

# Seasonal Monitoring for *Drosophila suzukii* (Diptera: Drosophilidae) in California Commercial Raspberries

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**ABSTRACT** Native to Southeast Asia, *Drosophila suzukii* (Matsumura) prefer to oviposit on ripe fruit and have become an important pest of California raspberries (*Rubus idaeus* L.) since their detection in Santa Cruz County, CA, in 2008. Preliminary management guidelines included *D. suzukii* monitoring recommendations, though there was little available information on seasonal occurrence and potential lures for use in raspberries. To address this issue, we trapped adult *D. suzukii* weekly for 2 yr (including both spring and fall harvests) in multiple raspberry varieties using apple cider vinegar and a yeast–sugar–water mixture as liquid lures, and measured fruit infestation when commercially ripe fruit were available. *D. suzukii* pressure as measured by larval infestation and adult trap captures was higher during the fall raspberry harvest season. The yeast lure captured significantly more *D. suzukii* during the fall harvest than the apple cider vinegar, and while both lures tended to capture more females than males, this varied by month of the year and was more pronounced for the yeast lure. Trap captures from each lure correlated well to one another, and often exhibited significant correlation to larval infestation. However, during all seasons and under both conventional and organic management, worrisome outliers were present (high larval infestation with low trap captures) that call into question the reliability of using the systems presented here as a basis for management decisions at this time.

**KEY WORDS** spotted wing drosophila, sex ratio, apple cider vinegar, yeast–sugar–water, phenology

California raspberry (*Rubus idaeus* L.) acreage has almost doubled over the past decade, with production concentrated along the California coast (Goodhue et al. 2011). In 2009, US\$293 million worth of raspberries were produced statewide, with US\$104 million of gross crop value produced in Santa Cruz County alone (Goodhue et al. 2011). In 2008, the first mainland United States detection of the Southeast Asian invasive pest *Drosophila suzukii* (Matsumura) occurred in Santa Cruz County (Hauser 2011). Unlike most other *Drosophila* spp., *D. suzukii* possess a large ovipositor with many sclerotized teeth and exhibit a preference for intact ripe and ripening fruit in lieu of overripe and blemished fruit as an oviposition substrate (Hauser 2011, Lee et al. 2011b, Burrack et al. 2013). Since that detection, significant crop losses have been reported among growers of berry crops (e.g., raspberry) and soft-skinned stone fruits (e.g., cherry [*Prunus avium* L.]) throughout the United States, Canada, and Europe (Lee et al. 2011a, Calabria et al. 2012, Cini et al. 2012, Kiss et al. 2013). Efforts to estimate the potential for economic damage have been difficult, but in California alone (at 20% damage) an estimated US\$300 million could be lost annually (Bolda et al. 2010, Walsh

et al. 2011) and revenues from raspberries could be decreased by 37% if *D. suzukii* is not managed (Goodhue et al. 2011).

*D. suzukii* was first described as a pest of cherries in Japan, and was found to oviposit most often on ripe cherries, peaches (*Prunus persica* (L.)), plums (*Prunus* spp.), persimmons (*Diospyros* spp.), strawberries (*Fragaria* × *ananassa* Duchesne) and grapes (*Vitis* spp.) with opportunistic infestation reported on other overripe and blemished fruits (Kanzawa 1936, 1939; Walsh et al. 2011). Little is known about the biology and seasonal occurrence of *D. suzukii* in raspberries, though *D. suzukii* has become one of the pests of greatest concern for raspberry growers and raspberries appear to be a highly attractive host. In fact, a host potential index developed from *D. suzukii* larval performance, flight bioassay, and oviposition data on different postharvest fruits indicates that raspberries have the greatest potential to serve as a postharvest host when compared with blackberries (*Rubus* spp.), blueberries (*Vaccinium* spp.), cherries, grapes, peaches, and strawberries (Bellamy et al. 2013). In laboratory no-choice experiments, raspberries had the highest *D. suzukii* oviposition rate as compared with blackberries, blueberries, and strawberries (Burrack et al. 2013). Also, raspberries showed greater field infestation than blackberries (Burrack et al. 2013).

Initial management guidelines recommended monitoring adults to ensure pest presence before initiating

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**Table 1.** Commercial harvest dates as reported by individual grower records for each management and variety

| Season      | Variety | Conventional  |               | Organic       |               |
|-------------|---------|---------------|---------------|---------------|---------------|
|             |         | First harvest | Final harvest | First harvest | Final harvest |
| Spring 2011 | G314.8  | 20 April 2011 | 4 July 2011   | 16 April 2011 | 9 July 2011   |
|             | S846.1  | 7 May 2011    | 16 July 2011  | 23 April 2011 | 27 Aug.2011   |
|             | S810.5  | 14 May 2011   | 3 Sept 2011   | 30 April 2011 | 3 Sept. 2011  |
| Fall 2011   | S846.1  | 23 July 2011  | 15 Oct. 2011  | 23 July 2011  | 3 Dec. 2011   |
|             | Z135.1  | 23 July 2011  | 29 Oct. 2011  | 13 Aug. 2011  | 3 Dec. 2011   |
|             | S810.5  | 30 July 2011  | 26 Nov. 2011  | 23 July 2011  | 3 Dec. 2011   |
| Spring 2012 | Z135.1  | 5 May 2012    | 7 July 2012   | 5 May 2012    | 22 July 2012  |
|             | S810.5  | 12 May 2012   | 18 Aug. 2012  | 5 May 2012    | 22 July 2012  |
|             | S846.1  | 2 June 2012   | 8 Sept 2012   | 7 July 2012   | 25 Aug. 2012  |
| Fall 2012   | Z135.1  | 21 July 2012  | 3 Nov. 2012   | 4 July 2012   | 20 Oct. 12    |
|             | Z321.1  | 28 July 2012  | 1 Dec. 2012   | 2 June 2012   | 8 Sept. 2012  |
|             | S810.5  | 28 July 2012  | 1 Dec. 2012   | 17 June 2010  | 30 Oct. 2012  |

control measures, with fruit purees, distillates such as apple cider vinegar or wine, chemicals such as acetic acid and ethanol, and fermentation mixtures of yeast, sugar, and water as potential lures (Walsh et al. 2011). An active fermentation that continuously produced volatiles (yeast–sugar–water mixture) was thought to be highly attractive, and apple cider vinegar became widely recommended because of its field longevity and clarity (Walsh et al. 2011). In Japan, grape wine mixtures were found to be more attractive in cherry orchards, while rice wine mixtures were more attractive in grapes (Kanzawa 1936, 1939), suggesting that background host odors may influence lure attractiveness, and highlighting the importance of evaluating a potential lure's efficacy in multiple host crops. As yeast–sugar–water mixture and apple cider vinegar have been the most commonly used and recommended lures in California cropping systems, we sought to compare trap captures, sex ratio bias, and correlation with infestation of these lures in California coastal raspberries during both the spring and fall harvest seasons. This study is also a first look at seasonal patterns and population levels in spring and fall harvested raspberries.

### Materials and Methods

**Horticultural Practices in California Raspberry.** Raspberry canes usually have an 18-mo commercial lifespan, with first fruiting for these canes in the fall following planting. Briefly, canes are grown under plastic high tunnels most of the year, and the plastic is removed during the rainiest part of the winter (usually late December through early February). Tunnels are open at the ends and semicircular at the top. Most tunnels are  $\approx 7.3$  m in width at the base, 6.0–6.7 m in height at the peak, and 85.3–91.4 m in length. Canes are winter-planted in December (December 2009, 2010, and 2011 in this study) prior to the first fruiting (fall of the following year) and then either pruned or mowed during the second winter. Horticultural management (including pruning or mowing) and variety dictate fruit ripening time during the following spring (Table 1). A final spring fruiting would then be harvested and the raspberry planting would be removed after that harvest, usually in mid-August.

**Site Selection.** All sites were in Santa Cruz County near Watsonville, CA, and managed according to common commercial raspberry production practices. One conventional management (Table 2) and one organic management (Table 3) planting typical of production in the Monterey Bay area were trapped simultaneously for the duration of this study. Six separate plantings (three plantings each of organic and conventional) were used to allow continuous monitoring from January 2011 and continuing through December 2012. Monitoring began in each planting before "first fruiting" (fall harvest) and continued through to the "second fruiting" (spring harvest). The canes were removed after the second fruiting each August, and a duplicate set of traps was placed at a nearby new raspberry planting grown using similar management practices in August just before the removal of the old canes to allow capture overlap for the 2 wk between the termination of harvest in the older planting and the initiation of harvest in the newer planting. However, overlap did not always occur as canes were sometimes removed unexpectedly. Plantings were selected to include commonly used varieties, maintain close proximity to the old planting (for new plantings), and retain similar insecticide management practices (either organic or conventional). Three raspberry varieties were chosen for each planting, representing the early, mid, and late ripening varieties that were most abundantly planted that year. For the first planting, this was the S810.5, S846.1, and G314.8 variety. G314.8 was replaced with Z135.1 for the second planting, so it consisted of S810.5, S846.1, and Z135.1. S846.1 was replaced with Z321.1 for the third planting, which consisted of S810.5, Z321.1, and Z135.1. As horticultural practices interact with variety ripening time, the variety that ripened earliest at each planting was not necessarily the same. Therefore, the commercial harvest period for each harvest and variety at each planting was used to determine weeks of harvest (Table 1).

**Adult Sampling.** Traps were made of 473-ml glass Ball Wide Mouth Jars (TMS Ball Co., Daleville, IN) with four 0.5-cm holes punched in a square (6.35 by 6.35 cm) pattern at the center of the lid. A 1.5-mm hole was punched in the center of the lid and a 1-mm-diameter plastic coated steel wire (The Hillman

**Table 2.** Conventional insecticide spray records (excluding Bt applications) as reported by individual growers for each planting (applied in  $\approx 1,871$  liters of carrier water per hectare)

| Harvest     | Date          | Product                          | AI                | Rate (AI/ha) |
|-------------|---------------|----------------------------------|-------------------|--------------|
| Spring 2011 | 21 April 2011 | Delegate WG <sup>a</sup>         | Spinetoram        | 52.8 g       |
|             | 11 July 2011  | Malathion 8 Aquamul <sup>b</sup> | Malathion         | 2.24 kg      |
| Fall 2011   | 30 Aug. 11    | Delegate WG <sup>a</sup>         | Spinetoram        | 52.8 g       |
|             | 16 Sept. 2011 | Malathion 8 Aquamul <sup>b</sup> | Malathion         | 2.24 kg      |
|             | 16 Sept. 2011 | Mustang Max <sup>c</sup>         | Zeta-cypermethrin | 33.6 g       |
| Spring 2012 | 12 Oct. 2011  | Delegate WG <sup>a</sup>         | Spinetoram        | 52.8 g       |
|             | 18 May 2012   | Delegate WG <sup>a</sup>         | Spinetoram        | 52.8 g       |
|             | 9 June 2012   | Delegate WG <sup>a</sup>         | Spinetoram        | 52.8 g       |
|             | 19 July 2012  | Malathion 8 Aquamul <sup>b</sup> | Malathion         | 2.24 kg      |
|             | 19 July 2012  | Mustang Max <sup>c</sup>         | Zeta-cypermethrin | 33.6 g       |
| Fall 2012   | 20 Aug. 2012  | Malathion 8 Aquamul <sup>b</sup> | Malathion         | 2.24 kg      |
|             | 20 Aug. 2012  | Mustang Max <sup>c</sup>         | Zeta-cypermethrin | 33.6 g       |
|             | 12 Sept. 2012 | Malathion 8 Aquamul <sup>b</sup> | Malathion         | 2.24 kg      |
|             | 12 Sept. 2012 | Mustang Max <sup>c</sup>         | Zeta-cypermethrin | 33.6 g       |
|             | 5 Oct. 2012   | Malathion 8 Aquamul <sup>b</sup> | Malathion         | 2.24 kg      |
|             | 5 Oct. 2012   | Mustang Max <sup>c</sup>         | Zeta-cypermethrin | 33.6 g       |

<sup>a</sup> Dow AgroSciences, LLC, Indianapolis, IN.

<sup>b</sup> Loveland Products, Inc., Greeley, CO.

<sup>c</sup> FMC Corporation, Philadelphia, PA.

Group, Cincinnati, OH) was threaded through as a hanging device ("Jar" trap). One hundred milliliter of liquid lure was used to attract and capture adults and the lure was changed weekly. A "Jar" trap of each liquid lure type (three types: apple cider vinegar [Safeway, Inc., Pleasanton, CA], a liquid yeast mixture, or water alone) was placed into each raspberry

tunnel (4 replicate tunnels were used for each variety, for a total of 12 traps per variety). The yeast-sugar-water mixture was prepared the evening before trap change by mixing 80 ml of active dry yeast (Giusto's Active Dry Yeast, South San Francisco, CA), 195 g sugar (C&H Sugar Company, Inc., Crockett, CA), and 4,800 ml of water. Before completing this study, a trap

**Table 3.** Organic insecticide spray records (excluding Bt applications) as reported by individual growers for each planting (applied in  $\approx 1,122$  liters of carrier water per hectare from Spring 2011 to Spring 2012, and  $\approx 937$  liters of carrier water per hectare for Fall 2012)

| Harvest     | Date          | Product                     | AI         | Rate (AI/ha) |
|-------------|---------------|-----------------------------|------------|--------------|
| Spring 2011 | 31 Mar. 2011  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 21 April 2011 | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 21 April 2011 | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 14 June 2011  | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 14 June 2011  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 16 July 2011  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
| Fall 2011   | 7 Aug. 2011   | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 29 Aug. 2011  | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 29 Aug. 2011  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 18 Sept. 2011 | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 18 Sept. 2011 | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 9 Oct. 2011   | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 6 Nov. 2011   | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 6 Nov. 2011   | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 16.4 ml      |
|             | 30 Mar. 2012  | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
| Spring 2012 | 30 Mar. 2012  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 28 April 2012 | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 13 May 2012   | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 18 May 2012   | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 18 May 2012   | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 12 June 2012  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 26 June 2012  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 14 July 2012  | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 14 July 2012  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
| Fall 2012   | 1 Aug. 2012   | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 1 Aug. 2012   | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 24 Aug. 2012  | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 24 Aug. 2012  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 1 Sept. 2012  | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |
|             | 21 Sept. 2012 | Entrust 80 WG <sup>b</sup>  | Spinosad   | 112.1 g      |
|             | 21 Sept. 2012 | Pyganic 1.4 EC <sup>a</sup> | Pyrethrins | 18.4 ml      |

<sup>a</sup> McLaughlin Gormley King (MGK), Co., Minneapolis, MN.

<sup>b</sup> Dow AgroSciences, LLC, Indianapolis, IN.

comparison study (Lee et al. 2012) was published that suggested that the "Haviland" trap (a Rubbermaid container with 8.5-cm-diameter opening covered with a 0.32-cm metal mesh hardware cloth and rain tent [further described in Lee et al. 2012]) captured more *D. suzukii* in most crops and locations than the five other traps evaluated (Lee et al. 2012). All of these traps were compared using apple cider vinegar as a lure; because the liquid lure plus Jar trap trapping system used in our study was not included in the published trap comparison, we performed a trapping system calibration experiment that is included in the Supp Materials (Supp Figs. 1 and 2, Supp Table 1 [online only]) to address the omission. Commercial raspberry canes are staked approximately every 5.5 m, so we hung our traps from the stakes located at 11, 16.5, and 22 m into the field within the center of the canes at canopy height in the middle row of the tunnel. Traps were randomized after each weekly lure replacement to one of these three distances to mitigate the effect of the varying distance into the tunnel on trap captures. Traps containing each lure type were deployed in four replicate tunnels for each of the three raspberry varieties at both the conventional and organic planting, for a total of 72 traps. Trapped adult *D. suzukii* were identified, sexed, and counted under a dissecting microscope. The data for the water controls (which caught four flies over the entire 2-yr trapping period) were excluded from all analyses.

Monthly vacuum samples were conducted for the duration of the study. The north row of canes in each of the four replicate tunnels was vacuumed with a D-VAC Vacuum Insect Net model 122 (Rincon-Vitova Insectaries, Inc. Ventura, CA) for the entire height of the canes along a 5.5-m segment from 11 to 16.5 m into the field. Samples were bagged in 3.79-liter self-sealing bags (Ziploc Freezer Bags, S.C. Johnson & Son, Inc., Racine, WI) and returned to the laboratory where adult *D. suzukii* were identified, sexed, and counted under a dissecting microscope.

**Larval Sampling.** When fruit was available during the commercial harvest period, 40 raspberries (as close to marketable ripeness as possible) were pooled from each variety for each planting with 10 raspberries collected per replicate tunnel. Fruit were held for 2–5 d to allow larvae to grow and then gently crushed. The crushed berries were then soaked in a salt water solution of 473 ml of water mixed with 22.5 ml of non-iodized salt for  $\approx 10$  min to allow the larvae to float. This mixture was gently agitated and poured through 6-mm wire mesh into a series of four sieves with the coarsest at the top and the finest at the bottom (2.36-mm mesh, 500-, 300-, and 250- $\mu$ m mesh) 76.2 mm in diameter and 50.8 mm in height sieves (W.S. Tyler Industrial Group, Mentor, OH). Total *Drosophila* larvae per 40 raspberry sample were counted by inspecting both sides of the sieves under a dissecting microscope. The vast majority of *Drosophila* larvae are *D. suzukii* in commercially ripe raspberries at commercial harvest timing; therefore, larvae were not reared to verify species (Author's observations resulting from data collection for Hamby et al. 2012).

**Statistical Analyses.** Analysis of lures was limited to the data obtained in the first 10 wk of fall harvest for both years, as this was the only time period when sufficient *D. suzukii* were captured to permit a statistical comparison. Only trap data from the S810.5 variety was used because it was the only variety present in all plantings. Comparisons of total *D. suzukii* captured were made using the Glimmix procedure of SAS 9.3 statistical software (SAS Institute Inc., Cary, NC) with Lure, Management, Year, Week (harvest week), and all two-way interactions of these fixed effects included in a Poisson mixed model. Computational issues precluded running a full factorial model, so the model was reduced to include only two-way interactions, thus reducing the number of random effects that had to be estimated. A random statement with the appropriate random effects was included to deal with the repeated measures design of this experiment and ensure replication was treated correctly. Effect means were separated using an LSmeans statement with a Tukey–Kramer adjustment for multiple comparisons.

Capture sex ratios were compared between the apple cider vinegar and yeast lure. As vacuum samples were collected monthly, fly captures were summed across all traps that were deployed for both liquid lure types for each management approach and month for each year (2011 and 2012). Because vacuum sample captures were so much lower than trap captures and contained numerous 0 values, the vacuum samples were not included in the sex ratio statistical analysis. A logistic regression was performed using PROC GLIMMIX in SAS 9.3 (SAS Institute Inc., Cary, NC) for the organic plantings and conventional plantings separately. The generalized linear mixed model with binomial distribution was composed of the response variable Females by Total and included Month, Lure, and Month  $\times$  Lure as fixed effects. Effect means were separated using an LSmeans statement with a Tukey–Kramer adjustment for multiple comparisons.

A Pearson correlation was performed in SAS 9.2 using PROC CORR to associate fruit infestation with apple cider vinegar and yeast captures. Because fruit infestation was measured as a sum of the larvae collected from 40 fruit total (10 collected from each tunnel for each variety) at each site, the four replicate trap captures for each corresponding week were totaled for each lure type in each variety at each site. Both infestation and total flies from each trap were  $\log_{10}(x + 0.25)$ -transformed to compress the values being correlated into a smaller range and reduce outlier effects. Data from 2011 and 2012 were used from both organic and conventional plantings for all varieties and weeks during commercial harvest that had fruit samples. The data were sliced by harvest season and management as *D. suzukii* occurrence and infestation is very different in the fall versus the spring harvest and the difference between organic and conventional insecticide programs is thought to impact the number of adult flies present in the fields (see Tables 2 and 3).

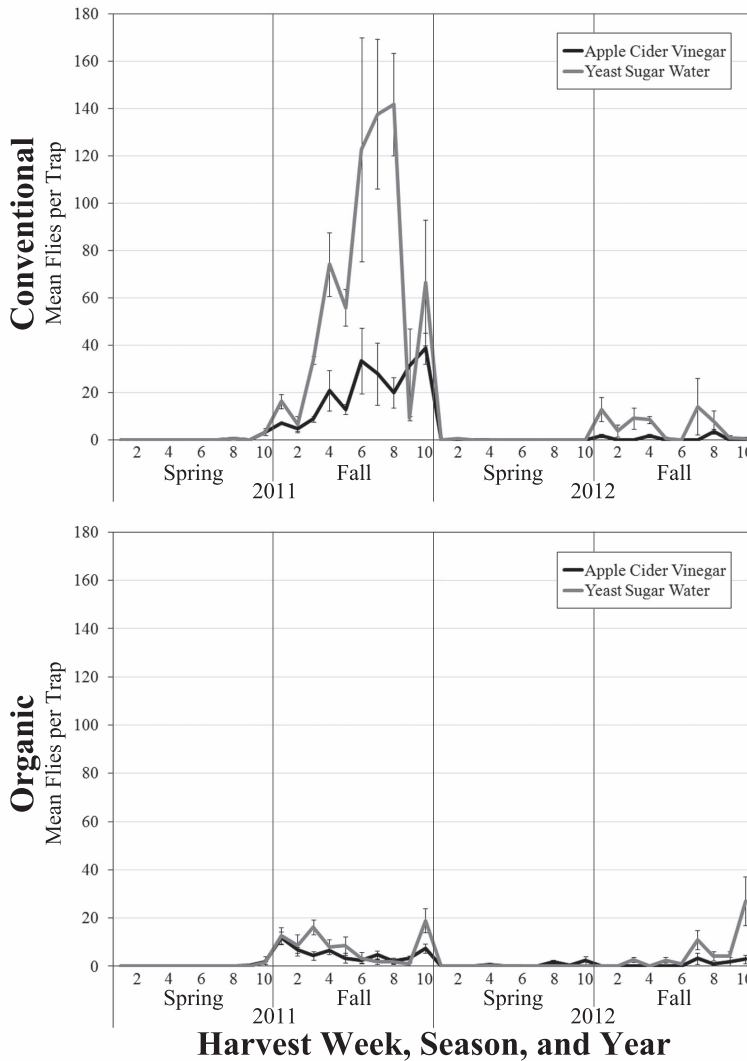


Fig. 1. Mean *D. suzukii*  $\pm$  SE captured per trap per week in the first 10 wk of commercial spring and fall harvest for variety S810.5 in 2011 and 2012.

### Results and Discussion

**Adult Trapping.** The seasonal abundance of adult *D. suzukii* captured during the spring and fall harvest was strikingly different (Fig. 1). Very few *D. suzukii* adults were captured during the spring; in fact, most weeks no flies were caught in either lure type (Table 4). Because of the low spring adult captures, lures could only be compared in the fall. Lure ( $F_{1,13} = 78.29$ ;  $P < 0.0001$ ), Management ( $F_{1,12} = 18.81$ ;  $P = 0.0010$ ), Year ( $F_{1,12} = 114.94$ ;  $P = 0.0015$ ), Week ( $F_{9,117} = 3.13$ ;  $P = 0.0020$ ), and the interactions of Lure  $\times$  Year ( $F_{1,13} = 15.95$ ;  $P = 0.0015$ ), Lure  $\times$  Week ( $F_{9,135} = 29.58$ ;  $P < 0.0001$ ), Management  $\times$  Year ( $F_{1,12} = 10.34$ ;  $P = 0.0074$ ), and Management  $\times$  Week ( $F_{9,117} = 2.44$ ;  $P = 0.0140$ ) had a significant effect on total flies captured in the fall.

It is unsurprising that management exhibited a significant effect on *D. suzukii* adult trap captures,

though the authors would have expected the organic plantings to exhibit greater *D. suzukii* captures than the conventional plantings because the insecticides used in the conventional plantings (malathion, spinetoram, and zeta-cypermethrin) are generally considered to be more efficacious than those used in the organic planting (pyrethrin and spinosad). For instance, Bruck et al. (2011) field-applied pyrethrin (Pyganic EC 5.0, McLaughlin Gormley King Co., Minneapolis, MN) at 62 g (AI)/ha and found adult *D. suzukii* captured in a sweep net to remain significantly fewer than the control for only 1 d posttreatment (when sampled at 1, 5, 10, and 14 d), whereas field application of malathion (Malathion 8 EC, Arysta Life-Science North America, LLC, Cary, NC) at 2.8 kg (AI)/ha showed significant reduction relative to control for up to 10 d posttreatment (Bruck et al. 2011).

**Table 4.** Weekly and yearly mean  $\pm$  SE *D. suzukii* adult trap captures for each apple cider vinegar and yeast lures during spring and fall harvest

| Week | N  | Spring              |                 | Fall <sup>a</sup>   |                   | $t_{135}$ | P value <sup>b</sup> |
|------|----|---------------------|-----------------|---------------------|-------------------|-----------|----------------------|
|      |    | Apple cider vinegar | Yeast           | Apple cider vinegar | Yeast             |           |                      |
|      |    | Mean $\pm$ SE       | Mean $\pm$ SE   | Mean $\pm$ SE       | Mean $\pm$ SE     |           |                      |
| 1    | 16 | 0.00 $\pm$ 0.00     | 0.06 $\pm$ 0.06 | 5.25 $\pm$ 1.33     | 10.56 $\pm$ 2.22  | -5.42     | <0.0001              |
| 2    | 16 | 0.13 $\pm$ 0.09     | 0.25 $\pm$ 0.14 | 2.88 $\pm$ 0.83     | 4.88 $\pm$ 1.63   | -3.98     | 0.0153               |
| 3    | 16 | 0.00 $\pm$ 0.00     | 0.00 $\pm$ 0.00 | 3.44 $\pm$ 1.06     | 15.50 $\pm$ 3.26  | -9.12     | <0.0001              |
| 4    | 16 | 0.19 $\pm$ 0.19     | 0.13 $\pm$ 0.13 | 7.31 $\pm$ 2.86     | 22.81 $\pm$ 8.33  | -8.07     | <0.0001              |
| 5    | 16 | 0.00 $\pm$ 0.00     | 0.00 $\pm$ 0.00 | 4.19 $\pm$ 1.51     | 16.94 $\pm$ 6.19  | -8.97     | <0.0001              |
| 6    | 16 | 0.00 $\pm$ 0.00     | 0.00 $\pm$ 0.00 | 9.06 $\pm$ 4.81     | 31.81 $\pm$ 17.21 | -9.27     | <0.0001              |
| 7    | 16 | 0.00 $\pm$ 0.00     | 0.06 $\pm$ 0.06 | 9.00 $\pm$ 4.16     | 41.25 $\pm$ 16.31 | -10.62    | <0.0001              |
| 8    | 16 | 0.69 $\pm$ 0.28     | 0.19 $\pm$ 0.14 | 6.75 $\pm$ 2.48     | 38.88 $\pm$ 16.13 | -10.94    | <0.0001              |
| 9    | 16 | 0.25 $\pm$ 0.17     | 0.06 $\pm$ 0.06 | 9.19 $\pm$ 4.85     | 3.88 $\pm$ 1.03   | 3.32      | 0.1161               |
| 10   | 16 | 2.00 $\pm$ 0.74     | 1.19 $\pm$ 0.45 | 12.44 $\pm$ 4.28    | 28.31 $\pm$ 8.96  | -6.44     | <0.0001              |
| Year |    |                     |                 |                     |                   |           |                      |
| 2011 | 80 | 0.34 $\pm$ 0.15     | 0.31 $\pm$ 0.11 | 13.00 $\pm$ 1.77    | 37.34 $\pm$ 5.96  | -3.88     | 0.0090               |
| 2012 | 80 | 0.31 $\pm$ 0.11     | 0.08 $\pm$ 0.04 | 0.90 $\pm$ 0.21     | 5.63 $\pm$ 1.11   | -8.27     | <0.0001              |

<sup>a</sup> Due to the significant Lure\*Week ( $F_{9, 135} = 29.58$ ;  $P < 0.0001$ ) and Lure\*Year ( $F_{1, 13} = 15.95$ ;  $P = 0.0015$ ), interactions comparisons were made between the lure trap captures during fall harvest for each week and year separately comparing apple cider vinegar against yeast.

<sup>b</sup> Values were Tukey-Kramer adjusted for multiple comparisons.

Also, when *D. suzukii* females were exposed to fruits that had been treated with malathion (Malathion 5 EC, Arysta LifeScience North America, LLC, Cary NC) at 599 g/liter, spinetoram (Delegate 25WG, Dow AgroSciences LLC, Indianapolis, IN) at 250 g/kg, and spinosad (Entrust 80 WP, Dow AgroSciences LLC, Indianapolis, IN) at 800 g/kg, the spinetoram treatment resulted in fewer remaining adults and eggs than did either spinosad or malathion (Beers et al. 2011). However, during the first 10 wk of harvest in the fall of both years, *D. suzukii* captures were greater in the conventional plantings than in the organic plantings (Fig. 1). This could be because of the more frequent insecticide applications in the organic fields (five application dates each fall) relative to the conventional fields (three application dates each fall). Alternatively, this trend may be because of a repellent effect of the more efficacious conventional insecticides that increases lure attractiveness relative to fruit host odors when applied to the fruit.

Because of the significant interactions of Lure with Week and Year, it is difficult to discern overall trends. However, when data are compared week to week and year to year, the yeast lure exhibits significantly higher trap captures than the apple cider vinegar lure for all weeks except week 9, where there is no significant difference between the lures (Apple Cider vs. Yeast Week 9  $t(135) = 3.32$ ;  $P = 0.1161$ ) and also exhibits significantly greater trap captures in both years (Table 4). Because yeasts are believed to be an important source of nutrients for many species of *Drosophila*, and *Drosophila* spp. have preferences for specific yeast species (Cooper 1960, Starmer 1981, Vacek et al. 1985), it is unsurprising that yeast fermentations are highly attractive. In fact, yeast-produced volatiles have been found to be the more important attractant relative to fruit volatiles, and sufficient to attract *Drosophila melanogaster* (Meigen) on their own (Becher et al. 2012). Like other *Drosophila* spp., *D. suzukii* is potentially associated with a specific yeast species, *Hanseniaspora uvarum* (Hamby et al. 2012).

Monthly vacuum samples began to capture low levels of flies in June and exhibited no captures after December in both years, with many fewer captured when compared with the monthly trap captures (Fig. 2; Tables 5 and 6). Because the monthly vacuum sample captures were too few for statistical analysis, they were excluded. We split this analysis by the two management approaches, as there was a sex bias in the efficacy of some of the insecticides with females generally exhibiting lower mortality than males (Bruck et al. 2011). Month was included as an effect in the model to account for seasonal differences due to *D. suzukii* phenology and effects of temperature and weather, because temperature would affect the rate of yeast growth (Arthur and Watson 1976) and presumably affect attractiveness of the lure as well. At the conventional plantings, both Month ( $F_{11, 27} = 47.25$ ;  $P < 0.0001$ ) and the interaction of Lure  $\times$  Month ( $F_{11, 27} = 4.63$ ;  $P = 0.0006$ ) had a significant impact on the sex ratio of the flies (females by total) captured, though the Lure ( $F_{1, 27} = 0.23$ ;  $P = 0.6338$ ) was not significant (Fig. 2). However, at the organic plantings Lure ( $F_{1, 28} = 4.43$ ;  $P = 0.0444$ ), Month ( $F_{11, 28} = 26.92$ ;  $P < 0.0001$ ), and the interaction Lure  $\times$  Month ( $F_{11, 28} = 25.85$ ;  $P < 0.0001$ ) were significant. Because of the significant interaction of Lure  $\times$  Month, interpretation of these data becomes more complicated. However, when the lures are compared across each month, apple cider vinegar is significantly less female biased than the yeast lure in July and September–December (Table 6) and significantly more female biased than the yeast lure in January and February (Table 6). In general, more females were captured in lure-based traps than males, and more males were captured in vacuum samples than females, though this trend is more pronounced in the higher capturing organic plantings (Tables 5 and 6).

Traps and lures have been found to have sex-specific biases in capture rates and are thought to be inaccurate in determining natural sex ratio (Harper

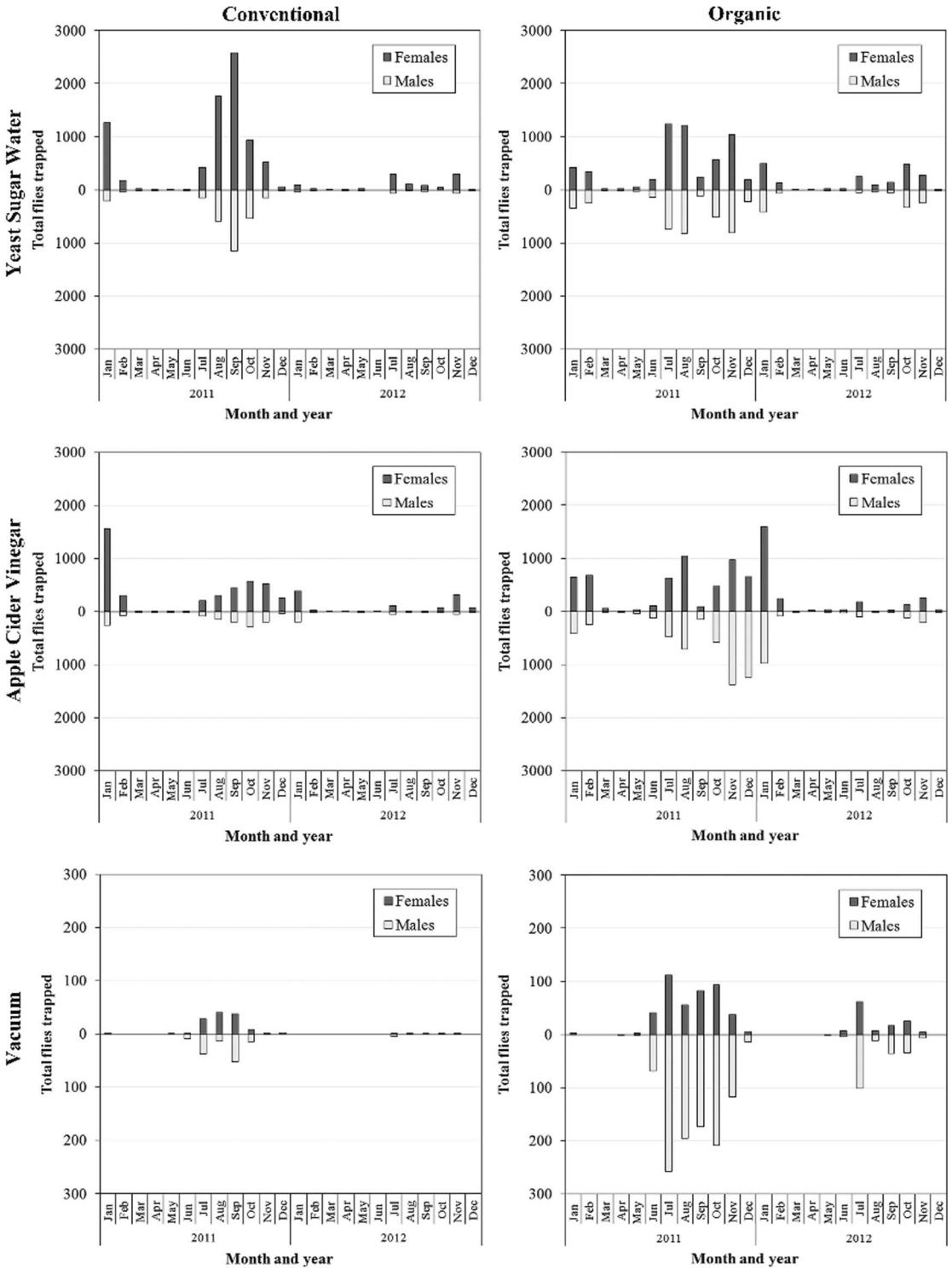


Fig. 2. Total male and female *D. suzukii* captured per month for each management with each lure (four sampling dates) and vacuum sample (one sampling date).

**Table 5. Monthly adult mean  $\pm$  SE *D. suzukii* total trap captures, sex ratio (Females by Total), and larvae in 40 berries  $\pm$  SE for both years ( $n = 2$ ) at the conventional plantings**

| Month | Apple cider vinegar |                    | Yeast               |                    | Vacuum           |                    | Larvae in 40 berries |  |
|-------|---------------------|--------------------|---------------------|--------------------|------------------|--------------------|----------------------|--|
|       | Mean $\pm$ SE       | Sex ratio $\pm$ SE | Mean $\pm$ SE       | Sex ratio $\pm$ SE | Mean $\pm$ SE    | Sex ratio $\pm$ SE | Mean $\pm$ SE        |  |
| Jan.  | 401.67 $\pm$ 111.75 | 0.76 $\pm$ 0.05    | 267.17 $\pm$ 131.23 | 0.78 $\pm$ 0.05    | 0.17 $\pm$ 0.17  | 1.00 $\pm$ NA      |                      |  |
| Feb.  | 67.83 $\pm$ 28.23   | 0.84 $\pm$ 0.04    | 39.17 $\pm$ 16.56   | 0.82 $\pm$ 0.07    | 0.00 $\pm$ 0.00  | -                  |                      |  |
| Mar.  | 6.00 $\pm$ 1.86     | 0.74 $\pm$ 0.13    | 6.83 $\pm$ 2.94     | 0.71 $\pm$ 0.16    | 0.00 $\pm$ 0.00  | -                  |                      |  |
| April | 2.83 $\pm$ 1.14     | 0.95 $\pm$ 0.05    | 4.00 $\pm$ 1.69     | 0.77 $\pm$ 0.11    | 0.00 $\pm$ 0.00  | -                  |                      |  |
| May   | 3.00 $\pm$ 1.29     | 0.67 $\pm$ 0.19    | 4.50 $\pm$ 4.10     | 0.63 $\pm$ 0.32    | 0.17 $\pm$ 0.17  | 1.00 $\pm$ NA      | 2.0 $\pm$ 1.0        |  |
| June  | 3.50 $\pm$ 3.30     | 0.73 $\pm$ 0.28    | 1.50 $\pm$ 1.50     | 0.67 $\pm$ NA      | 1.67 $\pm$ 1.48  | 0.11 $\pm$ 0.11    | 15.5 $\pm$ 7.5       |  |
| July  | 38.17 $\pm$ 9.23    | 0.72 $\pm$ 0.07    | 77.50 $\pm$ 20.49   | 0.75 $\pm$ 0.04    | 7.10 $\pm$ 5.37  | 0.47 $\pm$ 0.07    | 623.0 $\pm$ 477.0    |  |
| Aug.  | 64.43 $\pm$ 32.74   | 0.62 $\pm$ 0.05    | 352.71 $\pm$ 156.91 | 0.83 $\pm$ 0.04    | 9.17 $\pm$ 5.39  | 0.75 $\pm$ 0.10    | 259.0 $\pm$ 139.0    |  |
| Sept. | 112.67 $\pm$ 70.96  | 0.58 $\pm$ 0.15    | 640.17 $\pm$ 290.86 | 0.76 $\pm$ 0.05    | 15.17 $\pm$ 7.58 | 0.55 $\pm$ 0.15    | 657.0 $\pm$ 657.0    |  |
| Oct.  | 154.33 $\pm$ 65.60  | 0.82 $\pm$ 0.08    | 252.83 $\pm$ 132.26 | 0.84 $\pm$ 0.09    | 4.17 $\pm$ 3.00  | 0.62 $\pm$ 0.14    | 273.5 $\pm$ 270.5    |  |
| Nov.  | 154.57 $\pm$ 45.37  | 0.82 $\pm$ 0.04    | 171.00 $\pm$ 37.32  | 0.82 $\pm$ 0.05    | 0.83 $\pm$ 0.48  | 0.56 $\pm$ 0.29    | 11.0 $\pm$ 8.0       |  |
| Dec.  | 61.83 $\pm$ 16.35   | 0.88 $\pm$ 0.03    | 13.67 $\pm$ 9.35    | 0.77 $\pm$ 0.10    | 0.50 $\pm$ 0.34  | 1.00 $\pm$ 0.00    | 2.0 $\pm$ NA         |  |

and Story 1962, Scheirs et al. 1997, Leong and Thorp 1999, Thomas et al. 2001). Our study found both liquid lures tended to capture more females than males, while the vacuum samples tended to catch more males than females (Table 4; Fig. 2). The increased male capture in the vacuum samples may be because of differences in *D. suzukii* activity by sex, as male *D. suzukii* were found to be more active in laboratory activity bioassays (Hamby et al. 2013) and research observations in Japan described females as more “passive” (Kanzawa 1939). It is unsurprising that time of year would impact the sex ratio of trap captures, as *D. suzukii* may overwinter as mated females and males exhibit greater mortality at cold temperatures (Kanzawa 1936, 1939; Dalton et al. 2011). As mentioned previously, there may be an interaction with season and lure efficacy due to the active fermentation of the yeast that may also contribute to the significant interaction between month of the year and lure.

**Larval Infestation.** Larval infestation did not show as strong a seasonal trend as adults captures, though populations did build-up over the course of the harvest season, and spring harvest tended to exhibit fewer larvae than fall (Fig. 3; Tables 5 and 6). The fall harvest season would be expected to have greater pest pres-

sure because the *D. suzukii* population would have been able to build-up during the spring harvest and then transition to fall harvest plantings in mid-August. In addition to a natural population increase, a shortage in hourly labor at the end of the spring harvest may also contribute to increased fall populations. Hourly labor to perform horticultural practices is limited in August as this is the time period where simultaneously the fall harvest plantings are being tunneled (high plastic tunnels are replaced after the harshest part of winter), spring harvest is concluding, and in some cases fall harvest is beginning (Personal communication Patrick Kingston, Garrouette Farms, Inc.). One result of this labor shortage is a lag in fruit removal between the cessation of spring harvest and the removal of the remaining spring canes. Many ripe and overripe fruit remain in the spring harvest plantings at this time and equipment cannot enter the field to spray as the cane support stakes have been removed. Random samples of fruit taken from the fallen brambles we have taken reveal that they can contain very high numbers of larvae (e.g., 682 larvae in a 40 berry sample) that become a source for *D. suzukii* infesting the fall harvest plantings.

**Table 6. Monthly adult mean  $\pm$  SE *D. suzukii* total trap captures, sex ratio (Females by Total), and larvae in 40 berries  $\pm$  SE for both years ( $n = 2$ ) at the organic plantings with a comparison of sex ratios for apple cider vinegar and yeast lures**

| Month | Apple cider vinegar |                    | Yeast               |                    | Vacuum            |                    | Larvae in 40 berries | Sex ratio <sup>a</sup> |                        |
|-------|---------------------|--------------------|---------------------|--------------------|-------------------|--------------------|----------------------|------------------------|------------------------|
|       | Mean $\pm$ SE       | Sex ratio $\pm$ SE | Mean $\pm$ SE       | Sex ratio $\pm$ SE | Mean $\pm$ SE     | Sex ratio $\pm$ SE | Mean $\pm$ SE        | $t_{25}$               | $P$ value <sup>b</sup> |
| Jan.  | 606.67 $\pm$ 56.80  | 0.61 $\pm$ 0.02    | 278.67 $\pm$ 59.26  | 0.52 $\pm$ 0.03    | 0.50 $\pm$ 0.34   | 1.00 $\pm$ NA      |                      | 4.87                   | 0.0064                 |
| Feb.  | 210.33 $\pm$ 69.63  | 0.71 $\pm$ 0.02    | 130.50 $\pm$ 42.99  | 0.63 $\pm$ 0.02    | 0.00 $\pm$ 0.00   | -                  |                      | 6.28                   | 0.0002                 |
| Mar.  | 13.33 $\pm$ 6.44    | 0.82 $\pm$ 0.06    | 7.50 $\pm$ 2.45     | 0.79 $\pm$ 0.10    | 0.00 $\pm$ 0.00   | -                  |                      | 1.09                   | 1.000                  |
| April | 6.50 $\pm$ 3.69     | 0.88 $\pm$ 0.07    | 4.17 $\pm$ 2.48     | 0.78 $\pm$ 0.11    | 0.17 $\pm$ 0.17   | 0.00 $\pm$ NA      |                      | 1.08                   | 1.000                  |
| May   | 17.67 $\pm$ 0.50    | 0.64 $\pm$ 0.14    | 18.17 $\pm$ 9.07    | 0.76 $\pm$ 0.09    | 1.00 $\pm$ 0.63   | 0.25 $\pm$ 0.25    | 11.0 $\pm$ 3.0       | -1.71                  | 0.98                   |
| June  | 48.50 $\pm$ 25.27   | 0.58 $\pm$ 0.12    | 62.67 $\pm$ 43.33   | 0.75 $\pm$ 0.11    | 20.17 $\pm$ 17.82 | 0.48 $\pm$ 0.21    | 304.0 $\pm$ 217.0    | -3.36                  | 0.1920                 |
| July  | 116.33 $\pm$ 62.28  | 0.59 $\pm$ 0.04    | 189.17 $\pm$ 85.77  | 0.71 $\pm$ 0.08    | 53.40 $\pm$ 28.41 | 0.37 $\pm$ 0.14    | 1336.0 $\pm$ 20.0    | -4.48                  | 0.0167                 |
| Aug.  | 254.86 $\pm$ 80.64  | 0.61 $\pm$ 0.09    | 309.00 $\pm$ 172.43 | 0.68 $\pm$ 0.03    | 38.43 $\pm$ 31.79 | 0.48 $\pm$ 0.10    | 108.0 $\pm$ 78.0     | -0.67                  | 1.000                  |
| Sept. | 48.50 $\pm$ 24.41   | 0.56 $\pm$ 0.12    | 89.50 $\pm$ 26.80   | 0.71 $\pm$ 0.03    | 51.17 $\pm$ 20.44 | 0.37 $\pm$ 0.05    | 351.5 $\pm$ 103.5    | -7.28                  | <0.0001                |
| Oct.  | 218.33 $\pm$ 08.55  | 0.43 $\pm$ 0.03    | 317.50 $\pm$ 116.89 | 0.55 $\pm$ 0.02    | 60.17 $\pm$ 25.96 | 0.39 $\pm$ 0.06    | 1576.0 $\pm$ 146.0   | -5.43                  | 0.0015                 |
| Nov.  | 469.67 $\pm$ 205.51 | 0.51 $\pm$ 0.05    | 398.50 $\pm$ 152.21 | 0.58 $\pm$ 0.05    | 27.67 $\pm$ 13.60 | 0.36 $\pm$ 0.08    | 460.0 $\pm$ 43.0     | -8.99                  | <0.0001                |
| Dec.  | 324.00 $\pm$ 43.03  | 0.47 $\pm$ 0.05    | 71.17 $\pm$ 35.40   | 0.57 $\pm$ 0.10    | 3.33 $\pm$ 2.06   | 0.27 $\pm$ 0.15    | 99.0 $\pm$ NA        | -11.12                 | <0.0001                |

<sup>a</sup> Due to the significant Lure  $\times$  Month ( $F_{11, 25} = 25.85$ ;  $P < 0.0001$ ) interaction comparisons were made between the lure sex ratios at the organic planting for each month separately comparing apple cider vinegar against the yeast lure.

<sup>b</sup> Values were Tukey-Kramer adjusted for multiple comparisons.



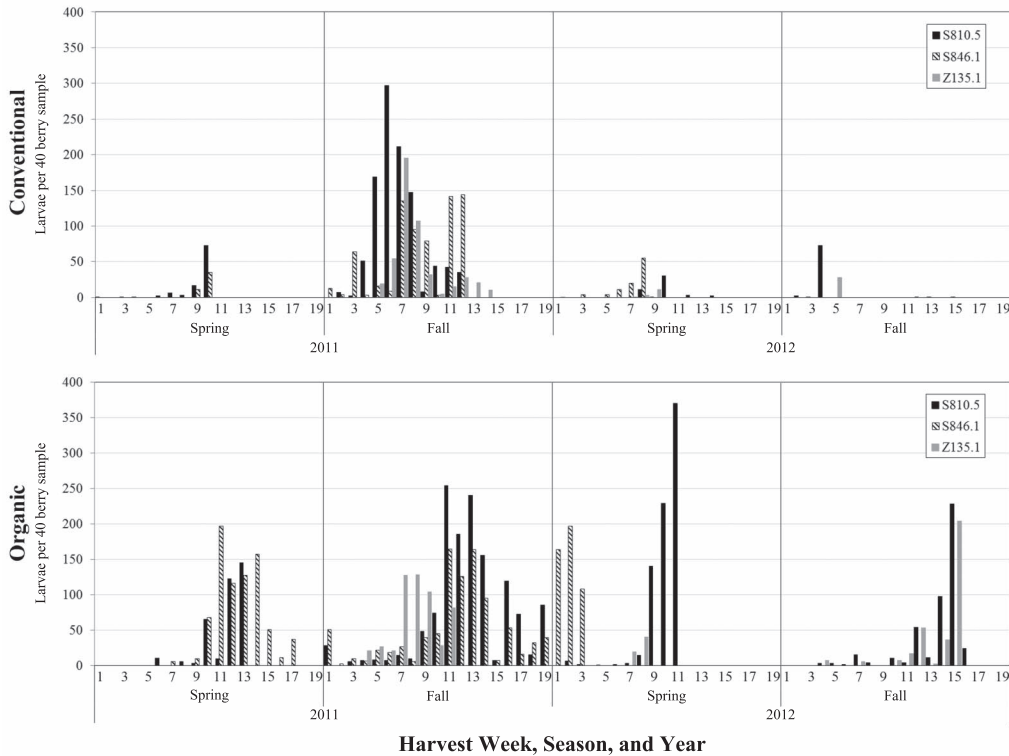


Fig. 3. Total *D. suzukii* larvae in a 40 raspberry sample (S810.5, S846.1, and Z135.1 varieties) per commercial harvest week (not all varieties were harvested for all 19 wk) for spring and fall harvest 2011 and 2012.

**Correlation of Adult Trapping and Larval Infestation.** Pearson correlation coefficients for the spring trap captures (sum of all replicates for each lure type deployed in each raspberry variety each week) of the conventional plantings versus larval infestation data (larvae in 40 berries from each raspberry variety each week) were not significant for either lure. However, there was a significant correlation between the adult trap captures of the apple cider vinegar lure and the yeast lure ( $r = 0.59$ ;  $P < 0.0001$ ). At the organic plantings, the correlation between spring trap captures and infestation was higher, with a significant correlation between apple cider vinegar and infestation ( $r = 0.69$ ;  $P < 0.0001$ ) and a slightly poorer correlation between yeast and infestation ( $r = 0.58$ ;  $P < 0.0001$ ). Again both lures correlate better with one another than either do to number of larvae in 40 berries ( $r = 0.78$ ;  $P < 0.0001$ ). Despite low spring trap captures in the S810.5 variety, larvae were encountered in the first 10 wk of spring harvest in both conventional and organic plantings in both years of the study (Figs. 1 and 3).

A Pearson correlation of fall trap captures (sum of all replicates in each variety for each week) at the conventional planting for each lure type against the larval infestation data (number of larvae in 40 berries of each variety) showed a significant correlation between infestation and the apple cider vinegar lure ( $r = 0.59$ ;  $P < 0.0001$ ) and the yeast lure ( $r = 0.70$ ;  $P <$

$0.0001$ ). However, the trap captures with the apple cider vinegar lure correlate better to the trap captures with the yeast lure than either lure type does to the larval infestation ( $r = 0.80$ ;  $P < 0.0001$ ). For the fall captures at the organic planting, the apple cider vinegar captures versus infestation correlated slightly better ( $r = 0.63$ ;  $P < 0.0001$ ), and the yeast captures correlated more poorly, though remaining statistically significant ( $r = 0.44$ ;  $P < 0.0001$ ). Again, captures associated with the two lures correlate better with one another than either does to infestation ( $r = 0.79$ ;  $P < 0.0001$ ).

Though correlations were generally significant, sufficient numbers of low trap capture with high larval count outlying data points that would likely result in unacceptable damage for commercial management were present in all correlations. Yeast and apple cider vinegar captures correlated well in all situations, and better than either did with larval infestation. This indicates that the total trap captures were similar enough, despite the fact that yeast captured significantly more *D. suzukii* in the fall, and that either lure could be used to provide adult capture estimates during the commercial harvest seasons. Although traps baited with liquid lures provide useful information about *D. suzukii* population fluctuations, especially in the fall, their utility for making control decisions such as initiation of insecticide

sprays in spring is limited because of their reliability in predicting fruit infestation.

In conclusion, current *D. suzukii* control tactics rely heavily on insecticide use because little is known about *D. suzukii* biology and phenology. *D. suzukii* pressure as measured by larval infestation and adult trap captures was higher during the fall raspberry harvest season than the spring. The yeast lure captured significantly more *D. suzukii* during the fall harvest. While both liquid lures tended toward a female bias, the degree of bias varied by month of the year and was more pronounced for the yeast lure. Vacuum samples tended to be male biased, with the caveat that vacuum sample sex ratios were based on far fewer flies than the liquid lure captures. Vacuum sampling is very labor intensive and unlikely to be adopted by raspberry growers as a measure of *D. suzukii* field populations; though there has been some interest in developing vacuuming as a control measure for organic raspberries. Yeast and apple cider vinegar captures (when totaled across the replicate traps) correlated well with one another in all seasons and managements; therefore, either lure could be used as a qualitative population measure. The authors speculate that the trap and lures tested in this study do not compete well with raspberry host fruit odors to attract *D. suzukii* during the spring because larvae were present in our berry samples during spring commercial harvest while the adult trap captures were very low. Indeed, neither lure correlated well with larval infestation in the spring for the conventional management despite significant correlation with larvae in other management settings and seasons. However, all correlations had outliers that represent a commercially unacceptable outcome such as low adult trap captures with high larval counts. We have presented the first study of *D. suzukii* seasonal occurrence and monitoring in California coastal raspberries, and provide a comprehensive comparison of adult captures using apple cider vinegar and yeast lures in raspberries.

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