Research Article

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Adding yeasts with sugar to increase the number of effective insecticide classes to manage *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in cherry

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Abstract

BACKGROUND: *Drosophila suzukii* is a major pest of cherry in the western United States. We evaluated whether the addition of sugary baits could improve the efficacy of two classes of insecticides not considered to be sufficiently effective for this pest, diamides and spinosyns, in laboratory and field trials in cherry.

RESULTS: Adding cane sugar alone or in combination with the yeasts *Saccharomyces cerevisiae* or *Aureobasidium pullulans* significantly improved insecticide efficacy. However, the significance of adding yeasts to the sugar plus insecticide on fly mortality varied with respect to both the insecticide and yeast species. The addition of *S. cerevisiae* to sugar also did not significantly reduce egg densities in fruit compared with sugar alone. The addition of a yeast plus sugar significantly reduced egg densities in three field trials with cyantraniliprole and in two out of three trials with spinosad.

CONCLUSION: The addition of cane sugar with or without yeast can improve the effectiveness of diamide and spinosyn insecticides for *D. suzukii* in cherry. Inclusion of these two insecticides in *D. suzukii* management programs may alleviate the strong selection pressure currently being imposed on a few mode-of-action insecticide classes used by growers to maintain fly suppression over long continuous harvest periods of mixed cultivars.

Keywords: spotted-wing drosophila; yeast; sugar; pest management

1 INTRODUCTION

Drosophila suzukii is a new pest in North America and Europe,[1,2] requiring strict management programs to allow growers to produce maggot-free fruit at harvest in a number of crops, including cherry, *Prunus avium* (L.).[3,4] Growers have been forced to adopt calendar spray programs to manage this pest and currently rely primarily on two classes of broad-spectrum insecticides, organophosphates and synthetic pyrethroids.[5] The effect of these new management programs (types, rates and timings of insecticide sprays) on the variable limits established for pesticide residues by different international trading partners can be problematic for some cherry growers to target certain export markets.[6]

The development of integrated pest management programs for *D. suzukii* has several aspects, including understanding its phenology, host range, overwintering and dispersal characteristics.[7] Populations of *D. suzukii* continue to build during the summer, with peak densities occurring after the latest summer harvest of cherries and berry crops.[8,9] The short generation time of *D. suzukii* and the exposure to insecticide residues over much of the summer likely promote the selection for insecticide resistance within managed fields.[10] However, the continuous reintroduction of susceptible flies from unmanaged habitats (refugia) outside orchards probably slows this selection pressure.[11] Nevertheless, the implementation of an effective resistance management strategy for *D. suzukii* should consider a rotation of insecticides with different modes of action.[12]

The key to developing management programs in tree fruits with an enhanced biological control component has been the growers' switch from broad-spectrum insecticides to the use of newer classes of insecticides, such as spinosyns and diamides.[13,14] Diamide insecticides are particularly safe for aphid predators of the black cherry aphid, *Myzus cerasi* (Fabricius), a major pest concern in

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young orchards.[15] However, the diamides chlorantraniliprole and cyantraniliprole were not found to be effective for *D. suzukii*.[5,16] Spinosyns in these same studies were shown to be able to inflict high levels of fly mortality, but were moderately effective when compared with a synthetic pyrethroid insecticide in reducing the numbers of eggs oviposited in fruit and subsequent survival of larvae and in suppressing fly population densities in field plots.[5] Thus, these two classes of insecticides have not been adopted by growers to manage *D. suzukii* in conventional cherry orchards. Conversely, spinosad is registered and widely used in certified organic cherry production owing to the lack of efficacious alternatives.

One option to increase the number of insecticide classes and use more selective chemistries in management programs for *D. suzukii* is to add feeding stimulants.[17,18] Adult drosophilids are strongly attracted to food cues provided by the simple microbial fermentation of sugars in fruits to acetic acid and ethanol.[19,20] Multicomponent synthetic blends that mimic the odor of active yeasts have been identified that are as attractive as the uncharacterized fermenting fruit substrates or the use of wine and vinegar for both *D. melanogaster* Meigen and *D. suzukii*.[21,22] Yeasts also serve as an important nutritional source for drosophilids, as well as influencing reproduction, fecundity and niche separation.[23–25]

The yeast *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Ascomycota, Saccaromycetes) in combination with sugar has been used effectively as bait in traps for *D. suzukii*.[3,26] The addition of brown cane sugar and *S. cerevisiae* to spinosad was shown to be more effective in killing adults and reducing the number of eggs laid on cherry than the use of a spinosad-laced protein bait spray applied as large droplets or the addition of a sugary protein bait in an aqueous solution in laboratory bioassays.[17] Recently, the addition of 1.2–2.4% sucrose alone was shown to improve the efficacy of a number of insecticides, including spinosad applied in highbush blueberry, *Vaccinium corymbosum* L., and strawberry, *Fragaria* × *ananassa* Duchesne.[18]

A survey of the natural yeast assemblage found to be associated with *D. suzukii* in cherry and raspberry (*Rubus* spp.) in California included 28 species.[27] The yeast *Hanseniaspora uvarum* (Niehaus) (Ascomycota, Saccaromycetes) was the most commonly isolated species in this study, and the authors suggested this might be a better candidate for selective monitoring of *D. suzukii* than the use of S. *cerevisiae*. The potential use of *H. uvarum* instead of *S. cerevisiae* as a feeding stimulant with adulticides for *D. suzukii* has not been reported.

Yeasts and yeast-like organisms are common inhabitants of the phyllosphere in fruit trees.[28] In an unrelated research project at the Washington laboratory, three common yeast species, *Metschnikowia pulcherrima* Pitt and Miller, *Cryptococcus tephrensis* Vishniac and the yeast-like fungus *Aureobasidium pullulans* (de Bary) Arnaud, were collected from codling moth, *Cydia pomonella* (L.), larval guts and feeding tunnels in apple and assessed as feeding stimulants with the use of the codling moth granulosis virus.[29–31] Based on the success of these studies with *C. pomonella*, all three yeast isolates in combination with sugar were also evaluated with *D. suzukii*.

Herein, we report the results of laboratory and field studies conducted over several years in cherry with the use of sugar, sugar with live yeasts and unpasteurized corn steep liquor as baits to improve the efficacy of spinosyn and diamide insecticides for *D. suzukii*. The latter material is marketed to both organic and conventional growers as an adjuvant to increase the effectiveness of insecticides, but has not been previously evaluated. Laboratory studies assessed the performance of these baits under controlled conditions. Field and field/laboratory studies were then conducted to assess the efficacy of the most efficacious baits that would be available for both organic and conventional cherry growers.

2 MATERIALS AND METHODS

2.1 Materials

Insecticides included two formulations of spinosad (IRAC Group 5) 800 g AI kg^{-1} (Entrust 80WP; Dow AgroSciences LLC, Indianapolis, IN) and 225 g AI L^{-1} (Entrust SC) and cyantraniliprole (IRAC Group 28) 102 g AI L^{-1} (Exirel; E.I. Dupont de Nemours, Co., Wilmington, DE). The treatment concentrations of active ingredients (AI) L^{-1} tested in our studies were 0.124 and 0.084–0.105 g for the WP and EC formulations of spinosad and 0.004–0.127 g for cyantraniliprole respectively. Cane sugar (C&H Dark Brown Cane Sugar, Domino Foods, Yonkers, NY) was tested at 3.6 g L^{-1}. Monterey Insect Bait (Brandt Inc., Fresno, CA), a non-stabilized 99.7% corn steep liquor, was used at 0.5% in all trials. *S. cerevisiae* was provided by Red Star (Milwaukee, WI) as a 454 g compressed cake with $6–10 \times 10^9$ cfu g^{-1} and was tested at 3.6 g L^{-1}.

Yeasts were isolated from laboratory-reared *D. suzukii* by streaking flies onto the surface of yeast extract peptone dextrose (YPD) agar plates (20 g L^{-1} of peptone, 20 g L^{-1} of glucose, 10 g L^{-1} of yeast extract, 20 g L^{-1} of agar) for 5 min. After 48 h at 25 °C, each colony-forming unit of unidentified species was replated several times to assess purity and to distinguish isolated colonies for identification. Three isolates that were common and morphologically homogeneous were subjected to genetic identification. These three isolates were identified as the yeast *H. uvarum* with standard methods for DNA isolation and sequencing of genes for domains 1 and 2 (D1/D2) of large subunit (LSU) rRNA and ITS.[32] The PCR products were purified using GeneJET PCR Purification kit (catalog number K0702; Fermentas, Glen Burnie, MD) and sequenced using PCR primers by MWG Operon (Ebersberg, Germany). The sequences were identified by comparison with the GenBank database of non-redundant sequences using BLAST.[33]

Four yeast species, including *H. uvarum*, *M. pulcherrima*, *C. tephrensis* and *A. pullulans*, were grown on a liquid YPD medium, and following centrifugation the density of yeast cells was estimated with a hemacytometer (EMS, Hatfield, PA) to be between 6×10^7 and 10^8 cells mL^{-1}. All four yeasts were tested in the laboratory at 3.6 g L^{-1}. Yeasts were stored at 5 °C up to 14 days prior to use.

Blossom Protect (Westbridge Ag Products, Vista, CA) is a 51.6% binary blend of two strains of the yeast-like fungus *Aureobasidium pullulans* (de Bary) (Ascomycota, Sordariomycetes) with $\geq 8.8 \times 10^8$ cfu g^{-1}, and is registered for control of fireblight *Erwinia amylovora* (Burrill) (Proteobacteria, Enterobacteriaceae). Blossom Protect was tested at 1.5 g (0.77 g AI) L^{-1} in the laboratory and in a 1:7 combination with Buffer Protect (Westbridge Ag Products), which includes citric acid and disodium phosphate as its active ingredients (65.4%), to maintain a pH of 3.67 in the field.

2.2 Laboratory bioassays

Studies were conducted in plastic containers evaluating the response of flies to dried residues placed either on the container alone, the fruit alone or on both substrates. These three bioassays allowed us to examine in the laboratory the relative activity of residues with or without the oviposition site and when distributed in discrete drops or uniformly within the test arena.

Flies were reared on a synthetic drosophilid diet (Carolina Biological, Burlington, NC). Flies (3–4 days old) were anesthetized with CO_2, sexed and added to containers with a fine-tipped brush. The general protocol for each of these assays was that three male and female flies were added to each container with a water wick. Studies were conducted at 24 °C. Fly mortality was recorded at 2 and 6 h after flies had recovered from the anesthesia. Cherries were either harvested from the unsprayed USDA Research Farm located near Moxee, Washington (46° 33′ N, 120° 20′ W), or were organic fruit purchased from local stores. All fruits were washed and dried prior to treatments being applied. Adult flies were removed after 48 h, and the numbers of eggs laid on fruit were determined with a microscope. Cherries were incubated for an additional 5 days after flies had been removed from containers. Larvae were extracted from macerated fruit with a standard 10% NaCl flotation method.[34] Larvae were counted and expressed as number per container.

The first bioassay was a series of dual comparisons with cyantraniliprole ($N = 40–105$) to compare adult mortality between two treatments at a concentration of 0.107 g AI L^{-1}. Five 10 µL droplets of each treatment were placed equidistant and 25 mm apart within individual 90 mm plastic petri plates and allowed to dry. Nine comparisons were made, including distilled water versus insecticide, insecticide alone versus the insecticide plus yeast and insecticide alone versus insecticide plus sugar. The remaining six tests compared the insecticide plus sugar versus the insecticide plus each of the five yeasts plus sugar, and the insecticide plus corn steep liquor.

The second bioassay was also designed to measure levels of fly mortality in dual treatment comparisons ($N = 20–108$). However, the experimental design was changed from the first bioassay to increase fly exposure to the insecticide treatments. These bioassays were conducted in 250 mL clear plastic cups (11 cm i.d.) with a single ripe 'Bing' cherry. The interior of the plastic cups and their lids were entirely sprayed with a 500 µL solution of individual treatments (Aerosol Spray Bottle; Thermo Scientific, Rochester, NY). Cherries were dipped in a stirred 500 mL beaker with each treatment for 3 s. Cups and fruit were air dried for >3 h before flies were added. Five and four comparisons were made with cyantraniliprole and spinosad respectively. With both insecticides, a distilled water control was compared with the insecticide alone, each insecticide alone was compared with insecticide plus sugar and insecticide plus sugar was compared with insecticide plus *S. cerevisiae* and sugar and insecticide plus corn steep liquor. In addition, cyantraniliprole plus sugar was compared with the insecticide plus *A. pullulans* and sugar.

A third type of laboratory bioassay was developed to reduce the contact exposure of flies to the insecticide before they contacted fruits. This bioassay was also conducted in 250 mL clear plastic containers with two dipped fruits, and the containers were not sprayed. Two different experiments were conducted with each insecticide. In the first, a water control was compared with the insecticide alone or in combination with sugar or sugar plus *S. cerevisiae*. The second experiment compared the water control with each insecticide alone and with sugar plus *S. cerevisiae*.

2.3 Field/laboratory studies

Studies were conducted in a 0.25 ha 'Bing' orchard at the USDA Research Farm from 2012 to 2014 and in a 0.44 ha 'Bing' orchard situated near Parker, Washington (46° 29′ N, 120° 26′ W), in 2013 and 2014. All studies were established as completely randomized designs with replicate single-tree plots ($N = 6–12$) separated by 10 m. Sprays in the Moxee orchard were applied at 689 kPa with a handgun sprayer equipped with a D-6 nozzle (GunJet, Model 43; TeeJet Technologies, Wheaton, IL). Sprays in the Parker orchard were applied with a backpack mist sprayer (SR200; STHL Inc., Norfolk, VA) with a maximum air velocity of 81 m s^{-1}. Sprays in both orchards were applied to individual trees, based on a timed calibration of spray in order to deliver treatments at 935 L ha^{-1}. A water control was included in the Parker orchard in 2013; otherwise, controls were untreated trees.

Treatments were applied each year, beginning when fruit turned straw color (near the beginning of June). Three fruits and two leaves were collected arbitrarily at 2.0–2.5 m height from around the perimeter of the canopy of each tree on each bioassay date and placed in an untreated 250 mL plastic cup with a lid. The same experimental protocol as that used in the laboratory bioassays was followed with regard to the number and age of flies added to the containers, length of assay, temperature and methods used to count eggs and larvae.

Samples of mature fruit were collected each year from orchards following the last bioassay to determine natural infestation levels of *D. suzukii* and the western cherry fruit fly, *Rhagoletis indifferens* Loew, within treatments. A total of 220 fruits in the Moxee orchard from 2012 to 2014 and in the Parker orchard in 2014 were arbitrarily sampled from the canopy of each tree replicate. All fruits uninjured by birds were harvested from trees in the Parker orchard in 2013 (mean numbers per treatment ranged from 110 to 182 per tree). Subsamples of 20 fruits from each replicate were macerated and floated in the salt solution to count the number of *D. suzukii* larvae in each replicate. The remaining fruits were placed on wire screens inside plastic totes and stored outdoors in a covered structure for 2–3 weeks. The number of *R. indifferens* pupae was then counted in each tote.

The study in 2012 was conducted in the Moxee orchard, with eight replicates of five treatments randomized in the block. Treatments included an untreated control, cyantraniliprole (0.106 g AI L^{-1}) alone and in combination with the commercial formulation of *A. pullulans* and sugar and spinosad (0.124 g AI L^{-1}, WP formulation) alone and in combination with *S. cerevisiae* and sugar. Treatments were applied on 11 and 21 June and on 2, 12 and 19 July. Six identical bioassays were conducted on 26 June and on 3, 10, 14, 17 and 21 July.

Two similar studies were conducted in 2013. Ten replicates of eight treatments were randomized within the Parker orchard. Treatments included a water control, spinosad (0.084 g AI L^{-1}, SC formulation) and cyantraniliprole alone at two rates (0.063 and 0.127 g AI L^{-1}) or in combination with either *S. cerevisiae* and sugar or corn steep liquor. The organosilicone adjuvant Silwet L-77 (Helena Chemical Co., Collierville, TN) was added at 0.03% to all sprays. Sprays were applied on 24 and 31 May and on 12 June. All fruits were collected from trees on 23–25 June. Owing to the low fruit set on some of the selected trees, six replicates of each treatment were used in the analysis. The second study was conducted in the Moxee orchard with 12 replicates of three treatments. Treatments included an untreated control and spinosad (0.084 g AI L^{-1}, SC formulation) alone and in combination with *S. cerevisiae* plus sugar. Sprays were applied on 30 May, on 10, 20 and 27 June and on 11 July, and bioassays were conducted on 28 June, 5 July and 12 July.

Studies were again conducted in both orchards during 2014. The study in the Parker orchard included seven replicates of three treatments. Treatments included an untreated control and spinosad (0.105 g AI L^{-1}, SC formulation) applied either alone or in combination with *S. cerevisiae* and sugar. Sprays were applied on

28 May and on 5, 10 and 17 June. Bioassays were run on 14, 19 and 23 June. The Moxee orchard study included 12 replicates of three treatments, including an untreated control and cyantraniliprole alone (0.127 g AI L^{-1}) and in combination with *S. cerevisiae* plus sugar. Sprays were applied on 12, 19 and 26 June and on 2 July. Bioassays were conducted on 24, 27 and 30 June and on 3 July.

2.4 Statistical analysis

The mean counts of eggs and larvae of *D. suzukii* from field/laboratory studies repeated on several dates each year were calculated and used in the subsequent analyses. These count data were transformed with square root [x], and only data from untreated control treatment sets could be normalized (Shapiro–Wilks test, Statistix 9; Analytical Software, Tallahassee, FL). Thus, datasets were analyzed with the non-parametric Kruskal–Wallis test of ranks. Treatments were not compared with the untreated controls owing to the severe lack of homogeneity among variances. Data from the water control were included in the 2013 Parker study to evaluate the efficacy of each insecticide against both pest species. An all-pairwise Mann–Whitney *post hoc* test was used to compare pairs of treatments when the Kruskal–Wallis test was significant, $P < 0.05$.[35] Data for the proportion of dead flies in the binary laboratory assays were compared with Fisher's exact test, $P < 0.05$.

3 RESULTS

3.1 Laboratory bioassays

Significant differences were found for the mean proportion of dead flies after 2 and 6 h in binary trials with cyantraniliprole alone and with the addition of several food baits (Table 1). Cyantraniliprole alone caused low levels of fly mortality in this bioassay, which was no different from the water control after 2 h and less than 10% after 6 h. The proportion of dead flies was <0.10 in each set of bioassays comparing cyantraniliprole alone versus cyantraniliprole in combination with each of the six yeasts without sugar, and no significant differences were found. Adding sugar to cyantraniliprole significantly increased its toxicity, threefold at 2 h and fivefold after 6 h. The further addition of either *S. cerevisiae* or the four laboratory-reared yeasts to the sugar did not increase fly mortality in these bioassays (Table 1). In contrast, the addition of the commercial formulation of *A. pullulans* with sugar significantly increased fly mortality (twofold compared with sugar alone). The addition of corn steep liquor to cyantraniliprole did not significantly increase fly mortality compared with the use of sugar.

Significant differences were found among treatments with food baits added in bioassays using sprayed cups with a single dipped fruit (Table 2). In general, levels of mortality were higher in this type of bioassay compared with the previous test using five 10 µL drops of insecticide plus bait (Table 1). Both insecticides caused significant increases in fly mortality compared with the water control. The addition of sugar to each insecticide also significantly increased fly mortality at both time periods. The addition of *S. cerevisiae* did not further increase the mortality level achieved with cyantraniliprole plus sugar. However, the addition of *S. cerevisiae* significantly increased the activity of spinosad with sugar. The addition of the commercial yeast *A. pullulans* with sugar significantly increased fly mortality compared with sugar after 2 h with cyantraniliprole, but this difference was not significant after 6 h. The addition of corn steep liquor with both insecticides was significantly less effective than the addition of sugar (Table 2).

Significant differences in the numbers of eggs and larvae were found among treatments in the four sets of bioassays conducted with cyantraniliprole- and spinosad-dipped cherries in unsprayed containers (Table 3). The addition of the sugar plus yeast bait significantly reduced both egg and larval counts with each insecticide. The addition of the yeast and sugar was only significantly more effective than the use of sugar alone with cyantraniliprole and not spinosad for egg density. However, larval density was significantly lower with the addition of yeast and sugar versus sugar with both insecticides. The only treatment among these four bioassays with zero larvae recovered was the use of spinosad with *S. cerevisiae* and sugar in bioassay 1 (Table 3).

3.2 Field/laboratory studies

In the Moxee orchard during 2012, the addition of the commercial formulation of *A. pullulans* with sugar to cyantraniliprole significantly reduced eggs and larvae compared with the insecticide alone. Similarly, the addition of *S. cerevisiae* and sugar to the WP formulation of spinosad significantly reduced egg and larval counts compared with spinosad alone. *R. indifferens* pupae were only recovered from harvested fruit in the untreated control [mean (SE) = 0.5 (0.5) per 100 fruits].

The mean proportion of cherries injured by *D. suzukii* in the Parker orchard in 2013 was not significantly different between the water control and spinosad alone, the two rates of cyantraniliprole alone and cyantraniliprole plus the corn steep liquor bait (Table 4). The higher rate of cyantraniliprole with both *S. cerevisiae* and sugar or the corn steep liquor had significantly fewer *D. suzukii* larvae than the water control. Although larval recovery of *D. suzukii* was reduced by >80% with the addition of these baits compared with the high rate of cyantraniliprole alone, means were not significantly different among treatments. The addition of *S. cerevisiae* and sugar to the lower rate of cyantraniliprole significantly reduced the number of *D. suzukii* larvae recovered compared with the insecticide alone.

All cyantraniliprole treatments significantly reduced levels of fruit injury by *R. indifferens* compared with the water control (Table 4). All cyantraniliprole treatments except the low rate plus *S. cerevisiae* and sugar had significantly lower levels of infestations of *R. indifferens* than the spinosad treatment. The effectiveness of cyantraniliprole for *R. indifferens* was not significantly increased with the addition of the food baits at either insecticide rate in this study. However, the only treatment in this study that did not have any *R. indifferens* pupae recovered from harvested fruit was the high rate of cyantraniliprole plus *S. cerevisiae* with sugar (Table 4).

The effectiveness of the seasonal spray program of spinosad in the Moxee orchard during 2013 was similar to the 2012 results, in spite of the change in the insecticide's formulation (Table 5). The addition of *S. cerevisiae* and sugar significantly improved the insecticide's performance. Mean counts of *D. suzukii* eggs and larvae were decreased 73 and 54% with the bait added compared with a 97 and 98% reduction found the previous year. *R. indifferens* pupae were only recovered from harvested fruits in the untreated control [mean (SE) = 1.0 (0.8) per 100 fruits].

The effectiveness of adding *S. cerevisiae* and sugar to spinosad was less in the Parker orchard in 2014 than it had been in the Moxee orchard the previous year (Table 5). The addition of *S. cerevisiae* with sugar significantly reduced the densities of eggs but not larvae compared with the insecticide alone (67 and 45% reduction respectively). The addition of *S. cerevisiae* and sugar to cyantraniliprole also significantly reduced the density of eggs and larvae in bioassays conducted with Moxee fruit and foliage in 2014

Table 1. Mean proportions of adult *D. suzukii* mortality after 2 and 6 h in paired bioassays with water, the diamide insecticide cyantraniliprole (IC) alone and the insecticide mixed with one or more food baits in plastic petri plates with five equidistant 10 µL drops

Treatment comparisons[a]		Replicates	Mean proportion of dead flies[b] after 2 h	Mean proportion of dead flies[b] after 6 h
Water	I_C	90	0.03 versus 0.05, n.s.	0.05 versus 0.08*
I_C	I_C + sugar	105	0.05 versus 0.17***	0.08 vs. 0.43***
I_C + sugar	I_C + *Sc*/sugar	45	0.18 versus 0.22, n.s.	0.42 versus 0.43, n.s.
I_C + sugar	I_C + *Hu*/sugar	65	0.19 versus 0.16, n.s.	0.46 versus 0.44, n.s.
I_C + sugar	I_C + *Ap*/sugar	25	0.20 versus 0.23, n.s.	0.47 versus 0.50, n.s.
I_C + sugar	I_C + *Mp*/sugar	25	0.20 versus 0.27, n.s.	0.47 versus 0.58, n.s.
I_C + sugar	I_C + *Ct*/sugar	25	0.20 versus 0.16, n.s.	0.47 versus 0.49, n.s.
I_C + sugar	I_C + *Ap**/sugar	40	0.13 versus 0.25***	0.36 versus 0.54***
I_C + sugar	I_C + CSL	40	0.06 versus 0.13, n.s.	0.26 versus 0.28, n.s.

[a] Cyantraniliprole (I_C) was applied at 0.107 g AI L^{-1}. Brown cane sugar was added at 3.6 g L^{-1}. Five yeasts, *Saccharomyces cerevisiae* (*Sc*), *Hanseniaspora uvarum* (*Hu*), *Aureobasidium pullulans* (*Ap*), *Metschnikowia pulcherrima* (*Mp*) and *Cryptococcus tephrensis* (*Ct*), were tested at 3.6 g L^{-1}. A commercial formulation of *A. pullulans* (*Ap**) was tested at 0.77 g L^{-1}. A commercial corn steep liquor (CSL) formulation was added at a 0.5% rate.
[b] Data were analyzed with Fisher's exact test with two proportions: 'n.s.' denotes a non-significant comparison of proportions, $P > 0.05$; * denotes $P < 0.05$; ** denotes $P < 0.01$; *** denotes $P < 0.001$.

Table 2. Mean proportions of adult *D. suzukii* mortality after 2 and 6 h in paired bioassays with water, the insecticides cyantraniliprole (I_C) and spinosad (I_S) alone and insecticides mixed with one or more food baits in insecticide-sprayed deli cups with a single insecticide-dipped cherry fruit

Treatment comparisons[a]		Replicates	Mean proportion of dead flies[b] after 2 h	Mean proportion of dead flies[b] after 6 h
Water	I_C	108	0.02 versus 0.17***	0.03 versus 0.39***
I_C	I_C + sugar	55	0.14 versus 0.38***	0.31 versus 0.78***
I_C + sugar	I_C + *Sc*/sugar	30	0.37 versus 0.41, n.s.	0.58 versus 0.65, n.s.
I_C + sugar	I_C + *Ap**/sugar	40	0.38 versus 0.55**	0.79 versus 0.84, n.s.
I_C + *Sc*/sugar	I_C + CSL	20	0.53 versus 0.24***	0.78 versus 0.63**
Water	I_S	93	0.01 versus 0.26***	0.01 versus 0.73***
I_S	I_S + sugar	58	0.23 versus 0.45***	0.69 versus 0.84***
I_S + sugar	I_S + *Sc*/sugar	50	0.42 versus 0.52*	0.74 versus 0.82*
I_S + *Sc*/sugar	I_S + CSL	20	0.83 versus 0.45***	0.98 versus 0.94, n.s.

[a] The insecticides cyantraniliprole (I_C) and spinosad (I_S) were applied at 0.107 and 0.124 g AI L^{-1} respectively. Brown cane sugar was added at 3.6 g L^{-1}. The yeasts *Saccharomyces cerevisiae* (*Sc*) and the commercial formulation of *Aureobasidium pullulans* (*Ap**) were tested at 3.6 and 0.77 g L^{-1} respectively. A commercial corn steep liquor (CSL) formulation was added at a 0.5% rate.
[b] Data were analyzed with Fisher's exact test with two proportions: n.s. denotes a non-significant comparison of proportions, $P > 0.05$; * denotes $P < 0.05$; ** denotes $P < 0.01$; *** denotes $P < 0.001$.

(Table 5). No *D. suzukii* larvae were recovered from harvested fruit in either orchard across all treatments. Also, no *R. indifferens* were recovered from fruit collected in the Parker orchard. *R. indifferens* pupae in the Moxee orchard were only recovered from the unsprayed treatment [mean (SE) = 1.4 (0.4) per 100 fruits].

4 DISCUSSION

Many growers of high-value fruit crops, such as cherry, in the United States are now required to use calendar-based prophylactic insecticide spray programs to protect their crop from attack by *D. suzukii*. To avoid an annual retreading on the 'pesticide treadmill', cherry growers need more effective management strategies formulated around an integrated approach, including both cultural and insecticidal tactics. Practices that can minimize the occurrence and proximity of extra-orchard sources of *D. suzukii*[36] and the post-harvest retention of host material within orchards are key considerations to achieve and maintain pest suppression. The concept of planting early-season cultivars and reducing the occurrence of contiguous fields of hosts with overlapping harvest dates has been suggested as a management practice for *D. suzukii*, and this may be possible to achieve for some growers.[37] Hampton *et al.*[37] suggest that the interception of flies outside the orchard via mass trapping and attract-and-kill tactics may have promise, but both strategies likely require better sanitation to reduce the size of potential immigrant populations, and the use of more effective baits.

The relative efficacy of different classes of insecticides for *D. suzukii* across a number of crops, including cherry, has been reported in both laboratory and field trials.[5,16,38] The most toxic materials for *D. suzukii* in these studies have been the synthetic pyrethroids, carbamates and organophosphates. Spinosad is the most effective insecticide for certified organic orchards.[38] Spinosad has been effective in killing flies but somewhat less effective in reducing the numbers of eggs deposited on and flies produced from sprayed fruits compared with synthetic

Table 3. Mean numbers of eggs and larvae of *D. suzukii* from dipped cherry fruit in unsprayed containers in a series of laboratory bioassays with cyantraniliprole (I_C) and spinosad (I_S) alone and with either sugar or the yeast *S. cerevisiae* (*Sc*) plus sugar added

Bioassay number (number of replicates)	Treatment[a]	Mean (SE) number per container[b]	
		Eggs	Larvae
1 ($N = 104$)	I_S + sugar	0.19 (0.07)	0.04 (0.02) a
	I_S + *Sc*/sugar	0.11 (0.05)	0.00 (0.00) b
ANOVA		$H = 0.68$, $P = 0.41$	$H = 4.29$, $P < 0.05$
2 ($N = 75$)	I_S	1.25 (0.22) a	0.53 (0.14) a
	I_S + *Sc*/sugar	0.57 (0.13) b	0.16 (0.06) b
ANOVA		$H = 7.83$, $P < 0.01$	$H = 6.48$, $P < 0.05$
3 ($N = 54$)	I_C + sugar	0.33 (0.26) a	0.13 (0.05) a
	I_C + *Sc*/sugar	0.17 (0.08) b	0.04 (0.02) b
ANOVA		$H = 4.19$, $P < 0.05$	$H = 6.95$, $P < 0.01$
4 ($N = 60$)	I_C	2.35 (0.60) a	0.75 (0.25) a
	I_C + *Sc*/sugar	0.85 (0.48) b	0.40 (0.21) b
ANOVA		$H = 13.32$, $P < 0.001$	$H = 5.77$, $P < 0.05$

[a] The insecticides cyantraniliprole (I_C) and spinosad (I_S) were applied at 0.107 and 0.124 g AI L^{-1} respectively. Sugar and *S. cerevisiae* were added at 3.6 g L^{-1}. The mean (SE) number of eggs and larvae per container in the untreated controls was 12.9 (1.1).
[b] Column means within each experiment followed by a different letter were significantly different, $P < 0.05$.

Table 4. Mean levels of cherry fruit injury from *D. suzukii* and *R. indifferens* across seasonal treatments of a water control, spinosad (I_S) and two rates of cyantraniliprole (I_C) alone and with baits in the Parker orchard, 2013, $N = 6$

Treatment[a] (insecticide rate, g AI L^{-1})	Mean (SE) number per 100 fruits[b]	
	D. suzukii	*R. indifferens*
Water control	3.5 (0.6) ab	14.5 (5.3) a
I_S (0.105)	6.0 (1.7) a	5.3 (2.1) ab
I_C (0.064)	5.5 (1.2) a	0.1 (0.1) c
I_C (0.064) + *Sc*/sugar	0.9 (0.5) bc	0.7 (0.4) bc
I_C (0.064) + CSL	5.4 (1.7) a	0.1 (0.1) c
I_C (0.127)	2.0 (0.6) abc	0.2 (0.1) c
I_C (0.127) + *Sc*/sugar	0.4 (0.3) c	0.0 (0.0) c
I_C (0.127) + CSL	0.3 (0.2) c	0.2 (0.1) c
ANOVA	$H = 9.13$, $P < 0.0001$	$H = 13.17$, $P < 0.0001$

[a] Treatments included a water control, the insecticides spinosad and cyantraniliprole alone and cyantraniliprole at two rates in combination with the yeast *S. cerevisiae* with sugar or with corn steep liquor (CSL). Silwet L-77 (0.03%) was added to all sprays. Sprays were applied on 24 and 31 May and on 12 June, and fruits were harvested on 23–25 June 2013.
[b] Column means followed by a different letter were significantly different, $P < 0.05$.

pyrethroids.[5] The performance of the diamide insecticides chloroantraniliprole and cyanotraniliprole for *D. suzukii* adults has been poorer than that of the more broad-spectrum classes of insecticides.[5,16] However, cyanotraniliprole has been relatively effective in reducing the numbers of surviving larvae from treated fruits, in spite of often high levels of egg deposition.[5,18]

Efforts to improve the effectiveness of spinosad and diamide insecticides have included increasing their labelled application rates and adding a feeding stimulant. Cowles *et al.*[18] evaluated the addition of sucrose with spinosad, cyantraniliprole and several other insecticides. It is presumed that the sugar stimulates fly feeding and uptake of the insecticide. Among nine field/laboratory bioassays with blueberry foliage and fruit (three field-aged residues evaluated at three time periods), the addition of sucrose significantly increased fly mortality with cyantraniliprole in two tests. Also, the addition of sucrose did not significantly increase fly mortality with spinosad on grape foliage or the numbers of emerged flies from treated strawberry. Among the seven other insecticides evaluated, the addition of sucrose significantly increased fly mortality in at least half of the assays only with acetamiprid and bifenthrin, and only significantly reduced the numbers of larvae produced per fruit with fenpropathrin.[18] Thus, the addition of sugar alone may not be adequate substantially to improve insecticide activity or add new classes of insecticides to manage *D. suzukii*. Instead, a more complex bait utilizing yeast and sugar may have greater potential to enhance insecticides.

The coevolution and mutualistic interactions of drosophilids and yeasts have been studied in various natural and managed ecosystems, and findings suggest that the use of yeasts should be considered.[24,39] Yeasts are ubiquitous in orchards utilizing sugary food sources provided by ripe and overripe fruit,[40] and are major protein sources for drosophilid larval development.[41] Yeast volatiles are important sensory cues for drosophilids and have been shown to mediate attraction, oviposition and larval development.[21] Thus, yeast baits using *S. cereviase* are often used to monitor *D. suzukii*.[26,37] Interestingly, our results suggest that significant variability may exist among yeast species in their ability to enhance the toxicity of sugary insecticide baits.

Previously, the yeast *S. cerevisiae* was selected to improve the activity of spinosad because it is the most common commercially available yeast and thus would be the least expensive for growers to use in pest management.[17] This two-component bait significantly increased fly mortality and reduced numbers of eggs and larvae in cherry bioassays compared with spinosad alone (60 and 71% respectively).[17] In the present study, the addition of *S. cerevisiae* with sugar to spinosad significantly increased fly mortality and reduced larval density in fruit compared with the addition of sugar only, but the density of eggs laid was not significantly reduced. Similarly, the addition of *S. cerevisiae* to sugar provided some improvement in the efficacy of cyantraniliprole plus sugar.

Table 5. Mean counts of eggs and larvae of *D. suzukii* with the insecticides cyantraniliprole (I_C) and spinosad (I_S) sprayed alone or in combination with the yeasts *S. cerevisiae* (*Sc*) or *A. pullulans* (*Ap*) with sugar in field/laboratory bioassays with field-sprayed cherry fruit and foliage from 2012–2014

Bioassay number, year, orchard (number of replicates)	Treatments[a]	Mean (SE) count per cup[b]	
		Eggs	Larvae
1 2012, Moxee, ($N = 8$)	I_C	1.74 (0.45) a	1.50 (0.41) a
	I_C + *Ap**/sugar	0.02 (0.29) b	0.00 (0.00) b
	I_S	2.10 (0.36) a	1.71 (0.31) a
	I_S + *Sc*/sugar	0.06 (0.06) b	0.04 (0.04) b
	ANOVA	$H = 8.26$, $P < 0.001$	$H = 9.25$, $P < 0.001$
2 2013, Moxee ($N = 12$)	I_S	1.23 (0.78) a	0.54 (0.31) a
	I_S + *Sc*/sugar	0.33 (0.33) b	0.25 (0.13) b
	ANOVA	$H = 6.45$, $P < 0.05$	$H = 10.40$, $P < 0.001$
3 2014, Parker ($N = 7$)	I_S	2.71 (0.61) a	0.95 (0.27)
	I_S + *Sc*/sugar	0.90 (0.37) b	0.52 (0.25)
	ANOVA	$H = 5.70$, $P < 0.05$	$H = 2.28$, $P = 0.14$
4 2014, Moxee ($N = 12$)	I_C	1.77 (0.41) a	0.03 (0.02) a
	I_C + *Sc*/sugar	0.12 (0.07) b	0.00 (0.00) b
	ANOVA	$H = 23.30$, $P < 0.0001$	$H = 4.27$, $P < 0.05$

[a] Cyantraniliprole (I_C) was applied at 0.106 g AI L^{-1} in 2012 and 0.127 g AI L^{-1} in 2013 and 2014. Spinosad (I_S) was applied as a WP formulation at 0.124 g AI L L^{-1} in 2012 and as a SC formulation at 0.084 g AI L^{-1} in 2013 and 0.105 g AI L^{-1} in 2014. Sugar and the yeast *S. cerevisiae* were added at 3.6 g L^{-1} in all three years. A commercial formulation of the yeast *A. pullulans* (*Ap**) was applied at 1.5 g (0.77 g AI) L^{-1} in combination with 10.5 g L^{-1} of Buffer Protect and 3.6 g L^{-1} of sugar in 2012.
[b] Column means within each year–orchard study followed by a different letter were significantly different, $P < 0.05$. The mean (SE) number of eggs and larvae per container in the untreated controls across the four sets of bioassays was 32.0 (2.7), 55.4 (9.4), 22.4 (2.7) and 39.0 (2.6) eggs, and 24.6 (2.2), 16.6 (6.0), 16.1 (2.6) and 12.8 (2.0) larvae respectively.

However, we anticipated that other yeasts combined with sugar could further enhance these insecticides.

Isolation of yeasts from adults and larvae of *D. suzukii* and from infested and uninfested cherry fruits found a number of yeast species; *H. uvarum* was the most common species, and the only one identified from both life stages and from both types of fruit.[27] Interestingly, *H. uvarum* was also the most common yeast collected from adult flies sampled from our laboratory colony. In spite of the close association of *H. uvarum* with *D. suzukii*, its addition with sugar in our bioassays did not increase the activity of cyantraniliprole in our laboratory bioassays.

We also evaluated the potential of using an available yeast-like microbe, *A. pullulans*, to enhance the activity of selected insecticides. The commercial formulation of *A. pullulans* (Blossom Protect) is marketed for control of fireblight, caused by *Erwinia amylovora* (Burrill), in pear and apple. *A. pullulans* was not collected from adult or larval *D. suzukii* in cherry in California,[27] or from adults in our laboratory colony. However, *A. pullulans* is rather ubiquitous in many habitats,[42] and was isolated from both maggot-infested and uninfested cherry fruits.[27] Unlike with *S. cerevisiae*, the addition of the commercial formulation of *A. pullulans* to sugar significantly increased fly mortality with cyantraniliprole. In comparison, we did not see any activity when our laboratory-reared isolate of *A. pullulans* was added. We decided not to test the commercial isolate of *A. pullulans* with spinosad because this product would not be available for organic growers. Also, because the price of the commercial product when used for fire blight control is relatively high, its repeated application in cherry to improve the control of *D. suzukii* would likely be cost prohibitive, and we discontinued our testing after 2012.

Owing to its commercial availability, we evaluated corn steep liquor, a byproduct of the corn wet-milling industry.[43] The liquor is an unstabilized brew including proteins and simple and complex carbohydrates (15% w/w), with the monosaccarides glucose and fructose accounting for nearly 75% of this total.[43] The liquor is not stabilized, and changes in its color and odor occurred during storage (5 °C) in our laboratory. The attractiveness of corn steep liquor for adult *D. suzukii* changed significantly over a 2 year period in storage at 20–25 °C, likely owing to microbial activity (Knight AL, unpublished data). Surprisingly, while corn steep liquor can be an effective attractant for *D. suzukii* when placed in traps, its addition did not increase the toxicity of either insecticide in our trials.

The additional cost of adding feeding stimulants to insecticides should be reasonable to growers and accounted for by either improved control or reduced rates of insecticide, while not interfering with other management issues. We established here that the addition of *S. cerevisiae* and sugar can significantly improve control of *D. suzukii* with either spinosad or cyantraniliprole. Secondly, the addition of *S. cerevisiae* with sugar significantly reduced the effective rate of cyantraniliprole. Whether the addition of this bait could reduce the effective rate of spinosad was not tested because spinosad at the full rate seemed to be less effective than cyantraniliprole, and at this rate it was also not very effective for *R. indifferens*. Conversely, cyantraniliprole was effective for *R. indifferens*, and adding the bait did not affect this level of control.

Grower adoption of the use of yeast and sugar baits can also be fueled by improved management of other non-dipteran pests in the orchard. The initial premise for combining yeasts and sugar together to improve cherry pest management was multifaceted, and we hypothesized that the control of insects, plant pathogens and birds could all be improved.[17] Antagonistic yeasts, such as *S. cerevisiae* and *A. pullulans*, do not always provide commercially acceptable pre- or post-harvest control of fruit disease when used alone,[44,45] but their application can help to suppress inoculum levels and reduce the potential for the development of fungicide resistance.[45,46] *D. suzukii* has an unusual biology among

drosophilids, as females oviposit in ripening fruit using a serrated ovipositor. Oviposition, larval development, pupation and fly emergence in cherry can release fruit juices rich in sugar and could promote secondary disease infections to occur in the clustered fruits. This would further increase the economic loss incurred by the grower.[3] We hypothesized that the use of yeast and sugar baits could have a second beneficial effect by protecting fruit from fruit rot organisms.[17]

Cherry growers in the Pacific Northwest industry have begun to use cane sugar for arthropod pest management.[17] These growers are making multiple applications of cane sugar (1–3%) to repel birds from orchards beginning a few weeks before and through harvest. Sprays in the Pacific Northwest primarily target American robins, *Turdus migratorius* L., and common starlings, *Sturnus vulgaris* L., which are both sickened if they ingest food with high concentrations of sucrose owing to their lack of the sucrase enzyme.[47,48] Thus, many cherry orchards are being treated with concentrations of sugar much higher than those that have been evaluated for *D. suzukii*.[17,18] For example, laboratory studies found a significant increase in *D. suzukii* adult mortality with sucrose concentrations as low as 0.1% added to spinosad.[18] Our laboratories have evaluated either 0.125 or 0.375% concentrations of sugar and have primarily used the higher rate in our trials.[17] The potential interactions of the use of sugar for bird management on the efficacy of insecticide programs for either *D. suzukii* or *R. indifferens* have not been evaluated. The effect with which growers were initially most concerned when applying ≥1% sugar for bird management – sticky fruit with the potential for increased fruit rot – has either not occurred or has not been a constraint hindering adoption of this practice. The ecological impacts of sugar applications to cherry canopies on the competitive interactions of pathogenic fungi and antagonistic yeasts have not yet been considered. The impact of various agrichemicals, including fungicides and sunburn protectants but not sugar, on the phyllosphere in apple have been detailed.[49–52] The ultimate effect of this sugary stimulation on yeast communities in cherry and the potential impacts on *D. suzukii* management should be addressed.

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