

Exploring the Efficacy and Mechanisms of a Crop Sterilant for Reducing Infestation by Spotted-Wing *Drosophila* (Diptera: Drosophilidae)

Steven Van Timmeren,^{1,✉} Philip D Fanning,^{1,✉} Torsten Schöneberg,² Kelly Hamby,² Jana Lee,^{3,✉} and Rufus Isaacs^{1,4}

¹Department of Entomology, Michigan State University, 202 CIPS, 578 Wilson Road, East Lansing, MI 48824, ²Department of Entomology, University of Maryland, College Park, MD 20742, ³USDA-ARS Horticultural Crops Research Unit, Corvallis, OR 97331, and ⁴Corresponding author, e-mail: isaacsr@msu.edu

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Abstract

Vinegar flies (Diptera: Drosophilidae) are well known to be associated with yeasts, which provide important nutrients and emit attractive semiochemicals. *Drosophila suzukii* (Matsumura) has become a major pest of berries and cherries around the world, requiring intensive management to maintain fruit quality. Although insecticides remain a dominant control approach, disruption of fly–yeast–host interactions remains a promising avenue for reducing the economic impact of this pest. We conducted field and laboratory experiments to explore whether a crop sterilant (peroxyacetic acid and hydrogen peroxide) developed for disease control can affect *D. suzukii*. In 2 yr of field tests in highbush blueberries, we found significantly lower infestation by *D. suzukii* in plots treated with the crop sterilant, both alone and in a rotation program with zeta-cypermethrin. When shoots from treated plots were tested in no-choice bioassays, crop sterilant treatments did not affect adult mortality or oviposition, but they reduced infestation. To explore the mechanisms in the laboratory, we found that the crop sterilant did not affect adult mortality, nor oviposition on treated fruit under no-choice settings, but adult flies settled and oviposited less on treated fruit in choice settings. When the crop sterilant was applied to colonies of *Hanseniaspora uvarum* (Niehaus) (Saccharomycetales: Saccharomycodaceae) and *Issatchenkia terricola* (Van der Walt) (Saccharomycetales: Saccharomycetacea) yeasts that are attractive and provide nutrition to *D. suzukii*, there was a dose-dependent inhibition of their growth. We highlight the potential for microbial management as a component of integrated pest management programs and prioritize research needs to incorporate this approach into control programs.

Key words: yeast, integrated pest management, microbial control, peroxyacetic acid, hydrogen peroxide

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), has been present in North America for more than a decade (Asplen et al. 2015), and management in commercial fruit systems continues to be challenging (Del Fava et al. 2017, Farnsworth et al. 2017). Growers have adapted to this pest primarily by increasing the number of insecticide applications to fields (Van Timmeren and Isaacs 2013, Rodriguez-Saona et al. 2019). Current management practices are not economically (Goodhue et al. 2011, Farnsworth et al. 2017) or environmentally (Lee et al. 2019) sustainable over the long term, and organic producers face an even greater challenge as limited effective insecticide options are available (Van Timmeren and Isaacs 2013), requiring greater adoption of cultural approaches for control. Spinosyn is currently the most effective insecticide available for organic production, but recent detections of resistance to this insecticide in California (Gress and Zalom 2018) highlight the need for alternative approaches to control this pest.

Many insects have strong associations with yeasts (Vega and Blackwell 2005), and *Drosophila* spp. feed on yeasts (Phaff et al. 1956) and transmit them to their host substrates (Gilbert 1980). In one study, Morais et al. (1994) isolated 28 yeast species from the external surface of *Drosophila serido* (Vilela and Sene) and 18 species, including some not found on external surfaces, internally from their crop. This example is not unique, with numerous other drosophila species exhibiting strong associations with yeasts in both the adult and larval stages (Shihata and Mrak 1952, Fogleman et al. 1982, Starmer et al. 1986, Morais et al. 1994). The association and importance of yeasts for growth and reproduction of *D. suzukii* has been highlighted recently (Harris and Shaw 2014, Bellutti et al. 2018, Lewis and Hamby 2019), and *Hanseniaspora uvarum* (Niehaus) (Saccharomycetales: Saccharomycodaceae) predominates in isolations from larval frass and adult alimentary canals

(Hamby et al. 2012, Lewis et al. 2019). Yeast volatiles are highly attractive to *D. suzukii* (Scheidler et al. 2015), and yeast mixed with sugar and other fermenting media is used in monitoring traps for the species (Burrack et al. 2015, Huang et al. 2017, Jaffe et al. 2018). Because yeasts affect *D. suzukii* fitness and attraction, control strategies that disrupt fungal communities have the potential to reduce *D. suzukii* fruit infestation.

Peroxyacetic acid is the product of a reaction between hydrogen peroxide and acetic acid and is commonly used as a postharvest sanitizer to control pathogens such as bacteria, molds, and yeasts that colonize the surface of fruits and vegetables (Rodgers et al. 2004, Bachelli et al. 2013, Van de Velde et al. 2016). In addition, peroxyacetic acid is effective at controlling diseases such as *Botrytis* in tomato (Ayoub et al. 2018) and sour rot in grapes (Hall et al. 2018) in field settings. Hydrogen peroxide is also effective as an antimicrobial sanitizer and is used in a similar manner as peroxyacetic acid (Oliveira et al. 2018). Both of these products are marketed for reducing fungal infections, with a benefit that very little residue is left on the surface of fruits and vegetables after the product is applied, and what little remains has negligible human health risks (Mahajan et al. 2014, Feliziani et al. 2016). Because of this, several commercial products are now available that can be used in organically certified production systems. One such product is Jet-Ag (Jet Harvest Solutions, Longwood, FL) which is a mixture of peroxyacetic acid (4.9% v/v) and hydrogen peroxide (26.5% v/v), marketed as a crop sanitizer. Three recent studies (Roubos et al. 2019a,b; Sial et al. 2019) tested two peroxyacetic acid/hydrogen peroxide (PAA-HP) products as part of larger studies into the effectiveness of organic insecticides, phagostimulants, and adjuvants against *D. suzukii*. These laboratory-based studies found PAA-HP to reduce infestation of berries by *D. suzukii* in some assays, but the results were inconsistent and did not explore the mode of action for products that were tested. Therefore, there is still a need to evaluate the efficacy of PAA-HP in replicated field trials and to better understand the mode of action of this product.

The objectives of this study were to evaluate a commercial peroxyacetic acid/hydrogen peroxide crop sanitizer product in a replicated field setting for its potential to control *D. suzukii*. In addition, we explored the potential mechanisms for the effectiveness of crop sanitizers against this pest. In particular, we evaluated effects on 1) adult and egg survivorship, 2) propensity of adults to settle on fruit, 3) sublethal effects on oviposition and success of offspring, and 4) growth of yeasts.

Materials and Methods

Field Efficacy Trials

Replicated trials were set up in highbush blueberry, *Vaccinium corymbosum* L., during the summers of 2017 and 2018 at the Trevor Nichols Research Center in Fennville, MI. Rows within this planting contained 12 bushes spaced 1.2 m apart, with black weed fabric

under the bushes, and maintained with a 0.8-m herbicide strip on either side of the row. Four replicates of three half-row plots were set up in a 'Bluecrop' variety section of this planting. Spray treatments were applied to both sides of all rows in each plot using an FMC 1029 airblast sprayer calibrated to deliver 468 liters of water per hectare. Treatment applications were first applied in mid-July of each year (18 July 2017 and 13 July 2018) and ended in mid-August of each year (8 August 2017 and 10 August 2018). In 2017, a commercial PAA-HP product (Jet-Ag, 4.9% peroxyacetic acid and 26.5% hydrogen peroxide, applied at a 1% v/v rate) was tested as a stand-alone treatment, and compared with a conventional insecticide rotation, as well as a zeta-cypermethrin/PAA-HP/PAA-HP rotation (Table 1). In the conventional insecticide rotation, weekly applications were made of zeta-cypermethrin, methomyl, and then phosmet. The same treatments were tested in 2018, but with one additional treatment where PAA-HP was applied twice a week.

Infestation was assessed using a filter salt test method (Van Timmeren et al. 2017) using a strong salt solution to extract larvae from ripe fruit samples collected once a week. Ripe fruit samples (89–148 ml) were collected from the exterior and interior of bushes from the middle row within each plot and the total number of small, medium, and large larvae in the filtered sample were counted under a microscope for each sample. Fruit samples were collected the day before or immediately prior to the next scheduled weekly spray. In addition, berry samples were collected from the ground directly under bushes within each plot because these berries reflect the performance of the applications applied the week before. One ground sample was collected near the end of the trial in 2017 (8 August) and two samples were collected in 2018 (10 and 13 August) and assessed for larvae using the same method.

In 2018, additional bioassays were conducted during the trial. Foliage and fruit bioassays, as well as fruit only bioassays, were conducted by collecting fruit and foliage on 1 August, 1 d after treatments were applied to plots. Foliage and fruit bioassays were set up by collecting five ripe berries and shoots containing 10 leaves and placing them in water picks inside 0.95-liter clear plastic deli cups (see Van Timmeren and Isaacs 2013). Twelve adult *D. suzukii* (6 male, 6 female) that were 3–5 d old were placed in the cups and flies were recorded as alive, moribund, or dead after 24 h. Flies used in experiments came from laboratory colonies established from collections in 2014 and 2017. After 7 d, berries were removed from cups and infestation was assessed using a filter salt test. To compare choice and no-choice scenarios, fruit only bioassays were conducted by placing 10 ripe berries inside 0.47-liter clear plastic deli cups on a 2.5-cm-thick slice of moistened floral foam (Smithers-Oasis Co., Kent, OH). Forceps were used to pick berries using the pedicel of each berry to avoid affecting any treatment residue on the berries. Divots were made in the floral foam in a circle around the outside edge to hold the berries, with 10 berries placed in each cup. For the no-choice trials, all 10 berries in the cup were collected from the treatment plot. For choice trials, five berries were collected from treatment

Table 1. Insecticides, manufacturers, and rates used in laboratory and field experiments in Michigan highbush blueberry

Active ingredient (AI)	Trade name	Manufacturer	Rate (g AI/ha ⁻¹)
Methomyl	Lannate 2.4 LV	Corteva Agriscience, Indianapolis, IN	1,008.8
Hydrogen peroxide (26.5%) + peroxyacetic acid (4.9%)	Jet-Ag	Jet Harvest Solutions, Longwood, FL	1,240.2 229.2
Phosmet	Imidan 70WP ^a	Gowan Company LLC, Yuma, AZ	1,043.5
Zeta-cypermethrin	Mustang Maxx 0.8EC	FMC Corporation, Philadelphia, PA	26.9

^aSolution pH buffered using TriFol (0.625 ml per 1 liter).

plots and placed on one side of the cup and another five berries were collected from unsprayed bushes directly adjacent to the trial bushes and placed in the cups. Fifteen adult *D. suzukii* (5 male, 10 female) were released in the center of the cups, and removed 24 h later. The total number of oviposition holes were counted on each berry, and then berries were allowed to incubate for an additional 6 d before infestation was assessed using a filter salt test. All bioassay containers were maintained in an environmental chamber at 25°C, 75% RH, and a 16:8 (L:D) h cycle for the duration of the experiment.

Statistical analyses for field efficacy trials were conducted using Systat 13 (Systat Software, Inc., Chicago, IL). Data were tested for homogeneity of variances using a Levene's test and for normality using a Shapiro–Wilk test. Non-normal data were $\log(X + 1)$ transformed to achieve normality; data that did not meet the assumptions of normality after transformation were subsequently analyzed using nonparametric tests. For weekly fruit infestation, the average total number of *Drosophila* larvae per 30 ml of fruit data in both years were analyzed using either analysis of variance (ANOVA) followed by a Fisher's least significant difference (LSD) test for means separation or a Kruskal–Wallis test followed by a Conover–Inman test for post hoc comparisons, and each sampling date was analyzed separately. For foliage and fruit bioassays as well as fruit only bioassays, adult mortality data were analyzed using a generalized linear model with a binomial distribution using JMP version 14.0 (SAS Institute 2018). For fruit only choice and no-choice tests, one-way ANOVA was conducted followed by Fisher's LSD for means separation for oviposition holes per berry and larvae per berry responses.

Adult Efficacy Experiments

A laboratory experiment was conducted to determine the effects of direct and residual contact of PAA-HP on *D. suzukii* adults. Male and female flies were exposed to PAA-HP using a Potter spray tower (Burkard Scientific, Middlesex, United Kingdom) either by spraying flies with PAA-HP directly or by applying PAA-HP to 100 × 20 mm Petri dishes and placing the flies in the dishes once residue had dried. For the direct contact treatment, 10 adult (5 male, 5 female) 2–7 d old flies were anesthetized using brief exposure to CO₂, placed inside Petri dishes, and sprayed with 2 ml of a 1% v/v PAA-HP solution, applied at a pressure of 103.4 kPa. Flies were transferred to a new unsprayed Petri dish that contained a small cube of *Drosophila* diet (see Van Timmeren et al. 2018). Dishes were subsequently placed in an environmental chamber at 25°C and 75% RH and a 16:8 (L:D) h cycle. For the residual contact treatment, Petri dishes were sprayed with the same treatment, and untreated dishes served as controls. Residues were allowed to dry for 1 h before 5 male and 5 female CO₂ anesthetized flies were added to the dish along with a small cube of diet and their mortality was assessed at 24 h. Each exposure method was replicated fifteen times. During fly health assessments flies were classified as alive, moribund, or dead, following the classification criteria used in Van Timmeren et al. (2019).

A second experiment was conducted to determine possible sublethal effects of PAA-HP on adult *D. suzukii* after exposure via direct contact. Twenty adult (10 male, 10 female) 3- to 5-d-old flies were sprayed directly with either a 1 or 5% v/v PAA-HP solution using a Potter spray tower in the same manner as described previously, and distilled water served as the control. After sprays were applied, all flies were subsequently transferred to new 100 × 20 mm Petri dishes containing 20 ml of cornmeal-based *Drosophila* diet spread evenly across the entire bottom surface of the dishes. The diet was dyed using blue food coloring (0.05 ml per dish, McCormick & Co., Inc., Hunt Valley, MD) to make eggs more visible. Flies were left in

Petri dishes for 4 h on the day applications were made before being transferred to new dishes. Subsequently, flies were transferred to new dishes every 24 h for 7 d after applications were made. Each time flies were transferred to new dishes fly mortality was assessed using the same criteria as mentioned earlier, and the number of eggs laid in the diet was counted. Dishes with adult flies and developing larvae were maintained in an environmental chamber with the same settings as mentioned previously. After 10 d, the total number of pupae were counted in each of the Petri dishes.

Data from both adult efficacy experiments were tested for normality and homogeneity of variances as described previously. The percent adult mortality data in the direct and residual exposure experiment were analyzed to compare treated and untreated for male and female flies using a Wilcoxon test using JMP version 14.0 (SAS Institute 2018). To analyze the sublethal responses, the total number of pupae over 7 d were $\log(X + 1)$ transformed before being analyzed using ANOVA. The total number of eggs laid over 7 d met the assumptions of normality and the untransformed data were analyzed using ANOVA.

Settling Behavior and Oviposition Experiments

Laboratory experiments were conducted at the USDA-ARS Horticultural Research Unit in Corvallis, OR, in 2018–2019 to investigate the effect of PAA-HP on *D. suzukii* settling behavior on berries, oviposition behavior of flies, and egg survival after exposure to PAA-HP. Experiments investigating fly settling behavior took place in October 2018 using store-bought organic blueberries. Berries were either dipped in distilled autoclaved water or in PAA-HP (1.2% v/v) and allowed to dry for 30 min before being placed in Petri dishes. Ten dipped berries were placed in a 90 × 23 mm Petri dish containing either all water-dipped berries or all PAA-HP-dipped berries. One dish of both treatments was placed inside a 23 × 23 × 25 cm mesh cage, and 30 adult *D. suzukii* (15 males, 15 females) were released in the cages. Adult flies were 4–10 d old and were F₁ generation flies from wild-collected parents. At 10, 30, 60, and 120 min, flies that settled on berries were removed and counted; flies that were removed from cages were not replaced. Choice cages were replicated 10 times over the course of 3 d. The number of settled male and female flies per treatment was summed by cage and compared using paired *t*-tests. Here and in this section, data were checked to fit parametric models and tested using JMP 14.0 (SAS Institute 2018).

To determine the effect of PAA-HP on oviposition and larval survival, *D. suzukii* were placed in cages containing 10 berries treated with either PAA-HP (1.2% v/v) or distilled autoclaved water in no-choice cages, or five treated plus five control berries in choice cages. Blueberries for the no-choice study ('Elliott' variety) were collected from an unsprayed field where fruit clusters were covered with mesh bags to prevent infestation, and blueberries for the choice study were store-bought. All berries were dipped and dried as described previously and placed in cages in 90 × 23 mm Petri dishes. Ten *D. suzukii* adults (five males, five females) were allowed to lay eggs in the cages for 24 h at which point berries were removed from cages and the number of eggs laid with visible filaments per berry were counted. Adult flies were given access to water and a small streak of honey water was placed in a Petri dish for the duration of the experiment. After counting eggs, berries were subsequently placed in a 30 ml plastic cup with a mesh lid and adult emergence was recorded 2 wk later. Each treatment was replicated 15 times for the no-choice study in July–August 2018 and for the choice study in March 2019. Flies used in experiments were F₁–F₃ generation flies

from wild-collected adults. For the no-choice study, the number of eggs laid or adults developing was tested separately with treatment as a fixed effect and trial date as a random effect. For the choice study, the same outcomes were compared in paired *t*-tests.

An experiment was conducted to determine whether PAA-HP affects survival of eggs laid in berries. Blueberries ('Reka' and 'Elliott' varieties) were collected from an unsprayed field and placed in cages with ~100 adult *D. suzukii*. Berries were removed from cages after 2–6 h, eggs were counted, and berries were assigned to a treatment such that each treatment included berries with few eggs to many eggs with an average of 4.4–4.6 eggs per berry. Pre-infested berries were then dipped in solution and dried using the same methods, treatments, and rates as the previous two experiments. Dipped berries were placed in mesh-covered 30-ml cups, and adult emergence was recorded 2 wk later. Each treatment was replicated in 44–45 berries over 5 d in July–August 2018. The number of adults developing from eggs was tested for the effect of the treatments.

Efficacy against Microbes

Three strains of *H. uvarum* and *Issatchenkia terricola* (Van der Walt) (Saccharomycetales: Saccharomycetaceae) (synonym: *Pichia terricola*) previously isolated from *D. suzukii* larval frass (Hamby et al. 2012, Lewis et al. 2019) were used to test for efficacy against yeast species. *Hanseniaspora uvarum* strains were isolated on 20 August 2015 (strain 179), 27 August 2015 (strain 200), and 8 September 2015 (strain 308), whereas *I. terricola* strains were isolated on 20 August 2015 (strain 188), 10 September 2015 (strain 270), and 1 October 2015 (strain 290). Yeast strains were revived from cryostorage and cultivated on potato dextrose agar (PDA; Fisher Scientific Co., Hampton, NH). To prepare a yeast spore suspension, the cultures were scraped with a sterile L-shaped cell spreader (Fisher Scientific Co.) and flushed with sterile distilled water into a sterile tube. To standardize yeast quantity for the subsequent growth inhibition assays, the yeast concentration was adjusted to 1×10^5 cells per ml using a hemocytometer, and 300 μ l were distributed on PDA 100 \times 25 mm Petri dishes using a sterile L-shaped cell spreader.

To test the ability of PAA-HP to prevent yeast growth, we simultaneously introduced PAA-HP and yeast cells to PDA media. A sterile cork borer (16.07 mm diameter) was used to remove an agar plug from the center of thick (~25 ml per 100-mm-diameter dish) PDA in Petri dishes 60 min after the yeast suspension was inoculated. The hole was filled with 500- μ l PDA, which was amended with PAA-HP at concentrations of 0, 0.5, 1.0, or 1.5% v/v. The PAA-HP was directly incorporated into liquid PDA agar (temperature approximately 60°C) and stirred for 10 min to homogenize the solution prior to filling the central hole. After the added agar solidified, all Petri dishes were sealed with parafilm, transferred into a growth chamber set to 26°C, and arranged in a randomized complete block design.

To determine whether PAA-HP can suppress yeast growth, yeast suspensions were allowed to grow for 24 h prior to plug removal and the introduction of the PAA-HP. The diameter of the inhibition zone was measured at 24, 48, 72, and 96 h after adding PAA-HP using a caliper (Fowler Ultra-Cal II., Crissier, Switzerland) at two perpendicular points (Supp Fig. 1 [online only]) for both experiments. The inhibition zone was defined as the area where no yeast growth was visible (same day inoculation) or the area with reduced growth of yeast cells (24-h yeast growth). Three plates were used to test each strain, PAA-HP concentration, and yeast inoculation time point on each experimental date for a total of 72 plates per experiment. The entire experiment was replicated on three separate dates.

Statistical analysis was conducted with R version 3.5.2 (R Core Team 2018) and R Studio (R Studio Team 2015) version 1.1.463. The data were analyzed using a linear mixed-effects model (package 'nlme'; Pinheiro et al. 2018) with PAA-HP concentration and hours after inoculation as fixed factors, and the random factor treatment replicate was nested within the random factor experimental date. Data were tested for homogeneity of variances using a Levene's test and for normality using a Shapiro–Wilk test. Due to heterogeneous variances, a nonparametric Games–Howell post hoc test (package 'userfriendlyscience'; Peters 2015) was used. The analysis was done separately for each strain.

Results

Field Efficacy Trials

Few larvae were found in fruit samples during the first 2 wk of the trials during 2017 (Fig. 1) and 2018 (Fig. 2), and there were no significant differences among treatments for berries sampled from the interior of the blueberry bushes (2017: $H < 6.5$, $df = 3, 12$, $P > 0.09$; 2018: $H < 2.32$, $df = 4, 15$, $P > 0.68$) or from the exterior of the bushes (2017: $H < 3.0$, $df = 3, 12$, $P > 0.39$; 2018: $H < 4.0$, $df = 4, 15$, $P > 0.41$). During the third week of the 2017 trial, larvae were detected in all treatments and still no significant differences were found among treatments in either sampling location ($H < 2.83$, $df = 3, 12$; $P > 0.42$). Infestation increased during the fourth week of the 2017 trial and at this time, fewer larvae were found in fruit collected from the PAA-HP and zeta-cypermethrin/PAA-HP rotation treatments when compared with the untreated treatment. These differences were not significant for exterior fruit samples, but were significant for the interior fruit samples ($H = 3.90$, $df = 3, 12$; $P = 0.037$). During the 2018 trial, infestation increased during the third week of the trial with fewer larvae detected in the conventional rotation, zeta-cypermethrin/PAA-HP rotation, and PAA-HP once a week treatments when compared with the untreated treatment. As during the 2017 trial, these differences were not significant for exterior fruit samples, but were highly significant for interior fruit samples ($F = 5.6$, $df = 4, 14$; $P = 0.007$). During the fourth week of the trial, infestation increased in all treatments and no significant differences were found among treatments in either sampling location ($H < 0.68$, $df = 4, 15$; $P > 0.95$).

For berries sampled from the ground under the bushes during 2017, fewer larvae were found in berries from the treated plots (untreated: 0.74 ± 0.14 , conventional rotation: 0.15 ± 0.097 , zeta-cypermethrin/PAA-HP rotation: 0.10 ± 0.059 , PAA-HP once a week: 0.56 ± 0.36); however, this was not significant ($H = 6.83$, $df = 3, 12$; $P = 0.078$). During 2018, fewer larvae were present in fruit collected from treated plots on 10 August (untreated: 4.91 ± 3.12 , conventional rotation: 0.60 ± 0.47 , zeta-cypermethrin/PAA-HP rotation: 0.68 ± 0.48 , PAA-HP once a week: 0 ± 0 , PAA-HP twice a week: 0 ± 0) as well as on 13 August (untreated: 14.71 ± 3.38 , conventional rotation: 1.65 ± 0.43 , zeta-cypermethrin/PAA-HP rotation: 6.59 ± 3.23 , PAA-HP once a week: 3.17 ± 1.61 , PAA-HP twice a week: 2.92 ± 1.25). These results were also not significant on both dates (10 August: $H = 7.85$, $df = 4, 15$, $P = 0.097$; 13 August: $H = 2.96$, $df = 4, 15$, $P = 0.055$).

In semifield bioassays, adult mortality after 24 h was highest in the conventional rotation cups and lowest in the untreated cups (untreated: $14.4 \pm 2.2\%$, conventional rotation: $68.8 \pm 21.6\%$, zeta-cypermethrin/PAA-HP rotation: $60.4 \pm 7.1\%$, PAA-HP once a week: $39.6 \pm 7.1\%$, PAA-HP twice a week: $41.7 \pm 20.1\%$), although there were no significant differences among treatments ($\chi^2 = 1.31$, $df = 4$,

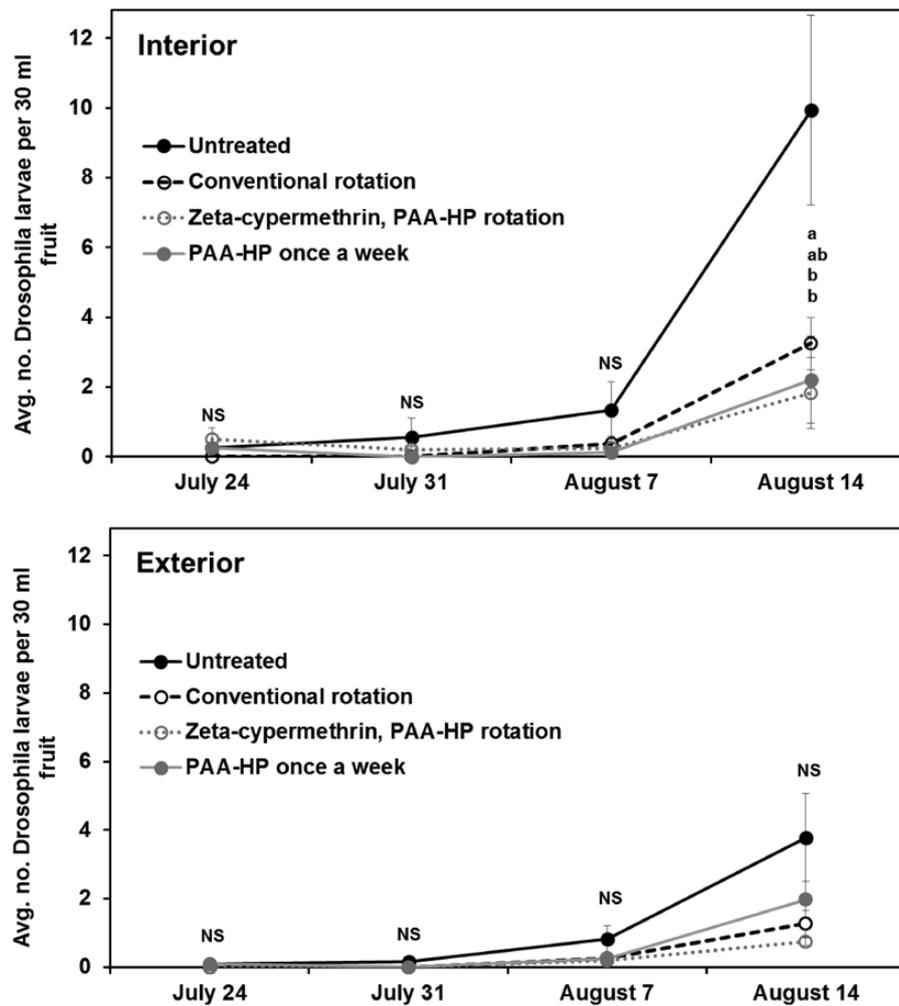


Fig. 1. The average total number of *Drosophila* larvae per 30 ml of fruit collected from treatment plots within a small plot trial conducted in July–August 2017. Blueberry bushes received one of three insecticide spray programs or were left untreated, and fruit were sampled once a week for *Drosophila* larvae using a filter salt test method. Interior and exterior refer to the position within the bushes from which the berries were sampled. Averages are presented as number of larvae per 30 ml of collected fruit. Averages are presented \pm SE, and values with the same letters within a week are not significantly different.

15; $P = 0.85$). When fruit were assessed for infestation, there were fewer larvae in the four chemical treatments than the untreated treatment. The conventional rotation and zeta-cypermethrin/PAA-HP rotation treatments had significantly lower infestation than the untreated (Fig. 3; $F = 3.95$, $df = 4, 15$; $P = 0.022$).

In no-choice bioassays of fruit, adult mortality after 24 h was low in all treatments, ranging from 1.7 to 5%, and there were no significant differences among treatments ($\chi^2 = 0.12$, $df = 4, 15$, $P = 0.99$). There were more oviposition holes present on untreated berries than the other treatments, although this was not significant (Fig. 4; $H = 7.42$, $df = 4, 15$; $P = 0.12$). When berries were assessed for larvae, there were more larvae in the untreated berries than all the other treatments (Fig. 4; $F = 3.37$, $df = 4, 15$; $P = 0.037$).

In choice bioassays of fruit, adult mortality after 24 h was low in all treatments (untreated: $1.7 \pm 1.7\%$, conventional rotation: $0 \pm 0\%$, zeta-cypermethrin/PAA-HP rotation: $11.7 \pm 1.7\%$, PAA-HP once a week: $3.3 \pm 1.9\%$, PAA-HP twice a week: $3.3 \pm 1.9\%$), and there was numerically higher mortality in the zeta-cypermethrin/PAA-HP rotation treatment than the other treatments ($\chi^2 = 0.81$, $df = 4, 15$; $P = 0.93$). There were no significant differences among insecticide treatments in the number of oviposition holes per berry (Fig. 5; $F = 0.80$, $df = 4, 15$; $P = 0.53$). When berries were assessed for larvae there were significantly fewer larvae in the treated berries than

in the untreated controls (Fig. 5; $F = 3.33$, $df = 4, 15$; $P = 0.023$). In addition, there were significantly fewer larvae detected in the berries treated with crop sterilant than in the untreated berries in the cups ($F = 14.11$, $df = 1, 6$; $P = 0.001$).

Adult Efficacy Experiments

There was no adult mortality for either male or female *D. suzukii* in any of the treatments after 8 h. After 24 h, there was some mortality for both males and females (Table 2). However, mortality was at or below 20% for all treatments, and there were no significant differences among treatments (Table 2).

In the adult sublethal experiment, there was 0% adult mortality after 4 h. After 7 d, the total percent adult mortality was low in all treatments, and there were no significant differences among treatments (Table 2). There were no significant differences among treatments in the total number of eggs laid per female over the 7 d of the trial. Likewise, there were no significant differences in the number of pupae that developed in the Petri dishes over the 7 d of the trial (Table 2).

Settling Behavior and Oviposition Experiments

In the fly settling behavior experiment, a lower percentage of *D. suzukii* adults settled on berries dipped in PAA-HP as opposed to

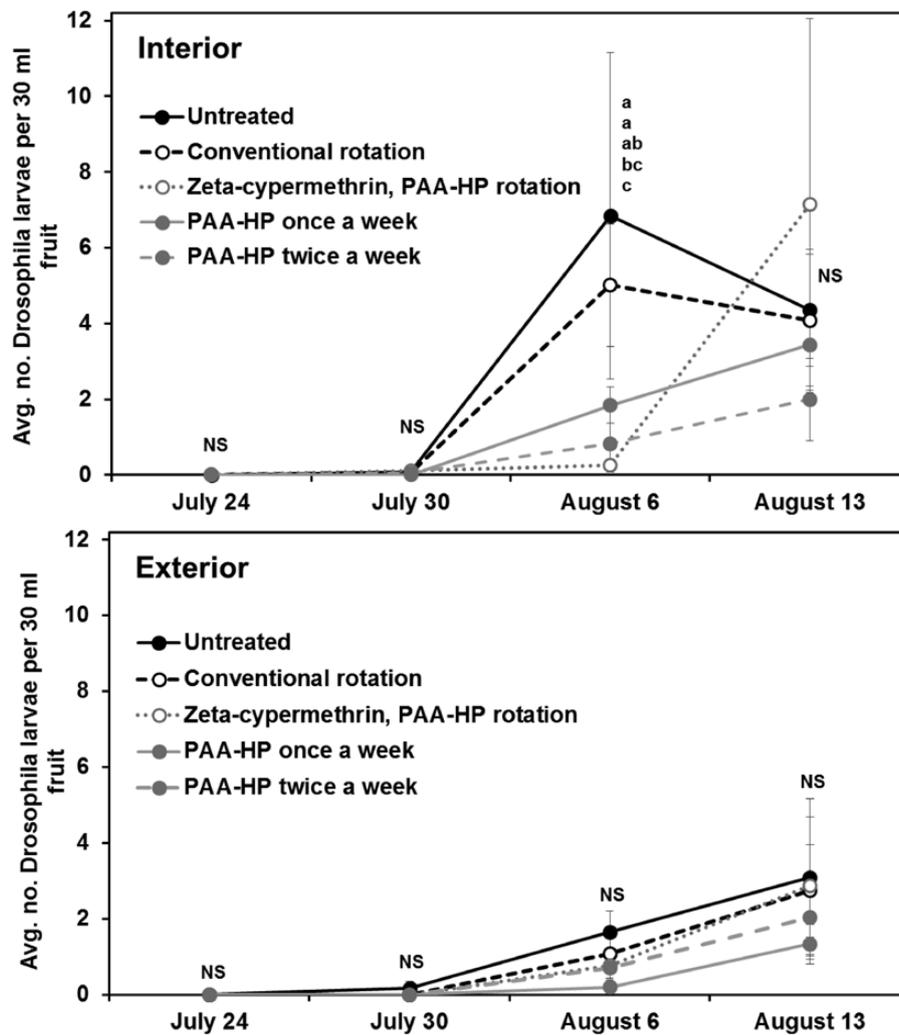


Fig. 2. The average total number of *Drosophila* larvae per 30 ml of fruit collected from treatment plots within a small plot trial conducted in July–August 2018. Blueberry bushes received one of three insecticide spray programs or were left untreated and fruit were sampled once a week for *Drosophila* larvae using a filter salt test method. Interior and exterior refer to the position within the bushes from which the berries were sampled. Averages are presented as number of larvae per 30 ml of collected fruit. Averages are presented \pm SE, and values with the same letters within a week are not significantly different.

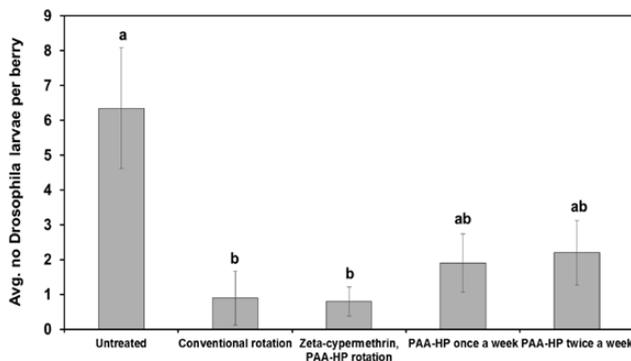


Fig. 3. The average number of *Drosophila* larvae per berry in foliage and fruit bioassays. Blueberry bushes were sprayed with one of four treatments or left untreated and fruit and foliage were collected 1 d after treatment and placed in bioassay containers along with 6 male and 6 female *D. suzukii* adults. Averages are presented \pm SE, and values with the same letters are not significantly different.

berries dipped in water, a result that was significant for both males and females (Table 2). However, in the oviposition no-choice experiment, there were no significant differences in the number of eggs

laid between the two treatments, nor were there any significant differences in the number of adults that emerged from treated berries (Table 2). In the oviposition choice experiment, fewer eggs were laid in berries dipped in PAA-HP than in water, but there was no difference in the number of adults that emerged from berries (Table 2). In the egg survival experiment, there was no difference between the number of adults that emerged from infested berries that were dipped in PAA-HP or water (Table 2).

Efficacy against Microbes

In experiments where PAA-HP was added to dishes on the same day as yeast inoculation, PAA-HP significantly reduced yeast growth, regardless of the strain or species 24 h post-exposure (Fig. 6; strain 179: $F = 827$, $df = 3, 24$, $P < 0.001$; strain 200: $F = 515$, $df = 3, 24$, $P < 0.001$; strain 308: $F = 302$, $df = 3, 24$, $P < 0.001$; strain 188: $F = 241$, $df = 3, 24$, $P < 0.001$; strain 270: $F = 303$, $df = 3, 24$, $P < 0.001$; strain 290: $F = 255$, $df = 3, 24$, $P < 0.001$). Stronger inhibition was observed in the 1 and 1.5% concentrations compared with the 0.5% concentration for all *H. uvarum* strains (Fig. 6). For *I. terricola*, only the growth of strain 270 varied with PAA-HP concentration, with 1.5% more strongly inhibiting growth than 0.5% (Fig. 6). We measured a 1- to 2-mm reduction of the size of the inhibition zone after 48 h and again

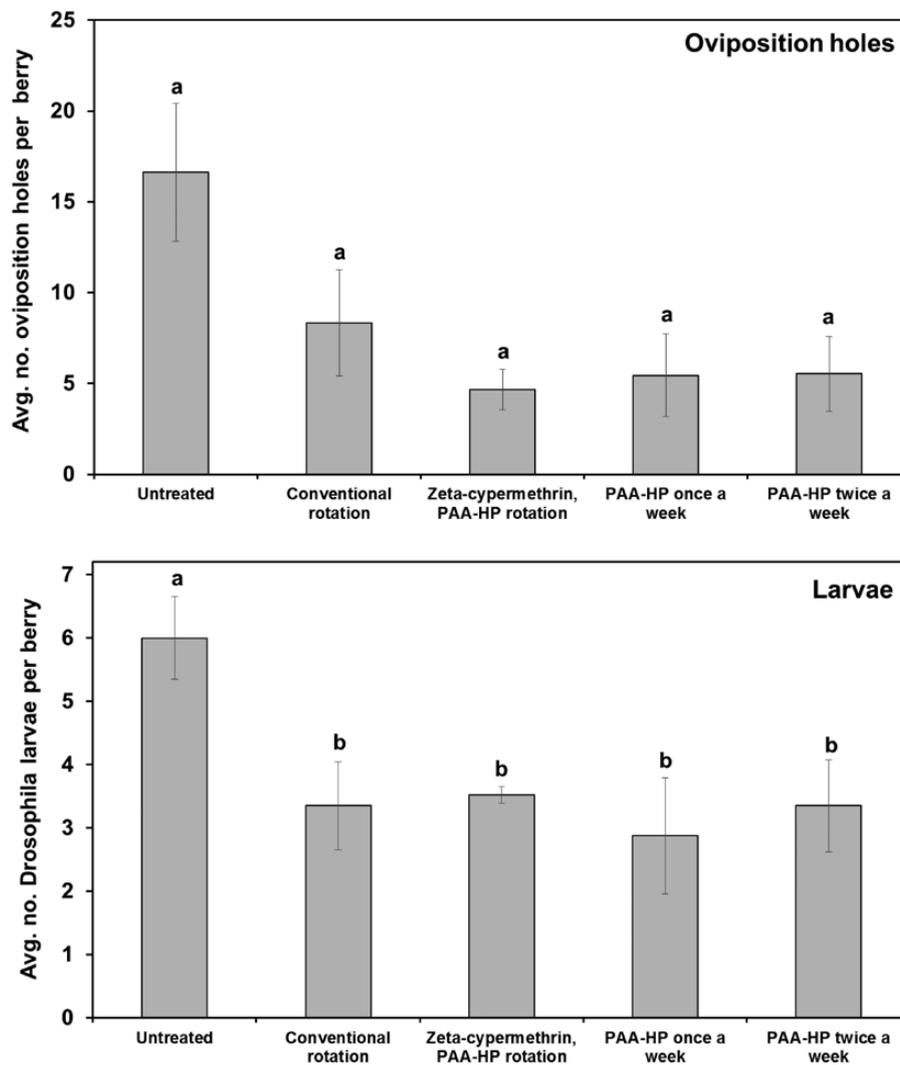


Fig. 4. The average number of oviposition holes per berry (top graph) and the average number of larvae per berry (bottom graph) in no-choice fruit bioassays. Blueberry bushes were sprayed with one of four treatments or left untreated and fruit were collected 1 d after treatment and placed in bioassay containers containing moistened floral foam along with 5 male and 10 female *D. suzukii* adults. Flies were removed from containers after 24 h, and oviposition holes were counted. Berries were assessed for larvae 6 d later using a filter salt test method. Averages are presented \pm SE, and values with the same letters are not significantly different.

after 72 h for both *H. uvarum* and *I. terricola*, indicating the yeast was able to slightly recolonize the area of inhibition (Supp Fig. 2 [online only]). Although *H. uvarum* inhibition was significantly affected by time (strain 179: $F = 8.5$, $df = 3, 120$, $P < 0.001$; strain 200: $F = 3.0$, $df = 3, 120$, $P = 0.032$; strain 308: $F = 3.8$, $df = 3, 120$, $P = 0.012$), the post hoc analyses could not separate the area of inhibition between time points. Inhibition of *I. terricola* strains did not vary with time (strain 188: $F = 1.5$, $df = 3, 120$, $P = 0.209$; strain 270: $F = 1.5$, $df = 3, 120$, $P = 0.210$; strain 290: $F = 2.3$, $df = 3, 120$, $P = 0.077$). Thus, only the 24 h measurements are presented.

In experiments where PAA-HP was added 24 h after yeast inoculation, PAA-HP also significantly reduced yeast growth 24 h post-exposure (Fig. 7; strain 179: $F = 876$, $df = 3, 24$, $P < 0.001$; strain 200: $F = 684$, $df = 3, 24$, $P < 0.001$; strain 308: $F = 587$, $df = 3, 24$, $P < 0.001$; strain 188: $F = 1578$, $df = 3, 24$, $P < 0.001$; strain 270: $F = 1991$, $df = 3, 24$, $P < 0.001$; strain 290: $F = 4121$, $df = 3, 24$, $P < 0.001$); however, the inhibition zone was 2–3 times smaller compared with the plates where the PAA-HP agar was added the same day. In addition, the inhibition zone stopped yeast growth rather than eliminating the yeasts from the area. The highest *H. uvarum* inhibition

was observed in the 1.5% treatment, which was significantly higher than the 1.0% concentration for strain 179 and strain 200, as well as the 0.5% concentration for all *H. uvarum* strains (Fig. 7). Greater inhibition was also observed in the 1.0% concentration compared with the 0.5% concentration for *H. uvarum* strain 200. For *I. terricola*, the highest inhibition was observed in the 1.5% concentration, which was significantly higher than the 1.0% treatment and the 0.5% treatment, irrespective of the strain (Fig. 7). A significant difference between the 1.0% treatment and the 0.5% treatment was only observed for *I. terricola* strains 188 and 290. Time did not affect the size of the inhibition zone for either of the species (strain 179: $F = 0.07$, $df = 3, 120$, $P = 0.975$; strain 200: $F = 0.03$, $df = 3, 120$, $P = 0.994$; strain 308: $F = 0.02$, $df = 3, 120$, $P = 0.995$; strain 188: $F = 0.4$, $df = 3, 120$, $P = 0.780$; strain 270: $F = 1.6$, $df = 3, 120$, $P = 0.190$; strain 290: $F = 1.3$, $df = 3, 120$, $P = 0.268$; Supp Fig. 3 [online only]).

Discussion

In the 2 yr of field trials, we found that applying PAA-HP on a weekly basis in field trials reduced infestation by *D. suzukii*. Infestation was

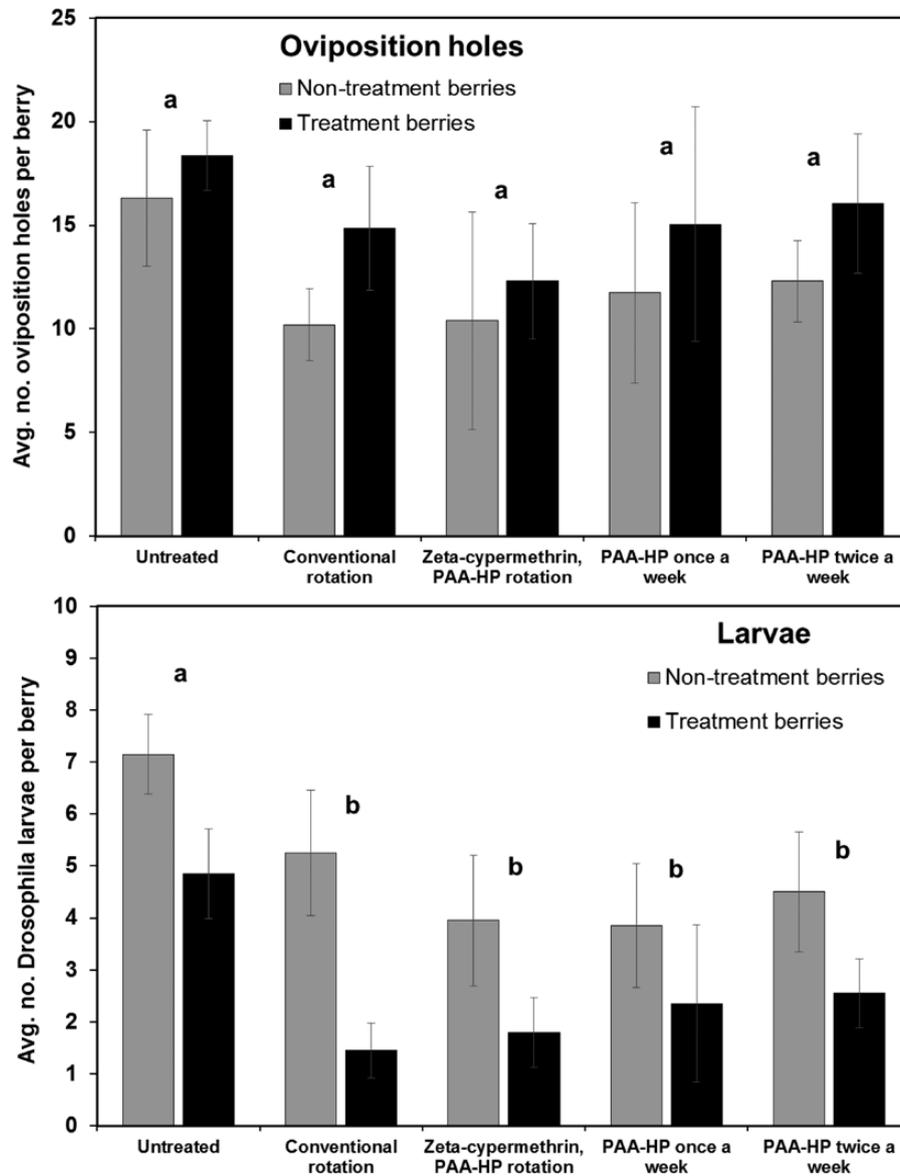


Fig. 5. The average number of oviposition holes per berry (top graph) and the average number of larvae per berry (bottom graph) in choice fruit bioassays. Blueberry bushes were sprayed with one of four treatments or left untreated and fruit were collected 1 d after treatment from treatment plots and adjacent untreated bushes. Five treated and five untreated berries were placed on opposite sides of a bioassay container containing moistened floral foam along with 5 male and 10 female *D. suzukii* adults. Flies were removed from containers after 24 h, and oviposition holes were counted. Berries were assessed for larvae 6 d later using a filter salt test method. Averages are presented \pm SE, and values with the same letters are not significantly different.

significantly affected once it started to occur, but only for a short period before *D. suzukii* pressure became too high. However, it is important to consider the size of the plots that were used in the field trials when drawing conclusions regarding these results. Each treatment replicate consisted of three rows of six blueberry bushes, with the entire spray trial consisting of 0.0963 ha (2017) and 0.161 ha (2018). There were no unsprayed buffer rows in the spray trials; however, because *D. suzukii* adults can disperse (Wong et al. 2018), it is entirely possible that flies in the field trials were preferentially choosing berries on untreated bushes for egg laying. It will be important to test the efficacy of PAA-HP on a larger scale to determine how *D. suzukii* acts in a no-choice situation in the field. Adult females may choose to lay their eggs on PAA-HP-treated berries in the absence of other available oviposition resources. PAA-HP treatments only impacted fruit infestation in the interior of the blueberry bushes. This is likely due to the higher pest pressure experienced

in this part of the canopy; *D. suzukii* exhibit higher adult activity and larval infestation in the center of the bushes (Diepenbrock and Burrack 2017, Rice et al. 2017, Jaffe and Guedot 2019).

Follow-up laboratory studies investigating possible modes of action for PAA-HP indicate that adult mortality does not explain the results observed in field trials. No significant mortality occurred when adults were exposed to PAA-HP, either by direct contact or contact with residues and there was no indication that exposure to PAA-HP reduces population growth. In no-choice cage trials, there were no significant differences in the number of eggs laid between PAA-HP and distilled water treatments. Also, exposing newly laid eggs to PAA-HP did not reduce the number of *D. suzukii* that emerged. However, in choice settling and choice oviposition experiments, adult flies landed and laid more eggs on water versus PAA-HP-treated berries, indicating adult choice may play a role in the efficacy of PAA-HP in the field. In foliage and fruit as well as fruit

Table 2. Percentages, averages, and statistics from adult mortality and attraction and egg-laying experiments

Experiment	Outcome	Treatments			Z/F/t	df	P
		Untreated	Direct	Residual			
Adult exposure	Male mortality	1.3 ± 0.9%	2.0 ± 1.1%	2.0 ± 1.1%	<0.44	1, 13	0.65
	Female mortality	1.3 ± 0.9%	2.0 ± 1.1%	1.3 ± 0.9%	0.44	1, 13	0.65
Sublethal exposure		<u>Water</u>	<u>PAA-HP 1%</u>	<u>PAA-HP 5%</u>			
	Adult mortality	5.8 ± 3.3%	5.0 ± 3.4%	3.3 ± 1.7%	0.086	2, 15	0.92
	Eggs laid	5.9 ± 0.6	8.2 ± 1.1	5.7 ± 1.1	2.13	2, 15	0.15
	Pupae developed	60.8 ± 8.1	77.1 ± 6.3	60.3 ± 9.4	2.03	2, 15	0.17
Attraction		<u>Water</u>	<u>PAA-HP 1.2%</u>				
	Male settling	39.3 ± 5.7%	24.0 ± 2.5%		2.44	1, 18	0.038
	Female settling	34.0 ± 5.7%	13.3 ± 2.8%		4.60	1, 18	0.0013
Oviposition no-choice	Eggs laid	12.1 ± 3.0	11.2 ± 3.6		0.07	1, 24	0.80
	Adults emerged	6.1 ± 1.9	5.4 ± 1.5		0.13	1, 24	0.73
Oviposition choice	Eggs laid	16.6 ± 2.2	8.6 ± 2.4		2.6	1, 14	0.020
	Adults emerged	5.9 ± 1.0	4.1 ± 1.3		1.3	1, 14	0.21
Egg survival	Adults emerged	1.9 ± 0.2	2.1 ± 0.3		0.03	1, 92	0.88

Bold P-values indicate significant differences at $P < 0.05$.

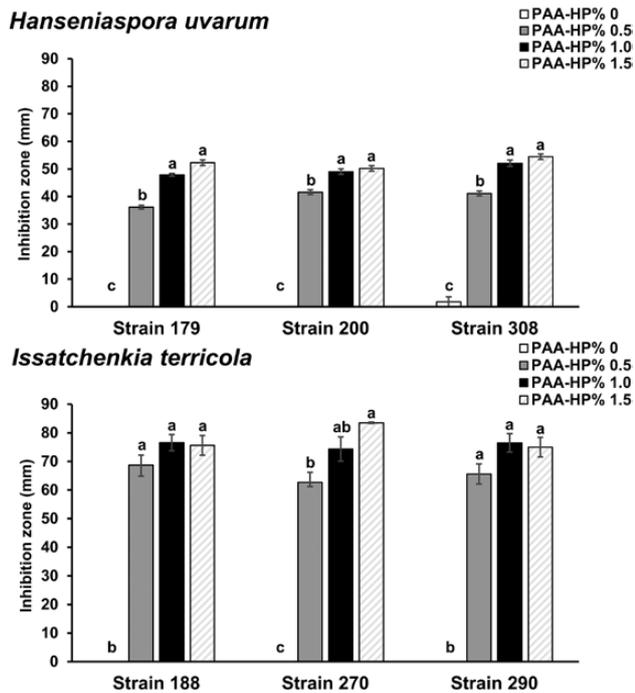


Fig. 6. Growth inhibition 24 h after adding PAA-HP at four concentrations (0, 0.5, 1.0, 1.5%) to Petri dishes inoculated with either *Hanseniaspora uvarum* (top) or *Issatchenkia terricola* (bottom), when PAA-HP was added to the dish 60 min after yeast inoculation. Means with the same letter for each strain are not significantly different. Figures in bold represent significant differences.

only bioassays, there were fewer larvae in PAA-HP treated berries than in untreated berries. These results are consistent with some of the foliage and fruit bioassays conducted in other studies (Roubos et al. 2019a,b). Additional research is needed to determine the role of adult *D. suzukii* behavior in the efficacy of PAA-HP.

PAA-HP is able to inhibit the growth of *H. uvarum* and *I. terricola* yeasts both before and after inoculation on an agar plate. These species of yeasts are fed on by *D. suzukii* adults and larvae in raspberry fields (Hamby et al. 2012, Lewis et al. 2019), and yeasts may be important sources of nutrition for adult egg-laying (Mori et al. 2017). Yeasts also impact *D. suzukii* larval survival and adult oviposition preferences (Bellutti et al. 2018). Because PAA-HP

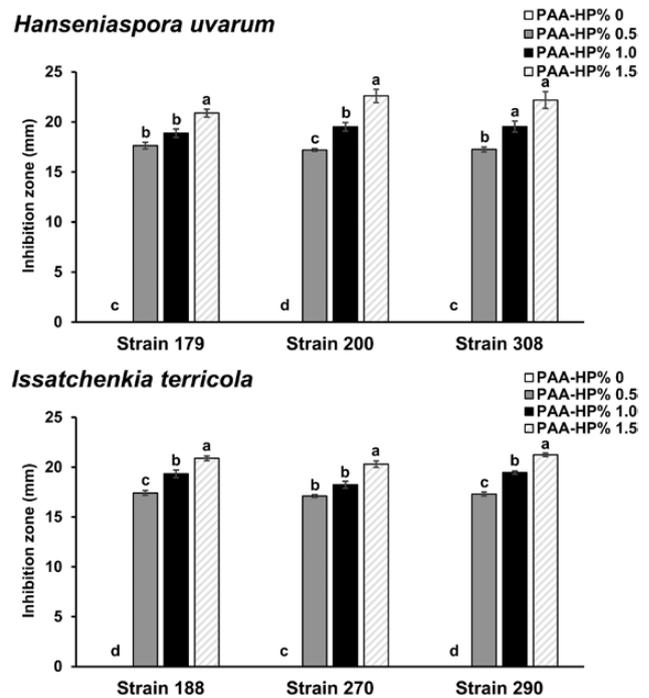


Fig. 7. Growth inhibition 24 h after adding PAA-HP at four concentrations (0, 0.5, 1.0, 1.5%) to Petri dishes inoculated with either *Hanseniaspora uvarum* (top) or *Issatchenkia terricola* (bottom), when yeasts were allowed to grow for 24 h before PAA-HP was added to the dish. Means with the same letter for each strain are not significantly different.

impacts the growth of *D. suzukii*-associated yeasts, changes in the fruit fungal communities may be affecting female oviposition choices and the health of larvae in treated berries. Additional research is needed to determine the specific impact of PAA-HP on fruit fungal communities (including fungal pests of this crop) and to evaluate how this could alter *D. suzukii* behavior and survival.

Symbiotic bacteria may also play a role in how *D. suzukii* decide where to feed and lay eggs, and influence larval health. Analysis of gut bacteria in *D. suzukii* adults has revealed acetobacteria and enterobacteria make up the highest percentage of bacteria present (Martinez-Sañudo et al. 2018). Acetobacteria are commonly found

on plants (Matsushita et al. 2016) and improve larval growth and development time in *Drosophila melanogaster* Meigen (Staubach et al. 2018). *Drosophila suzukii* adults are attracted to certain acetobacteria (Mazzeto et al. 2016), although detailed studies to determine the nature of this relationship have not been conducted. Peroxyacetic acid and hydrogen peroxide are both effective at controlling acetobacteria in postharvest sanitation scenarios (Alasri et al. 1993, Osaili et al. 2018, Rood et al. 2018). Future studies should explore the possible role that bacterial communities may play in the efficacy of PAA-HP on *D. suzukii*.

An important consideration with PAA-HP is that it degrades into chemical components that are harmless to microorganisms, thus limiting the residual effects. Rather than being a complete crop sterilant, yeasts found on plants and elsewhere in the environment may be able to recolonize rapidly after treatment, thus potentially limiting the effectiveness of PAA-HP in controlling *D. suzukii*. However, in our experiments where PAA-HP was added 24 h after yeast inoculation, there was no evidence of changes in the size of the inhibition zone over time, possibly indicating that yeasts may not have been able to recolonize or recover from the PAA-HP treatment.

Future studies are needed to determine whether there are ways to optimize the performance of PAA-HP beyond what was tested in this study. The 1% v/v rate of PAA-HP in this study was 490 parts per million (ppm) and the 1.5% rate was 735 ppm. Rood et al. (2018) found PAA to be ineffective against various microbes at 100 ppm, but effective at 500 ppm. In our study, there were certain instances where *H. uvarum* and *I. terricola* growth was significantly more inhibited at the 1.5% rate than at the 1% rate, so it is possible that using a higher rate of PAA-HP may improve efficacy against *D. suzukii*. In addition, there is some indication that adjuvants may increase the effectiveness of PAA against microbes (Asensio et al. 2015) and possibly against *D. suzukii* as well (Roubos et al. 2019b). Future studies should investigate the effectiveness of PAA and HP separately to determine which specific compound is responsible for observed effects on *D. suzukii* or if both compounds work together.

An important component of future trials will be investigating the effect of PAA and HP on pesticide residues already present on fruit or in spray solutions where PAA-HP is tank mixed with other pesticides. Previous studies have found PAA and HP can degrade pesticide residues both in solution and on the surface of the fruit (Hwang et al. 2001, Crowe et al. 2006, Cengiz and Certel 2012). This could be beneficial for growers wanting to decrease pesticide residues of harvested fruit; however, there is also a risk that efficacy of other pesticides could be compromised by applications of PAA-HP.

This study represents the first test of the efficacy of PAA-HP against *D. suzukii* in a field setting and investigates possible mechanisms behind the results from field trials. Many questions remain to be answered regarding the relationship between PAA-HP and *D. suzukii*, and larger-scale trials are needed to determine whether the effectiveness translates to a larger scale and ultimately whether PAA-HP may be appropriate in commercial control programs. This includes organic systems where spinosyn remains the primary means of control. Having another effective product could reduce the amount of spinosyn growers need to apply each season, which in turn could potentially reduce the risk of spinosyn resistance developing in fields. The combined benefits of disease and insect suppression from crop sterilants that can be used close to harvest warrant further investigation by researchers and growers interested in reducing dependence on synthetic pesticides for managing *D. suzukii*.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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