviposition sites, thus increasing the overall risk of *D. suzukii* infestations in areas where these plants are present. Forty-two plant species did not have any *D. suzukii* emerge even though 18 have been reported as hosts in the field or in laboratory trails (Lee et al. 2015, Poyet et al. 2015, Kenis 2016). These negative results may be due to the low number of plant samples from a limited number of collecting sites. Mulberry, a known host of *D. suzukii* was collected from the green / red stage (May) to ripe berries (June) and no *D. suzukii* emerged. This suggested that *D. suzukii* populations in early spring may be too low to detect even when host plants are available.

The seasonality of available host plants around the vineyard is important to investigate since grapes ripen late in the growing season when *D. suzukii* populations have had time to increase in the neighboring habitats. These six host plants that were identified around the vineyards produce fruit annually and none of the fruit make it through the winter in a state that can be consumed by larvae or adult *D. suzukii*. Consequently, *D. suzukii* must find new suitable nutrient sources as well as oviposition sites each season beginning in the spring when they emerge from overwintering habitats. Early flowering plants such as bird cherry and tatarian honeysuckle may provide a nutrient source for these emerging adults early in the spring when other food sources are absent (Tochen et al. 2016). Once oviposition sites become available, such as tatarian honeysuckle and wild caneberries, the *D. suzukii* populations should start to increase. Our data provided evidence that *D. suzukii* uses and emerges from wild host plants early in the season before they are detected in traps in the vineyards.

Caneberries were present at all vineyards. *Drosophila. suzukii* occurrence rates in caneberry fruit were high, and the plants produce fruit over an extended period. This suggests that they are an important wild host plant in spring and early summer. Tatarian honeysuckle also appears to be an important spring fruiting plant, but was only collected at one location. At that location wild caneberries and tatarian honeysuckle were the only spring host plants that yielded *D. suzukii* adults. Pokeweed, Japanese honeysuckle, cherry and mock strawberries supported *D. suzukii* in the late summer and autumn.

These fruits begin to ripen around the same time as grapes (August), so there is the potential to have a large increase in *D. suzukii* populations closely synchronized with grape susceptibility to *D. suzukii* oviposition. After grapes are harvested *D. suzukii* may be utilizing these late season wild host plants as nourishment before selecting overwintering habitats. Pelton et al. (2016) concluded that larger woodland areas around cultivated crops may increase survivorship of overwintering adults and gave rise to earlier emergence of *D. suzukii* in the spring.

By determining if vineyards are at higher risk for *D. suzukii* infestation based upon the incidence and prevalence of these six host plant species, it may be possible to employ cultural integrated pest management strategies to keep population levels low. *Drosophila suzukii* populations may be kept at low numbers through the removal of the known wild host plants immediately surrounding the field margins and edges of the woodlands that are adjacent to vineyards. European corn borer populations were shown to increase in weedy fields and cause more damage to sweet corn field than in weed-free fields (Shurr 1970, Showers et al. 1980, Weber et al. 1990). Furthermore, removal of these hosts may also destroy *D. suzukii* refugia that these flies may use when insecticidal sprays are applied in the vineyards. This may not be feasible in all locations, however this cultural control tactic may have some impact on population levels on a pest in which the only other control strategy available is to apply insecticidal sprays. Pokeweed, wild blackberries, Japanese honeysuckle and tatarian honeysuckle are conspicuous and can be easily mowed when they appear in the landscape. Controlling mock strawberries may prove difficult due to the inconspicuous nature of the plant growing amongst grass, and removing wild bird cherry trees may be economically unfeasible. If early season host plants are removed, the decrease in the initial population, even if slight, may be enough to decrease the overall population later in the season.

Vineyards with the host plants identified in this survey are at higher risk of *D. suzukii* infestations than those vineyards that do not have these plant species. Furthermore, the rate of occurrence for these plants allows for further risk assessment. These four vineyards surveyed and those vineyards throughout Virginia where tatarian honeysuckle and caneberries are present in the landscape are at the highest risk for *D. suzukii* infestations based upon this survey and should be highly scrutinized for *D. suzukii*

when grapes begin to ripen. Future work should focus on evaluating *D. suzukii* populations in vineyards in which wild and ornamental host are unmanaged versus fly populations in fields from which all host plants have been removed. It may also be beneficial to develop a risk assessment of *D. suzukii* population levels based upon the prevalence of wild host plants in a given landscape across several cultivated crops.

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Table 2.1. Geographical location and landscape composition (hectares) surrounding four Virginia vineyards within a 500m diameter circle radiating from a central point at each site.

Site	County Location	GPS Coordinates		Grape Area	Woodlands	Grasslands	Other Agriculture	Pond
1	Amherst County	37.712064,	-79.173887	0.8	72.2	2.9		
2	Nelson County	37.822940,	-79.017156	12.1	36.5	18.4	5.4	2.7
3	Albemarle County	38.051443,	-78.746242	1.52	30.9	31.4		
4	Orange County	38.234451,	-78.102461	23.5	8.5	16.9	22.6	

Table 2.2. Wild and ornamental plant species that were sampled for *Drosophila suzukii* adult emergence in four Virginia vineyards 2013 and 2014.

Family	Species	Number of sites where fruits were sampled* 2013	2014	*D. suzukii* Host
Amaranthaceae				
	Amaranthus viridis L.		1/4	
Anacardiaceae				
	Rhus coriaria L.	2/4		
	Toxicodendron radicans L.	2/4		
Asteraceae				
	Achillea millefolium L.		1/4	
	Ambrosia psilostachya L.	1/4	1/4	
	Bellis perennis L.	1/4	2/4	
	Cirsium vulgare (Savi) Ten.	1/4	1/4	
	Mikania scandens L.		1/4	
	Solidago spp. L.		1/4	
Brasicaceae				
	Brassica rapa L.		1/4	
	Capsella bursa-pastoris L.		1/4	
Caprifoliaceae				
	[1]*Lonicera japonica* Thunb.	3/4	3/4	Yes
	[1]*Lonicera tatarica* L.	1/4	1/4	Yes
	[1]*Sambucus nigra* L.	1/4	1/4	
	[1]*Symphoricarpos orbiculatus* L.	1/4	1/4	
Compositae				
	Snaphalium purpureum L.		1/4	
	Xanthium strumarium L.	1/4	1/4	
Convolvulaceae				
	Calystegia sepium L.	1/4	2/4	
	Ipomoea hederacea Jacq.	1/4	1/4	

Family	Species	Number of sites where fruits were sampled* 2013	2014	*D. suzukii* Host
Cornaceae				
	[1]*Cornus mas* L.	1/4	1/4	
Cupressaceae				
	Juniperus spp. L.	1/4	1/4	
Ericaceae				
	[1]*Vaccinium angustifolium* Aiton	1/4	1/4	
Euphorbiaceae				
	Acalypha virginica L.		1/4	
	Euphorbia dentata Michx.		1/4	
Fabaceae				
	Vicia villosa Roth		1/4	
Lamiaceae				
	Nepeta cataria L.	1/4	1/4	
	Callicarpa dichotoma L.	1/4	1/4	
Malvaceae				
	Abutilon theophrasti Medik.		1/4	
Moraceae				
	[1]*Morus nigra* L.	1/4	1/4	
Oxalidaceae				
	Oxalis stricta L.		1/4	
Passifloraceae				
	Passiflora spp. L.	1/4	1/4	
Phytolaccaceae				
	Phytolacca americana L.	4/4	4/4	Yes
Plantaginaceae				
	Veronica americana Schwein. ex Benth.	1/4		
Polygonaceae				
	Persicaria maculosa S.F.Gray		1/4	

Family	Species	Number of sites where fruits were sampled* 2013	2014	*D. suzukii* Host
Roseaceae				
	[1]*Duchesnea indica* Th. Wolf.	1/4	2/4	Yes
	[1]*Prunus avium* L.	2/4	3/4	Yes
	[1]*Prunus padus* L.	1/4	1/4	
	[1]*Rosa acicularis* Lindl.	3/4	3/4	
	[1]*Rosa rubiginosa* L.	2/4	2/4	
	[1]*Rubus* spp. L.	4/4	4/4	Yes
	[1]*Rubus phoenicolasius* Maxim	1/4	1/4	
Scrophulariaceae				
	Verbascum thapsus L.	1/4	1/4	
Solanaceae				
	Datura stramonium L.	1/4	1/4	
	[1]*Solanum carolinense* L.	2/4	2/4	
	[1]*Solanum nigrum* L.	1/4	1/4	
Vitaceae				
	[1]*Parthenocissus quinquefolia* L.	2/4	2/4	
	[1]*Vitis* spp. L.	2/4	2/4	

[1]Known wild and ornamental host plants of *Drosophila suzukii* based on reviews of Lee et al. 2015, Kenis et al. 2016.
*Number of vineyard sites where plant species was collected / total vineyard sites.

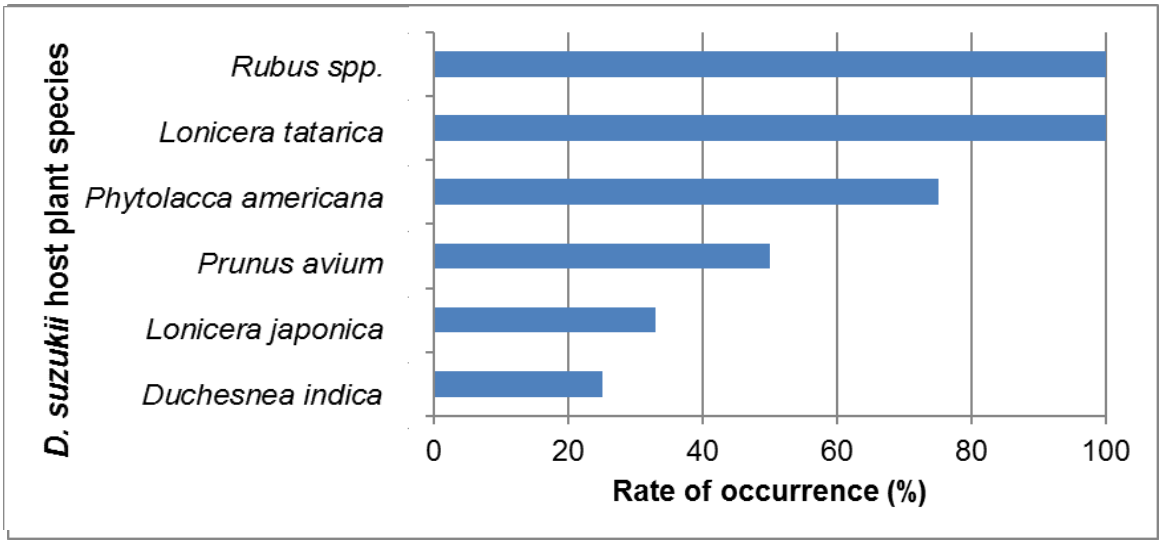

Figure 2.1. Rate of occurrence of *Drosophila suzukii* in the host plants that gave rise to adults over two years at four vineyards.

Table 2.3. Seasonal occurrence, location and plant species sampled that were positive for *Drosophila suzukii* adult emergence in 2013 at four Virginia vineyards.

Foliage Collection Date	[a]Site #	Sample Species
6/26/13	4	Tatarian honeysuckle
6/26/13	4	Tatarian honeysuckle
6/26/13	4	Tatarian honeysuckle
7/8/13	4	Tatarian honeysuckle
7/8/13	4	Tatarian honeysuckle
7/8/13	4	Wild blackberries
7/8/13	3	Wild blackberries
7/23/13	2	Wild blackberries
7/23/13	1	Wild blackberries
7/30/13	2	Wild blackberries
7/30/13	2	Wild blackberries
7/30/13	2	Wild black raspberries
7/30/13	1	Wild blackberries
8/5/13	3	Pokeweed
8/5/13	3	Wild raspberries
8/6/13	2	Pokeweed
8/20/13	4	Pokeweed
8/29/13	4	Pokeweed
10/10/13	4	Pokeweed
10/10/13	4	Pokeweed
10/10/13	4	Pokeweed
10/18/13	3	Pokeweed
10/18/13	3	Japanese honeysuckle
10/18/13	3	Mock strawberry
10/18/13	3	Japanese honeysuckle
10/18/13	3	Japanese honeysuckle
10/18/13	3	Pokeweed

[a]Site 1, Amherst, Amherst Co., Site 2, Tyro, Nelson Co., Site 3, Crozet, Albemarle Co., Site 4, Gordonsville, Orange Co.

Table 2.4. Seasonal occurrence, location and plant species sampled that were positive for *Drosophila suzukii* adult emergence in 2014 at four Virginia vineyards.

Foliage Collection Date	Vineyard #	Sample Type
7/7/14	2	Wild blackberry
7/14/14	4	Tatarian honeysuckle
7/14/14	4	Tatarian honeysuckle
7/14/14	4	Tatarian honeysuckle
7/14/14	4	Tatarian honeysuckle
7/14/14	4	Tatarian honeysuckle
7/14/14	4	Wild blackberries
7/14/14	3	Wild blackberries
7/14/14	3	Wild blackberries
7/14/14	4	Wild blackberries
7/14/14	3	Wild blackberries
7/21/14	1	Wild blackberries
7/21/14	2	Wild blackberries
7/21/14	1	Wild blackberries
7/30/14	4	Wild blackberries
7/30/14	4	Tatarian honeysuckle
7/30/14	4	Wild blackberries
7/30/14	3	Wild blackberries
7/30/14	3	Pokeweed
7/30/14	3	Pokeweed
7/30/14	4	Wild cherry
7/30/14	3	Wild cherry
7/30/14	3	Pokeweed
8/4/14	1	Wild blackberries
8/11/14	4	Pokeweed
8/11/14	3	Pokeweed
8/11/14	3	Pokeweed
8/11/14	3	Wild cherry
8/11/14	3	Wild cherry
8/11/14	3	Wild cherry
8/11/14	4	Wild cherry
8/11/14	4	Wild cherry
8/11/14	4	Japanese honeysuckle
8/18/14	2	Wild cherry
8/18/14	2	Wild cherry
8/18/14	2	Pokeweed

[a]Site 1, Amherst, Amherst Co., Site 2, Tyro, Nelson Co., Site 3, Crozet, Albemarle Co., Site 4, Gordonsville, Orange Co.

CHAPTER 3: COMPARISON OF TRAPPING BAITS USED IN THE MONITORING OF *DROSOPHILA SUZUKII* (MATSUMURA) (DIPTERA: DROSOPHILIDAE) IN VIRGINIA VINEYARDS

Meredith Shrader

Abstract

Drosophila suzukii (Matsumura), is a pest of berries, cherries and grapes, and has recently been expanding its distribution globally, significantly affecting the economy of fruit growers. The cornerstone of any *D. suzukii* monitoring program is to correctly identify this pest species, determine the presence or absence of it within a fruit production region, determine fruit infestation levels, and monitor its population growth. This information has been difficult to obtain due to *D. suzukii* populations rapidly increasing, the possibility that fruit may be infested before flies are captured in traps and the lack of correlation between trap captures and fruit injury levels. Many baits and traps have been developed to attract and capture *D. suzukii* to gather this information from small fruit production areas. However, baits and trapping systems have not been extensively studied in vineyards. I compared several homemade *D. suzukii* baits and traps as well as commercially available baits, synthetic lures and traps to determine the efficacy and selectivity of these products in two Virginia vineyards for three consecutive years. Several homemade baits were more efficient at attracting *D. suzukii* than the commonly used apple cider vinegar bait, with the apple cider vinegar plus Merlot bait capturing the most *D. suzukii*; however it was not selective for this pest. The synthetic plum-scented attractant from Alpha Scents and the yeast plus sugar bait were more selective than any other homemade bait, but their attractiveness varied based upon location and year. The Biobest trap and bait caught the most *D. suzukii* of the commercially tested attractants, but over half the flies captured were not *D. suzukii* and it was statistically similar to the apple cider vinegar and Merlot bait. The commercial synthetic lures targeting *D. suzukii* were not effective at attracting the flies to traps.

Introduction

Drosophila suzukii (Matsumura), an invasive and polyphagous frugivore native to eastern Asia, was first detected in the continental U.S. in 2008 in a raspberry planting in Santa Cruz, California (Lee et al. 2011). By 2009, it had been detected throughout the west coast of the US and into Canada and Florida. *Drosophila suzukii* was detected in Virginia in 2011 in a raspberry field and has been collected in monitoring traps every year since (Pfeiffer 2011). In 2012, *D. suzukii* was detected in a vineyard in the Piedmont region of Virginia. Of the invasive insects that have been introduced in the past decade, spotted wing drosophila has been the most severely detrimental to the small fruit industry (Bolda et al. 2010, Goodhue et al. 2011), inflicting high levels of damage to a wide range of fruiting crops, including grapes (Walsh et al. 2010, Ioriatti et al. 2015).

Wine grapes are an important crop in Virginia, which was the nation's fifth largest wine grape producer in 2013, with 1,366 ha in production (Wolf 2014). A 2010 economic impact study estimated that the Virginia wine industry employs more than 4,700 people and contributes almost $750 million to the Virginia economy on an annual basis (Frank et al. 2012). Wine grape growers are concerned about the potential for *D. suzukii* to spread sour rot and other pathogens, such as *Acetobacter* spp. and lay eggs in grapes (Hamby et al. 2012, Chandler et al. 2014, Ioriatti et al. 2015, Rombaut et al. 2017). This combination of pathogens can lead to sour rot in grapes, which causes whole clusters to become unsuitable for wine production. It is essential to have a good integrated pest management (IPM) strategy for *D. suzukii* to reduce populations within the field, ideally including biological control, cultural control, and insecticide spray tactics. However, the most effective control strategy for protecting cultivated fruits from *D. suzukii* oviposition has been insecticidal sprays. Effective monitoring tools that correlate to damage risk are the basis for all IPM strategies.

Monitoring pest populations is a basic element of IPM and can help wine growers determine if their vineyards and grapes are at risk of *D. suzukii* infestation. Vineyards that trap *D. suzukii* are at greatest risk for grape infestations than those that do not capture this pest, however trapping numbers are not always indicative of population levels. Various traps, baits and lures for *D. suzukii* have been evaluated, but none attract *D. suzukii* exclusively (Cha et al. 2012, Landolt et al. 2012a, Landolt et al. 2012b, Cha et al.

2013, Burrack et al. 2015). When monitoring began in the U.S. in 2008, traps were simple plastic cups baited with apple cider vinegar (ACV) (Steck et al. 2009). While traps and baiting solutions have evolved, there is still no standard attractant or trap for this pest. Trapping numbers and trapping selectivity for *D. suzukii* has varied between trapping baits and trap designs, and efficacy has also differed depending upon the crop in which traps were placed (Burrack et al. 2015). Monitoring traps for *D. suzukii* have not been extensively evaluated in vineyards. The trapping baits that have attracted the most flies in vineyards have utilized wine as part of the baiting solution (Shrader and Pfeiffer personal observation). Our hypothesis is no homemade or commercially available bait or lure will catch *D. suzukii* within Virginia vineyards. These experiments were conducted to determine the efficacy and selectivity of homemade and commercially available baits and trapping systems. In 2013 and 2014, alternative trap baits were compared to determine if they were more effective for capturing *D. suzukii* compared to the standard ACV bait. In 2015, we compared traps for *D. suzukii* using homemade baits in deli cup traps and commercially available trapping systems to determine if the commercially available trapping systems were more effective and selective in capturing *D. suzukii* than the homemade ACV plus Merlot bait and deli cup trap.

Materials and Methods

Trapping Locations. For all studies conducted between 2013 through 2015, traps were deployed in two vineyards in the Piedmont region of Virginia, one in Orange Co. (Site 1) (Coordinates: 38.234451, -78.102461) and one in Albemarle County (Site 2) (Coordinates: 38.051443, -78.746242). Traps were placed in a block Petit Verdot at both sites in 2013 and 2014. Site 1 in 2013 and 2014 was 0.65 ha with vineyard rows measuring 260 m. The grape panels for Petit Verdot were 8.5 m long with vines spaced every 1.2 m (4 vines per panel) and 3.7 m between rows. In 2015, traps at Site 1 were placed in a 1.07 ha block of Cabernet Franc with vineyard rows measuring 130 m, the grape panels were 6 m long with vines every 2.4 m (2 vines per panel) and 3.7 m between rows.

Site 2 (2013- 2105) (Albemarle Co.) was 0.6 ha with vineyard rows measuring 170 m, grape panels were 6 m long with vines every 1.5 m (3 vines per panel) and 3 m

between rows. The vineyard blocks at both locations were subject to standard pesticide spray regimens for all trapping years. During the trapping period at both sites and in all years, fungicides as well as the insecticides were applied, including captan, acetamiprid, clothianidin, spirotetramat, kaolin clay and malathion, and may have affected fly captures.

Traps and Baits. Trap efficacy and selectivity experiments in 2013 and 2014 were conducted using1-liter translucent plastic deli cups (model: TD41032-A01, Solo, Urbana, IL) with eight 7-mm diameter holes positioned equidistantly around the cup at approximately 2 cm below the rim. A translucent plastic lid was used to cover the top of the cup. To hang the traps in the vines, white string was fed through two of the holes in the cup and tied to make a loop. The trapping treatments are summarized in Table 3.1 and included, 1) ACV alone, 2) ACV plus Merlot wine (60:40; adapted from Landolt et al. 2011), 3) yeast plus sugar plus water, 4) a commercial sachet lure containing a synthetic pad saturated with a synthetic chemical blend of plum-scented liquid designed to disperse the fruit scent (volatiles) combined with low-toxicity antifreeze drowning solution (Prestone® Low Tox®, Lake Forest, IL), and 5) antifreeze (Prestone® Low Tox®, Lake Forest, IL) as the control. A drop (0.25 ml) of unscented, clear hand detergent (Softsoap Advanced Clean Liquid Hand Soap, Colgate-Palmolive, New York, NY) was added to all liquid baits to aid in capturing flies by breaking the surface tension of the liquid. All five trapping treatments were replicated four times at both Sites.

In 2015, an experiment comparing the effectiveness of commercially available traps and baits with the homemade ACV plus Merlot bait and plastic deli cup was conducted at two Sites in Virginia. Trap and bait treatments were deployed at both Sites and included, 1) Pherocon trap and bait (Trécé Inc., Adair, OK), 2) Biobest trap (Droso-trap) and Dros'Attract bait (Biobest, Oevel, Belgium), 3) Alpha Scents trap (DROSUZ) composed of a sticky card and *D. suzukii* attractant sachets (Alpha Scents, West Linn, OR), 4) ACV plus Merlot wine in the plastic deli trap (60:40; adapted from Landolt et al. 2011), and a 5) blank consisting of low-toxicity antifreeze (Prestone® Low Tox®, Lake Forest, IL) in a plastic deli cup (Solo, Urbana, IL) (Table 3.2). A drop (0.25 ml) of odorless, clear hand detergent (Softsoap Advanced Clean Liquid Hand Soap, Colgate-Palmolive, New York, NY) was added to all liquid baits to aid in capturing flies by

breaking the surface tension of the liquid. All five trapping treatments were replicated five times at both sites.

Trap Deployment and Monitoring. Traps were deployed after véraison to coincide with the grape ripening period. In 2013, trapping began on 29 August and 6 September at Sites 1 and Site 2, respectively and continued through 26 September. In 2014, traps were deployed at both Sites on 25 August and removed on 22 September. A total of four replicates of each treatment were evaluated at each location in both years. In 2015, traps were placed in the vineyards on 27 July and checked weekly until 8 September, with five replicates per treatment at both Sites. Treatments were arranged in a randomized complete block design. At Site 1, treatments were separated by one panel within rows (17 m between traps) and a buffer row between trap rows (7.4 m). At Site 2, treatments were separated by one panel within rows (12 m between traps) and a buffer row between trap rows (6 m). For both Sites and all years, the position of traps within trapping rows was randomly rotated weekly to control for potential positional effects. Traps were hung in the middle of the vineyard blocks and at least 24 m from the end of the row and at least 2 rows (> 10 m) from the edge of the varietal block, thus decreasing the potential of vineyard edge effect on fly captures. Traps were hung near grape clusters (~1.4 m off the ground) in the canopy of vines in the middle of a grape panel. Traps were serviced weekly, flies were collected from the traps and the *D. suzukii* captured and transported back to the laboratory. The number of *D. suzukii* were counted and sexed for each trap using a dissecting microscope. All non-target Diptera were counted but not identified.

In 2013 and 2014, the yeast plus sugar plus water bait and the plum volatile sachets were changed weekly, while the ACV and ACV plus Merlot baits were changed biweekly. In 2015, the ACV plus Merlot mix, Pherocon lure and Biobest trap with Dros'Attract bait were changed biweekly. The Alpha Scents sachet was also changed biweekly and the sticky cards were changed weekly. To ascertain when *D. suzukii* flies became active in each vineyard, a homemade deli cup trap with the ACV plus Merlot bait was hung in a tartarian honeysuckle (*Lonicera tatarica* L.) plant on 3 June at Site 1 and in a bird cherry tree (*Prunus padus* L.) at Site 2.

Statistical Analyses. Statistical analysis was conducted using JMP® Pro version 13 (SAS Institute Inc., Cary, NC, 2016) and outcomes were considered significant at $P \leq 0.05$. The capture of adult *D. suzukii* from each trap of each vineyard block was averaged weekly. Traps were evaluated based upon the efficacy and selectivity of *D. suzukii* and other non-targets captured. Trapping data was Log (1+x) transformed to fit the assumptions of normality. Trap capture data for all three field seasons were analyzed by a two-way ANOVA with trapping week, trap treatments, and the interaction effects of trapping week and trapping treatments. Two-way ANOVA interactions for mean weekly total *D. suzukii* (males and females) captures were further analyzed with a Tukey's HSD was used to separate the means for trapping week and trapping treatments. If interaction were significant ($P \leq 0.05$) a slice test was performed to look at the simple effects. A Slice Test was used to determine the simple effects and ascertain weather trapping week or trapping treatments was responsible for the interaction effects. Trapping locations and trapping years were analyzed separately due to different trapping dates and total trap captures. *D. suzukii* male, female, and non-target flies captured were analyzed separately via a one-way ANOVA, blocked by date and a Tukey's HSD was used to separate the means. To compare selectivity among the different treatments, captures of *D. suzukii* were expressed as a proportion of all captured Diptera, excluding instances when no *D. suzukii* were captured in the traps. Trap selectivity was analyzed via a one-way ANOVA and a Tukey's HSD was used to separate the means.

Results

Effect of Trap Bait on *D. suzukii* Captures in 2013 and 2014.

In 2013, a two-way ANOVA showed a significant effect of trapping treatment on fly captures of both sexes at both Sites. There was no significant effect of fly captures over the trapping period, nor were there any interactions of trapping week and trapping treatment at either Site (Table 3.3). At Site 1 in 2013, traps baited with ACV plus Merlot caught significantly more female and male *D. suzukii* than ACV alone (Table 3.4). Traps containing yeast plus sugar plus water bait and the Alpha Scents plum sachet also caught more female *D. suzukii* than ACV, but were not significantly different from one another or from the ACV plus Merlot bait. All traps caught a substantial number of non-target

flies, with the yeast + sugar + water catching the least. In 2013 at Site 2, traps containing ACV plus Merlot caught significantly more female and male *D. suzukii* than the other baits (Table 3.4). In 2013 at both Sites, none of the baits tested were selective for *D. suzukii*. The Alpha Scents plum sachet and yeast plus sugar plus water bait captured the greatest percentage of *D. suzukii* and the plum scented bait was more attractive to female over male *D. suzukii* numerically (Table 3.4).

In 2014, a two-way ANOVA showed a significant effect of trapping treatment, but neither trapping week or the interactions of trapping week and trapping bait were significant at Site 1 (Table 3.3). However, at Site 2 male captures showed a significant effect of trapping week and trapping treatment as well as significant interactions of trapping week and trapping treatment on fly captures (Table 3.3). Female and total *D. suzukii* captures were significantly affected by trapping treatment, but trap capture did not vary based upon trapping dates nor were there any interaction effects of trapping week and trapping treatment. In 2014 at Site 1, traps with the ACV plus Merlot wine bait caught significantly more female and male flies than the other treatments. There was also a significant effect of treatment on the number of non-target flies caught, with most non-targets caught in traps with ACV plus Merlot treatment. In 2014 at Site 2, traps with ACV plus Merlot caught significantly more female and male flies than the other treatments. The ACV and ACV plus Merlot mix caught the most non-target flies at Site 2 (Table 3.4). In 2014 for both Sites, the Alpha Scents plum sachet captured the highest percentage of *D. suzukii*. No *D. suzukii* were captured in control traps at either Site in either year. Total *D. suzukii* captures suggested that Site 1 had a higher *D. suzukii* population than Site 2.

Effect of Trap Type and Bait on *D. suzukii* Captures in 2015.

The first *D. suzukii* male flies were captured on 24 June at Site 2 in a wild bird cherry tree and on 29 June at Site 1 in a tartarian honeysuckle using a homemade plastic deli cup trap with ACV plus Merlot. In 2015 season, a factorial ANOVA was employed to determine the effects of trapping week and trapping treatments at both trapping Sites for total flies, female and male *D suzukii* captured. The main effects of trapping week and trapping treatments were statistically significantly as well as the interactions of

trapping week and trapping treatment for total, female and male *D. suzukii* (Table 3.5). Fly captures declined on 25 August at Site 1 and trapping numbers remained low for the remainder of the trapping dates (Fig. 3.1). Site 2 had trapping numbers decline on the 25 August, but trapping number increased on 31 August and 8 September for Biobest and ACV plus Merlot (Fig. 3.2), thus trapping date rather than trapping treatment was the effect responsible for the interactions. The main effect of trapping week on total trap captures at Site 1 was analyzed with a Tukey's HSD to separate the means and the most *D. suzukii* were captured in traps on 3 and 10 August, and the fewest on 25 August and 8 September (Table 3.6). The traps that captured the most *D. suzukii* as determined by the Tukey HSD at Site 1 were the ACV plus Merlot and the Biobest trap with Dros'Attract bait (Table 3.6). The Slice Test for simple effects showed a significant difference in trap captures for the 3, 10, 17, 31 August trapping dates for Site 1 (Table 3.7). The Slice Test for trapping treatment showed a significant difference in total *D. suzukii* trap captures for the ACV plus Merlot ($F = 24.771$, $P < 0.0001$, $df = 5, 119$) and the Biobest trap with Dros'Attract bait ($F = 22.4201$, $P < 0.0001$, $df = 5, 119$). The main effect of trapping week on total *D. suzukii* trap captures at Site 2 was analyzed with a Tukey's HSD to separate the means and the most *D. suzukii* were captured on 3, 10 and 31 August and the fewest captured on 17, 25 August and 8 September (Table 3.8). The Tukey's HSD showed a significant difference of *D. suzukii* trap captures for the ACV plus Merlot and Biobest trap and baits (Table 3.8). The Slice Test for simple effects showed a significant difference in total *D. suzukii* trap captures based upon trapping date (Table 3.9). The Slice test for trapping treatment showed a significant difference in total *D. suzukii* trap captures for the ACV plus Merlot ($F = 10.1577$, $P < 0.0001$, $df = 5, 120$) and the Biobest trap with Dros'Attract bait ($F = 7.3568$, $P < 0.0001$, $df = 5, 120$).

At Site 1, there were significantly more female and male *D. suzukii* captured in the ACV plus Merlot and Biobest trap with Dros'Attract bait (Table 3.10). The chemical attractants from Alpha Scents and Pherocon caught significantly fewer flies than the ACV plus Merlot and Biobest baits. Site 1 had also had significantly more non-target flies captured in traps with ACV plus Merlot and the Biobest trap with the Dros'Attract bait than other treatments. Site 2 had significantly more female and male *D. suzukii* captures in the ACV plus Merlot and the Biobest trap with the Dros'Attract bait (Table

3.10). These baits also had a large number of non-target flies captured. The Pherocon trap and lure and the Alpha Scents lure captured more non-target flies than and were statistically similar for both male and female *D. suzukii* captures (Table 3.10). In 2015 at both Sites, the large number of non-target fly captures in all traps showed that these traps and baits/lures were not specific for *D. suzukii* (Table 3.10). More non-target flies were captured in all traps than *D. suzukii* with the Biobest trap capturing at most 40% *D. suzukii* compared to total fly captures.

Discussion

This is the first study that compared *D. suzukii* attractants exclusively in wine grape vineyards on the east coast over multiple years. The trapping periods began just after véraison, when grapes started to sequester sugar and the penetration force decreased, at which point (< 20 cN, Chapter 4) grapes became susceptible to *D. suzukii* oviposition (Burrack et al. 2013, Ioratti et al. 2015). It is also after véraison until harvest when grapes need to be most intensively protected from *D. suzukii*, so it was critical to assess these baits' attractiveness to *D. suzukii* in the presence of ripening grapes and fermentative volatiles associated with sour rot, for population monitoring purposes. Our findings from the studies in 2013 and 2014 indicated that the addition of Merlot wine to the ACV bait in the plastic deli cup traps increased captures of *D. suzukii* males and females. Several other baits also attracted more *D. suzukii* than the ACV alone bait, further demonstrating that ACV is not an ideal attractant for monitoring *D. suzukii* populations. There was also only one instance when trap captures were affected by date (male *D. suzukii* counts at Site 2 in 2014), so the overall efficacy with the homemade baits for total *D. suzukii* trap captures and female *D. suzukii* captures did not vary over years or locations. These trapping results were similar to those from studies conducted in raspberry and blackberry plantings when trapping efficacy increased with the addition of Merlot wine (Landolt et al. 2012a, Landolt et al. 2012b). The Alpha Scents plum sachet attracted several *D. suzukii* and was more effective in attracting female *D. suzukii* compared to the ACV plus Merlot bait in 2013 (Table 3.4). This bait was more effective at Site 1 compared to Site 2 and may have been more efficacious when *D. suzukii* populations were higher. None of the baits tested were exclusively selective for *D.*

suzukii and attracted many non-target flies. Nevertheless, the attraction of *D. suzukii* to a synthetic fruit scented lure in the field demonstrated the potential to develop a more *D. suzukii*-specific synthetic chemical lure.

Our trapping results with homemade baits in Virginia vineyards were different from those in blueberry cropping systems, where the yeast plus sugar plus water bait performed better than the ACV plus Merlot bait (Walsh et al. 2011, Iglesias et al. 2014). These findings suggested that a monitoring system for *D. suzukii* may need to be crop-specific and that grape vineyards in particular may require different baiting and trapping systems compared with other fruit cropping sites. Grapes must stay on the vine in order to reach desired levels of sugars and acids needed for wine-making. Thus, a long-term monitoring bait that would be attractive in the presence of ripening grape berries would be especially valuable in vineyards. Burrack et al. (2015) further demonstrated that there was an interaction of trapping crop and the bait/trap efficacy in *D. suzukii* captures. Trapping studies indicated that *D. suzukii* trap captures in blueberries were lower than those seen in caneberries and that the fermenting cup (water/whole wheat flour/yeast/ACV/sugar) treatment suspended over ACV performed better in caneberries compared to blueberries. Trap captures may vary based upon fly population, fruit phenology, host preference, surrounding habitats and even plant architecture (Lee et al. 2011, Burrack et al. 2013, Burrack et al. 2015). Grape trellises in particular make trap placement for monitoring *D. suzukii* difficult because a North to South orientation of the rows allows for very little shading, which is desired over direct sunlight for trap placement. Additionally, grape leaves are removed from the vines in order to maximize grape cluster exposure to the sunlight, which aids in the ripening process. Traps must be placed into the canopy of the vines as close to the grapes as possible without being in direct sunlight to increase trapping efficacy.

In 2015, commercial *D. suzukii* trapping systems were compared with the deli cup trap containing ACV plus Merlot, with varying results. The Biobest trap with Dros'Attract bait and the ACV plus Merlot bait caught significantly more *D. suzukii* than the other treatments at both trapping Sites. This was expected because one of the components in the Dros'Attract bait is wine (proportions proprietary) and baits containing wine performed well in 2013 and 2014. The Biobest trap was also red in

color, which may have added to the attractiveness of the trap to *D. suzukii* (Rice et al. 2016). The synthetic *D. suzukii* bait and traps from Alpha Scents and Pherocon did not perform well at either site, attracting very few *D. suzukii*. The Alpha Scents trapping system utilized a yellow sticky card for fly capture, which resulted in captures of non-Dipteran insects, including beetles and wasps. Yellow sticky cards also caused flies captured on the cards to be exposed for long periods before collection, and desiccation made identification of insects more difficult when forceps were used to manipulate flies under dissecting microscopes in the lab. Iglesias et al. (2014) encountered similar difficulties when counting fly specimens on sticky cards. This may have also caused male *D. suzukii* to be counted in higher numbers than females due to the more conspicuous markings on male versus female *D. suzukii*. Fly captures could also have been affected by the sticky cards becoming stuck to grape leaves, thus decreasing the trap surface area. On 25 August 2015, there was a significant decrease in captures at both Sites, which may account for the interaction of trapping treatments and trapping dates on *D. suzukii* captures. Spray records indicated that insecticides had been sprayed throughout the trapping period, but the insecticides used targeted piercing and sucking insects and were not considered efficacious against *D. suzukii* (van Timmerman and Isaacs 2013). Furthermore, malathion was applied in the vineyards after 25 August and so was not responsible for this decline. Thus, insecticide applications did not appear to be responsible for the reduced captures at Site 1, but captures may have been influenced by the phenological state of the grapes or the age of female *D. suzukii*. This date was when several varieties of wine grapes experienced a pronounced physiological and morphological change. The Slice Test for simple effects also demonstrated that trapping date was more responsible for the interaction effects than trapping treatments. The sugar levels in the grapes increased significantly from 15 to 22 ºBrix and the penetration force decreased from 19.55 cN to 11 cN in the trapping blocks (See Chapter 4). Burrack et al. (2013) demonstrated that penetration force was a key factor affecting fruit susceptibility to *D. suzukii* oviposition and when penetration force decreases oviposition into fruits increases. *Drosophila suzukii* may have been more attracted to the ripening fruit than to the traps. Female *D. suzukii* captured in traps when fruit was available had lower egg loads than females captured when fruit was not ripe suggesting females were orienting to

fruits to lay eggs rather than fermenting baits, assumed to be food sources (Burrack et al. 2015).

None of the commercially available or homemade baits and traps was selective for *D. suzukii* for all trapping years and locations. The plum scented sachet by Alpha Scents was consistent at capturing between 60 – 90% *D. suzukii* over non-target flies, however trapping numbers varied by location. The benefits of *D. suzukii*-specific baits could help tremendously in fly identification and ease of trap counts, but this goal remains difficult to achieve (Landolt et al. 2011). The 4-component chemical lure, marketed by Trécé Inc, captured as many *D. suzukii* as ACV plus Merlot, but resulted in regional differences in captures (Cha et al. 2013). Cha et al. (2015) further tested this lure in various cropping systems and showed some *D. suzukii* selectivity in blackberries grown in Oregon but that it failed in cherries in Washington. Further testing in additional fruit production regions throughout the growing season is needed.

It is probable that synthetic chemical lures may be more useful to stakeholders than fermentative baits due to the longevity and selectivity of formulated chemical attractants (Cha et al. 2013). These chemical baits can also be deployed without fear of spoilage or decreased attractiveness over time as seen with homemade baits. Reduced sample identification and sorting time would also be welcomed with *D. suzukii* specific chemical lures. Female *D. suzukii* need to be targeted for a selective *D. suzukii* lure because they directly injure fruit. Being able to estimate the population of *D. suzukii* in the field, especially the percentage of females and their egg laying potential through trap capture numbers can be useful for developing a risk analysis protocol for managing D. suzukii in small fruit and grape (Burrack et al. 2015). Female *D. suzukii* must be identified under a dissecting microscope and can be mistaken for other *Drosophila* even by trained technicians. *D. suzukii* specific traps could help commercial growers more easily identify females by assuming all flies in the traps without spots are female and flies with spots are males, but no bait or trapping system has been developed to selectively attract *D. suzukii* across all fruit cropping systems.

After conducting *D. suzukii* bait and trap design research in Virginia vineyards and until more research is conducted on the synthetic lures, the ACV plus Merlot bait with plastic deli cup or the Biobest trap with Dros'Attract bait should be deployed in

vineyards on the east coast. If *D. suzukii* are captured in traps then the grapes present are at a high risk of *D. suzukii* attack and fly infestation within the vineyards and should be managed appropriately to control this pest. Even though these baits are not *D. suzukii*-specific they have shown to be attractive in vineyard environments and have consistently captured *D. suzukii* at both high and low densities. They have also been effective over the extended ripening period up to harvest when baits are changed biweekly.

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Table 3.1. Description of trap baits and trap designs used for monitoring *Drosophila suzukii* in (2013 and 2014) in Virginia vineyards.

Trap Name	Cup Vol (ml)	Bait Vol (ml)	Headspace Area (ml)
ACV	1,000	300 ml Apple cider vinegar (5% acidity; Kroger, Cincinnati, OH)	700
Blank	1,000	300 ml low-toxicity antifreeze (Prestone® Low Tox®, Lake Forest, IL)	700
Yeast + Sugar + Water	1,000	12.5 g white granulated cane sugar (Kroger, Virginia) + 2.8 g yeast (Fleishmann's RapidRise Yeast, ACH Food Companies, Inc., Cordova, TN) + 300 ml water	700
Alpha Scents plum sachet	1,000	Plum sachet (Alpha Scents, West Linn, OR) suspended over 300 ml low-toxicity antifreeze (Prestone® Low Tox®, Lake Forest, IL)	700
ACV + Merlot	1,000	180 ml Apple cider vinegar (5% acidity; Kroger, Cincinnati, OH) + 120 ml Merlot wine (Franzia, California)	700

Table 3.2. Description of trap baits and trap designs used for monitoring *Drosophila suzukii* in 2015 in Virginia vineyards.

Trap Name	Trap Design	Bait Type and Volume (ml)
Biobest	Round red bottom with clear top, holes (1 cm^2) on side	300 ml Dros'Attract
Blank	Clear plastic deli cup (Solo, Urbana, IL)	300 ml low-toxicity antifreeze (Prestone® Low Tox®, Lake Forest, IL)
Pherocon	Clear plastic deli cup with 2 mesh holes on side (45 cm^2)	7.6 cm long lure strip with 3 liquid wells (Trécé Inc., Adair, OK) suspended over 150 ml low-toxicity antifreeze (Prestone® Low Tox®, Lake Forest, IL)
Alpha Scents	Yellow Sticky Card (240 cm^2)	103 cm^2 *D. suzukii* lure (Alpha Scents, West Linn, OR)
ACV + Merlot	1-liter clear plastic deli cup (Solo, Urbana, IL)	180 ml Apple cider vinegar (5% acidity; Kroger, Cincinnati, OH) + 120 ml Merlot wine (Franzia, California)

Table 3.3 Two-way ANOVA results for *Drosophila suzukii* captured in five trapping systems at Vineyard Site 1 (Orange Co., VA) and Vineyard Site 2 (Albemarle Co., VA) in 2013 and 2014.

			Year					
Site	Count	Parameter	2013			2014		
			F	df	P	F	df	P
1	Total	Week	0.7972	3, 3	0.5015	0.2045	3, 3	0.8929
		Trapping Treatment	3.4815	4, 4	0.0228	13.9359	4, 4	<0.0001
		Week*Treatment	0.6604	12, 12	0.7399	0.6410	12, 12	0.7987
	Female	Week	1.0877	3, 3	0.3633	0.7869	3, 3	0.5059
		Trapping Treatment	2.8489	4, 4	0.0471	22.8287	4, 4	<0.0001
		Week*Treatment	0.7017	12, 12	0.7041	0.2578	12, 12	0.2578
	Male	Week	0.4101	3, 3	0.7464	0.2344	3, 3	0.8720
		Trapping Treatment	5.3758	4, 4	0.0028	10.5029	4, 4	<0.0001
		Week*Treatment	0.6319	12, 12	0.7641	0.5605	12, 12	0.8647
2	Total	Week	0.5953	2, 2	0.5953	2.1886	3, 3	0.0987
		Trapping Treatment	43.6812	3, 3	<0.0001	66.6303	4, 4	<0.0001
		Week*Treatment	1.2714	6, 6	0.3073	1.5334	12, 12	0.1375
	Female	Week	0.9013	2, 2	0.4193	0.7487	3, 3	0.5274
		Trapping Treatment	30.2593	3, 3	<0.0001	41.9767	4, 4	<0.0001
		Week*Treatment	1.4529	6, 6	0.2363	0.7163	12, 12	0.7297
	Male	Week	0.0828	2, 2	0.9208	4.0240	3, 3	0.0113
		Trapping Treatment	17.8429	3, 3	<0.0001	47.4107	4, 4	<0.0001
		Week*Treatment	0.8047	6, 6	0.5762	3.1097	12, 12	0.0018

Table 3.4. Effect of trap bait on captures of female and male *Drosophila suzukii* and non-target flies in plastic deli cup traps in two commercial vineyards in Virginia in 2013 and 2014.

		Site 1[2]			Site 2[2]			Selectivity[3]
		Female	Male	Other Flies	Female	Male	Other Flies	Total *D. suzukii*
2013	Blank	0.0 c	0.0 c	0.0 b	0.0 c	0 b	0 b	-
	Plum Sachet	25.7 ± 6.9 a	3.4 ± 1.3 bc	53.9 ± 15.5 a	3.1 ± 0.7 b	0.2 ± 0.2 b	0.1 ± 0.1 b	0.8 ± 0.07 ab
	Yeast + Sugar + Water	27.9 ± 10.9 a	6.1 ± 2.5 b	3.8 ± 2.1 b	2.7 ± 0.8 b	0.9 ± 0.5 b	1.2 ± 1.2 b	0.9 ± 0.04 a
	Apple Cider Vinegar + Merlot	32.8 ± 7.2 a	18.8 ± 5.5 a	56.6 ± 16.4 a	16.2 ± 2.4 a	6.3 ± 1.2 a	27.2 ± 9.1 a	0.6 ± 0.06 bc
	Apple Cider Vinegar	3.8 ± 1.3 b	2.6 ± 0.9 bc	61.1 ± 21.9 a	1.1 ± 0.4 bc	0.3 ± 0.2 b	0.4 ± 0.2 b	0.4 ± 0.08 c
	F	19.1917	11.2223	7.6590	38.0643	28.9305	23.6012	10.2364
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	df	4, 75	4, 75	4, 75	4, 40	4, 40	4, 40	3, 88
		Female	Male	Other Flies	Female	Male	Other Flies	Total *D. suzukii*
2014	Blank	0.0 d	0.0 d	0.0 d	0.0 c	0.0 c	0.0 c	-
	Plum Sachet	23.3 ± 5.7 b	4.9 ± 1.9 c	10.6 ± 3.7 c	1.9 ± 0.7 b	0.0 c	3.4 ± 0.8 b	0.6 ± 0.06 a
	Yeast + Sugar + Water	1.8 ± 0.5 c	1.9 ± 0.3 cd	43.4 ± 12.8 c	0.5 ± 0.3 c	0.4 ± 0.3 c	1.7 ± 0.9 bc	0.5 ± 0.06 ab
	Apple Cider Vinegar + Merlot	63.3 ± 9.9 a	114.8 ± 30.0 a	95.8 ± 16.4 a	10 ± 1.2 a	8 ± 1.3 a	46.2 ± 8.8 a	0.5 ± 0.03 ab
	Apple Cider Vinegar	24.9 ± 4.2 b	35.8 ± 10.7 b	49.4 ± 12.2 b	1.5 ± 0.3 b	1.2 ± 0.3 b	43.4 ± 12.8 a	0.4 ± 0.06 b
	F	82.6039	78.4494	56.6780	61.5671	72.2677	61.1942	13.8997
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	df	4, 75	4, 75	4, 75	4, 75	4, 75	4, 75	4, 155

[1] Means within column followed by the same letter are not significantly different by Tukey's HSD test.
[2] Traps checked weekly from 29 August - 6 Sept, 2013 and from 25 August – 22 September, 2014 at Sites 1 and 2, respectively.
[3]Trap selectivity, expressed as *Drosophila suzukii* captures as a proportion of total Drosophilidae (nontarget and *Drosophila suzukii* combined for both Sites) captured per trap per week

Table 3.5. Site 1 (Orange Co.) and Site 2 (Albemarle Co.) two-way ANOVA results in 2015 for *Drosophila suzukii* captured in five trapping systems.

			Year		
Site	Count	Treatment Parameter	2015		
			F	df	P
1	Total	Week	24.3621	5, 5	<0.0001
		Trapping Treatment	48.2405	4, 4	<0.0001
		Week*Treatment	6.3468	20, 20	<0.0001
	Female	Week	19.3243	5, 5	<0.0001
		Trapping Treatment	56.9708	4, 4	<0.0001
		Week*Treatment	6.2764	20, 20	<0.0001
	Male	Week	21.2900	5, 5	<0.0001
		Trapping Treatment	25.4623	4, 4	<0.0001
		Week*Treatment	4.7001	20, 20	<0.0001
2	Total	Week	7.9760	5, 5	<0.0001
		Trapping Treatment	36.1465	4, 4	<0.0001
		Week*Treatment	2.6890	20, 20	<0.0001
	Female	Week	5.2344	5, 5	<0.0001
		Trapping Treatment	39.0627	4, 4	<0.0001
		Week*Treatment	2.9116	20, 20	<0.0001
	Male	Week	8.5663	5, 5	<0.0001
		Trapping Treatment	23.5372	4, 4	<0.0001
		Week*Treatment	2.0077	20, 20	<0.0001

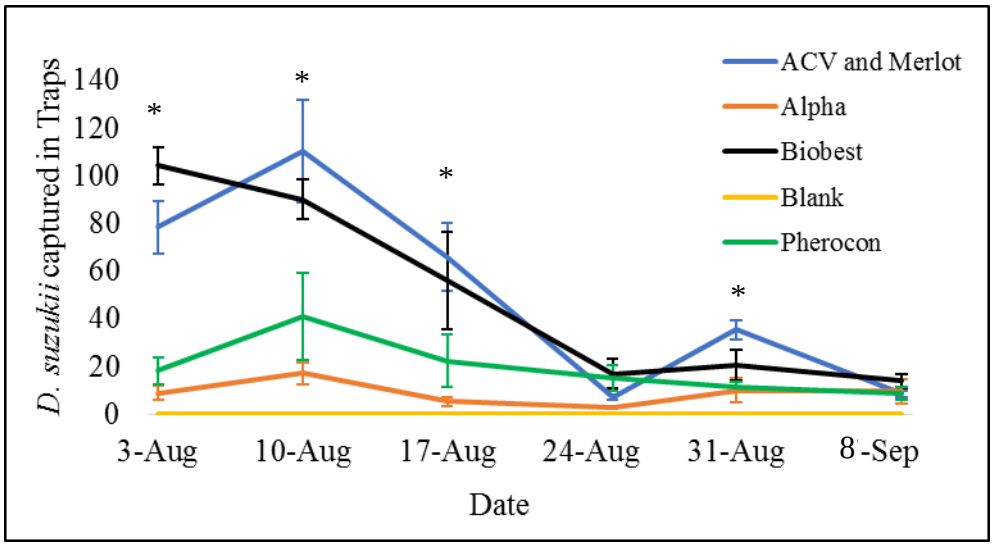

Figure 3.1. Mean (± SE) weekly *Drosophila suzukii* (males and females) trap captures at Site 1 (Orange Co.) in 2015. Dates for which there were significant different by a Slice Test are indicated by asterisk.

Table 3.7. Slice Test analysis for simple effects on mean total captures of *Drosophila suzukii* at Site 1 in 2015.

	Trapping Week					
	3 August	10 August	17 August	25 August	31 August	8 September
F	31.5110	32.5648	12.9323	0.8179	2.5664	0.3803
P	< 0.0001	< 0.0001	< 0.0001	0.5162	0.0416	0.8223
df	4, 119	4, 119	4, 119	4, 119	4, 119	4, 119

Table 3.6. The main effect of trapping date and trap treatment on total trap captures of *Drosophila suzukii* at Site 1 in 2015.

	Trapping Week					
	3 August	10 August	17 August	25 August	31 August	8 September
Mean[1] *D. suzukii*	41.96 ± 8.9 ab	51.8 ± 10.2 a	30 ± 7.4 bc	8.44 ± 2.1 d	15.3 ± 3 cd	8.24 ± 1.5 d
	Trapping Treatment					
	ACV and Merlot	Alpha Scents	Biobest	Blank	Pherocon	
	51 ± 8.2 a	9 ± 1.6 bc	51.4 ± 7.9 a	0 c	19.6 ± 4 b	

[1]Means (± SE) within rows followed by the same letter are not significantly different by Tukey's HSD test

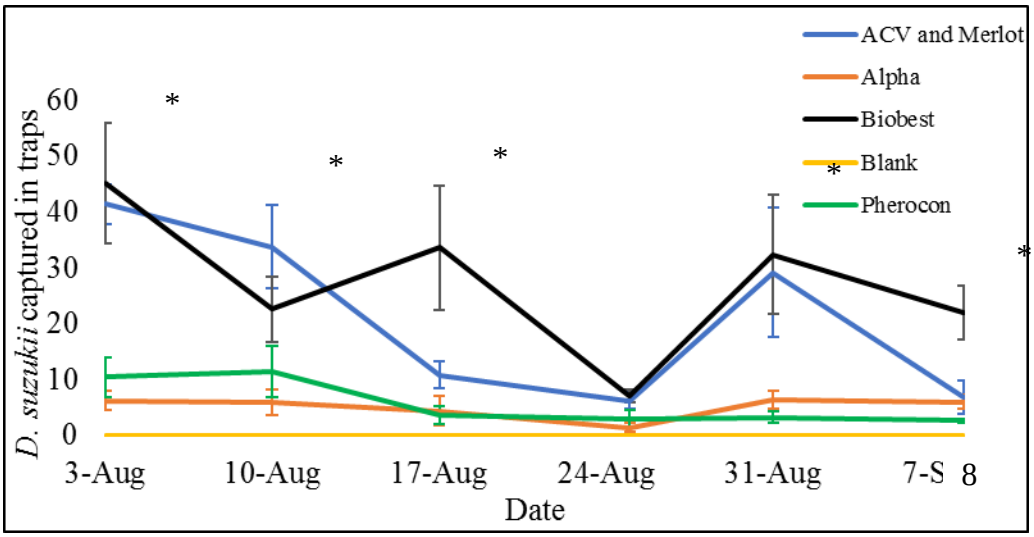

Figure 3.2. Mean (± SE) weekly *Drosophila suzukii* (males and females) trap captures at Site 2 (Albemarle Co.) in 2015. Dates for which there were significant different by a Slice Test are indicated by asterisk.

Table 3.9. Slice Test analysis for simple effects on mean total captures of *Drosophila suzukii* at Site 2 in 2015.

	Trapping Week					
	3 August	10 August	17 August	25 August	31 August	8 September
F	19.2281	7.9310	7.9010	0.3952	10.1880	3.1793
P	< 0.0001	< 0.0001	< 0.0001	0.8118	< 0.0001	< 0.0001
df	4, 120	4, 120	4, 120	4, 120	4, 120	4, 120

Table 3.8. The main effect of trapping date and trapping treatment on total trap captures of *Drosophila suzukii* at Site 2 in 2015.

	Trapping Week					
	3 August	10 August	17 August	25 August	31 August	8 September
Mean[1] *D. suzukii*	41.96 ± 8.9 a	51.8 ± 10.2 ab	30 ± 7.4 bc	8.44 ± 2.1 c	15.3 ± 3 ab	8.24 ± 1.5 bc
	Trapping Treatment					
	ACV and Merlot	Alpha Scents	Biobest	Blank	Pherocon	
	21.4 ± 3.4 a	5.1 ± 0.8 b	27.1 ± 3.8 a	0 b	5.7 ± 1.2 b	

[1]Means within rows followed by the same letter are not significantly different by Tukey's HSD test

Table 3.10. Effect of trap bait and trap type on captures of female and male *Drosophila suzukii* and non-target flies at two commercial vineyards in Virginia in 2015.

	Mean total ± SE number of flies captured[1]						
	Site 1[2]			Site 2[2]			Selectivity[3]
	Female	Male	Other Flies	Female	Male	Other Flies	Total *D. suzukii*
Blank	0 d	0 c	0.07 ± 0.1 c	0 c	0 d	0 c	-
Alpha Scents	2.8 ± 0.5 c	6.3 ± 1.3 b	30.7 ±4.4 b	1.1 ± 0.2 b	3.9 ± 0.6 bc	13.0 ± 2.0 b	0.3 ± 0.02 b
Biobest	29.2 ± 4.3 a	22.1 ± 3.8 a	127.1 ± 20.8 a	13.4 ± 2.1 a	13.7 ± 2.0 a	52.9 ± 10.1 a	0.4 ± 0.02 a
Apple Cider Vinegar + Merlot	34.9 ± 5.5 a	16.1 ± 2.9 a	124.7 ± 22.5 a	9.8 ± 1.9 a	11.3 ± 2.3 ab	94.4 ± 21.7 a	0.3 ± 0.02 ab
Pherocon	10.9 ± 1.9 b	8.6 ± 2.6 b	51.0 ± 10.5 b	3.8 ± 0.9 b	4.9 ± 1.4 c	29.0 ± 7.6 b	0.3 ± 0.03 ab
F	123.8940	66.6424	134.9362	73.7498	36.1375	84.5319	2.7656
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0427
df	4, 146	4, 146	4, 146	4, 143	4, 143	4, 143	3, 225

[1]Means within rows followed by the same letter are not significantly different by Tukey's HSD test.
[2] Traps checked weekly from 27 July - 8 September, 2015 at Sites 1 and 2, respectively.
[3]Trap selectivity, expressed as *D. suzukii* captures as a proportion of total Drosophilidae (nontarget and *Drosophila suzukii* combined for both Sites) captured per trap per week.

CHAPTER 4: *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) OVIPOSITION AND ADULT EMERGENCE IN SIX WINE GRAPE VARIETIES GROWN IN VIRGINIA

Meredith Shrader

Abstract

Drosophila suzukii (Matsumura) is a pest of small fruits and grapes in the US and in its home range of Japan. Physiological and morphological laboratory testing was performed on six commonly grown wine grape varieties in Virginia. Skin thickness, penetration force and ºBrix were analyzed to determine ovipositional preferences. Experiments were performed for three consecutive years from grapes collected at one Virginia vineyard. More eggs were laid in intact Viognier grapes than any other variety. Oviposition into intact grapes was not affected by skin thickness or ºBrix, however oviposition increased when penetration force decreased. An ovipositional choice test determined no varietal preferences. Survivorship from egg to adulthood using uninjured and injured grapes was also assessed to determine varietal suitability as *D. suzukii* hosts, with more flies emerging from injured grapes than uninjured. However, *D. suzukii* adults did emerge from intact grapes and at higher percentages than previously recorded in other wine grape studies. All varieties had eggs oviposited into them when injured and oviposition increased as ºBrix increased, but not significantly for any variety. Determining the time at which each grape variety became susceptible to oviposition was determined using a *D. suzukii* bioassay spanning 12 weeks using grapes from the green pea stage until ripe. Susceptibility to *D. suzukii* oviposition was based upon ripening period and penetration force. Early ripening varieties may be more susceptible to *D. suzukii* oviposition in the field with later maturing, harder fleshed varieties avoiding *D. suzukii* oviposition.

Keywords: wine grapes, penetration force, susceptibility, survivorship, skin thickness, Brix

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), spotted wing drosophila, is economically damaging to small fruits, cherries and grapes in areas where these crops are produced (Goodhue et al. 2011, Walsh et al. 2011). *Drosophila suzukii* can develop in both wild and cultivated grapes, including both table and wine grapes (Ioriatti et al. 2015, Kim et al. 2015). The serrated ovipositor of female *D. suzukii* facilitates oviposition in ripening fruit that other drosophilid species cannot utilize (Lee et al. 2011a). Larvae developing within the fruit reduce fruit quality and marketability (Walsh et al. 2011). Furthermore, female *D. suzukii* can render grapes susceptible to secondary pathogens, such as *Acetobacter* spp., *Gluconobacter* spp. and *Hanseniaspora uvarum*, and facilitate sour rot outbreaks in vineyards through piercing and wounding the fruit during oviposition (Barata et al. 2012, Hamby and Becher 2016, Rombaut et al. 2017). If sour rot is found in a few grapes within a cluster, the whole cluster may be culled. In addition to the loss of individual clusters in the field, sour rot in grapes can cause whole packing crates of grapes to be rejected by processors and wine makers.

Grapes are suitable hosts for *D. suzukii*. In Japan, Kanzawa (1939) demonstrated that *D. suzukii* reared on grapes had larger pupae than those reared on cherries. However, recent studies have shown that *D. suzukii* lay fewer eggs in grapes and have a longer developmental time and lower survivorship, 0 to 9%, compared with larva that develop in other fruits, such as raspberries and cherries (Lee et al. 2011b, Linder et al. 2014). Relative ranking of crop hosts suggest that grapes are poor hosts for *D. suzukii* (Bellamy et al. 2013), however, flies are able to complete their life cycle on this fruit. *Drosophila suzukii* ovipositional preferences have been linked to fruit characteristics such as fruit firmness and ºBrix, with more *D. suzukii* eggs being laid in fruit with low penetration force and higher ºBrix (Burrack et al. 2013, Ioriatti et al. 2015). It may be possible to determine if certain varieties are at greater risk of *D. suzukii* infestations based upon the morphological and physiological characteristics for each grape variety within a vineyard.

Wine grapes become susceptible to *D. suzukii* oviposition after véraison, with each grape variety ripening at different points during the growing season. During ripening, grapes undergo characteristic morphological and physiological changes associated with this process. Determining which grape varieties become susceptible to *D.*

suzukii oviposition through morphological or physiological testing could determine which varieties are more susceptible and at a higher risk to *D. suzukii* oviposition. Those varieties that become susceptible earlier and at higher risk for infestation can then be more intensely managed. Our hypothesis is that there is no varietal difference in susceptibility to *D. suzkiii* oviposition based upon morphological factors and that no *D. suzukii* will be able to complete development within these grapes. This study was undertaken to determine the physiological and morphological characteristics that make wine grape cultivars susceptible to *D. suzukii* oviposition, specifically skin thickness, skin penetration force and ºBrix, and to evaluate the resulting adult emergence from the grapes.

Materials and Methods

The *D. suzukii* laboratory populations for these studies were from the offspring of adults collected from caneberry plantings in Montgomery County, Virginia during the summers of 2012-2015 and maintained on a commercially available medium, Nutri-Fly MF (molasses formulation) (Genesee Scientific Corporation, San Diego, CA). Six varieties of wine grapes, *Vitis vinifera* L., were used to evaluate varietal effects on *D. suzukii* oviposition and larval survivorship, including Petit Manseng, Petit Verdot, Vidal Blanc (hybrid: Ugni blanc x Rayon d'Or), Viognier, Cabernet Franc, and Pinotage. Field-collected clusters of each variety came from a single vineyard in the Piedmont region (Orange County) of Virginia. The vineyard block size from which grapes of each variety were collected was; Petit Verdot 0.65 ha, Cabernet Franc 1.07 ha, Pinotage 0.2 ha, and Vidal Blanc 0.75 ha, Viognier 0.3 ha, and Petit Manseng 0.3 ha. Six clusters were randomly collected from the middle of each grape varietal block at least 3 rows (> 11 m) away from the block edge and from the middle of the selected row (> 50m). The clusters had received standard applications of fungicides and insecticides, including captan, acetamiprid, clothianidin, spirotetramat, kaolin clay and malathion. Clusters were ice-cooled and transported to Blacksburg for laboratory testing, which began within 24 hours of their collection from the field. Grapes used in ovipositional bioassay experiments were randomly selected and cut from the clusters using scissors; leaving the peduncle attached prevented access of *D. suzukii* adults to any exposed flesh created by removing

the stem. Each year, laboratory-assays were conducted over the course of several weeks during which grape physiological and morphological properties changed for each variety during the ripening process. While these changes may have affected ovipositional rates for the varieties tested, these experiments were an attempt to mirror grape physiological and morphological properties within the vineyard in which the grape varieties ripened at different rates. Thus, grapes were not held in refrigerated conditions until all grape varieties reached the same physiological or morphological state.

Physiological and Morphological Characteristics. Grapes undergo several physiological changes throughout the growing season. These changes were recorded for each experimental date by measuring the sugar content, skin thickness, and penetration force of 25 healthy, randomly-selected grape berries for each of the six grape varieties. °Brix were determined by pressing the juice from a 20 g sample of randomly-selected grapes of each variety and then placing the juice on a handheld, temperature-compensated refractometer (Zoro, Buffalo Grove, IL). Skin thickness (mm) was measured with a digital caliper (resolution 0.01 mm, Mitutoyo, Kanagawa, Japan). Penetration force measurements (centi-newtons, cN) were conducted using a technique adapted from Burrack et al. (2013), and involved placing a dulled #2 (2-mm) insect pin (BioQuip Products, Rancho Dominguez, CA) on a piece of cork attached to a centi-newton gauge (Haag-Streit USA, Mason, OH). Measurements were performed with grapes still on the cluster in case pressure from neighboring grapes affected readings. The pin was then pressed onto the equatorial midline of the grape skin until it punctured the surface of the grape.

***Drosophila suzukii* Oviposition and Adult Emergence.** To investigate the effects of grape physiological characteristics on ovipositional preference and larval survivorship of *D. suzukii* on wine grapes, three laboratory-based experiments were performed: 1) no-choice trials using intact grapes, 2) no-choice trials using intact and manually damaged grapes, and 3) choice trials using intact grapes.

No-choice trial using intact grapes 2014. No-choice assays were conducted to compare differences in *D. suzukii* infestation rates between six wine grape varieties commonly grown in Virginia. Grapes for this experiment were collected on 27 August, 2, 9 and 16 September and experiments began within 24 hours of their collection. Grapes were collected on these dates because all varieties had undergone véraison and were entering the ripening period. Grapes were checked for wounds under a dissecting microscope before the experiment; grapes with wounds were not used. No-choice tests involved placing 10 g of grapes from each of the six varieties in individual 355 ml clear plastic cups (Solo, Urbana, IL). A constant mass was used to reduce effects of fruit size. Grapes were weighed individually for each variety so an approximate number of grapes per variety could be determined. A quantity of 10 g was typically equivalent to 5-6 Viognier, 6-7 Cabernet Franc, 13-14 Petit Verdot, and 8-9 Pinotage, Petit Manseng, or Vidal Blanc grapes. Twelve rearing cups for each of the six grape varieties were used each week for four consecutive weeks after véraison (28 August – 16 September). Uninjured grapes were then placed in 355 ml plastic rearing cups and five females and five males (between 0-14 days old) were added. Cups were covered with plastic wrap (Saran, Oakland, CA) and placed in a growth chamber at 23^{o} C, 16:8 (L:D) and 50-80% RH. Flies were exposed to the grapes for 48-hr and then removed from the cups. Individual grapes were examined under a dissecting microscope and eggs were counted. All grapes were then returned to the cups, covered with plastic wrap (Saran, Oakland, CA) and returned to the growth chamber. Rearing cups were checked daily for 21 days and any emerging flies were collected and counted. Laboratory-assays were conducted over the course of 4 weeks in which grape physiological and morphological properties had changed for each variety, which may have affected ovipositional rates.

Wine grape varietal susceptibility 2015. During 2015, bioassays to determine the physiological and morphological point that each grape variety became susceptible to *D. suzukii* oviposition using uninjured and injured wine grapes were conducted. Experiments were also conducted to compare *D. suzukii* oviposition varietal preferences in injured and uninjured grapes. When grapes became susceptible to *D. suzukii* oviposition, the numbers of eggs as well as the number of adults emerging were counted

for uninjured and injured grapes. The ovipositional rates and resulting adult emergence observations were conducted from 10 August - 31 August, which encompassed the period when grapes became susceptible to *D. suzukii* oviposition.

Uninjured grapes. Oviposition experiments in uninjured grapes were conducted for a total of 12 weeks (18 June - 9 September). Grapes were checked for wounds under a dissecting microscope before the experiment; grapes with wounds were not used. Five uninjured grapes from each of the six varieties were placed in individual 355 ml clear plastic cups (Solo, Urbana, IL) on each experimental date. Five replicates per week of each grape variety were compared from the green pea stage (18 June) until véraison (20 July) when the replicates were increased to eight a week for each variety. Five females and five males (between 0-14 days old) were added, cups were covered with plastic wrap (Saran, Oakland, CA) and placed in a growth chamber at 23^{o}C, 16:8 (L:D) and 50-80% RH. Flies were exposed to the grapes for a 48-hour period and then removed from the cups. Individual grapes were examined for oviposition wounds and eggs under a dissecting microscope and the date of the first oviposition was noted for each variety as well as the physiological characteristics of that grape variety at the time.

Injured grapes. Oviposition experiments were conducted using injured grapes for a total of 12 weeks (18 June - 9 September). Grapes were checked for wounds under a dissecting microscope before the experiment; grapes with wounds were not used. Three intact grapes were then pierced three times around the equatorial midline of the grape with pointed metal forceps. The wounds created by the forceps were 2-3 mm wide and ~2 mm deep. Five male and five female *D. suzukii* flies (0-14 days old) were placed into a 355 ml clear plastic cup with three injured grapes. Three replicates for each grape variety were performed weekly. Fruit were exposed to flies for a 48-hour period and removed. Grapes were examined for oviposition wounds and eggs under a dissecting microscope and the date of first oviposition was noted as well as the physiological characteristics of that grape variety at the time of first oviposition.

Oviposition and adult emergence 2015. Once grapes became susceptible to *D. suzukii* oviposition (3 August) the number of eggs laid for each variety and grape condition were recorded. The experiments were conducted weekly between 10 and 31 August. The number of eggs laid for each variety and grape condition were counted

using a dissecting microscope. After eggs were counted, all grapes were returned to the cups, covered with plastic wrap (Saran, Oakland, CA) and held for 21 d in a growth chamber at 23ºC, 16:8 (L:D) and 50-80% RH. The grapes were checked daily by a visual inspection for any adults. Adults emerging were counted and collected from the plastic rearing cups, so an approximate number emerging from each grape variety and berry condition could be tabulated for each replicate.

Choice and no-choice trials using intact grapes 2013. No-choice and choice assays were conducted to compare differences in *D. suzukii* infestation rates between six wine grape varieties commonly grown in Virginia. Grapes for this experiment were collected from the field on 6, 14 and 22 September and experiments began within 24 hours of their collection. Grapes were collected on these dates because all varieties had undergone véraison and were entering the ripening period. Grapes were checked for wounds under a dissecting microscope before the experiment, and grapes with wounds were not used. Cage no-choice tests involved placing a 20 g sample of individual grapes, cut off the cluster with pedicle attached, from one of the six varieties into 0.30 m^3 collapsible mesh cages (BioQuip, Salinas CA). A constant mass was used to reduce effects of fruit size. Grapes were weighed individually for each variety so an approximate number of grapes per variety could be determined. A quantity of 20 g was typically equivalent to 10-11 Viognier, 13-14 Cabernet Franc, 26-28 Petit Verdot, and 17- 18 Pinotage, Petit Manseng or, Vidal Blanc grapes. Fifteen male and 15 female *D. suzukii* flies (between 0-14 days old) were released into the center of the cages with grapes. Cages were placed on laboratory benches at room temperature of 23ºC and exposed to indirect sunlight, RH was not measured. Cage positions on the benches were re-randomized for each experimental date. Grapes were exposed to flies for 4 h, then the grapes were removed and placed in 355 ml clear plastic rearing cups (Solo, Urbana, IL), covered with plastic wrap (Saran Wrap, Oakland, CA) and held in a growth chamber at 23ºC, 16:8 (L:D) and 50-80% RH. The number of eggs per grape was not counted. Cups were checked daily for 21 d and emerging flies were collected and counted. Twelve treatment replicates were run for each experimental date, with 3 experimental days in 2013. Choice experiments were performed using the same methodology as above using 20 g of each grape variety placed in a random arrangement within each cage. Eggs were

not counted after the 4 h fly exposure period for the no-choice experiments. There were a total of twelve treatment replicates for each variety over the three experimental dates.

Statistical Analysis. Physiological characteristics for surface penetration force and skin thickness were analyzed using a full factorial ANOVA with testing dates (2013-2015) and wine grape variety as main effects. If interactions of grape variety and date were determined to be significant, each year was analyzed separately using one-way ANOVA, blocked by date, followed by Tukey's HSD to separate the means for the six grape varieties. Linear regression was used to examine the relationships between oviposition, skin thickness and penetration force of the grapes for each experimental year. In 2013, choice cage tests using the six grape varieties were arranged in a completely randomized design within each cage, using the date of the experiment as a blocking factor. The 20 g grape samples for each variety were re-randomized within each cage for every experimental date. The ovipositional and adult emergence data for no-choice and choice tests for 2013, 2014 and 2015 for intact grape experiments could not be normalized, therefore a nonparametric Wilcoxon Test was used as well as a Wilcoxon Each Pair test to determine statistical significance. The 2015 injured grape oviposition preference and adult emergence data were analyzed using a one-way ANOVA, blocked by date, followed by Tukey's HSD to separate the means. Survivorship to adulthood in 2014 and 2015, using both uninjured and injured grapes, was analyzed using a mixed-model ANOVA (JMP SAS, Cary, NC) with grape variety as a fixed effect and replicate number within grape variety and experimental date as random effects.

Results

Physiological and Morphological Characteristics. There were significantly different penetration forces and skin thicknesses for all six grape varieties in all three years as well as a significant interaction between penetration force, skin thickness and testing date (Table 4.1). Due to testing dates being significantly different, each experimental date for each year was analyzed separately. During the testing period, penetration force decreased, ºBrix increased, and skin thickness showed little variation for most grape varieties.

In 2013, while there were some differences as berries matured, Petit Manseng required the highest force to penetrate, while Viognier required the least (Table 4.2). In 2014 and 2015, Vidal Blanc and Petit Manseng required the most force to penetrate while Viognier required the least (Table 4.2). Skin thickness for all years tested did not show an overall decrease for all varieties over the ripening period (Table 4.3). In 2013, the thickest skin was shown to be Petit Manseng, while Petit Verdot had the thinnest. In 2014, Vidal Blanc had the thickest skin while Petit Verdot and Viognier had the thinnest. In 2015, Petit Manseng and Vidal Blanc possessed the thickest skin and Viognier the thinnest. Degrees Brix were tracked throughout the growing season and increased for most varieties over the testing period for each year (Table 4.4).

No-Choice Trial Using Intact Grapes 2014. The greatest number of eggs was laid in Viognier grapes and the fewest in Vidal Blanc (Table 4.5). The total number of adults emerging and the survivorship of eggs to adulthood were not significantly different among the six varieties. Low egg to adult survivorship in Viognier may have been due to larval competition. Significantly more eggs were laid when penetration force decreased (Figure 4.1), but the correlation value was low ($R^2 = 0.033$). There was no correlation between skin thickness and oviposition.

Wine Grape Varietal Susceptibility 2015.

Uninjured grapes. Penetration force for the six grape varieties varied significantly by date over the twelve-week period. No oviposition was observed during the first six weeks of testing of uninjured grapes. Uninjured grapes became susceptible to *D. suzukii* oviposition on 3 August 2015, with Viognier being the first attacked when its average penetration force was 16.15 cN (Table 4.6). Cabernet Franc had oviposition directly into the flesh two weeks later (17 August 2015), when its average penetration force was 19.55 cN. Pinotage was the last grape variety to have eggs laid directly into the flesh (31 August 2015), with an average penetration force of 13.75 cN. Petit Manseng, Petit Verdot and Vidal Blanc had no eggs laid directly into the flesh in uninjured grapes.

Injured grapes. The first egg laid on the manually injured grapes was on 18 June 2015 on Viognier (5º Brix) and Cabernet Franc (5.8º Brix), with one and two eggs laid, respectively. Of those eggs laid on the Cabernet Franc, one adult emerged (data not shown). The average penetration resistance values for uninjured Viognier and Cabernet France were 32.9 and 24.35 cN respectively (Table 4.6). Petit Manseng and Vidal Blanc had eggs laid on the injured grapes on 29 June with average penetration forces of 26.7 and 32.5 cN in the uninjured grapes, respectively. Petit Verdot and Pinotage were the last to have eggs laid in the injured grapes (8 July) with an average penetration force of 20.6 and 28.2 cN in the uninjured grapes, respectively. This indicated that *D. suzukii* females would lay eggs on unripe fruit in which the skin had been damaged.

Oviposition and Adult Emergence 2015. The Wilcoxon test showed that significantly more eggs were laid on uninjured Viognier and Cabernet Franc than on any other varieties (Table 4.7). Few eggs were laid on Pinotage and no eggs were laid on the Vidal Blanc, Petit Manseng or Petit Verdot grapes (Table 4.7). Grape varieties had significantly different average penetration forces ($df = 5$, $F = 66.6314$; $P < 0.0001$) with Viognier being the lowest, measuring 9.23 cN (Table 4.7). The firmest varieties were Vidal Blanc and Petit Manseng and the thickest-skinned variety was Petit Manseng. Penetration force and oviposition in intact grapes were evaluated by a linear regression and found to be significant, however the R^2 value was low ($R^2 = 0.088$) (Figure 4.2). There was no linear correlation between skin thickness and oviposition. Survivorship was between 25-29% based upon variety, with significantly more *D. suzukii* surviving in Cabernet Franc and Viognier (Table 4.7).

The number of eggs laid in injured grapes was significantly different among the six varieties tested. The greatest numbers of eggs laid were in the Vidal Blanc and Pinotage grapes, with 15.8 and 11.2 eggs per replicate. The fewest eggs were laid on the Petit Manseng. Neither adult emergence nor survivorship (18-38%) was significantly different among varieties (Table 4.7).

Choice and No-Choice Trails Using Intact Grapes 2013. There was no significant difference in the number of adult *D. suzukii* emerging among the six grape varieties in the

choice tests (Table 4.8). There was no significant linear correlation between penetration force or skin thickness and number of adults emerging. There were also no significant differences in the number of adult *D. suzukii* emerging from the no-choice tests (Table 4.8).

Discussion

Testing in 2014 and 2015 demonstrated an ovipositional preference for Viognier, which also had the lowest penetration force. Linear regression performed in 2014 and 2015 demonstrated that oviposition increased in grapes with lower penetration forces, but the linear fit of the lines was poor. Our experimental observations were supported by previous similar studies showing more eggs laid in fruits when penetration forces decreased as well as no oviposition in grapes with high penetration forces (Lee et al. 2011b, Ioriatti et al. 2015). There was no linear relationship between skin thickness and penetration force nor was there a relationship between skin thickness and oviposition from comparisons in 2014 and 2015. These results enabled us to determine that *D. suzukii* risk of infestation was not based upon the skin thickness of the varieties. It had been feared that thin-skinned varieties would be more susceptible to *D. suzukii* oviposition. The choice and no-choice tests in 2013 showed no differences in adult emergence for any of the six varieties tested. These findings were similar to those of Lee et al. (2011a), when no significant difference was seen in ovipositional preference for four wine grape varieties.

The 12-week uninjured grape susceptibility experiment determined that *D. suzukii* oviposition in grapes appeared to be based upon when each variety underwent véraison and began the ripening process. This was also the period during which the grapes began to sequester sugar. Thus, the early maturing grape varieties such as Viognier became susceptible to *D. suzukii* oviposition a month sooner than other varieties tested. The lack of oviposition seen in Vidal Blanc and Petit Manseng may have been due to the high penetration force of these varieties. Our laboratory findings suggested that physical factors could be used to determine which grape varieties within a vineyard might be at higher risk from *D. suzukii* oviposition. These measurements could be used to determine when peak susceptibility will occur for each variety as well as determine a varietal risk

table for oviposition based upon ripening period (early or late), although laboratory findings are not always representative of what occurs in the field. Ioriatti et al. (2015) showed similar results with respect to penetration force and *D. suzukii* susceptibility when they observed that grape varieties with consistently high penetration force (< 40 cN) had no infestations of *D. suzukii*. However, the results of Pelton et al. (2017), working in Oregon, differed from our findings in that *D. suzukii* larvae were present in all grape varieties, regardless of penetration force in the vineyards surveyed, but at low presence and abundance (15%) and that the likelihood of larval presence increased as the season progressed with no significant effect of variety.

The intact grape oviposition assay further demonstrated that penetration force was a limiting factor for *D. suzukii* susceptibility in wine grapes. Our observations were substantiated by the injured grape experimental results. Observations from the 12-week oviposition bioassay for injured grapes showed that oviposition would occur in grapes that were damaged despite low ºBrix. Furthermore, all grape varieties in this experiment had *D. suzukii* oviposit in wounds regardless of the level of soluble sugars (ºBrix). Lee et al. (2011b) also demonstrated that *D. suzukii* could develop on strawberries with low ºBrix. *Drosophila suzukii* laid more eggs in the injured grape varieties with higher ºBrix, but differences in egg numbers were not statistically significant. Every grape variety tested had adults emerge, but an increase in eggs laid did not result in more adults emerging. This suggested that there was a carrying capacity based upon grape variety (See Chapter 5). The overall suitability of grapes may have influenced survivorship as well; Bellamy et al. (2015) determined that grapes were a poor host for *D. suzukii*, which may have explained the low survivorship of eggs to adulthood within the grapes.

The fact that *D. suzukii* can utilize wine grapes as a host plant has a two-fold impact on growers. First, when *D. suzukii* oviposit into grapes and adults emerge, the fly population could increase within the vineyard. D*rosophila suzukii* populations in vineyards may also increase when flies utilize alternative host plants around the vineyards migrate into the vineyard blocks once grapes have ripened. Increased populations of *D. suzukii* are likely to injure more grapes. Second, the wounding of grapes by the serrated ovipositor of *D. suzukii* increases the likelihood of invasion by secondary pathogens, such as those causing sour rot, throughout the vineyard (Barata et

al. 2012, Atallah et al. 2014, Rombaut et al. 2017). The whole cluster may be culled when only a few grapes have sour rot causing a loss of product and increasing management costs due to paying workers to cull clusters in the field. Based on our results, it appeared that Viognier is at the highest risk for oviposition early in the growing season than other later maturing varieties due to low penetration force needed to puncture the grapes. It was also an early maturing variety, which could make it a primary target for *D. suzukii* emigrating from areas around the vineyard, having developed on wild host plants. However, later maturing varieties (Cabernet Franc) may be at greatest risk of *D. suzukii* oviposition due to these grapes ripening later in the summer when *D. suzukii* populations are larger than those populations that occur in the spring. Determining which varieties are most susceptible and at highest risk of *D. suzukii* oviposition would help viticulturists plan what varieties to plant as well as design spray programs based upon physiological and morphological characteristics of each grape variety instead of spraying whole vineyards. This may decrease the management costs for these later maturing and harder fleshed varieties by eliminating unwarranted insecticidal sprays based upon the morphological and physiological state of the grapes.

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Table 4.1. Full factorial analysis for penetration force (cN) and skin thickness (mm) for six wine grape varieties, across all testing dates (years and weeks) combined

	Penetration Force (cN)			Skin Thickness (mm)		
	F	df	P	F	df	P
Date	70.3854	10	< 0.0001	98.5465	10	< 0.0001
Grape Variety	146.5846	5	< 0.0001	95.9302	5	< 0.0001
Date*Grape Variety	8.0515	50	< 0.0001	9.9689	50	< 0.0001

Table 4.2. Mean (± SE) penetration force (centi-newtons, cN) from 25 randomly selected grapes representing six wine grape varieties exposed to *Drosophila suzukii* in choice (2013) and no-choice bioassays (2014, 2015).

Year		Experimental Date			
2013	**Variety**	**7 September**	**14 September**	**23 September**	
	Petit Manseng	19.1 ± 0.7 a	19.4 ± 0.8 a	21.1 ± 0.9 a	
	Vidal Blanc	17.4 ± 0.6 ab	20.1 ± 0.7 a	17.0 ± 0.5 bc	
	Viognier	14.9 ± 0.4 b	15.6 ± 0.5 b	13.5 ± 0.6 d	
	Petit Verdot	15.6 ± 0.7 b	16.2 ± 0.9 b	15.6 ± 0.6 bcd	
	Cabernet Franc	15.6 ± 0.58 b	19.2 ± 0.7 a	14.3 ± 0.8 cd	
	Pinotage	16.6 ± 0.7 ab	19.1 ± 0.8 a	17.9 ± 0.6 b	
	F	5.7941	6.8029	16.7790	
	P	< 0.0001	< 0.0001	< 0.0001	
	df	5, 149	5, 149	5, 149	
		28 August	**3 September**	**10 September**	**17 September**
2014	Petit Manseng	18.2 ± 0.6 ab	17.0 ± 0.8 ab	15.9 ± 0.6 a	16.9 ± 0.7 a
	Vidal Blanc	20.9 ±0.8 a	19.5 ± 0.6 a	16.5 ± 0.6 a	14.9 ± 0.3 a
	Viognier	12.8 ± 0.7 d	12.1 ± 0.6 c	10.6 ± 0.6 b	10.4 ± 0.6 b
	Petit Verdot	16.4 ± 0.8 bc	15.8 ± 0.67 b	11.2 ± 0.6 b	9.8 ± 0.4 b
	Cabernet Franc	15.6 ± 0.6 bcd	17.6 ± 0.9 ab	11.7 ± 0.5 b	10.9 ± 0.4 b
	Pinotage	14.8 ± 0.7 cd	16.2 ± 0.7 b	11.3 ± 0.6 b	10.1 ± 0.4 b
	F	16.0873	11.4257	20.3718	37.5043
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	df	5, 149	5, 149	5, 149	5, 149
		10 August	**17 August**	**25 August**	**31 August**
2015	Petit Manseng	18.6 ± 0.5 ab	17.9 ± 0.3 ab	15.3 ± 0.6 a	14.7 ± 0.4 a
	Vidal Blanc	19.6 ± 0.6 ab	17.5 ± 0.4 ab	15.2 ± 0.3 a	14.2 ± 0.4 a
	Viognier	10.8 ± 0.6 d	7.0 ± 0.4 d	10.0 ± 0.3 b	9.2 ± 0.3 c
	Petit Verdot	17.9 ± 0.6 b	17.3 ± 0.6 b	14.1 ± 0.4 a	11.2 ± 0.3 b
	Cabernet Franc	20.8 ± 0.7 a	19.6 ± 0.6 a	11.0 ± 0.4 b	14.3 ± 0.3 a
	Pinotage	15.4 ± 0.6 c	14.8 ± 0.4 c	14.3 ± 0.3 a	13.8 ± 0.4 a
	F	36.0143	79.9381	37.5827	40.8402
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	df	5, 149	5, 149	5, 149	5, 149

[1]Values within a column followed by the same letter are not significantly different (α =0.05, Tukey-Kramer adjustment)

Table 4.3. Mean (± SE) skin thickness (mm) representing 25 randomly selected grapes from six wine grape varieties exposed to *Drosophila suzukii* in choice (2013) and no-choice bioassays (2014, 2015).

Year		Experimental Date			
2013	**Variety**	**7 September**	**14 September**	**23 September**	
	Petit Manseng	0.16 ± 0.005 ab	0.15 ± 0.004 a	0.21 ± 0.011 a	
	Vidal Blanc	0.17 ± 0.009 a	0.13 ± 0.008 ab	0.13 ± 0.008 bc	
	Viognier	0.13 ± 0.006 c	0.12 ± 0.006 bc	0.14 ± 0.007 bc	
	Petit Verdot	0.10 ± 0.004 d	0.08 ± 0.003 d	0.09 ± 0.004 d	
	Cabernet Franc	0.10 ± 0.006 d	0.11 ± 0.007 c	0.11 ± 0.005 cd	
	Pinotage	0.14 ± 0.005 bc	0.14 ± 0.005 ab	0.16 ± 0.008 b	
	F	23.1004	17.3075	31.8142	
	P	< 0.0001	< 0.0001	< 0.0001	
	df	5, 149	5, 149	5, 149	
		28 August	**3 September**	**10 September**	**17 September**
2014	Petit Manseng	0.11 ± 0.004 ab	0.08 ± 0.005 b	0.14 ± 0.006 a	0.16 ± 0.005 a
	Vidal Blanc	0.16 ± 0.009 a	0.11 ± 0.005 a	0.14 ± 0.008 a	0.12 ± 0.005 c
	Viognier	0.08 ± 0.002 b	0.06 ± 0.004 c	0.09 ± 0.004 c	0.09 ± 0.005 d
	Petit Verdot	0.07 ± 0.004 b	0.06 ± 0.003 c	0.07 ± 0.002 d	0.09 ± 0.004 d
	Cabernet Franc	0.15 ± 0.03 a	0.10 ± 0.004 b	0.11 ± 0.003 b	0.15 ±0.004 ab
	Pinotage	0.13 ±0007 a	0.09 ± 0.004 b	0.11 ± 0.005 b	0.13 ± 0.004 bc
	F	8.0112	27.9790	30.2302	44.5660
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	df	5, 149	5, 149	5, 149	5, 149
		10 August	**17 August**	**25 August**	**31 August**
2015	Petit Manseng	0.11 ± 0.006 b	0.09 ±0.004 b	0.09 ± 0.003 a	0.08 ± 0.004 a
	Vidal Blanc	0.14 ± 0.005 a	0.10 ± 0.006 ab	0.06 ± 0.005 b	0.06 ± 0.004 bc
	Viognier	0.05 ± 0.005 c	0.11 ± 0.007 a	0.07 ± 0.005 b	0.02 ±0.002 d
	Petit Verdot	0.10 ± 0.004 b	0.09 ± 0.004 b	0.04 ± 0.002 c	0.04 ± 0.003 c
	Cabernet Franc	0.11 ± .004 b	0.09 ± 0.004 b	0.08 ± 0.003 ab	0.06 ± 0.003 b
	Pinotage	0.10 ± 0.004 b	0.10 ± 0.004 b	0.07 ± 0.005 b	0.05 ± 0.003 bc
	F	33.5571	3.9352	16.1847	37.9970
	P	< 0.0001	0.0025	< 0.0001	< 0.0001
	df	5, 149	5, 149	5, 149	5, 149

[1]Values within a column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer adjustment)

Table 4.4. Soluble solids (ºBrix) of six wine grape varieties used in a study comparing oviposition by *Drosophila suzukii*, based on 30 g grape samples.

Year	Grape Variety	Experimental Date			
		7 September	**14 September**	**23 September**	
2013	Petit Manseng	15.6	22.8	18.0	
	Petit Verdot	15.4	20.4	16.0	
	Viognier	19.2	23.2	21.8	
	Vidal Blanc	16.0	16.0	15.0	
	Cabernet Franc	13.2	16.2	19.2	
	Pinotage	17.2	24.2	23.2	
		28 August	**3 September**	**10 September**	**17 September**
2014	Petit Manseng	14.8	20.2	21.4	23.8
	Petit Verdot	11.9	12.8	17.6	16.2
	Viognier	17.5	17.0	20.0	21.0
	Vidal Blanc	14.5	19.8	20.0	21.0
	Cabernet Franc	16.7	19.2	19.2	21.4
	Pinotage	17.9	18.4	22.0	21.2
		10 August	**17 August**	**25 August**	**31 August**
2015	Petit Manseng	7.0	19.2	21.8	21.8
	Petit Verdot	12.4	17.0	18.4	18.8
	Viognier	14.0	17.4	19.0	23.0
	Vidal Blanc	18.8	18.0	21.0	21.4
	Cabernet Franc	11.0	15.0	19.0	19.0
	Pinotage	21.2	22.0	20.6	24.0

Table 4.5. Mean (± SE) grape penetration force, *Drosophila suzukii* oviposition, adult emergence and egg to adulthood survival in laboratory no-choice bioassays in uninjured wine grapes (20 g) 2014.

Variety	Penetration Force[1] (cN)	SWD eggs laid[2]	Adult SWD emergence[2]	% Survival[1]
Petit Manseng	17.0 ± 0.4 a	2.8 ± 0.6 a	0.6 ± 0.1	11
Petit Verdot	13.3 ± 0.4 b	3.1 ± 0.8 a	0.7 ± 0.2	12
Viognier	11.5 ± 0.3 c	4.8 ± 1.3 a	0.4 ± 0.1	9
Vidal Blanc	17.9 ± 0.4 a	0.7 ± 0.2 b	1.5 ± 0.4	49
Cabernet Franc	13.9 ± 0.4 b	1.1 ± 0.5 b	0.9 ± 0.2	28
Pinotage	13.1 ± 0.4 b	3.6 ± 0.6 a	1.0 ± 0.2	24
F	42.7375	-	-	2.1064
P	< 0.0001	-	-	0.0699
Prob > ChiSquare	-	0.0007	0.2678	
df	5, 149	5	5	5, 110

[1]Values within a column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer adjustment)
[2]Values within a column followed by the same letter are not significantly different ($\alpha = 0.05$, Wilcoxon Each Pair Test)

Table 4.6. [a]Penetration force (cN) (mean ± SE) representing 25 randomly selected grapes from six grape varieties exposed to a *Drosophila suzukii* oviposition bioassay in 2015.

Date	Cabernet Franc	Petit Verdot	Petit Manseng	Viognier	Vidal Blanc	Pinotage
18-Jun	24.4 ± 0.6	22.3 ± 0.6	27.0 ± 0.5	32.9 ± 0.5	30.7 ± 0.6	31.3 ± 0.6
29-Jun	25.3 ± 0.7	28.6 ± 0.5	26.7 ± 1.1	26.6 ± 0.8	32.6 ± 0.5	30.9 ± 0.6
8-Jul	26.7 ± 0.7	20.6 ± 0.6	28.6 ± 0.5	24.7 ± 0.7	29.4 ± 0.9	28.2 ± 0.7
13-Jul	24.0 ± 0.5	22.9 ± 0.6	25.5 ± 0.3	22.9 ± 0.06	31.5 ± 0.6	24.9 ± 0.4
20- July	22.3 ± 0.6	20.3 ± 0.6	22.6 ± 0.5	24.9 ± 0.7	31.4 ± 0.6	24.3 ± 0.4
27-Jul	25.1 ± 0.5	18.8 ± 0.4	26.1 ± 0.4	12.4 ± 0.4	28.7 ± 0.5	18.2 ± 0.5
3-Aug*****	24.2 ± 0.8	23.5 ± 0.7	25.3 ± 0.6	16.2 ± 0.6*****	20.1 ± 0.6	14.8 ± 0.5
10-Aug	20.8 ± 0.7	17.9 ± 0.6	18.6 ± 0.5	10.8 ± 0.6	19.5 ± 0.6	15.4 ± 0.6
17-Aug*****	19.6 ± 0.6*****	17.3 ± 0.6	17.9 ± 0.6	7.0 ± 0.4	17.5 ± 0.4	14.8 ± 0.4
25-Aug	11.0 ± 0.4	14.1 ± 0.4	15.3 ± 0.6	10.0 ± 0.3	15.2 ± 0.3	14.3 ± 0.3
31-Aug*****	14.3 ± 0.3	11.2 ± 0.3	14.7 ± 0.4	9.2 ± 0.3	14.2 ± 0.4	13.8 ± 0.4*****
9-Sept	11.1 ± 0.3	10.2 ± 0.2	13.8 ± 0.5	9.7 ± 0.6	13.9 ± 0.3	10.2 ± 0.2

[a]Dates marked by * when direct oviposition occurred.

Table 4.7. Mean (± SE) grape penetration force, *Drosophila suzukii* oviposition, adult emergence and egg to adulthood survival in laboratory no-choice bioassays from uninjured and injured wine grapes 2015.

		Uninjured Grapes			Injured Grapes		
Variety	Penetration Force[1] (cN)	SWD eggs laid[2]	Adult SWD emergence[2]	% Survival[1]	SWD eggs laid[1]	Adult SWD emergence[1]	% Survival[1]
Petit Manseng	16.6 ± 0.3 a	0 b	0 b	-	3.9 ± 1.8 b	1.0 ± 0.5	26
Petit Verdot	15.1 ± 0.4 b	0 b	0 b	-	7.3 ± 1.3 ab	2.1 ± 0.5	28
Viognier	9.2 ± 0.26 c	4.38 ± 1.4 a	1.14 ± 0.34 a	26 a	7.8 ± 1.8 ab	2.5 ± 0.6	32
Vidal Blanc	16.6 ± 0.3 a	0 b	0 b	-	14.5 ± 2.9 a	2.6 ± 0.8	18
Cabernet Franc	16.4 ± 0.5 a	2.07 ± 0.9 a	0.59 ± 0.27 a	29 a	6.2 ± 1.9 ab	2.3 ± 0.8	38
Pinotage	14.6 ± 0.2 b	0.14 ± 0.1b	0.04 ± 0.04 b	25 b	11.2 ± 2.6 ab	3.0 ± 0.9	27
F	66.6314	-	-	6.4228	3.1163	1.0003	2.6618
P	< 0.0001	-	-	0.0070	0.0138	0.4246	0.0848
Chi-Squared	-	< 0.0001	< 0.0001		-	-	
df	5, 149	5	5	2, 20.1	5, 66	5, 66	5, 10.5

[1]Values within a column followed by the same letter are not significantly different (α = 0.05, Tukey-Kramer adjustment)
[2]Values within a column followed by the same letter are not significantly different (α = 0.05, Wilcoxon Each Pair Test)

Table 4.8. Penetration force (mean ± SE) and adult *Drosophila suzukii* emergence (mean ± SE) from each experimental arena in laboratory choice and no-choice assays of six wine grape varieties 2013.

		Adult SWD emergence[2] from 20g grapes	
Variety	Penetration Force[1] (cN)	No-choice	Choice
Petit Manseng	19.9 ± 0.5 a	0.9 ± 0.4	0.4 ± 0.2
Petit Verdot	15.8 ± 0.4 de	0.3 ± 0.1	0.8 ± 0.4
Viognier	14.7 ± 0.3 e	1.1 ± 0.5	0.8 ± 0.4
Vidal Blanc	18.2 ± 0.4 b	0.06 ± 0.1	0.5 ± 0.3
Cabernet Franc	16.4 ± 0.5 cd	0.4 ± 0.2	0.3 ± 0.1
Pinotage	17.9 ± 0.4 bc	0.4 ± 0.3	0.6 ± 0.2
F	20.6976	-	-
P	< 0.0001	-	-
Prob > ChiSquare	-	0.1319	0.6755
df	5, 149	5	5

[1]Values within a column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey-Kramer adjustment)

[2]Values within a column followed by the same letter are not significantly different (Wilcoxon Each Pair Test)

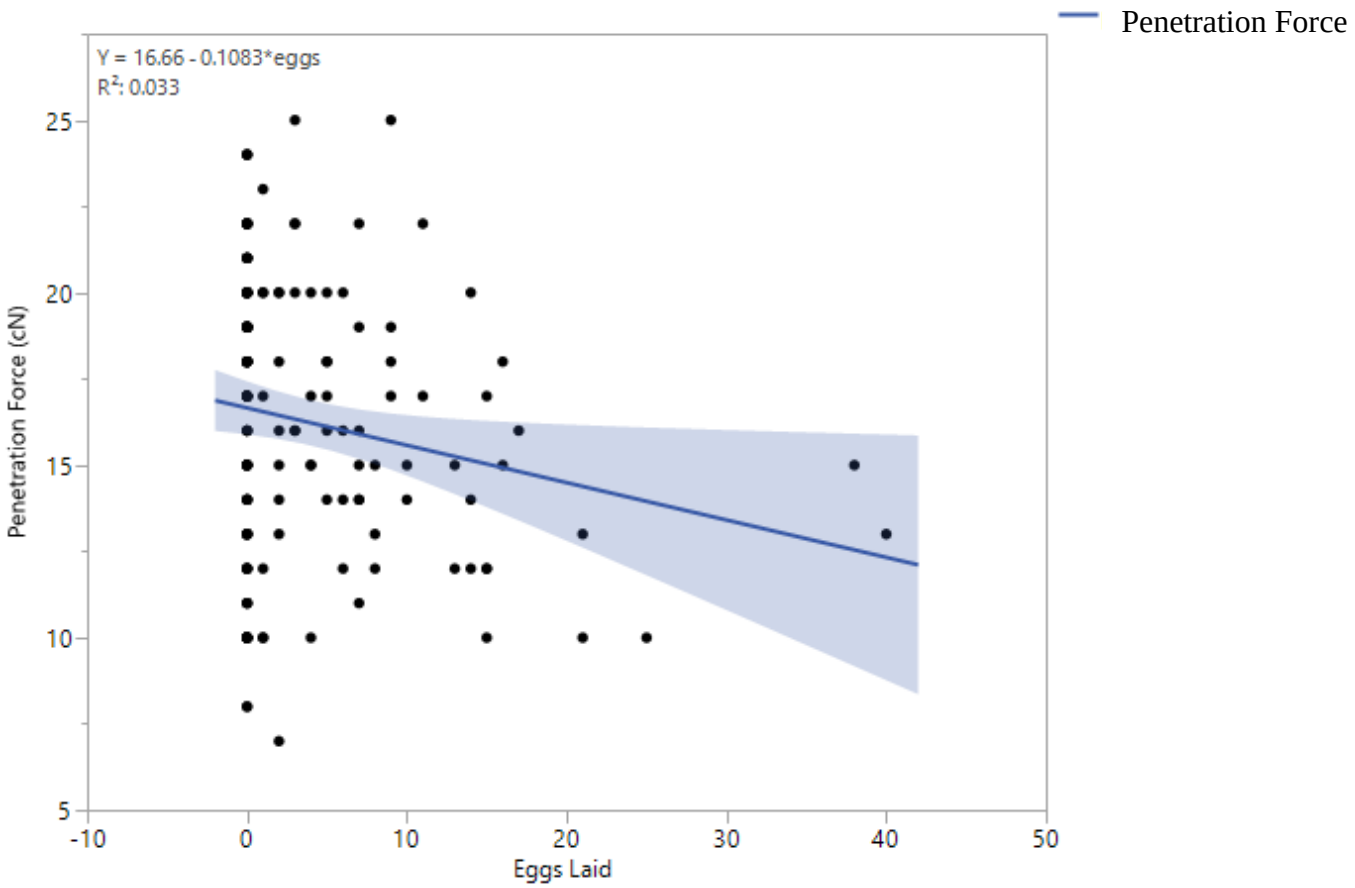

Figure 4.1. Mean penetration force for all grapes and resulting oviposition prevalence by *Drosophila suzukii* 2014.

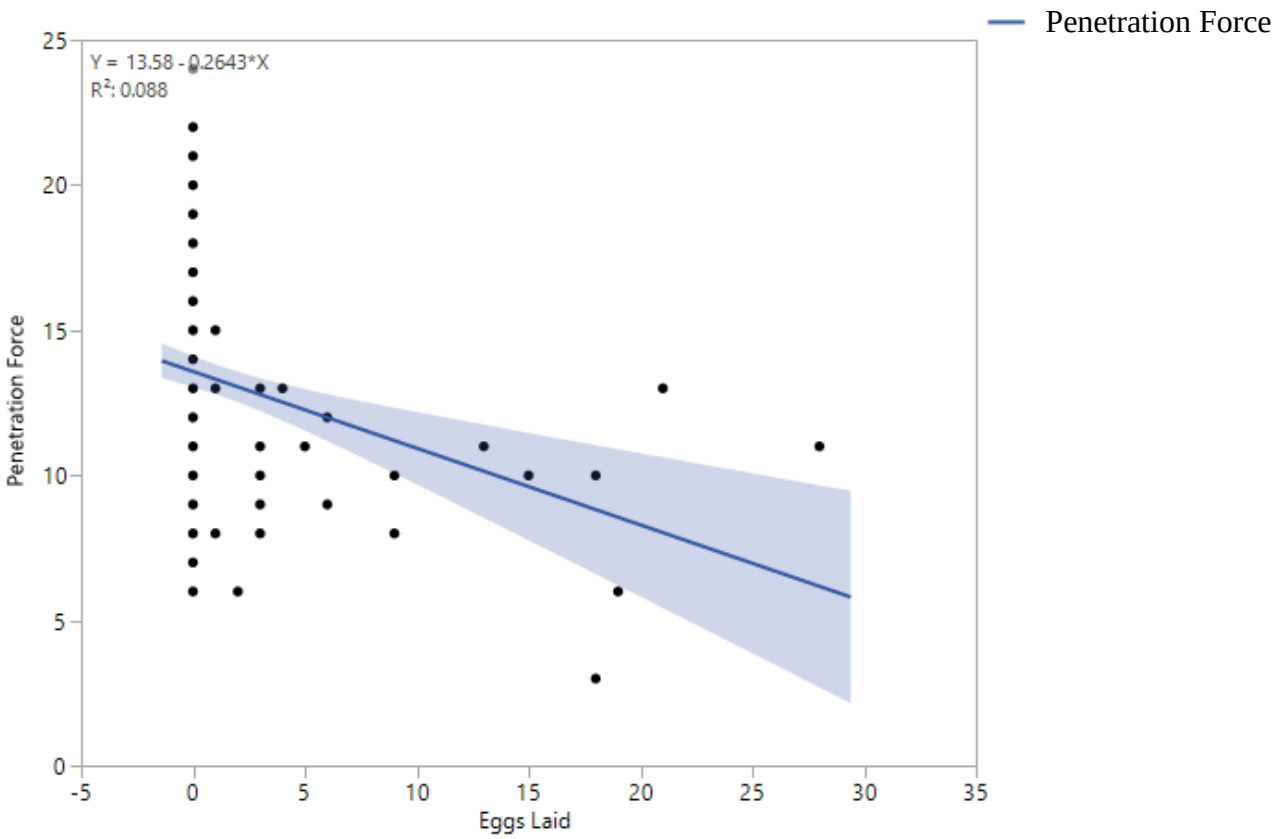

Figure 4.2. Mean penetration force for all grapes and resulting oviposition prevalence by *Drosophila suzukii* 2015.

CHAPTER 5: EFFECTS OF INTERSPECIFIC LARVAL COMPETITION ON DEVELOPMENTAL PARAMETERS IN NUTRIENT SOURCES BETWEEN *DROSOPHILA SUZUKII* (MATSUMURA) (DIPTERA: DROSOPHILIDAE) AND *ZAPRIONUS INDIANUS* GUPTA

Meredith Shrader

Abstract

Two invasive drosophilids, *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* Gupta are expanding their geographic distribution and cohabiting grape production in the Mid-Atlantic. The ecological and economic impact of these two species within vineyards is currently unknown. It was logical to assume that *Z. indianus* is not capable of ovipositing directly into grapes because they lack a serrated ovipositor, and further, that *Z. indianus* may use *D. suzukii* oviposition punctures as deposition sites for their own eggs. Therefore, an interspecific larval competition assay was performed at varying larval densities using commercial medium and four commonly grown wine grapes in Virginia to investigate the impact *Z. indianus* larvae may have on the mortality and developmental parameters of *D. suzukii* larvae. *Zaprionus indianus* did not affect *D. suzukii* mortality or development parameters even at the highest interspecific densities tested when reared in commercial medium. However, *Z. indianus* did cause higher *D. suzukii* mortality when competition took place within grapes. Mortality was also influenced by the variety of grape in which the larvae were reared, with smaller grapes having the highest *D. suzukii* mortality. *Zaprionus indianus* also increased development time to pupariation and adult emergence for most interspecific competition levels compared to the intraspecific *D. suzukii* controls. Pupal volume was marginally affected at the highest interspecific larval densities. Studies also indicated that *D. suzukii* larvae reared in Viognier grapes, even when in competition with *Z. indianus*, had higher survivorship rates to adulthood and developmental parameters and pupal volume were not affected.

Keywords: Interspecific competition, intraspecific competition, wine grapes, medium, pupal volume, *Drosophila suzukii*, *Zaprionus indianus*, development days

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, Gonzalalez-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, Gonzalalez-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, Gonzalalez-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 5.1). 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 5.1).

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 5.1). 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 5.2). 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 5.2). 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 5.2).

There was a significant relationship between competition level and grape variety on larvae surviving to pupariation based upon the nominal logistic regression analysis (Table 5.3) 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 5.4). 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 5.2). 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 5.4).

The binary nominal logistic regression showed a significant relationship between competition level and grape variety on larvae surviving to adulthood. (Table 5.3). 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 5.5). 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 5.5). 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 5.5).

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 not effected 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 5.4). 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 5.6, Fig. 5.1A). Larval developmental days at the 2:2 competition level and 4 *D. suzukii* alone control were significantly impacted by both grape variety and competition level (Table 5.7). There was also a significant interaction between competition level and grape variety on larval developmental days (Table 5.7).

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 5.8). Larval developmental time was longest in Viognier at the 2:2 competition level, but was only significantly different when compared to Petit Manseng (Fig. 5.2A). The shortest larval developmental time was seen in the Petit Verdot grapes at the 4 *D. suzukii* alone competition level (Fig. 5.2A). The Slice Test for larval development was significantly different for Petit Manseng and Petit Verdot, but not Viognier or Cabernet Franc (Table 5.8). 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 5.4) with no adults emerging from the Petit Verdot (Fig. 5.1B). There was no effect of competition level on total development, nor was there an interaction of grape variety and competition level (Table 5.6). 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 5.7). 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. 5.2B). The Slice Test for total development was significantly different for the 2:2 density, but not at the 4 *D. suzukii* density (Table 5.8). 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. 5.2B). 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 5.9). 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. 5.1C), 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 5.7). Pupal volumes were smallest when larvae were reared on the Viognier grapes, at both competition levels (Fig 5.2C). There were no significant interactions between competition level and grape variety on the volume of the pupae (Table 5.7).

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. 5.3). Larval development time was not affected at the 2:2 competition level compared to the 4 *D. suzukii* alone controls (P= 0.9423) (Fig. 5.4).

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. 5.3). Total development time was not affected at the 2:2 competition level and 4 *D. suzukii* alone controls (P= 0.4804) (Fig. 5.4).

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. 5.3). 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. 5.4).

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 5.1. 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 5.2. 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 5.3. 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 5.4. 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 5.5. 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 5.6. 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 5.7. 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 5.8. 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 5.9. 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

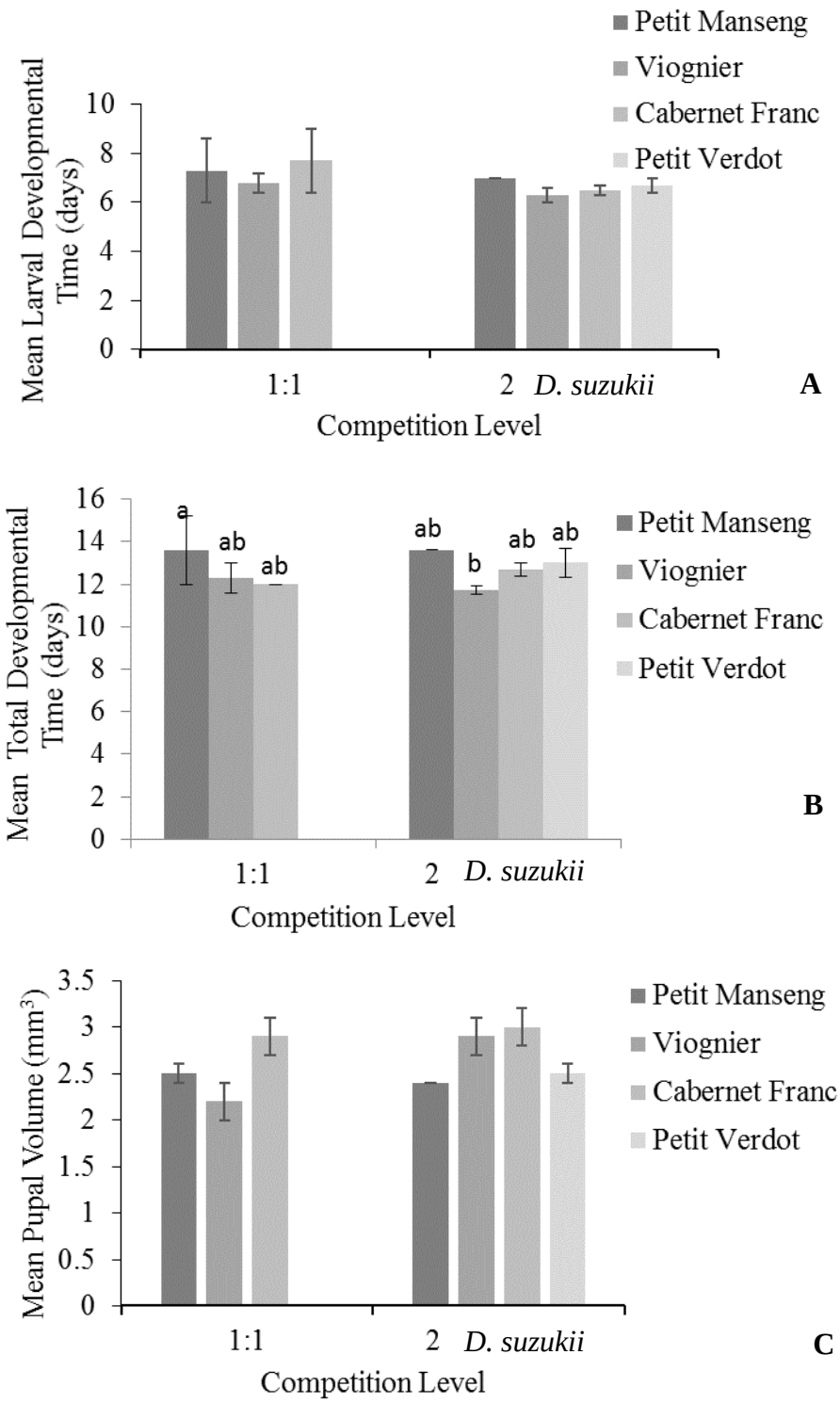

Figure 5.1. Mean (± SE) (A) larval development time, (B) total development time and (C) pupal volume (mm^3) of *Drosophila suzukii* on four wine grape varieties. Means sharing the same letter are not significantly different. The number of larvae of each species on a single grape: 1:1 = 1 *D. suzukii* / 1 Z. *indianus* larvae.

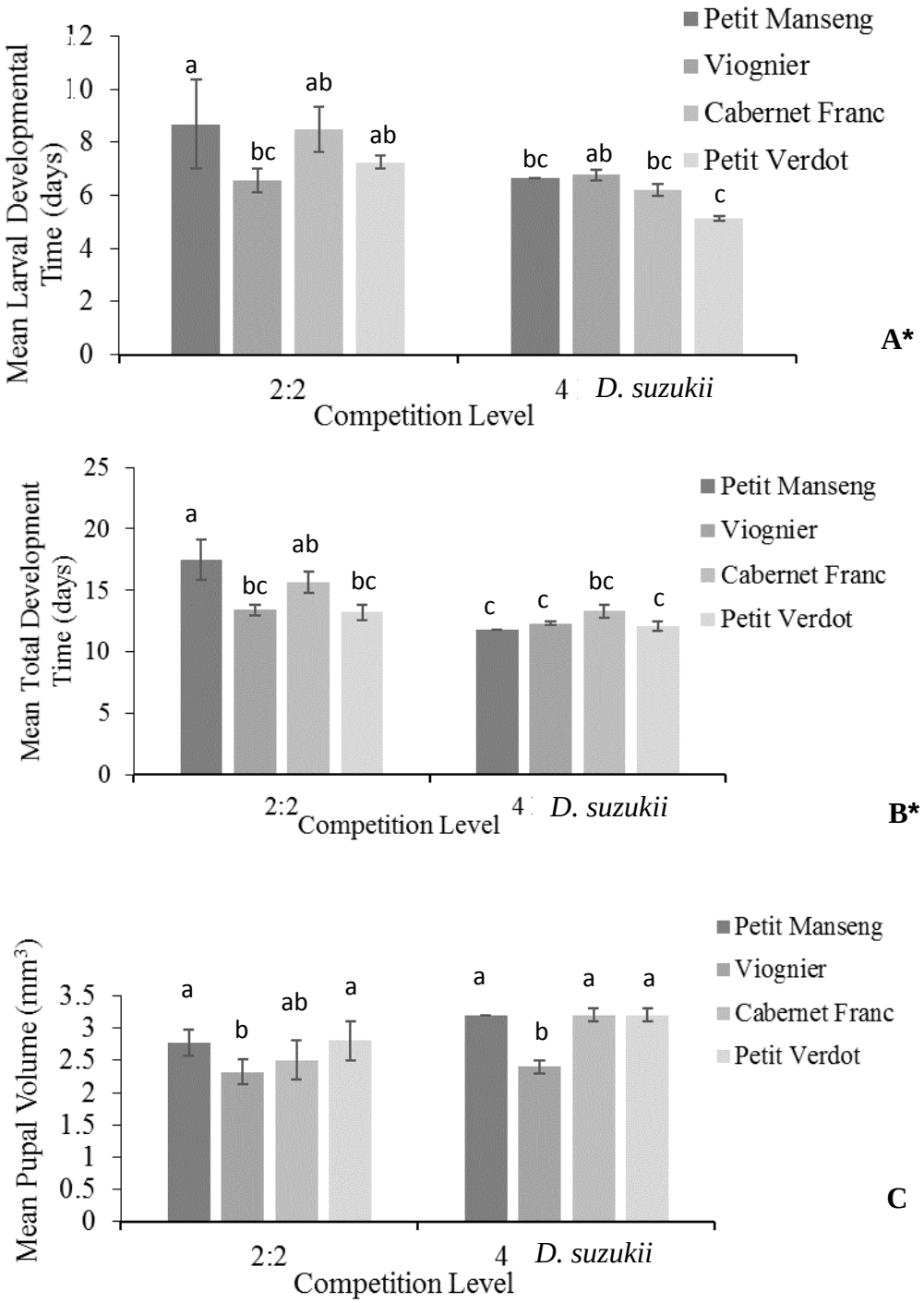

Figure 5.2. Mean (± SE) (A) larval development time, (B) total development time and (C) pupal volume (mm^3) of *Drosophila suzukii* on four wine grape varieties. Means sharing the same letter are not significantly different. *Indicates interactions of competition level and grape variety. The number of larvae of each species on a single grape: 2:2 = 2 *Drosophila suzukii* / 2 *Zaprionus indianus* larvae.

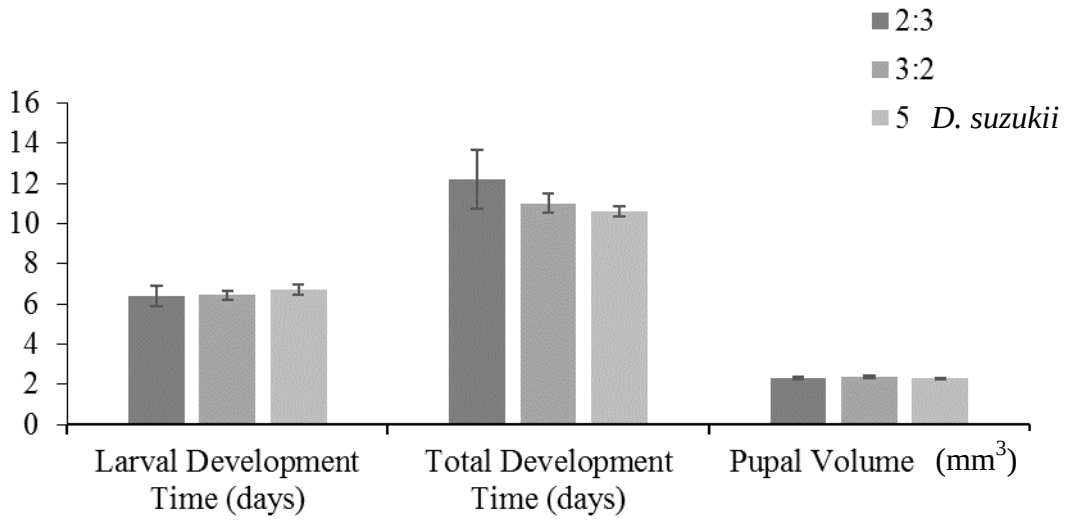

Figure 5.3. Mean (± SE) larval development time, total development time and pupal volume (mm^3) of *Drosophila suzukii* on Viognier grapes. Means sharing the same letter are not significantly different. Indicates the number of larvae of each species on a single Viognier grape: 2:3 = 2 *Drosophila suzukii* / 3 *Zaprionus indianus* larvae, 3:2 = 3 *Drosophila suzukii* / 2 *Zaprionus indianus* larvae.

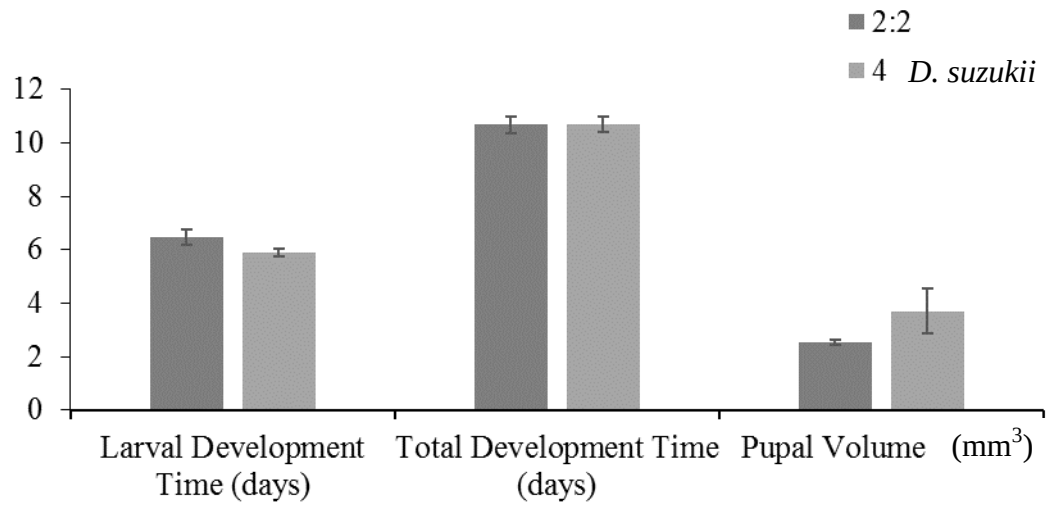

Figure 5.4. Mean (± SE) larval development time, total development time and pupal volume (mm^3) of *Drosophila suzukii* on Viognier grapes. Means sharing the same letter are not significantly different. Indicates the number of larvae of each species on a single Viognier grape: 2:2 = 2 *Drosophila suzukii* / 2 *Zaprionus indianus*.

CHAPTER 6: SUMMARY AND CONCLUSIONS

My research revealed important insights into risk factors that may make certain Virginia vineyards more likely to have infestations of *D. suzukii*. The abundance and seasonal availability of host plants, trapping bait efficacy and selectivity, wine grape susceptibility to *D. suzukii* oviposition and the ecological impacts of interspecific larval competition with *Z. indianus* in wine grapes are important factors when accessing the possibility of *D. suzukii* establishment within the vineyard. Investigation of these factors and interpretation of the results may help improve our understanding of the pest status of *D. suzukii* in Virginia vineyards. Moreover, this may contribute to the development of cultural control tactics and improved vineyard management programs, such as reduced spray applications based upon grape varietal susceptibility to *D. suzukii* attack.

Results Summary and Implications

Drosophila suzukii is highly polyphagous species, known to utilize a wide range of plant species. However, my data from sampling over 24 plant families (Chapter 2) suggested that there were a limited number of suitable hosts immediately surrounding four Virginia vineyards. Six host plant species were identified from three plant families, Rosaceae, Phytolaccaceae, and Caprifoliaceae. The seasonality of these plants was evaluated to determine when *D. suzukii* utilized each. The seasonal availability of these hosts and their rate of occurrence in the vineyard landscape may impact early season populations of *D. suzukii*. Growers will be able to scout the areas immediately surrounding their vineyards and determine what *D. suzukii* host plants are present. By scouting these plants they can determine if their vineyard is at a high risk of *D. suzukii* occurrence. The more host plant species present surrounding the vineyards, the higher the risk to the vineyard for presence. However, risk should also take seasonality and rate of occurrence into account.

The four counties surveyed for *D. suzukii* hosts (Albemarle, Amherst, Nelson and Orange Co.), yielded six host plant species utilized as oviposition sites at varying times throughout the summer in landscapes surrounding the vineyards (Chapter 2). Wild caneberries and tatarian honeysuckle were the only host plants available early in the

growing season (June). Pokeweed, bird cherry trees, Japanese honeysuckle and mock strawberries were utilized late in the growing season (July – October). This survey demonstrated the season-long abundance of these host plants available to *D. suzukii* adults as well as the importance of wild caneberries. Wild caneberries were located at all four vineyards and had a prolonged fruiting period. This provided *D. suzukii* with ovipositional sites throughout the growing season up to when grapes became susceptible to oviposition. It may be possible to influence *D. suzukii* populations in vineyards if these important host plants are removed from the immediate vicinity. This survey allowed me to infer the risk of *D. suzukii* occurrence in vineyards based upon the number of host plant species present and their abundance in the landscape. Vineyards that have caneberries and tatarian honeysuckles may be at highest risk for *D. suzukii* infestations since these host plants had a 100% rate of occurrence around the vineyard and were present for long periods during the grape ripening period.

Risk of *D. suzukii* infestations within the vineyard may be assessed via monitoring. Determining the presence or absence of *D. suzukii* within a cultivated crop is crucial for timing management strategies such as insecticidal sprays. Thus, effective and selective baits and trapping systems are a critical tool for *D. suzukii* management. My research confirmed that the addition of Merlot wine to apple cider vinegar improved bait and trap efficacy compared to a trap containing apple cider vinegar alone. These results in vineyards differed from those in blueberry plantings, where a yeast plus sugar bait captured the most flies. Different traps and baits may need to be used in different fruit production systems. I also demonstrated that the apple cider vinegar and Merlot bait was attractive to *D. suzukii* when grapes were ripening (Chapter 3). It is important for a bait to be competitive with the fruit crop in which the trap is deployed to monitor populations of *D. suzukii* when fruit is ripening. My trapping data also showed that the homemade baits and traps captured as many *D. suzukii* as the commercially available trapping systems evaluated. None of the baits or traps tested was highly selective for *D. suzukii*, however these baits and trapping systems did capture flies when grapes were susceptible to *D. suzukii* oviposition. These monitoring data should allow growers to time sprays based upon the presence or absence of flies within the vineyards when grapes become susceptible to *D. suzukii* oviposition.

My analysis of the physical and morphological characteristics of six wine grapes allowed me to determine when each grape variety became susceptible to *D. suzukii* oviposition (Chapter 3). Based upon these data, I was able to determine which grape varieties were at highest risk of *D. suzukii* oviposition. The grape varieties that matured earlier like Viognier became susceptible to *D. suzukii* oviposition sooner than the later maturing varieties such as Vidal Blanc. Thus, early maturing varieties were at greater risk of *D. suzukii* oviposition early in the growing season than later maturing varieties. However, all grape varieties became susceptible to *D. suzukii* oviposition once ripening occurred. Also, later maturing varieties may be at greater risk of *D. suzukii* attack because they are ripening when *D. suzukii* populations are larger in late summer compared to lower early spring *D. suzukii* populations. My study supported previous work that susceptibility is based upon penetration force with oviposition increasing as penetration force decreased. However, my research was the first to demonstrate that skin thickness did not play a role in ovipositional preference (Chapter 4). Thin-skinned varieties were not at a greater risk of *D. suzukii* oviposition than thick-skinned varieties. In 2013, choice and no-choice bioassays of *D. suzukii* oviposition in intact grapes showed no significant difference in adult emergence among the six wine grape varieties tested. In 2014 and 2015, no-choice bioassays using intact grapes demonstrated that most eggs were laid in Viognier, whereas manually damaged grapes had the most eggs laid in Vidal Blanc. These experiments demonstrated that all varieties were susceptible and at risk of *D. suzukii* oviposition at varying degrees, with more eggs laid as the penetration force of the skin decreased. Survival of eggs to adulthood varied based upon grape variety. However, my study demonstrated a much higher survival rate of *D. suzukii* reared from intact grapes than has been documented in previous studies. Larvae from eggs laid in intact Viognier had higher survival rates to adulthood compared with other varieties (Chapter 4). Based upon these data, I determined that Viognier was at the highest risk of *D. suzukii* oviposition due to an early ripening period and low penetration force needed to pierce the skin.

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 larval density in the grapes increased. These developmental impacts were exacerbated by the grape variety in which the two species resided (Chapter 5). The larvae reared in smaller grapes showed 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, perhaps suggesting that the fecundity of any females emerging would not have been negatively affected. This study demonstrated that Viognier grapes were a more suitable grape variety for *D. suzukii* survival and should be monitored and managed more closely for this pest than the other 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, 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 in competition with *Z. indianus*.

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 hypothesis that *Z. indianus* can oviposit in grapes at wound sites from *D. suzukii* oviposition. 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.

Suggestions for Future Research

Future studies examining *D. suzukii* populations in vineyards and impacts on grape production will continue to be of importance for vineyard managers. Results from the wild host plant survey suggested that certain plants next to vineyards may be of importance for population dynamics of *D. suzukii*. My host plant survey investigating four vineyards (Albemarle, Amherst, Nelson and Orange Co.) is likely not representative of all grape growing regions in Virginia. Thus, it may be beneficial to investigate host plant species in additional vineyards throughout the grape production regions of Virginia. Further studies should be conducted to investigate the seasonal abundance of these wild

hosts and to determine if *D. suzukii* populations in vineyards can be reduced through removal of these plants. An examination of these host plants on fitness parameters, such as survivorship, developmental time and female fecundity could also help determine which host plants are most suitable for *D. suzukii* viability.

My three-year trapping study determined that homemade baits such as ACV plus Merlot were as effective at trapping *D. suzukii* as the commercially available baits and trapping systems. Future experiments should investigate the Alpha Scents plum sachet or similar synthetic baits to develop a *D. suzukii* selective attractant for use in traps. This would decrease sorting time of flies captured and the chances of *D. suzukii* misidentification. This trapping research also demonstrated that these baits were competitive while fruit was ripening, but that the most effective baits in grape vineyards differed from the most efficacious baits in blueberry plantings. Ideally, trapping baits should attract *D. suzukii* across several cropping systems; thus, further testing of new baits should be conducted in vineyards and other crops to determine and compare their effectiveness.

While my wine grape susceptibility study using six varieties determined that Viognier grapes had the lowest penetration force and became vulnerable to *D. suzukii* oviposition early in the grape ripening period, further studies are needed on other varieties, since there are more than two dozen major varieties grown in Virginia. It may be possible to develop a *D. suzukii* susceptibility table based on varietal penetration force and ripening period, enabling viticulturists to know when each variety becomes susceptible to *D. suzukii* and to plan a spray schedule accordingly.

The interspecific larval competition study investigating the impact of *Z. indianus* on the developmental parameters of *D. suzukii* in wine grapes revealed an interesting hypothesis; *Zaprionus indianus* may hinder *D. suzukii* population growth in vineyards where both species are present. Future studies should investigate whole clusters of grapes in cages with both fly species present, a scaled-up version of the interspecific competition study within a single grape. A whole cluster bioassay should more closely represent actual field conditions than my study, in which larvae were transferred to individual grapes. After a substantial period has passed in cages, allowing for three or more generations of *D. suzukii*, all flies should be collected and counted to determine

population levels for each species. Furthermore, grape clusters should be collected from fields where both species are present and any resultant flies should be identified to determine the occurrence of co-infestation in the vineyard.

These investigative studies on *D. suzukii* should be the framework for a *D. suzukii* risk analysis profile for vineyards. It may be possible in the future to use the presence and abundance of host plants as well as the variety of wine grapes grown to determine the risk of *D. suzukii* oviposition and population growth potential within the vineyard. This risk assessment should be used in conjunction with *D. suzukii* specific bait that could correlate trapping numbers to actual infestation rates in the grapes.

Appendix A

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. 1). *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. 2) 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. 3). *Zaprionus indianus* eggs were also observed sharing the same ovipositional punctures in red grapes as *D. suzukii* eggs (Fig. 4). 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. 5). Upon rearing the larvae to adults, a total of eight *Z. indianus* and four *D. suzukii* were present in the rearing cup (Fig. 6).

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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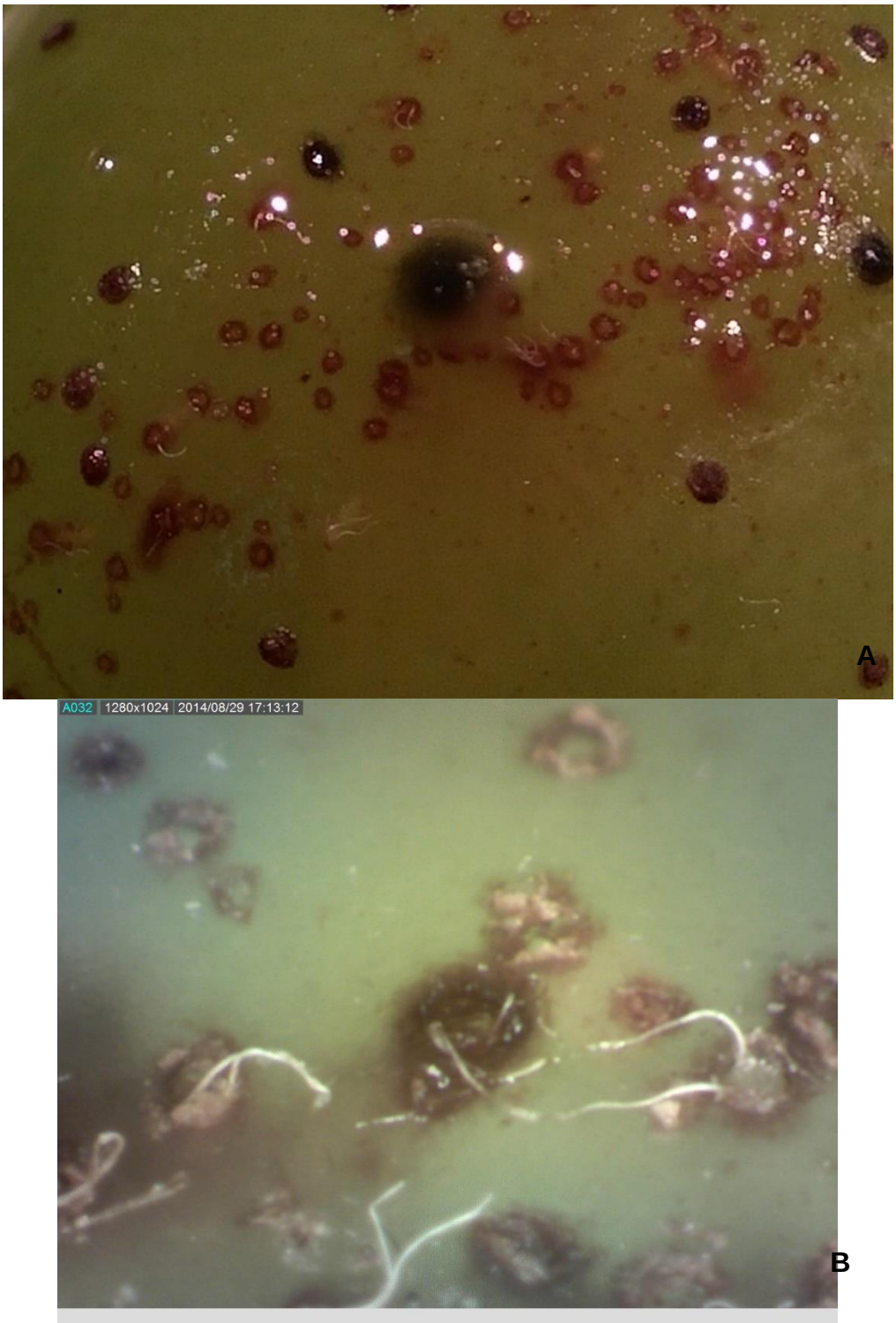

Figure 1. (A) *Drosophila suzukii* eggs and ovipositional punctures on Viognier grapes. (B) *Drosophila suzukii* egg filaments extending from a Viognier grape.

Figure 2. *Zaprionus indianus* eggs laid en masse over *Drosophila suzukii* oviposition punctures with eggs in a Viognier grape.

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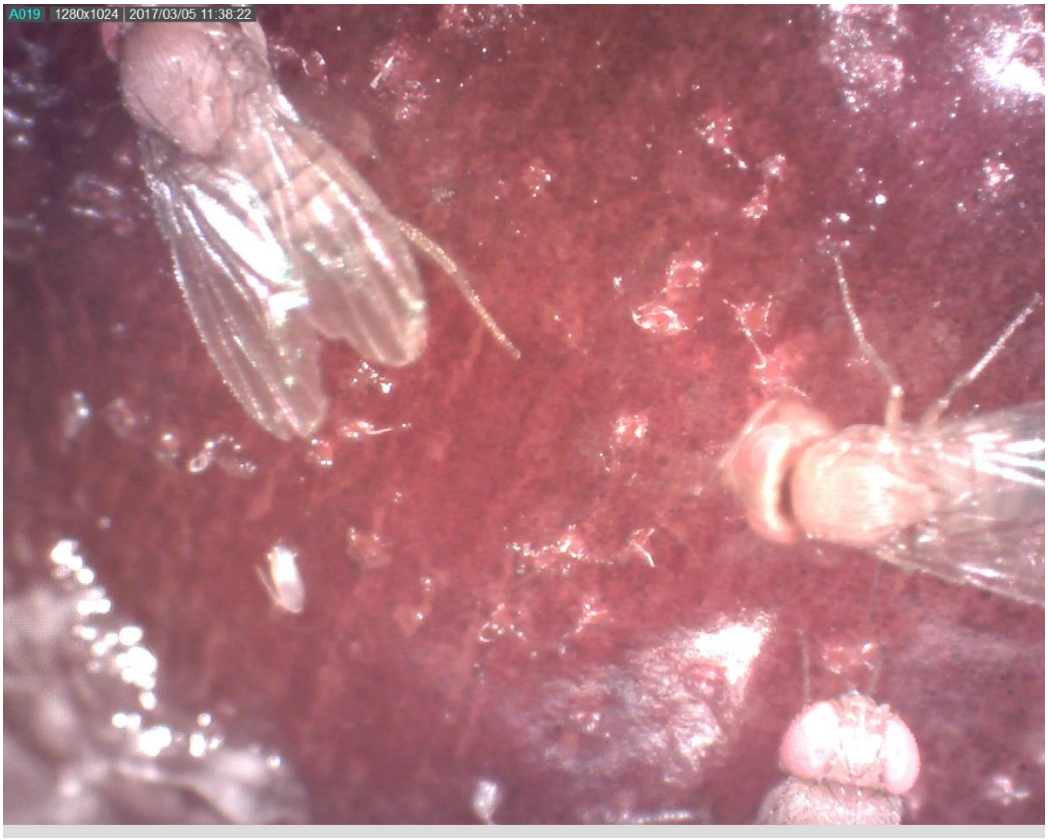

Figure 3. *Drosophila suzukii* females, oviposition punctures and eggs in a red grape.

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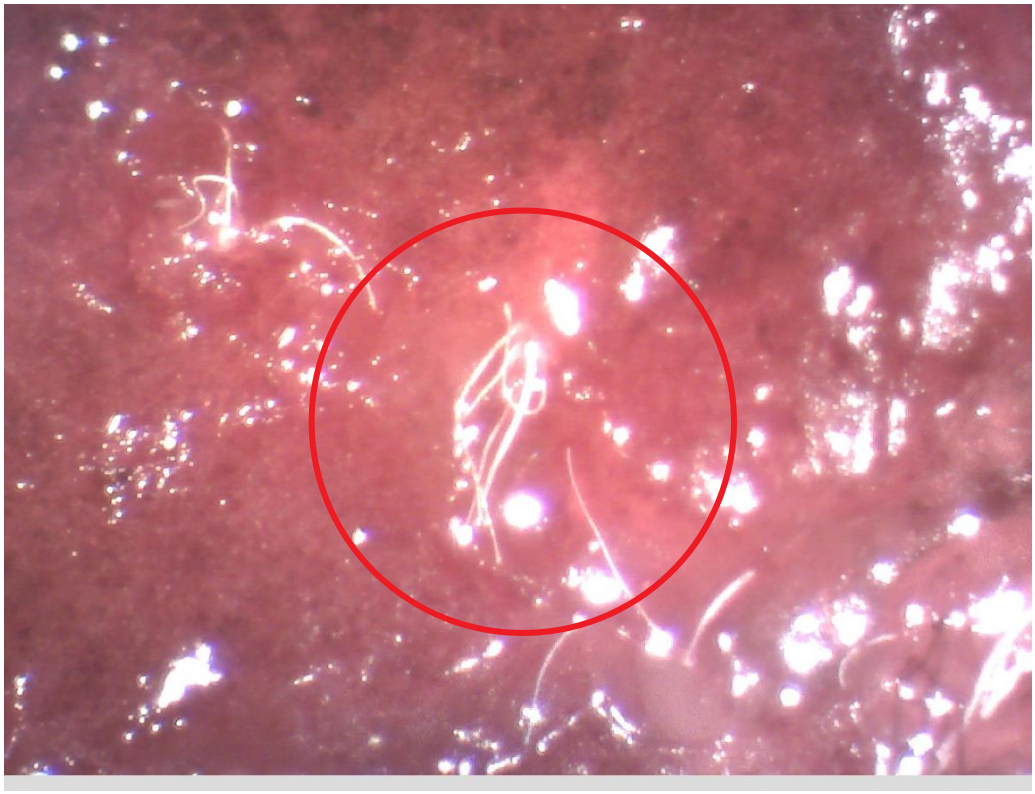

Figure 4. Combined respiratory filaments of a *Zaprionus indianus* and *Drosophila suzukii* egg in a common oviposition puncture in a red grape.

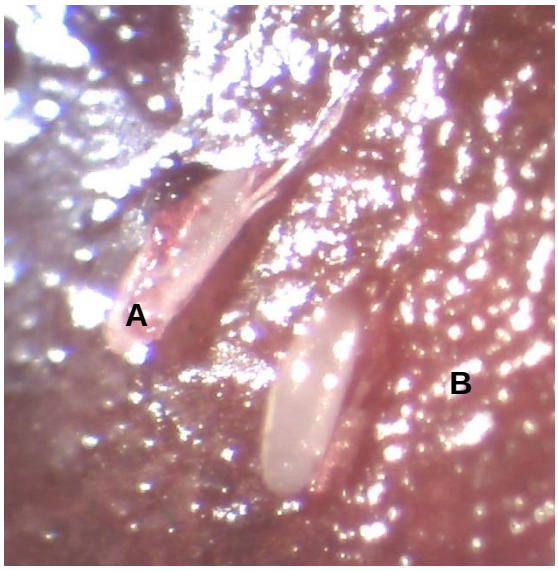

Figure 5. (A) *Zaprionus indianus* and (B) *Drosophila suzukii* eggs dissected out of the single ovipositional wound in red grape.

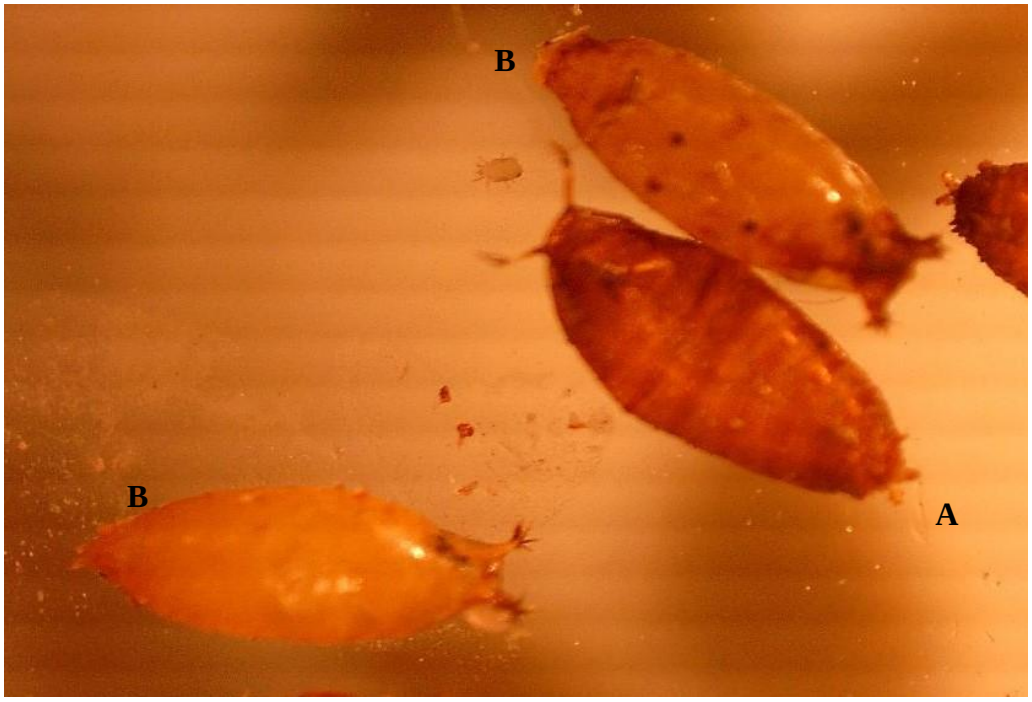

Figure 6. (A) *Zaprionus indianus* and (B) *Drosophila suzukii* pupae reared from red grapes in 2017.