

AN ABSTRACT OF THE THESIS OF

Joseph R. Kleiber for the degree of Master of Science in Horticulture presented on June 6, 2013

Title: Comparison of Baits for Monitoring the Spotted Wing Drosophila, *Drosophila suzukii*

Abstract approved:

Denny J. Bruck

Spotted wing Drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is a pest of small and stone fruits that is widely distributed across much of the United States, Canada, Europe and Asia. Unlike other members of the Drosophilidae that only lay eggs in overripe or rotting fruit, SWD infest ripening and ripe fruit. The female SWD has a serrated ovipositor that allows her to lay eggs under the skin of a wide range of fruits, where the eggs hatch into larvae and they consume the inside of the fruit. This feeding results in fruit collapse and deterioration, and the hole created by the oviposition puncture allows for secondary infection by microorganisms, yield losses, reduced fruit quality and degrades. The current control is to apply an insecticide treatment when the fruit begins to color and continue to keep the crop protected with chemical treatments

until harvest is complete. The current monitoring tools are not sensitive enough to attract SWD at the critical time to establish economic thresholds, appropriately time treatments and allow growers to make good management decisions.

The objective of this research project was to determine if a more sensitive attractant than the currently standard 5% acidity apple cider vinegar traps could be defined. A number of food products and commercially available lures were tested in greenhouse and field experiments for their attractiveness to *D. suzukii*. There were no bait treatments that captured significantly more *D. suzukii* than the currently standard 5% acidity apple cider vinegar(ACV)-baited traps. Balsamic vinegar, soy sauce, balsamic honey vinegar, a *D. melanogaster* lure, Monterey Insect Bait, rice vinegar, seasoned rice vinegar, and NuLure® performed similarly to ACV, broadening the field of starting material that could be investigated for their use as an SWD attractant. A number of fermentation compounds were also tested in greenhouse and field trials for their attractiveness to SWD. Four classes of compounds were tested: short chain alcohols, short chain carboxylic acids, low molecular weight acetates, and esters of 2-phenylethanol. In the greenhouse trials, some of the compounds alone were determined to be attractive and subsequently used in field trials. However, none of the compounds or combinations of the compounds tested improved attractiveness of apple cider vinegar traps when compared to the standard 5% acidity apple cider vinegar-baited trap.

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Comparison of Baits for Monitoring the Spotted Wing Drosophila, *Drosophila suzukii*

by

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

Joseph R. Kleiber, Author

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Comparison of Baits for Monitoring the Spotted Wing Drosophila, *Drosophila suzukii*

CHAPTER 1.

General Introduction

Joseph R. Kleiber

History

The spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumura), is a pest of economic importance for growers of small fruits and some tree fruits. Kanzawa (1935) wrote a comprehensive report about the first findings of the fly, its general biology, and initial investigations into attractants. The first confirmation of SWD was in 1916, and its name was translated from Japanese as Cherry Drosophila. By 1930, the emergent cherry production in Japan had been impacted by SWD and damage was reported in several varieties of European grapes as well. From Japan, the flies spread around the oriental region and by 1977, infestations were reported in China, India, Thailand, and Korea (Delfinado and Hardy 1977). A few years later, in 1980, they had made their way across the Pacific to Hawaii (Kaneshiro 1983). Infestations in the continental United States were first reported in 2008 when a sample of a Drosophilid fly collected in a raspberry field was received by the California Department of Food and Agriculture in Sacramento, CA (Hauser *et al.* 2009). That same year, the first SWD in Europe was recorded in Spain (Calabria *et al.* 2012). In 2009, SWD was detected on both coasts of North America in Florida, Oregon, Washington, and British Columbia (Dreves *et al.* 2009, Steck *et al.* 2009) and subsequently spread to Utah, Michigan, North Carolina, South Carolina, and Louisiana in 2010. Currently, greater than 35 states report the presence of SWD (Beers *et al.* 2010, NAPIS and Purdue University 2012). In Europe, populations established in France and Italy in 2009 (Calabria *et al.* 2012) and had spread to Croatia, Germany, Russia, Slovenia, and Switzerland by 2011 (EPPO 2012).

Biology

Drosophila suzukii belong in the *suzukii* subgroup of the *melanogaster* group, being closely related to the common vinegar fly *D. melanogaster* (van Der Linde and Houle 2008). Within the *Drosophila* genus, the only other species known to oviposit in intact fruit is *D. pulchrella*, a pest of tree fruit in Japan. *D. suzukii* and *D. pulchrella* have similar ovipositors and ecological behavior, but can be differentiated by the male wing markings; *D. suzukii* only have a spot at the tip of the leading edge whereas *D. pulchrella* have other markings in addition to the spot (Takamori *et al.* 2006). Other species within the *Drosophila* genus have smaller, less sclerotized ovipositors and tend to lay their eggs on rotting plant matter or overripe fruits and vegetables (Fellows and Heed 1972, Jaenike 1983, Markow and O'Grady 2005). Within the family Drosophilidae, *Zaprionus indianus* is another direct pest of a variety of fruits including figs and citrus, but are easily distinguishable from *D. suzukii* due to distinctive dorsal white stripes that extend from the head to the tip of the thorax (Steck 2005).

Extensive work on *D. suzukii* was done by Kanzawa (1935) on the morphology of adults, eggs, and pupae as well as exploration of life stage timing, longevity of adults, fecundity of females and surveys of host range in Japan. SWD adults are small (2-3 mm) with red eyes and a tan thorax and abdomen with unbroken brown bands at the end of each abdominal segment. The males have a characteristic spot near the end of the wing on the leading edge. Seasonal variation of the size and darkness of the spot have been observed (Beers *et al.* 2011). Two sex combs running parallel to the length of the front legs are visible as dark bands. The females tend to be larger than the males and lack the wing spot and combs on the front legs. They can be

identified by their large saw-like ovipositor positioned at the end of the abdomen. The tan ovipositor consists of two halves, each with a row of black or brown teeth that increase in size toward the posterior end.

The eggs are milky white and oblong, 0.5-0.7 mm in length, about 0.2 mm in width with two filaments at one end. When the egg is laid under the skin of the fruit, all that is visible from the exterior is the two 0.4 mm filaments projecting from the oviposition scar (Kanzawa 1935).

The larvae are typical of *Drosophila*; they grow from about 0.6 mm in length when first emerged from the egg and develop through three instars to 5.5 mm in length and 0.8 mm in width. The larvae are a translucent white with distinguishable yellowish entrails. The black mouthparts are visible in the head. Two tan respiratory organs protrude from the posterior end and curve upwards. The larvae live inside the host fruit and feed on the fruit and the yeast present as the fruit deteriorates (Kanzawa 1935).

When the larva is ready to pupate it may leave the host fruit, but pupation inside the fruit is more common (Walsh et al. 2011). The resulting pupa is brown and oblong, with respiratory organs on the anterior and posterior sides. The posterior respiratory organs are similar to the posterior respiratory organs of the larva. The anterior respiratory organs are visible as two protrusions, from either side of the head, with a whorl of 7-8 spikes around the termination of the spiracle (Kanzawa 1935).

Kanzawa (1935) observed the life stages of multiple generations of SWD in captivity over three years of research. The length of generation time varied from a minimum of eight days to 23 days at ambient temperature and ambient humidity in Japan. The eggs hatched after 1-4 days, and were in their larval stage for about five

days. Adults emerge after 4-10 days in the pupal stage and start laying eggs as little as one day after imago. Adult females and males live approximately 30 and 20 days, respectively. Overwintered adults can survive for more than 100 days, with a particular individual female surviving for 234 days. A female can lay over 300 eggs in her lifetime, with an average of 140 eggs and maximum of 362 eggs. The short generation time can lead to an observed 15 generations in a year in the experimentally raised colony. The number of field generations per year in the northwest U.S. and southwest Canada is estimated to be 4-5 (Coop and Dreves 2013), and the number in California is estimated to be ten generations per year (Walsh *et al.* 2011).

The host range of SWD is widely varied and includes both crop and non-crop hosts. Kanzawa (1935) reported that SWD can infest cherries, grapes, gooseberries, raspberries, peaches, plums, persimmons, tomatoes, olives, mulberries, and loquats. Infestation levels in intact ripe fruit were compared to the levels in fruit left to shrivel or rot after harvest date, which indicated higher infestation levels in ripe fruit rather than overripe or rotting fruit (Kanzawa 1935). Masatake (2005) states there are 20 species of host plants, listing pokeweed in addition to others previously named. In recent studies of susceptibility of some fruits at varying stages, oviposition began with fruit coloration and increased as the fruit ripened to maturity (Lee *et al.* 2011). The mobility of the fly and the wide range of hosts allow SWD to fully utilize a landscape of crop and wildland hosts that ripen throughout the year.

Part of the interaction of many fruit-feeding insects with their fruit hosts involves yeast growing on the host and yeast transferred to the host by the insect. Yeast-insect interactions have been widely studied, and exist across many orders and a diverse range of substrates (Ganter 2006). Beetles are associated with yeasts on

fungi, flowers, wood, seeds and fruits and use the yeasts as nutrition or to detoxify the substrate it is propagated onto. Termites, ants, neuropterans, hemipterans, bees, wasps, and flies all have been shown to have associations with yeasts in some way (Ganter 2006). A common reason for the association is that the yeast supplements a diet lacking essential amino acids; *Drosophilid* adults utilize the presence of yeast on plant material to increase the nutritional value of the substrate. Larvae also benefit, for example, a higher number of *Drosophila melanogaster* larvae developed on grapes and artificial substrate inoculated with yeast than on grapes that were free of yeast (Becher et al. 2012). Associations go beyond just feeding on yeast; many *Drosophila* have live yeast cells present in their gut and frass, and distribute yeast to places they feed and oviposit (Gilbert 1980). *D. melanogaster* adults and larvae have been shown to influence the construction of yeast communities in banana (Stamps et al. 2012). In some cases, flies become a pest by vectoring yeast that contributes to the rotting of fruit (de Camargo and Phaff 1957) or to the spoilage of wine (Mortimer and Polsinelli 1999, Loureiro 2003). SWD have an association with *Hanseniaspora uvarum*, a widespread yeast most often identified from mature fruit, wine fermentation, and spoilage (Hamby et al. 2012). *H. uvarum* was isolated from adults and larvae more than any other yeast. It was also isolated from both infested and uninfested cherries and raspberries. Adults can transfer the yeasts to the exterior of hosts, and larvae will propagate them throughout the fruit interior while moving and feeding.

Pest Status and Management

A majority of commercial small and tree fruit production in the US occurs in the western states (USDA-ERS 2013). Since SWD use these berries, cherries, and grapes as a host, there is a great potential for economic impact from SWD, ranging

from no damage to complete crop loss. Although SWD has been documented infesting a very wide range of hosts, the growers of blueberry, blackberry, raspberry, strawberry and cherry on the west coast are most concerned (Walsh *et al.* 2011). Bolda *et al.* (2010) analyzed the value of small fruit production in California, Oregon and Washington and the potential losses due to SWD infestation. Virtually all raspberry and blackberry production, 84% of cherry production, 83% of strawberry production, and 26% of blueberry production occurred in California, Oregon and Washington in 2008. The total value of these crops was \$2.6 billion for these three states. An estimated 20% yield loss from SWD infestation would result in a \$511 million revenue loss for the western states and will vary greatly across regions and crops. A more recent analysis by Goodhue *et al.* (2011) showed that the cost of controlling SWD in California raspberries and strawberries is far less than the potential yield loss if even a slight amount of SWD damage were incurred. The end use of the crop also determines the economic loss; fresh market fruit has a higher value and a lower damage threshold than fruit to be processed. In 2011, Oregon red raspberries were valued at a price of \$1.80/pound for fresh berries and \$0.92/pound for processed, and Oregon blackberries were \$1.56/pound and \$0.75/pound for fresh and processed berries, respectively (USDA NASS 2012).

In the Pacific Northwest, blackberry production in the northwest occurs throughout the summer and early fall, depending on the cultivar and region in which the berries are grown. The fruiting season lasts about four weeks. *Rubus ursinus*, a species of trailing blackberries native to western US, includes the popular “Marion” cultivar. The fruit ripens early, from mid-June to August, and grows well in western Oregon and southwest Washington. *Rubus laciniatus*, another species of trailing

blackberry introduced to the US from Europe in the late 1800s, includes the well-known “Himalaya” cultivar that has become naturalized throughout the northwest as an invasive weed (Caplan and Yeakley 2006). Other blackberry cultivars that are important commercially include the blackberry-raspberry hybrids “Boysen” and “Logan” (Finn and Strik 2013b). The historical pest problems associated with blackberry production include cane spot, purple blotch, downy mildew, red berry mite, and Botrytis. Unless the field has a large amount of pest pressure to begin with, weevils, nematodes, and Phytophthora root rot are not usually a problem (Strik 1996). There are controls available to manage these pests and diseases, although they can become problematic at times (Pscheidt and Ocamb 2013).

Although red raspberries, *Rubus idaeus*, are native to North America, cultivated types were introduced to the US in 1771. Yellow raspberries are genetically mutated red raspberries and do not develop their red color. Black raspberries are native to the US as well, *R. occidentalis* being native to eastern North America and *R. leucodermis* being native to the west. The fruiting season for raspberries is from mid-June to mid-September depending on the cultivar and production region (Finn and Strik 2008c). Climbing cutworm, weevils, raspberry crown borer, western raspberry fruitworm, spider mites, leafrollers, spur blight, cane blight, Phytophthora root rot, yellow rust, nematodes and Botrytis are all potential problems in the production of raspberries. Monitoring for these pests and managing them early greatly reduces the impact (Coyne *et al.* 2013).

Cultivated strawberries are a cross between two species, *Fragaria virginiana* and *F. chiloensis*, both native to North America. Most of the strawberry production in Oregon is of June-bearing cultivars that are ripe from early June to mid-July. They

have about a three week harvest period annually. A small amount of production is everbearing, which fruit in June/July and again in the fall, or day-neutral berries that continue to fruit throughout the summer (Finn and Strik 2008a). Historical problems for strawberry producers include mites, cutworms, armyworms, aphids, thrips, whiteflies, Botrytis, powdery mildew, leaf spot, Anthracnose, leaf blotch, Phytophthora root rot, red stele root rot, verticillium wilt, and cabbage looper (Bolda et al. 2013).

The most common type of blueberry grown worldwide and in Oregon is the northern highbush blueberry, *Vaccinium corymbosum*. This species is native to the eastern US and grows well in western Oregon and Washington thriving in mild winters and long growing seasons. The fruiting season in the Northwest is from late June through September depending on the cultivar. Most cultivars bloom within about a week of each other and the fruit requires between two and five weeks to ripen (Strik and Finn 2008). Historical problems with blueberry production include midges, symphylans, cherry fruitworm, winter moths, leafrollers, root weevils, scale, mummy berry, phytophthora root rot, Botrytis, Alternaria, Anthracnose, shock and scorch viruses and bacterial canker (DeFrancesco et al. 2013).

Cherry production on the west coast is dominated by sweet cherries such as Bing and Rainier, with Washington, California, and Oregon being the number one, two and three producers of sweet cherries in the US. The season for cherries is mid-June for early fruiting varieties until mid-August for later maturing varieties (Gugino 2013). Although an increasing amount of cherries are being grown for fresh market fruit, cherries are still grown mostly for processing. The harvest window for fresh market and different processing techniques is different; brining cherries are harvested

at 14-18% sugar content, measured in Brix, fresh market are harvested between 16-18% Brix and canning cherries are harvested above 20 or 21% Brix (Long and Olsen 2013). In laboratory tests, female SWD laid more eggs or increased numbers of SWD developed on fruit with increasing sugar levels (Lee et al. 2011). Since canning cherries are harvested at higher sugar levels, they are more likely to be negatively affected by SWD than fresh market or brining cherries. The historical key pest in cherries is the cherry fruit fly, which growers currently spray for 3-4 times per season. Other problems including black cherry aphid, leafrollers, shothole borer, powdery mildew, bacterial canker, and verticillium wilt (Smith 2013).

Integrated pest management (IPM) involves using multiple tools and practices in addition to chemical treatments to control a pest. Cultural controls must be used to relieve pest pressure along with maintaining the natural balance of predators (biological control agents) and prey in the agroecosystem. Judicious use of pesticides is only one part of an integrated system of controls meant to lessen the impact of pest control on the environment (Kogan 1998). Area-wide IPM expands the idea of integrating different controls of a pest to treat a whole area, not just one farm. Highly mobile and very destructive pests, like SWD, that are being treated on a farm-to-farm basis have the ability to move to areas of refuge and build up populations only to return to treated areas. Area-wide management can limit resurgence of pests by decreasing the numbers in adjacent farms or regions (Hendrichs *et al.* 2007). Since SWD have been shown to travel between crops with different ripening times in order to utilize all available hosts (Walsh *et al.* 2011), the control of SWD could potentially benefit from an area-wide IPM program with a multiple-tactic approach.

An important element of managing SWD is chemical control. For conventional growers, there are many formulations with different modes of action that are effective when sprayed directly on the insects as well as have residual effects of protecting the crop for up to 14 days after application. Conventional insecticides in the spinosyn, organophosphate and pyrethroid classes have performed well in field trials with residual effects of 5-14 days but organic growers have limited effective options of spinosad and pyrethrin with no residual effect (Bruck et al. 2011). The current recommendation to protect blueberries, caneberries and strawberries from SWD infestation is with the application of cover sprays when fruit begins to color and every 5-10 days until harvest is complete. Rotating chemistries and resistance management classes is extremely important when treating for SWD since they have a short generation time. This is especially important for organic farms that have limited chemical options. Spinosyns are nicotinic acetylcholine receptor agonists, organophosphates are acetylcholine esterase inhibitors, and pyrethroids are sodium channel modulators (IRAC 2013). Since the three modes of action are in different resistance classes, the chemical classes can be rotated to limit resistance development. In some cases, resistance in the population is not only developed for the insecticide being applied, but cross resistance to other classes of insecticides can develop (Liu and Yue 2000). Resistance to insecticides is not uncommon in agricultural pests due to the frequent applications of treatments, and much work has gone into developing insecticide resistance management. Some ways to combat resistance development in a population are reducing selection pressure (Bielza 2008), using rotations of different chemistries, and using a combination of different chemistries in the same application (Zhao et al. 2010).

Biological control plays an important role in the control of insect pests in agricultural systems with the ability to permanently suppress pest populations below economic thresholds rather than only providing a temporary control of pests as chemical control does (Stern et al. 1959). In recent times, biological control has been a focus for many people. First, it was taken as a safe alternative to the pesticides that Rachel Carson's Silent Spring illuminated as dangerous to human and environmental safety (Carson 1962). After a wave of biological control agent releases in the 1960s and 1970s, the criticism of introducing exotic species to new localities increased due to the documentation of the adverse effects introduced species can have on non-target species in some cases (Barratt et al. 2010). For biological control with parasitoids or predators to be effective, three guidelines must generally be met: the pest is exotic, the predator is able to establish in the new location, and the predator must be limited in its non-target interactions (van Den Bosch 1971). Since SWD is an invasive pest, the first requirement is met, and control by predators from its native region may be possible. Work with *Pachycrepoideus vindemiae* (Rondani), an ectoparasitoid of SWD pupae, shows that it thrives when grown on SWD and since *P. vindemiae* is native to the Northwest of the United States, it should remain established (Beers et al. 2012). In addition to parasitoids, predators such as *Orius* species are being investigated for their ability to manage SWD populations (Cini et al. 2012). Other biological controls are the use of insect pathogens; viruses, fungi, nematodes, and bacteria all can have an effect on insect populations. The nematode *Howardula aoronymphium* parasitizes the mycophagous *D. putrid* and *D. testacea* (Jaenike 1991) and the nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* infect *D. melanogaster* (Dobes et al. 2012). *Beauveria* and *Metarhizium* bacteria are both been

used in the control of fruit flies and have a wide host range (Toledo *et al.* 2000). A virus also exists for *Drosophila*, Drosophila C Virus that induces a loss of fecundity and decreased lifespan in *D. innubila* (Unckless 2011). A successful pathogen must be able to thrive in the environment of the pest, be transmitted across and between generations, and be specific enough to minimize the non-target effects (Lacey *et al.* 2001). A complication of using biological control strategies with SWD is the low damage threshold of infestation. While they may not serve as standalone control, biologicals can be important mortality factors that have the potential to attenuate SWD populations.

Cultural control of pests is effective in decreasing pest pressure or even eliminating a problem. Some caneberry production in the Northwest utilizes the cultural practice of alternate-year fruiting to control diseases such as leaf rust, purple blotch, and others (Pscheidt and Ocamb 2013). Cultural controls include trapping, erecting physical barriers such as netting, trenches or fences, hot and cold treatments, and flooding (Vincent *et al.* 2003). Mass trapping already plays a role in the long term control of various pests as well as in the eradication of some invasive species (El-Sayed *et al.* 2006), and is a potential component of the management of SWD. Physical barriers in the form of insect netting are used in blueberry production in Japan and have been shown to fully eliminate infestation without affecting new shoot growth or yield over the three year experiment (Shinzo *et al.* 2007). Other cultural controls involve removing dropped or split fruit from the field, pruning to maximize harvested fruit, and selecting cultivars that are harvested outside the peak SWD infestation period. In addition to those practices, solarizing fruit by bagging it in clean plastic solarizing fruit under plastic in the sun is recommended for berries that are

already infested or will not be utilized (Dreves et al. 2011). Since the fruit is more susceptible to infestation at later ripening stages (Lee et al. 2011), harvesting fruit early minimizes exposure to SWD in the field.

Volatile Attraction

An insect's environment is made up of not only the tactile landscape that it encounters, but largely of chemical signals given off by other insects, plants, and animals. Volatile chemical cues indicate the presence of mates, food, oviposition sites, danger and other important factors in the surroundings. Carey and Carlson (2011) review in detail the mechanisms by which odors are sensed, how insect behavior is altered by odor, and how olfaction is utilized in insect management. Insects sense chemical cues with their antennae, which are covered in sensory hairs called sensilla (Shanbhag *et al.* 1999). These sensilla respond to different stimuli by producing an electrical signal that is sent to the mushroom body and lateral horn regions of the insect's brain. The mushroom body is responsible for olfactory learning and memory, and the lateral horn is responsible for innate olfactory behaviors (Masse *et al.* 2009). Depending on the species and chemical cues to be detected by the antennae, the number of sensilla can range from about 400 in *D. melanogaster* to more than 100,000 in *Manduca sexta* (Sanes and Hildebrand 1976, Shanbhag *et al.* 1999). There can also be sexual dimorphism in the number of sensilla arising due to the differences in necessity of host- or mate- seeking (Zwiebel and Takken 2004).

Along with the olfactory system that insects possess, they also have a gustatory system for sensing chemical cues that they come in contact with. In *Drosophila*, this system is comprised of the two labial palps covered with sensilla on the proboscis, taste pegs in the pharynx that make contact with food as it passes, and

taste bristles along the legs and the anterior margin of the wings (Amrein and Thorne 2005). The sensilla of the gustatory system function similarly to those of the olfaction system, sending electrical responses to the fly's brain when detectable compounds are encountered (Stocker 1994). The two chemosensory systems do not always work together to influence the behavior of the insect. *D. melanogaster* females have been shown to have an egg-laying preference for substrates containing acetic acid that is mediated by the gustatory system in conjunction with a positional avoidance of substrates containing acetic acid that is driven by the olfaction of the fly (Joseph et al. 2009).

Insect behavior is driven by taking in chemical cues present in the air or on the substrate it is in contact with and responding to those signals. The first step in determining the behavioral response of an insect to a chemical cue is determining if the compound is biologically active to the insect. Since the sensilla respond to chemicals with an electrical signal, that signal can be measured using an electroantennogram (EAG) (Mayer et al. 1984). An insect is immobilized and electrodes are attached to an antenna, one at the severed tip of the antenna and another at the base of the antenna or into the base of the decapitated head. The antenna is exposed to various odors and an electrical response corresponds to the insect's detection of a compound (Arn et al. 1975). Compounds can be presented singly or in series from a mixture of compounds that has been separated by gas chromatography (GC-EAD) (Struble and Arn 1984). This technique elucidates which compounds the insect can detect, but not the behavioral response it will elicit. Therefore, further testing of the EAG-active compounds is required to determine if it has an attractive, deterrent, or no effect.

Plant-insect interactions are mediated by semiochemicals that can have many different effects on insect behavior. Kairomones are chemical cues released from the plant that changes insect behavior with no benefit to the plant. Three types of kairomones are attractants (which draw the insect to the plant), arrestants (that slow down or stop the movement of the insect) and excitants (that cue the insect to feed or oviposit) (Metcalf and Metcalf 1992). Leaf volatiles act as attractants, dispersing through air to attract insects from long-range. Once the insect is near or in contact with the attractive plant, close range volatiles elicit an arrestant or excitant effect to stop movement and induce feeding or oviposition. Fruit flies of the family Tephritidae use fruit odors to find suitable hosts for oviposition; females seek out ripe fruit and use their ovipositor to create a cavity where between four and ten eggs are laid (Ioannou et al. 2012).

Another level in the interactions between plants and insects is mediated by the role that yeast plays in modifying insect behavior. Yeasts have their own volatile profile, and as they rot or ferment plant material, the compounds produced are changed significantly as well. Since yeast can be propagated by insects that feed on decaying matter, the volatiles produced by the yeast benefit both the producer and receiver of the chemical cues, known as synomones. Some bark beetles are attracted to trees that are infested and emitting yeast volatiles, and two Hymenopteran parasitoids, *Roptrocercus xylophagorum* and *Spathius pallidus*, which use the beetles as hosts are attracted to the volatiles emitted by trees infested by beetles as well (Ganter 2006). The attraction of *D. melanogaster* to fruit hosts is due to the yeast on the fruit surface and even yeast alone attracts more flies than fruit cleaned of surface yeast (Becher et al. 2012). To take advantage of drosophilids' attraction to yeast

volatiles, the Solomon's lily has evolved an odor more similar to wine and vinegar than any fruit volatile (Stöckl et al. 2010). Flies are lured to the plant by the fermentation volatiles, only to become unwilling pollinators. Since yeasts increase the nutritional content of plant material, yeast colonies are sought after by many insects.

For communication between insects of the same species, pheromones are produced by individuals to elicit a response from another individual of the same species. Female-produced sex pheromones disperse very far, and the males of the species can detect tiny amounts of the pheromone and are directed toward the point source by following the concentration gradient (Carde and Knols 2000). Long distance male-produced pheromones are less common, usually attracting both females and males, and are referred to as aggregation pheromones (Landolt 1997). These aggregation pheromones signal that the male is seeking a mate and also tend to identify sites that are appropriate for feeding or oviposition. *Drosophila* species have been shown to release aggregation pheromones to increase the density of oviposition at a site, which increases larval survival (Wertheim et al. 2002). Along with volatile pheromones, species of the *D. melanogaster* subgroup utilize contact and close range pheromones, which are expressed on the insect's cuticle (Cobb and Jallon 1990). Female produced pheromones orient males to females and induce male courtship behaviors such as touching and wing vibrations (Shorey and Bartell 1970). The *D. melanogaster* male produced pheromone 11-*cis*-vaccenyl acetate promotes aggressive behavior between males and is transferred to females during mating to deter multiple mating with a mated female (Wang and Anderson 2010). Another pheromone produced by males, 7-tricosene, increases receptiveness of females to male courtship (Grillet et al. 2006).

Monitoring

Monitoring of insect populations is a very important part of any integrated pest management program, allowing the timely treatment of pests to minimize detrimental effects on crops (Cohnstaedt et al. 2012). Sampling of the population can take the form of active sampling of larvae (Hammack et al. 2003) or adults (Turnipseed 1974), passive trapping without attractants (Boiteau 2000), and trapping with attractants (Byers 1992, Casaña-Giner et al. 2001, El-Sayed et al. 2009, Landolt et al. 2012). Insects respond to various stimuli that can be used alone or in combination to attract them: thermostimuli, photostimuli, mechanostimuli, and chemostimuli (Dethier 1947). Research into trapping of *Drosophila* tends to be in manipulating color (Hottel 2011) and odor (Hutner et al. 1937, Cha et al. 2012).

Pheromones, plant-based kairomones and combinations of the two are used as attractants in traps for monitoring and mass-trapping purposes. Pheromone lures mimic female calling signals and are widely used in the trapping of moths, beetles, and some hymenoptera species (Dethier 1947). Kairomone attractants are used to draw insects to plant odors that signal food or oviposition site rather than the direct signal of a mate (Metcalf and Metcalf 1992). In some cases, the combination of pheromone and kairomone is needed to achieve adequate attraction (Landolt and Phillips 1997) or a combination of a food odor and oviposition host kairomone is most attractive (Landolt et al. 2007). Attractants are also used for the direct control of pests in similar programs of mass trapping and attract and kill by luring insects to either a trap where they are contained or a surface from which they can feed with an insecticide applied to it (El-Sayed et al. 2006).

In order to incorporate monitoring into an integrated pest management program, a relationship between pest density and crop damage must be made (Shotwell 1935). An economic threshold, defined by Stern *et al.* (1959) as “the density at which control measures should be determined to prevent an increasing pest population from reaching an economic injury level,” is established. The economic threshold of an insect pest varies depending on the region, crop and end use for the crop (Stern 1973). Controls are taken only once the economic threshold has been surpassed, not by calendar or plant physiology. Applying insecticides only when needed reduces pesticide residue on the crop, saves time and money by limiting applications, decreases the chance of secondary pest infestations, reduces the effect of the pesticide on the environment and promotes human health and safety (Kogan 1998). Monitoring insects allows the observation and analysis of populations and establishment of models and life tables (Kuno 1991).

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CHAPTER 2

Attraction of the Spotted Wing Drosophila (Diptera: Drosophilidae) to Vinegars and Other Commercially Available Baits

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Abstract

Spotted wing Drosophila, *Drosophila suzukii*, is becoming a significant problem in many countries, negatively impacting fruit industries. This work was undertaken to determine the relative attractiveness of different products including vinegar, soy sauce, and commercially available lures. Twenty-two types of vinegar and other attractants were tested in 0.6 m³ cages in greenhouse bioassays, as well as in field trials to determine attractiveness of products to *D. suzukii*. In field trials between June and September 2011, no differences were shown in trap catch between 5% acidity apple cider vinegar (ACV), balsamic vinegar, soy sauce, balsamic honey vinegar, a *D. melanogaster* Lure, Monterey Insect Bait, rice vinegar, seasoned rice vinegar, and NuLure®. ACV is the current standard for *D. suzukii* trapping, and we still recommend it because it's ease of use, clarity to see fly captures and low cost. An explanation for the consistent performance of apple cider vinegar as an attractant to *D. suzukii* may be that its odor profile is distinct for the *D. suzukii* to discern from the background scents in berry production fields.

Keywords: Spotted Wing Drosophila; *Drosophila suzukii*; attractants; monitoring; baits

Introduction

The spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) was first found in Japan in 1916 and first described in detail in 1934 (Kanzawa 1934). It was first discovered in the United States in Hawaii in the 1980s, and then in California in 2008 and Oregon, Washington and Florida in 2009 where it was rapidly identified as an invasive pest of a wide range of small and stone fruits (Bolda et al. 2010). The spread of *D. suzukii* continued to more than 35 of the United States (NAPIS and Purdue University, 2013). The first reports of *D. suzukii* in Europe occurred in 2008 in Spain, and populations have since spread to much of Europe, including Italy, France, Switzerland, Slovenia, Croatia, Austria, Germany, Russia and Belgium (Cini et al. 2012).

Unlike other members of the Drosophilid family, *D. suzukii* is capable of infesting intact ripe and ripening fruit. The female has a unique serrated ovipositor, which she uses to cut a small hole in the skin of fruit (Mitsui et al. 2006). After a hole has been created in the fruit, the female lays 1-3 white eggs. The eggs have two white filaments that are visible with a magnifying glass on the outside of the fruit. The eggs hatch within the fruit and resulting larvae feed inside the fruit. As the larvae feed, the fruit starts to collapse, shelf life is reduced, aesthetic value decreases, and secondary infection can occur (de Camargo and Phaff 1957). Larvae will then pupates inside or outside the fruit, on the exterior of the fruit or drops to the ground (Walsh et al. 2011). Female *D. suzukii* lay an average of 380 eggs in their lifetime (Kanzawa 1939), and there can be between 3 and 9 generations in a single summer (Coop et al. 2010), depending on the climate (Walsh et al. 2011). In the Pacific Northwest, adult *D. suzukii* captures in ACV-baited traps between

February and May are generally low. Late season (September-November) catches tend to be much higher than during the production period of most susceptible crops grown in the PNW. The number of adult *D. suzukii* remains high until December, when freezes and colder weather either kill *D. suzukii* or initiate overwintering behavior. The theoretical host range of *D. suzukii* is wide, with varying infestation levels on hosts depending on season (Walsh et al. 2011).

The value of production loss is much higher than the cost of control measures (Goodhue et al. 2011). In the United States, conventional fruit growers are managing *D. suzukii* by applying chemical applications of primarily organophosphates, pyrethroids, and spinosyns. Because of the lack of confident monitoring methods or thresholds, current recommendations are for growers to protect their crops with insecticides beginning at first color when fruit becomes susceptible (Lee et al. 2011a), and continuing spray until harvest (Bruck et al. 2011, Haviland and Beers 2012). Current management strategies are based solely on fruit susceptibility stage and not on the presence and levels of *D. suzukii* in the crop, resulting in a spray schedule determined by the calendar and not by the presence or abundance of *D. suzukii*. Improved monitoring for determining the need for applications and timing treatment decisions is important. A more sensitive attractant is necessary to catch *D. suzukii* early in the season, compete with fruit, and aid in studies of movement and spatial population modeling. More powerful attractants for *D. suzukii* could potentially be useful to alert a grower of its arrival, which would allow early detection and facilitate timely action, before a significant population is established.

Although proprietary blends have been developed for other *Drosophila* species (Baker *et al.* 2003), there is a lack of data on potential successful *D. suzukii* attractants. Wines, vinegars, sugars, and fermenting baits have all been used with some success (Kanzawa 1935, Adams *et al.* 2012, Landolt *et al.* 2012). Kanzawa (1935) performed baiting experiments in 1931 with a variety of wines, sugars, and other attractants. Rice wine, red wine, and cherry wine were found to be attractive, as well as molasses mixed with rice wine, red wine, cherry wine, acetic acid, citronella oil, bergamot oil, or a mix of rice wine and acetic acid. Three-part combinations of molasses with wine and vinegar in different ratios yielded the conclusion that the most flies were caught when the ratio of wine is the highest. Landolt *et al.* (2012) showed that the combination of a merlot wine and 5% acidity ACV (in a 60:40 wine:vinegar ratio) was more attractive than either the merlot wine diluted to 7.2% ethanol or ACV diluted to 2% acetic acid. The combination of ethanol and acetic acid (10% ethanol and 2% acetic acid in water) was also attractive than either a 10% ethanol in a water solution or 2% acetic acid in a water solution. The combination of wine and vinegar was more attractive than the combination of acetic acid and ethanol, indicating other volatile components in wine and vinegar other than ethanol and acetic acid are key ingredients of attractiveness. Other work demonstrated that the combination of a merlot wine with ACV was more attractive than combinations of other wines and ACV (Adams *et al.* 2012). The combination of rice wine vinegar with a merlot wine was more attractive than other combinations of vinegars and a merlot. Some researchers report that a sugar water and yeast solution attracts more *D. suzukii* sooner than ACV in the spring and during harvest season when weather is warm (Dreves *et al.*

2010, Isaacs et al. 2012). Bait type may be seasonal, necessitating multiple attractants in a single trap, or single baits at certain times of the year to maintain the sensitivity of the attractant throughout the season.

To optimize the monitoring capability, trap design also requires consideration. Landolt et al. (2012) found that McPhail-like yellow Dome traps (Dome; Agrisense, BCS Ltd., UK) captured more *D. suzukii* than homemade cup traps (946-ml clear Solo cups with four 1-cm-diameter holes drilled near the top). Lee et al. (2012) determined that traps with a greater entry area, such as mesh openings instead of holes, catch more flies. Lee et al. (submitted, 2013) found that traps with red or yellow color, a larger bait surface area and entry point on the side rather than the top were features that improved *D. suzukii* captures. Dreves (unpublished, 2012) reported traps with small headspace and large bait surface area captured significantly more flies. Basoalto et al. (2013) determined in choice cages that traps with red and black pattern attracted more SWD than solid red or black traps. An optimum trap with good design and appropriate bait would facilitate a standardized trapping system, enabling realistic comparisons of *D. suzukii* captures and infestation levels, thus development of thresholds.

Experiments below were carried out in cages in a greenhouse and in the field to evaluate and compare existing and novel attractants for *D. suzukii*. Greenhouse bioassays were used initially, to find treatments suitable for field trapping. In all field experiments, ACV (5% acidity) served as the standard attractant (i.e. positive control) to which the various treatments were compared.

Materials and Methods

Source of Insects. A laboratory colony of *D. suzukii* was started and maintained as described by Bruck et al (2011). Approximately 200 *D. suzukii* of mixed sex, between five and 12 days old, were used in all greenhouse bioassays.

Greenhouse Bioassays. To test the attractiveness of different products, bioassays were performed in 0.6 x 0.6 x 0.6 m mesh cages (Lumite Screen Collapsible Cage, BioQuip Products, Rancho Dominguez, CA) in a greenhouse with high humidity. A box fan was placed on top of each cage to generate upwards airflow. Water and sucrose were provided *ad libitum* in the arena to maximize *D. suzukii* survival. Three or four treatments were compared simultaneously in each cage along with an unbaited soapy water control. Treatments were randomly located in each cage in either one of the four corners or cage center. Control traps were an uncovered clear cup (236 ml,) containing 100 ml of soap water prepared by adding 4 ml of Ultra Pure + Clean dish soap (Colgate-Palmolive Co., New York, NY) to 3.78 L of water to break water surface tension and facilitate capture by drowning. Treatment traps consisted of five ml of each treatment placed in a small glass vial (8 ml, Wheaton, Millville, NJ, USA) that was then placed inside an uncovered clear 236 ml plastic cup containing 100 ml soap water. The experiments were performed for 24 hrs at which time the flies were collected from each cup and the number of males and female *D. suzukii* captured enumerated. Treatment placement was rerandomized each consecutive day.

To determine which baits from the cage bioassays performed best in each experiment and were subsequently used in field experiments, the percent of flies captured

by each treatment was compared to the percentage of flies each treatment would have captured if all treatments in the experiment performed the same. In experiments with two, four and five treatments, the potential percentage of flies captured in each treatment would be 50, 25 and 20%, respectively. The actual percentage of flies that each treatment captured in the experiment was calculated by: $(\text{number of flies in treatment trap} / \text{total number of flies caught by all traps}) \times 100$. The treatments with a higher actual percentage than potential percent were subsequently used in field experiments.

Greenhouse Experiment 1. Two commercially available lures were tested for their attractiveness to *D. suzukii*. Treatments were Insect Bait (Monterey Insect Bait, Monterey AgResources, Fresno, CA) and Bird Shield (Bird Shield Repellent Concentrate, Bird Shield Repellent Corporation, Pullman, WA). Insect Bait is an insecticide spray adjuvant consisting of 99.7% corn steep liquor and 0.3% constituents that are ineffective as spray adjuvant. The main component in Bird Shield is methyl anthranilate, a major constituent in the flavor profile of grapes (Mattick et al. 1963). Four cages containing Insect Bait and a water control were maintained for 24 hrs for four consecutive days. Four cages of Bird Shield versus a water control were maintained for 24 hrs for three consecutive days. At the conclusion of each 24 hr period in this experiment and subsequent experiments, the number of SWD captured in each treatment was enumerated.

Greenhouse Experiment 2. Five common off the shelf vinegars were tested for their attractiveness to *D. suzukii*. Treatments included: ACV (Fred Meyer Apple Cider Vinegar, Inter-American Products Inc. Cincinnati, OH), balsamic vinegar (Safeway Select Balsamic Vinegar of Modena, Safeway Inc, Pleasanton, CA), raspberry vinegar

(Red Raspberry Vinegar, Kozlowski Farms, Forestville, CA), red wine vinegar (Safeway Select Red Wine Vinegar, Safeway Inc, Pleasanton, CA), and white wine vinegar (Safeway Select White Wine Vinegar, Safeway Inc, Pleasanton, CA). The five treatments were placed at random positions in the cage, replicated in four cages. Traps were serviced and positions rerandomized every 24 hours for five days, for a total of 20 replicates.

Greenhouse Experiment 3. Four fermented food products were tested for their attractiveness to *D. suzukii*. Treatments were: ACV (Fred Meyer Apple Cider Vinegar, Inter-American Products Inc. Cincinnati, OH), balsamic vinegar (Safeway Select Balsamic Vinegar of Modena, Safeway Inc, Pleasanton, CA), malt vinegar (London Pub Malt Vinegar, World Finer Foods Inc, Bloomfield, NJ), ume plum vinegar (Ume Plum Vinegar, Eden Foods Inc, Clinton, MI), and kombucha (Organic Raw Kombucha, Millennium Products Inc, Beverly Hills, CA). Balsamic vinegar is made from white grape juice that is concentrated by heating before being subjected to alcohol then acetic fermentation. Malt vinegar is vinegar made from the acetification of brewed malted barley. Ume plums are related to plums and apricots; ume vinegar is the by-product of the preservation process of those fruits (Itoh 2012). Kombucha is the product of the fermentation of sweetened tea, yielding acetic acid and some ethanol along with other components (Dufresne and Farnworth 2000). The five treatments were placed in either a cage corner or center in four replicate cages on each of three consecutive days for a total of 10 replicates with the treatments placed in each position in the cage twice.

Greenhouse Experiment 4. The four treatments tested for their attractiveness to *D. suzukii* were nutritional yeast (Nutritional Yeast Flakes, KAL Inc, Park City, UT)

dissolved in water, soy sauce (Kikkoman Soy Sauce, Kikkoman Foods Inc, Walworth, WI), and liquid amino acids (Bragg's Liquid Amino Acids, Bragg Live Foods Inc, Santa Barbara, CA), and a negative water control. The treatments were positioned randomly in four cages. Traps were serviced and positions in the cages rerandomized every 24 hrs totalling eight replicates.

Field Trials. Field trials were performed at three trial sites in cultivated raspberry crops at unsprayed farms in Benton County, OR as well as in adjacent vegetation of unmanaged Himalayan blackberries adjacent to fruiting crops. Traps were constructed from 946 cl clear plastic cups with a lid and fifteen 4.8 mm diameter holes drilled around the side near the top. With the exception of NuLure® and Monterey Insect Bait, 150 ml of each bait was poured into the traps with an additional .2 ml of soap to diminish the surface tension and facilitate capture by drowning. NuLure® and Monterey Insect Bait were diluted 50%. Treatments were replicated in linear blocks, with traps placed 10 m apart and blocks separated by 20 m. Traps were hung in the canopies of each crop to achieve similar exposure at all trap positions and to maximize SWD capture. The traps were serviced weekly by collecting captured flies, refreshing the attractant, and rotating the traps to a new position. Treatments were randomly positioned within each block.

Field Experiment 1. This experiment compared the most attractive treatments from greenhouse experiments 2 and 3 in the field. The five treatments chosen based on greenhouse experiments were ACV, balsamic vinegar, ume plum vinegar, raspberry vinegar, and a water control. Baits were placed at two trial sites, cultivated raspberry and

cultivated blackberry crops, on 22 June 2011. Both started on the same day and maintained in the field for six weeks.

Field Experiment 2. This experiment tested the attractiveness of soy sauce compared to ACV in the field. The treatments were ACV, water control, and soy sauce. The three treatments were placed in four blocks along a fence bordering wild Himalayan blackberries on 18 Jul 2011. The traps were serviced and rotated weekly and maintained in the field for four weeks.

Field Experiment 3. This experiment compared additional commercial vinegars that had not been tested previously in the greenhouse with ACV and balsamic vinegar, which had both performed well in the greenhouse. The treatments were ACV, balsamic vinegar, cherry vinegar (Wild Cherry Red Wine Vinegar, The All Spice Co, Eugene, OR), balsamic cherry vinegar (Dark Cherry Balsamico, Lucini Italia, Bolgheri, Tuscany, Italy), balsamic honey vinegar (Balsamic Honey Vinegar, Honey Ridge Farms, Brush Prairie, WA), and a water control. Baits were placed in wild Himalayan blackberries and cultivated raspberries on 3 Aug 2011. Three blocks at the two trial sites were maintained in the field for one week due to the limited supply of the baits.

Field Experiment 4. This experiment compared attractive products from greenhouse experiment 1, Monterey Insect Bait and Bird Shield, and another commercially available lure, a *D. melanogaster* lure (ChemTica Internacional, S. A., Heredia, Costa Rica) to ACV, balsamic vinegar and a negative water control. The six treatments were randomly placed in five blocks at two trial sites, cultivated raspberry and

Himalaya blackberry, on 3 Aug 2011. The traps were serviced and rotated weekly and maintained in the field for four weeks.

Field Experiment 5. Rice vinegar (Rice Vinegar, Marukan Vinegar Inc, Paramount, CA), seasoned rice vinegar (Seasoned Rice Vinegar, Marukan Vinegar Inc, Paramount, CA), an additional corn-based attractant (Nu-Lure Insect Bait, Miller Chemical & Fertilizer Corporation, Hanover, PA) not screened in the greenhouse, ACV, and a water control were tested for their attractiveness to *D. suzukii*. The five treatments were randomly placed in four blocks in two trial sites, cultivated raspberry and Himalaya blackberry, on 31 Aug 2011. The traps were serviced and rotated weekly and maintained in the field for four weeks.

Statistical Analysis. Field trap catches of males and females were square root transformed before being initially subjected to analysis of variance with treatment, block, week collected, and all two way interactions. The interactions were removed due to non-significance. The final model fit included treatment, block, and week collected terms. The performance of each of the experimental attractants was compared to ACV as the positive control by Dunnett's correction, and significance shown at $P \leq 0.05$ (R Development Core Team 2012).

Results

Greenhouse Experiments (Table 2.1). *Greenhouse Experiment 1 (Figures 2.1-2.2).* Both Bird Shield (57.4% ♂; 57.9% ♀) and Insect Bait (70.4% ♂; 70.7% ♀) were found to be attractive compared to the water control when compared by percentage caught.

Greenhouse Experiment 2 (Figure 2.3). Apple cider vinegar caught more than the 20% of the total males caught in the cage (21.8%), but proportionally fewer females (19.6%). The other treatments caught similar proportions of both males and females. Balsamic vinegar (26.1%; 22.8% ♀) and raspberry vinegar (25.2% ♂; 31.0% ♀) both caught a proportionally higher number of flies. Red wine vinegar (18.2% ♂; 17.6% ♀) and white wine vinegar (8.7% ♂; 9.0% ♀) caught proportionally fewer.

Greenhouse Experiment 3 (Figure 2.4). More than 20% of the captured flies were caught by each balsamic vinegar (22.2% ♂; 26.4% ♀) and ume plum vinegar (22.0% ♂; 20.7% ♀). Kombucha showed a difference in attraction between males, which accounted for 20.9% of the trapped flies, and females that were only 19.0%. ACV (15.7% ♂; 14.3% ♀) and malt vinegar (19.3% ♂; 19.7% ♀) caught fewer proportionally than expected.

Greenhouse Experiment 4 (Figure 2.5). All treatments showed the same trends in male and female catches. Soy sauce was the only treatment to catch more than the expected proportion of 25% (53.3% ♂; 52.6% ♀). Less than 25% of the captures were caught by each yeast (15.8% ♂; 16.2% ♀), liquid amino acids (23.6% ♂; 24.7% ♀), and the water control (7.2% ♂; 6.5% ♀).

Field Experiments (Table 2.2). *Field Experiment 1 (Figure 2.6).* No flies were caught in the water control traps. Although promising in greenhouse trials, significantly less males and females were caught in ume plum vinegar and raspberry vinegar than ACV. Balsamic vinegar did catch a similar amount of flies as the ACV.

Field Experiment 2 (Figure 2.7). When compared to ACV in the field, soy sauce was found to catch a similar amount of flies. Some flies were caught in the water control, but significantly less than ACV.

Field Experiment 3 (Figure 2.8). In this experiment, the total catches were low for all treatments, and only balsamic honey vinegar had similar catches as ACV for both males and females. Balsamic vinegar, cherry vinegar, and balsamic cherry vinegar all had different results for males and females, possibly due to the low catch numbers and high variability in the experiment. The water control caught significantly less than ACV for both males and females.

Field Experiment 4 (Figure 2.9). All the treatments showed similar trends of male and female catches. Similar numbers of flies were caught in ACV as balsamic vinegar, *Drosophila* Lure, and Insect Bait. Very few flies were caught in Bird Shield and the water control.

Field Experiment 5 (Figure 2.10). All the treatments showed similar trends in the number of male and female flies caught. The water control was the only treatment to have significantly fewer catches than ACV. Rice vinegar, seasoned rice vinegar, and NuLure all caught similar numbers of flies to ACV.

Discussion

No baits tested proved to be better performers of attracting *D. suzukii* than ACV. Discrepancies in bait attractiveness between greenhouse assays and field trapping are common in the study of fruit flies (Lee et al. 1997, Zhu et al. 2003). An apparent reduction in the attractiveness of ume plum vinegar, raspberry vinegar and Bird Shield in

the field compared to the greenhouse may be due to the differences in background odors present in the greenhouse and fruit production fields where testing took place. In the cages, the odors encountered by the flies are baits in the traps and ripening strawberry and vegetation volatiles of plants being grown in the same greenhouse (Solanaceous and *Brassica* vegetable starts, *Thuja*, *Taxus*, *Pieris*, *Fuchsia*, and some other houseplants). In contrast, the production fields are filled with odors of assorted small fruits, tree fruits, vegetables, pollen and nectar. All the odors from ripe and decomposing fruit could possibly mask the baits, negatively impacting the captures of flies during the harvest season.

It was expected that raspberry and cherry vinegars would be attractive, since both raspberries and cherries are attractive hosts to SWD (Lee et al. 2011a). When placed in the field though, raspberry vinegar and cherry vinegars have odors similar to what the flies would encounter at a berry production farm. This similarity in odor profiles between the bait and competing fruiting crop might conceivably make it difficult for the flies to orient to the lure when fruit is present in the field.

Female *D. melanogaster* seek out protein sources preferentially after mating (Vargas et al. 2010). If *D. suzukii* undergo the same shift in dietary requirements, the protein in Monterey Insect Bait and NuLure® may be the key ingredient in the attraction. Corn protein similar to what constitutes Monterey Insect Bait has been shown to attract fruit flies in the family of Tephritids such as *Ceratitis capitata* and *Anastrepha ludens* as a feeding stimulant (Lee et al. 1997, Casaña-Giner et al. 2001). Since it attracts a range of flies, it may not be a good candidate for a highly *D. suzukii* specific attractant. The

attraction of *D. suzukii* to NuLure is consistent with findings that Tephritid flies which also utilize ripe fruit hosts are attracted to corn protein (Lee et al. 1997).

Balsamic vinegar has a different odor profile than the white wine vinegar bait because of the heating and aging process that white grape juice goes through during the acidification process of balsamic vinegar (Natera et al. 2003). The compounds added by these processes may be what are responsible for the numerical increase in response to balsamic vinegar bait over the white wine vinegar bait in the greenhouse trial.

Soy sauce is a fermentation product of soybeans, salt and enzymes, and it contains a number of volatile organic acids (Yang and Choong 2001, Schueller 2006). *D. suzukii* is attracted to acetic acid (Landolt et al. 2012), and other insects have been shown to be attracted to other short-chain acids (Hibbard et al. 1997), so the mixture of such acids in soy sauce may be the reason for the attractiveness. As a fermentation product of soy beans, the odors in soy sauce are very different than the fruit odors of the field sites. In contrast to fruit vinegar baits being put in fruit production fields, the uniqueness of the soy sauce lure odor profile may allow this lure to stand out from the background of other odors and allow the flies to more easily orient to the lure. The rice vinegars may be attractive in the field for the same reason, and are corroborative of the findings of Kanzawa (1935).

Since the yeast tested in the greenhouse was just dissolved in water and not fermenting, it was not creating volatiles or CO₂ for the flies to be attracted to. The liquid amino acid solution is a hydrolyzed vegetable protein mix, made by acid-catalyzed hydrolysis of non-fermented soybeans (Schueller 2006). Since it is not fermented, it lacks

the fermentation products present in soy sauce that are thought to be attractive. The mild attraction to kombucha may be from the acetic acid and ethanol content. The combination of the two has been shown to be attractive to *D. suzukii* (Landolt et al. 2012).

Previous work shows that there are many types of fermented baits that are attractive to the *D. suzukii*. More promising is products from different fermented materials than small fruit hosts of *D. suzukii*. Kanzawa (1935) showed that wine vinegar is less attractive than rice vinegar in when vinegar-baited traps were placed in grape vineyards, potentially from competition with the grape odors already in the field. Balsamic vinegar was the only small fruit-based bait that performed as well as ACV in these experiments; the other baits that performed as well as ACV are made from raw materials other than fruit.

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Table 2.1: Mean ($\pm$ SE) of *D. suzukii* male and female caught in traps placed in greenhouse cage bioassays. Treatments in bold caught more than an even proportion of flies (50%, 20%, or 25%), so were tested further in field experiments.

		Mean no. of flies ($\pm$ SE)		Percent captured	
Treatment		Male	Female	Male	Female
Greenhouse Experiment 1a	Water Control	22.5 $\pm$ 2.6	31.1 $\pm$ 3.7	42.6	42.1
	Bird Shield	30.3 $\pm$ 3.7	42.7 $\pm$ 4.9	57.4	57.9
Greenhouse Experiment 1b	Water Control	23.6 $\pm$ 5.0	26.2 $\pm$ 4.6	29.6	29.3
	Monterey Insect Bait	56.1 $\pm$ 5.7	63.1 $\pm$ 4.0	70.4	70.7
Greenhouse Experiment 2	ACV control	18.8 $\pm$ 3.0	19.6 $\pm$ 2.4	21.8	19.6
	Raspberry Vinegar	21.6 $\pm$ 2.7	31.0 $\pm$ 3.2	25.2	31.0
	Red Wine Vinegar	15.6 $\pm$ 3.0	17.6 $\pm$ 2.7	18.2	17.6
	White Wine Vinegar	7.5 $\pm$ 1.1	9.0 $\pm$ 1.2	8.7	9.0
	Balsamic Vinegar	22.4 $\pm$ 3.2	22.8 $\pm$ 3.6	26.1	22.8
Greenhouse Experiment 3	ACV	12.9 $\pm$ 1.9	15.5 $\pm$ 2.4	15.7	14.3
	Balsamic Vinegar	18.3 $\pm$ 2.6	28.7 $\pm$ 3.4	22.2	26.4
	Malt Vinegar	15.9 $\pm$ 2.1	21.4 $\pm$ 3.8	19.3	19.7
	Ume Plum Vinegar	18.1 $\pm$ 2.3	22.5 $\pm$ 3.4	22.0	20.7
Greenhouse Experiment 4	Kombucha	17.2 $\pm$ 3.1	20.6 $\pm$ 4.8	20.9	19.0
	Yeast	12.8 $\pm$ 3.1	17.5 $\pm$ 3.3	15.8	16.2
	Soy Sauce	43.1 $\pm$ 6.2	57.0 $\pm$ 7.7	53.3	52.6
	Liquid Amino Acids	19.1 $\pm$ 2.5	26.8 $\pm$ 3.0	23.6	24.7
	Control	5.8 $\pm$ 1.5	7.0 $\pm$ 1.2	7.2	6.5

Table 2.2: Mean ($\pm$ SE) numbers of male and female SWD caught in traps placed in the field from 22 Jun 2011 to 28 Sep 2011. Means followed by an asterisk are statistically lower than ACV by Dunnett's correction. Treatments in bold performed similarly to ACV.

	Treatment	Males	Females	t, P values for males	t, P values for females
Field Experiment 1	ACV	4.1 $\pm$ 1.6	3.4 $\pm$ 1.1		
	Balsamic Vinegar	2.9 $\pm$ 1.2	2.8 $\pm$ 1.0	t = 1.49; P = 0.375	t = -1.23; P = 0.548
	Ume Plum Vinegar	0.2 $\pm$ 0.1 *	0.3 $\pm$ 0.3 *	t = -5.02; P < 0.001	t = -5.53; P < 0.001
	Raspberry Vinegar	0.3 $\pm$ 0.1 *	0.6 $\pm$ 0.3 *	t = -4.26; P < 0.001	t = -4.59; P < 0.001
	Control	0 $\pm$ 0 *	0 $\pm$ 0 *	t = -5.23; P < 0.001	t = -6.30; P < 0.001
Field Experiment 2	ACV	3.8 $\pm$ 1	2.2 $\pm$ 0.8		
	Soy Sauce	4.2 $\pm$ 1.7	3.8 $\pm$ 1.4	t = -0.75; P = 0.673	t = 1.18; P = 0.399
	Control	0 $\pm$ 0 *	0 $\pm$ 0 *	t = -4.45; P < 0.001	t = -3.63; P < 0.001
Field Experiment 3	ACV	4.7 $\pm$ 2.0	4.7 $\pm$ 1.9 *		
	Balsamic	0.3 $\pm$ 0.3 *	1.0 $\pm$ 0.6 *	t = -3.14; P = 0.032	t = -2.28; P = 0.144
	Cherry Vinegar	0.3 $\pm$ 0.3 *	1.7 $\pm$ 0.7	t = -3.14; P = 0.033	t = -1.46; P = 0.479
	Balsamic Cherry Vinegar	1.0 $\pm$ 0.6	0.3 $\pm$ 0.3 *	t = -2.27; P = 0.148	t = -3.16; P = 0.032
	Balsamic Honey Vinegar	1.0 $\pm$ 0.6	1.3 $\pm$ 0.9	t = -2.27; P = 0.148	t = -2.08; P = 0.198
	Control	0 $\pm$ 0 *	0 $\pm$ 0 *	t = -3.76; P = 0.011	t = -3.78; P = 0.011
Field Experiment 4	ACV	27.4 $\pm$ 9.3	22.1 $\pm$ 5.5		
	Balsamic	24.5 $\pm$ 8.2	17.3 $\pm$ 5.6	t = -0.77; P = 0.902	t = -0.75; P = 0.912
	Drosophila Lure	18.1 $\pm$ 4.6	16.7 $\pm$ 4.2	t = 1.10; P = 0.704	t = 2.47; P = 0.062
	Insect Bait	36.9 $\pm$ 13.2	39.8 $\pm$ 12.2	t = -0.53; P = 0.978	t = -0.57; P = 0.970
	Bird Shield	0 $\pm$ 0 *	0.2 $\pm$ 0.1 *	t = -5.70; P < 0.001	t = -6.27; P < 0.001
	Control	0 $\pm$ 0 *	0 $\pm$ 0 *	t = -5.70; P < 0.001	t = -6.47; P < 0.001

Table 2.2 continued. Mean ($\pm$ SE) numbers of male and female SWD caught in traps placed in the field from 22 Jun 2011 to 28 Sep 2011. Means followed by an asterisk are statistically lower than ACV by Dunnett's correction. Treatments in bold performed similarly to ACV.

Treatment		Males	Females	t, P values for males	t, P values for females
Field Experiment 5	ACV	57.9 $\pm$ 12.4	36.7 $\pm$ 9.7		
	Rice Vinegar	46.9 $\pm$ 12.4	31.0 $\pm$ 8.1	t = -0.97; P = 0.731	t = -0.85; P = 0.808
	Seasoned Rice Vinegar	55.0 $\pm$ 16.4	43.0 $\pm$ 14.4	t = -0.55; P = 0.951	t = 0.01; P = 1
	NuLure	34.9 $\pm$ 7.6	29.4 $\pm$ 6.6	t = -1.82; P = 0.215	t = -0.47; P = 0.971
	Control	0 $\pm$ 0 *	0 $\pm$ 0 *	t = -8.06; P < 0.001	t = -6.92; P < 0.001

Figure 2.1: Percent of male and female SWD caught in greenhouse bioassay 1a traps unbaited (Control) and baited with Bird Shield in a vial above a soap water moat. The dashed line indicates the even proportion of flies (50%), and treatments that captured more flies were used in subsequent experiments.

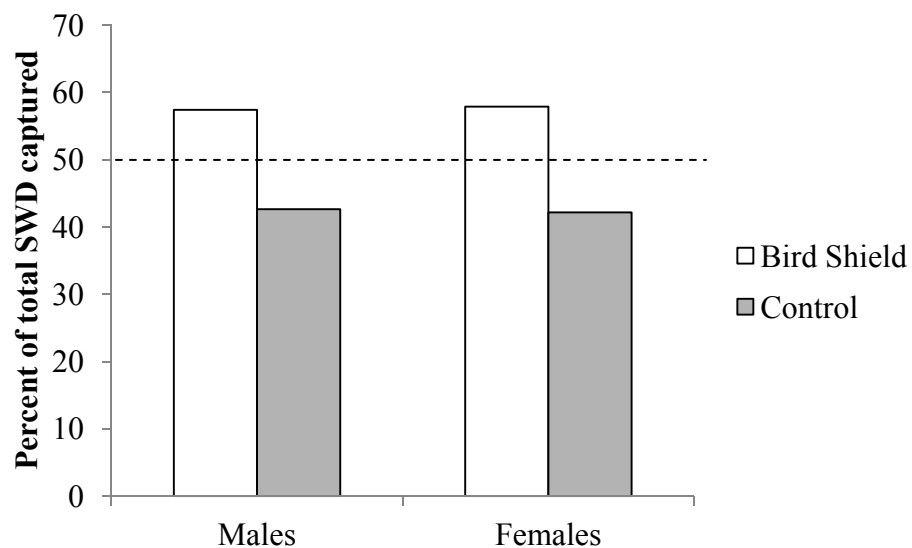


Figure 2.2: Percent of male and female SWD caught in greenhouse bioassay 1b traps unbaited (Control) and baited with Insect Bait in a vial above a soap water moat. The dashed line indicates the even proportion of flies (50%), and treatments that captured more flies were used in subsequent experiments.

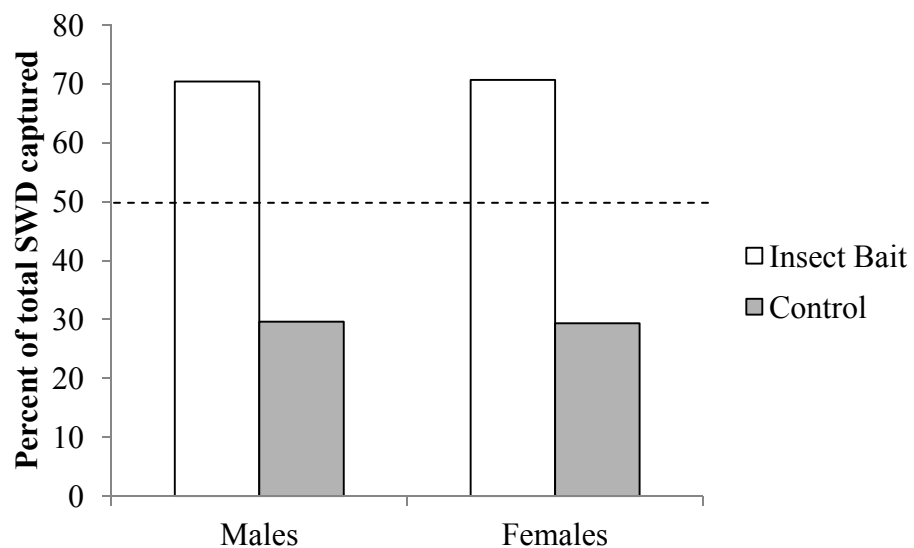


Figure 2.3: Percent of male and female SWD caught in greenhouse bioassay 2 traps baited with ACV, balsamic vinegar, raspberry vinegar, red wine vinegar or white wine vinegar in a vial above a soap water moat. The dashed line indicates the even proportion of flies (20%), and treatments that captured more flies were used in subsequent experiments.

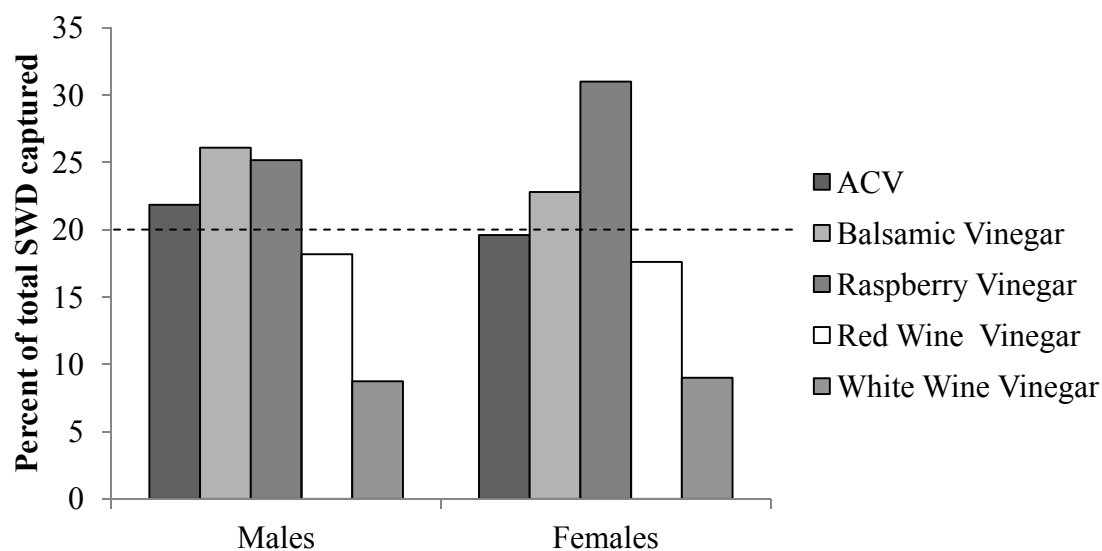


Figure 2.4: Percent of male and female SWD caught in greenhouse bioassay 3 traps baited with ACV, balsamic vinegar, kombucha, malt vinegar, or ume plum vinegar in a vial above a soap water moat. The dashed line indicates the even proportion of flies (20%), and treatments that captured more flies were used in subsequent experiments.

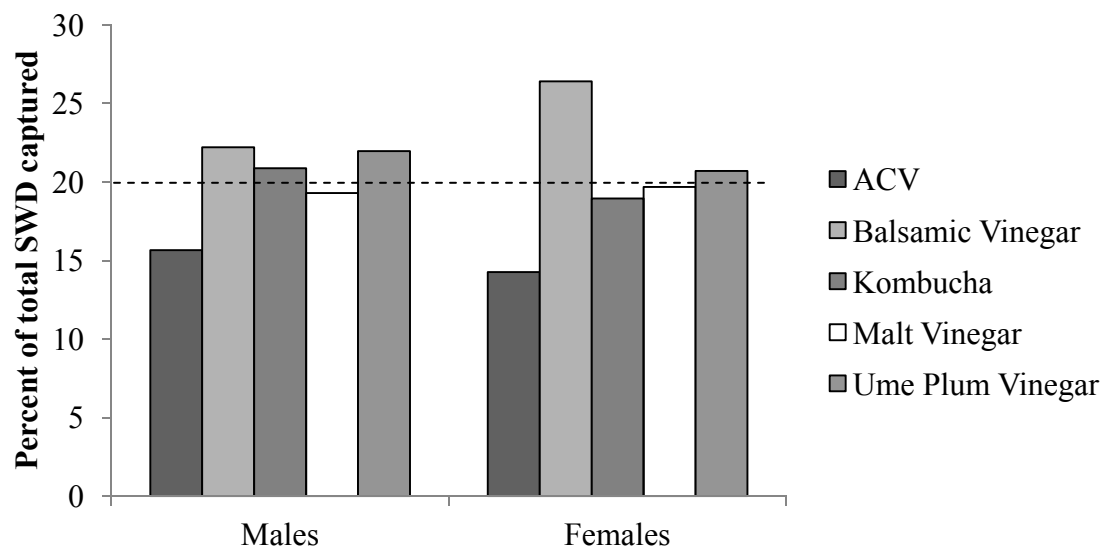


Figure 2.5: Percent of male and female SWD caught in greenhouse bioassay 4 traps unbaited (Control) or baited with liquid amino acids, soy sauce, or dissolved nutritional yeast in a vial above a soap water moat. The dashed line indicates the even proportion of flies (25%), and treatments that captured more flies were used in subsequent experiments.

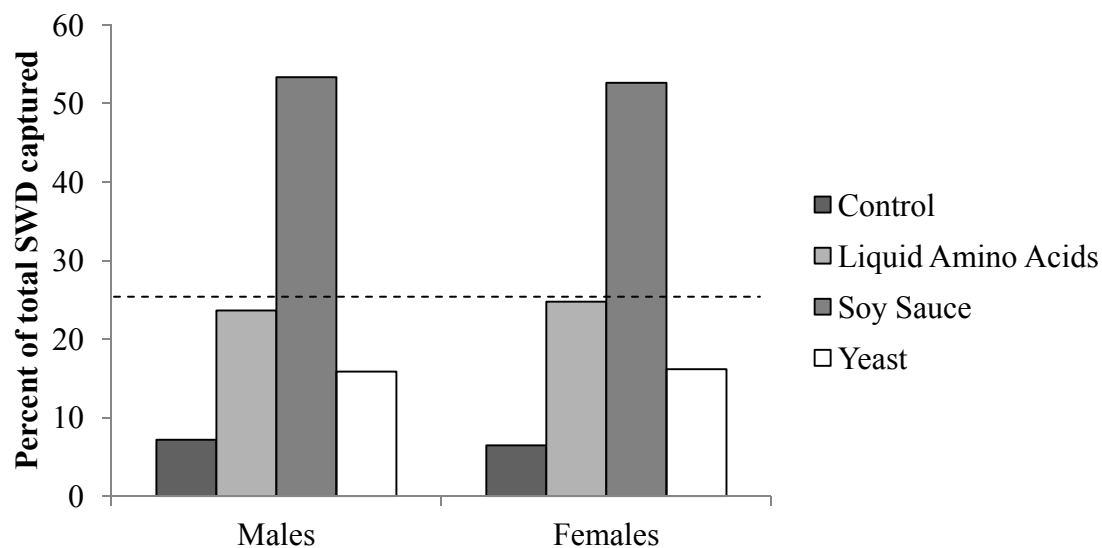


Figure 2.6: Mean (+ SE) numbers of male and female SWD caught in field experiment 1 traps placed in the field from 22 Jun 2011 to 3 Aug 2011. Treatments marked by an asterisk are statistically lower than ACV by Dunnett's correction.

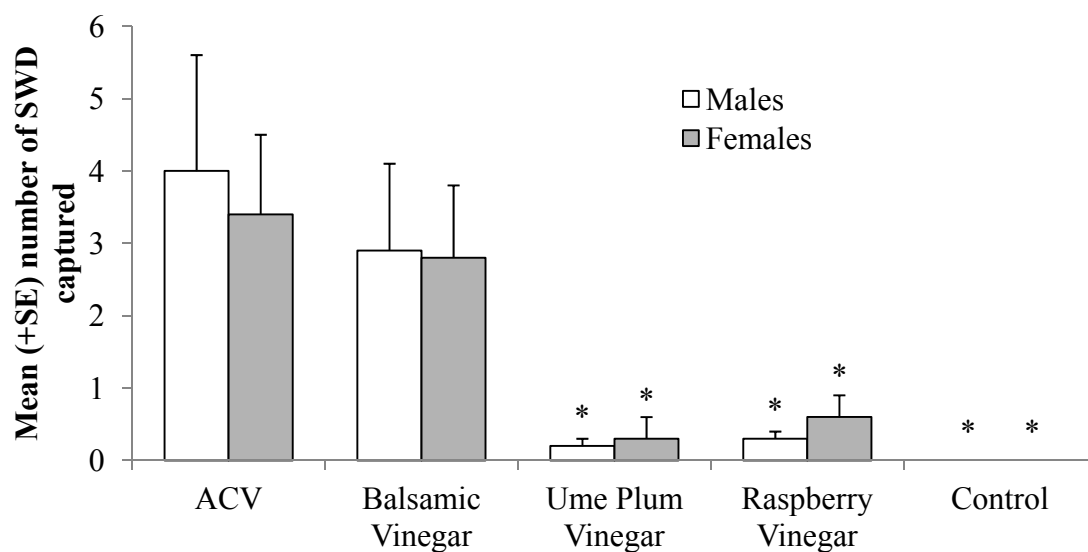


Figure 2.7: Mean (+ SE) numbers of male and female SWD caught in field experiment 2 traps placed in the field from 18 Jul 2011 to 15 Aug 2011. Treatments marked by an asterisk are statistically lower than ACV by Dunnett's correction.

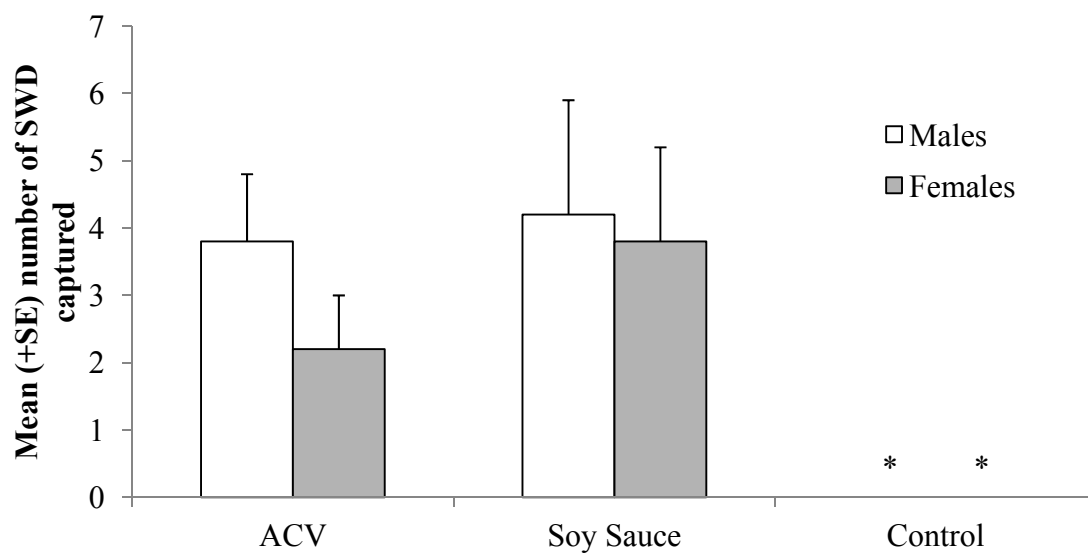


Figure 2.8: Mean (+ SE) numbers of male and female SWD caught in field experiment 3 traps placed in the field from 3 Aug 2011 to 10 Aug 2011. Treatments marked by an asterisk are statistically lower than ACV by Dunnett's correction.

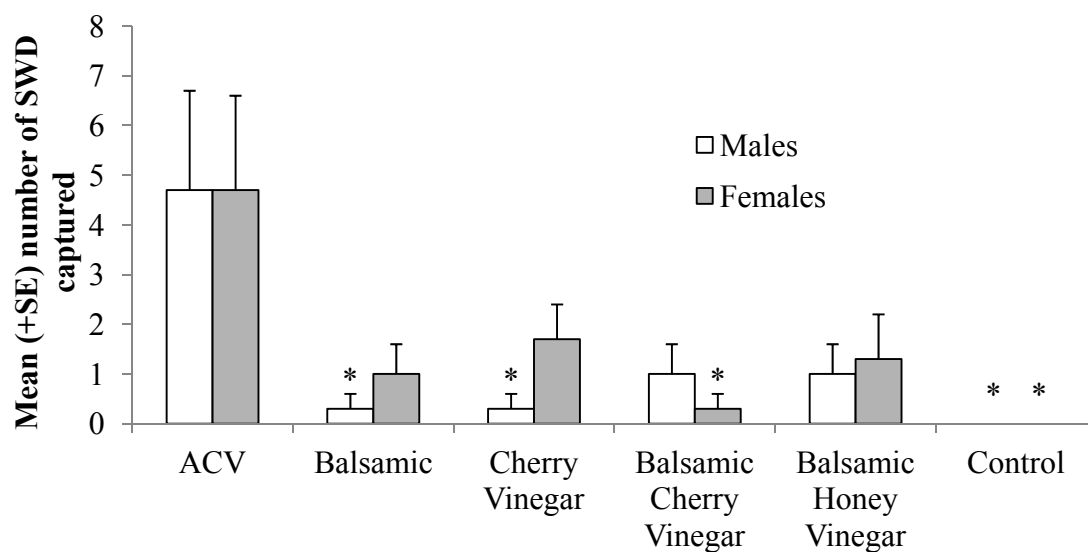


Figure 2.9: Mean (+ SE) numbers of male and female SWD caught in field experiment 4 traps placed in the field from 3 Aug 2011 to 17 Aug 2011. Treatments marked by an asterisk are statistically lower than ACV by Dunnett's correction.

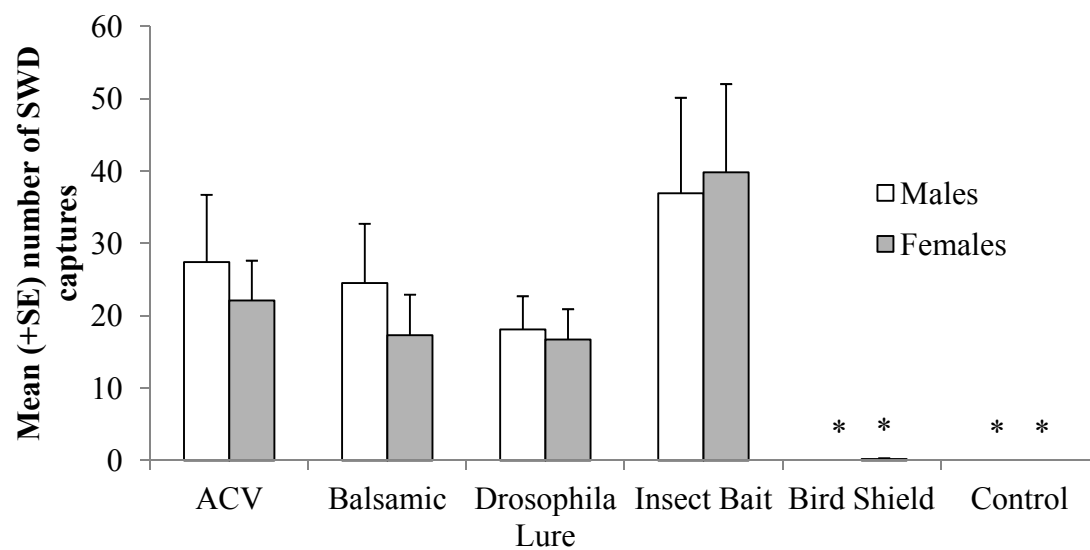
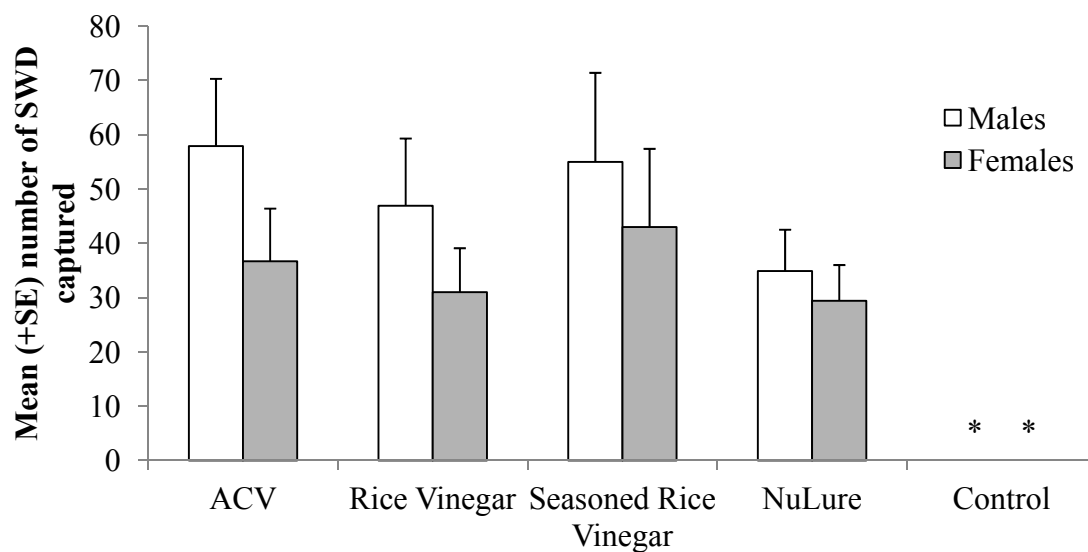


Figure 2.10: Mean (+ SE) numbers of male and female SWD caught in field experiment 5 traps placed in the field from 31 Aug 2011 to 28 Sept 2011. Treatments marked by an asterisk are statistically lower than ACV by Dunnett's correction.



CHAPTER 3.

Attractiveness of Fermentation Products to Spotted Wing *Drosophila* (Diptera: Drosophilidae)

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Abstract

Laboratory screening bioassays and field trapping experiments of spotted wing *Drosophila* flies, *Drosophila suzukii* were conducted to determine the attractiveness of 17 potentially attractive compounds as well as compare attractant efficiency during peak fruit ripeness and postharvest captures late in the season. Compounds structurally similar to each of the fermentation products acetic acid, ethanol, ethyl acetate and 2-phenethyl alcohol were screened for attractiveness in greenhouse cage bioassays. The compounds determined to be attractive in the greenhouse bioassay (methanol, ethanol, propanol, formic acid, acetic acid, ethyl acetate, propyl acetate, phenethyl acetate, phenethyl propionate, phenethyl butyrate) were subsequently tested individually in the field as a volatile supplement to apple cider vinegar (ACV) or neutralized apple cider vinegar (NACV) (pH~7) bait traps as well as in combination in NACV. The numbers of captures in ACV traps were not increased by the addition of any of the compounds tested, although differences in catches between supplemented compounds were observed. Compounds that are most prevalent in wine and vinegar (methanol, ethanol, acetic acid, ethyl acetate) as well as phenethyl propionate and phenethyl butyrate had less of a negative impact on the captures in ACV traps than other compounds tested in the field. Comparing the captures of the same treatments during peak fruit ripeness and postharvest late in the season when no fruit hosts were available revealed that although the total number of flies captured late in the season was lower, the trends in treatment performance were similar. This is promising for the consistent performance of baits from peak fruit ripeness through harvest.

Introduction

The spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is a widely distributed pest of small and stone fruit production, found in North America, Europe, and Asia (Walsh et al. 2011). Unlike other *Drosophila* species that only lay eggs in overripe or rotting fruit, female *D. suzukii* have a characteristic serrated ovipositor that allows them to lay eggs in ripe and ripening fruit (Lee et al. 2011). The damage caused by the oviposition scar and larvae that hatch from the eggs results in unmarketable fruit and crop loss, in some cases up to 80% (Walsh et al. 2011). The production of strawberries, blueberries, caneberries, and cherries in the western US is threatened by the presence of *D. suzukii*, and potential losses are significant (Bolda et al. 2010). A number of treatment programs for *D. suzukii* exist and are economically sound since the loss from yield reduction far outweighs the cost of control. Goodhue et al. (2011) performed an economic analysis weighing the costs and benefits of controlling SWD in California's raspberry and strawberry crops. Control cost per treatment in raspberries ranges from \$9.65/acre for Mustang EW to \$81.34/acre for the organic product Entrust. For one representative farm in California, the total cost of controlling *D. suzukii* along with light brown apple moth (*Epiphyas postvittana*, Lepidoptera: Tortricidae) and the two spotted spider mite (*Tetranychus urticae*, Trombidiformes: Tetranychidae) was \$334 per acre. At a per ton price of at least \$3779 and a yield of roughly 13 tons per acre, the control cost of those three pests for raspberries on California's central coast would be justified for even a small increase in the yield above an untreated field.

Although *D. suzukii* is a pest of ripe fruit, fermented products play an important role in monitoring, with recommended attractants including wine, vinegar, and fermenting yeast baits (Walsh et al. 2011). Work has been done testing the most attractive combinations of wine, vinegar, acetic acid, and ethanol (Landolt et al. 2012) and different combinations of wines and vinegars (Adams et al. 2012), with data suggesting a rice vinegar and a merlot wine are more co-attractive than other tested combinations to date. Biologically active compounds in wine and vinegar as determined by GC-EAD have been combined and shown to be as attractive to *D. suzukii* as a wine and vinegar blend (Cha et al. 2012). A close association between *D. suzukii* and the yeast *Hanseniaspora uvarum* was discovered (Hamby et al. 2012), stressing the link between SWD and fermentation products. Other *Drosophila* have associations with yeast (Gilbert 1980) and *D. melanogaster* are attracted to fermentation products, more so than to fruit volatiles alone (Zhu et al. 2003, Becher et al. 2012). The links between *Drosophila* and yeast or fermented products illuminates the importance of fermentation products in the attraction of *D. suzukii*.

This work focused on four groups of fermentation products for their attractiveness to *D. suzukii*. Acetic acid, ethanol, ethyl acetate, and 2-phenylethanol have already been identified in both wine and vinegar (Ough and Amerine 1988, Guerrero et al. 2006) and make up part of a *D. melanogaster* lure, ethyl acetate being optional (Baker et al. 2003). Specifically acetic acid and ethanol have recently been shown to be important in attracting *D. suzukii* (Adams et al. 2012, Landolt et al. 2012) and acetic acid, ethanol and 2-phenylethanol are included in a mix of compounds that attracted *D. suzukii* as well as a

mix of wine and vinegar (Cha et al. 2012). Compounds similar to these four were screened in the greenhouse and field to determine their effectiveness as *D. suzukii* attractants.

Another component of trapping explored by this work is the seasonal variation in attractiveness of baits which has not been addressed previously. The presence and composition of *D. suzukii* hosts change throughout the year, progressing from no fruit in the field in the early season to ripening fruit, ripe fruit, and overripe fruit over the course of the season and again to no fruit available in the late season. Insects have been shown to change throughout the year too; there are changes numerically (Escudero-Colomar et al. 2008), physiologically (Robb and Forbes 2005), and with regard to sexual selection (Vélez and Brockmann 2006). *D. suzukii* show differences in attraction over the course of the season with numerically more *D. suzukii* captured in the wine and vinegar traps in February than in April (Cha et al. 2012). By determining if attractiveness of baits is seasonal or not, trapping experiments can determine more precisely their scope of inference and baits can be deemed effective for a part of the year or as a consistent attractant. The effectiveness of the attractant is most important during the time of fruit ripening and peak fruit ripeness in order to monitor the populations of *D. suzukii* in the fields and confidently make management decisions.

The current recommendation for *D. suzukii* control is for growers to apply prophylactic insecticide sprays beginning at first color when the fruit becomes susceptible (Lee et al. 2011), and follow a regime based on the ripening of the fruit (Haviland and Beers 2012). Although monitoring is not a direct management method, it

is the first step in an IPM system and important to integrate with a host of other management practices (Cini et al. 2012) to keep SWD numbers low. To utilize monitoring data, an economic threshold level of infestation needs to be established (Stern 1973), which may be the mere presence of *D. suzukii* based on the control costs versus loss of yield costs. Since the current monitoring tool cannot accurately reveal even the presence of *D. suzukii* in a field, the attractants need to be improved upon to permit moving away from prophylactic treatment towards an integrated management of SWD.

Materials and Methods

Insects. A laboratory colony of *D. suzukii* was started and maintained as described by Bruck et al. (2011). The adult flies used in greenhouse experiments were between five and 12 days old.

Greenhouse bioassays. Compounds were screened in the greenhouse using a two choice cage assay. All bioassays were conducted in 0.6 x 0.6 x 0.6 m mesh cages inside a greenhouse with 16L:8D photoperiod and temperature between 13 and 24°C. Within each cage were two clear cup traps, treatment and control, positioned near opposite corners on the cage bottom (~20 cm from the corner, ~45 cm from each other). The clear cup trap was a 946 ml clear plastic cup with a clear plastic lid and fifteen 4.8 mm holes punched around the perimeter of the cup near the top. Control traps contained 100 ml of the soap water drowning solution made by adding 4 ml of dish soap (Dawn Ultra, Proctor & Gamble, Cincinnati, OH, USA) to 3.78 L of water. Soap was added to diminish the water surface tension and facilitate capture by drowning. Treatment traps consisted of the odor being pipetted onto a cotton roll placed in a small glass vial (8 ml, Wheaton, Millville,

NJ, USA) that was set in the center of the cup surrounded by soap water. Moist cotton in a Petri dish and two small agar-based diet cups were placed in the middle of the cage to ensure survivorship of the flies. Approximately 200 SWD of mixed sex were put in each of the cages with the traps for 24 h and the number of flies in each trap enumerated. The difference between the treatment and control traps was compared to the total number of flies captured in both traps by an attractivity index (AI): $(\# \text{ of flies in treatment trap} - \# \text{ of flies in control trap}) / \text{total number of flies trapped}$. This gives a ratio of the difference between the traps to the total number of flies captured, where an attractive treatment has a positive value and a deterrent has a negative value.

Determination of attractive concentrations. These bioassays were conducted to determine the concentration of each class of chemical that was most attractive to *D. suzukii*. Concentrations of 10, 100 and 1000 ppm of ethyl and phenethyl acetate were compared to a water control. These ranges encompass the typical occurrence of ethyl acetate and phenethyl alcohol in wine (Nykanen and Suomalainen 1983). Treatment traps contained the test compound in the center vial surrounded by 100 ml of soap water. At least two replicates of each two choice test were performed. The treatment with the highest AI was determined to be the most attractive concentration.

Greenhouse bioassay 1. The objective of this experiment was to determine the attractiveness of several short chain alcohols to SWD. The odors of methanol, ethanol, propanol, butanol and pentanol were each compared to a water control in this series of two-choice assays. The treatment trap contained 7.2 ml of the lure in the center vial surrounded by 93 ml of soap water. This yielded a concentration of 7.2% alcohol by

volume as the attractant, which was used and attractive in previous trapping studies (Adams et al. 2012, Landolt et al. 2012). Each two-choice test was replicated seven times.

Greenhouse bioassay 2. The objective of this experiment was to determine the attractiveness of short chain acids to the SWD. Formic acid, acetic acid, propionic acid, butyric acid and valeric acid were each compared to a water control in these bioassays. The treatment trap contained 2 ml of the acid in the center vial surrounded by 98 ml of soap water. The resulting concentration of 2% acid was chosen because of its use and attractiveness in previous trapping studies (Adams et al. 2012, Landolt et al. 2012). Seven replicates of each test were performed.

Greenhouse bioassay 3. This experiment was performed to test the attractiveness of several phenethyl esters to the SWD. The compounds were presented at rates of 880 µl phenethyl acetate, 980 µl phenethyl propionate and 1070 µl phenethyl butyrate, corresponding to 1000 ppm of each compound. The treatment trap contained one of the compounds in the center vial surrounded by 100 ml of soap water. Each two-choice test was replicated seven times.

Greenhouse bioassay 4. This experiment was performed to test the attractiveness of low molecular weight acetates to the SWD. The compounds were presented at rates of 5.5 µl ethyl acetate, 6.4 µl propyl acetate, 7.3 µl butyl acetate and 8.3 µl pentyl acetate, corresponding to a concentration of 10 ppm of each compound. The treatment trap contained one of the listed compounds in the center vial surrounded by 100 ml of the soap water. Each two-choice experiment was replicated seven times.

Field experiments. Field tests were performed in cultivated small fruit fields in Benton County, Oregon, USA. The same clear cup trap used in the greenhouse bioassays was used in these field tests. The drowning solution was made by adding 4 ml of dish soap to 3.78 L of apple cider vinegar (ACV) or ACV neutralized to ~pH 7 with sodium hydroxide. The odor tested in each treatment was pipetted onto a cotton roll in a vial suspended by wire into the drowning solution of the trap. The vials were used to keep the presentation of the odors consistent in the greenhouse and field trials and to prevent side reactions that might occur if the compounds were added directly to ACV. Ethyl acetate content in vinegar is influenced by the amount of ethanol in the vinegar (Tesfaye et al. 2004), so an increase of ethanol to an ACV trap would be associated with an increase of ethyl acetate. The use of vials also facilitated the approximation of the release rates of the attractants. Traps were placed at least 10 m apart in replicated linear blocks. The blocks of traps were separated by at least 20 m. Traps were hung in the canopies of each crop to achieve similar exposure at all trap locations to maximize consistency and SWD capture. Traps were placed in the field for five days and the number of SWD captured determined. After each five-day period, no attractants were placed in the field for two days allowing odors from the previous experiment to dissipate and not influence the next experiment.

Evaporation rates of the odors from the dispenser vials were initially tested by dispensing 1 ml of a compound onto a cotton roll in a microcentrifuge tube and weighing at 0.5, 1, 2, 3, 4, 5 and 6 days after loading. Observations of attractant levels in the field experiments supported the initial testing of the attractant evaporation and ensured that the attractant was still present through the time it was refreshed.

Field experiment 1. This experiment was performed to test the effect of adding alcohol odors to an ACV drowning solution. Trapping was conducted in cherries from 2 Jun 2012 to 27 Jun 2012 and in blackberries from 16 Jul 2012 to 10 Aug 2012. The alcohols selected for testing in the field were based on the results of greenhouse bioassay 1; methanol, ethanol and propanol were the odors attractive in the greenhouse. 7.2 ml of the alcohol to be tested was dispensed into a small glass vial with a cotton roll inside. The vial was then hung by a wire into 93 ml of ACV drowning solution. The traps were placed in the field in a Latin square design for five days and the attractants were renewed daily. After each two-day non-testing period, the traps were rotated within four blocks.

Field experiment 2. This experiment was based on the results of greenhouse bioassay 2 to test the effect of adding acid odors to the drowning solution of neutralized ACV. Trapping was conducted in cherries from 9 Jul 2012 to 3 Aug 2012 and in blackberries from 30 Jul 2012 to 24 Aug 2012. The drowning solution was ACV neutralized to a pH between 6 and 8 by the addition of sodium hydroxide pellets (>98%, CAS No. 1310-73-2). 4 ml of dish soap were added to 3.78 L of neutralized ACV. The ACV was neutralized to decrease the acetic acid odor profile of the ACV and to allow the acid being tested to be the more dominant acidic odor. Formic acid, acetic acid and valeric acid were presented in the traps by dispensing 2 ml of each onto a cotton roll in a plastic vial (2 ml, Corning Inc., Corning, NY, USA) which was suspended by a wire into 98 ml of neutralized ACV drowning solution. The attractants were renewed every other day. In the cherries, the three treatments and an ACV standard were arranged in a Latin

square design. A neutralized ACV standard was added to the trial in the blackberries and the treatments were randomized within four blocks and re-randomized weekly.

Field experiment 3. The treatments in this experiment were based on the results of greenhouse bioassay 3. Trapping was conducted in raspberries from 16 Jul 2012 to 10 Aug 2012 and in cherries from 30 Jul 2012 to 24 Aug 2012. An ACV standard was used in this experiment, and the treatment traps contained 100 ml of the ACV drowning solution with a microcentrifuge tube (1.5 ml, Brand Tech Scientific, Inc., Essex, CT, USA) with cotton and the treatment compound suspended by a wire above the drowning solution. The compounds were presented at rates of 880 µl phenethyl acetate, 980 µl phenethyl propionate and 1070 µl phenethyl butyrate. The traps were placed in the field in a Latin square design and maintained in the field for five days. After each two-day non-testing period, the treatments were rotated within the blocks.

Field Experiment 4. The objective of this experiment was to determine the attractiveness of an ACV drowning solution containing the attractive acetates tested in greenhouse bioassay 4. Trapping was conducted in raspberries from 16 Jul 2012 to 10 Aug 2012 and in cherries from 6 Aug 2012 to 31 Aug 2012. An ACV standard was used in this experiment, and the treatment traps contained 100 ml of the ACV drowning solution with a microcentrifuge tube with cotton and the attractant suspended by wire above the drowning solution. The compounds were presented at rates of 5.5 µl ethyl acetate and 6.4 µl propyl acetate. The traps were randomized within blocks in the field and maintained for five days and the attractants were renewed daily. After each two-day non-testing period, the trap position was re-randomized within the blocks.

Field experiment 5 (Table 3.1). The objective of this experiment was to determine if combinations of the most attractive compounds from field experiments 1-4 elicited a higher response by *D. suzukii* to the traps than the individual compounds alone. This experiment was performed in blackberries and blueberries from 3 Sep 2012 to 5 Oct 2012. The compounds used in this experiment were the treatments from field experiments 1-4 that did not have a negative effect on the attractiveness of the ACV drowning solution. Four vials, each containing one compound from a different field experiment, were suspended by a wire above 91 ml of neutralized ACV drowning solution. A trap with 100 ml of neutralized ACV drowning solution was used as the control. The four treatments were: 1) 2 ml acetic acid, 5.5 µl ethyl acetate, 7.2 ml methanol and 980 µl phenethyl propionate; 2) 2 ml acetic acid, 5.5 µl ethyl acetate, 7.2 ml ethanol and 980 µl phenethyl propionate; 3) 2 ml acetic acid, 5.5 µl ethyl acetate, 7.2 ml methanol and 1070 µl phenethyl butyrate; 4) 2 ml acetic acid, 5.5 µl ethyl acetate, 7.2 ml ethanol and 1070 µl phenethyl butyrate. The traps were randomized within blocks in the field and maintained for five days. After each two-day non-testing period, the trap position was re-randomized within the blocks.

Comparison of trapping time. This experiment was performed to compare the catch data of the same treatments during peak fruit ripeness and post harvest. From 8 Oct 2012 to 2 Nov 2012, the treatments used in field experiments 1-4 were maintained in one of the same fields the first experiment was performed in (June-August, 2012). Field experiment 1 was repeated in the blackberry field, field experiment 2 in the cherry orchard, field experiment 3 in the cherry orchard and field experiment 4 in the raspberry

field. Experiments 1, 3 and 4 used the same treatment layout as was used during peak ripeness trapping. The neutralized ACV trap was added to the treatment list in experiment 2, so a randomized block design was used in post-harvest trapping rather than the Latin square design used during peak ripeness trapping.

Statistical analysis. For field trapping experiments, male and female counts were combined because the trends were similar and the five day total catch numbers were $\log_{10}(x + 1)$ - transformed. The data was analyzed using an analysis of variance (ANOVA) with treatment, block and date collected as terms (R Development Core Team 2012). The means were compared using the Tukey-Kramer test. For comparison of trapping time, the interaction of treatment $\times$ trapping time was analyzed.

Chemicals. Phenethyl acetate ($\geq 99\%$, CAS No. 103-45-7), phenethyl butyrate ($\geq 98\%$, CAS No. 103-52-6), phenethyl propionate ($\geq 98\%$, CAS No. 122-70-3), ethyl acetate ($\geq 99.7\%$, CAS No. 141-78-6), propyl acetate ($\geq 98\%$, CAS No. 109-60-4), methanol ($\geq 99.9\%$, CAS No. 67-56-1), propanol (99.5%, CAS No. 67-63-0), pentanol ($\geq 99\%$, CAS No. 71-41-0), butanol ($\geq 99.4\%$, CAS No. 71-36-3), acetic acid ($\geq 99\%$, CAS No. 64-19-7), butyric acid ($\geq 99\%$, CAS No. 107-92-6), propionic acid ($\geq 99.5\%$, CAS No. 79-09-4), and valeric acid ($\geq 99\%$, CAS No. 109-52-4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (95%, CAS No. 64-17-5) and formic acid ($\geq 88\%$, CAS No. 64-18-6) were purchased from Oregon State University Chemistry Stores. Propyl acetate (99%, CAS No. 109-60-4), butyl acetate (97%, CAS No. 123-86-4), and pentyl acetate (95%, CAS No. 628-63-7) were synthesized by Joe Kleiber as described in Williamson (1999).

Results

Greenhouse Bioassays. (Table 3.2). *Determination of attractive concentrations* (Figures 3.1-3.2). Ethyl acetate dispensed at 5.5 µl was determined to be the most attractive concentration tested with an AI of 36. Dispensed at 55 and 550 µl, ethyl acetate was less attractive with AIs of seven and eight, respectively. Phenethyl acetate was most attractive at a rate of 880 µl with an AI of 53. Dispensed at 8.8 and 88 µl, phenethyl acetate was less attractive with AIs of 20 and 39, respectively.

Greenhouse bioassay 1 (Figure 3.3). Treatment traps baited with methanol, ethanol, and propanol caught more mean adult SWD in the cage bioassays than the control traps. Traps baited with butanol and pentanol caught fewer flies than the control traps in the bioassays.

Greenhouse bioassay 2 (Figure 3.4). Treatment traps baited with formic acid, acetic acid and valeric acid caught more adult SWD than the control traps. Traps baited with butyric acid and propionic acid caught fewer flies than the controls.

Greenhouse bioassay 3 (Figure 3.5). Treatment traps baited with ethyl acetate and propyl acetate caught more adult SWD than the control traps. Traps baited with butyl acetate and amyl acetate caught fewer flies than the controls.

Greenhouse bioassay 4 (Figure 3.6). Phenethyl acetate, propionate, and butyrate all caught more adult SWD than the control traps.

Field Experiments. (Table 3.3). *Field experiment 1* (Figure 3.7). The numbers of male and female flies caught in the field showed similar trends within each experiment, and were pooled for analysis. The total number of SWD captured in traps baited with

different alcohols were significantly different in both cropping systems ($F_{3, 54}=25.06$, $P < .001$ in cherries; $F_{3, 54}=42.93$, $P < .001$ in blackberries). In both crops, the treatment traps with propanol captured significantly fewer adults than the ACV standard. Methanol and ethanol containing traps in both crops captured a similar number of total flies as ACV.

Field experiment 2 (Figure 3.8). Numbers of flies caught in the traps containing different acid treatments were significantly different in both cropping systems ($F_{3, 54}=38.13$, $P < .001$ in cherries; $F_{4, 69}=7.30$, $P < .001$ in blackberries). In cherries, all treatments caught significantly fewer adult SWD than the ACV standard. In blackberries, traps containing acetic acid and the neutralized ACV captured similar amounts of flies. The formic acid and valeric acid treatments captured significantly fewer flies than the ACV but a similar amount of flies as the traps containing neutralized ACV.

Field experiment 3 (Figure 3.9). The total number of flies captured in the traps baited with various acetates did not differ significantly in raspberries ($F_{2, 39}=1.15$, $P = .328$). However, in the cherry orchard, the total number of flies captured differed significantly between treatments ($F_{2, 39}=4.21$, $P = .022$). Both ethyl acetate and propyl acetate captured similar numbers of flies as ACV. Traps baited with ethyl acetate captured significantly more flies than traps baited with propyl acetate.

Field experiment 4 (Figure 3.10). The total numbers of flies captured in traps baited with different phenethyl esters differed significantly when placed in both cherry orchards ($F_{3, 54}=5.14$, $P = .003$) and raspberry fields ($F_{3, 54}=5.60$, $P = .002$). In raspberries, traps baited with phenethyl propionate captured a similar number of flies as the ACV. Traps baited with phenethyl acetate and phenethyl butyrate captured fewer flies than the

ACV. When placed in the cherry orchard, the traps baited with phenethyl butyrate captured a similar number of flies as ACV. Phenethyl acetate and phenethyl propionate captured fewer flies than ACV.

Field experiment 5 (Figure 3.11). The total numbers of flies captured in traps baited with combinations of attractants differed significantly in both blackberries ($F_{5, 83} = 4.60$, $P = .001$) and blueberries ($F_{5, 84} = 8.35$, $P < .001$). A summary of the attractant combinations tested are detailed in Table 2. In the blackberries, traps baited with combinations 3 and 4 as well as the neutralized ACV captured as many flies as the ACV standard. Traps baited with combinations 1 and 2 captured significantly fewer flies than ACV. All the combination treatments captured statistically similar numbers of flies as the neutralized ACV. In the blueberry field, only the neutralized ACV and traps baited with combination 2 captured as many flies as the ACV control.

Comparison of Trapping Time. The total number of flies captured during post-harvest trapping with alcohol baits was significantly lower than the number caught during peak blackberry ripeness ($P < .001$). The treatments showed similar trends in the harvest and post harvest seasons as indicated by a non-significant treatment $\times$ season interaction ($P = .660$). Acid baits were significantly more attractive during peak blackberry ripeness than during post-harvest trapping ($P = .007$) but the treatment $\times$ season interaction was not significant ($P = .230$). Traps baited with phenethyl esters captured significantly more SWD during peak cherry ripeness than during post-harvest trapping ($P = .007$), but the treatment $\times$ season interaction was not significant ($P = .517$). Traps baited with acetates

were significantly more attractive during peak raspberry ripeness than during post-harvest trapping ($P < .001$) but the treatment $\times$ season interaction was not significant ($P = .516$).

Discussion

The results of the greenhouse concentration testing were not consistent with the levels of candidate compounds naturally found in wine and vinegar, indicating a difference between the biological activity of the compounds and their presence in commonly used baits. Under the conditions of our studies, the most attractive concentration of ethyl acetate was 10 ppm while ethyl acetate occurs in wine at concentrations as low as 15 ppm up to 384 ppm in souring wine (Nykanen and Suomalainen 1983). The range of ethyl acetate present in wine vinegar is 10-100 mg/L (Blanch et al. 1992), encompassing the concentration found to be most attractive in the greenhouse bioassays. The concentration of phenethyl acetate determined to be most attractive in the greenhouse screening was 1000 ppm, much higher (10-100 fold) than its average concentration of 460 ppb in wine and 1220 ppb in apple cider vinegar (Ough and Amerine 1988, Natera et al. 2003). The difference in concentrations between the attractive concentrations determined in the greenhouse screening and in the natural products may be a true attraction to the concentration used in these experiments, but it also may be due to the release rate. Cha et al. (2012) approximated vials of other fermentation odors to release at rates 6.7 times greater than a 60% wine solution and 24.5 times greater than a 40% vinegar solution. Another factor that influences the attractiveness of compounds is synergy with other odors in the bait. The synergistic effect of ethanol was shown by Landolt et al. (2011), who demonstrated that ethanol is not

attractive to SWD without the presence of acetic acid. A similar phenomenon has been seen in bark beetles; ethanol alone is only weakly attractive, but increases attractiveness of a mix of monoterpenes (Byers 1992). The synergistic effects of the different compounds would need to be considered when developing a mix of attractive compounds rather than testing individual compounds.

The alcohols tested in the greenhouse bioassays were presented at the same volume per volume concentration of 7.2% yielding different molecular concentrations because of the different weights of the compounds. There is also a decrease in volatility of the compounds as the molecular weights increase, leading to different concentrations of the odors sensed by the insects in the trials. Dependence of biological activity on the concentration of the odors presented is shown by the optimal concentration determination experiments run in this study. If the concentration of the attractant is different than the optimal concentration, it could elicit a different response. The differences in chemical composition of the acetates and alcohols systematically influenced the attractiveness to SWD, since the attractiveness of compounds in both classes decreases as the molecular weight of the compound tested increased (see Table 1). Biological activity of similar compounds has been shown to change with molecular weight in *Milichiella lacteipennis* (Diptera: Milichiidae) (Dorner and Mulla 1963) as well as in *Vespula vulgaris* (Hymenoptera: Vespidae) (El-Sayed et al. 2009). The differences between similar compounds could be due to either differences in release rate due to the different molecular weights or the insect could be detecting the compounds with different receptors. The alcohols and acetates are also the two most volatile groups, followed by

the acids and then the phenethyl esters. Changes in the molecular weight of the acids were not accompanied by a systematic change in the attractiveness to SWD like the alcohols and acetates; the carboxylic acids with 1, 2, and 5 carbons were attractive while the acids with 3 and 4 carbons were not. The prevalence of isovaleric acid, but not propionic or butyric acids, in vinegar (Yang and Choong 2001) may be one explanation of the difference in the pattern of acid attractiveness.

Data from field experiment 1 suggest that adding an ethanol odor to an ACV trap does not increase the attractiveness of the trap to SWD. This is consistent with findings from Landolt et al. (2011) who showed that ethanol dispensed from vials had no effect on capture rate. This fact along with the increase of attraction from ethyl acetate is an important set of findings. They indicate that the synergism between ethanol and acetic acid in baits seen in the same paper may not be from the individual odors, but from the formation of ethyl acetate, which is influenced by the level of ethanol in vinegar (Tesfaye et al. 2004). Since butyl acetate and amyl acetate were deterrents in the greenhouse screening and propyl acetate did not add to the attractiveness of the ACV traps, combining other alcohols with acetic acid would likely not have the same effect as ethanol. Combining ethanol, and other alcohols, with the acids in the attractant would also likely not be very effective given the deterrent effect of propionic and butyric acids in the greenhouse and formic and valeric acids in the field.

No combination of attractants captured significantly more flies than the ACV or neutralized ACV traps, indicating that the combinations are not additive or synergistic when the vials of the attractants are combined in a single trap. The odors of the best

performing attractants in each category were combined to test if there would be any additive effect of the combination, but results revealed that there was no more attraction to the traps baited with a combination of vials when compared to the ACV control than to any of the traps baited with individual lure vials. When trapping with the combinations was performed in blueberries, three of the four treatments actually captured significantly fewer flies than the neutralized ACV trap, indicating a deterrent effect of the combinations of vials in the traps. This makes the transition from individual compound testing to combination testing complicated due to complex interactions in response to attractants.

The amount of lure dispensed into the vials used in individual and combination experiments in both the greenhouses and field experiments may not produce the most attractive concentration of each compound in the trap since all the compounds in each class were tested at the same rate. The acids were tested at a rate of 2%, the concentration of acetic acid in a mixture of wine and vinegar used by others (Adams et al. 2012, Landolt et al. 2012), which may have resulted in screening abnormally high concentrations of other acids. In wines and vinegars, there are much lower levels of other acids than of acetic acid (Nykanen and Suomalainen 1983, Yang and Choong 2001). The use of the other acids at elevated concentrations could have resulted in a deterrent effect, similar to what had been observed when *D. melanogaster* were exposed to increasing concentrations of acetic acid in a bait (Reed 1938). The compound most abundant in wine and vinegar from each class was selected as the benchmark for concentration testing, leading to the possibility that most of the remaining compounds were presented at a

concentration too high for attraction. Other alternatives to testing the most abundant compound in wine or vinegar would have been to test one of the other compounds in the class to determine the concentration at which the other compounds in the class should have been presented or to test all the compounds at a range of concentrations.

Vinegar is a recommended attractant for SWD and the standard to which attractants in these experiments were compared. Although ethyl acetate dispensed from a vial in an ACV trap and a combination of vials of acetic acid, ethanol, ethyl acetate, and phenethyl butyrate in a neutralized ACV trap captured numerically more flies than ACV and neutralized ACV respectively, no treatment in this experiment statistically increased the attraction of ACV or neutralized ACV. Therefore, more work is needed to determine which compounds could be used to enhance SWD captures.

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Table 3.1. Combinations of attractants in vials suspended in a neutralized ACV trap placed in the field between 3 Sept 2012 and 5 Oct 2012.

	Combo 1	Combo 2	Combo 3	Combo 4
Acetic Acid	2 ml	2 ml	2 ml	2 ml
Ethyl Acetate	5.5 μ l	5.5 μ l	5.5 μ l	5.5 μ l
Methanol	7.2 ml	-	7.2 ml	-
Ethanol	-	7.2 ml	-	7.2 ml
Phenethyl Propionate	985 μ l	985 μ l	-	-
Phenethyl Butyrate	-	-	1074 μ l	1074 μ l

Table 3.2: Mean and standard deviation (SE) of summed male and female *Drosophila suzukii* captured in greenhouse bioassays, with attractivity index (AI)

		Treatment		Control		AI*
		Catch	SE	Catch	SE	
Determination of Attractive Concentration 1	Phenethyl Acetate					
	8.8 µl	125	8.6	83	3.2	20
	88 µl	157	3.9	69	3.7	39
	880 µl	187	6.0	58	3.7	53
Determination of Attractive Concentration 2	Ethyl Acetate					
	5.5 µl	122	12.0	57	11.5	36
	55 µl	54	0.0	47	0.5	7
	550 µl	46	5.2	39	2.9	8
Greenhouse Bioassay 1	Alcohols					
	Methanol	1037	14.1	90	5.4	84
	Ethanol	1005	35.7	95	3.8	83
	Propanol	263	7.2	79	4.0	54
	Butanol	29	1.5	115	4.2	-60
	Pentanol	6	0.3	71	3.1	-84
Greenhouse Bioassay 2	Acids					
	Formic Acid	184	4.3	85	3.3	37
	Acetic Acid	448	10.5	202	4.4	38
	Propionic Acid	87	2.8	149	2.4	-26
	Butyric Acid	249	4.1	385	5.0	-21
	Valeric Acid	306	11.4	148	4.9	35
Greenhouse Bioassay 3	Phenethyl Esters					
	Phenethyl Acetate	242	2.8	102	3.2	41
	Phenethyl Propionate	328	9.2	142	3.7	40
	Phenethyl Butyrate	202	5.7	81	3.5	43
Greenhouse Bioassay 4	Acetates					
	Ethyl Acetate	225	3.4	160	4.5	17
	Propyl Acetate	207	5.0	154	3.8	15
	Butyl Acetate	69	0.6	108	5.6	-22
	Amyl Acetate	34	1.4	76	3.7	-38

* AI = $\Sigma(\text{treatment captures} - \text{control captures}) / (\text{treatment captures} + \text{control captures})$ *

Table 3.3. Mean and standard error (SE) of summed male and female *Drosophila suzukii* captured in traps placed in the field from 2 Jun 2012 to 2 Nov 2012. The letters signify statistical separation of means within chemical classes for each crop using Tukey's honestly significant difference test.

		Mean ¹	SE	HSD ²	Mean	SE	HSD
Field Experiment 1		Cherries			Blackberries		
	ACV	48.7	7.12	A	49.1	13.99	A
	Methanol	52.4	6.03	A	47.0	12.85	A
	Ethanol	51.9	7.93	A	71.3	23.96	A
	Propanol	18.5	4.42	B	10.3	3.73	B
Field Experiment 2		Cherries			Blackberries		
	ACV	45.1	3.93	A	17.1	2.70	A
	Formic Acid	11.9	2.39	B	6.8	1.32	B
	Acetic Acid	11.7	2.41	B	11.8	3.77	AB
	Valeric Acid	11.3	2.53	B	6.3	1.67	B
	Neutralized ACV				11.5	2.79	AB
Field Experiment 3		Cherries			Raspberries		
	ACV	56.2	9.56	A	7.3	1.61	A
	Phenethyl Acetate	27.1	7.44	B	2.4	0.99	B
	Phenethyl Propionate	47.5	15.33	B	4.4	1.66	AB
	Phenethyl Butyrate	45.0	8.74	AB	3.5	1.68	B
Field Experiment 4		Cherries			Raspberries		
	ACV	39.2	7.20	AB	25.3	14.20	ns
	Ethyl Acetate	60.3	10.16	A	25.3	10.49	ns
	Propyl Acetate	37.3	5.78	B	18.0	7.82	ns
Field Experiment 5		Blackberries			Blueberries		
	ACV	94.1	22.98	A	10.4	2.52	A
	Combination 1	28.8	5.01	B	2.9	0.66	B
	Combination 2	49.6	14.72	B	4.8	1.03	AB
	Combination 3	50.1	13.00	AB	3.1	0.68	B
	Combination 4	71.1	26.68	AB	3.1	0.86	B
	Neutralized ACV	67.3	24.03	AB	9.9	2.05	A

¹ Mean number of SWD adults per trap per five-day trapping period

² Treatments followed by different letters are significantly different by a Tukey-Kramer post-hoc analysis at the 0.05 level.

Figure 3.1: Attractivity index of phenethyl acetate concentrations screened for attractiveness to *Drosophila suzukii* in greenhouse cage bioassays.

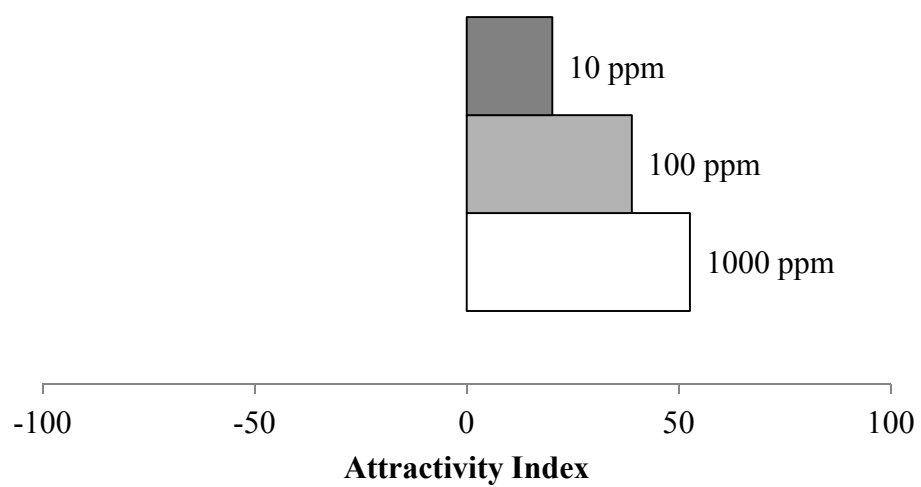


Figure 3.2. Attractivity index of ethyl acetate concentrations screened for attractiveness to *Drosophila suzukii* in greenhouse cage bioassays.

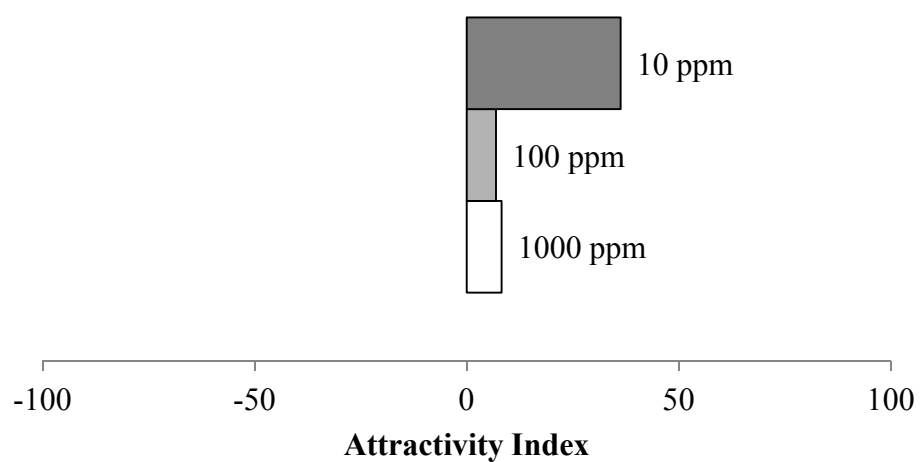


Figure 3.3: Attractivity index of treatments screened in greenhouse bioassay 1 for attractiveness to *Drosophila suzukii*.

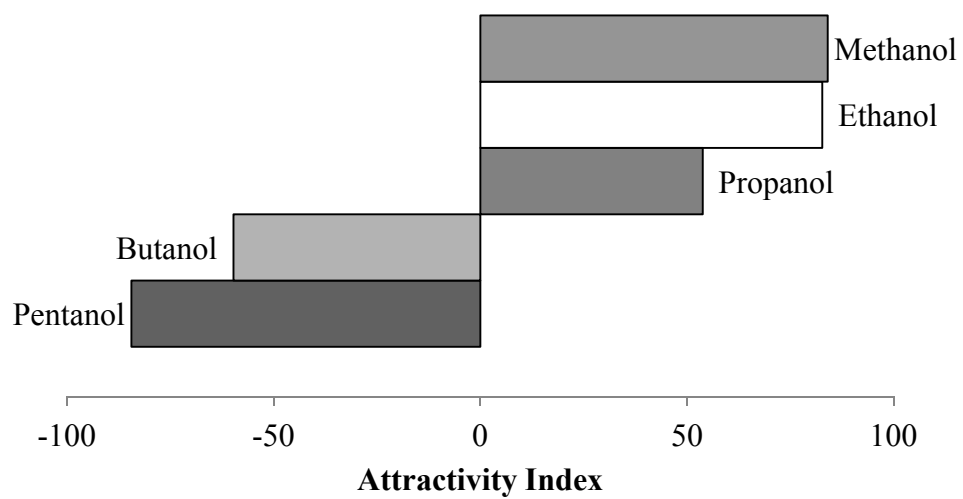


Figure 3.4: Attractivity index of treatments screened in greenhouse bioassay 2 for attractiveness to *Drosophila suzukii*.

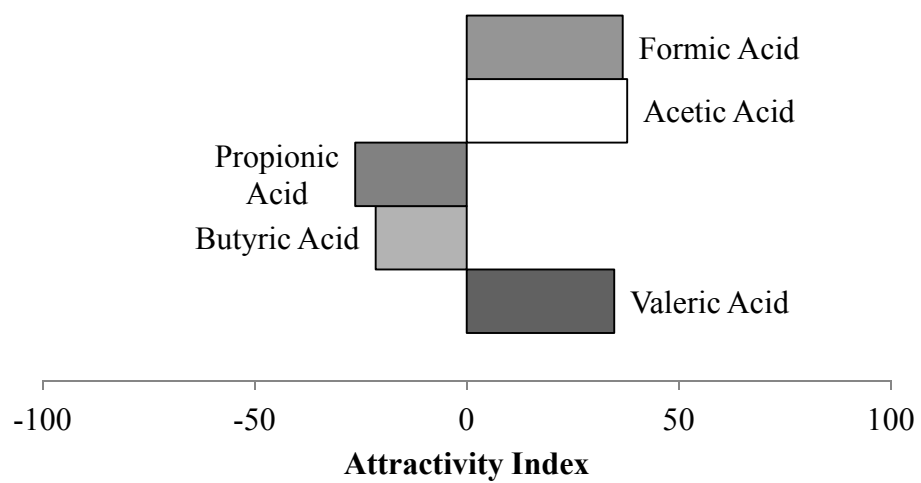


Figure 3.5: Attractivity index of treatments screened in greenhouse bioassay 3 for attractiveness to *Drosophila suzukii*.

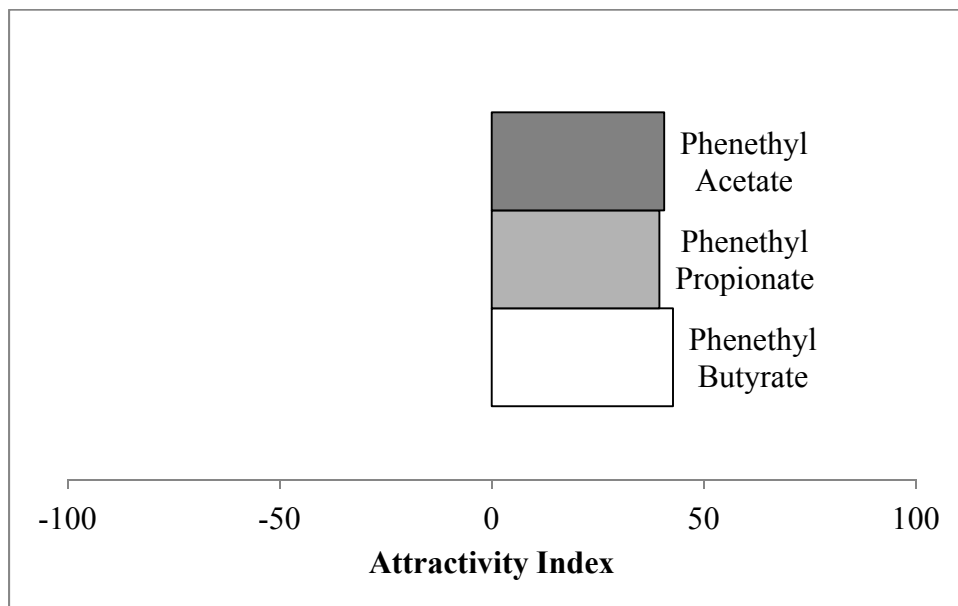


Figure 3.6: Attractivity index of treatments screened in greenhouse bioassay 4 for attractiveness to *Drosophila suzukii*.

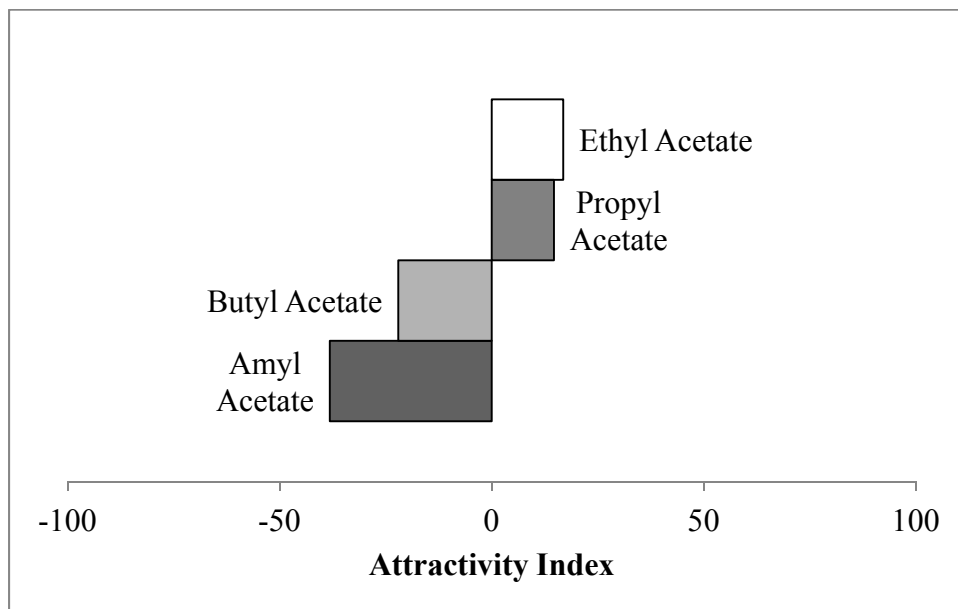


Figure 3.7: Mean (+SE) number of *Drosophila suzukii* caught in field experiment 1 traps placed in a cherry and blackberry field. Treatments in the same crop with different letters captured a significantly different number by a Tukey-Kramer post-hoc analysis at the 0.05 level.

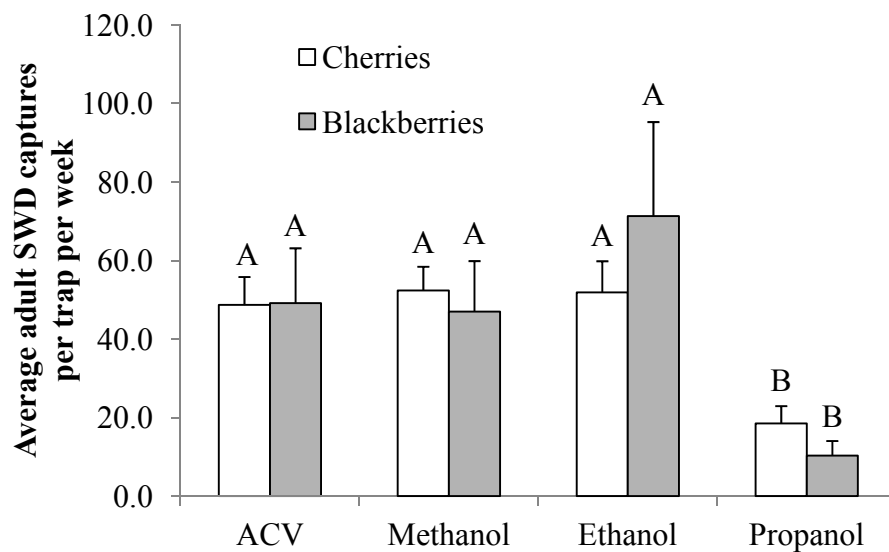


Figure 3.8: Mean (+SE) number of *Drosophila suzukii* caught in field experiment 2 traps placed in a cherry and blackberry field. Treatments in the same crop with different letters captured a significantly different number by a Tukey-Kramer post-hoc analysis at the 0.05 level.

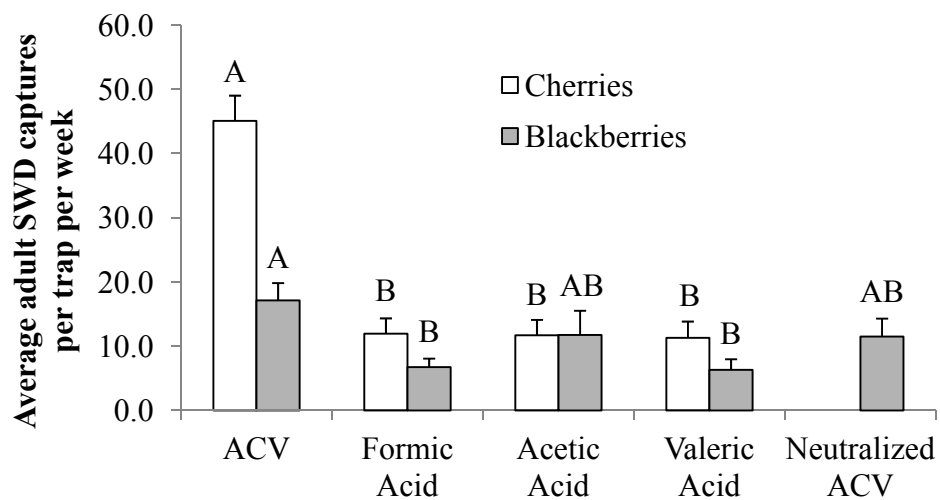


Figure 3.9: Mean (+SE) number of *Drosophila suzukii* caught in field experiment 3 traps placed in a cherry and raspberry field. Treatments in the same crop with different letters captured a significantly different number by a Tukey-Kramer post-hoc analysis at the 0.05 level.

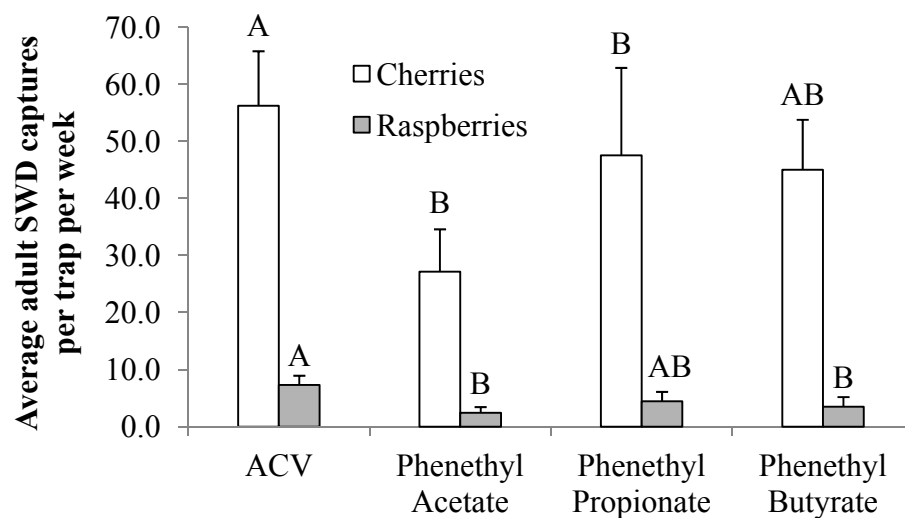


Figure 3.10: Mean (+SE) number of *Drosophila suzukii* caught in field experiment 4 traps placed in a cherry and raspberry field. Treatments in the same crop with different letters captured a significantly different number by a Tukey-Kramer post-hoc analysis at the 0.05 level.

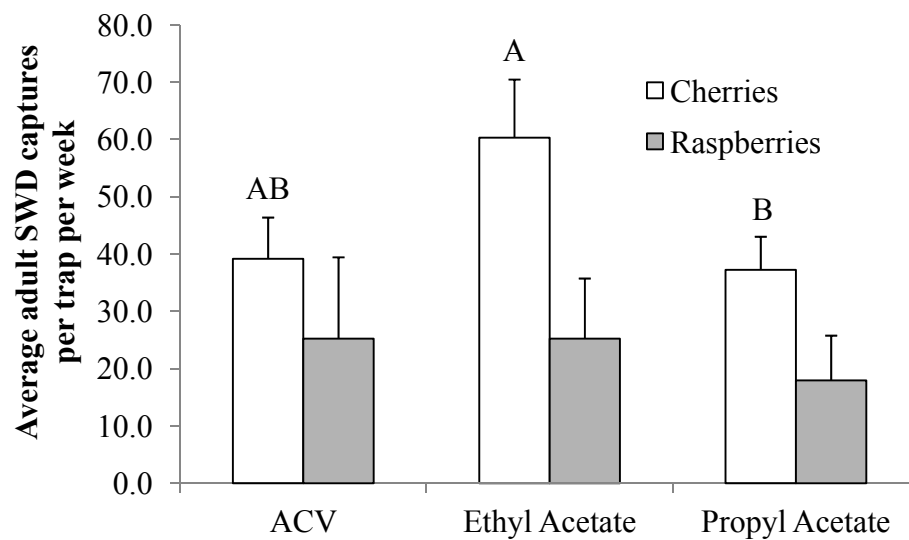
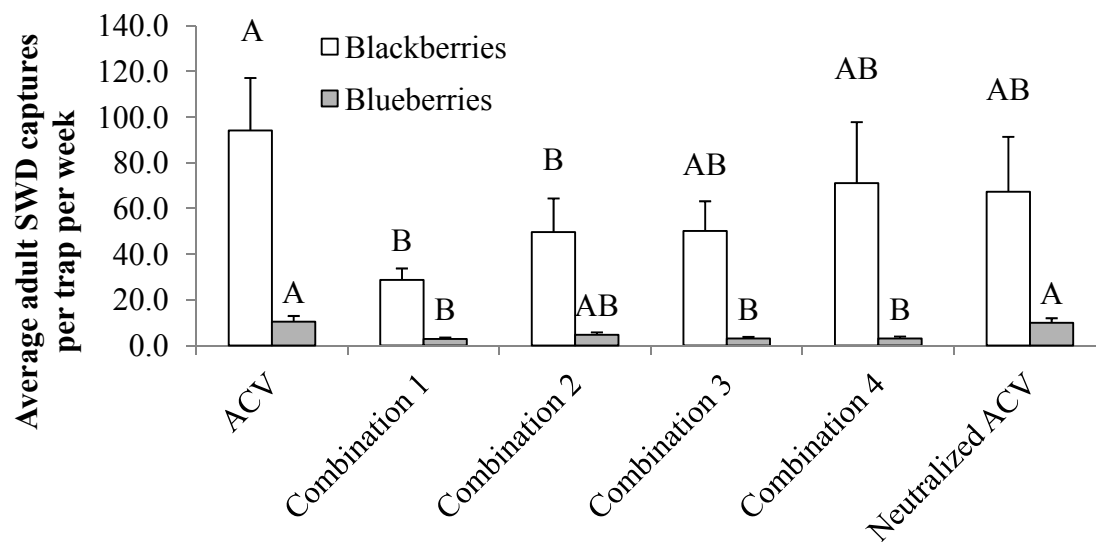


Figure 3.11: Mean (+SE) number of *Drosophila suzukii* caught in field experiment 5 traps placed in a blackberry and blueberry field. Treatments in the same crop with different letters captured a significantly different number by a Tukey-Kramer post-hoc analysis at the 0.05 level.



CHAPTER 4.

General Conclusions

Joseph R. Kleiber

Spotted wing *Drosophila* (SWD) is a widespread pest of small and stone fruits, negatively impacting some of the most important fruit production regions around the world. Populations can currently be controlled with a variety of conventional chemical insecticides or limited organic insecticides (Bruck et al. 2011) with the insecticides applied according to the ripeness of the fruit (Haviland and Beers 2012). Since the insecticides are applied as a prophylactic measure, the current control techniques do not align with the integrated pest management ideals of establishing an action threshold and only applying treatment once it is needed (Stern 1973). In order to establish action and economic thresholds, a robust monitoring technique is needed, which is not currently available for SWD using the current standard of 5% acidity apple cider vinegar (ACV), wine and fermenting bait traps. This work was performed to both expand the range of attractants available and to determine the attractive components of the baits.

The first part of this research was exploring other baits that could be used as attractants to SWD. Vinegars, wines and fermenting baits are all used as monitoring tools (Walsh et al. 2011) so additional fermented food products were tested for their attractiveness to SWD. In addition, other commercially available insect baits were tested for their attractiveness to SWD. Twenty different potential baits were screened for their attractiveness in greenhouse bioassays and subsequently in field trapping experiments. In field trapping experiments, the SWD captures of the treatments were compared to an ACV trap positive control. Balsamic vinegar, soy sauce, balsamic honey vinegar, *Drosophila* Lure, Insect Bait, rice vinegar, seasoned rice vinegar and NuLure all generally captured similar numbers of SWD as ACV. Although none of the treatments

captured more SWD than ACV, the range of baits that are now known to be attractive to SWD has been expanded. With a wider range of attractants, their combination may prove to be more effective than ACV or their components can be analyzed to create a defined bait composed of specific compounds known to be attractive to SWD.

The second part of this research was to explore the attractiveness of some specific compounds found in fermented bait. Ethanol, acetic acid, 2-phenylethanol and ethyl acetate are all components of wine and vinegar (Ough and Amerine 1988, Guerrero et al. 2006) and have been shown to be effective in a *Drosophila melanogaster* lure (Baker et al. 2003). Compounds similar to these four were tested for their attractiveness in greenhouse bioassays as well as in field trapping experiments. Experiments were divided up by the class of compounds: short chain alcohols, short chain carboxylic acids, phenethyl esters, and small molecular weight acetates. In greenhouse bioassays, 17 compounds were screened for their attractiveness to SWD by comparing them to a soap water control trap. The 11 compounds that were determined to be attractive were then tested in the field for their added attractiveness to SWD when presented in combination with ACV in a clear cup trap. A few of the attractive odors in the greenhouse bioassays acted as deterrents when presented with ACV in the field. This illuminated some discrepancies between laboratory experiments and field experiments that have been noticed before in other insects (Zhu et al. 2003, Knudsen et al. 2008). Background odors are quite different between a greenhouse with very few ambient berry odors and a berry production field that is full of fruit and plant odors and other stimuli and may play a role in the perception of different odors by the insects. Differences in attractiveness of the

compounds were still able to be established and the most attractive compounds were presented together as combination treatments in a field setting. No combination of compounds captured more flies than the positive control. The overall ineffectiveness of the compounds to add to the attractiveness of ACV in the field may be purely a result of the odors not being attractive to the SWD. Other alternatives are that the added odors are redundant to odors already in the ACV or the concentration of odors may have not been ideal for attractiveness and rather had a deterrent effect to the SWD.

More research can be done in both the areas of exploring the attractiveness of other baits as well as determining what compounds in the baits are acting as the attractants. The baits tested in the first part of this project are only a small number of potential baits when considering how wide the host range of SWD is. The compounds in each bait are also numerous since fruits can contain hundreds of volatile compounds (Aprea et al. 2009), and different yeast impart characteristic odors when introduced to fermentable material (Ugliano et al. 2006). Future work focused on determining which compounds are attractive in combination with one another may lead to a defined bait that is robust enough to effectively monitor for SWD and allow control of the pest in an integrated manner.

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