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

JOSEPH R. KLEIBER,[1] C. RIKARD UNELIUS,[2,3] JANA C. LEE,[4,5] DAVID MAXWELL SUCKLING,[2] MICHAEL C. QIAN,[6] AND DENNY J. BRUCK[7]

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ABSTRACT Laboratory screening bioassays and field trapping experiments of spotted wing drosophila flies, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), were conducted to determine the attractiveness of 17 compounds as well as to compare attractant efficiency during peak fruit ripeness and postharvest captures late in the season. Compounds structurally related to each of the fermentation products acetic acid, ethanol, ethyl acetate, and 2-phenethyl alcohol were screened for attractiveness compared with a soap water control in greenhouse cage bioassays. The compounds determined to be attractive in the greenhouse bioassay (methanol, ethanol, propanol, formic acid, acetic acid, ethyl acetate, propyl acetate, phenethyl acetate, phenethyl propionate, and phenethyl butyrate) were individually tested in the field added to apple cider vinegar (ACV). The acids were also tested individually in neutralized ACV (NACV; pH ≈7). Combinations of the compounds were tested in NACV. The capture numbers in ACV traps were not significantly increased by the addition of any of the compounds tested, although significant deterrent effects of some of the compounds allowed differences between treatments to be observed. Compounds that are most prevalent in wine and vinegar (methanol, ethanol, acetic acid, and ethyl acetate) as well as phenethyl propionate and phenethyl butyrate were less deterrent than the other compounds tested in the field. Captures during peak fruit ripeness were compared with the postharvest period when fruit hosts were not available or were overripe. Although the total number of flies captured late in the season was lower, the trends in treatment performance were similar, indicating a consistent performance of these baits from peak fruit ripeness through postharvest.

KEY WORDS apple cider vinegar, bait, monitoring, spotted wing drosophila, trapping

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a widely distributed pest of small and stone fruit production, found in North America, Europe, and Asia (Walsh et al. 2011). Unlike other *Drosophila* species that lay eggs only in overripe or rotting fruit, female *D. suzukii* have a characteristic serrated ovipositor that allows them to lay eggs in ripe and ripening fruit (Lee et al. 2011). The damage caused by the oviposition scar and larvae that hatch from the eggs results in unmarketable fruit and crop loss, in some cases up to 80% (Walsh et al. 2011). The production of strawberries, blueberries, caneberries, and cherries in the western United States is threatened by the presence of *D. suzukii*, and potential losses are significant (Bolda et al. 2010). A number of treatment programs for *D. suzukii* exist and are economically sound because the loss from yield reduction far outweighs the cost of control. Goodhue et al. (2011) performed an economic analysis weighing the costs and benefits of controlling *D. suzukii* in California's raspberry and strawberry crops. Control cost per treatment in raspberries ranges from US$9.65 per acre for zeta-cypermethrin (Mustang EW) to US$81.34 per acre for the organic insecticide spinosad (Entrust, Dow AgroSciences, Indianapolis, IN).

A common grower practice for *D. suzukii* control in Oregon is to apply an insecticide spray when *D. suzukii* are detected in monitoring traps and the fruit has begun to ripen, which is when the fruit becomes susceptible (Lee et al. 2011). Once *D. suzukii* are present and the fruit is susceptible, the fruit should be protected with follow-up sprays based on monitoring data and the residual effectiveness of the chosen insecticide (Haviland and Beers 2012). Although monitoring alone does not reduce pest pressure, it is the first step in an integrated pest management system and important to integrate with a host of other management practices (Cini et al. 2012) to keep *D. suzukii* numbers low. An economic threshold level of infestation needs

[1] Department of Horticulture, Oregon State University, 4017 ALS, Corvallis, OR 97331.
[2] The New Zealand Institute for Plant & Food Research Ltd., PB 4704, Christchurch, 8140, New Zealand.
[3] Department of Chemistry and Biomedical Sciences, Linnaeus University, Smalandsgatan 24, Kalmar, Sweden SE-391-82.
[4] Horticulture Crops Research Unit, USDA–ARS, 3420 Orchard Ave, Corvallis, OR 97331.
[5] Corresponding author, e-mail: jana.lee@ars.usda.gov.
[6] Food Science and Technology, Oregon State University, 100 Weigand Hall, Corvallis, OR 97331.
[7] DuPont Pioneer, 7300 NW 62nd Ave., Johnston, IA 50131.

to be established to use monitoring data (Stern 1973), which may be the mere presence of *D. suzukii* based on the control costs vs. loss of yield costs. Because there are no monitoring tools that can definitively reveal the presence of *D. suzukii* in a field, attractants need to be improved to move away from prophylactic treatment toward an integrated management of *D. suzukii.* Therefore, the effectiveness of the attractant is most important during the time of fruit ripening and peak fruit ripeness to monitor the populations of *D. suzukii* in the field and confidently make management decisions.

Drosophila have associations with yeast (Gilbert 1980, Palanca et al. 2013), and *Drosophila melanogaster* Meigen are attracted to fermentation products, more so than to fruit volatiles alone (Zhu et al. 2003, Becher et al. 2012). The association between *Drosophila* and yeast or fermented products illuminates the potential importance of fermentation products in the attraction of *D. suzukii.* Fermented products are already known to play an important role in the monitoring of *D. suzukii*, with recommended attractants including wine, vinegar, and fermenting yeast baits (Walsh et al. 2011). An association between *D. suzukii* and the yeast *Hanseniaspora uvarum* was discovered, stressing the link between *D. suzukii* and fermentation products (Hamby et al. 2012). Recent work has tested combinations of wine, vinegar, acetic acid, and ethanol (Landolt et al. 2011) and different combinations of wines and vinegars (Landolt et al. 2012), with data suggesting a rice vinegar and a merlot wine are more coattractive than other tested combinations. Physiologically active compounds in wine and vinegar, as determined by gas chromatography coupled with electroantennographic detection (GC–EAD), have been combined and shown to be as attractive to *D. suzukii* as a wine and vinegar blend (Cha et al. 2012). Acetic acid, ethanol, acetoin, and methionol were recently recommended for a four-component bait (Cha et al. 2014).

This work presented here focused on four groups of fermentation products to determine attractiveness to *D. suzukii.* Acetic acid, ethanol, ethyl acetate, and 2-phenylethanol have already been identified in both wine and vinegar (Ough and Amerine 1988, Guerrero et al. 2006) and make up part of a *D. melanogaster* lure, with ethyl acetate and 2-phenylethyl acetate being optional (Baker et al. 2003). Acetic acid and ethanol have recently been shown to be important in attracting *D. suzukii* (Landolt et al. 2011, 2012), and acetic acid, ethanol and 2-phenylethanol are included in a mix of compounds that attracted *D. suzukii* similar to a mix of wine and vinegar (Cha et al. 2012). Based on these reports, we decided to evaluate structurally related analogs to already identified attractants in search for compounds with an enhanced attractivity. With this aim, a series of compounds with varying carbon chain lengths in the classes of alcohols (methanol to pentanol), carboxylic acids (methanoic to pentanoic), and acetates (ethyl to pentyl) as well as three esters of 2-phenylethanol (acetate to butyrate) were tested for their attractiveness to *D. suzukii.*

Compounds were first screened in greenhouse cage bioassays to identify any compound within each series that was attractive to *D. suzukii* under greenhouse conditions. The field trapping experiments used attractive compounds from the greenhouse cage bioassays and evaluated the attractiveness of these compounds with apple cider vinegar (ACV) or neutralized ACV (NACV) in an environment and time where the traps will actually be used. Further field tests also determined the attractiveness of combinations of the compounds that elicited the best response when tested alone in the field. NACV was used when trapping with the acids and combination treatments to decrease the acetic acid odor profile of the ACV, and to allow the acid being tested to be the more dominant acidic odor.

Another component of trapping explored by this work is the seasonal variation in attractiveness of baits, which has not been addressed previously. The presence and composition of *D. suzukii* hosts change throughout the year in the field, progressing from no fruit in the early season to ripening fruit, ripe fruit, and overripe fruit, to no fruit available in the late season. Insects have also been shown to change seasonally, with changes occurring numerically (Escudero-Colomar et al. 2008), physiologically (Robb and Forbes 2005), and with regard to sexual selection (Vélez and Brockmann 2006). Numerically, more *D. suzukii* were captured in wine and vinegar traps in February than in April (Cha et al. 2012). We aimed to determine whether attractiveness of baits to *D. suzukii* varied seasonally because this knowledge would enable trapping experiments to determine a more precise scope of inference. Further, such results would help to determine the effect of season on bait effectiveness. One experiment from each class of compounds was repeated in the same field as summer trapping after harvest season of the crops. The fruit was either harvested or overripe, and decaying fruit was present in the field. Trapping allowed for the comparison between seasons of the number of flies captured by treatments as well as if compounds performed differently between the two seasons.

Materials and Methods

Insects. A laboratory colony of *D. suzukii* was started and maintained as described by Bruck et al. (2011). The colony was started with adults emerging from infested fruits collected from grower fields in Oregon and Washington in 2009 and 2010. The colony was propagated by exposing *Drosophila* cornmeal diet (San Diego Drosophila Stock Center, San Diego, CA) in petri dishes (VWR International, Radnor, PA) to the colony and rearing out the resulting eggs to adult flies to join the colony. On multiple occasions each year, wild *D. suzukii* are added to the colony to decrease the likelihood of inbreeding and increase the genetic similarity of the colony population to the wild population.

Greenhouse Bioassays. Compounds were screened in the greenhouse using a two-choice cage assay. All

bioassays were conducted in 0.6- by 0.6- by 0.6-m mesh cages inside a greenhouse with photoperiod of 16:8 (L:D) h and temperature between 13 and 24°C. Within each cage were two clear cup traps, treatment and control, positioned near opposite corners on the cage bottom (≈20 cm from the corner and ≈45 cm from each other). The clear cup trap was a 946-ml clear plastic cup (32 oz Clear Plastic Beverage Cup, Solo Cup Company, Lake Forest, IL) with a clear plastic lid (Lid with Straw Slot, Solo Cup Company, Lake Forest, IL) and 15, 4.8-mm holes punched around the top perimeter of the cup. All control traps contained 100 ml of the soap water drowning solution made by adding 4 ml of dish soap (Dawn Ultra, Proctor & Gamble, Cincinnati, OH) to 3.78 liters of water. Treatment traps consisted of one of the 17 volatiles (see below for rates) pipetted onto a cotton roll placed into individual small glass vials (8 ml, Wheaton, Millville, NJ) that were set in the center of the cup surrounded by soap water. Each trap was tested with only one volatile type. Moist cotton in a petri dish and two small agar-based diet cups were placed in the middle of the cage to ensure survivorship of flies. Adult flies used in greenhouse experiments were between 5 and 12 d old. Approximately 200 *D. suzukii* of mixed sex were put in each of the cages with traps for 24 h, and the number of flies in each trap was enumerated. The difference in catch between treatment and control traps was compared with total number of flies captured in both traps using an Attractivity Index (AI): 100 × (number of flies in treatment trap − number of flies in control trap) divided by total number of flies trapped. A potential attractant has a positive value and a potential deterrent has a negative value. Treatments with a positive average AI were subsequently used in field experiments. The AI used here is a modified version of the antifeedant index used in feeding bioassays of the *Hylobius abietis* (L.) (Unelius et al. 2006).

Selection of Test Compound Concentrations. These bioassays were conducted to aid in the selection of test compound concentrations. Ethyl acetate (8.8, 88, and 880 μl) and phenethyl acetate (5.5, 55, and 550 μl) were dispensed onto a cotton roll in a glass vial in the center of 100 ml of soap water in a clear cup trap, and each treatment was compared with a soap water control. Volumes used correspond to 10, 100, and 1,000 ppm of ethyl and phenethyl acetate in water, which encompasses typical occurrence of ethyl acetate and phenethyl alcohol in wine (Nykänen and Suomalainen 1983). Between two and six replicates of each two-choice test were performed. The concentration that gave the highest AI was selected as the test concentration.

Greenhouse Bioassay 1. The objective of this experiment was to determine the attractiveness of several short-chain alcohols to *D. suzukii.* The odors of methanol, ethanol, propanol, butanol, and pentanol were each compared with a water control in this series of two-choice assays. The treatment trap contained one of the five alcohols (7.2 ml, neat) in the center vial surrounded by 93 ml of soap water. A volume of 7.2 ml of each alcohol was chosen because of its use and attractiveness in previous trapping studies (Landolt et al. 2011, 2012). These two-choice tests and all subsequent choice tests were replicated seven times.

Greenhouse Bioassay 2. The objective was to determine the attractiveness of short-chain acids to the *D. suzukii.* Formic acid, acetic acid, propionic acid, butyric acid, and valeric acid were each compared with a water control in these bioassays. The treatment trap contained one of the five acids (2 ml, neat) in the center vial surrounded by 98 ml of soap water. A volume of 2 ml of each acid was chosen because of its use and attractiveness in previous trapping studies (Landolt et al. 2011, 2012).

Greenhouse Bioassay 3. This experiment tested the attractiveness of three phenethyl esters to the *D. suzukii.* The compounds were presented at rates of 880 μl of phenethyl acetate, 980 μl of phenethyl propionate, and 1,070 μl of phenethyl butyrate, corresponding to 1,000 ppm of each compound. The treatment trap contained one of the compounds (neat) in the center vial surrounded by 100 ml of soap water.

Greenhouse Bioassay 4. This experiment tested the attractiveness of four low molecular weight acetates to the *D. suzukii.* The compounds were presented at rates of 5.5 μl of ethyl acetate, 6.4 μl of propyl acetate, 7.3 μl of butyl acetate, and 8.3 μl of pentyl acetate, corresponding to a concentration of 10 ppm of each compound. The treatment trap contained one of the listed compounds (neat) in the center vial surrounded by 100 ml of the soap water.

Field Experiments. Field tests were performed in cultivated small fruit fields in Benton County, OR. The same type of clear cup trap used in the greenhouse bioassays was used in these field tests. The drowning solution was made by adding 4 ml of dish soap to 3.78 liters of 5% acidity ACV (Fred Meyer Apple Cider Vinegar, Inter-American Products Inc., Cincinnati, OH) or ACV neutralized to ≈pH 7 with sodium hydroxide. The ACV was neutralized to decrease its acetic acid profile and to allow the acid being tested to be the dominant acidic odor. The odor tested in each treatment (see below for compounds and rates) was pipetted onto a cotton roll in an appropriately sized vial with a 10-mm opening suspended by wire into the drowning solution of the trap. The vials were used to keep the presentation of the odors consistent in the greenhouse and field trials and to prevent side reactions that might occur if the compounds were added directly to ACV. Ethyl acetate content in vinegar is influenced by the amount of ethanol in the vinegar (Tesfaye et al. 2004), so an increase of ethanol to an ACV trap would be associated with an increase of ethyl acetate. Each trap was tested with only one volatile or combination treatment. Traps were placed at least 10 m apart in four replicated linear rows (blocks). The rows were separated by at least 20 m. Traps were hung in the canopies of each crop to achieve similar exposure at all trap locations to maximize consistency and *D. suzukii* capture. In all five experiments, traps were placed in the field for 5 d, and the number of *D. suzukii* captured was determined. After each 5-d period, no attractants were placed in

Table 1. Combinations of attractants dispensed onto cotton rolls in separate 10-mm opening vials suspended in a NACV trap placed in the field between 3 September 2012 and 5 October 2012

Compound	Vial type	Combo 1	Combo 2	Combo 3	Combo 4
Acetic acid	2-ml PP	2 ml	2 ml	2 ml	2 ml
Ethyl acetate	1.5-ml PP	5.5 μl	5.5 μl	5.5 μl	5.5 μl
Methanol	8-ml glass	7.2 ml	–	7.2 ml	–
Ethanol	8-ml glass	–	7.2 ml	–	7.2 ml
Phenethyl propionate	1.5-ml PP	985 μl	985 μl	–	–
Phenethyl butyrate	1.5-ml PP	–	–	1,074 μl	1,074 μl

PP, polypropylene.

the field for 2 d, allowing odors from the previous week to dissipate and not influence the next trapping week when odors were placed in a new position.

Field Experiment 1. This experiment was based on results from greenhouse bioassay 1 to test the effect of adding alcohol odors to an ACV drowning solution. Four weeks of trapping was conducted in cherries from 2 June 2012 to 27 June 2012 and in blackberries from 16 July 2012 to 10 August 2012. An ACV standard and three alcohols were selected for testing in the field based on the results of greenhouse bioassay 1; methanol, ethanol, and propanol were the odors with positive AI in the greenhouse. The alcohol to be tested (7.2 ml, neat) was dispensed into a small glass vial with a cotton roll inside. The vial was then hung by a wire into 93 ml of ACV drowning solution. Sixteen traps (four per treatment) were placed in the field in a Latin square design with four rows and four distances (column) from the field edge. During the 5-d trapping period, attractants were renewed daily because of the high evaporation rate of alcohols. After each 2-d nontesting period, the traps were placed into a new Latin square design; each treatment was present at each row × column position once during the 4 wks.

Field Experiment 2. This experiment was based on results from greenhouse bioassay 2 to test the effect of adding acid odors to the drowning solution of NACV. Four weeks of trapping was conducted in cherries from 9 July 2012 to 3 August 2012 and in blackberries from 30 July 2012 to 24 August 2012. The drowning solution was ACV neutralized to a pH between 6 and 8 by the addition of sodium hydroxide pellets (>98%, CAS no. 1310-73-2). Dish soap (4 ml) was added to 3.78 liters of NACV. Formic acid, acetic acid, and valeric acid (2 ml, neat) were presented in the traps by dispensing each onto a cotton roll in a plastic vial (2 ml, Corning Inc., Corning, NY), which was suspended by a wire into 98 ml of NACV drowning solution. The attractants were renewed every other day. In cherries, the three treatments and an ACV standard were arranged in a new Latin square design each week. An NACV standard was added to the trial in blackberries, and the five treatments were set up in a randomized complete block design (RCBD) with four blocks (20 traps total) and rerandomized weekly.

Field Experiment 3. The treatments in this experiment were based on results from greenhouse bioassay 3 to test the effect of phenylethyl esters. Four weeks of trapping was conducted in raspberries from 16 July 2012 to 10 August 2012 and in cherries from 30 July 2012 to 24 August 2012. An ACV standard was used in this experiment, and treatment traps contained 100 ml of the ACV drowning solution with a microcentrifuge tube (1.5 ml, Brand Tech Scientific, Inc., Essex, CT) with cotton and the treatment compound suspended by a wire above the drowning solution. The compounds were presented at the test rate of 1,000 ppm in water (880 μl of phenethyl acetate, 980 μl of phenethyl propionate, and 1,070 μl of phenethyl butyrate), and renewed each week. The traps were placed in the field in a new Latin square design each week.

Field Experiment 4. The objective of this experiment was to determine the attractiveness of an ACV drowning solution containing the attractive acetates tested in greenhouse bioassay 4. Trapping was conducted in raspberries for 5 wk from 9 July 2012 to 10 August 2012 and for 4 wk in cherries from 6 August 2012 to 31 August 2012. An ACV standard was used in this experiment, and the treatment traps contained 100 ml of the ACV drowning solution with a microcentrifuge tube with cotton and the attractant suspended by wire above the drowning solution. The compounds were presented at rates of 5.5 μl of ethyl acetate and 6.4 μl of propyl acetate, and renewed daily. The traps were set up in an RCBD with four blocks in the field and rerandomized weekly.

Field Experiment 5. The objective of this experiment was to determine if combinations of compounds from field experiments 1–4 would act in synergy and elicit a higher response by *D. suzukii* than individual compounds. Four weeks of trapping was conducted in blackberries and blueberries from 3 September 2012 to 5 October 2012. The compounds used in this experiment were acetic acid, ethyl acetate, methanol, ethanol, phenethyl propionate, and phenethyl butyrate. Four vials, each containing one compound from a different field experiment, were suspended by a wire above 91 ml of NACV drowning solution. Alcohols and acetates (ethyl and propyl) were renewed daily, acids every other day, and phenethyl esters every week. A trap with 100 ml of NACV drowning solution was used as the control. A summary of the attractant combinations tested is found in Table 1. The traps were set up in an RCBD with four blocks in the field and rerandomized weekly.

Comparison of Trapping Season. This experiment was performed to compare the catch data of the same treatments during peak fruit ripeness and postharvest. From 8 October 2012 to 2 November 2012, field experiments 1–4 were repeated in one of the same fields

in which the first experiment was performed (June–August 2012). Field experiment 1 was repeated in the blackberry field, field experiment 2 in the cherry orchard, field experiment 3 in the cherry orchard, and field experiment 4 in the raspberry field.

Statistical Analysis. For all laboratory experiments, the AI was tested for being >0 with a one-sided *t*-test. All statistics were tested in JMP 7.0.1 (SAS Institute, Cary, NC). For field trapping experiments, male and female counts were combined because the trends were similar and the 5-d total catch numbers were transformed ($\mathrm{Log}_{10}(x+1)$) to homogenize variances. The data from each experiment were analyzed separately per crop type. For Latin square designs, treatment, date collected, and treatment × date were fixed terms, and row and position were random terms. For RCBD, treatment, date collected, and treatment × date were fixed terms, and block was a random term. All means were compared using the Tukey–Kramer test. For comparison of trapping season, the data from each repeated experiment were analyzed separately using treatment, season, treatment × season interaction as fixed terms, and block as a random term. The date collected was specific to each season and not included as a term. A significant treatment × season interaction would be evidence for different trends between in-season and late-season trapping.

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

Results

Greenhouse Bioassays. Selection of Test Compound Concentrations.

Ethyl acetate dispensed at 5.5 μl was selected as the concentration to be used, with an AI of 36 (Table 2). Dispensed at 55 and 550 μl, ethyl acetate was less attractive with AIs of seven and eight, respectively. Phenethyl acetate dispensed at a rate of 880 μl was selected to be the test concentration because of an AI of 53. Dispensed at 8.8 and 88 μl, phenethyl acetate was less attractive with AIs of 20 and 39, respectively.

Greenhouse Bioassay 1. Treatment traps baited with methanol, ethanol, and propanol caught more mean adult *D. suzukii* in the cage bioassays than the water control traps (positive AI value, significantly >0, Table 2). Traps baited with butanol and pentanol caught fewer flies than the control traps in the bioassays (negative AI value).

Greenhouse Bioassay 2. Treatment traps baited with formic acid and acetic acid caught more adult *D. suzukii* than the control traps (Table 2). Traps baited with valeric acid did not catch significantly more *D. suzukii* than the control ($P = 0.06$), but this chemical was still included in field trials. Traps baited with butyric acid and propionic acid caught fewer flies than the controls.

Greenhouse Bioassay 3. All treatments caught more adult *D. suzukii* than the control traps (Table 2).

Greenhouse Bioassay 4. Treatment traps baited with ethyl acetate caught more adult *D. suzukii* than the control traps (Table 2). Traps baited with propyl acetate did not catch significantly more flies than the controls ($P = 0.15$), but this chemical was still included in field trials. Traps baited with butyl acetate and pentyl acetate caught fewer flies than the controls.

Field Experiments. *Field Experiment 1.* The numbers of male and female flies caught in the field showed similar trends within each experiment and were pooled for analysis (Fig. 1a). The total number of *D. suzukii* captured in traps baited with different alcohols was significantly different in both cropping systems (treatment $F_{3, 24} = 11.2$, $P < 0.001$ in cherries; $F_{3, 24} = 51.5$, $P < 0.001$ in blackberries; *F*-values for date and treatment × date not reported for any field experiments). In both crops, the treatment traps with propanol captured significantly fewer adults than the ACV standard. Traps containing methanol and ethanol captured a similar number of total flies as ACV in both crops.

Field Experiment 2. Numbers of flies caught in the traps containing different acid treatments were significantly different in both cropping systems (Fig. 1b; $F_{3, 24} = 27.9$, $P < 0.001$ in cherries; $F_{4, 57} = 7.5$, $P < 0.001$ in blackberries). In cherries, all treatments caught significantly fewer adult *D. suzukii* than the ACV standard. In blackberries, traps containing acetic acid and the NACV captured similar amounts of flies as ACV. The formic acid and valeric acid treatments captured significantly fewer flies than ACV, but a similar amount of flies as the traps containing NACV.

Field Experiment 3. The total numbers of flies captured in traps differed significantly between phenethyl esters when placed in both cherry orchards (Fig. 1c; $F_{3, 24} = 3.0$, $P = 0.05$) and raspberry fields ($F_{3, 24} = 4.7$, $P = 0.010$). In raspberries, traps baited with phenethyl propionate captured a similar number of flies as ACV. Traps baited with phenethyl acetate and phenethyl butyrate captured fewer flies than ACV. When placed in the cherry orchard, the traps baited with phenethyl butyrate captured a similar number of flies as ACV. Phenethyl acetate and phenethyl propionate captured fewer flies than ACV.

Field Experiment 4. The total number of flies captured in the traps baited with various acetates did not differ significantly in raspberries (Fig. 1d; $F_{2, 42} = 0.91$, $P = 0.410$). However, in the cherry orchard, the total

Table 2. Mean (+SE) of male and female *D. suzukii* captured in greenhouse bioassays, with (AI)

Test, compound/volume	Treatment[a]		Control[a]		AI*	SE
	Catch	SE	Catch	SE		
Selection of test compound concentration 1						
Phenethyl acetate						
8.8 μl	20.8	8.6	13.8	3.2	−3.0	20.8
88 μl	26.2	3.9	11.5	3.7	41.1	13.1
880 μl	31.2	6.0	9.7	3.7	52.9	11.3
Selection of test compound concentration 2						
Ethyl acetate						
5.5 μl	61.0	12.0	28.5	12	36.2	26.1
55 μl	27.0	0	23.5	0.5	6.9	1.1
550 μl	15.3	5.2	13.0	2.9	3.3	30.4
Greenhouse bioassay 1						
Alcohols						
Methanol	172.8	15.2	15.0	5.8	84.9*	4.6
Ethanol	167.5	38.5	15.8	4.1	82.8*	2.9
Propanol	43.8	7.7	13.2	4.2	55.9*	11.1
Butanol	4.8	1.7	19.2	4.6	−59.4	9.5
Pentanol	1.0	0.4	11.8	3.3	−83.8	5.8
Greenhouse bioassay 2						
Acids						
Formic acid	30.7	4.6	14.2	3.6	41.5*	11.4
Acetic acid	64.0	10.5	28.9	4.4	33.4*	11.8
Propionic acid	12.4	2.8	21.3	2.4	−32.3	12.4
Butyric acid	20.8	3.1	32.1	3.8	−22.7	10.8
Valeric acid	51.0	12.3	24.7	5.3	25.9[b]	13.9
Greenhouse bioassay 3						
Phenethyl esters						
Phenethyl acetate	34.6	2.8	14.6	3.2	42.4*	9.4
Phenethyl propionate	46.9	9.2	20.3	3.7	33.7*	10.1
Phenethyl butyrate	28.9	5.7	11.6	3.5	43.8*	13.8
Greenhouse bioassay 4						
Acetates						
Ethyl acetate	32.1	3.4	22.9	4.5	20.3*	8.1
Propyl acetate	29.6	5.0	22.0	3.8	13.4[c]	11.8
Butyl acetate	9.9	0.6	15.4	5.6	−6.6	12.2
Pentyl acetate	4.9	1.4	10.9	3.7	−27.2	11.1

[a] Treatment = chemical in vial + surrounding soapy water; Control = soapy water; n = 7.
[b] $P = 0.0604$.
[c] $P = 0.150$.
* Asterisk indicates that value is significantly >0 by *t*-test.
AI = 100 × (treatment captures − control captures)/(treatment captures + control captures).

number of flies captured differed significantly between these treatments ($F_{2, 33} = 4.0$, $P = 0.027$). Both ethyl acetate and propyl acetate captured similar numbers of flies as ACV. Traps baited with ethyl acetate captured significantly more flies than traps baited with propyl acetate.

Field Experiment 5. The total numbers of flies captured in traps baited with several combinations of attractants differed significantly in both blackberries (Fig. 2; $F_{5, 68} = 5.4$, $P < 0.001$) and blueberries ($F_{5, 69} = 8.3$, $P < 0.001$). In blackberries, traps baited with combination 4 as well as NACV captured as many flies as the ACV standard. Traps baited with combinations 1, 2, and 3 captured significantly fewer flies than ACV. All the combination treatments captured statistically similar numbers of flies as NACV. In the blueberry field, only the NACV and traps baited with combination 2 captured as many flies as the ACV control.

Comparison of Trapping Season. The total number of flies captured during postharvest trapping was significantly lower than the number caught during peak ripeness at all four sites as indicated by a significant season effect (Table 3). However, the treatments showed similar trends during the harvest and postharvest seasons, as indicated by a nonsignificant treatment × season interaction (Table 3).

Discussion

None of the compounds tested in the field trapping experiments increased the attractiveness of the ACV traps when presented individually or in combination. Some of the compounds had a deterrent effect that allowed us to discern differences in the performance of compounds. Testing the series for acids and acetates revealed that compounds other than the known fly attractants (acetic acid and ethyl acetate) had a deterrent effect when added to an ACV trap.

No combination of odors presented with NACV captured significantly more flies than the ACV or NACV traps. The tested combinations are not additive or synergistic when the vials of the attractants are combined in a single trap, and may be redundant or not behaviorally active. Odors from each field experiment were combined to test if there would be any additive effect of the combination, but results re-

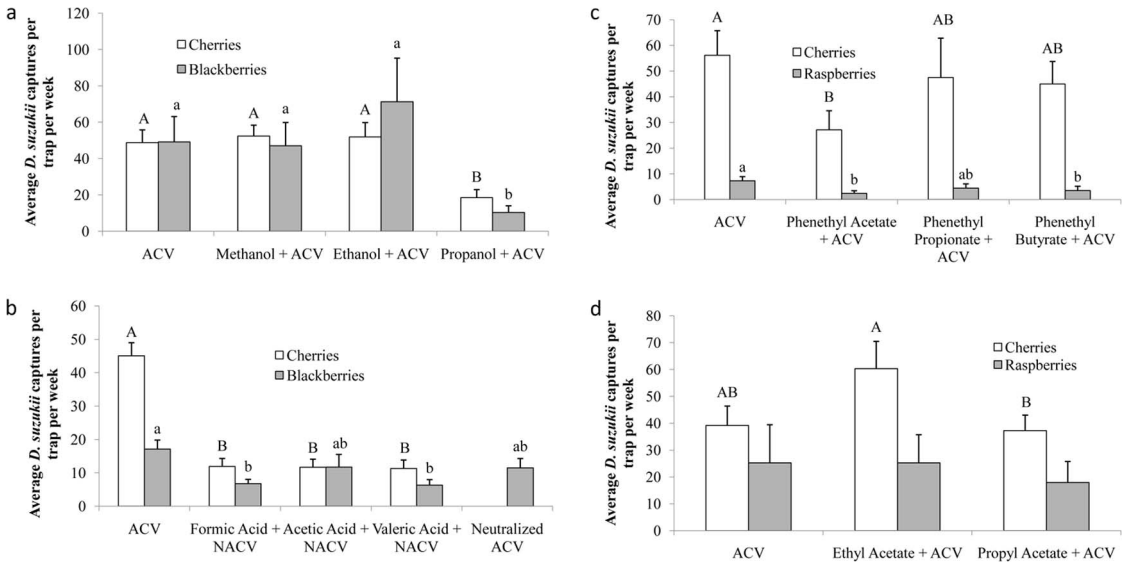

Fig. 1. Mean (+SE) number of *D. suzukii* caught in traps of (a) field experiment 1, (b) field experiment 2, (c) field experiment 3, and (d) field experiment 4. Treatments in the same crop with different letters captured a significantly different number by a Tukey–Kramer post hoc analysis at the 0.05 level.

vealed that there was no more attraction to the traps baited with a combination of vials when compared with the ACV control than to any of the traps baited with individual compound vials. When trapping with the combinations was performed in blueberries, three of the four treatments actually captured significantly fewer flies than the NACV trap, indicating a deterrent effect. This makes the transition from individual compound testing to combination testing complicated because of complex interactions in response to attractants when presented in varying crops.

In the greenhouse cage assays preceding the field trials, the alcohols were presented at the same volume per volume concentration of 7.2% yielding different molecular concentrations because of the different weights of the compounds. There is also a decrease in volatility of the compounds as the molecular weights increase, leading to different concentrations of the odors sensed by the insects in the trials. Dependence of biological activity on the concentration of the odors presented is shown by the optimal concentration determination experiments run in this study. If the concentration of the attractant is different than the optimal concentration, it could elicit a different response. The attractiveness to *D. suzukii*, decreased with increased chain length of the acetates and alcohols (Table 2). Biological activity of structural analogs has also been shown to change with chain length in *Milichiella lacteipennis* Loew (Diptera: Milichiidae) (Dorner and Mulla 1963) and *Vespula vulgaris* (L.) (Hymenoptera: Vespidae) (El-Sayed et al. 2009). The differences in attractiveness between the tested analogs could result from differences in release rate as well as attraction. The alcohols and acetates are also the most volatile test compounds, followed by the acids and then the phenethyl esters. The attractiveness to *D. suzukii* of the acids was not correlated with the chain length; the carboxylic acids with 1, 2, and 5 carbons were attractive, while the acids with 3 and 4 carbons were not. The prevalence of isovaleric acid,

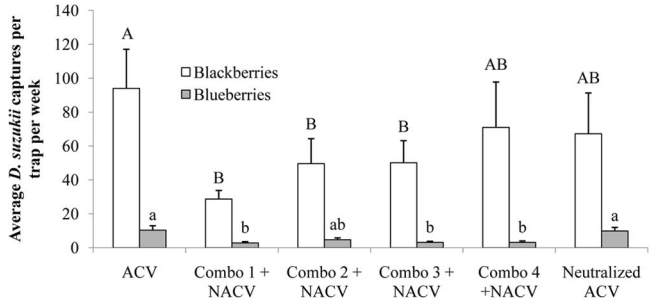

Fig. 2. Mean (+SE) number of *D. suzukii* caught in field experiment five traps placed in a blackberry and blueberry field. Treatments in the same crop with different letters captured a significantly different number by a Tukey–Kramer post hoc analysis at the 0.05 level.

Table 3. Statistical analyses of average captures of *D. suzukii* between the ripening and postharvest season

Field experiment	Source of variation	df	*F*	*P*
1—Alcohols in blackberries	Treatment	3, 117	16.22	<0.001
	Season	1, 117	61.01	<0.001
	Treatment × season	3, 117	0.54	0.659
2—Acids in cherries	Treatment	3, 117	13.04	<0.001
	Season	1, 117	7.22	0.008
	Treatment × season	3, 117	1.38	0.251
3—Phenethyl esters in cherries	Treatment	3, 117	3.20	0.026
	Season	1, 117	27.47	<0.001
	Treatment × season	3, 117	0.54	0.653
4—Acetates in raspberries	Treatment	3, 117	0.04	0.956
	Season	1, 117	25.54	<0.001
	Treatment × season	3, 117	0.39	0.676

but not propionic or butyric acids, in vinegar (Yang and Choong 2001) may be an explanation of this.

The amount of lure dispensed into the vials used in individual and combination experiments in both the greenhouses and field experiments may not produce the most attractive concentration of each compound in the trap because all the compounds in each class were tested at the same rate. The acids were tested at a rate of 2%, the concentration of acetic acid in a mixture of wine and vinegar used by others (Landolt et al. 2011, 2012). In wines and vinegars, there are much lower levels of other acids than of acetic acid (Nykänen and Suomalainen 1983, Yang and Choong 2001). The use of the other acids at elevated concentrations could have resulted in a deterrent effect, similar to what had been observed when *D. melanogaster* were exposed to increasing concentrations of acetic acid in baits (Reed 1938). The compound most abundant in wine and vinegar from each class was selected as the benchmark for concentration testing, leading to the possibility that most of the remaining compounds were presented at a concentration too high for attraction.

In the trapping season experiment, the season by treatment interaction was not significant, indicating a consistent relative performance of the tested compounds when added to ACV traps. The effect of these compounds on *D. suzukii* did not change between these two seasons, so trap effectiveness can be interpolated between the two seasons: traps that are attractive at the season at the beginning of ripeness should remain effective through the postharvest period.

Vinegar is a commonly used attractant for *D. suzukii* and the standard to which attractants in these experiments were compared. Although ethyl acetate dispensed from a vial in an ACV trap and a combination of vials of acetic acid, ethanol, ethyl acetate, and phenethyl butyrate in a NACV trap captured numerically more flies than ACV and NACV respectively, no treatment in this experiment statistically increased the attraction of ACV or NACV. Therefore, more work is needed to determine compounds to enhance *D. suzukii* captures, possibly incorporating results of Cha et al. (2012, 2014) with volatiles from wine and vinegar or other sources of attractants.

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References Cited

Baker, T. C., J. Zhu, and K.-C. Park. 2003 Fruit fly attractant compositions. U.S. Patent 6,543,181. Iowa State University Research Foundation, Inc., Ames, IA.

Becher, P. G., G. Flick, E. Rozpêdowska, A. Schmidt, A. Hagman, S. Lebreton, M. C. Larsson, B. S. Hansson, J. Piškur, P. Witzgall, et al. 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. Resour. Econ. Update 13: 5–8.

Bruck, D. J., M. Bolda, L. Tanigoshi, J. Klick, J. Kleiber, J. DeFrancesco, B. Gerdeman, and H. Spitler. 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manag. Sci. 67: 1375–1385.

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.

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.

Dorner, R. W., and M. S. Mulla. 1963. Lower aliphatic amines as chemical attractants for *Milichiella lacteipennis* (Diptera: Milichiidae). Ann. Entomol. Soc. Am. 56: 1–2.

El-Sayed, A. M., L. A. Manning, C. R. Unelius, K. C. Park, L. D. Stringer, N. White, B. Bunn, A. Twidle, and D. M. Suckling. 2009. Attraction and antennal response of the common wasp, *Vespula vulgaris* (L.), to selected synthetic chemicals in New Zealand beech forests. Pest Manag. Sci. 65: 975–981.

Escudero-Colomar, L. A., M. Vilajeliu, and L. Batllori. 2008. Seasonality in the occurrence of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] in the north-east of Spain. J. Appl. Entomol. 132: 714–721.

Gilbert, D. G. 1980. Dispersal of yeasts and bacteria by *Drosophila* in a temperate forest. Oecologia 46: 135–137.

Goodhue, R., M. Bolda, D. Farnsworth, J. Williams, and F. Zalom. 2011. Spotted wing Drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manag. Sci. 67: 1396–1402.

Guerrero, E. D., R. N. Marín, R. C. Mejías, and C. G. Barroso. 2006. Optimisation of stir bar sorptive extraction applied to the determination of volatile compounds in vinegars. J. Chromatogr. A 1104: 47–53.

Hamby, K. A., A. Hernandez, 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.

Haviland, D. R., and E. H. Beers. 2012. Chemical control programs for *Drosophila suzukii* that comply with international limitations on pesticide residues for exported sweet cherries. J. Integr. Pest Manage. 3: 1–6.

Landolt, P., T. Adams, and H. Rogg. 2011. 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.

Landolt, P., T. Adams, T. Davis, and H. Rogg. 2012. Spotted wing Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), trapped with combinations of wines and vinegars. Fla. Entomol. 95: 326.

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

Nykänen, L., and H. Suomalainen. 1983. Aroma of beer, wine, and distilled alcoholic beverages. D. Reidel Publishing Company, Dordrecht, Germany.

Ough, C. S., and M. A. Amerine. 1988. Methods for analysis of musts and wines, 2nd ed. Wiley-Interscience, New York, NY.

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. (doi:10.1371/journal.pone.0075332).

Reed, M. R. 1938. The olfactory reactions of *Drosophila melanogaster* Meigen to the products of fermenting banana. Physiol. Zool. 11: 317–325.

Robb, T., and M. R. Forbes. 2005. On understanding seasonal increases in damselfly defence and resistance against ectoparasitic mites. Ecol. Entomol. 30: 334–341.

Stern, V. M. 1973. Economic thresholds. Annu. Rev. Entomol. 18: 259–280.

Tesfaye, W., M. Morales, B. Benítez, M. García-Parrilla, and A. Troncoso. 2004. Evolution of wine vinegar composition during accelerated aging with oak chips. Anal. Chim. Acta 513: 239–245.

Unelius, C. R., G. Nordlander, H. Nordenhem, C. Hellqvist, S. Legrand, and A.-K. Borg-Karlson. 2006. Structure-activity relationships of benzoic acid derivatives as antifeedants for the pine weevil, *Hylobius abietis.* J. Chem. Ecol. 32: 2191–2203.

Vélez, M. J., and H. J. Brockmann. 2006. Seasonal variation in female response to male calling song in the field cricket, *Gryllus rubens.* Ethology 112: 1041–1049.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. 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. 2: G1–G7.

Williamson, K. L., and D. M. Masters. 1999. Macroscale and microscale organic experiments, 3rd ed. Houghton Mifflin, Boston, MA.

Yang, M. H., and Y. M. Choong. 2001. A rapid gas chromatographic method for direct determination of short-chain (C2–C12) volatile organic acids in foods. Food Chem. 75: 101–108.

Zhu, J., K. C. Park, and T. C. Baker. 2003. Identification of odors from overripe mango that attract vinegar flies, *Drosophila melanogaster.* J. Chem. Ecol. 29: 899–909.

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