Bait and Trap Design Preferences for *Drosophila suzukii*

By

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**Abstract**

Knowledge of *Drosophila suzukii's* preference for an attractive trap design and bait can be used to improve detection and management of this new invasive fruit pest. Desired trap and bait attributes include: high *D. suzukii* capture and species specificity; early-season detection prior to significant crop damage; higher capture of females than males; limit escapage from trap; and a positive response to seasonal changes in population, landscape, and weather.

Bait trials were replicated 3-4 times in 4 different host crops, including: 'Spartan' cultivar blueberries on a no-spray farm (Benton Co. Oregon); a wild 'Himalaya' blackberry perimeter adjacent to a diversified, organic-certified farm (Benton Co., Oregon); no-spray Early 'Burlette' cultivar cherries on a diversified, organic-certified farm (Benton Co., Oregon); and organic 'Meeker' cultivar raspberries on an organic-certified farm (Whatcom Co., Washington). Baits included: apple cider vinegar, Chinkiang vinegar, *Saccharomyces cerevisiae* yeast, *Hanseniaspora uvarum* yeast, BioLure®, Torula Yeast Pellets®, Monterey Insect Bait®, Suzukii Trap®, and a 4-compound (acetic acid, methionil, acetoin, and ethanol) lure. Each bait was placed in a 950 mL side-mesh entry clear cup trap. Chinkiang vinegar and *H. uvarum*-baited traps had consistently high *D. suzukii* counts in all crop types tested. Suzukii Trap® baited traps had promising results; however, factors such as humidity and bait evaporation affected captures in blueberry and blackberry crops. Consistently, Torula Yeast Pellets® baited traps had low efficacy, and BioLure® and Monterey Insect Bait® baited traps showed little to no efficacy. Traps with the 4-compound lure yielded moderate trap catch relative to other baits, but showed the highest specificity to *D. suzukii* across the season. During the late-season period, traps baited with apple cider vinegar showed increased *D. suzukii* capture compared to observed early-season counts. Early-season *Drosophila* species captures were primarily other *Drosophila* species (66 – 100%), whereas late-season captures were 90 – 95.5% *D. suzukii*. The gender distribution of *D. suzukii* shifted from higher counts of females early-season to higher counts of males late-season.

Trap designs varied in color, entry type (mesh vs. hole), trap volume, bait surface area, and headspace (area between bait surface and entry holes). Designs were replicated 4 times and tested in the wild 'Himalaya' blackberry perimeter described above. Each trap contained a *Saccharomyces cerevisiae* yeast/sugar solution. Traps with higher trap captures of *D. suzukii* had a low volume between the surface of the bait and the entry holes and a high volume between the entry holes and the trap top. Three traps that captured the highest numbers of *D. suzukii* overall were: 1) "Clear 20-Hole" (950 mL; 20-holes; clear cup), 2) "Squatty Botty Fly Trap" (1183 mL; side-mesh; red-yellow-black vertical stripe cup), and 3) "Lucky 13" (530 mL 13-hole; red cup). Increased entry area improved trap catch, and color did not appear to impact capture. However, traps supplemented with a killing agent placed inside the lid revealed significantly more *D. suzukii* than traps without.

Key Words: *Drosophila suzukii*, spotted wing drosophila, preferences, trap, design, bait, monitoring, fruit pest, invasive species

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**CHAPTER 1: INTRODUCTION**

**1.1 The Pest.** *Drosophila suzukii* (Matsumura) is an invasive berry and stone fruit pest that originated in East Asia (Kanzawa, 1939). After initially arriving to the United States in Hawaii in the 1980's, *D. suzukii* was first detected in the mainland United States in 2008 (California), and has since spread to over 45 states (Figure 1).

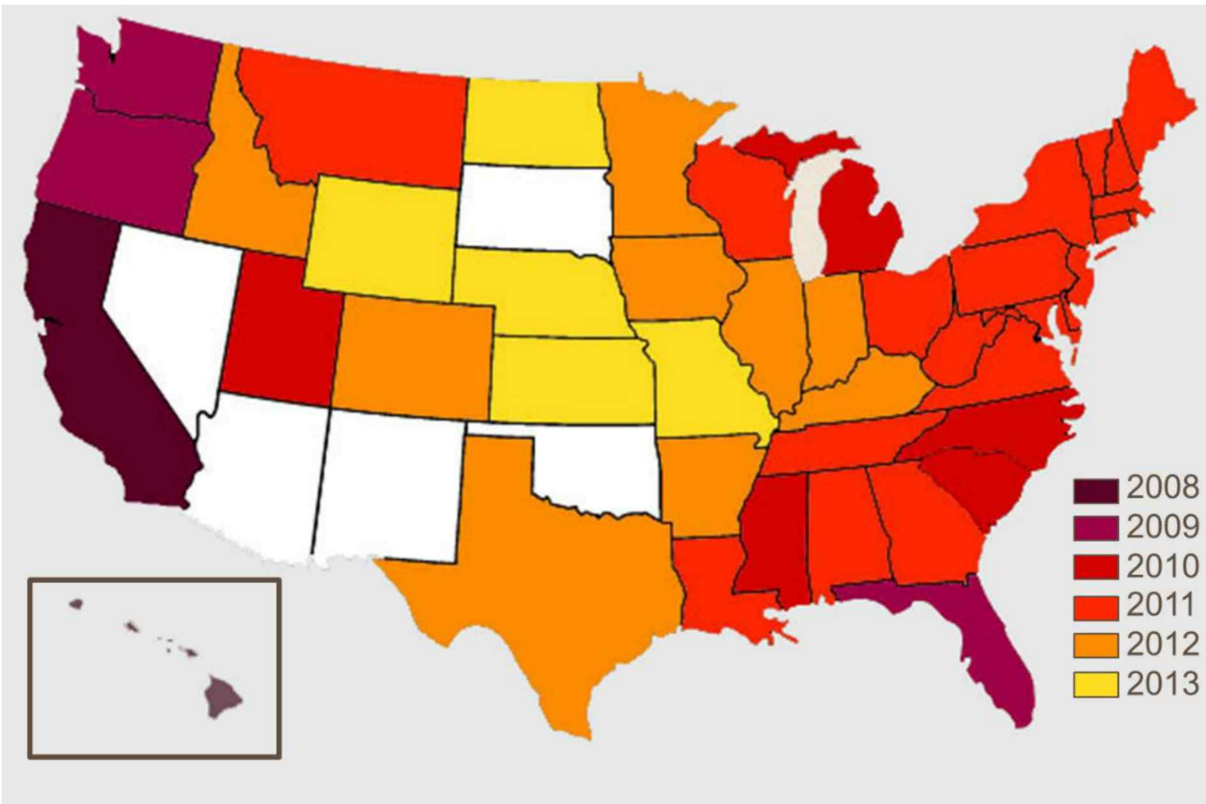



Figure 1. *D. suzukii* distribution in the mainland United States by year of entry.

Globally, *D. suzukii* is present in Mexico, Canada, and is distributed throughout Europe (Invasive Species Compendium). The first South American report cited a significant number of *D. suzukii* collected from southern Brazil in 2012 and 2013 (Depra´, Poppe, Schmitz, De Toni, & Valente, 2014) (Figure 2).

*D. suzukii* have been identified/noted in the following countries from the current literature available, but are not limited to:

Asia: Japan, North Korea, South Korea, Taiwan, Thailand, China, Bangladesh, Myanmar, India, Pakistan.

Europe: Spain, France, Italy, Belgium, Netherlands, Portugal, Slovenia, Russia, Croatia, etc. (Invasive Species Compendium)

Americas: United States, Canada, Brazil, Mexico.

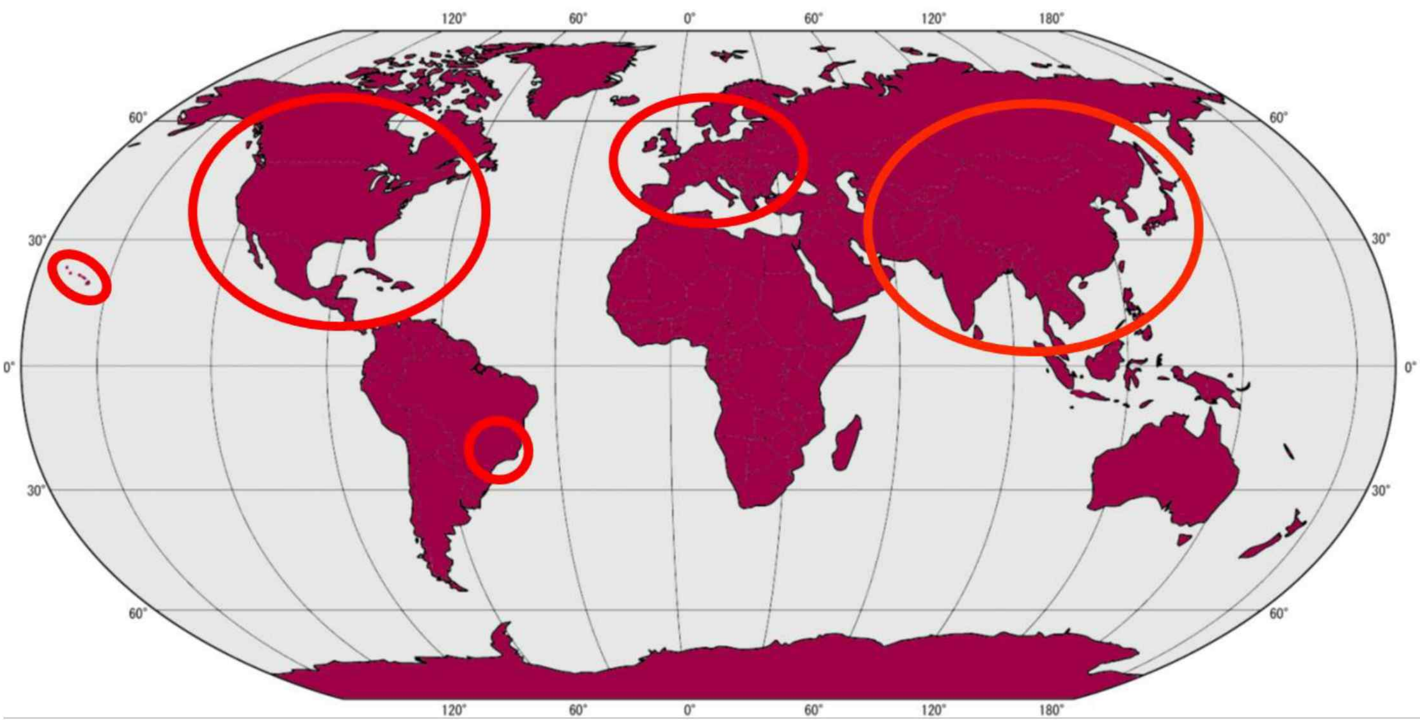



Figure 2. Global distribution *D. suzukii*. *D. suzukii* presence indicated by red circles.

**1.2 Identification & Life Cycle.** *D. suzukii* resemble other small vinegar flies, but are distinctive for their yellowish-brown bodies, red eyes, and dark, unbroken bands encircling the abdominal segments (Dreves, Walton, & Fisher, 2009). Male *D. suzukii* are identifiable by the presence of a dark spot on the leading top edge of each wing, and two dark combs (each with 3-6 teeth) running parallel on each front leg (Figure 3).


Figure 4. Male *D. suzukii*. Arrows pointing to sex combs. Note single dark and smoky "spot" on leading edge of each wing. Photo courtesy A. Ohrn, Oregon State University 2013

The female of the species is identifiable by her ovipositor, which is distinctive for its large size relative to other *Drosophila* species, saw-like serrations and dark scleritization (Figure 4).

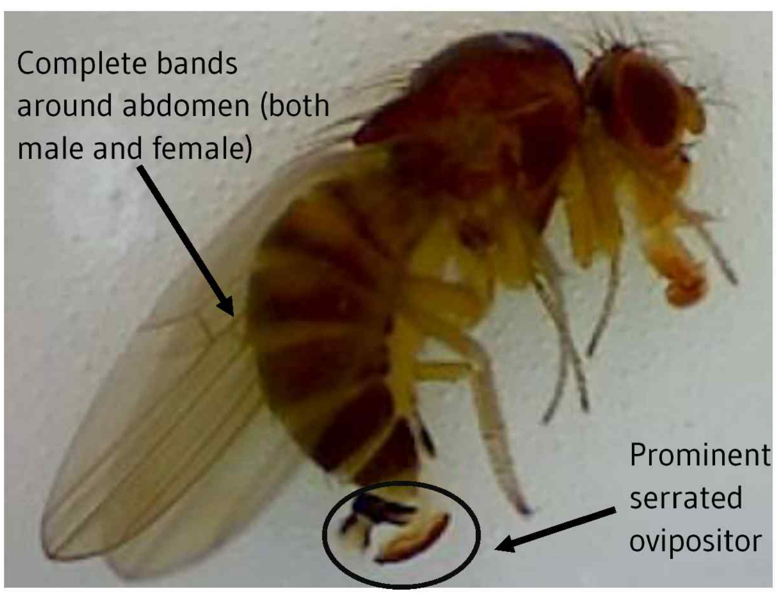



Figure 3. Female *D. suzukii*. Note prominent ovipositor (circled).
Photo courtesy A. Ohrn, Oregon State University 2013.

This prominent ovipositor allows *D. suzukii* to lay eggs in fresh fruit, which sets the species apart from many other *Drosophila* species which oviposit in rotten or damaged fruit. These other *Drosophila* species do not need to pierce the firm flesh of a fresh fruit with their ovipositors and therefore lack the distinctive scleritization, pointed shape, and serrations of the *D. suzukii* ovipositor (Figure 5)

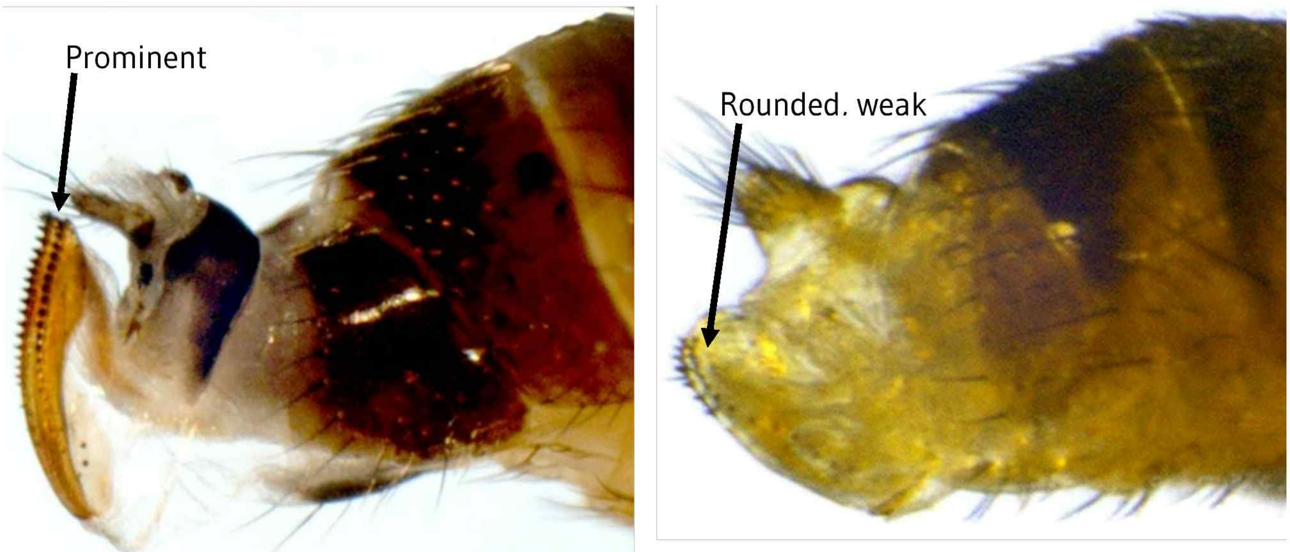



Figure 5. *D. suzukii* ovipositor (left) compared to *D. simulans* (right). Photos courtesy M. Hauser, California Department of Food & Agriculture.

The female *D. suzukii* cuts through the fruit's surface, to clear the path to lay one to three eggs per fruit, totaling several hundred eggs in her lifetime (Kanzawa, 1939) (Figure 6). The eggs hatch and legless and headless larvae emerge after one to three days depending on temperature (Kanzawa, 1939).

Figure 6. Egg with two long filaments laid by female *D. suzukii*.
Photo courtesy E. Beers, Washington State University 2010.

Larvae begin to feed on the inner flesh of the fruit, undergoing three instar stages. This feeding often results in damage which includes scarring, bruising, softening, exuding of juice through the oviposition hole, and liquefaction of fruit body (Walton et al., 2010; Figure 7).

Figure 7. Heavily infested blueberry showing *D. suzukii* larvae and damage.
Photo courtesy A.J. Dreves, Oregon State University.

The larvae pupate after three to 13 days, resting in or outside fruit for four to five days before emerging as adult *D. suzukii*. Generation time will vary based on temperature; in Asia, 3-13 generations have been observed annually (Kanzawa, 1939), whereas in the West Coast of the United States, 3 -9 generations have been predicted per year (Walsh, et al., 2011) which is currently being validated. A degree-day model has been developed based on the lower threshold of 10°C and an upper threshold of 30°C which predicts the generational oviposition and emergence (Coop, Dreves 2010-2014).

**1.3 Impact.** *D. suzukii* is a danger to fruit industries across the world for many reasons, including: the ability to infest fruit that are hanging on the plant which reduces marketable quality; rapid reproduction leading to multiple generations each season; and high dispersal ability. A related species, *D. pseudoobscura*, has demonstrated the capability to travel 15 km within 24 hours (Coyne, et al., 1982). Some of the most economically important hosts for *D. suzukii* in the United States include cherries, caneberries (blackberries, raspberries, and their cultivars and hybrids), blueberries, and strawberries (National Agricultural Statistics Service, 2012). Yield and quality losses in blueberries, strawberries, and raspberries have been observed as high as 20-50% in California (Goodhue, Bolda, Farnsworth, Williams, & Zalom, 2011). The potential economic loss of $520 million or more per year across three states (California, Oregon, and Washington) resulting from *D. suzukii* damage make the fly a major threat to many small and stone fruit industries (Bolda, Goodhue, & Zalom, 2010). In 2012, Oregon had 29,450 acres of blueberries, caneberries, and cherries in production, with a farm-gate value of approximately $236.3 million according to the USDA-National

Agricultural Statistics Service, “Non-citrus Fruits and Nuts Summary” (2012). The large viable small fruit industry has been threatened by this pest over the past five years.

Other consequences of *D. suzukii* include reliance on insecticide use (Bruck, et al., 2011), additional financial burden for monitoring and treatment, and limitations and complications for worldwide trade. Countries establish their own regulations and levels for the maximum level of pesticide residues allowed for human consumption, called Maximum Residue Limits (MRLs). Consequently, a crop that meets the MRL for the United States may be above the legal limit for a different country, and the shipment will be rejected. With crops like berries, there is a very narrow window of time in which an alternative purchaser of the crop can be found for a rejected shipment, often resulting in substantial economic loss (Haviland & Beers, 2012).

Since *D. suzukii* is a recent pest, effective and specific pest management strategies are currently being tested to help reduce *D. suzukii* populations. Many growers rely on insecticides, including those in the families: organophosphate, pyrethroid, carbamate, and spinosyn (Beers, Van Steenwyk, Shearer, Coats, & Grant, 2011). These chemicals have been effective at controlling populations when timed and applied appropriately. However, current management relies on broadcast application, which is not a suitable long-term solution since over usage can lead to resistance, affect non-target organisms, and pose a negative impact to the environment (Desneux, Decourtye, & Delpuech , 2007).

**1.4 Integrated Pest Management**. Integrated pest management (IPM) (Dent, 1995) offers a counter strategy for pest management that reduces the risks of broadcast insecticide spraying and a single tool management system. An IPM plan strives to develop and promote the use of multiple tools working together to create a pest management strategy that is ecologically and economically sound with the smallest environmental impact. The best practices to help manage a pest population are being identified by understanding: the pest's life cycle, biology, behavior and movement in the landscape, seasonal phenology, and criteria for host preference. IPM strategies have been used successfully in a variety of agricultural settings but are not currently available for managing *D. suzukii*, a new pest.

A key IPM tool used in agriculture is monitoring, which evaluates the presence and level of pest populations (Dent, 1995). Monitoring a pest can help determine the timing and necessity of treatments, and strive to predict potential infestations. Traps designs and baits are currently being tested to monitor for *D. suzukii* that have sensitivity to low populations, capture higher numbers of females, attract higher numbers of *D. suzukii* than the fruit crop, trap flies effectively without escape, and detect the arrival of surviving overwintering populations. Growers are in need of a durable, easy-to service, economical way to effectively detect, identify, and limit the population of *D. suzukii*. In the future, a good trap design with an attractive bait can be used to manage *D. suzukii* populations by utilizing mass trapping (El-Sayed, Suckling, Wearing, & Byers, 2006) or attract and kill technology (El-Sayed, Suckling, Byers, & Wearing, 2009), which is using a baited trap that contains a low dose of insecticide.

**1.5 Efficacious Trap Design**. Lee et al. (2012, 2013), who led a regional trapping study across the United States, and Basoalto et al. (2013) found that traps that incorporate the colors red, yellow, and black, have a larger fly entry area, and a large bait surface area attracted higher numbers of *D. suzukii.* Unpublished research by Whitener and Beers (2014) indicates that trap capture does not have a linear increase with surface area – intermediate surface area traps brought in higher counts of *D. suzukii* than both smaller and larger surface area. However, they did find that trap capture increased with bait volume. Malmon et al. (2008) determined that flying *Drosophila* are attracted to long vertical objects. Another trap component that appears to impact *D. suzukii* trap capture is the headspace, or the volume of air between the surface of the bait and the entry holes. 950 mL clear deli cup traps with a low headspace (418.47 $cm^3$) brought in higher numbers of *D. suzukii* than similar traps with additional collection container, resulting in a high headspace (892.61 $cm^3$) (Figure 8) (Marek & Dreves, 2014).

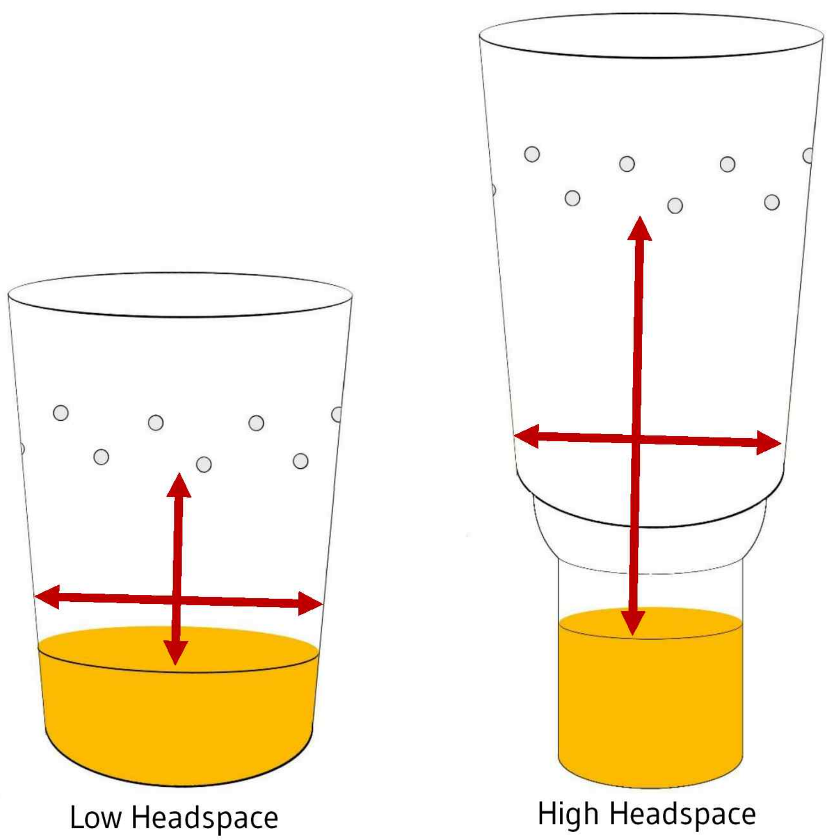



Figure 8. A clear 10-hole trap with low headspace (418 $cm^3$) caught significantly more *D. suzukii* than a clear 10-hole trap with a high headspace (893 $cm^3$).

Past research has shown several trap types to have the potential to catch *D. suzukii*. One commercially available trap, CAPtiva "Zorro", had exceptionally high numbers of *D. suzukii* for such a small trap - 250 mL, the size of a spice jar (Basoalto, Hilton, & Knight, 2013). In addition to the small total trap volume, the trap has an increased number of entry holes. Another commercially available trap, a bottom entry yellow McPhail-type or MultiLure® dome traps, was used in Oregon with success (Landolt, Adams, & Rogg, 2011). In Hawaii, the Multi-Lure trap yielded promising results, catching high numbers of *D. suzukii* in a tephritid fruit fly food lure trial (Leblanc, Vargas, & Rubinoff, 2010). However, Lee et al. (2013) noted that significantly more *D. suzukii* were caught in traps with side entry than traps with bottom entry, like the McPhail, as well as higher numbers of *D. suzukii* in traps with a mesh entry compared to entry holes.

**1.6 Preliminary Bait Preference.** The standard bait initially used in most traps across the United States upon arrival of *D. suzukii* is pure apple cider vinegar (5% acetic acid). According to Dreves (unpublished data 2010-2013) a solution of sugar water and dry active baker's yeast, *Saccharomyces cerevisiae,* attracted significantly higher numbers of *D. suzukii*, especially in warmer months (April through November), than the standard apple cider vinegar in years 2011 - 2013. Recent research indicates that the yeast *Hanseniaspora uvarum* is present in *D. suzukii* larval frass, adult midgut, and on several host fruit in higher quantity than any other yeast variety, including *S. cerevisiae* (Hamby, Hernandez, Boundy-Mills, & Zalom, 2012). Additional research performed by Leblanc (2010, 2011) using commercialized baits including Torula Yeast Pellets® bait and BioLure® (consisting of three chemical sachets: ammonium acetate, trimethylamine hydrochloride, and putrescine), had high *D. suzukii* catch in traps placed in warm and humid climates of Hawaii (Leblanc, Vargas, & Rubinoff, 2010). However, research from southern France, which typically has less humidity than Hawaii, reveals contradictory results with the use of Torula Yeast Pellets®, showing a consistently poor performance as a bait for *D. suzukii* (Botta, et al., 2013). Unpublished research by Yang et al. (2012) reported that Chinkiang vinegar, a glutinous rice based vinegar, consistently brought in higher counts of *D. suzukii* compared to apple cider vinegar, even when apple cider vinegar included additives. A lure was developed based on known *D. suzukii* attractants using four chemical compounds: acetic acid, methionil, acetoin, and a drowning solution of 95% ethanol (Cha, et al., 2013). Researchers are striving to find an ideal bait with high specificity to *D. suzukii*, especially females, the ability to persist in the field so it does not need to be changed every week, is easy to service, and has high visibility of flies to a viewer in the field.

**1.7 *D. suzukii* Cues.** *Drosophila* utilize multiple sensory cues in order to hone in on food or oviposition sites, the use being determined based on availability with or without the presence of other cues. The ability of *Drosophila* to find a food or oviposition source depends on factors including olfactory and visual cues, the presence of wind, and the density of the population.

Becher and colleagues (2010) described *D. melanogaster*, a close cousin of *D. suzukii*, responding to chemical signals such as food and baits using long range upwind flight because of *Drosophila*'s rather narrow or close range attraction capabilities. Stimuli such as visual, color, shape, and capacity of a bait to volatize and release odors play an important role in attracting flies. *Drosophila* are attracted to odors from yeast and vinegar. Acetic acid volatiles, present in vinegar, are reported to attract *D. melanogaster* (Becher, Bengtsson, Hansson, & Witzgall, 2010). *Drosophila* get a significant portion of their diet from yeast, and Becher et al. (2012) found this amounted to a strong attraction of *D. melanogaster* to yeast.

*Drosophila* have demonstrated the ability to orient, by flying and walking in a zigzag motion or by flying upward, into odor plumes that are continuous or at high frequencies, but do not respond to low frequencies. *Drosophila* utilize vision as well as antennae to determine the direction of wind, flying crosswind in a constant wind and flying up or downwind in a shifting wind environment (Gaudry, Nagel, & Wilson , 2012). Egg-laying female *D. melanogaster* lay more eggs in fruit that has already been infested, and Rockwell and Grossfield (1978) found that as the population density of *D. melanogaster* increases, resulting in an increase in aggregation pheromone, each female lays a significantly higher number of eggs (Rockwell & Grossfield, 1978). The sensory receptors most prevalent in flies are associated with fruit, such as yeast and vinegar volatiles, and social odors, including the aggregation pheromone (Gaudry, Nagel, & Wilson , 2012), allowing *Drosophila* to cue in for oviposition with sensitivity to low concentrations of odor.

**1.8 Research Goal and Objectives.**

The goal of this research is to contribute to the development of IPM tools for *D. suzukii*, including detection and eradication, in order to ultimately reduce insecticide usage.

The three objectives include: 1) test baits for high trap counts, specificity to *D. suzukii* with female preference, 2) improve trap design for monitoring adult presence, and 3) deliver results to researchers, students, growers and the backyard gardeners via educational outreach and engagement.

## CHAPTER 2: Materials & Methods

All field research was conducted in 2013.

### 2.1 Experimental Sites

Site 1. Blueberry site

A 2.3 ha monoculture, u-pick, no-insecticide spray, drip-irrigated multi-cultivar blueberry farm in Corvallis, Oregon (Benton County, OR; mid-Willamette valley. 44.6, -123.3) (Figure 9) was chosen for a bait trial site. Conventional fertilizers and herbicides were used. This trial was placed in 'Spartan' cultivar blueberry bushes which were planted in 1999 and 2000. The baited traps, each placed three meters apart, were set in four replicated blocks separated by 40 meters within a randomized complete block design.


Figure 9. A 2.3 ha u-pick blueberry farm, 'Spartan' cultivar, bait study. Four replicates are indicated in purple lines.

Site 2. Wild 'Himalaya' blackberry site

A mature, wild 'Himalaya' blackberry (*Rubus armeniacus*) bramble adjacent to an 8 ha diversified, organic farm in Corvallis, Oregon (Benton County, OR; mid-Willamette valley. 44.6,-123.3) (Figure 10) was selected for the trap design and late-season bait studies. Four replicates in a randomized block design were implemented. The blackberry bramble was situated 10 – 100 meters from hoop houses.

For details of vegetation diversity and abundance within the 'Himalaya' blackberry bramble, see Appendix 1- Table 1.

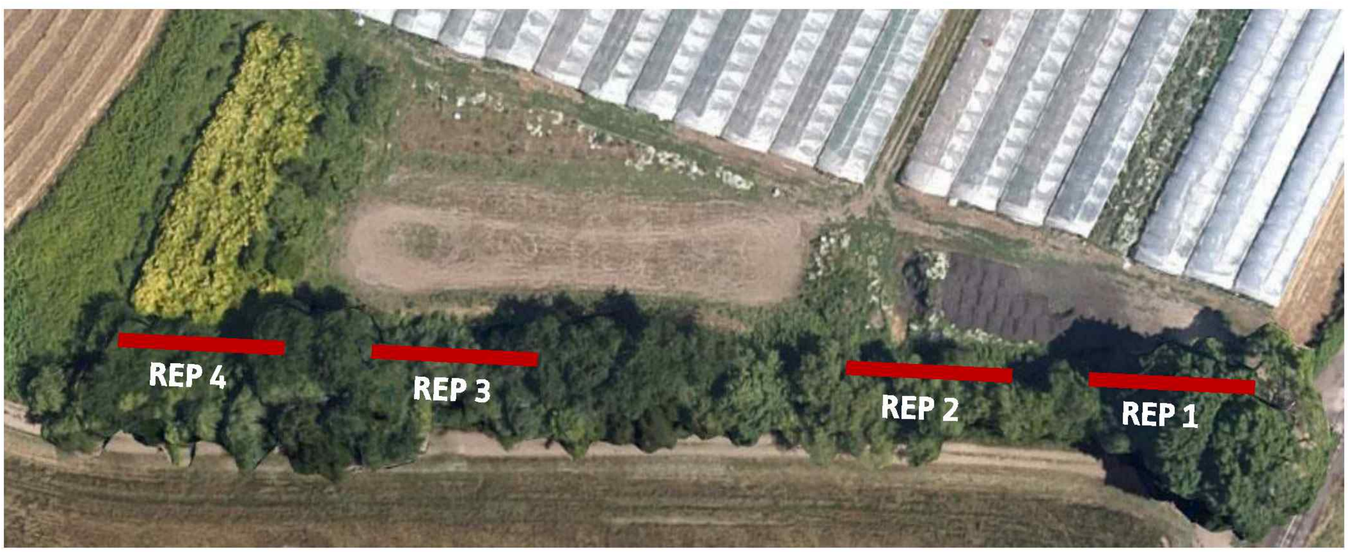



Figure 10. Wild 'Himalaya' Blackberry border adjacent to 8 ha organic diversified farm.

Site 3. Cherry site (Dr. Jana Lee, USDA-ARS, Horticultural Crops Research Unit)

A mature 0.5 ha cherry orchard, 'Early Burlette' cultivar, on an 8 ha diversified, organic farm in Corvallis, Oregon, described in site 2 (Benton County, OR; mid-Willamette valley. 44.6,-123.3; Figure 11) was selected for the bait study. Four replicates in a randomized block design were implemented.  Traps were placed in alternating trees, 8.5 meters apart. Rows were separated by 5.5 meters.

Figure 11. 0.5 ha of cherry on an 8 ha organic diversified farm.

Site 4. Raspberry site (Colleen Burrows, Washington State University Whatcom County Extension)

Drip-irrigated raspberries, 'Meeker' cultivar, on an organic farm in Whatcom County, Washington, (48.8, -121.9) were selected for the bait study. Three replications of traps were placed approximately 3 meters from the field edge on the crop border and each trap spaced approximately 9 meters apart.

## 2.2 Environmental Conditions

Temperature (°C) and precipitation (cm) were obtained throughout the study period May 21 – October 31, 2013 from the US Bureau of Reclamation AgriMet weather data from the Corvallis, Oregon AgriMet Weather Station (CRVO) (Benton County, OR; mid-Willamette valley. 44.63416, -123.19) located 4 km north of the wild 'Himalaya' blackberry site and 4.8 km north of the blueberry site.

## 2.3 Test Baits.

To learn more about a bait's attractiveness to *D. suzukii*, two field tests were conducted. Nine baits were compared over 12 weeks in 'Spartan' cultivar blueberry (site 1). The resulting top four baits with highest trap catch were selected for a five-week late-season trial across wild 'Himalaya' blackberry (site 2).

Traps were baited and assessed in early-season (May 23-July 11); mid-season (July 18-August 22); and late-season (September 12-October 10). The study period was divided based on abundance shifts (generation changes), also predicted by a degree-day model (Coop et al., 2014). Nine baits were tested during the early-season and mid-season period: apple cider vinegar (5% acetic acid), Chinkiang vinegar, a yeast and sugar water solution of *S. cerevisiae*, a *H. uvarum* concentration with sugar, BioLure® (3 sachets as described in the introduction), Torula Yeast Pellets® bait, Monterey Insect Bait®, Suzukii Trap®, and a 4-compound hanging lure, which is comprised of had two 2 mL vials containing acetic acid, methionil, and acetoin and a 95% ethanol drowning solution. This lure will be referred to as the "Cha-Landolt" lure. Details of bait composition and manufacturer can be found in Appendix 1 - Table 2. The sample of *H. uvarum*, isolated from grape skins in Oregon by members of Dr. James Osborne's enology lab at Oregon State University, was prepared fresh every week (see Appendix B *H. uvarum* Bait Preparation). The BioLure®, Torula Yeast Pellets® Bait, and "Cha-Landolt" lures were prepared in large batches for two weeks of use. Each bait was placed in a side-mesh trap (two 4 cm by 9 cm mesh side entry areas on a standard 950 mL clear deli cup with lid) with three vertical red stripes, based on Oregon

trials in which a clear side mesh trap caught more flies than other trap types tested (Lee, et al., 2012). The vertical red stripes were added to increase attractiveness (Figure 12), as the color red was found to increase trap capture.

Figure 12. Side mesh trap with vertical stripes used in all bait trials. Photo courtesy A. J. Dreves, Oregon State University 2013.

All baited traps were rotated spatially between eight trap positions each week in each of 4 replications, all of which were placed in rows of 'Spartan' cultivar, an early blooming blueberry cultivar.

Each trap contained 160 mL of selected bait pre-season (May 30 – July 3), but bait volume was consistently low at collection due to high evaporation from high temperature and wind was increased mid-season (July 2 – August 27, blueberry crop). Bait volume was increased to 175 mL and traps were placed deeper within the plant canopy where they were protected from wind and sun to reduce evaporation.  Traps were serviced weekly by collecting contents into collection containers which were transported to the lab. New bait, lure, or drowning solution was added weekly to the traps, with Torula Yeast Pellets® bait being an exception as it was prepared fresh every other week and week-old solution stored in the lab was used on the alternative weeks. BioLure® and "Cha-Landolt" lure baits were replaced every other week. Torula Yeast Pellets®, Chinkiang vinegar, BioLure®, Monterey Insect Bait®, and Suzukii Trap® were commercially available, apple cider vinegar, Chinkiang vinegar,  were commercially available products reconstituted as baits, and "Cha-Landolt" lure, *H. uvarum,* and *S. cerevisiae*, were prepared in the lab. Each trap was evaluated for male and female *D. suzukii* and other *Drosophila* species.

The late-season trial utilized the same spacing between traps and reps, and took place at site 2 (wild 'Himalaya' blackberry). The baits used were: *H. uvarum*/sugar/water solution, "Cha-Landolt" lure, apple cider vinegar, Suzukii Trap® bait, and Chinkiang vinegar. Each was prepared and used as discussed earlier.

Corresponding studies took place in cherry (Jana Lee, USDA-ARS, Horticultural Crops Research Unit) and in raspberry (Colleen Burrows, Washington State University Whatcom County Extension). These studies also utilized side-mesh traps 160-175 mL of selected bait. Treatments were randomized, traps were serviced weekly as described above, and contents were assessed for *D. suzukii* females and males, as well as other *Drosophila* species.

The cherry trial compared the following baits: apple cider vinegar, BioLure®, "Cha-Landolt" lure, Chinkiang vinegar, *H. uvarum*, Monterey Insect Bait®, *S. cerevisiae*/sugar/water solution, Suzukii Trap®, and Torula Yeast Pellets® bait. Trap positions were randomized and serviced weekly, contents evaluated for *D. suzukii* females and males and other *Drosophila* species.

The raspberry trial compared the following baits: apple cider vinegar, BioLure®, *S. cerevisiae*/sugar/water solution, Monterey Insect Bait®, Suzukii Trap® bait, and Torula Yeast Pellets® bait.

### 2.4 Improve Trap Design

**2.4.1.** Promising Trap Designs

In order to improve the understanding of *D. suzukii* preferences to a trap design, eight promising trap designs were placed in the bramble of wild 'Himalaya' blackberry (site 2). Traps were selected by taking into account efficacious trap qualities including a small headspace, increased fly entry area for greater volatilization, and a combination of red, yellow, and black colors. Figure 13 shows the trap types selected for this study. See Appendix 1 - Table 2 for detailed parameters on all trap types.

"Clear 20-Hole", "Lucky 13"-Hole, and the "Squatty Botty" fly trap all had similar, small headspaces. The standard "Clear 10-Hole", "Clear 20-Hole", Red "6 Hole", "Lucky 13" Hole, and CAPtiva "Zorro" had entry areas made up of multiple 5mm diameter holes. "Dreves Side Mesh", "Squatty Botty" fly trap, and MultiLure® had an entry with a mesh grid of 2x2 mm entry areas.


Figure 13. The 8 trap designs tested in wild 'Himalaya' blackberry adjacent to an 8 ha diversified, organic farm in Corvallis, OR.

Each trap type was separated from one another by 3 meters. The eight trap designs were replicated four times, blocks separated by 40 meters. This study occurred over three time periods: early-season (4 weeks; May 21-July 9), mid-season (5 weeks; July 30 - August 27), and late-season (4 weeks; September 12- October 10).

Each trap contained 175 mL of a yeast and sugar water solution of *S. cerevisiae* prepared 1-2 hours before servicing. Each trap was serviced weekly by collecting contents into containers which were transported to the lab. New *S. cerevisiae* bait was replaced in traps at time of weekly service. Male and female *D. suzukii* and other *Drosophila* were recorded.

A new 2013 trap design created in the Dreves' lab, referred to as the "Squatty Botty Fly Trap" also referred to as "Squatty Botty", took into consideration the physical features that appears to attract more *D. suzukii*. This new trap was tested along with the standard and successful traps, including: "Dreves Side-Mesh©" 950 mL clear deli, the standard "Clear 10-Hole" 950 mL clear deli, Red "6-Hole©" 530 mL red cup, "Clear 20-Hole" 950 mL clear deli, "Lucky 13"-Hole 530 mL red cup, CAPtiva "Zorro", and McPhail-type MultiLure®.

**2.4.2.** "Clear 20-Hole" Headspace

A lab trial was initiated to compare trap catch in low and high entry holes relative to the surface area of the bait within in a standard "Clear 20-Hole" trap in January 2014. Each trap contained 175 mL of apple cider vinegar. The low entry holes were placed 2.5 cm above the bait surface, with 140 $cm^3$ between bait and entry holes and 420 $cm^3$ between entry holes and top of trap.

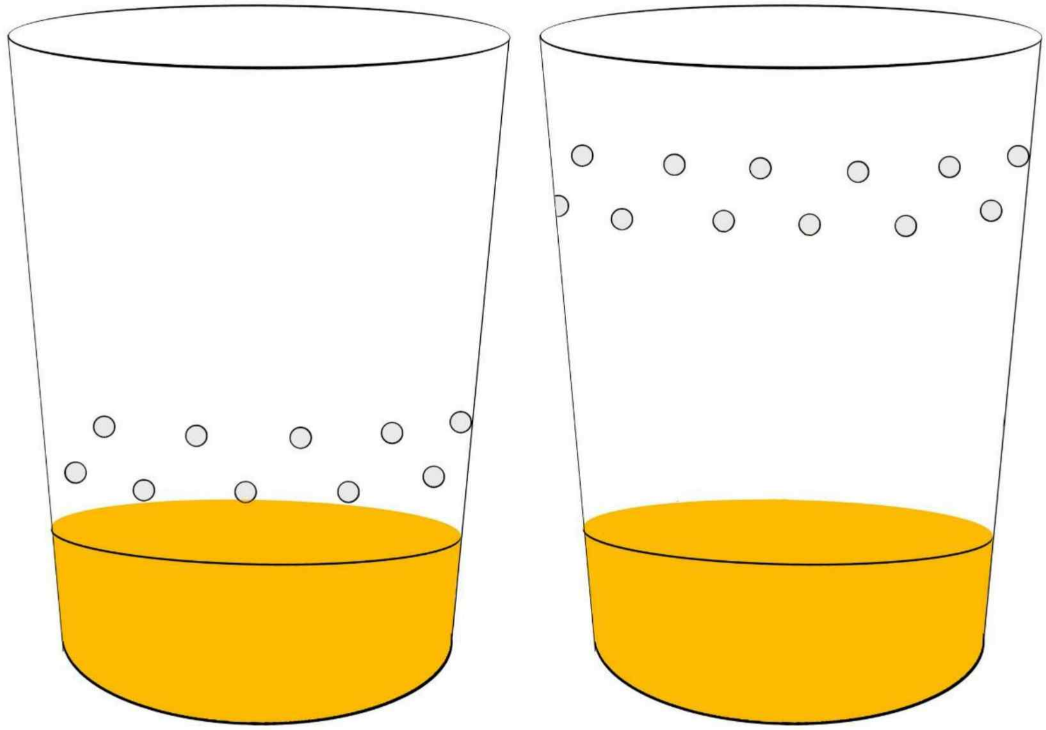


Figure 14. Graphic representation of "Clear 20-Hole" trap with 165 mL of bait with low headspace (left) and high headspace (right). Headspace volume calculation: Distance from bait surface to fly entry (x) Trap top surface area

The high entry holes were placed approximately 8 cm above the bait surface, with 520 $cm^3$ between bait and entry holes and 38 $cm^3$ between entry holes and trap top. Two hundred *D. suzukii* taken from the USDA-ARS Horticulture research unit laboratory in Corvallis were placed in a 1 m by 1 m screened observational cage (Bioquip, CA).Two replications of each trap type were replicated over a nine day period, totaling 18 replications. A Petri dish with water and a sponge was placed in each cage to provide hydration and moisture for the flies. Trap contents were collected and evaluated for total *D. suzukii* trap catch.

**2.4.3.** Attract-and-Kill

An additional study was conducted to determine the effect of adding a killing agent inside a "Squatty Botty Fly Trap" and a "Clear 20-Hole" trap. We observed that many of the flies entering the traps also exited the traps. This trial took place across the wild 'Himalaya' blackberry (site 2) and each was rotated and serviced weekly. Five treatments were tested: "Squatty Botty Fly Trap" with and without an organophosphate vapor-type insecticidal strip, "Clear 20-Hole" traps with and without the insecticidal strip, and "Clear 20-Hole" traps with soapy water only. "Squatty Botty Fly Trap" was tested three weeks mid-season (August 13-27) and five weeks late-season (September 12-October 10), and "Clear 20-Hole" was tested over three weeks late-season (October 10 – 31). The insecticidal strip was adhered to the top inner surface of the trap lid using aluminum tape and contents were assessed for male & female *D. suzukii* and other *Drosophila* species and compared against contents of the same trap type without a killing agent. A yeast sugar-water solution bait (175 mL) was added to all traps except for "Clear 20-Hole" traps which contained soapy water only.

## 2.5 Statistical Analysis

Trap count data (flies/trap/week) for the trap design study were analyzed by ANOVA using PROC GLM in SAS 9.2 (2003). Trap counts were transformed using Log (x + .50) or Sqrt (x + 0.50) to homogenous variances, when necessary. Bait study data were analyzed using the PROC Mixed model using trap counts as a random variable and collection dates, trap positions, and treatment as fixed variables. Means were separated using Tukey's HSD or Fisher's LSD means separation test. Means with the same letters, as designated in figures, are not significantly different. Significant block (replications), dates, and position effects were analyzed for significance in the studies ($p < 0.05$).

Proportions of male/female/other *Drosophila* species were calculated using Excel by adding total fly catch for each category per week and dividing by the overall total of all *Drosophila* species. Standard errors were determined by dividing the standard deviation of the data in question by the square root of the sample size. Additional statistics will be reviewed for analyzing datasets when preparing a future refereed publication.

# CHAPTER 3: RESULTS

## 3.1 Environmental Conditions

Temperature and precipitation throughout the three seasonal periods are presented in Figure 15. The temperature during the early-season period (May 21 – July 2) revealed lower temperatures (ranging from 7ºC to 21ºC, averaging 15.9ºC) and higher rainfall (accumulated 9.7 cm) than mid-season (July 2 – August 27) which had temperatures ranging from 11.75ºC to 30.5ºC, averaging 20ºC; and a low 0.28 cm of accumulated rainfall. The temperature over late season (September 5 – October 31) began to decline, with temperatures ranging from 5.6ºC to 27.4ºC, averaging 14.4ºC, while rainfall significantly increased to an accumulation of 17.8 cm. The photoperiod, which is number of hours of sunlight within a 24 hour period, declined as the season progressed. Early-season, the photoperiod was 15:02 – 15:29 hours, mid-season it was 15:21 – 13:28 hours, and late-season it was less than 13 hours (U.S. Naval Observatory, n.d.).

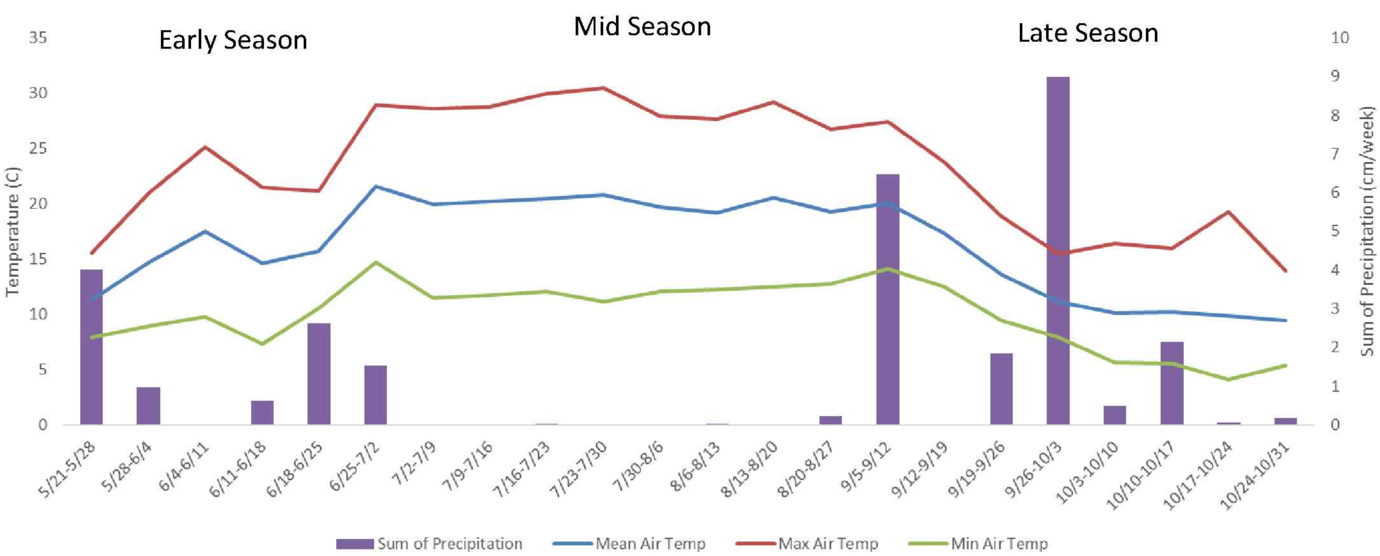



Figure 15. Weekly maximum, minimum, and mean temperature (°C) with the weekly sum of precipitation (cm) for the entire study period (May 21 – October 30) in Corvallis, Oregon.

Trap captures of *D. suzukii* increased as the growing season progressed, with the maximum trap captures occurring in early September, before decreasing from mid-September as mean temperatures dropped from 20ºC and above to 15ºC and below (Figure 16). The late-season increase in *D. suzukii* capture occurring in early September corresponds to a small decrease in temperature, a significant increase in precipitation, and a reduction in light hours. Precipitation increased from an accumulated 0.28 cm mid-season to 17.8 cm late-season.

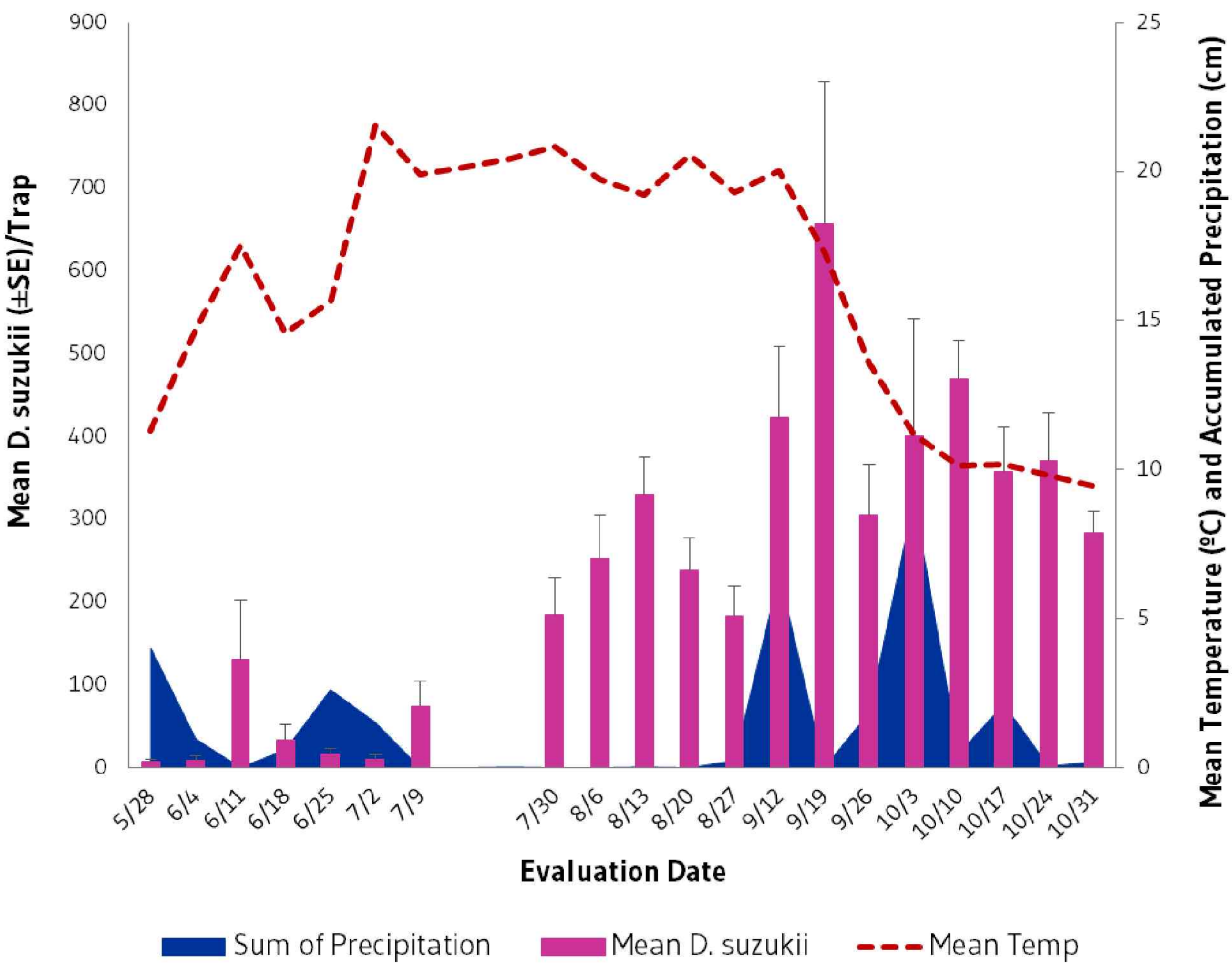



Figure 16. Seasonal mean *D. suzukii* catch in the "Clear 20-Hole" trap compared to accumulated precipitation (cm) and average weekly temperature (ºC).

Trap capture increased as host fruit reached maturity, with the greatest number of *D. suzukii* captures observed as the 'Himalaya' blackberry plant reached stages 8 – 9, which is when the berries are ripe to overripe (Figure 17, 18).


Figure 17. Photographic representation of the phenological stage 1 - 8 of wild 'Himalaya' blackberry. Stage 9 (overripe) and 10 (deteriorated) not pictured.

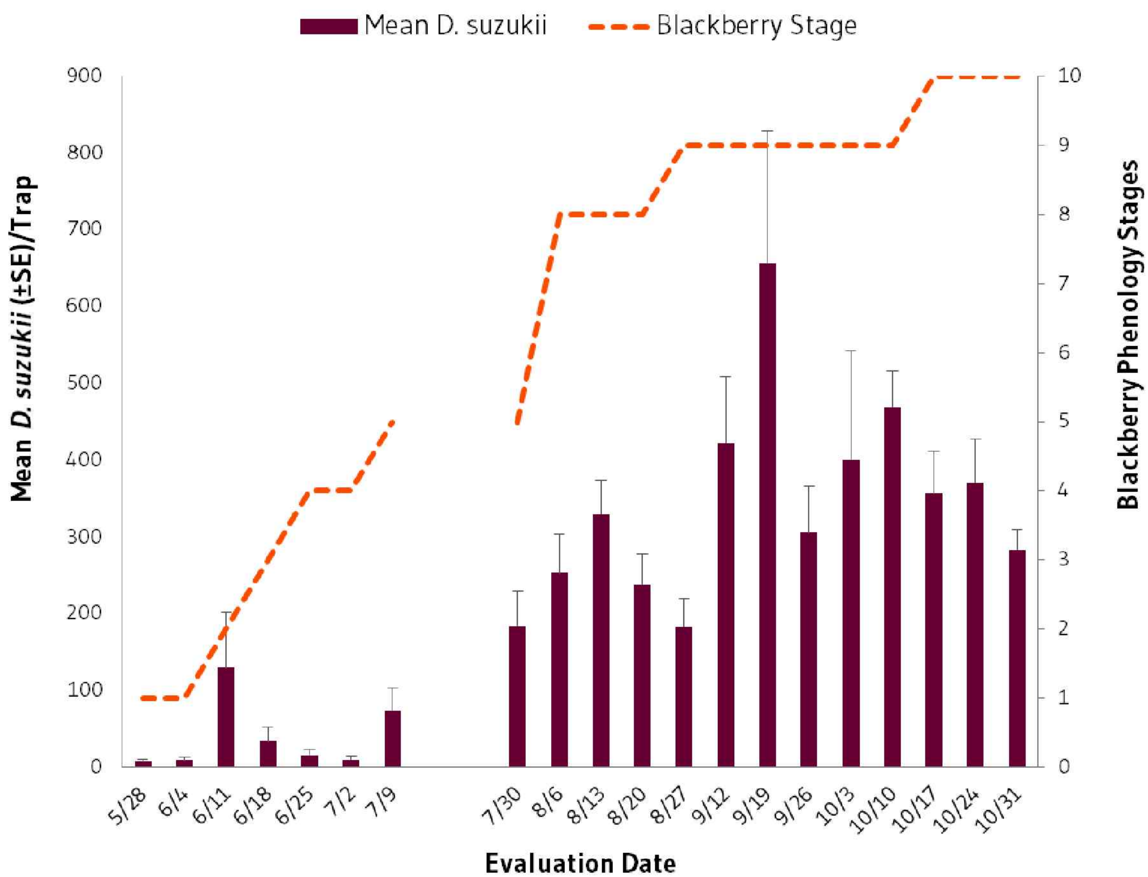



Figure 18. Weekly mean *D. suzukii* catch in yeast-baited "Clear 20-Hole" trap (bars) overlaid with blackberry phenology plant stage of 'Himalaya' blackberry (line). (Note: No data collected between 7/9-7/30)

### 3.2 Test baits

Chinkiang vinegar attracted numerically and consistently higher numbers of *D. suzukii* (0 - 75% higher than other baits) in the three fruiting crop sites: blueberry, wild "Himalaya" blackberry, and cherry (Figures 19 – 21; 24 – 27). When comparing yeast types in the blueberry crop, *H. uvarum* baited traps caught higher numbers of *D. suzukii* than *S. cerevisiae* (0 – 75% higher) all weeks except: May 30, in which only one *D. suzukii* was captured in all three yeast baits; June 20, which utilized an *H. uvarum* bait that failed to reach its maximum population due constraints within the lab; and August 22 (Figures 22 – 23). This final collection date in blueberry (August 22) showed a spike in Torula Yeast Pellets® bait efficacy, despite low collections throughout the season. Apple cider vinegar showed increased efficacy at the end of late-season period, catching higher numbers, jumping from 2.8% of all *D. suzukii* captured in the top four baits and apple cider vinegar mid-season in blueberries, to 16.2% of all *D. suzukii* in the same baits late season in blackberries. Late-season captures of apple cider vinegar baited traps placed in blackberry were lower than Chinkiang vinegar and *H. uvarum*-baited traps. Monterey Insect Bait® was ineffective at attracting *D. suzukii* early-season at all three sites (blueberry, cherry, raspberry) and was discontinued mid-season for blueberry and cherry after catching only one *D. suzukii* in the side-mesh trap in blueberry between the start date (May 30) and the final collection prior to being discontinued (July 3).

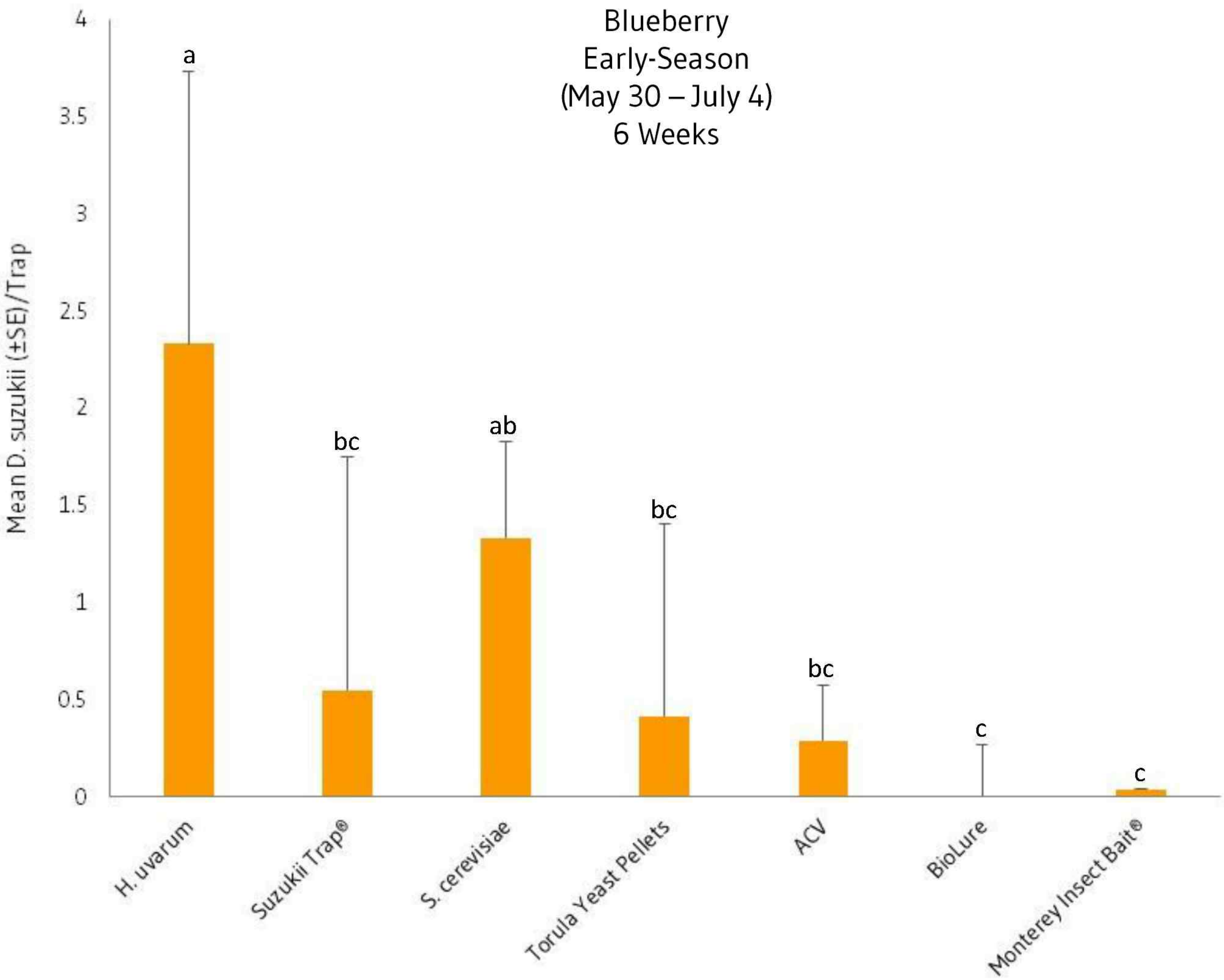



Figure 19. Comparison of seasonal mean *D. suzukii* catch of eight baits during early season (May 30 – July 4) in a blueberry crop. Note: "Cha-Landolt" lure not included because of a delayed setup of June 28 (F test: 7.79, df: 6, p-value: <0.0001).

The mean trap catch of *D. suzukii* of four baits in blueberry was compared to apple cider vinegar during mid-season period (July 11 – August 1). Chinkiang vinegar, *H. uvarum*, and "Cha-Landolt" lure caught the highest number of *D. suzukii* with no statistically significant difference. Chinkiang vinegar yielded a numerically higher catch, followed by *H. uvarum* (Figure 20).

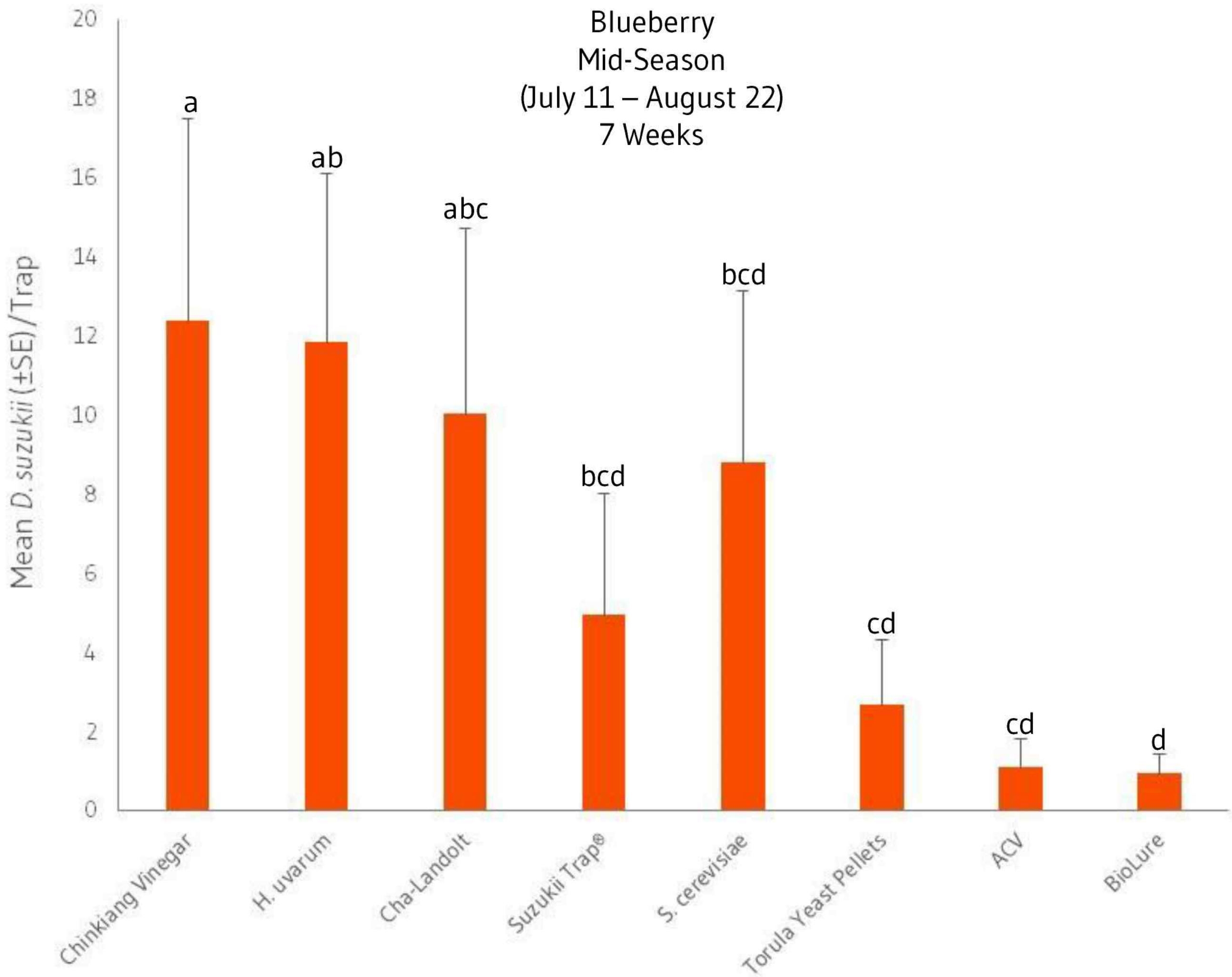



Figure 20. Mean *D. suzukii* (±SE) catch of all baits during seven weeks of mid-season (July 11 – August 1) in a blueberry crop, 'Spartan' cultivar (F test: 6.78, df: 7, p-value: <.0001). (Note: Suzukii Trap® bait was only tested from 7/11 – 8/1, due to delayed shipment from Spain).

A similar trend in trap catch where Chinkiang vinegar baited traps caught numerically but not statistically significantly the highest number of *D. suzukii* of all bait types. *H. uvarum* and apple cider vinegar baited traps caught high numbers of *D. suzukii* (Figure 18).

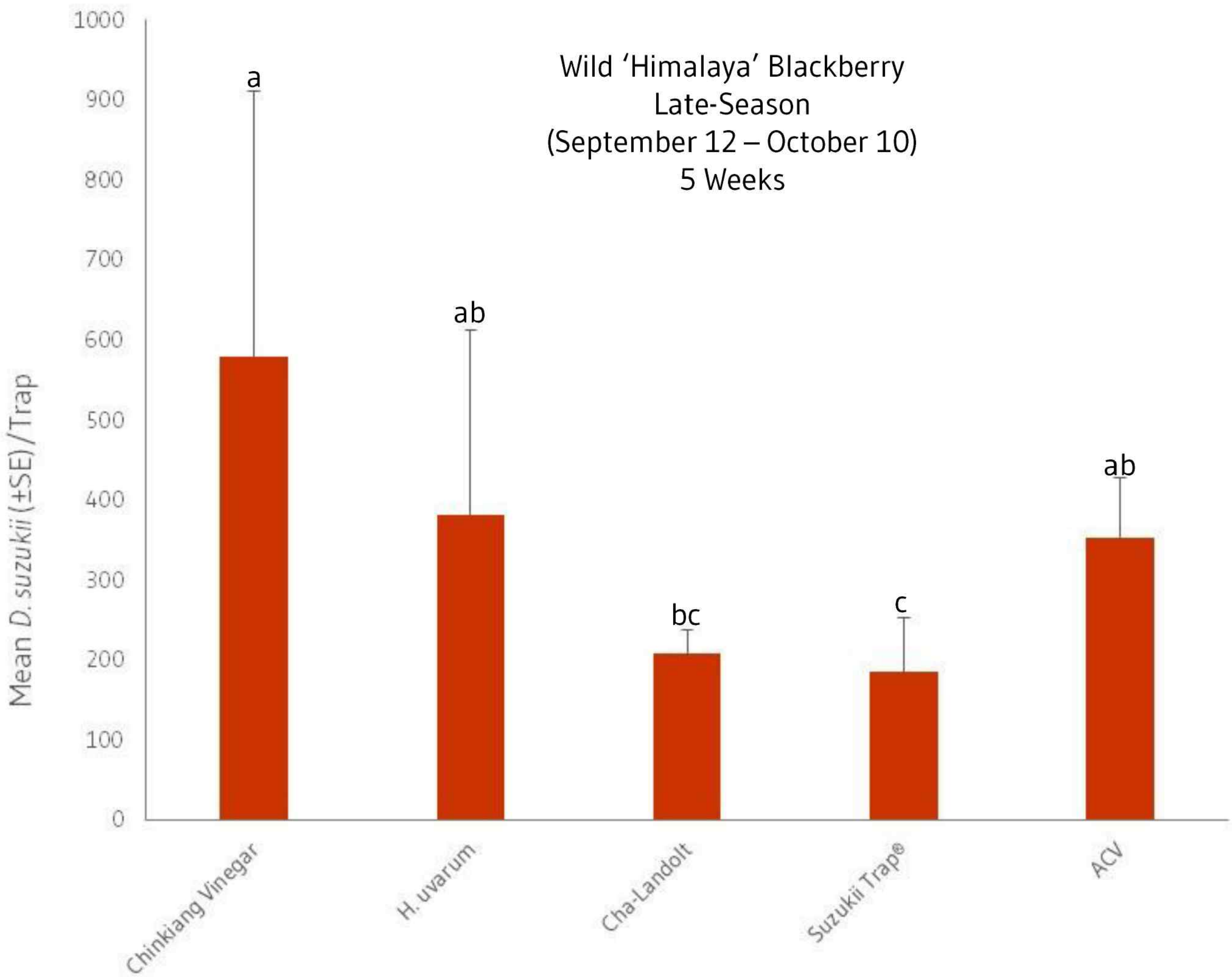



Figure 21. Mean *D. suzukii* catch for all baits during late season (9/12 – 10/10) in wild 'Himalaya' blackberry bramble (F test: 6.33, df: 4, p-value: 0.0005).

The mean *D. suzukii* capture using yeast baits (*S. cerevisiae*, *H. uvarum*, and Torula Yeast Pellets® bait) shows higher numbers caught by H. uvarum-baited traps most collection dates, and consistently low catches in Torula Yeast Pellets® baited traps. Four of the six weeks of early-season collections had higher numbers of *D. suzukii* using *H. uvarum*-baited traps than *S. cerevisiae* baited traps (Figure 22), however numbers are low and variable.

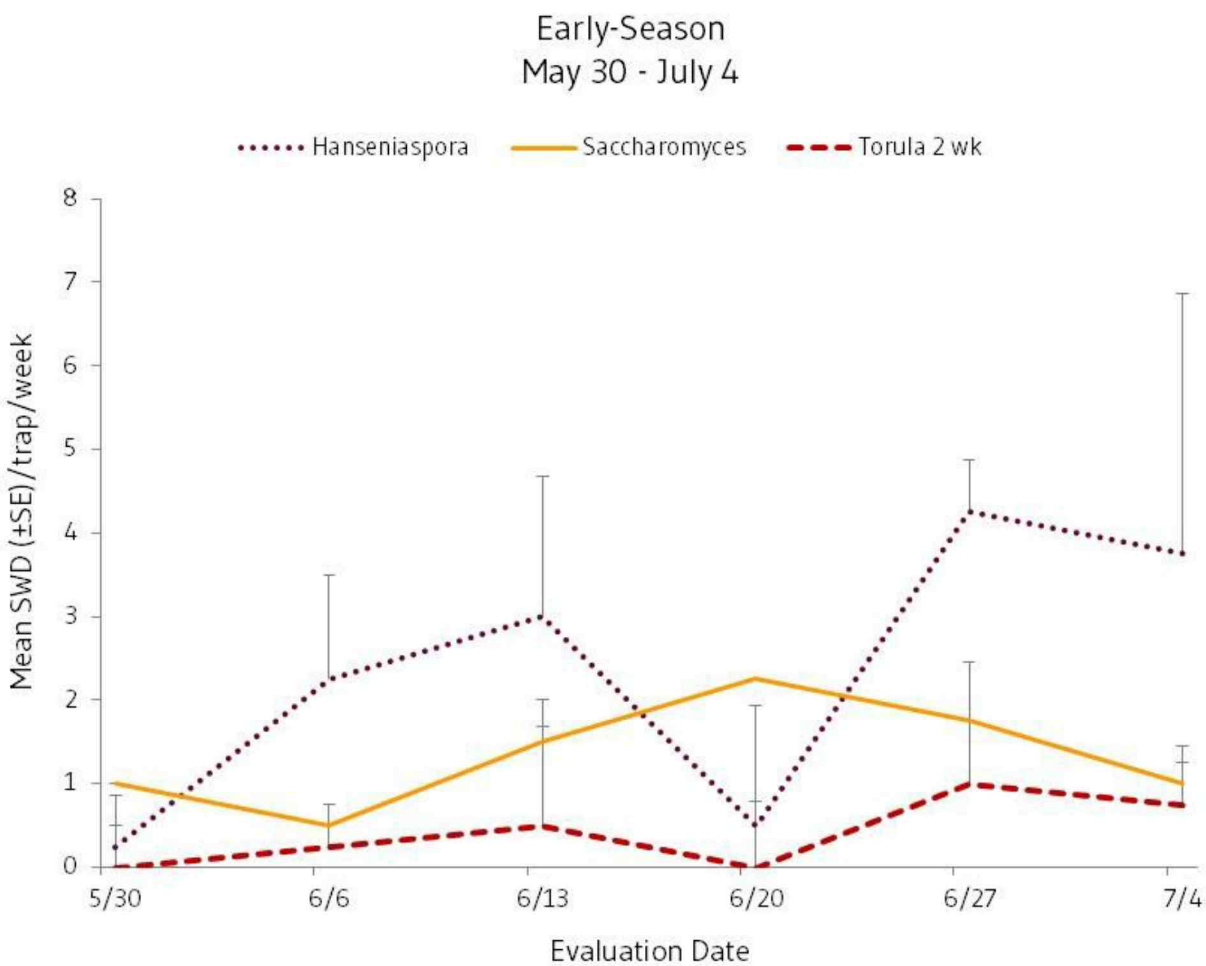



Figure 22. Comparison of mean trap catch for yeast baited traps during early-season (5/30 – 7/4) in a blueberry crop, 'Spartan' cultivar.

The yeast bait *D. suzukii* captures during the mid-season period (Figure 23) follow the same trends as early-season, where Torula Yeast Pellets® baited traps typically catching the lowest number of *D. suzukii* (0 to 16, averaging 3.3) and *H. uvarum*-baited traps typically catching the highest number of *D. suzukii* (1 to 7, averaging 4.6). However, at the final collection date (August 22) Torula Yeast Pellets® baited traps caught significantly more *D. suzukii* while *H. uvarum*-baited traps caught the least *D. suzukii*. This was an interesting result, however the pellets were not further tested after this collection.

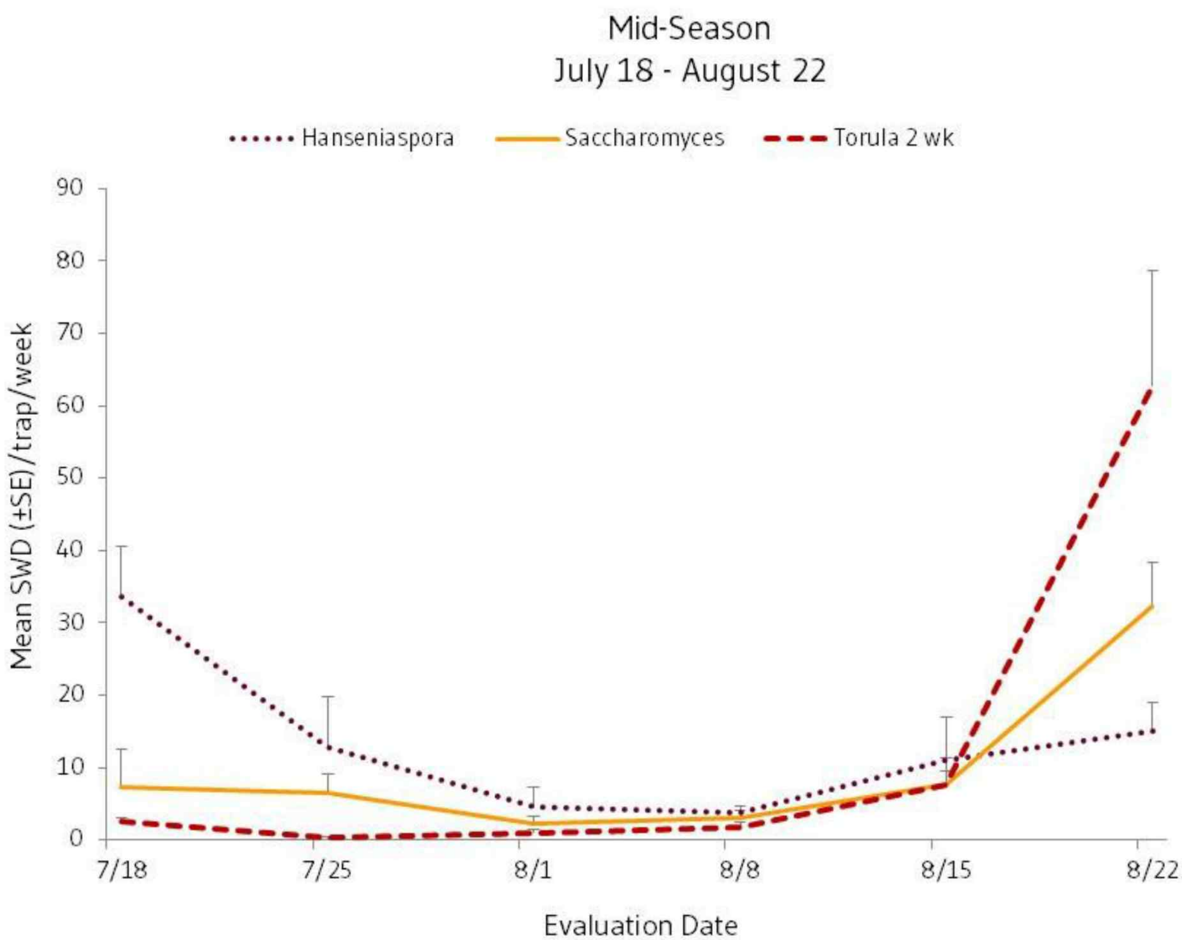



Figure 23. Comparison of weekly mean trap catch using yeast-baited traps during mid-season (7/18 – 8/22) in a blueberry crop, 'Spartan' cultivar.

Trap captures from the cherry trial led by Jana Lee (USDA-ARS, Corvallis) had similar proportions as those found in blueberry and blackberry, showing high catch in Chinkiang vinegar and *H. uvarum*-baited traps for the entire trial period. An increased efficacy was observed using the Suzukii Trap® bait. Numbers caught in cherries early-season (Figure 24) which were significantly higher than in blueberries (10 – 50 times greater trap catch) during the same time period.

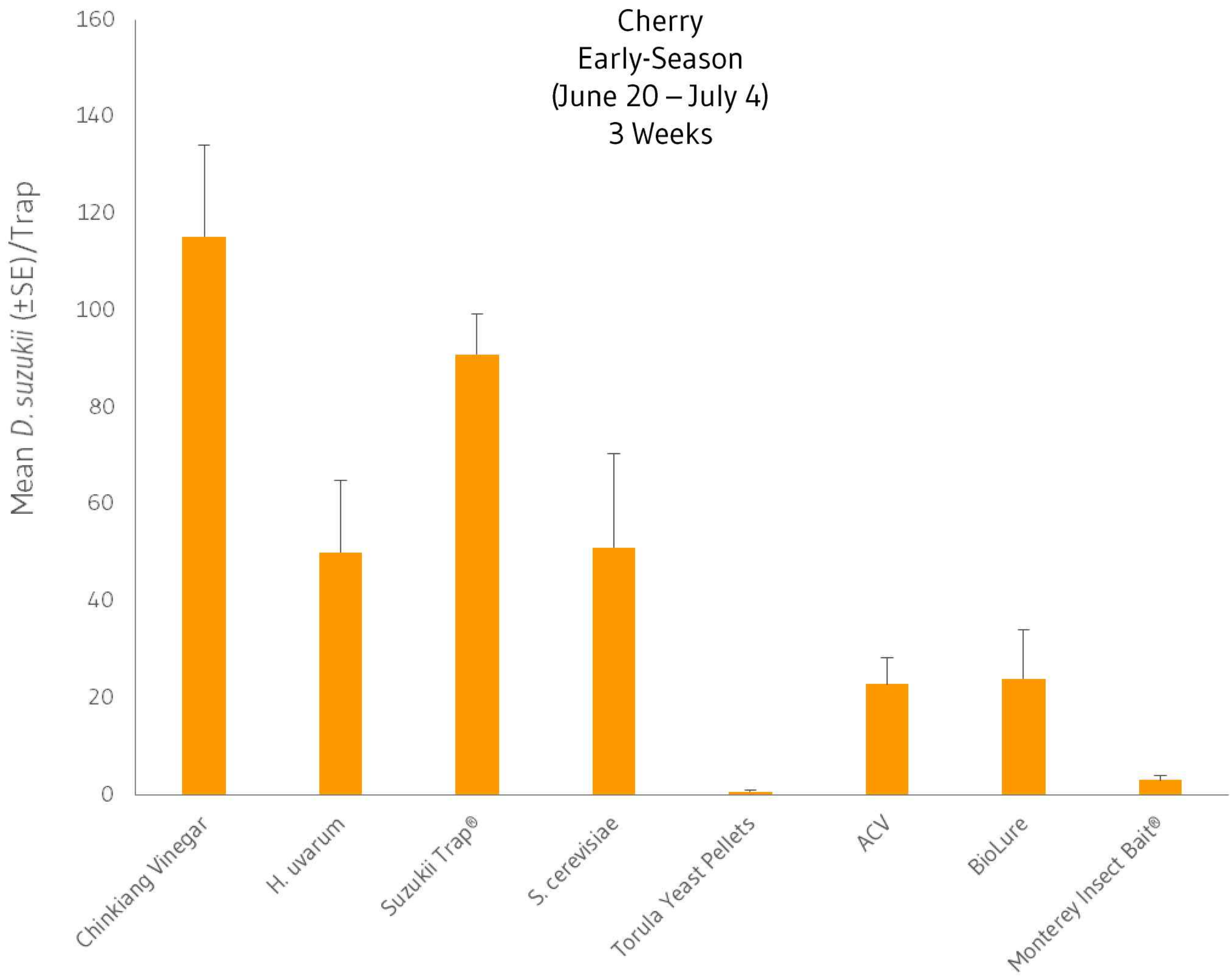



Figure 24. Comparison of seasonal mean trap catch for nine baited traps early-season (6/20 – 7/4) in a cherry crop, 'Early Burlette' cultivar. (Note: Suzukii Trap® bait was not present for July 4 collection, so no statistics have been done on this data set).

Mid-season traps had increased captures using Suzukii Trap® baited traps, catching significantly higher number of *D. suzukii* than all other baits (ranging from 40 to 428, averaging 215; Figure 25). After Suzukii Trap®, Chinkiang vinegar and *H. uvarum*-baited traps caught the highest numbers of *D. suzukii* (Chinkiang vinegar-baited trap catch ranged from 21 to 389 and averaged 174; *H. uvarum*-baited trap catch ranged from 24 to 331 and averaged 147). Moderate numbers of *D. suzukii* were captured in *S. cerevisiae*-baited traps (ranging 16 to 239, averaging 122) and "Cha-Landolt" lure-baited traps (ranging 14 to 120, averaging 70). Few to no *D. suzukii* were captured in traps containing apple cider vinegar (ranging from 3 to 63, averaging 30), Torula Yeast Pellets® bait (ranging from 0 to 1, averaging 0.3), and BioLure® (ranging 3 to 16, averaging 6).

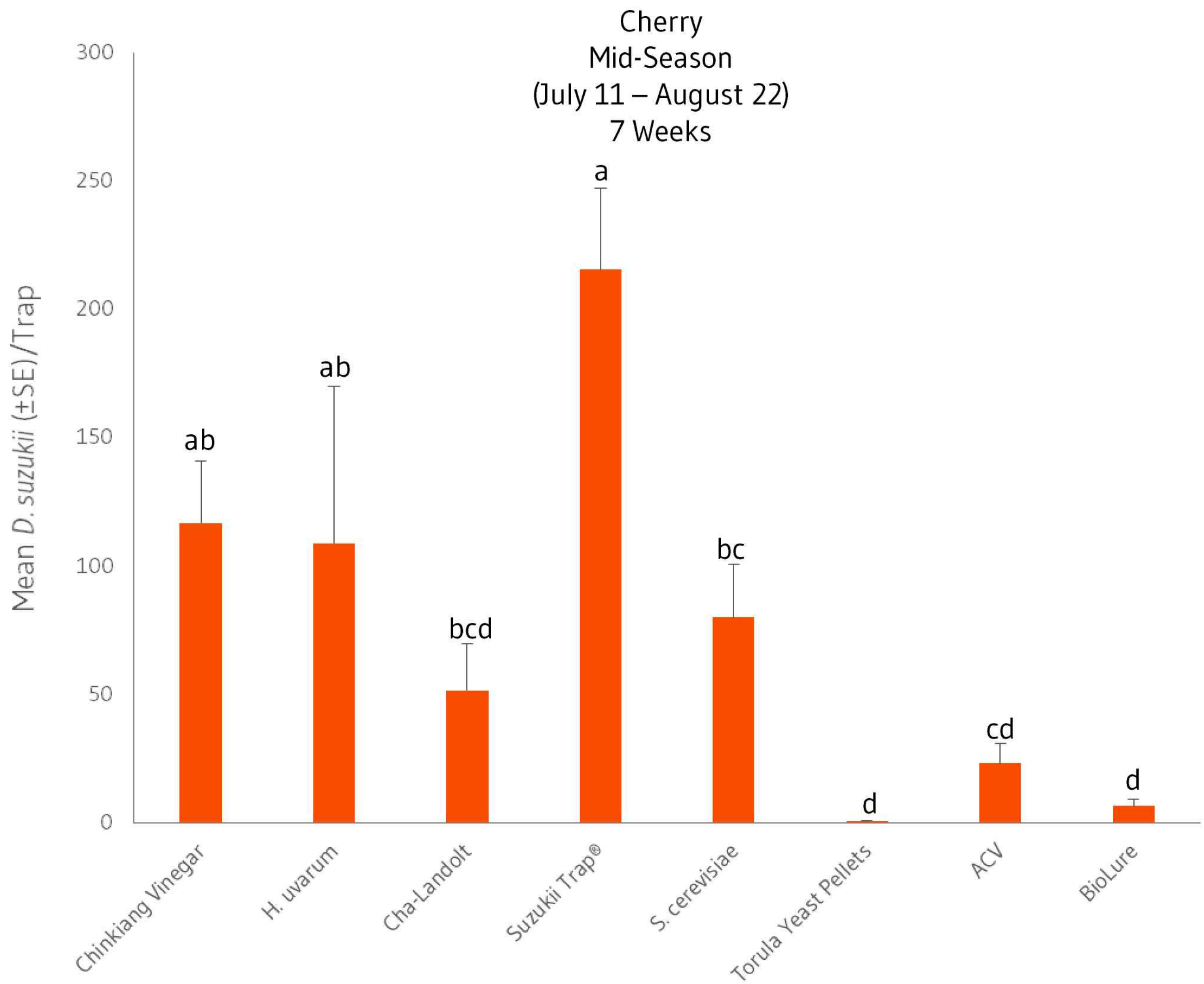



Figure 25. Comparison of mean trap catch using eight baited traps during mid-season (7/11 – 8/22) in a cherry crop, 'Early Burlette' cultivar (F test: 10.72, df: 7, p-value: <0.0001).

Like the blueberry and blackberry trials, the late-season cherry traps show increased trap catch in apple cider vinegar relative to mid-season captures (Figure 24). Chinkiang vinegar continued to attract the highest numbers of *D. suzukii* (ranging from 20 to 352, averaging 219), followed by *H. uvarum* (ranging from 125 to 193, averaging 158), Suzukii

Trap® (ranging from 43 to 200, averaging 108), and apple cider vinegar (ranging from 57 to 156, averaging 109). "Cha-Landolt" lure baited traps had the lowest number of *D. suzukii* captured (ranging from 8 to 266, averaging 86). The final collection date (10/3) showed a decreased capture for all baits other than Chinkiang vinegar.

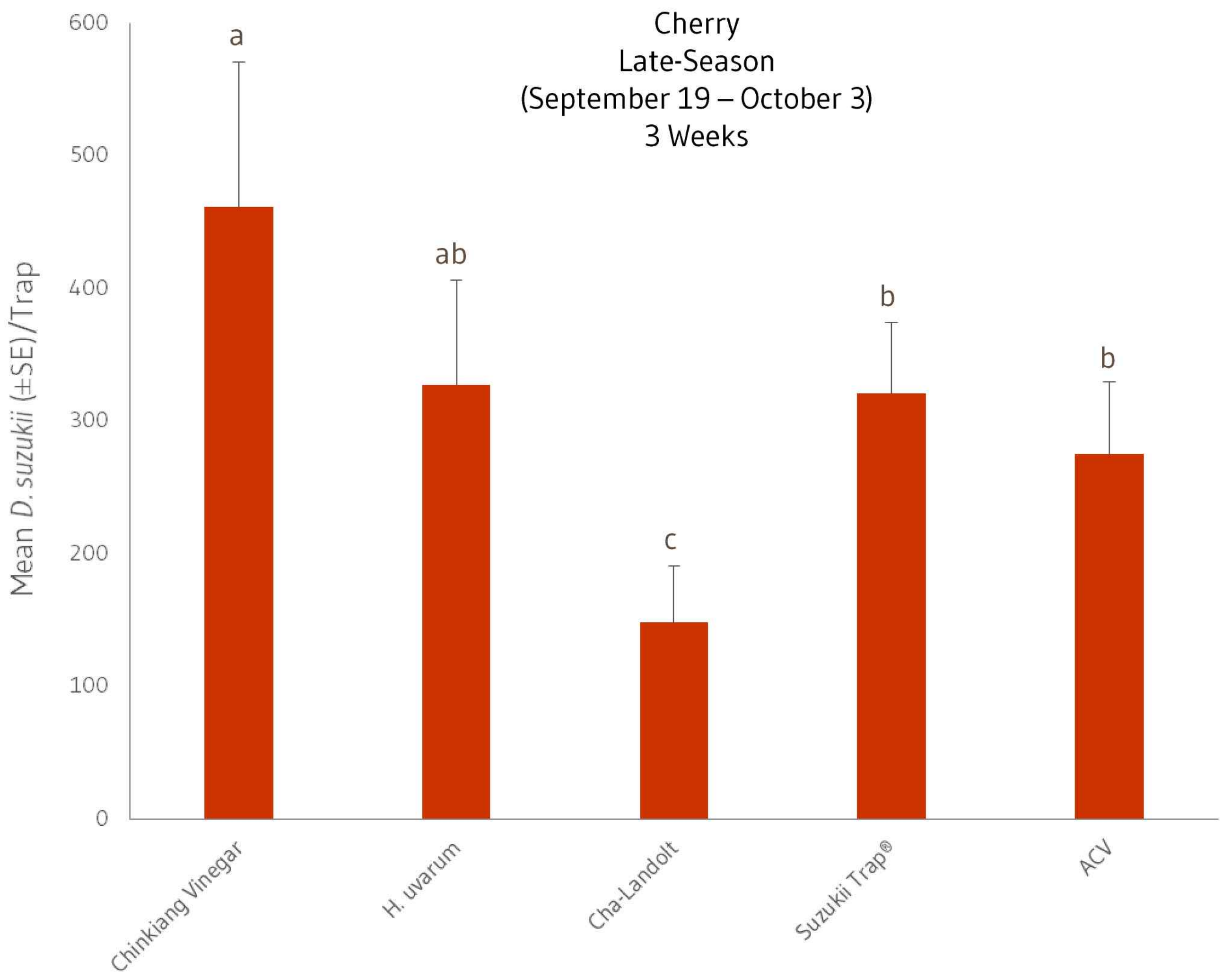



Figure 26. Comparison of mean trap catch using five baited traps late-season (9/12 – 10/3) in a cherry crop, 'Early Burlette' cultivar (F test: 10.23, df: 4, p-value: <0.0001).

A bait study in traps placed in raspberries led by Colleen Burrows (WSU Whatcom County Extension), showed high early-season and mid-season trap catch efficacy using

Suzukii Trap® bait (Figure 28). *S. cerevisiae* yeast baited traps caught higher numbers of *D. suzukii* than Torula Yeast Pellets® bait throughout the early-season. Monterey Insect Bait® and BioLure® once again performed poorly, catching 0-1 flies throughout the season.

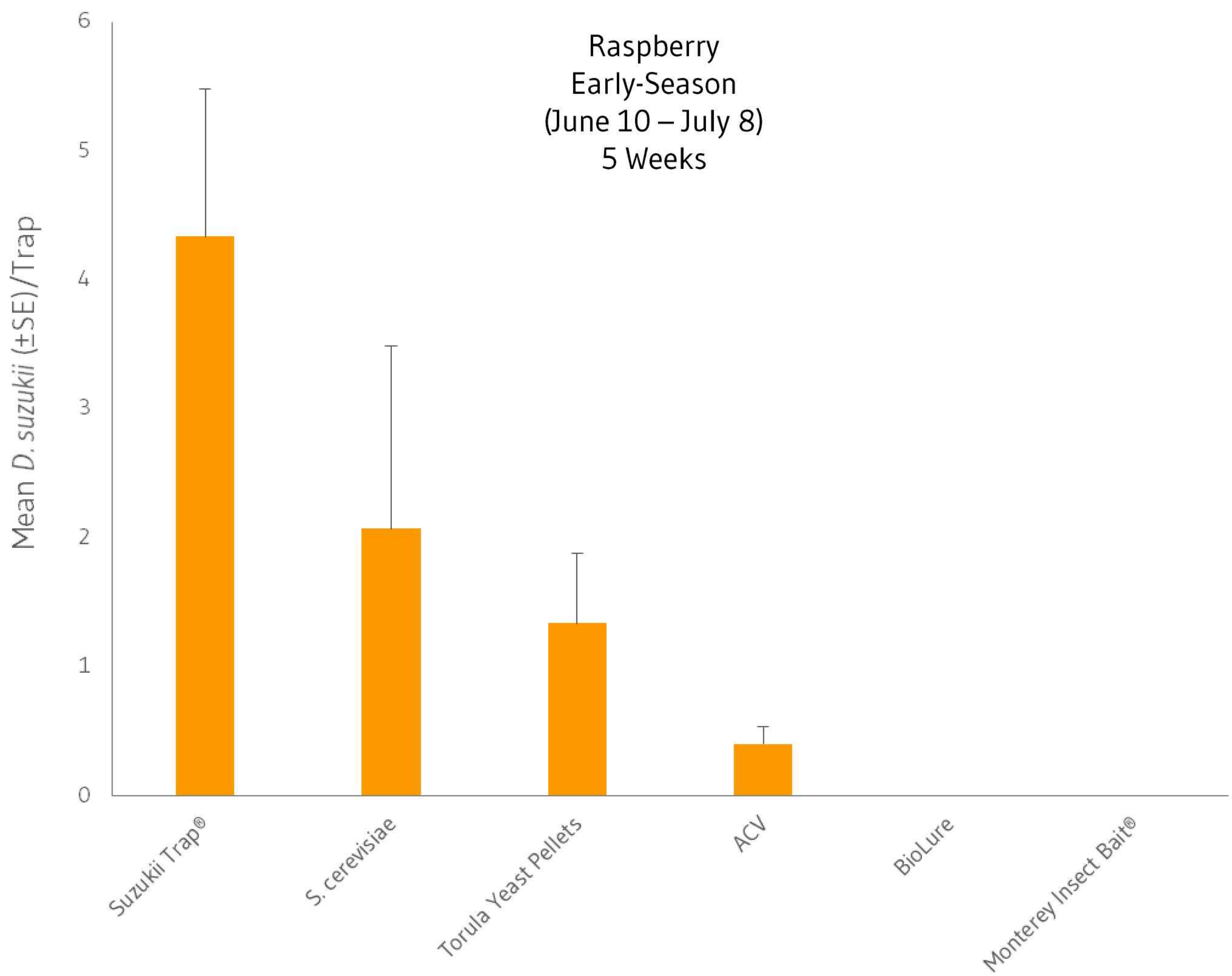



Figure 27. Comparison of mean trap catch using six baited traps early-season (6/10 – 7/8) in a raspberry crop, 'Everson' cultivar (F test: 3.26, df: 5, p-value: <0.01).

Mid-season captures followed the same pattern (Figure 28), however in the final collection Torula Yeast Pellets® baited traps capture (ranging from 0 to 7, averaging 1) increased to approximately the same count as *S. cerevisiae* (ranging from 0 to 8, averaging 2). Suzukii Trap® baited traps brought in significantly more *D. suzukii* than any other bait (ranging from 0 to 16, averaging 4). Few to no *D. suzukii* were captured by apple cider vinegar (ranging from 0 to 1, averaging 0.5), BioLure® (0 total captures), and Monterey Insect Bait® (0 total captures).

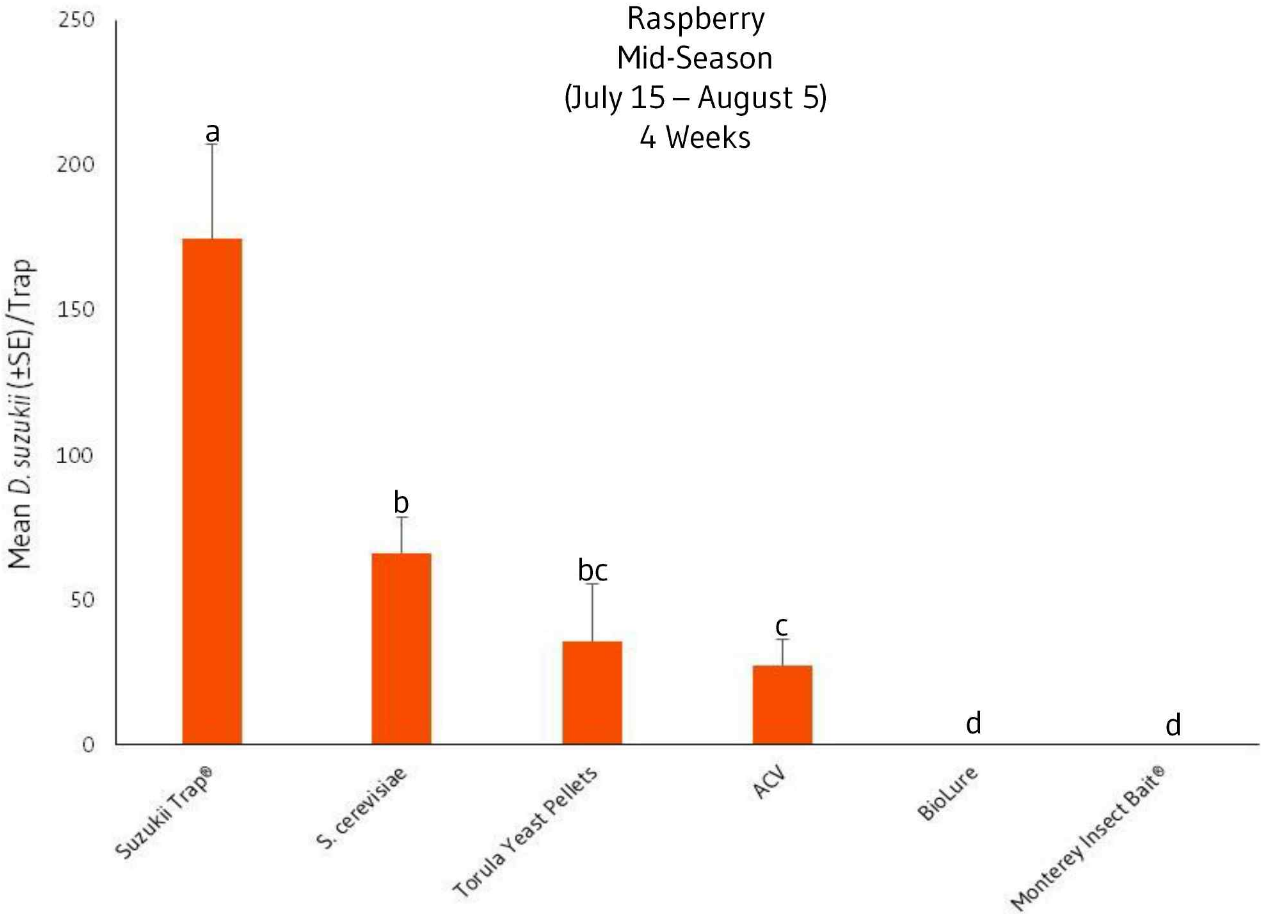



Figure 28. Comparison of mean trap catch for using six baited traps mid-season (7/15 – 8/5) in a raspberry crop, 'Everson' cultivar (F test: 52.5, df: 5, p-value: <0.0001). (Note: Due to uncharacteristically high numbers of Torula Yeast Pellets® the last week of mid-season, this graph does not include 8/12 collection data)

The *Drosophila* distribution of trap contents from traps placed in blueberry and wild 'Himalaya' blackberry between other *Drosophila* as well as male and female *D. suzukii* revealed seasonal shifts in all bait varieties. Early-season traps showed a significantly higher percentage of other *Drosophila* species (66 - 100%), whereas late-season counts shifted in favor of *D. suzukii* catch, which made up (90 – 95.5%) of trap catch. Proportion graphs for the standard apple cider vinegar (Figure 29), the top performing vinegar bait, Chinkiang vinegar (Figure 30), and the top performing yeast bait, *H. uvarum* (Figure 31), showed high counts of male *D. suzukii* at late-season trapping, and higher female catches during mid-season, though this did vary by bait. *H. uvarum* shifted from a majority within the *D. suzukii* population of female to majority male on July 18, whereas apple cider vinegar and Chinkiang vinegar both shifted from majority female to majority male on September 12th.

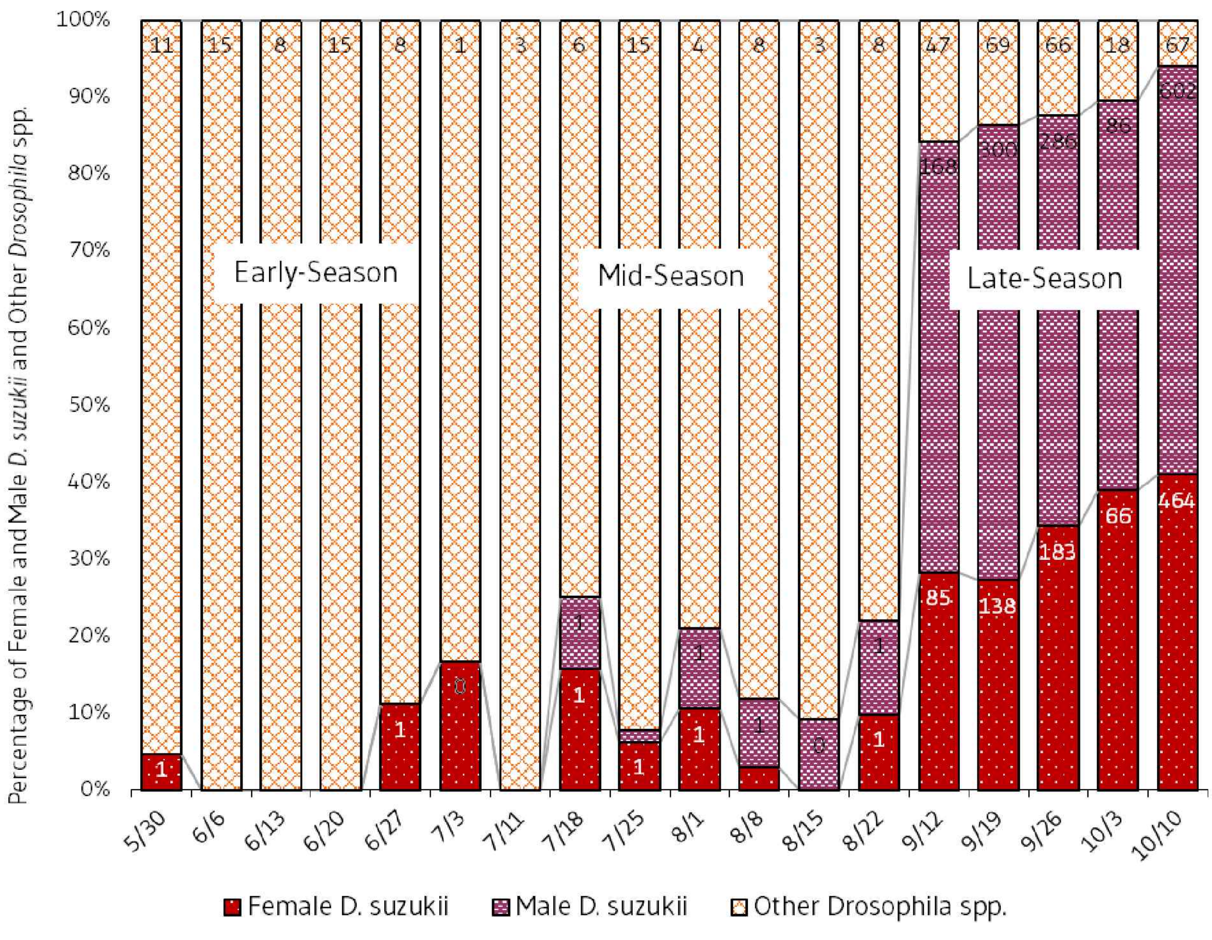



Figure 29. Shift in percentage of female/male *D. suzukii* and other *Drosophila* species caught in apple cider vinegar-baited traps shown over 3 seasons. Total numbers caught are included in the bars.

The distribution of *Drosophila* species in Chinkiang vinegar-baited traps follows the same trend as apple cider vinegar-baited traps, but a more even distribution of trap catch was observed during the mid-season (Figure 30).

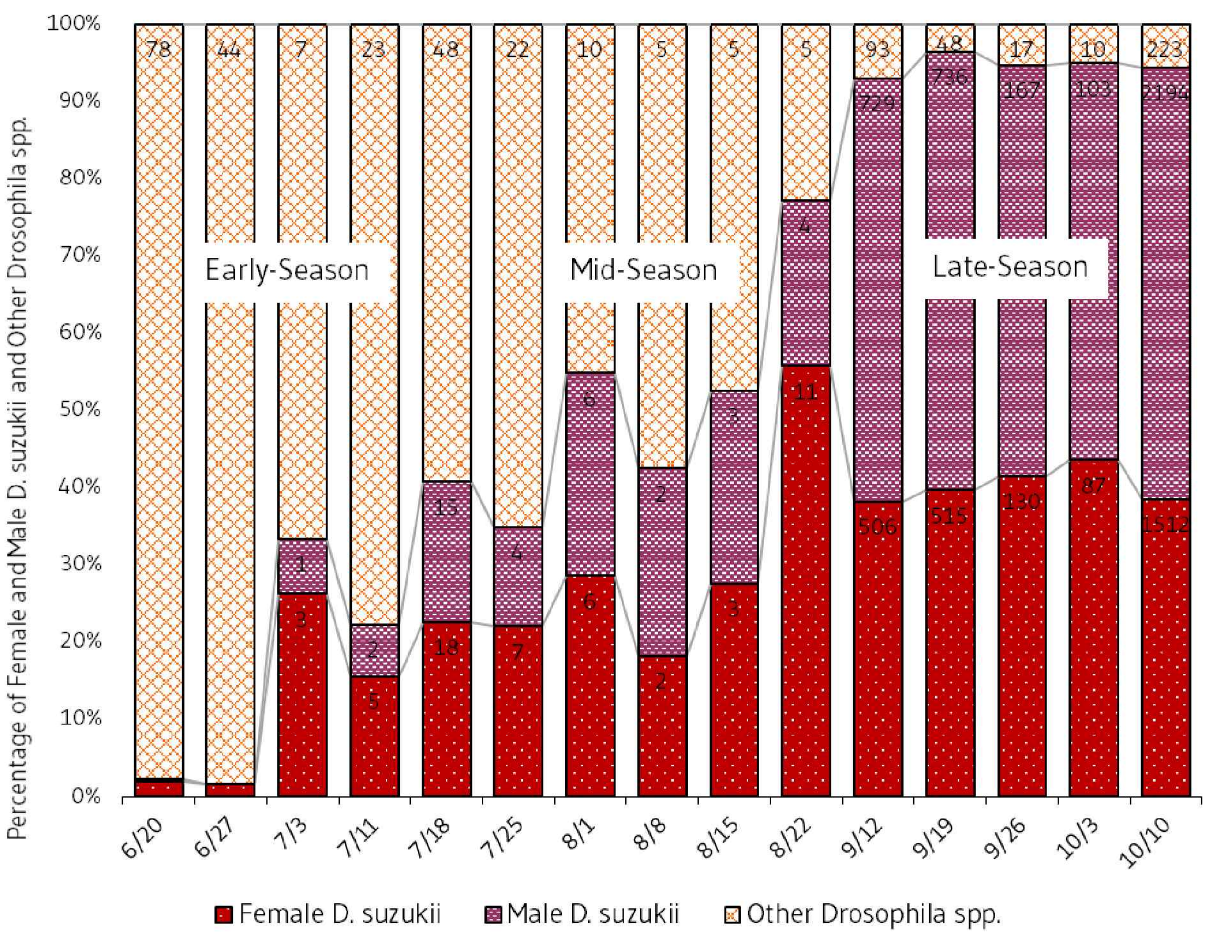



Figure 30. Shift in percentage of female/male *D. suzukii* and other *Drosophila* species caught in Chinkiang vinegar-baited traps shown over 3 seasons. Total numbers caught are included in the bars.

*H. uvarum*-baited traps captured a similar proportion of *D. suzukii* to other *Drosophila* species as Chinkiang vinegar, but the shift to higher male capture than female occurs between 7/11 and 7/18, whereas in Chinkiang vinegar-baited traps the shift occurs more than six weeks later, between 8/22 and 9/12 (Figure 31).

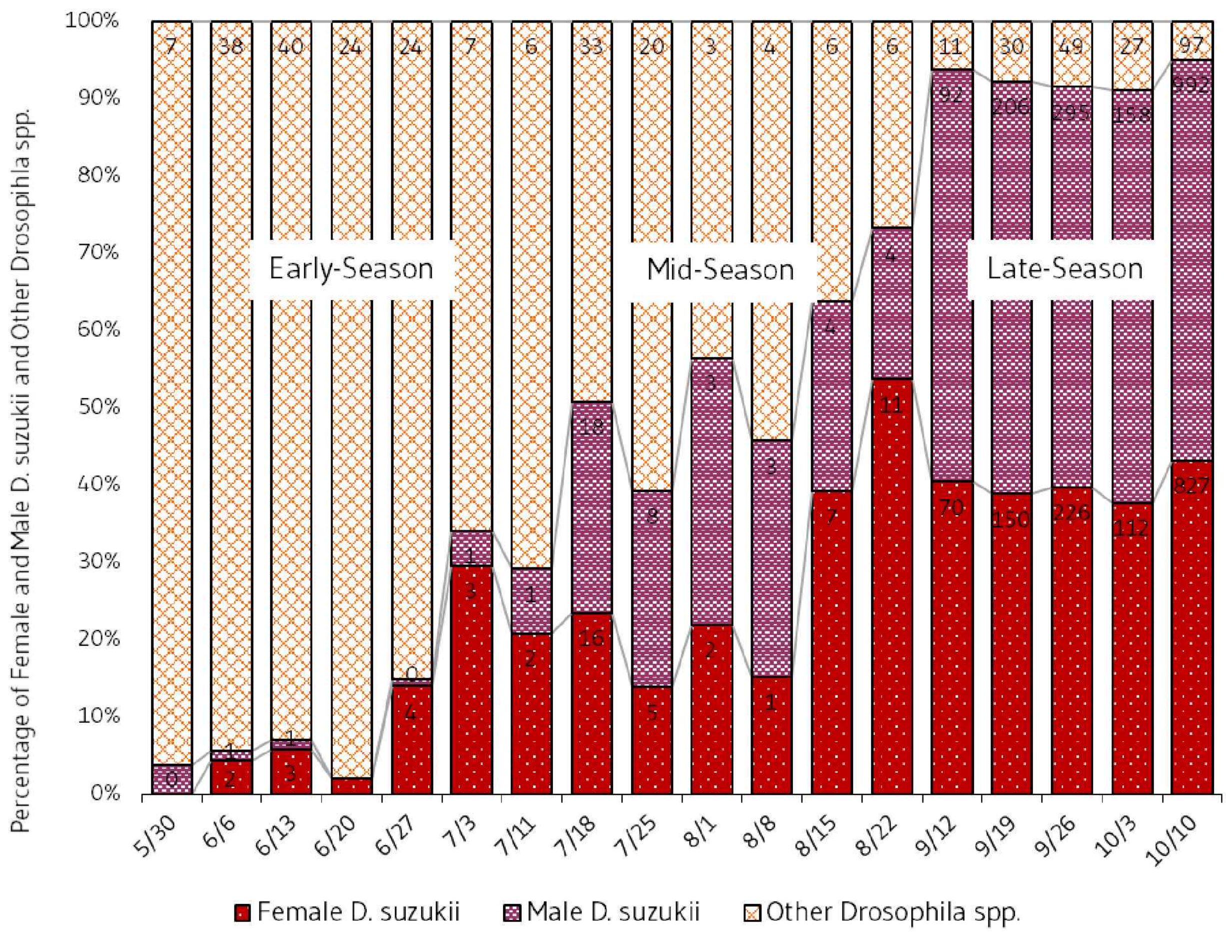



Figure 31. Shift in percentage of female/male *D. suzukii* and other Drosophila species caught in *H. uvarum*–baited traps shown over three seasons. Total numbers caught are included in the bars.

This shift of population demographics between *D. suzukii* and other *Drosophila* species is consistent in Suzukii Trap® bait and "Cha-Landolt" lure bait (Figure 33). Early-season shows a majority of other *Drosophila* species (66 – 100% "Other"), a shift to a more even distribution of *Drosophila* during mid-season, and in late-season the majority of *Drosophila* species captured are *D. suzukii* (90-95.5% "Other"). Within *D. suzukii*, there is a shift towards higher numbers of males late season. Suzukii Trap® bait has higher specificity for *D. suzukii* than the standard apple cider vinegar bait, catching 17% *D. suzukii* early-season, 21% mid-season, and 95.5% late-season, compared to 0% *D. suzukii* early-season, 12.5% in mid-season, and 90% late-season in apple cider vinegar-baited traps. "Cha-Landolt" lure has the highest specificity early-season and mid-season, catching 44% *D. suzukii* early-season and 59% in mid-season, and sustained high sensitivity late-season, 95% *D. suzukii.*

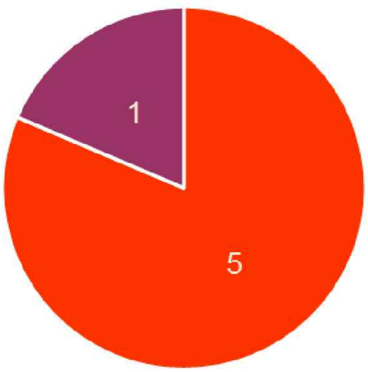
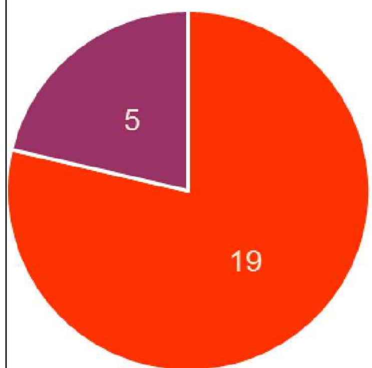
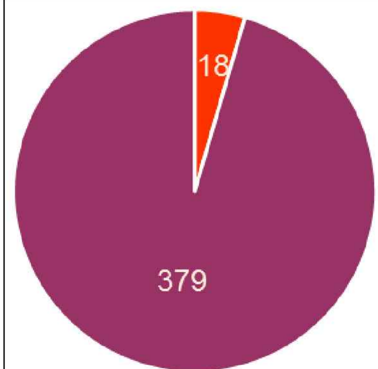
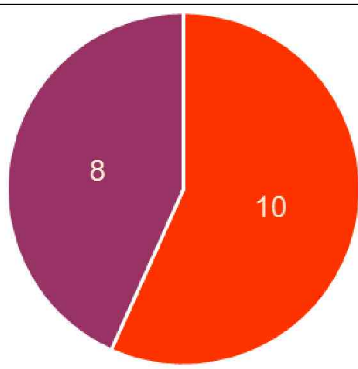
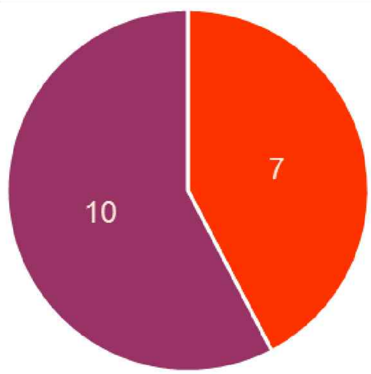
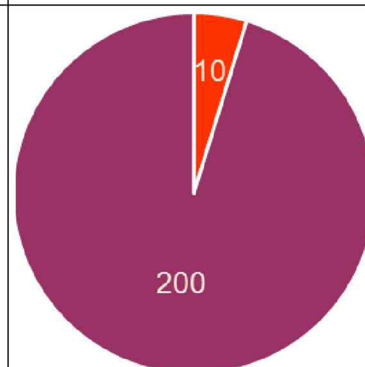
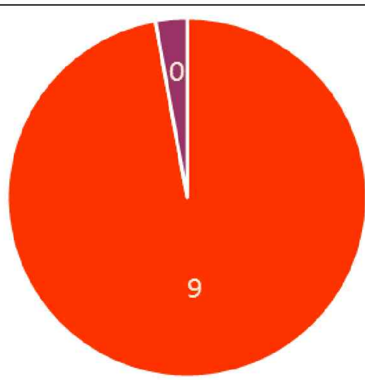
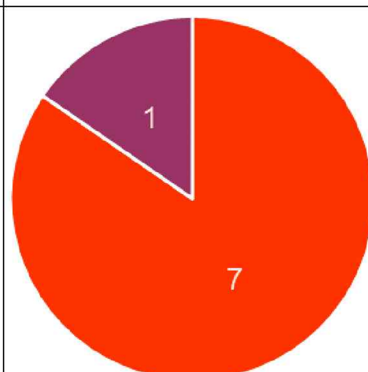
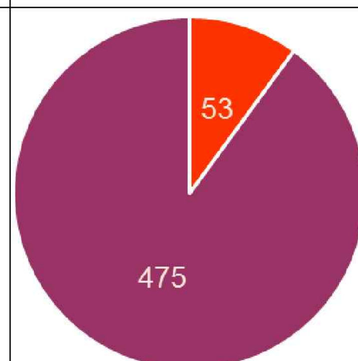
| **Baits** | | **Early-Season (5/30 –7/4)** | **Mid-Season (7/11 –8/22)** | **Late-Season (9/12 –10/10)** |
|---|---|---|---|---|
| Suzukii Trap ® | |   |   |   |
| "Cha-Landolt" lure | |   |   |   |
| Apple cider vinegar | |   |   |   |
| *D. suzukii*<br>Other Drosophila spp. | | | | |

Figure 32. Seasonal specificity of Suzukii Trap® bait and "Cha-Landolt" lure compared to the standard apple cider vinegar. Numbers in charts show mean trap counts during seasonal periods (early-, mid-, and late-season).

## 3.3 Improve Trap Design

**3.3.1** Promising Trap Designs

Despite differences in trap catch preference between the seasonal periods, "Clear 20-Hole" and "Squatty Botty©" fly trap were both consistently high performers for catching *D. suzukii*. One major difference in trap catch between the three seasons was the difference in numbers of *D. suzukii* in the field. Early-season traps caught 150 *D. suzukii*, and catch grew to almost 500 *D. suzukii* late-season. However, as *D. suzukii* exponentially increased in population levels, the MultiLure® trap consistently caught very low numbers of *D. suzukii* (ranging from 3 – 46 May 28 to August 27, averaging 20 flies) compared to the standard "Clear 10-Hole" which caught 7 – 150 flies (averaging 72). For the same early-season time period, the "Clear 20-Hole" trap caught 7 – 329 flies (averaging 122).

Early-season *D. suzukii* capture (Figure 33) shows no significant difference between trap types. However, the "Clear 20-Hole" trap caught the highest number of *D. suzukii*, which was closely followed by the standard "Clear 10-Hole" trap catch. Fly catch was low and variable for all trap types.

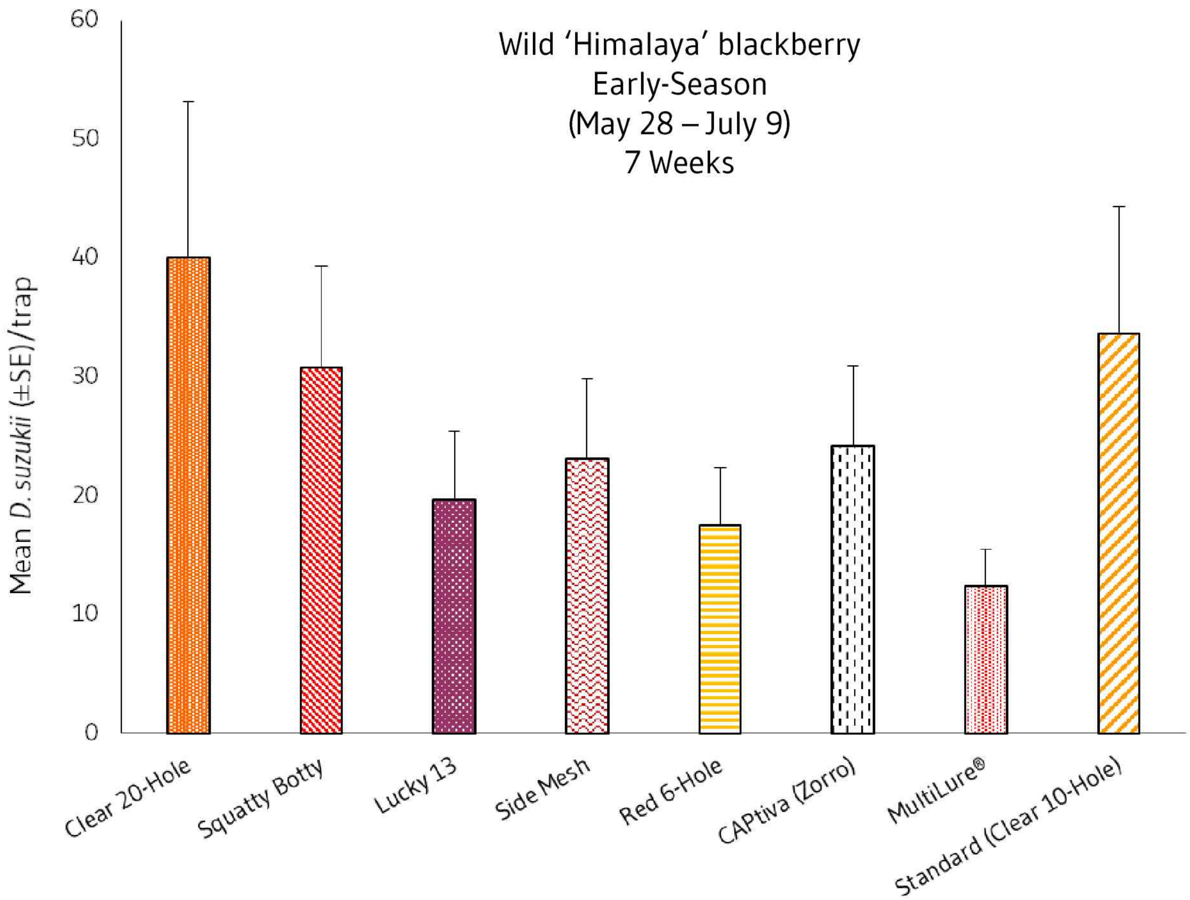



Figure 33. Seasonal comparison of mean trap catch of *D. suzukii* (±SE) for eight different trap designs early-season (5/28 – 7/9) in a wild 'Himalaya' blackberry border (F test: 1.48, df: 7, p-value: 0.179, n: 192; There are date differences in trap catch requiring individual analysis of each date).

Trap catch during the mid-season period (7/30 – 8/27; Figure 34) yielded significant differences in *D. suzukii* trap catch, with all traps catching more flies than MultiLure® and the standard "Clear 10-Hole". However, no significant difference was observed between the "Clear 10-Hole", "Lucky 13"-Hole, "Red 6-Hole", and "Side Mesh". "Squatty Botty" fly trap caught the highest number of *D. suzukii*, but the difference was not statistically significant from the "Clear 20-Hole", "Lucky 13"-Hole, "Red 6-Hole", and "Side Mesh".

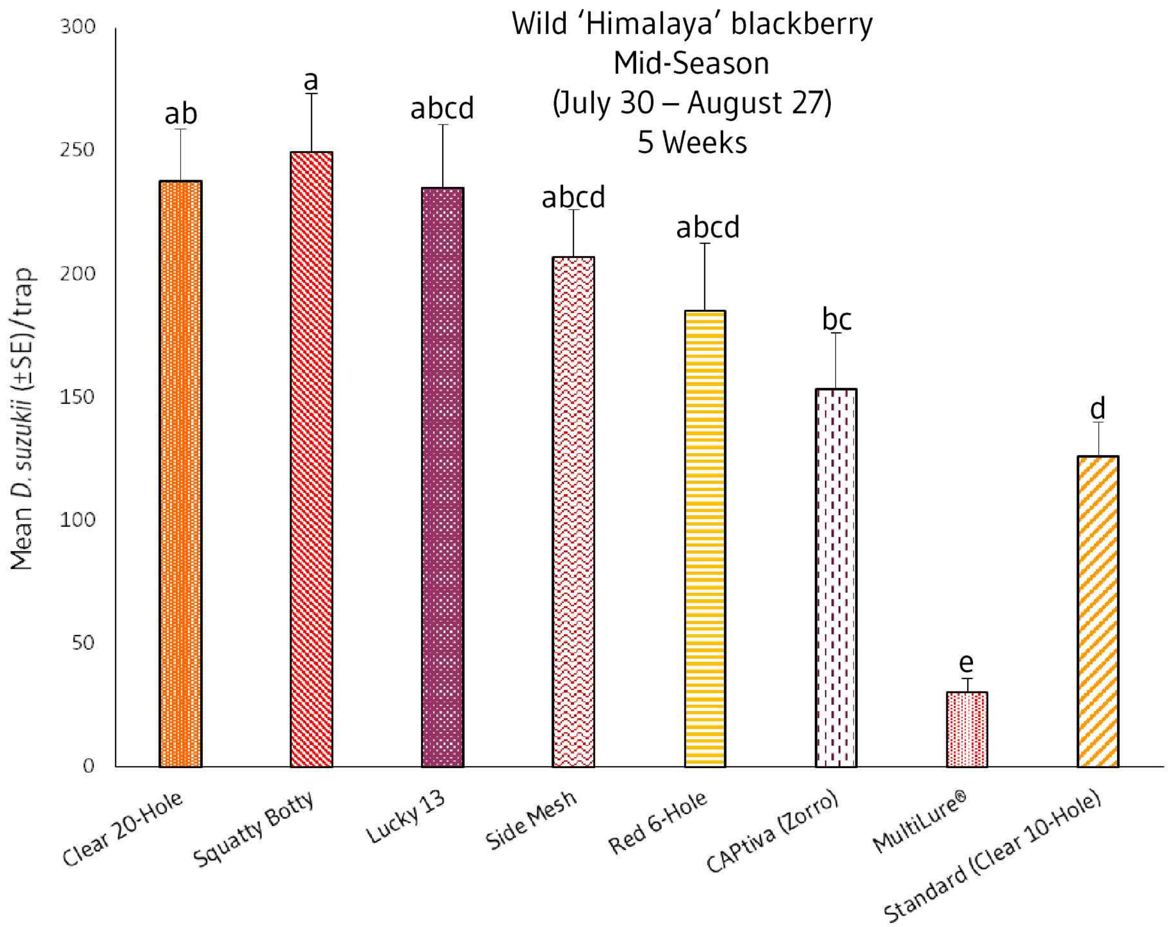



Figure 34. Comparison of mean trap catch (±SE) for eight different trap designs mid-season (7/30 – 8/27) in a wild 'Himalaya' blackberry border (F test: 14.68, df: 7, p-value: <0.0001, n: 192).

Comparison of trap catch from four top-performing traps ("Clear 20-Hole", "Squatty Botty" fly trap, "Lucky 13"-Hole, and "Side Mesh") during the late season period (9/12 – 10/10) is presented in Figure 35. "Clear 20-Hole" caught a numerically higher number of *D. suzukii* than all traps. "Clear 20-Hole", "Squatty Botty" fly trap, and "Lucky 13"-Hole were not significantly different in trap catch. The "Clear 20-Hole" trap caught significantly more flies than the “Side Mesh” trap (115-290 more flies caught in the “Clear 20-Hole”, averaging 236).

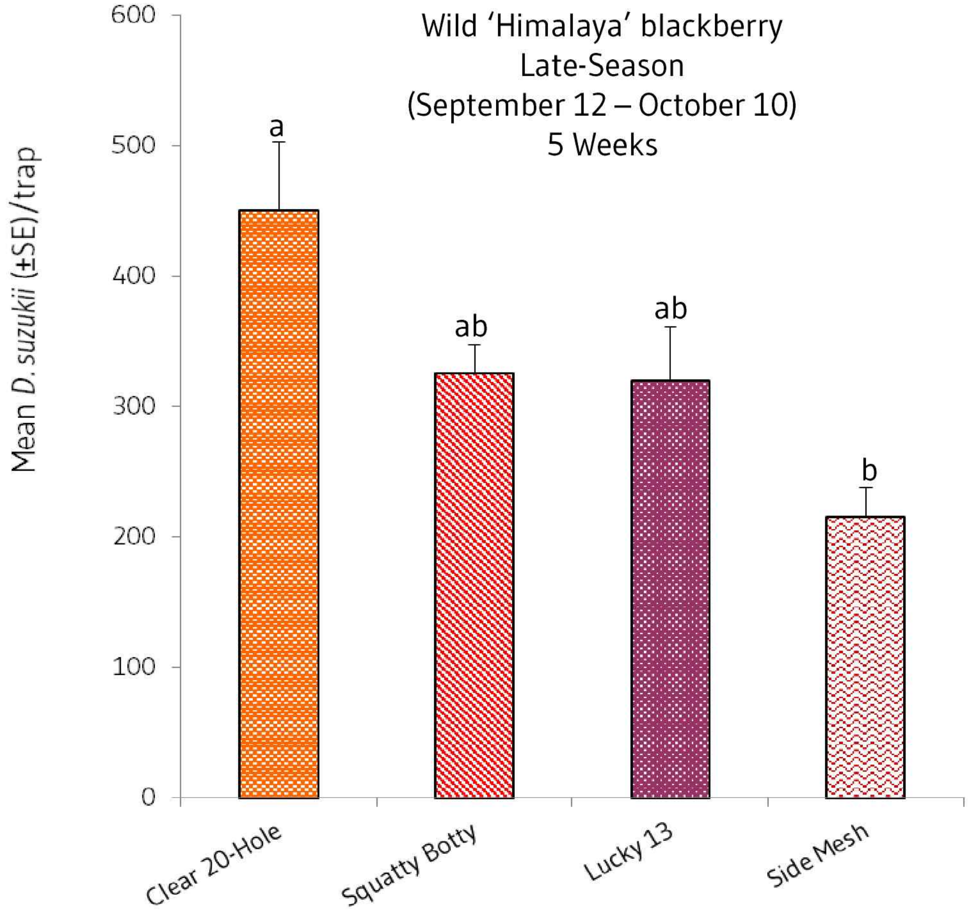



Figure 35. Comparison of mean trap catch (±SE) for four different trap designs late-season (9/12 – 10/10) in a wild ‘Himalaya’ blackberry border (F-test: 6.73, df: 3, p-value: <0.0004, n: 80)

**3.3.2** Seasonal trap catch in replicated block.

Within the four replications of the trap design study there were visible shifts in the highest *D. suzukii* capture. Figure 36, 37, and 38 show the mean (±SE) *D. suzukii* caught in each replicated block within blackberry by season (early, mid, and late). A shift in trap catch was observed from season to season. The highest trap catch in early to early-mid season was recorded in replicate 3 and 4 (Figure 36). Replicates two and three yielded the highest counts in in mid- to late-season (Figure 37). The highest counts in late-season were found in replicate 1 in late-season (Figure 38).

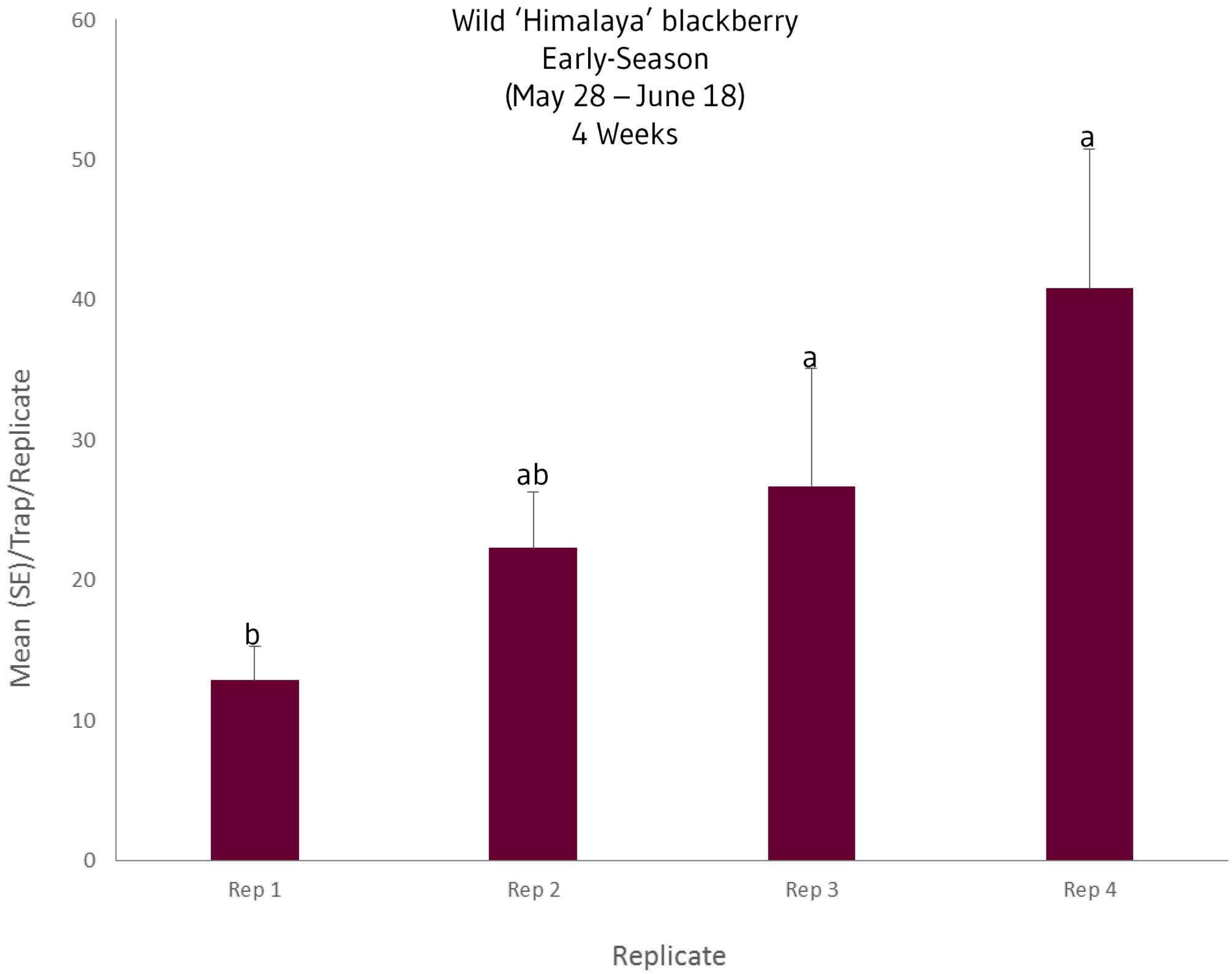



Figure 36. The mean (±SE) trap catch of *D. suzukii* recorded in each replicated block during early-season (5/28 – 6/18) (F test: 27.26, df: 3, p-value: <0.0001).

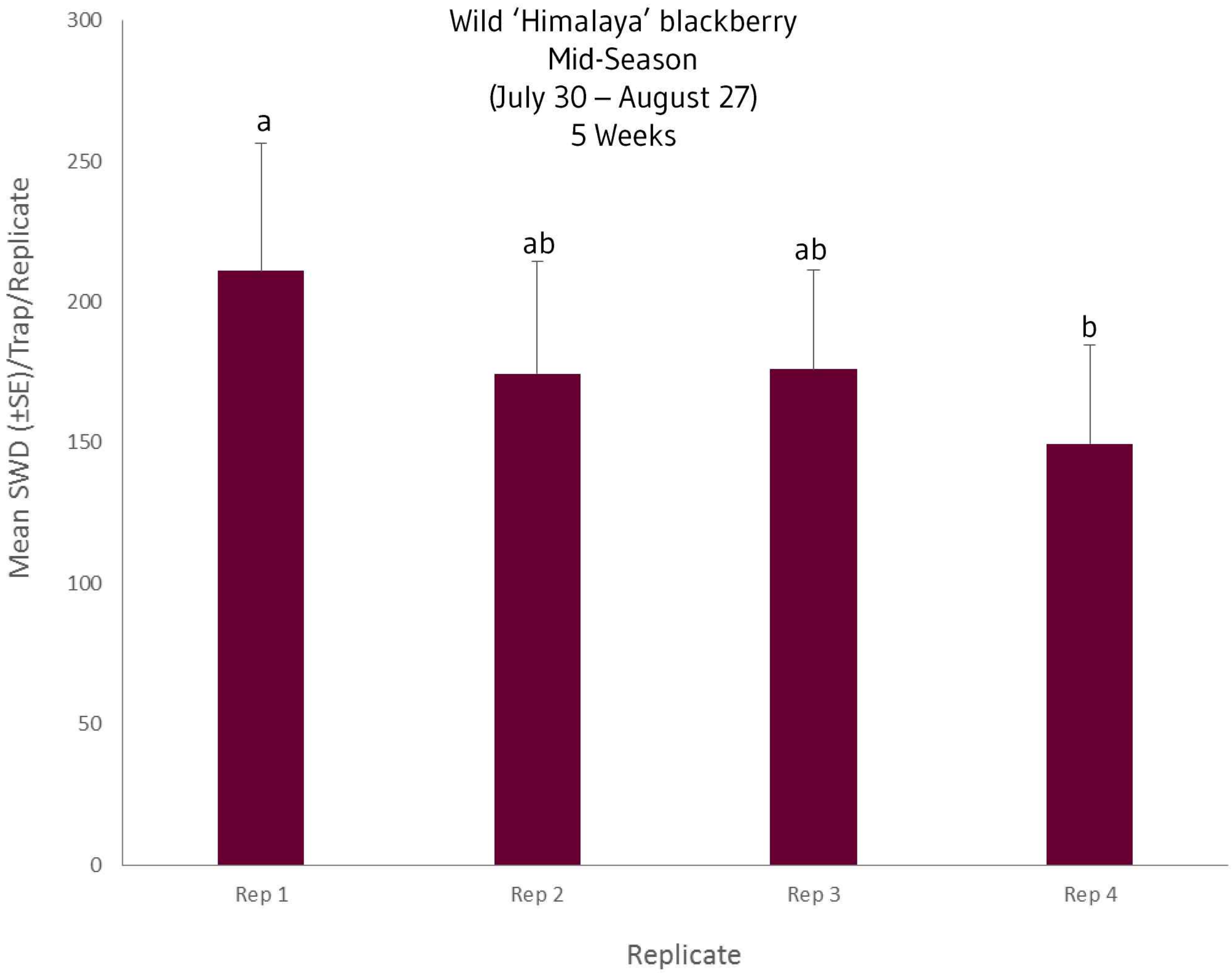



Figure 37. The mean (±SE) trap catch of *D. suzukii* recorded in each replicated block during mid-season (7/30-8/27) (F test: 2.71, df: 3, p-value: 0.04; Note: Square root transformation and Fisher's LSD mean separation test)

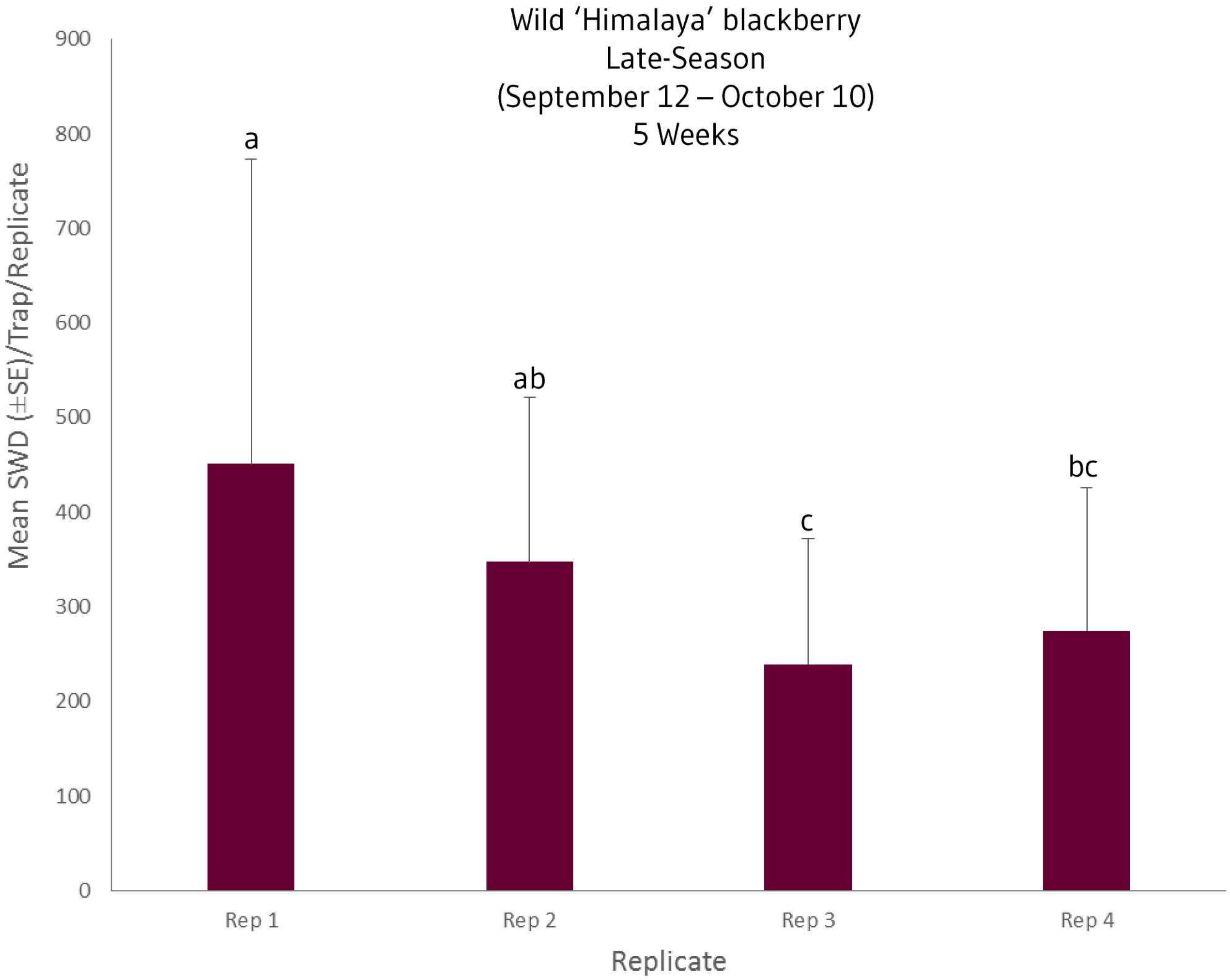



Figure 38. The mean (±SE) trap catch of *D. suzukii* recorded in each replicated block during late-season (9/12-10/10) (F test: 9.61, df: 3, p-value: <0.0001).

**3.3.3** "Clear 20-Hole" Headspace

Data collected from the trap design study shows a clear relationship between the volume above and below entry holes and the number of *D. suzukii* captured. Mean trap from May 28 – October 10 showed traps with a small volume below entry holes and a large volume above entry holes typically catching the highest numbers of *D. suzukii* (Figure 39).

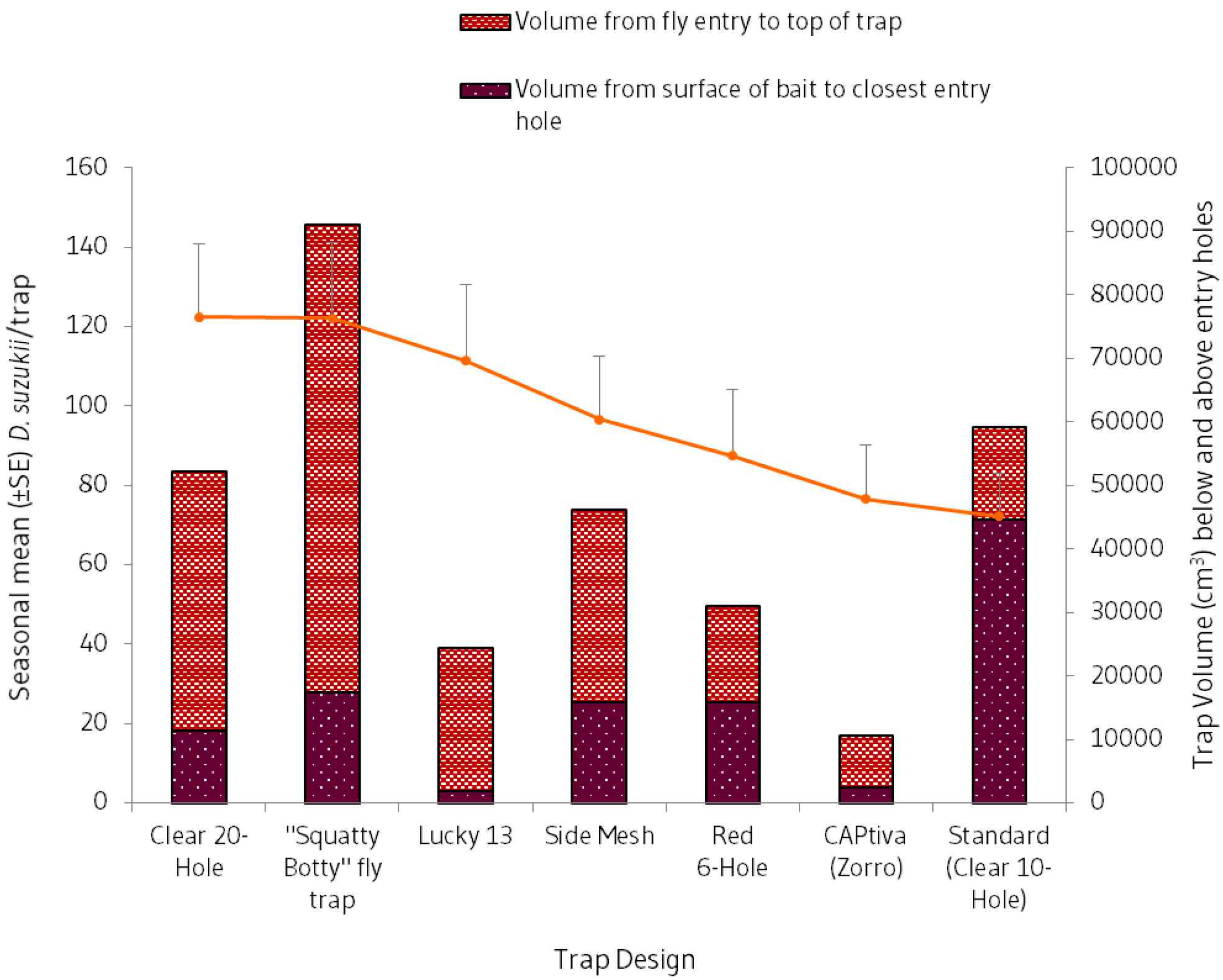



Figure 39. Comparison of seven trap designs listed from highest mean (±SE) *D. suzukii* catch to lowest from 5/28 – 10/10. The volume below and above entry holes of each trap is represented by the line graph.

A no-choice laboratory comparison of trap catch between traps with holes placed close to the bait surface (2.2 cm) and holes placed high on the cup (8.2 cm) and further away from the bait surface (Figure 40). The difference in trap catch between low and high holes was significant. The low-hole traps caught an average of 47% more *D. suzukii* than the high-hole traps.

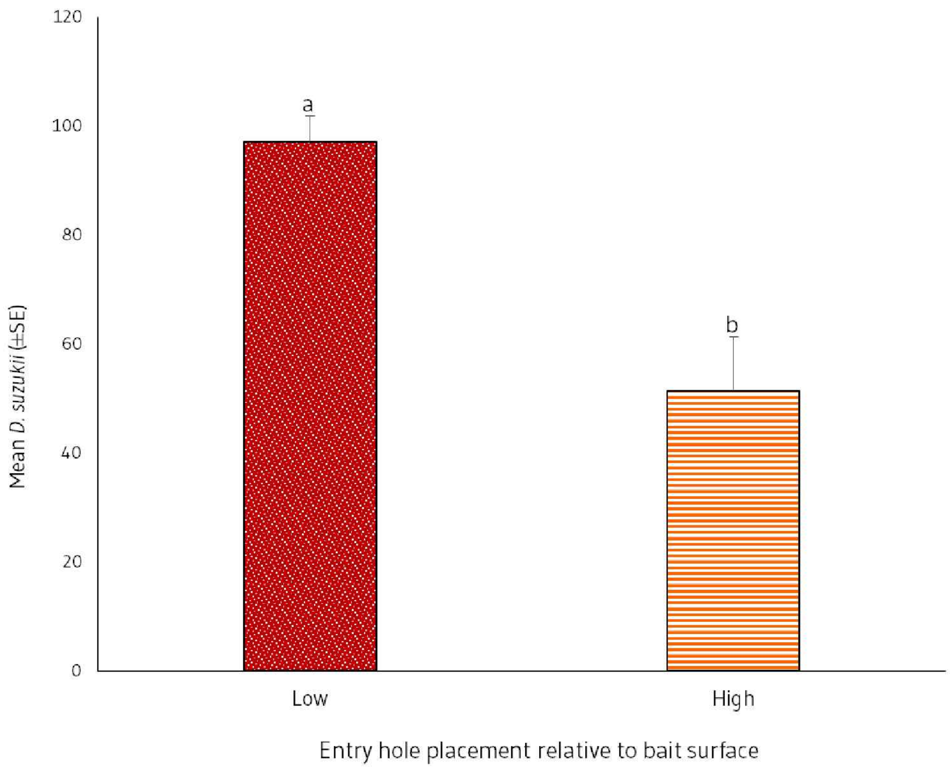



Figure 40. Mean trap catch in “Clear 20-Hole” trap with low-positioned holes versus high-positioned holes relative to the bait surface for entire collection period (1/15/2014 – 1/29/2014). F test: 9.69; df: 1; p-value: 0.

**3.3.4** Attract-and-Kill

The attract-and-kill study revealed a significant difference in *D. suzukii* catch between yeast-baited traps ”Squatty Botty” fly trap and ”Clear 20-Hole” with a killing agent and without in a wild ‘Himalaya’ blackberry crop. In both mid- and late-seasons for “Squatty Botty” fly trap and late-season for “Clear 20-hole”, there were higher numbers with a killing agent present than without (Figure 41). Yeast-baited “Squatty Botty” fly traps with kill agent had 104% more *D. suzukii* than without killing agent in mid-season. Late-season yielded 290% more with kill agent.

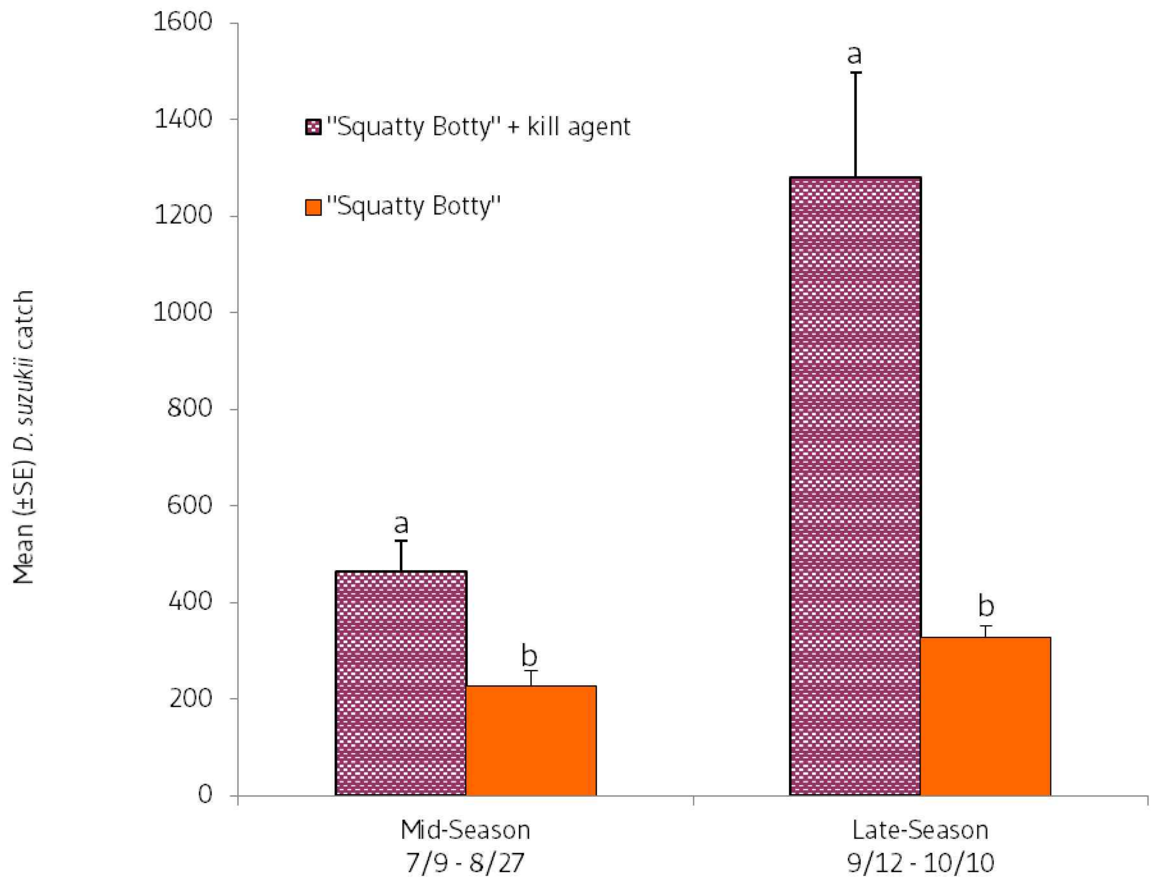



Figure 41. Mid-season (7/9 – 8/22) and Late-Season (9/12 – 10/10) comparison of "Squatty Botty" fly trap with and without presence of organophosphate killing agent (Mid-Season: F test: 3.45, p-value: <0.07; Late-Season: F test: 19.46, p-value: <0.0001).

Comparison of "Clear 20-Hole" yeast-baited traps with and without the presence of an organophosphate killing agent were assessed late season (10/10 – 10/31) and the presence of a killing agent was related to higher *D. suzukii* catch than "Clear 20-Hole" traps with the killing agent present in lid caught an average of 50% more *D. suzukii* than "Clear

20-Hole" traps without (Figure 43).

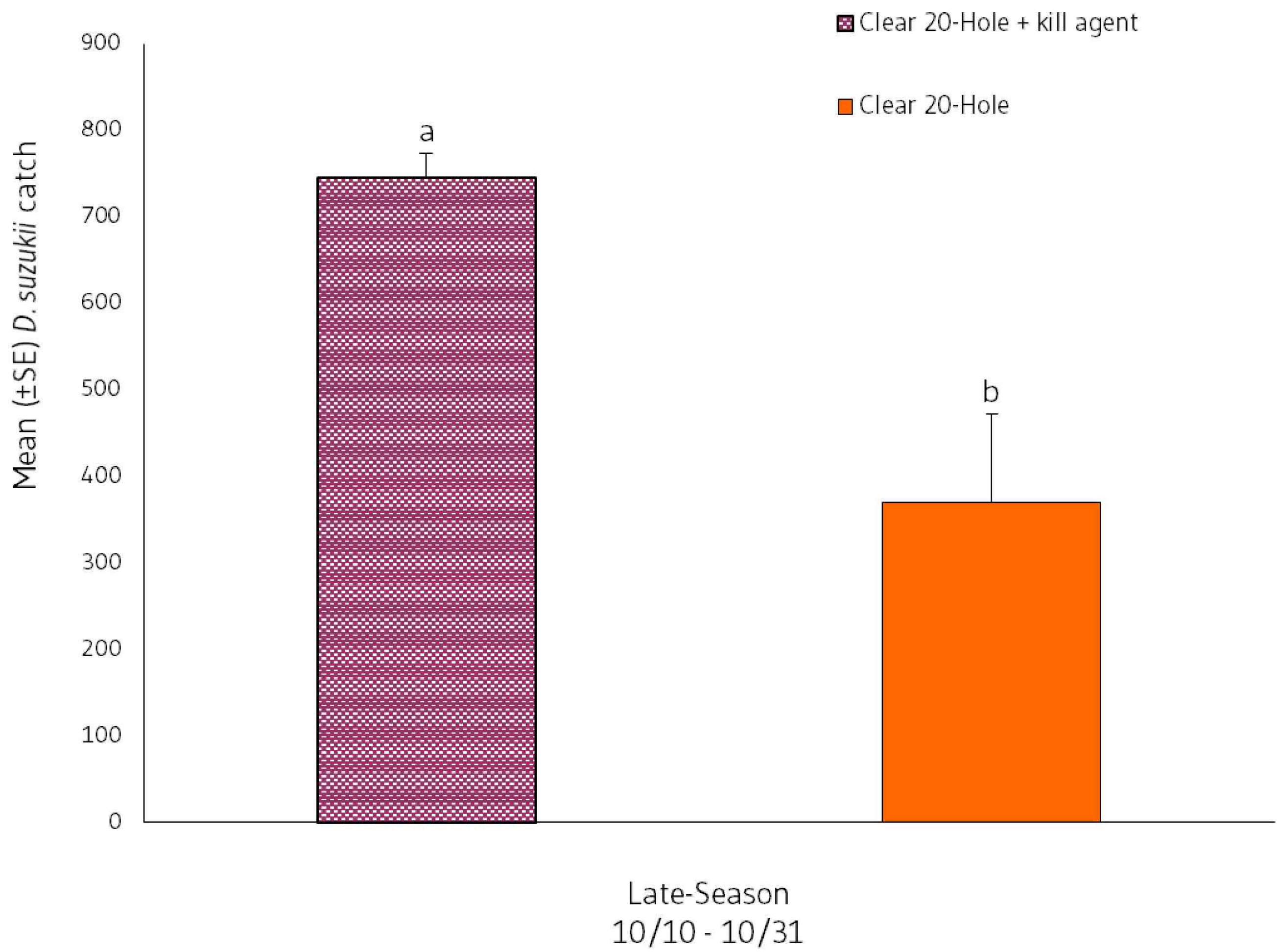



Figure 42. Late-season (10/10 – 10/31) comparison of "Clear 20-Hole" trap with and without presence of organophosphate killing agent (F test: 20.31, p value: < 0.0003).

### 3.4 Education and Outreach

The third and final objective for this *D. suzukii* management project was to educate others about the research findings. This is important for a variety of reasons, particularly to increase public understanding about this pest. There is often a disconnect between research being conducted and the results being communicated to laypeople, for whom the research implications can be enormously important. Additionally, for the *D. suzukii* project, growers both backyard and commercial have contributed to the academic load, making it important for them to stay apprised to current preference information on the pest. The following is a list of the presentations I have completed with data from this project:

- Thesis proposal and final results were presented for evaluation and feedback at the Student Research in Entomology Symposium at Oregon State University in Corvallis, Oregon to an audience of approximately 25 at the beginning (February 23, 2013) and end (March 1, 2014) of the research process.
- A poster titled "Can Improved *Drosophila suzukii* trap designs and baits predict damage?" which included data from this research was presented by A.J. Dreves and H. Andrews at the 67$^{th}$ National Entomological Society of America meeting in Austin, Texas (November 13, 2013).
- Trap design and bait study results and conclusions were presented in a talk titled, "Spotted Wing Drosophila Preferences: Traps and Food" at the 73$^{rd}$ Annual Pacific Northwest Insect Management Conference in Portland, Oregon. I received 1st Place Undergraduate/3rd Place Overall in student competition (January 6, 2014)
- A poster highlighting the trap design and bait study results titled, "Bait and Trap Design Preferences for *Drosophila suzukii*" was presented for the BioResource Research Interdisciplinary Program, Bioenergy Education and Horticulture Poster Fair in Corvallis, Oregon. I was voted "People's Choice" (January 27, 2014).
- A 10-minute paper presentation titled, "Bait and Trap Design Preferences for *Drosophila suzukii*" was delivered to a Bioresource Research 406: Projects class (February 17, 2014).

- Data and conclusions from trap design and bait trials were presented in a talk titled, "Bait and Trap Design Preferences for *Drosophila suzukii*" at the 98th Annual Meeting of the ESA Pacific Branch in Phoenix, Arizona. I received 1st place in the undergraduate student paper competition (April 7, 2014).
- Poster titled "Bait and Trap Design Preferences for *Drosophila suzukii*" presented at the Oregon State University Celebrating Undergraduate Excellence poster fair in Corvallis, Oregon (May 15, 2014).
- Discussed current understanding of *D. suzukii* trap and bait preferences in a presentation titled, "Current Traps and Baits", for an audience of 50 at "A Warm-up: Management Tools for SWD", a workshop on *D. suzukii* management for Pacific Northwest growers which was conducted by the North Willamette Research and Extension Center in Aurora, Oregon (May 22, 2014).
- Final seminar presentation "Bait & Trap Design Preferences for *Drosophila suzukii*" (June 13, 2014).
- I was filmed for a segment on *D. suzukii* and management for the home gardener with "Garden Time" TV show on August 15, 2014 (Projected to air in September 2014).
- Projected:
    - Compete in the Undergraduate Student Ten-Minute Paper Competition with on the topic "Detecting and controlling *D. suzukii* with traps" at the National Entomological Society of America 62nd Annual Meeting in Portland, Oregon (November 17, 2014). I received a scholarship for registration.

# CHAPTER 4: Discussion

The results from the bait and trap design trials suggest that many improvements can be made to the current trapping system. Almost all of the baits caught higher numbers of *D. suzukii* than the standard apple cider vinegar bait used from the onset of invasion, and all trap designs other than the MultiLure® caught consistently higher numbers than the standard "Clear 10-hole" trap.

## 4.1 Major Impacts of Research

- Chinkiang vinegar caught the highest number of *D. suzukii* across most seasons and crop types.
- *H. uvarum*-baited traps consistently caught higher numbers of *D. suzukii* than other yeast baits (*S. cerevisiae* and Torula Yeast Pellets® bait). However, *H. uvarum* is not currently available or convenient for commercial use.
- Suzukii Trap® baited traps caught high numbers of *D. suzukii* when placed in densely canopied regions of crops (cherry, raspberry), out-catching Chinkiang vinegar-baited traps.
- "Cha-Landolt" lure revealed the highest specificity for *D. suzukii* across all seasons.
- Traps with a small-to-moderately sized volume below entry holes and a large volume above entry holes caught higher numbers of *D. suzukii*.
- Addition of a killing agent to the inside of the lid of a *S. cerevisiae* baited trap revealed significant increase in *D. suzukii* catch, showing promise for *D. suzukii* management via an attract-and-kill strategy.
- Trap placement is key for determining the presence of D. suzukii.

### 4.2 Environmental Conditions

The population of *D. suzukii* was affected by the changing environment: temperature, precipitation, and host fruit phenology. Early-season populations were low as *D. suzukii* was first starting to appear and repopulate after a significant decline in population over fall, winter, and spring. The combination of warming temperatures and precipitation were ideal for *D. suzukii*, but fruit had not reached the phenological stages preferred by *D. suzukii*: ripe and overripe, suggesting that an increase in early-season *D. suzukii* success may be limited by available host sites in addition to low numbers. Mid-season, a fruit ripening period, was consistently warm and dry which are not necessarily favorable conditions for *D. suzukii*. However, conditions within a crop canopy, such as protection from the sun and increased moisture retention resulting in higher humidity, could favor *D. suzukii*. The decrease in *D. suzukii* between mid- and late-season may have occurred due to a condensed cropping season and high temperatures mid-season, resulting in a compromised population of flies in the previous generation. The perfect storm for *D. suzukii* hits at the beginning of the late-season, as host crops reach full maturity, temperatures decrease slightly, and precipitation increases significantly. Luckily, populations reached maximum levels when fruits were overripe but not desiccated, which is after the peak harvesting season. However, this could pose a problem for farms with multiple hosts that have different ripening times, and means it is important to remain vigilant with regards to clearing away older and damaged crops which provide oviposition sites (this is the IPM practice known as "Sanitation")..

### 4.3 Test Baits

In terms of bait success, this research did not find the "silver bullet" that most fruit growers desire. Chinkiang vinegar, *H. uvarum*, and Suzukii Trap ® showed potential as effective baits, increasing efficacy over the use of the original standard apple cider vinegar, however, factors such as cost, lack of convenience, and variation in results between crop site, season, and environmental conditions indicate that more work is necessary to understand the limitations/attributes of the baits. This variation represents many of the limitations of the study, chief among them the high susceptibility of baits to evaporation in blueberry and blackberry, which was most likely the result of: an entry area that was too large; plants that were young, small, and exposed; and warm, dry, windy conditions that were seasonally and regionally atypical. The mid-season procedure modification to increase bait from 150 to 170 mL and placing traps deeper within the canopy corresponded to higher and more consistent trap captures as well as decreased loss of bait from evaporation. Evaporation was not a problem in raspberry or cherry, where fruit was growing under a dense canopy, maintaining a humid and protected area for *D. suzukii* to thrive. Increasing bait levels to 200-250ml might compensate for the loss that can occur in areas of high wind and temperature, and low humidity.

Chinkiang vinegar, despite consistently catching high numbers of *D. suzukii* all seasons in all crops, may not be a practical option due to the high cost ($3/550 mL), poor clarity (dark color), and thick consistency when exposed to evaporation. Identification of *Drosophila* in the field is not possible with use of Chinkiang vinegar bait as it is dark and stains the entire fly body, although whether or not this is a concern depends on the goal of the trapping system; a grower will want to see his/her catch in the field, but a researcher will strain the contents, making the characteristics visible again. Additionally, a management or eradication system may rely less heavily on identification than a monitoring and detection system. However, these concerns may be alleviated by using a lower concentration of vinegar. The blueberry and blackberry trials used 100% Chinkiang vinegar, while research performed at the cherry site used Chinkiang vinegar at 50% concentration. The 50% concentration did not appear to limit the numbers of *D. suzukii* caught and it also had fewer problems with evaporation causing a sludgy bait as seen in the blueberry trial. There was a numerically smaller number of *D. suzukii* found at the cherry site in Chinkiang bait than the Suzukii Trap® bait, but the difference between Chinkiang vinegar-baited traps and Suzukii Trap® baited traps was not significant (p-value >0.05), and Chinkiang vinegar caught significantly more *D. suzukii* late-season than Suzukii Trap®. Therefore, a smaller percentage of Chinkiang vinegar (25 - 50%) may be more economical and practical for catching *D. suzukii*, and finding an ideal Chinkiang vinegar concentration represents an area of future research.

*H. uvarum* consistently out-captured *D. suzukii* in both other yeast varieties, *S. cerevisiae* and Torula Yeast Pellets® bait. However, *H. uvarum* cultivation is currently both a time sensitive and a time consuming process; each week 3-6 hours were spent solely on *H. uvarum* preparation and inoculation, split between two or more days. The resulting yeast population was reliant on the success of this process, as evidenced by the observation that the week of June 13-20, one of only two weeks that *H. uvarum* caught significantly lower numbers than the *S. cerevisiae* bait, the yeast culture was in the growth chamber for one day less than usual due to a temporary lack of access to preparatory facilities. For both Chinkiang vinegar and *H. uvarum,* future research could be directed towards making the baits more user friendly – in the case of Chinkiang vinegar, this could mean synthesizing active ingredients so that the resulting lure is clear with a thin consistency. *S. cerevisiae* and Torula Yeast Pellets® bait are both available in a dried form that is accessible and easy to use for consumers, so if a manufacturer could mass produce a similarly simple form of *H. uvarum* then it would be an improvement over both apple cider vinegar and *S. cerevisiae.* Torula Yeast Pellets® yielded increased trap capture the last week of collections for mid-season in both blueberries and raspberries relative to *H. uvarum* and *S. cerevisiae*. Due to no further data it is uncertain if these bumps in capture represent a late-season shift in *D. suzukii* yeast preference or if it simply represents an anomaly in data. Regardless, as *D. suzukii* late-season trapping is not the stage most important for management of D. suzukii and trap captures were incredibly low most of the season (catching 0-1 flies per trap most weeks), the potential benefits of this bait are outweighed by its overall poor efficacy.

The use of Suzukii Trap® bait in traps shows definite potential, outcatching the standard, apple cider vinegar, in all trials. Suzukii Trap ® was particularly effective in the cherry and raspberry trials, where it caught significantly more *D. suzukii* than apple cider vinegar, however it was not as effective in the blueberry and blackberry mid-season, catching a numerically but not significantly higher number of *D. suzukii* than apple cider vinegar. This may be due to the environmental conditions and susceptibility of the bait to evaporation – the raspberry and cherry trials took place in areas of dense canopy with a higher humidity, whereas the blueberry and blackberry trials were exposed to the elements and the liquid lost to evaporation was at times visible to the naked eye. Visual observations noted that Suzukii Trap® baited traps lost more bait to evaporation than traps containing other baits. By mid-season, trap placement was adjusted to minimize evaporation by placing traps as deep in the plant canopy as possible and increasing bait volume, but the conditions of a hotter dryer area continued to be less than desirable to *D. suzukii*. Trap catch using apple cider vinegar as the bait over the late-season increased in efficacy compared to its use early season and relative to counts from other baits, with no observed statistical difference between apple cider vinegar and Chinkiang vinegar in blackberry, and a much smaller difference in cherries.

The "Cha-Landolt" 4-compound hanging lure also showed potential, as it consistently had higher specificity for *D. suzukii* than other baits. The trap captures were not numerically high, but the solution was stored improperly over the summer. Further research using proper storage can show if this lure has the specificity and high trap capture ideal for *D. suzukii* management.

Apple cider vinegar, the standard, captured low numbers throughout the growing season, but in each late-season trial (blackberry and cherry) showed a dramatic increase in capture relative to other baits. This suggests that a late-season shift in bait to apple cider vinegar-baited traps may draw in higher numbers of *D. suzukii* than the bait most effective early- and mid-season.

A consistent trend in *Drosophila* trap catches was seen across bait types and across the seasons. High numbers of other *Drosophila* species were recorded early-season with a late-season shift in September to high numbers of *D. suzukii*. The consistency between trends of *Drosophila* catches amongst the baits supports that this shift is occurring in the environment as a whole. This suggests that even a highly specific bait to *D. suzukii* may not have good efficacy for catching *D. suzukii* until midway through the season when numbers increase. Survival of *D. suzukii* adults is low in the spring from winter mortality, and accumulation is slow through May and June in the Willamette Valley before exponentially increasing mid- and late-season. This results in higher early-season counts of other *Drosophila* species who are more acclimated to winter cold and those that overwinter as larvae. Variation between the same treatments over different replicates indicates that trap placement is an important factor for trap capture, demonstrating a higher difference in yield than differences between individual treatments. Therefore early-season trapping would be most effective if traps and baits are placed based on hotspot locations where overwintering *D. suzukii* first emerge, with trap design and bait being of secondary importance. Interestingly, the distribution of male and female *D. suzukii* varied between the baits: early- to mid-season were largely female, but *H. uvarum* had higher numbers of males. This indicates that landscape-wide a greater proportion of the *D. suzukii* population was female in early- and mid-seasons, and *H. uvarum* was specifically more attractive to males at this point. As females have the capability to store male sperm over winter and are responsible for oviposition to repopulate as the growing season starts, it would make sense that a greater proportion of overwintering *D. suzukii* are female, which appears to be represented in the data by the larger female population. Successful early-season management is a critical area for future research as capturing these females could cripple *D. suzukii* repopulation efforts. All three baits show higher numbers of male *D. suzukii* compared to female *D. suzukii* late-season, indicating again that this is a landscape-wide trend that occurs as populations reach their peak, oviposition sites are reduced, and female *D. suzukii* begin to prepare for overwintering.

The bait trial initially started as a small-scale lab trial, in which lab-reared *D. suzukii* were placed in 1m x 1m mesh observation cages containing traps baited with various attractants to observe which baits caught the highest number of *D. suzukii*. After several weeks of data collection, it was clear that the trial would not yield significant results. This was due in large part to high mortality of the *D. suzukii* (age dependent) as well as no significant differences between baits. The cage effect of enclosing bait volatiles and close proximity of baits to each other may have caused interference by mixing volatiles within the cage. The high mortality of adults before entering traps may be counteracted by consistent use of older flies, which have a lower mortality rate than younger flies, as well as careful placement of the study to avoid direct sunlight in the summer.

**4.4 Improve Trap Design**

The trap design study showed design preferences for *D. suzukii*. Bottom-entry McPhail style traps such as the Multi-Lure brought in very low numbers of *D. suzukii* relative to other traps, consistent with 2011 and 2012 trap studies. Increased volatile diffusion into the environment and cross ventilation due to a side entry design may result in higher *D. suzukii* trap catch than traps with bottom entry. This conclusion needs further study.

Another observation was a correlation between the distribution of volume above the bait level and the trap catch of *D. suzukii*. The traps that caught the highest numbers overall of *D. suzukii* had low volume between the bait and the entry holes and a high volume above the entry holes. The headspace trial showed a significant difference between low entry holes and high entry holes within the same trap. It is speculated that the low entry holes draw higher numbers due to greater bait volatilization resulting from the small area between the bait and entry holes, and the high volume retains these flies due to reduced escapage. As a fly enters a trap, it will fly to the top of the trap. Having a large area between entry holes and the top of the trap lowers the risk of walking or flying down to the holes and escaping. Observational reports support this theory, with larger *D. suzukii* congregations present in traps with low holes and a large volume above them, such as the "Clear 20-Hole" and the "Squatty Botty" fly trap. This element of design may have a larger impact than color, as the overall top trap was a clear trap, despite past research that indicated D. suzukii preference for yellow, red, and black over clear colored traps. Future research will explore a modified "Clear 20-Hole", using the same volume proportions and entry area, but with the addition of thick vertical red stripes.

The most striking result from the study was the effect that the inclusion of a killing agent on the inside lid of a trap had on *D. suzukii* catch. The number of *D. suzukii* caught in traps with the killing agent were consistently higher than the number caught in traps without. This indicates that the fumes from the killing agent were not interfering with the attractiveness of the bait in the "Clear 20-Hole" and "Squatty Botty" fly traps used in this study. Based on the conclusions drawn from the headspace trial, an ideal attract and kill system may have a low volume between the bait surface area and entry holes to promote bait volatilization, with a large volume above entry holes to serve the dual purpose of containing killing agent fumes and preventing escapage prior to death.

Research was limited to late and post-season and in only one trap variety, so future work should target the pre-season and mid-season months when it is most important to minimize *D. suzukii*, as well as comparing other trap types and alternative killing agents. The significant effect that the killing agent appears to have means further studies would benefit by comparing efficacy among several of the top traps from the trap design study, because the results may show that a trap other than "Clear 20-Hole" draws the highest numbers of *D. suzukii* if escapage is no longer a concern. Additionally, the killing agent used in this trial was used in an experimental capacity (not registered), so future research will compare similar products that have recently been registered for use in Oregon. An attract-and-kill management strategy for *D. suzukii* shows potential for use of an in-trap killing agent or an external kill spray on a trap to kill the 10 - 30% of *D. suzukii* that land on the trap but do not enter (Cowles 2014). Depending on the insecticide used, this may or may not be usable for organic growers, but it is appropriate in a no-spray capacity. One organic insecticide currently being explored is a boric acid sugar solution and a Truvia® solution.

Further analyses using other statistical methods (e.g., non-parametric statistics) will be explored to make sure all statistical assumptions are met and toexamine date-to-date and replication variation in trap catch, season-to-season and crop type variations between trap catch from various designs and baits.

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## Appendix A

Table 1. Vegetation at site 2, wild 'Himalaya' blackberry site by replicate.

| Common Name | Scientific Name | Rep 1 | Rep 2 | Rep 3 | Rep 4 |
|---|---|---|---|---|---|
| Wild 'Himalaya' blackberry | *Rubus armeniacus* | Y | Y | Y | Y |
| Big Leaf Maple | *Acer macrophyllum* | 3 | 0 | 0 | 0 |
| Indian Plum | *Oemleria cerasiformis* | 2 | 1 | 0 | 0 |
| Black Hawthorn | *Crataegus douglasii* | 8 | 10 | 8 | 4 |
| Poison Oak | *Toxicodendron diversilobum* | 1 | 0 | 0 | 0 |
| Cascara Buckthorn | *Rhamnus purshiana* | 0 | 2 | 0 | 0 |
| Wild Cherry | *Prunus avium* | 0 | 0 | 6 | 11 |
| Persimmon | *Diospyros kaki* | 0 | 0 | 0 | 5 |
| Figs | *Ficus carica* | 0 | 0 | 0 | 1 |
| Fallow | Weeds, grass, bare ground | Y | Y | Y | N |

Table 2. Bait Study Bait Descriptions

| Bait | Active Ingredients | Manufacturer | Lot no. | Price | Prep |
|---|---|---|---|---|---|
| *Hanseniaspora uvarum* | *H. uvarum* | Oregon State University Enology Lab | N/A | N/A | See protocol in Appendix B. Add 5.52 g white sugar per 160 mL of liquid before usage |
| *Saccharomyces cerevisiae* | *S. cerevisiae* | Red Star Baker's yeast | 3042 | $10/900 g | Measure out 14g Red Star baker's yeast and 32.66g white table sugar. Add sugar to empty container, cover with 2 cups hot water. Stir. Add 2 additional cups of cooler water to bring the temperature slightly above room temperature. Sprinkle yeast on sugar water and let sit. After 10 minutes, gently stir the solution. |
| AT800 Torula Yeast Pellets® | Torula Yeast, Borax | ISCA Technologies, Inc. | 245599 0 | $60/180 pellets | Add 4 Torula pellets to 5.4 cups of water and 44.167 g of white sugar. Make fresh every other week, prepare enough for two weeks. |
| Monterey Insect Bait® | 99.7% Corn steep liquor | Monterey AgResources | 5062201 110 | | Add 80 ml of 100% Monterey bait and 80 ml water to each trap |
| BioLure® | 3 Membranes: ammonium acetate (black packet), trimethylamine hydrochloride (Red packet), putrescine (small black) | Suterra LLC. | | | Stick large black packet under one of the panels of mesh and red packet under the other panel. Stick the small black packet between the panels on the thin side of trap. Packets are changed every other week. 160 ml water + 1 drop pure unscented soap added to each trap. |
| Apple Cider Vinegar | 5% Acetic Acid | The Kroger Co. | | $8/1 gal | Add 160 ml ACV + 1 drop of pure unscented soap to each trap |
| Suzukii Trap® | 7% p/p Peptides<br>2% p/p Organic Acids | Bioiberica S.A. | 13/0002 | Not Yet Available in the USA | Add 160 ml Suzukii Trap® to each trap + 1 drop of pure unscented soap to each trap |
| Chinkiang vinegar | 5.8g/100 mL acid. See Wei Yang for full composition | Jiangsu Cereals, Oils & Foodstuffs IMP. | N/A | $3/500 mL | Add 160 ml Chinkiang vinegar + 1 drop of pure unscented soap to each trap |

| | | | | | |
|---|---|---|---|---|---|
| | | & Exp. Group Corps | | | |
| "Cha-Landolt" lure | Vials: Acetic acid, methionil, acetoin<br>Drowning solution: 95% ethanol. In 1L of drowning solution: 908 ml H2O, 16 ml (1.6%) acetic acid, 76 ml 95% ethanol, 0.2 ml dish soap | USDA-ARS | N/A | Trece lure (based on "Cha-Landolt" lure) $4.50/lure through Great Lakes IPM, Inc. | Vials are prepared by USDA and replaced every other week. They hang from the lid of the trap. 160 ml ethanol drowning solution added to each trap. |

Table 3. Bait Study Trap Parameters

| Trap Design | Trap Body Volume | Trap Color | Entry Area Shape | Entry Area Type | Location of Entry | # Entry Areas | Trap Cup Type | Manufacturer |
|---|---|---|---|---|---|---|---|---|
| Side Mesh with stripes | 1000 mL | Clear with 3 red vertical stripes | Rectangle | Mesh Grid 2x2 mm | Side | 2 | Deli | Solo™ (Urbana, IL) |
| Side Mesh with stripes for BioLure® | 1000 mL | Clear with 3 red vertical stripes | Rectangle | Mesh Grid 2x2 mm | Side | 2 | Deli | Solo™ (Urbana, IL) |

| Trap Design | Individual Entry Size | Trap Height (mm) | Area of a Single Entry ($mm^2$) | Total Entry Area ($mm^2$) | Distance from bait surface to fly entry (mm) | Diameter at bait surface (mm) | Bait Surface Area ($mm^2$) | Headspace ($mm^3$) |
|---|---|---|---|---|---|---|---|---|
| Side Mesh with stripes | (2) 5.7x9.7 cm rectangles | 145 | 1300 | 2600 | 18 | 90 | 6362 | 114511 |
| Side Mesh with stripes for BioLure® | (2) 5.7x9.7 cm rectangles | 145 | 1300 | 2600 | 48 | 90 | 6362 | 305363 |

Table 4. Trap Design Parameters

| Trap Design | Trap Body Volume | Manufacturer | Trap Color | Entry Area Shape | Entry Area Type | Location of Entry | # Entry Areas | Trap Cup Type | Individual Entry Size | Trap Height (mm) |
|---|---|---|---|---|---|---|---|---|---|---|
| "Squatty Botty" fly trap | 1500 mL | Homemade, Oregon State University | Yellow Body, Red & Black Sleeve | Rectangle | Mesh Grid 2x2 mm | Side | 4 | PVC Pipe | (4) 1.3x5.3 cm rectangles | 175 |
| "Side Mesh" | 1000 mL | Solo™ (Urbana, IL) | 100% Clear | Rectangle | Mesh Grid 2x2 mm | Side | 2 | Deli | (2) 5x9.5 cm rectangles | 145 |
| "Red 6-Hole" | 500 mL | Chinet® Kirkland Signature™ (Sacramento, CA) | 100% Red | Circle | Hole | Side | 6 | Party | 5 mm diameter | 115 |
| "Clear 20-Hole" | 1000 mL | Solo™ (Urbana, IL) | 100% Clear | Circle | Hole | Side | 20 | Deli | 5 mm diameter | 145 |
| "Clear 10-Hole" | 1000 mL | Solo™ (Urbana, IL) | 100% Clear | Circle | Hole | Side | 10 | Deli | 5 mm diameter | 145 |
| "Lucky 13"-Hole | 500 mL | Chinet® Kirkland Signature™ (Sacramento, CA) | Red body with horizontal black and yellow stripe | Circle | Hole | Side | 13 | Party | 5 mm diameter | 115 |
| CAPtiva (Zorro) | 250 mL | Marginal Design (Oakland, CA) | Bottom half Clear, Top half Red, Black, horizontal red stripe | Circle | Hole | Side | 10 | Spice Jar | 5 mm diameter | 130 |
| International Multilure™ | 700 mL | Better World Manufacturing (Fresno, CA) | Bottom half Yellow Top half Clear | Circle | Mesh Grid 2x2 mm | Bottom | 1 | McPhail-type | 50 mm diameter circle | 150 |
| "Squatty Botty" + Killing Agent | 1500 mL | Homemade, Oregon State University | Yellow Body, Red & Black vertical stripes | Rectangle | Mesh Grid 2x2 mm | Side | 4 | PVC Pipe | (4) 1.3x5.3 cm rectangles | 175 |

| Trap Design | Area of a single entry (mm²) | Total entry area (mm²) | Distance from bait surface to fly entry (mm) | Diameter at bait surface (mm) | Bait surface area (mm²) | Headspace (mm³) | Distance from top of fly entry to top of trap (mm) | Diameter at fly entry (mm) | Surface Area at fly entry (mm²) | Volume from fly entry to top of trap (mm³) | Extra Details |
|---|---|---|---|---|---|---|---|---|---|---|---|
| "Squatty Botty" fly trap | 238 | 952 | 20 | 105 | 8659 | 173180 | 85 | 105 | 8659 | 736016 | |
| "Side Mesh" | 1300 | 2600 | 25 | 90 | 6362 | 159043 | 40 | 98 | 7543 | 301719 | |
| "Red 6-Hole" | 19 | 118 | 41 | 70 | 3848 | 157786 | 25 | 88 | 6082 | 152053 | Clear tape seals the straw hole |
| "Clear 20-Hole" | 19 | 393 | 18 | 90 | 6362 | 114511 | 60 | 93 | 6793 | 407574 | |
| "Clear 10-Hole" | 19 | 196 | 70 | 90 | 6362 | 445321 | 18 | 102 | 8171 | 147083 | Standard Trap |
| "Lucky 13"-Hole | 19 | 255 | 5 | 70 | 3848 | 19242 | 55 | 72 | 4072 | 223933 | Clear tape seals the straw hole |
| CAPtiva (Zorro) | 19 | 196 | 10 | 55 | 2376 | 23758 | 35 | 55 | 2376 | 83154 | Electrical tape is used for red attraction. Top of spice container is also red |
| International Multilure™ | 491 | 0 | N/A | 145 - 50 | 1455 | N/A | N/A | N/A | N/A | N/A | |
| "Squatty Botty" + Killing Agent | 238 | 952 | 20 | 105 | 8659 | 173180 | 85 | 105 | 8659 | 736016 | Piece of killing agent adhered to inner roof of trap with Nashua® Aluminum tape |

## Appendix B

### *Hanseniaspora uvarum* Bait Protocol

Preparation Protocol 9-12-2013 – Monica Marcus

**Preparation of the YPD media:**

- 1750 mL DI water
- 17.78 g yeast
- 35 g Peptone (Bacto-Peptone)
- 35 g Dextrose/Glucose

Measure out yeast, peptone, and dextrose in weighing dish using small scale.

Add 1000 mL DI water and stir bar to large Erlenmeyer flask, place on heat pad. Turn on "stir" function (no heat!)

Add yeast, peptone, and dextrose to flask, use remaining 400 mL DI water to clear any solids left on sides of flask. At this point, all 1400 mL of DI water should be in the flask.

Stir for five minutes, or until all solids are integrated into liquid.

Measure out 165 mL into four large glass containers using a large graduated cylinder. Add 5-20 mL of liquid into small glass vials. These will be kept for inoculating the next week's media. Lightly screw on all lids, so barely attached to the base. This is now ready to be autoclaved.

**Autoclave the media:**

Instructions are attached to the machine, which is in the Pilot Plant on the first floor of Wiegand Hall, Oregon State University. Make sure to fill out the sign up sheet with the date, time, your name, room 224, "slow", and 30 min. If the machine is currently in use, you can store the media on the desk next to the machine or in the refrigerator in the lab.

Follow directions for "liquid" (If autoclaving without liquid you can use the fast release).

To remove from the Autoclave, open door from behind to allow steam to escape. Use large orange gloves from Wiegand 224 to remove the containers from the autoclave, as they will be extremely hot. There is a cart in Wiegand 224 which may be available for use.

Allow containers to cool until handleable.

**Inoculating the media with *H. uvarum*:**

- Bunsen burner & starter
- Inoculation Loop
- *H. uvarum* prepared the previous week
- YPD media

In the fume hood (Wiegand 224), spray surface with ethanol and wipe down with paper towels. Turn on the fan and light (not UV light).

Place Bunsen burner in hood and start. Sanitize the inoculation loop by heating entire wire section until red-hot.

Use vortexer to incorporate sediment in *H. uvarum* from previous week into the liquid.

Open one of the new containers of YPD media and run the Bunsen burner over the top. Dip the loop in the *H. uvarum* and immediately dip the loop into the fresh media.

Run the Bunsen burner over the opening of the newly inoculated container and place the lid on tightly. Place the loop over the Bunsen burner until red-hot. Repeat for each container of media, making sure to clean the loop between each. Replace lid of *H. uvarum* if taking a break.

After complete, return Bunsen burner and loop and clean the surface with ethanol and paper towels. *H. uvarum* from the past week can be dumped down the drain, and the vial can be washed.

**Growth chamber**

Place inoculated YPD media in the growth chamber (25$^{o}$C) adjacent to the fume hood.

After 2-3 days, the *H. uvarum* will reach its maximum population, which is approximately 1 x $10^7$ CFU (colony forming unit) /mL. Remove from the growth chamber and store in a refrigerator until use. There will be a whitish sediment present in each container.

**If at any point in the preparation of YPD media and *Hanseniaspora uvarum* needs to stop, place closed containers in the fridge.