"SHOO FLY": Reduce Pesticide Dependence with Knowledge about *Drosophila suzukii*

by

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Charlene Marie Marek for the degree of Bachelor of Arts in International Studies in Environmental Science presented on May 29, 2014.
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Abstract approved: _____
Dr. Amy J. Dreves

Drosophila suzukii was a known pest of berries, grapes, and stone fruits in East Asia for almost a century. Yet in recent years it has successfully migrated throughout the United States, Mexico, and Europe due to globalized fruit trade. The invasive success of *D. suzukii* is causing unrest for fruit growers in these regions who face severe economic loss from infestation. An ovarian maturity study was conducted by dissecting field-collected females. Study results show mid-Willamette Valley, Oregon *D. suzukii* females to be most oviposition-ready between June and September. A trap design study was conducted to quantify features of a successful trap for monitoring and eradication. Headspace, the volume between the liquid bait surface and the closest entry hole, was found to be a significant design feature. Successful trap designs, such as the Side Mesh trap used during summer 2012, can be used to monitor field presence and population accumulation. Knowledge of *D. suzukii* ovarian maturity in combination with trap catch, fruit phenology and weather patterns can be used to predict *D. suzukii* activity and help time treatment before infestation. These results contribute to an integrated pest management strategy for *D. suzukii* to reduce pesticide dependence.

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Bachelor of Arts in International Studies in Environmental Science thesis of Charlene Marie Marek presented on May 29, 2014

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I understand that my thesis will become part of the collection of Oregon State University. My signature below authorizes release of my thesis to any reader upon request. I also affirm that the work represented in this thesis is my own work.

_____
Charlene Marie Marek, Author

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CHAPTER 1 INTRODUCTION

1.1 Sustainability in International Environmental Politics

Sustainable development is a general paradigm of modern international environmental politics (Chasek et al. 2010). A growing global population increases consumption of natural resources, making creative solutions for responsible environmental management ineluctable. Sustainability is defined by the United Nations as creating "a decent standard of living for everyone today without compromising the needs of future generations" (United Nations, 2014). Moving toward sustainability means addressing the triple bottom line by focusing on the environment, equity and economy through development, business and consumptive decisions (UNEP, 2014).

Agenda 21 was signed at the 1992 Earth Summit in Rio de Janiero, Brazil and serves as an outline for addressing sustainability internationally (Chasek et al. 2010). Chapter 14 of Agenda 21, Promoting Sustainable Agriculture and Rural Development (SARD), discusses the importance of agrarian reform, land conservation and the improved management of inputs (fertilizers and pesticides). In Chapter 14, integrated pest management is introduced in subsection I, Integrated Pest Management and Control in Agriculture. According to subsection I, "chemical control of agricultural pests has dominated the scene, but its overuse has adverse effects on farm budgets, human health and the environment, as well as on international trade" (Agenda 21, 1993). Subsection I also mentions developing an International Code of Conduct on the Distribution and Use of Pesticides, which was later signed in 2003 by the United Nations Food and Agriculture Organization (FAO).

Article 2 of the International Code of Conduct on the Distribution and Use of Pesticides, Terms and Definitions, defines Integrated Pest Management (IPM) as "the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment… and encourages natural pest control mechanisms" (FAO, 2003). There are five overarching categories of IPM methods: biological control (implementation of predators), cultural control (such as harvesting fruits early), mechanical control (trapping), physical control (using barriers to prevent pest contact with a plant) and chemical control (such as effectively timing pesticide applications to reduce treatments) (UC IPM, 2013). IPM is multidisciplinary, and can include genetics, repellants, understanding pest life history, quantifying plant susceptibility in correlation with fruit phenology (development timing), and more. This thesis focuses on ovarian maturity and trapping, which are categorized as chemical and mechanical controls within IPM.

1.2 Arguments for minimizing pesticide applications

Due to lack of knowledge on the new pest *D. suzukii*, United States farmers resorted to pesticides for crop protection during initial mainland infestation in 2008 (Dreves, 2014 personal communication). Lambda-cyhalothrin (Warrior II), fenpropathrin (Danitol), zeta-cypermethrin (Mustang), spinetoram (Delegate), spinosad (Success or Entrust) and malathion (Malathion) are six commonly used pesticides for *D. suzukii* control (Haviland and Beers 2012). Two factors that exacerbate pesticide use in addressing *D. suzukii* are zero tolerance for infested fruit in the fresh/export markets (Bruck et al. 2011) and crop assurance (Dreves, 2014 personal communication). From a farmer's perspective, it is crucial to protect

crops and secure profits to support themselves and their families. Thus ownership of final treatment decision-making belongs entirely to the farmer. However, pesticides may not be the most viable option for preventing *D. suzukii* infestation. Although the negative environmental and health effects of pesticides are outside the scope of this thesis, they are in no way insignificant or irrelevant (Galt 2014). Pesticides may not always be the most effective method of control, specifically by posing challenges in meeting international trade regulations, properly timing treatments in accordance with rain periods and avoiding resistance development in pest populations. These concerns could be addressed by implementing IPM in crop production.

International import regulations must be considered when applying pesticides to fresh fruits for export outside of the United States. A Maximum Residue Limit (MRL) is "a measurement of the maximum level of pesticide residues that are allowed on a commodity for human consumption" (Haviland and Beers 2012). An acceptable MRL in the United States for domestic consumption may not be acceptable in importing countries. This was true of May 2011 sweet cherries from the United States bound for Canada, Japan, South Korea, Taiwan, Australia and the European Union, which had comparable or lower pesticide MRLs for imported fresh fruit. If fresh fruit is rejected, an alternative market must be found immediately to avoid a total economic loss (Haviland and Beers 2012). Strategically timing pesticide treatments with fruit development would allow for less applications and a decreased chance of non-compliance with international MRLs while preventing *D. suzukii* infestation. *D. suzukii* lays a majority of its eggs in marketable fruit (colored and ripe), and in some cases can't complete its lifecycle using green fruit (Lee et al. 2011).

Climate is important to consider when applying pesticides. Timmeran & Isaacs (2013) found that the "efficacy of most treatments (chemical) was reduced greatly after exposure to just over 2 cm of rain." Rain causes pesticides to wash away and redistribute, creating areas of higher and lower concentrations on the plant. Pesticide redistribution from rain puts the plant at risk for infestation until reapplication, because *D. suzukii* can lay eggs on temporarily unprotected fruit. Since pesticide efficacy is reliant on weather conditions, chemical control in the Pacific Northwest United States and in other temperate regions may not be the most ideal strategy for preventing *D. suzukii* infestation.

D. suzukii could possibly develop resistance to these pesticides due to its short generation time (Haviland and Beers 2012), and it is argued that preventing resistance development will be crucial in protecting fruit markets (Bruck et al. 2011). Italian scientists Grassi and Pallaoro (2012) discussed the difficulties posed by pesticide use (including meeting MRLs, facing negative environmental and health impacts, as well as possible resistance development in *D. suzukii* populations), and found multi-method approaches to be most viable in sustainably managing *D. suzukii* in the Trentino region of Italy.

Trade regulations, climate conditions and possible pest resistance development are difficulties facing chemical control. Fruit phenology and susceptibility can be considered in timing pesticide applications to reduce spraying frequency. Considering possible pesticide resistance development in *D. suzukii* populations, an IPM approach is necessary for ensuring long-term crop protection.

CHAPTER 2 BACKGROUND/LITERATURE REVIEW

2.1 Distribution and Impact

Drosophila suzukii (Matsumura) is a highly invasive species threatening small fruit production in many countries around the world (Cini et al. 2012). The United States Environmental Protection Agency defines an invasive species as "a plant or animal that is non-native to an ecosystem, which is likely to cause economic, human health or environmental damage in that ecosystem, and which is extremely difficult to control their spread" (EPA, 2014). The spread of *D. suzukii* has been difficult to control due to international fruit trade, and infestation has proven costly. Figure 1 shows male and female *D. suzukii*. *Drosophila suzukii* has earned the common name of "Spotted Wing" in the United States due to the black spots on the wings of (most) males.

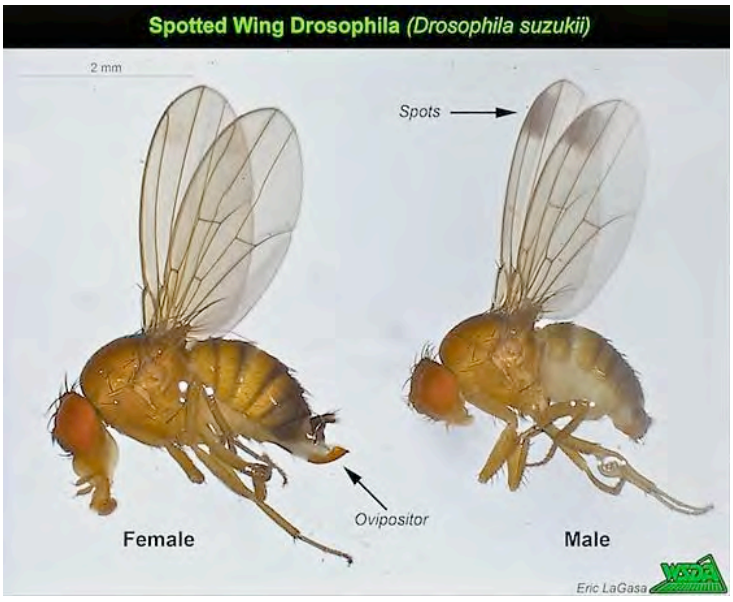

Figure 1. Female and male *D. suzukii*. Photo credit to Eric LaGasa.

D. suzukii has expanded its geographic range considerably in the past six years, spreading from Asia to North America to Europe and recently to Latin America. It was first described in Japan in 1931 before arriving to Hawaii in the 1980's and California in 2008 (Lee et al. 2011). *D. suzukii* has since migrated throughout mainland North America and is currently confirmed by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) to be present in Mexico (NAPPO 2011), Canada (NAPPO 2010) and more than forty states within the United States (Figure 2), including Oregon in 2009 (USDA APHIS, 2014). By 2009, *D. suzukii* was present in Europe (Lee et al. 2011, Calabria et al. 2012) and is currently impacting agriculture in Austria, Belgium, Croatia, France, England, Germany, Italy, the Netherlands, Portugal, Slovenia, Spain, Switzerland and Wales (Figure 3), as well as viticulture in Trentino, Italy (Grassi et al. 2011, Grassi & Pallaoro 2012). *D. suzukii* is also found in many Asiatic countries including: China, India, Japan, North and South Korea, Myanmar, Pakistan, Russia, Taiwan and Thailand (Figure 4). As of 2014, *D. suzukii* has been confirmed present in southern Brazil as well (Deprá et al. 2014).

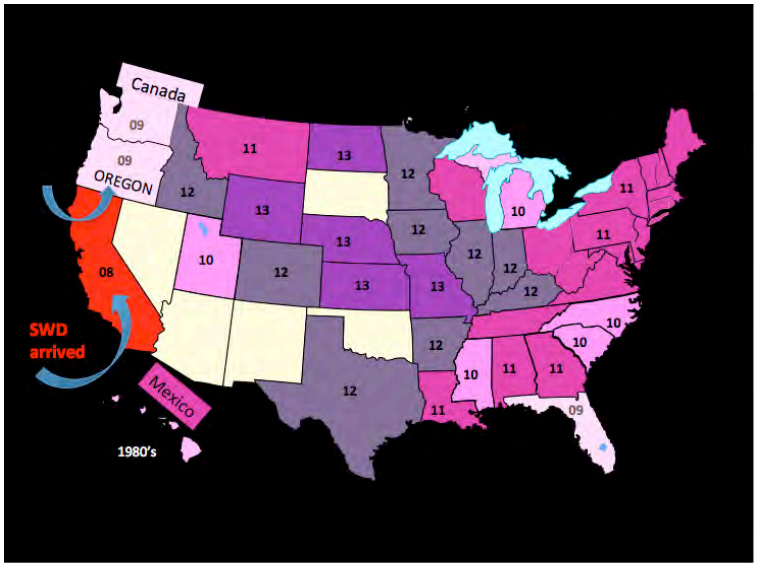

Figure 2. *D.suzukii* United States distribution as of 2014. Colors and numbers show areas of detection and years respectively.

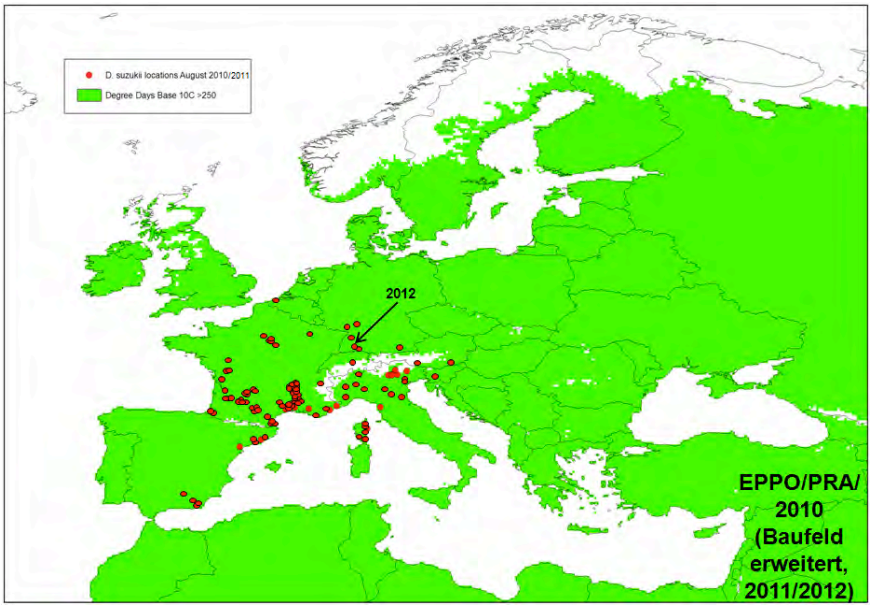

Figure 3. *D. suzukii* European distribution as of 2012. Modified diagram credit to Dr. Peter Baufeld, Julius Kühn-Institut, Braunschweig, Germany.

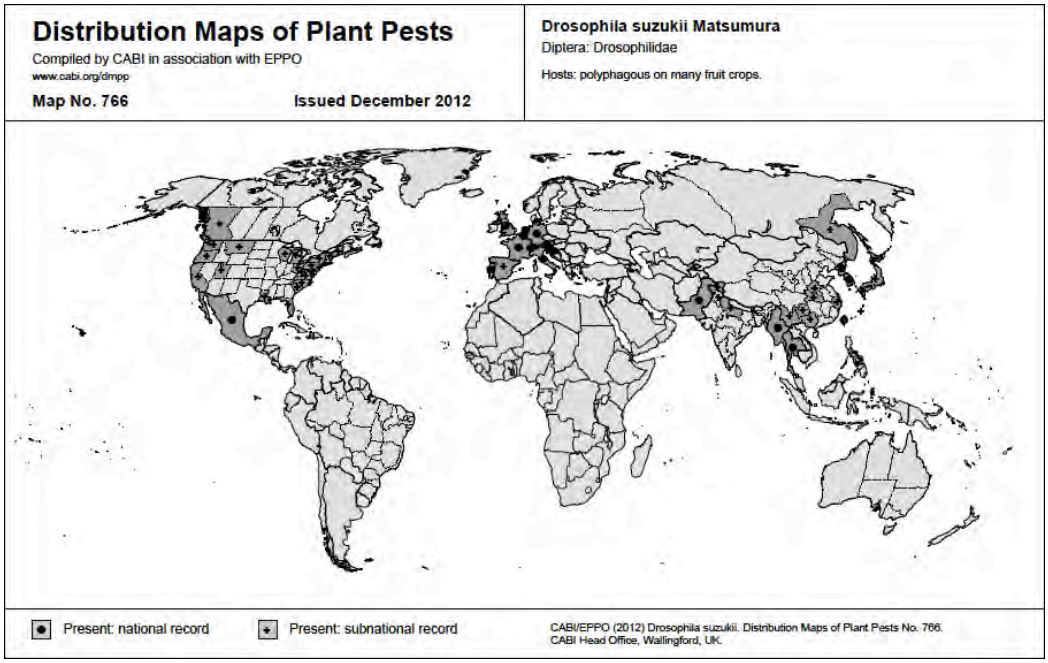

Figure 4. *D. suzukii* global distribution as of 2012. *D. suzukii* was distributed throughout North America, Europe and Asia (Distribution Maps of Plant Pests, 2012). It has since been confirmed present in southern Brazil as well (Deprá et al. 2014).

The fruit market impacts of infestation are substantial. Goodhue et al. (2011) reported that a yield loss of 20% from 2008 production values of raspberry, blackberry, cherry, strawberry & blueberry in California, Oregon and Washington could incur an estimated $511.3 million in damages in the United States alone. Figure 5 shows typical damage on a cherry. Wounds left from oviposition (egg-laying) expose fruits to pathogens and cause rapid degradation of quality and market value (EPPO, 2014). This pest fits comfortably in Lincoln's head on the US penny (one cent coin), and has immense potential for crop damage and economic loss (Figure 6).

Figure 5. Fruit damage caused by *D. suzukii* oviposition (egg-laying). Photo credit to Dr. Martin Hauser, California Dept. of Food & Agriculture, Sacramento, USA.

Figure 6. Pest in perspective. Photo credit to Jimmy Klick, Oregon State University, Horticulture Dept.

The spreading of *D. suzukii* throughout the world is worrisome, because *D. suzukii* will likely continue to find new convential crop and alternative non-crop hosts in various environments (Burrack et al. 2014). Ometto et al. (2013) described *D. suzukii* as 'highly polyphagous': infesting and feeding upon a wide variety of fruits. Lee et al. (2011) exposed fruits to *D. suzukii* in a laboratory setting to confirm strawberries, blueberries, raspberries, blackberries and cherries as susceptible to infestation. Yu et al. (2013) added figs and mulberries to this list and Steffan et al. (2013) found 'wounded' (broken skin) cranberries to be suitable *D. suzukii* hosts as well. Lee et al. (2011) reported fruits with coloration as more susceptible to infestation than green, unripe fruits, which coincides with Lee et al. (2012) and Basoalto et al. (2013) finding *D. suzukii* to be attracted by colors on a spectrum from red to black.

There are many reasons for the invasive success of *D. suzukii*: one being its large temperature tolerance ranging from 10˚C to 30˚C (Kanzawa 1939). Because *D. suzukii* now occurs in regions where it did not until recently, it has few natural predators within its new geographic distribution to control its abundance (Rota-Stabelli et al. 2013). Additionally, *D. suzukii* is especially mobile and can navigate regions quickly by its own flight and passive introduction to new regions through the global fruit trade. Rota-Stabelli et al. (2013) described the *D. suzukii* ovipositor as a "key adaptation" enhancing its invasive success. Most females of the Drosophilidae family have dull, short, underdeveloped ovipositors (an external egg-laying organ), inhibiting egg-laying in intact, healthy, firm fruit. However, the serrated *D. suzukii* ovipositor allows females to penetrate ripening fruits, specifically market-ready fruit (Figure 7), which is an ecological niche not filled by many other *Drosophila* species.

Figure 7. The *D. suzukii* ovipositor. The *D. suzukii* ovipositor is saw-like and robust. Photo credit to Dr. Martin Hauser, California Dept. of Food & Agriculture, Sacramento, USA.

Figure 8. *D. suzukii* female ovipositing in fruit. *D. suzukii* females use their ovipositors to pierce fruit and lay eggs in marketable fruit. Photo credit to Dr. Elizabeth Beers, *Orchard Pest Management Online*, Washington State University.

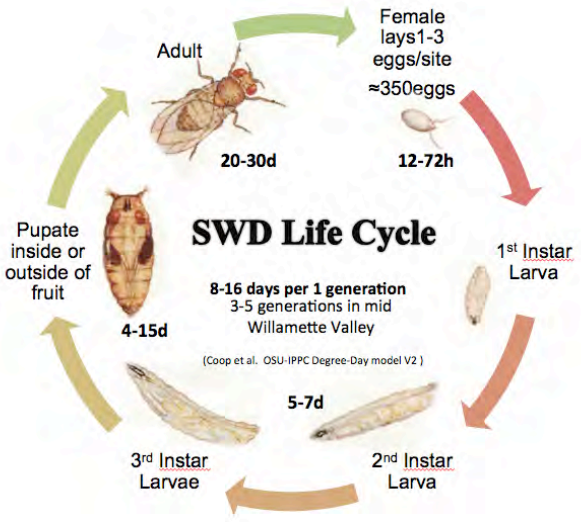

Figure 9. The *D. suzukii* lifecycle comprises four main stages. Diagram credit to Tanya Telshow, Oregon State University, Crop & Soil Science Dept.

2.2 Lifecycle and Overwintering

D. suzukii lives 1.5 - 2 months on average and completes four stages of development: egg, larvae, pupae and adult. Kanzawa (1939) found females to have a high reproductive capability, laying hundreds of eggs during their short lifespan. Eggs hatch into larvae, which travel inside the soft flesh of the fruit and consume nutrients for development (Marek observations).

Figure 10. *D. suzukii* larvae feeding on a blueberry. Photo credit to Dr. Amy J. Dreves, Oregon State University, Crop & Soil Science Dept.

Larvae enter a pupal (cocoon) stage after nutrient consumption before beginning an adult life (Gilbert 2010). The length of this lifecycle is dependent on seasonal conditions. Coop and Dreves (2013) reported that, "depending upon the temperature, the whole cycle can take from only a couple of weeks, to about a month, and much longer in locations with cooler weather." *D. suzukii* is predicted to complete no more than four or five generations in the Pacific Northwest US, while there may be 8 or 9 generations in Southern California (Coop & Dreves 2013).

D. suzukii reproduces continuously throughout the year, but survival rates vary depending on seasonal temperatures (Dalton et al. 2011, Thistlewood et al. 2012). Mitsui et al. (2010) suggested that adults are capable of overwintering (surviving the winter), because reproductively immature flies were prominent in autumn populations. It is hypothesized that robust *D. suzukii* females can survive the winter by feeding on alternative (non-crop) fruit hosts (Walsh et al. 2011). Studies conducted by Dalton et al. (2011) have shown overwintering survival in California and Oregon. There is significant population die-off when temperatures reach below freezing (Dreves et al., in prep). However, this doesn't ensure eradication, because the pest is firmly established in Hokkaido, Japan where average winter temperatures range from -12˚C to -4 ˚C (Walsh et al. 2011). Gerdeman and Tanigoshi (2012) noted that some *D. suzukii* females are inseminated (mated) prior to the winter, which may increase winter survivorship. Walsh et al. (2011) stated, "monitoring of *D. suzukii* in areas where it is well established, such as in the Willamette Valley in Oregon, provides growers with an early assessment of overwintering population density." This knowledge will be important for estimating early season population growth as well as timing the start of oviposition in fruiting crops. A *D. suzukii* female is shown in Figure 11 with a mature egg leaving her ovipositor.

Figure 11. Female *D. suzukii* with egg exiting ovipositor. Photo credit to Dr. Elizabeth Beers, *Orchard Pest Management Online*, Washington State University.

2.3 The Drosophila Body, Ovaries and Eggs

Drosophila ovaries are located in the abdomen, or the largest section of the fly body (Figure 12). The spermatheca, seminal receptacle, and uterus are connected to the ovaries. Sperm is stored in both the spermatheca and seminal receptacle. These organs gradually release sperm for egg fertilization once the ovaries are fully developed and oviposition-ready (King, 1970).

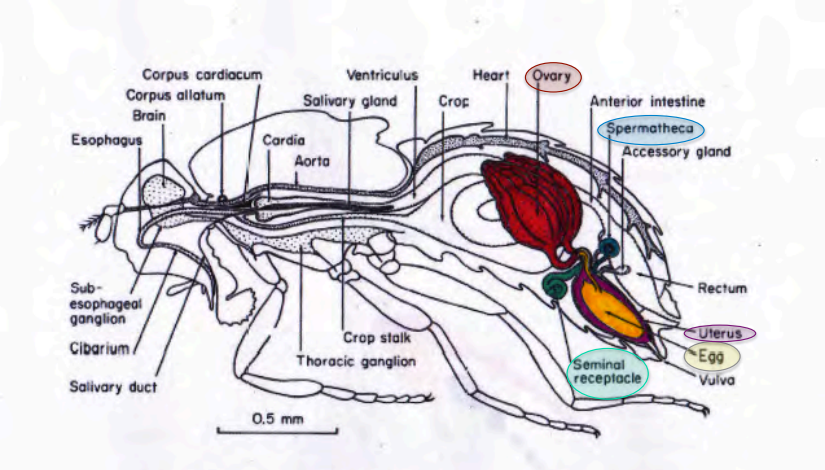

Figure 12. Female *Drosophila* ovaries are located in the abdomen. Diagram modified by Charlene Marek from King 1970.

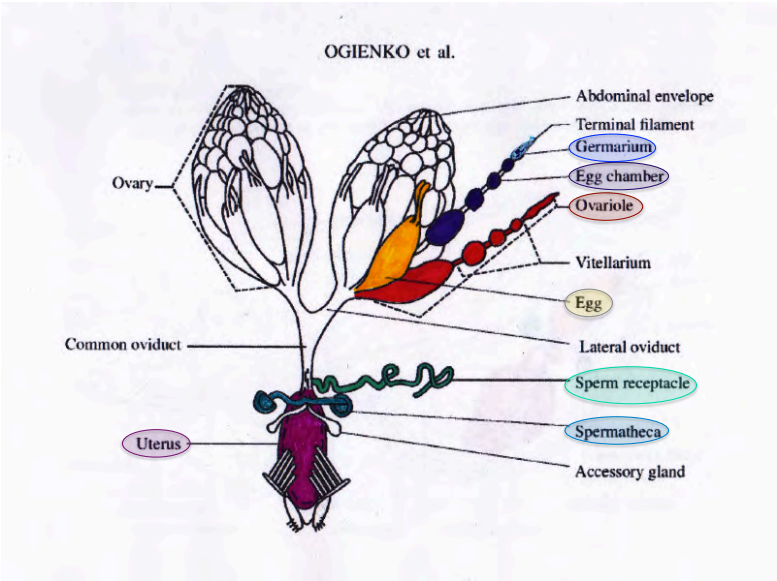

Figure 13. *Drosophila* ovaries are strands of developing eggs. Developed eggs are found closest to the oviducts. Diagram modified by Charlene Marek from Ogienko et al. 2007.

Drosophila ovaries are strands of ovarioles (egg chambers) through which developing eggs travel (Ogienko et al. 2007), as shown in Figure 13. Oogonia (immature egg cells) are located at the ovarian apex known as the germarium. Oogonia travel from the germarium through a collection of egg chambers, the vitallarium. This process is known as vitellogenesis during which the egg yolks develop and grow. Mature eggs have chorions (shells), and are found closest to the oviducts. This positions the eggs for release into the uterus for fertilization and oviposition (Gilbert, 2010). A chorion has two respiratory filaments, which extend outside of the fruit skin to enable gas exchange for the still-developing ovum (Gilbert, 2010). Figure 14 shows these filaments as well as the micropyle (channel into the ovum) through which sperm travel during egg fertilization (Gilbert, 2010).

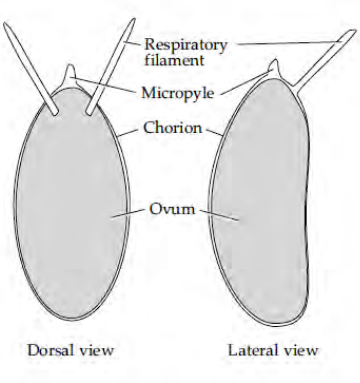

Figure 14. Mature eggs have chorions (shells) with respiratory filaments. Diagram credit to Gilbert 2010.

Figure 15. Oviposited *D. suzukii* eggs. Egg respiratory filaments are visible from outside this blueberry's skin. Photo credit to Dr. Amy J. Dreves, Oregon State University, Crop & Soil Science Dept.

2.4 Integrated Pest Management (IPM) Research

Knowledge of the *D. suzukii* lifecycle in connection to fruit development is crucial for treatment timing and method of control decision-making. Understanding when *D. suzukii* produce the most viable eggs can help predict reproduction timing with fruit susceptibility (Gerdeman & Tanigoshi, 2012). For this reason, degree-day models are being developed to track activity of *D. suzukii* populations and correlate when seasonal harvests are threatened (Coop 2014). Other IPM research includes evaluating the effectiveness of organic and traditional pesticides (Timmeren & Isaacs 2013), implementing parasitoids to control field population sizes (Chabert et al. 2012, Poyet et al. 2013, Rossi Stacconi et al. 2013), quantifying host potentials to identify *D. suzukii*'s preferred host fruits (Bellamy et al. 2013), as well as genetically modifying and releasing *D. suzukii* to decrease mating success, a strategy called the sterile insect technique (SIT) (Schetelig & Handler 2013).

Additional *Drosophila* research discusses the motivators for flight and attraction to food sources. *Drosophila* spp. have two olfactory sensory organs on the head, the antenna and maxillary pulp, which allow for recognition of food sources (Laissue & Vosshall 2008). Becher et al. (2012) reported that *Drosophila* spp. rely on transported volatile compounds to track food sources upwind. Acetic acid volatiles (found in apple cider vinegar) have been found to be attractive to *Drosophila melanogaster* adults and larvae (Becher et al. 2010). This suggests that acetic acid is a cue for *Drosophila* food sources and oviposition sites (Becher et al. 2012). Yeast has been identified as a beneficial food source for larvae development and adult survival (Hamby et al. 2012, Becher et al. 2012), as well as egg production and viability (Simmons & Bradley 1997, Chippendale et al. 1993, 1997).

Monitoring for pest life stages is a key part of an integrated pest management program. A large component of this thesis research focuses on detecting the presence of adult *D. suzukii*. Colors that visually mimic ripening fruit have been found to be a significant feature in trap design for *D. suzukii* attraction (Lee et al. 2013, Basoalto et al. 2013). Lee et al. (2013) found side mesh entry to be more successful than top mesh entry, and a larger bait surface area to slightly increase trap catch.

To detect *D. suzukii* field entry, traps must be placed on the perimeters of the field, where *Drosophila* are hypothesized to spend most of their time to avoid predators (Soibam et al. 2012). Mitsui et al. (2010) reported that *Drosophila* migration is dependent on the abundance of food resources. Dobzhansky, Powell and Taylor observed in 1979 that open meadows are unfavorable territory for *Drosophila* species and flies move quickly from it; the dense moist woods is favorable so relatively few flies leave it." Powell and Dobzhansky (1976) found that flies in favorable territory travel slower and/or less than those in hostile areas.

Tables 2, 3 and 4 in Appendix A provide a summary of selected articles on research in the United States, Japan and Europe as guides for informative *D. suzukii* literature. Most of current published research is taking place within the United States, Japan and Europe (especially Italy and France). Some articles had a larger research scope than only their respective category in each table. However, articles were listed under a single category for simpler organization. These articles are limited to those written in English or with English abstracts.

Bait research focuses on luring *D. suzukii* into traps for eradication, while barriers focus on physically blocking the pest from coming in contact with fruit. Not all "chemical control" literature is advocating for pesticide use, but rather determining how pesticides can be moderately and most effectively applied. Distribution literature discusses the introduction of *D. suzukii* to a region and/or its tracked dispersal throughout a region. Host fruit articles focus on determining which crops are at risk for infestation while market impact discusses the potential and/or actual economic losses from infestation. Outreach literature reports on initiatives to involve and educate communities about the pest. Overwintering resources focus on *D. suzukii* ability to survive harsh winter conditions. Articles labeled "IPM" focus on integrating multiple methods of control to reduce pesticide dependence.

Thesis Objectives

This thesis focuses on synthesizing two studies, ovarian maturity (a form of chemical control) and trap design (mechanical control), to effectively manage the invasive crop pest *Drosophila suzukii* in the mid-Willamette Valley, Oregon. Knowledge of seasonal fruit phenology (ripening stages) and *D. suzukii* oviposition in accordance with ovarian maturity and weather conditions aids in better timing crop treatments to reduce unnecessary pesticide applications. Female *D. suzukii* dissections intended to provide insight into oviposition timing to determine when crops are most susceptible to infestation. Trapping intended to monitor field activity and to observe relative population sizes between early, mid- and late seasons. Studying trap designs helps determine attractive characteristics to increase *D. suzukii* catches, and contributes to the development of an effective future mass trapping design for eradication and crop protection. It is respected that the final control method decision belongs to the grower. This thesis attempts to empower farmers by discussing additional methods to support long-term cropland health and farmer economic stability. Knowledge gained from the two studies of this thesis could be useful for farmers and scientists in controlling this invasive internationally.

CHAPTER 3 METHODS

This methods chapter is divided into two sections with twelve subsections.

3.1 Ovarian Maturity

3.1.1 Specimen Collection

3.1.2 Dissection Tools

3.1.3 Dissection Technique

3.1.4 Ovarian Maturity Rating Scale

3.1.5 Ovarian Maturity Data Analysis

3.2 Trap Design

3.2.1 Farm Site Location

3.2.2 Trap Designs

3.2.3 Replication Plot Descriptions

3.2.4 Weekly Trap Servicing Procedure

3.2.5 Drosophila Identification and Counting

3.2.6 Trap Design Data Analysis

3.2.7 Trap Design Statistical Data Analysis

3.1 Ovarian Maturity

3.1.1 Specimen Collection

A total of 794 females were dissected and evaluated for ovarian maturity. This study was conducted in collaboration with Dr. Amy J. Dreves, and USDA-ARS personnel in Corvallis, Oregon including Dr. Jana Lee. Female *D. suzukii* were collected both dead and alive from the field. Dead specimens were trapped throughout years 2011, 2012 and 2013. Live specimens were collected only during the summer of 2012. Some dissected females were a year or older while others were just a few days old.

Dead traps were baited with apple cider vinegar (ACV) (5% acetic acid; Fred Meyer Brand, manufactured in Cincinnati, Ohio, USA 45202), and live traps (not pictured) were baited with fresh bananas, cherries and dry yeast. *D. suzukii* was collected from live traps in the research farm's cherry orchard and raspberry hoop houses using an aspirator (Figure 16). Specimens were collected in the field by inhaling through the yellow flexible tube and aiming the metal tube.

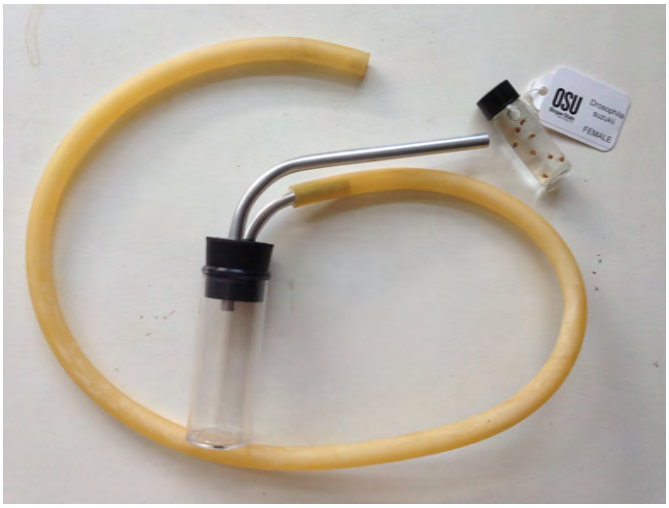

Figure 16. An aspirator used for live collections. Photo credit to Dr. Amy J. Dreves, Oregon State University, Crop & Soil Science Dept.

Dead-trap-caught females were stored in 60-70% ethanol until dissection in the lab. Not all ethanol-stored females had intact reproductive systems, and so were not evaluated. Females caught by live traps were stored live for 1-2 days in test tubes with media for food, as seen in Figure 17.

Figure 17. Choosing a female for dissection. Photo credit to Dr. Amy J. Dreves, Oregon State University, Crop & Soil Science Dept.

3.1.2 Dissection Tools

Various materials were used to conduct female dissections and ovarian analysis, including: fine point 5-INOX Rubis tweezers (Switzerland), minutins, Phosphate Buffered Solution (PBS) pH 7, a light dissecting stereo microscope, a microscope camera, gel-bottom dissecting dishes, clear dissecting wells and a black backdrop (Figures 17 & 18), as well as specialized, homemade tools created by Rich Little, a retired California Agriculture Commissioner (Figure 18). Tool blades were made from minutins to be sharp, small-scale and effective for dissections.

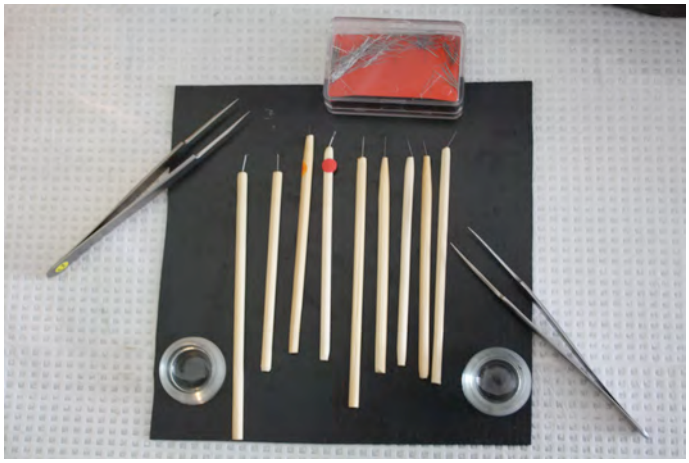

Figure 18.Tools for *D. suzukii* dissections. Photo credit to Charlene Marek.

3.1.3 Dissection Technique

Vials with live flies were placed in a freezer for 3-5 minutes before dissection to prevent escape when choosing a female for ovarian analysis. A gel-bottom dissecting dish was placed on top of a black backdrop for contrast (between white/clear *D. suzukii* tissues and organs) and to prevent light microscope glare. A single female (dead or live) was placed inside the dissecting dish with 1-2 drops of PBS pH 7. The light microscope was used at 80x magnification during dissections. Fine point tweezers were used to secure the female by the thorax (chest section) in the dissecting dish. Tools created by Rich Little were used to separate the female's thorax and abdomen. One of these tools was inserted between the cuticle (thick skin) fold of the thorax and abdomen to separate the body in two sections. One hand secured the detached abdomen with tweezers while the other hand gently tugged the abdomen cuticle apart with a dissecting tool to reveal the ovaries. Figure 19 shows a dissected female.

Figure 19.The *D. suzukii* reproductive system after dissection. Photo credit to Charlene Marek.

3.1.4 Ovarian Maturity Rating Scale

Ovarian maturity was determined using a 4 stage rating scale. Ovaries were categorized by size relative to the fly abdomen. Oogonia (immature egg cells) are mostly present during the earliest stage of development (Stage 1). Drosophila eggs reach sexual maturity by developing egg yolks, a process known as vitellogenesis (Stage 2). Eggs are mature (oviposition-ready) if enclosed by a chorion with two breathing filaments (Stage 3). Degenerative ovaries (Stage 4) are over mature and have lost the fullness from the previous stage. The ovarian development rating scale used during this study is displayed in Table 1.

Table 1. Ovarian maturity rating scale for *D. suzukii*.

Stage #	Stage Name	Description
1	Oogonia	Ovaries are small in comparison to the abdomen. Oogonia eggs are clear, circular granules.
2	Vitellogenesis	Egg yolks are developing. There is distinction between darker and clear regions of the eggs. No filaments present.
3	Chorion	Ovaries fill the abdominal cavity. Breathing filaments are present on eggs stationed at the bottom of the ovary and in the uterus.
4	Degenerative	Eggs are overmature and losing their intact structure. Filaments present.

3.1.5 Ovarian Maturity Data Analysis

The count of females with Chorion Stage 3 ovaries was recorded for each month to quantify monthly ovarian maturity. Dissected females had varying levels of maturity. Thus other maturity stages were recorded during dissections to understand yearly ovarian maturity progression. This data helps estimate the most oviposition-intense periods of the year in the Pacific Northwest United States.

3.2 Trap Design

3.2.1 Farm Site Location

A 12-week trap design study was conducted at a 20-acre (8 ha) certified organic, diversified (fruits and vegetables) farm in the mid-Willamette Valley, Benton County, Oregon from June 11, 2012 to September 4, 2012. The research site had 40 hoop houses (Figure 20) on the south half of the farm bordered by invasive *Rubus armeniacus* (wild Himalayan Blackberry) on the east, south and west farm borders. The trap design study was broken into 3 periods (early, mid- and late seasons) each four weeks: June 12th – July 9th, July 16th – August 6th, and August 13th – September 4th. These seasonal divisions were based on abundance shifts and generation changes predicted and confirmed by a degree-day model (Coop 2014), based on a lower and upper developmental threshold of 50˚F (10˚C) and 88˚F (30˚C) respectively, starting January 1; and substantial increases in *D. suzukii* population sizes.

Figure 20. Aerial map of research farm site in the mid-Willamette Valley. Replications 1, 2 and 3 are labeled in their respective positions on the 20-acre organic farm with adjacent landscape. Image modified from Google Maps, accessed December 2013.

3.2.2 Trap Designs

Eleven trap designs (of which 10 are shown in Figure 21) were tested and rotated between eleven flagged positions (A through K) in each plot. Each position was located one meter above ground, and spaced three meters from neighboring positions to prevent interference. There were 33 traps total, with three traps of each design, one design present in each replication plot. Trap design names were Red Mesh, Spaceship, Side Cone, Side Mesh with cup, Red cup, Red McPhail, Captiva, Side Mesh, Clear 10-Hole and Clear 10-Hole with cup. Some design parameters included color (primarily red and clear), differing fly entry types (holes vs. mesh), headspace (small versus large volume between bait surface area and

entry/exit area) shown in Figure 22, and attached collection cups for quicker field servicing. A chart of all individual trap characteristics is included in Appendix B. This study was intended to test previously successful trap characteristics by examining a design's ability to consistently capture high numbers of *D. suzukii*.

Figure 21. Ten trap designs were used for data analysis. Their names from top left clockwise are Red Mesh, Spaceship, Side Cone, Side Mesh with cup, Red cup, Red McPhail, Captiva, Side Mesh, Clear 10-Hole and Clear 10-Hole with cup. Photo credits to Charlene Marek.

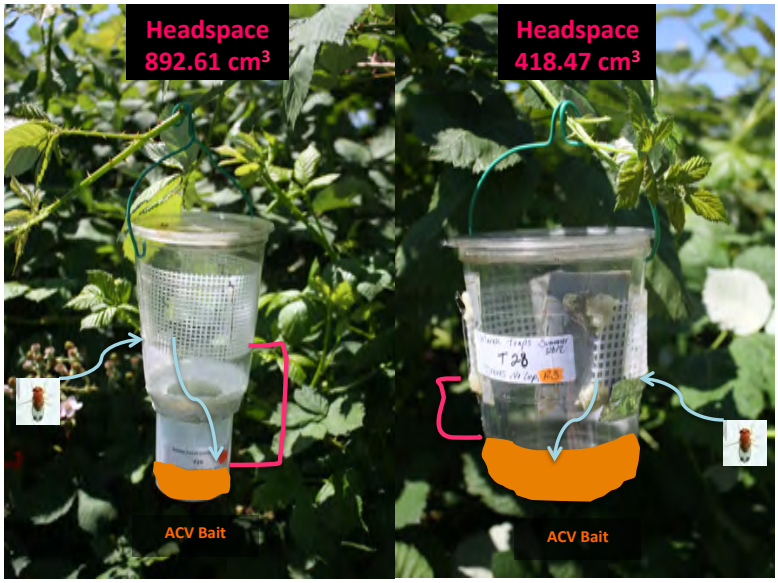

Figure 22. Trap diagram, cup vs. no cup. Differences in headspace volume are shown. Photo and figure credits to Charlene Marek.

All traps were used throughout the entire study except for three designs: Green Cone, Marek and Side Mesh. Green Cone was replaced during week 3 of the study with the Side Mesh. The Marek trap (Figure 23) was created by Charlene Marek and first hung during week 5. Trap features included red for visual attractiveness, an oil lamp wick for volatilization, small body size for easy trap placement and small entry holes for increased *D. suzukii* catch. However, the trap was removed and rehung during week 6 due to malfunctions. The Marek trap had variable results due to flaws in design (such as leakage throughout the study) and is not included in data analysis.

Figure 23. The Marek Trap. Photo credit to Charlene Marek.

3.2.3 Replication Plot Descriptions

The study was replicated three times in the invasive blackberry hedges on the east, west and south farm borders. Although all replications had wild Himalaya blackberry, the replication plots differed in presence of overhead stories (larger, taller trees which shade younger trees and the wild Himalaya blackberry bush), as well as abundance and diversity of ground cover

below. Each replication plot exhibited unique characteristics described in the following section.

Replication 1 plot

Replication 1 plot was located at the east edge of the farm. Trap designs were hung on the west side of the wild blackberry hedge at 1.8 m in height, 3 m wide about 4 meters from the hoop houses. The outer east border of the wild blackberry faced railroad tracks and a road was directly south of the replication. Due to a lack of overstory, except for a young lone tree on the north end, the replication was exposed to heat, sunlight, wind and summer rainstorms. Tall pasture grasses and weeds between the hedge and the hoop houses were mowed during mid-season of the study, changing the ecology of the replication.

Figure 24. A photo of Replication 1 plot taken during summer 2012. Photo credit to Charlene Marek.

Replication 2 plot

Replication 2 plot was located at the south edge of the farm and had a variety of unkempt underbrush and plant diversity. The site was located in a riparian zone and shielded by a wild cherry over story with elderberry (an alternative *D. suzukii* host fruit), which protected traps from sunlight, heat, wind and rain. Other plant species included maple, alder, and Cascara spp. with persimmons and figs located north of the plot's southwest corner. There were hoop houses about 20-40 m from the Replication 2 plot, which included crops such as blackberries, grapes, raspberries, strawberries, as well as vegetables green bell pepper, tomatoes, greens, onions and garlic.

Figure 25. A photo of Replication 2 plot with the cherry overstory taken during summer 2012. Photo credit to Monica Marcus.

Replication 3 plot

Replication 3 plot was located on the west edge of the farm. The majority of the site was openly exposed to weather conditions due to a lack of over story. However, there was a small cluster of three trees in the middle of the replication plot, which provided shade and some weather protection. The inner-border of the plot (east-facing) was located 2 meters

from a tomato hoop house. The outer-border of the plot was adjacent to a perennial grass seed field. There was a small persimmon and fig orchard about 3.5-5 meters from trap positions A, B, and C. The persimmon and fig orchard bordered the southwest corner and west ends of respective Replication 2 and 3 plots.

Figure 26. A photo of Replication 3 plot taken during summer 2012. Photo credit to Charlene Marek.

Figure 27. Persimmon and fig orchard adjacent to Replication 3 plot. Photo credit to Charlene Marek.

3.2.4 Weekly Trap Servicing Procedure

Traps were serviced weekly by pouring bait samples into labeled collection containers and refilling each trap with 150 mL of ACV bait according to a filler line. A teaspoon of unscented dish soap (Planet Ultra Dishwashing Liquid, manufactured in Victoria, British Columbia) was added per gallon ACV. This decreased bait surface tension and allowed *D. suzukii* to sink to the trap bottom. After all bait samples were collected, traps were re-randomized and placed in new positions in their respective replication plots according to a position randomization chart (Appendix D). Percentage of fruit development for the wild blackberry in each replication plot was estimated according to a phenology chart (Figure 28).

Figure 28. Blackberry Phenology. The blackberry fruit development stages 1-8 are pictured and described. Photo credits to OSU Spotted Wing Action Team (SWAT) members, Oregon State University, Crop & Soil Science Dept.

3.2.5 Drosophila Identification and Counting

At the laboratory, each bait sample was strained of ACV and contents by using a fine-netted screen over a large container. Flies and other organisms (e.g., parasitoids, house flies, sap beetles, midges, other *Drosophila* spp.) caught by the net were placed in a white shallow container and examined under the scope. Contents were sorted and the number of *D. suzukii*

males, females, and other *Drosophila* spp., were counted and recorded using a 40x dissenting scope. Ovipositors were examined to identify *D. suzukii* females. Males were identified by spots on their wings. When no spots were present, the two sex-combs running parallel with front leg were confirmed for correct identification.

3.2.6 Trap Design Data Analysis

Unless otherwise noted, trap design study data was analyzed according to early, mid- and late seasons. The weekly total count of *D. suzukii* (male and female trapped in all replication plots for the week) caught per design were averaged across the replication plots to calculate the mean and standard error of *D. suzukii* per trap per week.

The weekly mean (± SE) catches of the Clear 10-Hole were compared to the mean (± SE) catches (± SE) of all traps with cups (Side Mesh with cup, Half-cone, Red Cup, Red Mesh, Clear 10-Hole with cup and Spaceship). Comparing the standard trap against traps with cups allowed the significance of headspace to be evaluated in trap design. The Clear 10-Hole was used for this analysis, because it is the standard trap and was used throughout the entire study.

The Clear 10-Hole and Clear 10-Hole with cup weekly mean (± SE) catches were compared to further analyze headspace significance. The Side Mesh and Side Mesh with cup weekly mean (± SE) catches were also compared. Both the Clear 10-Hole and Side Mesh weekly mean (± SE) catches were compared to speculate which trap characteristics (other than headspace), could be significant in trap design.

The weekly mean (± SE) catches of the four traps without cups (Captiva, Clear 10-Hole, Side Mesh and Red McPhail) were compared during mid- and late seasons only, because the Side Mesh entered the trap design study halfway through the early season (at the start of week 3). These four traps were compared to determine the most successful trap of the study.

Trap placement was evaluated by examining weekly average Clear 10-Hole *D. suzukii* replication plot catches. Weekly Clear 10-Hole catches were examined to determine population fluctuations and weekly catch in all replication plots. These totals were averaged across the replications to determine overall population fluctuation throughout the study in comparison to blackberry phenology. Total replication plot catches for the entire 12-week study were compared to determine the plot with the highest trap catches.

Seasonal proportions of male and female *D. suzukii* and other *Drosophila* spp. were assessed from weekly mean replication plot catches. The weekly total male, female *D. suzukii* and *Drosophila* spp. trapped were averaged across replications to find the weekly mean plot catches for each. The proportion of other *Drosophila* spp. to *D. suzukii* was analyzed to determine when *D. suzukii* field prominence occurs. The proportion of male/female *D. suzukii* was evaluated to determine if gender ratio is equivalent or skewed depending on the season. The weekly proportions of male to female *D. suzukii* were evaluated to determine when females comprise most of the *D. suzukii* population, as this would indicate oviposition activity and fruiting crop susceptibility.

Historical hourly access weather data collected from the Corvallis, Oregon Agrimet Weather Station (CRVO) from June 11th to September 4th 2012 was used to analyze cumulative weekly

precipitation (mm), as well as high, average and low temperatures (˚C) for the early, mid- and late seasons. The CRVO station is located at 44.63416˚N 123.19˚W at an elevation of 230 ft (70.1 m).

3.2.7 Trap Design Statistical Data Analysis

Statistical analysis was performed by analysis of variance (PROC General Linear Model-ANOVA, SAS Institute Inc. SAS 2002-2007). The number of adults data that did not meet normality and equality of variance assumptions were transformed as appropriate when model assumptions were not met using square root ($x + .5$) before analysis (Sokal and Rohlf 1998). Means were separated using Tukey's HD test with a 95% significance level ($p = .05$). Interactions between trap position and blocks were analyzed. Fly counts are presented as mean ± SE flies/week or season (when no differences were found between dates).

CHAPTER 4 RESULTS

This results chapter is divided into two sections with four subsections. *D. suzukii* is referred to as "SWD" (Spotted Wing *Drosophila*) in all figures and text in this section.

4.1 Ovarian Maturity

i) Mid-Willamette Valley, Oregon January - July Ovarian Maturity

ii) Monthly Percentages of Females with Mature Ovaries

4.2 Trap Design

4.2.1 Headspace

i) Clear 10-Hole vs. Traps with Cups

ii) Clear 10-Hole vs. Clear 10-Hole with cup

iii) Side Mesh vs. Side Mesh with cup

iv) Clear 10-Hole vs. Side Mesh

v) Traps Without Cups

4.2.2 Trap Placement

i) Weekly Total Replication Plot Catches

ii) Seasonal Replication Plot Catches

iii) Mean Weekly SWD Catch with Blackberry Phenology

iv) Total Replication Plot Catches

4.2.3 Species and Gender Shifts

i) Species and Gender Shifts

ii) SWD Gender Shift

4.2.4 Weekly Precipitation (mm) and Temperature (˚C)

4.1 Ovarian Maturity

Mature ovaries fully filled the female abdomen and appeared as those shown in Figure 29. Oogonia Stage 1 ovaries were most prominent in the winter months of January (100%) and February (80%). Fly bodies were darker and ovaries were small in comparison to the abdomen. There was a 43% decrease in Oogonia Stage 1 ovaries between February and March, with a 43% increase in Vitellogenesis Stage 2 ovaries (Figure 30). Vitellogenesis Stage 2 ovaries were observed from February through July with varying prominence. Most Vitellogenesis Stage 2 ovaries were observed during March (43%) and May (52%). Figure 31 shows Chorion Stage 3 ovaries to be most abundant from April (.52) through September, although the proportion in May was relatively low (.30). The largest proportions of Chorion Stage 3 ovaries were observed during June (.62), July (.60), August (.45) and September (.70). Degenerative Stage 4 ovaries were observed in June and July at 7% (Figure 30).

Figure 29. Chorion Stage 3 *D. suzukii* ovaries. Photo credit to Dr. Amy J. Dreves, Oregon State University, Crop & Soil Science Dept, and Dr. Jana Lee (USDA-ARS-Hort unit).

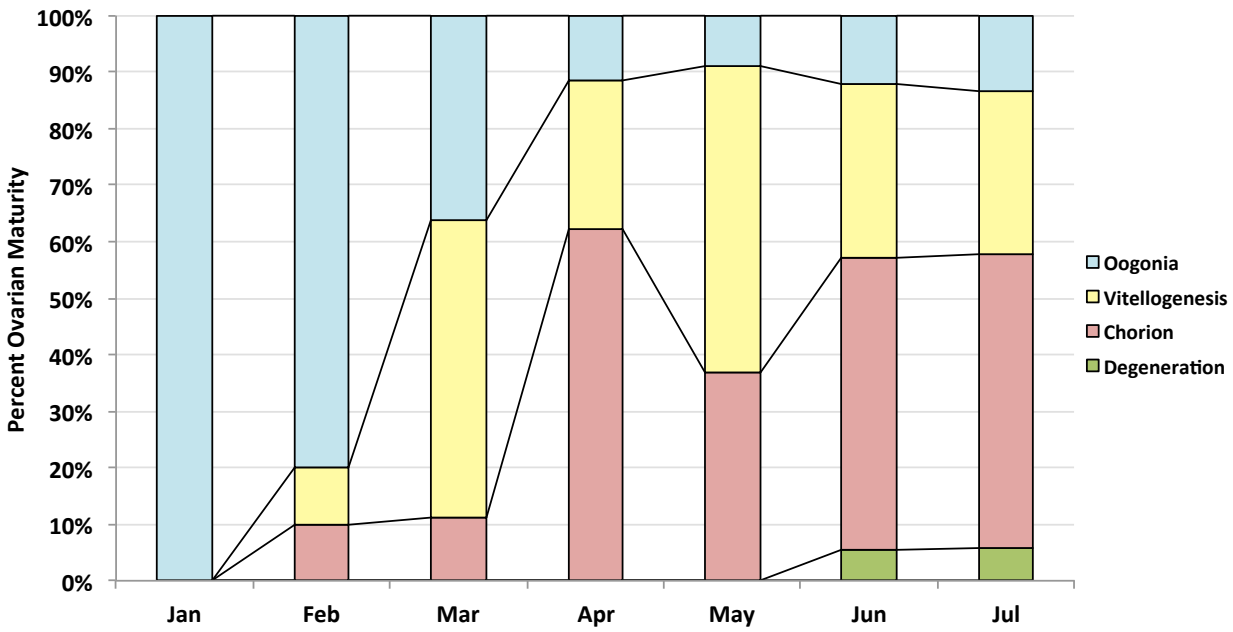

Figure 30. Ovarian maturity 4 stage ratings from January to July in mid-Willamette Valley, Oregon.

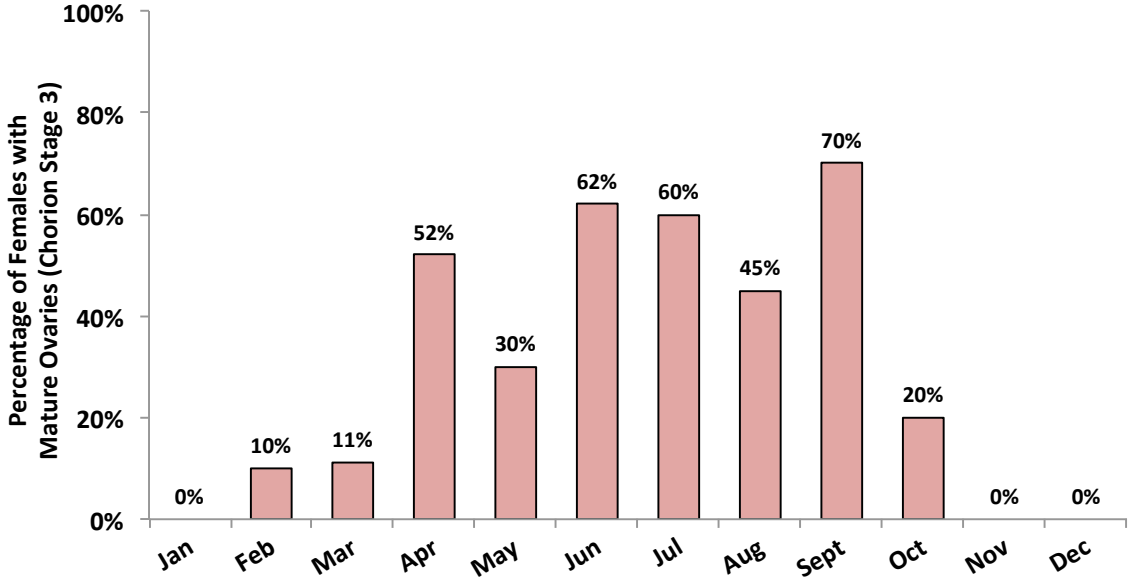

Figure 31. Monthly percentages of females with mature ovaries.

4.2 Trap Design

4.2.1 Headspace

The concept of headspace and its significance were discovered through this design study. Figures 32-36 show mean seasonal or weekly trap catches with standard error. The seasonal differences in SWD abundance can be seen in the y-axes of the individual graphs in Figure 32 (as well as Figures 33-36). The Clear 10-Hole was found to have numerically higher mean trap catches each season than any of the traps with cups. The early season Clear 10-Hole mean catch was 2.7 SWD, with mid- and late season mean catches, 25.5 and 98 SWD respectively.

Although the Clear 10-Hole caught twice as much SWD as any of the traps with cups during the early season, catches were low and no significant difference was found ($F=1.44$; $df=6$, $p=0.2111$). However, there were significant differences in trap catches between the Clear 10-Hole and traps with cups during the mid-season ($F=3.39$, $df=6$, $p<.0051$) and late season ($F=7.35$, $df=6$, $p<.0001$). In the mid-season, the Clear 10-Hole had significantly higher catches than Half Cone, Clear 10-Hole with cup and Spaceship. However, the Clear 10-Hole did not have significantly higher trap catches than the Side Mesh with cup, Red Cup or Red Mesh (which were not significantly different from Half Cone, Clear 10-Hole with cup or Spaceship). Further analysis was necessary to determine headspace as an explanatory variable in trap catch differences between traps with and without cups.

The weekly mean catches of the Clear 10-Hole were compared with the weekly mean catches of the Clear 10-Hole with cup. The early season had a range in trap catch from 0 to 28 SWD, with an average of 4.88 SWD. The mid- and late season ranges in catches were

9-105 SWD and 88-326 SWD, with 52.88 SWD and 211.88 SWD averages respectively. The Clear 10-Hole and Clear 10-Hole with cup had respective headspaces 779.25 cm^3 and 1141.43 cm^3. The headspace difference was 362.18 cm^3. The Clear 10-Hole had consistently, numerically higher weekly catches than the Clear 10-Hole with cup, but the differences in early season trap catches were not significantly different (F=1.71; df=1; p=0.2042). However, the mid- (F=6.48; df=1; p=<.0155) and late (F=8.67; df=1; p<.0075) seasons showed significant differences in trap catches between the two designs. Figure 33 shows a significant difference in weekly mean catches between the Clear 10-Hole and the Clear 10-Hole with cup.

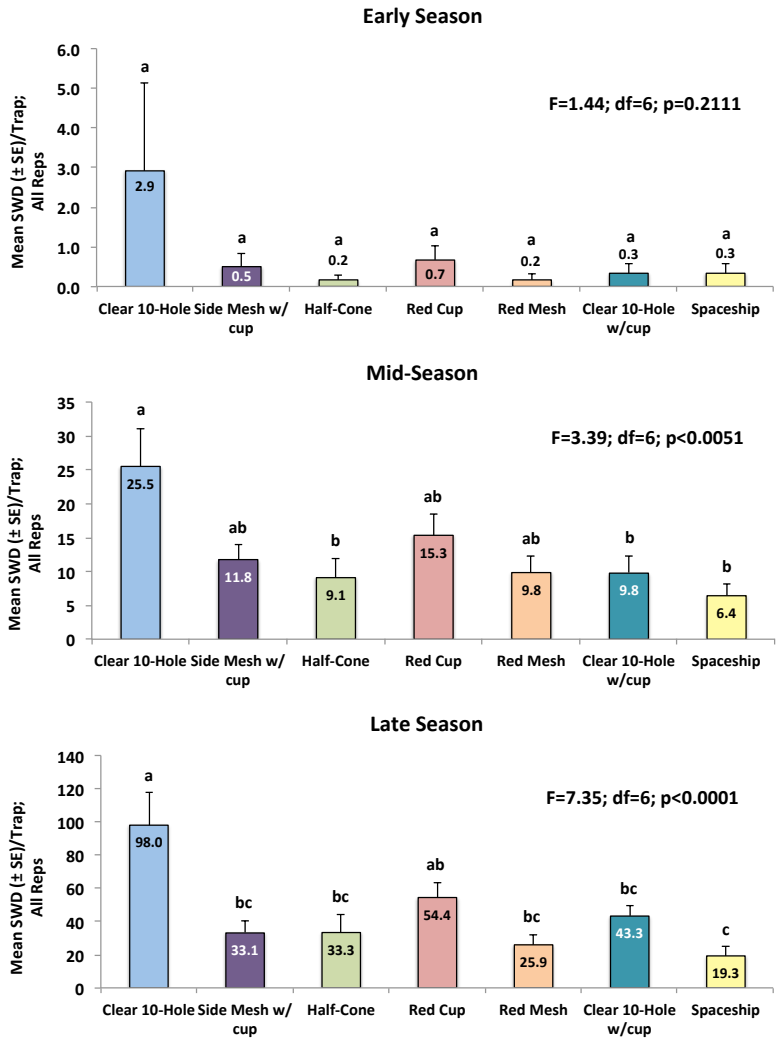

Figure 32. Seasonal headspace comparisons, Clear 10-Hole vs. traps with cups. Means with the same letter are not significantly different. Note mean seasonal catch magnitude differences.

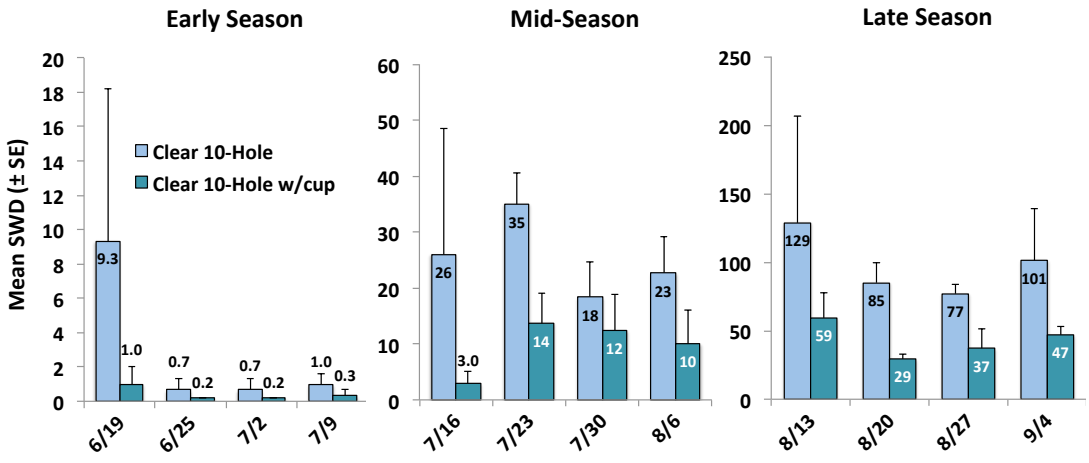

Figure 33. Seasonal headspace comparisons, Clear 10-Hole vs. Clear 10-Hole with cup. Note mean seasonal catch magnitude differences.

The Side Mesh and the Side Mesh with cup had respective headspaces 418.47 cm^3 and 892.61 cm^3. The headspace difference was 474.14 cm^3. The number of SWD trapped in the mid-season ranged from 13 to 130 SWD, and in the late season from 58 to 688 SWD. The average SWD caught during the mid-season was 73.5 SWD and 295.88 SWD in the late season. The Side Mesh was not implemented until July 2nd. Figure 34 shows numerical differences in weekly mean catches between the Side Mesh and the Side Mesh with cup. Once implemented, the Side Mesh caught consistently more SWD than the Side Mesh with cup. Mid-season catches for the Side Mesh were consistently more than three times greater than those for the Side Mesh with cup. During the late season, the Side Mesh had significantly higher counts than the Side Mesh with cup. The Side Mesh caught 4.87x more SWD than the Side Mesh with cup during week 9, and 3.96x, 6.89x, 4.8x more SWD during weeks 10, 11 and 12 respectively. The early season did not show significant differences in trap catches between the two designs ($F=2.79$; $df=1$; $p=0.1259$). However, the mid- ($F=12.65$; $df=1$; $p=0.0018$) and late ($F=24.61$; $df=1$; $p<.0001$) seasons did show significant differences in trap catches.

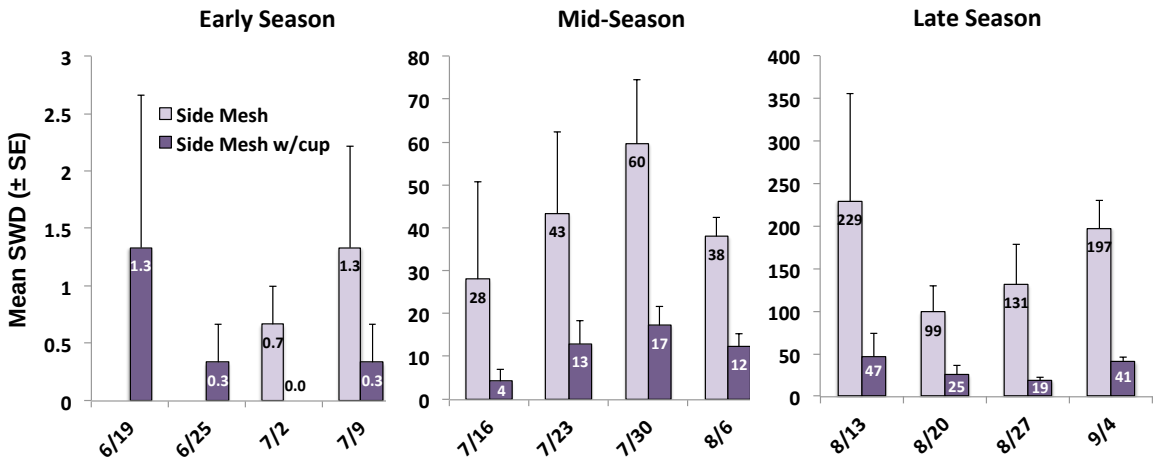

Figure 34. Seasonal headspace comparisons, Side Mesh vs. Side Mesh with cup. Side Mesh was not implemented until week 3. Note mean seasonal catch magnitude differences.

The Clear 10-Hole and the Side Mesh weekly catches were not significantly different during the early (F=.02; df=1; p=.8783), mid- (F=2.6; df=1; p=.1211) or late (F=3.16; df=1; p=.0893) seasons (Figure 35). The headspace difference was 360.78 cm^3. The Clear 10-Hole and the Side Mesh caught a total 2 SWD during week 3 (early season), but the Side Mesh had consistent numerically higher weekly mean trap catches throughout the rest of the study. There was not a large difference in mean catches between the Clear 10-Hole and Side Mesh during weeks 5 and 6: only 2 and 8 SWD respectively. However, there was a very large difference in mean catches during week 7, when the Side Mesh caught 3.33x more than the Clear 10-Hole. Weeks 8, 9, 10, 11 and 12 had catch differences 15, 100, 14, 54 and 96 SWD respectively.

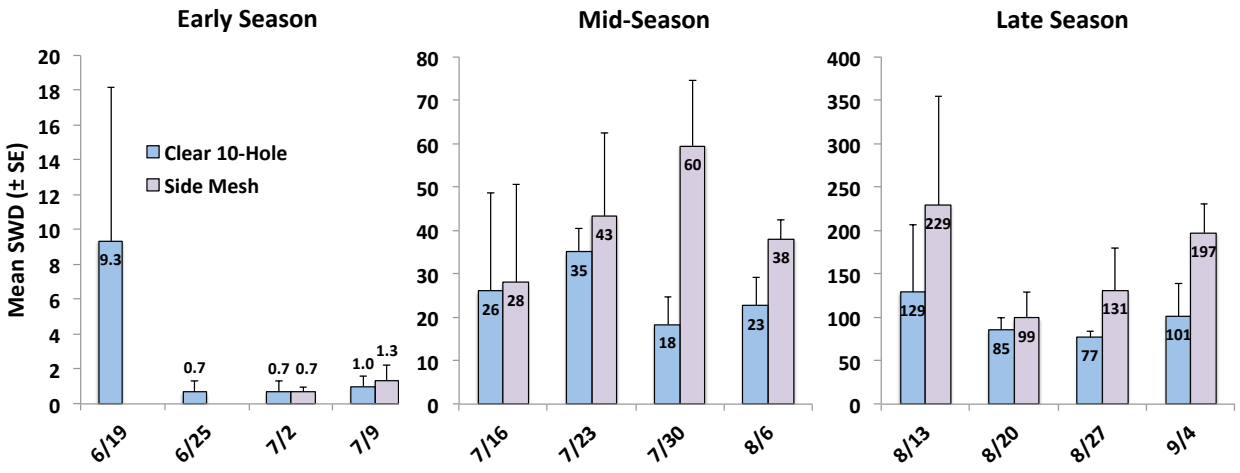

Figure 35. Seasonal headspace comparisons, Clear 10-Hole vs. Side Mesh. The Side Mesh was introduced two weeks late into the study (June 25th). Note mean seasonal catch magnitude differences.

Traps without cups had the same catch trends during the mid- and late seasons. Abundance differences in SWD are visible on the y-axes of the two individual graphs in Figure 36. A range of 17 to 130 SWD were caught from during the mid-season, with a trap average of 70.63 SWD. The traps without cups caught a range of 111 to 688 SWD during the late season, and had a trap average of 293.38 SWD. The Side Mesh had the highest seasonal mean catches, followed by the Clear 10-Hole, Captiva and Red McPhail. The seasonal mean Side Mesh catch was numerically higher than all other mean catches of traps without cups during the mid- and late seasons. The Side Mesh had significantly higher trap catches than the Red McPhail and the Captiva during both the mid- ($F=5.03$; $df=3$; $p=0.0044$) and late seasons ($F=5.02$; $df=3$; $p=0.0045$). However, there was no significant difference between the Side Mesh and the Clear 10-Hole during the mid- or late seasons.

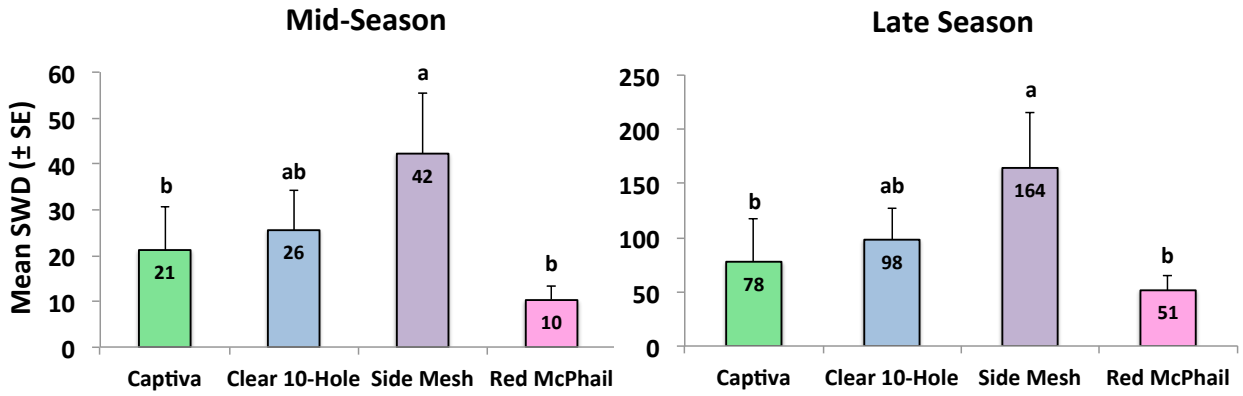

Figure 36. Mid- and late season comparison of traps without cups. The early season is not shown, because Side Mesh entered the study during week 3. Note mean seasonal catch magnitude differences.

4.2.2 Trap Placement

Trap placement was found to be a significant factor for trap success. The weekly catch peaks reveal three *D. suzukii* generations, or substantial increases in population described in the degree day model. Population growth throughout the study is shown by the y-axis scale magnitude differences of the three individual graphs. The replication 2 plot had the greatest *D. suzukii* catches during most weeks. During week 9 (August 13th), the replication 2 plot caught 3.18x more SWD than the replication 3 plot. The replication 3 plot was the second most successful replication plot and replication 1 plot was the least successful. Weekly total SWD catches are shown in Figure 37. The replication 2 plot (south) was found to be significantly different than replication 1 and 3 plots during both the early ($F=11.14$; $df=2$; $p<.0001$) and mid-($F=5.76$; $df=2$; $p<.0041$) seasons. During the late season, both replication 2 and 3 plots had significantly higher catches than the replication 1 plot ($F=6.06$; $df=2$; $p<.0031$), and replication 2 plot was not significantly different than the replication 3 plot. There was no significant catch difference ($p>0.05$) between trap positions within the replication 1, 2 or 3 plots during any of the three seasons.

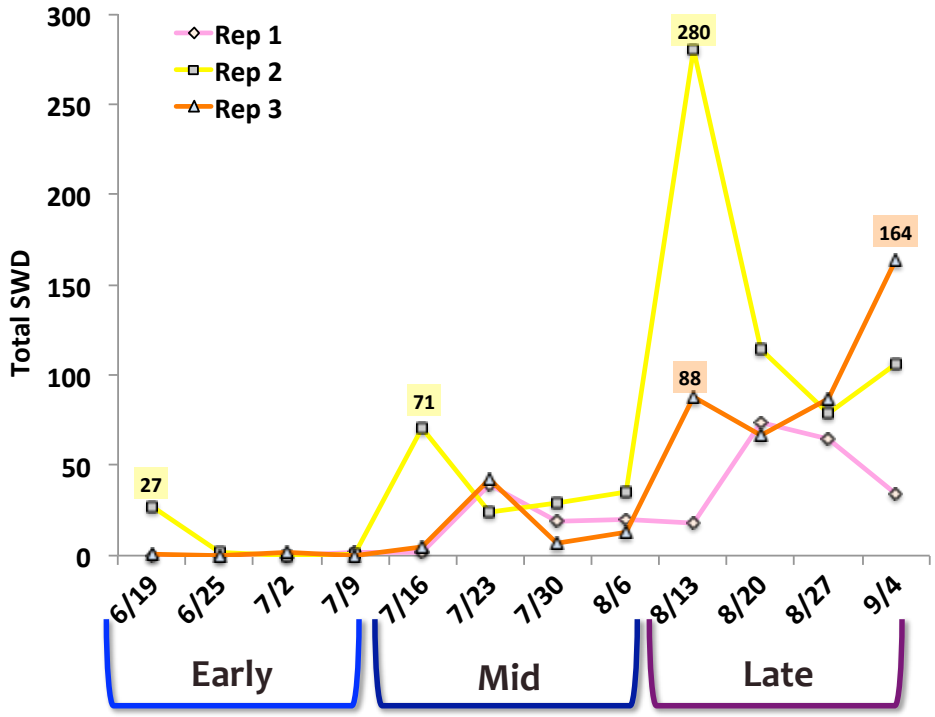

Figure 37. Weekly total replication plot catches. Each season had a distinct generation.

Figure 38 shows weekly replication plot trap catches divided into three numerically-different seasons: early, mid- and late. The replication 3 plot had higher catches than the replication 2 plot on July 2nd, July 23rd, August 27th & September 4th, with respective catch differences: 2, 18, 8 and 58 SWD. The replication 1 plot had higher catches than replication 2 plot on July 9th and July 23rd. Figure 39 displays the mean weekly SWD catch. There is a general positive population growth trend shown. Catches were low in the early season weeks 1, 2, 3 and 4 (June 19th, 25th, July 2nd, 9th), and grew during the mid-season weeks 5 and 6 (July 16th, 23rd). The population declined during week 7 (July 30th), increased slightly during week 8 (August 6th), and then rapidly increased from 22.7 to 128.7 mean catches during week 9 (August 13th). Mean catches declined during weeks 10 and 11 (August 20th, 27th), and began to rise again during week 12 (September 4th) to around 100 mean SWD

catches. Figure 40 shows the cumulative SWD catches for each replication plot from the 12-week study. Traps placed in replication 2 plot had high SWD catches. The replication 2 plot caught 4,349 SWD total, which is nearly half of all flies caught during this study. Replication 1 and 3 plots caught 1,892 and 3,020 SWD respectively.

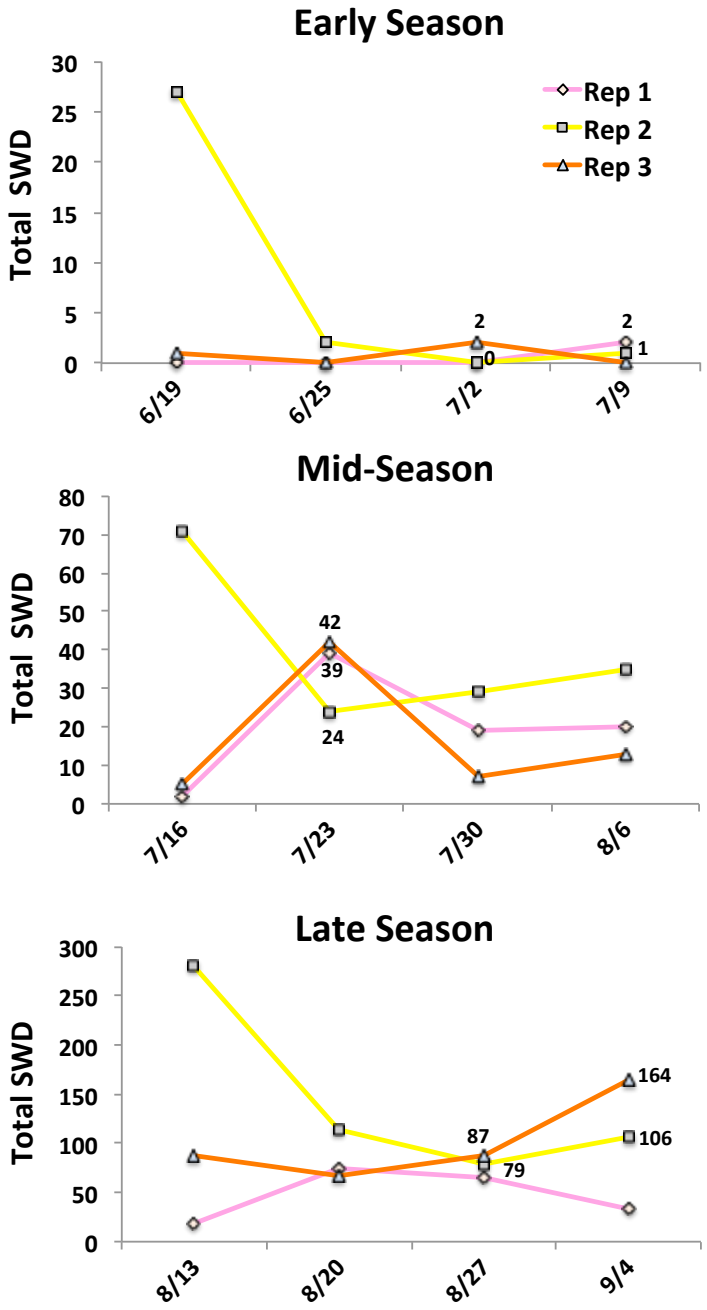

Figure 38. Seasonal replication plot catches. Population growth can be seen in the y-axis scale differences between seasons.

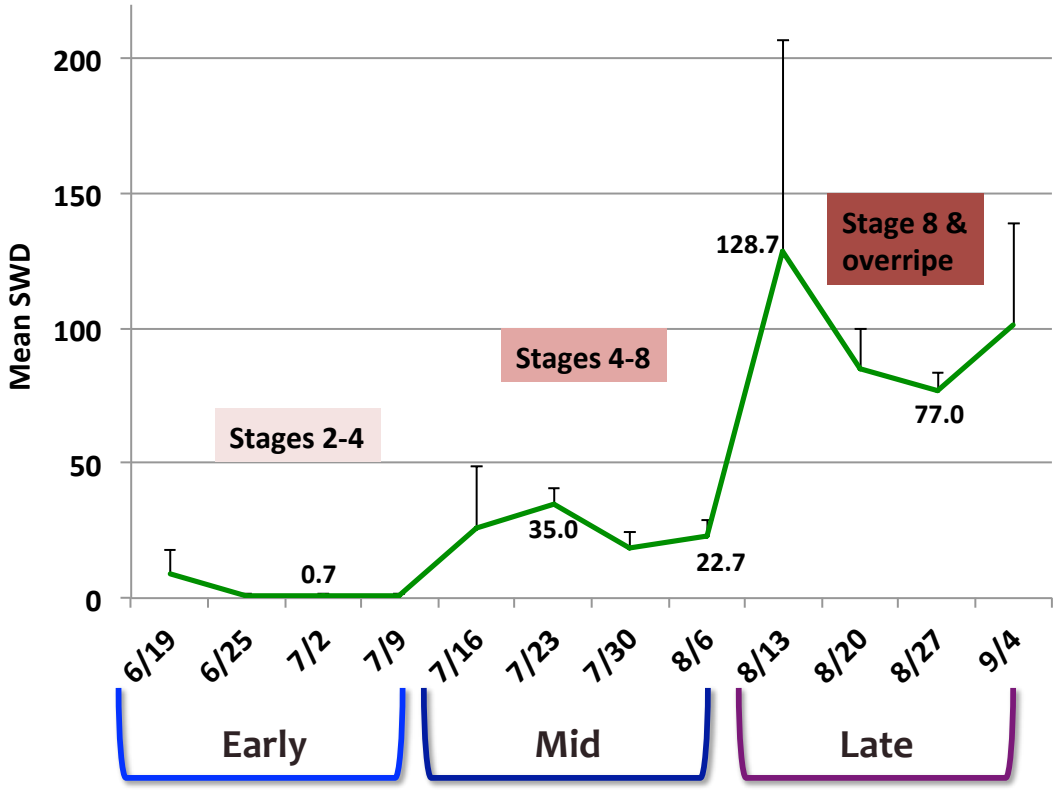

Figure 39. Mean weekly SWD catch with blackberry phenology.

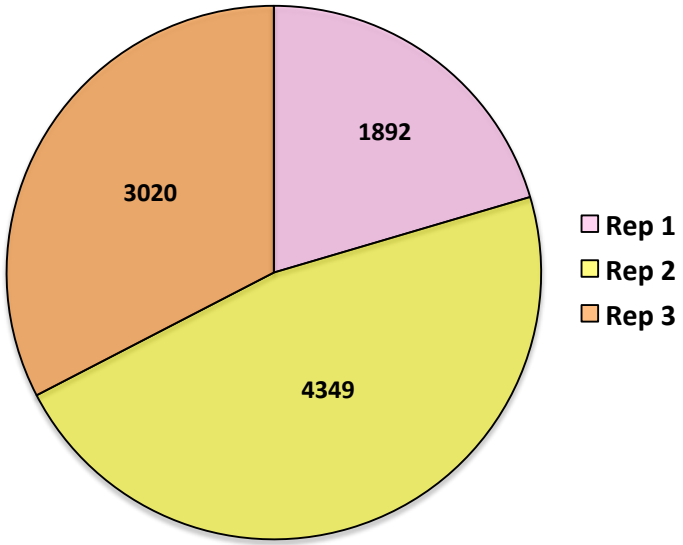

Figure 40. Total replication plot catches. This chart totals all *D. suzukii* caught in all traps in each replication plot.

4.2.3 Species and Gender Shifts

The proportions in Figure 41 show field population shifts between other *Drosophila* spp. and SWD. Figures 41 & 42 show gender shifts in SWD. Data labels in both figures represent mean weekly catches across all replication plots and trap designs. There were very low catches during the early season weeks 1, 2, 3 and 4, and most catches were of other *Drosophila* spp. Most SWD trapped during the early season were females, although the difference in mean catches between male and female SWD was very low.

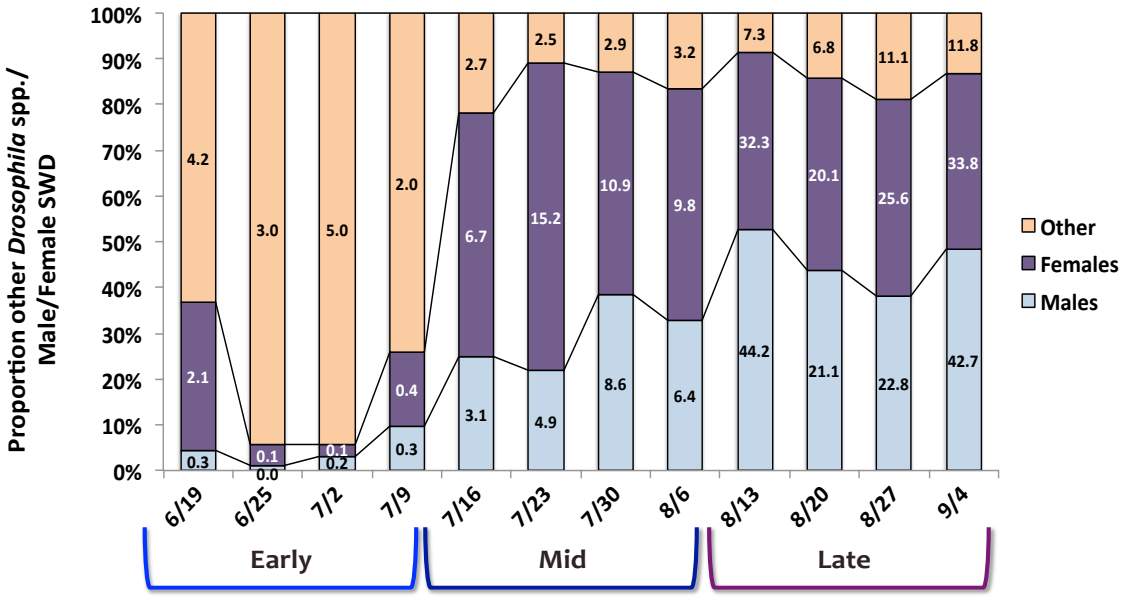

Figure 41. *Drosophila* spp./SWD species shift. The early season was dominated by other *Drosophila* spp., the mid-season by female SWD and the late season by male SWD. The data labels show average catches per week across all replication plots.

Between weeks 4 (July 9th) and 5 (July 16th) there was a species shift from other *Drosophila* spp. to SWD. Mostly SWD were caught after July 9th. A gender shift from female dominated in the mid-season (July 16th - August 6th) to a male dominated late season (August 13th – September 4th) is also visible in Figure 41. Figure 42 shows the gender shift from female to male SWD. The magnitude of weekly mean other *Drosophila* spp. catches during this study stayed relatively constant, with a range of 2.0 to 11.8 SWD. However, the magnitude of

weekly mean female and male SWD did not remain relatively constant, and significant increases in abundance are seen in both the mid- and late seasons in comparison to the early season (Figures 41 & 42).

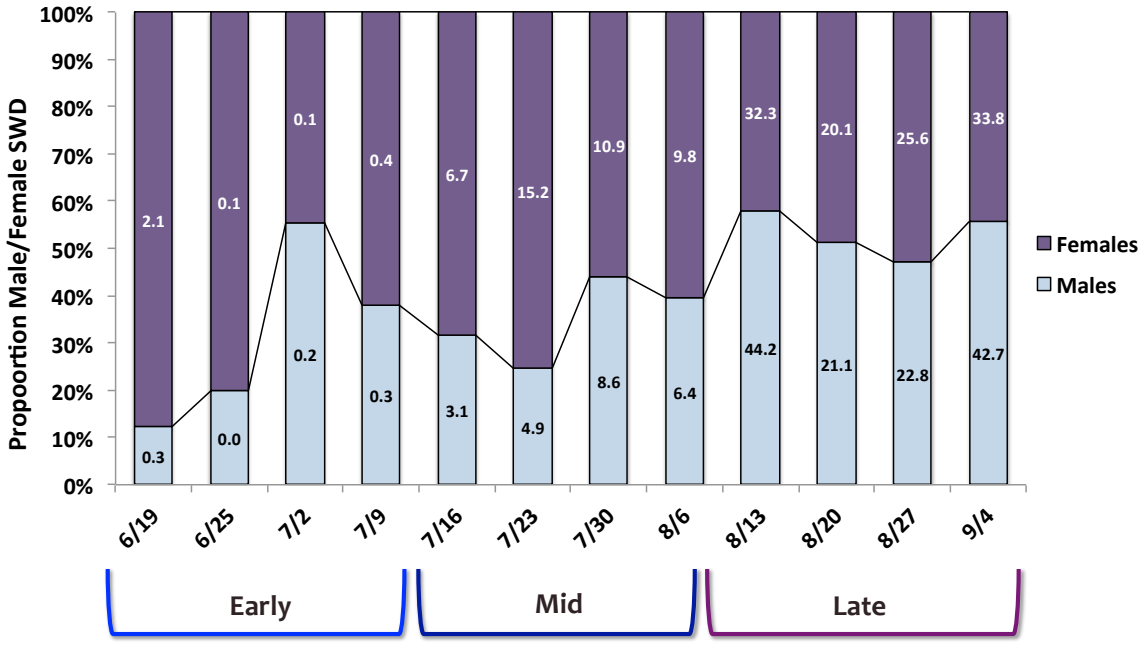

Figure 42. SWD gender shift.

4.2.4 Weekly Precipitation (mm) and Temperature (˚C)

Figure 43 shows total weekly precipitation (mm) as well as weekly high, average and low temperatures (˚C). The early season received the most precipitation of the study (33.78 mm), with most (21.3 mm) occurring during week 2. The coolest temperatures were observed during the early season, with highs ranging from 24˚C to 30˚C. The early season average temperature was 16˚C. Temperature peak highs occurred between weeks 4 (30˚C) and 5 (31˚C), 8 (38˚C) and 9 (38˚C). Warmer temperatures and lower precipitation rates characterized the mid- and late seasons. The mid-season received 3.3 mm precipitation during week 6 only. The average mid-season temperature was 19˚C with a range in high

temperatures from 29˚C to 38˚C. The late season received the least precipitation of the study, .8 mm, which occurred during weeks 11 and 12. The late season average temperature was 19.8˚C with a range in high temperatures from 28˚C to 38˚C. There was no precipitation during weeks 5, 7, 8, 9 and 10.

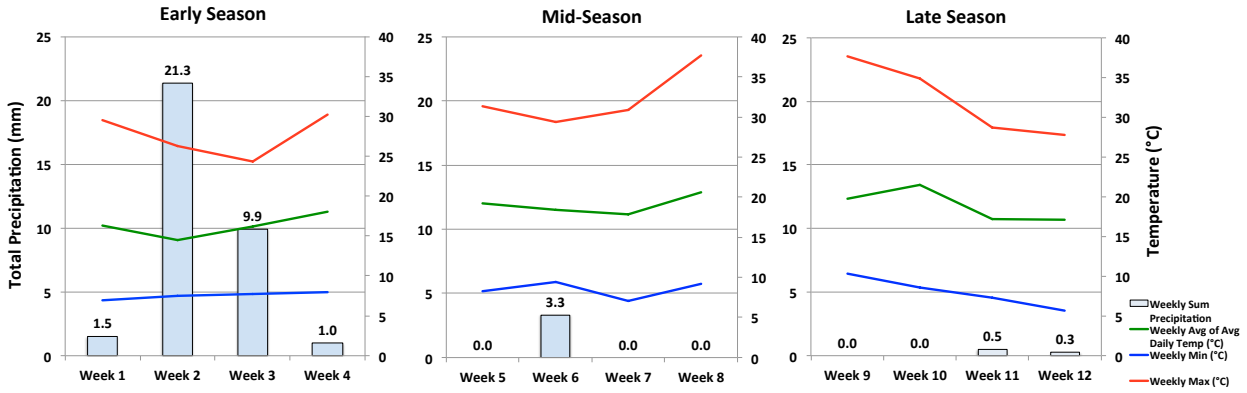

Figure 43. Weekly precipitation (mm) and temperature (˚C). Data are from June 12th (week 1) to September 4th (week 12). Bars show sum precipitation (mm) and lines show temperature maximums, averages and minimums (˚C).

CHAPTER 5 DISCUSSION/ANALYSIS

This discussion/analysis chapter is divided into three sections with four subsections.

5.1 Ovarian Maturity

5.2 Trap Design

5.2.1 Headspace

5.2.2 Marek Trap Design Improvements

5.2.3 Trap Placement

5.2.4 Species and Gender Shifts

5.3 Synthesis of Knowledge and IPM Methods

5.1 Ovarian Maturity

It is speculated that Oogonia Stage 1 ovaries are most prominent in January and February due to *D. suzukii* female overwintering capabilities and a lack of fruit hosts during this time of year. There was a large increase in Chorion Stage 3 ovaries in April. However, there are no commercial ripening crops during this time of year. It is speculated that this spike in ovarian maturity is due to utilization of alternative crop hosts such as elderberry. It may be pro-active for farmers to monitor and control for *D. suzukii* in neighboring alternative host plants to suppress early season population growth.

Ovarian maturity knowledge can be combined with other IPM strategies to control *D. suzukii* while minimizing pesticide applications. Understanding the coincidence of *D. suzukii* ovarian maturity with fruit phenology and field temperatures educates farmers about infestation timing. Oviposition (egg-laying) is greatly reduced in the Pacific Northwest United States in late fall when females prepare for winter (Dreves, Lee personal communication), which reduces justification for chemical treatment during this time. Regularly picking ripe fruit, a strategy known as sanitation, can help reduce oviposition sites for *D. suzukii* and remove eggs, larvae and pupae from the field to prevent adult population growth. Ripening fruit coincides with warming weather and increased food and oviposition resources for *D. suzukii* to complete its lifecycle. In the mid-Willamette Valley, Oregon, this takes place during June, July and August.

5.2 Trap Design

5.2.1 Headspace

The significant difference in seasonal mean *D. suzukii* catches between the Clear 10-Hole and traps with cups demonstrates the importance of headspace for trap design. Red Cup, Side Mesh with cup and Red Mesh were not significantly different from the Clear 10-Hole, which is probably due to their comparably shorter headspaces to other traps with cups. The success of the Side Mesh in comparison to the Clear 10-Hole is probably due to its smaller headspace, which allowed ACV volatiles to better disperse outside the trap to lure *D. suzukii* inside. It is speculated that smaller distances between the ACV liquid bait line and an entry point allow stronger bait volatiles to exit the trap and attract *D. suzukii*.

It is possible that the mesh side entry also contributed the Side Mesh's large trap catches, but further studies will be necessary to determine if there is significant catch difference between mesh and open-hole side entries. The Clear 10-Hole is currently known as the "standard trap" for catching *D. suzukii*, but based on results from this study, the Side Mesh may be a more effective standard trap. Traps need to be more attractive than surrounding fruiting crop to eradicate *D. suzukii* and prevent infestation.

5.2.2 Marek Trap Design Improvements

A defining feature of the Marek trap was smaller entry-holes for selective *D. suzukii* attraction, but the trap was unsuccessful. Although non-*Drosophila* spp. were unable to enter the trap, it is possible that the entry holes were too small for *Drosophila* spp. as well. It is probable that small entry holes also prevented bait volatilization, significantly lowering

the trap's attractiveness. Trap success may be improved by implementing a side mesh entry. Mesh sizes should be tested to find an optimal size for preventing entry of non-*Drosophila* spp. while allowing bait volatilization and convenient entrance for *D. suzukii*. Due to the bottleneck design, it is possible that bait volatiles were contained within the top cap area and unable to dissipate outside the trap. If this trap were to be redesigned, entry holes should probably be placed at the top of the bottleneck to allow maximal volatilization.

An oil lamp wick was placed inside the trap to absorb bait for increased volatilization. However, wicks needed to be replaced weekly to prevent disintegration and made trap servicing tedious. The small headspace also made servicing difficult, because ACV would spill out of the entry holes during bait collection and rehanging. This resulted in a lower bait line, which is believed to have further decreased the trap's attractiveness. The trap was also prone to leaks due to its super-glued bottom.

5.2.3 Trap Placement

Trap placement was determined to be a significant factor for trap success. In order for a trap to be effective, it must be placed in favorable *Drosophila* spp. habitat conditions. This was shown through replication plot catch differences and detected population increases. Catch differences are most likely due to habitat differences at replication 1, 2 and 3 plots. Replication 1 plot had an outer-bordering of railroad tracks and a service road, with hoop houses on the farm inner-border and no overstory. These characteristics allowed direct exposure to sunlight, wind, rain and traffic, creating unfavorable conditions for *D. suzukii*. Almost half of all captured *D. suzukii* were caught in the replication 2 plot. This plot was

located in a riparian zone and had a cherry overstory, which provided additional host fruits, shelter, shade, increased moisture and weather protection. These characteristics provide favorable habitat for *Drosophila* spp. (Dobzhansky et al. 1979). This may have led to less emigration from the replication 2 plot, and instead more immigration from replication 1 and 3 plots, as well as the surrounding open field. *Drosophila* spp. is known to stay within favorable habitats and this may explain the population accumulation in the replication 2 plot. Mating activity increases with more individuals, which leads to higher trap catches.

Significant *D. suzukii* activity was first detected in replication 2 plot, and was probably due to the relatively early ripening of the cherry overstory. However, the replication 3 plot was the highest catching during the last two weeks of the study. The cherries and blackberries in the replication 2 plot may have been over-exploited while the blackberries in the replication 3 plot were ripe and healthy. This might have caused a migration from the replication 2 plot to the replication 3 plot for intact resources. *D. suzukii* might have used the replication 3 plot blackberries for oviposition sites and food sources while seeking refuge from late season direct sunlight and high temperatures in the persimmon orchard.

There was also a substantial population crash during late August. It is speculated this is due to exceptional hot and dry conditions during August 2012, which may have degraded fruit viability for *D. suzukii* feeding and oviposition. This study found *D. suzukii* to be more active during the late season than during the early or mid-seasons in the mid-Willamette Valley, Oregon.

5.2.4 Species and Gender Shifts

There was an observed species shift from *Drosophila* spp. to *D. suzukii*, as well as a gender shift from female to male *D. suzukii*. Given this knowledge, it is vital for farmers to protect fruiting crops in the early and mid-seasons when the *D. suzukii* population is female dominated. During this time, fruits are beginning to ripen and 62% of June and 60% of July females examined were oviposition-ready. If *D. suzukii* populations are minimized during the early and mid-seasons, population sizes and infestation rates could be reduced. Further research should be conducted to determine if females could be targeted to prevent oviposition and crop loss. Although males are vital for sustaining populations, only females pose a direct threat to fruiting crops. Studies should be conducted on females to change their mating and/or oviposition behavior.

5.3 Synthesis of Knowledge and IPM Methods

Monitoring farmland borders and surrounding lands is important when developing IPM programs. Neglected farm-bordering wild blackberry pose a great threat to fruiting crops by allowing *D suzukii* populations to persist and grow. *D suzukii* can infest bordering hedges and migrate into a farm once additional oviposition sites and food sources are discovered in fruiting crops. Warmer temperatures may lead to more field activity from increased release of volatile compounds from ripening fruit, attracting flies to fruit in search of food and mates. Traps with reduced headspace may increase trap effectiveness and catch success to eradicate *D. suzukii*. Traps can be set throughout fruiting crops, but must be regularly serviced to remove captured flies and replenish bait.

Implementing effective IPM programs for *D. suzukii* internationally will be dependent on local weather, farm and landscape conditions. This study was conducted in the mid-Willamette Valley, Oregon where crops ripen in June, July and August. Other regions may have different harvest periods due to climate differences, which will need to be accounted for when initiating IPM programs. However, knowledge of *D. suzukii* lifecycle timing in coincidence with ripening crops is essential for determining when fruits are most susceptible to infestation. Trapping removes adult *D. suzukii* from field populations while sanitation removes eggs, larvae, and pupa from the fruiting crop. Eradicating *D. suzukii* at all life stages can assure its successful management while reducing pesticide dependence.

CHAPTER 6 LIMITATIONS/FUTURE STUDY

Due to time limitations, the trap design study included only three replications. Additional replication plots may help in reducing variation in fly captures to increase sensitivity to differences. Additional analysis will have to be conducted to isolate and better understand other parameters such as color, bait surface area and volume, etc. This design study wanted to also analyze trap sensitivity during the early season, but trap captures in designs with cups were found to be inconclusive. Comparing these designs without their cups would have been informative. This thesis was also unable to focus on the environmental and health impacts of the pesticides commonly used to control *D. suzukii*.

This study did not focus on baits, but they are an important aspect of trap design. ACV was used throughout the trap design study, but different baits may be more successful at varying times during a fruiting crop's season. It is known that *Drosophila* spp. are dependent on yeasts as food sources while developing (Hamby et al., 2012). This suggests that utilizing yeast baits during the early season of the study could have resulted in higher early season *D. suzukii* catches. Using baits in accordance with seasonal fruit phenology may increase trap attractiveness. ACV may be most effective during the mid- and late seasons when fruit is ripe. However, ACV is a relatively weak bait and could be enhanced to lure *D. suzukii* from greater distances.

Traps must be able to lure *D. suzukii* in without allowing them to escape after trap entry. Dish soap was diluted in ACV bait to pull *D. suzukii* into the liquid after coming into contact

with the ACV. However, the traps in this study allowed flies to enter and exit freely if they did not touch the bait. For this reason, attract n' kill baits will be essential in future studies and IPM implementation to eradicate *D. suzukii* from fruiting crops.

CHAPTER 7 CONCLUSION

Farmers in the United States were forced to resort to pesticides for crop protection when initially controlling *D. suzukii* infestation. However, pesticides may not the most viable method for preventing infestation. Environmental and health impacts (Galt 2014), meeting Maximum Residue Level regulations for international fruit trade, effectively timing applications in rainy climates and preventing *D. suzukii* pesticide resistance are circumstances to consider when implementing chemical control. Ovarian maturity and trapping are two methods, which can be synthesized into an effective Integrated Pest Management program. Understanding *D. suzukii* ovarian maturity timing in coordination with seasonal weather and fruit phenology allows farmers to efficiently schedule their crop treatments to minimize pesticide use. Implementing traps with small headspaces will increase trap catches, and further studies can maximize favorable trap characteristics for *D. suzukii* eradication. Traps must be placed in favorable *Drosophila* spp. habitat along and outside farm borders to effectively protect fruiting crops.

Drosophila spp. are more abundant than *D. suzukii* in the mid-Willamette Valley, Oregon during June. The *D. suzukii* population explodes with females from early July until early August when the population shifts to male prominent. It is crucial to know when female *D. suzukii* are most abundant in the field, because their ovarian maturity aligns with fruit phenology for oviposition. Although this thesis only focuses on ovarian maturity and trapping, many strategies and fields of study can contribute to IPM programs to reduce pesticide dependence for controlling *D. suzukii* globally.

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APPENDIX A

Table 2. Summary of *D. suzukii* management research in the United States.

Research Focus	Year	Author(s)	Article Title
Baits	2014	Cha et al.	A four-component synthetic attractant for Drosophila suzukii (Diptera: Drosophilidae) isolated from fermented bait headspace.
	2013	Cha et al.	Comparison of a synthetic chemical lure and standard fermented baits for trapping Drosophila suzukii (Diptera: Drosophilidae).
	2012	Landolt et al.	Trapping spotted wing drosophila, Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol.
	1996	Kido et al.	Nontarget insect attraction to methyl eugenol traps used in male annihilation of the oriental fruit fly (Diptera: Tephritidae) in riparian Hawaiian stream habitat.
Barriers	2013	Hanson et al.	High Tunnels for Organic Raspberry Production in the Midwest US
	2013	Demchak & Hanson	Small Fruit Production in High Tunnels in the US
Chemical control	2013	Hamby et al.	Integrating Circadian Activity and Gene Expression Profiles to Predict Chronotoxicity of Drosophila suzukii Response to Insecticides
	2013	Timmeren & Isaacs	Control of spotted wing drosophila, Drosophila suzukii, by specific insecticides and by conventional and organic crop protection programs.
	2012	Haviland & Beers	Chemical Control Programs for *Drosophila Suzukii* That Comply With International Limitations on Pesticide Residues for Exported Sweet Cherries
	2012	Walse et al.	Postharvest treatment of strawberries with methyl bromide to control spotted wing drosophila, Drosophila suzukii.
	2012	Yee & Alston	Behavioral responses, rate of mortality, and oviposition of western cherry fruit fly exposed to malathion, zeta-cypermethrin, and spinetoram
	2011	Beers et al.	Developing Drosophila suzukii management programs for sweet cherry in the western United States.
	2011	Bruck et al.	Laboratory and field comparisons of insecticides to reduce infestation of Drosophila suzukii in berry crops
	2011	Gerdeman et al.	Biology and management of spotted wing drosophila, Drosophila suzukii (Matsumura) in small fruits in the Pacific Northwest.
Distribution	2011	Hauser	A historic account of the invasion of Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification.
	2011	Walsh et al.	Drosophila suzukii (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential.
Host Fruits	2013	Bellamy et al.	Quantifying Host Potentials: Indexing Postharvest Fresh Fruits for Spotted Wing Drosophila, Drosophila suzukii
	2013	Burrack et al.	Variation in selection and utilization of host crops in the field and laboratory by Drosophila suzukii Matsumara (Diptera: Drosophilidae), an invasive frugivore.
	2013	Steffan et al.	Susceptibility of cranberries to Drosophila suzukii (Diptera: Drosophilidae).
	2013	Yu et al.	Host Status and Fruit Odor Response of Drosophila suzukii (Diptera: Drosophilidae) to Figs and Mulberries
	2012	Hamby et al.	Associations of Yeasts with Spotted-Wing Drosophila (Drosophila suzukii; Diptera: Drosophilidae) in Cherries and Raspberries
	2011	Lee et al.	The susceptibility of small fruits and cherries to the spotted-wing drosophila, Drosophila suzukii
Market Impact	2013	Dean et al.	Development and impact of the spotted wing drosophila, Drosophila suzukii, in Florida strawberries.
	2011	Lee et al.	In Focus: spotted wing drosophila, Drosophila suzukii, across perspectives.
	2011	Goodhue et al.	Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs.
Trapping	2013	Basoalto et al.	Factors affecting the efficacy of a vinegar trap for Drosophila suzikii (Diptera; Drosophilidae).
	2013	Freda & Braverman	Drosophila suzukii, or spotted wing Drosophila, recorded in Southeastern Pennsylvania, U.S.A.
	2012	Lee et al.	Evaluation of monitoring traps for Drosophila suzukii (Diptera: Drosophilidae) in North America.
Outreach	2012	Burrack et al.	Using volunteer-based networks to track Drosophila suzukii (Diptera: Drosophilidae) an invasive pest of fruit crops.
	2011	Dreves	IPM program development for an invasive pest: coordination, outreach and evaluation.
Overwintering	2012	Thistlewood et al.	Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), a new pest of stone fruits in western North America.
	2011	Dalton et al.	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

Table 3. Summary of *D. suzukii* management research in Japan. Some articles found during literature research were available only in Japanese. Unfortunately, these articles couldn't be categorized by the author or included in this table.

Research Focus	Year	Author(s)	Article Title
Barriers	2005	Kawase & Uchino	Effect of mesh size on Drosophila suzukii adults passing through the mesh.
Biological Control	2013	Kasuya et al.	Ecological, morphological and molecular studies on Ganaspis individuals (Hymenoptera: Figitidae) attacking Drosophila suzukii (Diptera: Drosophilidae)
	2013	Poyet et al.	Resistance of Drosophila suzukii to the larval parasitoids Leptopilina heterotoma and Asobara japonica is related to haemocyte load.
Habitat	2004	Kimura	Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions.
Host Fruits	2013	Kinjo et al.	Oviposition efficacy of Drosophila suzukii (Diptera: Drosophilidae) on different cultivars of blueberry.
	2010	Mitsui et al.	Seasonal life cycles and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan.
	2006	Mitsui et al.	Spatial distributions and clutch sizes of Drosophila species ovipositing on cherry fruits of different stages
	2005	Uchino	Distribution and seasonal occurrence of cherry drosophila Drosophila suzukii (Diptera: Drosophilidae) injurious to blueberry in Chiba Prefecture.
	1939	Kanzawa	Studies on Drosophila suzukii Mats.
Trapping	1993	Sasaki & Abe	Occurrence of Drosophila in cherry orchards. (I) Species and their seasonal prevalence obtained from bait traps.

Table 4. Summary of *D. suzukii* management research in Europe.

Research Focus	Year	Author(s)	Article Title
Biological control	2012	Chabert et al.	Ability of European parasitoids (Hymenoptera) to control a new invasive Asiatic pest, Drosophila suzukii
Chemical control	2013	Gargani et al.	Notes on *Drosophila suzukii* Matsumura (Diptera Drosophilidae): field survey in Tuscany and laboratory evaluation of organic products.
	2012	Angeli et al.	The products effective against *Drosophila suzukii*.
	2012	Profaizer et al.	*Drosophila suzukii*: evaluation of chemical pesticides and recommendation on positioning.
Distribution	2013	Marongiu et al.	First record of *Drosophila suzukii* in Sardinia (Italy).
	2010	Süss & Constanzi	Presence of *Drosophila suzukii* (Matsumura, 1931) (Diptera Drosophilidae) in Liguria (Italy).
Economic Impact	2013	Ros et al.	The potential economic impact of *Drosophila suzukii* on small fruits production in Trentino (Italy).
Host Fruits	2012	Vogt et al.	A new pest, the spotted wing drosophila, *Drosophila suzukii* (Matsumura), is threatening fruit-growing and viticulture (Diptera, Drosophilidae).
	2010	Baufeld et al.	The Cherry vinegar fly - *Drosophila suzukii* - an emerging risk for fruit and wine growing.
Invasion Tracking	2012	Calabria et al.	First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe.
IPM	2013	Simoni et al.	DROSKII: a transnational attempt for insight on the damage potential of *Drosophila suzukii* and on the development of risk management and control measures.
	2012	Cini et al.	A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management.
	2012	Grassi & Pallaoro	*Drosophila suzukii* (Matsumura), a revolution for soft fruits in Trentino.
	2012	Ioriatti et al.	*Drosophila suzukii* (Matsumura), a new invasive species harmful to crops with small fruits.
	2012	Warlop & Filleron	Controlling cherry fruit fly under organic farming in France: hopes and despairs.
	2011	Grassi et al.	*Drosophila* (*Sophophora*) *suzukii* (Matsumura), new pest of soft fruits in Trentino (North-Italy) and in Europe.
Sterile Insect Technique (SIT)	2013	Schetelig & Handler	Germline transformation of the spotted wing drosophilid, *Drosophila suzukii,* with a *piggyBac* transposon vector
Trapping	2012	Boselli et al.	Monitoring of *Drosophila suzukii* in Emilia Romagna.
	2012	Rouzes et al.	First occurrence of *Drosophila suzukii* in the Sauternes vineyards

APPENDIX B

Trap Design	Trap #	Entry Area Shape	Entry Area Type (Hole or mesh grid)	Location of Entry	Trap Cup Type	# Entry Areas	Individual Entry Size (Diameter or dimension of specific hole)	Area of a Single Entry
Red Mesh	1,12, 23	Rectangle	Mesh Grid (Coarse Mesh)	Side	Deli	2	7.5x5 cm^2 (2 mm grid)	37.5 cm^2 = 375 mm^2
Red Cup	2, 13, 24	Circle	Hole	Side	Party	6	5 mm Diameter	19.635 mm^2
Red McPhail	3, 14, 25	Circle	Mesh Grid (Coarse Mesh)	Side	Bucket	4	(3) 2.5 cm side hole (2 mm grid); (1) 2.5 cm bottom hole (2 mm grid)	19.6350 cm^2 = 196.35 mm^2
Side Mesh w/cup	4, 15, 26	Rectangle	Mesh Grid (Coarse Mesh)	Side	Deli	2	(2) 4x8 cm^2 rectangle (2x2 mm grid)	4 x 8 cm^2 = 32 cm^2 * 2 = 64 cm^2 = 640 mm^2
Half-cone	5, 16, 27	Teapot spout	Double Mesh Grid (Coarse & Fine Mesh Layer)	Side	Deli	2	2 entrances, triangle 3.5cm sides, length tea spout 5.5 high x 4	31.7 cm^2 = 317 mm^2
Green Cone	6, 17, 28	Cone	Coarse & Fine Mesh	Side	Trap & Toss	3, then enter a hole at the bottom of the three panel cone, hole is 1.5 cm	6 x 11.5 cm; 3 windows	69 cm^2 = 690 mm^2
Side Mesh	6, 17, 28	Rectangle	Mesh Grid (Coarse Mesh)	Side	Deli	2	5x7.5 cm side hole (2 mm grid)	37.5 cm^2 = 375 mm^2
Spaceship	7, 18, 29	Circle	Mesh Grid (Coarse Mesh)	Top	Deli	1	10.5 cm (2 mm grid), then enter a hole at the bottom of the three panel cone, hole is 1.5 cm	95.726 cm^2 = 967.26 mm^2
Clear 10-Hole	8, 19, 30	Circle	Hole	Side	Deli	10	4 mm	12.566 cm^2 = 125.66 mm^2
Captiva	9, 20, 31	Circle	Hole	Side	Spice Container	12	4 mm Diameter	12.566 cm^2 = 125.66 mm^2
Marek	10, 21, 32	Circle	Hole	Side	Sobi Bottle	10	2 mm Diameter	3.142 mm^2
Clear 10-Hole w/cup	11, 22, 33	Circle	Hole	Side	Deli	10	5 mm Diameter	19.6350 cm^2 = 196.35 mm^2

Trap Design	Total Entry Area	Bottom Collection Cup (Influences Head Space Volitalization)	Total Free Space Height (Distance from liquid line to first entry way, cm)	Trap Specific Free Space (Distance from top of collection container to first entry way)	Trap Body Volitalization (Volume of distance from top of collection container (if present- otherwise, just liquid line) to first trap entry way) (Calc: Area of Trap Top*Trap Specific Free Space)	Collection Cup Volitalization (Area of bottom of container*2.7cm (height of freespace of collection container))	Headspace - Total Volitalization (Volume from vinegar line to closest entry hole (Dis from liquid line to top of collection container*Area of Collection conatiner bottom + Area of Trap bottom*trap body distance from top of collection container to entry, cm))
Red Mesh	75 cm^2 = 750 mm^2	Yes	10.2 cm = 102 mm	7.5 cm = 75 mm	848.25 cm^3 = 8482.5 mm^2	89.6 cm^3 = 896 mm^3	937.85 cm^3 = 9378.5 mm^3
Red Cup	117.81 mm^2	Yes	11.6 cm = 116 mm	8.9 cm = 89 mm	698.65 cm^3 = 6986.5 mm^2	89.6 cm^3 = 896 mm^3	788.25 cm^3 = 7882.5 mm^3
Red McPhail	14.73 cm + 4.91 cm = 19.63cm = 196.3 mm^2	No	8.5 cm = 85 mm	8.5 cm = 85 mm	1216.35 cm^3-161.89 cm^3 (the middle, hollow frustrum volume) = 1054.46 cm^3 = 10544.6 mm^3	0	1054.46 cm^3 = 10544.6 mm^3
Side Mesh w/cup	64 cm^2 = 640 mm^2	Yes	9.8 cm = 98 mm	7.1 cm = 71 mm	803.01 cm^3 = 8030. 1 mm^3	89.6 cm^3 = 896 mm^3	892.61 cm^3 = 8926.1 mm^3
Half-cone	63.4 cm^2 = 634 mm^4	Yes	9.5 cm = 95 mm	6.8 cm = 68 mm	769.08 cm^3 = 7690.8 mm^3	89.6 cm^3 = 896 mm^3	858.68 cm^3 = 8586.8 mm^3
Green Cone	69 * 3 = 207 cm^2 = 2070 mm^2	Yes	17 cm = 170 mm	14.3 cm = 143 mm	2360.93 cm^3 = 23609.3 mm^3	89.6 cm^3 = 896 mm^3	2450.53 cm^3 = 24505.3 mm^3
Side Mesh	37.5 * 2 = 75 cm^2 = 750 mm^2	No	3.7 cm = 37 mm	3.7 cm = 37 mm	418.47 cm^3 = 4184.7 mm^3	0	418.47 cm^3 4184.7 mm^3
Spaceship	95.726 cm^2 = 957.26 mm^2	Yes	18 cm = 180 mm	15.3 cm = 153 mm	1730.43 cm^3 = 17304.3 mm^3	89.6 cm^3 = 896 mm^3	1820.03 cm^3
Clear 10-Hole	113.097 cm^2 = 1130.97 mm^2	No	Dis. To bottom holes from ACV = 7.5 cm = 75 mm; Dis. To top holes from ACV = 9 cm = 90	Dis. To bottom holes from ACV = 7.5 cm = 75 mm; Dis. To top holes from ACV = 9 cm = 90 mm	779.25 cm^3 = 7792.5 mm^3 (to bottom holes); 935.1 cm^3 = 9351 mm^3 (to top holes)	0	779.25 cm^3 (to bottom holes); 935.1 cm^3 (to top holes) = 9351 mm^3
Captiva	125.664 cm^2 = 1256.64 mm^2	No	2 mm	2 mm	47.6 cm^3 = 476 mm^3	0	47.6 cm^3 = 476 mm^3
Marek	31.416 mm^2	No	Dis. To bottom holes from ACV = 7 mm; Dis. To top holes from ACV = 1.5 cm = 15 mm	Dis. To bottom holes from ACV = 7 mm; Dis. To top holes from ACV = 1.5 cm = 15 mm	269.5 mm^3 = 2695 mm^3 from liquid to bottom holes; 57.75 cm^3 = 577.5 mm^3 from liquid to top holes	0	269.5 mm^3 from liquid to bottom holes; 57.75 cm^3 = 577.5 mm^3 from liquid to top holes
Clear 10-Hole w/cup	196.175 cm^2 = 1961.75 mm^2	Yes	12 cm = 120 mm	9.3 cm = 93 mm	1051.83 cm^3 = 10518.3 mm^3	89.6 cm^3 = 896 mm^3	1141.43 cm^3 = 11414.3 mm^3

Trap Design	Trap Height (Lengthwise top to bottom)	Trap Width (diameter of trap, measured across the top of the container)	Area of Trap Top (cm) pi((.5)(Trap Width))^2	Trap Body Volume	Trap Overall Color	Amount of Red (quantified; refer to key)	Amount of Green (quantified; refer to key)	Amount of Clear (quantified; refer to key)	Extra Details
Red Mesh	23.6 cm	12 cm = 120 mm	113.1 cm^2 = 1131 mm^2	32 oz	Clear	20%	0%	90%	5x14 (cm?) loose red mesh
Red Cup	20.5 cm	10 cm = 100 mm	78.5 cm^2 = 785 mm^2	18 oz	Red	80%	0%	10%	Clear tape seals the straw hole
Red McPhail	18.6 cm	13.5 cm = 135 mm	143.1 cm^2 = 1431 mm^2	64 oz	Red	85%	0%	15%	
Side Mesh w/cup	23.6 cm	12 cm = 120 mm	113.1 cm^2 = 1131 mm^2	32 oz	Clear	0%	0%	100%	
Half-cone	24.6 cm	12 cm = 120 mm	113.1 cm^2 = 1131 mm^2	32 oz	Clear	0%	0%	100%	
Green Cone	34.5 cm	~14.5 cm = 145 mm, top is rounded, difficult to accurately measure	165.1 cm^2 = 1651 mm^2	72 oz	Clear	15% (Peanut Butter Cap)	15%	85%	2 passages need to be cleared in order to enter collection cup *Peanut butter jar for bottom collection cup
Side Mesh	14.5 cm	12 cm = 120 mm	113.1 cm^2 = 1131 mm^2	32 oz	Clear	0%	0%	100%	
Spaceship	30 cm	12 cm = 120 mm	113.1 cm^2 = 1131 mm^2	32 oz	Clear/White	0%	15%	70%	2 passages need to be cleared in order to enter collection cup
Clear 10-Hole	14 cm	11.5 cm = 115 mm	103.9 cm^2 = 1039 mm^2	32 oz	Clear	0%	0%	100%	Standard Trap
Captiva	12.5 cm	5.5 cm = 55 mm	23.8 cm^2 = 238 mm^2	250 mL	Clear	40%	0%	60%	Electrical tape is used for red attraction. Top of spice container is also red
Marek	13.5 cm	Variable, different than other traps, measurement taken from bottom of container: 7 cm = 70 mm, across top: 3.6 cm = 36 mm	38.5 cm^2 = 385 mm^2 (Used Bottom Diameter Measurement)	300 mL	50% Red, 50% Clear	45%	0%	55%	Smallest entry area, could be problematic..
Clear 10-Hole w/cup	23.6 cm	12 cm = 120 mm	113.1 cm^2 = 1131 mm^2	32 oz	Clear	0%	0%	100%	Same as the clear 10 hole, but has bottom collection cup

Amt of Red Key	Quantified
Red Container Top	10%
Red Container	80-90%
Captiva Red Strip	15%
Haviland Red Top Perimeter	20%
Red Mesh	20%
Peanut Butter Container Cap	15%

Amt of Green Key	Quantified
Green Funnel	15%

Amt of Clear Key	Quantified
Clear Container Top	10%
Clear Container	90%
Clear Middle Section Haviland	5%
Clear Section of Captiva	60%

APPENDIX C

Rep 1

A	Open edge/end of the field; Near Telephone pole
B	Continuous BWY
C	Continuous BWY
D	Continuous BWY
E	Continuous BWY and slender Rose bush branch
F	Continuous BWY and slender Rose bush branch
G	Continuous BWY
H	Continuous BWY
I	Continuous BWY
J	Continuous BWY
K	End of Rep 1, Continuous BWY

Rep 2

A	Cherry overstory, Hawthorne tree too.
B	Cherry overstory, Hawthorne tree too.
C	Cherry overstory
D	Cherry overstory, direct contact with WBY for half
E	Cherry overstory, mostly WBY
F	No cherry overstory, but Hawthorne overstory present.
G	WBY recesses upward, bush is tallest at this position
H	WBY still recesses upward, and taller than the rest of the bush
I	WBY pushes outward of the overalll form of the bush
J	Tucked corner, sort of like a cave. Trap hangs on a Hawthorne branch.
K	Beginning of another Cherry overstory. Another Hawthorne is to the right. WBY continues. End of the Rep.

Rep 3

A	Edge of WBY, Tall grass and more WBY
B	Level WBY, some tall grass growing through the WBY
C	Tall grass separates C from B. C is down a little hill.
D	Bush is lowest with taller shoots of WBY
E	Continuous WBY
F	Edge of Hawthorne andWBY
G	Hawthorne overstory, forms a cave.
H	Edge of Hawthorne and WBY
I	Curve/Indent in continuous row of WBY
J	Another Curve/Indent
K	Continuous WBY, high branchs of WBY, end of Rep.

APPENDIX D

Please note the following trap name changes

Old → New
Dreves Side Mesh → Side Mesh with cup
Dreves No Cup → Side Mesh
Screw Bottom 10-Hole → Clear 10-Hole with cup

East Side

Rep 1	6/19/12	6/25/12	7/2/12	7/9/12	7/16/12	7/23/12						
Position	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**	**Week 12**
A	Captiva	Dreves Side Mesh	Spaceship	Dreves No Cup	Screw Bottom 10-Hole	Red Mesh	Red Cup	Half-cone	Red McPhail	Clear 10-Hole	Marek	Red Cup
B	Dreves Side Mesh	Green Cone	Half-cone	Red Mesh	Spaceship	Screw Bottom 10-Hole	Screw Bottom 10-Hole	Red Mesh	Screw Bottom 10-Hole	Spaceship	Dreves Side Mesh	Dreves No Cup
C	Clear 10-Hole	Red McPhail	Screw Bottom 10-Hole	Red McPhail	Red Mesh	Spaceship	Marek	Spaceship	Captiva	Red McPhail	Red McPhail	Red McPhail
D	Marek	Red Mesh	Red Cup	Half-cone	Red Cup	Dreves Side Mesh	Red McPhail	Red Cup	Red Cup	Screw Bottom 10-Hole	Spaceship	Marek
E	Red McPhail	Red Cup	Dreves No Cup	Spaceship	Clear 10-Hole	Captiva	Captiva	Dreves No Cup	Half-cone	Dreves No Cup	Red Mesh	Screw Bottom 10-Hole
F	Red Mesh	Half-cone	Dreves Side Mesh	Clear 10-Hole	Captiva	Marek	Clear 10-Hole	Dreves Side Mesh	Clear 10-Hole	Marek	Dreves No Cup	Dreves Side Mesh
G	Green Cone	Captiva	Red McPhail	Marek	Half-cone	Clear 10-Hole	Dreves Side Mesh	Captiva	Dreves Side Mesh	Red Mesh	Half-cone	Clear 10-Hole
H	Red Cup	Clear 10-Hole	Captiva	Red Cup	Dreves Side Mesh	Red McPhail	Spaceship	Red McPhail	Marek	Half-cone	Clear 10-Hole	Captiva
I	Screw Bottom 10-Hole	Screw Bottom 10-Hole	Clear 10-Hole	Screw Bottom 10-Hole	Marek	Half-cone	Dreves No Cup	Screw Bottom 10-Hole	Dreves No Cup	Dreves Side Mesh	Captiva	Red Mesh
J	Spaceship	Spaceship	Marek	Captiva	Dreves No Cup	Dreves No Cup	Half-cone	Marek	Spaceship	Red Cup	Screw Bottom 10-Hole	Half-cone
K	Half-cone	Marek	Red Mesh	Dreves Side Mesh	Red McPhail	Red Cup	Red Mesh	Clear 10-Hole	Red Mesh	Captiva	Red Cup	Spaceship

South Side

Rep 2	6/19/12	6/25/12	7/2/12	7/9/12	7/16/12	7/23/12						
Position	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**	**Week 12**
A	Spaceship	Half-cone	Red Mesh	Screw Bottom 10-Hole	Clear 10-Hole	Marek	Red Mesh	Dreves No Cup	Red McPhail	Spaceship	Captiva	Marek
B	Green Cone	Marek	Captiva	Dreves Side Mesh	Red McPhail	Red Cup	Dreves Side Mesh	Clear 10-Hole	Red Mesh	Captiva	Half-cone	Red Cup
C	Screw Bottom 10-Hole	Clear 10-Hole	Marek	Red Cup	Dreves Side Mesh	Clear 10-Hole	Dreves No Cup	Red McPhail	Half-cone	Red Mesh	Red McPhail	Red McPhail
D	Dreves Side Mesh	Spaceship	Screw Bottom 10-Hole	Captiva	Dreves No Cup	Dreves No Cup	Screw Bottom 10-Hole	Marek	Spaceship	Red Cup	Red Cup	Spaceship
E	Red McPhail	Green Cone	Half-cone	Red Mesh	Spaceship	Screw Bottom 10-Hole	Captiva	Spaceship	Screw Bottom 10-Hole	Half-cone	Screw Bottom 10-Hole	Screw Bottom 10-Hole
F	Marek	Red Cup	Dreves Side Mesh	Clear 10-Hole	Half-cone	Captiva	Red McPhail	Dreves Side Mesh	Clear 10-Hole	Dreves No Cup	Dreves Side Mesh	Clear 10-Hole
G	Red Mesh	Red Mesh	Dreves No Cup	Spaceship	Red Cup	Dreves Side Mesh	Clear 10-Hole	Half-cone	Red Cup	Screw Bottom 10-Hole	Red Mesh	Dreves Side Mesh
H	Red Cup	Captiva	Red McPhail	Marek	Captiva	Half-cone	Spaceship	Captiva	Dreves Side Mesh	Marek	Clear 10-Hole	Red Mesh
I	Captiva	Screw Bottom 10-Hole	Clear 10-Hole	Half-cone	Marek	Red McPhail	Red Cup	Screw Bottom 10-Hole	Dreves No Cup	Dreves Side Mesh	Dreves No Cup	Dreves No Cup
J	Half-cone	Dreves Side Mesh	Spaceship	Dreves No Cup	Screw Bottom 10-Hole	Red Mesh	Marek	Red Mesh	Captiva	Clear 10-Hole	Marek	Half-cone
K	Clear 10-Hole	Red McPhail	Red Cup	Red McPhail	Red Mesh	Spaceship	Half-cone	Red Cup	Marek	Red McPhail	Spaceship	Captiva

West Side

Rep 3	6/19/12	6/25/12	7/2/12	7/9/12	7/16/12	7/23/12						
Position	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**	**Week 12**
A	Half-cone	Green Cone	Spaceship	Clear 10-Hole	Dreves Side Mesh	Red Mesh	Marek	Red McPhail	Screw Bottom 10-Hole	Spaceship	Red Mesh	Half-cone
B	Clear 10-Hole	Red McPhail	Half-cone	Red McPhail	Red Mesh	Spaceship	Dreves No Cup	Red Cup	Captiva	Red McPhail	Captiva	Dreves No Cup
C	Screw Bottom 10-Hole	Spaceship	Red Cup	Captiva	Dreves No Cup	Dreves No Cup	Half-cone	Marek	Spaceship	Red Cup	Screw Bottom 10-Hole	Red McPhail
D	Dreves Side Mesh	Half-cone	Marek	Red Mesh	Spaceship	Screw Bottom 10-Hole	Clear 10-Hole	Spaceship	Half-cone	Half-cone	Red Cup	Clear 10-Hole
E	Red Mesh	Captiva	Screw Bottom 10-Hole	Marek	Captiva	Marek	Spaceship	Captiva	Dreves Side Mesh	Marek	Dreves No Cup	Marek
F	Red Cup	Screw Bottom 10-Hole	Red McPhail	Screw Bottom 10-Hole	Marek	Red McPhail	Captiva	Screw Bottom 10-Hole	Dreves No Cup	Dreves Side Mesh	Clear 10-Hole	Screw Bottom 10-Hole
G	Red McPhail	Red Cup	Clear 10-Hole	Half-cone	Clear 10-Hole	Captiva	Dreves Side Mesh	Dreves Side Mesh	Clear 10-Hole	Dreves No Cup	Red McPhail	Dreves Side Mesh
H	Green Cone	Clear 10-Hole	Dreves Side Mesh	Red Cup	Half-cone	Clear 10-Hole	Red Mesh	Half-cone	Marek	Red Mesh	Dreves Side Mesh	Captiva
I	Spaceship	Marek	Captiva	Dreves Side Mesh	Red McPhail	Red Cup	Red McPhail	Clear 10-Hole	Red Mesh	Captiva	Marek	Red Cup
J	Marek	Red Mesh	Red Mesh	Spaceship	Red Cup	Dreves Side Mesh	Red Cup	Dreves No Cup	Red Cup	Screw Bottom 10-Hole	Spaceship	Spaceship
K	Captiva	Dreves Side Mesh	Dreves No Cup	Dreves No Cup	Screw Bottom 10-Hole	Half-cone	Screw Bottom 10-Hole	Red Mesh	Red McPhail	Clear 10-Hole	Half-cone	Red Mesh