4-2015

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Environ. Entomol. 44(2): 356–367 (2015); DOI: 10.1093/ee/nvv013

ABSTRACT Native to Southeast Asia, the spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), has become a serious pest of soft-skinned fruit crops since its introduction into North America and Europe in 2008. Current monitoring strategies use baits based on fermentation products; however, to date, no fruit-based volatile blends attractive to this fly have been identified. This is particularly important because females are able to cut into the epicarp of ripening fruit for oviposition. Thus, we conducted studies to: 1) investigate the behavioral responses of adult *D. suzukii* to volatiles from blueberry, cherry, raspberry, and strawberry fruit extracts; 2) identify the antennally active compounds from the most attractive among the tested extracts (raspberry) using gas chromatography (GC)–mass spectrometry and coupled gas chromatography –electroantennographic detection (GC-EAD); and 3) test a synthetic blend containing the EAD-active compounds identified from raspberry extract on adult attraction. In olfactometer studies, both female and male *D. suzukii* were attracted to all four fruit extracts. The attractiveness of the fruit extracts ranks as: raspberry ≥ strawberry > blueberry ≥ cherry. GC analyses showed that the fruit extracts emit distinct volatile compounds. In GC-EAD experiments, 11 raspberry extract volatiles consistently elicited antennal responses in *D. suzukii*. In choice test bioassays, a synthetic EAD-active blend attracted more *D. suzukii* than a blank control, but was not as attractive as the raspberry extract. To our knowledge, this is the first report of a behaviorally and antennally active blend of host fruit volatiles attractive to *D. suzukii*, offering promising opportunities for the development of improved monitoring and behaviourally based management tools.

KEY WORDS Olfactometer, GC-EAD, fruit, volatile organic compound, attractant

The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a fruit-infesting fly native to Southeast Asia (Kanzawa 1939, Calabria et al. 2010, Walsh et al. 2011). Since its introduction in 2008, *D. suzukii* has become a devastating pest of soft-skinned fruit crops, such as raspberries, blueberries, and strawberries, in the continental United States of America (Beers et al. 2011, Hauser 2011); although it has been in the Hawaiian Island as far back as the 1980s (Kaneshiro 1983). In Europe, *D. suzukii* flies were first collected in Rasquera, Spain, in 2008 (Calabria et al. 2010) and also in Pisa, Italy (Cini et al. 2012). Unlike most drosophilid flies that feed and oviposit on overripe fruit, *D. suzukii* can feed and oviposit on ripening fruit (Mitsui et al. 2006, Calabria et al. 2010). The females possess a serrated ovipositor to cut through the epicarp of their hosts. Fruit infestation by *D. suzukii* larvae results in significant financial losses to farmers (Walsh et al. 2011, Cini et al. 2012, Vitagliano et al. 2013).

Because of its economic impact on fruit crops (Goodhue et al. 2011), farmers usually resort to calendar-based applications of organophosphate, pyrethroid, and spinosyn insecticides to manage *D. suzukii* (Beers et al. 2011, Lee et al. 2011a). Early detection of this fly on farms is essential for quick management measures that could lead to reductions in the rate and amount of insecticide applications. To date, *D. suzukii* populations are monitored by traps that use fermentation products such as apple cider vinegar, wine, or yeast as baits (Beers et al. 2011; Lee et al. 2011a, 2012, 2013; Landolt et al. 2012a,b). In particular, apple cider vinegar is commonly used because it is easily available and is relatively cheap (Beers et al. 2010; Lee et al. 2012, 2013). However, apple cider vinegar is not selective. Lee et al. (2012), in a study testing different trap designs using apple cider vinegar as bait, found that only 26–31% of the total numbers of *Drosophila* spp. caught in traps were *D. suzukii*. Also, apple cider vinegar baits are not very efficient at attracting flies prior to fruit injury (Burrack et al. 2015); thus, farmers cannot properly time protective treatments (C. Rodriguez-Saona, personal observation). To develop alternative baits, Landolt

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et al. (2012a,b) baited traps with wine and vinegar and found higher *D. suzukii* numbers in traps baited with a mixture of wine and vinegar than those baited with either product alone; the higher numbers were attributed mainly to the presence of ethanol and acetic acid. Further studies showed that *D. suzukii* antennae respond to 13 volatiles present in headspace collections from Merlot wine and rice vinegar (Cha et al. 2012). Three of these volatiles, acetoin, ethyl lactate, and methionol, together with ethanol and acetic acid, increased *D. suzukii* captures in traps compared with a mixture of acetic acid and ethanol alone (Cha et al. 2014). Moreover, a synthetic lure with these five compounds captured slightly more *D. suzukii* than a mixture of wine and vinegar; and a four-component lure containing acetoin, methionol, acetic acid, and ethanol provided similar results (Cha et al. 2014).

Because *D. suzukii* invade farms in early stages of fruit maturation, it is reasonable to hypothesize that females utilize fruit volatiles during host location. In fact, attraction to fruit volatiles is common for other flies that attack fruit. For example, the apple maggot, *Rhagoletis pomonella* Walsh, and the Asian fruit fly, *Bactrocera invadens* Drew, Tsura & White, (Diptera: Tephritidae) were attracted to host fruit volatiles in flight tunnel and field studies (Zhang et al. 1999, Kimbokota et al. 2013). Similarly, McPhail traps baited with host fruit volatiles attracted the oriental fruit fly, *Bactrocera dorsalis* Hendel, in the field (Cornelius et al. 2000, Siderhurst and Jang 2006). Furthermore, *Drosophila melanogaster* Meigen (a sister species of *D. suzukii*) and several other *Drosophila* spp. possess many (~60) odor receptors on their antennae, which are associated with ~1,300 olfactory receptor neurons that enable them to perceive and respond to many different odorants (Hallem et al. 2004, Kreher et al. 2008). Recently, Revadi et al. (2012) showed that *D. suzukii* females are attracted to odors from fresh blackberry, blueberry, cherry, raspberry, and strawberry in Y-tube olfactometer assays. In coupled gas chromatography–electroantennographic detection (GC-EAD) experiments, it was demonstrated that *D. suzukii* antennae respond to odors from fermentation products (Cha et al. 2012) and fruit such as blueberries (Maistri 2012) and raspberries (Revadi et al. 2012). However, studies have yet to identify a blend of fruit volatiles attractive to *D. suzukii*.

To improve the baits currently used to monitor *D. suzukii* and identify potential behaviorally based tools to manage this pest, we investigated the attraction of *D. suzukii* to volatiles from four of its hosts: highbush blueberry (*Vaccinium corymbosum* L.), cherry (*Prunus cerasus* L.), raspberry (*Rubus idaeus* L.), and strawberry (*Fragaria* × *ananassa* Duchesne). These four hosts were chosen because Lee et al. (2011b) showed, in no-choice and choice tests, that blueberry, cherry, raspberry, and strawberry fruit are susceptible to *D. suzukii* attack. Specifically, we conducted studies to: 1) determine the behavioral response of *D. suzukii* to volatiles from fruit extracts; 2) identify the antennally active compounds in the most attractive fruit extract among tested (raspberry); and 3) evaluate the behavioral activity of *D. suzukii* to a synthetic blend formulated with the antennally active compounds from the raspberry extract.

Methods and Materials

Insect Rearing. In July and August 2012, *D. suzukii*-infested blueberry (*V. corymbosum* 'Bluecrop') fruits were collected from commercial farms in Burlington County, NJ, and incubated on a thin layer of sand in deli cups (diameter 10 cm, height 4.5 cm; Prime Source, Bunzl Distribution USA Inc., MO) at 24–26°C for 20 d. Upon emergence, adults were identified under an optical stereomicroscope (Nikon, Tokyo, Japan) and subsequent generations were used for our experiments. The colony was reared on cornmeal diet (Dalton et al. 2011) in an incubator chamber (Percival Scientific Inc., IA) at 23°C, 70% relative humidity (RH), and a photoperiod of 14:10 (L:D) h.

Preparation of Fruit Extracts. We obtained fresh blueberries from Naturipe Farms LLC (Salinas, CA), pitted red tart cherries in water from Oregon Fruit Products (Salem, OR), and fresh raspberries and strawberries from Driscoll Strawberry Associates Inc. (Watsonville, CA). Batches of 500 g of fruit each were homogenized using a laboratory blender (Torrington, CT); in the case of cherries, the water was drained before blending. The pulp and seeds were strained with a nylon strainer and the resulting extracts were centrifuged (Sorvall RC 5B Plus; Thermo Fisher Scientific Inc., MA) at 3,470 g for 12 min. The extracts containing water-soluble (polar) substances were stored in a freezer (−10°C), in quantities of 10 ml in 12-ml vials (Cole-Parmer, Vernon Hills, IL). Vials with fruit extracts were selected randomly from the freezer for behavioral bioassays and headspace volatile collections.

Behavioral Bioassays. We investigated the attraction of adult female and male *D. suzukii* to volatiles from blueberry, cherry, strawberry, and raspberry fruit extracts in two-, three-, and five-choice assays using an eight-arm olfactometer (Plus Labs Inc., Lansing, MA), as described by Gökçe et al. (2005) but with some modifications. For our study, the size of the arm openings in the olfactometer was reduced by placing cotton corks that had a hole drilled in the middle and an inserted drinking straw (diameter ~5 mm, length 2.5 cm). This modified set up enabled *D. suzukii* to fly from the central chamber to a satellite chamber containing the odor source through the arm of the olfactometer but difficult for it to fly back, i.e., leave the satellite chamber. Cotton corks without perforations were used to completely seal off olfactometer arms not in use. The olfactometer was connected to a vacuum pump (GAST manufacturing Inc., MI), which pulled air at a rate of 2 liters/h from each satellite chamber through the connecting arm and into the central chamber. Each bioassay ran for 24 h and tested the attractiveness of 5 ml of each of the four fruit extracts versus 5 ml of distilled water (controls). Fruit extracts and water were placed in 29.6-ml plastic cups (WNA, Chelmsford, MA). The exterior of the plastic cups were

wrapped with aluminum foil to exclude any possible visual cues and were placed in the satellite chambers. Treatments were randomly assigned to satellite chambers to avoid positional biases. Flies ($N = 15$ in two-choice tests, and $N = 40$ in three- and five-choice tests) were released at once in the central chamber of the olfactometer. All flies were 3–5 d old and were used only once. Four olfactometers ran concurrently with two of them testing the attraction of females and two the attraction of males. Bioassays were conducted in the laboratory at 25°C, 60% RH, and a photoperiod of 16:8 (L:D) h, with ~1,700 lux light illuminance. The olfactometers were cleaned with ethanol, odor-free soap, and distilled water after each test.

For the two-choice tests (one fruit extract vs. a control), only two opposite satellite chambers of the olfactometer were used: a chamber for the fruit extract and the other for the control. There were 10 replicates for each of the four fruit extracts and for each sex. For the three-choice tests (two different fruit extracts vs. control), we used four alternating satellite chambers of the olfactometer so that in two opposite chambers we placed the fruit extracts and in the other opposite chambers the controls. Based on the results from the two-choice tests (see Results section), in one set of experiments, we paired the two most attractive fruit extracts (i.e., raspberry and strawberry) against two controls, and in another set of experiments, we paired the two least attractive fruit extracts (i.e., blueberry and cherry) against two controls. These experiments were replicated 12 times for both sexes. For the five-choice tests (all four fruit extracts vs. control), all eight satellite chambers of the olfactometer were used so that the four alternating chambers had one of the four fruit extracts, while the other four alternating chambers had controls. This experiment was replicated 10 times for both sexes.

Data recorded for the control in the three- and five-choice tests were the mean number of flies found in the two and four control satellite chambers of the olfactometer, respectively. We expressed the number of *D. suzukii* in the satellite chambers as a percentage of the total number of flies tested. We performed Wilcoxon signed-rank tests (IBM SPSS, version 20; Armonk, NY) to determine whether there were differences in the attractiveness of the fruit extracts compared with the controls in two-choice tests. For the three- and five-choice tests, we performed Kruskal–Wallis tests (IBM SPSS); followed by Mann–Whitney *U* test (IBM SPSS), if statistically significant.

Volatile Collections. We collected the headspace volatiles from 10 ml of each of the four fruit extracts in 22-ml clear glass vials (Sigma-Aldrich, St. Louis, MO) at a time. The lid of the vial had two perforations, one for air inlet and the other for air outlet. We inserted a Pasteur pipette filled with activated charcoal in the inlet to filter the air. A Super-Q trap (30 mg; ARS, Gainesville, FL) was inserted into the outlet such that the volatiles were pulled at ~1.5 liters/min onto it using a 12 V pump (Sensidyne, Clearwater, FL) for 4 h. Trapped volatiles were eluted with 150 µl of dichloromethane (Sigma-Aldrich) with the help of a gentle stream of N_2. Headspace volatiles from an empty 22 ml glass vial were collected concurrently as controls. Samples were stored in a freezer at −10°C until gas chromatography with flame ionization detector (GC-FID), coupled gas chromatography-mass spectrometry (GC-MS), and GC-EAD studies were conducted. We collected eight replicates for each fruit extract and control.

Headspace volatile samples were run in a Hewlett-Packard (HP) 6890 series GC-FID with an Agilent HP-1 column (10 m by 0.53 mm ID by 2.65 µm film thickness). The carrier gas was helium, flowing at a constant rate of 5 ml/min. The oven temperature was programmed at 40°C and held for 1 min; then increased to 180°C at 14°C/min and held for 2 min, increased again to 200°C at 40°C/min and held for 2 min, and then to 220°C and held for 5 min. We used these GC-FID data to investigate differences in the blend composition among the four fruit extracts. For analyses of volatiles, prior to GC analysis, we added 400 ng of *n*-octane to each sample as internal standard. The area of all peaks in the chromatogram was then calculated based on the *n*-octane peak area. Data on total amount of volatiles were subjected to analysis of variance (IBM SPSS), followed by Tukey's honestly significant difference test, if significant differences were detected. To determine whether distinct blends of volatiles were emitted from the four fruit extracts, we performed principal components analysis (PCA; Minitab, version 16; Minitab Inc., State College, PA) based on the relative peak areas from the GC-FID analyses.

In addition, we used GC-MS to identify the specific headspace volatiles within fruit blends. The headspace volatiles from the fruit extracts were analyzed in an HP 6890 GC equipped with an HP 5973 mass selective detector with a DB-Waxetr column (J&W Scientific Inc., Folsom, CA, 60 m by 0.25 mm internal diameter [ID], 0.25-µm film thickness; temperature programmed at 40°C for 2 min, then to 260°C at 15°C/min and held for 10 min) in the splitless mode, with helium as carrier gas and linear velocity of 36 cm/s. A 70-eV electron beam was used for sample ionization. The hydrocarbon mixture (*n*C7–*n*C30) was used as the external standard. The volatile compounds were identified by comparing their mass spectra with the NIST 11 (Gaithersburg, MD) and Wiley 7N (John Wiley, NY) mass spectral libraries.

GC-EAD Experiments. The coupled GC-EAD system used was as previously described (Zhang et al. 1997; Zhang and Polavarapu 2003). Headspace volatile samples from the raspberry extract (the most attractive extract among tested; see Result section) were tested with female and male *D. suzukii* antennae ($N = 30$). A HP 6890 gas chromatograph equipped with a 60 m by 0.25 mm ID, 0.25-µm film thickness DB-Waxetr capillary column (J&W Scientific Inc.) in the splitless mode with hydrogen as carrier gas (1.4 ml/min) was used for GC-EAD analysis (40°C for 2 min, then programmed to 260°C at 15°C/min and held for 10 min). The capillary column effluent and nitrogen makeup gas (10 ml/min) were split (~1:1) by a fixed outlet splitter (SGE Inc., Austin, TX) to the FID and EAD. The head of a

D. suzukii fly was excised from the body and both antennae were positioned between two gold wire electrodes, which were immersed in saline-filled (0.9% NaCl) wells (1.25 mm in diameter; ~3 mm apart) in a small acrylic plastic holder (8 cm in length by 0.8 cm in width by 0.6 cm in breadth). The output recording electrodes were connected to a high-impedance 1:100 amplifier with automatic baseline drift compensation. The airstream flowing over the antennae (~500 ml/min) was humidified by bubbling through distilled water before entering the EAD interface. The antennal preparation was cooled to ~5°C inside a condenser by circulating near 0°C water from a bench-top refrigeration unit (RTE-100, NESLAB instruments Inc., Portsmouth, NH) through the insulation layer of the modified condenser containing the acrylic plastic holder mounted on top of the GC. The flame ionization and electrophysiological output signals were recorded using the HP ChemStation software. GC-MS analyses of raspberry volatile extracts were conducted, as described above (see Volatile Collections section), to identify the GC-EAD-active compounds.

Dispenser Formulation. All GC-EAD-active compounds [1) butyl acetate, 99.5%; 2) hexanal, 98%; 3) 2-heptanone, 99%; 4) 3-methyl-1-butanol, 99%; 5) *trans*-2-hexenal, 98%; 6) 3-methyl-2-butyl acetate, 98%; 7) 2-heptanol, 98%; 8) hexanol, 99%; 9) *cis*-3-hexenol, 98%; 10) 6-methyl-5-hepten-2-ol, 98%, and 11) linalool, 97%)] were purchased from Sigma-Aldrich and mixed into an 11-component blend (compounds 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 in a ratio [by volume] of 1:36:3:5:10:2:7:8:9:2:8, respectively). The chemical blend was mixed with twice the amount of mineral oil (Fischer Scientific, Fairlawn, NJ) for slow release of these compounds. To absorb and hold liquid oil mixture, a small cotton ball was inserted into a transparent polypropylene centrifuge tube (1.5 ml snap-cap, VWR Corp., Radnor, PA), which was then impregnated with 500 µl of the chemical–mineral oil mixture. The same amount of mineral oil was loaded as the blank control. After loading, the cap was tightly closed. The dispensers were kept in a freezer at −10°C until deployed. For behavioral assay (see Evaluation of GC-EAD-Active Blend section), the cap of the tube was opened and then placed into a sealed plastic zip bag (7.6 cm by 12.7 cm, PGC Scientific, Frederic, MD) as a kairomone dispenser.

Dispenser Release Rate Study. A release rate study was conducted in the laboratory. Six polypropylene centrifuge tube dispensers were placed into a sealed plastic zip bag with the cap opened, separately, and suspended on hooks in a fume hood (temperature: 20–25°C, face velocity: 0.6553 m/s). All dispensers were removed from the fume hood and weighted every 4 h; the experiment was conducted for 48 h.

Evaluation of GC-EAD-Active Blend. Two choice test experiments were conducted to test the attraction of *D. suzukii* flies to a synthetic blend of the eleven GC-EAD-active compounds (see Results section). First, we used the eight-arm olfactometer described in the Behavioral Bioassays section to test fly choice between: 1) raspberry extract versus control; 2) an 11-component synthetic blend versus control; and 3) raspberry extract versus an 11-component synthetic blend. Similar studies as those described in the Behavioral Bioassays section were conducted to test the attraction of *D. suzukii* to the raspberry extract. Briefly, 5 ml of the raspberry extract was placed in a 29.6-ml plastic cup with a cotton ball to absorb and hold the liquid, the cup was then covered with aluminum foil. Subsequently, the cup was placed in one satellite arm of the olfactometer and in the opposing satellite arm we placed a 29.6-ml plastic cup covered with aluminum foil and containing a cotton ball soaked with 5 ml of distilled water (control). The other arms of the olfactometer were blocked as described in the Behavioral Bioassays section. To test the attraction of *D. suzukii* to the EAD-active blend, the pure synthetic blend (11 components [see Dispenser Formulation section], 460 µl) was diluted with ethyl acetate into 1 ml. A water solution of the synthetic blend was made by diluting 40 µl of the synthetic blend solution with 10 ml d-H_2O and adding one drop of surfactant Tween 80 (Sigma-Aldrich). One milliliter of this solution containing the synthetic blend was placed in a 1.5-ml polypropylene centrifuge tube; the centrifuge tube was placed inside a 29.6-ml plastic cup to keep it upright. This cup was then placed in one satellite arm of the olfactometer, while a control (cup with a centrifuge tube containing 1 ml of water) was placed in an opposite arm, as described in the Behavioral Bioassays section. Similarly, to test the attractiveness of the synthetic volatile mixture against the raspberry extract, the synthetic volatile blend and the raspberry extract were placed in opposite arms of the olfactometer. For each test, female or male *D. suzukii* ($N = 10–15$) were released in the central chamber of the olfactometer. All flies used in the experiment were 2–8 d old and obtained from a laboratory colony, as previously described in the Insect Rearing section. Experimental conditions were according to those described in the Behavioral Bioassays section. Each choice test was replicated 6–10 times

In addition, we conducted cage experiments to test fly choice between the 11-component EAD-active dispenser (see Dispenser Formulation section) and a blank control, and between two blank controls. These experiments were performed in 30 by 30 by 30 cm cages, with two sides consisting of clear Plexiglas, one side of fine white nylon mesh, and one side with a cloth sleeve that provided access to the interior; the top and bottom were made of Plexiglas. Two 1-liter clear plastic containers (Prime Source Deli Containers, Chattanooga, TN) were placed inside the cages, diagonally opposed to each other, and 12 cm apart. A 29.6-ml cup containing a cotton ball saturated with 10% sugar—water solution was placed between the containers. The containers had four 7-mm-diameter holes drilled at equal distance around the container and 30 mm from the top edge to provide access to flies. In each container, we added 150 ml of a drowning fluid that consisted of 4 ml of unscented liquid soap (Free & Clear, Seventh Generation Inc., Burlington, VT) and 38 g of Borax (Henkel Co., Scottsdale, AZ) in 3.8 liters of water (after Landolt et al. 2012a). Also, each container had a

7.5 by 8.5 cm yellow sticky card (Great Lakes IPM, Vestaburg, MI) hung above the drowning fluid from a paperclip hook attached to the lid. A polypropylene centrifuge tube dispenser (described in the Dispenser Formulation section) was hung from a second hook in each container. In one cage, the dispenser in one of the containers had the GC-EAD-active synthetic raspberry blend while the other container had a blank control (mineral oil) dispenser. Another cage had two containers both with blank control (mineral oil) dispensers. Twenty *D. suzukii* flies (10 males and 10 females) were released at the center of each cage. Cages were placed on a laboratory bench under 25–28°C and a photoperiod of 14:10 (L:D) h, with ~950 lux light illuminance provided by two fluorescent tubes located above the cages. Flies were released between 1400 and 1700 hours, and the total number of flies in each container, i.e., sum of flies on sticky card and in drowning liquid, was counted and sexed 48 h after release. The experiment was repeated three times with new lures and flies.

Choice data were analyzed using *G* tests (Sokal and Rohlf 1995), with the null hypothesis that flies would have a 1:1 distribution over the two satellite chambers containing the synthetic blend/dispenser, raspberry extract, or distilled water/mineral oil. Only flies that made a choice were used in the analysis and data from all replicate experiments were pooled prior to analysis.

Results

Behavioral Bioassays. In two-choice tests, more female and male *D. suzukii* were attracted to fruit extracts than the control (blueberry–females: $Z = 2.81$, $P = 0.005$, males: $Z = 2.81$, $P = 0.005$; cherry–females: $Z = 2.81$, $P = 0.005$, males: $Z = 2.69$, $P = 0.007$; raspberry–female: $Z = 2.81$, $P = 0.005$, male: $Z = 2.81$, $P = 0.005$; strawberry–female: $Z = 2.81$, $P = 0.005$, male: $Z = 2.81$, $P = 0.005$; Fig. 1).

In three-choice tests, when we paired the two least attractive extracts, female *D. suzukii* attraction differed among the blueberry and cherry extracts and the control ($\chi^2 = 13.13$; df = 2; $P = 0.001$; Fig. 2a). Females were more attracted to the blueberry extract than the control ($U = 18.00$; $P = 0.002$) and to the cherry extract than the control ($U = 13.00$; $P = 0.004$); however, there was no difference in attraction between the blueberry and cherry extracts ($U = 48.00$; $P = 0.163$). A similar pattern was found for males ($\chi^2 = 11.24$; df = 2; $P = 0.004$; Fig. 2a).

When we paired the two most attractive extracts, female *D. suzukii* attraction differed among the raspberry and strawberry extracts and the control ($\chi^2 = 20.99$; df = 2; $P < 0.001$; Fig. 2b). Females were more attracted to the raspberry extract than the control ($U = 4.00$; $P < 0.001$) and to the strawberry extract than the control ($U = 4.50$; $P < 0.001$); however, there was no difference in attraction between the raspberry and strawberry extracts ($U = 67.50$; $P = 0.795$). A similar pattern was found for males ($\chi^2 = 17.19$; df = 2; $P < 0.001$; Fig. 2b).

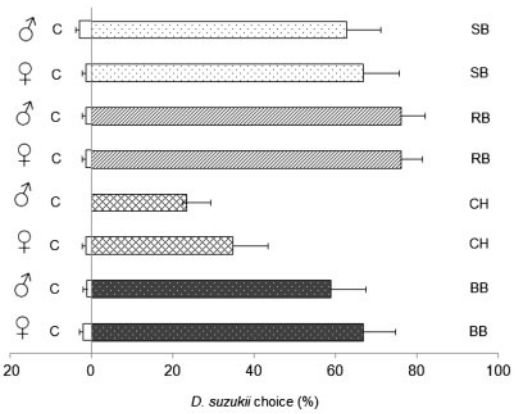

Fig. 1. Mean percent (±SE) of adult *D. suzukii* males and females that chose fruit extracts (shaded bars) and control (unshaded bars) in a two-choice test. SB, strawberry; RB, raspberry; CH, cherry; BB, blueberry; C, control. Each bar is the mean of 10 replicates. In each replicate, $N = 15$ *D. suzukii* flies were tested. All fruit extracts were statistically more preferred than control (Wilcoxon signed-ranks test, $P < 0.05$).

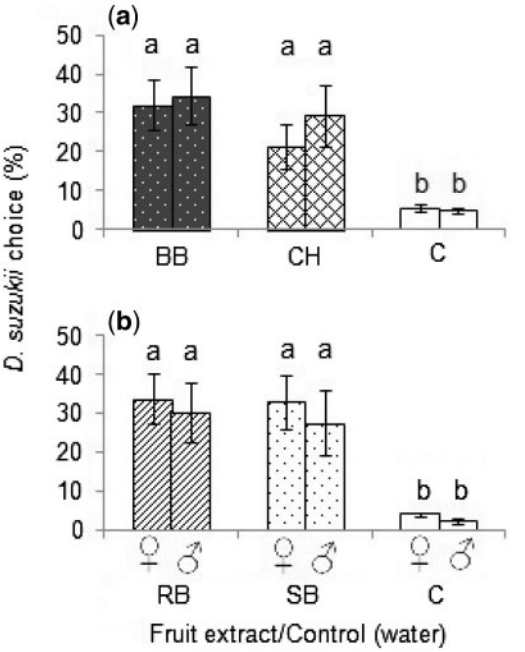

Fig. 2. Mean percent (±SE) of adult *D. suzukii* females and males that chose blueberry (BB) and cherry (CH): (a) and raspberry (RB) and strawberry (SB), (b) extracts and control in a three-choice test. Each bar is the mean of 12 replicates. In each replicate, $N = 40$ *D. suzukii* flies were tested. Statistical differences are indicated by different letters based on Mann–Whitney *U* tests at $P < 0.05$.

In five-choice tests, where we evaluated the flies' attraction to all four fruit extracts simultaneously, female *D. suzukii* were more attracted to the raspberry and strawberry extracts than to the blueberry and

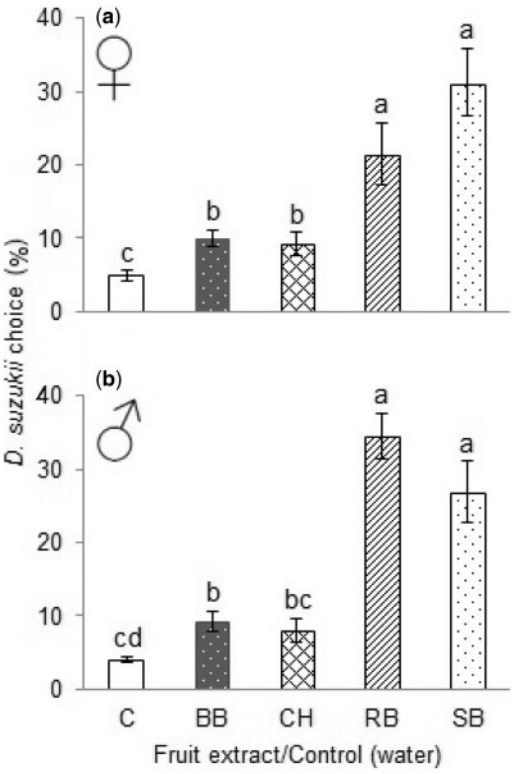

Fig. 3. Mean percent (±SE) of adult *D. suzukii* females (a) and males (b) that chose blueberry (BB), cherry (CH), raspberry (RB), and strawberry extracts (SB) and control (C) in a five-choice test. Each bar is the mean of 10 replicates. In each replicate, $N = 40$ *D. suzukii* flies were tested. Statistical differences are indicated by different letters based on Mann–Whitney U tests at $P < 0.05$.

cherry extracts, and the attraction to all fruit extracts was more than the control ($\chi^2 = 27.67$; df = 4; $P < 0.001$; Fig. 3a). A similar pattern was found for male *D. suzukii*; however, male attraction to the cherry extract was not significantly different from the control ($P > 0.05$; Fig. 3b).

Volatile Collections. In the GC-FID run there were differences in the total amount of volatiles emitted from raspberry, strawberry, cherry, and blueberry fruit extracts ($F = 8.09$; df = 3; $P < 0.001$; Supp Tables 1–5 [online only]). The raspberry extract emitted higher amounts of volatiles (mean ± SE: 1.6 ± 0.20 µg/h) than the cherry extract (0.3 ± 0.03 µg/h; $P < 0.001$). The amount of volatiles emitted from the blueberry extract (1.2 ± 0.26 µg/h) was also higher than the cherry extract ($P = 0.011$); however, there were no significant differences in the amounts of volatiles emitted from raspberry, strawberry (0.9 ± 0.18 µg/h), and blueberry extracts ($P > 0.05$), or between the strawberry and cherry extracts ($P = 0.172$; Supp Table 1 [online only]).

The PCA resulted in a model with the first two PCs explaining 40.8% of the total variation in volatile blends of the fruit extracts. Although the score plot of PC1 versus PC2 shows, for the most part, that distinct

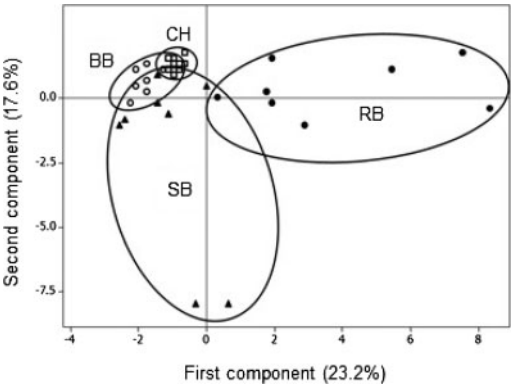

Fig. 4. Cluster plot of relative peak areas of volatile compounds from blueberry, cherry, raspberry, and strawberry extracts following PCA. SB, strawberry; RB, raspberry; CH, cherry; BB, blueberry.

blends were emitted from all four fruit extracts, it also shows some degree of overlap among them (Fig. 4). The first PC explained 23.2% of the variation in volatile blends and separated the blend from the raspberry extract from the blends of the other fruit extracts, while the second PC explained 17.6% of the variation in the data and separated the blend from the strawberry extract from the blends of blueberry and cherry extracts (Fig. 4).

In the GC-MS run, no GC peaks were detected in control vials (data not shown). In contrast, 55 peaks were identifiable in the chromatograms of all four fruit extracts; of these, 47, 35, 29, and 24% were found in the strawberry, raspberry, blueberry, and cherry fruit extracts, respectively. Moreover; 31, 22, 11, and 9% were unique to the strawberry, raspberry, cherry, and blueberry extracts, respectively. None were common to all four fruit extracts, indicating qualitative differences among volatile blends. Additionally, 25% of the volatiles overlapped among two or three of the fruit extracts (Table 1).

GC-EAD Analyses. The GC-EAD experiments consistently revealed 11 antennally active volatiles from the raspberry extract (Fig. 5a; Table 2). A natural ratio of a synthetic blend of the compounds identified also elicited similar antennal responses (Fig. 5b).

Release Rate of Dispenser. Dispenser release rate was studied in a laboratory fume hood conditions for 48 h and remaining kairomone residues were determined by weight loss method. The kairomone components desorbed from the polypropylene centrifuge tube over time was best described by the equation: $Y = 511.37\,e^{-0.0025t}$, indicating that the volatile ingredients evaporated from the mineral oil formulation in polypropylene centrifuge tube dispensers following first order kinetics ($r^2 = 0.9714$). Under laboratory conditions, ~30 mg of the kairomone components were released during the first day and ~28 mg were released during the second day.

Evaluation of GC-EAD-Active Blend. In choice tests with the olfactometer, both female and male *D. suzukii* were highly attracted to the raspberry extract

Table 1. Volatile organic compounds identified from extracts of blueberry, cherry, raspberry, and strawberry fruits

#	RT (min)	Compounds	Compounds indentified (%)			
			BB	CH	RB	SB
1	5.28	Methyl butyrate	–	–	–	5.6
2	5.43	2-Methyl-3-buten-3-ol	–	19.0	–	19.1
3	5.75	Ethyl butanoate	–	–	–	10.2
4	5.50	2-Methyl-2-butanol	–	–	8.8	2.9
5	5.91	Ethyl 2-methyl-butanoate	–	–	–	2.4
6	6.10	Butyl acetate	–	–	0.2	–
7	6.26	Hexanal	2.6	–	18.4	1.7
8	6.80	2-Methyl-4-pentanol	–	–	1.8	–
9	6.99	3-Methyl-2-buten-1-ol	–	1.9	–	–
10	7.01	3-Penten-2-ol	1.5	–	–	–
11	7.24	2-Heptanone	–	–	0.8	–
12	7.45	3-Methyl-1-butanol	–	–	2.0	–
13	7.63	*trans*-2-Hexenal	57.5	–	3.7	0.8
14	7.69	Ethyl caproate	–	–	–	1.8
15	7.91	3-Methyl-2-butanol acetate	–	–	1.0	–
16	8.07	*n*-Hexyl ocatoate	–	–	–	0.6
17	8.21	3-Hydroxy-2-butanone	–	–	–	0.1
18	8.26	Octanal	–	–	–	2.0
19	8.43	2-Hexanol	0.6	–	–	0.4
20	8.44	2-Heptanol	–	–	4.0	–
21	8.48	2-Methyl-2-buten-1-ol	–	–	1.2	–
22	8.64	*trans*-2-Hexenyl acetate	–	–	–	0.6
23	8.75	1-Hexanol	6.8	–	5.3	2.1
24	8.87	*trans*-3-Hexen-1-ol	0.5	–	–	–
25	9.07	*cis*-3-Hexen-1-ol	0.8	–	7.0	0.7
26	9.25	*Trans*-2-Hexen-1-ol	8.8	2.8	–	3.9
27	9.68	Acetic acid	–	–	–	1.8
28	9.73	6-Methyl-5-hepten-2-ol	–	–	0.8	–
29	9.90	Furfural	–	2.0	–	–
30	10.00	Methyl 3-hydroxy butanoate	–	–	–	0.9
31	10.11	Ethyl 3-hydroxy butanoate	–	–	–	0.5
32	10.45	Linalyl propionate	–	–	–	1.5
33	10.46	Linalool	1.7	0.6	8.1	–
34	10.49	Benzaldehyde	–	42.3	–	0.6
35	10.50	1-octanol	0.6	–	–	–
36	10.67	2-Methyl propanoic acid	–	–	–	1.5
37	10.99	Mesifuranne	–	–	–	8.8
38	11.16	Butanoic acid	–	–	–	0.8
39	11.45	α-Terpineol	–	2.2	–	–
40	11.50	2-Methyl-1-butyric acid	–	–	–	4.9
41	12.00	4-Ethyl benzaldehyde	–	–	–	1.3
42	12.80	Hexanoic acid	–	1.1	–	–
43	12.81	*trans*-Geraniol	1.6	–	11.5	–
44	12.82	Caproic acid	–	–	–	5.2
45	12.88	Dihydro-β-ionone	–	–	0.2	–
46	12.90	*p*-Cymen-8-ol	0.3	–	–	–
47	13.01	α-Ionone	–	–	4.4	–
48	13.13	Benzyl alcohol	–	4.1	–	–
49	13.14	α-Ionol	–	–	1.0	–
50	13.64	β-Ionone	–	–	7.8	–
51	14.28	Octanoic acid	0.5	4.2	–	–
52	14.96	Nonanoic acid	0.3	–	–	–
53	15.12	Eugenol	0.4	1.5	–	–
54	15.61	Decanoic acid	–	0.4	–	–
55	16.56	Methyl jasmonate	0.4	0.4	–	–

#, Peak number; RT, retention time (DB-Waxetr column); BB, blueberry; CH, cherry; SB, strawberry; RB, raspberry; (–), not present.

(as shown above in Behavioral Bioassays; Fig. 6a). Female, but not male, *D. suzukii* were also attracted to the 11-component synthetic blend (Fig. 6a). However, when flies were given a choice between the raspberry extract and the 11-component synthetic blend, over 95% of the flies preferred the raspberry extract.

In choice tests using cages, significantly more *D. suzukii* males (86%), but not females, preferred the dispensers containing the 11 antennally active raspberry volatile compounds in mineral oil compared with the control (mineral oil) dispensers (Fig. 6b). There was no preference when flies were offered a choice between two control (mineral oil) dispensers (Fig. 6b).

Discussion

This study demonstrates that: 1) volatiles from homogenized blueberry, cherry, raspberry, and strawberry fruit are attractive to adult spotted wing drosophila, *D. suzukii*, a serious pest of small fruit crops; 2) *D. suzukii* antennae responds to 11 water-soluble volatiles found in raspberry fruit; and 3) a blend of antennally active raspberry fruit compounds attracts more *D. suzukii* adults compared with a blank control in laboratory bioassays; however, the results were inconsistent between sexes depending on the type of assay. Also, our EAD-active blend was much less attractive than the raspberry extract, indicating that some components in the blend are missing.

Many insects utilize plant volatile compounds as olfactory cues in host location (e.g., Cornelius et al. 2000, Bruce et al. 2005, Siderhurst and Jang 2006, Bruce and Pickett 2011, von Arx et al. 2011). Among vinegar flies, it is known that fruit volatiles are involved in *Drosophila* spp. orientation (e.g., Lebreton et al. 2012, Faucher et al. 2013) and oviposition behaviors (e.g., Stensmyr et al. 2012, Linz et al. 2013). Revadi et al. (2012) showed recently that *D. suzukii* flies are attracted to odors from intact raspberry, blackberry, blueberry, cherry, and strawberry fruit, indicating that fruit volatiles are important in *D. suzukii* host location. Similarly, we showed that, when given a choice between fruit extracts and water, female and male *D. suzukii* are attracted to volatiles from blueberry, cherry, strawberry, and raspberry fruit extracts. Moreover, in multiple choice trials, we showed that volatiles from raspberry and strawberry extracts are more attractive to *D. suzukii* than volatiles from blueberry and cherry extracts, while the behavioral response towards volatiles from blueberry and cherry extracts was similar. Based on our behavioral data, *D. suzukii* responses to the four fruit extracts can be ranked as follows: raspberry $\geq$ strawberry $>$ blueberry $\geq$ cherry; the raspberry fruit extract being the most attractive and the cherry extract the least attractive. Lee et al. (2011b) showed that raspberry and strawberry fruit are better hosts for *D. suzukii* development than blueberry and cherry fruit. When comparing blackberry, blueberry, raspberry, and strawberry fruits, Burrack et al. (2013) reported the highest *D. suzukii* oviposition in raspberry fruit and the lowest in blueberry fruit. Altogether, previous studies and our study suggest a positive relationship among adult *D. suzukii* attraction, female oviposition, and offspring performance, particularly for raspberries.

When performing GC-EAD studies, Revadi et al. (2012) showed that *D. suzukii* antennae respond to several compounds emitted from intact raspberry fruit;

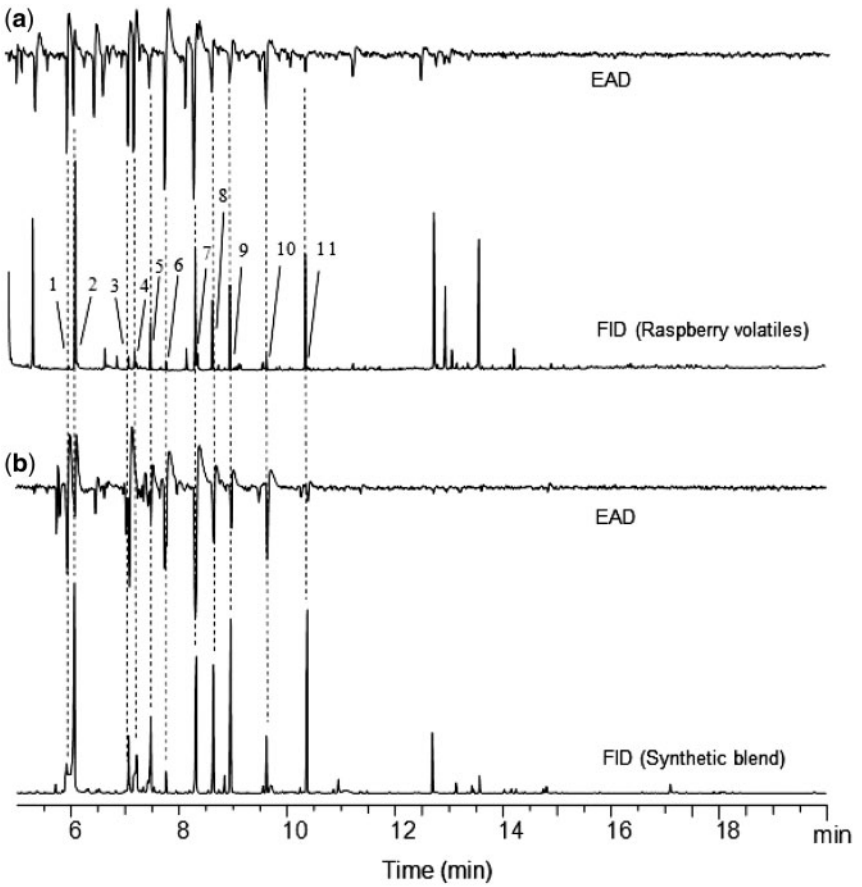

Fig. 5. Reconstructed simultaneous GC-EAD and FID responses of an adult female *D. suzukii* antenna to a volatile extract of raspberry (a) and a synthetic chemical blend (b) of identified compounds. Numbered peaks refer to compounds listed in Table 2.

Table 2. GC-EAD active compounds identified from the raspberry volatile extract

Peak no.	RT (min)[a]	Kovats index		Compounds	Ratio
		DB-Waxetr	DB-5MS		
1	6.10	1,089	811	Butyl acetate	1
2	6.26	1,105	800	Hexanal	30
3	7.24	1,201	888	2-Heptanone	3
4	7.45	1,221	733	3-Methyl-1-butanol	5
5	7.63	1,241	855	*trans*-2-Hexenal	10
6	7.91	1,269	919	3-Methyl-2-butenyl acetate	2
7	8.43	1,324	900	2-Heptanol	7
8	8.75	1,358	868	Hexanol	8
9	9.07	1,393	854	*cis*-3-Hexenol	9
10	9.73	1,467	992	6-Methyl-5-hepten-2-ol	2
11	10.46	1,553	1,102	Linalool	8

[a]DB-Waxetr column; RT, retention time.

however, these compounds were not identified. Maistri (2012) identified 16 antennally active compounds to female *D. suzukii* from blueberries (*V. corymbosum* 'Duke'): ethyl acetate, butyl acetate, cunene (isopropylbenzene), isocumene (propylbenzene), 1,8-cineole, anhydrolinalool oxide, α-terpinene, tridecane, octanal, tetradecane, *trans*-caryophyllene, methyl salicylate, hexanoic acid, δ-3-carene, geraniol, and α-ionone but their behavioral activity was not tested. In our GC-EAD studies, we identified 11 compounds, butyl acetate, hexanal, 2-heptanone, 3-methyl-1-butanol, *trans*-2-hexenal, 3-methyl-2-butenyl acetate, 2-heptanol, hexanol, *cis*-3-hexenol, 6-methyl-5-hepten-2-ol, and linalool, from the raspberry fruit extract that consistently elicit strong antennal responses in female and male *D. suzukii*.

Our behavioral responses are largely in agreement with the headspace volatile analyses and GC-EAD data, which show differences among fruit extracts especially with regards to the EAD-active compounds. For example, the strawberry extract contained only four of the EAD-active compounds present in the raspberry extract (hexanal, *trans*-2-hexenol, hexanol, and *cis*-3-hexen-1-ol). Cherry extracts were the least attractive to *D. suzukii* of all extracts in our behavioral assays, likely because these extracts contained only one of the EAD-active compounds (linalool). Yet, other factors, such as

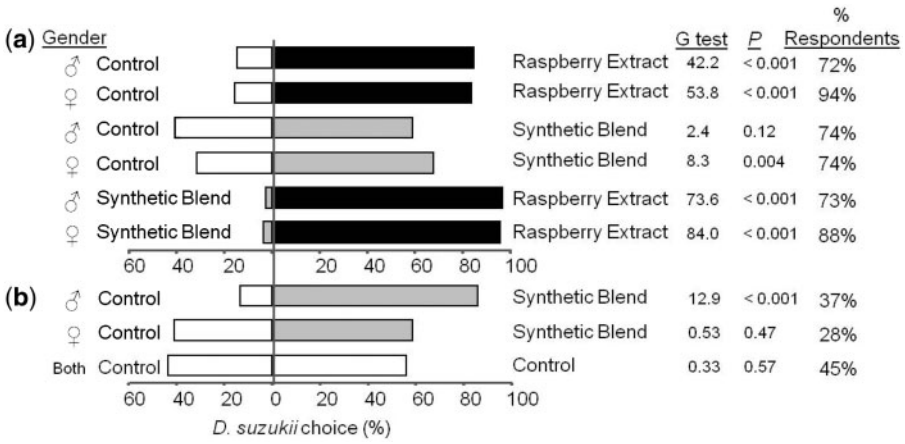

Fig. 6. Adult *D. suzukii* response (males and females) to raspberry extract against distilled water (control), an 11-compoment synthetic blend against a control, and 11-component synthetic blend against raspberry extract in an olfactometer (a). Data are percent of flies choosing one of the olfactometer arms containing the test substance 24 h after release. Experiments were replicated 6–10 times with 10–15 flies of each sex ($N = 90$–110), and adult *D. suzukii* response to dispensers containing either the 11 antennally active raspberry volatiles in mineral oil or mineral oil alone (control), and two control (mineral oil) dispensers, in cages (30 by 30 by 30 cm) (b). Data are percent of flies choosing one of the containers 48 h after release. Experiments were replicated three times with 20 flies each ($N = 60$).

quantitative differences among volatiles present, might have also played a role in the attraction because strawberry and blueberry fruit extracts emitted a similar number of EAD-active volatiles; however, in behavioral bioassays, both females and males of *D. suzukii* were more attracted to strawberry volatiles than those from blueberries.

Many of the antennally active volatile compounds we identified in the raspberry fruit extract from GC-EAD experiments on *D. suzukii* are commonly found in the volatile profiles of several small fruits including raspberries, blackberries, blueberries, strawberries, and cherries. For example, Malowicki et al. (2008a,b) found hexanal, 2-heptanone, *trans*-2-hexenal, 6-methyl-5-hepten-2-ol, and linalool in the volatile profile of raspberry fruit. All compounds found in our raspberry fruit extract, with the exception of 3-methyl-1-butanol and 3-methyl-2-butenyl acetate, were also present in the blackberry fruit volatile profile (Du et al. 2010). In addition, Levaj et al. (2010) found that butyl acetate is emitted from blueberry, strawberry, and raspberry fruit; linalool from blueberries, cherries, strawberries, and raspberries; *cis*-3-hexenol from blueberries; hexanal and 2-heptanone from cherries; and hexanol from strawberries. Although these previous studies and our results indicate that other small fruits may emit some of same volatiles found in the raspberry fruit extract, it is likely that not all of these fruits emit them at the right amounts and ratios attractive to *D. suzukii*. For our studies, we used fruit extracts; however, we expect that intact fruit may emit some of the same but also other unique volatiles. Now that a set of raspberry volatiles, both attractive and antennally active to *D. suzukii*, has been identified, future studies in our laboratory will investigate whether these volatiles are also emitted from intact fruit at different stages of maturation.

A formulated blend containing all of the 11 EAD-active compounds was more attractive than a blank control to adult *D. suzukii* in our behavioral studies. However, there were gender differences in the attraction based on the type of behavioral assay and volatile-mixture formulation: females were more attracted to the synthetic blend in olfactometer assays, while males were more attracted to baited dispensers in cage studies. The reasons for these inconsistencies are unclear, but it could be due to differences in the physiological state of the flies and/or environmental conditions, even when we tried to keep these factors as similar as possible in both behavioral assays. Despite these discrepancies in our results, it is clear that *D. suzukii* responds to the 11-component synthetic blend; however, this blend is not optimal as our results show that it is weaker in attracting flies than the raspberry extract, indicating that some components might be missing. It is also likely that the synthetic blend attracted fewer flies than the raspberry extract due to differences in release rates between them. Furthermore, as a first step in identifying attractive volatiles from raspberry, we made aqueous extraction of homogenized fruits (water-soluble compounds), which expectedly was highly efficient in collecting very polar compounds but had very low efficiency in collecting less polar compounds. This is evidenced by the presence of many polar compounds (i.e., alcohols, aldehydes, and ketones) among the 11 EAD-active compounds. Further studies are underway to identify other behaviorally and EAD-active volatile compounds (polar and non-polar) from raspberry to optimize our blend.

Some of the compounds in our synthetic blend have been shown to elicit antennal responses in other fruit-attacking flies, suggesting some shared perception of fruit odors. For example, in a recent study, Linz et al. (2013) showed that 2-heptanone, 3-methyl-2-butenyl

acetate, and linalool elicit antennal responses from *D. melanogaster*, *Drosophila yakuba* Burla, *Drosophila orena* Tsacas & David, and *Drosophila erecta* Tsacas & Lachaise. Linalool has also been shown to elicit antennal responses from the Mexican fruit fly *Anastrepha ludens* Loew (Diptera: Tephritidae), and is now an integral component of a synthetic lure used to monitor this pest (González et al. 2006, Rasgado et al. 2009). Hexanol elicited antennal responses from *A. ludens* and *Anastrepha oblique* Macquart (Malo et al. 2005, Cruz-López et al. 2006), while *cis*-3-hexenol also elicited antennal responses from *A. ludens* (Malo et al. 2005). Both hexanol and *cis*-3-hexenol are compounds used in the formulation of synthetic lures attractive to *A. ludens* and *A. oblique*.

In the present study, we developed an 11-component synthetic kairomone for *D. suzukii* based on antennally active compounds of a homogenized raspberry fruit extract. Use of synthetic lures to monitor adult *D. suzukii* flight activity might be more practical (i.e., easy to use) and economical than currently available baits (Burrack et al. 2015). Deploying an attractive lure from a controlled-release dispenser has the advantage of releasing a constant amount of volatiles over a long period of time. For example, apple volatile lures at a release rate of 0.4 ± 0.02 mg/h have been used to monitor the apple maggot fly, *R. pomonella*, for 86 d (Jones 1988). A powerful lure for *D. suzukii* is one that can detect the first fly, and could thus determine when to initiate control measures, and has high selectivity. Recently, Cha et al. (2013) developed a synthetic lure, based on fermentation products, to monitor *D. suzukii*. This synthetic lure captured more *D. suzukii* flies than an apple cider vinegar bait, and was also more selective, i.e., captured fewer non-target flies. Burrack et al. (2015) also found that this lure captures flies earlier than apple cider vinegar. Because *D. suzukii* attacks ripening fruit, it is likely that in addition to volatiles from fermentation products, this pest utilizes fruit volatiles in host location. For example, Toledo et al. (2009) used lures based on host fruit volatiles to monitor *Anastrepha obliqua* (Macquart) in the field and found that these lures caught fewer nontarget insects than the standard hydrolyzed protein bait. Thus, combining volatiles from fruit and fermentation products could improve current monitoring lures in terms of early detection of adult *D. suzukii* in farms and catching of less nontarget flies. It is also likely that fruit-based volatile lures might be more specific to *D. suzukii* and attract them earlier than fermentation-based lures. Our studies so far have been under controlled laboratory conditions. Future studies will test the performance of different release devices and formulations of the identified 11-component lure or an optimized lure from the raspberry fruit extract under field conditions. Additional laboratory studies will determine if the lure can be simplified by eliminating any redundant compound(s).

Moreover, the decrease of kairomone components in mineral oil formulation from polypropylene centrifuge tube under laboratory conditions follows first order kinetics ($Y = 511.37\, e^{-0.0025t}$), and the rate constant (k) is equal to 0.0025. Our bioassay data indicate that the release rate from the current kairomone dispenser is suitable for laboratory experimentation. However, the dispenser formulation and release rate for use under field conditions need to be evaluated because of the higher volatilities of kairomone components expected in the field.

In conclusion, raspberry extracts were more attractive to adult female and male *D. suzukii* than the other tested extracts, and thus their volatiles probably play an important role in the fly's host-seeking process probably for food and oviposition. To our knowledge, this is the first identification of antennally active fruit-based volatiles with behavioral activity for *D. suzukii*. Indeed, when 11 antennally active volatiles from raspberries were formulated into a synthetic lure in a natural ratio, the resultant blend attracted adult *D. suzukii* flies. However, further research including the identification of additional volatiles from raspberry fruit that elicit antennal and behavioral activity to improve the attractiveness of the synthetic blend and dose response experiments to optimize the synthetic blend for field application is needed. After optimization under laboratory and field conditions, this fruit-based lure could be used to improve current monitoring efforts and also to develop behavior-based control strategies such as attract-and-kill for *D. suzukii*, an important and devastating pest of small fruit crops.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

We are grateful to Vera Kyryczenko-Roth for help in rearing the *D. suzukii* colony, and to Robert Holdcraft for assistance with bioassays. We would like to thank Elvira de Lange, Tracy Leskey, Anne Nielsen, and two anonymous reviewers for comments on an early draft of the manuscript. This project was funded by the New Jersey Blueberry Research Council, a New Jersey Specialty Crop Block Grant, a U.S. Department of Agriculture (USDA) Nebraska Integrated Pest Management (IPM) grant (2013-34103-21468) and a Free University of Bozen-Bolzano overseas grant for PhD students.

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Received 15 September 2014; accepted 29 January 2015.