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Multistate Comparison of Attractants and the Impact of Fruit Development Stage on Trapping *Drosophila suzukii* (Diptera: Drosophilidae) in Raspberry and Blueberry

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Abstract

Spotted-wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is an invasive pest of soft-skinned fruits across the globe. Effective monitoring is necessary to manage this pest, but suitable attractants are still being identified. In this study, we combined lures with fermenting liquid baits to improve *D. suzukii* trapping specificity and attractiveness. We also measured the efficiency and specificity of baits/lures during different times of the season; the reproductive status of females among baits/lures; and the effects of locations and crop type on these response variables. We developed a metric that combined mating status and fat content to determine differences in types of females attracted. Lures utilizing yeast and sugar-based volatiles trapped the most *D. suzukii*. The addition of a commercial lure to yeast and sugar-based lures increased catches in most locations, but was also the least specific to *D. suzukii*. Apple juice-based chemical lures tended to be most specific to *D. suzukii*, while lures comprised of a singular attractant tended to trap more *D. suzukii* with a higher reproductive potential than combinations of attractants. Trap catch and lure specificity was lower during fruit development than fruit ripening. While catch amounts varied by geographic location and crop type, attractants performed similarly relative to each other in each location and crop. Based on the metrics in this study, the yeast and sugar-based attractants were the most effective lures. However, further work is needed to improve early season monitoring, elucidate the effects of physiological status on bait attraction, and understand how abiotic factors influence bait attraction.

Key words: fermentation bait, integrated pest management, invasive pest species, monitoring, soft-skinned fruit

Spotted-wing drosophila, *Drosophila suzukii* Matsumaru (Diptera: Drosophilidae), is an invasive pest in Europe (Calabria et al. 2012; Cini et al. 2012, 2014), North America (Steck et al. 2009, Bolda et al. 2010, Abraham et al. 2015), and South America (Deprá et al. 2014). Unlike other Drosophilidae, these flies exploit maturing and ripe fruits, destroying the fruit's marketability (Mitsui et al. 2006, Walsh et al. 2011). Their propensity to attack undamaged fruit, along with the absence of effective biocontrol agents, has made *D. suzukii* a major pest of soft-skinned fruits (Bolda et al. 2010, Lee et al. 2011, Cini et al. 2014, Asplen et al. 2015, Burrack et al. 2015).

For almost a decade, researchers and growers have worked to develop an integrated pest management (IPM) program to limit the damage and losses associated with *D. suzukii* (Dreves 2011, Haye et al. 2016). A key component of a successful IPM program is to monitor for pests, which allows for early detection, more efficient timing of treatments, accurate risk assessment, and quantitative evaluation of implemented methods (Wall 1990). However, early and accurate detection is critical for monitoring to be successful (Wall 1990, Kogan 1998, Dreves 2011, Hauser 2011, Cha et al. 2015). While many cultural and chemical control methods have been adapted to improve management, *D. suzukii* monitoring still relies on nonspecific and sometimes cumbersome attractants (Iglesias et al. 2014, Burrack et al. 2015, Haye et al. 2016).

The most successful baits to date are fermented odors based on different combinations of wine, sugar, fruit vinegar, yeast, and flour (Walsh et al. 2011, Landolt et al. 2012, Lee et al. 2012, Hampton et al. 2014, Burrack et al. 2015, Cha et al. 2015, Haye 2016, Huang et al. 2017). The abundance of nontarget Drosophilae trapped by these attractants increases the difficulty in identifying *D. suzukii* without the aid of magnification, and is a significant drawback for practical monitoring (Landolt et al. 2012, Hamby and Becher 2016, Wang et al. 2016). The corresponding trap catch numbers utilizing these attractants are also poor predictors of fruit infestation levels, which is problematic because larvae are generally protected inside the fruit from chemical management (Burrack et al. 2015, Pelton et al. 2017). Furthermore, none of these fermentations or synthetic attractants have been particularly successful in trapping *D. suzukii* early in the season (Burrack et al. 2015, (but see Cha et al. 2018)). The relative attraction to different lures can vary depending on the physiological state of the fly, and it appears that attraction to a lure can also vary over the course of a growing season (Burrack et al. 2015, Wong et al. 2018). If physiological and abiotic factors are affecting attraction, it is important to consider these effects when assessing the attractiveness of lures and improve on the deficiencies of current attractants.

In addition to assessing the efficacy of fermentation baits and a commercial lure, we tested fruit volatiles to determine if the addition of fruit-specific volatiles improves the specificity and attractiveness of the baits over the course of the growing season. Since *D. suzukii* is known to be attracted to apple cider vinegar, as well as damaged and rotting fruits, headspace volatiles from fresh and fermented apple juices were collected and analyzed by gas chromatography–mass spectrometry (A. Zheng, unpublished data). Special attention was given to the compounds produced and/or enriched during the fermentation process. A laboratory two-choice bioassay indicated that *D. suzukii* were strongly attracted to ethyl octanoate, a chemical produced during the fermentation process. During a preliminary field test in 2014, a 7-component blend identified from apple juice trapped more *D. suzukii* than an 11-compound blend identified from raspberry (Abraham et al. 2015). Subsequent bioassays showed that three volatiles: acetoin, ethyl octanoate, and acetic acid, distinguished themselves as particularly attractive to *D. suzukii* (A. Zhang, unpublished data). These three chemicals have also been identified in the odor complex of raspberries (Pabst et al. 1991, Klesk et al. 2004, Aprea et al. 2015). As a result, they served as the foundation for our fruit volatile treatment.

In this study, we developed reproductive metrics to better understand how different types of flies, at different times in the growing season, respond to varying attractants. Herein, we calculated a female reproductive potential (RP) based on fat content and the presence of immature eggs (see *Materials and Methods*). This rationale was based on the assumption that the presence of immature eggs can be used as an analog for ongoing reproductive activity, and fat content is indicative of nutritional levels and future reproductive capabilities (McIntyre and Gooding 2000, Arrese and Soulages 2010, Smith et al. 2011). Previous studies have shown that female Drosophilidae in different reproductive states respond differently to attractants (Terashima and Bownes 2004, Swoboda-Bhattarai et al. 2017). Since drosopholid egg production is linked to the nutritional status of females via the yolk proteins synthesized in fat bodies, fat content in individual flies could provide insight into the relative physiological status of each fly (Gelti-Douka et al. 1974, Terashima and Bownes 2004). By developing a metric that accounts for both of these variables together, we can identify attractants that target flies with different reproductive capabilities, during different times of the growing season, and have a better understanding of local population dynamics.

Our five primary objectives were to compare: 1) the efficacy of individual baits/lures and assess any additive effects of combining baits with lures; 2) the relative efficiency of baits/lures during different times of the season; 3) the specificity of baits/lures to *D. suzukii*; 4) the reproductive status of females between baits/lures; and 5) any geographic and crop type effects on objectives 1–4.

Materials and Methods

Experimental Design

We compared *D. suzukii* trap catches of various baits and lures by individual states (Georgia, Florida, Michigan, Oregon, and Wisconsin; Table 1) and pooled across all states. Organically managed Southern Highbush (Florida and Georgia) and mixed varieties of blueberry (Michigan) and mixed varieties of raspberry (Oregon and Wisconsin) crops were used.

The experimental period was divided into two, 4-wk-long collection periods that coincided with fruit development and fruit ripening within each state. With the exception of Florida and Georgia, the initial experimental period (fruit development) began once the first *D. suzukii* were trapped in monitoring traps using a commercial lure. Data were only collected in Florida and Georgia during fruit ripening. Traps were processed and baits were replaced every week; trap catches were filtered and stored in 70% ethanol. The commercial lure was replaced before the start of the fruit ripening collection period, and the apple juice chemicals were replaced every 2 wk. Within each location, each treatment was replicated four times as part of a randomized complete block design and re-randomized each week. The distance between each trap and each block was at least 10 m. *Drosophila suzukii* commercial style traps (Scentry Biologicals, Inc., Billing, MT) were used for every treatment. Traps were hung at fruit level; approximately ¾ of the total height of the plant from soil to canopy.

Table 1. Locations, types of crops, trapping periods, and experimental weeks used in this study

State	Fruit stage	Trapping period	Fruit infestation assessment
Florida *blueberry*	Developing	na	na
	Ripening	29 April–27 May	29 April–27 May
Georgia *blueberry*	Developing	na	na
	Ripening	17 May–14 June	17 May–14 June
Michigan *blueberry*	Developing	10 June–8 July	na
	Ripening	20 July–17 August	20 July–17 August
Oregon *raspberry*	Developing	25 May–15 June	25 May–15 June
	Ripening	15 June–20 July	22 June–20 July
Wisconsin *raspberry*	Developing	7 June–5 July	7 June–5 July
	Ripening	12 August–9 September	12 August–9 September

Treatments

Each treatment was comprised of either a single bait/lure or a combination of liquid baits and lures (Table 2). Four single baits and lures used were: yeast and sugar (YS); a fermenting bait composed of yeast, flour, and apple cider vinegar (YFV), a Scentry brand commercial lure (SC), and a lure composed of apple juice chemicals (AJ). The four combination baits and lures were created by hanging either a SC or AJ lure over YS or YFV bait solutions (Table 2). The SC lure is comprised of chemicals identified by an optimization study of chemical attractants first reported by Cha et al. (2012). The AJ lure was constructed of a micro centrifuge tube containing 1 ml of a 1:5 mixture of acetoin:ethyl octanoate. The micro centrifuge tube was then attached to the underside of the trap lid with wire, approximately 2 cm below the lid. An additional 1 ml acetic acid was then added to the drowning solution (Zhang and Feng 2017).

Data Collection

Trap catch

Each week male and female *D. suzukii*, and nontarget Drosophilidae were counted from each trap. Trap catches were subsampled if the numbers of *D. suzukii* were >100 by counting six 3.5 cm^2 cells in an 8 × 6 cell gridded tray, and then estimating a sample total. Flies were identified and stored in 70% ethanol. The specificity of individual baits to *D. suzukii* was calculated as the proportion of total *D. suzukii* trapped by the total number of drosophilids trapped.

First *D. suzukii* capture

For each bait, the week when *D. suzukii* were first trapped was recorded. Data were pooled across all states; Oregon was excluded from analysis since *D. suzukii* were trapped in every bait within the first week of the experiment.

Fruit infestation

At each location, and during each week of the experiment, two 100 g samples of ripe marketable fruit were collected. One subsample was sealed in a plastic bag (Ziploc) and stored in a refrigerator (2°C). This collection method provided a snapshot of infestation levels relative to trap catches, but did not allow us to directly compare trap catches from individual bait treatments with infestation levels. Within 48 h, the fruit was slightly crushed and placed in a 1% salt-water solution for 1 h. After 1 h, the number of larvae present was counted and the infestation rate (# of larvae/100 g of fruit) was calculated. The second 100 g subsample was used to confirm infestation species. Subsamples were placed in sealed deli container (11 cm diameter) with a 5 cm diameter hole cut into the lid and replaced with fiberglass window screen mesh. The deli container was placed in a growth chamber under 12:12 (L:D), 20°C, 70% RH conditions for 14 d to allow larvae inside the fruit to complete their development to adulthood. Emerged adult flies were positively identified as either *D. suzukii* or nontarget drosophila. Samples from Florida and Georgia were excluded from analysis due to the absence of larvae in all fruit samples. Data from Michigan and Georgia were only collected during weeks 5–8, during fruit ripening.

Reproductive status of captured flies

The reproductive status of *D. suzukii* female flies was determined twice during the experiment: during fruit development and fruit ripening. Captured females were subsampled in the first week and again in the fifth week of the trapping period (in Florida, females were dissected only during fruit ripening, the equivalent of the fifth week at the other locations). Six *D. suzukii* females from each treatment within each block were dissected. If fewer than six females in any given block were trapped, then flies from the following week were included as well. In Florida, flies collected in week 2 and week 4 were included. Flies were stored in 70% ethanol until they were dissected. The total number of mature eggs was counted, the presence of immature eggs was determined, and one of the paired spermathecae was dissected to determine the mating status (Avanesyan et al. 2017). We adapted a method of assessing abdominal fat content initially utilized for codling moth, where flies were scored on the relative absence ('*low*'), presence ('*medium*'), or abundance ('*high*')

Table 2. Recipe components for each lure and bait used alone or in combination

Treatment	Components
Lures	
Apple juice chemicals lure	*Lure:* 1 ml of a 1:5 ratio of acetoin and ethyl octanoate (Sigma-Aldrich, St. Louis, MO) 1 ml of acetic acid (Sigma-Aldrich, St. Louis, MO), *Drowning Solution*
Scentry lure	*Lure:* Scentry lure (Scentry Biologicals, Inc., Billings, MT) *Drowning Solution*
Apple cider vinegar and flour baits	
Fermenting bait	3.55 g of dry active yeast (Red Star), 51.8 g of whole wheat flour (Gold Medal), 6.14 ml of apple cider vinegar (Great Value), *Drowning Solution*
Apple juice chemicals lure over fermenting bait	*Lure:* 1 ml of a 1:5 ratio of acetoin and ethyl octanoate, 1 ml of acetic acid, 3.55 g of dry active yeast, 51.8 g of whole wheat flour, 6.14 ml of apple cider vinegar, *Drowning Solution*
Scentry lure over fermenting bait	*Lure:* Scentry lure 3.55 g of dry active yeast, 51.8 g of whole wheat flour, 6.14 ml of apple cider vinegar, *Drowning Solution*
Yeast and sugar baits	
Yeast and sugar bait	1.69 g of dry active yeast, 8.45 g of sugar (Great Value), *Drowning Solution*
Apple juice chemicals lure over yeast and sugar bait	*Lure:* 1 ml of a 1:5 ratio of acetoin and ethyl octanoate, 1 ml of acetic acid, 1.69 g of dry active yeast, 8.45 g of sugar, *Drowning Solution*
Scentry lure over yeast and sugar bait	*Lure:* Scentry lure 1.69 g of dry active yeast, 8.45 g of sugar, *Drowning Solution*

Apple juice chemicals had the following purities: acetoin, 99%; ethyl octanoate, 99≥%; acetic acid, 99.7≥%. The drowning solution was comprised of 150 ml of water, 0.16 ml of unscented dish soap (Seventh Generation), and 1.5 g of boric acid (Fisher Scientific, Hampton, NH). Boric acid was not included in the drowning solutions when using the yeast and sugar, or fermenting baits. Brands for each product purchased are listed at their first mention.

of abdominal fat (Landolt and Guédot 2008 (Supp Fig. 1 [online only]). The RP of each dissected fly was rated from 1 to 6: '1' was a fly with low fat content and no immature eggs; '2' was fly with medium fat content and no immature eggs; '3' was a fly with high fat content and no immature eggs; '4' was a fly with low fat content and immature eggs; '5' was a fly with medium fat content and immature eggs; and '6' was a fly with high fat content and immature eggs. Flies were subsequently grouped into *Low* (RP: 1–2), *Medium* (RP: 3–4), and *High* (RP: 5–6) reproductive statuses to simplify the statistical analyses and improve the interpretability of the results.

Statistical Analysis

All statistical analyses were performed using PROC GLIMMIX (SAS 9.4 Windows; Schabenberger 2005, Lee et al. 2012). *Drosophila suzukii* capture data, infestation levels, and specificity ratios were log-transformed ($\log_{10}(x + 1)$) to meet the assumptions of normality. The square root of the number of mature eggs was also taken to meet the assumptions of normality. The proportion of female *D. suzukii* trapped data was arcsine(sqrt(y)) transformed to meet the assumptions of normality and maintain meaningful proportions of 0 and 1 (McDonald 2009). If fewer than five flies were caught in any given trap, that trap was excluded from data analyses. Degrees of freedom were estimated using the Kenward–Roger method. Means were estimated using restricted maximum likelihood and adjusted according to the Kenward–Roger method.

Several comparisons of *D. suzukii* trap captures were made with mixed models. When *D. suzukii* from all five states were pooled, states were considered random effects. This model examined the effects of bait types on trap catches by including treatment, treatment * week, and week as fixed effects, and block nested within week, and state, and week nested within state as random effects. A second model for the pooled data on trap catches included treatment, fruit development period, and treatment * fruit development period as fixed effects, with state and fruit development period nested within state as random effects. Since flies in Florida and Georgia were only trapped during fruit ripening, these states were excluded from analyses when fruit stage was considered. A third model tested each individual state and included treatment, state, treatment * week, and week as fixed effects, and block nested within week, and state, and week nested within state as random effects. A fourth model tested each fruit type independently (similar to the third model) from state since each state only contained one fruit type which confounded fruit type with geographic location. To isolate any interaction between crop type and treatment, we calculated the proportion of *D. suzukii* trapped by each treatment relative to the total number of *D. suzukii* trapped within a specific block, in each week, at each geographic location.

Fruit infestation

We modeled the effects of week and state on fruit infestation levels by including week, state, and week * state as fixed effects, and treating block nested within week, and state, and fruit development period nested within state as random effects. We also examined any correlations between male and female trap catch numbers by calculating the relative proportion of each treatment's male and female trap catches, within a block and week. Relative proportion for each sex, and each treatment was calculated by taking the total number of flies trapped by that treatment in a given block and week, and dividing it by the total number of flies trapped in that same block and week. We developed a model to determine the relationship between relative female trap catch and fruit infestation levels that included female trap catch, state, week, and state * female trap catch.

RP, reproductive status, presence of immature eggs, and number of mature eggs

For RP, a proportional-odds cumulative logit model with mixed effects was used. This model included bait treatment as the explanatory variable (there was no significant effect of fruit development period, or any interactional effect of fruit development period and treatment on RP), and location with block nested within location as random effects. The estimation method was the maximum likelihood with the 'Laplace' approximation (Stroup 2013, Kemmitt et al. 2015). The estimated values of cumulative probabilities were compared with an odds ratio test, giving the relative proportions of the fat content and RP rankings for each treatment (Stroup 2013).

For the effect of treatment on the presence of immature eggs and reproductive status, a binary logistic regression with mixed effects was used. The model included treatment as the explanatory variable (effect of fruit development period was not significant and was not included in the final model), and location and block nested within location as random effects. Pairwise comparisons were conducted using a Tukey's adjustment for multiple comparisons with the lsmeans option.

The model that examined the effect of treatment on the number of mature eggs included treatment, treatment * fruit development period, and fruit development period as fixed effects, and block nested within fruit development period and state, and fruit development period nested within state as random effects.

Results

First Trap Catch

First week of *D. suzukii* trap catch varied by treatment (F = 3.42; df = 7, 24; P < 0.05). The first trap catch for the apple juice treatment was significantly later than all other treatments except YFV+AJ and YFV (P < 0.05; Table 3).

Trap Catch by Treatment

Trap catch varied with bait treatment for males (F = 30.9; df = 7, 805.7; P < 0.0001), females (F = 42.3; df = 7, 805.8; P < 0.0001), and total *D. suzukii* (F = 48.1; df = 7, 808.2; P < 0.0001; Table 3). We also found a significant interaction between bait treatment and week (males: F = 2.99; df = 49, 805.5; P < 0.0001; females: F = 2.75; df = 49, 805.4; P < 0.0001; total *D. suzukii*: F = 2.36; df = 49, 805.8; P < 0.0001; Fig. 1) and bait treatment and state (males: F = 6.93; df = 28, 805.8; P < 0.0001; females: F = 7.99; df = 28, 805.8; P < 0.0001; total *D. suzukii*: F = 6.66; df = 28, 808.2; P < 0.0001; Supp Table 1 [online only]). In the majority of states, the Scentry lure hung over the yeast and sugar bait trapped the most flies (Supp Table 1 [online only]).

There were significantly more males (F = 13.1; df = 1, 126; P < 0.0005), females (F = 22.0; df = 1, 125.9; P < 0.0001), and total *D. suzukii* (F = 20.5; df = 1, 126; P < 0.0001) trapped in raspberry than blueberry crops. There was also a significant interaction between crop type and bait treatment for male (F = 11.8; df = 7, 875; P < 0.0001), female (F = 8.39; df = 7, 875; P < 0.0001), and total *D. suzukii* (F = 10.4; df = 7, 877; P < 0.0001; Table 4). There were significantly more male (F = 25.1; df = 1, 546.4; P < 0.0001), female (F = 43.2; df = 1, 272.2; P < 0.0001), and total *D. suzukii* (F = 39.2; df = 1, 332.9; P < 0.0001) trapped during fruit ripening than fruit development. There was a significant interaction effect between fruit development period and bait treatment on trap catches for male (F = 3.36; df = 7, 882.5; P < 0.005), female (F = 5.55; df = 7, 735.5; P < 0.0001), and total *D. suzukii* (F = 4.39; df = 7, 750.1; P < 0.0001; Fig. 2).

Table 3. Average male, female, total *D. suzukii* trapped per week (± SE), as well as the average week (± SE) the first *D. suzukii* were trapped after initial trap placement (first capture)

Treatment abbreviation	Male	Female	Total	First capture
AJ	18.4 ± 3.66^{e}	25.5 ± 4.58^{j}	43.1 ± 7.92^{o}	2.0 ± 0.4^{p}
SC	29.1 ± 4.99^{bcd}	26.9 ± 4.39^{hi}	56.1 ± 9.16^{mn}	1.0 ± 0.0^{q}
YFV	28.3 ± 6.34^{d}	44.1 ± 8.01^{i}	72.4 ± 14.1^{n}	1.5 ± 0.3^{pq}
YS	66.9 ± 13.3^{bc}	86.8 ± 16.3^{g}	154 ± 29.2^{lm}	1.0 ± 0.0^{q}
YFV+AJ	37.1 ± 6.47^{cd}	55.0 ± 9.18^{hi}	90.5 ± 15.0^{n}	1.5 ± 0.3^{pq}
YFV+SC	37.9 ± 5.93^{b}	44.9 ± 6.36^{g}	78.1 ± 10.6^{l}	1.0 ± 0.0^{q}
YS+AJ	59.4 ± 10.9^{bc}	76.0 ± 12.3^{gh}	134 ± 22.1^{lm}	1.0 ± 0.0^{q}
YS+SC	**94.2 ± 14.9^{a}**	**82.6 ± 10.7^{f}**	**176 ± 24.7^{k}**	1.0 ± 0.0^{q}

Statistical analyses were completed using a ($\log_{10}(x + 1)$) transformation of the values presented here. Values within each column followed by different letters are significantly different from each other within the mixed model (Tukey's HSD test; $P < 0.05$), and bold values represent the highest number of flies trapped. Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

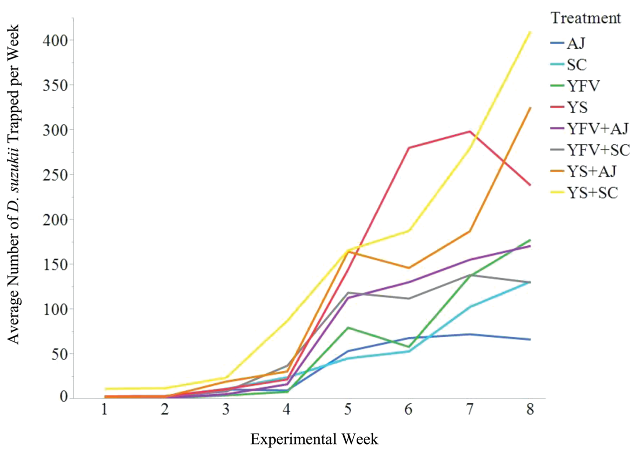

Fig. 1. Average number of *D. suzukii* trapped in each bait treatment during each week of the experiment. SEs were excluded from the figure for clarity. Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

Trap Specificity by Treatment

The specificity of each bait to *D. suzukii* (as defined by EQ1) was significantly different between bait treatments ($F = 12.3$; df = 7, 802.1; $P < 0.0001$), across states ($F = 177$; df = 4, 72.9; $P < 0.0001$), and for crop type ($F = 112$; df = 1, 853; $P < 0.0001$) (Table 5). The specificity of each bait to *D. suzukii* also varied from week to week ($F = 201$; df = 7, 772.6; $P < 0.0001$), and was lower during fruit development than during ripening ($F = 1084$; df = 7, 824.8; $P < 0.0001$). There were also a significant interaction effects between bait treatment and week ($F = 1.63$; df = 4, 772; $P < 0.005$), bait treatment and state ($F = 5.39$; df = 28, 802.1; $P < 0.0001$), and bait treatment and stage of fruit development ($F = 7.13$; df = 7, 515.4; $P < 0.0001$) on trap specificity.

The relative number of male ($F = 13.7$; df = 7, 240; $P < 0.0001$) and female ($F = 28.4$; df = 7, 240; $P < 0.0001$) *D. suzukii* trapped was significantly different between treatments (Supp Fig. 2 [online only]). In males, the YS+AJ treatment had the highest relative trap catch compared to the other treatments (Tukey's HSD; $P < 0.05$). In females, the YS+SC treatment had a significantly higher relative trap catch compared to the other treatments (Tukey's HSD; $P < 0.05$). There was also significant interaction between fruit crop and treatment on the relative proportion of males ($F = 2.86$, df = 7, 240; $P < 0.01$), and females ($F = 3.53$; df = 7, 240; $P < 0.005$; Supp Fig. 2 [online only]). In both males (YS+AJ) and females (YS+SC), the attractant that trapped the highest relative proportion of flies also trapped a higher relative proportion in raspberries than blueberries (Tukey's HSD; $P < 0.05$).

Proportion of Female *D. suzukii* Trapped

The proportion of females trapped was significantly different between bait treatments ($F = 3.81$; df = 7, 697.2; $P < 0.0005$), states ($F = 5.22$; df = 4, 30.4; $P < 0.005$), and for the interactions between treatment and state ($F = 4.21$; df = 28, 653.8; $P < 0.0001$) and treatment and crop type ($F = 4.64$; df = 7, 729.5; $P < 0.0001$). The proportion of female *D. suzukii* trapped did not vary by crop type alone ($F = 1.99$; df = 1, 7.83; $P = 0.22$). The proportion of female *D. suzukii* decreased from fruit development to fruit ripening ($F = 87.4$; df = 1, 637.6; $P < 0.0001$; Fig. 3) and generally decreased from week to week ($F = 25.7$; df = 7, 720.6; $P < 0.0001$; Table 6). The proportion of females that were mated compared to not mated also varied by bait treatment ($F = 2.96$; df = 7, 1134; $P < 0.005$; Table 7).

Fruit Infestation

All of the emerged insects from the fruit samples in each state were positively identified as *D. suzukii*. A model of fruit infestation levels that included state, week, and the interaction between the two was statistically significant ($F = 114$; df = 31, 95; $P < 0.0001$). Each individual variable significantly affected fruit infestation levels (state: $F = 29.0$; df = 3, 123; $P < 0.0001$; week: $F = 126$; df = 7, 119; $P < 0.0001$; state * week: $F = 55.1$; df = 21, 105; $P < 0.0001$). Fruit infestation was highest in Oregon (Tukey's HSD; $P < 0.05$), but generally increased over time across all states (Supp Fig. 3 [online only]). A model of fruit infestation that included average female trap catches in addition to state, week, and the interaction between the state and female trap catches showed no significant correlation between fruit infestation and female trap catches ($F = 0.17$; df = 1, 73; $P = 0.68$) but a significant interaction effect of state and female trap catch on infestation levels ($F = 9.99$; df = 2, 73; $P < 0.0005$; Supp Fig. 4 [online only]). Fruit infestation levels tended to increase with female trap catch in Oregon and Wisconsin, but not Michigan.

Reproductive Status

Reproductive status (mated or unmated) of dissected female *D. suzukii* varied by state ($F = 87.4$; df = 3, 981; $P < 0.001$), where

Table 4. Average number of male, female, and total *D. suzukii* trapped per week (± SE) within each crop

Treatment abbreviation	Males		Females		Total	
	Blueberry	Raspberry	Blueberry	Raspberry	Blueberry	Raspberry
AJ	4.11 ± 1.34^{b}	32.6 ± 6.76^{z}	6.27 ± 1.99^{c}	44.8 ± 8.31^{yz}	10.4 ± 3.14^{c}	75.8 ± 14.5^{yz}
SC	11.7 ± 3.81^{ab}	46.5 ± 8.72^{yz}	9.51 ± 2.36^{bc}	44.4 ± 7.90^{yz}	21.3 ± 5.97^{bc}	90.9 ± 16.3^{xz}
YFV	35.3 ± 11.8^{ab}	21.5 ± 4.83^{z}	48.6 ± 13.7^{abc}	39.6 ± 8.44^{z}	83.9 ± 25.0^{abc}	61.1 ± 13.1^{z}
YS	27.4 ± 8.23^{ab}	106 ± 24.1^{xz}	37.2 ± 9.60^{abc}	136 ± 29.7^{xy}	64.7 ± 16.7^{abc}	241 ± 53.6^{xy}
YFV+AJ	20.5 ± 6.5^{ab}	52.8 ± 10.7^{z}	32.4 ± 9.70^{bc}	76.5 ± 15.0^{xyz}	52.1 ± 14.9^{bc}	128 ± 25.1^{yz}
YFV+SC	32.5 ± 8.65^{a}	43.1 ± 8.16^{xz}	36.0 ± 9.32^{ab}	53.8 ± 8.59^{xyz}	68.5 ± 17.0^{ab}	96.9 ± 16.5^{yz}
YS+AJ	38.4 ± 13.9^{ab}	80.0 ± 16.4^{xz}	50.6 ± 15.4^{abc}	101 ± 18.7^{xyz}	87.6 ± 26.9^{abc}	181 ± 34.2^{xyz}
YS+SC	51.2 ± 16.6^{a}	137 ± 23.5^{x}	46.8 ± 10.5^{a}	118 ± 17.5^{x}	97.9 ± 25.6^{a}	256 ± 40.0^{x}
Average	27.6 ± 3.6	65.0 ± 5.42	33.4 ± 3.61	76.7 ± 5.82	60.8 ± 6.75	141 ± 11.0

Values within a crop (column) followed by different letters are significantly different from each other (Tukey's HSD test; $P < 0.05$). Statistical analyses were completed using a ($\log_{10}(x + 1)$) transformation of the values presented here. Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

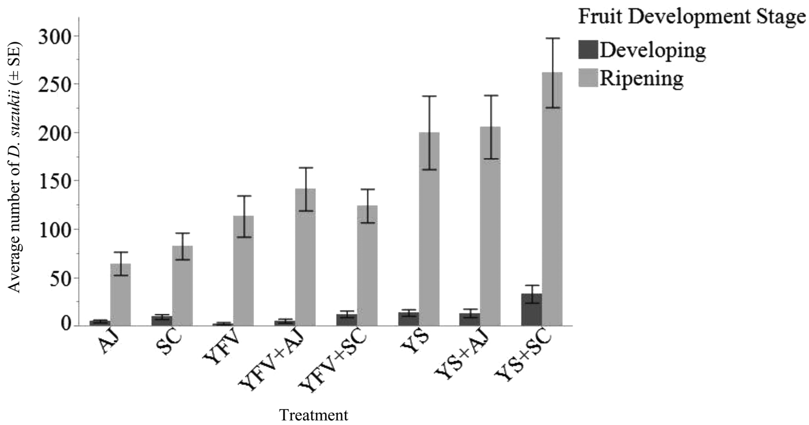

Fig. 2. Average number of *D. suzukii* (± SE) trapped as a function of bait treatment and fruit developmental stage. Statistical analyses were performed on transformed data and differences between treatments are detailed in Table 6. Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

the percent of mated *D. suzukii* in Georgia (32.4%) was significantly lower than Wisconsin (86.5%), Michigan (91.6%), and Oregon (94.8%), and the percent mated *D. suzukii* in Wisconsin was significantly lower than Oregon (Tukey's HSD test; $P < 0.05$). The percent mated did not vary by treatment ($F = 1.89$; df = 7, 977; $P = 0.07$), but significantly more females were mated during fruit development than during fruit ripening ($F = 9.52$; df = 1, 983; $P < 0.005$), and there was a significant interaction between treatment and fruit development period ($F = 2.84$; df = 7, 969; $P < 0.01$; Table 7). The number of mature eggs differed by treatment ($F = 3.20$; df = 7, 1120; $P < 0.005$), fruit development period ($F = 64.8$; df = 1, 1122; $P < 0.0001$), and there was a significant interaction effect between fruit development period and treatment ($F = 4.13$; df = 7, 1120; $P < 0.0005$; Table 7).

Reproductive Potential

RP of female *D. suzukii* varied by treatment ($F = 15.4$; df = 7, 988.8; $P < 0.0001$), and *D. suzukii* had a significantly higher RP during fruit development than fruit ripening ($F = 18.8$; df = 1, 985.1; $P < 0.0001$). The differences in RP between individual treatments were complex (Fig. 4; Supp Table 3 [online only]). In general, the YFV+AJ and YFV+SC attractants trapped more flies with a lower RP than the other treatments (Fig. 4).

Discussion

The bait comprised of the Scentry lure over a yeast and sugar bait (YS+SC) trapped the most *D. suzukii*. The number of flies trapped was higher during fruit ripening than fruit development, which is consistent with previously reported *D. suzukii* population dynamics (Hamby et al. 2016, Pelton et al. 2016, Wang et al. 2016). YS+SC trapped the most *D. suzukii* during both of these periods, although there were weeks during fruit ripening when the yeast and sugar (YS) bait and the yeast and sugar bait with the addition of apple juice chemicals (YS+AJ) trapped more flies. Despite trapping the most flies, the YS+SC attractant was also among the least specific to *D. suzukii*. It is unclear if the low ratio of *D. suzukii* to nontarget Drosophilidae, particularly early in fruit development, is a result of low overall *D. suzukii* abundance relative to nontarget Drosophilidae, attraction to different volatiles or hosts across the growing season, or the nonspecific nature of the yeast and sugar volatiles. The SC was a strong nontarget drosophilid attractant, so the low specificity we observed in the YS+SC attractant may just reflect the overall population levels of *D. suzukii*.

During fruit development, none of the traps were particularly specific to *D. suzukii* (<33% of all Drosophilidae), which is consistent with other studies (Cha et al. 2018). However, the number

Table 5. Average number of *D. suzukii* trapped and the percentage of total drosophilids that were *D. suzukii* trapped per week (± SE) during fruit development, fruit ripening, and across the duration of the experiment

Treatment abbreviation	Fruit development		Fruit ripening		Total season	
	Weeks 1–4		Weeks 5–8		Weeks 1–8	
	Average no. *D. suzukii*	Percent *D. suzukii*	Average no. *D. suzukii*	Percent *D. suzukii*	Average no. *D. suzukii*	Percent *D. suzukii*
AJ	5.88 ± 1.47^{c}	28.6 ± 5.19^{f}	65.4 ± 12.0^{e}	64.5 ± 4.93^{f}	43.1 ± 7.91^{e}	53.0 ± 4.03^{f}
SC	10.5 ± 2.50^{b}	19.4 ± 3.20^{fg}	83.4 ± 13.7^{d}	36.9 ± 3.61^{g}	56.1 ± 9.16^{cd}	31.9 ± 2.83^{h}
YFV	3.54 ± 1.27^{c}	28.1 ± 6.41^{fg}	114 ± 21.3^{cd}	59.7 ± 4.17^{f}	72.4 ± 14.0^{de}	51.2 ± 3.75^{f}
YS	14.6 ± 3.42^{b}	25.4 ± 4.40^{fg}	201 ± 37.9^{b}	53.9 ± 4.18^{f}	154 ± 27.6^{b}	45.6 ± 3.45^{fg}
YFV+AJ	6.35 ± 2.14^{c}	18.0 ± 3.92^{fg}	142 ± 22.3^{bcd}	54.7 ± 4.32^{f}	90.5 ± 14.3^{d}	43.3 ± 3.63^{g}
YFV+SC	13.3 ± 3.38^{b}	18.2 ± 3.28^{fg}	125 ± 17.3^{b}	42.2 ± 3.69^{g}	82.8 ± 11.9^{b}	35.2 ± 2.96^{h}
YS+AJ	14.1 ± 4.35^{b}	19.6 ± 3.16^{fg}	206 ± 32.7^{bc}	55.7 ± 4.18^{f}	134 ± 22.1^{bc}	45.3 ± 3.48^{fg}
YS+SC	34.0 ± 9.19^{a}	15.6 ± 3.29^{g}	263 ± 35.9^{a}	35.8 ± 3.15^{g}	177 ± 24.7^{a}	30.0 ± 0.02^{h}

Florida and Georgia were not included in any the data for fruit development, and Michigan was excluded from the ratio data during fruit development. Values within a development period (column) followed by different letters are significantly different from each other (Tukey's HSD test; $P < 0.05$). Statistical analyses were completed using a ($\log_{10}(x + 1)$) transformation of the values presented here. Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

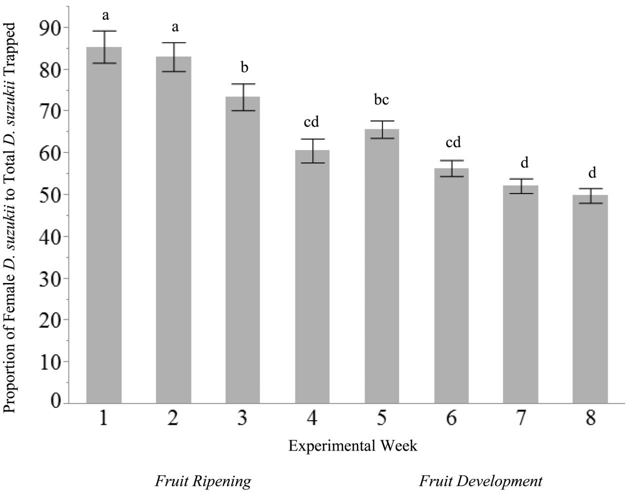

Fig. 3. Proportion of female *D. suzukii* to total *D. suzukii* (± SE) trapped averaged across all bait treatments during each week of the experiment. Weeks not connected by the same letter are significantly different from each other (Tukey's HSD; $P < 0.05$).

of *D. suzukii* relative to nontarget Drosophilidae increased substantially as the growing season progressed. During fruit ripening the apple juice chemicals lure (AJ) was the most specific to *D. suzukii*, but when considered across all collection periods, this lure was not significantly more specific to *D. suzukii* than the fermenting bait (YFV) or the fermenting bait with the apple juice chemicals (YFV+AJ). While the addition of AJ to the YS bait improved overall *D. suzukii* specificity, it was also associated with a significantly lower *D. suzukii* trap catch. These results suggest that fruit volatiles may be important for *D. suzukii* to initially find hosts but the alightment onto oviposition sites may be mediated by a yeast and sugar complex. Previous research has found a negative synergistic effect of fruit and fermentation volatiles on the attraction of *D. melanogaster* (Becher et al. 2012), so it is possible that a similar effect is occurring with *D. suzukii* and other Drosophilidae at our collection sites. The AJ treatment included the addition of acetic acid to the drowning solution, which may be the critical component for *D. suzukii* attraction to the AJ treatment (Cha et al. 2012, 2014). However, effectively trapping *D. suzukii* is more complex than previously reported, as abiotic and biotic factors may be strongly influencing *D. suzukii* trap catches (Burrack et al. 2015, Tochen et al. 2016, Swoboda-Bhattarai et al. 2017). Although the addition of apple juice volatiles decreased the YS trap catch, we found that attraction to fruit volatiles varied across time and fruit development period. As a result, a chemical combination derived from apple juice volatiles may improve the specificity and attractiveness of a yeast-based bait during certain times of the year. Further research is needed to determine if the addition of acetic acid, or other fruit volatiles, to a yeast and sugar bait, can improve *D. suzukii* trap catch without attracting other Drosophilidae.

The metrics to assess the reproductive status of the flies (mating status, number of mature eggs and RP) indicated a difference in the types of flies trapped by each attractant. In general, female flies trapped in the AJ, SC, and YS treatments tended to be similar to each other, more likely to carry more mature eggs, and have a higher RP than the flies from the treatments containing combinations of lures and baits or YFV-based attractants. We also trapped a higher percentage of mated females relative to unmated flies in the AJ treatment, although this treatment was not significantly different than the YS, YS+AJ, SC, or YFV+AJ attractants. It is unclear why singular attractants trapped flies with different reproductive statuses compared to the combination bait and lures, and the YFV-based attractants. Less complex attractants have been shown to be more selective to *D. suzukii* (Cha et al. 2015), and flour-based fermentation volatiles may indicate lower quality oviposition locations via damaged fruit and thus more larval competition from other Drosophilidae (Utrio and Eriksson 1977, Burrack et al. 2015). If these odor types are indicative of poorer quality oviposition sites, then females with higher RP may avoid these fruits. As a result, simpler attractants based on odorants that indicate a ripe (Kassim et al. 2009), but not overripe fruit may be necessary to improve *D. suzukii* trap catches, at least during fruit development.

Drosophila suzukii trap catches varied across states and crops. The specific influence of the crop and state was difficult to discern because each state only conducted the experiment within a single crop: Florida,

Table 6. Percent of total *D. suzukii* trapped that were female (± SE) by treatment and state

Treatment abbreviations	Florida	Georgia	Michigan	Oregon	Wisconsin
n	44	198	204	224	235
AJ	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	66.3 ± 4.60^{c}	59.6 ± 4.22^{de}	77.7 ± 3.35^{f}
SC	60.0 ± 20.0^{a}	72.8 ± 6.57^{b}	51.5 ± 6.22^{c}	55.1 ± 4.56^{de}	61.1 ± 4.23^{f}
YFV	0.00 ± 0.00^{a}	46.8 ± 10.2^{b}	63.4 ± 5.75^{c}	72.1 ± 4.82^{d}	71.4 ± 3.24^{f}
YS	25.0 ± 17.1^{a}	63.9 ± 0.05^{b}	60.1 ± 6.49^{c}	65.3 ± 4.01^{de}	74.2 ± 3.64^{f}
YFV+AJ	0.00 ± 0.00^{a}	55.6 ± 11.7^{b}	57.5 ± 6.61^{c}	66.5 ± 5.66^{de}	69.3 ± 3.39^{f}
YFV+SC	69.3 ± 11.2^{a}	62.4 ± 4.66^{b}	57.9 ± 4.72^{c}	69.2 ± 3.40^{de}	63.5 ± 3.51^{f}
YS+AJ	22.2 ± 16.5^{a}	53.0 ± 11.4^{b}	65.9 ± 5.33^{c}	60.7 ± 4.37^{de}	75.2 ± 3.34^{f}
YS+SC	53.5 ± 8.73^{a}	66.6 ± 4.83^{b}	63.0 ± 4.07^{c}	51.8 ± 0.04^{e}	64.9 ± 3.86^{f}
Average	41.8 ± 6.09^{i}	60.0 ± 3.06^{h}	60.5 ± 1.94^{h}	62.3 ± 1.58^{h}	69.6 ± 1.32^{g}

Treatment values within a state followed by different letters are significantly different from each other (Tukey's HSD test; $P < 0.05$). Average percent female between states followed by different letters are significantly different (Tukey's HSD test; $P < 0.05$). Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

Table 7. Average number of mature eggs in female *D. suzukii* and percent of dissected females that were mated from each treatment during fruit development and ripening (± SE)

Treatment abbreviation	Fruit development		Fruit ripening		Total	
	Mature eggs	Percent mated	Mature eggs	Percent mated	Mature eggs	Percent mated
AJ	14.5 ± 2.16^{ab}	96.7 ± 0.03^{g}	5.78 ± 0.80^{c}	91.7 ± 3.28^{hi}	8.32 ± 0.93^{e}	93.1 ± 2.53^{j}
SC	9.68 ± 1.77^{ab}	100 ± 0.00^{g}	6.32 ± 0.74^{c}	80.5 ± 4.40^{hi}	7.35 ± 0.76^{ef}	86.6 ± 3.14^{j}
YFV	16.2 ± 2.86^{a}	96.3 ± 3.70^{g}	4.77 ± 0.76^{cd}	78.8 ± 4.46^{hi}	7.50 ± 1.00^{ef}	83.0 ± 3.56^{j}
YS	7.95 ± 1.35^{ab}	93.0 ± 3.93^{g}	6.37 ± 0.83^{c}	85.4 ± 3.93^{hi}	6.89 ± 0.71^{ef}	88.0 ± 2.92^{j}
YFV+AJ	9.62 ± 1.58^{ab}	84.4 ± 5.46^{g}	3.18 ± 0.74^{d}	94.4 ± 2.72^{h}	5.64 ± 0.81^{ef}	90.6 ± 2.71^{j}
YFV+SC	9.15 ± 1.42^{ab}	82.6 ± 5.65^{g}	4.00 ± 0.75^{cd}	82.6 ± 4.11^{hi}	5.74 ± 0.72^{ef}	82.6 ± 3.31^{j}
YS+AJ	11.9 ± 1.17^{ab}	93.6 ± 3.60^{g}	3.71 ± 0.60^{cd}	94.7 ± 2.61^{h}	6.83 ± 0.77^{ef}	94.2 ± 2.11^{j}
YS+SC	8.71 ± 1.23^{b}	92.4 ± 3.28^{g}	2.82 ± 0.42^{d}	75.6 ± 4.56^{i}	5.21 ± 0.60^{f}	82.7 ± 3.00^{j}

Flies from Florida were excluded from analyses. Treatments in each column followed by different letters are significantly different from each other (Tukey's HSD test; $P < 0.05$). Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

Georgia, and Michigan in blueberries, Oregon and Wisconsin in raspberries. The YS+SC lure was consistently among the most attractive across all five states. In Wisconsin, Michigan, and Oregon, the YS and the YS+AJ lure also performed well; while the YFV+SC lure performed well in every state except Wisconsin. The yeast and sugar-based lures also trapped *D. suzukii* in the first week of the experiment, which preceded fruit infestation detection in each state by at least 1 wk. With the exception of Oregon, fruit infestation levels were generally poorly correlated with female trap catches (Supp Fig. 4 [online only]). Despite trapping *D. suzukii* each week in Georgia and Florida, there were no observed infestation levels. The average number of female *D. suzukii* trapped in Florida (0.53 ± 0.15) and Georgia (5.11 ± 0.51) was lower relative to the other states. Georgia also trapped a significantly smaller percentage of mated flies relative to the other states (Supp Fig. 2 [online only]). These results suggest that the reproductive composition of the populations in Georgia and Florida was different than the other states, and that abiotic factors could either be limiting larval infestation rates or promoting infestation rates in different geographic locations. The differences in the observed fruit infestation between states could reflect differences in stage of fruit development or be symptomatic of the abiotic conditions typical of those geographic regions (e.g., warmer and more humid conditions in the southern states) (Haviland et al. 2016, Tochen et al. 2016). The *D. suzukii* trap catch in Florida and Georgia also coincided with significantly smaller female/male *D. suzukii* ratios. The subtropical climate found in Florida and Georgia permits flies to bypass the reproductive diapause characteristic of *D. suzukii* found in the oceanic and temperate climates typical of the other study locations (Price et al. 2009, Hamby et al. 2016). Flies from these subtropical climates are active outside of the primary fruit-growing season, which may be driving some of the differences we observed between the attractant trap catches of climatic regions. While the YS+SC trap performed well across a wide geographic range, the smaller female/male ratios observed in Florida and Georgia, and higher trap catch of YFV+SC outside of Wisconsin, reiterate the importance of tailoring monitoring programs to reflect regional climates and cultural practices.

The success of the yeast and sugar-based lures in this study contradicts the findings of previously reported trap catches using yeast and sugar baits (Burrack et al. 2015, although see Iglesias et al. 2014). They reported that the fermenting bait cup, as well as a synthetic lure hung over apple cider vinegar, both trapped significantly more *D. suzukii* than the other treatments including yeast and sugar. While the addition of the Scentry lure to the yeast and sugar bait improved the overall trap catch and specificity in our study, the yeast and sugar bait alone performed nearly as well and is more economical for growers. However, unlike the fermenting bait in Burrack et al (2015), the YFV lure used in our study did not have sugar or ethanol included as a component. Although the base components were similar (yeast,

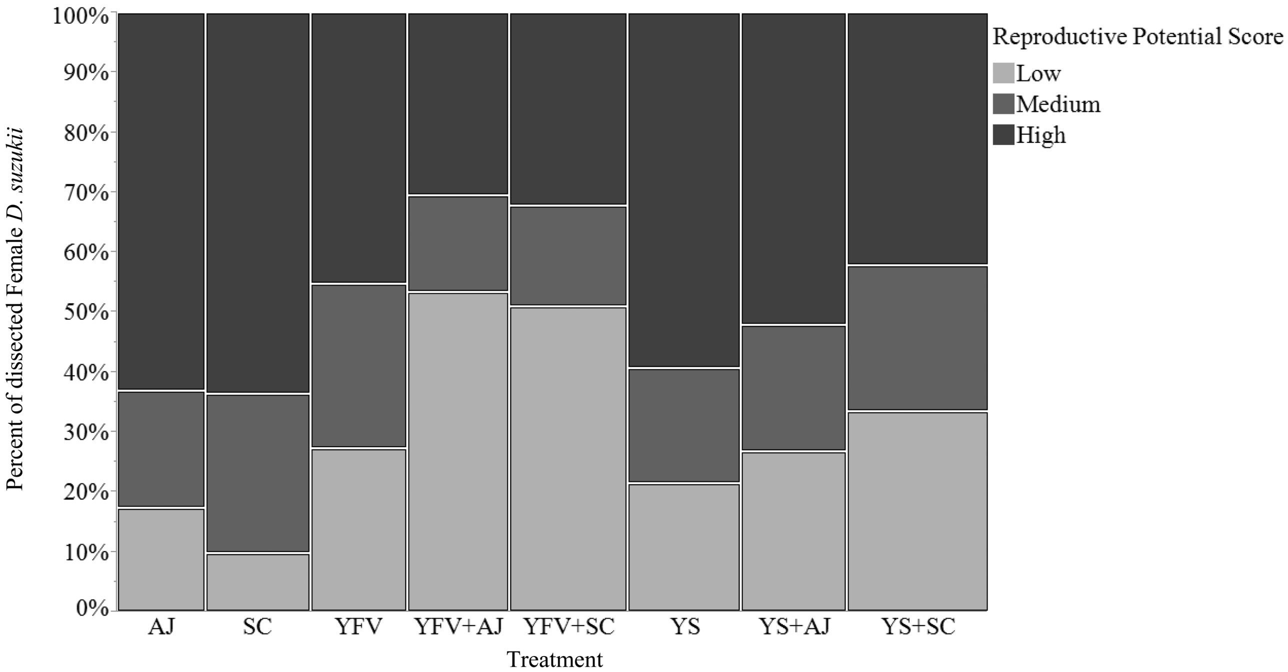

Fig. 4. Percent of dissected female *D. suzukii* with a given RP score for each treatment. Individual statistical comparisons are detailed in Supp Table 3 [online only]. Treatment abbreviations correspond to the following treatments: AJ—apple juice chemicals lure; SC—Scentry lure; YFV—vinegar and flour fermenting bait; YS—yeast and sugar; YFV+AJ—apple juice chemicals lure over fermenting bait; YFV+SC—Scentry lure over vinegar and flour fermenting bait; YS+AJ—apple juice chemicals lure over yeast and sugar bait; YS+SC—Scentry lure over yeast and sugar bait.

flour, apple cider vinegar), the addition of sugar and ethanol likely resulted in different odor plumes between the two lures, thus making it difficult to draw concrete comparisons. Regardless, the attractiveness of the yeast-based baits in this study and the fermenting baits in Burrack et al. (2015) support a growing body of evidence that microbial volatiles are important olfactory cues for many Drosophilidae, including *D. suzukii* (Becher et al. 2012, Hamby et al. 2012, Hamby et al. 2014, Kleiber et al. 2014, Scheidler et al. 2015, Huang et al. 2017). The yeast used in our study was a commercially available baker's yeast (*Saccharomyces cerevisiae*). Yeast species isolated directly from *D. suzukii* and raspberries (e.g., *Hanseniaspora uvarum*) have been shown to be attractive to *D. suzukii*, but more work needs to be done to assess how ecologically relevant yeast volatiles improve trap catches (Hamby et al. 2012, Palanca et al. 2013, Huang et al. 2017), and how different components can influence fermentation profiles.

One of the major hurdles in implementing an effective *D. suzukii* lure continues to be trapping emerging *D. suzukii* early in the season, at least in areas where *D. suzukii* undergo reproductive diapause in winter (Wallingford et al. 2016). It is unclear if the lower *D. suzukii* trap catches, and associated low *D. suzukii* specificity, seen early in the season are indicative of low overall abundance, or shifts in attraction to the baits early in fruit development. The majority of *D. suzukii* trapped during fruit development are females, and mated, which supports the hypothesis that overwintering *D. suzukii* and in other drosophilid populations are predominantly females storing sperm from fall matings (Collett and Jarman 2001, Rossi-Stacconi et al. 2016). If female flies emerging from overwintering are focused on oogenesis and are nutrient deficient, it is possible that they are attracted to a different suite of volatiles than when looking for oviposition sites later in the growing season. As work on *D. suzukii* attractants continues, the influences of a female's reproductive and physiological state over time, the stage of fruit development, and regional climatic factors need to be considered to fully optimize fly attractants and understand the factors driving individual behavior and population dynamics.

Supplementary Data

Supplementary data are available at Environmental Entomology online.

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References Cited

Abraham, J., A. Zhang, S. Angeli, S. Abubeker, C. Michel, Y. Feng, and C. Rodriguez-Saona. 2015. Behavioral and antennal responses of spotted wing drosophila, *Drosophila suzukii*, to volatiles from fruit extracts. Environ. Entomol. 44: 356–367.

Aprea, E., F. Biasioli, and F. Gasperi. 2015. Volatile compounds of raspberry fruit: from analytical methods to biological role and sensory impact. Molecules. 20: 2445–2474.

Arrese, E. L., and J. L. Soulages. 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55: 207–225.

Asplen, M. K., G. Anfora, A. Biondi, D. S. Choi, D. Chu, K. M. Daane, P. Gibert, A. P. Gutierrez, K. A. Hoelmer, W. D. Hutchison, et al. 2015. Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. J. Pest Sci. 88: 469–494.

Avanesyan, A., B. D. Jaffe, and C. Guédot. 2017. Isolating spermathecae and determining mating status of *Drosophila suzukii*: a protocol for tissue dissection and its applications. Insects. 8: 32.

Becher, P. G., G. Flick, E. Rozpędowska, A. Schmidt, A. Hagman, S. Lebreton, M. C. Larsson, B. S. Hansson, J. Piškur, P. Witzgall, and M. Bengtsson. 2012. Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. Funct. Ecol. 26: 822–828.

Bolda, M. P., R. E. Goodhue, and F. G. Zalom. 2010. Spotted wing drosophila: potential economic impact of a newly established pest. Agric. Resoure Econ. Update, Univ. Calif. Giannini Foundation 13: 5–8.

Burrack, H. J., M. Asplen, L. Bahder, J. Collins, F. A. Drummond, C. Guédot, R. Isaacs, D. Johnson, A. Blanton, J. C. Lee, et al. 2015. Multistate comparison of attractants for monitoring *Drosophila suzukii* (Diptera: Drosophilidae) in blueberries and caneberries. Environ. Entomol. 44: 704–712.

Calabria, G., J. Máca, G. Bachli, L. Serra, and M. Pascual. 2012. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J. Appl. Entomol. 136: 139–147.

Cha, D. H., T. Adams, H. Rogg, and P. J. Landolt. 2012. Identification and field evaluation of fermentation volatiles from wine and vinegar that mediate attraction of spotted wing Drosophila, *Drosophila suzukii*. J. Chem. Ecol. 38: 1419–1431.

Cha, D. H., T. Adams, C. T. Werle, B. J. Sampson, J. J. Adamczyk, Jr., H. Rogg, and P. J. Landolt. 2014. A four-component synthetic attractant for *Drosophila suzukii* (Diptera: Drosophilidae) isolated from fermented bait headspace. Pest Manag. Sci. 70: 324–331.

Cha, D. H., S. P. Hesler, S. Park, T. B. Adams, R. S. Zack, H. Rogg, G. M. Loeb, and P. J. Landolt. 2015. Simpler is better: fewer non-target insects trapped with a four-component chemical lure vs. a chemically more complex food-type bait for *Drosophila suzukii*. Entomol. Exp. Appl. 154: 251–260.

Cha, D. H., S. P. Hesler, A. K. Wallingford, F. Zaman, P. Jentsch, J. Nyrop, and G. M. Loeb. 2018. Comparison of commercial lures and food baits for early detection of fruit infestation risk by *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 111: 645–652.

Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insectol. 65: 149–160.

Cini, A., G. Anfora, L. A. Escudero-Colomar, A. Grassi, U. Santosuosso, G. Seljak, and A. Papini. 2014. Tracking the invasion of the alien fruit pest *Drosophila suzukii* in Europe. J. Pest Sci. 87: 559–566.

Collett, J. I., and M. G. Jarman. 2001. Adult female *Drosophila pseudoobscura* survive and carry fertile sperm through long periods in the cold: populations are unlikely to suffer substantial bottlenecks in overwintering. Evolution. 55: 840–845.

Deprá, M., J. L. Poppe, H. J. Schmitz, D. C. De Toni, and V. L. Valente. 2014. The first records of the invasive pest *Drosophila suzukii* in the South American continent. J. Pest Sci. 87: 379–383.

Dreves, A. J. 2011. IPM program development for an invasive pest: coordination, outreach and evaluation. Pest Manag. Sci. 67: 1403–1410.

Gelti-Douka, H., T. R. Gingeras, and M. P. Kambysellis. 1974. Yolk proteins in Drosophila: identification and site of synthesis. J. Exp. Zool. 187: 167–172.

Hamby, K. A. and P. G. Becher. 2016. Current knowledge of interactions between *Drosophila suzukii* and microbes, and their potential utility for pest management. J. Pest Sci. 89: 621–630.

Hamby, K. A., A. Hernández, K. Boundy-Mills, and F. G. Zalom. 2012. Associations of yeasts with spotted-wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. Appl. Environ. Microbiol. 78: 4869–4873.

Hamby, K. A., M. P. Bolda, M. E. Sheehan, and F. G. Zalom. 2014. Seasonal monitoring for *Drosophila suzukii* (Diptera: Drosophilidae) in California commercial raspberries. Environ. Entomol. 43: 1008–1018.

Hamby, K. A., D. E. Bellamy, J. C. Chiu, J. C. Lee, V. M. Walton, N. G. Wiman, R. M. York, and A. Biondi. 2016. Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. J. Pest Sci. 89: 605–619.

Hampton, E., C. Koski, O. Barsoian, H. Faubert, R. S. Cowles, and S. R. Alm. 2014. Use of early ripening cultivars to avoid infestation and mass trapping to manage *Drosophila suzukii* (Diptera: Drosophilidae) in *Vaccinium corymbosum* (Ericales: Ericaceae). J. Econ. Entomol. 107: 1849–1857.

Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag. Sci. 67: 1352–1357.

Haviland, D. R., J. Caprile, S. Rill, K. A. Hamby, and J. A. Grant. 2016. Phenology of spotted wing drosophila in the San Joaquin Valley varies by season, crop and nearby vegetation. Calif. Agric. 70: 24–31.

Haye, T., P. Girod, A. G. S. Cuthbertson, X. G. Wang, K. M. Daane, K. A. Hoelmer, C. Baroffio, J. P. Zhang, and N. Desneux. 2016. Current *D. suzukii* IPM tactics and their practical implementation in fruit crops across different regions around the world. J. Pest Sci. 89: 643–651.

Huang, J., L. Gut, and M. Grieshop. 2017. Evaluation of food-based attractants for *Drosophila suzukii* (Diptera: Drosophilidae). Environ. Entomol. 46: 878–884.

Iglesias, L. E., T. W. Nyoike, and O. E. Liburd. 2014. Effect of trap design, bait type, and age on captures of *Drosophila suzukii* (Diptera: Drosophilidae) in berry crops. J. Econ. Entomol. 107: 1508–1518.

Kassim, A., J. Poette, A. Paterson, D. Zait, S. McCallum, M. Woodhead, K. Smith, C. Hackett, and J. Graham. 2009. Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative traits loci (QTL). Mol. Nutr. Food Res. 53: 625–634.

Kemmitt, G., P. Valverde-Garcia, A. Hufnagl, L. Bacci and A. Zotz. 2015. The impact of three commonly used fungicides on *Typhlodromus pyri* (Acari: Phytoseiidae) in European vineyards. J. Econ. Entomol. 108: 611–620.

Kleiber, J. R., C. R. Unelius, J. C. Lee, D. M. Suckling, M. C. Qian, and D. J. Bruck. 2014. Attractiveness of fermentation and related products to spotted wing Drosophila (Diptera: Drosophilidae). Environ. Entomol. 43: 439–447.

Klesk, K., M. Qian, and R. R. Martin. 2004. Aroma extract dilution analysis of cv. Meeker (*Rubus idaeus* L.) red raspberries from Oregon and Washington. J. Agric. Food Chem. 52: 5155–5161.

Kogan, M. 1998. Integrated pest management: historical perspectives and contemporary developments. Annu. Rev. Entomol. 43: 243–270.

Landolt, P. J., and C. Guédot. 2008. Field attraction of codling moths (Lepidoptera: Tortricidae) to apple and pear fruit, and quantitation of kairomones from attractive fruit. Ann. Entomol. Soc. Am. 101: 675–681.

Landolt, P. J., T. Adams, and H. Rogg. 2012. Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. J. Appl. Entomol. 136: 148–154.

Lee, J. C., D. J. Bruck, H. Curry, D. Edwards, D. R. Haviland, R. A. Van Steenwyk, and B. M. Yorgey. 2011. The susceptibility of small fruits and cherries to the spotted-wing drosophila, *Drosophila suzukii*. Pest Manag. Sci. 67: 1358–1367.

Lee, J. C., H. J. Burrack, L. D. Barrantes, E. H. Beers, A. J. Dreves, K. A. Hamby, D. R. Haviland, R. Isaacs, T. A. Richardson, P. W. Shearer, et al. 2012. Evaluation of monitoring traps for *Drosophila suzukii* (Diptera: Drosophilidae) in North America. J. Econ. Entomol. 105: 1350–1357.

McDonald, J. H. 2009. Handbook of biological statistics, vol. 2. Sparky House Publishing, Baltimore, MD.

McIntyre, G. S., and R. H. Gooding. 2000. Egg size, contents, and quality: maternal-age and -size effects on house fly eggs. Can. J. Zool. 78: 1544–1551.

Mitsui, H., H. K.Takahashi, and M. T. Kimura. 2006. Spatial distributions and clutch sizes of Drosophila species ovipositing on cherry fruits of different stages. Popul. Ecol. 48: 233–237.

Pabst, A., D. Barron, P. Etievant, and P. Schreier. 1991. Studies on the enzymic hydrolysis of bound aroma constituents from raspberry fruit pulp. J. Agric. Food Chem. 39: 173–175.

Palanca, L., A. C. Gaskett, C. S. Günther, R. D. Newcomb, and M. R. Goddard. 2013. Quantifying variation in the ability of yeasts to attract *Drosophila melanogaster*. Plos One. 8: e75332.

Pelton, E., C. Gratton, R. Isaacs, S. Van Timmeren, A. Blanton, and C. Guédot. 2016. Earlier activity of *Drosophila suzukii* in high woodland landscapes but relative abundance is unaffected. J. Pest Sci. 89: 725–733.

Pelton, E., C. Gratton, and C. Guédot. 2017. Susceptibility of cold hardy grapes to *Drosophila suzukii* (Diptera: Drosophilidae). J. Appl. Entomol. 141: 644–652.

Price, J. F., O. E. Liburd, C. R. Roubos, and C. A. Nagle. 2009. Spotted wing drosophila in Florida berry culture. University of Florida, Florida Cooperative Extension Service. Publication ENY-861. http://edis.ifas.ufl.edu/in839 [accessed 3 April 2017].

Rossi-Stacconi, M. V., R. Kaur, V. Mazzoni, L. Ometto, A. Grassi, A. Gottardello, O. Rota-Stabelli, and G. Anfora. 2016. Multiple lines of evidence for reproductive winter diapause in the invasive pest *Drosophila suzukii*. J. Pest Sci. 89: 689–700.

Schabenberger, O. 2005. Introducing the GLIMMIX procedure for generalized linear mixed models. Proceedings of the 30th Annual SAS Users Group International Conference. SAS Institute, Cary, NC. http://www2.sas.com/proceedings/sugi30/196-30.pdf [accessed 23 December 2016].

Scheidler, N. H., C. Liu, K. A. Hamby, F. G. Zalom, and Z. Syed. 2015. Volatile codes: correlation of olfactory signals and reception in Drosophila-yeast chemical communication. Sci. Rep. 5: 14059.

Smith, C. R., A. V. Suarez, N. D. Tsutsui, S. E. Wittman, B. Edmonds, A. Freauff, and C. V. Tillberg. 2011. Nutritional asymmetries are related to division of labor in a queenless ant. PLoS One. 6: e24011.

Steck, G. J., W. Dixon, and D. Dean. 2009. Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera, Drosophilidae), a fruit pest new to North America. Pest Alerts. http://www.freshfromflorida.com/content/download/66350/1600760/Pest_Alert_-_Spotted_Wing_Drosophila_-_Drosophila_*suzukii*.pdf) [accessed 30 September 2016].

Stroup W. W. 2013. Generalized linear mixed models – Modern concepts, methods and applications. CRC Press, Taylor & Francis Group, A Chapman & Hall Book, Boca Raton, FL, 529 p.

Swoboda-Bhattarai, K. A., D. R. McPhie, and H. J. Burrack. 2017. Reproductive status of *Drosophila suzukii* (Diptera: Drosophilidae) females influences attraction to fermentation-based baits and ripe fruits. J. Econ. Entomol. 110: 1648–1652.

Terashima, J., and M. Bownes. 2004. Translating available food into the number of eggs laid by *Drosophila melanogaster*. Genetics. 167: 1711–1719.

Tochen, S., J. M. Woltz, D. T. Dalton, J. C. Lee, N. G. Wiman, and V. M. Walton. 2016. Humidity affects populations of *Drosophila suzukii* (Diptera: Drosophilidae) in blueberry. J. Appl. Entomol. 140: 47–57.

Utrio, P., and K. Eriksson. 1977. Volatile fermentation products as attractants for Macrolepidoptera. Ann. Zool. Fenn. 14: 98–104.

Wall, C. 1990. Principles of monitoring, pp. 9–23. *In* R. L. Ridgway, S. M. Silverstein, and M. N. Inscoe (eds.), Behaviour-modifying chemicals for insect management. Marcel Dekker, Inc., New York.

Wallingford, A. K., Lee, J. C. and G. M. Loeb. 2016. The influence of temperature and photoperiod on the reproductive diapause and cold tolerance of spotted-wing drosophila, *Drosophila suzukii*. Entomol. Exp. Appl. 159: 327–337.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. C. Lee, D. J. Bruck, V. M. Walton, S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integr. Pest Manage. 106: 289–295.

Wang, X. G., T. J. Stewart, A. Biondi, B. A. Chavez, C. Ingels, J. Caprile, J. A. Grant, V. M. Walton, and K. M. Daane. 2016. Population dynamics and ecology of *Drosophila suzukii*. J. Pest Sci. 89:701–12.

Wong, J., A. K. Wallingford, G. M. Loeb, and J. C. Lee. 2018. Physiological status of *Drosophila suzukii* (Diptera: Drosophilidae) affects their response to attractive odors, J. Appl. Entomol. doi:10.1111/jen.12497.

Zhang, A. and Feng, Y. 2017. Methods of attracting *Drosophila suzukii* using acetoin blend. U.S. Patent 20170251664 A1.