Sucrose Improves Insecticide Activity Against *Drosophila suzukii* (Diptera: Drosophilidae)

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ABSTRACT The addition of sucrose to insecticides targeting spotted wing drosophila, *Drosophila suzukii* (Matsumura), enhanced lethality in laboratory, semifield, and field tests. In the laboratory, 0.1% sucrose added to a spray solution enhanced spotted wing drosophila feeding. Flies died 120 min earlier when exposed to spinosad residues at label rates enhanced with sucrose. Added sucrose reduced the LC_{50} for dried acetamiprid residues from 82 to 41 ppm in the spray solution. Laboratory bioassays of spotted wing drosophila mortality followed exposure to grape and blueberry foliage and/or fruit sprayed and aged in the field. On grape foliage, the addition of 2.4 g/liter of sugar with insecticide sprays resulted in an 11 and 6% increase of spotted wing drosophila mortality at 1 and 2 d exposures to residues, respectively, averaged over seven insecticides with three concentrations. In a separate experiment, spinetoram and cyantraniliprole reduced by 95–100% the larval infestation of blueberries, relative to the untreated control, 7 d after application at labeled rates when applied with 1.2 g/liter sucrose in a spray mixture, irrespective of rainfall; without sucrose infestation was reduced by 46–91%. Adding sugar to the organically acceptable spinosyn, Entrust, reduced larval infestation of strawberries by >50% relative to without sugar for five of the six sample dates during a season-long field trial. In a small-plot field test with blueberries, weekly applications in alternating sprays of sucrose plus reduced-risk insecticides, spinetoram or acetamiprid, reduced larval infestation relative to the untreated control by 76%; alternating bifenthrin and phosmet (without sucrose) reduced infestation by 65%.

KEY WORDS *Drosophila suzukii*, spotted wing drosophila, phagostimulant, insecticide, adjuvant

Introduction

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), native to Asia, has rapidly colonized fruit growing areas in North America since its discovery in California in 2008 (Hauser 2011, Walsh et al. 2011). Customers are unlikely to tolerate the presence of larvae within fruit, and so fruit growers have had to adopt intensive spray programs to protect fruit from infestation. Spotted wing drosophila is difficult to manage because it has a short life cycle and high fecundity, and fruit must be protected just as they are ripening, which limits suitable insecticides to those that have short preharvest intervals.

Currently, effective insecticides suggested for managing spotted wing drosophila are principally conventional broad-spectrum products inimical to integrated pest management programs, such as advanced generation pyrethroids and organophosphates (Beers et al. 2011, Haviland and Beers 2012, Van Timmeren and Isaacs 2013). Neonicotinoids have been used to a limited extent because they are perceived to be less effective (Bruck et al. 2011), and they can also be anticipated to have broad-spectrum effects and negative consequences to beneficial arthropods, if used in foliar sprays (James 2003, He et al. 2012). The exception regarding broad spectrum impacts for insecticides effective against spotted wing drosophila are spinosyns (spinosad and spinetoram; Beers et al. 2011, Bruck et al. 2011, Haviland and Beers 2012), which, for resistance management, are limited in the number of applications per year permitted per crop and on each farm.

Because the current effective pesticide options for managing spotted wing drosophila are limited, it is important to make the best use of those insecticides. Behavior-modifying chemicals could improve the efficiency of insecticides. For example, improved attractants could concentrate fly activity where they may encounter insecticides; such an attract-and-kill approach could use insecticides applied directly to the outside of an attractant trap, or to the trap and surrounding vegetation and/or fruit (Hampton et al. 2014). Phagostimulants combined with insecticides could also improve their effectiveness. Using phagostimulants with insecticides may then permit insecticides that

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require ingestion to be effective, such as boric acid (Xue and Barnard 2003) and some insect growth regulators (Casaña-Giner et al. 1999). It is unclear, without empirical evidence, what effect the addition of phagostimulants may have on the performance of contact-acting insecticides.

Lapping mouthparts found within Diptera are an ancient trait that allows flies to exploit extrafloral nectaries and deposits of honeydew (Yeates and Wiegmann 1999). On detection of sweet substances with their tarsal taste receptors, flies respond with the proboscis extension reflex, in which the sponging mouthparts are lowered to the substrate, the labellar lobes are extended and the flies proceed to taste the surface (Gordesky-Gold, et al. 2008). If the fly is hungry, sugars are solubilized through regurgitation of crop liquids, and then ingested (Dethier 1976). We investigated which sweeteners were readily consumed by spotted wing drosophila adults, quantified the minimum amount of sucrose that, when added to a spray mixture, is required to elicit enhanced spotted wing drosophila feeding on residues, and through laboratory, field–laboratory, and field tests determined whether the addition of sucrose to insecticide sprays enhances spray residue toxicity to spotted wing drosophila adults.

Materials and Methods

Threshold Response to Sucrose and Sucrose Combinations. A series of no-choice tests explored the threshold feeding responses of spotted wing drosophila to known *Drosophila melanogaster* Meigen phagostimulants: sucrose, sodium chloride (NaCl), or their combination. For these experiments, candidate materials were diluted in distilled water containing 2.4 g/liter of erythrosin B red dye (Sigma-Aldrich, St. Louis, MO). An atomizer (Model BRF4AB, Specialty Bottle, Seattle, WA) fitted onto a 15-ml centrifuge tube (Fisher Scientific, Pittsburgh, PA) sprayed this mixture onto an 18-mm-diameter glass coverslip, which was then allowed to dry. The coverslips were sprayed so that there were discrete droplets present on the surface (i.e., they were not sprayed to run-off). Each coverslip was then placed in the bottom of a 30-ml single-serving plastic container (P100 Solo Soufflé cup, Dart Container, Mason, MI), to which 20 unsexed *D. suzukii* flies were added. After 2 h, the flies were chilled to 4°C in a refrigerator, and then placed on a chill plate under 25 × magnification to determine whether there was dye present in the digestive system.

Test 1. Sucrose was sprayed at concentrations of 0, 0.01, 0.032, 0.1, 0.32 and 1% onto coverslips for no-choice assessment of the threshold response to sucrose.

Test 2. Sucrose was diluted to 0, 0.01, 0.1 and 1.0% in distilled water containing 620 μl/liter of Silwet L-77 surfactant (Momentive Performance Materials, Albany, NY) and sprayed onto glass coverslips for no-choice assessment of feeding.

Test 3. Sucrose at 0.032% was compared with a water check, 5 mMol NaCl, and the combination of 0.032% sucrose plus 5 mMol NaCl in a no-choice trial. All treatments were diluted in distilled water containing surfactant (see Test 2).

Test 4 followed the same procedure as Test 3, except that the sucrose concentration was increased to 0.1%.

The number of flies feeding and not feeding in each treatment group were summed over replicates (Test 1, $n = 6$; Tests 2–4, $n = 5$) and compared with the same categories in the untreated (water plus dye) control using Fisher's exact test (Microsoft Research 2014). Additional pairs of treatments were analyzed using the same approach, where questions arose regarding enhancement of feeding response from stimulus combinations. For Test 1, the percentage of flies feeding was also subjected to analysis of variance to determine if there was a linear trend of feeding with increasing sucrose concentration. Data presented are the percentage of flies responding by feeding, of the total flies tested.

Laboratory Assessment of Insecticide Enhancement with Sweeteners. Two laboratory experiments were performed to investigate whether the addition of sucrose would enhance the performance of insecticides. In Test 1, water was compared with spinosad (Entrust, Dow AgroScience, Indianapolis, IN) at a concentration of 0.12 g/liter of active ingredient (2 oz/100 gal of formulated product) and spinosad at the same concentration combined with 0.3% sucrose. Each material was sprayed onto the interior surfaces of five 30-ml plastic cups with the previously described atomizer. After the spray dried, 20 female spotted wing drosophila flies were added per cup and the containers were tightly capped. Flies were not provided additional water, as a wet cotton swab could absorb the spray deposited on the side of the plastic container, which could then be imbibed. The numbers of dead flies were observed every 20 minutes for 5.5 h, and the mortality over time was compared through regression analysis to determine if the slopes or intercepts differed significantly among treatments (Analytical Software 2008).

In Test 2, the dose–response of adult spotted wing drosophila to acetamiprid (Assail 30 SG, United Phosphorus, King of Prussia, PA) was compared when sprayed by itself, or when applied in combination with 0.3% sucrose, to coverslips in a 24-h mortality assay. Concentrations of acetamiprid were chosen to be evenly spaced on a log scale, with five concentrations between and including 40 and 400 ppm, when acetamiprid was presented without sucrose, and six between and including 4 and 80 ppm, when presented with sucrose. Glass coverslips (18 mm in diameter) were sprayed with the previously described atomizer to provide distinctly separated droplets on the surface, which were allowed to dry. Fifteen female flies were enclosed within tightly capped 30-ml plastic cups, to which the sprayed coverslip acted as the floor. To avoid excessive check mortality from dehydration, a cotton swab soaked in distilled water was provided in each container, with the swab positioned to not touch the treated coverslip. The number of live and dead flies was determined after 24 h of exposure. Data were combined over three sets of cups for each concentration,

on each of the two days, resulting in two sets of results with 45 flies each. Abbott's formula (Abbott 1925) was used to correct for the untreated check mortality, and the data were averaged over the two dates. Regression analysis (Analytical Software 2008) of logit-transformed proportion mortality data determined if the slopes or intercepts differed significantly among treatment groups; mortality values <10 or >90% were excluded from analysis, based on their low value for estimating the LD_{50} (Finney 1947, p. 83), but are presented in Fig. 2. An alternative regression included all data and was run with logit-transformed data weighted according to Finney (1947, p. 41).

Semifield Experiment, 2012. This experiment compared effectiveness of insecticide residues on grape foliage against spotted wing drosophila when applied with and without a phagostimulant (sucrose added at 2.4 g/liter), conducted at the Valley Laboratory of the Connecticut Agricultural Experiment Station in Windsor, CT. The treatments were based on labeled rates of insecticides per unit area, with an assumption that they would be applied at 470 liter/ha (50 gal/ac) in fruit crops. Products tested were Assail 30 SG at 350 g/ha, Malathion 5 EC (malathion, Dragon Corp., Roanoke, VA) at 2.3 liter/ha, Entrust (spinosad, Dow AgroSciences, Indianapolis, IN) at 140 g/ha, Delegate 30WG (spinetoram, Dow AgroSciences) at 420 g/ha, AmTide Imidacloprid 2 F (imidacloprid, AmTide, Irvine, CA) at 234 ml/ha, Belay (255 g/liter a.i. clothianidin, Valent USA, Walnut Creek, CA) at 292 ml/ha, and Actara 25WDG (thiamethoxam, Syngenta Crop Protection, Greensboro, NC) at 210 g/ha. Products were applied at these indicated rates (1×), and at 0.5 × and 0.25×, with and without the addition of sucrose, in a full $7 \times 3 \times 2$ factorial randomized design. In addition to these 42 treatment combinations, samples were collected to evaluate toxicity to adult spotted wing drosophila at 1, 2, 4, and 8 d after treatment (DAT), amounting to 168 samples per trial. To keep the experiment manageable, four individual grape leaves were sprayed with each insecticide × rate combination, and the leaf was flagged with vinyl ribbon indicating the treatment. Leaves then were clipped as needed, placed in an aluminum foil packet, and immediately frozen until the bioassay could be conducted.

Spraying was conducted with the previously described atomizer, with five pumps applied to the underside of each leaf. A separate set of 10 leaves were individually weighed, sprayed with water in the same manner that insecticides had been applied, and immediately re-weighed to determine gravimetrically the amount of spray applied to the leaf surface. The leaves were scanned and areas quantified to the nearest square millimeter with SigmaScan Image (Systat Software, San Jose, CA) so that the average spray deposit per square centimeter could be determined. The amount of wet spray delivered to the leaf surfaces in this experiment was 2.2 ± 0.2 (mean ± SE) $\mu l/cm^2$.

The entire factorial experiment was repeated three times, by spraying leaves on 25 July, 1 August, and 29 August to obtain three replicates. All three spray dates were followed at some time during the 8 d posttreatment sampling protocol by significant rain events, which may have eroded some insecticide residues. The first replicate was followed 3, 4, and 5 d later with 0.6, 4.8, and 1.2 cm of precipitation, respectively. The second replicate was followed 5 d later with 2.3 cm of precipitation, and replicate 3 on the spray date and 5 d later with 1.3 and 1.5 cm of precipitation, respectively.

Two sections of each grape leaf were cut to fit within the bottom and sides of a 30-ml plastic container, and held in place with double-sided adhesive tape so that the sprayed undersurface of the leaf was directed toward the interior of the container. This procedure left the inside of the lid and ~30% of the side of the cup uncovered by the grape leaf. A cotton swab dipped in distilled water was added, and 10 spotted wing drosophila adults (5 male and 5 females) were placed in the container. Mortality was assessed 24 and 48 h after adding the flies. The numbers of dead flies per container were subjected to analysis of variance (ANOVA).

Semifield Experiment, 2013. The objective of this experiment was to compare the efficacy of insecticides with and without a phagostimulant (sucrose at 1.1 kg/ha) against spotted wing drosophila on highbush blueberry. The treatments and rates were: Exirel 10SE (cyantraniliprole, DuPont, Newark, DE) at 1.50 liter/ha (plus 0.25% Dyne-Amic adjuvant; Helena Chemical, Collierville, TN), Assail 30SG at 370 g/ha (acetamiprid, United Phosphorous Inc., King of Prussia, PA), Imidan liquid formulation (phosmet, Gowan, Yuma, AZ) at 2.34 liter/ha, Malathion 8 Aquamul (malathion, Loveland Products, Inc., Loveland, CO) at 2.92 liter/ha, Bifenture 10DF (bifenthrin, United Phosphorus, King of Prussia, PA) at 1.1 kg/ha, Danitol 0.83EC (fenpropathrin, Valent USA, Walnut Creek, CA) at 782 ml/ha (plus 0.25% Dyne-Amic adjuvant), Delegate 30WG (spinetoram, Dow AgroSciences, Indianapolis, IN) at 420 g/ha, and Movento (spirotetramat, Bayer Crop Science, Research Triangle Park, NC) at 731 ml/ha (plus 0.25% MSO adjuvant). Rates and use of adjuvants were based on manufacturers' recommendations. The study was conducted in a blueberry field, 'Bluecrop,' located at the P. E. Marucci Blueberry/Cranberry Center in Chatsworth, New Jersey. The experimental area consisted of 12 rows, at 2.7 m between rows, of 23–116 bushes each spaced 1.2 m apart. Each treatment was repeated on five randomly assigned bushes (each bush was considered a replicate; total $n = 90$ bushes). A 1–3 bush buffer was used between treatments within rows.

Applications were made on 5 July with an R&D CO_2 backpack sprayer (Opelousas, LA). The sprayer was calibrated to deliver 470 liter/ha (equivalent to 50 gal/ac) at 240 kPa, using a single ConeJet TXVS 4 nozzle (Spraying Systems, Wheaton, IL), yielding 156 ml (5.3 fl oz) per bush. Sucrose at 1.1 kg/ha in 470 liter/ha translated to 1.2 g/liter in the spray mixture. Treated terminals with 2–3 clusters of berries and two leaves were taken from each treated bush 1, 3 and 7 DAT on 6, 8, and 12 July, respectively. The terminals were placed in assay containers consisting of a 950-ml deli

container with a hole cut in the bottom into which a florist's water pick was tightly fit. Terminals were supplied with water and were kept in the laboratory during the length of the experiment. Before flies were added, the number of berries was counted. Flies were added to the assay containers within 2–3 h after terminals were clipped from bushes. Total precipitation during this study (7–12 July) was 1 cm (NJ Weather & Climate Network; http://www.njweather.org/data, last accessed 8 January 2015). To evaluate the effect of precipitation on the length of residual activity of treatments additional treated terminals were taken on 1 DAT, before any rain events. These terminals were aged in a greenhouse, exposed to the sun but protected from rain until they were needed at 3 and 7 DAT. They were then placed in assay containers, as described above, and the number of berries counted. Ten adult spotted wing drosophila, five females and five males, were removed from a laboratory colony and kept in clean rearing tubes in a 25°C incubator for 2–3 h before being released into the assay containers. Sexually mature flies (3–4 d old) were used in the experiment. Flies were anesthetized with small puffs of CO_2 gas injected into the rearing tubes to facilitate handling and placement in the assay containers. After flies were added to the assay containers, the containers were placed on a bench in the laboratory under a photoperiod of 14:10 (L:D) h and at 25–28°C. Adult fly mortality data were collected following 1, 3, and 7 d after initial exposure to the treated fruit. Any flies still alive were removed during the last evaluation on day 7. Fruits were allowed to incubate under the same laboratory conditions for seven more days following the last adult observation, at which point larval data were collected using the salt water extraction method (Yee 2014). Fruit samples were submerged in warm salt water (~2,160 g of salt in 19 liter of water) causing larvae to exit the fruit. Larvae and pupae were then collected using a 30-mesh sieve and counted. The number of larvae and pupae normalized per 10 berries was calculated ([no. larvae + pupae/no. ripe fruit] × 10).

Data for residues aged 1 d in the field were of a 9 × 2 factorial design (nine levels for insecticide treatments and two levels for presence or absence of sucrose), with mortality measured repeatedly for five groups of 10 flies, on 1, 3, or 7 d following initial exposure. Data for the remainder of the experiment were structured as a 9 × 2 × 2 factorial design, with the same design as above, but with the additional factor of rain (or not) and field aging of residues (3 vs. 7 d), with mortality measured repeatedly for three groups of 10 flies, on 1, 3, or 7 d following initial exposure. Mortality data were assessed with two statistical methods. For each insecticide × residue age treatment combination, the samples were aggregated to provide total live and dead flies (from groups of 50 flies for 1 d of residue aging, 30 flies for the other residue age groups) × presence or absence of sucrose, which formed a 2 × 2 contingency table suitable for analysis with Fisher's exact test. The second statistical method separately analyzed the totals for dead flies for 1, 3, and 7 d field aging of residues, as repeated measures ANOVA (considering mortality at 1, 3, and 7 d of exposure) with the highest order interaction sums of squares and degrees of freedom as a conservative error term for calculating the *F*-statistic and statistical significance. The numbers of larvae and pupae reared from blueberries used for insecticide bioassays were subjected to ANOVA, following square root $(x + 0.5)$ transformation.

Field Test of Phagostimulants to Protect Day-Neutral Strawberries. This field trial was conducted using a new planting of day-neutral strawberry (alternating rows of 'Seascape' and 'Tribute') planted on plastic at NYSAES in Geneva, NY, in June 2012 using double rows with 30-cm spacing between plants and 1.5 m between rows. Flowers were removed by hand until mid-summer. As fruit began ripening, the following six insecticide treatments were applied to individual plots (2 m of double row) with five replicates in a randomized complete block design: 1) water only control, 2) water plus sucrose at 2.4 g/liter, 3) Entrust SC at a rate of 140 g a.i./ha once per week, 4) Entrust SC at a rate of 140 g a.i./ha plus sucrose at a rate of 2.4 g/liter, 5) Brigade WSB (bifenthrin, FMC, Philadelphia, PA) at a rate of 110 g a.i./ha once per week, and 6) Brigade WSB 110 g a.i./ha applied twice per week. Materials were applied with a spray volume of 700 liter/ha of water using a backpack sprayer (Solo Model 475, Newport News, VA), with flat fan nozzle, applied at 280 kPa. The experiment started on 1 September and continued until 19 October. The weekly sprays were applied on 1, 7, 14, 21 and 28 September and 5, 12 and 19 October. After two successive applications of Entrust, Brigade WSB (110 g a.i./ha) was applied once on 14 September and once on 5 October, in place of Entrust to follow label restrictions that require insecticide rotation for resistance management.

Ripe strawberry fruits were collected from each plot once per week starting in September for a total of six sample dates. Fruit were weighed, examined for disease (*Colletotrichum acutatum* and *Botrytis cinerea*) and other damage, and marketable fruit was placed in plastic deli cups with yellow sticky cards for rearing out *Drosophila*. The quantity of fruit per sample ranged from 46 to 172 g (98.3 ± 24.9 g, mean ± SD). No flies were reared from the first sample date on 10 September and, therefore, this date was not included in the analysis. Specifically, data from 14, 19, and 26 September and 3, 10, and 17 October were included. Flies reared from fruit were sorted as male or female *D. suzukii*, or other drosophilids (data not presented). Data were normalized by calculating the number of flies per kg of fruit prior to using square root $(x + 0.5)$ transformation to establish homogeneity of variance and conducting analysis of variance. Repeated measures analysis of variance was performed with the General ANOVA feature of Statistix 9 (Analytical Software, 2008).

Field Test of Phagostimulants to Protect Blueberries. The objective of this experiment was to determine the efficacy of using three programs against spotted wing drosophila on the late to mid-season 'Bluecrop' variety of highbush blueberries: 1) alternating Delegate 30WG and Assail 30SG (reduced-risk

program), 2) alternating Delegate 30WG and Assail 30SG, each with the addition of sugar at 1.1 kg/ha (reduced-risk program plus phagostimulant), and 3) Imidan alternating with Bifenture (standard program). Treatments in each rotation program were alternated by week. For weeks 1 and 3: Delegate 30WG at 410 g/ha (6 oz/ac), Delegate 30WG at 410 g/ha plus sugar, and Imidan at 2.3 liter/ha (32 fl oz/ac). For weeks 2 and 4: Assail 30SG at 360 g/ha (5.3 oz/ac), Assail 30SG at 360 g/ha plus sugar, and Bifenture 10DF at 1.1 kg/ha (16 oz/ac). The experiment was conducted in an abandoned blueberry farm located in Chatsworth, NJ. Plots consisted of two rows of ~16–20 bushes each, replicated four times in a randomized complete block design. Treatment rows were separated by single buffer rows, and treated plots were separated by 4–6 bushes, spacing between rows and bushes was the same as described above. Applications were made with a tractor-mounted Pak-Blast model MBICO28 Sprayer (Rear's Mfg. Co., Eugene, Oregon). The sprayer was calibrated to deliver 370 liter/ha (equivalent to 40 gal per acre) at 210 kPa using five nozzles with D3 orifices, or ~125 ml per bush. Sprays were applied on 10, 16, 22, and 30 July 2013. Samples were taken on 6 August from treated plots (7 d after the final spray) by picking five 470 ml (one pint) samples from each plot. Samples were kept in an incubator under a photoperiod of 14:10 (L:D) h at 24°C for 10–13 d and then evaluated from 16–19 August. Samples were evaluated by submerging samples in warm water containing 70 g/liter of salt, causing larvae to leave fruit. Larvae and pupae caught by a 30-mesh sieve were counted and the total number (larvae + pupae) per 470 ml sample was calculated. Data were analyzed using ANOVA and means separated by Tukey's honestly significant difference (HSD) test at $P = 0.05$.

Results

Threshold Response to Sucrose and Sucrose Combinations. Spotted wing drosophila responded and fed to a greater extent on dried spray droplets containing sucrose, sodium chloride or their combination than they did to the dye control (Table 1). Fisher's exact test (FET) revealed that the threshold for eliciting a response was at or below the spray concentration of 0.01% sucrose in Test 1 ($P < 0.0001$; FET). However, without a surfactant, the droplets contracted while drying to leave highly concentrated spots on the glass surface, and so the concentration of the sucrose at these point sources greatly increased. When a surfactant was added, the threshold concentration for sucrose to elicit a significant feeding response increased to 0.1% ($P < 0.0001$; FET; Test 2, Table 1). As the concentration of sucrose increased, so did the percentage of flies responding by feeding (Test 1: $F = 39.4$; df = 1, 35; $P < 0.0001$; Test 2: $P = 0.045$; FET; comparison between sucrose concentrations of 0.01 and 0.1%).

In Test 3, 5 mMol sodium chloride sprayed onto glass elicited a small response ($P = 0.012$; FET). Sodium chloride also increased the sensitivity to sucrose (Table 1): when presented alone, sucrose (0.032%) was not fed upon, but it became detectable when presented with sodium chloride, and the combination elicited greater feeding than sodium chloride presented alone ($P = 0.036$; FET). The enhancement of feeding from the presence of sodium was not statistically significant in Test 4 ($P = 0.13$; FET), and the presence of sucrose, with or without sodium, had an overwhelming influence on feeding ($P < 0.0001$; FET).

Table 1. Response of groups of 20 *D. suzukii* adults presented spray droplets dried onto a glass surface and allowed to feed in a no-choice test for 2 h

Test 1.	Sucrose (%)		Feeding (%, mean ± SE)[a]
	0		0.76 ± 0.76
	0.01		25.1 ± 5.6*
	0.032		12.2 ± 4.5*
	0.1		36.7 ± 5.6*
	0.32		49.9 ± 3.4*
	1		46.9 ± 9.7*
Test 2.	Sucrose (%)		Feeding (%, mean ± SE)
	0		0.0 ± 0.0
	0.01		0.0 ± 0.0
	0.1		9.5 ± 2.7*
	1		19.4 ± 2.4*
Test 3.	Sucrose (%)	NaCl (mMol)	Feeding (%, mean ± SE)
	0	0	0.0 ± 0.0
	0	5	5.8 ± 1.8*
	0.032	0	0.0 ± 0.0
	0.032	5	14.0 ± 5.5*
Test 4.	Sucrose (%)	NaCl (mMol)	Feeding (%, mean ± SE)
	0	0	4.3 ± 3.2
	0	5	8.6 ± 2.7
	0.1	0	40.5 ± 1.5*
	0.1	5	47.6 ± 3.3*

[a] An asterisk indicates significant difference within the test from the dye control (0 concentration), Fisher's exact test, $P < 0.05$)

Laboratory Assessment of Insecticide Enhancement with Sweeteners. In Test 1, the addition of sucrose shifted the intercept ($F = 17.06$; df = 1, 127; $P = 0.0001$), but not the slope ($F = 0.32$; df = 1, 126; $P = 0.57$), of the relationship between fly mortality and duration of exposure (Fig. 1). Thus, flies exposed to the spinosad plus sucrose combination interacted with these residues faster, and started dying sooner, than when the spinosad was presented alone. Using the different intercepts and the common slope of these lines, equivalent mortality in the no-sucrose group occurred ~120 min later than the group exposed to the combination of spinosad plus sucrose.

In Test 2, the addition of sucrose shifted the intercept ($F = 24.1$; df = 1, 5; $P = 0.005$), but not the slope of the dose response ($F = 0.05$; df = 1, 4; $P = 0.83$), so that the LC_{50} shifted from 82.4 to 41 ppm (Fig. 2). The alternative weighted regression including all data found no statistical differences in slopes ($F = 0.76$; df = 1,7; $P = 0.41$) or intercepts ($F = 1.96$; df = 1,8; $P = 0.20$). However, the LC_{50} estimates from this regression were essentially unchanged (83.9 and 46.3 for acetamiprid without and with sugar, respectively).

Semifield Experiment, 2012. Application of insecticides to the undersides of grape foliage resulted in residues for some of these insecticides that remained toxic to the adult flies over the course of this

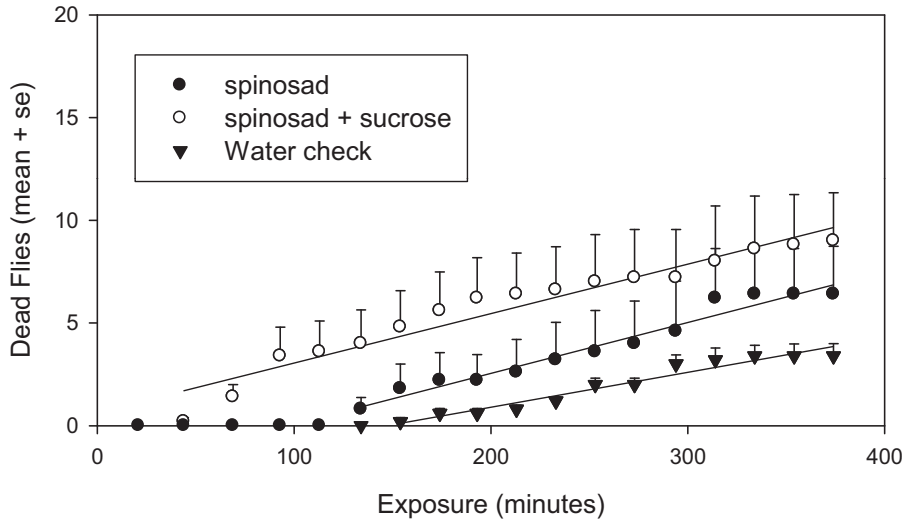

Fig. 1. Number of dead flies (mean + SE) of the 20 female spotted wing drosophila exposed to dried residues of Entrust applied with or without 0.3% sucrose. Regression lines were fitted through data from 130–370 minutes following initial exposure ($n = 5$).

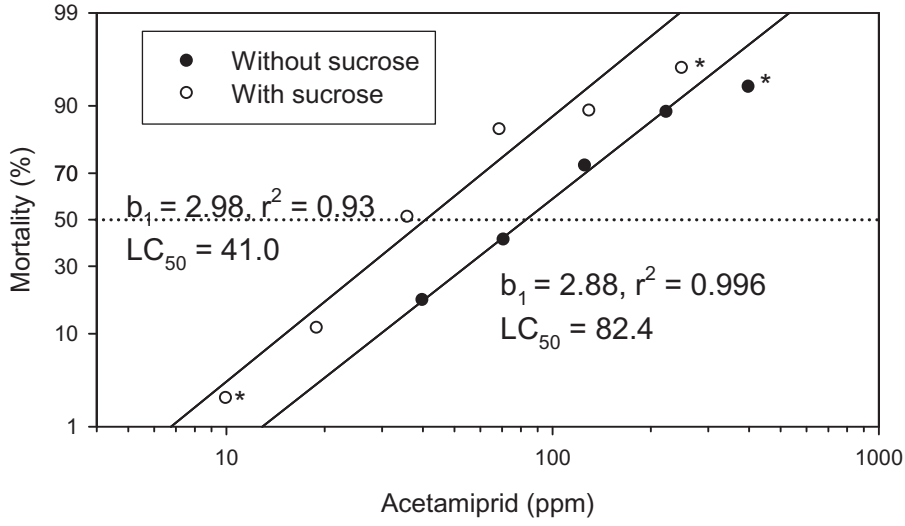

Fig. 2. Dose–response relationships for spotted wing drosophila female flies exposed to acetamiprid applied with and without 0.3% sucrose. Spray droplets on a glass coverslip were allowed to dry before flies were exposed ($n = 90$ individuals per datum). Data marked with asterisks were excluded from the logit-transformed regression analysis estimating the LC_{50}.

experiment (Fig. 3). Main effects of residue age ($F = 26.3$ and 9.9; df = 3, 329; $P < 0.0001$), insecticide ($F = 40.5$ and 34.2; df = 6, 329; $P < 0.0001$), rate ($F = 10.8$ and 12.3; df = 2, 329, $P < 0.0001$), and the addition of sugar ($F = 24.1$ and 6.9; df = 1, 329, $P < 0.0001$ and 0.009) were all highly significant for mortality at 1 and 2 d exposure to residues, respectively. Insecticide × residue age was the only statistically significant interaction ($F = 3.48$ and 3.42; df = 18, 329, $P < 0.0001$) for mortality at 1 and 2 d, respectively. As would be expected, the efficacy of insecticides significantly decreased with decreasing rate and as residues aged. However, with the exception of a rapid decrease in efficacy with residue age seen with malathion (Fig. 3), which is probably responsible for the significant insecticide × residue age interaction, the efficacies of the remaining insecticides were remarkably stable over the course of this experiment. In particular, the neonicotinoids (Fig. 3A) appeared to not lose efficacy until after 4 d, and the spinosyns (spinosad and spinetoram, Fig. 3B) retained their efficacy through 8 d of field residue aging. Longer than anticipated residual activity may have resulted from application of the insecticides to the undersides of grape leaves, where they were protected from exposure to UV light and erosion by rain. Overall, the spinosyns were the most

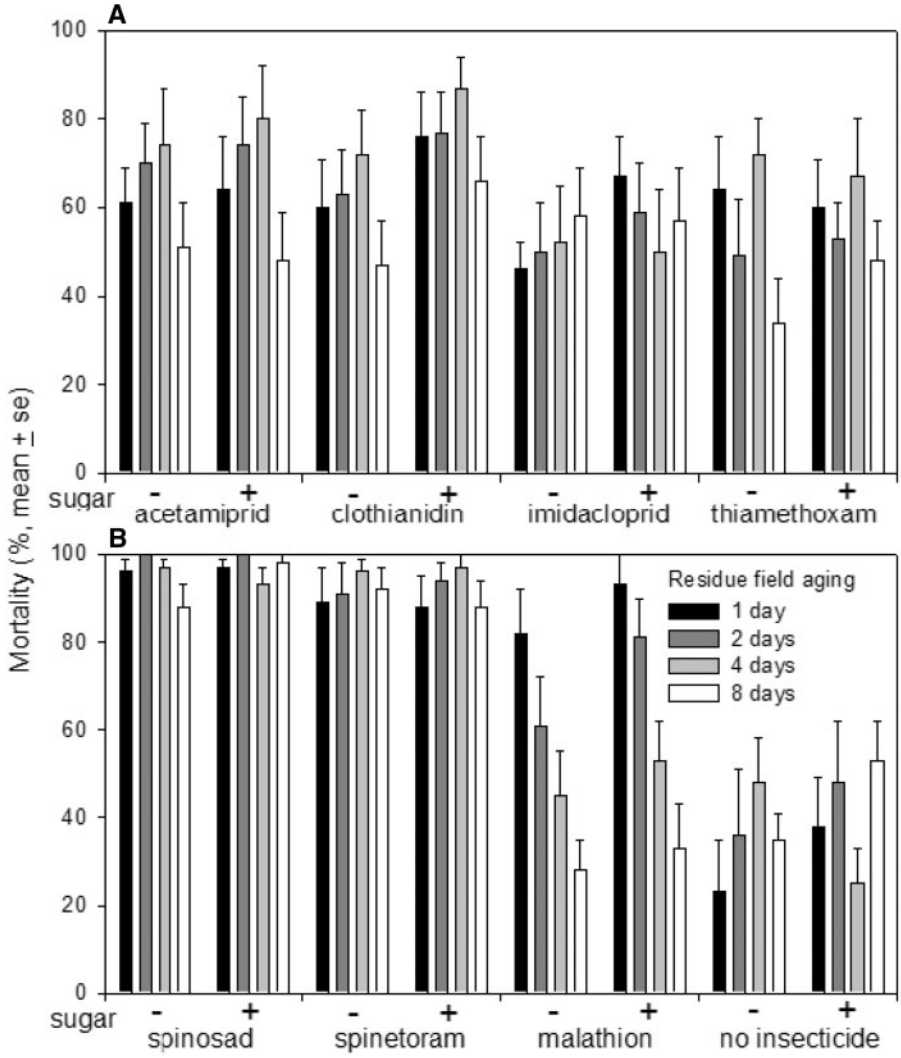

Fig. 3. Mortality (mean ± SE) for groups of 10 adult spotted wing drosophila held with grape foliage sprayed in the field with various insecticides 1–8 d earlier. Data have been averaged over three insecticide rates and three replicates (insecticides, $n = 9$; no-insecticide, $n = 6$).

Table 2. Main effect means (±SE) for insecticides and sucrose combined with insecticides for percentage mortality of adult spotted wing drosophila, and rate effects for insecticides tested in the 2012 semifield study

	Mortality (%) at 24 h exposure[a]				Mortality (%) at 48 h exposure[a]			
Insecticide	Rate: 0.25×	0.5×	1×	Average	0.25×	0.5×	1×	Average
Acetamiprid	40 ± 7.9	52 ± 7.5	54 ± 7.9	48 ± 4.5 b	53 ± 7.1	77 ± 5.3	69 ± 7.0	66 ± 3.9bc
Clothianidin	40 ± 6.4	46 ± 6.7	53 ± 6.8	47 ± 3.9bc	59 ± 6.2	70 ± 5.8	76 ± 6.3	68 ± 3.6 b
Imidacloprid	20 ± 4.7	16 ± 4.3	25 ± 6.3	21 ± 3.0 d	49 ± 6.0	53 ± 7.0	62 ± 6.5	55 ± 3.8 c
Malathion	35 ± 7.5	52 ± 8.2	56 ± 8.7	48 ± 4.8 b	48 ± 7.2	66 ± 6.4	66 ± 7.4	60 ± 4.1bc
Spinetoram	64 ± 8.2	71 ± 7.6	75 ± 7.3	70 ± 4.4 a	87 ± 4.6	95 ± 2.6	94 ± 2.2	92 ± 1.9 a
Spinosad	66 ± 7.5	75 ± 6.3	85 ± 4.4	75 ± 3.7 a	92 ± 2.6	97 ± 1.4	99 ± 0.4	96 ± 1.1 a
Thiamethoxam	34 ± 7.0	32 ± 6.9	39 ± 8.0	35 ± 4.1 c	53 ± 6.9	56 ± 6.3	58 ± 7.1	56 ± 3.8 c
Sucrose								
Absent		43.5 ± 2.4 b				67.5 ± 2.1 b		
Present		54.6 ± 2.4 a				73.2 ± 2.0 a		

Groups of 10 spotted wing drosophila adults were enclosed with previously sprayed grape foliage. Formulations of insecticides and the field rate (1 ×) concentrations are detailed in the text. Data are averaged over 1, 2, 4, and 8 d of aging in the field; $n = 12$ groups of flies for insecticide × rate combinations; $n = 252$ for sucrose main effects.

[a] Means followed by the same letter in a column do not significantly differ (Tukey's HSD test, $P < 0.05$).

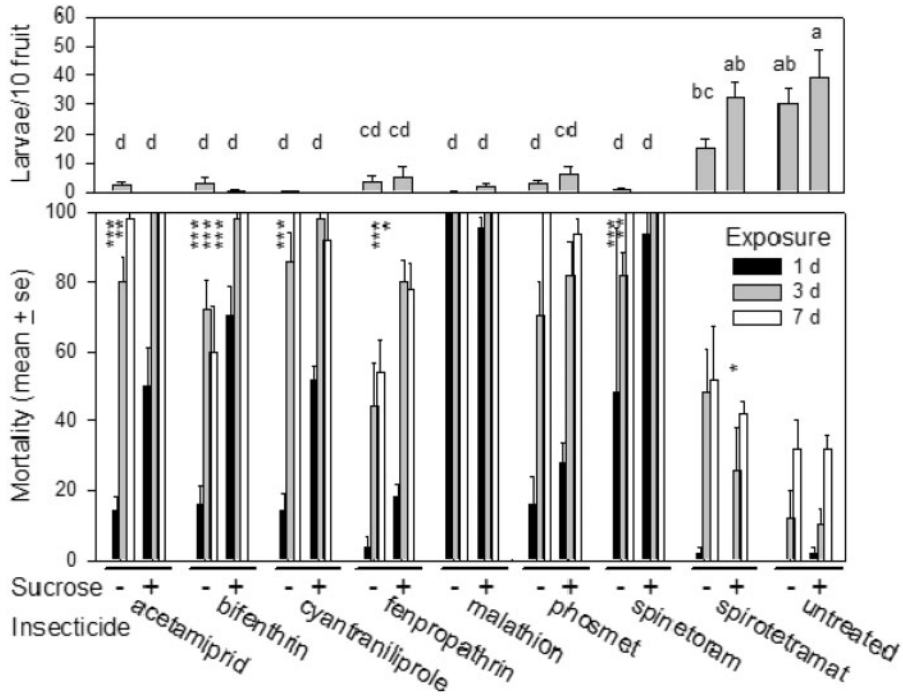

Fig. 4. Lower panel: average (±SE) mortality for groups of 10 adult spotted wing drosophila ($n = 5$) held with blueberry foliage and fruit sprayed in the field with various insecticides 1 d earlier. Mortality of flies was recorded following 1, 3, and 7 d of exposure. Mortality data without and with sucrose are presented in paired sets of three bars for each insecticide, for which the statistical significance for a sucrose effect is indicated above the bar: *, **, and *** signify $P < 0.05$, 0.01, and 0.001, respectively (Fisher's exact test for live and dead flies, with and without sucrose, totaled for the five replicates). Upper panel: the number of larvae infesting the fruit following 7 d of exposure, one bar for each insecticide × sucrose treatment combination. Bars with the same letter do not significantly differ (Tukey's HSD, $P = 0.05$).

effective insecticides (Table 2), followed by acetamiprid, malathion, and clothianidin. Imidacloprid and thiamethoxam were ranked as the least effective insecticides. The addition of sucrose increased mortality by 11 and 6%, overall, for the 1 and 2 d exposure intervals, respectively. Although the insecticide × sucrose interaction was not statistically significant, there was little room for improvement with the spinosyns, and so the enhanced mortality from the addition of sucrose was principally due to improved performance of the neonicotinoids and malathion.

Semifield Experiment, 2013. Addition of sucrose to insecticides had effects that varied with insecticide, residue aging, rainfall, and duration of exposure (Figs. 4–6). For example, at 1 d following application, and following 1 d of exposure to the residues, the addition of sugar significantly enhanced the lethality of acetamiprid, bifenthrin, cyantraniliprole, and spinetoram, but not fenpropathrin, malathion, phosmet, or spirotetramat (Fig. 4). There was a significant enhancement of mortality with fenpropathrin with the addition of sucrose by 3 d of exposure to residues (Fig. 4). Malathion at 1 d post treatment was highly lethal with or without sugar, suggesting why there was not any enhancement with the addition of sucrose for this active ingredient. Spirotetramat was an ineffective insecticide for spotted wing drosophila; its performance was not enhanced by the addition of sucrose. The lack of enhanced insecticidal effect for combining sucrose with phosmet may require further study to understand. Phosmet has high inherent contact and unusually long residual toxicity to spotted wing drosophila when presented alone (Van Timmeren and Isaacs 2013), which could disrupt feeding behaviors and thus interfere with phagostimulatory enhancement.

The number of larvae developing from fruits exposed to spotted wing drosophila during the pesticide bioassay generally mirrors the fly mortality. When flies were quickly killed, there was little opportunity for eggs to be deposited in fruits and the number of resulting larvae was greatly reduced. There may be an element of either sublethal effects on the flies' ability to lay eggs or systemic effects for three of the best performing insecticides (based on protection of fruit from infestation): the addition of sucrose to acetamiprid, cyantraniliprole, and spinetoram resulted in no successful development of larvae, even though the adult mortality with these treatment combinations was not as rapid as with malathion.

The benefit gained by combining insecticides with sucrose diminished with longer duration of field aging of residues and with rain (Figs. 4–6). There were significant insecticide ($F = 12.05$; df = 8, 8; $P = 0.001$), and sucrose ($F = 8.58$; df = 1, 8; $P = 0.044$) main effects of spotted wing drosophila mortality with residues aged for 1 d, and days of exposure greatly influenced

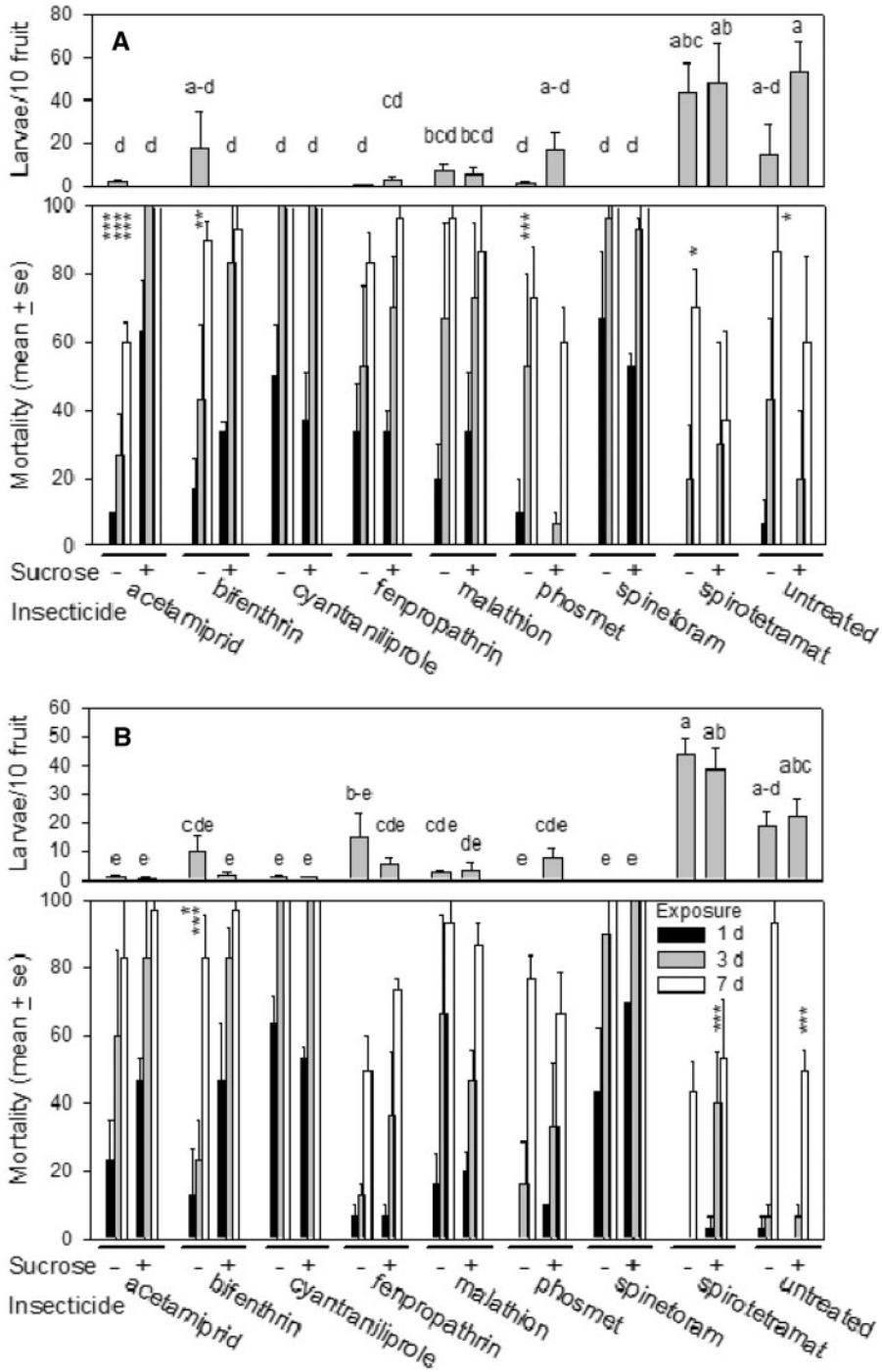

Fig. 5. Lower panel: Average (±SE) mortality for groups of 10 adult spotted wing drosophila ($n = 3$) held with blueberry foliage and fruit sprayed in the field with various insecticides 3 d earlier. Mortality of flies was recorded following 1, 3, and 7 d of exposure. Mortality data without and with sucrose are presented in paired sets of three bars for each insecticide, for which the statistical significance for a sucrose effect is indicated above the bar: *, **, and *** signify $P < 0.05$, 0.01, and 0.001, respectively (Fisher's exact test for live and dead flies, with and without sucrose, totaled for the three replicates). Upper panel: the number of larvae infesting the fruit following 7 d of exposure, one bar for each insecticide × sucrose treatment combination. Bars with the same letter do not significantly differ (Tukey's HSD, $P = 0.05$). (A) blueberry shoots held in a greenhouse and protected from rainfall; (B) blueberry shoots exposed in the field to rainfall.

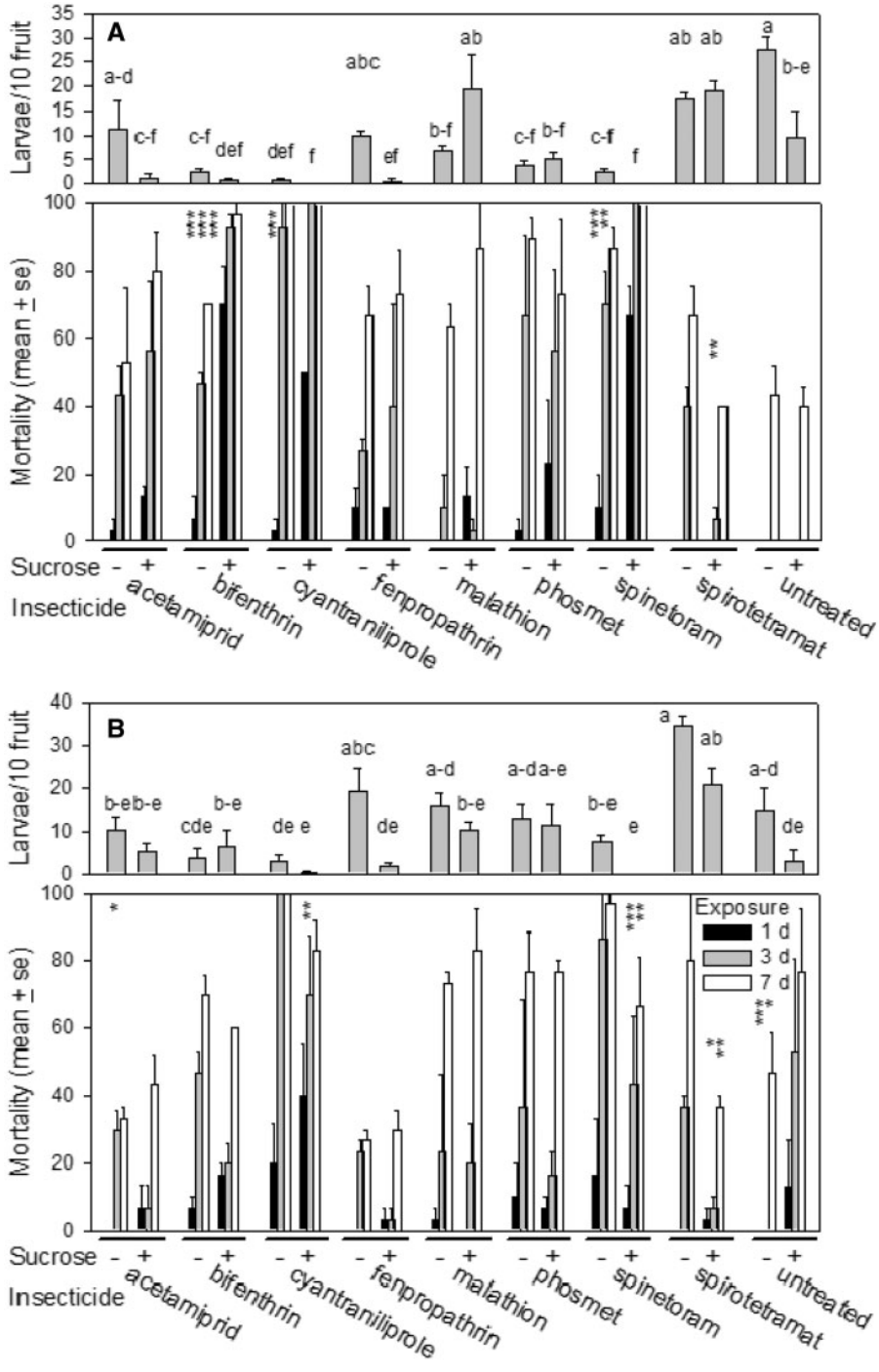

Fig. 6. Lower panel: Average (±SE) mortality for groups of 10 adult spotted wing drosophila ($n = 3$) held with blueberry foliage and fruit sprayed in the field with various insecticides 7 d earlier. Mortality of flies was recorded following 1, 3, and 7 d of exposure. Mortality data without and with sucrose are presented in paired sets of three bars for each insecticide, for which the statistical significance for a sucrose effect is indicated above the bar: *, **, and *** signify $P < 0.05$, 0.01, and 0.001, respectively (Fisher's exact test for live and dead flies, with and without sucrose, totaled for the three replicates). Upper panel: the number of larvae infesting the fruit following 7 d of exposure, one bar for each insecticide × sucrose treatment combination. Bars with the same letter do not significantly differ (Tukey's HSD, $P = 0.05$). (A) Blueberry shoots held in a greenhouse and protected from rainfall, (B) blueberry shoots exposed in the field to rainfall.

mortality ($F = 135.5$; df = 2, 16; $P < 0.0001$; Fig. 4). There were significant interactions between days of exposure to residues and insecticide ($F = 4.61$; df = 16, 16; $P = 0.002$), and days of exposure and sucrose ($F = 4.4$; df = 2, 16; $P = 0.03$). There were differences among insecticides following 3 d of residue aging ($F = 18.88$; df = 8, 8; $P = 0.0002$; Fig. 5). The addition of sugar was no longer significant ($F = 3.31$; df = 1, 8; $P = 0.11$) as a main effect; however, interactions between days of exposure and sucrose remained significant ($F = 5.70$; df = 2, 50; $P = 0.006$). The main effects and various interactions for rain were not significant (main effect $F = 2.1$; df = 1, 8; $P = 0.19$). At 7 d of residue aging (Fig. 6), there were differences among insecticides ($F = 6.15$; df = 8, 8; $P = 0.009$); the sugar main effect was not significant ($F = 0.45$; df = 1, 8; $P = 0.52$), but the interaction between days of exposure and sucrose remained significant ($F = 6.82$; df = 2, 50; $P = 0.002$). The main effects and various interactions for rain were not significant (main effect $F = 4.34$; df = 1, 8; $P = 0.07$).

Analysis with Fisher's exact test reveals details for the effect of the addition of sucrose to insecticide performance. The greatest enhancement in efficacy was observed with bifenthrin, for which statistically significant improvement with the addition of sucrose was evident for residues aged for 3 d, with or without rain, and for 7 d of aging when not exposed to rain. The same group of insecticides that benefitted from the addition of sucrose at 1 d posttreatment showed some benefit at 3 d. For example, acetamiprid caused significantly greater mortality when combined with sucrose with 3 d of field aging as compared with acetamiprid without sugar, but only when the residues were not exposed to rain. Spinetoram and cyantraniliprole did not show significant improvement with the addition of sucrose at 3 d of residue aging. These insecticides caused greater mortality of spotted wing drosophila from the addition of sucrose at 7 d of field aging when not exposed to rain, but exhibited poorer toxicity to adult flies with the addition of sucrose, relative to their application without sugar, when residues were exposed to rain.

Larval development following bioassays of foliage and fruit with residues aged 3 d demonstrated statistically significant pesticide differences ($F = 27.07$; df = 8, 72; $P < 0.0001$) and pesticide × sucrose interaction ($F = 3.27$; df = 8, 72; $P = 0.0031$; Fig. 5). Other main effects and interactions were not significant. At 7 d of residue aging, the number of larvae developing was influenced by sucrose ($F = 40.88$; df = 1, 72; $P < 0.0001$), pesticide ($F = 25.84$; df = 8, 72; $P < 0.0001$), and rain ($F = 8.33$; df = 1, 72; $P = 0.0051$) main effects and sucrose × pesticide ($F = 5.58$; df = 8, 72; $P < 0.0001$) and pesticide × rain ($F = 2.98$; df = 8, 72; $P = 0.006$) interactions (Fig. 6). No other interactions were statistically significant. Most notably, malathion rapidly lost efficacy over time, as reflected in the poor performance at 7 d of residue aging. The addition of sucrose to phosmet at 3 d residue aging resulted in significantly greater survival of adults and more larvae in fruit.

Overall, both spinetoram and cyantraniliprole treatments (with and without sucrose) prevented development of larvae at 3 d of residue age, and the addition of sucrose prevented nearly all larval establishment for 7 d residue aging too, irrespective of rain; these products reduced infestation relative to the untreated check by 95–100% when combined with sucrose, versus 46–91% when applied without sucrose (Fig. 6). Acetamiprid performed well with or without sucrose up until 3 d of residue age, and then only continued to be effective at preventing larval development through 7 d of residue aging when combined with sucrose and not exposed to rainfall. The enhanced performance of bifenthrin when combined with sucrose persisted through 3 d of residue aging, and for 7 d when not subjected to rain.

Field Test of Phagostimulants to Protect Day-Neutral Strawberries. The middle portion of harvest had the highest numbers of spotted wing drosophila reared from fruit, and there were significant differences in the number of flies reared on different sampling dates ($F = 17.76$; df = 5, 100; $P < 0.001$). There was also a significant insecticide treatment effect ($F = 10.57$; df = 5, 20; $P < 0.0001$). Overall, the fewest flies were reared from fruit treated twice per week with Brigade WSB (an experimental program that would exceed allowed use for this product), although this was not statistically distinguishable from Brigade once per week or Entrust plus sucrose once per week. Entrust without sucrose was not statistically different from the untreated control and there were no differences between control plots and plots treated with water plus sucrose. There was a significant interaction between treatment and time ($F = 2.15$; df = 25, 100; $P = 0.004$), and so we present results for each sampling date included in the study (Fig. 7). For five of the six sample dates, there were fewer flies reared from fruit treated with Entrust plus sucrose than for the Entrust applied alone.

Field Test of Phagostimulants to Protect Blueberries. There were significant differences among treatments in the number of larvae recovered from blueberry samples ($F = 6.15$; df = 3, 9; $P = 0.015$; Table 3). The reduced risk program (alternating Assail with Delegate) plus sucrose was ranked as having the fewest larvae (76% reduction relative to the untreated check), followed by the conventional standard program (rotating Bifenture with Imidan, with 65% fewer larvae); both of these treatments significantly differed from the untreated check. The reduced risk program without sugar added as a phagostimulant had an intermediate number of larvae and did not significantly differ from either these treatments or the untreated check. While not statistically significantly different, addition of sucrose to the spray mixture reduced by 50% the number of larvae recovered from blueberry samples taken from plots in which Assail and Delegate were applied without sucrose.

Discussion

Spotted wing drosophila flies are sensitive to and able to detect relatively low concentrations of sucrose presented on surfaces in their environment. Their

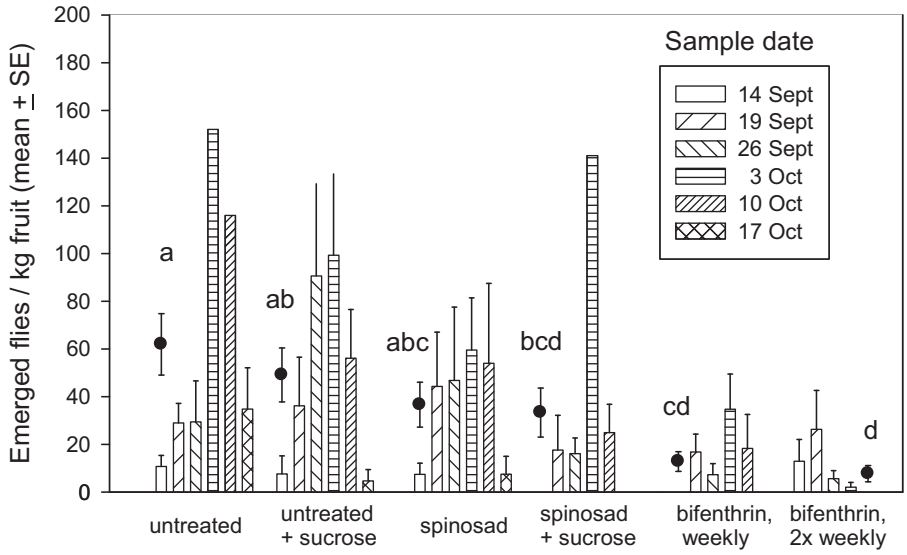

Fig. 7. Numbers of *D. suzukii* flies reared from day-neutral strawberry samples (46–172 g per sample) on six sampling dates in Geneva, NY, 2012. All insecticides sprays were applied weekly; the Brigade 2 × weekly was sprayed twice per week; details for the spray programs are given within the text. Means separations are not given for the individual sample dates (bars) as there were significant treatment × date interactions ($n = 5$). Averages for spray programs (filled circles) followed by the same letter do not significantly differ (Tukey HSD, $P < 0.05$, $n = 30$).

Table 3. Number of larvae reared from 470 ml samples of 'Bluecrop' blueberries following various 4-wk spray programs; $n = 4$.

Spray Program	Materials	Rate[a] a.i./ha	Dates applied	Spotted wing drosophila per sample[b,c] (mean ± SE)
Standard	Imidan	690 g	10 July, 22 July	8.7 ± 2.3 b
	Bifenture	110 g	16 July, 30 July	
Reduced risk	Delegate	120 g	10 July, 22 July	12.1 ± 8.1ab
	Assail	108 g	16 July, 30 July	
Reduced risk + phagostimulant	Delegate + sucrose	120 + 1,100 g	10 July, 22 July	6.1 ± 1.6 b
	Assail + sucrose	108 + 1,100 g	16 July, 30 July	
Untreated check	–	–	–	25.1 ± 6.1 a

[a] a.i., active ingredient.
[b] Total number of larvae and pupae.
[c] Means in the same column followed by different letters are significantly different (Tukey's HSD Test, $P \leq 0.05$).

feeding on these deposits provides an opportunity that we can exploit for increasing their exposure to insecticides directed to manage their populations. During this work, we had anticipated that those insecticides believed to perform best through ingestion (e.g., cyantraniliprole) would benefit more when presented in combination with sucrose than would pyrethroids (e.g., bifenthrin), which are known to act through contact (Waddill 1978, Dinter et al. 2008). Our experiments demonstrated that both contact insecticides and those acting principally through ingestion benefitted from the addition of sucrose as a phagostimulant.

All of the insecticides we tested affect the insect nervous system. The addition of sucrose to insect growth regulators may allow these products to be effective against spotted wing drosophila, even when requiring ingestion (De Clercq et al. 1995). However, it is likely that spotted wing drosophila adults incorporate taste while exploring their environment, or may accidentally ingest material during grooming behaviors, and so use of phagostimulants may not be essential for insecticides requiring ingestion to be effective.

Sugar added to an insecticide spray mixture is regulated in the United States as an adjuvant, a product that enhances the insecticide's performance. It is exempt from tolerance (U.S. Environmental Protection Agency [U.S. EPA] 2002) and is not itself insecticidal, and so it does not require use of a labeled product to be added to sprays. The intent in this work was to identify a low enough concentration that could inexpensively enhance the performance of insecticides while not presenting sucrose at a high enough concentration on plant surfaces to increase nontarget impacts for natural enemies or plant pathogens. We can anticipate nontarget effects for other dipterans, such as syrphids, which have sponging mouthparts and presumably also would exploit low but detectable surface residues of sucrose. Impacts from using sucrose with insecticides on syrphids, nondipteran natural enemies, and pollinators have not yet been investigated in fruit crops, but

such studies would be advisable. Also, the 0.1–0.3% concentration of sucrose used in foliar sprays can be anticipated to present at least a transient effect in stimulating fungal spores to germinate. Whether this will lead to additional infection of plants or fruits remains to be investigated. However, growers in New England had routinely adopted the addition of 2.4 g/liter (2 lb/100 gal) of sucrose with their insecticide sprays to target spotted wing drosophila in 2013, and did not report increased disease problems. Similarly, in our 2012 field study with day-neutral strawberries, we did not detect any increase in fruit rots associated with the addition of sugar.

While phagostimulants have a place for enhancing insecticide effectiveness in foliar sprays, their use may be essential for more complex behavioral manipulation strategies. Field tests of trapping in 2013 revealed that only 10–30% of flies contacting the outside of standard red cup traps subsequently entered and drowned, evident from the differences between traps without and with insecticides applied to the outside of traps (Hampton et al. 2014). Insecticides placed on the outside of attractant traps convert traps to an attract-and-kill system. It would be unfortunate for the same insecticides that are being used and are currently effective in foliar sprays to be overused by relying on these same products to kill flies visiting attractant traps. Use of phagostimulants with attractant traps broadens the array of potentially useful insecticides to target spotted wing drosophila to include products that require ingestion to be effective, such as boric acid (Xue and Barnard 2003), insect growth regulators (Casaña-Giner et al. 1999), or photoactive insecticides (Heitz 1997), and could enable advanced insecticide resistance management strategies involving synergistic insecticide mixtures, insecticide spatial mosaics, high dose strategies, entomopathogens, or products that otherwise would be too expensive to use in foliar and fruit sprays. This wider array of insecticides could target spotted wing drosophila adults in wooded habitat or at perimeters of fields, so that there would be no risk of exposure of fruit to insecticide residues.

When phagostimulants are used with attractant traps, the potential exposure to nontarget organisms should be greatly limited, and so much more potent phagostimulants than those suggested for foliar sprays could be deployed. The full array of phagostimulants could include the materials tested in this work (sucrose and low concentrations of sodium chloride), perhaps using sucrose at a very high concentration, as well as other "sweeteners" to which drosophilids may be more specifically tuned, such as glycerol and trehalose (Hallem et al. 2006).

The present work demonstrates that the addition of sucrose as a phagostimulant improves the activity of several insecticides to target spotted wing drosophila adults and improve protection of fruit from infestation. The enhancement in activity of several reduced risk insecticides, such as spinosyns, cyantraniliprole, and acetamiprid, provided equivalent or superior protection of blueberry and strawberry fruits when compared with application of conventional insecticides. Adoption of insecticide programs based on reduced risk insecticides applied with phagostimulants should reduce the broad spectrum environmental toxicity associated with the over reliance on organophosphates and pyrethroids for managing this pest.

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