CHEMICAL CONTROL OF *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) IN GEORGIA BLUEBERRIES

by

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(Under the Direction of ASHFAQ A. SIAL)

ABSTRACT

Drosophila suzukii Matsumura is an invasive pest originating from Southeast Asia and has become a worldwide pest of soft-skinned fruits. The fruit market has a zero-tolerance for this pest, which resulted in the move from reactionary integrated pest management (IPM) to preventative IPM. Management in Georgia, USA, blueberries is most achieved by prophylactic applications of broad-spectrum insecticides. This study explored the effectiveness of using reduced-risk insecticides for resistance mitigation and market expansion, in comparison to practice seen in the region at present. The inclusion of the reduced risk chemicals in rotation with broadspectrum insecticides resulted in statistically equivalent efficacy. In conjunction, the effects of various insecticides on immature life stages of *D. suzukii* were also explored to better understand the full effectiveness of insecticides deployed for *D. suzukii* management. All insecticides tested yielded ovicidal and larvicidal activity, but to varying degrees.

INDEX WORDS: Integrated pest management, *Drosophila suzukii*, insecticides, life stage

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Importance and cultivation of blueberries in Georgia. The blueberry industry of Georgia began in 1925 when seedlings from Florida were planted at the University of Georgia Tifton Campus (G Krewer & NeSmith, 2000). The industry began its initially slow ascent in production in the 1950s with 40 hectares (ha) after the development of commercially viable cultivars (Gerard Krewer, 2018; G Krewer & NeSmith, 2000). In the 1970s, federal grants allowed for the purchase of mechanical harvesters, resulting in a leap to 600ha. Steady growth continued through the 1990s (Gerard Krewer, 2018; G Krewer & NeSmith, 2000) and in the past few decades, Georgia has become a top blueberry producing state. According to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), Georgia produced approximately $74 million in blueberries in 2017.

Origin and arrival of *Drosophila suzukii*: a major threat to blueberry production. *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), commonly known as spotted-wing drosophila (SWD), is in the subgenus Sophorhora and species group Melanogaster (Hauser, 2011). *D. suzukii* was first described in 1916, first studied in the 1930s, and classified as a non-threat to crop production (Asplen et al., 2015; Cini, Ioriatti, & Anfora, 2012; Haye et al., 2016). It has been established in the United States (US) on the islands of Hawaii since the 1980s (Walsh et al., 2011). However, within the past decade, *D. suzukii* has established in both the continental US and Europe as well as in South America (Asplen et al., 2015; Cini et al., 2012; Vilela &

Mori, 2014). *D. suzukii's* rapid spread to various regions of the world has been attributed to human activities, primarily global distribution of infested fruit and a lack of natural regulatory factors within those new regions (Haye et al., 2016). Due to near homogeneous presence of *D. suzukii* in the Nearctic, Palearctic, Indomalaya, and Neotropic realms - which includes the Vavilov's centers - has resulted in substantial problems for producers of small fruits (F Andreazza et al., 2017; Harlan, 1971).

Morphological features. *Drosophila suzukii* is a vinegar fly that has key morphological adaptations relevant to its pest status. They range in coloration from dark brown in the winter morphs to a golden brown in the summer morphs, both of which have red eyes (Shearer et al., 2016). *D. suzukii* body ranges from 2-3mm in length. *D. suzukii* is sexually dimorphic, with females typically being larger than males, resulting in distinct identifications. Males' most notable feature is the subdistal black spot extending from Costal to R4+5 longitudinal veins, which are easily seen unaided (Vilela & Mori, 2014). Females' wings don't have any marking or colorations. Additionally, males have two apically positioned rows of sex combs on each foreleg, the larger set on the first tarsomere and the smaller set on the second (Vilela & Mori, 2014). Female identification characteristics, unlike their male counterparts, are restricted to the genital region. The terminalia, tergite VIII, gives rise to the epiproct, the hypoproct, and the apically pointed oviscapt valves with strong marginal peg-like ovisensilla (Vilela & Mori, 2014). The peg-like ovisensilla coupled with high dexterity of the oviscapt valves allows for the piercing of ripening fruit and the subsequent oviposition beneath the exocarp, the primary reason for its pest status (Asplen et al., 2015; Vilela & Mori, 2014).

Lifecycle. As with most vinegar flies, *D. suzukii* have a relatively short life cycle and high fecundity. Depending on environmental conditions, up to 15 generations occur per year (Cini et al., 2012). Females normally oviposit a single egg per penetration site and in some cases multiple eggs per berry. Each egg has two respiratory filaments that remain external to the fruit; larvae emerge from eggs within 12-48 hours (hrs) and begin consuming the surrounding tissues (F Andreazza et al., 2017). To complete development, *D. suzukii* has three instars followed by the pupa stage and then the adult. Pupariation commonly occurs fully outside the fruit; however, occasionally in the wild and especially under laboratory conditions partially or fully within the fruit. After adult emergence, an additional 1-2 days are required for sexual maturity (F Andreazza et al., 2017). Adult total lifespan can reach upwards of 153 days while the average is 86 days; females have an average lifespan of 79 days and males 93 days (Emiljanowicz, Ryan, Langille, & Newman, 2014). Like most vinegar flies *D. suzukii* exhibit extreme fecundity. On cherries, females produced 219 to 563 eggs and those on Drosophila media produce 92-868 eggs. Fecundity seems to be host-dependent (Cini et al., 2012; Emiljanowicz et al., 2014).

Activity. *D. suzukii* is a highly durable species, having both heat and cool tolerances that allow for survival in regions whose temperatures range from -7.8°C to 37°C (Dalton et al., 2011; Enriquez & Colinet, 2017; Toxopeus, Jakobs, Ferguson, Gariepy, & Sinclair, 2016) although oviposition occurs within a more restricted range of (10°C to 30°C) and sexual activity peaks at 25°C. Humidity is also an important factor in *D. suzukii* survivability. According to Tochen et al 2015, at a tested temperature of 20.6 ± 0.2°C, the reproductively active humidity range is 33-100% with the higher end providing benefits like longer life spans, decreases in time needed for generational cycling, and developmental success. Wild populations in Georgia are crepuscular, limiting activities to the more moderate temperature and humidity ranges (Evans, Toews, & Sial, 2017).

Damage. *D. suzukii* has achieved its pest status exclusively through the activity of the female's oviposition site selection. This species of vinegar fly is highly polyphagous, one of the many traits that has allowed hyper-expansion of their geographical range. While polyphagous, *D. suzukii* primarily targets thin-skinned fruits. Agriculturally important hosts include blueberries, blackberries, raspberries, cherries, grapes, plums, peaches, and strawberries (Andreazza et al., 2017). The initial damage the fruit experiences is inflicted by the female's saw-like ovipositor. All other damage is the direct result of larvae feeding on the living tissues of the fruit, or secondary infestation by other fruit-feeding species (Mortelmans, Casteels, & Beliën, 2012). The larvae remain within the fruit, periodically surfacing for very short times throughout the various instar, only completely emerging as third instars just before pupation. The continuous consumption and waste excretion of the larvae leads to rapid degradation of the fruit quality.

Monitoring of *D. suzukii*. Monitoring is an important component for both growers and consumers; growers for the sake of management and consumers for the sake of quality and later usage. The most lucrative market for blueberries is the fresh market, which has a zero-tolerance for *D. suzukii* infestation (Bruck et al., 2011). Having a system in place to determine the presence and abundance of *D. suzukii* would benefit growers, the first of which allows them to determine when to implement management protocols. Monitoring systems have two basic requirements of equal importance: the ability to attract and quantify the target. To meet the first requirement, all *D. suzukii* traps have attractants, which come in many forms, but all exploit the target's proclivities. Those attractants include apple-cider vinegar, sugar yeast water solution, fermenting fruit juice/purees, and synthetic lures (Burrack et al., 2015; Landolt, Adams, & Rogg, 2012). Currently there are no highly selective lures for *D. suzukii*; however, the yeast sugar water solution and the synthetic lure in conjunction with the apple cider vinegar did show a higher degree of selectiveness compared to the others (Lee et al., 2012). Attractants are not limited to a single sense; trap colors play a big role in their effectiveness. Colors such as yellow, red, and black have continuously yielded higher captures, with conflicting results on which is more effective (Basoalto, Hilton, & Knight, 2013; Lee et al., 2013). There are other important factors that contribute to the effectiveness of traps which include size and location of entry holes, surface area of attractant, volatility of the attractant, life span of the attractant, overall size, and the location of the traps (Basoalto et al., 2013; Renkema, Buitenhuis, & Hallett, 2014).

Management of *D. suzukii*. Due to the sudden emergence of *D. suzukii* as a pest, growers attempting to protect fruit from infestation have shifted from the application of selective insecticides only in response to various monitoring techniques to broad-spectrum insecticide applications initiated by the stage of crop development, with treatments continuing weekly (Mishra et al., 2018; Vilela & Mori, 2014). Currently, trap capture has not been correlated to population size and, thus, an economic threshold has not been developed for this pest. Due to a recent shift in cultural norms, there is a zero-tolerance for SWD, currently mitigating a need for the development of an economic threshold. Most growers in Georgia apply a single insecticide with a short preharvest interval as needed to allow maximum harvest time.

Cultural controls. Cultural control includes sanitation, short harvest intervals, solarization, bagging, exclusion netting, cold treatment, crushing, mass trapping, and spraying (Asplen et al., 2015; Cini et al., 2012; Lee et al., 2011; Walsh et al., 2011). Sanitation means the removal, and most often the destruction, of pest refuges and includes the methods of solarization, bagging, and crushing. Solarization is a process by which the material is placed in a pile and tightly sealed with 1-2mm thick clear plastic to both entrap and kill pests via solar energy (Lee et al., 2011). Bagging is similar to solarization with the only difference being the material is placed in a clear or black bag. Spraying refers to the treatment of over-ripened or downed fruit that will not be harvested for sale. This was initially implemented to discourage *D. suzukii* colonization; however, it is known that some chemicals have curative effects on infested fruits (Walsh et al., 2011; Wise, Vanderpoppen, Vandervoort, O'donnell, & Isaacs, 2015). The exclusion net is a method by which a mesh enclosure is used to cover the plants before the fruit is susceptible. This method has shown extreme success in both Japan and Canada, with a mesh grid size range of 0.98mm to 1.00mm respectively (Asplen et al., 2015; Lee et al., 2011). Cold treatment is a post-harvest method that functions as a curative approach. If harvested fruit is stored at or below 5°C for 24-72 hrs, the larvae and pupae have a reduced chance of survivorship to adulthood (Aly, Kraus, & Burrack, 2017). Crushing is a method that destroys the structural integrity of the fruit, removing the evolutionary advantage that *D. suzukii* has over other members of the Drosophila genus as well as fruit-feeding arthropods ubiquitous in the environment and thus making the fruit open for all to compete. Mass trapping is a method by which 60 to 100 traps are placed in a single acre, emptied and recharged regularly, with the express purpose of reducing the population. Most cultural control techniques are only applicable for small niche growers due to the labor-intensive implementation and/or maintenance.

Chemical Control. Many insecticides, most of which are broad-spectrum, have been shown to have insecticidal activity on *D. suzukii* (Van Timmeren & Isaacs, 2013). Treatments in Georgia like most other regions begin during the ripening period and continue until just before the final harvest. Treatments are often applied with a great lack of prudence, thus contributing to the risk of resistance development.

Many efficacy studies have been conducted to determine the efficacy of different insecticides. However, some of those studies did not limit themselves to just conventional insecticides they include sulfur-based fungicides, azadirachtin, and chlorfenapyr (Felipe Andreazza et al., 2017). Those non-conventional insecticides have a relatively low adulticidal and larvicidal activity, with potential for use in organic system functioning as repellence or oviposition mitigation (Felipe Andreazza et al., 2017; Andreazza et al., 2018). Due to the great success Bacillus thuringiensis on lepidopteran pest followed by the success of B. thuringiensis serovar. *Israelensis* (B.t.i) with respect to the mosquitoes; promoted the test of B.t.i on *D. suzukii* resulting in no toxic activity (Biganski, Jehle, & Kleespies, 2018). Exploration of natural crop protection products such as natural insecticides, microorganisms, oils, and mineral dust, yielding a variety of effects on *D. suzukii* (Cahenzli, Strack, & Daniel, 2018). Of all the products tested by the Cahenzli, Strack, et al. (2018), spinosad and quassia extract were the only products to have economically significant adulticidal activity; however, the quassia extract was only effective upon direct contact. Microorganisms had no adulticidal function, but the medium of application lead to fermentation, which resulted in elevated oviposition (Cahenzli et al., 2018). Most other test products yield results of no important significance, whilst oils had no adulticidal activities, paraffin, hemp, and glycine betaine oils yielded 53%, 40%, and 51% reduction in oviposition (Cahenzli et al., 2018).

Project goals and objectives. The purpose of this project was to determine whether alternative practices that are both future-oriented and market-expanding are equivalent inabilities to manage *D. suzukii* population compared to the current practices. This thesis focused on the following objectives:

1. Evaluate the effectiveness of resistance mitigating insecticide rotations in comparison to that of the current rotations
2. Evaluate the curative effects of various insecticides with respect to immature life stages of *Drosophila suzukii*

References

Aly, M. F., Kraus, D. A., & Burrack, H. J. (2017). Effects of postharvest cold storage on the development and survival of immature *Drosophila suzukii* (Diptera: Drosophilidae) in artificial diet and fruit. Journal of economic entomology, 110(1), 87-93.

Burrack, H. J., Asplen, M., Bahder, L., Collins, J., Drummond, F. A., Guédot, C., ... & Loeb, G. (2015). Multistate comparison of attractants for monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in blueberries and caneberries. Environmental entomology, 44(3), 704-712.

Lee, J. C., Shearer, P. W., Barrantes, L. D., Beers, E. H., Burrack, H. J., Dalton, D. T., ... & Isaacs, R. (2013). Trap designs for monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Environmental entomology, 42(6), 1348-1355.

Lee, J. C., Burrack, H. J., Barrantes, L. D., Beers, E. H., Dreves, A. J., Hamby, K. A., ... & Stanley, C. A. (2012). Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. Journal of Economic Entomology, 105(4), 1350-1357.

Basoalto, E., Hilton, R., & Knight, A. (2013). Factors affecting the efficacy of a vinegar trap for *Drosophila suzukii* (Diptera; Drosophilidae). Journal of Applied Entomology, 137(8), 561-570.

Landolt, P. J., Adams, T., & Rogg, H. (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(1-2), 148-154.

Renkema, J. M., Buitenhuis, R., & Hallett, R. H. (2014). Optimizing trap design and trapping protocols for *Drosophila suzukii* (Diptera: Drosophilidae). Journal of economic entomology, 107(6), 2107-2118.

Shearer, P. W., West, J. D., Walton, V. M., Brown, P. H., Svetec, N., & Chiu, J. C. (2016). Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BMC ecology, 16(1), 11.

Aly, M. F., Kraus, D. A., & Burrack, H. J. (2017). Effects of postharvest cold storage on the development and survival of immature *Drosophila suzukii* (Diptera: Drosophilidae) in artificial diet and fruit. Journal of economic entomology, 110(1), 87-93.

Andreazza, F., Bernardi, D., Baronio, C. A., Pasinato, J., Nava, D. E., & Botton, M. (2017). Toxicities and effects of insecticidal toxic baits to control *Drosophila suzukii* and Zaprionus indianus (Diptera: Drosophilidae). Pest management science, 73(1), 146-152.

Andreazza, F., Bernardi, D., Dos Santos, R., Garcia, F., Oliveira, E., Botton, M., & Nava, D. (2017). *Drosophila suzukii* in southern neotropical region: current status and future perspectives. Neotropical entomology, 46(6), 591-605.

Andreazza, F., Vacacela Ajila, H. E., Haddi, K., Colares, F., Pallini, A., & Oliveira, E. E. (2018). Toxicity to and egg-laying avoidance of *Drosophila suzukii* (Diptera: Drosophilidae) caused by an old alternative inorganic insecticide preparation. Pest management science, 74(4), 861-867.

Asplen, M. K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K. M., . . . Hutchison, W. D. (2015). Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. Journal of Pest Science, 88(3), 469-494.

Basoalto, E., Hilton, R., & Knight, A. (2013). Factors affecting the efficacy of a vinegar trap for *Drosophila suzukii* (Diptera; Drosophilidae). Journal of Applied Entomology, 137(8), 561-570.

Biganski, S., Jehle, J., & Kleespies, R. (2018). Bacillus thuringiensis serovar. israelensis has no effect on *Drosophila suzukii* Matsumura. Journal of Applied Entomology, 142(1-2), 33-36.

Bruck, D. J., Bolda, M., Tanigoshi, L., Klick, J., Kleiber, J., DeFrancesco, J., . . . Spitler, H. (2011). Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest management science, 67(11), 1375-1385.

Burrack, H. J., Asplen, M., Bahder, L., Collins, J., Drummond, F. A., Guédot, C., . . . Lee, J. C. (2015). Multistate comparison of attractants for monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in blueberries and caneberries. Environmental entomology, 44(3), 704-712.

Cahenzli, F., Strack, T., & Daniel, C. (2018). Screening of 25 different natural crop protection products against *Drosophila suzukii*. Journal of Applied Entomology, 142(6), 563-577.

Cini, A., Ioriatti, C., & Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management.

Dalton, D. T., Walton, V. M., Shearer, P. W., Walsh, D. B., Caprile, J., & Isaacs, R. (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(11), 1368-1374.

Emiljanowicz, L. M., Ryan, G. D., Langille, A., & Newman, J. (2014). Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet. Journal of economic entomology, 107(4), 1392-1398.

Enriquez, T., & Colinet, H. (2017). Basal tolerance to heat and cold exposure of the spotted wing drosophila, *Drosophila suzukii*. PeerJ, 5, e3112.

Evans, R. K., Toews, M. D., & Sial, A. A. (2017). Diel periodicity of *Drosophila suzukii* (Diptera: Drosophilidae) under field conditions. PloS one, 12(2), e0171718.

Harlan, J. R. (1971). Agricultural origins: centers and noncenters. Science, 174(4008), 468-474.

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(11), 1352-1357.

Haye, T., Girod, P., Cuthbertson, A., Wang, X., Daane, K., Hoelmer, K., . . . Desneux, N. (2016). Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. Journal of Pest Science, 89(3), 643-651.

Krewer, G. (2018). Blueberries and Strawberries. New Georgia Encyclopedia. Retrieved from https://www.georgiaencyclopedia.org/articles/business-economy/blueberries-and-strawberries

Krewer, G., & NeSmith, D. (2000). The Georgia blueberry industry: Its history, present state, and potential for development in the next decade. Paper presented at the VII International Symposium on Vaccinium Culture 574.

Landolt, P., Adams, T., & Rogg, H. (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(1-2), 148-154.

Lee, J. C., Bruck, D. J., Dreves, A. J., Ioriatti, C., Vogt, H., & Baufeld, P. (2011). In focus: spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest management science, 67(11), 1349-1351.

Lee, J. C., Burrack, H. J., Barrantes, L. D., Beers, E. H., Dreves, A. J., Hamby, K. A., . . . Shearer, P. W. (2012). Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. Journal of economic entomology, 105(4), 1350-1357.

Lee, J. C., Shearer, P. W., Barrantes, L. D., Beers, E. H., Burrack, H. J., Dalton, D. T., . . . Haviland, D. R. (2013). Trap designs for monitoring *Drosophila suzukii* (Diptera: Drosophilidae). Environmental entomology, 42(6), 1348-1355.

Mishra, R., Chiu, J. C., Hua, G., Tawari, N. R., Adang, M. J., & Sial, A. A. (2018). High throughput sequencing reveals *Drosophila suzukii* responses to insecticides. Insect science, 25(6), 928-945.

Mortelmans, J., Casteels, H., & Beliën, T. (2012). *Drosophila suzukii* (Diptera: Drosophilidae): A pest species new to Belgium. Belg J Zool, 142(2), 143-146.

Renkema, J. M., Buitenhuis, R., & Hallett, R. H. (2014). Optimizing trap design and trapping protocols for *Drosophila suzukii* (Diptera: Drosophilidae). Journal of economic entomology, 107(6), 2107-2118.

Shearer, P. W., West, J. D., Walton, V. M., Brown, P. H., Svetec, N., & Chiu, J. C. (2016). Seasonal cues induce phenotypic plasticity of *Drosophila suzukii* to enhance winter survival. BMC ecology, 16(1), 11.

Toxopeus, J., Jakobs, R., Ferguson, L. V., Gariepy, T. D., & Sinclair, B. J. (2016). Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. Journal of insect physiology, 89, 37-51.

Van Timmeren, S., & Isaacs, R. (2013). Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection, 54, 126-133.

Vilela, C. R., & Mori, L. (2014). The invasive spotted-wing Drosophila (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil). Revista Brasileira de Entomologia, 58(4), 371-375.

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., . . . Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. Journal of Integrated Pest Management, 2(1), G1-G7.

Wise, J., Vanderpoppen, R., Vandervoort, C., O'donnell, C., & Isaacs, R. (2015). Curative activity contributes to control of spotted-wing drosophila (Diptera: Drosophilidae) and blueberry maggot (Diptera: Tephritidae) in highbush blueberry. The Canadian Entomologist, 147(1), 109-117.

CHAPTER TWO

EFFECTIVENESS OF REDUCED-RISK INSECTICIDES IN SEASON-LONG RESISTANCE MANAGEMENT PROGRAMS FOR *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE)

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Abstract

Drosophila suzukii Matsumura is a key invasive insect pest on small fruits across the world. Current control programs consist of weekly application of broad-spectrum insecticides with short re-entry and harvest intervals. To expand the market opportunities and mitigate resistance, alternative modes of action must be used and rotated. This study examines the outcomes of two programs, the farmer management program (FMP) and the best management program (BMP). The FMP represents standard growers practices within the Georgia blueberry industry. The BMP represents the market expanding and resistance mitigating programs that can function as an alternative to FMP. All insecticide applications were applied at the maximum field rate and the programs were compared via adult mortality in semi-field bioassays and the progeny production within those assays. The findings of this study show that the adulticidal activity of BMP is statistically equivalent to that of FMP.

1. Introduction

Spotted-wing drosophila (SWD), *Drosophila suzukii* Matsumura, is a recently established invasive pest species of a large variety of economically important crops, especially berries and stone fruits. This species of vinegar fly was first described in 1916 and determined in the 1930's as a non-threat to crop production (Asplen et al., 2015; Cini, Ioriatti, & Anfora, 2012; Haye et al., 2016). However, within the past decade, SWD has established in both the US, Europe, and South America. SWD's rapid spread has been attributed to human activities, primarily via the global distribution of infested fruit, and a lack of natural regulatory factors within those new regions (Haye et al., 2016). Most drosophilid species invasive or not never achieve economic pest status because they require damaged fruit before infestation. This, however, is not true of SWD and some of its sister taxa, which have a highly sclerotized ovipositor allowing oviposition beneath the exocarp of the fruit without prior damage (Asplen et al., 2015; Vilela & Mori, 2014).

Due to the relatively recent invasion of SWD in conjunction with most affected markets having a zero-tolerance fruit damage policy, efforts at sustainable management have largely been abandoned. Growers attempting to prevent fruit infestation and maintain high annual yields have shifted from the application of selective insecticides only in response to various monitoring techniques have shifted to broad-spectrum insecticide applications initiated by fruit ripening and continued weekly (Mishra et al., 2018; Vilela & Mori, 2014). Insecticide selection and application rotations were accompanied by some cultural changes ranging from a reduction in the harvest intervals to physical exclusion with netting; not all of which are applicable in every setting or system (Asplen et al., 2015).

SWD, like most other vinegar flies, have relatively short generation times with high fecundity and, depending on environmental conditions, up to 15 generations can occur per year

(Cini et al., 2012). This coupled with proactive insecticide applications, results in an increased risk of the development of resistance, which is further exacerbated when growers constrict their management to only one to two chemicals per harvest season. Malathion is the preferred conventional insecticide for use in south Georgia due to its short preharvest interval and is coupled with Mustang Maxx (Zeta-cypermethrin) as a hammer when populations become too high. This type of pest management is a great example of a practice that could lead to the rapid development of resistant populations further reducing the arsenal for effectively managing SWD. Resistance is an example of real-time evolution due to artificial pressures in artificial settings. The basic premise is a novel insecticide introduced to a new population of a pest, killing the majority of the population. However, those few individuals survive due to having some novel, genetically-based resistance. This resistance is then passed to the next generation and the process repeats until the majority of the individuals in that population are resistant. As a result, the effectiveness of that insecticide is completely lost against that population and its application is no longer economically feasible.

Insect resistance management (IRM) is an extremely important aspect of integrated pest management (IPM), this is both economically and ecologically critical. Field evolved resistance is the primary target of IRM and is defined as a genetically based decrease in susceptibility of a population to an artificially introduced toxin caused by persistent exposure of the population to the toxin in the field (Tabashnik, Van Rensburg, & Carrière, 2009). IRM consist of any number of strategies with the specific objective of preventing or retarding the pest population decrease in susceptibility. Refuge and mode of action (MoA) strategies will be implemented in this study. In a population, most mutations that result in resistance occur on recessive genes meaning the only heterozygous individuals will exhibit true resistance (Tabashnik et al., 2009). The refuge strategy takes advantage of this by offering an adjacent population that is not under selective pressure due to toxin presence. Once the adults emerge, the populations mix thus inoculating the gene pool with non-resistance genes. MoA refers to the physiological pathway targeted by the toxin and by alternating or rotating the insecticides with different MoAs resistance development is further retard resistance develops by periodically altering the selection pressure (Sparks & Nauen, 2015).

The goal of this study was to evaluate reduced-risk insecticides with different modes of action into season-long programs to improve *D. suzukii* control and reduce non-target effects on beneficials and the likelihood of resistance development. To achieve this goal, we compared the effectiveness of existing farmer management programs with best management programs that included more reduced-risk insecticides on season-long management of *D. suzukii*. The findings will help farmers implement more sustainable management programs for *D. suzukii*. Two different programs were evaluated, the farmer management program (FMP) and the best management program (BMP).

2. Material & Methods:

2.1. Insects

The laboratory colony was established from SWD adults collected from a local homeowner's garden in Clarke County, Georgia during the summer of 2013. The colonies were sustained in accordance with Jaramillo et al. (2015) on a standard cornmeal-molasses-yeast media with slight modifications. Adults placed in 177 ml square bottom polypropylene bottles (Genesee Scientific, San Diego, CA) filled with ~ 50 ml of media and capped with cotton plugs (Genesee Scientific, San Diego, CA). The bottles were housed in an environmental chamber (Model I36VLCB, Percival Scientific, Perry, IA) with light: dark cycle of 14:10 and at 24±1°C; 50-60% RH.

Population maintenance occurred routinely, two to three times per week, by transferring adults to new media and the discarding of unfit media bottles. Three to seven-day-old adult (mature) males and females were used for bioassays.

2.2. Experimental Plots

Field studies were conducted in commercial fields of rabbiteye blueberries (*Vaccinium ashei*) located in southeastern Georgia during the harvest season (May-July) of 2017, 2018, and 2019. The same commercial field was used in 2017 and 2018. The layout remained the same with a simple reduction in plot size. In 2019 a different commercial field was used as well as a complete randomized block design. The field used in 2017 and 2018 was composed of ‘Alapaha’, ‘Brightwell’, and ‘Austin’ cultivars. The blueberry bushes were 12 years old and received regular maintenance including annual hedging and irrigation. The field was divided into three main blocks: the untreated control, best management program (BMP), and the farmer management program (FMP). The two rotation programs were evaluated with and without Nu Film P® as an adjuvant. The field used in 2019 was composed of ‘Powder Blue’, ‘Brightwell’, and ‘Climax’ cultivars. The blueberry bushes were approximately 10 years old and received regular maintenance including annual pruning and irrigation. The field was organized in complete randomized block design. The blocks included: the untreated control, best management program, and the farmer management program. The sticker Scanner was used in all chemically treated plots to control for variation in plots were chemical labels suggest the use of a sticker for best results. The data collected from 2017and 2018 was analyzed a two-way ANOVA and the data from 2019 was analyzed using a one-way ANOVA. Arcsin transformation was performed on proportion data to meet the assumptions of normal distribution. Field infestation data was analyzed using a generalized linear model with Poisson distribution.

2.3.Chemical Treatments

Two management programs called FMP and BMP were evaluated. The insecticides and their affiliated practice and rate for this study are presented in Table 1, Table 2 provides a list of all insecticides used in conjunction with active ingredient, manufacturer, chemical class, and Insecticide Resistance Action Committee (IRAC) classification. A total of four applications were made weekly, a combination of the presence or absence of the adjuvant Nu Film P®, and two insecticides. The applications were made with a ~946 liter John Bean air blast sprayer calibrated to deliver ~467L/ha at a tractor speed of ~5.1 KPH and 1300 RPM. After treatment application, the bushes were left to dry for ~35 minutes before sampling.

2.4.Sampling

2.4.1. Semi-Field Bioassays

Semi-field bioassays were conducted on samples collected 0 and 7 days after treatment (DAT). Each bioassay chamber contained a blueberry shoot with at least 5 leaves and 5 ripe berries. The chamber themselves were designed according to Van Timmeren, S., & Isaacs, R. (2013) with some modifications. The bioassay chambers consisted of a 32oz deli cup and lid (Fabri-Kal®, Kalamazoo, MI), a 1oz soufflé portion cup and lid (Solo Cup Co., Lake Forest, IL), a mesh boat (Jackson Wire International Inc. Houston, TX), and green 4 ¾” (~12cm) water pick (SpringHill Floral Supply, Akron, OH). The 32oz cup is the primary body of the bioassay chamber and houses all other components. The lid has two holes: one is ~2x3cm and covered with mesh fabric and the other has an ~2cm diameter that allows for the introduction of flies, after which is closed off with 1-2 cotton balls. The 1oz deli cup functions as a source of diet and water, the cup is filled with filtered water and the lid with a hole made by a soldering iron is just large enough so that a non-sterile cotton roll (Defend® by Mydent International, Hauppauge, NY) will fit snug leaving ~1/4 of the wick remains on the outside of the cup; which is wrapped with a medium filter paper (Scientific Equipment of Houston, Navasota, TX) strip that was soaked in a yeast-sugar water diet. The diet is 1:3:2 ratio by weight of sugar, water, and yeast, and it's allowed to sit for 24 hours at room temp. The wire mesh boat is made from a 3x5 cm cutting that has been folded in half and hot glued to the base of the chamber. The water pick functions to provide both water and support the bushes shoot; the pick is inserted through a hole at the bottom of the chamber so that the lid is flush against the base and hot glue.

The chambers were labeled and taken to the field in a custom holder for sample collection, with the holder placed in a shaded area to protect freshly harvested samples from direct sunlight (UV radiation) and heat. The untreated control was sampled once per week, while the treated plots were sampled before treatment (0 DAT) and after treatment (7 DAT), except for the first and last week. Once all samples had been collected, they were taken immediately to the lab to preserve quality.

Five pairs of female and male *D. suzukii* (3-7days old) were aspirated from culture bottles into glass vials and introduced into bioassay chambers via a hole in the lid then plugged with 1-2 cotton balls. The chambers remain on the lab bench at 23±1°C and 40-50% RH (relative humidity). Mortality was recorded at 24, 72, 120, and 168h after exposure in the 2017 study, with the 120h exposure observation omitted in the 2018 study.

2.4.2. Progeny Counts

After the 168h observation, the berries were removed from the bioassay chamber and placed in a 2oz soufflé portion cup and lid (Fabri-Kal®, Kalamazoo, MI) for a week to allow progeny to develop. In 2017 a folded paper towel, and in 2018 a non-sterile cotton roll, was placed in the bottom to wick-up excess liquid generated due to progeny development. The lids had a hole of ~2cm diameter covered with mesh fabric for venting. After a week of development, the berries were frozen and dissected to count SWD larvae, pupae, and adults if any had emerged.

2.4.3. Field Infestations

Field infestation samples were collected in conjunction with the bioassay samples and placed in a 16-ounce deli cup with lid (Choice Paper Company, Brooklyn, NY), the cup was filled halfway with berries. The lids had an ~2x3cm hole covered with fabric mesh for venting. The samples were left on the lab bench for two weeks to allow emergence after which they were frozen and then processed via salt-extraction as described in (Van Timmeren & Isaacs, 2013) with modifications. The field infestation cups were emptied into a 1gal Ziploc bag and lightly crushed by hand just enough pressure to ensure that all the berries had been ruptured. A salt solution of 1cup per gal. of water was added to the bag, enough to completely emerge the crushed fruit. The bags remained undisturbed for at least 30 minutes before being strained with a ¼inch mesh screen sifting pan (Sona Enterprises (SE®), Santa Fe Springs, CA) and sprayed with a hand pressure sprayer (Solo Cup Co., Lake Forest, IL), the content was captured with a pan and strained by a permanent basket coffee filter (Medelco Inc., Bridgeport, CT). The content that remained in the filter was used to generate the counts using a dissecting scope.

Table 1 Insecticide rotation with respect to practice, time, and application rates.

Treatment Program 2017		Week1	Week2	Week3	Week4
BMP	With Nu Film P®	Phosmet	Spinetoram	Malathion	Fenpropathrin
	Without Nu Film P®	1.3lbs/acre	6oz/acre	2.5pt/acre	16oz/acre
FMP	With Nu Film P®	Malathion	Fenpropathrin	Malathion	Fenpropathrin
	Without Nu Film P®	2.5pt/acre	16oz/acre	2.5pt/acre	16oz/acre
Treatment Program 2018		**Week1**	**Week2**	**Week3**	**Week4**
BMP	With Nu Film P®	Phosmet	Spinetoram	Malathion	Cyantraniliprole
	Without Nu Film P®	1.3lbs/acre	6oz/acre	2.5pt/acre	20.5oz/acre
FMP	With Nu Film P®	Malathion	Malathion	Malathion	Malathion
	Without Nu Film P®	2.5pt/acre	2.5pt/acre	2.5pt/acre	2.5pt/acre

Note: Nu Film P® was applied at 6oz per acre. The malathion used was Malathion 8F

Treatment Program 2019		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
BMP	Scanner	Malathion 2pt/acre	Spinetoram 6oz/acre	Chromobacterium 3lb/acre	Cyantraniliprole 20.5oz/acre	Chromobacterium 3lb/acre
FMP	Scanner	Zeta-cypermethrin 4oz/acre	Malathion 2pt/acre	Zeta-cypermethrin 4oz/acre	Malathion 2pt/acre	

Note: Scanner was applied at 4oz per acre. The malathion used was Malathion 5EC

Table2 Commercial names of insecticides used couple with active ingredients, class, and MoA using IRAC (Insecticide Resistance Action Committee) classification.

Trade Name	Active ingredient	Manufacturer	Class	Mode of Action
Imidan 70W	Phosmet	Gowan Company 370 S. Main Street Yuma, AZ 85366-5569	Organophosphate	1B
Delegate™ 25WG	Spinetoram	Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268	Spinosyns	5
Malathion 8F	Malathion	Gowan Company 370 S. Main Street Yuma, AZ 85366-5569	Organophosphate	1B
Malathion 5EC	Malathion	Drexel Chemical Company P.O. Box 13327 Memphis, TN 38113	Organophosphate	1B
Grandevo® WDG	Chromobacterium Subtsugae Strain PRAA4-1^{T}	Marrone Bio Innovation Inc. 1540 Drew Ave. Davis, CA 95618	-	-
Mustang® Maxx	Zeta-cypermethrin	FMC Corporation 2929 Walnut Street Philadelphia, PA 19104	Pyrethroids/ Pyrethrins	3A
Danitol 2.4EC	Fenpropathrin	Valent U.S.A Corporation 1600 Riviera Ave Ste 200 Walnut Creek, CA 94596	Pyrethroids/ Pyrethrins	3A
Exirel™10SE	Cyantraniliprole /Cyazypyr	DuPont 1007 Market Street Wilmington, DE 19898	Diamides	28

3. Results

3.1. Adult Mortality

Adult mortality refers to the bioassay where adult flies were exposed to treated plant material. There are significant differences in adult mortality between each of the practices compared to the control. However, the two practices, BMP and FMP were not significantly different from one another (Table 3). All chemicals, regardless of practice, yielded a significant difference in adult mortality for the 0 DAT bioassays at 24h compared to the UTC. The adjuvant Nu Film P® treatment plots were not significantly different from the plots lacking Nu Film P® at 0 DAT. There was a significant difference for Nu Film P® at 7 DAT. However, this difference is not economically important because neither the presence nor absence of Nu Film P® are significantly different from that of the untreated control.

Table 3 Adult Mortality

Treatment		**2017**	**2018**	**2019**
UTC		15.11 ± 3.74 a	13.75 ± 3.27 a	14.29 ± 3.75 a
BMP	**With Adjuv.**	76.67 ± 4.12 b	77.50 ± 8.85 b	52.37 ± 9.65 b
	Without Adjuv.	80.56 ± 3.74 b	85.00 ± 5.97 bc	
FMP	**With Adjuv.**	82.47 ± 3.95 b	100.00 ± 0.00 c	62.88 ± 11.53 b
	Without Adjuv.	86.11 ± 3.70 b	91.66 ± 8.33 bc	
Program: df,F,*p*		2:185, 89.39, <.0001	2:92, 143.334, <.0001	2:48, 9.932, 0.0002
NuFilm: df,F,*p*		1:185, 0.147, 0.372	1:92, 0.003, 0.956	

3.2. Progeny

There was a significant difference in the number of progenies produced with respect to each of the two practices compared to the control, but the two practices themselves were not significantly different (Table 4). There is a significant difference in the amount of progeny produced with respect to 0 DAT and the control. However, there is no significant difference between the 7 DAT and control, thus confirming the results of the adult mortality. There were no significant differences in the production of offspring in regard to the absence and presence of Nu Film P® with respect to 0 DAT and 7 DAT. There were no significant differences among the selected insecticides at 0 DAT, but there was a significant difference when each is compared to the control. There were no significant differences at any level for the 7 DAT progeny with respect to each insecticide.

Table 4 Progeny

Treatment		**2017**	**2018**	**2019**
UTC		39.45 ± 3.70 a	36.30 ± 2.34 a	10.86 ± 1.40 a
BMP	**With Adjuv.**	10.83 ± 1.63 b	15.00 ± 3.27 b	8.42 ± 2.16 ab
	Without Adjuv.	13.16 ± 2.51 b	17.00 ± 3.62 b	
FMP	**With Adjuv.**	11.00 ± 2.41 b	15.62 ± 3.60 b	2.83 ± 1.16 b
	Without Adjuv.	10.34 ± 1.86 b	20.17 ± 4.21 b	
Program: df, F, *p*		2:307, 40.01, <.0001	2:136, 17.92, <.0001	2:42, 5.46, 0.008
NuFilm: df, F, *p*		1:307, 0.137, 0.712	1:136, 1.29, 0.258	

3.3.Field Infestation

Natural *D. suzukii* infestation in the experimental field was negligible each year of the study. Whilst differences can be seen in Table 5, those differences are not statically significant.

Table 5 Infestation

Treatment		**2017**	**2018**	**2019**
UTC		0.0043 ± 0.00	0.1358 ± 0.04	0.0000 ± 0.00
BMP	**With Adjuv.**	0.0000 ± 0.00	0.0372 ± 0.04	0.0000 ± 0.00
	Without Adjuv.	0.0008 ± 0.00	0.0030 ± 0.00	-
FMP	**With Adjuv.**	0.0003 ± 0.00	0.0325 ± 0.03	0.0000 ± 0.00
	Without Adjuv.	0.0155 ± 0.01	0.0043 ± 0.00	-
Program: df, χ^2, *p*		2, 0.65, 0.723	2, 4.39, 0.111	2, <0.001, 1
NuFilm: df, χ^2, *p*		1, 0.72, 0.397	1, 0.68, 0.410	-

4. Discussion/Conclusion:

These studies document the efficacy of season-long insecticide programs with weekly application for *D. suzukii* control in conventional blueberry growing system in Georgia, USA. The two management programs compared: FMP (a traditional growers management program) and BMP (a resistance mitigating and market expanding management program). The 24 h exposure showed a higher percentage of adult mortality in the treated plots (Table 3). It can also be seen in Table 3 that FMP and BMP are statistically equivalent, suggesting that the two programs are interchangeable with respect to *D. suzukii* management. There were no notable residual effects at 7 DAT substantiating the results of (Diepenbrock, Rosensteel, Hardin, Sial, &

Burrack, 2016). The adjuvant NuFilm P had no significant effect on the efficacy of either management program in 2017 or 2018 resulting in the removal of the adjuvant variable from the 2019 study.

Whilst BMP does have an equivalent effect on *D. suzukii* management, the major concern from the grower perspective is the cost difference. For 2017, implementation of the BMP would cost 158% more than that of the FMP, for 2018 its 381% more, and for 2019 it is 806%. These cost differences can be very steep in the short term. However, investment on the front end can prevent great financial loss on the tail end resulting from loss of cheap conventional insecticides due to resistance development. BMP is resistance mitigating by alternating modes of actions, reducing the likelihood of resistance development to any single chemical class. The MoA alternation with *D. suzukii*'s highly polyphagous nature, whose host-range includes non-crops resulting in the near homogeneous presence (Andreazza et al., 2017), theoretically will complement one another extremely well due to much of the surrounding terrain functioning as a refuge and inoculating the field population with unselected gene pool.

The BMP utilizes reduced risk chemicals, which convey both ecological and economic benefits. The ecological benefit of these chemicals is that they are less likely to affect non-targets (Liu, Zhang, Peng, Rojas, & Trumble, 2012). The economic benefits are a direct result of the ecological benefits; unaffected non-targets result in stabilized and sustained beneficial communities further suppressing the pest populations.

Alternating modes of action can also have market expanding effects by allowing fruit to be sold in markets with stricter residue standard. This is theoretically achieved by preventing unripe fruit from accumulating residues of a single compound during ripening and exceeding the maximum residue limits (MRL) of some markets. Fruit exceeding the MRL of their intended market result in the rejection of the entire shipment, resulting in the grower having additional shipping fee and a loss of income (Diepenbrock et al., 2016).

BMP yield statistically equivalent control of *D. suzukii* to that of FMP while also have market expanding and resistance mitigating properties. While there is an expense difference with respect to the implementation of BMP it is well worth the difference. The market expansion can translate into a real-time increase in profit, as the resistance mitigation will ensure a long-lived means of uninterrupted *D. suzukii* control.

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6. References

Andreazza, F., Bernardi, D., Dos Santos, R., Garcia, F., Oliveira, E., Botton, M., & Nava, D. (2017). *Drosophila suzukii* in southern neotropical region: current status and future perspectives. Neotropical entomology, 46(6), 591-605.

Asplen, M. K., Anfora, G., Biondi, A., Choi, D.-S., Chu, D., Daane, K. M., . . . Hutchison, W. D. (2015). Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. Journal of Pest Science, 88(3), 469-494.

Cini, A., Ioriatti, C., & Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management.

Diepenbrock, L. M., Rosensteel, D. O., Hardin, J. A., Sial, A. A., & Burrack, H. J. (2016). Season-long programs for control of *Drosophila suzukii* in southeastern US blueberries. Crop Protection, 81, 76-84.

Haye, T., Girod, P., Cuthbertson, A., Wang, X., Daane, K., Hoelmer, K., . . . Desneux, N. (2016). Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. Journal of Pest Science, 89(3), 643-651.

Liu, T.-X., Zhang, Y.-M., Peng, L.-N., Rojas, P., & Trumble, J. T. (2012). Risk assessment of selected insecticides on Tamarixia triozae (Hymenoptera: Eulophidae), a parasitoid of Bactericera cockerelli (Hemiptera: Trizoidae). Journal of Economic Entomology, 105(2), 490-496.

Mishra, R., Chiu, J. C., Hua, G., Tawari, N. R., Adang, M. J., & Sial, A. A. (2018). High throughput sequencing reveals *Drosophila suzukii* responses to insecticides. Insect science, 25(6), 928-945.

Sparks, T. C., & Nauen, R. (2015). IRAC: Mode of action classification and insecticide resistance management. Pesticide biochemistry and physiology, 121, 122-128.

Tabashnik, B. E., Van Rensburg, J. B. J., & Carrière, Y. (2009). Field-Evolved Insect Resistance to Bt Crops: Definition, Theory, and Data. Journal of Economic Entomology, 102(6), 2011-2025. DOI:10.1603/029.102.0601

Van Timmeren, S., & Isaacs, R. (2013). Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection, 54, 126-133.

Vilela, C. R., & Mori, L. (2014). The invasive spotted-wing Drosophila (Diptera, Drosophilidae) has been found in the city of São Paulo (Brazil). Revista Brasileira de Entomologia, 58(4), 371-375.

CHAPTER THREE

CONTROLLING IMMATURE LIFE STAGES TO REDUCE POPULATIONS OF *DROSOPHILA SUZUKII* (DIPTERA: DROSOPHILIDAE) MATSUMURA

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Abstract

The spotted-wing drosophila, (*Drosophila suzukii*) (Matsumura), is a key invasive pest on small fruits. The control of *D. suzukii* is crucial for profitable production because there is a zero-tolerance for infested fruit. Current control programs are based on insecticides targeting adults. Certain insecticides, however, can be used after infestation as curative control. This study was conducted to assess the effectiveness of selected insecticides on immature life stages of *D. suzukii*. Flies were allowed to oviposit on blueberries, the eggs were allowed to develop to the various instars, and the fruit containing the instars was sprayed with cyantraniliprole, fenpropathrin, malathion, phosmet, spinetoram, spinosad, and zeta-cypermethrin. The study was conducted under pristine laboratory conditions and confirmed the curative control. Examination under field conditions is the next step to further confirm the results for their implementation in the refining of the integrated pest management programs.

1. Introduction

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an invasive pest that causes detrimental damage through infestation in soft-skinned fruits i.e., cherry, blueberry, raspberry, and strawberry (Goodhue, Bolda, Farnsworth, Williams, & Zalom, 2011; Lee et al., 2011). *Drosophila suzukii* is widely distributed throughout all major fruit production regions worldwide (Miller et al., 2015; Walsh et al., 2011; Wiman et al., 2014). Infestation of susceptible fruit can significantly affect fruit marketing and have a negative influence on local economies (Farnsworth et al., 2017; Goodhue et al., 2011).

Spotted-wing drosophila attacks ripe and ripening fruit by the adult female laying eggs within 1mm of the fruit surface (Lee et al., 2011). *Drosophila suzukii* has a unique serrated ovipositor that can penetrate the skin of the fruit (Dalton et al., 2011; Lee et al., 2011; Wiman et al., 2014). Hatching larvae burrow into the fruit flesh and develop within the fruit (Walsh et al., 2011). The egg and larval stages are typically protected from any of harmful agents (Dalton et al., 2011; Hamby et al., 2016; Plantamp et al., 2016) such as biocontrol agents and insecticides (Miller et al., 2015; Stacconi et al., 2015; Stacconi et al., 2017). Insecticides are currently the most often-used method for *D. suzukii* management (Diepenbrock, Rosensteel, Hardin, Sial, & Burrack, 2016; Fanning, Grieshop, & Isaacs, 2018; Van Timmeren & Isaacs, 2013). Most management programs recommend insecticides to target the adult life stage through direct contact with residues applied to the fruit (Cuthbertson, Collins, Blackburn, Audsley, & Bell, 2014; Fanning et al., 2018; Smirle, Zurowski, Ayyanath, Scott, & MacKenzie, 2017; Van Timmeren & Isaacs, 2013). However, limited data is available regarding the effects of insecticides on immature life stages (Shawer and Wise).

The objective of this study was to evaluate the efficacy of commonly used insecticides from different chemical classes on immature life stages of *D. suzukii* in blueberry.

2. Materials and Methods

Laboratory experiments with insecticides were conducted during the periods of fruit ripening and harvest for rabbiteye blueberry in Georgia. *Drosophila suzukii* used for this experiment were maintained in a laboratory colony as previously described (Grant and Sial 2016; Evans, Toews, et al. 2017), The blueberries purchased from local food market were rinsed on a sieve and then dried using paper towels until the surface was entirely dry. A total of 30 healthy blueberries were then placed into 473 ml plastic deli containers with lids (Fabri-Kal Corp., Kalamazoo, MI). A set of 30 female and 30 male *D. suzukii* adults were released into each container for oviposition. Each container was covered with a lid that had a hole, approximately 3.8 cm x 5 cm, covered with finely woven mesh cloth. Adult flies were left in the containers for approximately 10-48 h for egg laying on blueberries. After egg-laying, *D. suzukii* adults were removed, and eggs were counted using a stereomicroscope. The infested blueberries were divided into groups based on the number of eggs laid on them, ranging from zero to seven. The infested blueberries were placed into observation chambers, after dividing them into groups of 10 berries containing a total of 20 eggs. The observation chambers were made of 473 ml plastic deli containers (Fabri-Kal Corp., Kalamazoo, MI) that had a wire mesh boat hot glued to the bottom. The lids had a mesh-covered hole similar to the oviposition chambers with the addition of the yellow sticky card glued in a vertical orientation. The infested blueberries remained in the observation chambers until they reached the desired life stage, as outlined in Table 3.1. The infested berries were then removed from the observation chambers and placed in Petri dishes for treatment with insecticides. The infested berries in Petri dishes were sprayed with insecticides at maximum field rate using a Potter spray tower. The insecticide treatments included cyantraniliprole, fenpropathrin, malathion, phosmet, spinetoram, spinosad, and zeta-cypermethrin (Table 3.2). Once the spray residues dried, the treated berries were returned to the observation chambers, where they remained for two weeks. The remainder of the berries from oviposition chambers were used to validate the timing of *D. suzukii* life stages. Observations were made twice per week until the end of the 14-day observation period and the total number of adult flies were recorded.

Table 3.1 Temporal oviposition delay to achieve the desired life stage.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
3rd instar		2nd instar	1st instar			egg	Treatment/Harvest

Table 3.2 Trade names of insecticides used, coupled with active ingredients, chemical class, and field rate

Trade Name	Chemical Class	Active ingredient	Rate per acre	g(AI)/ha	Company
Danitol 2.4EC	Pyrethroids/Pyrethrins	Fenpropathrin	16 fl oz	1119.8	Valent U.S.A Corporation
Delegate™ 25WG	Spinosyns	Spinetoram	3-6 oz	209.8-419.6	Dow AgroSciences LLC
Entrust	Spinosyns	Spinosyns A & D	4-6 fl oz	280.2-420.3	Dow AgroSciences LLC
Exirel™10SE	Diamides	Cyantraniliprole /Cyazypyr	13.5-20.5 fl oz	946.9-1437.3	DuPont
Imidan 70W	Carbamates	Phosmet	1.3lb	1456.9	Gowan Company
Malathion 8F	Carbamates	Malathion	2.5 pt	2490.9	Gowan Company
Mustang Max 0.8EC	Pyrethroids/Pyrethrins	Zeta-cypermethrin	4 fl oz	280.7	FMC Corporation

All preparations were based on 467.7liters/ha spray volume (50gal/acre)

3. Statistical Analysis

Each life stage was analyzed independently of one another for an insecticide level comparison, as well as a group analysis for comparison of life stages. To determine the impact of insecticides on different immature life stages, two-way analysis of variance (ANOVA) was applied to the percent mortality data and Post hoc Tukey HSD (Honest Significance Difference) was performed to obtain the comparison of significance among the insecticides. R studio (R Development Core Team, 2018) was used with the "agricolae" package (Mendiburu, 2015) to determine the significance of the difference among treatments. Figures were generated using the "ggplot" package (Wickham, 2016). The *P-value* was set to 0.05 to determine the statistical difference among the treatments.

4. Results

Cyantraniliprole, malathion, phosmet, spinetoram, and spinosad had higher mortality while fenpropathrin and zeta-cypermethrin had medium level mortality on the egg stage of *D. suzukii* ($F_{7, 92}$=43.44, *P*= <0.001) (Figure 3.1). Similar mortality was observed on first instars as in the eggs, with the exception of zeta-cypermethrin, which was not statistically different compared to the control ($F_{7,102}$=27.0 *P*= <0.001) (Figure 3.2). All compounds except fenpropathrin and zeta-cypermethrin were found to cause significant mortality (~80% mortality) of the second instar stage ($F_{7,102}$=39.71, *P*= <0.001) (Figure 3.3). Cyantraniliprole mortality on the third instar stage was not significantly different from the control. All other insecticides provided significantly higher mortality than the control ($F_{7,112}$=13.46, *P*= <0.001) (Figure 3.4).

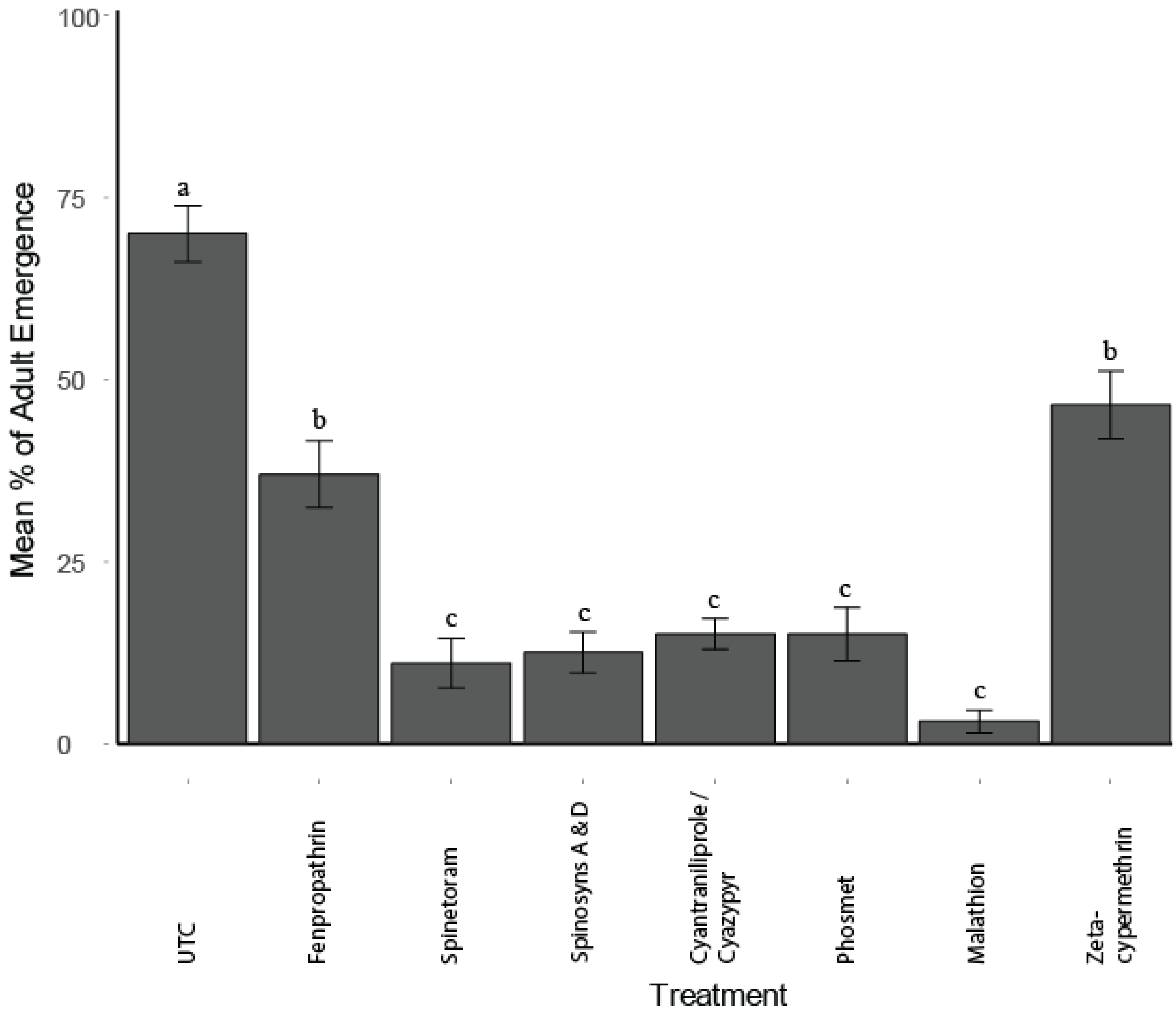

Figure 3.1.
Mean SWD adult emergence from treated berries infested with egg. $F(7,92) = 43.33$, $p<0.001$.

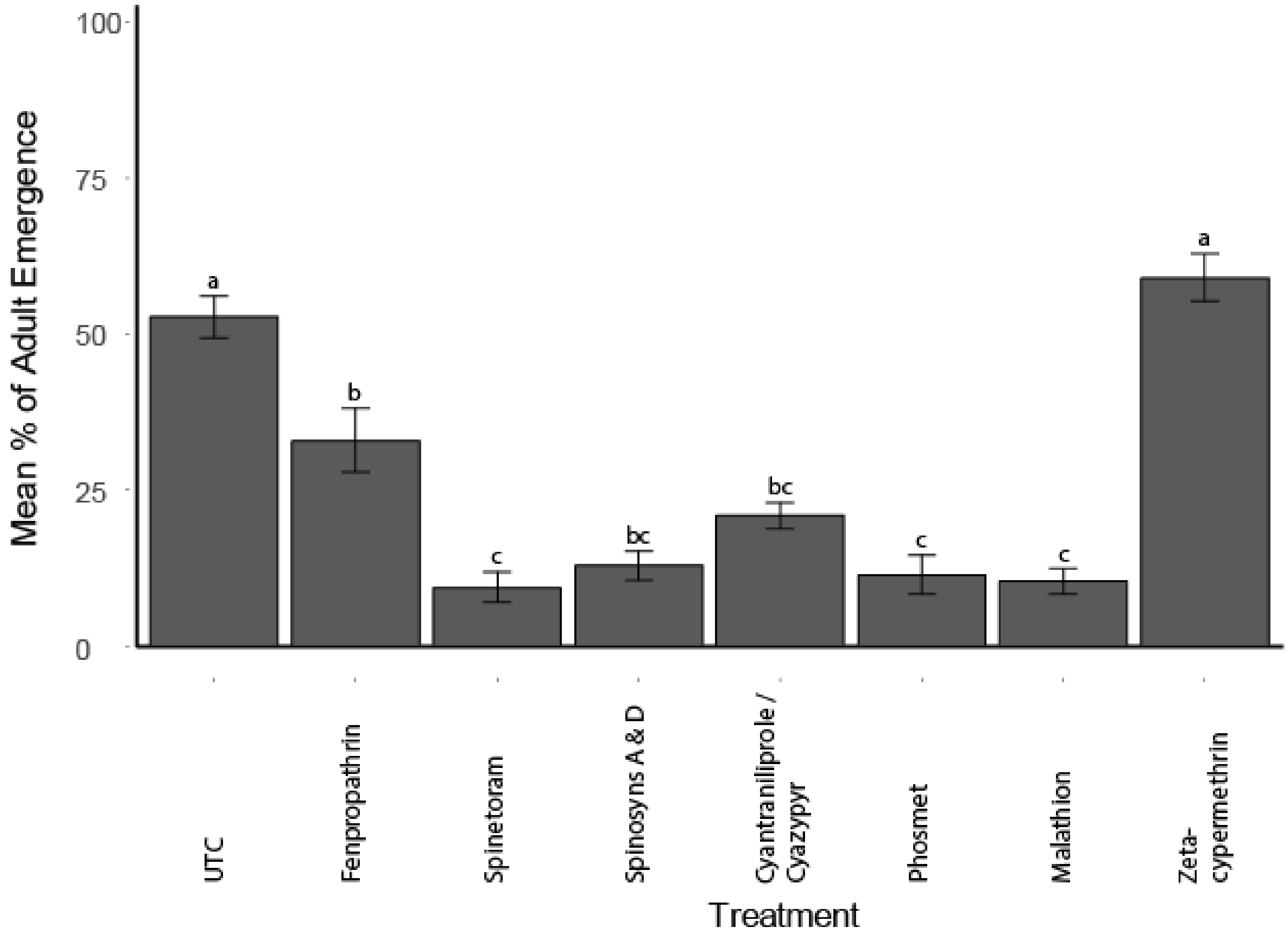

Figure 3.2.
Mean SWD adult emergence from treated berries infested with first instar. $F(7,102) = 27$, $p < 0.001$.

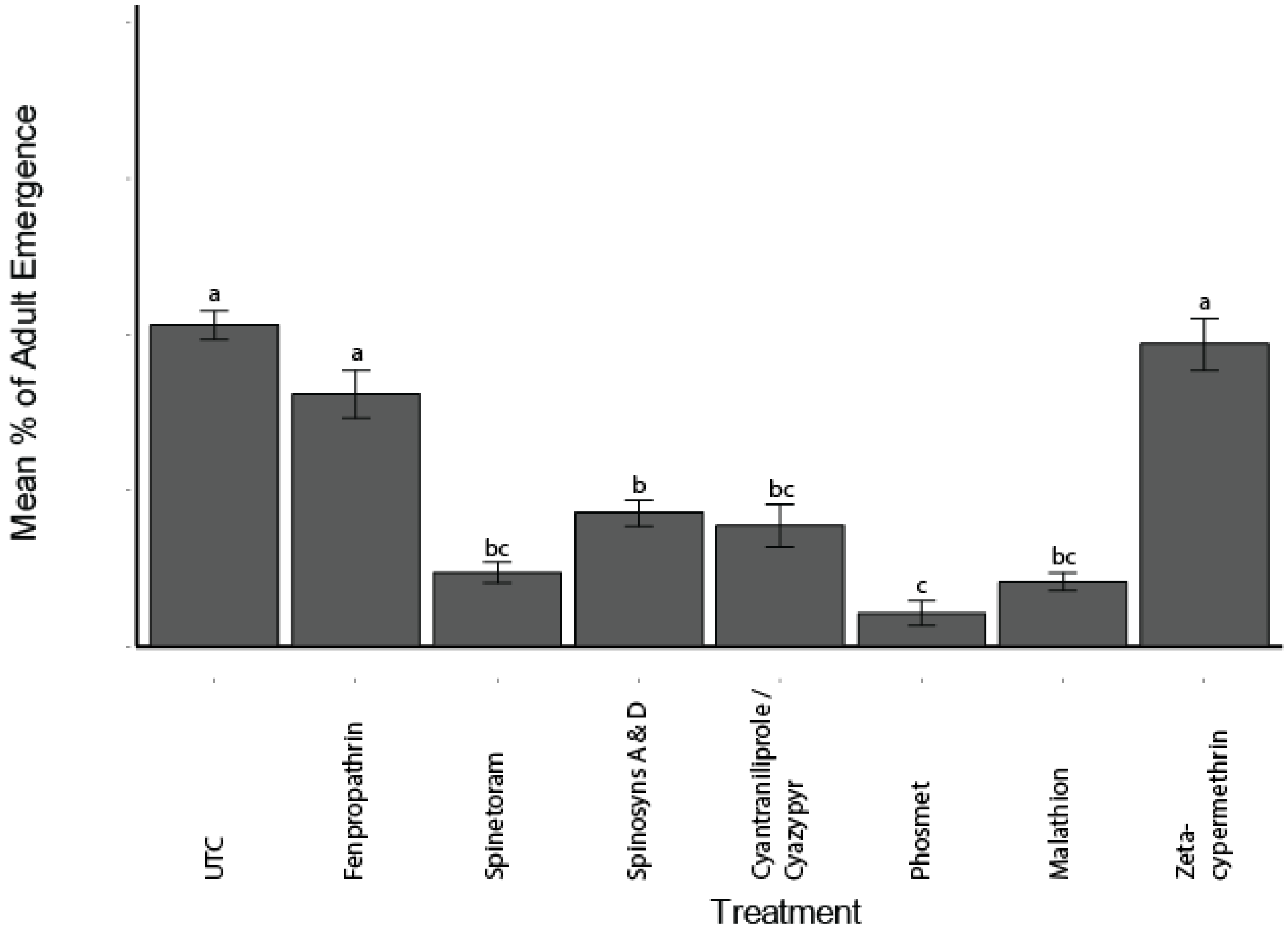

Figure 3.3
Mean SWD adult emergence from treated berries infested with second instar. F(7,112) = 39.71, p<0.001.

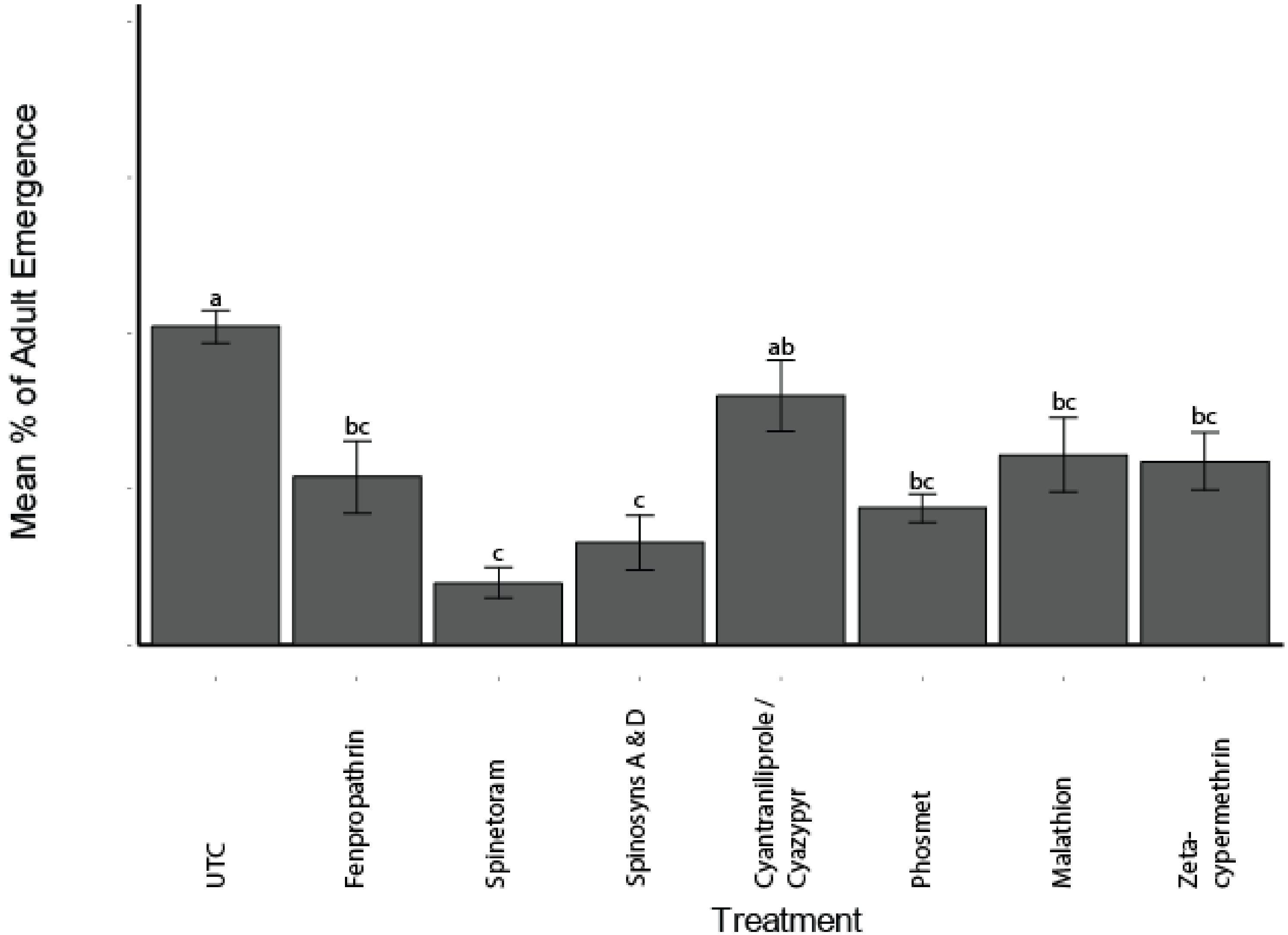

Figure 3.4
Mean SWD adult emergence from treated berries infested with third instar. $F(7,102) = 13.46$, $p<0.001$.

5. Discussion/Conclusion:

This study shows varying degrees of curative larvicidal and ovicidal activity of insecticides against SWD in blueberry fruit. Most insecticides show consistent effectiveness regardless of the life stage (temporal delay in treatment), further confirming the findings in Wise et al. 2015. The exceptions include cyantraniliprole/cyazypyr, malathion, and zeta-cypermethrin. Cyantraniliprole/cyazypyr and malathion have a significantly higher survival rate of third instars compared to the eggs, confirming the findings of Wise et al. 2015. This is attributed to the belief that larger larvae dwell deeper within fruits resulting is less exposure, coupled or not with the older larvae having larger bodies that require larger doses for lethal effects (JC Wise, Vanderpoppen, Vandervoort, O'donnell, & Isaacs, 2015). Zeta-cypermethrin has a significantly lower survival rate of third instar compared to the first instar, contrary to the findings of Wise et al. 2015. Further exploration into plant-chemical and insect-plant interactions (John Wise & Whalon, 2009) would provide greater insight into why the reverse trend occurs with zeta-cypermethrin.

Some insecticides used are temporally effective while others are effective independent of timing (JC Wise et al., 2015). It is currently believed that those insecticides that are temporally influenced is due to behavioral or toxicological phenomena, all of which interact with the penetration power of the chemical (JC Wise et al., 2015; John Wise & Whalon, 2009). In this study, the best example of a temporally effective insecticide is zeta-cypermethrin.

Zeta-cypermethrin has ovicidal activity as well as larvicidal activity with respect to the third instar only. When *D. suzukii* behavior/activity is overlaid on the graphs, it is easily seen that zeta-cypermethrin yields significant differences while the immature stages are closest to the surface. Eggs are near the surface due to them being non-motile and the nature of the oviposition.

The third instars, however, are near the surface because they are preparing to pupate, which is often done partially or fully emerged from the fruit. On the contrary, the first instar resides deeper in the fruit and the second instar is even further, which provides an increasingly thick fleshy barrier from the outside world. Thus, the penetration power of the insecticide becomes more important with respect to the larval age up to the middle-aged third instars (John Wise & Whalon, 2009). The best example of an insecticide penetration power being overcome by the behavioral activity is fenpropathrin.

Fenpropathrin lacked a significant difference to the control group with respect to only the second instar. This suggests that the depth at which the second instar normally resides is deeper than the penetration power of the chemical. However, this could be attributed to the larger body size or a combination thereof. Unlike the aforementioned insecticides, cyantraniliprole is significantly different with respect to all life stages except for the third instar. It is theorized, that this is an example of an increased body size functioning to mitigate the lethal effects via simple dilution.

This study confirms that all the insecticides used have a curative effect on *D. suzukii* in blueberry systems and can be deployed in any rotation combination. All life stages are present during peak harvest due to the high fecundity and extreme generational overlapping of *D. suzukii*. spinetoram, spinosyns A & D, phosmet, and malathion would be excellent choices for starting a rotation due to their activity being temporally independent. The results of the study add to the general body of knowledge that will aid in the development of future IPM protocols that ideally will become more reactionary rather than preventative, thereby sustaining the ecological balance within the fields and promoting the overall health of the ecosystem.

6. References

Cuthbertson, A. G., Collins, D. A., Blackburn, L. F., Audsley, N., & Bell, H. A. (2014). Preliminary Screening of Potential Control Products against *Drosophila suzukii*. Insects, 5(2), 488-498. DOI:10.3390/insects5020488

Dalton, D. T., Walton, V. M., Shearer, P. W., Walsh, D. B., Caprile, J., & Isaacs, R. (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(11), 1368-1374. Retrieved from <Go to ISI>://WOS:000296427600004

Diepenbrock, L. M., Rosensteel, D. O., Hardin, J. A., Sial, A. A., & Burrack, H. J. (2016). Season-long programs for control of *Drosophila suzukii* in southeastern blueberries (vol 81, pg 76, 2016). Crop Protection, 84, 171-171. Retrieved from <Go to ISI>://WOS:000374801200022

Evans, R. K., Toews, M. D., & Sial, A. A. (2017). Diel periodicity of *Drosophila suzukii* (Diptera: Drosophilidae) under field conditions. Plos One, 12(2). Retrieved from <Go to ISI>://WOS:000394244300040

Fanning, P. D., Grieshop, M. J., & Isaacs, R. (2018). Efficacy of biopesticides on spotted wing drosophila, *Drosophila suzukii* Matsumura in fall red raspberries. Journal of Applied Entomology, 142(1-2), 26-32. Retrieved from <Go to ISI>://WOS:000419830900003

Farnsworth, D., Hamby, K. A., Bolda, M., Goodhue, R. E., Williams, J. C., & Zalom, F. G. (2017). Economic analysis of revenue losses and control costs associated with the spotted wing drosophila, *Drosophila suzukii* (Matsumura), in the California raspberry industry.

Pest Management Science, 73(6), 1083-1090. Retrieved from <Go to ISI>://WOS:000400767400005

Goodhue, R. E., Bolda, M., Farnsworth, D., Williams, J. C., & Zalom, F. G. (2011). Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Management Science, 67(11), 1396-1402. Retrieved from <Go to ISI>://WOS:000296427600007

Grant, J. A., & Sial, A. A. (2016). Potential of Muscadine Grapes as a Viable Host of *Drosophila suzukii* (Diptera: Drosophilidae) in Blueberry-Producing Regions of the Southeastern United States. Journal of Economic Entomology, 109(3), 1261-1266. Retrieved from <Go to ISI>://WOS:000378041400037

Hamby, K. A., E. Bellamy, D., Chiu, J. C., Lee, J. C., Walton, V. M., Wiman, N. G., . . . Biondi, A. (2016). Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. Journal of Pest Science, 89(3), 605-619. DOI:10.1007/s10340-016-0756-5

Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Management Science, 67(11), 1358-1367. Retrieved from <Go to ISI>://WOS:000296427600003

Mendiburu, F. D. (2015). Agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.2-3. http://CRAN.R-project.org/package=agricolae.

Miller, B., Anfora, G., Buffington, M., Daane, K. M., Dalton, D. T., Hoelmer, K. M., . . . Walton, V. M. (2015). Seasonal occurrence of resident parasitoids associated with

Drosophila suzukii in two small fruit production regions of Italy and the USA. Bulletin of Insectology, 68(2), 255-263. Retrieved from <Go to ISI>://WOS:000364491800012

Plantamp, C., Salort, K., Gibert, P., Dumet, A., Mialdea, G., Mondy, N., & Voituron, Y. (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. Retrieved from <Go to ISI>://WOS:000376211300004

R Development Core Team. (2018). R: A language and environment for statistical computing, Vienna, Austria.: R Foundation for Statistical Computing,

Smirle, M. J., Zurowski, C. L., Ayyanath, M. M., Scott, I. M., & MacKenzie, K. E. (2017). Laboratory studies of insecticide efficacy and resistance in *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) populations from British Columbia, Canada. Pest Management Science, 73(1), 130-137. Retrieved from <Go to ISI>://WOS:000389408100017

Stacconi, M. V. R., Buffington, M., Daane, K. M., Dalton, D. T., Grassi, A., Kacar, G., . . . Anfora, G. (2015). Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas. Biological Control, 84, 28-35. Retrieved from <Go to ISI>://WOS:000352509000004

Stacconi, M. V. R., Panel, A., Baser, N., Ioriatti, C., Pantezzi, T., & Anfora, G. (2017). Comparative life history traits of indigenous Italian parasitoids of *Drosophila suzukii* and their effectiveness at different temperatures. Biological Control, 112, 20-27. Retrieved from <Go to ISI>://WOS:000406003200003

Van Timmeren, S., & Isaacs, R. (2013). Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Protection, 54, 126-133. Retrieved from <Go to ISI>://WOS:000327578800017

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., . . . Zalom, F. G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): Invasive Pest of Ripening Soft Fruit Expanding its Geographic Range and Damage Potential. Journal of Integrated Pest Management, 2(1), G1-G7. DOI:10.1603/IPM10010

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag New York.

Wiman, N. G., Walton, V. M., Dalton, D. T., Anfora, G., Burrack, H. J., Chiu, J. C., . . . Ioriatti, C. (2014). Integrating temperature-dependent life table data into a matrix projection model for *Drosophila suzukii* population estimation. Plos One, 9(9), e106909. DOI:10.1371/journal.pone.0106909

Wise, J., Vanderpoppen, R., Vandervoort, C., O'donnell, C., & Isaacs, R. (2015). Curative activity contributes to control of spotted-wing drosophila (Diptera: Drosophilidae) and blueberry maggot (Diptera: Tephritidae) in highbush blueberry. The Canadian Entomologist, 147(1), 109-117.

Wise, J., & Whalon, M. (2009). A systems approach to IPM integration, ecological assessment and resistance management in tree fruit orchards. In Biorational Control of Arthropod Pests (pp. 325-345): Springer.

CHAPTER 4

CONCLUSION

After its initial landfall in 2008, *D. suzukii* quickly spread throughout the continental USA in less than 10 years. This rapid dissemination is primarily due to the highly polyphagous nature of the pest. The swift expansion and ubiquitous presence of *D. suzukii* in nearly every environment has ensured the continued presence of the pest in the foreseeable future. Ensuring that *D.suzukii* management will remain a high priority in all systems where it has obtained pest status is critical. IPM programs require a basic understanding of pest biology and physiology. Those findings are then utilized in conjunction with chemicals to manage the populations in specific locations. Under ideal conditions, highly targeted chemicals would be implemented to reduce the environmental fallout of destabilizing the naturally occurring biological control agents.

Existing management programs in Georgia often consist of organophosphate and pyrethroids/pyrethrins. In some cases, these two classes are rotated throughout the harvest season. In other cases, only a single class is used for the duration of the season. Use of only one class is highly risky, as the possibility of resistance development and residue build-up could limit access to some markets. Programs that utilize a larger variety of MoAs substantially reduce the likelihood of resistance development to any one MoA within the rotation. Resistance development is further mitigated by utilizing MoAs that target different pathways. The BMP program utilized the following chemical classes: spinosyns, diamides, and a chromobactrium (which is not classified). The BMP changed each year and utilized one of the aforementioned chemicals in conjunction with organophosphate and pyrethroids/pyrethrins in rotation. For each year BMP was tested, there was not a significant difference when compared to FMP. This means the methods are interchangeable, although BMP does provide additional benefits. These benefits include increased resistance mitigation properties and market expansion. However, these benefits do come at an increased cost, ranging from 158% to 806% of the cost incurred with the implementation of FMP per acre.

Most management programs explore the efficacy of chemicals with respect to adulticidal activities. However, due to short life cycles and the longevity of adults, there is extreme overlapping of generations resulting in all life stages being present simultaneously. Understanding the efficacy of the chemicals with respect to ovicidal and larvicidal activities can provide additional insight in regards to developing IPM programs. All chemicals tested had ovicidal and larvicidal activity. However, the larvicidal activity was not consistent in chemicals with respect to each instar. Examples include zeta-cypermethrin, fenpropathrin, and cyantraniliprole. Zeta-cypermethrin does not have an effect on the 1st and 2nd instar; this is hypothesized to be due to a lack of systemic activity on the fruits. Fenpropathring does not have an effect on the 2nd instar, suggesting that systemic activity is not efficient enough to yield a toxic dosages at the appropriate depth. Cyantraniliprole does not have an effect on the 3rd instar, suggesting that the dosage is not sufficient for the body size.

Future studies should include continued exploration into resistance mitigation and market expanding rotations. Additionally, testing of the effects of ovicidal and larvicidal activities of chemicals should be studied under field conditions. Further exploration into chemical plant interactions with respect to the larvicidal activities and sequestration within fruits should be explored. Together, these studies will result in a greater understanding of the pest as a whole and lead to the development of more refined IPM programs.