AN ABSTRACT OF THE THESIS OF

Samantha Tochen for the degree of Master of Science in Horticulture presented on November 13, 2014.

Title: Influence of Abiotic and Biotic Factors on Developmental Parameters of *Drosophila suzukii* (Diptera: Drosophilidae).

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Drosophila suzukii (Diptera: Drosophilidae), a pest of thin-skinned fruits, has been detected worldwide recently, with new locations of establishment determined annually since it first became a concern on continental North America in 2008. *Drosophila suzukii* females are different from most other drosophilids as being one of two known species capable of economic injury in fruit. This is achieved via a serrated ovipositor that pierces the skin of ripening susceptible fruit, depositing eggs that will then hatch into larvae, utilizing the fruit as a food source. Current control methods involve the application of pesticides on a weekly basis upon the coloring of fruit, ending when harvest is complete. To shift back towards IPM, knowledge of *D. suzukii* development under different environmental factors is necessary to improve upon current management options.

The first objective of this research project was to determine the developmental period, longevity, and fecundity of *D. suzukii* when exposed to seven constant temperatures and separately, five constant relative humidities (RHs) at 22 ± 2 ºC. Temperature had an influence on these parameters, with decreasing developmental periods as temperatures increased from 10 to 28ºC. Developmental period increased above 28ºC. The highest net reproductive rate and intrinsic rate of population increase was 22 ºC. Estimations using linear and nonlinear fit for the minimum, optimal and maximum were 7.2, 28.1, and 42.1 ºC, respectively. The minimal, optimal, and maximal temperatures for intrinsic rate of population increase were 13.4, 21.0, and 29.0 ºC . Relative humidity also had a significant influence on these parameters. The estimated

minimum, optimal and maximum development thresholds for RH were 27, 79, and 100% RH, respectively. Fecundity appeared to increase with RH, with a maximum observed at 93%, however ovary dissections indicated that no differences in ovary maturation occurred between 78 and 93% RH.

The second objective of this research was conducted to determine the effect of floral resource feeding on nutrient levels in female *D. suzukii* when provided cherry or blueberry blossoms as well as lifespan over an observed feeding period. When adults were provided cherry blossoms, they had significantly longer lifespans than their water-fed counterparts over a 41-day observation period. Adults provided blueberry blossoms exhibited longer lifespans, however, this observation could not be statistically compared with the cherry and water treatments due to availability of blossoms and a staggered start date. A nutritional assay showed higher levels of glycogen and sugar levels amongst female flies provided either blossom type or sucrose over water-fed flies. Lipid levels tended to be the same across treatments.

Overall, this research discovered new information about *D. suzukii* biology that can be incorporated into future management strategies. The temperature-related development and fecundity study contributed to the development of a population prediction model. The humidity work will be incorporated into the *D. suzukii* model to refine estimates across different locations. The model will provide a tool for growers to determine management timing. The nutritional work may aide in the development of a bait spray and timing of bait spray application.

Influence of Abiotic and Biotic Factors on Developmental Parameters of *Drosophila suzukii* (Diptera: Drosophilidae).

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Samantha Tochen, Author

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CONTRIBUTION OF AUTHORS

Dr. Vaughn Walton and Danny Dalton were instrumental in all aspects of the development and writing of Chapter 2. Dr. Vaughn Walton assisted with the statistical analysis of Chapter 3. Dr. Jana Lee was instrumental in the development and writing for the nutritional assay as well as providing statistical and writing guidance for nutritional lifespan in Chapter 4.

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CHAPTER 1.

Introduction.

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Background

       Drosophila suzukii Matsumura (Diptera: Drosophilidae) was first described in 1916, having been linked to maggots found in cherry and subsequently called Cherry Drosophila in Japan. By 1930, *D. suzukii* had greatly impacted cherry production in Japan as well as certain grape varieties (Kanzawa 1935). Since then, it has become an economic pest in small and stone fruits. By the 1970s, *D. suzukii* could be found throughout mainland Asia (Delfinado and Hardy 1977). The first known detection of *D. suzukii* in North America was in Hawaii by the 1980s (Kaneshiro 1983). However, it wasn't until 2008 that *D. suzukii* was first recorded on mainland North America, in raspberries in California. By 2009, *D. suzukii* had been identified in Oregon, Washington, British Columbia, and Florida, and continued to spread across North America in subsequent years (Hauser et al. 2009). Nearly all states in the United States have identified the presence of *D. suzukii*, however not all have observed crop loss (National Agricultural Pest Information System [NAPIS] 2013). Europe has not been immune to *D. suzukii*, having detected its presence in 2008 in Spain and later France and Italy (Cini et al. 2012). *Drosophila suzukii* continued its spread throughout Europe, having since been detected in Switzerland, Germany, Austria, Belgium, and the UK (Baroffio et al. 2013). As of 2013, *D. suzukii* moved southwest to be discovered in Bosnia and Herzegovina (Ostojic et al. 2014). In 2011, *D. suzukii* was found in Mexico (Cini et al. 2012). While there were speculations of *D. suzukii* being present in South America as early as the 1990's, its presence was not confirmed until a survey on drosophilids in Brazil was conducted in 2013 (Deprá et al. 2014).

Morphology

       Male *D. suzukii* have a single black spot on the tip of the leading edge of their wing, and two sex combs arranged on the first and second tarsal segments. Females possess a modified ovipositor that is lined with sclerotized teeth, resulting in a serrated edge that allows them to lay eggs in fruit. Their body size is around 2.5 mm and is a dark, yellowish brown (Kanzawa 1935). Variations in appearance can be seasonal, with a larger, darker phenotype appearing as temperature and photoperiod decreases (personal observation). Different fruit diets can result in size differences (Kanzawa 1935).

Drosophila suzukii eggs are milky white and oblong with two string like projections from one end which protrude out of the fruit.   Eggs have an overall width of 0.5mm, and the projections extend 0.4mm in length. The small, white larvae will go through three instars until they are 5.5 mm in length and 0.8mm wide. Larvae have black scraping mouthparts that curve inward as is found in drosophilids. Larval *D. suzukii* feeding results in economic losses as they feed within the fruit pulp. Pupae are oblong, 3.5 mm in length and 1.2 mm wide, and a reddish brown color, with two horn shaped protrusions that divide into 7-8 tipped stars at the distal end. Protrusions are off the posterior end of the pupal case, coinciding with the posterior respiratory organs of the larvae (Kanzawa 1935).

Drosophila suzukii is in the subgroup *suzukii*, which is part of the *melanogaster* species group, found in the subgenus *Sophophora* (Hauser et al. 2009).   The subspecies, *Drosophila subpulchrella*, can also cause economic loss. It possesses a serrated ovipositor capable of piercing fruit and oviposition. The host range of *D. subpulchrella* does not appear as broad as that of *D. suzukii*; it can be found in blackberries, Japanese raspberries and *Prunus buergeriana* (Fukushima 1993).   Sasaki and Sato (1995) have observed *D. subpulchrella* as a cherry pest, but did not report *D. subpulchrella* as possessing *D. suzukii*'s ability to pierce through the fruit. In contrast, Atallah et al. (2014) were able to observe *D. subpulchrella* oviposition in cherries and raspberries, but not grapes which *D. suzukii* can infest. Males of *D. suzukii* and *D. subpulchrella* share the morphological feature of having a single spot on their wings (Takamori et al. 2006). Another drosophilid capable of economic loss is *Zaprionus indianus* Gupta and is distinct with its white striped head and thorax. *Zaprionus indianus* originates from Africa and has resulted in an estimated 50% loss in the commercial fig production in Brazil (Vilela et al. 2001) though it is known to infest 73 different hosts in its native range (Lachaise and Tsacas 1983). Other drosophilids are not considered pests, as they lack the sclerotized, serrated ovipositor and are limited to laying eggs on rotting organic matter.

Host range

Their host range in Japan initially included strawberries, cherries, grapes, gooseberries, raspberries, peaches, plums, persimmons, apples, mulberries, tomatoes, loquats, and autumn-

olives. Kanzawa (1935) reported finding the aforementioned fruits infested naturally while in either perfect condition or damaged. Mulberries and figs are suitable hosts, however, other drosophilid larvae provide competition in figs (Yu et al. 2012). Cranberries have been shown to be unsuitable hosts when fruit is intact (Steffan et al. 2013). Levels of infestation can vary amongst cultivars as has been shown in *Rubus* species and blueberries, with females successfully laying eggs in cultivars with thinner skin over thicker skin cultivars (Burrack et al. 2013, Kinjo et al. 2013). Susceptibility of certain fruits will vary at different stages of ripeness and coloration, with a preference for increased brix (Lee et al. 2011). Work by Lee et al. (2014) has elucidated non-crop alternative hosts infested by *D. suzukii* in Oregon and Michigan, with *Lonicera sp.* and *Sarcococca confusa* being highly susceptible in addition to the ubiquitous 'Himalaya' blackberry, *Rubus armeniacus* Focke and wild sweet cherry, *Prunus avium* (L.). In Oregon, *S. confusa* bears fruit in the spring, providing an early season host for *D. suzukii* whereas *P. avium* and *R. armeniacus* provide a late season host (Lee et al. 2014).

Pest status and management in blueberries and cherries

In Oregon, many commercial small and tree fruit productions are susceptible to *D. suzukii* infestation leading to concern over potential economic losses. Sweet cherry and blueberry production are of particular concern as Oregon ranks as third in production for both crops in the United States. Yield losses in 2009 ranged from minimal to 80% depending on crop and locality (Bolda et al. 2010). Estimates by Bolda et al. (2010) predicted a 20% yield loss would result in the loss of nearly $20 million from the combined total value of blueberry and cherry costs in Oregon ($108.1 million).

Northern highbush blueberry, *Vaccinium corymbosum*, originating from the eastern United States, comprises the majority of blueberries grown in Oregon and is grown globally. Supply of blueberries has not matched demand, leading to increase in acreage annually. The United States is the largest producer of blueberries in the world, and Oregon ranks fifth and third in acreage and production, respectively. This production occurs primarily in the Willamette Valley region and, as of 2013, has resulted in a $94 million industry (NASS 2014). In the western states, it is estimated that 9 tons of blueberries are produced per acre. Overhead

irrigation is commonly found in new plantings of blueberries in Oregon. Harvesting is carried out primarily through the use of machinery. Growers are planting highbush blueberries at higher densities to increase yield and reduce production cost (Strik and Yarborough 2005). With an increase in density, canopy cover increases as well as leaf production, leading towards higher irrigation demands (Bryla and Strik 2007). Blossoms will begin to appear in late April and continue through May, with subsequent fruit set taking 2 to 5 weeks to ripen. Harvest can begin as early as June and continue through September, depending on the varieties present (Strik and Finn 2008). Prior to *D. suzukii*'s introduction, insect pest concerns were limited to aphids serving as disease vectors in blueberry production. In other growing regions of the United States, blueberry maggot, cranberry fruit worm, cherry fruitworm, thrips, blueberry gall midge, and Japanese beetle have led to significant pest problems. Pathogens such as mummy berry, Botrytis, Alternaria, Anthracnose, shock and scorch viruses, bacterial canker, and phytophthora root rot have elicited more concern in Oregon blueberry production (Strik and Yarborough 2005).

Cherry production, consisting of primarily sweet cherries, *Prunus avium*, is also important in Oregon, being the third largest producer in the United States, just after California and Washington (NASS 2012). The majority of sweet cherry production is found in the Mid-Columbia region with a small portion in the Willamette Valley grown for processing, resulting in a $91 million industry (NASS 2014). Harvest begins in mid June and can continue into August depending on the variety. Previously, the main pest was *Rhagoletis indefferens* and was managed with applications of a spinosad bait (GF-120 NF; Dow AgroSciences, Indianapolis, IN) in Oregon and Washington (Beers et al. 2011). Other pests and pathogens in sweet cherry production include black cherry aphid, leafrollers, shothole borer, powdery mildew, bacterial canker, and verticillium wilt (Smith 2013).

Monitoring

Monitoring of *D. suzukii* began with Kanzawa's efforts in the 1930's. Kanzawa (1935) trapped *D. suzukii* adults with mixtures of different fruit wines, rice wine, molasses, and acetic acid. A rice wine and honey solution in plastic bottles with mesh was later developed and used to detect presence of flies (Sasaki 2005).   Landolt et al. (2012) confirmed the attraction of *D.*

suzukii to vinegar and wine, suggesting a combination of the two to be superior than either bait alone. A blend of volatile compounds acetic acid, ethanol, acetoin, and methionol, have been identified as being attractive to adult *D. suzukii* in the field (Cha et al. 2013). Lee et al. (2013) found that red and yellow traps, traps with a higher bait surface area (90 cm^2), and side-entry traps caught overall more *D. suzukii* than their trap counterparts. Monitoring for presence of larvae in fruit can be obtained by submersing crushed fruit in either a saltwater solution or sugar water solution but this method is inefficient and time consuming and no current levels of sampling error are known (Wiman et al. 2014). Other methods have been explored utilizing sieves or boiling the fruit slurry to varying success. Initial methods submersed fruit in tap water for 1 hour, leading to an approximately 80% detection rate (Kawase et al. 2007). Through the use of PCR-RFLP, molecular diagnostics may now identify all stages of *D. suzukii*, eliminating the need to rear out larvae for identification purposes (Kim et al. 2014). Unfortunately, current monitoring efforts are not accurate at predicting presence of *D. suzukii* in the field and damage can be incurred before traps catch reflect adults. By understanding how environmental conditions affect *D. suzukii*, prediction models can be established, reducing the reliance on current trapping systems to indicate presence, and potentially allowing for the reduction of chemical applications.

Management

In Japan, control methods have included cultural control methods involving the removal of damaged or leftover fruit in field during and after the harvest season, pruning to limit amount of fruit left on bushes, monitoring for infestations, and utilizing early season cultivars over later season cultivars as *D. suzukii* populations would peak at that time (Shinzo et al. 2007). Failure to remove fruit left on bushes or trees may be heavily infested, providing a source for new flies for the rest of the season or subsequent seasons should they overwinter. Removal of host plants surrounding the field can reduce incoming populations (Sasaki 2005). Insect netting with openings of 0.98mm has also been used to prevent infestation by *D. suzukii*. However, there exists a tradeoff of reducing light intensity and subsequently reducing anthocyanin content (Shinzo et al. 2007), being a logistical challenge to set up around tree fruits (Yamada 2008), and

causing sun damage to fruit (personal observation). Post-harvest irradiation of fruit can result in no production of F_1 adults, however the use of radiation on fresh commodities current limits where these products may be sold (Follett et al. 2014).

Chemical control has been the method of control in the United States, with more formulations available to growers. In Japan, chemical control has been limited to a flowable permethrin and acetamiprid SP that can only be applied three times a year (Kawase et al. 2007). The application of sprays is applied when fruit reaches the coloring stage (Sasaki 2005). In the Pacific Northwest, field and lab assays indicated organophosphate and pyrethroid will provide between 7-10 and 10-14 days of control, respectively, while spinetoram provides 5-7 days of control (Beers et al. 2011, Bruck et al. 2011). Semi-field residual bioassays have supported earlier reports of the effectiveness of these formulations (Van Timmeren and Isaacs 2013). In organic productions, spinosad has so far been the only product to provide residual activity (5-7 days control). Rotation of chemicals is recommended in order to mitigate the possibility of resistance development (Denholm and Rowland 1992, Tabashnik and Croft 1982). Laboratory trials in the UK have shown spinosads, deltamethrin, and a new experimental product, TZ2674, as being effective at achieving adult mortality upon direct application, exposure to treated fruit, and potential ovicidal/larvicidal effects (Cuthbertson et al. 2014). Application of sprays also begins with fruit color, but can be applied on a more frequent basis as the chemical restrictions are not as severe as in Japan. This method of control however only targets a small percentage of the total *D. suzukii* population (Wiman et al. 2014)

Biocontrol is an important and often effective component to IPM programs. There is limited research available regarding biocontrol of *D. suzukii*. Hymenopteran parasitoids from the families Figitidae, Pteromalidae, Diapriidae, and Braconidae have been shown to attack *D. suzukii* (Cini et al. 2012). *Pachycrepoideus vindemiae* has been identified as a parasitoid of *D. suzukii* in Italy and Oregon (Rossi et al. 2013). Parasitism rates of *P. vindemiae* are highly variable in a laboratory setting, and the effects of which appear to be negligible in field situations. *Drosophila suzukii* has been shown to be significantly more resistant to wasp parasitism due to increased hemocyte production (Kacsoh and Schlenke 2012). Generalist predators may be

limited in their ability to provide control of *D. suzukii* due to decreased access to pests with life cycles in cryptic locations (Symondson et al. 2002). The entomopathogenic fungi strain, *Isaria fumosorosea* Pf21, was able to provide 85% mortality in adult *D. suzukii* under laboratory conditions (Naranjo-Lázaro et al. 2014). Further research would need to be conducted to determine how this strain would perform under field conditions. Fungi within this species complex have proven to be successful against other pests under field conditions, notably white flies (Zimmermann 2008). In terms of sterile insect technique, preliminary work involving genetically modified *D. suzukii* individuals shows promise and would lead to female mortality during rearing and adult male sterility (Schetelig and Handler 2013).

Abiotic factors

Kariya (unpublished) was the first to examine generation time of *D. suzukii*, observing 15 generations in a year. Kanzawa (1935) conducted a more comprehensive study at ambient temperature and humidity, indicating up to 2-3 generations on cherries, and up to 15 generations in a year when utilizing multiple hosts. The generation period ranged from 8 to 23 days. Sasaki (2005) found 3 to 4 generations on cherries and only ten generations a year. From Kanzawa's work, eggs hatched between day 1 and 4 post-oviposition, followed by 5 days in the larval stage with eclosion occurring after 4 to 10 days. The lifespan for an adult is approximately 20-56 days, however, increased dramatically to 234 days under overwintering conditions. Females lay between 11 to 362 eggs, with an average of 38 eggs per day (Kanzawa 1935). Yamada (2008) observed an average oviposition count in the field of five eggs per fruit. It is speculated that *D. suzukii* overwinters as adults, with adults using fallen leaves or other suitable habitats as a buffer from the elements (Kanzawa 1935, Sasaki 2005). Adults will mate after 1-2 days post-eclosion and lay eggs singularly immediately, with a preference for fully intact ripe fruit. Females were found to lay eggs in fruit found in lower branches and out of the wind. Females will lay eggs in unripe fruit, however development tends to be insufficient due to the acidic environment (Kanzawa 1935).

Drosophila suzukii temperature-related development shows that egg hatch can occur within 24 hours when temperatures are below 25°C (Sasaki 2005). In Japan, populations are low

at the beginning of the growing season and increase over the season, peaking at the end of harvest (Sasaki 2005) and these trends are confirmed by a population model that was developed from abovementioned work (Wiman et al. 2014), which helped to better explain *D. suzukii* seasonal phenology in different climates. Environmental conditions have been important in determining where and when *D. suzukii* populations would become problematic to susceptible crops, with warmer regions in California and North Carolina capable of supporting year round population stage-structure versus the cooler region of Oregon (Wiman et al. 2014). *Drosophila suzukii* does not appear to be capable of surviving long periods of extreme cold temperatures (Dalton et al. 2011).

As is clearly seen above, *D. suzukii* life history characteristics are notably influenced by abiotic factors such as temperature and humidity, as well as biotic factors, such as nutritional value of food sources. As mentioned previously, Kanzawa examined the development and generation time in *D. suzukii*. However, this work was conducted at ambient temperature and humidity and other temperature ranges were not explored. Additional work by Sasaki (2005) determined that *D. suzukii* experiences a decrease in pre-eclosion development time as temperatures approach 25°C. At 32°C and warmer, oviposition may occur however development was incomplete. Oviposition could not be observed at the lower temperature extreme of 10°C , and the minimum development temperature was estimated to be 9°C. *Drosophila suzukii* was found to be relatively cold-intolerant when compared to other Japanese drosophilids (Mitsui et al. 2010). Oviposition was not observed at 10°C and may be limited when temperatures are above 32°C with subsequent development incomplete. This information reflects the Japanese population of *D. suzukii* and although work by Mitsui et al. (2010) showed that different geographic strains of *D. suzukii* showed no difference to extreme temperature tolerances, however, it should not assume this represents *D. suzukii* populations in Oregon for two reasons. One, it is unknown as of this writing the initial source of *D. suzukii* North American population. And two, in cosmopolitan species such as *D. simulans*, *D. melanogaster*, *D. immigrans* and *D. serrata*, acclimation responses to environmental conditions may occur quickly. *Drosophila melanogaster* populations can increase in resistance to unfavorable

conditions within ten generations (Chown et al. 2011). However, this acclimation may have disadvantages such as decreased fertility or fecundity due to redirection of cell resources (Hoffmann et al. 2003).

Another abiotic factor that should be considered is relative humidity and its effect on *D. suzukii*. Humidity's effect is not often explored on biological parameters of pest species, yet water loss, influenced by ambient humidity and temperature, in insects is a vital consideration to their survival, such that insects divert resources away from other aspects of fitness. Due to a small body size and disproportionate cost of gas exchange, insects constantly battle maintaining water balance (Chown et al. 2011). However, just as low humidity leads to desiccation, high humidity may favor fungal pathogens and disrupt osmoregulation (Contreras et al. 2013, Roca and Lazzari 1994). Diptera are known to avoid areas of high humidity when coupled with high temperatures (Chown et al. 2011). Studies conducted on Olive fruit fly demonstrate that females fail to develop mature oocytes at low relative humidity, gaining the ability as humidity is increased. However, both longevity and fecundity peaked at 75% RH before decreasing as relative humidity approached 100% (Broufas et al. 2009).   One study involving *Apolygus lucorum* (Hemiptera: Miridae) looked at the effect of relative humidity on intrinsic rate of population increase, oviposition rate, and reproductive success in order to predict population increase and time control methods. The mirids were tested at 40, 50, 60, 70 and 80% RH and declined in intrinsic capacity between 70 and 80% RH (Lu and Wu 2011). Humidity also plays a role on nutrient availability, decreasing bacteria and yeasts on leaf surfaces that flies rely on for nitrogen. Additionally, relative humidity alters nectar concentrations, which may in turn alter foraging behavior, as nectar is a source of sugars for insects (Contreras et al. 2013). Relative humidity is important to consider in microclimates, as local distribution can vary greatly with gradients in RH, as was observed in willow flea beetles, *Altica subplicata* (Coleoptera: Chrysomelidae)*,* on the shore of the Great Lakes in northern Michigan (Bach 1993).

Floral resources

A biotic factor to be considered in the survival and longevity of *D. suzukii* is the presence of floral nectaries as they are likely to encounter this food source at some point while moving

through agricultural landscapes. *Drosophila suzukii* larval diet is well known, as it is the destructive stage that consumes the host fruit. However, there is little known on nutritional uptake of *D. suzukii* adults. Hamby et al. (2012) isolated associated yeast species from *D. suzukii* larvae, adults, and infested fruit, which are believed to be an important food source for Drosophila. Adults of many insect species require sugar meals to achieve maximum longevity, utilizing meals to generate energy immediately or store later as trehalose (body sugar) or glycogen (fat body) (Olson 2000). When allocating resources from sugar meals, *Drosophila melanogaster* females will utilize the dietary carbon to develop and lay eggs, only a small portion (12%) of which are carried over from sugar meals consumed as larvae (Min et al. 2006). *D. melanogaster* is capable of utilizing fructose, maltose, sucrose, and glucose, all of which can be found in floral nectar (Hassett 1948). Most Diptera are completely dependent upon adult feeding for egg maturation (Waeckers 2007), necessitating the understanding of adult *D. suzukii* nutritional uptake as it can influence the development of improved trapping systems or bait sprays (and timing thereof if competing with floral resources as has been the situation with fruit availability and trapping). Also of importance is the altering of foraging behavior by many insects, where adults will stay in areas that provide both a food source and sites for oviposition. This is observed in *Rhagoletis pomonella* (Prokopy 1993) and *Drosophila melanogaster* (Jaenike 1986). *Drosopila suzukii* populations may not need to migrate to the extent of their Japanese counterparts (Mitsui 2010) due to the presence of multiple hosts and food sources in the Oregon agricultural landscape.

References cited:

Atallah, J., Teixeira L., Salazar R., G. Zaragoza, and A. Kopp. 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. Proc. R. Soc. B. 281: 20132840. doi: 10.1098/rspb.2013.2840

Averill, A. L., and R. J. Prokopy. 1993. Foraging of *Rhagoletis pomonella* flies in relation to interactive food and fruit resources. Entomol. Exp. Appl. 66: 179–185.

Bach, C. E. 1993. Effects of microclimate and plant characteristics on the distribution of a willow flea beetle, *Altica subplicata*. Am. Midl. Nat. 193–208.

Baroffio, C. A., Richoz P., Fischer S., Kuske S., C. Linder, and P. Kehrli. 2014. Monitoring *Drosophila suzukii* in Switzerland in 2012. J. Berry Res. 4: 47–52.

Beers, E. H., Van Steenwyk R. A., Shearer P. W., W. Coates, and J. Grant. 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag. Sci. 67: 1386–1395. doi: 10.1002/ps.2279

Bolda, M. P., R. E. Goodhue, and F. G. Zalom. 2010. Spotted wing drosophila: potential impact of a newly established pest. Agricultural Res Econ Update. 13: 5–8.

Broufas, G. D., M. L. Pappas, and D. S. Koveos. 2009. Effect of relative humidity on longevity, ovarian maturation, and egg production in the Olive Fruit Fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 102: 70–75. doi: 10.1603/008.102.0107

Bruck, D., Tanigoshi L. K., Gerdeman B. S., Hollis-Spitler G., J. DeFrancesco, and A. J. Dreves. 2011. Current recommendations for managing Spotted Wing Drosophila (SWD), *Drosophila suzukii*, in PNW Blueberries.

Bryla, D. R., and B. C. Strik. 2007. Effects of cultivar and plant spacing on the seasonal water requirements of highbush blueberry. J. Amer. Soc. Hort. Sci. 132: 270–277.

Burrack, H. J., Fernandez G. E., T. Spivey, and D. A. Kraus. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: Drosophilidae), an invasive frugivore. Pest. Manag. Sci. 69: 1173–1180. doi: 10.1002/ps.3489

Cha, D. H., Adams T., Werle C. T, Sampson B., Adamczyk J., H. Rogg, and P. Landolt. 2014. A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace. Pest. Manag. Sci. 70: 324–331. doi: 10.1002/ps.3568

Chown, S. L., J. G. Sørensen, and J. S. Terblanche. 2011. Water loss in insects: An environmental change perspective. J. Insect Physiol. 57: 1070–1084. doi: 10.1016/j.jinsphys.2011.05.004

Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insect. 65: 149–160.

Contreras, H. L., Goyret J., von Arx M., Pierce C., Bronstein J., R. Raguso, and G. Davidowitz. 2013. The effect of ambient humidity on the foraging behavior of the hawkmoth *Manduca sexta*. J. Comp. Physiol. 199: 1053–1063.

Cuthbertson, A. G. S., Collins D. A., Blackburn L. F., L. Blackburn, and H. Bell. 2014. Preliminary screening of potential control products against *Drosophila suzukii*. Insects. 5: 488–498. doi: 10.3390/insects5020488

Denholm, I., and M. W. Rowland. 1992. Tactics for managing pesticide resistance in arthropods: theory and practice. Annu. Rev. Entomol. 37: 91–112. doi: 10.1146/annurev.en.37.010192.000515

Deprá, M, Poppe J. L., Schmitz H. J., D. Toni, and V. Valente. 2014. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J. Pest. Sci. 87: 379–383. doi: 10.1007/s10340-014-0591-5

Emiljanowicz, L. M., G. D. Ryan, and A. Langille. 2014. Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. J. Econ. Entomol. 107: 1392–1398.

Follett, P. A., A. Swedman , and D. K. Prices. 2014. Postharvest irradiation treatment for quarantine control of *Drosophila suzukii* (Diptera: Drosophilidae) in fresh commodities. J Econ Entomol. 107: 964–969.

Fukushima Prefecture Tree Fruit Research Institute. 1993. Host plants of SWD.

Hamby, K. A., Hernández A., K. Boundy-Mills, and F. G. Zalom. 2012. Associations of yeasts with Spotted-Wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. Appl. Environ. Microbiol. 78: 4869–4873. doi: 10.1128/AEM.00841-12

Hassett, C. C. 1948. The utilization of sugars and other substances by *Drosophila*. Biol. Bull. 114–123.

Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest.

Manag. Sci. 67: 1352–1357. doi: 10.1002/ps.2265

Hoffman, A. A., Hallas R. J., J. A. Dean, and M. Schiffer. 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science. 301: 100–102.

Jaenike, J. 1986. Feeding behavior and future fecundity in *Drosophila*. Am. Naturalist. 127: 118–123.

Kacsoh, B. Z., and T. A. Schlenke. 2012. High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. PLoS One. doi: 10.1371/journal.pone.0034721

Kaneshiro, K. Y. 1983. *Drosophila* (Sophophora) *suzukii* (Matsumura). Proc. Hawaiian Ent. Soc. 24: 179.

Kanzawa, T. 1939. Studies on *Drosophila suzukii* Mats. Kofu. Rev. Appl. Ent. 29: 622.

Kim, S. S., Tripodi A. D., D. T. Johnson, and A. L. Szalanski. 2014. Molecular Diagnostics of *Drosophila suzukii* (Diptera: Drosophilidae) using PCR-RFLP. J. Econ. Entomol. 107: 1292–1294.

Kinjo, H., Kunimi Y., T. Ban, and M. Nakai. 2012. Oviposition efficacy of *Drosophila suzukii* (Diptera: Drosophilidae) on different cultivars of blueberry. J. Econ. Entomol. 106: 1767–1771.

Lachaise, D., and L. Tsacas. 1983. Breeding sites in tropical African drosophilids. The Genetics and Biology of Drosophila. Academic Press, London, pp 221–332

Landolt, P. J., Adams T., T. S. Davis, and H. Rogg. 2012. Spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), trapped with combinations of wines and vinegars. Fla. Entomol. 95: 326–332.

Lee, J. C., Dreves A. J., Cave A. M., Kawai S., Isaacs R., Miller J. C., S. Van Timmeren, and D. J. Bruck. 2014. Infestation of wild and ornamental non-crop fruits by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Ann. Entomol. Soc. Am. In Review.

Lee, J. C., Shearer P. W., Barrantes L. D., Beers E. H., Burrack H. J., Dalton D. T., A. J. Dreves, and D. J. Bruck. 2013. Trap designs for monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 42: 1348–1355.

Lee, J. C., Bruck D. J., Curry H., Edwards D., Haviland D. R., R. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest. Manag. Sci. 67: 1358–1367. doi: 10.1002/ps.2225

Lu, Y., and K. Wu. 2011. Effect of relative humidity on population growth of *Apolygus lucorum* (Heteroptera: Miridae). Jap. J. Appl. Entomol. Zool. 46: 421–427.

Mercedes, D., and D. E. Hardy. 1977. A catalog of the Diptera of the Oriental Region. Suborder Cyclorrphapha (excluding division Aschiza). University Press of Hawaii.

Min, K.-J., Hogan M. F., M. Tatar, and D. M. O'Brien. 2006. Resource allocation to reproduction and soma in *Drosophila*: A stable isotope analysis of carbon from dietary sugar. J. Insect Physiol. 52: 763–770. doi: 10.1016/j.jinsphys.2006.04.004.

Mitsui, H., K. Beppu, and M. T. Kimura. 2010. Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol. Sci. 13: 60–67. doi: 10.1111/j.1479-8298.2010.00372.

Naranjo-Lázaro, J. M., Mellín-Rosas M. A., González-Padilla V. D., Sánchez-González G., G. Moreno-Carrillo, and H. Arredondo-Bernal. 2014. Susceptibility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) to Entomophatogenic Fungi. Southwest. Entomol. 39: 201–203. doi: 10.3958/059.039.0119.

National Agricultural Pest Information System (NAPIS). 2013. Pest tracker, reported pest status of spotted wing drosophila *Drosophila suzukii*. (http://pest.ceris.purdue.edu/pestlist.php).

Olson, D. M., Fadamiro H. Y., J. G. Lundgren, and G. E. Heimpel. 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. Physiol. Entomol. 25: 17–26.

Ostojic´, I., M. Zovko, and D. Petrovic´. 2014. First record of spotted wing *Drosophila suzukii* (Matsumura, 1931) in Bosnia and Herzegovina. Radovi Poljoprivrednog Fakulteta Univerziteta u Sarajevu (Works of the Faculty of Agriculture University of Sarajevo) 59: 127–133.

Roca, M. J., and C. R. Lazzari. 1994. Effects of relative humidity on the haematophagous bug *Triatoma infestans*: Hygropreference and eclosion success. J. Insect Physiol. 40: 901–907.

Sasaki, M., and R. Sato. 1996. Bionomics of *Drosophila pulchrella* Tan, Hus et Sheng (Diptera: Drosphilidae) in Fukushima prefecture. Tohoku Agricultural Research (Japan).

Schetelig, M. F., and A. M. Handler. 2013. Germline transformation of the spotted wing drosophilid, *Drosophila suzukii*, with a piggyBac transposon vector. Genetica. 141: 189–193. doi: 10.1007/s10709-013-9717-6.

Shinzo, K., U. Ken, and T. Kyoko. 2007. Control of Cherry Drosophila *Drosophila suzukii*

injurious to blueberry. Plant Prot. 61: 205–209.

Smith, T. J. 2013. Overview of tree fruit production in the Pacific Northwest United States of America and southern British Columbia, Canada.

Stacconi, M. R., Grassi A. L., Dalton D. T., Miller B., Ouantar M., Loni A., Ioriatti C., V. M. Walton, and G. Anfora. 2013. First field records of *Pachycrepoideus vindemiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas. Entomologia 1: e3.

Steffan, S. A., Lee J. C,, Singleton M. E., Vilaire A., Walsh D. B., L. S. Lavine, and K. Patten. 2013. Susceptibility of cranberries to *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 106: 2424–2427.

Strik, B. C., and C. E. Finn. 2008. Blueberry cultivars for Oregon. EC 1308-E. Oregon State University Extension Service. March 2008. Corvallis, OR.

Strik, B. C., and D. Yarborough. 2005. Blueberry production trends in North America, 1992 to 2003, and predictions for growth. HortTech. 15: 391–398.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561.

Tabashnik, B. E., and B. A. Croft. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. Environ. Entomol. 11: 1137–1144.

Takamori, H., Watabe H., Fuyama Y., Z. Ya-ping, and T. Aotsuka. 2006. *Drosophila subpulchrella*, a new species of the *Drosophila suzukii* species subgroup from Japan and China (Diptera: Drosophilidae). Entomol. Sci. 9: 121–128. doi: 10.1111/j.1479-8298.2006.00159.

Tochen, S., Dalton D. T., Wiman N. G., Hamm C., P. W. Shearer, and V. M. Walton. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ. Entomol. DOI: http://dx.doi.org/10.1603/EN13200.

USDA N. 2014. Oregon agriculture: facts & figures. (http://www.nass.usda.gov/Statistics_by_State/Oregon/Publications/facts_and_figures/facts_and_figures.pdf). Retrieved March 20, 2014.

Van Timmeren, S., and R. Isaacs. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop

Prot. 54: 126–133. doi: 10.1016/j.cropro.2013.08.003.

Vilela, C. R., Teixeira E. P., and C. P. Stein. 2001. Mosca-africana-dofigo, *Zaprionus indianus* (Diptera: Drosophilidae). Histórico e Impacto das Pragas Introduzidas no Brasil. Holos, Ribeirão Preto SP, pp 48–52.

Wäckers, F. L., Romeis J., and P. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. Ann. Rev. Entomol. 52: 301–323.

Walsh, D. B., Bolda M. P., Goodhue R. E., Dreves A. J., Lee J., Bruck D. J., Walton V. M., S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integ. Pest Manag. 2: 1–7. doi: 10.1603/IPM10010.

Wiman, N. G., Walton V. M., Dalton D. T., Anfora G., Burrack H. J., Chiu J. C., Daane K. M., Grassi A., Ioriatti C., Miller B., S. Tochen, and X. G. Wang. 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLoS ONE 9(9): e106909. doi:10.1371/journal.pone.0106909.

Yamada, Y. 2008. Oviposition inhibitor trials on *Drosophila suzukii*. Undergraduate, Yamagata University

Yu, D., F. G. Zalom, and K. A. Hamby. 2012. Host status and fruit odor response of *Drosophila suzukii* (Diptera: Drosophilidae) to figs and mulberries. J. Econ. Entomol. 106: 1932–1937.

Zimmermann, G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Sci. Techn. 18: 865–901. doi: 10.1080/09583150802471812.

CHAPTER 2.

Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry.

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Abstract

Temperature-related studies were conducted on *Drosophila suzukii* Matsumura (Diptera: Drosophilidae: Drosophilini) using seven temperatures on blueberries, *Vaccinium corymbosum* L. (Ericales: Ericaceae) and cherries, *Prunus avium* (L.) L. 1755 (Rosales: Rosaceae), important commercial hosts of *D. suzukii*. Temperature had a significant influence on *D. suzukii* developmental period, survival and fecundity, with decreasing developmental periods as temperatures increased to 28 ºC. Above 28 ºC, developmental periods increased, indicating that higher temperatures approached the developmental extremes for the species. *Drosophila suzukii* females laid less eggs on blueberries than on cherries when reproduction occurred. The highest net reproductive rate (R_o) and intrinsic rate of population increase (r_m) was recorded on cherries at 22 ºC and was 195.1 and 0.22, respectively. Estimations using linear and nonlinear fit for the minimum, optimal and maximum temperatures where development can take place were 7.2, 28.1 and 42.1 ºC, respectively. The r_m values for minimal, optimal, and maximal population increase were 13.4, 21.0 and 29.3 ºC, respectively

Introduction

 Drosophila suzukii Matsumura (Diptera: Drosphilidae) is an economic pest of small and stone fruit species and is now established across production areas of North America, Asia, and Europe (Cini et al. 2012, Goodhue et al. 2011, Kanzawa 1939, Kawase et al. 2007, Lee et al. 2011, Walsh et al. 2011). Two key economically important fruit produced in these regions include blueberries, *Vaccinium corymbosum* L. (Ericales: Ericaceae) and cherries, *Prunus avium* (L.) L. 1755 (Rosales: Rosaceae). Female *D. suzukii* oviposit in ripening fruit using a serrated ovipositor as opposed to other drosophilids such as *D. melanogaster*, which oviposit in overripe or previously damaged fruit (Lee et al. 2011, Walsh et al. 2011). Developing larvae subsequently consume the fruit flesh, making infested fruit unmarketable for fresh consumption. Larval feeding degrades quality of fruit, which may further lead to fruit rejection at processing facilities. It is estimated that *D. suzukii* damage may lead to $500 million in annual losses in Western USA production areas assuming 30% damage levels (Goodhue et al. 2011). Mitsui et al. (2006, 2007) described high fecundity of *D. suzukii* on wild Japanese fruits that are closely related to American cultivated berry species, but no clear developmental or reproductive parameters were given in those studies.

 In North American commercial production areas of small and stone fruit, pesticide applications have been the primary control tactic against *D. suzukii* (Beers et al. 2011, Bruck et al. 2011). The most effective sprays include pyrethroids, carbamates, and spinosyns targeting ovipositing adult females. Current spray programs are timed to prevent oviposition in ripening susceptible crops (Beers et al. 2011, Bruck et al. 2011). Traps baited with apple cider vinegar or a combination of sugar-water and yeast are used to monitor for *D. suzukii* (Cha et al. 2012, Landolt et al. 2012, Lee et al. 2012) but are limited as a warning tool against *D. suzukii* attack.

 Current control methods can be improved by gaining knowledge of the basic mechanisms of *D. suzukii* population increase (Price 1997). The influence of long-term insect survival includes favorable temperatures during the overwintering period (Dalton et al. 2011) and most likely the availability of essential food resources. Such factors may drive early season *D. suzukii*

population increase and resultant elevated pest pressure on susceptible host crops (Kanzawa 1939, Mitsui et al. 2006, Mitsui et al. 2007).

Studies to determine the impact of temperature on insect pests have been used to better understand the underlying mechanisms for population increase as temperature influences insect emergence, development, fecundity, and mortality rates (Carey 1993, 2001). The effect of temperature on development of *D. suzukii* was originally investigated in Japan, but this work was conducted under fluctuating ambient (Kanzawa 1939) and outdoor temperatures (Kimura 2004). These research areas demand attention in order to gain an understanding of local *D. suzukii* population dynamics for use in IPM forecasting. The goals of this study were to estimate net reproductive rate (R_o) and intrinsic rate of population increase (r_m) of a laboratory colony of *D. suzukii* reared across a range of constant temperatures and on two commercially valuable fruit crops, blueberry and cherry. These crops represent fruit-producing species of significant economic value that are attacked by this pest (Goodhue et al. 2011, Lee et al. 2011, Walsh et al. 2011).

Materials and Methods

Collection and rearing. Initial stock cultures of *D. suzukii* adults and pupae were provided to Oregon State University (OSU) in 2009 by the USDA-ARS Horticultural Crops Research Laboratory (HCRL), Corvallis, Oregon, and routinely augmented by field-collected *D. suzukii* that were obtained from weekly field collections made in the Willamette Valley from 2010 to 2012.

The OSU stock colony for these studies were maintained in the laboratory at 22 ± 2 ºC, 65% relative humidity (RH) and a photoperiod of 16 L: 8 D. Each rearing cage contained a plastic container modified to hold a sponge and water. An insect diet medium was added to the cage as an artificial food source (Dalton et al. 2011) and provided a site for oviposition.

Rearing containers for experimental units were constructed as previously described (Dalton et al. 2011). Containers consisted of 163 ml plastic soufflé cups (Solo, Urbana, IL) equipped with a moistened filter paper strip (Whatman International, Ltd, Maidstone, UK) and a cotton square (US Cotton, LLC, Rio Rancho, NM) to act as a water source for the insects. Adult

mated female flies were secured in cups with tight-fitting lids that were pierced to provide gas exchange between the cups and the temperature cabinets.

Fresh unsprayed sweet cherries, *P. avium* ('Rainier', 'Bing' cv) and blueberries *V. corymbosum* ('Duke', 'Bluecrop', and 'Jersey' cv) were regularly sourced from the OSU Mid-Columbia Research and Extension Center (MCAREC) in Hood River, Oregon as well as the OSU Lewis-Brown and Botany Plant Pathology research farms in Corvallis, Oregon. Equivalent numbers of fruit were collected with stems intact and stored in cold rooms at 4 ºC until they were exposed to *D. suzukii*. Individual fruit were closely examined under a stereomicroscope to ensure that they were free of any eggs or larvae before being exposed to flies.

Fruit was exposed to *D. suzukii* females for thirty-minutes at 22 ± 2 °C, 65% RH and were subsequently removed, examined under the stereomicroscope, and grouped by the number of eggs present. A single infested fruit containing no more than five eggs was subsequently placed into rearing containers and placed into the temperature cabinets within one hour of initial egg deposition. For both fruit types, each temperature treatment consisted of at least twenty containers with one fruit.

Rearing units containing infested fruit were placed in temperature-controlled growth chambers (Model E-30BHO; Percival Scientific, Perry, IA) at 10, 14, 18, 20, 24, 28 and 30 ºC and 60-70% RH under a 16 L: 8 D photoperiod. Each container was observed every 48 h (three lowest temperatures) or 24 h (four highest temperatures) until pupae became apparent on fruit or adult females were found. Pupae were marked once detected. Attempts to track individual larvae were made, but due to the propensity of larvae to stay within fruit until the final instar, these developmental data are not presented. Data are presented as time (days) taken from egg to pupal stage, pupal stage to adult, and adult to mortality.

To allow controlled mating, females were removed upon emergence and placed individually along with one male in separate cups containing uninfested fruit. Missing or dead male flies were replaced throughout the oviposition period to allow continued mating and ensure optimal reproduction of *D. suzukii* females. Missing or dead females were excluded from data analysis. Fruit in each container was replaced every 24 h and examined to determine daily

oviposition activity and survival period for each female *D. suzukii*. Data from these observations were plotted over time in order to display survival and oviposition trends for each of the fruit types and treatment temperatures.

Statistical analysis

Influence of temperature on survival and developmental period and fecundity. Impact of temperature on duration of each life stage was analyzed using ANOVA with fruit, gender and temperature as independent factors. Differences of the means were separated using Tukey's HSD. Nonparametric Kruskal-Wallis ANOVA rank-sum tests were used to compare adult female survival for each temperature and fruit type. Analyses were conducted using Statistica (Statsoft 7.1, Tulsa, Oklahoma).

Life table parameters and threshold determination. The mean generation times (T) of *D. suzukii* on cherries and blueberries were estimated using the equation, $T = \Sigma\ l_x m_x x / R_o$ (Price 1997), where x = age in days, l_x = the proportion of females surviving on day x and m_x = the mean number of eggs produced on day x and R_o is the net reproductive rate. The mean survival and fecundity values for each temperature were then used to estimate the intrinsic rate of population increase (r_m) at each temperature. These values were determined by using the equation, $r_m = \log_e R_o / T$ (Price 1997), where R_o is the net reproductive rate and T is the mean generation time. Net reproductive rate was obtained using the equation, $R_o = \Sigma\ l_x m_x$, and rate of development was estimated by using linear and nonlinear regression. Linear and non-linear regression was performed on the data of both fruit types by using the reciprocal of development time in days ($1/T$) on temperature. The lower developmental threshold was subsequently determined by solving the regression equation for $1/T = 0$. A non-linear model adapted from Briere et al. (1999) for temperature-dependent development was used to estimate upper and optimal thresholds. The expression of the user-specified regression model is: $r(T) = nT(T - T_b)(T_L - T)\exp(1/m)$, where $r(T)$ is the rate of development at temperature T; T_L is the upper temperature developmental threshold; T_b is the lower temperature developmental threshold and n and m are empirical constants.

The thermal constant (k) in degree-days (DD°) from oviposition to adult was estimated by

using $k = 1/b$, where represents the slope of the estimated relationship between temperature and the rate of development (Campbell et al. 1974, Liu and Meng 1999). The lower and upper thresholds for intrinsic rate of population increase (r_m) were estimated by fitting the nonlinear model to the r_m values obtained from blueberries and cherries. Fitting of non-linear models were conducted using Statistica (Statsoft 7.1).

Results

  Influence of temperature on life stages. Drosophila suzukii developed and eclosed at all temperatures in infested fruit within experimental containers. Subsequent survival of adults occurred at all temperatures except at 30 °C for males on cherries (Table 2a) and 30 °C for females on blueberries (Table 2b). Survival to adulthood at the remaining temperatures was lowest at the extreme temperatures of 10 and 30 °C in blueberries (10% and 1% survival, respectively). In cherries, survival from egg to adulthood was 10% at 10 °C and 2% for 30 °C respectively. The lowest percent mortality occurred at 26 °C (40%) in blueberries and 41% mortality at 22 °C in cherries. In blueberries, similar proportions of males developed to adulthood at 10-14 °C. Numerically more females developed to adulthood at 18-22 °C, and more males developed to adulthood at 26-30 °C. In cherries, relatively more females developed to adulthood at 14, 18, 22, 26 and 30 °C and relatively more males developed to adulthood at 10 and 28 °C. Fruit ($F_{4, 607} = 3.2$, $P = 0.011$) had an impact on survival periods from adult to mortality. Survival periods were shorter in blueberries compared to cherries at 10 and 14 °C (Table 2, Fig. 1A and 1B). No survival periods were statistically different when compared by gender, except for the period from adulthood to mortality at 10 and 14 °C. At these temperatures, male longevity was shorter than that of females ($F_{4, 607} = 2.79$, $P = 0.025$). Life span for adult females ranged from 2 to 35 days. In general, temperature increase resulted in a decrease in survival period (Table 2 a, b, Fig. 1A and B). The shortest survival period (2 days) for adult flies was recorded at 30 °C in cherry.

  Median rank-sum tests of survival rates of reproducing females on cherries (median = 51 days) were significantly higher than those on blueberries (median = 47 days, $\chi^2_{1,4} = 3.61$, p = 0.05; Fig 1). Rank-sum tests for adult female survival displayed significant differences for

cherries ($H_{4,\ 124}$ = 76.97, p = 0.0001, Fig 1A). Multiple comparisons of p-values for days survived showed that adult females exposed to 14, 18 and 22 °C displayed similar longevity, and longevity comparing 26 and 28 °C was statistically similar. The higher two temperatures resulted in a shorter adult lifespan compared to the lower three temperatures. Survival periods in cherry were significantly different depending on temperature ($\chi^2_{1,4}$ = 12.91, p = 0.03), and median adult female survival times for 14, 18, 22, 26 and 28 °C were 40.7, 44.2, 14.9, 6.4 and 5 days, respectively.

Rank-sum tests for adult female survival curves displayed significant differences in blueberries ($H_{4,\ 83}$ = 50.24, P = 0.0001, Fig. 1B). Multiple comparison of p-values for days survived showed that adult females exposed to 14 and 18 °C displayed similar longevity, and comparison between 22, 26 and 28 °C treatments produced statistically similar longevity. The three higher temperatures resulted in shorter adult lifespan compared to the two lower temperatures. Survival periods in blueberry were significantly different based on temperature ($\chi^2_{1,4}$ = 32.96; P = 0.0001). Median adult female survival times on blueberry for 14, 18, 22, 26, and 28 °C were 34.3, 28.3, 13.8, 3.4 and 2 days respectively.

Influence of temperature on developmental periods. Most statistical comparisons were non-significant at similar developmental stages in both fruit at 26 and 28 °C. In cherries and blueberries, developmental periods of all life stages were significantly affected by temperature ($F_{20,\ 1934}$ = 6.35, P < 0.0001; Table 1). The numerically largest differences were recorded between 10 and 14 °C. Statistical differences were found in virtually all cases for developmental times required by each of the recorded life stages from 10 to 26 °C. However, no statistical difference was observed in the duration of the developing stages of surviving flies from the 30 °C treatments compared to survivors at the 26 and 28 °C treatments. The longest development period from egg to adult occurred at 10 °C (79.4 ± 4.1 days) in cherry and the shortest (11.0 ± 0.2 days) in blueberry at 30 °C (Table 2). Immature flies spent the largest proportion of time in the feeding (larval) stage compared to all other developmental stages at all temperatures except for 26 and 28 °C in both fruits.

Influence of temperature on fecundity. No eggs were laid at 10 or 30 °C. Statistical comparison of *D. suzukii* fecundity showed that significantly more eggs were laid in cherries compared to blueberries at all temperatures ($F_{5, 170} = 6.1$, p < 0.0001; Table 3). Within cherries, the highest numbers of eggs were laid at 18 °C, followed in order by 22, 26, 14 and 28 °C. In blueberries, differences were less pronounced with statistically similarly numbers of eggs laid at 22 and 18 °C. Lower numbers of eggs were laid at 26 °C followed by 28 and 14 °C. The lowest fecundity was recorded at 14 °C in blueberry. The oviposition period was longest (~38 days) at 14 °C on cherry (Fig 2). Oviposition on cherries at 14 °C started at 29 days post-emergence and continued to 64 days post-emergence in one case. Pre-oviposition periods decreased as temperatures increased in both fruit treatments. At all temperatures, age-specific fecundity assumed a normal curve shape typical of insects (Carey 1993). The maximum fecundity of 141 eggs per female was recorded at 18 °C on cherry.

Life table parameters and threshold determination. Because both cherries and blueberries produced flies with similar developmental times from egg to adult, data of both fruit types were combined to determine lower threshold, upper threshold, and optimal developmental temperatures. The function obtained by linear regression using 10, 14, 18 and 22 °C was y= 0.0048x- 0.0344 ($R^2 = 0.997$, $F_{1, 8} = 4289$, $P = 0.0001$) with an estimated lower developmental threshold of 7.2 °C (Fig 3). The thermal constant, $k = 208.33$ DD, was obtained using the minimum threshold temperature from linear regression. The upper developmental threshold temperature (42.1 °C) and optimal temperature for development (28.1 °C) were obtained using the non-linear model. The non-linear equation is y=(0.156e-6)x(x-(6.513))((42.0577)- x)exp(1/0.235)), ($R^2 = 0.98$, $F_{1, 9} = 26.12$, $P = 0.00028$). It is estimated that there will be 7.1 generations per season using 20-year daily means for Corvallis, Oregon (USDI – Bureau of Reclamation).

The developmental times spent in each life stage on cherries and blueberries showed similarities (Table 3). Generation time (*T*) for *D. suzukii* ranged from 12 to 43.9 days and decreased with an increase in temperature. The net reproductive rate (R_o) and intrinsic rate of population increase (r_m) for both fruit increased as temperature increased to peak at 22 °C, and

then decreased at 28 °C. The rate of increase was above zero in all cases, indicating positive population growth over this range of temperatures. The numerically highest population developmental parameters were found from *D. suzukii* on cherries at 22 °C, R_o = 195.1 eggs per female, r_m = 0.22, and generation time of 24.2 days. The corresponding parameters on blueberries were lower. The lowest R_o and r_m for *D. suzukii* were recorded at 28 °C on blueberries with estimated values R_o = 0.6 and r_m = 0.01.

For cherries, the lower (13.4 °C) and upper (29.33 °C) threshold for r_m and optimal temperature (21 °C) were obtained using non-linear estimation. The non-linear equation is y=(0.0000161)x(x-(13.4))((29.33)-x)exp(1/0.33)), (R^2 = 0.81, $F_{1, 9}$ = 13.04, P = 0.024).

Discussion

The data presented in this study provide a comprehensive set of survival, developmental and reproductive parameters for a *D. suzukii* colony that continues to be maintained in Oregon. Age–related female survival and oviposition at each temperature provide an indication of which temperatures are most suitable for longevity and reproduction of *D. suzukii*. Both survival and reproductive potential are summarized by the r_m values and indicate optimal population increase at 21 °C using non-linear estimation. The lower and upper estimated thresholds for population increase are realistic in comparison to Tephritids (Quesada-Moraga et al. 2012) and initial data on *D. suzukii* (Kanzawa 1939, Kimura 2004). The fact that no adult males were found at 30 °C for cherries and no females were found at 30 °C for blueberries suggests that *D. suzukii* has low survival in the field at these temperatures. In the present study, only three individuals (1.5%) survived to adulthood at this upper temperature. The relatively low survival rates and slow developmental rates recorded on both fruit at 10 and 30 °C are an indication that these temperatures are close to the developmental extremes for *D. suzukii*. A recent study has shown high mortality for flies subjected to even more severe cold temperatures (Dalton et al. 2011). Data out of the scope and lacking from this study are temperature-related survival rates of the individual developmental stages of eggs and larvae.

The developmental period from egg to adult ranged from 28.8 days at 14 °C to 10.8 days at 26 °C. These findings are similar to earlier research that showed developmental periods from

egg to adults at 37 days under fluctuating cold temperatures (10-14 °C), and 11 days at fluctuating mild temperatures (24-26 °C) (Kanzawa 1939). The life history traits identified under constant temperatures in the current study are not possible to directly compare with the decades-old research conducted on *D. suzukii* at fluctuating temperatures in Japan (Kanzawa 1939). In our study, developmental periods decreased in an approximately linear fashion from 14 to 26 °C before increasing again at the hottest temperature of 30 °C.

Reproduction of *D. suzukii* was facilitated in cherries and blueberries at temperatures ranging from 14-28 °C. The mean fecundity per female increased with increasing temperatures up to 22 °C, and decreased at temperatures above this for both fruit types. Fecundity rates of the closely related *D. simulans* over a ten-day period ranged from 350 to 500 eggs per female (Weeks et al. 2007). Fecundity of *D. suzukii* on cherries, as recorded by Kanzawa (1939), was 172 eggs per female. The highest mean oviposition recorded in this study was 141 eggs per female at 18 °C over 45 days, a much lower value than found on *D. simulans* (Weeks et al. 2007). However, our fecundity data are relatively similar to that found by earlier research (Kanzawa 1939). Direct comparisons between studies cannot be made because of lacking environmental data from the earlier work. The fecundity of *D. suzukii* on blueberries was significantly lower than that found in cherries, with the highest fecundity of 19.8 eggs per female recorded at 22 °C. In a separate unpublished study (Jana Lee, unpublished data) the fecundity rate of *D. suzukii* was higher at 148.4 ± 19.1 eggs over a four-week period on 'Elliot' blueberries and 160.5 ± 46.8 eggs on artificial rearing medium. This may be a possible indication that the cultivars used in our study were comparably less suitable than 'Elliot' for oviposition and development.

The fact that no reproduction was recorded in the present study at 10 and 30 °C does not mean that reproduction cannot occur at these temperatures, but rather that these temperatures may approach the lower and upper extremes for development. Future work at extreme developmental boundaries may be useful to explain possible field reproduction under these conditions. Such work should include large experimental populations, cold- or warm-adapted *D. suzukii* populations, and fluctuating temperatures that include the suspected physiological limits.

Our data suggest that *D. suzukii* has short generation times and high reproductive levels compared to other Dipteran pests found in fruit in the United States (Reissig et al. 1979, Texeira and Polavarapu 2001, Quesada-Moraga 2012). The apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Reissig et al. 1979), and blueberry maggot, *R. mendax* (Curran) (Texeira and Polavarapu 2001), both complete fewer generations per season than *D. suzukii*.   It is more important to predict first emergence in the case of these pests. For the Lepidopteran pests such as *Cydia pomonella* L. (Lepidoptera: Tortricidae), another severe fruit pest with relatively discrete generations, the same is true (Jones and Wiman 2012). The thermal constant for *C. pomonella* were estimated to range from 510-615 DD (Rock and Shaffer 1983, Aghdam et al. 2009) and the intrinsic rate of natural increase (r_m) was 0.08 (Rock and Shaffer 1983). The thermal constant and r_m values are 208 DD and 0.2, respectively for *D. suzukii*, allowing more rapid population increase in comparison to these insects. It is therefore believed that pest models using only DD have limited value for *D. suzukii* for three reasons. Firstly, *D. suzukii* has relatively short generation times, and generations begin to overlap relatively early during the growing season. Secondly, *D. suzukii* has a high reproductive potential and population increase is more rapid at ideal temperatures. Thirdly, fluctuating temperatures dramatically impact both reproduction and survival for *D. suzukii*, and DD models do not estimate the impact of these fluctuations on insect population levels and resultant population pressure.

Our study is an attempt to understand the impact of temperature on *D. suzukii* developmental, survival and reproductive parameters. Information from this work is intended to identify temperature factors favorable to *D. suzukii* but by no means provides a comprehensive picture of all factors influencing *D. suzukii* population levels. More detailed work is needed on the mechanisms of winter survival, the impact of humidity on development and population increase parameters under controlled fluctuating temperatures, and the impact of temperature on the immature developmental stages of eggs and larvae.

References Cited:

Aghdam, H. R., Fathipour Y, G. Radjabi, and M. Rezapanah. 2009. Temperature-dependent development and temperature thresholds of codling moth (Lepidoptera: Tortricidae) in Iran. Environ. Entomol. 38: 885-895.

Beers, E. H., Van Steenwyk R. A., Shearer P. W., W. W. Coates, and J. A. Grant. 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manage. Sci. 67: 1386–1395.

Briere, J. F., Pracros P., A. Y. Le Roux, and J. S. Pierre.1999. A novel rate model of temperature-dependent development for arthropods. Environ. Entomol. 28: 22-29.

Bruck, D. J., Bolda M., Tanigoshi L., Klick J., Kleiber J., DeFrancesco J., B. Gerdeman and H. Spitler. 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manage. Sci. 67: 1375–1385.

Campbell, A., Frazer B. D., Gilbert N., A. P. Gutierrez, and M. Mackauer. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11:431-438.

Carey, J. R. 1993. Reproduction. Applied Demography for Biologists with Special Emphasis on Insects (ed. by JR Carey) Oxford University Press, New York, NY, USA, pp. 43-76.

Cha, D. H., Adams T., H. Rogg and P. J. Landolt. 2012. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. J. Chem. Ecol. 38: 1419-1431.

Cini, A., C. Ioriatti and G. Anfora 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insectology 65: 149–160.

Dalton, D. T., Walton V. M., Shearer P. W., Walsh D.. B., J. Caprile, and R. Isaacs. 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manage. Sci. 67: 1368–1374.

Goodhue, R. E., Bolda M., Farnsworth D., J. C. Williams, and F. G. Zalom. 2011. Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manage. Sci. 67: 1396–1402.

Jones, V. P., and N. G. Wiman. 2012. Modeling the interaction of physiological time, seasonal weather patterns, and delayed mating on population dynamics of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Pop. Ecol. 54: 421-429.

Kanzawa, T. 1939. Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi Agricultural Experiment Station 49 pp. (abstr.). Rev. Appl. Ent. 29: 622.

Kawase, S, K. Uchino, and K. Takahashi. 2007. Control of cherry drosophila, *Drosophila suzukii*, injurious to blueberry. Plant Protection. 61: 205–209.

Kimura, M. T. 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. Oecologia. 140: 442–449.

Landolt, P. J., T. Adams, and H. Rogg. 2012. Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. J. Appl. Entomol. 136: 148–154.

Lee, J. C. Burrack, H. J., Barrantes, L. D., Beers, E. H., Dreves, A. J., Hamby, K. A., Haviland, D. R., Isaacs, R, Richardson, T. A., Shearer, P. W., Stanley, C. A., Walsh, D. B., Walton, V. M., F. G. Zalom, and D. J. Bruck. 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. J. Econ. Entomol.105: 1350–1357.

Lee, J. C., Bruck, D. J., Curry, H., Edwards, D. L., Haviland, D., R. Van Steenwyk, and B. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted wing drosophila, *D. suzukii*. Pest Manage. Sci. 67: 1358–6137.

Liu, S.S., and X. D. Meng. 1999. Modeling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. Bull. Entomol. Res. 89: 53-63.

Mitsui, H., K. H. Takahashi, and M. T. Kimura. 2006. Spatial distributions and clutch sizes of *Drosophila* spp. ovipositing on cherry fruits of different stages. Pop. Ecol. 48: 233–237.

Mitsui, H., K. Van Achterberg, and G. Nordlander. 2007. Geographical distributions and host associations of larval parasitoids of frugivorous Drosophilidae in Japan. J. Nat. Hist. 41: 1731–1738.

Price, P. W. 1997. Insect Ecology 3rd Edition. Population Dynamics John Wiley & Sons, New York, pp. 169-197.

Quesada-Moraga, E., P. Valverde-García and I. Garrido-Jurado. 2012. The effect of temperature and soil moisture on the development of the preimaginal Mediterranean fruit fly (Diptera: Tephritidae). Environ. Entomol. l41: 966-970.

Reissig, W. H., Barnard, J., Weires, R. W., E. H. Glass and R. W. Dean. 1979. Prediction of apple maggot fly emergence from thermal unit accumulation. Environ. Entomol. 8: 51-54.

Rock, G. C. and P. L. Shaffer. 1983. Developmental rates of codling moth (Lepidoptera: Olethreutidae) reared on apple at four constant temperatures. Environ. Entomol. 12: 831-834.

R Development Core Team 2012 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0.

Statistica 7.1. Statsoft Inc. 2005. 2300 East 14th Street, Tulsa, OK, 74104.

USDI Bureau of Reclamation. (http://www.usbr.gov/pn/agrimet/agrimetmap/crvoda.html). Retrieved April 4, 2012

Walsh, D. B. , Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., Walton, V. M., S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integr. Pest Manage. 2: G1–G7.

Table 2.1. *Drosophila suzukii* mean developmental periods (day ± SEM) at seven constant temperatures on 'Rainier' and 'Bing' cherries. Means within a row followed by different lowercase letters are significantly different and means within a column followed by different uppercase letters are significantly different. Means were separated using Tukey's HSD (p<0.05).

				Temperature ºC			
Life Stage							
Sex	10	14	18	22	26	28	30
Females	n = 3	n = 38	n = 27	n = 37	n = 48	n = 19	n = 2
Egg to pupa	47 ± 4a	14.7 ± 0.3b	10.8 ± 0.2c	8.0 ± 0.2d	6.8 ± 0.2f	6.2 ± 0.2f	7.5 ± 0.5e
Pupa to adult	32.4 ± 2.3a	14.1 ± 0.2b	7.6 ± 0.2c	6.0 ± 0.2d	4.0 ± 0.1e	3.7 ± 0.2e	4.5 ± 0.5de
Egg to adult	79.3 ± 4.1a	28.8 ± 0.3b	18.2 ± 0.3c	14 ± 0.1d	10.8 ± 0.1e	9.9 ± 0.3e	12.0 ± 1.0e
Adult longevity	35 ± 12.5aA	27.3 ± 3.9bA	18.2 ± 2.9c	10.5 ± 4.2d	12.8 ± 4.9d	10.7 ± 3.5d	2.0 ± 0e
Males	n = 7	n = 28	n = 22	n = 31	n = 23	n = 20	
Egg to pupa	43.1 ± 2.5a	14.4 ± 0.4b	10.8 ± 0.3c	8.5 ± 0.2d	6.9 ± 0.2e	6.1 ± 0.2f	-
Pupa to adult	35.1 ± 2.9a	14.3 ± 0.3b	8.1 ± 0.6c	5.5 ± 0.2d	4.2 ± 0.1e	4.0 ± 0.1e	-
Egg to adult	78.3 ± 3.1a	28.7 ± 0.5b	18.9 ± 0.3c	14.0 ± 0.2d	11.1 ± 0.2e	10.0 ± 0.1f	-
Adult longevity	31.0 ± 4.3aB	20.8 ± 3.2aB	16.8 ± 3.2b	13.2 ± 7.7a	12.8± 8.2b	10.1 ± 6.8b	-

Table 2.2. *Drosophila suzukii* mean developmental rates (day ± SEM) at seven constant temperatures on 'Duke', 'Bluecrop' and 'Jersey' blueberries. Means within a row followed by different lowercase letters are significantly different and means within a column followed by different uppercase letters are significantly different. Means were separated using Tukey's HSD (p<0.05).

Life Stage	Temperature ºC						
Sex	10	14	18	22	26	28	30
Females	n = 5	n = 26	n = 22	n = 41	n = 48	n = 19	
Egg to pupa	43.6 ± 2.3a	16.2 ± 0.4b	11.7 ± 0.3c	8.2 ± 0.2d	6.6 ± 0.1e	5.9 ± 0.3f	
Pupa to adult	33.8 ± 1.1a	12.7 ± 0.5b	8.5 ± 0.2c	6.0 ± 0.2d	4.6 ± 0.1e	4.2 ± 0.3e	-
Egg to adult	77.4 ± 2.4a	28.3 ± 0.4b	20.1 ± 0.3c	14 ± 0.2d	10.9 ± 0.1e	10.1 ± 0.3e	-
Adult longevity	24.4 ± 4.3aA	16.6 ± 2.1bA	17 ± 2.1c	11.7 ± 0.4d	12.1 ± 0.7d	12 ± 0.5d	-
Males	n = 5	n = 16	n = 16	n = 39	n = 52	n = 20	n = 1
Egg to pupa	45 ± 2.9a	14 ± 0.5b	11.1 ± 0.2c	8.1 ± 0.2d	6.8 ± 0.2e	6.0 ± 0.2f	6.0 ± 0.2f
Pupa to adult	42.4 ± 10.4a	14.1 ± 1.4b	8.4 ± 0.1c	6.2 ± 0.3d	4.6 ± 0.3e	4.1 ± 0.1f	4.0 ± 0.2f
Egg to adult	78.4 ± 2.8a	27.3 ± 0.8b	19.5 ± 0.2c	14.1 ± 0.2d	11.1 ± 0.1e	10.1 ± 0.1g	10.0 ± 0.2f
Adult longevity	22.2 ± 4.7aB	11.5 ± 1.2bB	14.2 ± 2.9c	11.9 ± 2d	12.1± 1.9e	13.2 ± 0.8e	10.2f

Table 2.3. Temperatures where comparison of *Drosophila suzukii* fecundity (eggs per adult female life stage ± SEM) was measured on cherry and blueberry. Means within a row followed by different lowercase letters are different and means within a column followed by different uppercase letters are significantly different. Means were separated using Tukey's HSD (p<0.05).

	Temperature ºC				
Fruit	14	18	22	26	28
Cherry	18.6 ± 2.1cA	141 ± 20.3aA	62 ± 15.2bA	20.3 ± 2.1cA	12 ± 0.5dA
Blueberry	6.6 ± 2.1eB	17 ± 2.1cB	19.8 ± 7.9cB	12.1± 4.9dB	7.3 ± 0.8eB

Table 2.4. Parameters of temperatures where population increase for *Drosophila suzukii* was measured (R_o = the net reproductive rate; T = mean generation time in days; and r_m = intrinsic rate of population increase) on cherry and blueberry.

Temperature	Parameter and Fruit					
°C	R_o		T		r_m	
	Cherry	Blueberry	Cherry	Blueberry	Cherry	Blueberry
14	8.1	7.0	43.9	39	0.05	0.05
18	140.8	33	39	28.3	0.13	0.12
22	195.1	79.1	24.2	25.1	0.22	0.17
26	13.4	17.2	12.5	13.9	0.21	0.20
28	2.1	0.6	12.7	12	0.02	0.01
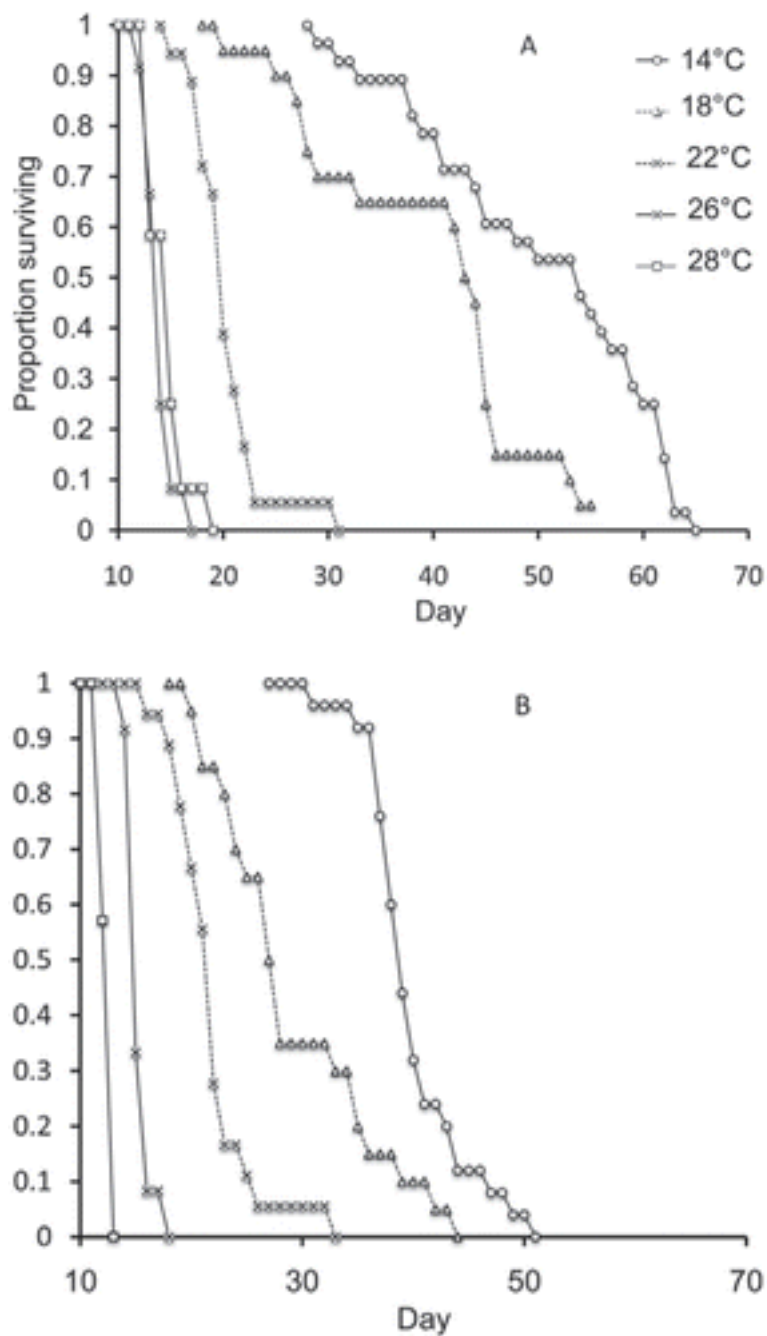
Figure 2.1. Proportion of adult *Drosophila suzukii* survival at five constant temperatures on cherry (A) and blueberry (B).
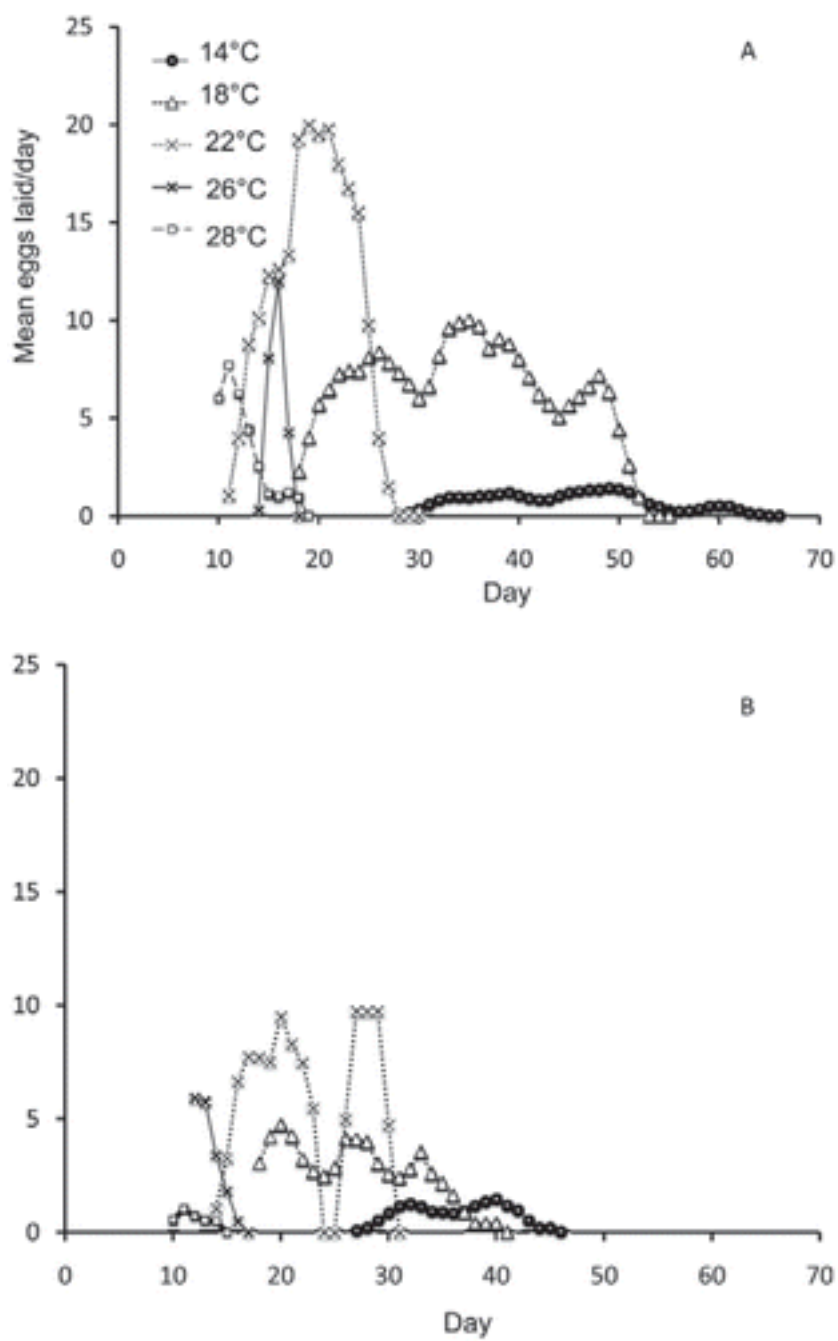
Figure 2.2 Mean daily *Drosophila suzukii* egg production at five temperatures on cherry (A) and blueberry (B).
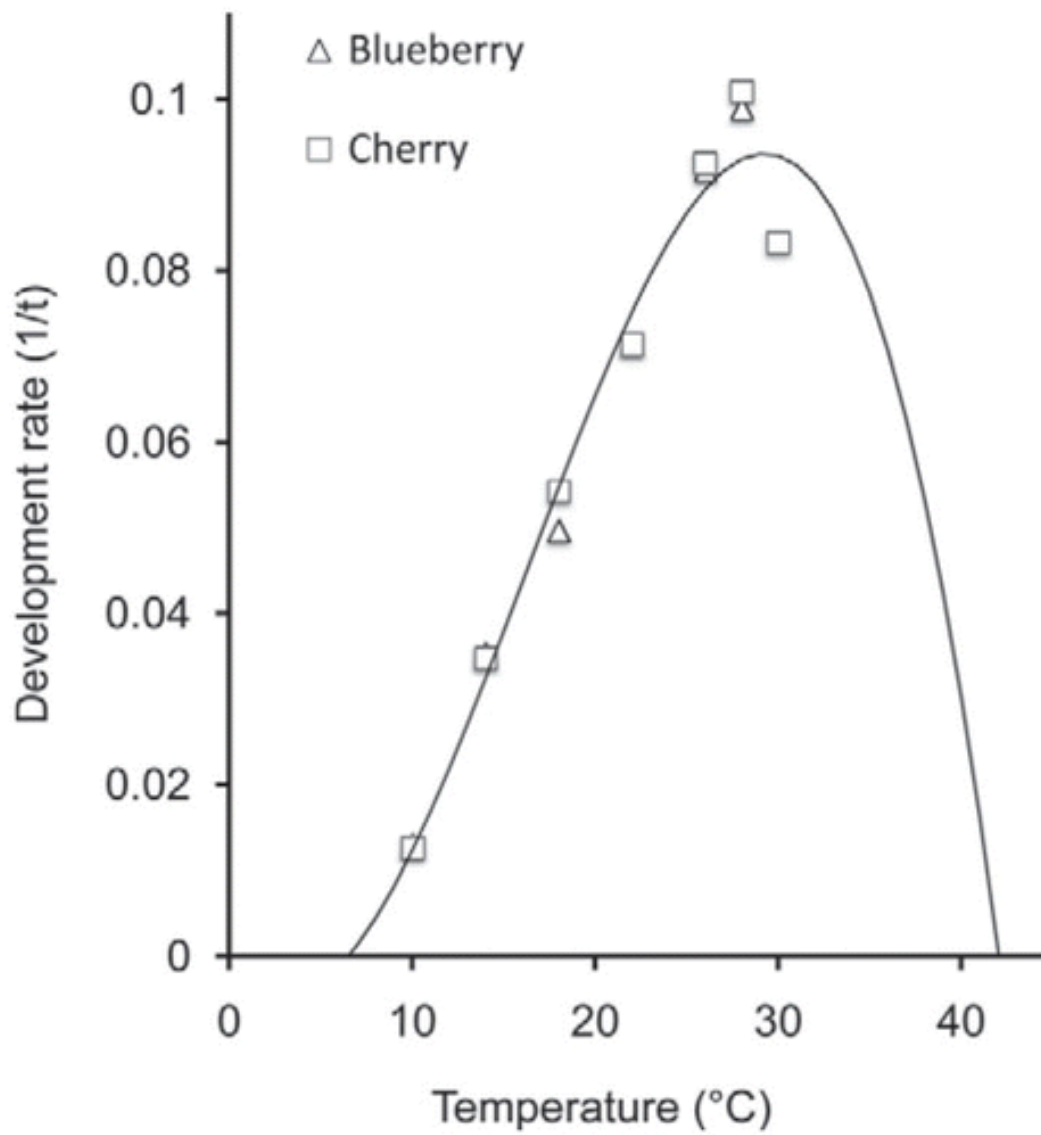
Figure 2.3. Optimum temperature for development and minimum and maximum developmental thresholds for *Drosophila suzukii* as a function of temperature.

CHAPTER 3.

Humidity-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on blueberry.

Samantha Tochen and Vaughn Walton

Abstract:

Drosophila suzukii is a worldwide economic pest, capable of infesting stone and small fruits. Previous work has examined the role of temperature in order to develop a model to predict population levels. However, the role of humidity was not examined at that time. The current study was conducted at 17, 33, 65, 78 and 93% relative humidity on commercially available blueberries. Humidity had a significant impact on survival and fecundity. Above 65% RH, survival and fecundity increased. The shortest mean generation occurred at 17% RH at 0 days and the longest mean generation time was at 93% RH at 9.226. The estimated lower development threshold is 27% RH, the upper development threshold is 100% RH, and the optimal humidity for development is 79% RH. Egg laying increased with RH, with a maximum observed at 93%. Dissection of ovaries indicated that there was no difference in ovary maturation between 93% and 78% RH.

Introduction

Insects are capable of colonizing wide ranges of environments including extremes in temperature and humidity. Patterns in insect abundance and distributional pattern are limited by water availability and temperature, as insects are prone to water loss due to small body size and inherent cost of gas exchange (Addo-Bediako et al. 2001, Chown et al. 2011). To overcome thermal extremes and potential water loss, insects utilize physiological components, such as heat shock proteins (Bubliy et al. 2013); behavior via reorientation in an environment in response to temperature and humidity gradients, moving to preferred conditions in order to maintain water balance (Tichy 2003); and physical adaptations to minimize water loss (Bradley et al. 2009). However, osmoregulation must be maintained counter to water balance (Contreras et al. 2013). Regulation of temperature and water balance may divert resources away from other aspects of fitness as this takes precedence to maintain homeostasis (Chown et al. 2011). Humidity affects diverse physiological parameters i.e. low humidity can lead to water loss thereby inhibiting reproduction. Excessively high humidity can result in proliferation of fungal pathogens (Roca and Lazzari 1994). Drosophilids exhibit multiple strategies to survive low humidity.

The effect of humidity is not often explored on biological parameters of pest species, yet water loss, influenced by ambient humidity and temperature, is a vital consideration to determine the success of such insects. Diptera are known to avoid areas of high humidity when coupled with high temperatures (Chown et al. 2011). Studies conducted on Olive fruit fly, *Bactrocera oleae*, demonstrate females fail to develop mature oocytes at low relative humidity. However, both longevity and fecundity of *B. oleae* peaked at 75% RH before decreasing as relative humidity approached 100% (Broufas et al. 2009). Lu and Wu (2011) studied the effect of relative humidity on intrinsic rate of population increase, oviposition rate, and reproductive success on the mirid, *Apolygus lucorum*. These authors found a decline in these parameters between 70 and 80% RH.

As temperature and humidity are both important factors in successful establishment, knowledge of the extremes and optimums for population growth as it pertains to *D. suzukii* reproduction is essential. As *D. suzukii* can cause economic loss, determining survival

parameters is vital in understanding its biology and ultimately, developing adequate control methods (Hendrichs et al. 2007). Temperature related development has been established (Tochen et al. 2014). However, no scientific documentation has examined the effect of humidity on developmental parameters and overall, population growth in *D. suzukii*.

This study has the aim to determine the effect of humidity on the longevity and reproductive potential by examination of egg production and ovarian maturation of *D. suzukii*. This information can then be incorporated into currently available prediction models (Wiman et al. 2014) in order to provide greater accuracy to the models that currently exist.

Material and Methods

Drosophila suzukii stock colonies: The initial laboratory colony was established from *D. suzukii* adults and pupae provided to Oregon State University (OSU) in 2009 by the USDA-ARS Horticultural Crops Research Laboratory (HCRL), Corvallis, Oregon with annual addition of wild-caught *D. suzukii* collected from Himalayan blackberries in the Willamette Valley. These cultures were maintained in the laboratory at 22 ± 2 ºC, 65% relative humidity (RH) and a photoperiod of 16:8 (L:D) , and provided artificial diet (Dalton et al. 2011) that served as a food source and allowed oviposition and a water wick. Adult rearing containers consisted of 163 mL plastic soufflé cups (Solo, Urbana, IL) and were modified to include two 2 by 1.5 inch windows with hot-glued fabric mesh screens. Microcentrifuge tubes (1mL) filled with water and a cotton wick and artificial food media were provided.

Relative humidity: Bugdorm rearing units (30X30X30 cm, DP1000, MegaView Science, Taiwan) were modified using plastic sheets to cover the existing screened portions and the mesh sleeves were replaced with plastic sleeves and closed with a clamp. For humidity regulation, different saturated salt solutions were prepared by boiling 100 mL water and adding 50 grams salt (Winston and Bates 1960), and upon cooling, more salt was added to the solution. The solutions were subsequently placed in petri dishes at the bottom of the cages to allow for maximal surface exposure of the solution to achieve the required humidity levels (Table 3.1). Egg crate lighting panel (Home Depot LP2448EGG-5, Corvallis, OR) was placed on top of the petri dishes to provide a platform for the rearing containers. A calibrated hygrometer (VWR

35519-050, Friendswood, TX) was used to ensure that the relative humidity within cages was at the required level. The levels of relative humidity were 17, 33, 65, 78 and 93%. Each treatment was continuously recorded using data loggers (HOBO U12-012, Bourne, MA) within each cage.

Experimental flies: Commercial organic blueberries were exposed to *D. suzukii* individuals for 30-minute increments in order to obtain 250 eggs for experimental purposes. Berries containing a maximum of 5 eggs were then placed in the egg rearing containers, allowing for 50 eggs per RH cage chamber. All replicates were run at once with start date of December 15, 2012. Cages were monitored every 48 hours until eclosion occurred in order to minimize RH fluctuations. Upon eclosion, adults were allowed to reach sexual maturity for three days upon which one male and one female were placed in an adult rearing container. These containers each had a single blueberry fruit to which oviposition was allowed. Blueberries were replaced every 48 hours until female mortality. Dead males were replaced in order to facilitate adequate mating during the experimental process. The number of eggs and longevity of each female was recorded. Fruit with eggs laid by females were placed in separate egg-rearing containers for 5 days to allow for larval development. Egg rearing was conducted in 30 mL plastic soufflé cups (Solo, Urbana, IL) that had modified lids consisting of a fabric mesh cover. Egg hatch (resultant larvae) was observed through a combination of saltwater dunk and dissection of fruit. A salt-water solution was prepared by combining ¼ cup table salt with 4 cups of water. This was added to a 100 mL beaker containing the crushed berries. Floating larvae were counted and removed. Individual berries in the salt solution were dissected with tweezers to count remaining larvae.

In order to determine the maturation of eggs within ovaria of adult female *D. suzukii*, a subset of the eclosed females were placed together in an adult rearing container without fruit for 7 days. These individuals were then dissected in order to determine eggs maturation for each of the humidity treatments. Mature eggs (Fig. 3.1) were identified by the presence of a formed chorion as the outer surface (Klowden 2002). Due to the limited survival of adults at 17, 33% RH, a subset of adults was used to examine the state of mature oocyctes was not established, however dead females were dissected and those results were incorporated.

Statistical analysis: ANOVA was utilized to measure development from egg to eclosion of adults, longevity of adult flies, and total number of eggs laid over a lifetime. Linear regression was used to estimate means. Chi-square was used to analyze proportional egg hatch. The effect of RH on the percentage of females with mature oocytes was analyzed using a chi-square test. ANOVA was utilized to analyze the effect of RH on the mean number of oocytes per female during first week of adult life. Linear regression was used to estimate means. When significance was detected ($p < 0.05$), Tukey's Honest Significant Difference test was conducted to compare means. Transformations were used when necessary to correct for normality. Developmental rate was determined by using non-linear regression (Briere 1999) in order to estimate optimal, lower and upper thresholds for intrinsic rate of population increase (Price, 1997) using Statistica (Statsoft 7.1). The mean survival and fecundity values for each RH were used to estimate the intrinsic rate of population increase (r_m) at each temperature. These values were determined by using the equation, $r_m = \log_e R_o/T$ (Price 1997), where R_o is the net reproductive rate and T is the mean generation time. Net reproductive rate was obtained using the equation, $R_o = \Sigma\, l_x m_x$. Non-linear regression was performed using the reciprocal of development time in days ($1/T$) on RH. All other analysis was conducted using R (R Development Core Team 2012).

Results:

Development, Longevity and egg production:

Relative humidity did not affect development from egg to eclosion (F=0.933, df=4, 234; p=0.4455). Female flies were able to develop and eclose at all RH levels, with subsequent survival greatly limited at the lowest RH. There was significant difference in survival between RH levels (F=33.55, df=4,179; p<0.001) with females at the two lowest RH levels surviving less than two days. Females at the three higher RHs survived for significantly longer periods (mean of 20 days). The longevity of females at the three higher RH levels, however, was statistically similar (Table 3.3).

There was also a significant difference in survival of males between RH levels (F=9.524, df=4,76; p<0.001). Generally, males survived for shorter periods at low humidity compared to longer periods at higher humidity (Table 3.4).

Egg production was significantly affected by RH (F=45.47, df=5, 97; p <0.001) with a trend of increased egg laying at increasing humidity (Table 3.3). Relative humidity had a significant effect on the proportion of eggs hatched (x^2=58.67, df=3, p<0.001).

Ovarian maturation: Relative humidity affected both the percentage of females with mature oocytes (x^2=78.20, df=4, p<0.001) and the number of oocytes in the ovaries (F=32.96, df=4, 102; p<0.001) (Table 3.2). Analysis of square root transformed data indicated that the lowest two RH levels resulted in the fewest mature oocytes. At 65% RH the percentage of females with mature oocyctes was numerically higher than at 33% and 17% RH. At 78 and 93% RH, nearly all females had mature oocytes present and had significantly more eggs than the other three levels.

Threshold determination: The function, y=(0.234e-8)*x*(x-(27.7232))*((110.437)-x)*exp(1/(0.131095)), obtained by fitting the non-linear Brierre (1999) model describes the role of humidity on reproduction (Figure 3.6). The estimated lower reproductive threshold is 27% RH, and the upper development threshold is 100% RH, and the optimal humidity for developmental tempo is 79% RH. Generation time (T) ranged from 0 to 9 days, with an initial decline as RH increases before maximizing at the highest RH. The net reproductive rate (Ro) increases with increasing RH, while the intrinsic rate of population increase (rm) increased to a peak at 78% RH and decreases thereafter. The intrinsic rate of population increase was negative at 37% RH. Generation time (T) was 4.495 at 78% RH and 7.771 at 37%. Generation time (T) and net reproductive rate were highest at 93% RH, T=9.226 and R_o=112.0.

Discussion

The experimental data indicated that increasing RH resulted in an increase in reproductive potential and longevity of *D. suzukii* to an optimal level of 78%RH. An exponential increase in egg laying was observed with increasing RH levels. However, when examining ovarian maturation, there was no difference between amount of mature oocytes present between 75% and 93% RH. The exponential increase in egg laying may have been impacted by a decrease in berry quality.

These results were consistent with results on unrelated insect species such as *Longitarsus bethae* (Chrysomelidae: Alticinae) (Simelane 2007).   Egg numbers laid by *L. bethae* were similar, irrespective of RH, however, eggs failed to develop at RH levels below 63%. The optimal RH for egg hatch was between 86 and 95% RH. For *L. bethae*, RH did not have a significant influence on duration of the pre-eclosion period (Simelane 2007). Similar trends were observed on *Bactrocera oleae* (Diptera: Tephritidae) (Broufas et al. 2009), where longevity and fecundity peaked at 75% and declined as RH approached 94%. Lu and Wu (2011) examined population increase in *Apolygus lucorum* (Hemiptera: Miridae) at different levels of RH and similarly found a decline in the intrinsic rate of population increase as RH decreased below the optimum of 70% RH. This phenomenon may, however, be the contrary for insects such as *Triatoma infestans* (Hemiptera: Reduviidae), where lower RH did not result in a reduction in reproductive capacity (Roca and Lazzari 1994).

Broufas et al. (2009) mentions the role of nutrition in their relative humidity study, indicating that the nutrient conditions may have affected results due to lack of access to proteins or bacteria on leaf surfaces. Relative humidity also impacts nutrient availability as it changes nectar concentration with varying levels, which can impact energy demands and, in turn, impact longevity and fecundity of adult flies (Contreras et al. 2013). Diet can have a significant effect on longevity in the presence of low humidity, as *D. melanogaster* larvae were able to increase their survival with a higher protein diet over those fed a higher carbohydrate diet (Chown et al. 2011). It is possible that nutritional deficiencies may have resulted with *D. suzukii* due to possible desiccation of the food source (Broufas et al. 2009). These deficiencies may have contributed to a decrease of survival and fecundity that may not be directly attributable to humidity, but further investigation into this phenomenon was not within the scope of the current study.

While this study examined the effects of RH and previous work covered the effects of temperature, the interaction between the two cannot be discounted and future studies should be conducted to determine how the combined effects might influence the reproductive capacity of *D. suzukii*. Mosquitoes show a reduction in oviposition rate as temperatures increase (de Almeida

Costa et al. 2010), however the measure of this reduction was strongly influenced by humidity, with greater reductions of egg laying when humidity was reduced at high temperatures, compared to when humidity was reduced at lower temperatures.

Likewise, further research should be undertaken to determine physiological capabilities to respond to water loss. Between *D. melanogaster* and *D. simulans*, cuticular hydrocarbons (CHC), which act primarily as anti-desiccation agents, seem to vary in an adaptive fashion. In *D. melanogaster*, this varies with breeding temperatures, while *D. simulans* does not appear to possess this trait. *Drosophila melanogaster* shows a gender difference in amount of CHC wheras *D. simulans* does not. Cuticular hydrocarbons are shown to act as pheromones, which may interfere with desiccation resistance. Brief non-lethal periods of exposure to low humidity can markedly improve desiccation resistance in *D. melanogaster*, which could be observed in *D. suzukii* (Chown et al. 2011). When reviewing acclimation responses in various Drosophilidae species, response is limited in rainforest species (*D. birchii*) but readily observed in cosmopolitan species such as *D. simulans*, *D. immigrans*, and *D. serrata*. This is not to say that *D. suzukii* will overcome all geographical boundaries, *D. serrata* expresses a similar acclimation response amongst populations from different habitats, overall limiting geographic variation for this trait (Chown et al. 2011).

This study was conducted under controlled laboratory conditions to determine the impact of lower and higher humidity on *D. suzukii*. These results show a profound impact under these conditions, but these results are by no means a comprehensive indication of how field humidity may impact these parameters, but do provide some measure of these fluctuations on *D. suzukii* field reproductive potential. Insects in the field are sensitive to changes in microclimate, and may, through behavioral adaptations, limit such impacts (Chown et al. 2011). Additional studies that may shed some light on these aspects should be conducted in order to determine the impact of such behavioral actions. In western flower thrips, larvae will drop off leaves at low humidity (Steiner et al. 2011). This behavior has been observed of *D. suzukii* larvae exiting fruit just prior to pupation, however the impetus behind this maneuver is unknown (personal observation). Future work should be conducted to see how *D. suzukii* responds to humidity within the field as

different microclimates exist. In a study of *Altica subplicata* at the Great Lakes of North Michigan, extent and distribution of herbivory damage was explained by the gradient of RH (Bach 1993). Orientation in the field may allow for maximizing survival during inclement periods of time as well as increasing economic damage in favorable conditions. The results from this research may lead to changes in management practices through manipulation of the field environment. Using drip irrigation or pruning the canopy to decrease humidity may obtain this objective.

References cited:

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2001. Revisiting water loss in insects: a large scale view. J. Insect Physiol. 47: 1377–1388.

Bach ,C.E. 1993. Effects of microclimate and plant characteristics on the distribution of a willow flea beetle, *Altica subplicata*. Am. Midl. Nat. 193–208.

Bradley, T. J. 2009. Animal Osmoregulation. Oxford University Press, Oxford.

Broufas, G. D., M. L. Pappas, and D. S. Koveos. 2009. Effect of relative humidity on longevity, ovarian maturation, and egg production in the olive fruit fly (Diptera: Tephritidae). Ann.Ent. Soc. Am. 102: 70–75. doi: 10.1603/008.102.0107.

Bubliy, O. A., T. N. Kristensen, and V. Loeschcke. 2013. Stress-induced plastic responses in *Drosophila simulans* following exposure to combinations of temperature and humidity levels. J Exp Biol 216: 4601–4607. doi: 10.1242/jeb.092502.

Chown, S. L., J. G. Sørensen, and J. S. Terblanche. 2011. Water loss in insects: An environmental change perspective. J. Insect Physiol. 57: 1070–1084. doi: 10.1016/j.jinsphys.2011.05.004.

Contreras, H. L., Goyret J., von Arx M., Pierce C. T., Bronstein J. L., R. A. Raguso, and G. Davidowitz. 2013. The effect of ambient humidity on the foraging behavior of the hawkmoth *Manduca sexta*. J. Comp.Physiol. A. 199: 1053–1063.

Costa, E. A. P. de A., Santos E. M. de M., J. C. Correia, and C. M. R. de Albuquerque. 2010. Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera, Culicidae). Rev. Bras. Entomol. 54: 488–493. doi: 10.1590/S0085-56262010000300021.

Klowden, M. J. 2002. Physiological systems in insects. Academic Press, San Diego, California.

Lu Y., and K. Wu. 2011. Effect of relative humidity on population growth of *Apolygus lucorum* (Heteroptera: Miridae). Appl. Entomol. Zool. 46: 421–427.

Ohtsu, T., M. T. Kimura, and C. Katagiri. 1998. How Drosophila species acquire cold tolerance. Eur. J. Biochem. 252: 608–611 (1998).

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roca, M. J., and C. R. Lazzari. 1994. Effects of relative humidity on the haematophagous bug *Triatoma infestans*: Hygropreference and eclosion success. J. Insect Physiol. 40: 901–907.

Simelane, D. O. 2007. Influence of temperature, photoperiod and humidity on oviposition and egg hatch of the root-feeding flea beetle *Longitarsus bethae* (Chrysomelidae: Alticinae), a natural enemy of the weed *Lantana camara* (Verbenaceae). Bull. Entomol. Res. 97: 111–116. doi: 10.1017/S0007485307004713.

Statistica 7.1. Statsoft Inc. 2005. 2300 East 14th Street, Tulsa, OK, 74104.

Steiner, M. Y., L. J. Spohr, and S. Goodwin. 2011. Relative humidity controls pupation success and dropping behaviour of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Aust. J. Entomol. 50: 179–186.

Tichy, H. 2003. Low rates of change enhance effect of humidity on the activity of insect hygrosensors. J. Comp. Physiol. A. 189: 175–179.

Tochen, S., Dalton D. T., Wiman N., Hamm C., P. W. Shearer, and V. Walton. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ. Entomol. 43: 501–510.

Vreysen, M. J. B., Robinson A. S., J. Hendrichs, and P. Kenmore. 2007. Area-Wide Integrated Pest Management (AW-IPM): Principles, Practice and Prospects. In: Vreysen MJB, Robinson AS, Hendrichs J (eds) Area-Wide Control of Insect Pests. Springer, Netherlands, pp 3–33.

Walsh, D. B., Bolda M. P., Goodhue R. E., Dreves A. J., Lee J., Bruck D. J., Walton V., S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integrated Pest Manag. 2: 1–7. doi: 10.1603/IPM10010.

Wiman, N. G., Walton V.M., Dalton D.T., Anfora G., Burrack H. J., J. C. Joanna, and K. M. Daane. 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLoS ONE 9: e106909.

Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology. 41: 232–237. doi: 10.2307/1931961.

Table 3.1. Saturated salt solutions used in experiment with corresponding relative humidity.

Solution	RH%
LiCl*H2O	17
MgCl2*6H2O	33
Mg(NO3)2*6H2O	65
NaCl	78
KNO3	93

Table 3.2. Presence and proportion of females with ovarian maturation in 1-week-old *Drosophila suzukii* at five constant relative humidities.

RH%	N	% females with mature oocytes	Mean no. of mature oocytes per female (±SE)
17	19	0	0±2.6b
37	24	4.2	0.3±1.7b
65	14	42.9	5.1±2.8b
78	22	95.5	13.9±2.5a
93	28	92.9	17.4±2.3a

Table 3.3. Mean longevity of *Drosophila suzukii* females and egg production on blueberry at five constant relative humidities.

RH%	N	Longevity (d) (± SE)	Mean no. of eggs per female (± SE)
17	19	1.5±3.3b	-1.4±6.9c
37	24	1.6±2.0b	-1.8±4.6c
65	19	19.8±2.9a	0.3±7.7b
78	20	18.8±3.0a	5.1±7.6b
93	21	23.6±2.9a	59.0±8.1a

Table 3.4. Mean longevity of *Drosophila suzukii* males at five constant relative humidities.

RH%	N	Longevity (d) ($\pm$ SE)
17	7	1.4±6.2b
37	21	0.6±3.1b
65	23	19.0±4.3a
78	14	15.5±5.0ab
93	17	18.7±4.6a
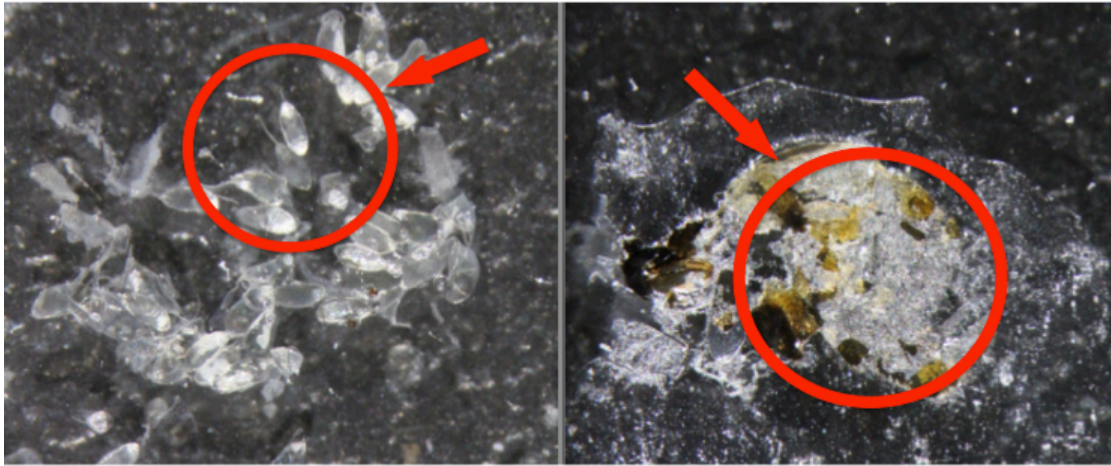
Figure 3.1. Depiction of mature oocytes vs. immature oocytes. In the left circle, example of four mature oocytes that were counted. The right circle depicts no mature oocytes suitable for counting.
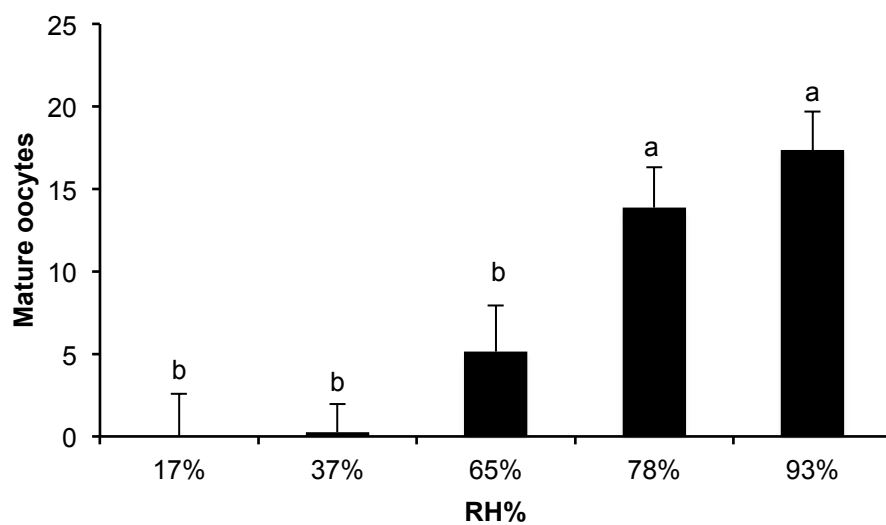
Figure 3.2. Mean number of mature oocytes per dissected female at five different relative humidities at 22 ± 2 ºC. Treatments with the same lowercase letter are not significantly different.
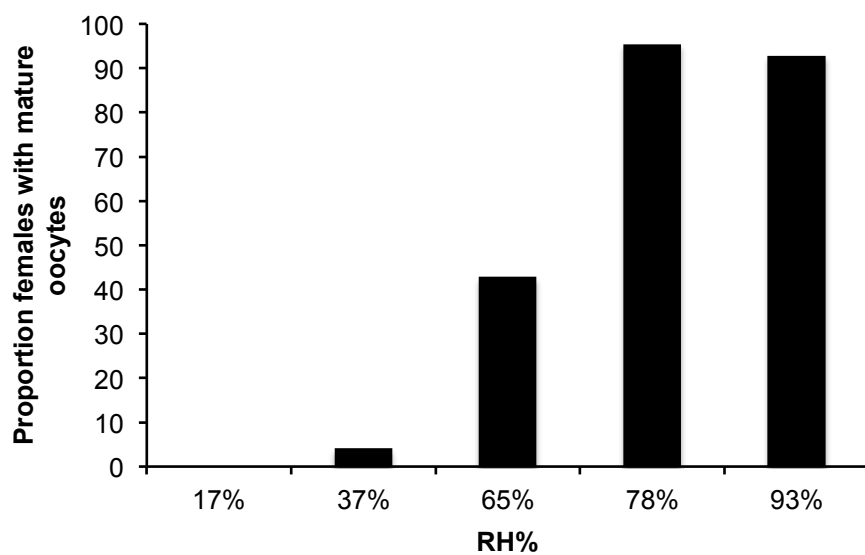
Figure 3.3. Proportion of dissected adult *Drosophila suzukii* females with mature oocytes at five different relative humidities.
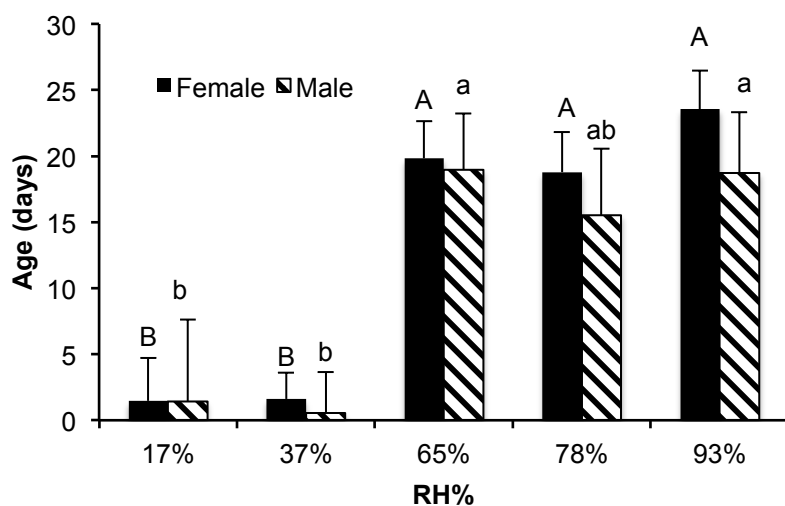
Figure 3.4. Mean adult *Drosophila suzukii* longevity (days) at five different relative humidities. Females and males were analyzed separately such that the same uppercase letters indicate no significant difference between female treatment group and the same lowercase letters indicate no significant difference between male treatment group.
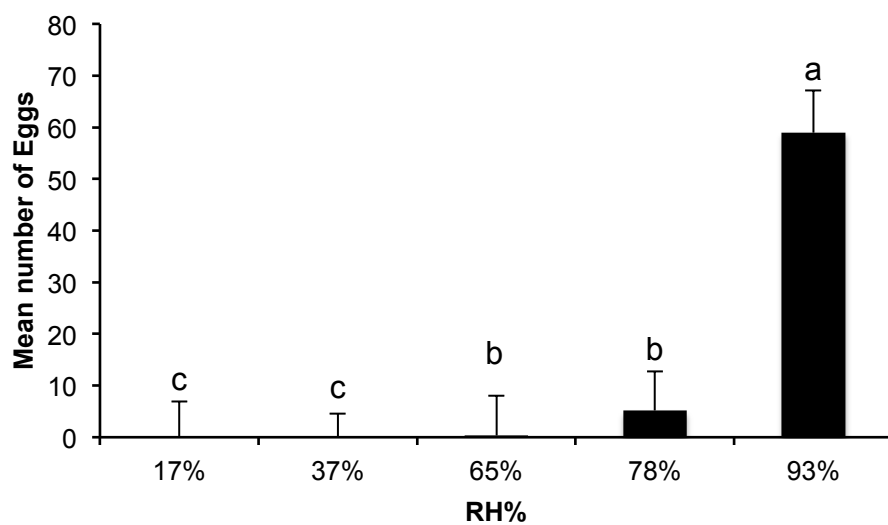
Figure 3.5. Mean number of eggs per female at five different relative humidities on blueberry.
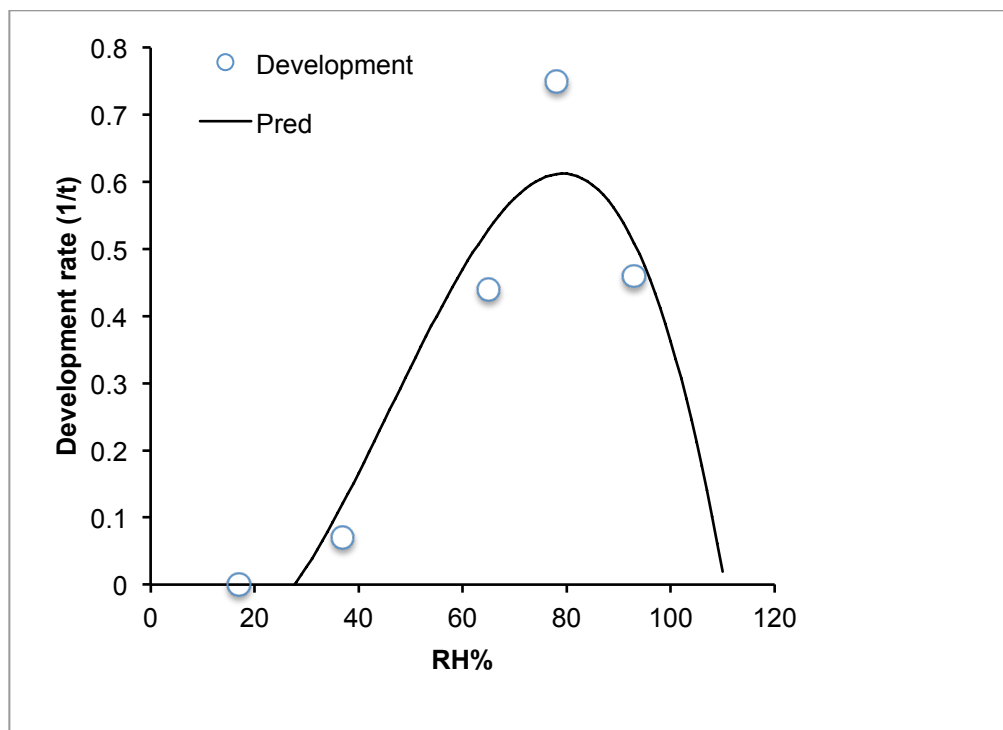
Figure 3.6. Optimum relative humidity (78%) for development and lower and upper thresholds (17 and 100%) for *Drosophila suzukii* as a function of relative humidity.

CHAPTER 4.

Drosophila suzukii use of floral resources and its impact on lifespan and nutrient status.

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Abstract:

We compared the effects of floral nectar from sweet cherry and blueberry on lifespan and nutrient levels of the economically important invasive *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Adult *D. suzukii* had an average lifespan of 3.4 ±0.5d and 11.1 ±0.6d on water and cherry, respectively, with 0% and 16% survival of cohort at the end of a 41-day observation period. Adult *D. suzukii* on blueberry had an average lifespan of 22.7 ± 0.6d with 50% survival of cohort at the end of a 33-day observational period. The nutritional assay showed higher levels of glycogen and sugar levels amongst flies exposed to both blossom types and sucrose. Sugar levels did not differ significantly in blossom and sucrose exposed flies during the 2013 trials. Lipid levels were often similar regardless of treatment.

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae) causes economic damage in small and stone fruit crops and was first found in mainland North America in 2008 (Walsh et al. 2011). *Drosophila suzukii* has since spread across the continent to all major affected production areas where susceptible crops are produced (Hauser 2011, NAPIS 2013). Current control efforts focus on repeated insecticide applications as soon as fruit starts to ripen (Beers et al. 2011, Bruck et al. 2011). There is increased evidence that surrounding vegetation may play an important role as a suitable *D. suzukii* habitat (Klick et al. 2014), but little information is available of how these habitats improve longevity and nutrient status. Little is known about the feeding and foraging behavior of adult *D. suzukii* and how this affects longevity. Hamby et al. (2012) isolated associated yeast species from *D. suzukii* larvae, adults, and infested fruit, which are believed to be an important food source for *Drosophila*. However, it is unknown what *D. suzukii* feeds upon early in the growing season before presence of fruit.

While some insect species do not feed post-eclosion, many must obtain carbohydrates and lipids to ensure optimal survival and reproduction rates (Chapman 1982, van Handel 1984, Nestel et al. 1985, Briegel 1990, Clements 1992, Fadamiro et al. 2005). Such resources are utilized and converted to energy sources, originating from monosaccharaides (fructose and glucose), disaccharides (trehalose), polysaccharides (glycogen), and triglycerides (lipid) in the insect body (Downer & Matthews 1976, Clements 1992, Rivero & Casas 1999). Synthesis of glycogen and lipids is carried out in the fat body of insects, however lipogenesis is not a trait that is widely used across all insect species as a means to convert energy (van Handel 1984, Clements

1992, Naksathit et al. 1999, Fadamiro et al. 2005). Amongst Dipterans, mosquitoes are capable

of lipogenesis yet the occurrence is limited in phorid flies (van Handel 1984, Clements 1992,

Naksathit et al. 1999, Fadamiro et al. 2005). *Drosophila melanogaster* will experience

lipogenesis under certain dietary conditions (Geer et al. 1985, Musselman et al. 2011,

Musselman et al. 2013). Little information is available on the use of floral and extrafloral

resources by drosophilids, as previous nutrition work has focused on yeast nutritional given the

majority of drosophilids being saprophytic (Anagnostou et al. 2010, Begon 1982, Hamby et al.

2012, Jones and Widemo 2005). Resources that include sugar or nectar can be used immediately

to generate energy for metabolic purposes or stored for later use by conversion to trehalose or

glycogen (Olson et al. 2000). Additional food resources are known to be essential for improved

fecundity (Chapman 1982, van Handel 1984, Nestel et al. 1985, Briegel 1990, Clements 1992,

Fadamiro et al. 2005). Sugar may be obtained from floral and extrafloral nectars, homopteran

honeydew and fruit (Bugg et al. 1989, Evans 1993, Jervis et al. 1993, 1996, Fadamiro et al.

2005). Nectar is primarily sugar-rich, and is comprised of 96% sucrose, glucose, and fructose,

but may contain low levels of amino acids, proteins, lipids, and other organic compounds

(Costero et al. 1998, Waeckers et al. 2007).

Most dipterans need to feed as adults in order to enable egg production (Waeckers et al.

2007). *Drosophila melanogaster* utilize nectar, fructose, maltose, sucrose and glucose that

originate from nectar, which improves survival (Baker & Baker 1983). When looking at

allocation of nutrients in *D. melanogaster* for oviposition, 35% of carbon in eggs comes from

adult sugar feeding and only 12% from larval reserves, with a shift to 100% of carbon coming

from adult sugar feeding after 10 days (Kyung Jin 2006). Tephritid dependence on such

resources examined how feeding behaviors affect longevity and reproduction (Averill and

Prokopy 1993, Chang et al. 1977, Lauzon 1993). One example of such a study demonstrated

Rhagoletis pomonella, will remain and inhabit areas where both nectar and oviposition sites are

readily available as opposed to habitats that contained fruit alone (Averill and Prokopy 1993).

From these studies, it appears that suitable oviposition media alone will not increase longevity

and fecundity (Averill and Prokopy 1993). *Drosophila melanogaster* lay eggs in batches and

therefore spend a considerable amount of time at suitable oviposition sites (Jaenike 1986). Such

sites were found to also contain a source of food and also suitable oviposition media. It was

hypothesized from that work that both resources are essential for optimal egg laying (Jaenike

1986). It is known that sugar supplementation changes the foraging behavior of coccinellids, as it

reduces pre-ovipositional period and increases survival while decreasing egg reabsorption during

reproductive diapause (Seagraves 2010).

    Drosophila suzukii immature stages are found in berries and are difficult to control

chemically; therefore, control methods target adults in commercial production units. Control

methods could be improved with increased knowledge of feeding behavior such as timing of

feeding or early season food sources, which may direct timing of spray applications or drawing

flies with poison baits. In order to determine the levels of nutrients in insect bodies derived from

food sources several methods have been utilized (Chang et al. 1977, Fadamiro et al. 2005,

Heimpel 2004, Olson et al. 2000, van Handel 1984) and includes anthrone reagant which has

been used to determine sugar presence in mosquitoes and Hymenopteran and Dipteran

parasitoids (Fadamiro et al. 2005, Heimpel 2004, Olson et al. 2000, van Handel 1984). Sugar

analysis on tephritid fruit flies have been conducted by using gas chromatography (Chang et al.

1977).

Drosophila suzukii are found in monitoring traps during the late dormant period (Dalton

et al. 2011, Wiman et al. 2014) when only blossoms and little to no fruit sources are available.

During the late dormant period, known food resources are limited, but *D. suzukii* are likely to

encounter blossoms (Lee 2004). Cherry blossoms are generally found earlier than those of

blueberries. Cherry blossoms can be found during early to late April (Hubbard, pers. comm.) and

blueberry blossoms are generally present in Oregon production systems during late April through

May (Strik and Finn 2008). We hypothesize that these floral resources are utilized by *D. suzukii*

during the late dormant period. The uptake of these resources is known to result in increases of

nutrient levels within the body of insects (Fadamiro et al. 2005, Heimpel 2004, Olson et al. 2000,

Lee 2004). We believe that the uptake of these resources by *D. suzukii* may enable increased

nutrient levels and survival. This hypothesis is tested by determining the lifespan and levels of

carbohydrates and lipids of *D. suzukii* after exposure to cherry and blueberry blossoms.

Materials and Methods

Drosophila suzukii adults were from a colony established at Oregon State University

(Tochen et al.2014) and supplemented seasonally with field-collected individuals from the

Willamette Valley. The stock colony was maintained at 22 ± 2 ºC, 65% relative humidity (RH),

a photoperiod of 16L:8D, and provided a water wick and artificial diet for feeding and

oviposition (Dalton et al. 2011).

Survivorship assays: A survivorship trial was initiated by placing 37-50 male and 38-50 female *D. suzukii* individuals into Bug dorm cages (DP1000, MegaView Science, Taiwan). All flies within treatments were supplied with a wicking sponge that dispensed water from a plastic ca. 100 mL water reservoir. Three treatments were replicated in six cages: 1) an untreated control, which contained water only; 2) blossoms from fresh unsprayed sweet cherries, *P. avium* (Rainier and Bing) and 3) blossoms from blueberries, *V. corymbosum* (Jersey). Blossoms were collected during the naturally flowering period in Oregon and replaced weekly until the conclusion of the study. All floral treatments included a freshly picked branch, which had at least twenty flowers secured with parafilm within a 100 mL beaker with of water. The control and cherry blossom treatments were run from April 14 to May 25, 2011 for 41 d, and blueberry treatments were run from April 29 to June 3, 2011 for 33-d.

All cages were placed in temperature-controlled growth chambers (Model E-30BHO; Percival Scientific, Perry, IA) which closely resembled the temperature and photoperiod during typical spring conditions of the Willamette Valley, Oregon. Because of the typically earlier flowering found with cherries, flies were exposed for 14 days to cherry blossoms at diurnal fluctuating temperatures of 3-13°C and a light: dark regime of 13L:11D. The temperature was subsequently increased to 4-16°C and a light: dark regime regime of 14L:10D for an additional 14 days (28 days total). Because blueberry blossom later in the year, flies were exposed to blueberry blossoms at diurnal fluctuating temperatures of 4-16°C and a light: dark regime of 14L:10D for 14 days. The temperature was subsequently increased to 7-20°C and a light regime

of 15L:9D for an additional 14 days (28 days total). Mortality of flies was observed every 48 hours and dead flies were removed and counted.

Glycogen, lipid and sugar assays: This experiment was conducted under conditions as with the stock colony. Prior to exposure, ~2-d-old adult females from the stock colony were starved for 8-h with the provision of water. In 2012, 20 females were then placed singularly into petri dishes with one of the treatments: 1) a 20% sucrose solution in a 1.5 mL microcentrifuge tube and cotton pad wick, 2) water in a similar configuration, 3) a single sweet cherry blossom, *P. avium* ('Rainier' and 'Bing'), and 4) a single blueberry blossom, *V. corymbosum* ('Jersey'). Flies experienced high mortality across treatments leading to a change in the experimental set-up. Cherry blossom, sucrose, and water were tested during April 26, 2012, and trials with blueberry blossom, sucrose, and water were tested during May 8, 2012. In 2013, between 18-20 females were placed into a Bugdorm with one of the treatments: 1) a 20% sucrose solution in a 100 mL beaker covered with Parafilm and cotton pad wick, 2) water in a similar configuration 3), blossoms from fresh unsprayed sweet cherries, *P. avium* ('Rainier' and 'Bing'), and 4) blossoms from blueberries, *V. corymbosum* ('Jersey') collected during the naturally flowering period in Oregon. Cherry blossom, sucrose, and water were tested during April 16 to 17, 2013, and trials with blueberry blossom, sucrose, and water were tested during April 30 to May 1, 2013. Flies were kept within feeding arenas for 24 or 48 hrs. All females were frozen individually in microcentrifuge tubes prior to wing removal and nutrient analyses. To standardize the effect of individual insect body size on food source uptake, the left wing of each female was removed and measured for wing length from the humeral-costal break to the end of L3 vein. Following the

protocol adapted for parasitic wasps from Olson et al. (2000), 50 µL of 2% sodium sulfate was pipetted into each 1.5 mL microcentrifuge tube with an individual fly. Flies were crushed well using a pestle, and 450 µL of chloroform-methanol (1:2) was added. Tubes were centrifuged for 2 minutes at 13000 rpm upon which a precipitate formed at the bottom. The supernatant was transferred into 12X75 mm glass test tubes and the precipitate was retained in the microcentrifuge tube for the glycogen assay. From the glass test tube, half of the solution was transferred into another glass tube to be used for the lipid assay and the remaining half of the solution was used for the fructose and total sugar assay. All tubes were heated to 90°C until the solution for lipid evaporated and 25 µL remained in the sugar tubes.

For the glycogen assay, 975 µL of anthrone reagent was added to each microcentrifuge tube, vortexed until the precipitate dissolved and heated at 90 °C for 10 minutes. Tubes were cooled on ice, and absorbance was read at 625 nm (Ultrospec 3100 pro, Amersham Biosciences, Piscataway, NJ). Absorbance readings were divided by 0.421 for 2012 assays, and 0.548 for 2013 assays based on a calibration with known standards. For the fructose assay, 975 µL of anthrone reagent was added to each tube, vortexed and heated at 30°C for 1 hr. Absorbance was read at 625 nm, and divided by 0.0533 in 2012, 0.0602 in 2013, and multiplied by two. For the total sugar assay, the same solution used for the fructose assay was then heated at 90°C for 7 min, cooled on ice. Absorbance was read at 625 nm, and divided by 0.0545 in 2012, and 0.056 in 2013, and multiplied by two to obtain total sugar levels on the whole insect. To estimate the level on other sugars, the amount of fructose was subtracted from total sugar. For the lipid assay, 40 µL of sulfuric acid was added to each glass tube and heated at 90°C for 2 minutes before

cooling on ice. Next, 960 µL of vanillan reagent was added, vortexed and left at room temperature for 25min. Absorbance was read at 525 nm, and divided by 0.038 in 2012, and 0.0292 in 2013, and multiplied by two to estimate lipid content in the whole fly [(Absorbance/0.038) x 2]. Samples were run in batches using 21 flies and a negative control consisting of the solutions described without the presence of an insect.

Statistical analyses

Proportional hazards model was used to determine differences between cherry and water treatment, sex and treatment x sex interaction on survivorship. The effects of diet, hour, and diet x hour interaction on the glycogen, lipids and body sugars were tested by ANOVA. Transformations (square root or log) were used when necessary to normalize distribution and equalize variances. Tukey HSD was used to compare between treatments. Statistical analysis was performed in JMP 8.01 (SAS Institute 2008). For simplification, ANOVA was used because statistical outcomes were similar with an ANCOVA with winglength as a covariate. The winglengths of females were similar among the treatment groups for the blueberry trial in 2012 ($F_{2,56}$ = 0.3, P = 0.7), cherry in 2012 ($F_{2,56}$ = 1.7, P = 0.2), blueberry in 2013 ($F_{5,107}$ = 1.5, P = 0.2), and cherry in 2013 ($F_{5,112}$ = 1.9, P = 0.1).

Results

Survivorship: Survivorship of male and female adult *D. suzukii* provided with cherry blossoms was enhanced when compared to individuals provided with water (x^2=89.42, d.f.=1, p<0.001). Sex did not have a role in lifespan (x^2=0.250, d.f.=1, p=0.617). Blueberry data was not be included in this analysis because it was run later when blueberry blossoms were naturally

available.   Adult *D. suzukii* given water had an average lifespan of 3.4 ±0.5d, with 0% survival

of the cohort during a 41-d observation period. Adults provided with cherry blossoms had a

lifespan of 11.1 ±0.6d, with 16% survival of cohort during the 41-d observation period. Flies

provided blueberry blossoms had a lifespan of 22.7 ±0.6d during a 33-d observation period with

50% survival of the cohort (Fig 4.1).

Glycogen, lipid, and sugar assays:

   Glycogen: In the 2012 cherry trial, there was no difference between glycogen levels of

females amongst the three treatments. During the blueberry trial in 2012, sucrose-fed females

had more glycogen than those with either water or blueberry blossoms. There was no difference

between glycogen levels between flies in the water or blueberry treatment (Table 4.2). When the

trial was repeated in 2013 after the modifications of the experimental setup, there was an

increase in glycogen levels among treatments containing blueberry blossoms, cherry blossoms,

and sucrose compared to the control. However, flies fed either blueberry and cherry blossoms

had lower levels of glycogen than flies fed sucrose. There were no differences in mean glycogen

levels between 24 h and 48 h fed flies (Table 4.3, 4.4).

   Fructose: In the 2012 cherry trial, flies fed sucrose had higher fructose levels than flies

fed blossoms or water. In the 2012 blueberry trial, flies fed sucrose had higher mean levels of

fructose than flies fed blossoms or water (Table 4.2). In the 2013 cherry trial, flies fed sucrose

for 24 and 48 h and cherry blossoms for 24 h had higher fructose levels than flies fed water for

24 or 48 h and cherry for 48 hr. Interestingly, flies fed cherry blossoms showed a drop in

fructose levels from 24 to 48 h (Table 4.3). In the 2013 blueberry trial, flies fed sucrose or

blossoms had higher fructose levels than flies fed water, with no difference between feeding periods (Table 4.4).

Other sugars: In the 2012 cherry and blueberry trial, there was no difference observed across treatments (Table 4.2). In the 2013 cherry and blueberry trial, flies fed blossoms or sucrose had higher levels of non-fructose sugar levels than the flies fed water, with no difference between feeding periods (Table 4.3, 4.4).

Lipid: In the 2012 cherry and blueberry trials, there was no difference in lipid levels across diet treatments (Table 4.2). In the 2013 cherry trial, flies fed any treatment for 48 h had abnormally high mean levels of lipids, indicating contamination or other user error, effectively confounding the cherry results for 2013 (Table 4.3). In the 2013 blueberry trial, there was generally no differences in lipid levels between treatments, except that flies fed water for 48 h had higher lipid levels than flies fed water for 48 h (Table 4.4).

Discussion

As expected *D. suzukii* exposed to cherry blossoms survived for longer periods than their counterparts that were exposed to water-only treatments. Flies exposed to blueberry blossoms in a separate trial, likewise showed high survivorship as expected. Adult flies were however provided with blossoms that were not surface washed. It may be possible for other nutrients indirectly derived from nectar such as yeasts (Hamby et al. 2014) may have contributed to nutrient uptake by flies during each of the exposure periods. *Rhagoletis indifferens* has been observed presenting feeding behavior on leaf surfaces of which leachates, bird feces, and bacteria may be present, and therefore these compounds may contribute towards a portion of the

nutrients present on the floral structures (Wee 2008). Presence of Sternorrynchan honeydew was also not accounted for despite the potential of being found in both agricultural situations (Smith 2013, Strik and Yarborough 2005). Tephritids primarily use Sternorrynchan honeydew as a food source (Lauzon 1993). This longevity trial may under estimate the effect of food under natural conditions as flies were caged, limiting dispersal and energy expenditure (Waeckers et al. 2007).

Glycogen, lipid and sugar assays

The results from the single feeding trial in 2013 reflected what has been observed in parasitoids, with an overall increase in carbohydrate levels amongst floral and sucrose treatments (Olson et al. 2000, Fadamiro 2005, Lee et al. 2004, 2006). Lipid levels were relatively constant across treatments contrary to what is expected given lipogenesis is common in non-parasitoid dipterans (Fadamiro 2005). In 2012, there was no increase in carbohydrate levels in the floral blossom trials, leading to the return of the longevity trial caged system. However, as mentioned above, other substrates cannot be discounted as providing nutrients.

Anthrone reacts to fructose and changes color at room temperature, allowing for a quick assessment of sugar feeding (Heimpel et al. 2004, van Handel 1972). Anthrone testing cannot distinguish the source of sugar, which may be limiting when testing field caught specimens or providing clusters of flowers. Gas chromatography allows for this distinction and has been used in tephritids and biting insects, however was not pursued for this study (Chang et al. 1977). Anthrone testing has the potential for false negatives due to an inability to detect sugar levels under 10 micrograms (Nunes 2008). Factors that can affect anthrone testing include the time it

takes to digest a sugar meal and volume of sugar ingested (Costero et al. 1998). As flies were not weighed before and after feeding, the uptake volume is unknown.

This study only examined carbohydrates and lipid values, yet amino acids and proteins are also found in nectar. Laboratory reared *Rhagoletis pomonella* displayed a higher protein and sucrose consumption over their wild caught counterparts, which in turn resulted in less fecund wild females (Webster et al. 1979). Pollen is another component of flowers not incorporated into this study, yet it provides nutrients in the form of nitrogen, lipids, and starchy carbohydrates (Waeckers et al. 2007). Pollen consumption has been observed in some Diptera, primarily pollinators and *Drosophila flavohirta* Malloch (Cowgill 1993, Nicolson 1994). In syrphids, females require amino acids from pollen to maturate their reproductive system (Cowgill 1993).

This study indicates that *Drosophila suzukii* can successfully utilize floral resources to enhance survivorship and consequently higher glycogen and sugar reserves. Further research should be conducted to determine the effect these resources have on field caught adults, utilizing gas chromatography to elucidate nutrient sources. Seasonal variations in diet sources should also be examined. Utilizing this information could lead to the improvement of attractants and baits as well as application location and timing.

References cited:

Averill, A. L., and R. J. Prokopy. 1993. Foraging of *Rhagoletis pomonella* flies in relation to interactive food and fruit resources. Entomol. Exp. Appl. 66: 179–185.

Baker, H. G., and I. Baker. 1983. A brief historical review of the chemistry of nectar. In: Bentley B, Elias T (eds) The Biology of Nectaries. Columbia University Press, New York, pp. 126–152.

Briegel, H. 1990. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. J. Med. Entomol. 27: 839–850.

Bruck, D., Tanigoshi L. K., Gerdeman B. S., Hollis-Spitler G., J. DeFrancesco, and A. J. Dreves. 2011. Current recommendations for managing spotted wing drosophila (SWD), *Drosophila suzukii*, in PNW blueberries.

Bugg, R. L., R. T. Ellis, and R. W. Carlson. 1989. Ichneumonidae (Hymenoptera) using extrafloral nectar of faba bean (*Vicia faba* L., Fabaceae) in Massachusetts. Biol. Agric. Hortic. 6: 107–114.

Cavicchi, S., Guerra D., G. Girogi, and C. Pezzoli. 1985. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. Genetics. 109: 665–689.

Chang, F., Winters N., Vargas R. I., S. L. Montogomery, and J. M. Takara. 1977. An analysis of crop sugars in the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), in Hawaii, and correlation with possible food sources. Proc. Hawaii. Entomol. Soc. 22: 461–468.

Chapman, T., and L. Partridge. 1996. Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. Proc. Biol. Sci. 263: 755–759.

Clements, A. N. 1992. The Biology of Mosquitoes. Chapman & Hall, New York.

Costero, A., Attardo G. M., T. W. Scott, and J. D. Edman. 1998. An experimental study on the detection of fructose in *Aedes aegypti*. J. Am. Mosq. Contr. Assoc.14: 234–242.

Dalton, D. T., Walton V. M., Shearer P. W., Walsh D. B., J. Caprile, and R. Isaacs. 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manag. Sci. 67: 1368–1374.

Downer, R. G. H., and J. R. Matthews. 1976. Patterns of lipid distribution and utilisation in insects. Amer. Zool. 16: 733–745. doi: 10.1093/icb/16.4.733.

Evans, E. W. 1993. Indirect interactions among phytophagous insects: aphids, honeydew and natural enemies. In: Watt AD, Leather SR, Walters KEF, Mills NJ (eds) Individuals, Populations and Patterns in Ecology. Intercept Press, U.K., pp 63–80.

Fadamiro, H. Y., Chen L., E. O. Onagbola , and L. Graham. 2005. Lifespan and patterns of accumulation and mobilization of nutrients in the sugar-fed phorid fly, *Pseudacteon tricuspis*. Physiol. Entomol. 30: 212–224. doi: 10.1111/j.1365-3032.2005.00449.

Hamby, K. A., Hernández A., K. Boundy-Mills, and F. G. Zalom. 2012. Associations of yeasts with spotted-wing drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. Appl. Environ. Microbiol. 78: 4869–4873. doi: 10.1128/AEM.00841-12.

Hassett, C. C. 1948 The utilization of sugars and other substances by *Drosophila*. Biol. Bull. 114–123.

Heimpel, G. E., Lee J. C., Wu Z., Weiser L., F. Wäckers, and M. A. Jervis. 2004. Gut sugar analysis in field-caught parasitoids: adapting methods originally developed for biting flies. Int. J. Pest Manag. 50: 193–198. doi: 10.1080/09670870410001731925.

Hendrichs, J., Lauzon C. R., S. S. Cooley, and R. J. Prokopy. 1993. Contribution of natural food sources to adult longevity and fecundity of *Rhagoletis pomonella* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 86: 250–264.

Jaenike, J. 1986. Feeding behavior and future fecundity in *Drosophila*. Am. Naturalist 127: 118–123.

Jervis, M. A., N. A. C. Kidd, and G. E. Heimpel. 1996. Parasitoid adult feeding and biological control - a review. BioControl News Inf. 17: 1N–22N.

Jervis, M. A., N. A. C. Kidd, and M. G. Fitton. 1993. Flower-visiting by hymenopteran parasitoids. J. Nat. Hist. 27: 67–105.

Lee, J. C., D. A. Andow, and G. E. Heimpel. 2006. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. Ecol. Entomol. 31: 470–480. doi: 10.1111/j.1365-2311.2006.00800.

Lee, J. C. H., G. Heimpel, and L. Gary. 2004. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. Entomol. Exp. Appl. 111 :189–199. doi: 10.1111/j.0013-8703.2004.00165.

Min, K-J, Hogan M.F., M. Tatar, and D. M. O'Brien. 2006. Resource allocation to reproduction

and soma in *Drosophila*: a stable isotope analysis of carbon from dietary sugar. J. Insect Physiol. 52: 763–770.

National Agricultural Pest Information System (NAPIS). 2013. Pest tracker, reported pest status of spotted wing drosophila *Drosophila suzukii*. (http://pest.ceris.purdue.edu/pestlist.php). Retrieved May 6, 2013.

Naksathit, A. T., J. D. Edman, and T. W. Scott. 1999. Amounts of glycogen, lipid, and sugar in adult females *Aedes aegypti* (Diptera: Culicidae) fed sucrose. J. Med. Entomol. 36: 8–12.

Nestel, D., R. Galun, and S. Friedman. 1985. Long-term regulation of sucrose intake by the adult Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). J. Insect Physiol. 31: 533–536. doi: 10.1016/0022-1910(85)90109-X.

Nicolson, S. W. 1994. Pollen feeding in the eucalypt nectar fly, *Drosophila flavohirta*. Physiol. Entomol. 19: 58–60.

Nunes, R. D., R. L. De Oliveira, and G. R. C. Braz. 2008. A novel method for measuring fructose ingestion by mosquitoes. J. Vect. Ecol. 33: 225–231.

Olson, D. M., Fadamiro H. Y., J. G. Lundgren, G. E. Heimpel. 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. Physiol. Entomol. 25: 17–26.

Rivero, A., and J. Casas. 1999. Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. Res. Popul. Ecol. 41: 39–45. doi: 10.1007/PL00011981.

Seagraves, M. P., Kajita Y., Weber D. C., J. J. Obrycki, and J. G. Lundgren. 2010. Sugar feeding by coccinellids under field conditions: the effects of sugar sprays in soybean. BioControl. 56: 305–314. doi: 10.1007/s10526-010-9337-3.

Smith, T. J. 2013. Overview of tree fruit production in the pacific northwest United States of America and southern British Columbia, Canada.

Strik, B. C., and C. E. Finn. 2008. Blueberry cultivars for Oregon. EC 1308-E. Oregon State University Extension Service. March 2008. Corvallis, OR.

Van Handel, E. 1972. Detection of nectar in mosquitoes. Mosquito News.

Van Handel, E., and L. O. Arden. 1984. Vitellogenin synthesis in blood-fed *Aedes aegypti* in the absence of the head, thorax and ovaries. J. Insect Physiol. 30: 871–875.

Wäckers, F. L., J. Romeis, and P. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. Ann. Rev. Entomol. 52: 301–323.

Webster, R. P., J. G. Stoffolano, and R. J. Prokopy. 1979. Long-term intake of protein and sucrose in relation to reproductive behavior of wild and laboratory cultured *Rhagoletis pomonella*. Ann. Entomol. Soc. Am. 72: 41–46.

Yee, W. L., and P. S. Chapman. 2008. Seasonal amounts of nutrients in Western cherry fruit fly (Diptera: Tephritidae) and their relation to nutrient availability on cherry plant surfaces. Environ. Entomol. 37: 1086–1098. doi: 10.1603/0046-225X(2008)37[1086:SAONIW]2.0.CO;2.

Table 4.1. Proportional hazards model testing for effect of treatment, sex, and treatment x sex interaction.

	df	X^2	P
Food	1	89.42	< 0.001
Sex	1	0.245	0.617
Food*sex	1	0.308	0.579

Table 4.2. ANOVA testing the effect of diet on absorbance values for estimated fructose, non-fructose sugars, glycogen and lipids of female *Drosophila suzukii* in 2012. Analysis was done on transformed values when necessary. Means presented on untransformed data.

Trial	Nutrient	Treatment	Mean ±SE	n	Effect	Df	F	P
Cherry 2012	Glycogen	Sucrose	45.7 ±5.4	20	Food	2,56	3.0	0.055
		Cherry	32.6 ±4.0	20				
		Water	29.9 ±5.1	19				
	Fructose	Sucrose	32.6 ±6.2 a	20	Food	2,56	3.6	0.035
		Cherry	19.7 ±4.6 ab	20				
		Water	14.3 ±3.7 b	19				
	Other sugars	Sucrose	18.0 ±4.2	20	Food	2,56	0.02	0.976
		Cherry	18.2 ±2.0	20				
		Water	17.3 ±2.0	19				
	Lipids	Sucrose	14.4 ±1.4	20	Food	2,56	0.6	0.565
		Cherry	15.6 ±1.9	20				
		Water	16.9 ±1.5	19				
Blueberry 2012	Glycogen	Sucrose	66.1 ±1.6 a	20	Food	2,56	9.4	<0.001
		Blueberry	43.0 ±4.5 b	19				
		Water	49.8 ±4.8 b	20				
	Fructose	Sucrose	45.6 ±7.4 a	20	Food	2,56	10	<0.001
		Blueberry	13.8 ±5.8 b	19				
		Water	13.2 ±3.7 b	20				
	Other sugars	Sucrose	36.6 ±4.4	20	Food	2,56	9.4	0.067
		Blueberry	24.7 ±2.9	19				
		Water	32.4 ±3.1	20				
	Lipids	Sucrose	15.0 ±1.3	20	Food	2,56	9.4	0.840
		Blueberry	16.1 ±1.7	19				

Table 4.3. ANOVA testing the effect of diet, hour, and the interaction between diet and hour on absorbance values for estimated fructose, non-fructose sugars, glycogen and lipids of female *Drosophila suzukii* on cherry blossoms in 2013. Analysis was done on transformed values when necessary. Means presented on untransformed data.

Trial	Nutrient	Treatment	Mean ±SE	n	Effect	Df	F	P
Cherry 2013	Glycogen	Sucrose 24 h	69.7 ±7.3 A[1]	20	Food	2, 112	56.1	<0.001
		Sucrose 48 h	53.0 ±1.3	20	Hour	1, 112	6.25	0.01
		Cherry 24 h	44.6 ±4.6 B	20	Food*H	2, 112	1.58	0.21
		Cherry 48 h	42.7 ±3.3	20				
		Water 24 h	20.0 ±3.5 C	20				
		Water 48 h	12.5 ±2.4	18				
	Fructose	Sucrose 24 h	50.3 ±10 a	20	Food	2, 112	52.8	<0.001
		Sucrose 48 h	71.6 ±6.8 a	20	Hour	1, 112	2.69	0.10
		Cherry 24 h	50.4 ±9.3 a	20	Food*H	2, 112	8.31	<0.001
		Cherry 48 h	21.9 ±7.0 b	20				
		Water 24 h	5.03 ±1.3 bc	20				
		Water 48 h	0.59 ±0.2 c	18				
	Other sugars	Sucrose 24 h	45.9 ±9.0 A	20	Food	2, 112	25.6	<0.001
		Sucrose 48 h	24.2 ±3.7	20	Hour	1, 112	44.0	<0.001
		Cherry 24 h	41.0 ±7.0 A	20	Food*H	2, 112	1.08	0.34
		Cherry 48 h	14.0 ±2.4	20				
		Water 24 h	13.9 ±1.3 B	20				
		Water 48 h	5.10 ±1.1	18				
	Lipids	Sucrose 24 h	12.1 ±1.1 c	20	Food	2, 112	5.59	0.005
		Sucrose 48 h	26.7 ±2.1 a	20	Hour	1, 112	83.1	<0.001
		Cherry 24 h	18.8 ±1.2 b	20	Food*H	2, 112	3.58	0.03
		Cherry 48 h	25.6 ±1.6 a	20				
		Water 24 h	11.4 ±1.4 c	20				
		Water 48 h	23.1 ±1.4 ab	18				

[1] Capitol letters denote that a means comparison was made with three treatment means, the 24 and 48 h data are combined.

Table 4.4. ANOVA testing the effect of diet, hour, and the interaction between diet and hour on absorbance values for estimated fructose, non-fructose sugars, glycogen and lipids of female *Drosophila suzukii* on blueberry blossoms in 2013. Analysis was done on transformed values when necessary. Means presented on untransformed data.

Trial	Nutrient	Treatment	Mean ±SE	n	Effect	Df	F	P
Blueberry 2013	Glycogen	Sucrose 24 h	67.0 ±5.7 A[1]	20	Food	2,107	194.5	<0.001
		Sucrose 48 h	69.0 ±5.6	20	Hour	1,107	6.5	0.01
		Blueberry 24 h	46.2 ±3.3 B	20	Food*H	2,107	1.1	0.34
		Blueberry 48 h	55.2 ±3.0	20				
		Water 24 h	3.63 ±0.6 C	20				
		Water 48 h	10.6 ±2.7	13				
	Fructose	Sucrose 24 h	55.3 ±7.0 A	20	Food	2,107	40.7	<0.001
		Sucrose 48 h	54.9 ±6.4	20	Hour	1,107	1.6	0.21
		Blueberry 24 h	37.6 ±6.6 A	20	Food*H	2,107	2.0	0.14
		Blueberry 48 h	58.2 ±8.5	20				
		Water 24 h	1.19 ±0.3 B	20				
		Water 48 h	0.42 ±0.1	13				
	Other sugars	Sucrose 24 h	33.4 ±5.3 A	20	Food	2,107	43.4	<0.001
		Sucrose 48 h	31.4 ±2.6	20	Hour	1,107	1.5	0.23
		Blueberry 24 h	27.6 ±4.0 A	20	Food*H	2,107	1.5	0.23
		Blueberry 48 h	40.7 ±6.5	20				
		Water 24 h	3.90 ±1.0 B	20				
		Water 48 h	3.83 ±0.9	13				
	Lipids	Sucrose 24 h	12.3 ±1.0 ab	20	Food	2,107	1.8	0.17
		Sucrose 48 h	10.1 ±1.0 b	20	Hour	1,107	3.0	0.09
		Blueberry 24 h	11.6 ±0.8 ab	20	Food*H	2,107	4.9	0.01
		Blueberry 48 h	14.1 ±1.1 ab	20				
		Water 24 h	10.8 ±0.8 ab	20				
		Water 48 h	14.8 ±1.6 a	13				

[1] Capitol letters denote that a means comparison was made with three treatment means, the 24 and 48 h data are combined.
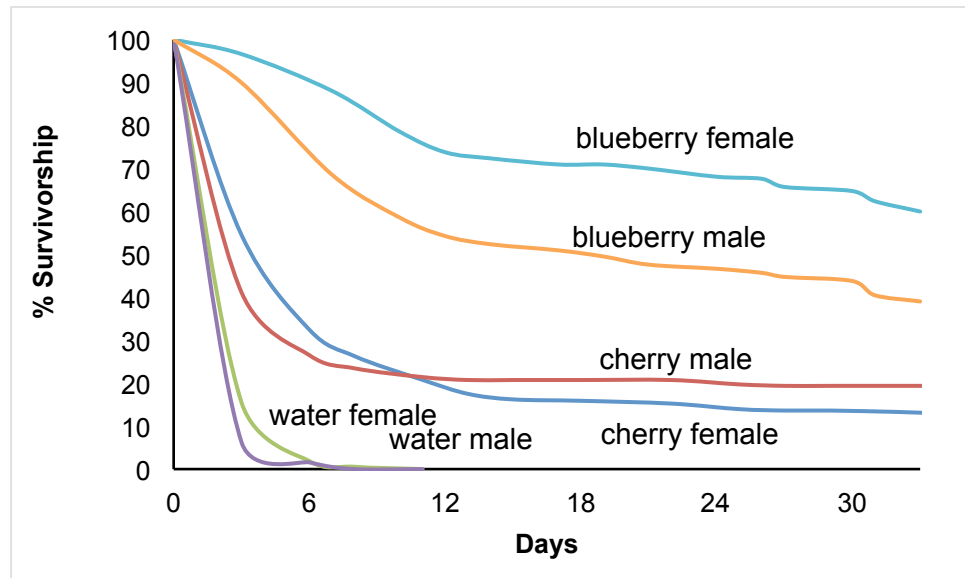
Figure 4.1. Proportion survivorship of male and female *Drosophila suzukii* provided cher blossoms, blueberry blossoms, or water only over a 41 or 33d observational period.

Figure 4.2. Amounts of glycogen (A), fructose (B), non-fructose sugars (C) and lipids (D) detected over the 48 hrs female *Drosophila suzukii* were provided sucrose, cherry blossoms, or water.
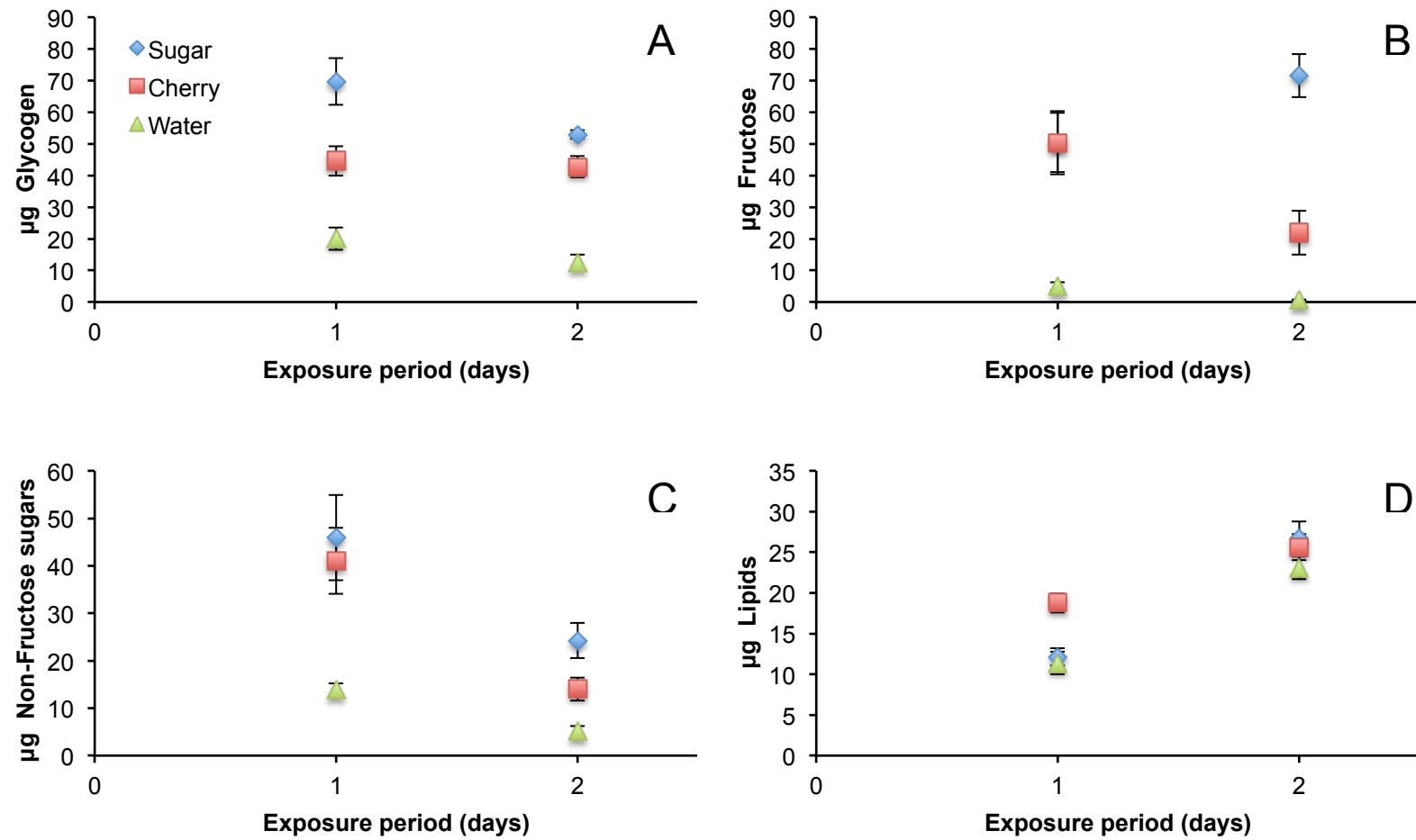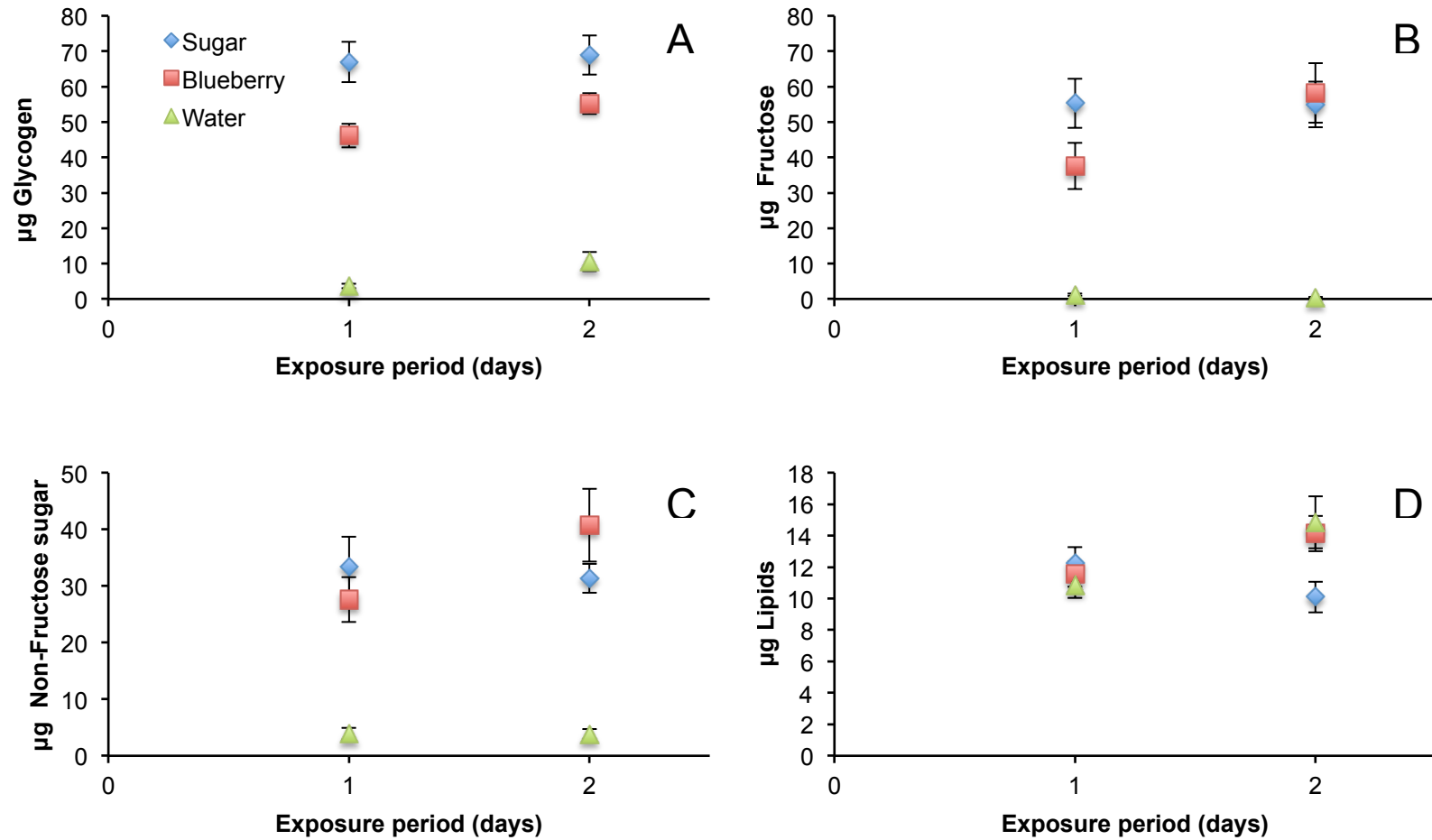
Figure 4.3. Amounts of glycogen (A), fructose (B), non-fructose sugars (C) and lipids (D) detected over the 48 hrs female *Drosophila suzukii* were provided sucrose, blueberry blossoms, or water.

CHAPTER 5.

General Conclusions.

Samantha Tochen

The arrival of *Drosophila suzukii* in California in 2008 and subsequent spread and detection throughout North America, Europe and South America, sparked concerned throughout integral fruit production regions (Cini et al. 2012, Deprá et al. 2014, Hauser et al. 2009). Its role as a pest of small and stone fruits has negatively impacted many of these areas through loss of yield and/or increased cost of production (Bolda et al. 2010). Growers rely on the use of insecticides to control populations, however they are applied as a prophylactic measure rather than guided by population levels. Control methods can be improved by gaining knowledge of the basic mechanisms of *D.suzukii* increase (Price 1997). Although previous research was conducted on *D. suzukii*'s phenology in Japan, it could not be assumed the Oregon population would be the same as cosmopolitan species of drosophlids that readily adapt to present environmental conditions (Chown et al. 2011). This work was performed to determine the phenology of *D. suzukii* in Oregon as well as elucidate the effect of early season floral feeding.

The first part of this research was to determine development rate, fecundity and longevity of *D. suzukii* on cherry and blueberry at seven constant temperatures (Tochen et al. 2014). Lab infested cherries and blueberries were exposed to constant temperatures ranging from 10 to 30 °C. Time till pupation, eclosion and mortality was tracked in addition to eggs laid per day by F^1 adults on either cherries or blueberries. Developmental period, survival and fecundity were all influenced significantly by temperature. Females exposed to blueberries had lower fecundity than those reared on cherries. Optimal temperature for development and intrinsic rate of population increase were 28.1 and 21.0 °C, respectively. This research has been incorporated into the development of a matrix projection model for population estimation (Wiman et al. 2014).

The second part of this research was to determine development rate, fecundity and longevity of *D. suzukii* on blueberry at five constant relative humidities. Lab infested blueberries were exposed to constant RHs ranging from 17 to 93% RH. While humidity had a significant effect on fecundity and longevity, however development was not influenced. Above 65% RH, fecundity and survival increased. Maximum egg laying was observed at 93% RH, however ovary dissections indicated no difference in ovary maturation between 78 and 93%, indicating

the potential other factors were influencing the maximum egg laying. Optimal RH for development was 79% RH.

The third part of this research was to determine lifespan and nutrient uptake of *D. suzukii* when provided cherry or blueberry blossoms as a food source. Long-term insect survival encompasses favorable temperatures during overwintering periods (Dalton et al. 2011) and the availability of essential food resources, which may lead to early season *D. suzukii* population increase and subsequently, increased pest pressure (Kanzawa 1939, Mitsui et al. 2006, Mitsui et al. 2007). Adult *D. suzukii* provided cherry blossoms survived significantly longer than those provided only water, with an average lifespan of 11.1 ±0.6d and 3.4 ±0.5d respectively. The nutritional assay showed higher levels of glycogen and sugar levels amongst female flies exposed to both blossom types and sucrose when compared against females fed water, with little difference between blossom and sucrose fed flies. Lipid levels tended to be the same regardless of treatment.

Future work should explore how relative humidity affects dispersal of adult *D. suzukii* in the field, as insects will reposition themselves in their environment to optimize their survival (Bach 1993, Chown et al. 2011).   Through this, cultural and management practices such as pruning or irrigation may be directed to make host areas less habitable for *D. suzukii*. This knowledge can also be incorporated into the matrix projection model to provide greater accuracy across different climatic conditions. Further research should also elucidate the effect of floral resources on adult longevity as opposed to lifespan. Beyond that, field caught adults should be studied, utilizing gas chromatography to elucidate nutrient sources. Given the experimental design, the presence of other substrates could not be discounted and gas chromatography could help discover alternative food sources. Through this work, the timing of bait spray applications may be influenced and aide in the development of a more attractive bait.

References cited:

Bach, C. E. 1993. Effects of microclimate and plant characteristics on the distribution of a willow flea beetle, *Altica subplicata*. Am. Midl. Nat. 193–208.

Bolda, M. P., R. E. Goodhue, and F. G. Zalom. 2010. Spotted wing drosophila: potential impact of a newly established pest. Agric. Res. Econ. Update. 13: 5–8.

Chown, S. L., J. G. Sørensen, and J. S. Terblanche. 2011. Water loss in insects: An environmental change perspective. J. Insect Physiol. 57: 1070–1084. doi: 10.1016/j.jinsphys.2011.05.004.

Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insect. 65: 149–160.

Dalton, D. T., Walton V. M., Shearer P. W., Walsh D. B., J. Caprile, and R. Isaacs. 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manag. Sci. 67: 1368–1374.

Deprá, M., Poppe J. L., Schmitz H. J., D. C. D. Toni, and V. L. S. Valente. 2014. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J. Pest Sci. 87: 379–383. doi: 10.1007/s10340-014-0591-5.

Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag. Sci. 67: 1352–1357. doi: 10.1002/ps.2265.

Ideo, S., Watada M., H. Mitsui, and M. T. Kimura. 2008. Host range of *Asobara japonica* (Hymenoptera: Braconidae), a larval parasitoid of drosophilid flies. Entomol. Sci. 11: 1–6. doi: 10.1111/j.1479-8298.2007.00244.

Kanzawa, T. 1939. Studies on *Drosophila suzukii* Mats. Kofu. Rev. Appl. Entomol. 29: 622.

Mitsui, H., K. Beppu, and M. T. Kimura. 2010. Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol. Sci. 13: 60–67. doi: 10.1111/j.1479-8298.2010.00372.

Price, P. W. 1997. Insect Ecology 3rd Edition. Population Dynamics John Wiley & Sons, New York, pp. 169-197.

Wiman, N. G., Walton V.M., Dalton D.T., Anfora G., Burrack H. J., J. C. Joanna, and K. M. Daane. 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLoS ONE 9: e106909.

Bibliography:

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2001. Revisiting water loss in insects: a large scale view. J. Insect Physiol. 47: 1377–1388.

Aghdam, H. R., Fathipour Y, G. Radjabi, and M. Rezapanah. 2009. Temperature-dependent development and temperature thresholds of codling moth (Lepidoptera: Tortricidae) in Iran. Environ. Entomol. 38: 885-895.

Atallah, J., Teixeira L., Salazar R., G. Zaragoza, and A. Kopp. 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. Proc. R. Soc. B. 281: 20132840. doi: 10.1098/rspb.2013.2840

Averill, A. L., and R. J. Prokopy. 1993. Foraging of *Rhagoletis pomonella* flies in relation to interactive food and fruit resources. Entomol. Exp. Appl. 66: 179–185.

Bach, C. E. 1993. Effects of microclimate and plant characteristics on the distribution of a willow flea beetle, *Altica subplicata*. Am. Midl. Nat. 193–208.

Baker, H. G., and I. Baker. 1983. A brief historical review of the chemistry of nectar. In: Bentley B, Elias T (eds) The Biology of Nectaries. Columbia University Press, New York, pp. 126–152.

Baroffio, C. A., Richoz P., Fischer S., Kuske S., C. Linder, and P. Kehrli. 2014. Monitoring *Drosophila suzukii* in Switzerland in 2012. J. Berry Res. 4: 47–52.

Beers, E. H., Van Steenwyk R. A., Shearer P. W., W. Coates, and J. Grant. 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag. Sci. 67: 1386–1395. doi: 10.1002/ps.2279

Bolda, M. P., R. E. Goodhue, and F. G. Zalom. 2010. Spotted wing drosophila: potential impact of a newly established pest. Agricultural Res Econ Update. 13: 5–8.

Bradley, T. J. 2009. Animal Osmoregulation. Oxford University Press, Oxford.

Briegel, H. 1990. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. J. Med. Entomol. 27: 839–850.

Briere, J. F., Pracros P., A. Y. Le Roux, and J. S. Pierre.1999. A novel rate model of temperature-dependent development for arthropods. Environ. Entomol. 28: 22-29.

Broufas, G. D., M. L. Pappas, and D. S. Koveos. 2009. Effect of relative humidity on longevity, ovarian maturation, and egg production in the Olive Fruit Fly (Diptera: Tephritidae). Ann.

Entomol. Soc. Am. 102: 70–75. doi: 10.1603/008.102.0107

Bruck, D. J., Bolda M., Tanigoshi L., Klick J., Kleiber J., DeFrancesco J., B. Gerdeman and H. Spitler. 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manage. Sci. 67: 1375–1385.

Bruck, D., Tanigoshi L. K., Gerdeman B. S., Hollis-Spitler G., J. DeFrancesco, and A. J. Dreves. 2011. Current recommendations for managing Spotted Wing Drosophila (SWD), *Drosophila suzukii*, in PNW Blueberries.

Bryla, D. R., and B. C. Strik. 2007. Effects of cultivar and plant spacing on the seasonal water requirements of highbush blueberry. J. Amer. Soc. Hort. Sci. 132: 270–277.

Bubliy, O. A., T. N. Kristensen, and V. Loeschcke. 2013. Stress-induced plastic responses in *Drosophila simulans* following exposure to combinations of temperature and humidity levels. J Exp Biol 216: 4601–4607. doi: 10.1242/jeb.092502.

Bugg, R. L., R. T. Ellis, and R. W. Carlson. 1989. Ichneumonidae (Hymenoptera) using extrafloral nectar of faba bean (*Vicia faba* L., Fabaceae) in Massachusetts. Biol. Agric. Hortic. 6: 107–114.

Burrack, H. J., Fernandez G. E., T. Spivey, and D. A. Kraus. 2013. Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: Drosophilidae), an invasive frugivore. Pest. Manag. Sci. 69: 1173–1180. doi: 10.1002/ps.3489

Campbell, A., Frazer B. D., Gilbert N., A. P. Gutierrez, and M. Mackauer. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11:431-438.

Carey, J. R. 1993. Reproduction. Applied Demography for Biologists with Special Emphasis on Insects (ed. by JR Carey) Oxford University Press, New York, NY, USA, pp. 43-76.

Cavicchi, S., Guerra D., G. Girogi, and C. Pezzoli. 1985. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. Genetics. 109: 665–689.

Cha, D. H., Adams T., Werle C. T, Sampson B., Adamczyk J., H. Rogg, and P. Landolt. 2014. A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace. Pest. Manag. Sci. 70: 324–331. doi: 10.1002/ps.3568.

Cha, D. H., Adams T., H. Rogg and P. J. Landolt. 2012. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. J. Chem. Ecol. 38: 1419-1431.

Chang, F., Winters N., Vargas R. I., S. L. Montogomery, and J. M. Takara. 1977. An analysis of crop sugars in the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), in Hawaii, and correlation with possible food sources. Proc. Hawaii. Entomol. Soc. 22: 461–468.

Chapman, T., and L. Partridge. 1996. Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. Proc. Biol. Sci. 263: 755–759.

Chiu, J. C., Jiang X., Zhao L., Hamm C. A., Cridland J. M., Saelao P., Hamby K. A., Lee E. K., Kwok R. S., Zhang G., Zalom F. G., V. M. Walton, and D. J. Begun. 2013. Genome of *Drosophila suzukii*, the Spotted Wing Drosophila. G3 (Bethesda). 9;3(12): 2257-71. doi: 10.1534/g3.113.008185.

Chown, S. L., J. G. Sørensen, and J. S. Terblanche. 2011. Water loss in insects: An environmental change perspective. J. Insect Physiol. 57: 1070–1084. doi: 10.1016/j.jinsphys.2011.05.004

Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insect. 65: 149–160.

Clements, A. N. 1992. The Biology of Mosquitoes. Chapman & Hall, New York.

Contreras, H. L., Goyret J., von Arx M., Pierce C., Bronstein J., R. Raguso, and G. Davidowitz. 2013. The effect of ambient humidity on the foraging behavior of the hawkmoth *Manduca sexta*. J. Comp. Physiol. 199: 1053–1063.

Costa, E. A. P. de A., Santos E. M. de M., J. C. Correia, and C. M. R. de Albuquerque. 2010. Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera, Culicidae). Rev. Bras. Entomol. 54: 488–493. doi: 10.1590/S0085-56262010000300021.

Costero, A., Attardo G. M., T. W. Scott, and J. D. Edman. 1998. An experimental study on the detection of fructose in *Aedes aegypti*. J. Am. Mosq. Contr. Assoc.14: 234–242.

Cuthbertson, A. G. S., Collins D. A., Blackburn L. F., L. Blackburn, and H. Bell. 2014. Preliminary screening of potential control products against *Drosophila suzukii*. Insects. 5: 488–498. doi: 10.3390/insects5020488.

Dalton, D. T., Walton V. M., Shearer P. W., Walsh D.. B., J. Caprile, and R. Isaacs. 2011. Laboratory survival of *Drosophila suzukii* under simulated winter conditions of the Pacific Northwest and seasonal field trapping in five primary regions of small and stone fruit production in the United States. Pest Manage. Sci. 67: 1368–1374.

Denholm, I., and M. W. Rowland. 1992. Tactics for managing pesticide resistance in arthropods: theory and practice. Annu. Rev. Entomol. 37: 91–112. doi: 10.1146/annurev.en.37.010192.000515

Deprá, M, Poppe J. L., Schmitz H. J., D. Toni, and V. Valente. 2014. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J. Pest. Sci. 87: 379–383. doi: 10.1007/s10340-014-0591-5

Downer, R. G. H., and J. R. Matthews. 1976. Patterns of lipid distribution and utilisation in insects. Amer. Zool. 16: 733–745. doi: 10.1093/icb/16.4.733.

Emiljanowicz, L. M., G. D. Ryan, and A. Langille. 2014. Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. J. Econ. Entomol. 107: 1392–1398.

Evans, E. W. 1993. Indirect interactions among phytophagous insects: aphids, honeydew and natural enemies. In: Watt AD, Leather SR, Walters KEF, Mills NJ (eds) Individuals, Populations and Patterns in Ecology. Intercept Press, U.K., pp 63–80.

Fadamiro, H. Y., Chen L., E. O. Onagbola , and L. Graham. 2005. Lifespan and patterns of accumulation and mobilization of nutrients in the sugar-fed phorid fly, *Pseudacteon tricuspis*. Physiol. Entomol. 30: 212–224. doi: 10.1111/j.1365-3032.2005.00449.

Follett, P. A., A. Swedman , and D. K. Prices. 2014. Postharvest irradiation treatment for quarantine control of *Drosophila suzukii* (Diptera: Drosophilidae) in fresh commodities. J Econ Entomol. 107: 964–969.

Fukushima Prefecture Tree Fruit Research Institute. 1993. Host plants of SWD.

Goodhue, R. E., Bolda M., Farnsworth D., J. C. Williams, and F. G. Zalom. 2011. Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manage. Sci. 67: 1396–1402.

Hamby, K. A., Hernández A., K. Boundy-Mills, and F. G. Zalom. 2012. Associations of yeasts with Spotted-Wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. Appl. Environ. Microbiol. 78: 4869–4873. doi: 10.1128/AEM.00841-12

Hassett, C. C. 1948. The utilization of sugars and other substances by *Drosophila*. Biol. Bull.

114–123.

Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest. Manag. Sci. 67: 1352–1357. doi: 10.1002/ps.2265

Heimpel, G. E., Lee J. C., Wu Z., Weiser L., F. Wäckers, and M. A. Jervis. 2004. Gut sugar analysis in field-caught parasitoids: adapting methods originally developed for biting flies. Int. J. Pest Manag. 50: 193–198. doi: 10.1080/09670870410001731925.

Hendrichs, J., Lauzon C. R., S. S. Cooley, and R. J. Prokopy. 1993. Contribution of natural food sources to adult longevity and fecundity of *Rhagoletis pomonella* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 86: 250–264.

Hoffman, A. A., Hallas R. J., J. A. Dean, and M. Schiffer. 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science. 301: 100–102.

Ideo, S., Watada M., H. Mitsui, and M. T. Kimura. 2008. Host range of *Asobara japonica* (Hymenoptera: Braconidae), a larval parasitoid of drosophilid flies. Entomol. Sci. 11: 1–6. doi: 10.1111/j.1479-8298.2007.00244.

Jaenike, J. 1986. Feeding behavior and future fecundity in *Drosophila*. Am. Naturalist. 127: 118–123.

Jervis, M. A., N. A. C. Kidd, and G. E. Heimpel. 1996. Parasitoid adult feeding and biological control - a review. BioControl News Inf. 17: 1N–22N.

Jervis, M. A., N. A. C. Kidd, and M. G. Fitton. 1993. Flower-visiting by hymenopteran parasitoids. J. Nat. Hist. 27: 67–105.

Jones, V. P., and N. G. Wiman. 2012. Modeling the interaction of physiological time, seasonal weather patterns, and delayed mating on population dynamics of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Pop. Ecol. 54: 421-429.

Kacsoh, B. Z., and T. A. Schlenke. 2012. High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii*, a relative of *D. melanogaster*. PLoS One. doi: 10.1371/journal.pone.0034721

Kaneshiro, K. Y. 1983. *Drosophila* (Sophophora) *suzukii* (Matsumura). Proc. Hawaiian Ent. Soc. 24: 179.

Kanzawa, T. 1939. Studies on *Drosophila suzukii* Mats. Kofu. Rev. Appl. Ent. 29: 622.

Kawase, S, K. Uchino, and K. Takahashi. 2007. Control of cherry drosophila, *Drosophila suzukii*, injurious to blueberry. Plant Protection. 61: 205–209.

Kim, S. S., Tripodi A. D., D. T. Johnson, and A. L. Szalanski. 2014. Molecular Diagnostics of *Drosophila suzukii* (Diptera: Drosophilidae) using PCR-RFLP. J. Econ. Entomol. 107: 1292–1294.

Kimura, M. T. 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. Oecologia. 140: 442–449.Lachaise, D., and L. Tsacas. 1983. Breeding sites in tropical African drosophilids. The Genetics and Biology of Drosophila. Academic Press, London, pp 221–332

Kinjo, H., Kunimi Y., T. Ban, and M. Nakai. 2012. Oviposition efficacy of *Drosophila suzukii* (Diptera: Drosophilidae) on different cultivars of blueberry. J. Econ. Entomol. 106: 1767–1771.

Klowden, M. J. 2002. Physiological systems in insects. Academic Press, San Diego, California.

Landolt, P. J., Adams T., T. S. Davis, and H. Rogg. 2012. Spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), trapped with combinations of wines and vinegars. Fla. Entomol. 95: 326–332.

Lee, J. C., Dreves A. J., Cave A. M., Kawai S., Isaacs R., Miller J. C., S. Van Timmeren, and D. J. Bruck. 2014. Infestation of wild and ornamental non-crop fruits by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Ann. Entomol. Soc. Am. In Review.

Lee, J. C., Shearer P. W., Barrantes L. D., Beers E. H., Burrack H. J., Dalton D. T., A. J. Dreves, and D. J. Bruck. 2013. Trap designs for monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 42: 1348–1355.

Lee, J. C. Burrack, H. J., Barrantes, L. D., Beers, E. H., Dreves, A. J., Hamby, K. A., Haviland, D. R., Isaacs, R, Richardson, T. A., Shearer, P. W., Stanley, C. A., Walsh, D. B., Walton, V. M., F. G. Zalom, and D. J. Bruck. 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. J. Econ. Entomol.105: 1350–1357.

Lee, J. C., Bruck D. J., Curry H., Edwards D., Haviland D. R., R. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest. Manag. Sci. 67: 1358–1367. doi: 10.1002/ps.2225

Lee, J. C., D. A. Andow, and G. E. Heimpel. 2006. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. Ecol. Entomol. 31: 470–480. doi: 10.1111/j.1365-2311.2006.00800.

Lee, J. C. H., G. Heimpel, and L. Gary. 2004. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. Entomol. Exp. Appl. 111 :189–199. doi: 10.1111/j.0013-8703.2004.00165.

Liu, S.S., and X. D. Meng. 1999. Modeling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. Bull. Entomol. Res. 89: 53-63.

Lu, Y., and K. Wu. 2011. Effect of relative humidity on population growth of *Apolygus lucorum* (Heteroptera: Miridae). Jap. J. Appl. Entomol. Zool. 46: 421–427.

Mercedes, D., and D. E. Hardy. 1977. A catalog of the Diptera of the Oriental Region. Suborder Cyclorrphapha (excluding division Aschiza). University Press of Hawaii.

Min, K.-J., Hogan M. F., M. Tatar, and D. M. O'Brien. 2006. Resource allocation to reproduction and soma in *Drosophila*: A stable isotope analysis of carbon from dietary sugar. J. Insect Physiol. 52: 763–770. doi: 10.1016/j.jinsphys.2006.04.004.

Mitsui, H., K. Beppu, and M. T. Kimura. 2010. Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. Entomol. Sci. 13: 60–67. doi: 10.1111/j.1479-8298.2010.00372.

Mitsui, H., K. Van Achterberg, and G. Nordlander. 2007. Geographical distributions and host associations of larval parasitoids of frugivorous Drosophilidae in Japan. J. Nat. Hist. 41: 1731–1738.

Mitsui, H., K. H. Takahashi, and M. T. Kimura. 2006. Spatial distributions and clutch sizes of *Drosophila* spp. ovipositing on cherry fruits of different stages. Pop. Ecol. 48: 233–237.

Naksathit, A. T., J. D. Edman, and T. W. Scott. 1999. Amounts of glycogen, lipid, and sugar in adult females *Aedes aegypti* (Diptera: Culicidae) fed sucrose. J. Med. Entomol. 36: 8–12.

Naranjo-Lázaro, J. M., Mellín-Rosas M. A., González-Padilla V. D., Sánchez-González G., G. Moreno-Carrillo, and H. Arredondo-Bernal. 2014. Susceptibility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) to Entomophatogenic Fungi. Southwest. Entomol. 39: 201–203. doi: 10.3958/059.039.0119.

National Agricultural Pest Information System (NAPIS). 2013. Pest tracker, reported pest status of spotted wing drosophila *Drosophila suzukii.* (http://pest.ceris.purdue.edu/pestlist.php). Retrieved May 6, 2013.

Nestel, D., R. Galun, and S. Friedman. 1985. Long-term regulation of sucrose intake by the adult Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). J. Insect Physiol. 31: 533–536. doi: 10.1016/0022-1910(85)90109-X.

Nicolson, S. W. 1994. Pollen feeding in the eucalypt nectar fly, *Drosophila flavohirta*. Physiol. Entomol. 19: 58–60.

Nunes, R. D., R. L. De Oliveira, and G. R. C. Braz. 2008. A novel method for measuring fructose ingestion by mosquitoes. J. Vect. Ecol. 33: 225–231.

Ohtsu, T., M. T. Kimura, and C. Katagiri. 1998. How Drosophila species acquire cold tolerance. Eur. J. Biochem. 252: 608–611 (1998).

Olson, D. M., Fadamiro H. Y., J. G. Lundgren, and G. E. Heimpel. 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. Physiol. Entomol. 25: 17–26.

Ostojic´, I., M. Zovko, and D. Petrovic´. 2014. First record of spotted wing *Drosophila suzukii* (Matsumura, 1931) in Bosnia and Herzegovina. Radovi Poljoprivrednog Fakulteta Univerziteta u Sarajevu (Works of the Faculty of Agriculture University of Sarajevo) 59: 127–133.

Price, P. W. 1997. Insect Ecology 3rd Edition. Population Dynamics John Wiley & Sons, New York, pp. 169-197.

Quesada-Moraga, E., P. Valverde-García and I. Garrido-Jurado. 2012. The effect of temperature and soil moisture on the development of the preimaginal Mediterranean fruit fly (Diptera: Tephritidae). Environ. Entomol. l41: 966-970.

R Development Core Team 2012 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0.

Reissig, W. H., Barnard, J., Weires, R. W., E. H. Glass and R. W. Dean. 1979. Prediction of apple maggot fly emergence from thermal unit accumulation. Environ. Entomol. 8: 51-54.

Rivero, A., and J. Casas. 1999. Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. Res. Popul. Ecol. 41: 39–45. doi: 10.1007/PL00011981.

Roca, M. J., and C. R. Lazzari. 1994. Effects of relative humidity on the haematophagous bug *Triatoma infestans*: Hygropreference and eclosion success. J. Insect Physiol. 40: 901–907.

Rock, G. C. and P. L. Shaffer. 1983. Developmental rates of codling moth (Lepidoptera: Olethreutidae) reared on apple at four constant temperatures. Environ. Entomol. 12: 831-834.

Sasaki, M., and R. Sato. 1996. Bionomics of *Drosophila pulchrella* Tan, Hus et Sheng (Diptera: Drosphilidae) in Fukushima prefecture. Tohoku Agricultural Research (Japan).

Schetelig, M. F., and A. M. Handler. 2013. Germline transformation of the spotted wing drosophilid, *Drosophila suzukii*, with a piggyBac transposon vector. Genetica. 141: 189–193. doi: 10.1007/s10709-013-9717-6.

Seagraves, M. P., Kajita Y., Weber D. C., J. J. Obrycki, and J. G. Lundgren. 2010. Sugar feeding by coccinellids under field conditions: the effects of sugar sprays in soybean. BioControl. 56: 305–314. doi: 10.1007/s10526-010-9337-3.

Shinzo, K., U. Ken, and T. Kyoko. 2007. Control of Cherry Drosophila *Drosophila suzukii* injurious to blueberry. Plant Prot. 61: 205–209.

Simelane, D. O. 2007. Influence of temperature, photoperiod and humidity on oviposition and egg hatch of the root-feeding flea beetle *Longitarsus bethae* (Chrysomelidae: Alticinae), a natural enemy of the weed *Lantana camara* (Verbenaceae). Bull. Entomol. Res. 97: 111–116. doi: 10.1017/S0007485307004713.

Smith, T. J. 2013. Overview of tree fruit production in the Pacific Northwest United States of America and southern British Columbia, Canada.

Stacconi, M. R., Grassi A. L., Dalton D. T., Miller B., Ouantar M., Loni A., Ioriatti C., V. M. Walton, and G. Anfora. 2013. First field records of *Pachycrepoideus vindemiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas. Entomologia 1: e3.

Statistica 7.1. Statsoft Inc. 2005. 2300 East 14th Street, Tulsa, OK, 74104.

Steffan, S. A., Lee J. C,, Singleton M. E., Vilaire A., Walsh D. B., L. S. Lavine, and K. Patten. 2013. Susceptibility of cranberries to *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 106: 2424–2427.

Steiner, M. Y., L. J. Spohr, and S. Goodwin. 2011. Relative humidity controls pupation success and dropping behaviour of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Aust. J. Entomol. 50: 179–186.

Strik, B. C., and C. E. Finn. 2008. Blueberry cultivars for Oregon. EC 1308-E. Oregon State University Extension Service. March 2008. Corvallis, OR.

Strik, B. C., and D. Yarborough. 2005. Blueberry production trends in North America, 1992 to 2003, and predictions for growth. HortTech. 15: 391–398.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561.

Tabashnik, B. E., and B. A. Croft. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. Environ. Entomol. 11: 1137–1144.

Takamori, H., Watabe H., Fuyama Y., Z. Ya-ping, and T. Aotsuka. 2006. *Drosophila subpulchrella*, a new species of the *Drosophila suzukii* species subgroup from Japan and China (Diptera: Drosophilidae). Entomol. Sci. 9: 121–128. doi: 10.1111/j.1479-8298.2006.00159.

Tichy, H. 2003. Low rates of change enhance effect of humidity on the activity of insect hygrosensors. J. Comp. Physiol. A. 189: 175–179.

Tochen, S., Dalton D. T., Wiman N. G., Hamm C., P. W. Shearer, and V. M. Walton. 2014. Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. Environ. Entomol. DOI: http://dx.doi.org/10.1603/EN13200.

USDA N. 2014. Oregon agriculture: facts & figures. (http://www.nass.usda.gov/Statistics_by_State/Oregon/Publications/facts_and_figures/facts_and_figures.pdf). Retrieved March 20, 2014.

USDI Bureau of Reclamation. (http://www.usbr.gov/pn/agrimet/agrimetmap/crvoda.html). Retrieved April 4, 2012

Van Handel, E., and L. O. Arden. 1984. Vitellogenin synthesis in blood-fed *Aedes aegypti* in the absence of the head, thorax and ovaries. J. Insect Physiol. 30: 871–875.

Van Handel, E. 1972. Detection of nectar in mosquitoes. Mosquito News.

Van Timmeren, S., and R. Isaacs. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Prot. 54: 126–133. doi: 10.1016/j.cropro.2013.08.003.

Vilela, C. R., Teixeira E. P., and C. P. Stein. 2001. Mosca-africana-dofigo, *Zaprionus indianus* (Diptera: Drosophilidae). Histórico e Impacto das Pragas Introduzidas no Brasil. Holos, Ribeirão Preto SP, pp 48–52.

Vreysen, M. J. B., Robinson A. S., J. Hendrichs, and P. Kenmore. 2007. Area-Wide Integrated Pest Management (AW-IPM): Principles, Practice and Prospects. In: Vreysen MJB, Robinson AS, Hendrichs J (eds) Area-Wide Control of Insect Pests. Springer, Netherlands, pp 3–33.

Wäckers, F. L., Romeis J., and P. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. Ann. Rev. Entomol. 52: 301–323.

Walsh, D. B., Bolda M. P., Goodhue R. E., Dreves A. J., Lee J., Bruck D. J., Walton V. M., S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integ. Pest Manag. 2: 1–7. doi: 10.1603/IPM10010.

Webster, R. P., J. G. Stoffolano, and R. J. Prokopy. 1979. Long-term intake of protein and sucrose in relation to reproductive behavior of wild and laboratory cultured *Rhagoletis pomonella*. Ann. Entomol. Soc. Am. 72: 41–46.

Wiman, N. G., Walton V. M., Dalton D. T., Anfora G., Burrack H. J., Chiu J. C., Daane K. M., Grassi A., Ioriatti C., Miller B., S. Tochen, and X. G. Wang. 2014. Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. PLoS ONE 9(9): e106909. doi:10.1371/journal.pone.0106909.

Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology. 41: 232–237. doi: 10.2307/1931961.

Yamada, Y. 2008. Oviposition inhibitor trials on *Drosophila suzukii*. Undergraduate, Yamagata University

Yee, W. L., and P. S. Chapman. 2008. Seasonal amounts of nutrients in Western cherry fruit fly (Diptera: Tephritidae) and their relation to nutrient availability on cherry plant surfaces. Environ. Entomol. 37: 1086–1098. doi: 10.1603/0046-225X(2008)37[1086:SAONIW]2.0.CO;2.

Yu, D., F. G. Zalom, and K. A. Hamby. 2012. Host status and fruit odor response of *Drosophila suzukii* (Diptera: Drosophilidae) to figs and mulberries. J. Econ. Entomol. 106: 1932–1937.

Zimmermann, G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Sci. Techn. 18: 865–901. doi: 10.1080/09583150802471812.