INVESTIGATION OF THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE BIOLOGY AND BEHAVIOR OF *DROSOPHILA SUZUKII*

by

C. KIRK GREEN

(Under the Direction of ASHFAQ A. SIAL)

ABSTRACT

Drosophila suzukii is a prolific pest of small and stone fruits. Current population monitoring and modeling efforts are not reliable enough to predict fruit infestation. Therefore, insecticides are most commonly applied prophylactically on a calendar rotation. We conducted laboratory and field trials to develop our understanding of *D. suzukii* thermal biology, natural habitats, and responses to management strategies. These are key areas of inquiry to refine population modeling and interpret monitoring data. We found that thermal stress during development decreases fertility in adulthood, but this can be mitigated if maternal parents were previously exposed to heat stress. We also found that evergreen foliage increased *D. suzukii* abundance during the winter, and conditions below leaf litter were favorable for overwintering flies. Finally, we evaluated management practice and population monitoring tools. These findings further our understanding of this pest's biology and will contribute to our ability to manage this key insect pest.

INDEX WORDS: Pest management, thermal biology, resources, overwintering ecology, population monitoring

INVESTIGATION OF THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE BIOLOGY AND BEHAVIOR OF *DROSOPHILA SUZUKII*

by

C. KIRK GREEN

B.I.S., GEORGIA STATE UNIVERSITY, 2015

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

INVESTIGATION OF THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE BIOLOGY AND BEHAVIOR OF *DROSOPHILA SUZUKII*

by

C. KIRK GREEN

Major Professor:	Ashfaq A. Sial
Committee:	Patricia J. Moore Michael D. Toews

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia

ACKNOWLEDGEMENTS

I would like to thank my parents who encouraged and supported me in my pursuit of education. I also want to thank my mentors: Ash Sial, Trish Moore, Brian Little, and Craig Robous. They have all volunteered sound advisement and/or technical support and influenced my development in this program. Additionally, Alexandria Talley proof-read this work and provided emotional support throughout the writing process. Finally, I want to thank all my lab mates, past and present, for working together with me on our projects and passing the time together in between.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is a highly prolific small fruit pest of Southeast Asian origin. First described in Japan in 1931 by Matsumura, *D. suzukii* has spread its range to a global scale, with distribution in Asia, Europe, Africa, South America, and North America (Asplen et al. 2015). Its first collection in the United States was in the Hawaiian island of Oahu in 1980, without any corresponding reports of damage. In the continental United States, the first collection was in 2008 in California, although it was only identified to the genus level at that time. A year later, the increasing amounts of strawberry damage by *Drosophila* larvae led to further investigation and its subsequent identification as *Drosophila suzukii* (Hauser 2011). In the next year 2010, *D. suzukii* was detected in seven Eastern U.S. states - a solid testament to its competence as an invader (Hauser 2011).

Just as troubling as its dispersal capabilities is its potential for economic damage; the 2012 estimated value for blueberry losses in the Eastern US was between $37-46 million. For the state of Georgia alone, losses were estimated to be between $9-$14 million, a 10-15% loss (Burrack et al. 2012). These losses are unacceptable for growers in the state of Georgia who produce over 77 million pounds of blueberries a year and in 2014 became the country's leading producers of blueberries (Georgia Info 2014). Rising management costs from SWD populations can be attributed to the cost of increased insecticide applications and the rejection of blueberry shipments infested with larvae. Entire shipments of blueberries are rejected if a single larva is detected post-harvest, resulting in an obscene amount of wasted food, resources, and labor (Bruck et al. 2011, Van Timmeren and Isaacs 2014). Additionally, the increased need for pesticide sprays can limit potential markets by exceeding maximum residue limits (Beers et al.

2011, Goodhue et al. 2011). Thus, it is critical to monitor the presence of *D. suzukii* and manage its population densities.

D. suzukii can be identified through distinct morphological traits present in the sexually dimorphic adults; females have a long and narrow ovipositor with many darkened, sclerotized teeth. The ovipositor is 6-7 times as long as the spermatheca, distinguishing it from other *Drosophila* which have a smaller ratio (Hauser 2011). Additionally, the last two abdominal segments of the females are darkened, whereas the abdomens of similar-looking *Drosophila* females have a more uniform coloration across segments. The males have a single black spot at the apex of each wing, hence the common name spotted wing drosophila (SWD). They also have a black sex comb on each first and second tarsal segment of the forelegs (Hauser 2011). The sex combs can be used to distinguish male *D. suzukii* from males of other similar looking species because each sex comb is arranged in a single row of spines, whereas similar species have at least one segment's combs composed of more than one row (Hauser 2011).

The generalist nature of this species and its high fecundity make it a particularly well-adapted invader. Unlike most species of *Drosophila,* which lay their eggs in decomposing plant matter and broken fruit (Basden 1955, Markow 2015), the female *D. suzukii* is able to oviposit inside of intact ripe and overripe fruits. *D. suzukii* derives this ability from its serrated ovipositor, which it uses to saw through the thin skin of the fruit. SWD are able to utilize a wide variety of commercially produced berries and stone fruits including cherries, strawberries, blackberries, raspberries, peaches, blueberries, and more (Walsh et al. 2011). A variety of non-crop ornamental and wild hosts have also been confirmed for *D. suzukii* through field collections and laboratory assays; these include many fruiting plants from the families Moraceae, Phytolaccaceae, Rosacea, Solanaceae, Rutaceae, and more (Lee et al. 2015). The eggs of this polyphagous pest hatch inside of the chosen fruit, inside of which the larvae feed and develop through their three larval instars. Pupation can occur inside of the fruit, or outside of it. (Asplen et al. 2015). Following emergence into adulthood, adults have a 1-3 day pre-oviposition period. Individuals can lay over 25 eggs per day (Asplen et al. 2015), and lifetime fecundity can range from less than 100 to more than 400 eggs (Hamby et al. 2016). The total development time from egg to adult can range from 10 to 79 days (Asplen et al. 2015), and estimates for the number of generations per season range from 7 to 13 (Tochen et al. 2014, Asplen et al. 2015). The variability of these life history traits is strongly influenced by humidity, temperature, and developmental substrate (Kinjo et al. 2014, Tochen et al. 2014, Hamby et al. 2016); it is therefore important to consider local environmental contexts when estimating SWD population parameters and devising management strategies.

Contemporary pest management strategies seek to implement all available pest suppression tools and knowledge of pest biology to inform management actions. The integrated pest management (IPM) paradigm has its roots in the concepts of pest management and integrated control. The integrated control concept acknowledges the natural biotic and abiotic processes, which are deterministic for pest population densities, whereas pest management is the human endeavor to interfere with those densities. IPM is the synthesis of these two concepts, wherein decision makers strive to keep pest populations below economic injury levels using multiple methods in as compatible a manner as possible, while minimizing interference with the control already afforded by natural processes (Stern et al. 1959, Kogan 1998). Central to the IPM dogma are economic injury levels and economic thresholds; economic injury levels are the lowest pest population densities capable of causing economic damage, and economic thresholds are the population densities at which management action is required to prevent populations from reaching the economic injury level (Stern et al. 1959). The formulation of effective decision rules based on economic thresholds is critical to the success and sustainability of IPM programs.

Control of a pest or pest complex, while reducing the rate of chemical control interventions, is commonly used as a measure of success for IPM programs (Kogan 1998). There have been many examples of successful IPM programs since the formulation of the IPM paradigm. In cropping systems affected by codling moth (*Cydia pomonella)*, knowledge of the host range, population dynamics, thermal tolerances, community interactions, and reproductive biology enabled the implementation of a very successful area-wide IPM program that greatly reduced damage by codling moth and reliance on organophosphates without significantly increasing production costs (Knight 2008). In cereal crops including rice, maize, sorghum, wheat, and millet, knowledge about life cycle, overwintering biology, host range, and reproductive biology of pest insects has helped reduce yield losses and dependency on chemical inputs across a wide range of pest complexes worldwide (E. Bragg et al. 2016). These programs utilized spatially and temporally targeted pesticide applications in a strategic manner to maximize efficacy while reducing the impact on natural enemies, combined with planting of resistant crop varieties, and modification of the local and landscape level environments to the detriment of pests and the benefit of their natural enemies. In all cases, a solid foundational understanding of the pests' biology and ecology, as well as the cropping systems and landscape they resided within, was necessary to make the implementation of an IPM program possible.

Currently, SWD management relies heavily on use of broad-spectrum chemical insecticides. Field and laboratory assays have demonstrated that pyrethroids, organophosphates, and spinosyns are effective adulticides against *D. suzukii*, and most of the insecticides tested from these classes have also been shown to provide 5-14 days of residual control as well (Beers et al. 2011, Bruck et al. 2011, Van Timmeren and Isaacs 2014); however, residual control has been shown to be reduced by rainfall events in various cropping systems (Van Timmeren and Isaacs 2014, Gautam et al. 2016). Neonicotinoids were demonstrated to have only moderate to low adulticidal activity (Beers et al. 2011, Bruck et al. 2011), but several neonicotinoids, an organophosphate, and a spinosyn have been shown to kill SWD eggs and larvae when applied topically post-infestation (Wise et al. 2015). Sprays, however, are typically done before fruit has ripened to limit adult population size and prevent fruit infestation, rather than post-infestation to cure the fruit. Unfortunately, traps placed for monitoring have not been shown to correlate with infestation levels and are not reliable for making decisions regarding spray applications (Beers et al. 2011, Kirkpatrick et al. 2018). As a result, most farmers spray for SWD on a calendar schedule determined by the periods of susceptibility for their crops rather than population monitoring efforts (Van Timmeren and Isaacs 2014, Elsensohn and Loeb 2018).

Ideally, IPM programs designed for *D. suzukii* should incorporate accurate population density modeling so that insecticide application can be targeted temporally and spatially for maximum efficacy. Additionally, integrating cultural control tactics informed by biological and ecological information could reduce the frequency at which economic thresholds are reached, and thereby further reduce the need for insecticide applications. Beyond decreasing costs for growers, reducing insecticide input is critical for delaying the onset of insecticide resistance (Brown 1953, Alyokhin et al. 2008, Scott et al. 2013), reducing negative environmental impacts (Ansari et al. 2014), and maximizing the benefits provided by natural enemies (Devine and Furlong 2007). Onset of insecticide resistance is an especially pressing concern in organic production systems, wherein growers are restricted to just two effective insecticides: spinosyn and pyrethrum (Van Timmeren and Isaacs 2014).

Improving population modeling and cultural control strategies require deeper knowledge about the ways in which environmental factors determine SWD population densities (Wiman et al. 2014, Kirkpatrick et al. 2018). Ambient temperatures, habitat resources, and host fruit phenology are among these potentially deterministic parameters. Additionally, it is important to consider how these same factors influence the activity, behavior, and relative spatial distributions of individual organisms, and how these affect the number of flies captured in trapping studies.

Temperature has a strong influence on SWD life history and population dynamics (Wiman et al. 2016). In *Drosophila spp.,* it has been shown that exposure to acute and chronic temperature extremes results in plastic phenotypic responses across multiple timescales, from as short as minutes to as long as years (Qin et al. 2005, Colinet and Hoffmann 2012, Bergland et al. 2014, Gerken et al. 2015, MacMillan et al. 2016). Furthermore, thermal tolerances vary along latitudinal clines in many *Drosophila spp.* (Kimura 2004). These responses lead to tradeoffs in critical life history traits such as development time, life span, and fecundity. In *D. suzukii*, the optimal range for development time is between 26° C and 28° C, and the optimal range for survivorship to adulthood is between 20 ° C and 26° C (Kinjo et al. 2014, Tochen et al. 2014, Hamby et al. 2016). Optimal temperature for maximizing adult lifespan varies depending on the host resources available, and adults can live for over two months at 14 °C on cherry (Tochen et al. 2014). *D. suzukii* is selective about the temperature of the oviposition substrate, with peak oviposition occurring at 22 °C (Zerulla et al. 2017). Oviposition is severely impaired below 10° C and above 30° C (Kinjo et al. 2014, Tochen et al. 2014).

Although the impacts of upper temperature extremes on the fertility and fecundity of adult SWD have recently been investigated in greater detail (Evans 2016), the impact on fertility and fecundity of adult flies that were exposed to upper temperature extremes during development is not known. This is especially important information to consider in *D. suzukii* because immatures are restricted to the interior of the fruit where they were deposited; the conditions they are exposed to during development could be strongly influential on their life history traits as adults and, consequentially, field population densities. The effects of thermal stress on fecundity and fertility are understudied relative to lethal effects, and yet are likely to be more precise predictors of local adaptation potential and population dynamics (Porcelli et al. 2017).

Given what is known about *D. suzukii* survival at the lower temperature extreme in laboratory studies, this species should not be able to survive over the winter in temperate climates (Kimura 2004, Dalton et al. 2011, Jakobs et al. 2015, Stephens et al. 2015). Yet, it can be captured year-round (Rossi-Stacconi et al. 2016, Thistlewood et al. 2018); a previous Georgia study has even reported peak population numbers in the winter months of December and January (Grant 2016). These findings suggest that *D. suzukii* have behavioral and physiological adaptations that allow them to persist in harsher climates. Indeed, female *D. suzukii* have a reproductive diapause characterized by less activity and higher cold tolerance (Zhai et al. 2016). Furthermore, *D. suzukii* exhibit a winter phenotype in higher latitudes with a darker coloration, increased sclerotization, longer wings, and larger body size. The winter morphology has been induced in laboratory by rearing larvae under simulated winter conditions and shown to confer increased cold tolerance (Wallingford and Loeb 2016), but the extent to which this tolerance increases survival in the field is unknown. How these adaptations combine with behavior in the field is unknown for this species, and a deeper understanding of this could of great benefit IPM programs and future research efforts.

In addition to ambient temperature, food and reproductive resources impact *D. suzukii* population densities and distributions. It has been demonstrated that *D. suzukii* is able to disperse from neighboring woodlands containing non-crop hosts into adjacent orchards, with statistically higher numbers captured in farm areas adjacent to non-crop hosts areas relative to areas lacking host plants (Klick et al. 2016). Furthermore, it has been shown that different hosts have different source and sink potentials for *D. suzukii*, and that these potentials vary from host to host throughout the seasons (Wang et al. 2016). Given the wide range of potential hosts and their varying phonologies, the population dynamics of SWD is likely to fluctuate in a complicated manner through time based on the availability of both cultivated and non-crop fruits (Lee et al. 2011). While it has been established that many individual species of plants have the potential to influence SWD populations in the field, less work has been done to characterize the effect of the habitats these plants exist in at the local level. Blueberry orchards are often bordered by commercial soft-wood pine stands, unmanaged deciduous forest, and bodies of water such as retaining ponds in addition to patches of wild *Rubus spp.* Despite how common these neighboring habitat types are, it has yet to be investigated whether SWD abundance varies from habitat to habitat. These different habitats are likely to have differing temperature and humidity patterns, host and feeding resource composition, and fauna, all of which could potentially impact the relative abundance of SWD among and within orchards.

Population monitoring and assessment is largely done through the use of hanging plastic containers baited with a bait or lure to attract adult flies. Numerous authors have evaluated the efficacy of various trap designs, homemade baits and commercial lures (Harris and Peifer 2005, Burrack et al. 2012, Landolt et al. 2012, Basoalto et al. 2013, Lee et al. 2013, Iglesias et al. 2014, Kleiber et al. 2014, Renkema et al. 2014, Cha et al. 2015, Frewin et al. 2017, Kirkpatrick et al. 2017). Studies demonstrate that the number, size, and location of entry holes on a trap affect *D. suzukii* captures as well as species composition. Results for location of entry hole (top or on the sides) vary across studies (Lee et al. 2013, Renkema et al. 2014). Increasing the cumulative entry area increases the number of *D. suzukii* captures (Basoalto et al. 2013), but increasing the size of individual holes past 156 mm^2 has a diminishing rate of turn while reducing selectivity (Renkema et al. 2014). Red, yellow, and black have been shown to be attractive trap colors with variable relative efficacies across studies (Lee et al. 2013, Iglesias et al. 2014, Renkema et al. 2014, Rice et al. 2016, Kirkpatrick et al. 2017), and a red and black striped pattern has also been shown to be effective (Basoalto et al. 2013). Earlier monitoring efforts often used apple cider vinegar as a bait, but various homemade bait solutions and commercial products capture *D. suzukii* earlier in the season, and at a higher rate (Burrack et al. 2015). Baits can be made at home using a combination of vinegar and merlot wine (Landolt et al. 2012), or with yeast and sugar in water (Iglesias et al. 2014). The key olfactory chemicals for *D. suzukii* in vinegar and merlot have been identified as acetic acid, ethanol, acetoin, and methionol (Cha et al. 2014), and used to formulate commercial lure products specifically for SWD. These have been demonstrated to be more selective for SWD than the homemade ingredients they are derived from (Cha et al. 2015) and are able to attract greater numbers of SWD than the other homemade baits (Frewin et al. 2017, Cha et al. 2018). When lure formulations and trap designs produced by Scentry Biologicals and Trécé Inc. were compared, the Scentry trapping system was found to be more attractive than Trécé Inc.'s (Frewin et al. 2017, Cha et al. 2018).

While plenty has been done to evaluate the relative efficacies of various trapping tools and methods, it is yet unknown how trapping numbers correspond to actual field population densities (Kirkpatrick et al. 2018). Among the information needed to translate capture numbers into absolute population density is the maximum distance that the pest can detect the trap from (Adams et al. 2017). This distance plausibly varies throughout the season as the abundance of various crop and non-crop fruits fluctuates (Lee et al. 2015, Klick et al. 2016), changing the olfactory environment and ability of the fly to detect traps. Furthermore, these effects are likely to vary among trap designs and attractants. However, no studies to date have specifically tested the effect of fruit presence on trapping efficacy for any of the commonly used *D. suzukii* trapping systems. Previous monitoring in Georgia blueberries using yeast-sugar-water traps has detected higher *D. suzukii* numbers in neighboring woodlands than in blueberry orchard interiors, and has also shown a greater peak in captures during the winter months than during the crop season (Grant 2016). Whether either or both of these monitoring trends can be partially explained by the effect of fruit phenology on trap efficacy remains to be seen.

Research questions to be addressed in this project include: (1) what is the impact of upper temperature extremes on *D. suzukii* survivorship and development to adulthood, and subsequent fertility and fecundity, (2) what are the relative distributions of *D. suzukii* in various habitats surrounding organic blueberry orchards, and how does this vary throughout the year, (3) what are the relative capacities of summer morph, winter morph, and pupal *D. suzukii* to survive the winter beneath leaf litter, and (4) how does the presence of blueberries in the field affect the efficiency of the commonly-used yeast-sugar-water solution baited trap?

The author hopes that the information generated here will further our understanding of biology and ecology of *D. suzukii*, help in increasing the resolution of population models that incorporate habitat, fruit phenology, and temperature parameters, and be useful for improving management practices. Ultimately, more reliable population models and cultural control could benefit *D. suzukii* IPM programs by making alternatives to calendar-based pesticide rotations more feasible, which would reduce costs to growers and non-target biota alike.

1. References

Adams, C. G., J. H. Schenker, P. S. McGhee, L. J. Gut, J. F. Brunner, and J. R. Miller. 2017. Maximizing Information Yield From Pheromone-Baited Monitoring Traps: Estimating Plume Reach, Trapping Radius, and Absolute Density of *Cydia pomonella* (Lepidoptera: Tortricidae) in Michigan Apple. Journal of Economic Entomology 110: 305-318.

Alyokhin, A., M. Baker, D. Mota-Sanchez, G. Dively, and E. Grafius. 2008. Colorado Potato Beetle Resistance to Insecticides. American Journal of Potato Research 85: 395-413.

Ansari, M. S., M. A. Moraiet, and S. Ahmad. 2014. Insecticides: Impact on the Environment and Human Health, pp. 99-123. In A. Malik, E. Grohmann and R. Akhtar (eds.), Environmental Deterioration and Human Health: Natural and anthropogenic determinants. Springer Netherlands, Dordrecht.

Asplen, M. K., G. Anfora, A. Biondi, D.-S. Choi, D. Chu, K. M. Daane, P. Gibert, A. P. Gutierrez, K. A. Hoelmer, W. D. Hutchison, R. Isaacs, Z.-L. Jiang, Z. Kárpáti, M. T. Kimura, M. Pascual, C. R. Philips, C. Plantamp, L. Ponti, G. Vétek, H. Vogt, V. M. Walton, Y. Yu, L. Zappalà, and N. Desneux. 2015. Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. Journal of Pest Science 88: 469-494.

Basden, E. B. 1955. XV.—The Distribution and Biology of Drosophilidæ (Diptera) in Scotland, including a New Species of *Drosophila*. Transactions of the Royal Society of Edinburgh 62: 603-654.

Basoalto, E., R. Hilton, and A. Knight. 2013. Factors affecting the efficacy of a vinegar trap for *Drosophila suzukii* (Diptera; Drosophilidae). Journal of Applied Entomology 137: 561-570.

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

Bergland, A. O., E. L. Behrman, K. R. O'Brien, P. S. Schmidt, and D. A. Petrov. 2014. Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*. PLOS Genetics 10: e1004775.

Brown, A. W. A. 1953. The Control of Insects by Chemicals. Canadian Journal of Public Health / Revue Canadienne de Sante'e Publique 44: 1-8.

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

Burrack, H., J. Ballington, J. Beam, D. Biddinger, B. Butler, B. Cline, R. Cowles, J. Cox, S. Dalton, D. Epstein, M. Evans, G. Fernandez, P. Fisher, H. Fraser, C. Geyer, R. Groves, J. Hampton, D. Horton, J. Jacobs, D. Johnson, G. Koehler, J. LaForest, O. Liburd, P. Liecsh, G. Loeb, D. Monks, P. Perkins-Veazie, D. Pfeiffer, D. Polk, J. Price, D. Robertson, S. Sambhav, B. Sampson, T. Schlenke, P. Shearer, M. Shrader, N. Singh, J. E. Smith, D. Stanaland, K. Swoboda, D. Trinka, S. Van Timmeren, J. VanKirk, J. Walgenbach, D. Weschler, and A. Wildes. 2012. Spotted wing *Drosophila* impacts in the eastern United States.

Burrack, H. J., M. Asplen, L. Bahder, J. Collins, F. A. Drummond, C. Guédot, R. Isaacs, D. Johnson, A. Blanton, J. C. Lee, G. Loeb, C. Rodriguez-Saona, S. van Timmeren, D. Walsh, and D. R. McPhie. 2015. Multistate Comparison of Attractants for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in Blueberries and Caneberries. Environmental Entomology 44: 704-712.

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

Cha, D. H., S. P. Hesler, A. K. Wallingford, F. Zaman, P. Jentsch, J. Nyrop, and G. M. Loeb. 2018. Comparison of Commercial Lures and Food Baits for Early Detection of Fruit Infestation Risk by *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 111: 645-652.

Cha, D. H., S. P. Hesler, S. Park, T. B. Adams, R. S. Zack, H. Rogg, G. M. Loeb, and P. J. Landolt. 2015. Simpler is better: fewer non-target insects trapped with a four-component chemical lure vs. a chemically more complex food-type bait for *Drosophila suzukii*. Entomologia Experimentalis et Applicata 154: 251-260.

Colinet, H., and A. A. Hoffmann. 2012. Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*. Functional Ecology 26: 84-93.

Dalton, D. T., V. M. Walton, P. W. Shearer, D. B. Walsh, 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 Management Science 67: 1368-1374.

Devine, G. J., and M. J. Furlong. 2007. Insecticide use: Contexts and ecological consequences. Agriculture and Human Values 24: 281-306.

E. Bragg, D., S. Rondon, J. Gavloski, U. Shankar, and D. P. Abrol. 2016. Integrated Pest Management in Tropical Cereal Crops.

Elsensohn, J. E., and G. M. Loeb. 2018. Non-Crop Host Sampling Yields Insights into Small-Scale Population Dynamics of *Drosophila suzukii* (Matsumura). Insects 9: 5.

Evans, R. 2016. Impact of Abiotic Factors on Behavior and Reproduction of *Drosophila suzukii* Under Field and Laboratory Conditions. Masters of Science Master's, University of Georgia Athens, Georgia.

Frewin, A. J., J. Renkema, H. Fraser, and R. H. Hallett. 2017. Evaluation of Attractants for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 110: 1156-1163.

Gautam, B., B. Little, M. Taylor, J. L. Jacobs, W. E. Lovett, R. M. Holland, and A. Sial. 2016. Effect of simulated rainfall on the effectiveness of insecticides against spotted wing drosophila in blueberries. 81: 122-128.

Georgia Info. 2014. Blueberries.

Gerken, A. R., O. C. Eller, D. A. Hahn, and T. J. Morgan. 2015. Constraints, independence, and evolution of thermal plasticity: Probing genetic architecture of long- and short-term thermal acclimation. Proceedings of the National Academy of Sciences 112: 4399-4404.

Goodhue, R. E., M. Bolda, D. Farnsworth, 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 Management Science 67: 1396-1402.

Grant, J. A. 2016. Investigation of Wild Flora Bordering Blueberry Fields to Quantify Their Potential as Viable Hosts of *Drosophila suzukii (*Diptera: Drosophilidae*)*. Master of Science, University of Georgia Athens, Georgia.

Hamby, K. A., D. E. Bellamy, J. C. Chiu, J. C. Lee, V. M. Walton, N. G. Wiman, R. M. York, and A. Biondi. 2016. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. Journal of Pest Science 89: 605-619.

Harris, T. J. C., and M. Peifer. 2005. The positioning and segregation of apical cues during epithelial polarity establishment in *Drosophila*. The Journal of Cell Biology 170: 813-823.

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 Management Science 67: 1352-1357.

Iglesias, L. E., T. W. Nyoike, and O. E. Liburd. 2014. Effect of Trap Design, Bait Type, and Age on Captures of *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Crops. Journal of Economic Entomology 107: 1508-1518.

Jakobs, R., T. D. Gariepy, and B. J. Sinclair. 2015. Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. Journal of Insect Physiology 79: 1-9.

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

Kinjo, H., Y. Kunimi, and M. Nakai. 2014. Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). 49: 297-304.

Kirkpatrick, D., P. McGhee, L. J. Gut, and J. R. Miller. 2017. Improving monitoring tools for spotted wing drosophila, *Drosophila suzukii*. 164.

Kirkpatrick, D. M., L. J. Gut, and J. R. Miller. 2018. Estimating Monitoring Trap Plume Reach and Trapping Area for *Drosophila suzukii* (Diptera: Drosophilidae) in Michigan Tart Cherry. Journal of Economic Entomology: toy062-toy062.

Kleiber, J. R., C. R. Unelius, J. C. Lee, D. M. Suckling, M. C. Qian, and D. J. Bruck. 2014. Attractiveness of Fermentation and Related Products to Spotted Wing Drosophila (Diptera: Drosophilidae). Environmental Entomology 43: 439-447.

Klick, J., W. Q. Yang, V. M. Walton, D. T. Dalton, J. R. Hagler, A. J. Dreves, J. C. Lee, and D. J. Bruck. 2016. Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation. Journal of Applied Entomology 140: 37-46.

Knight, A. L. 2008. Codling Moth Areawide Integrated Pest Management.

Kogan, M. 1998. Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annual Review of Entomology 43: 243-270.

Landolt, P., 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. Journal of applied entomology 136: 148-154.

Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Management Science 67: 1358-1367.

Lee, J. C., A. J. Dreves, A. M. Cave, S. Kawai, R. Isaacs, J. C. Miller, S. Van Timmeren, and D. J. Bruck. 2015. Infestation of Wild and Ornamental Noncrop Fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Annals of the Entomological Society of America 108: 117-129.

Lee, J. C., P. W. Shearer, L. D. Barrantes, E. H. Beers, H. J. Burrack, D. T. Dalton, A. J. Dreves, L. J. Gut, K. A. Hamby, D. R. Haviland, R. Isaacs, A. L. Nielsen, T. Richardson, C. R. Rodriguez-Saona, C. A. Stanley, D. B. Walsh, V. M. Walton, W. L. Yee, F. G. Zalom, and D. J. Bruck. 2013. Trap Designs for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Environmental Entomology 42: 1348-1355.

MacMillan, H. A., J. M. Knee, A. B. Dennis, H. Udaka, K. E. Marshall, T. J. S. Merritt, and B. J. Sinclair. 2016. Cold acclimation wholly reorganizes the *Drosophila melanogaster* transcriptome and metabolome. Scientific Reports 6: 28999.

Markow, T. A. 2015. The secret lives of *Drosophila* flies. eLife 4: e06793.

Porcelli, D., K. J. Gaston, R. K. Butlin, and R. R. Snook. 2017. Local adaptation of reproductive performance during thermal stress. Journal of Evolutionary Biology 30: 422-429.

Qin, W., S. J. Neal, R. M. Robertson, J. T. Westwood, and V. K. Walker. 2005. Cold hardening and transcriptional change in *Drosophila melanogaster*. Insect Molecular Biology 14: 607-613.

Renkema, J. M., R. Buitenhuis, and R. H. Hallett. 2014. Optimizing Trap Design and Trapping Protocols for *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 107: 2107-2118.

Rice, K. B., B. D. Short, S. K. Jones, and T. C. Leskey. 2016. Behavioral Responses of *Drosophila suzukii* (Diptera: Drosophilidae) to Visual Stimuli Under Laboratory, Semifield, and Field Conditions. Environmental Entomology 45: 1480-1488.

Rossi-Stacconi, M., R. Kaur, V. Mazzoni, L. Ometto, A. Grassi, A. Gottardello, O. Rota-Stabelli, and G. Anfora. 2016. Multiple lines of evidence for reproductive winter diapause in the invasive pest *Drosophila suzukii*: useful clues for control strategies. Journal of Pest Science 89: 689-700.

Scott, J. G., C. A. Leichter, F. D. Rinkevihc, S. A. Harris, C. Su, L. C. Aberegg, R. Moon, C. J. Geden, A. C. Gerry, D. B. Taylor, R. L. Byford, W. Watson, G. Johnson, D. Boxler, and L. Zurek. 2013. Insecticide resistance in house flies from the United States: Resistance levels and frequency of pyrethroid resistance alleles. Pesticide Biochemistry and Physiology 107: 377-384.

Stephens, A. R., M. K. Asplen, W. D. Hutchison, and R. C. Venette. 2015. Cold Hardiness of Winter-Acclimated *Drosophila suzukii* (Diptera: Drosophilidae) Adults, pp. 1619. ESA ENTOMOLOGICAL SOCIETY OF AMERICA, Great Britain.

Stern, V., R. Smith, R. Van den Bosch, and K. Hagen. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Hilgardia 29: 81-101.

Thistlewood, H. M. A., P. Gill, E. H. Beers, P. W. Shearer, D. B. Walsh, B. M. Rozema, S. Acheampong, S. Castagnoli, W. L. Yee, P. Smytheman, and A. B. Whitener. 2018.

Spatial Analysis of Seasonal Dynamics and Overwintering of *Drosophila suzukii* (Diptera: Drosophilidae) in the Okanagan-Columbia Basin, 2010-2014, pp. 221-232.

Tochen, S., D. T. Dalton, N. Wiman, C. Hamm, P. W. Shearer, and V. M. Walton. 2014. Temperature-Related Development and Population Parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. Environmental Entomology 43: 501-510.

Van Timmeren, S., and R. Isaacs. 2014. Corrigendum to "Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs" [Crop Prot. 54 (2013) 126–133]. Crop Protection 59: 81.

Wallingford, A. K., and G. M. Loeb. 2016. Developmental Acclimation of *Drosophila suzukii* (Diptera: Drosophilidae) and Its Effect on Diapause and Winter Stress Tolerance, pp. 1081. ESA ENTOMOLOGICAL SOCIETY OF AMERICA, Great Britain.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, 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. Journal of Integrated Pest Management 2: G1-G7.

Wang, X.-G., T. J. Stewart, A. Biondi, B. A. Chavez, C. Ingels, J. Caprile, J. A. Grant, V. M. Walton, and K. M. Daane. 2016. Population dynamics and ecology of *Drosophila suzukii* in Central California. Journal of Pest Science 89: 701-712.

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

Wiman, N. G., D. T. Dalton, G. Anfora, A. Biondi, J. C. Chiu, K. M. Daane, B. Gerdeman, A. Gottardello, K. A. Hamby, R. Isaacs, A. Grassi, C. Ioriatti, J. C. Lee, B. Miller, M. V. R. Stacconi, P. W. Shearer, L. Tanigoshi, X. Wang, and V. M. Walton. 2016. *Drosophila suzukii* population response to environment and management strategies. Journal of Pest Science 89: 653-665.

Wise, J. C., R. Vanderpoppen, C. Vandervoort, C. O'Donnell, and R. Isaacs. 2015. Curative activity contributes to control of spotted-wing drosophila (Diptera: Drosophilidae) and blueberry maggot (Diptera: Tephritidae) in highbush blueberry. The Canadian Entomologist 147: 109-117.

Zerulla, F. N., C. Augel, and C. P. W. Zebitz. 2017. Oviposition activity of *Drosophila suzukii* as mediated by ambient and fruit temperature. PLOS ONE 12: e0187682.

Zhai, Y., Q. Lin, J. Zhang, F. Zhang, L. Zheng, and Y. Yu. 2016. Adult reproductive diapause in *Drosophila suzukii* females. Journal of Pest Science 89: 679-688.

CHAPTER 2

IMPACTS OF HEAT STRESS ON THE DEVELOPMENT AND FERTILITY OF *DROSOPHILA* SUZUKII MATSUMURA (DIPTERA: DROSOPHILIDAE)

C. Kirk Green, Patricia J. Moore, and Ashfaq A. Sial*. Submitted to *Journal of Insect Physiology*.

Abstract

Drosophila suzukii is a globally invasive fruit pest that costs millions in yield losses and increased pest management costs. Management practices for *D. suzukii* currently rely heavily on calendar-based applications of broad-spectrum insecticides, but decision-based applications are theoretically possible with refined population modeling and monitoring. Temperature conditions are strongly deterministic of insect growth rates, fecundity, fertility, and resulting population densities. Therefore, information about the effects of temperature can be incorporated into population modeling to accurately predict *D. suzukii* population densities in the field, which is crucial to maximize pesticide application efficiency and improve sustainability. Here, the authors investigated the effects of heat stress during development on egg viability and fertility. We also investigated egg viability under heat stress after heat shock of the maternal parent. We found that heat stress during development resulted in lower egg viability, and reduced lifespan and fertility for surviving adults. However, heat-shock treatment of females prior to egg laying increased the viability of their eggs under heat stress. Female flies that developed at 30° C had smaller ovaries than the untreated group and male flies had less sperm in their testes, and no sperm in their seminal vesicles. We conclude that heat stress during development is likely to have negative effect on *D. suzukii* population abundance in the field. However, the intensity of such negative impact will depend on the phenotypic state of their maternal parents.

1. Introduction

Drosophila suzukii (Matsumura) is an invasive polyphagous fruit pest of global importance. Native to Southeast Asia, the host range of this dipteran has spread worldwide where it causes millions of dollars in economic losses to farmers of berries and small stone fruits (Del Fava et al. 2017). The adult female *D. suzukii* uses a serrated ovipositor to saw through the skin of intact ripe fruit and deposits its eggs in the interior. The eggs hatch into larvae that feed internally on the pulp of the fruit, rendering it damaged and unmarketable. Furthermore, oviposition wounds expose plants to secondary infection by bacteria, fungi, and other insects (Walsh et al. 2011). Economic losses are due to the increased cost of pest management, rejection of infested fruit shipments, and reduction of potential markets due to excessive insecticide residues (Bruck et al. 2011, Goodhue et al. 2011, Van Timmeren and Isaacs 2014).

Ideally, pest management programs should integrate all available tools to suppress pest populations at minimal cost and maximum efficiency. Among the foundational tools available to managers are insect traps, which are used to monitor for the presence of pest populations and estimate population density. These estimates can then be used to make informed decisions about when and where to apply insecticides and other control methods. Unfortunately, *D. suzukii* trap captures have not been shown to correlate strongly enough with fruit infestation to be a reliable basis for management decisions (Kirkpatrick et al. 2017). Because of this, management of *D. suzukii* is primarily done through the calendar-based application of broad-spectrum insecticides including pyrethroids, organophosphates, and spinosyns (Beers et al. 2011, Bruck et al. 2011, Van Timmeren and Isaacs 2014). Relying heavily on insecticide application to control insect populations accelerates the onset of insecticide resistance (Brown 1953, Alyokhin et al. 2008, Scott et al. 2013) and has adverse impacts on the environment and human health (Ansari et al.

2014). Additionally, broad-spectrum insecticide applications deplete the natural enemy populations that contribute to pest control (Devine and Furlong 2007). Therefore, an integrated approach to pest management is necessary to minimize insecticide application whenever possible to avoid these negative consequences.

One of the tools available for integrated pest management programs is population modeling. Population modeling that incorporates well-parameterized biotic and abiotic factors can be used to predict critical population density thresholds and time insecticide applications to maximize their effectiveness (Wiman et al. 2016). Temperature is among the most important abiotic factors that are known to be deterministic of *D. suzukii* developmental rates, viability, and fecundity. This is because *D. suzukii*, like all ectotherms, are unable to internally regulate their body temperature. Thus, their distribution is heavily influenced by climate, which varies considerably both regionally and within locales by microclimate (Sunday et al. 2014, Isaak et al. 2017). Optimal temperature for egg viability is known to be between 20° C and 26° C (Kinjo et al. 2014, Tochen et al. 2014, Hamby et al. 2016) and optimal temperatures for fecundity are around 22° C (Ryan et al. 2016, Zerulla et al. 2017). Reproduction and survival in this species are greatly reduced below 10° C and above 30° C (Kinjo et al. 2014, Tochen et al. 2014, Evans et al. 2018).

While the effects of temperature have been examined for some of its life history characters, the effects of heat stress during development on adult life history remain unexplored in *D. suzukii*. Adult flies can behaviorally regulate their temperature through movement, but their offspring are confined to the oviposition substrate. Immature *D. suzukii* are therefore likely to be subjected to suboptimal temperature conditions in the field. Larvae that survive these conditions into adulthood may have altered longevity and reproductive capacity. *D. suzukii* population modeling efforts would certainly need to incorporate these effects to be complete and accurate. Such effects have been documented among its congeners. For example, temperature fluctuations around the upper limit during development of *D. birchii* males resulted in reduced reproductive success in adulthood (Saxon et al. 2017). Additionally, *D. subobscura* have reduced sperm mobility and fertility following development in their upper temperature limits (Porcelli et al. 2017). In *D. ananassae*, development at the upper and lower temperature extremes resulted in adults with decreased thorax length, number of ovarioles, and testis length (Sisodia and Singh 2009) and *D. melanogaster* reared outside of their optimal temperature range have been found to have reduced adult longevity (Zwaan et al. 1991). Because of these studies and others (Cohet and David 1978, Crill et al. 1996, Huey et al. 1999), it is plausible to think that temperature during development could affect the viability and reproductive success of adult *D. suzukii*. Therefore, it is critical to explore the effects of temperature stress on the juveniles of this key pest in order to effectively manage it now, and in future climactic conditions.

Beyond the direct effects of temperature stress on juvenile development, it is also worth considering the effects of maternal temperature stress prior to egg laying. Such transgenerational phenotypic responses are documented in other organisms. *Drosophila melanogaster* reared at high temperatures produced offspring with increased fitness (Gilchrist and Huey 2001), and heat shocked adult springtails can have offspring with increased thermal resistance (Zizzari Zaira and Ellers 2014). Similar effects are also known in *Artemia* (Norouzitallab et al. 2014). It has been suggested that such transgenerational responses to stress can be possible through a variety of mechanisms including transfer of material to offspring, epigenetic inheritance, behavioral modification, and more (Badyaev 2005). While modified oviposition behavior has been demonstrated in *D. suzukii* in the presence of parasitoids (Poyet et al. 2017), the full potential for plastic transgenerational phenotypes in this species remains largely unexplored. Such effects could further contribute to our ability to model *D. suzukii* population densities and distributions and more accurately predict its ability to adapt to a changing climate.

The objectives of this study were to determine the effects of heat stress during development on egg viability and on adult fertility and reproductive organs in *D. suzukii*. We also investigated the potential for transgenerational phenotypic plasticity by exposing female *D. suzukii* to a heat shock treatment and then developing their eggs under heat stress. By exploring these questions, we will generate information that can be used to refine predictive population modeling for this species and contribute to the understanding of thermal tolerance in *Drosophila spp.* as a whole.

2. Materials and Methods

2.1 Insect rearing

Drosophila suzukii were obtained from a laboratory colony established from flies captured in Clarke County, GA in 2013. Flies were reared in 117-ml square bottom polypropylene bottles (model? ,Genesee Scientific, San Diego, CA) each containing 50 ml of standard fly diet as described in Jaramillo (Jaramillo, Mehlferber et al. 2015). A pinch of active dried baker’s yeast (part number, manufacturer, City, State) was sprinkled into each bottle. Bottles were capped with bonded dense-weave cellulose acetate plugs and placed on plastic trays in incubators (Model I36VLCB, Percival Scientific, Perry, IA) maintained at 24°C, 65% relative humidity, and a photoperiod of 14:10 (L:D) h. Adults between the ages of seven and fourteen days old were used for the experiment.

2.2 Egg viability experiment

In this experiment, we recorded the number of eggs that successfully developed into adulthood at the following three temperature treatments: 1) benign (24°C), 2) heat stress (30°C), or 3) maternal heat shock (30°C for four hours) + heat stress (30°C). Each temperature regulated incubator was kept at 65% relative humidity and a photoperiod of 14:10 (L:D). Eggs were collected by transferring adult flies between 10 and 21 days post emergence from stock cultures into ventilated 117-ml square bottom polypropylene bottles. The ventilated bottles were capped with a petri dish containing grape agar and a small amount of baker's yeast mixed in water. The bottles were inverted, then placed in an incubator overnight in benign conditions.

To assess the impact of heat shocking females on their eggs' hatching success, one group of mated females were separated from males and heat shocked at 30°C for four hours before being transferred into egg collection bottles with stock males. These bottles were then inverted and placed in the incubator set at benign conditions 24°C, 65% relative humidity, and a photoperiod of 14:10 (L:D) h. Following 12 hours of egg laying, adult flies were removed from all bottles and eggs were counted under a dissecting microscope. Groups of thirty eggs each were placed on a thin slice of grape agar and transferred to a polyethylene bottle containing standard fly media as described in section 2.1.

The bottles containing eggs produced by untreated females were placed in the incubators maintained at temperature treatments including benign (24°C) (n = 13) or heat stress (30°C) (n = 23) conditions and the bottles containing eggs produced by the heat shocked females were placed in incubator set at heat stress (30°C) (n = 10) conditions. The egg bottles were observed daily in each incubator, and every time an adult fly emerged it was transferred to a new bottle containing standard fly diet and placed in the benign incubator with other same-sex individuals that had emerged on the same day. The total number of male and female adults produced by each bottle was recorded. Adult flies were kept in this manner for six days and then used either for mating pairs to assess fertility in the second experiment (see fertility experiment below), or for dissections to assess reproductive organs. The egg bottles were observed until one week after the last adult had emerged before the bottles were discarded.

2.3 Fertility experiment

To assess the effects of development under heat stress on male and female reproduction, we used adult flies that survived the egg viability experiment to arrange mating pairs in a factorial design. Six days after adult emergence, one virgin male and one virgin female were paired in a new fly diet bottle. Males and females were paired treatment combinations for a total of four treatment groups including: benign male x benign female (BMBF) (n = 20), benign male x heat-stressed female (BMHF) (n = 10), heat-stressed male x benign female (HMBF) (n = 10), and heat-stressed male x heat-stressed female (HMHF) (n = 20). Pairs were left for five days in the benign incubator before being transferred to a new bottle. Pairs were left for another five days before being transferred to third bottle for a final five-day period, after which they were discarded. If the male died before a transfer this was recorded, and the female was transferred to a new bottle alone. If the female died this was recorded, and the male was discarded. The total number of male and female adults produced by each bottle was recorded, and bottles were observed for a week after the last adult had emerged before they were discarded.

2.4 Assessment of reproductive organs

A subset of the adult flies that survived the egg viability experiment was dissected to evaluate the status of their reproductive organs. Six days after eclosion, virgin adult females from the benign (n = 15) and heat stress (n = 20) treatment groups were chill anesthetized and their ovaries were dissected out in phosphate buffered saline (part number, ThermoFisher Scientific, City, State). Ovaries were held in their dissection well and photographed at 4x magnification with a Leica DFC295 stereomicroscope using Leica Application Suite morphometric software (LAS V4.1; Leica, Wetzlar, Germany). Ovary size was compared among samples by outlining the ovary and calculating a total area (mm^2) for each ovary using LAS V4.1 software. Likewise, virgin adult males from the benign (n = 10) and heat stress (n = 15) treatment groups were chill anesthetized and their reproductive system (testes, accessory glands, seminal vesicle, and ejaculatory duct) were dissected out in PBS. Whole reproductive systems were fixed in 4% formaldehyde in 1× PBS for 25 minutes. Fixed testes were stained with Hoechst 33342 (Sigma Aldrich) at 0.5 µg/ml. After fixing and rinsing, the ejaculatory duct and accessory glands were cut away in a dissection well in PBS, and each pair of testes was slide mounted with the seminal vesicle still attached.

Images of the testis tubules and seminal vesicles were captured using an EVOS fl Cell Imaging system (ThermoFisher Scientific) and DAPI filter set at 10x magnification for the tubule and 20x magnification for the seminal vesicle. The number of sperm nuclei clusters was counted in the tubule, and each replicate was assigned a rank based on the count (0 = None, 1 – 5 = Low, 6 – 15 = Medium, >15 = High). The presence or absence of tailed sperm was recorded in the seminal vesicle.

2.5 Data analysis

All data were analyzed in R version 3.4.1 (R Foundation for Statistical Computing; Vienna, Austria). Descriptive statistics were used to describe the outcomes for egg to adult viability and fertility for the different treatment groups. Responses were modeled from binomial or negative binomial distributions using generalized linear models (GLM), student's t-tests, and chi-squared tests of homogeneity to test for significant effects of experimental treatments. Analysis of deviance for GLM fit was used to identify significant explanatory variables, and insignificant variables were excluded from the models (McCullagh and Nelder 1989). Follow up Tukey's pairwise comparisons were made within significant explanatory variables to test for significant separations between or among means.

The egg to adulthood viability responses were modeled from a binomial distribution with a GLM and analysis of deviance was used to identify relevant explanatory factors. Tukey's pairwise comparisons were made to compare experimental temperature treatments. The fertility of the adult mating pairs was assessed with a GLM using a binomial distribution with the response variable of reproduction in 0, 1, 2, or 3 out of 3 vials as in Porcelli et al. (2016). The total number of progeny that survived into adulthood for each pair was modeled with a negative binomial GLM. The survivorship of adults in the pairs was modeled with a binomial GLM (Dowdy et al. 2005). The areas of the dissected ovaries were compared using student's t-test. The ranks given to the testis tubules based on number of sperm clusters were compared between temperature treatments using a chi-squared test of homogeneity. The presence or absence of tailed sperm in the males' seminal vesicles was compared using a chi squared test of homogeneity.

3. Results

3.1 Egg viability experiment

Heat stress during development had a significant effect on egg viability. Eggs developing in benign temperatures were the most likely to survive to adulthood. Analysis of deviance for GLM fits showed that temperature treatment explained a significant amount of the variance ($\chi^2 = 67.3$, $df = 2$, $P < 0.001$) in the development from egg to adult among experimental units. In pairwise comparisons, eggs developing under heat stress were significantly less likely to emerge compared to adults developing in benign conditions (z = -7.94, $P < 0.001$). Heat shock treatment of females prior to egg laying increased egg viability to more than the heat stress group (z = 3.18, P = 0.004) but still less than the benign group (z = -4.23, $P < 0.001$; Fig. 1).

3.2 Fertility experiment

The fertility of adult *D. suzukii* was significantly affected by heat stress during development. Flies that developed at 30°C had a drop from ~75% to ~50% egg viability. Reproductive success was lower in pairs that included heat-stressed males, i.e., HMBF and HMHF than the pairs that included only heat-stressed females (BMHF); 95% of BMBF pairs reproduced in at least one bottle, compared to 70% of BMHF, 20% HMBF, and 15% HMHF. Except for the benign male with benign female treatment (BMBF), all treatment combinations had a mean of less than two offspring (Fig. 2). Analysis of deviance for GLM fits showed that heat stress treatment explained a significant amount of the variance in whether a pair could reproduce ($\chi^2 = 134$, *df* = 3, $P < 0.001$). In pairwise comparisons between numbers of adult progeny produced by the treatment combinations, all treatments differed significantly except for HMHF and HMBF (z = -1.09, P = 0.674).

Heat stress during development also reduced the survivorship of flies that successfully emerged as adults (z = 3.31, $P < 0.001$). During the fertility trial, 100% of males that developed under heat stress had died by the end of day sixteen post-eclosion, as well as more than half of the females that developed under heat stress. No more than fifteen percent of the flies that developed under benign temperature died through the course of the trial (Fig. 3).

3.3 Assessment of reproductive organs

Female *D. suzukii* that developed under heat stress had a reduced ovary size compared to those that developed in benign conditions (Fig. 4). A two-tailed student's t-test comparing the summed area of the two outlined ovaries in each treatment showed a significant difference ($t = 7.944$, $P < 0.001$) between the means of the benign (mean = 1.31 mm^2 ± 0.141 95% CI) and high temperature regiments (mean = 0.64 mm^2 ± 0.084 95% CI).

Male *D. suzukii* that developed under heat stress had reductions in both the amount of sperm in the testis (Fig. 5) and the presence of sperm in the seminal vesicles (Fig. 6). A chi-squared test of homogeneity comparing the ranked number of sperm head clusters in the tubules against temperature treatment showed a significant difference between the treatment groups ($\chi^2 = 16.25$, $df = 3$, $P = 0.001$). A chi-squared test of homogeneity comparing the presence/absence of sperm in the seminal vesicle against temperature treatment also returned significance ($\chi^2 = 17.37$, $df = 1$, $P < 0.001$).

4. Discussion

Our findings demonstrate the importance of considering juvenile responses to temperature conditions when predicting insect population densities and species ranges. We observed a severe reduction in egg viability at 30°C, which expands on the results of previous studies that have found that the rate of development, survivorship, life span, and rate of reproduction in this species are all severely reduced or abolished above 30°C (Hamby et al. 2013, Kinjo et al. 2014, Tochen et al. 2014, Evans 2016, Evans et al. 2018). In insects, survival curves over time spent in high temperature stress typically have a point where they drop off rapidly, with the length of the shoulder of the curve inversely related to the temperature level (Hallman et al. 1998). The reduction in viability we see here is consistent with a damaging, but not lethal temperature stress; while some eggs were able to hatch and survive into adulthood, it was at a lower rate and with severe consequences for the survivors. Insects have various physiological mechanisms by which they can survive thermal damage. For example, heat stress is known to induce the production of heat shock proteins in animals through changes in the topology of DNA, RNA, and proteins and lipids, as well as through accumulation of denatured and damaged proteins. Heat shock proteins can mitigate thermal damage in both high and low temperature stress conditions by repairing damaged proteins and preventing denaturation (Richter et al. 2010). However, producing these proteins has an energetic cost and can result in trade-offs for juvenile and adult *Drosophila spp.* (Krebs and Loeschcke 1994, Krebs and Feder 1998).

This study, for the first time, demonstrated that exposure of *D. suzukii* females to heat shock prior to egg laying enhances the ability of their eggs to tolerate heat stress. Other *Drosophila spp.* are known to exhibit phenotypic plasticity in response to temperature extremes across a variety of timescales and using a wide array of physiological mechanisms (Qin et al. 2005, Colinet and Hoffmann 2012, Bergland et al. 2014, Gerken et al. 2015, MacMillan et al. 2016). Among these timescales are transgenerational responses with varying effects depending on the temperature conditions of parent and offspring. For example, the transgenerational effect of heat shocking females *D. melanogaster* was shown to reduce egg hatching success in benign conditions, signaling a trade-off between adult somatic maintenance and egg quality (Silbermann and Tatar 2000). Similarly, the offspring of *Tribolium castaneum* males exposed to heat shock were shown to have impaired reproductive performance (Sales et al. 2018). However, the effects of heat shock followed by juvenile heat stress was not investigated in either of these studies. It may be possible that while heat shocking adults reduces their egg viability and offspring fertility in benign conditions, it enhances those traits under heat stress. Lockwood *et al.* (2017) induced overexpression of the *Hsp23* gene in *D. melanogaster* ovaries and found increased thermal tolerances in their offspring. The increased egg viability at 30°C observed in this study suggests that the proximal cause of this effect in *D. suzukii* may be similar to that observed in *D. melanogaster*. Taking this effect into consideration, predictions about the viability of *D. suzukii* eggs based on ambient temperature may be inaccurate if they do not account for the phenotypic state of their maternal parents.

Larvae that were able to complete development under heat stress suffered a severe reduction in lifespan, fertility, and number of successful offspring compared to the benign group. These observations lend further support to the idea that the process of mitigating thermal damage has an energetic cost, which results in trade-offs for organisms (Krebs and Loeschcke 1994, Krebs and Feder 1998). Here, we are the first to observe these trade-offs following larval *D. suzukii* into adulthood. Interestingly, we observed that these effects were even more pronounced in males than females. Previous work on *Drosophila spp.* and *Tribolium castaneum* similarly found that males are more sensitive to stressful temperature conditions (Pétavy et al. 2001, David et al. 2005, Ryan et al. 2016, Porcelli et al. 2017, Sales et al. 2018), and here we are able to confirm this sensitivity holds true for male *D. suzukii* after development under heat stress. Comparing the developmental temperature combinations from the fertility experiment shows us that the thermal sensitivity of males during development can be a limiting factor for the ability of this species to reproduce and adapt to local climates. Other studies have pointed to the effects of heat stress during development of male *Drosophila spp.* as an important target for natural selection (Jørgensen et al. 2006, Porcelli et al. 2017, Saxon et al. 2017). Considering the sex-specific effects of heat stress during development of this species is important not just for modeling contemporary distributions, but also for predicting its adaptive potential in a rapidly changing climate. With consideration to the fact that juvenile *D. suzukii* are confined to the environment of their mother's choosing, these findings imply that population growth is limited by the availability of ovipositional substrate in suitable thermal environments.

When dissected, there were obvious differences in the reproductive structures for both sexes of *D. suzukii*. In females, a dramatic reduction in the average size of ovaries could be due to damage to the reproductive system and/or energetic trade-offs between reproductive output and homeostasis. Similarly, the number of visible sperm clusters in the testis tubules was much less than in the benign group for males, and the seminal vesicles lacked visible sperm. This inability to load sperm into the seminal vesicle provides a mechanistic explanation for the infertility of mating pairs which included a heat-stressed male. Porcelli *et al.* (2017) observed a similar effect in *D. subobscura* males after development at high temperatures, where there was a significant reduction in motile sperm found in the seminal vesicles. Overall, we found strong evidence for an organ-level effect of temperature on the ability of *D. suzukii* to reproduce. However, it is also possible that behavioral factors contributed to the differences in fertility and number of successful offspring observed among treatment groups. For example, acclimation to cold temperatures in *D. melanogaster* resulted in decreased courtship efficiency and mating success (Everman et al. 2018), and exposing three different *Drosophila spp.* to high temperature stress reduced courtship and mating success for all three species (Patton and Krebs 2001). Behavioral assays following development at upper temperature thresholds in *D. suzukii* may reveal a similar effect.

In conclusion, our results clearly demonstrate the negative effects of heat stress during larval development on egg viability and fertility of the surviving *D. suzukii* adults and the first to show organ-level evidence of these effects. Our studies also for the first time provide evidence for adult *D. suzukii* plastically imparting a phenotypic thermotolerance response onto its offspring. This information about the effects of high temperature stress during development can serve as a starting point for the refinement of predictive modeling of this key pest's populations by pointing to relevant explanatory variables that have not been included in previous models. As evidenced here and in other studies, limits on reproductive ability are arguably more relevant factors to consider than limits on survival when estimating population dynamics and predicting the adaptive potential of species in a changing climate (David et al. 2005, Jørgensen et al. 2006, Porcelli et al. 2017). Future studies should aim to investigate the effects of heat stress during development with even greater resolution and elucidate the mechanisms by which developmental plasticity is imparted to offspring by females exposed to high temperature stress.

5. References

Alyokhin, A., M. Baker, D. Mota-Sanchez, G. Dively, and E. Grafius. 2008. Colorado Potato Beetle Resistance to Insecticides. American Journal of Potato Research 85: 395-413.

Ansari, M. S., M. A. Moraiet, and S. Ahmad. 2014. Insecticides: Impact on the Environment and Human Health, pp. 99-123. In A. Malik, E. Grohmann and R. Akhtar (eds.), Environmental Deterioration and Human Health: Natural and anthropogenic determinants. Springer Netherlands, Dordrecht.

Badyaev, A. V. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proceedings of the Royal Society of London B: Biological Sciences 272: 877-886.

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

Bergland, A. O., E. L. Behrman, K. R. O'Brien, P. S. Schmidt, and D. A. Petrov. 2014. Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*. PLOS Genetics 10: e1004775.

Brown, A. W. A. 1953. The Control of Insects by Chemicals. Canadian Journal of Public Health / Revue Canadienne de Sante'e Publique 44: 1-8.

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

Cohet, Y., and J. David. 1978. Control of the Adult Reproductive Potential by Preimaginal Thermal Conditions. A Study in *Drosophila melanogaster*. Oecologia: 295.

Colinet, H., and A. A. Hoffmann. 2012. Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*. Functional Ecology 26: 84-93.

Crill, W. D., R. B. Huey, and G. W. Gilchrist. 1996. Within- and Between-Generation Effects of Temperature on the Morphology and Physiology of *Drosophila melanogaster.* Evolution 50: 1205-1218.

David, J. R., L. O. Ararpie, M. Chakir, H. Legout, B. Lemos, G. Petavy, C. Rohmer, D. Joly, and B. Moreteau. 2005. Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. Journal of Evolutionary Biology 18: 838-846.

Del Fava, E., C. Ioriatti, and A. Melegaro. 2017. Cost–benefit analysis of controlling the spotted wing drosophila (*Drosophila suzukii* (Matsumura)) spread and infestation of soft fruits in Trentino, Northern Italy. Pest Management Science 73: 2318-2327.

Devine, G. J., and M. J. Furlong. 2007. Insecticide use: Contexts and ecological consequences. Agriculture and Human Values 24: 281-306.

Dowdy, S., S. Wearden, and D. Chilko. 2005. Statistics for Research, 3 ed. John Wiley & Sons, Inc., Hoboken, New Jersey.

Evans, R. 2016. Impact of Abiotic Factors on Behavior and Reproduction of *Drosophila suzukii* Under Field and Laboratory Conditions. Masters of Science Master's, University of Georgia Athens, Georgia.

Evans, R. K., M. D. Toews, and A. A. Sial. 2018. Impact of short- and long-term heat stress on reproductive potential of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Journal of Thermal Biology 78: 92-99.

Everman, E. R., J. L. Delzeit, F. K. Hunter, J. M. Gleason, and T. J. Morgan. 2018. Costs of cold acclimation on survival and reproductive behavior in *Drosophila melanogaster*. PLoS ONE 13: 1-18.

Gerken, A. R., O. C. Eller, D. A. Hahn, and T. J. Morgan. 2015. Constraints, independence, and evolution of thermal plasticity: Probing genetic architecture of long- and short-term thermal acclimation. Proceedings of the National Academy of Sciences 112: 4399-4404.

Gilchrist, G. W., and R. B. Huey. 2001. Parental and Developmental Temperature Effects on the Thermal Dependence of Fitness in *Drosophila melanogaster*. Evolution 55: 209-214.

Goodhue, R. E., M. Bolda, D. Farnsworth, 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 Management Science 67: 1396-1402.

Hallman, G. J., D. L. Denlinger, J. P. Costanzo, D. J. Horn, C. W. Hoy, M. R. Lee, R. E. J. Lee, R. A. Leopold, R. K. Lindquist, R. L. Mangan, L. J. Mason, D. A. Reierson, M. K. Rust, A. C. Strait, and G. D. Yocum. 1998. Temperature sensitivity in insects and application in integrated pest management, Westview Press, United States.

Hamby, K. A., R. S. Kwok, F. G. Zalom, and J. C. Chiu. 2013. Integrating Circadian Activity and Gene Expression Profiles to Predict Chronotoxicity of *Drosophila suzukii* Response to Insecticides. PLOS ONE 8: e68472.

Hamby, K. A., D. E. Bellamy, J. C. Chiu, J. C. Lee, V. M. Walton, N. G. Wiman, R. M. York, and A. Biondi. 2016. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. Journal of Pest Science 89: 605-619.

Huey, R. B., D. Berrigan, G. W. Gilchrist, and J. C. Herron. 1999. Testing the adaptive significance of acclimation: a strong inference approach. American Zoologist 39: 323-336.

Isaak, D. J., S. J. Wenger, and M. K. Young. 2017. Big biology meets microclimatology: defining thermal niches of ectotherms at landscape scales for conservation planning. Ecological Applications 27: 977-990.

Jaramillo, S. L., E. Mehlferber, and P. J. Moore. 2015. Life-history trade-offs under different larval diets in *Drosophila suzukii* (Diptera: Drosophilidae). Physiological Entomology 40: 2-9.

Jørgensen, K. T., J. G. Sørensen, and J. Bundgaard. 2006. Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males. Journal of Thermal Biology 31: 280-286.

Kinjo, H., Y. Kunimi, and M. Nakai. 2014. Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). 49: 297-304.

Kirkpatrick, D., P. McGhee, L. J. Gut, and J. R. Miller. 2017. Improving monitoring tools for spotted wing drosophila, *Drosophila suzukii*. 164.

Krebs, R. A., and V. Loeschcke. 1994. Costs and Benefits of Activation of the Heat-Shock Response in *Drosophila melanogaster*. Functional Ecology 8: 730-737.

Krebs, R. A., and M. E. Feder. 1998. Hsp70 and larval thermotolerance in *Drosophila melanogaster:* how much is enough and when is more too much? Journal of Insect Physiology 44: 1091-1101.

MacMillan, H. A., J. M. Knee, A. B. Dennis, H. Udaka, K. E. Marshall, T. J. S. Merritt, and B. J. Sinclair. 2016. Cold acclimation wholly reorganizes the *Drosophila melanogaster* transcriptome and metabolome. Scientific Reports 6: 28999.

McCullagh, P., and J. A. Nelder. 1989. Generalized Linear Models, 2nd ed. CRC Press.

Norouzitallab, P., K. Baruah, M. Vandegehuchte, G. Van Stappen, F. Catania, J. Vanden Bussche, L. Vanhaecke, P. Sorgeloos, and P. Bossier. 2014. Environmental heat stress induces epigenetic transgenerational inheritance of robustness in parthenogenetic Artemia model. The FASEB Journal 28: 3552-3563.

Patton, Z. J., and R. A. Krebs. 2001. The effect of thermal stress on the mating behavior of three *Drosophila* species. Physiological And Biochemical Zoology: PBZ 74: 783-788.

Pétavy, G., J. R. David, P. Gibert, and B. Moreteau. 2001. Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. Journal of Thermal Biology 26: 29-39.

Porcelli, D., K. J. Gaston, R. K. Butlin, and R. R. Snook. 2017. Local adaptation of reproductive performance during thermal stress. Journal of Evolutionary Biology 30: 422-429.

Poyet, M., P. Eslin, O. Chabrerie, S. M. Prud'homme, E. Desouhant, and P. Gibert. 2017. The invasive pest *Drosophila suzukii* uses trans-generational medication to resist parasitoid attack. Scientific Reports 7: 43696.

Qin, W., S. J. Neal, R. M. Robertson, J. T. Westwood, and V. K. Walker. 2005. Cold hardening and transcriptional change in *Drosophila melanogaster*. Insect Molecular Biology 14: 607-613.

Richter, K., M. Haslbeck, and J. Buchner. 2010. The Heat Shock Response: Life on the Verge of Death. Molecular Cell 40: 253-266.

Ryan, G. D., L. Emiljanowicz, F. Wilkinson, M. Kornya, and J. A. Newman. 2016. Thermal Tolerances of the Spotted-Wing Drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 109: 746-752.

Sales, K., R. Vasudeva, M. E. Dickinson, J. L. Godwin, A. J. Lumley, Ł. Michalczyk, L. Hebberecht, P. Thomas, A. Franco, and M. J. G. Gage. 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nature Communications 9: 4771.

Saxon, A. D., E. K. O'Brien, and J. R. Bridle. 2017. Temperature fluctuations during development reduce male fitness and may limit adaptive potential in tropical rainforest *Drosophila*. Journal of Evolutionary Biology 31: 405-415.

Scott, J. G., C. A. Leichter, F. D. Rinkevihc, S. A. Harris, C. Su, L. C. Aberegg, R. Moon, C. J. Geden, A. C. Gerry, D. B. Taylor, R. L. Byford, W. Watson, G. Johnson, D. Boxler, and L. Zurek. 2013. Insecticide resistance in house flies from the United States: Resistance levels and frequency of pyrethroid resistance alleles. Pesticide Biochemistry and Physiology 107: 377-384.

Silbermann, R., and M. Tatar. 2000. Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. Evolution 54: 2038-2045.

Sisodia, S., and B. N. Singh. 2009. Variations in morphological and life-history traits under extreme temperatures in *Drosophila ananassae*. Journal of Biosciences 34: 263-274.

Sunday, J. M., A. E. Bates, M. R. Kearney, R. K. Colwell, N. K. Dulvy, J. T. Longino, and R. B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences 111: 5610-5615.

Tochen, S., D. T. Dalton, N. Wiman, C. Hamm, P. W. Shearer, and V. M. Walton. 2014. Temperature-Related Development and Population Parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. Environmental Entomology 43: 501-510.

Van Timmeren, S., and R. Isaacs. 2014. Corrigendum to “Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs” [Crop Prot. 54 (2013) 126–133]. Crop Protection 59: 81.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, 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. Journal of Integrated Pest Management 2: G1-G7.

Wiman, N. G., D. T. Dalton, G. Anfora, A. Biondi, J. C. Chiu, K. M. Daane, B. Gerdeman, A. Gottardello, K. A. Hamby, R. Isaacs, A. Grassi, C. Ioriatti, J. C. Lee, B. Miller, M. V. R. Stacconi, P. W. Shearer, L. Tanigoshi, X. Wang, and V. M. Walton. 2016. *Drosophila suzukii* population response to environment and management strategies. Journal of Pest Science 89: 653-665.

Zerulla, F. N., C. Augel, and C. P. W. Zebitz. 2017. Oviposition activity of *Drosophila suzukii* as mediated by ambient and fruit temperature. PLOS ONE 12: e0187682.

Zizzari Zaira, V., and J. Ellers. 2014. Rapid shift in thermal resistance between generations through maternal heat exposure. Oikos 123: 1365-1370.

Zwaan, B., R. Bijlsma, and R. Hoekstra. 1991. On the developmental theory of ageing. I. Starvation resistance and longevity in Drosophila melanogaster in relation to pre-adult breeding conditions. Heredity 66: 29.

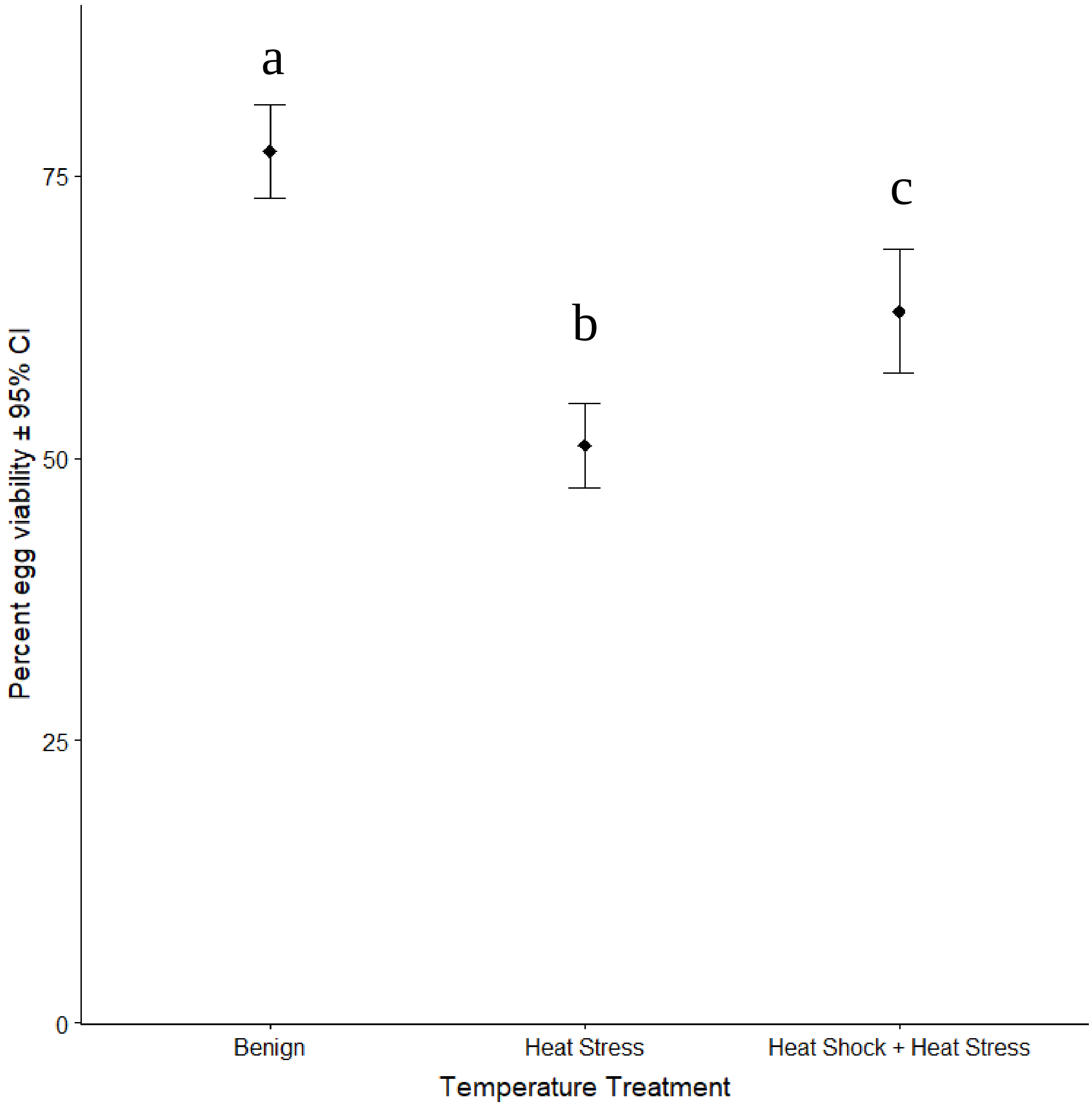

Fig. 2.1. Percent of *D. suzukii* eggs that survived into adulthood for each treatment group ± 95% confidence intervals. Benign and heat groups developed in 24°C and 30°C, respectively. The heat shock group was produced by adult female *D. suzukii* exposed to a brief heat shock prior to egg laying, and then their eggs were allowed to develop at 30°C.

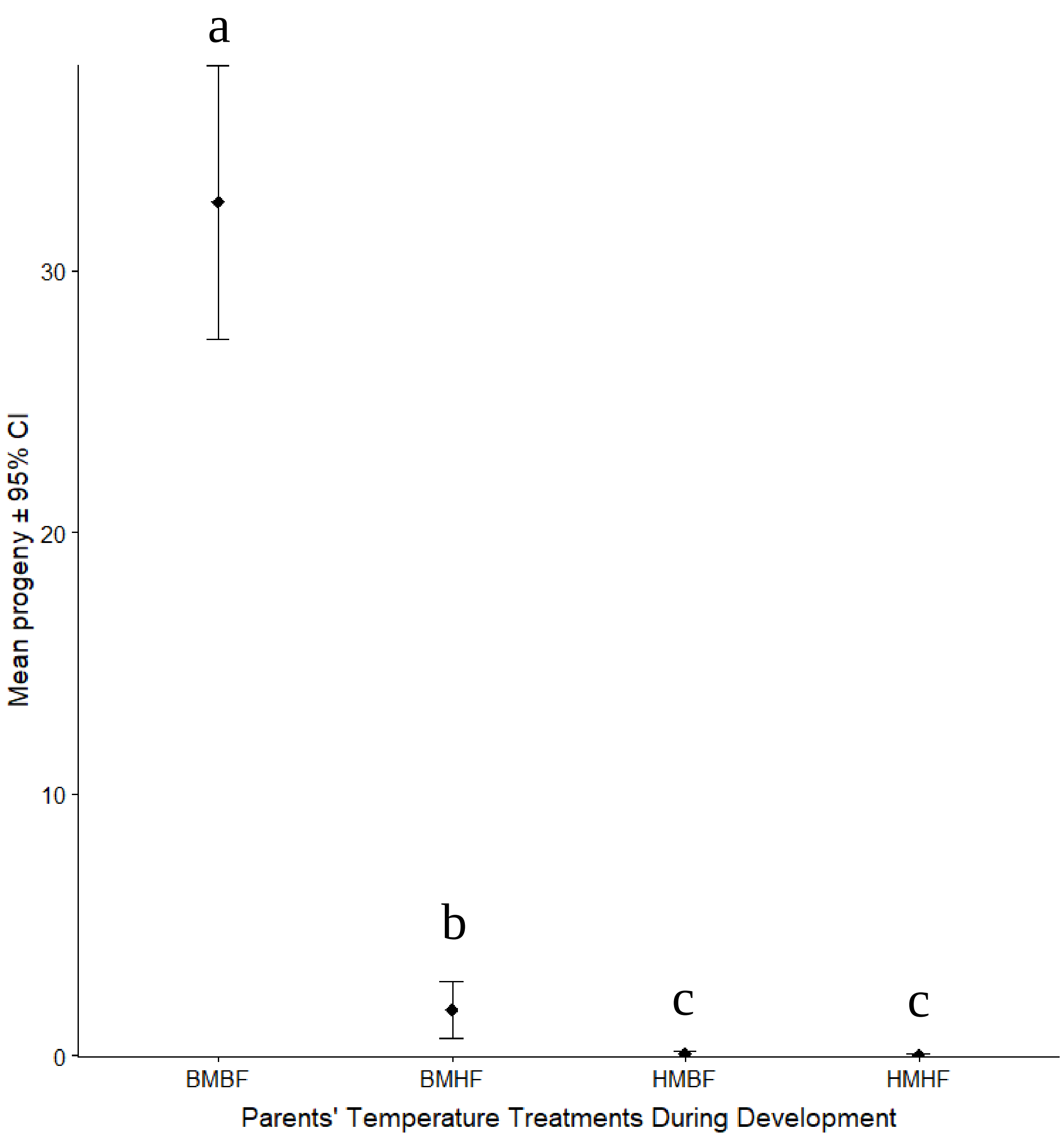

Fig. 2.2. Progeny for each of the adult treatment combinations (Mean ± 95% confidence interval). Mean is for all bottles in which a female fly was allowed to lay eggs. From left to right, treatment combinations are benign male x benign female (BMBF), benign male x heat-stressed female (BMHF), heat-stressed male x benign female (HMBF), and heat-stressed male x heat-stressed female (HMHF).

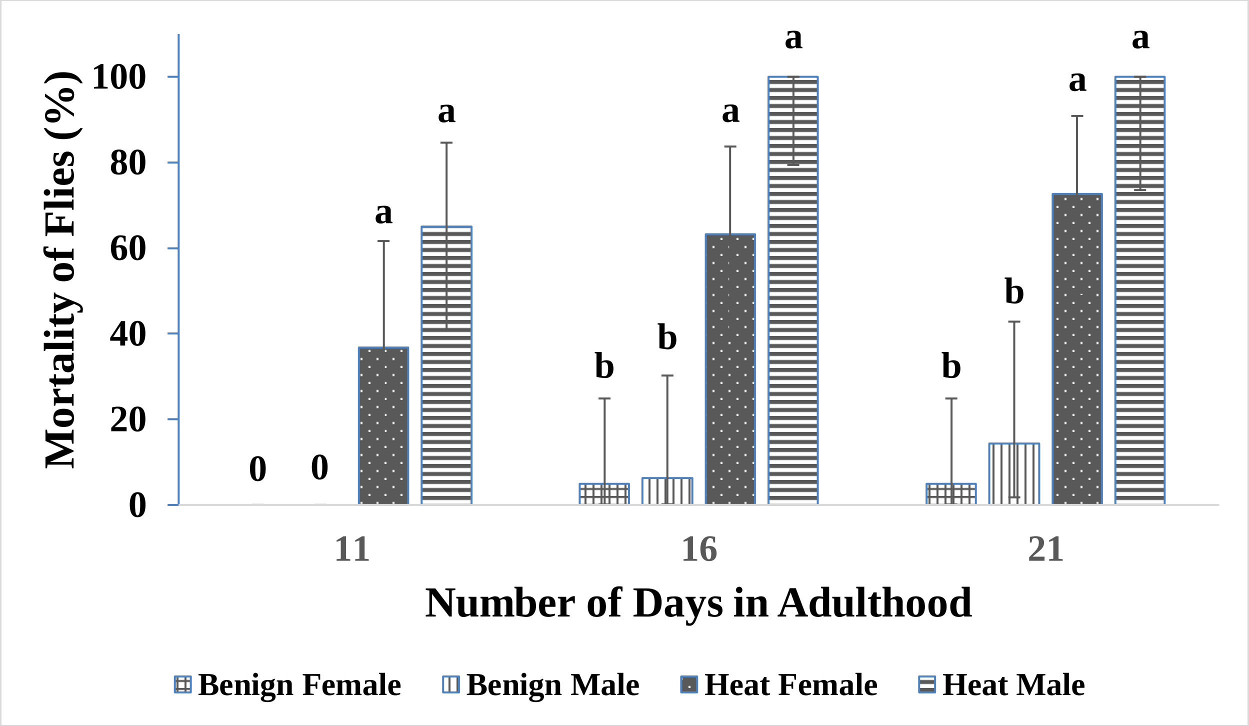

Fig. 2.3. Percent mortality of flies among *D. suzukii* at 11, 16, and 21 days old grouped by sex and temperature treatment during development. Living males that were discarded because their females had died were excluded from subsequent calculations. Letters denote significance within observation dates.

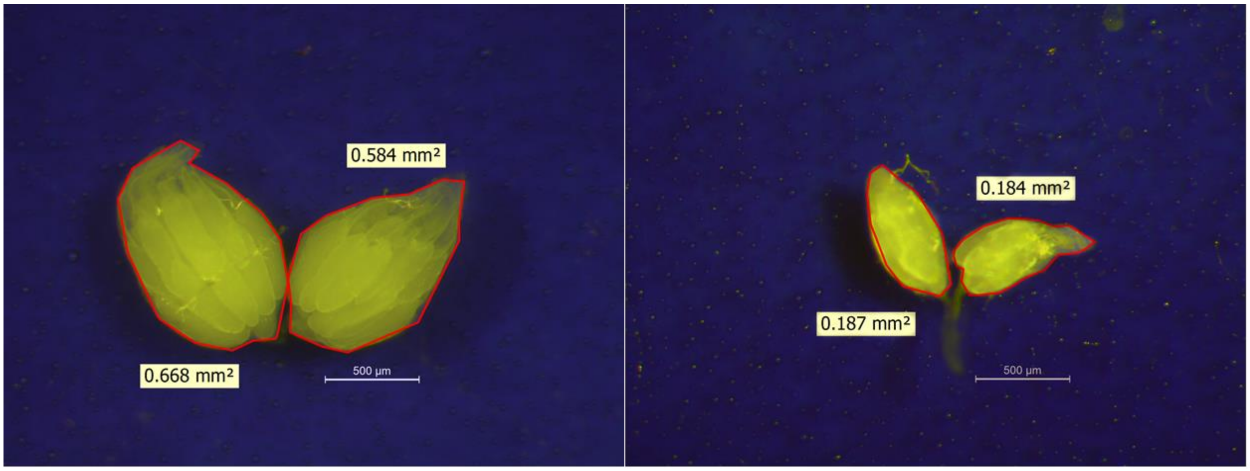

Fig. 2.4. Representative ovaries of the adult females from the benign group (left) and the heat stress treatment group (right)

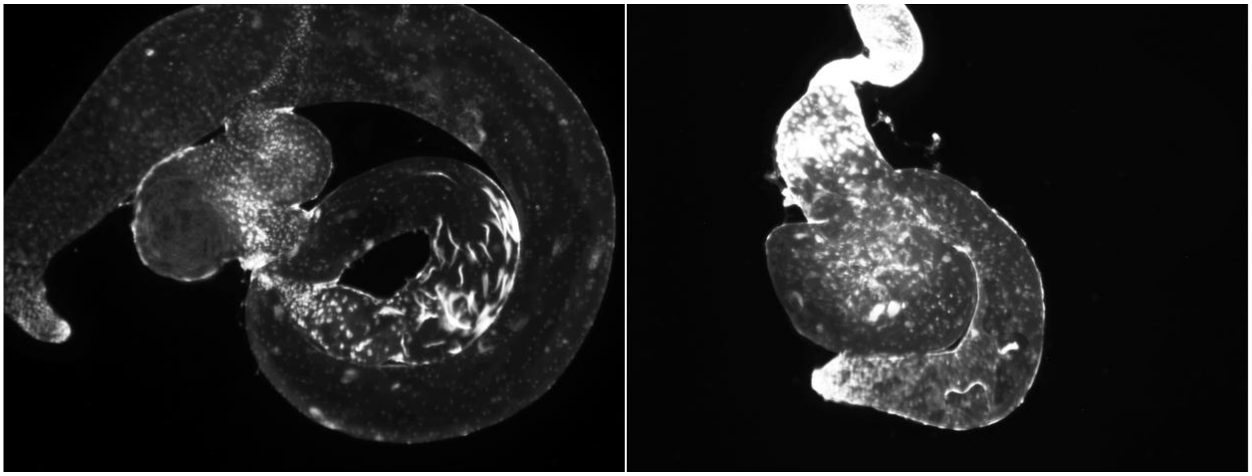

Fig. 2.5. Representative Hoechst-stained testis tubules of adult males from the benign group (left) and the heat stress treatment group (right).

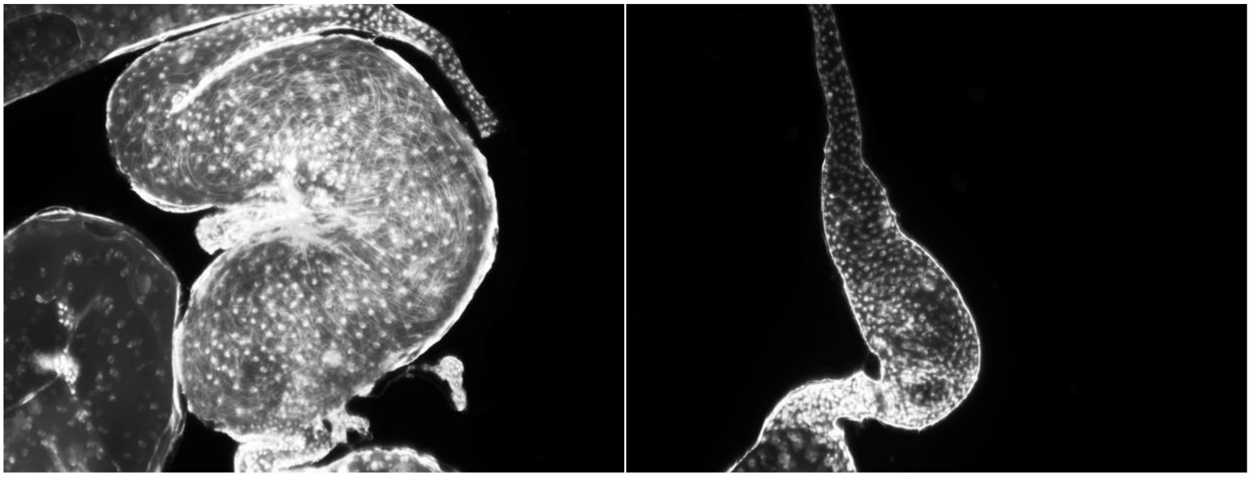

Fig. 2.6. Representative Hoechst-stained seminal vesicles of adult males from the benign (left) and heat stress treatment group (right).

CHAPTER THREE

INVESTIGATION OF YEAR-ROUND HABITAT RESOURCES AVAILABLE TO *DROSOPHILA SUZUKII* MATSUMURA (DIPTERA: DROSOPHILIDAE) IN GEORGIA BLUEBERRY ORCHARDS

C. Kirk Green and Ashfaq A. Sial. To be submitted to *Environmental Entomology*.

Abstract

Drosophila suzukii is a globally invasive fruit pest of economic importance. Invasion patterns for phytophagous insect species are influenced by the availability of suitable habitat including host plants and shelter from harmful ambient temperatures. While previous *D. suzukii* phenology work has been done in the state of Georgia, the yearly population trends among commonly found habitats surrounding blueberry orchards has not been examined. Additionally, the overwintering biology of *D. suzukii* remains poorly understood globally and in Georgia. In this study, we conducted year-round trapping in organic blueberry orchards and surrounding pine, deciduous forest, non-crop host, and riparian habitats. We also compared the relative overwintering survival abilities of *D. suzukii* summer morphs, winter morphs, and pupae using field bioassay chambers insulated by leaf litter. We found that in all habitats, the largest peak in *D. suzukii* captures was from June 28 – July 24. A second, smaller peak occurs for the deciduous and pine forests from October 13 – December 22. We also find that the male:female capture ratio changes throughout the year, with females dominating from mid- September to mid- March. In the overwintering bioassay, we found that ambient conditions were much more hospitable below the leaf litter than above; some pupae and female *D. suzukii* survived the full duration of the experiment. Winter morph survival did not differ significantly from that of summer morphs. Our findings corroborate studies from other regions, which suggest that *D. suzukii* take shelter in the woodlands during the winter, with evergreens providing the most protection. Our findings also support the idea that pupal and adult female *D. suzukii* may utilize leaf litter as an additional shelter resource to survive the winter.

1. Introduction

Global networks of trade and travel facilitate the invasion of non-native species and result in damage to both the environment and the economy (Meyerson and Mooney 2007). The ubiquity of commercial land use for agriculture further compounds the issue by creating large areas of monoculture; this simultaneously provides resources for herbivorous species of invasive arthropod and reduces native biodiversity, making ecosystems less resilient against invasion (With 2002). Indeed, simulation shows that availability of suitable habitat is the most important factor for determining both the stability and rate of spread for invasive insect populations (Lustig et al. 2017), and this is supported by empirical studies (Peterson 1997, Kamala Jayanthi and Verghese 2011). Thus, it is critical to understand habitat components that provide resources for invasive species when responding to the threats they pose at the local and regional level.

Habitat resources for insects include feeding, reproductive, and shelter resources. Identifying these resources at one point in time and in one location is not enough to form a full understanding of an insect's habitat; resource availability varies throughout time and seasons, shaping pest population densities both spatially and in absolute magnitude (Peterson 1997, Kamala Jayanthi and Verghese 2011, Uyi et al. 2018). Furthermore, insect populations have enormous adaptive potential, underlining the necessity of study at both the regional and local scales throughout time and space (Merrill and Robert 1998, Boll et al. 2006, Colinet and Hoffmann 2012, Bergland et al. 2014). Central to this concept is the need for insects to find food and shelter during time periods when their main crop host is unavailable and thermal conditions are inhospitable. Insects must contend with harsh conditions during winter and conditions vary with latitude, altitude, and proximity to bodies of water at the regional level. At the local level, vegetation, ground cover, and snow cover create even more variety in environmental conditions

(Leather et al. 1993). Insects that successfully survive the winter in off-season habitats are able to re-infest crops locally and then spread to higher latitudes where they are unable to overwinter. Overwintering habitats are therefore a central contributor to the persistence of invasive insect species in their introduced range.

Drosophila suzukii, also known as the spotted wing fruit fly (SWD), is one such invasive insect species. *D. suzukii* is native to Southeast Asia and has quickly become a costly economic burden of global importance (Del Fava et al. 2017). Females of this species are able to saw through the skin of berries and small stone fruits to deposit their eggs that hatch and feed on the fruit internally, rendering it unmarketable (Walsh et al. 2011). Its wide range of suitable host fruit combined with high dispersal abilities make this a particularly difficult pest to manage (Lee et al. 2011, Lee et al. 2015, Klick et al. 2016, Tait et al. 2018).

Drosophila suzukii phenology has been studied in various regions and cropping systems throughout the United States. These systems include blueberry (Grant 2016, Van Timmeren et al. 2017, Jaffe et al. 2018), cherry (Haviland et al. 2016, Wang et al. 2016), raspberry (Hamby et al. 2014, Klick et al. 2016, Jaffe et al. 2018), citrus (Wang et al. 2016), and more, including mixed-crop production systems (Harris et al. 2014, Grant 2016). Multiple studies have found that *D. suzukii* can migrate into and out of the field based on host fruit availability, and that fruiting non-crop plants and ornamentals act as a reservoir from which *D. suzukii* is able to re-infest crop fruits (Lee et al. 2011, Addesso et al. 2015, Lee et al. 2015, Klick et al. 2016). While most of these studies have taken place on the U.S. West coast and in the Northern states, the phenology of *D. suzukii* is relatively understudied in the southeast and even less in the state of Georgia, specifically. Previous work by Grant et al (2016) in Georgia used yeast-sugar-water solution traps to assess *D. suzukii* activity in blueberry fields and their surrounding margins, the gender ratios throughout time, and how ambient temperature affect their activity throughout the year. However, no work to date has examined the phenology of *D. suzukii* in relation to specific habitat types surrounding Georgia organic blueberry orchards. Furthermore, the previous year-round phenology work in Georgia has not used Scentry (Scentry Biologicals Inc., Billings, MO) trapping systems, which have since been demonstrated to catch more *D. suzukii* and with greater selectivity than other trapping systems (Cha et al. 2015, Frewin et al. 2017, Cha et al. 2018).

Another aspect of *D. suzukii* phenology that is poorly understood is their overwintering biology. Laboratory studies on *D. suzukii* survival at the lower temperature extreme suggest that the winter conditions in temperate climates would be too harsh for survival (Kimura 2004, Dalton et al. 2011, Jakobs et al. 2015, Stephens et al. 2015). Despite this, *D. suzukii* have been captured throughout the entire winter in several studies including in Georgia (Grant 2016, Rossi-Stacconi et al. 2016, Thistlewood et al. 2018). It has been suggested that *D. suzukii* could utilize man-made environments for shelter or potentially survive in leaf litter as sexually diapausing adults or pupae (Jakobs et al. 2015, Stephens et al. 2015, Rossi-Stacconi et al. 2016, Stockton et al. 2018). Furthermore, previous studies have reported a winter phenotype in *D. suzukii* in colder climates; this phenotype is characterized by a larger body size with darker coloration and longer wings (Stephens et al. 2015, Shearer et al. 2016, Wallingford and Loeb 2016). This phenotype has been induced in a laboratory setting by simulating winter conditions during immature development (Dalton et al. 2011, Shearer et al. 2016, Wallingford et al. 2016), and the resulting adults have been shown to have increased cold tolerance in the laboratory (Shearer et al. 2016, Wallingford et al. 2016). To date, it is still unclear what habitat resources *D. suzukii* utilize to survive over winter, or whether their winter morphology benefits them in a field setting.

In this study, we aim to build on previous *D. suzukii* year-round monitoring efforts in Georgia. We will increase the resolution of this work by using updated trapping techniques and by trapping in a wider variety of habitats than what was done previously. We also will explore an avenue not done before in Georgia: the overwintering biology of *D. suzukii*. For this, we will conduct in-field assays to evaluate the relative abilities of summer morphs, winter morphs, and pupae to survive the winter using leaf litter as shelter. Our objectives are to (1) provide population abundance data for various habitats surrounding organic blueberry orchards throughout time and (2) determine whether *D. suzukii* can survive overwinter in the leaf litter, and if so, what life stages and morphologies can do so. Through this work, we hope to contribute to a deeper understanding of the invasion biology of this species by identifying the habitat resources that facilitate its establishment and persistence throughout time.

2. Materials and Methods

2.1 Insect rearing

2.1.1. Summer morphology

The colony was reared from wild-caught *D. suzukii* from Clarke County, Ga on a standard fly diet substrate (Jaramillo, Mehlferber et al. 2015) portioned into 177-ml plastic square bottom bottles (Genesee Scientific, San Diego, CA). Bottles were kept in Percival (Model I36VLCB, Percival Scientific, Perry, IA) incubator at ~25°C, 50% relative humidity, and a 16:8 h (L:D) photoperiod. Incubator temperature was dropped to 10°C one week prior to the overwintering assay.

2.1.2. Winter morphology

This colony was reared from *D. suzukii* caught in Bacon County, GA in 2016. To create the winter morphs, 30 (15 male and 15 female) adults of reproductive age were transferred from the main colony into 177-ml plastic square bottom bottles with standard fly diet substrate and allowed to lay eggs for 24 hours in summer conditions (~25°C, 50% relative humidity, and a 16:8 h (L:D) photoperiod). After 24 hours, the F_0 adults were removed and the bottles with eggs were transferred to an incubator with 15°C and 12:12 (L:D) and allowed to develop into adulthood. One week before the beginning of the overwintering assay, the temperature of the incubator was lowered to 10°C.

2.1.3. Pupae

Eggs were produced in summer conditions and transferred to the winter incubator as described in section 2.1.2. One week before the beginning of the overwintering assay, pupae were carefully extracted from the bottles using forceps. Groups of 50 pupae were place on a Kimwipe (Kimberly-Clark Corporation, Neenah, WI) delicate task wipe and placed inside of a mesh bag. Mesh bags were stored in the winter incubator and temperature was lowered to 10°C until the beginning of the assay.

2.2 Experimental design

2.2.1 Habitat evaluation

Four experimental blocks were distributed evenly between two organic blueberry farms in Appling county (31.7122° N, 82.2583° W) and Bacon county (31.5412° N, 82.4319° W) in Southeast Georgia. Each block contained five distinct habitat types including organic blueberry, pine forest, deciduous forest, riparian, and non-crop host (*Rubus spp.*). Organic blueberry replicates were located central to each block. Each habitat was at least 40 square meters large, within 1000 m of the organic blueberry habitat, and at least 50 m away from other habitats. Blocks were all separated by at least 500m.

One Scentry (Scentry Biologicals Inc., Billings, MO) fly trap containing a hanging Scentry commercial lure packet above a water drowning solution was placed at the center of each habitat and collected once every two weeks. Scentless soap was added to the water to break surface tension and prevent flies from standing on the surface. Traps were hung in shaded areas to encourage fly visitations. Scentry lure packets were replaced once every four weeks. When collected, traps were rinsed out and the contents were stored for counting in the laboratory. Males and females were counted for each trap; if the number of flies was far in excess of 100, the sample was placed on a gridded petri dish and 25% of the sample was extracted for counting. The totals for the subsample were then multiplied by 4 to estimate the totals for the entire sample, and a note was made when this method was used.

2.2.2 Overwintering evaluation

Forty-eight holes were dug in a six by eight grid with each hole about 1 meter apart in the woods adjacent to a blueberry orchard in Alma, GA. Assay chambers were buried in these holes. The assay chambers consisted of a 32oz deli cup with ~2cm^2 holes drilled in the bottom for water drainage, a soil core filling most of the cup, a layer of leaf litter with an apple core on top, 100 (50M, 50F) chill-anesthetized *D. suzukii* adults or 50 *D. suzukii* pupae in a mesh bag (pupae chambers did not have an apple), and another layer of leaf litter on top. In the first year (2016-2017), half the chambers had summer morph adults and half had winter morph adults. In the second year (2017-2018), half the chambers had summer morph adults and half had pupae. These contents were all secured with a mesh cloth tied on with a rubber band, at which point the entire chamber was buried in one of the holes, and a layer of pine straw was laid over the top. A chicken-wire cage was hammered into the ground with tent stakes around each chamber to prevent wildlife intrusion. The position of each chamber in the grid was assigned using a random number generator in order to randomize the location of winter and summer morphs.

Each collection date, four summer morph and either four winter morph chambers (first year) or four pupae chambers (second year) were randomly selected to be brought back to the lab. For adult chambers, each was placed inside of screen cages at room temperature with a water wick, and the apple (or agar) was removed from the chamber. They were allowed to sit for one week, after which the number of flies which had exited the chamber was recorded in order to approximate survivorship. Pupae chambers were allowed to sit on a lab bench for one week, after which the mesh bags were extracted and the number of eclosed adults was counted. The first collection date was on the same day as deployment, and another followed every two weeks thereafter.

Two HOBO Pro v2 temp/RH monitors (Part No. U23-001) were placed at the site to monitor humidity and temperature. Data was collected every 15 minutes for the duration of the experiment. One was buried in a potted chamber exactly like the experimental treatments, and one was laid above ground and held in place with chicken wire.

2.3 Statistical analysis

Data was analyzed in R v 3.5.1. All count data were fit using a generalized linear model (GLM) using a Poisson error distribution and a log link function. Models were tested for overdispersion and were refit to a negative binomial GLM to correct for overdispersion, if it was detected. Analysis of deviance was used to screen for relevant explanatory variables and interactions. Variables were removed from the models if they were not significant. Tukey’s pairwise comparisons were made within significant explanatory variables to test for significant separation of means.

3.1 Habitat evaluation

All figures and analysis were done in R version 3.4.1 (R Foundation for Statistical Computing; Vienna, Austria). Summary statistics were computed to assess the population trends throughout the season, by habitat, and by sex of the captured flies. Data was assessed visually by plotting the aggregate mean captures across all the trapping periods, and the means by habitat across trapping periods. Mean trap captures for the entire span of the study were also plotted for each habitat along with their 95% confidence intervals. The proportion of males and females for each trapping period was plotted as a stacked bar chart to assess seasonal variation in sex ratios, and mean total captures were plotted as a grouped bar chart grouped by sex to evaluate seasonal variation in total captures for each sex. The dataset was modeled using a generalized linear model with a negative binomial distribution. The "lsmeans" package was used to compare habitat categories in presence of the interaction effect between habitat and sampling period.

3.2 Overwinter evaluation

All figures and analysis were done in R version 3.5.1 (R Foundation for Statistical Computing; Vienna, Austria). The number of surviving adults was averaged in each morphological group (summer, winter, pupae) for each length of time spent in the field and plotted as a line chart. The mean was calculated for each day that the HOBO loggers recorded temperature both above and below ground, and the means were plotted as a line chart for comparisons. Summary statistics were computed for the HOBO loggers above and below ground and they were compared. The number of surviving adults was modeled with a generalized linear model using a negative binomial distribution and the effects of morphology and length of time in the field as explanatory variables and blocked by the year of each trial. Analysis of deviance for linear models was then used to identify insignificant explanatory variables for exclusion.

Tukey's pairwise comparisons were used to determine significantly different treatments levels within significant explanatory variables.

3. Results

3.1 Habitat evaluation

Averaged across all habitats, peak *D. suzukii* captures were in the trapping period from June 28 through July 12, 2017 with an average of 681 per trap (± 241 95% CI). Trap captures began increasing sharply after June 14 leading up the July 12 peak. Captures fell quickly over the next month, and after July 24 averages dropped to below 100 per trap. All habitats followed this trend for this part of the year, although with varying magnitudes (Figure 3.1). For example, the traps collected from pine had a mean of 844 (±657 95% CI) *D. suzukii* per trap at the July 12 collection date, whereas the riparian traps had a mean of 474.5 (±403.5 95% CI). Later in the year, a second, smaller peak in average captures emerged. This peak was most pronounced in the pine and deciduous habitats, and to a lesser degree in the riparian and *Rubus spp.* habitats. After October 13, mean pine captures quickly shot up until December 10 where they peaked at 291.5 (±216.6 95% CI). After December 10 they began decreasing steadily until they were below 100 after February 2. Deciduous had trend of similar magnitude; their numbers shot up after November 25 and peaked at 247.3 (± 198.8 95% CI) on December 22. Average captures for deciduous dropped sharply at January 5 and bounced back up at January 19, dropped again at February 2 and stayed low until one final, smallest spike at March 17. From December 10 to December 22, riparian and *Rubus spp.* also had a small second peak, with numbers far outstripping blueberry at over 80 average captures per replicate (Figure 3.1). In Tukey's pairwise comparisons of the overall sample distribution, the only habitats that differed significantly were pine and riparian ($z = -3.07$, $P < 0.019$) (Figure 3.2).

Throughout the year, the ratio of total males captured relative to females varied considerably. From the beginning of the study until early August, the majority of the flies captured were male, with 78% being male in the first two weeks. From early August through mid-September, the sex ratio was roughly 1:1. Then from mid-September until mid-March, most flies captured were female (apart from December 10 through December 22, where the ratio was roughly 1:1), with females making up as high as 94% of captures from September 29 through October 13. Beginning in April, males suddenly became the vast majority of captured *D. suzukii* once again (Figure 3.3). In Tukey's pairwise comparisons between habitats, riparian habitat had significantly less flies captured than pine habitat for both males ($z = -2.964$, $P < 0.025$) and females ($z = -3.638$, $P < 0.002$). However, riparian habitat had significantly less captures than deciduous among females ($z = -2.506$, $P < 0.031$) but not in males ($z = -2.163$, $P < 0.194$).

3.2 Overwintering evaluation

In the first year (2016-2017), the experiment was cut short by the landowner removing the overwintering assay chambers from the ground. This was after the 4-week exposure period for winter morphs, and the 6-week period for summer morphs (these treatments were staggered so that there would be enough winter morphs to deploy). For both morphologies, the number of surviving adults dropped drastically between the 2-week and 4-week exposure periods (Figure 3.4). By this time, both groups had been exposed to ten days in which the below ground temperature dropped under 10°C (Figure 3.5). Winter morphs had numerically higher mean survival rates than summer morphs at two weeks, but did not differ statistically ($\chi^2 = 0$, $df = 1$, $P = 0.968$). The number of surviving adults was decreased significantly with increasing time spent in the field ($\chi^2 = 326$, $df = 5$, $P < 0.001$). In the second year, a similar trend occurred wherein after 4 weeks in the field the number of surviving adults dropped to almost zero in each chamber.

After four weeks, the adults had endured twelve days in which the below ground temperature dropped lower than 10°C (Figure 3.6). In the 8-week collection, no chambers had any surviving adults. However, in the 10-week sample, 7 adults emerged from one container (Figure 3.7). Among the pupal containers, the 2-week sample had the highest number of adults emerged, surpassing the control group which was brought back on the same day the experiment was deployed. The number of adults emerging from the pupal containers dropped sharply at the 4-week collection to an average of 5 per container and fluctuated around 5 for the rest of the collection dates (Figure 3.8). By week four, the pupae had endured twenty days in which the below ground temperature dropped under 10°C. In both the summer morph and pupae groups. In both years, the HOBO loggers placed underground had higher temperature minimums, lower maximums, and smaller standard deviations than their above-ground counterparts.

4. Discussion

In this study, we expand on previous work that monitored seasonal *D. suzukii* population trends in Georgia blueberry orchards. Additionally, we provide a first look at how those seasonal trends interact with the habitats surrounding Georgia blueberry farms. The first and largest spike in *D. suzukii* captures happened in late June and early July. This timing corresponds to the end of the blueberry harvest season in Georgia; thus, this spike in captures can potentially be explained by the growth of the *D. suzukii* population throughout the blueberry season combined with the end of insecticide applications intended to prevent berry infestation. Furthermore, the harvesting process leaves behind a considerable number of blueberries on the ground which can be utilized by *D. suzukii* as an ovipositional substrate. The availability of oviposition substrate combined with a significant degree-day accumulation by mid-July creates an ideal scenario for supporting large *D. suzukii* populations. Plant-feeding insect population densities and dispersal patterns are known to be strongly influenced by their host plant phenology, and here we confirm that *D. suzukii* is no exception (Peterson 1997, Hunter and Elkinton 2000, Kamala Jayanthi and Verghese 2011).

Interestingly, the *D. suzukii* population peaks observed here coincide with those observed in a previous Georgia study, but the relative magnitude of the first (summer) and second (winter) peak are reversed (Grant 2016). This discrepancy could potentially be explained by the trapping methodology; in the study by Grant et al. (2016), a standard yeast-sugar-water solution (Iglesias et al. 2014) was used as bait. Other *D. suzukii* monitoring efforts using apple cider vinegar solution as a lure have also reported higher peaks in the winter months than in the summer months (Harris et al. 2014, Haviland et al. 2016, Wang et al. 2016). The Scentry trapping system used in our study has since been demonstrated to be far more attractive and selective than the standard yeast trap and apple cider vinegar (Cha et al. 2015, Frewin et al. 2017, Cha et al. 2018). Therefore, the yeast traps may have been attractive enough to detect increases in *D. suzukii* population density, but not to accurately signal the magnitudes of those population increases relative to each other.

Among habitats, the pine and deciduous forests had population trends that did not line up with the other habitats; while all habitats had a similar peak in late June and early July, only pine and deciduous forests had a second, smaller peak from October through January. Previous work indicates that *D. suzukii* are trapped more frequently in woodland areas as compared to the adjacent crop fields (Grant 2016). One potential explanation is that there is less stimuli competing with traps for the flies' attention in the forests than in the orchards dominated by fruit-bearing plants; other studies have suggested that this effect influences their trapping patterns (Harris et al. 2014, Haviland et al. 2016). Insect traps have a limited range of attraction, and the more competing stimuli in the immediate environment, the less effective this range becomes. Despite their high efficacy relative to other trapping systems, even Scentry lures have been shown to have a short range of attraction (Kirkpatrick et al. 2018). However, if this were a main influence on the trend we observed here, one would expect to see lower captures in the blueberry orchards relative to the other habitats while fruit was present. Here, we didn't see any major differences until late October long after the blueberries were gone, at which point the forested habitats began to increase again while the other habitats remained at a low level.

An alternative hypothesis is that the temperature regulation provided by the shade of the forest canopy allows for more movement in *D. suzukii* and therefore, a higher frequency of trap captures. *D. suzukii* has been shown to be less active at temperatures above 28-30°C (Walsh et al. 2011, Kinjo et al. 2014, Tochen et al. 2014), and the unshaded areas between rows of blueberry bushes are much more likely to surpass this threshold. However, one would expect to see a higher count number in the forested habitats during the summer months and not the fall and winter months if high temperatures were driving the trend.

Other studies have also found a peak in captures over the winter in evergreen forests and have suggested that dropping temperatures stimulate *D. suzukii* to take flight in search of appropriate overwintering sites (Harris et al. 2014, Haviland et al. 2016). This explanation is compatible with the timing of the second peak in our trap captures, as well as the magnitude and timing of the deciduous habitat relative to the pine; while the deciduous habitat locations had a majority of deciduous plants, pine trees were present at these sites as well. The second peak in *D. suzukii* captures in deciduous forest occurred two weeks after the peak in pine had already occurred and numbers had begun to drop quickly. The magnitude of the second peak in pine was greater than that in deciduous forest, and the trap captures increased and decreased surrounding this peak much more gradually than in the deciduous forest, where captures peaked much more abruptly before crashing again. These patterns, in combination with the published literature, indicate that it is the pine trees which provided overwintering resources for the *D. suzukii* captured in our study; the scattered presence of pine trees in the deciduous stands allowed for an overwintering population to be detected, though with less stability and overall abundance than in the pure pine stands.

When examining the proportion of males and females captured throughout the year, we observed a trend wherein the majority of *D. suzukii* captured were male in the months from April until August. The ratio becomes more balanced in August and September, but then in late September and all the way until the end of March, females were captured in higher proportions on most collection dates. Notably, Haviland *et al.* found that females dominated in California cherry through the month of March while cherry was most susceptible to infestation, and Hamby *et al.* also found a higher proportion of females from January through September (Hamby et al. 2014, Haviland et al. 2016). The period of female dominancy observed in this study begins and ends earlier than in that of Hamby *et al.*, and encompasses that of Haviland et al.; however, our period of female dominance does not coincide with the period of blueberry susceptibility as it did for cherries in California. These results suggest that seasonal changes in *D. suzukii* sex ratios do not depend on fruit presence. Rather, our findings may lend support to the hypothesis that *D. suzukii* predominantly overwinter as females (Dalton et al. 2011, Hamby et al. 2014). Furthermore, we provide indirect field evidence for a greater male sensitivity to cold which has been observed in laboratory studies (Dalton et al. 2011, Tochen et al. 2014, Plantamp et al. 2016).

In this study, we also assessed the abilities of *D. suzukii* pupae, summer morphs, and simulated winter morphs to utilize detritus consisting of leaf litter and pine straw as an overwintering refuge. The reduced range and variability of below ground temperatures suggests that leaf litter can provide a valuable refuge for overwintering *D. suzukii*. Previous work has demonstrated that the survivorship of adult *D. suzukii* decreases significantly below 10℃ in the laboratory (Dalton et al. 2011). We found that the number of days where the average recorded temperature dropped below 10℃ was approximately double above ground compared to in our overwintering chambers, further underlining the value of leaf litter as a refuge. Furthermore, our sensors record no instances of the temperature dropping below freezing underground, whereas above ground we recorded approximately nine days of cumulative time below freezing. Considering these facts, any *D. suzukii* that successfully relocate beneath a leaf litter cover should doubtlessly be conferred a tremendous survival advantage. Indeed, a small number of female adults and unsexed pupae were able to survive in leaf litter chambers for the duration of the experiment.

Flies benefiting from this microhabitat advantage are still subject to challenging temperature stress, even in the relatively mild Georgia winters. Comparing the survival abilities of winter morphs, summer morphs, males, and females in our experimental chambers, we conclude that winter morph females are the most likely group to survive a harsh winter. Our field observations support previous work demonstrating the improved survival of winter morph *D. suzukii* in laboratory conditions (Shearer et al. 2016, Wallingford et al. 2016) and is internally consistent with the female-biased sex ratios in our trapping data. These data suggest that winter morph females should be considered the target for control strategies focused on overwintering *D. suzukii*.

In summary, we find that the seasonal trapping trend for this pest occurs with two peaks, one in early summer and beginning in late fall. The summer peak can likely be attributed to high population density caused by the cumulative time of blueberry availability combined with the cessation of insecticide application. The winter peak occurs exclusively in forested habitats, and with a greater magnitude in forests dominated by evergreen *Pinus spp.* We conclude that the additional foliage provided by evergreen plants mitigates the harsh thermal conditions of winter, attracts *D. suzukii* seeking an overwintering site, and causes a spike in trap captures. Furthermore, we provide the first field evidence that female and pupal *D. suzukii* are capable of surviving through the Georgia winter in detritus consisting of leaf litter and pine straw, with the winter morphology providing additional survival advantage. The overwintering survival results, habitat trapping data, and sex ratio trend throughout the year paint a convincing picture wherein female *D. suzukii* migrate from blueberry orchards to neighboring forests featuring evergreen foliage and detritus to take shelter for the winter. If reproduction occurs thereafter, it may also be possible for *D. suzukii* to survive the winter as pupae in the leaf litter. Future control strategies targeting overwintering *D. suzukii* may choose to focus on harvesting of pine straw in human-made pine stands to reduce overwintering refuge in these particularly habitable areas.

5. References

Addesso, K. M., J. B. Oliver, and P. A. O'Neal. 2015. Survey for spotted-wing drosophila (Diptera: Drosophilidae) in the five-county nursery production region of middle Tennessee, USA. Florida Entomologist.

Bergland, A. O., E. L. Behrman, K. R. O'Brien, P. S. Schmidt, and D. A. Petrov. 2014. Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*. PLOS Genetics 10: e1004775.

Boll, R., E. Lombaert, and L. Lapchin. 2006. Dispersal strategies of phytophagous insects at a local scale: adaptive potential of aphids in an agricultural environment. BMC Evolutionary Biology, Vol 6, Iss 1, p 75 (2006): 75.

Cha, D. H., S. P. Hesler, A. K. Wallingford, F. Zaman, P. Jentsch, J. Nyrop, and G. M. Loeb. 2018. Comparison of Commercial Lures and Food Baits for Early Detection of Fruit Infestation Risk by *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 111: 645-652.

Cha, D. H., S. P. Hesler, S. Park, T. B. Adams, R. S. Zack, H. Rogg, G. M. Loeb, and P. J. Landolt. 2015. Simpler is better: fewer non-target insects trapped with a four-component chemical lure vs. a chemically more complex food-type bait for *Drosophila suzukii*. Entomologia Experimentalis et Applicata 154: 251-260.

Colinet, H., and A. A. Hoffmann. 2012. Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*. Functional Ecology 26: 84-93.

Dalton, D. T., V. M. Walton, P. W. Shearer, D. B. Walsh, 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 Management Science 67: 1368-1374.

Del Fava, E., C. Ioriatti, and A. Melegaro. 2017. Cost–benefit analysis of controlling the spotted wing drosophila (*Drosophila suzukii* (Matsumura)) spread and infestation of soft fruits in Trentino, Northern Italy. Pest Management Science 73: 2318-2327.

Frewin, A. J., J. Renkema, H. Fraser, and R. H. Hallett. 2017. Evaluation of Attractants for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 110: 1156-1163.

Grant, J. A. 2016. Investigation of Wild Flora Bordering Blueberry Fields to Quantify Their Potential as Viable Hosts of *Drosophila suzukii* (Diptera: Drosophilidae). Master of Science, University of Georgia Athens, Georgia.

Hamby, K., M. Bolda, M. Sheehan, and F. J. E. e. Zalom. 2014. Seasonal monitoring for *Drosophila suzukii* (Diptera: Drosophilidae) in California commercial raspberries. 43: 1008-1018.

Harris, D. W., K. A. Hamby, H. E. Wilson, and F. G. Zalom. 2014. Seasonal monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) in a mixed fruit production system. Journal of Asia-Pacific Entomology 17: 857-864.

Haviland, D. R., J. Caprile, S. Rill, K. Hamby, and J. A. Grant. 2016. Phenology of spotted wing drosophila in the San Joaquin Valley varies by season, crop and nearby vegetation. California Agriculture 70: 24-31.

Hunter, A. F., and J. S. Elkinton. 2000. Effects of Synchrony with Host Plant on Populations of a Spring-Feeding Lepidopteran. Ecology 81: 1248-1261.

Iglesias, L. E., T. W. Nyoike, and O. E. Liburd. 2014. Effect of Trap Design, Bait Type, and Age on Captures of *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Crops. Journal of Economic Entomology 107: 1508-1518.

Jaffe, B. D., A. Avanesyan, H. K. Bal, Y. Feng, J. Grant, M. J. Grieshop, J. C. Lee, O. E. Liburd, E. Rhodes, C. Rodriguez-Saona, A. A. Sial, A. Zhang, and C. Guédot. 2018. Multistate Comparison of Attractants and the Impact of Fruit Development Stage on

Trapping *Drosophila suzukii* (Diptera: Drosophilidae) in Raspberry and Blueberry. Environmental Entomology 47: 935-945.

Jakobs, R., T. D. Gariepy, and B. J. Sinclair. 2015. Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. Journal of Insect Physiology 79: 1-9.

Jaramillo, S. L., E. Mehlferber, and P. J. Moore. 2015. Life-history trade-offs under different larval diets in *Drosophila suzukii* (Diptera: Drosophilidae). Physiological Entomology 40: 2-9.

Kamala Jayanthi, P. D., and A. Verghese. 2011. Host-plant phenology and weather based forecasting models for population prediction of the oriental fruit fly, *Bactrocera dorsalis* Hendel. Crop Protection 30: 1557-1562.

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

Kinjo, H., Y. Kunimi, and M. Nakai. 2014. Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). 49: 297-304.

Kirkpatrick, D. M., L. J. Gut, and J. R. Miller. 2018. Estimating Monitoring Trap Plume Reach and Trapping Area for *Drosophila suzukii* (Diptera: Drosophilidae) in Michigan Tart Cherry. Journal of Economic Entomology: toy062-toy062.

Klick, J., W. Q. Yang, J. C. Lee, and D. J. Bruck. 2016a. Reduced spray programs for *Drosophila suzukii* management in berry crops. International Journal of Pest Management 62: 368-377.

Klick, J., W. Q. Yang, V. M. Walton, D. T. Dalton, J. R. Hagler, A. J. Dreves, J. C. Lee, and D. J. Bruck. 2016b. Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation. Journal of Applied Entomology 140: 37-46.

Leather, S. R., K. F. A. Walters, and J. S. Bale. 1993. The Ecology of Insect Overwintering, Cambridge University Press, Great Britain.

Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Management Science 67: 1358-1367.

Lee, J. C., A. J. Dreves, A. M. Cave, S. Kawai, R. Isaacs, J. C. Miller, S. Van Timmeren, and D. J. Bruck. 2015. Infestation of Wild and Ornamental Noncrop Fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Annals of the Entomological Society of America 108: 117-129.

Lustig, A., D. B. Stouffer, C. Doscher, and S. P. J. L. E. Worner. 2017. Landscape metrics as a framework to measure the effect of landscape structure on the spread of invasive insect species. 32: 2311-2325.

Merrill, A. P., and F. D. Robert. 1998. The Influence of Dispersal and Diet Breadth on Patterns of Genetic Isolation by Distance in Phytophagous Insects. The American Naturalist: 428.

Meyerson, L. A., and H. A. Mooney. 2007. Invasive Alien Species in an Era of Globalization. Frontiers in Ecology and the Environment 5: 199-208.

Peterson, M. A. 1997. Host Plant Phenology and Butterfly Dispersal: Causes and Consequences of Uphill Movement. Ecology 78: 167-180.

Plantamp, C., K. Salort, P. Gibert, A. Dumet, G. Mialdea, N. Mondy, and Y. Voituron. 2016. All or nothing: Survival, reproduction and oxidative balance in Spotted Wing

Drosophila (*Drosophila suzukii)* in response to cold. Journal of Insect Physiology 89: 28-36.

Rossi-Stacconi, M., R. Kaur, V. Mazzoni, L. Ometto, A. Grassi, A. Gottardello, O. Rota-Stabelli, and G. Anfora. 2016. Multiple lines of evidence for reproductive winter diapause in the invasive pest *Drosophila suzukii:* useful clues for control strategies. Journal of Pest Science 89: 689-700.

Shearer, P. W., J. D. West, V. M. Walton, P. H. Brown, N. Svetec, and J. C. Chiu. 2016. Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival.

Stephens, A. R., M. K. Asplen, W. D. Hutchison, and R. C. Venette. 2015. Cold Hardiness of Winter-Acclimated *Drosophila suzukii* (Diptera: Drosophilidae) Adults, pp. 1619. ESA ENTOMOLOGICAL SOCIETY OF AMERICA, Great Britain.

Stockton, D. G., A. K. Wallingford, and G. M. Loeb. 2018. Phenotypic Plasticity Promotes Overwintering Survival in A Globally Invasive Crop Pest, *Drosophila suzukii*. Insects, Vol 9, Iss 3, p 105 (2018): 105.

Tait, G., A. Grassi, F. Pfab, C. Crava, D. Dalton, R. Magarey, L. Ometto, S. Vezzulli, V. Rossi Stacconi, A. Gottardello, A. Pugliese, G. Firrao, V. Walton, and G. Anfora. 2018. Large-scale spatial dynamics of *Drosophila suzukii* in Trentino, Italy.

Thistlewood, H. M. A., P. Gill, E. H. Beers, P. W. Shearer, D. B. Walsh, B. M. Rozema, S. Acheampong, S. Castagnoli, W. L. Yee, P. Smytheman, and A. B. Whitener. 2018. Spatial Analysis of Seasonal Dynamics and Overwintering of *Drosophila suzukii* (Diptera: Drosophilidae) in the Okanagan-Columbia Basin, 2010-2014, pp. 221-232.

Tochen, S., D. T. Dalton, N. Wiman, C. Hamm, P. W. Shearer, and V. M. Walton. 2014. Temperature-Related Development and Population Parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on Cherry and Blueberry. Environmental Entomology 43: 501-510.

Uyi, O. O., C. Zachariades, L. U. Heshula, and M. P. Hill. 2018. Developmental and reproductive performance of a specialist herbivore depend on seasonality of, and light conditions experienced by, the host plant. PLoS ONE 13: 1-19.

Van Timmeren, S., L. Horejsi, S. Larson, K. Spink, P. Fanning, and R. Isaacs. 2017. Diurnal Activity of *Drosophila suzukii* (Diptera: Drosophilidae) in Highbush Blueberry and Behavioral Response to Irrigation and Application of Insecticides. Environmental Entomology 46: 1106-1114.

Wallingford, A. K., and G. M. Loeb. 2016. Developmental Acclimation of *Drosophila suzukii* (Diptera: Drosophilidae) and Its Effect on Diapause and Winter Stress Tolerance, pp. 1081. ESA ENTOMOLOGICAL SOCIETY OF AMERICA, Great Britain.

Wallingford, A. K., J. C. Lee, and G. M. Loeb. 2016. The influence of temperature and photoperiod on the reproductive diapause and cold tolerance of spotted-wing drosophila, *Drosophila suzukii*. Entomologia Experimentalis et Applicata 159: 327-337.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, 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. Journal of Integrated Pest Management 2: G1-G7.

Wang, X.-G., T. Stewart, A. Biondi, B. Chavez, C. Ingels, J. Caprile, J. Grant, V. Walton, and K. Daane. 2016. Population dynamics and ecology of *Drosophila suzukii* in Central California. Journal of Pest Science 89: 701-712.

With, K. A. 2002. The Landscape Ecology of Invasive Spread. Conservation Biology 16: 1192-1203.

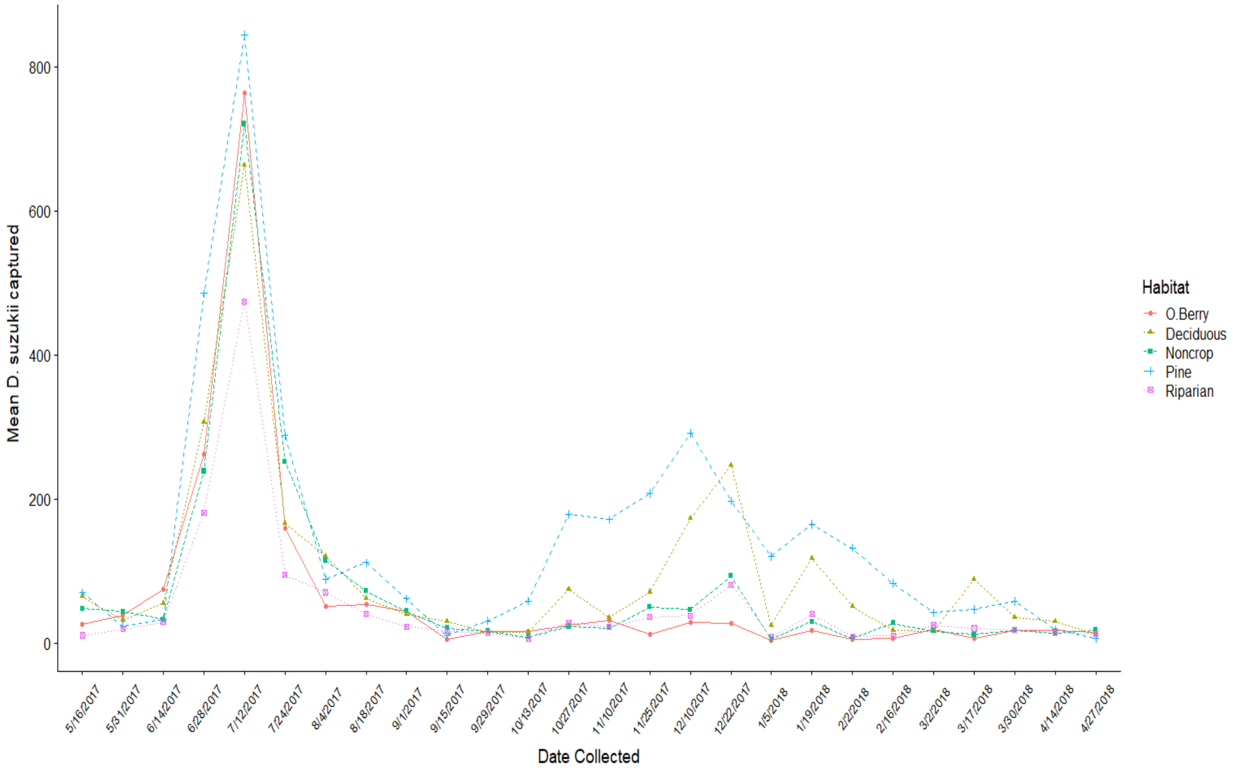

Fig. 3.1. Mean *D. suzukii* captures plotted by habitat and collection date.

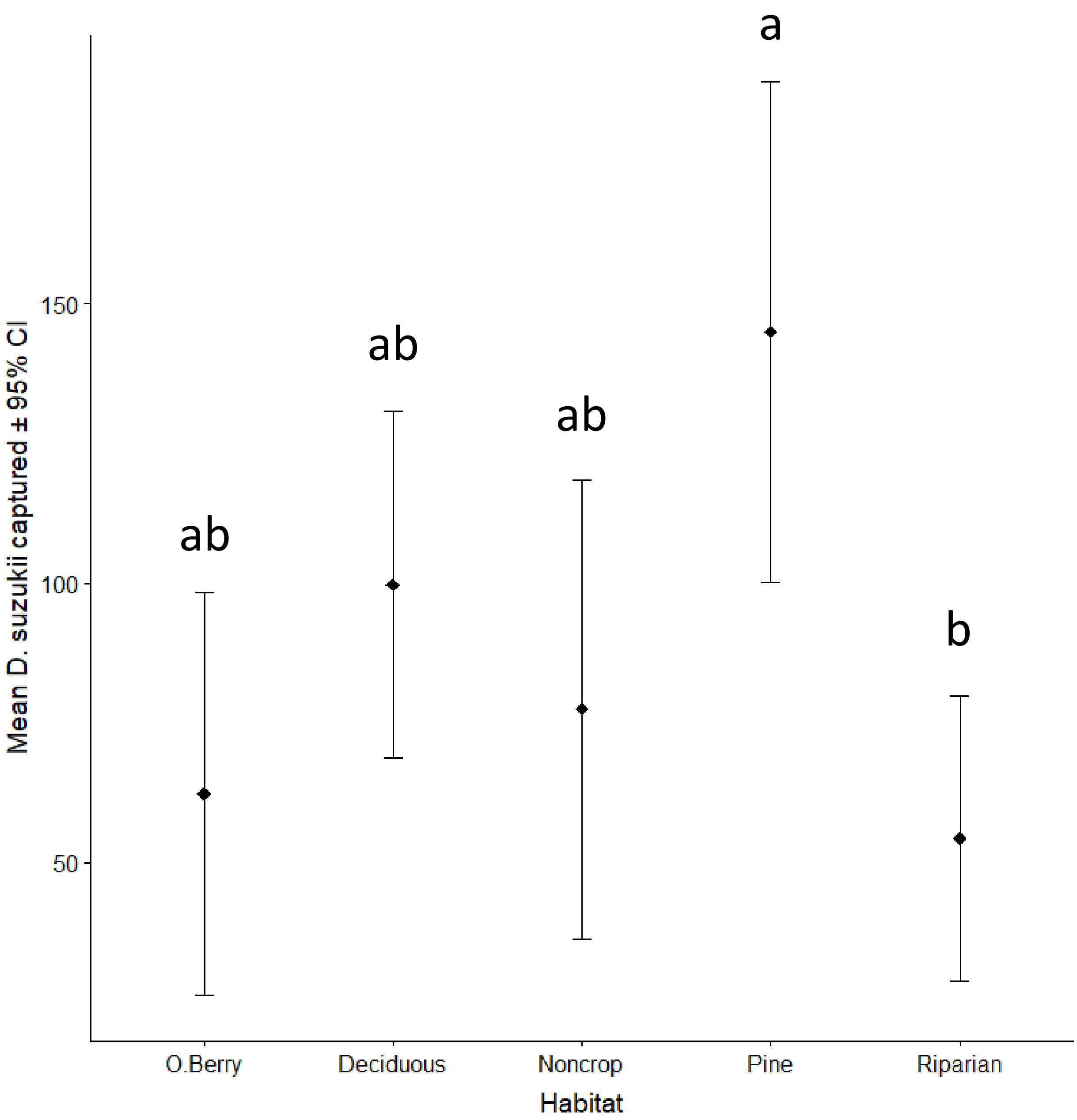

Fig. 3.2. Mean total *D. suzukii* captured in each habitat over the duration of the study.

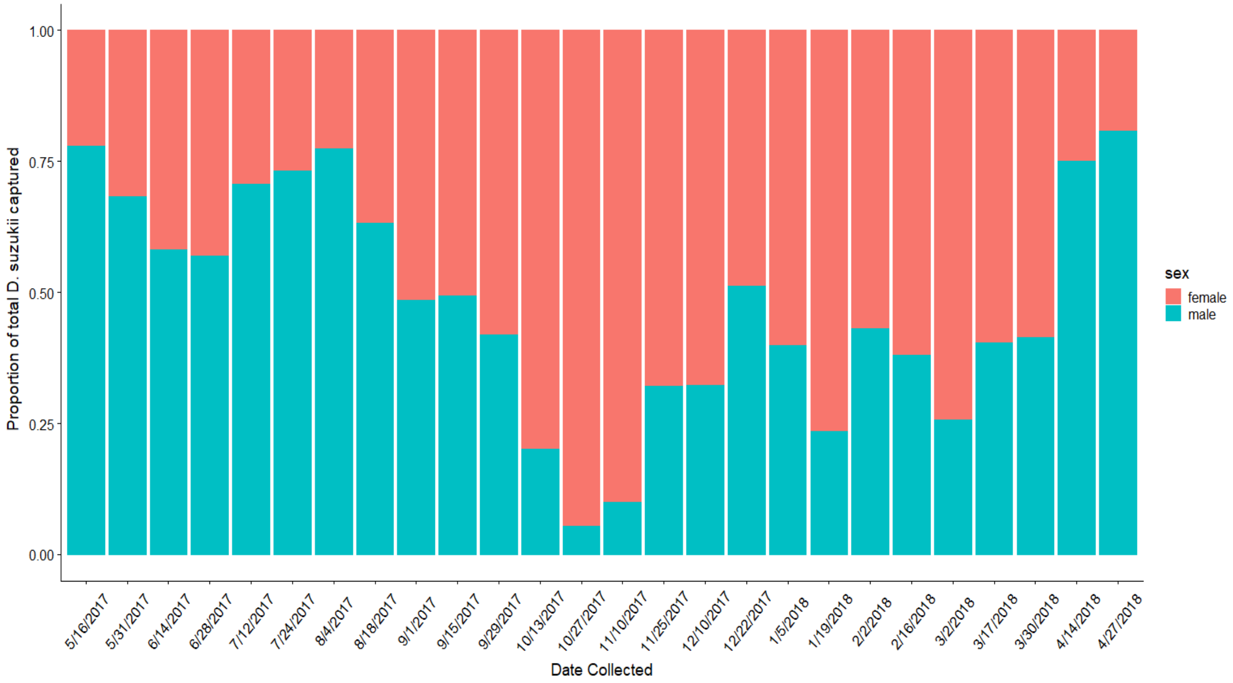

Fig. 3.3. Proportion of male and female *D. suzukii* captured over every two week trapping period.

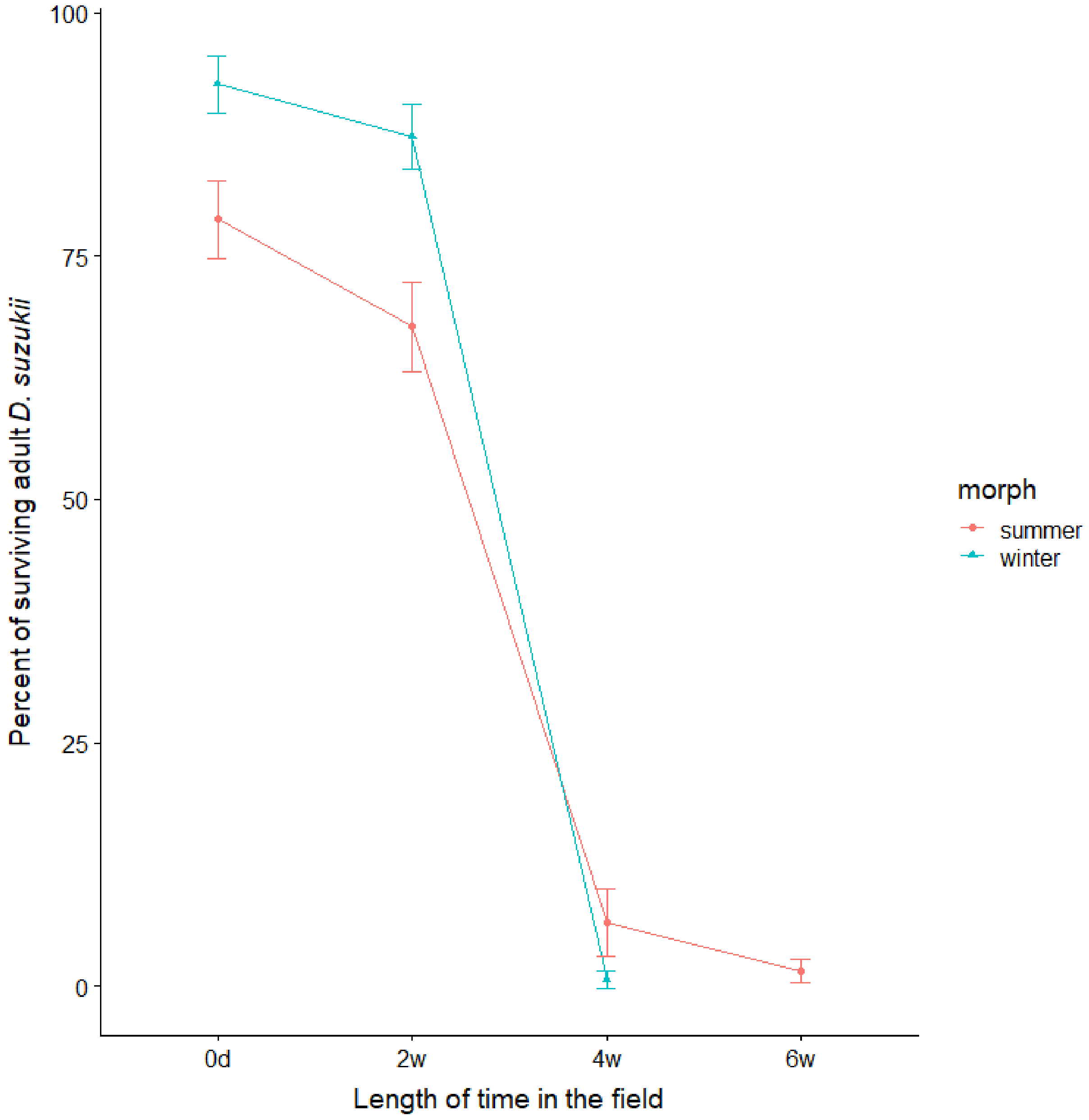

Fig. 3.4. Mean surviving adult *D. suzukii* after each length of time in the field for summer and winter morphs during the first year of the study (2017).

Fig. 3.5. Mean daily below-ground temperature during the experiment during the first year of the study (2017).

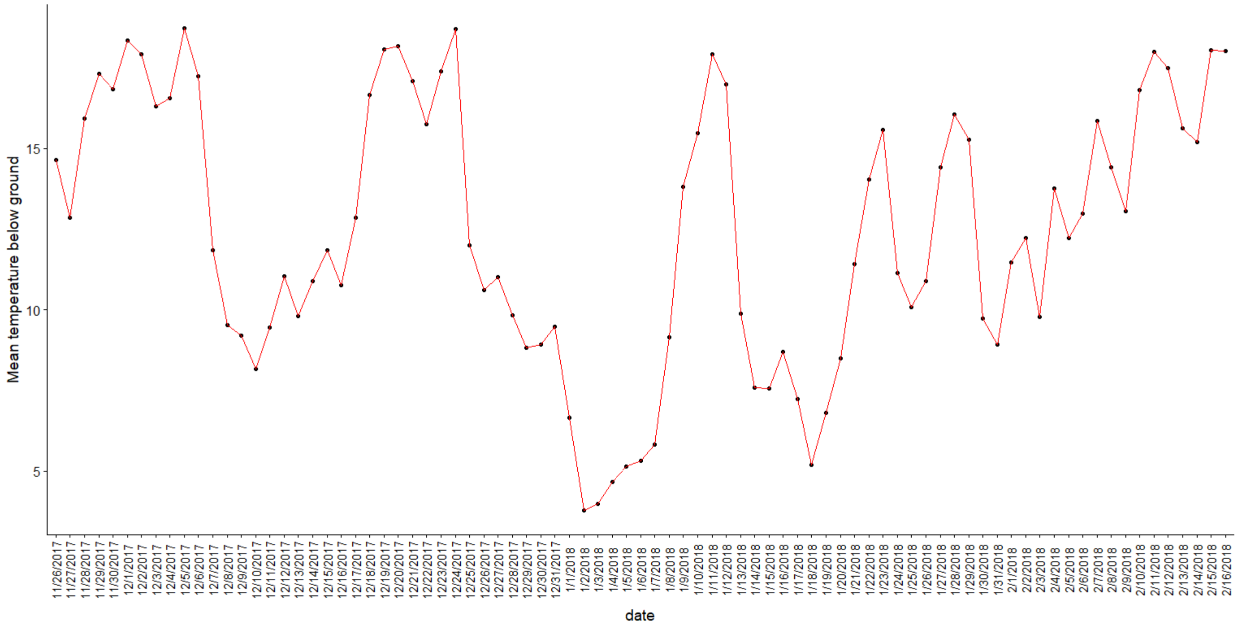

Fig. 3.6. Mean daily below-ground temperature during the experiment during the second year of the study (2018).

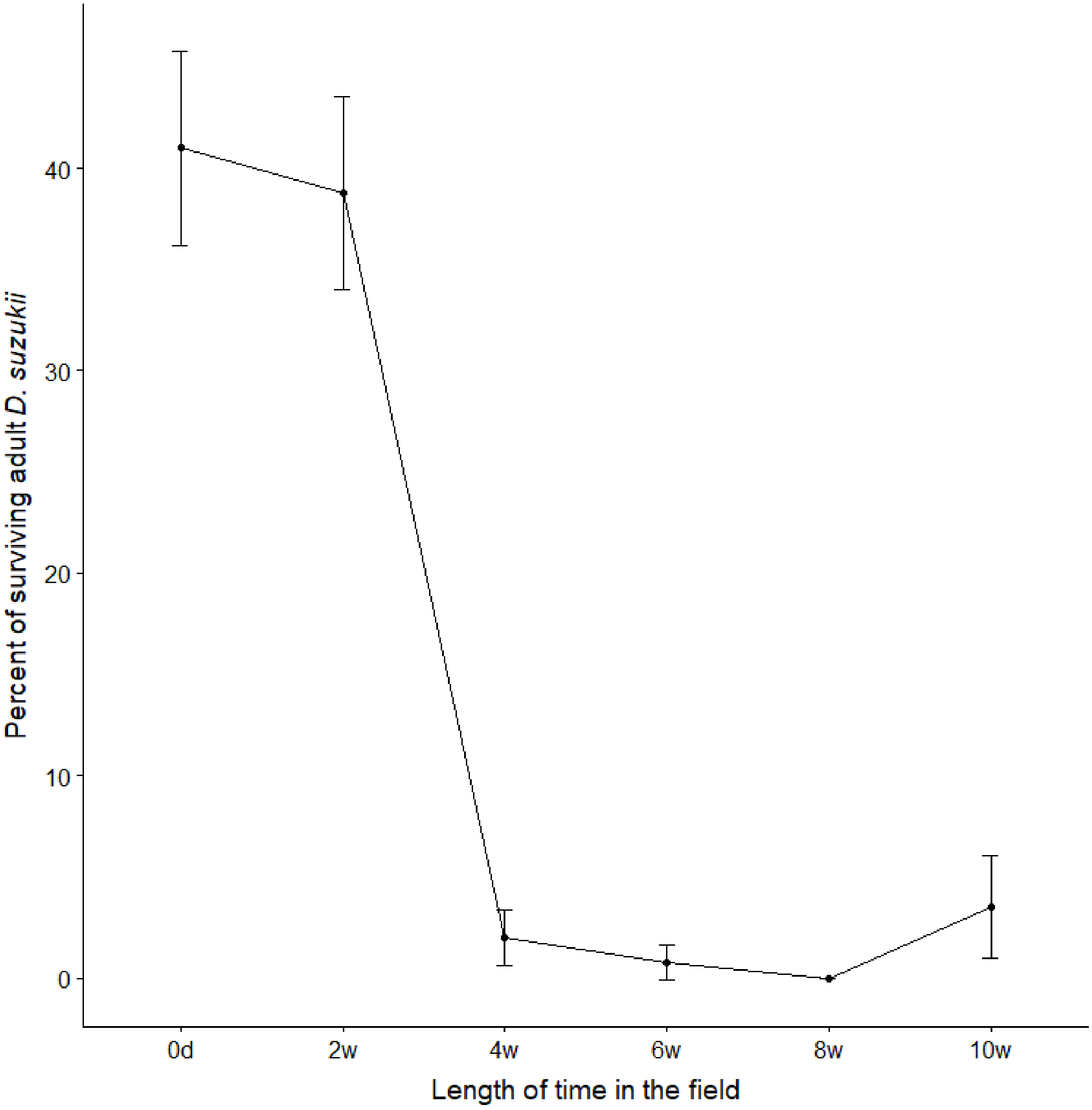

Fig. 3.7. Mean surviving adult *D. suzukii* after each length of time in the field during the second year of the study (2018).

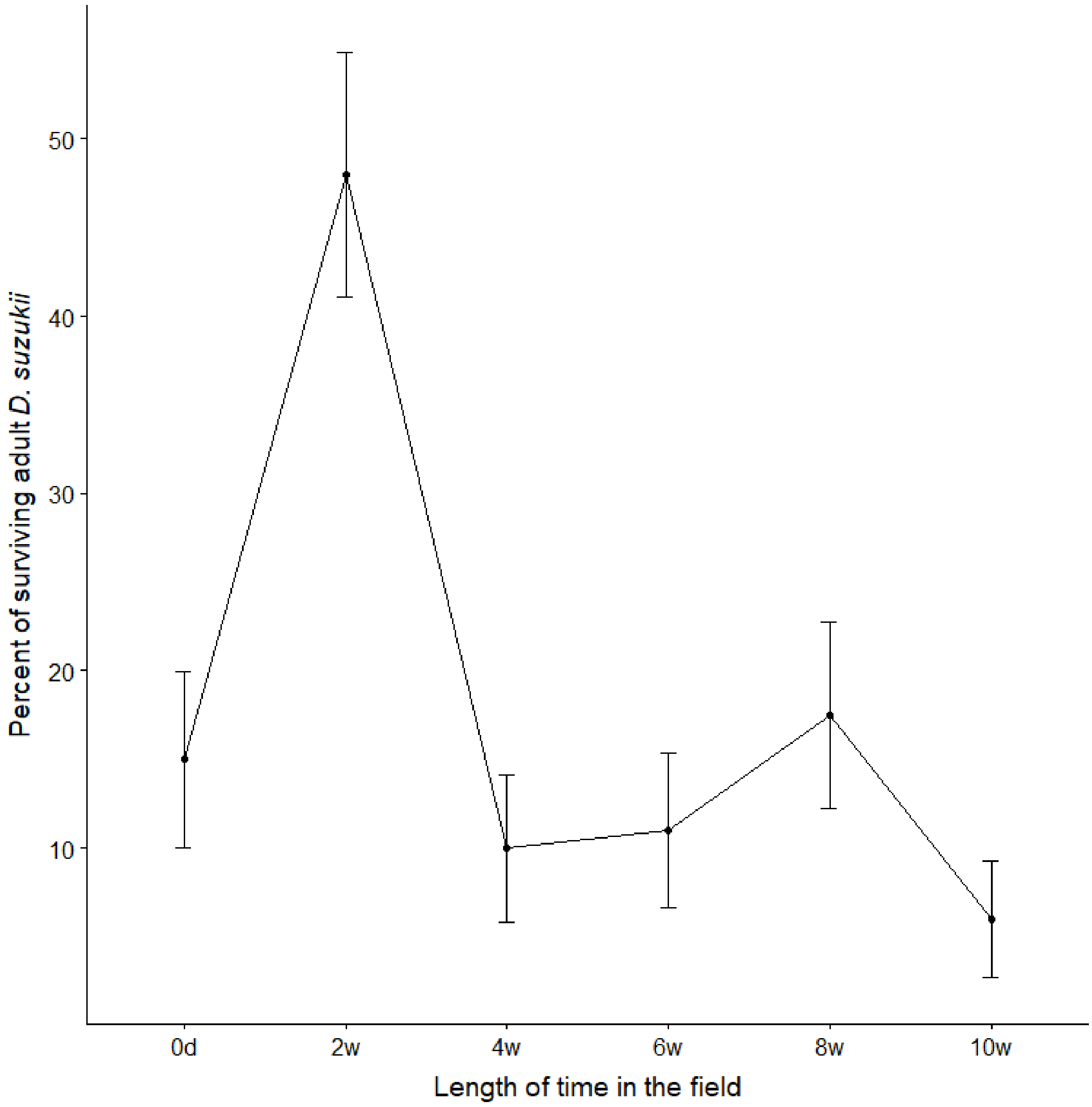

Fig 3.8. Mean pupae that eclosed as adult *D. suzukii* after each duration of time in the field (2018)

CHAPTER 4

EFFECTS OF BLUEBERRY FARM MANAGEMENT PRACTICE ON *DROSOPHILA SUZUKII* ABUNDANCE

C. Kirk Green and Ashfaq A. Sial. To be submitted to *Environmental Entomology*.

Abstract

Local factors such as farm management practice interact with landscape level factors such as heterogeneity and spatial configuration to influence beneficial and pest arthropods alike. Few studies have investigated these interactions, and a minority of those have been done so in perennial cropping systems. A study was conducted to investigate the influence of blueberry farm management practice (conventional, organic, or unmanaged) and landscape composition on both beneficial arthropods and the invasive vinegar fly, *Drosophila suzukii*. For this thesis, I present the portion of the study which was conducted by this lab group; this consists of all the *D. suzukii* trapping data through time and across management practice types. We found similar *D. suzukii* abundance in both management types, and that captures were typically higher in the woods at these farms than in the orchard interiors or at the orchard borders. We conclude that conventional and organic programs offer a similar level of protection to growers and that woodlands are more hospitable to *D. suzukii* than managed orchard interiors during blueberry season.

1. Introduction

Insects are generally seen as pests and an economic burden, but many insects and other arthropods are of great benefit to the economy and the ecosystem. Ecosystem services provided by insects include enriching of soil quality, plant pollination, and suppression of arthropod pest populations. One estimate places the total value of wild insect services at $60 billion per year (Losey and Vaughan 2006). Of this, pest suppression by native insects was estimated to save $4.49 billion annually in the United States (Losey and Vaughan 2006). Insects are not the only arthropods believed to provide pest suppression services; spider populations (order Araneae) have been found to be negatively correlated with many different pest populations across various agricultural systems (Liu et al. 2015, Susan E. Riechert 1999, Picchiet al. 2016).

Although many services are provided by beneficial arthropods, farmers still have to expend considerable effort and resources to reduce the damage caused by less helpful arthropods. Pesticide input into agricultural systems reduces the effectiveness of biological control and other ecosystem services, and simplification of landscapes surrounding these systems compounds on this issue (Grab et al. 2018, Tscharntke et al. 2005, Jonsson et al. 2014, Larsen et al. 2015). Landscape simplification also makes ecosystems more susceptible to invasive species (With 2002). Furthermore, large monocultures provide large patches of suitable habitat for pest insects, increasing their rate of spread and the stability of their populations (Lustig et al. 2017). The relationship between landscape simplification and arthropod populations is highly variable across temporal and spatial gradients. These differences could be caused by many mechanisms including varying climactic conditions, crop types and management practices (Larsen et al. 2015). The variability of these results suggests the need for further investigation into the local and landscape factors influencing pest and beneficial arthropod abundance and diversity.

Understanding these factors within specific climates, cropping systems, and management practices can help us design better agroecosystems and chemical application programs for a more integrative approach to pest management.

Local factors are defined by immediate effects that occur within the agricultural system, such as management practice or insecticide regime, and maintenance of vegetation (Chaplin-Kramer et al. 2011). At the local level, there is substantial evidence that habitat management can have a positive effect on agents of biological control and shape pest population densities. Multiple lines of evidence suggest that farms with adjacent semi-natural habitat or natural vegetation have higher species richness and diversity of natural enemies. These effects could potentially be due to increased amounts and variety of refuge and increased diversity of prey species (Langellotto and Denno 2004, Thomson and Hoffmann 2010, Sarthou et al. 2014). Adjacent natural habitats can also, however, provide reservoirs for pest species and help stabilize their populations around agricultural fields (Lee et al. 2015, Klick et al. 2016, Silva et al. 2018). In addition to habitat management, conventional and organic management practices may differ in their effects on local arthropod populations. The purpose of organic management practices is to reduce impacts on the environment and biodiversity by using softer pesticide formulations and fertilizers. Research suggests that while crop yields may be lower in organic systems (Mäder et al. 2002), they exhibit greater biodiversity and with reduced external inputs (Mäder et al. 2002, Asteraki et al. 2004, Oehl et al. 2004).

Landscape factors are defined by gradual effects that take place at a larger scale and incorporate the composition and configuration of the landscape (Fahrig et al. 2011). The composition of the landscape includes the proportions of different land cover types and the availability of non-crop habitat within the surrounding ecosystem. The configuration of the landscape includes the spatial arrangement and complexity of cover types (Veres et al. 2013). Landscape management through designing the surrounding agroecosystem structure towards higher diversity of suitable habitat has also shown to positively impact natural enemy abundances as well as pest suppression (Schmidt et al. 2008, Veres et al. 2013, Rusch et al. 2016). In many cases, landscape complexity is more important for predicting natural enemy abundance than local factors, although local management practices can compensate for a lack of landscape-level diversity to a large extent (Weibull et al. 2003, Chaplin-Kramer et al. 2011, Paredes et al. 2015).

In the current literature, there is a gap in our understand of how local and landscape environmental factors affect arthropod populations in perennial cropping systems. In recent reviews of the interactions between arthropod abundance and environmental factors, 36 of the 38 selected studies investigated annual cropping systems (Bianchi et al. 2006, Rusch et al. 2016). Perennial systems are believed to have different arthropod interactions compared to annual systems due to the year-round stability of vegetation within the agricultural system. For instance, natural enemies in the vineyards and cherry trees did not respond to higher availability of non-crop habitat (Stutz and Entling 2011, Alberto et al. 2012), yet higher landscape diversity in olive orchards caused a reduction in the primary pest of concern (Ortega and Pascual 2014).

The objective of this study was to assess the relative importance of local management practices and landscape factors on both natural enemies and the primary pest in blueberry cropping systems of Southeast Georgia. The primary, invasive pest we investigate is *Drosophila suzukii*, or spotted wing drosophila, which infests a range of cultivated and wild fruit hosts (Hauser 2011, Lee et al. 2015). The wide host range of *D. suzukii* has allowed this pest to successfully spread across landscapes using the abundance and diversity of fruit host resources available in the southeastern U.S. region. Multiple generations of *D. suzukii* can disperse between the adjacent forest to within blueberry orchards (Haviland et al. 2016, Klick et al. 2016), which creates a unique challenge for pest management strategies. Therefore, the blueberry system in the southeastern U.S. with its recent invasion by *D. suzukii* provides an interesting model system to evaluate the local and landscape factors that contribute to natural enemy and pest abundances. The information provided from this study help us understand the relationship of these variables in more stable, perennial systems, and specifically southeastern blueberries. In this chapter of my thesis, I present the portion of the work contributed by our laboratory; this includes the *D. suzukii* population monitoring across twenty blueberry orchards in Southeast Georgia including conventional and organic management practices, as well as unmanaged orchards.

2. Materials and Methods

2.1 Study Area

In 2017, using the University of Georgia extension network, 20 commercial blueberry orchards were selected in seven counties across South Georgia, U.S.A. (Coffee Co., Bacon Co., Appling Co., Pierce Co., Brantley Co., Ware Co., and Jeff Davis Co.). Blueberry production in the region harvests *Vaccinium ashei* (rabbiteye blueberry) and *Vaccinium corymbosum* (southern highbush blueberry). Our study area included a spatial extent covering approximately 8,846 km^2. Blueberry orchards were selected to vary in both local management and surrounding landscape. We selected a balanced number of management practices separated by at least 1 km, except two sites separated by 450 m. The different management systems among blueberry orchards included conventional (12), certified organic (5), and unmanaged (3). Management classifications were made based on intensity and type of insecticides applied. The twelve conventional sites utilized broad-spectrum synthetic insecticides, including primarily organophosphates and pyrethroids, and in some cases a spinosyn (i.e., Delegate^{TM}), and herbicides often applied between the blueberry rows for weed management. The five organic sites utilized reduced-risk organically certified (OMRI listed) insecticides and herbicides were not applied between blueberry rows. The three unmanaged sites varied from abandoned orchards to small scale harvesting with vegetation present between orchard rows mowed infrequently.

2.2 Sampling

Each orchard site was sampled every other week along a transect containing three stations for a total of sixty samples per sampling period. Transects included locations 25 m into the crop (orchard interior), along the orchard margin or first crop row (orchard border), and 15 m within the adjacent, non-crop forested habitat (forest). Yeast-sugar bait traps were deployed to monitor pest pressure (Landolt et al. 2012). After the two-week period, bait traps were collected and replaced, and the number of male and female *D. suzukii* in each was counted.

2.3 Data Analysis

All data were analyzed in R version 3.4.1 (R Foundation for Statistical Computing; Vienna, Austria). The total number of *D. suzukii* captured was modeled with a negative binomial generalized linear model (GLM) with trapping period, transect location, management practice, and the interaction of transect location and management practice used as explanatory variables. A negative binomial model was selected because the residuals using a Poisson error structure were over dispersed. Analysis of deviance for GLM fits was used to determine significant explanatory variables and interactions, and Akaike information criteria (AIC) scores were used to compare competing models. Tukey’s pairwise comparisons were used to detect significant separation of means within significant explanatory variables.

3. Results

Trapping period ($\chi^2 = 176$, $df = 6$, $P < 0.001$), management practice ($\chi^2 = 36.5$, $df = 3$, $P < 0.001$), location on the transect (field, border, or woods) ($\chi^2 = 30.3$, $df = 2$, $P < 0.001$), and the interaction of management practice and location on the transect ($\chi^2 = 28.2$, $df = 4$, $P < 0.001$) all significantly affected the number of *D. suzukii* captured. Compared by management practice, unmanaged orchards had the highest mean captures (8.7 ± 3.04 95% CI), followed by conventional (7.84 ± 3.15 95% CI) and then organic (5.37 ± 3.04 95% CI) (Figure 4.1). By location on the transect, traps placed in the woods had the highest mean captures (12.9 ± 5.45 95% CI), followed by field (4.84 ± 1.62 95% CI) and then border (4.184 ± 1.48 95% CI) (Figure 4.2). The mean number of captures was skewed towards the woods at conventional and organic farms and skewed towards the field at unmanaged farms (Figure 4.3). At conventional and organic farms, peak captures occurred on 6/27/2017 and 7/11/2017, respectively, with a noticeably higher peak in the woods than the field and border. Unmanaged farms also had a peak on 7/11/2017, but this occurred in both the woods and field with only border captures remaining relatively low. Unmanaged farms also had an additional peak on 5/15/2017 in the field and to a lesser extent on the border (Figure 4.4).

4. Discussion

In this study, we do not find evidence for a difference between conventional and organically managed fields in terms of *D. suzukii* population density, and both have lower capture numbers than unmanaged sites. Given this, we are able to conclude that organic management programs are equally effective at suppressing *D. suzukii* populations in the field when compared to their conventional counterparts. This conclusion is further validated by previous work in Michigan blueberries, which found that both organic and conventional insecticides provided significant control over *D. suzukii* compared to unmanaged plots (Van Timmeren and Isaacs 2014). Although both practices have equal efficacy against *D. suzukii*, their impacts on non-targets are unequal; compared to conventional management practices, organic practices have been shown to have a reduced impact on non-target communities including plants and beneficial arthropods (Vasconcelos and de Moura 2008, Geiger et al. 2010, Tofangsazi et al. 2018). Thus, utilization of organic blueberry management practices should be considered as an alternative to conventional practices that will have a reduced impact on the environment without a directly increased risk of susceptibility to *D. suzukii*. However, farmers may still be reluctant to adopt organic practices because of difficulties surrounding the control of other pests, and unfamiliarity with the organic market (Grieshop et al. 2012). Furthermore, pyrethrum and spinosyn are the only two insecticides available to organic growers, which increases the risk that *D. suzukii* will develop resistance to these chemicals (Van Timmeren and Isaacs 2014).

Looking at location along the transects running from the woods into the field, we find that the overall captures were significantly higher in the woods, with field and border captures being similar to each other. Many other studies have found higher *D. suzukii* captures in woodland areas relative to the adjacent crop fields (Harris et al. 2014, Haviland et al. 2016). This can potentially be explained by a combination of several factors. For one, cover provided by tree canopies and other foliage mitigate thermal extremes and unfavorable wind speeds, creating a more hospitable environment for insects (Gardiner and Dover 2008, Johns et al. 2012). Additionally, fruiting non-crop plants present in the woodlands can act as hosts for *D. suzukii* and serve as a population reservoir (Lee et al. 2015, Klick et al. 2016); these non-crop hosts are generally reduced or wiped out by the application of herbicides in the interiors of managed orchards. Interestingly, the trend of elevated captures in the woods does not hold for unmanaged fields during the early part of the season; here, we found increased captures in the orchard interiors relative to the woods and border. This result demonstrates that blueberry orchards are an ideal environment for *D. suzukii* in the absence of pesticide applications. This conclusion is further underlined by the sudden spike in captures across all management practices in mid-July, just towards the end of blueberry harvest season. This is likely a function of the cessation of insecticide sprays used to suppress *D. suzukii* populations combined with an abundance of leftover, fallen crop fruit littering the ground.

In conclusion, we find that organic and conventional management programs have equal efficacy for the control of *D. suzukii* in Georgia blueberry orchards. Given the low number of organic insecticides available, we surmise that the development of additional insecticides is warranted to delay the onset of insecticide resistance in organic production systems and make their adoption more feasible. As a result, we would hope to see a reduction in the non-target environmental impact caused by pest control.

5. References

Alberto, C. F., A. A. Hoffmann, and L. J. Thomson. 2012. Limited benefits of non-crop vegetation on spiders in Australian vineyards: regional or crop differences?, pp. 541. Springer Science + Business Media, Netherlands.

Asteraki, E. J., B. J. Hart, T. C. Ings, and W. J. Manley. 2004. Factors influencing the plant and invertebrate diversity of arable field margins. Agriculture, Ecosystems & Environment 102: 219-231.

Bianchi, F. J. J. A., C. J. H. Booij, and T. Tscharntke. 2006. Sustainable Pest Regulation in Agricultural Landscapes: A Review on Landscape Composition, Biodiversity and Natural Pest Control. Proceedings: Biological Sciences: 1715.

Chaplin-Kramer, R., M. E. O'Rourke, E. J. Blitzer, and C. Kremen. 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. Ecology Letters 14: 922-932.

Fahrig, L., J. Baudry, L. Brotons, F. G. Burel, T. O. Crist, R. J. Fuller, C. Sirami, G. M. Siriwardena, and J.-L. Martin. 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters 14: 101-112.

Gardiner, T., and J. Dover. 2008. Is microclimate important for Orthoptera in open landscapes? Journal of Insect Conservation 12: 705.

Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, P. Ceryngier, J. Liira, T. Tscharntke, C. J. B. Winqvist, and A. Ecology. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. 11: 97-105.

Grab, H., B. Danforth, K. Poveda, and G. Loeb. 2018. Landscape simplification reduces classical biological control and crop yield. Ecological Applications: n/a-n/a.

Grieshop, M. J., E. Hanson, A. Schilder, R. Isaacs, D. Mutch, C. Garcia-Salazar, M. Longstroth, and J. Sadowsky. 2012. Status Update on Organic Blueberries in Michigan. International Journal of Fruit Science 12: 232-245.

Harris, D. W., K. A. Hamby, H. E. Wilson, and F. G. Zalom. 2014. Seasonal monitoring of *Drosophila suzukii* (Diptera: Drosophilidae) in a mixed fruit production system. Journal of Asia-Pacific Entomology 17: 857-864.

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 Management Science 67: 1352-1357.

Haviland, D. R., J. Caprile, S. Rill, K. Hamby, and J. A. Grant. 2016. Phenology of spotted wing drosophila in the San Joaquin Valley varies by season, crop and nearby vegetation. California Agriculture 70: 24-31.

Johns, R. C., J. Boone, J. J. Leggo, S. Smith, D. Carleton, and D. W. Quiring. 2012. Temporal and Spatial Variations in Microclimate Influence the Larval Foraging Behaviors and Performance of a Conifer-Feeding Sawfly. Environmental Entomology 41: 594-602.

Jonsson, M., R. Bommarco, B. Ekbom, H. G. Smith, J. Bengtsson, B. Caballero-Lopez, C. Winqvist, and O. Olsson. 2014. Ecological production functions for biological control services in agricultural landscapes. Methods in Ecology and Evolution 5: 243-252.

Klick, J., W. Q. Yang, V. M. Walton, D. T. Dalton, J. R. Hagler, A. J. Dreves, J. C. Lee, and D. J. Bruck. 2016. Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation. Journal of Applied Entomology 140: 37-46.

Langellotto, G. A., and R. F. Denno. 2004. Responses of Invertebrate Natural Enemies to Complex-Structured Habitats: A Meta-Analytical Synthesis. Oecologia: 1.

Larsen, A. E., S. D. Gaines, and O. Deschênes. 2015. Spatiotemporal variation in the relationship between landscape simplification and insecticide use. Ecological Applications 25: 1976-1983.

Lee, J. C., A. J. Dreves, A. M. Cave, S. Kawai, R. Isaacs, J. C. Miller, S. Van Timmeren, and D. J. Bruck. 2015. Infestation of Wild and Ornamental Noncrop Fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Annals of the Entomological Society of America 108: 117-129.

Liu, S., Z. Li, Y. Sui, D. A. Schaefer, P. O. Alele, J. Chen, and X. Yang. 2015. Spider foraging strategies dominate pest suppression in organic tea plantations.

Losey, J. E., and M. Vaughan. 2006. The Economic Value of Ecological Services Provided by Insects. BioScience 56: 311-323.

Lustig, A., D. B. Stouffer, C. Doscher, and S. P. J. L. E. Worner. 2017. Landscape metrics as a framework to measure the effect of landscape structure on the spread of invasive insect species. 32: 2311-2325.

Mäder, P., A. Fließbach, D. Dubois, L. Gunst, P. Fried, and U. Niggli. 2002. Soil Fertility and Biodiversity in Organic Farming. Science 296: 1694-1697.

Oehl, F., E. Sieverding, P. Mäder, D. Dubois, K. Ineichen, T. Boller, and A. Wiemken. 2004. Impact of Long-Term Conventional and Organic Farming on the Diversity of Arbuscular Mycorrhizal Fungi. Oecologia: 574.

Ortega, M., and S. Pascual. 2014. Spatio-temporal analysis of the relationship between landscape structure and the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae). Agricultural & Forest Entomology 16: 14-23.

Paredes, D., L. Cayuela, G. M. Gurr, and M. Campos. 2015. Is Ground Cover Vegetation an Effective Biological Control Enhancement Strategy against Olive Pests? PLoS ONE 10: 1-13.

Picchi, M. S., G. Bocci, R. Petacchi, and M. H. Entling. 2016. Effects of local and landscape factors on spiders and olive fruit flies. Agriculture, Ecosystems and Environment 222: 138-147.

Rusch, A., R. Chaplin-Kramer, M. M. Gardiner, V. Hawro, J. Holland, D. Landis, C. Thies, T. Tscharntke, W. W. Weisser, C. Winqvist, M. Woltz, and R. Bommarco.

2016. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agriculture, Ecosystems and Environment 221: 198-204.

Sarthou, J.-P., A. Badoz, B. Vaissière, A. Chevallier, and A. Rusch. 2014. Local more than landscape parameters structure natural enemy communities during their overwintering in semi-natural habitats. Agriculture, Ecosystems and Environment 194: 17-28.

Schmidt, M. H., C. Thies, W. Nentwig, and T. Tscharntke. 2008. Contrasting responses of arable spiders to the landscape matrix at different spatial scales. Journal of Biogeography 35: 157-166.

Silva, R., J. P. Hereward, G. H. Walter, L. J. Wilson, and M. J. Furlong. 2018. Seasonal abundance of cotton thrips (Thysanoptera: Thripidae) across crop and non-crop vegetation in an Australian cotton producing region. Agriculture, Ecosystems & Environment 256: 226-238.

Stutz, S., and M. H. Entling. 2011. Effects of the landscape context on aphid-ant-predator interactions on cherry trees. Biological Control 57: 37-43.

Susan E. Riechert, a. 1999. The Hows and Whys of Successful Pest Suppression by Spiders: Insights from Case Studies. The Journal of Arachnology: 387.

Thomson, L. J., and A. A. Hoffmann. 2010. Natural enemy responses and pest control: Importance of local vegetation. Biological Control 52: 160-166.

Tofangsazi, N., A. Morales-Rodriguez, M. P. Daugherty, G. S. Simmons, and E. E. Grafton-Cardwell. 2018. Residual toxicity of selected organic insecticides to *Diaphorina citri* (Hemiptera: Liviidae) and non-target effects on *Tamarixia radiata* (Hymenoptera: Eulophidae) in California. Crop Protection 108: 62-70.

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. Ecology Letters 8: 857-874.

Van Timmeren, S., and R. Isaacs. 2014. Corrigendum to "Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs" [Crop Prot. 54 (2013) 126–133]. Crop Protection 59: 81.

Vasconcelos, S. D., and G. J. B. de Moura. 2008. PRELIMINARY SURVEY OF SOIL ANUROFAUNA IN A SEMI-ARID AGROECOSYSTEM IN PERNAMBUCO UNDER CONVENTIONAL AND ORGANIC MANAGEMENT. Biociências 16: 72-75.

Veres, A., S. Petit, C. Conord, and C. Lavigne. 2013. Does landscape composition affect pest abundance and their control by natural enemies? A review. Agriculture, Ecosystems and Environment 166: 110-117.

Weibull, A. C., O. Ostman, and A. Granqvist. 2003. Species richness in agroecosystems: the effect of landscape, habitat and farm management, pp. 1335. Kluwer Academic Publishers, Great Britain.

With, K. A. 2002. The Landscape Ecology of Invasive Spread. Conservation Biology 16: 1192-1203.

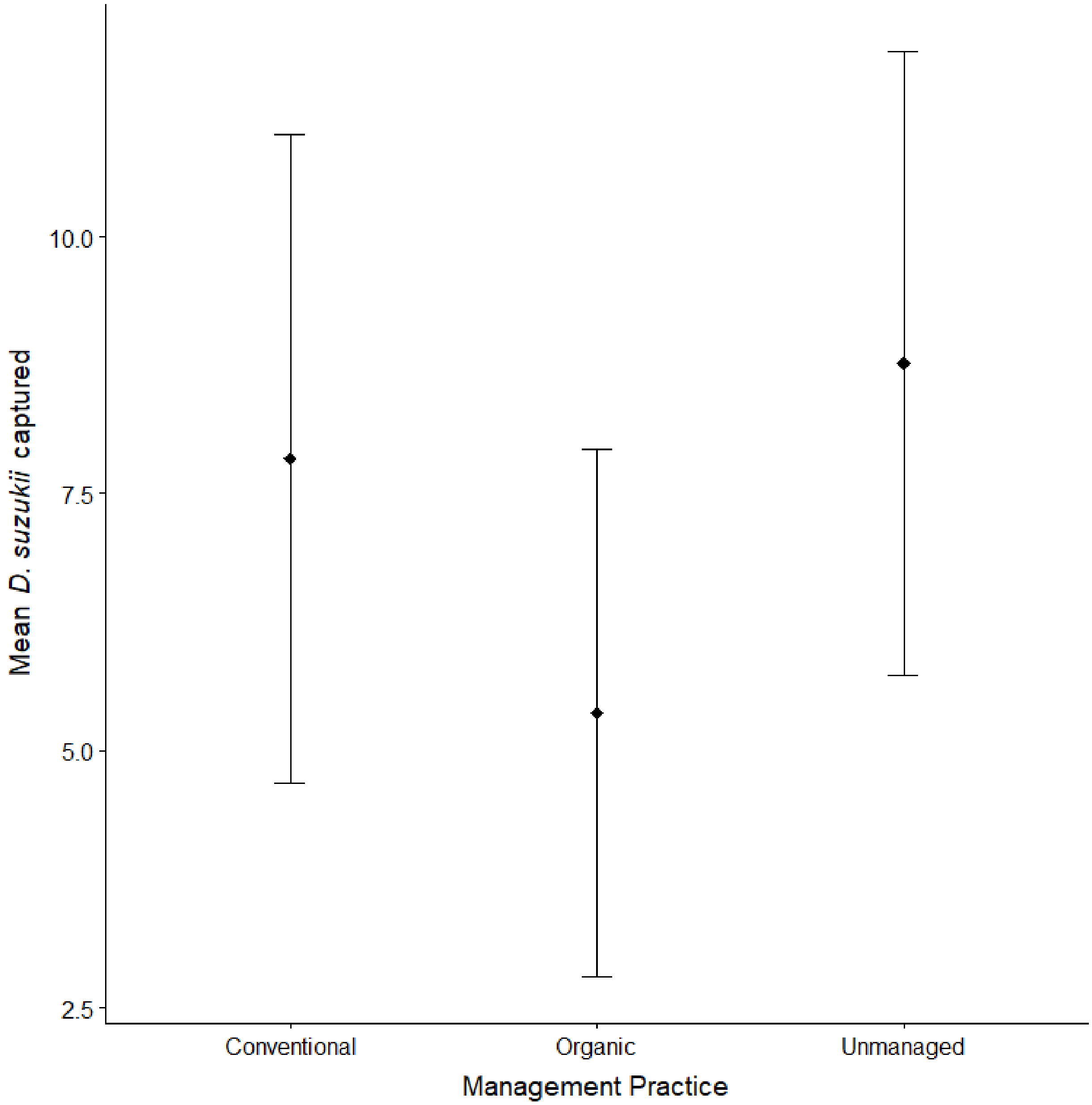

Fig. 4.1. Mean *D. suzukii* captured at farms with each type of management practice over the duration of the study.

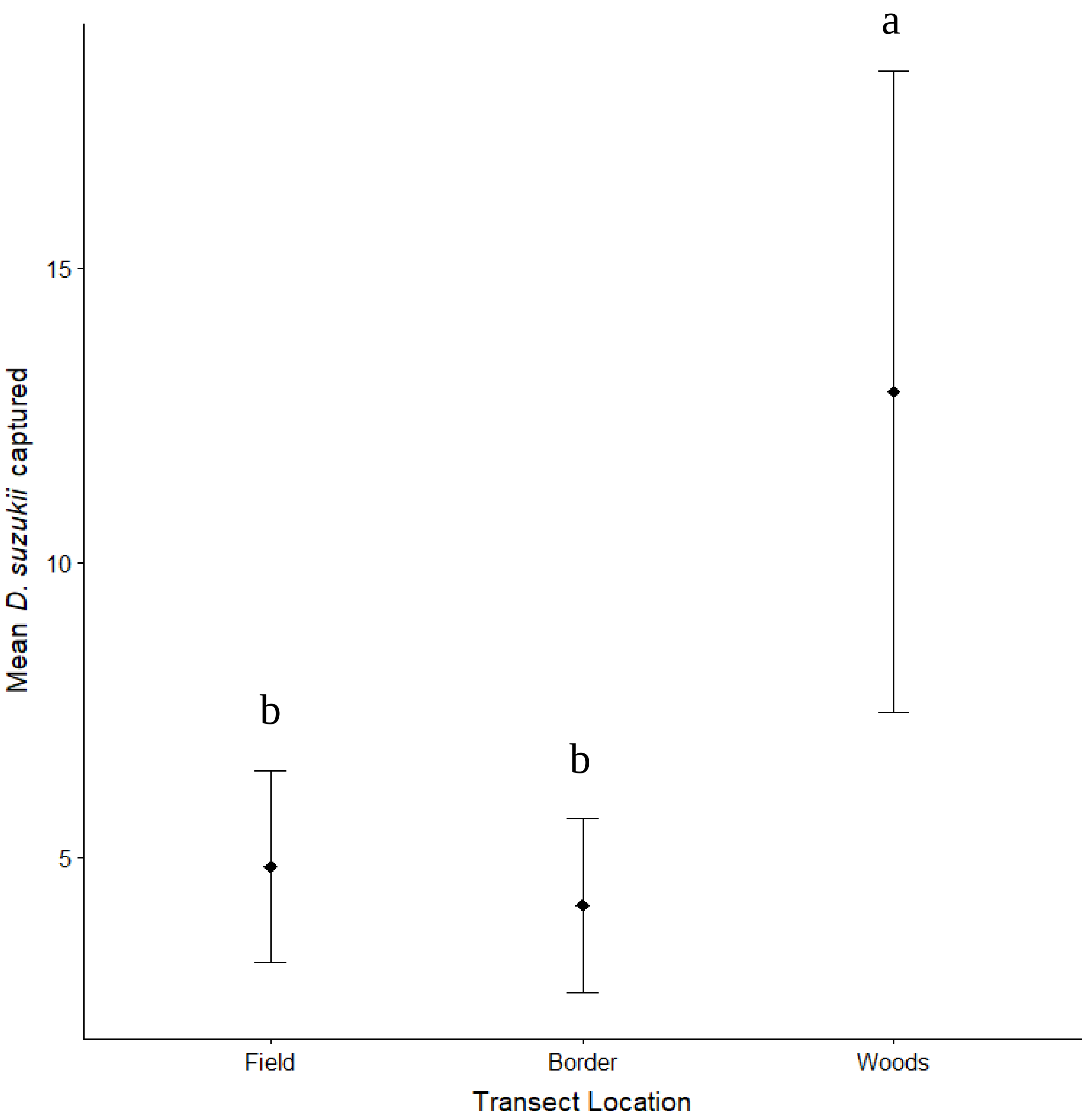

Fig. 4.2. Mean *D. suzukii* captured at each category of location along the transect over the duration of the study.

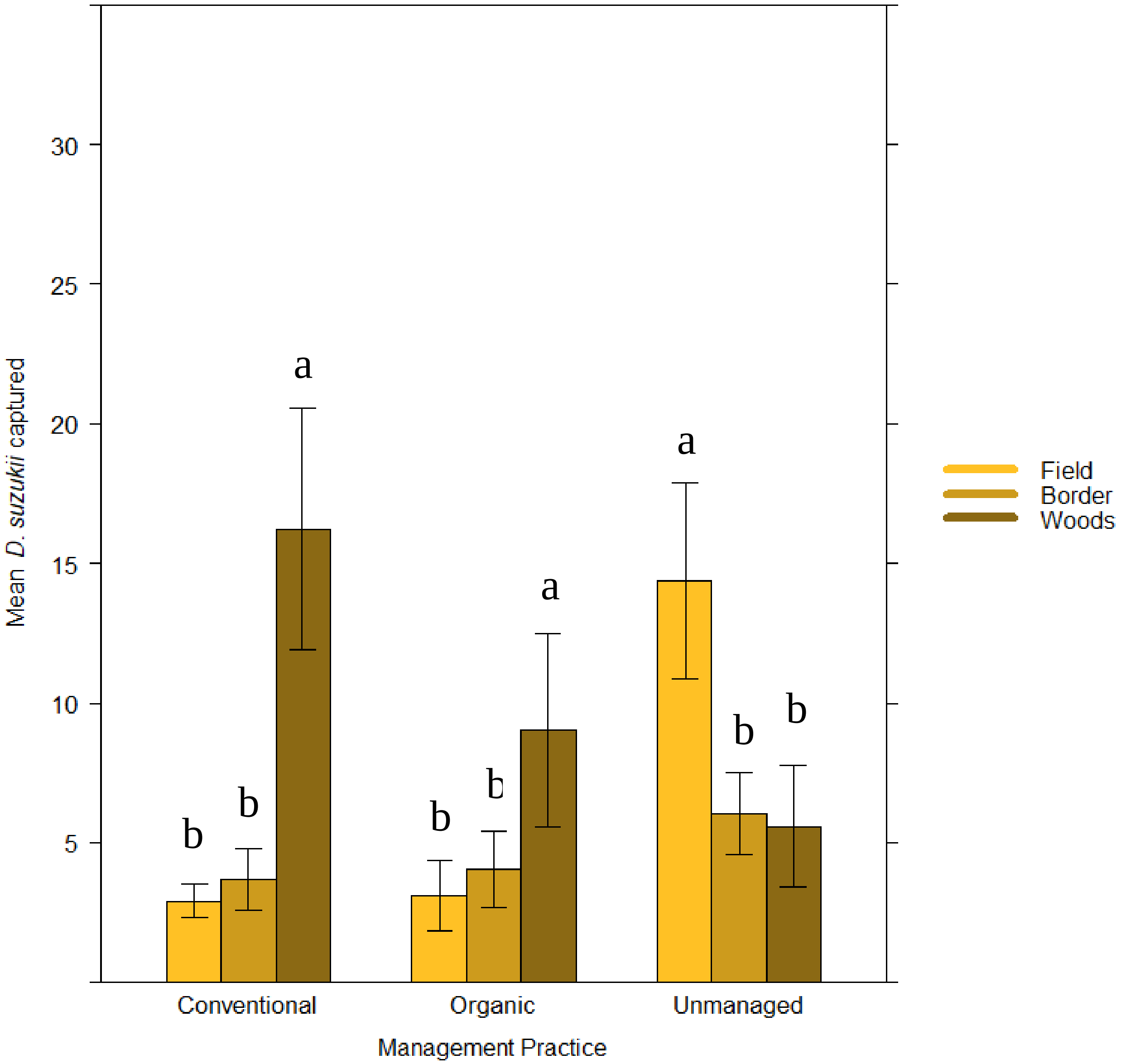

Fig. 4.3. Mean *D. suzukii* captured at farms at each location along the transect within each type of management practice. Letters denote significant separation of means within management practices at the 0.10 signficance level.

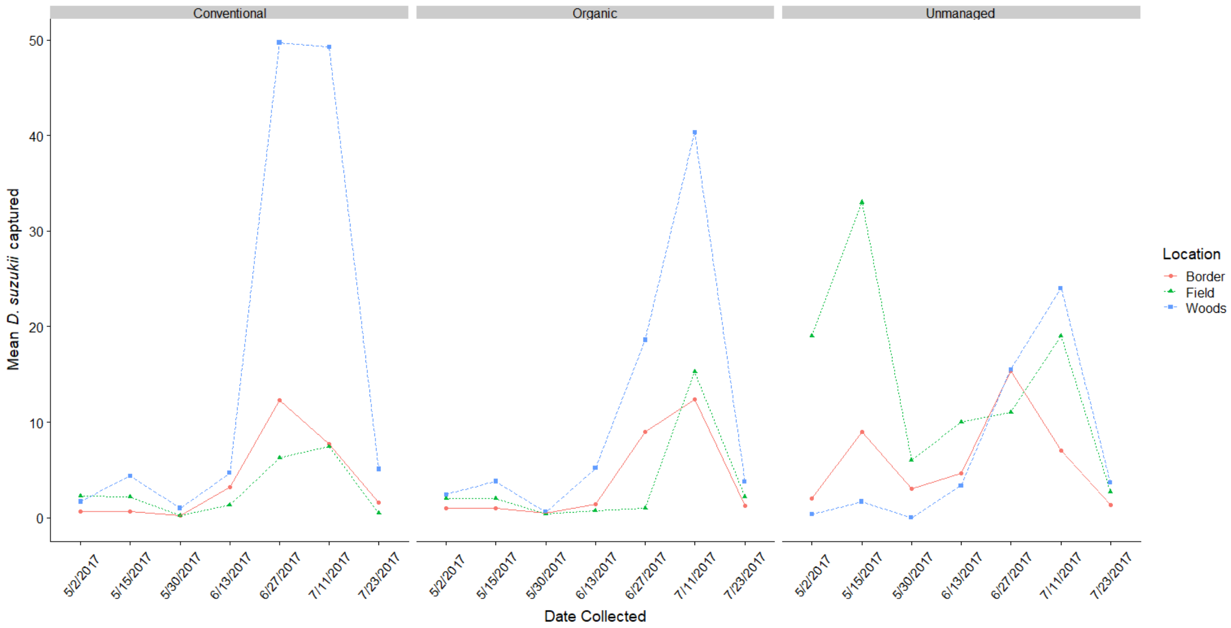

Fig. 4.4. Mean *D. suzukii* captured at farms at each location along the transect within each type of management practice at each collection date.

CHAPTER 5

INFLUENCE OF BLUEBERRY PRESENCE ON *DROSOPHILA SUZUKII* CAPTURES USING YEAST-SUGAR-WATER LURED TRAPS

C. Kirk Green and Ashfaq A. Sial. To be submitted to *Environmental Entomology*.

Abstract

Drosophila suzukii is an invasive vinegar fly which costs millions to Southeastern U.S. fruit growers. This pest is monitored primarily through the use of hanging yeast-sugar-water traps. Previous *Drosophila suzukii* monitoring work has reported peak captures during the winter months. This may be due to increased population density; alternatively, an absence of competing stimulus (wild and farmed fruit) may be artificially inflating trapping numbers. In this study, we investigated the effect of blueberry presence on the number of *D. suzukii* captured by a yeast-sugar-water trap within a mesh enclosure. We found that depending on the design of the enclosure, significantly more *D. suzukii* may be captured in the presence of blueberries, or the absence, or neither. We conclude that important variables to control for when isolating the effect of blueberry presence include proximity to neighboring fruit and the size of the enclosure. Our findings are mixed, but suggest that ripe fruit competes for the attention of *Drosophila suzukii* and influence trap captures. This effect is important to understand in order to correctly estimate *D. suzukii* population densities based on trap capture numbers.

1. Introduction

Drosophila suzukii is an invasive species native to Southeast Asia that has quickly spread its range across much of the world, as well as all the continental United States. Its success is due, in part, to the serrated ovipositor of the female which allows them to oviposit into intact fruits inaccessible to other *Drosophila spp* (Hauser 2011). Arrival of *D. suzukii* can spell disaster for farmers of thin-skinned and small stone fruits, including blueberries, figs, grapes, apricots, and cherries. In the Eastern United States, the losses due to crop rejection and increased management costs were estimated to be between $37-46 million (Burrack et al. 2012).

An important part of suppressing *D. suzukii* is monitoring their populations through trapping. The efficacy of a wide variety of trap designs, baits, and lures have been evaluated by many research efforts (Harris and Peifer 2005, Lee et al. 2012, Basoalto et al. 2013, Iglesias et al. 2014, Renkema et al. 2014, Burrack et al. 2015, Frewin et al. 2017, Cha et al. 2018). Due to the low cost and simplicity of design, many farmers and researchers hang plastic containers with holes poked in the sides and contain a yeast-sugar-water solution, which lures the flies in and drowns them (Landolt et al. 2012). Higher rates of capture should presumably correspond to higher population densities in the field. Unfortunately, trapping numbers have not been shown to reliably predict infestation in the field (Kirkpatrick et al. 2017). Estimating insect populations based on trapping numbers requires information about the distance from which the insect can detect the trap (Adams et al. 2017). To complicate matters, this trap detection distance is likely to fluctuate throughout the year for *D. suzukii* as the abundance of their many host fruits fluctuates (Lee et al. 2015, Klick et al. 2016). Previous *D. suzukii* monitoring work utilizing yeast-sugar-water traps in Georgia blueberry orchards has found the highest peak in captures in the winter months. Furthermore, trap captures were consistently higher in the neighboring woodlands compared to the orchard interiors (Grant 2016). While it is possible that these peaks and trends are due to a higher population density, it is plausible to think that higher captures in the winter could be due to less stimuli in competition with the traps. Likewise, lower captures in the blueberry orchards may be due to an abundance of ripe blueberries rather than a lower field population density compared to the neighboring woodlands.

The intention of these trials was to provide a potential explanation for these counter-intuitive trends. By doing so, we can improve the ability of farmers and researchers to predict *D. suzukii* population densities and infestation events based on trap captures. We conducted trials in the field and in a greenhouse to evaluate the effect of blueberry presence on the number of *D. suzukii* captured by yeast-sugar-water traps.

2. Materials and Methods

2.1 Site Selection

The greenhouse trial was conducted in a greenhouse at the Alma blueberry research and demonstration farm in Bacon County, Georgia. The mesh cube trials and the field enclosure trial were conducted in the Rabbit eye blueberry (*Vaccinium virgatum)* field at the same demonstration farm in Bacon County.

2.2 Insect Rearing

A laboratory colony was used to supply *D. suzukii* specimens for all trials. The colony was reared from wild-caught flies from Clarke County, Ga in 2013 on a standard fly diet (Jaramillo, Mehlferber et al. 2015) substrate portioned into 177-ml polypropylene square bottom bottles (Genesee Scientific, San Diego, CA) with 50mL of diet each. Bottles were capped with cellulose acetate plugs and kept in an incubator (Model I36VLCB, Percival Scientific, Perry, IA) at ~24°C, ~65% relative humidity, and a 14:10 h (L:D) photoperiod. Flies between the ages of six to twelve days old were used for the experiment to control for effects due to age.

2.3 Greenhouse Trial

Two 6x6x6 mesh cubes were laid on their sides and taped together inside of a greenhouse to create a sealed enclosure. A frame was constructed out of ½” PVC pipe to support the structure. For each trial, twenty-five male and twenty-five female *D. suzukii* were released inside of the enclosure along with one centrally positioned standard yeast-sugar-water lure plastic 473 mL (16oz) trap. Each standard 16oz trap contained two grams of active yeast, ten grams of sugar, a drop of scentless soap, and six ounces of water. Flies were selected to be between three and seven days old. Flies were always released at dusk and left alone for twenty-four hours before the trap was collected and the number of flies it caught was recorded. For control replicates, the enclosure contained only the trap and the flies. For treatment replicates, fourteen 8oz plastic containers holding 25g of store-bought blueberries were placed on the floor in a symmetrical, evenly spaced pattern. Additionally, ~125 grams of blueberries were scattered randomly along the ground. When the trap was collected at the end of the replicate period, all berries were collected and placed in vented containers to be reared for two weeks at room temperature, at which point the number of adult male and female *D. suzukii* in the container was recorded.

2.4 Field Trials

2.4.1 Six ft ^{3} mesh cages

Each day, four Rabbit eye blueberry bushes were selected. Each bush was pruned so that it could fit into a 6ft^3 beige mesh cage and then subjected to one of four treatments: all berries removed from the bush and ground, unripe (green) berries removed, ripe (blue) berries removed, or no berries removed. Any tall, non-blueberry vegetation in the immediate area was removed.

After the bushes were prepared, a 473mL(16oz) yeast-sugar-water trap with ~177mL (6oz) of lure was hung in the middle of each bush, and 50 *D. suzukii* (25M, 25F) were released inside. Flies were released at dusk to minimize exposure to high temperatures and left for twenty-four hours. After twenty-four hours, the traps were collected and the number of male and female *D. suzukii* captured was recorded. This procedure was repeated for four days.

2.4.2 Ten ft ^{3} mesh cages

Each day, one rabbiteye and one highbush blueberry bush were randomly selected and pruned to fit into a 10ft^3 white mesh cage. These trials were conducted in mid- June so that highbush blueberries would be past fruiting season and rabbiteye would be in the middle of it, simplifying the process of creating the berry-lacking control group. Once a highbush bush was selected, any remaining blueberries were removed from the bush and the ground. After the bushes were prepared, a 16oz yeast-sugar-water trap with ~6oz of lure was hung in the middle of both bushes, and 50 *D. suzukii* (25M, 25F) were released inside. Flies were released at dusk to minimize exposure to high temperatures and collected 24 hours later, after which the traps were collected and the number of male and female *D. suzukii* in the trap was recorded. This procedure was repeated for five days with a new pair of bushes each day.

2.4.3 24x24x8 ft^3 mesh enclosure

A 24x24x8 ft^3 enclosure was sewn together out of a white mesh fabric and used to enclose 8 Rabbiteye (*Vaccinium virgatum)* blueberry bushes in the middle of two rows. A frame was constructed out of 1.27cm (½”) PVC pipe to support the structure. For each trial, fifty male and fifty female *D. suzukii* were released inside of the enclosure along with one standard yeast-sugar-water lure plastic 16oz trap hung in the center. Each standard 16oz trap contained two grams of active yeast, ten grams of sugar, a drop of scentless soap, and six ounces of water. Flies were selected to be between three and seven days old. Flies were always released at dusk and left alone for twenty-four hours before the trap was collected and the number of flies it caught was recorded. For control replicates, the enclosure contained only the trap and the flies. For treatment replicates, each bush had five small boats made from chicken wire hung from it and each contained 25g of store-bought blueberries. Additionally, twenty 8oz plastic containers containing 25g of blueberries each were placed evenly along the ground under the bushes. When the trap was collected at the end of the replicate period, all berries were collected and placed in vented containers to be reared for two weeks at room temperature, at which point the number of adult male and female *D. suzukii* in the container was recorded. Because the thin material of the enclosure prevented removal to clear the flies, control and treatment replicates were alternated to prevent biasing the trap numbers towards either condition.

2.5 Statistical Analysis

Data were analyzed using R v.3.5.1. Data from each experiment was fit using a generalized linear model (GLM) with a Poisson distribution and a log link function. Each fitted model was tested for overdispersion and fit to a negative binomial GLM with a log link function to account for overdispersion if it was detected. Analysis of deviance was used to determine whether treatment condition explained significant deviance, and Tukey's pairwise comparisons were used to treatment means when analysis of deviance showed that treatment was significant.

3. Results

Significantly more *D. suzukii* were captured in the absence of blueberries (control) than in their presence in the greenhouse trial ($z = 2.14$, $P < 0.001$; Figure 5.1) and in the 24x24x8 ft^3 mesh enclosure ($z = 2.35$, $P = 0.019$; Figure 5.2). Berry presence did not have a significant effect on *D. suzukii* captures in the six ft^3 mesh cages ($\chi^2 = 2.16$, df = 3, $P = 0.539$; Figure 5.3).

In the ten ft ^{3} mesh cages, significantly less *D. suzukii* were captured in the absence of blueberries (control) than in their presence ($z = 2.14$, $P < 0.003$; Figure 5.4).

4. Discussion

After utilizing several different experimental designs, we have found mixed results. These differences can likely be attributed to the experimental designs. In the trial with the six ft^3 enclosures, we may have found no differences among groups because the enclosures were too small. Just outside of each enclosure were untreated blueberry bushes loaded with ripe blueberries, which certainly could have influenced the flies' olfactory environment and distracted them from the lure. However, we found the reverse of our expected outcome in the 10ft^3 enclosures. This may be a result of the different sizes of rabbiteye and highbush blueberry bushes. We utilized the smaller, highbush blueberry bushes after their fruiting season to facilitate the sanitation of the leftover blueberries and minimize the influence of ripe blueberries outside of the enclosure. However, the reduction in canopy cover compared to the rabbiteye bushes seems to have reduced the activity of the flies in their enclosures. It has been shown that *D. suzukii* are less active at temperatures above 28-30°C (Walsh et al. 2011, Kinjo et al. 2014, Tochen et al. 2014), so it is plausible that increased exposure to sunlight was enough to deter them from actively relocating within the control group enclosures.

In the two trials where we had higher captures in the absence of fruit (control group), we can see that these confounding factors are not present. In the greenhouse trial, experimental replicates were conducted in a controlled environment without exposure to wind, direct sunlight, and without proximity to blueberry bushes. In this scenario, we detected a strong signal for the negative effect of blueberry presence on trap captures. In the largest field enclosure, we saw the same signal as in the greenhouse. This trial had several advantages over the other two field trials.

First, the enclosure covered a much larger area and more realistically simulated the field environment. Second, replicates for this trial were conducted after the blueberry harvest season was over, minimizing the effect of the adjacent olfactory environment on the enclosure. Lastly, we conducted all replicates of this trial on the same eight rabbiteye blueberry bushes, minimizing variability due to bush size. This also, however, serves as a limitation on our ability to generalize this effect to other rabbiteye blueberry bushes. The greenhouse trial and the 24x24x8 ft^3 field trial were, arguably, our most robust designs and warrant further exploration into the potential effects of blueberry presence on *D. suzukii* captures.

The results of these trials could help explain why many monitoring efforts have detected peak numbers of *D. suzukii* in the field during the winter, and also in traps placed in the woods as compared to in the field (Grant 2016). Future experiments should seek to verify the effects seen here by replicating our trials with the proper controls. Once this trend effect has been established, researchers can then attempt to precisely quantify the effect of fruit presence on trap captures in various environmental conditions. This information in combination with precise information about local fruiting plant phenology could be very useful for the construction of models designed to estimate *D. suzukii* populations in the field. At the very least, farmers and researchers involved in monitoring efforts should take this information into consideration when noticing a high number of captures in areas and time periods with low fruit presence, as these numbers may be falsely inflated. Likewise, very low numbers during peak fruit presence may be deceptive, and cause growers to underestimate the threat posed by this invasive *Drosophila*.

5. References

Adams, C. G., J. H. Schenker, P. S. McGhee, L. J. Gut, J. F. Brunner, and J. R. Miller. 2017. Maximizing Information Yield From Pheromone-Baited Monitoring Traps: Estimating Plume Reach, Trapping Radius, and Absolute Density of *Cydia pomonella* (Lepidoptera: Tortricidae) in Michigan Apple. Journal of Economic Entomology 110: 305-318.

Basoalto, E., R. Hilton, and A. Knight. 2013. Factors affecting the efficacy of a vinegar trap for *Drosophila suzikii* (Diptera; Drosophilidae). Journal of Applied Entomology 137: 561-570.

Burrack, H., J. Ballington, J. Beam, D. Biddinger, B. Butler, B. Cline, R. Cowles, J. Cox, S. Dalton, D. Epstein, M. Evans, G. Fernandez, P. Fisher, H. Fraser, C. Geyer, R. Groves, J. Hampton, D. Horton, J. Jacobs, D. Johnson, G. Koehler, J. LaForest, O. Liburd, P. Liecsh, G. Loeb, D. Monks, P. Perkins-Veazie, D. Pfeiffer, D. Polk, J. Price, D. Robertson, S. Sambhav, B. Sampson, T. Schlenke, P. Shearer, M. Shrader, N. Singh, J. E. Smith, D. Stanaland, K. Swoboda, D. Trinka, S. Van Timmeren, J. VanKirk, J. Walgenbach, D. Weschler, and A. Wildes. 2012. Spotted wing drosophila impacts in the eastern United States.

Burrack, H. J., M. Asplen, L. Bahder, J. Collins, F. A. Drummond, C. Guédot, R. Isaacs, D. Johnson, A. Blanton, J. C. Lee, G. Loeb, C. Rodriguez-Saona, S. V. Timmeren, D. Walsh, and D. R. McPhie. 2015. Multistate Comparison of Attractants for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in Blueberries and Caneberries. Environmental Entomology 44: 704-712.

Cha, D. H., S. P. Hesler, A. K. Wallingford, F. Zaman, P. Jentsch, J. Nyrop, and G. M. Loeb. 2018. Comparison of Commercial Lures and Food Baits for Early Detection of Fruit Infestation Risk by *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 111: 645-652.

Frewin, A. J., J. Renkema, H. Fraser, and R. H. Hallett. 2017. Evaluation of Attractants for Monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 110: 1156-1163.

Grant, J. A. 2016. Investigation of Wild Flora Bordering Blueberry Fields to Quantify Their Potential as Viable Hosts of *Drosophila suzukii* (Diptera: Drosophilidae). Master of Science, University of Georgia Athens, Georgia.

Harris, T. J. C., and M. Peifer. 2005. The positioning and segregation of apical cues during epithelial polarity establishment in *Drosophila*. The Journal of Cell Biology 170: 813-823.

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 Management Science 67: 1352-1357.

Iglesias, L. E., T. W. Nyoike, and O. E. Liburd. 2014. Effect of Trap Design, Bait Type, and Age on Captures of *Drosophila suzukii* (Diptera: Drosophilidae) in Berry Crops. Journal of Economic Entomology 107: 1508-1518.

Jaramillo, S. L., E. Mehlferber, and P. J. Moore. 2015. Life-history trade-offs under different larval diets in *Drosophila suzukii* (Diptera: Drosophilidae). Physiological Entomology 40: 2-9.

Kinjo, H., Y. Kunimi, and M. Nakai. 2014. Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). 49: 297-304.

Kirkpatrick, D., P. McGhee, L. J. Gut, and J. R. Miller. 2017. Improving monitoring tools for spotted wing drosophila, *Drosophila suzukii*. 164.

Klick, J., W. Q. Yang, V. M. Walton, D. T. Dalton, J. R. Hagler, A. J. Dreves, J. C. Lee, and D. J. Bruck. 2016. Distribution and activity of *Drosophila suzukii* in cultivated raspberry and surrounding vegetation. Journal of Applied Entomology 140: 37-46.

Landolt, P., 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. Journal of applied entomology 136: 148-154.

Lee, J. C., A. J. Dreves, A. M. Cave, S. Kawai, R. Isaacs, J. C. Miller, S. Van Timmeren, and D. J. Bruck. 2015. Infestation of Wild and Ornamental Noncrop Fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Annals of the Entomological Society of America 108: 117-129.

Lee, J. C., H. J. Burrack, L. D. Barrantes, E. H. Beers, A. J. Dreves, K. A. Hamby, D. R. Haviland, R. Isaacs, T. A. Richardson, and P. W. Shearer. 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. Journal of economic entomology 105: 1350-1357.

Renkema, J. M., R. Buitenhuis, and R. H. Hallett. 2014. Optimizing Trap Design and Trapping Protocols for *Drosophila suzukii* (Diptera: Drosophilidae). Journal of Economic Entomology 107: 2107-2118.

Tochen, S., D. T. Dalton, N. Wiman, C. Hamm, P. W. Shearer, and V. M. Walton. 2014. Temperature-Related Development and Population Parameters for *Drosophila suzukii*

(Diptera: Drosophilidae) on Cherry and Blueberry. Environmental Entomology 43: 501-510.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, 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. Journal of Integrated Pest Management 2: G1-G7.

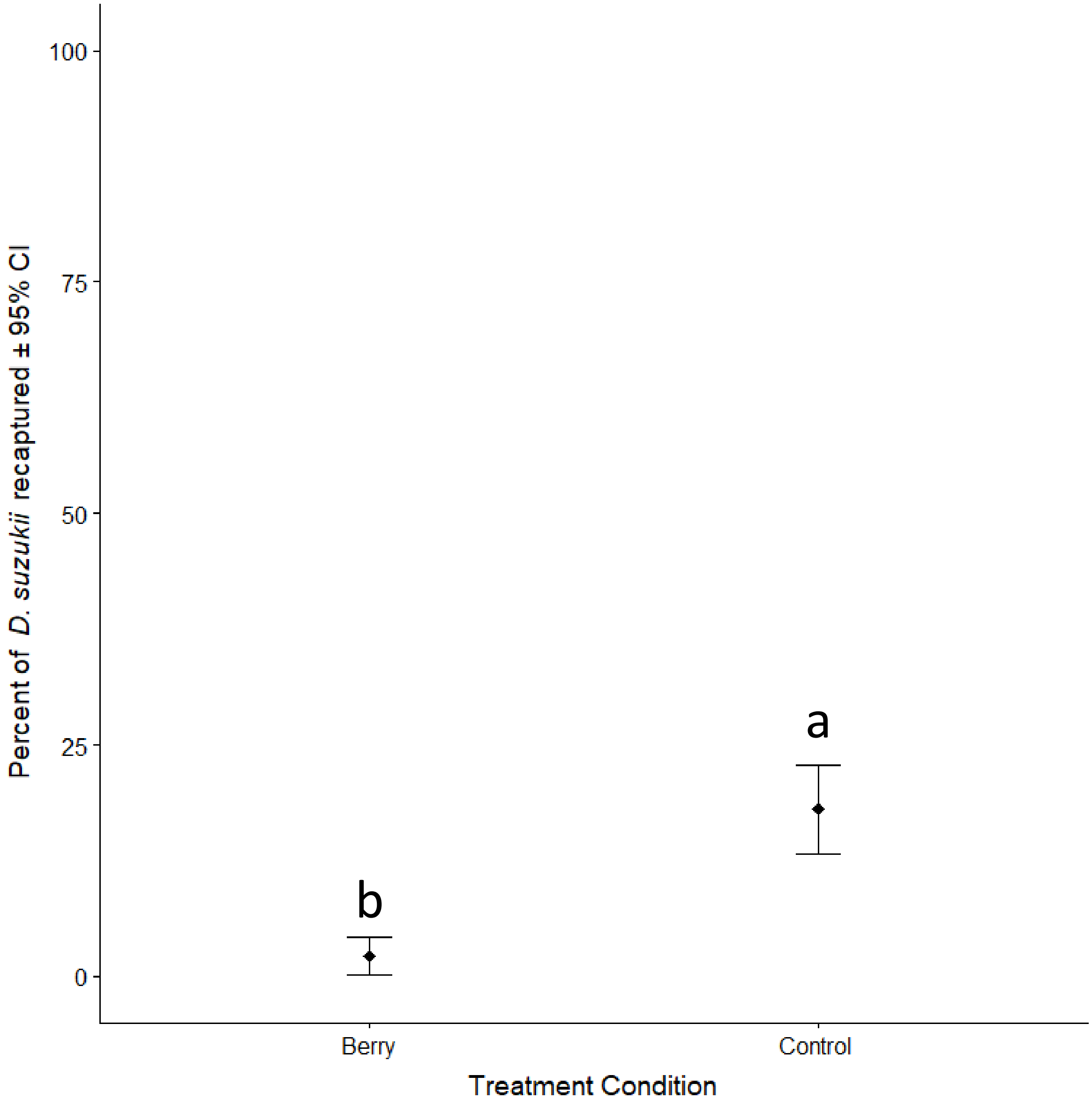

Fig. 5.1. Mean *D. suzukii* captured in each treatment condition during the greenhouse trial.

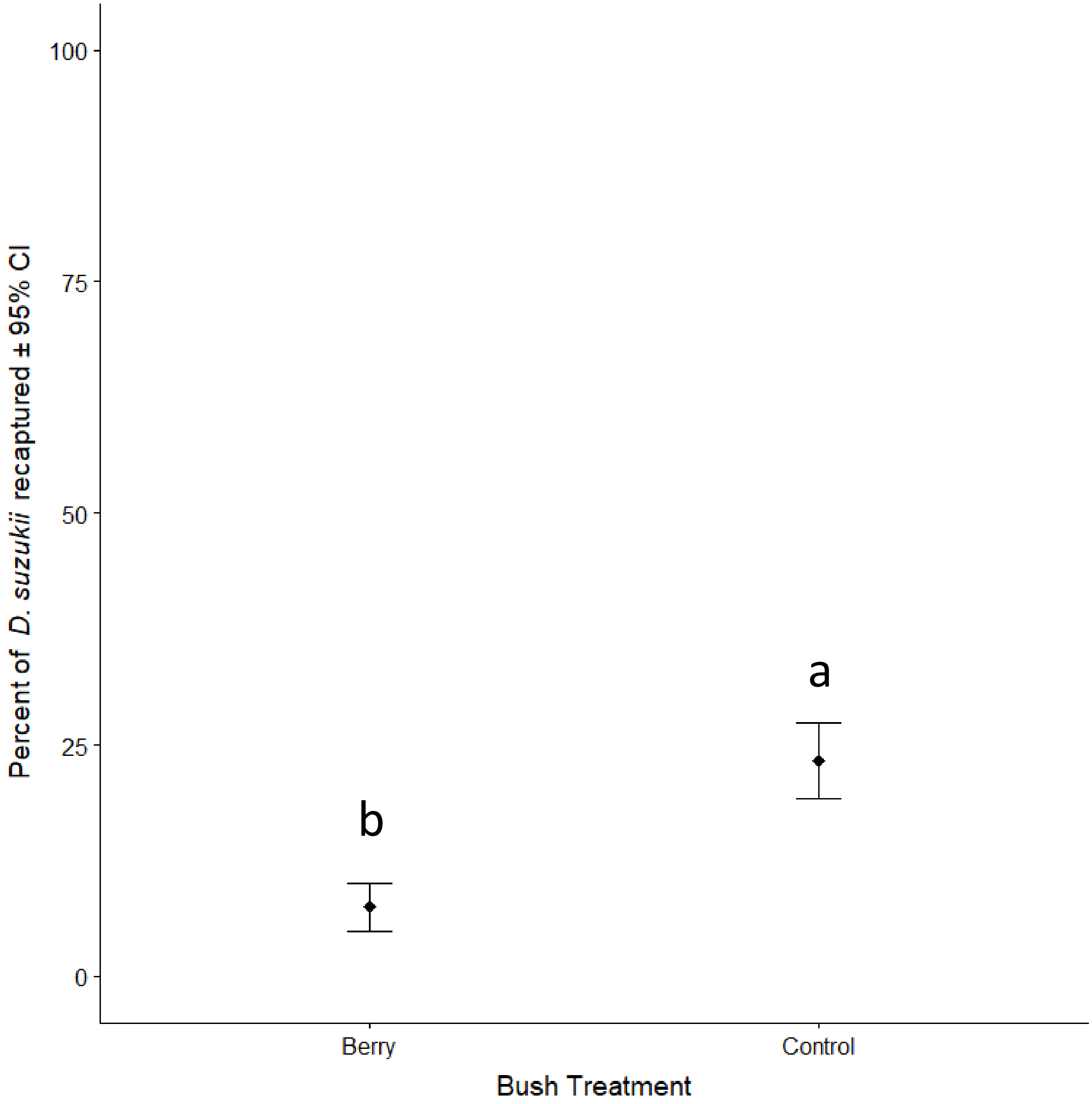

Fig. 5.2. Mean *D. suzukii* captured in each treatment conditions during the field trail using the 24x24x8 ft^3 mesh enclosure.

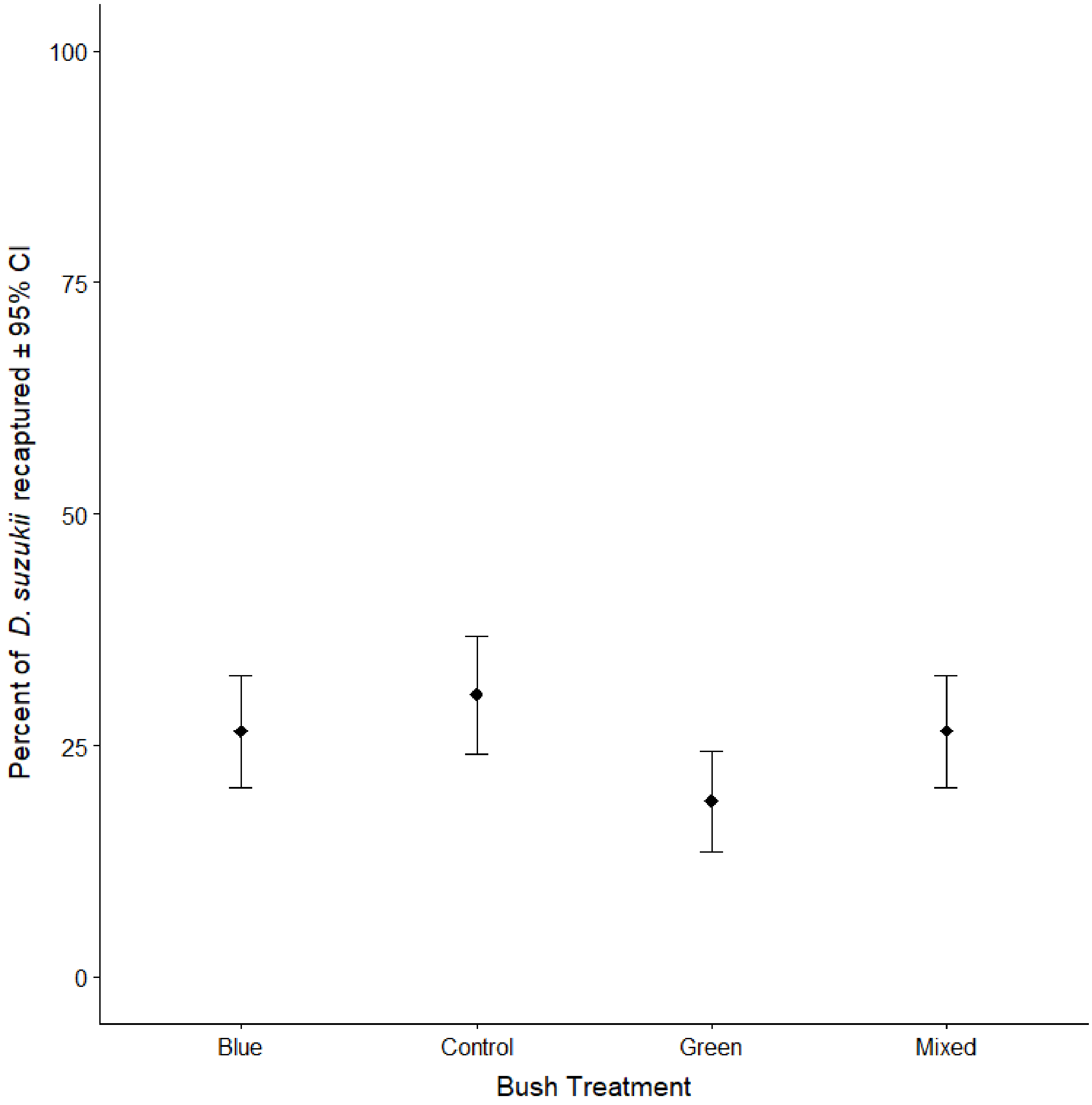

Fig. 5.3. Mean *D. suzukii* captured under each treatment condition during the field trial using the 6x6x6 ft^3 mesh enclosures.

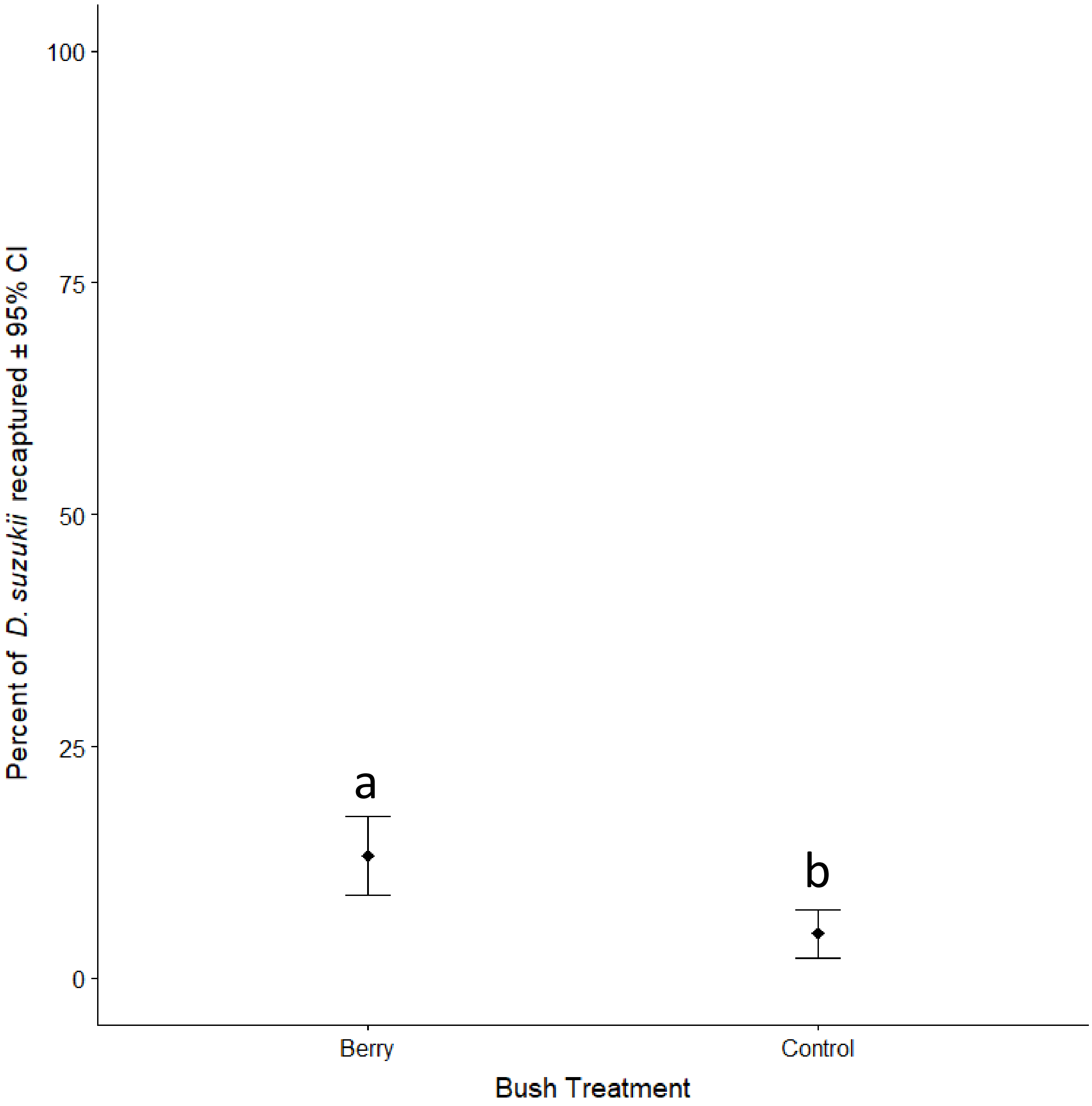

Fig. 5.4. Mean *D. suzukii* captured under each treatment condition during the field trial using the 10x10x10 ft^3 mesh enclosures.

CHAPTER 6

CONCLUSIONS

In these studies, one can find an overarching theme of adaptation. *Drosophila suzukii* is an animal with the ability to modify its phenotype and stretch the upper and lower bounds of its thermal tolerances. It is also able to utilize a wide variety of resources available to it in space and time to further improve its odds of survival and reproduction. The more we understand about how it physiologically responds to and overcomes inhospitable thermal conditions and what resources it is utilizing in the field, the better we can devise management strategies, model its populations, and inform future research efforts. It is essential that management tools and strategies continue to be devised and evaluated in order to reduce the costs incurred by this insect and delay the onset of insecticide resistance.

In our laboratory trials, we observed the effects of heat stress during development on egg viability and adult biology. We also observed evidence for a transmission of heat stress response from adult female to offspring. Both were previously undocumented for this species. Our contributions towards knowledge about the specific effects of heat stress during development is useful for the continued refinement of *D. suzukii* population models and understanding its adaptive potential.

In our field trials, we were able to observe the spatiotemporal changes in *D. suzukii* population density throughout time. We were able to determine that *D. suzukii* are more likely to be found in areas that retain foliage in the winter months. Our overwintering field assays suggest that *D. suzukii* are most likely to overwinter as females or pupae, and forest floor detritus may help insulate them during the cold winter months. These contributions will help shape our understanding of how *D. suzukii* persists in the climate of the southeastern United States.

We were also able to evaluate a variety of monitoring strategies and management practices. Pest management practice, including prophylactic chemical application and pest population monitoring, is an endeavor that can continually be refined. We found similar levels of control in conventional and organic programs, and we also identified a relevant factor (presence of ripe blueberries) which may affect trapping efficacy. By understanding impacts of conventional and organic practices on pest populations, and by critically evaluating the tools we use to monitor and model populations, we will be able to make better decisions about how to manage this key pest.