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ORIGINAL CONTRIBUTION

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Evaluation of organic insecticides for management of spotted-wing drosophila (*Drosophila suzukii*) in berry crops

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Abstract

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is an invasive pest affecting fruit production in many regions of the world. Insecticides are the primary tactic for controlling *D. suzukii* in organic as well as conventional production systems. Organic growers have a greater challenge because fewer insecticides are approved for use in organic agriculture. The most effective organically approved product is spinosad, but alternatives are needed because of label restrictions limiting the number of applications per year, toxicity to beneficial arthropods and the risk of developing resistance. We evaluated several organically approved insecticides against *D. suzukii* in laboratory assays and field trials conducted on organic blueberry and raspberry farms. Spinosad was consistently the most effective insecticide, but a few other insecticides such as azadirachtin + pyrethrins, *Chromobacterium subtsugae* and sabadilla alkaloids showed moderate activity. None of the treatments had long residual activity. Mortality started to decline by 3 days after treatment, and by 5 days after application, the treatments were not different from the controls. These products may be useful in rotation programmes, necessary for reducing reliance on spinosad and mitigating resistance. Cultural and biological control approaches are needed in fruit production for *D. suzukii* management, but insecticides will likely continue to be the dominant management tactic while these other approaches are being optimized and adopted.

KEYWORDS
bioassay, fruit, pest management, spinosad, toxicity

1 | INTRODUCTION

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is an invasive pest of soft-skinned fruits in Europe and North and South America (Calabria, Máca, Bächli, Serra, & Pascual, 2012; Deprá, Poppe, Schmitz, De Toni, & Valente, 2014; Hauser, 2011; Walsh et al., 2011). *D. suzukii* has quickly become a key pest of berry crops and stone fruits, shifting pest management programmes to prioritize this pest (Asplen et al., 2015; Lee, Bruck, Dreves et al., 2011). Most drosophilids infest rotting or overripe fruit because they cannot penetrate the skin of intact fruit (Attalah, Teixeira, Salazar, Zaragoza, & Kopp, 2014). However, *D. suzukii* is problematic because females possess a strongly sclerotized serrated ovipositor, which enables them to oviposit in ripening fruit (Attalah et al., 2014; Hauser, 2011). This causes direct injury by larval feeding within fruit and/or indirect injury whereby puncture wounds provide a route of entry for pathogens

(Rombaut et al., 2017; Ioriatti et al. 2018). *D. suzukii* has a wide host range including fruit crops of economic importance in North America such as blueberry, blackberry, cherry, raspberry, strawberry, peach and grape (Bellamy, Sisterson, & Walse, 2013; Lee, Bruck, Curry et al., 2011; Walsh et al., 2011). Growers risk the rejection of entire loads if infested fruit is detected during the marketing phase.

With zero tolerance for *D. suzukii* infestation, most growers have relied on frequent insecticide applications to control populations (Diepenbrock, Rosensteel, Hardin, Sial, & Burrack, 2016; Van Timmeren & Isaacs, 2013). Integrated pest management (IPM) programmes have often been abandoned for prophylactic insecticide use during the part of the season when fruit is ripe (Van Timmeren & Isaacs, 2013). Organic growers have a greater challenge in that fewer insecticides are approved for use in organic production (Iglesias & Liburd, 2017). A limited number of insecticide classes and products in organic agriculture could make resistance management more challenging as there are fewer chemical classes to rotate.

Biopesticides are products derived from naturally occurring living organisms such as animals, plants, fungi, bacteria and viruses (Senthil-Nathan, 2015). Microbial products based on the bacterium *Bacillus thuringiensis* serovar. *israelensis* (*B.t.i*) are specific to some Diptera. Biganski Jehle and Kleespies (2018) tested *B.t.i.* against *D. suzukii* but had very low mortality in their laboratory experiments. The bacterium *Chromobacterium subtsugae* sp. nov. showed lethal and sublethal effects against several chewing and sucking insects (Martin, Gundersen-Rindal, Blackburn, & Buyer, 2007). Since its development as a commercial product, *C. subtsugae* has been labelled for control of a broad spectrum of insect pests, including *D. suzukii*. Botanical pesticides, biopesticides of plant origin, include four major types (pyrethrum, rotenone, neem and essential oils) and three others in limited use (ryania, nicotine and sabadilla) (Isman, 2006). A number of these botanical pesticides have been included in recent studies evaluating chemical control options for *D. suzukii* and some have shown promise (Cahenzli, Strack, & Daniel, 2018; Fanning, Grieshop, & Isaacs, 2018; Shawer, Tonina, Tirello, Duso, & Mori, 2018).

Agricultural sanitizers, oxidizing agents used as fungicides or antimicrobials, are a potential supplement to insecticides. These sanitizers may affect naturally occurring yeasts on fruit. Yeasts are integral parts of *Drosophila* ecology, affecting physiology and behaviour, and are likely an important food resource for *D. suzukii* (Hamby & Becher, 2016). Hardin, Kraus, and Burrack (2015) found that fewer *D. suzukii* survived to adulthood and had longer development times when raised on a diet without yeast compared to a standard laboratory diet. Disrupting this yeast ecology could have a detrimental effect on the ability of *D. suzukii* to oviposit on and develop in fruit.

The goal of this study was to evaluate effectiveness of Organic Materials Review Institute (OMRI)-listed insecticides for *D. suzukii* control in organic berry crop production systems. Insecticide treatments were compared for their acute and residual efficacy against *D. suzukii* in laboratory and field bioassays. This study was part of a large research programme that aimed to develop, implement and evaluate system-based organic *D. suzukii* management strategies. Team members represent states with major fruit production industries impacted by *D. suzukii*.

2 | MATERIALS AND METHODS

A series of bioassays were performed to evaluate insecticide efficacy under laboratory and field conditions (Table S1). For the laboratory bioassays, we used various methods of exposing *D. suzukii* to insecticide residues: treated glass vials, treated blueberries, treated Petri dishes or direct spray on flies. Semi-field bioassays were conducted in multiple states to represent different growing regions and types of berry crops. Trials in Florida and Georgia were done on southern highbush blueberry (*Vaccinium corymbosum* L. × *V. darrowi* Camp) and rabbiteye blueberry (*V. virgatum* Aiton), trials in Michigan were done on northern highbush blueberry (*V. corymbosum*), and trials in Minnesota were done on raspberry (*Rubus idaeus* L.). Treatment efficacy in laboratory bioassays was determined based on adult fly mortality and, in the case of the fruit dip method only, the number of progeny able to develop on treated fruit. In the semi-field trials, efficacy was also based on adult fly mortality and the number of progeny able to develop on treated fruit as well as the amount of infestation in fruit collected from treatment plots.

2.1 | Insects

Drosophila suzukii adults used in bioassays were taken from separate laboratory colonies established by each collaborating university. Cultures were maintained on a standard cornmeal–molasses (or cane sugar)–yeast medium (Gautam et al., 2016; Jaramillo, Mehlferber, & Moore, 2015). Adults used in bioassays were 4–10 days old and were not starved prior to use in bioassays. Individuals were removed from colony rearing containers by aspiration or anesthetized using CO_2.

2.2 | Insecticide treatments

Insecticides and rates used in this study are listed in Table 1. All products were listed by OMRI as acceptable for use in certified organic production (OMRI Products List; https://www.omri.org/omri-lists, accessed 24 March 2016). The treatment list also includes two agricultural sanitizers registered as fungicides and algaecides.

2.3 | Laboratory experiments

The experiments conducted in 2016 included eight insecticide treatments (azadirachtin, azadirachtin + pyrethrins, spinosad, *Chromobacterium subtsugae*, hydrogen peroxide + peroxyacetic acid [PAA], hydrogen dioxide + PAA, pyrethrins and *Burkholderia* spp.) plus an untreated control. The experiments conducted in 2017 included six insecticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, *Burkholderia* spp. and sabadilla alkaloids) plus an untreated control.

TABLE 1 Organic Materials Review Institute-listed insecticide treatments, classes and rates used in laboratory and semi-field bioassays

Trade name	Active ingredient	Manufacturer	Rate (AI/ha)[a]
Aza-Direct®	Azadirachtin	Gowan Company LLC, Yuma, AZ	28.2 g
AzaGuard™	Azadirachtin	BioSafe Systems LLC, East Hartford, CT	39.2 g
Azera®	Azadirachtin (1.2%) + Pyrethrins (1.4%)	Valent USA Corporation, Walnut Creek, CA	49.2 g 54.1 g
Entrust® SC	Spinosad	Dow AgroSciences LLC, Indianapolis, IN	105.4 g
Grandevo®	*Chromobacterium subtsugae*	Marrone Bio Innovations, Davis, CA	1,005.9 g
Jet-Ag®[b]	Hydrogen peroxide (26.5%) + Peroxyacetic acid (4.9%)	Jet Harvest Solutions, Longwood, FL	12.4 g 2.3 g
OxiDate® 2.0[c]	Hydrogen dioxide (27.1%) + Peroxyacetic acid (2.0%)	BioSafe Systems LLC, East Hartford, CT	12.7 g 0.9 g
PyGanic® EC 1.4	Pyrethrins	McLaughlin Gormley King Co., Minneapolis, MN	61.6 g
Venerate™ XC	*Burkholderia* spp.	Marrone Bio Innovations, Davis, CA	17.7 kg
Veratran D®[d]	Sabadilla alkaloids	McLaughlin Gormley King Co., Minneapolis, MN	33.5 g

[a]Rate of formulated product applied at the equivalent of 467.5 L water/ha. [b]Agricultural sanitizer labelled as a fungicide, bactericide, algaecide. [c]Agricultural sanitizer labelled as a broad-spectrum algaecide/fungicide. [d]Currently not labelled on berry crops.

2.3.1 | Glass vial bioassay

A glass vial bioassay was conducted at the University of Florida in 2017. Insecticides were mixed with acetone to equal a total volume of 1 ml. Acetone was used as the solvent for quick and even evaporation of the solution (Smirle, Zurowski, Ayyanath, Scott, & MacKenzie, 2017). Spinosad, *C. subtsugae* and *Burkholderia* spp. did not mix well when added directly to acetone, resulting in uneven coverage of the vials. Therefore, these products were mixed with 100 µl deionized water before mixing with acetone. Insecticide solutions were poured into 250-ml graduated glass flasks (Fisher Scientific Company LLC, Pittsburgh, PA), which were rotated so that all sides were coated evenly. Excess solution was poured out after coating the vials, and vials were air-dried before adding flies. Treated vials were arranged in a completely randomized design with four replicates. The caps of the vials had five 4-mm holes for ventilation that were covered with a 0.8-mm fine mesh and affixed with a cotton wick saturated with 5% sugar solution. Ten *D. suzukii* adults (5 males and 5 females) were placed in each vial and stored in a growth chamber set at 24°C, 65% RH and a photoperiod of 14:10 [L:D] hr. Mortality was assessed at 72 hr post-exposure.

2.3.2 | Fruit dip bioassay

Fruit dip bioassays were conducted at the University of Georgia in 2016 and 2017. Treatments were applied to store-bought organic blueberries. Berries were rinsed 2–3 times in deionized water to wash off any insecticide residues, then rinsed in 2% propionic acid for 5 s to inhibit mould growth and finally dipped in the insecticide solutions for 5 s. All solutions were prepared using deionized water. The berries were air-dried after each step. Berries were then placed in 59.2-ml plastic deli cups containing a 1 cm deep layer of autoclaved sand. Openings in the deli cup lids were plugged with moistened cotton balls to minimize mortality due to desiccation. The cotton balls also served as a water source for the flies over the course of the experiment. Each deli cup received five berries and 10 *D. suzukii* adults (5 males and 5 females). Cups were placed in a reach-in environmental chamber at 24°C, 70% RH and a photoperiod of 14:10 [L:D] hr. Mortality was assessed at 72 hr post-exposure. After 72 hr, the berries were transferred to clean deli cups without flies and were held for 2 weeks to allow progeny to develop. Berries were then dissected, and the numbers of larvae, pupae and adults were recorded.

2.3.3 | Topical application bioassay

A topical application bioassay was performed at Michigan State University in 2016. Treatments were sprayed directly onto CO_2-anesthetized *D. suzukii* adults in Petri dishes (100 × 15 mm) using a Potter Spray Tower (Burkhard Scientific, Uxbridge, UK) set at 103.4 kPa with 2 ml of spray solution applied to each replicate (Van Timmeren, Mota-Sanchez, Wise, & Isaacs, 2018). All solutions were prepared using deionized water. Following treatment, the flies were transferred to untreated Petri dishes and provided a portion of

standard Drosophila diet for nutrition. The diet did not need to be renewed over the course of the experiment. Mortality was assessed at 72 hr post-application. Petri dishes were maintained in a growth chamber at 25°C, 75% RH and a photoperiod of 16:8 [L:D] hr.

2.3.4 | Residual contact bioassay

A residual contact bioassay was performed at Michigan State University in 2017. Treatments were sprayed onto plastic Petri dishes using a Potter Spray Tower (Burkhard Scientific) set at 103.4 kPa with 2 ml of spray solution applied to each replicate. All solutions were prepared using deionized water. Flies were placed in the dishes after residues dried and were provided a portion of standard Drosophila diet for nutrition. Mortality was assessed at 72 hr post-application. Petri dishes were maintained in a growth chamber at 25°C, 75% RH and a photoperiod of 16:8 [L:D] hr.

2.4 | Semi-field experiments

Semi-field experiments were performed in Florida, Georgia, Michigan and Minnesota. Insecticide treatments were applied to plants in the field; then, bioassay samples were collected from treatment plots and brought back to the laboratory. Each bioassay sample consisted of a single cut blueberry branch or raspberry cane containing 5–7 leaves. In the blueberry experiments, five ripe berries were also collected per sample. Each sample was placed in a 946-ml clear plastic container (Fabri-Kal®, Kalamazoo, MI) as described in Van Timmeren and Isaacs (2013). A 10-cm-long single anchor water pick (No. 1932; Smithers-Oasis Co., Kent, OH) was inserted through a hole on the bottom of the chamber. The cut branches were inserted into the picks to prevent desiccation during the experiment. Samples were placed directly into bioassay chambers in the field; then, the chambers were transported to the laboratory. The branches with leaves and berries were exposed to *D. suzukii* adults reared in the laboratory, mortality was assessed after 120 hr, and the number of progeny (larvae, pupae and adults) coming out of the berries was counted.

Field infestation was determined in the blueberry experiments by collecting fruit samples from each plot and extracting larvae using a salt solution and filter method as described in Van Timmeren, Diepenbrock, Bertone, Burrack, and Isaacs (2017).

2.4.1 | Florida

The Florida semi-field trial was conducted on an organic blueberry farm in Island Grove, FL, from 25 to 30 April 2017. Plots consisted of two cultivars of southern highbush blueberries ("Farthing" and "Meadowlark") planted in single rows with one cultivar per row. The experiment was a randomized complete block design with four replicates. Each treatment plot contained five blueberry bushes (6.1 m × 1.22 m), and each plot was separated by a buffer of three bushes (3.0 m). There were four insecticide treatments (spinosad, azadirachtin + pyrethrins, *C. subtsugae* and hydrogen peroxide + PAA) plus a control (water only). Treatments were applied using a handheld CO_2 sprayer with an output equivalent to 467.5 L/ha at 241.3 kPa. Samples for bioassays were collected at 0, 3 and 5 days after treatment (DAT). Each bioassay chamber received 10 *D. suzukii* adults (5 males and 5 females). After the mortality assessment, flies were removed and berries were kept for 7 days at 24°C and 65% RH; then, the number of progeny was determined using the filter salt extraction method (Van Timmeren et al., 2017).

To measure field infestation, 100 blueberries were collected from each replicate on 2 May 2017 (7 DAT). Berries were weighed, placed in plastic bags and held for 7 days at room temperature before assessment using the filter salt extraction method. Infestation was reported as the number of larvae per gram of blueberries.

2.4.2 | Georgia

The Georgia semi-field trials were conducted on certified organic blueberry farms in Appling County GA. Two experiments were run each year, one in southern highbush blueberry ("Star" variety) and one in rabbiteye blueberry ("Premier" variety). The 2016 experiment in southern highbush blueberry ran from 28 April to 6 May 2016, and the experiment in rabbiteye blueberry ran from 7 to 14 June 2016. The 2017 experiment in southern highbush blueberry ran from 21 to 26 April 2017, and the experiment in rabbiteye blueberry ran from 9 to 14 June 2017. All bushes were 6–8 years old and at least 1.5 m in height. Bushes were planted on 3.66 m row centres and were 0.91 m (southern highbush) to 1.22 m (rabbiteye) apart within rows. Sets of three or five bushes were treated in each replicate depending on space available. Samples for bioassays and field infestation were collected from the centre bushes. A buffer row on either side of the experimental plots was left untreated to limit drift from the rest of the field. Treatments were applied using handheld CO_2 sprayers with an output equivalent to 467.5 L/ha at 241.3 kPa. Each bioassay chamber received 10 *D. suzukii* adults (5 males and 5 females). After the mortality assessment, berries were transferred to clean deli cups without flies to allow progeny to develop. After an incubation period of 2 weeks on a laboratory bench at 23°C, the number of progeny was determined by dissecting berries.

To measure field infestation, 0.12–0.24 L of ripe blueberries was collected from each plot. Berries were weighed and then assessed using the filter salt extraction method. Infestation was reported as the number of larvae per gram of blueberries.

In the 2016 experiment in southern highbush blueberry, there were seven insecticide treatments (azadirachtin, azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, hydrogen dioxide + PAA and *Burkholderia* spp.) plus a control in four replicates. Samples for bioassays and field infestation were collected at 0, 3, 5 and 7 DAT. In the 2016 experiment in rabbiteye blueberry, there were four insecticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and pyrethrins) plus a control in three replicates. Samples for bioassays and field infestation were collected at 0, 3 and 7 DAT.

In both experiments, in 2017, there were four insecticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and hydrogen peroxide + PAA) plus an untreated control in three replicates.

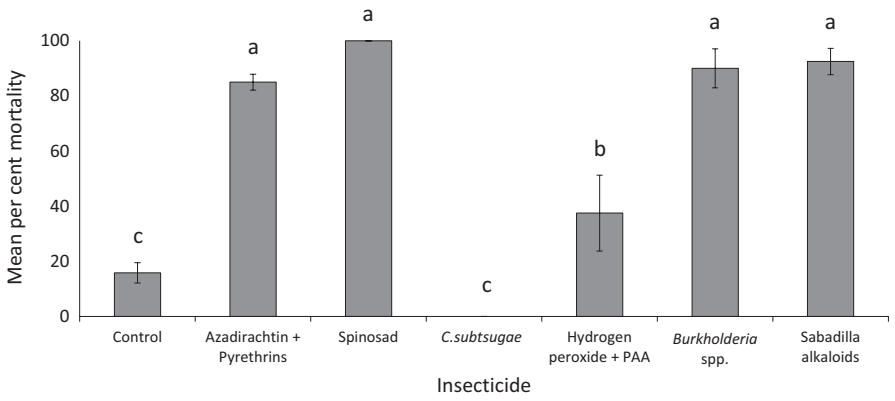


FIGURE 1 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment in glass vial laboratory bioassays at 72-hr exposure. Bioassays were conducted at the University of Florida in 2017. Means within each year followed by the same letter were not significantly different (LSD test, $p < 0.05$)

Each experiment received a single insecticide application, and samples for bioassays and field infestation were collected at 0, 3 and 5 DAT.

2.4.3 | Michigan

The Michigan semi-field trials were conducted at the Trevor Nichols Research Center in Fennville, MI. The 2016 experiment ran from 5 to 12 August 2016, and the 2017 experiment ran from 28 July to 07 August 2017. Each treatment plot consisted of six bushes. All bushes were 6–7 years old and an average of 1.2 m in height. Bushes were planted on 3.66 m row centres and were 1.2 m apart within rows. Treatments were applied using an FMC 1029 airblast sprayer set at an output of 467.5 L/ha. Treatments were applied to three adjacent rows of northern highbush blueberry bushes ("Bluecrop" variety), and samples for bioassays and field infestation were collected from the centre row of each plot. Each bioassay chamber received 12 *D. suzukii* adults (6 males and 6 females), and chambers were kept at 25°C, 75% RH and 16:8 [L:D] hr. After the mortality assessment, berries were left in the chambers for 7 days; then, the number of progeny was determined using the filter salt extraction method (Van Timmeren et al., 2017).

In the 2016 trial, there were seven insecticide treatments (azadirachtin, azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, hydrogen dioxide + PAA and *Burkholderia* spp.) plus an untreated control in four replicates. Samples were collected at 0 and 7 DAT. To measure field infestation, 0.12–0.24 L of ripe blueberries was collected in each plot on 04 August 2016 (7 DAT). Berries were weighed and then assessed using the filter salt extraction method. Infestation was reported as the number of larvae per gram of blueberries.

In the 2017 trial, there were four insecticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and hydrogen peroxide + PAA) plus an untreated control in three replicates. Samples for bioassays were collected at 0, 3 and 5 DAT. To measure field infestation, 0.12–0.24 L of ripe blueberries was collected from each plot at the end of the residue sampling period on 3 August 2017 (6 DAT). Berries were weighed and then assessed using the filter salt extraction method. Infestation was reported as the number of larvae per gram of blueberries.

2.4.4 | Minnesota

The Minnesota field trial was conducted at a commercial certified organic farm in Hastings, MN, on primocane fruiting raspberry (cultivar "Autumn Britten"). The 2016 trial ran from 19 to 24 August 2016, and the 2017 trial ran from 19 to 24 August 2017. The experimental plot consisted of four rows 35.97 m long on 1.83 m centres. There were three insecticide treatments (azadirachtin + pyrethrins, spinosad and *C. subtsugae*) plus an untreated control in five replicates. Each plot was 1.83 m long and contained about 18 canes. Insecticides were applied using 1-L pump pressure sprayers, delivering 571 L/ha at 206.8 kPa. Each bioassay chamber received 10 *D. suzukii* adults (5 males and 5 females), and chambers were kept at 25°C, 47% RH and 16:8 [L:D] hr. Samples for bioassays were collected at 0, 3 and 5 DAT. Fruit samples for assessing field infestation were not collected in this experiment.

2.5 | Statistical analysis

Laboratory data (mortality and progeny) and semi-field data (mortality, progeny and field infestation) were analysed using generalized linear mixed models (PROC GLIMMIX, SAS Institute, 2013) with insecticide as the main effect and replicate as a random effect. Degrees of freedom were adjusted using the Kenward–Roger approximation, and post hoc means separation tests were performed using Fisher's protected least significant difference (LSD) ($\alpha = 0.05$). Mortality of both sexes was pooled for analysis. Larvae, pupae and adults were pooled for progeny count analysis. Separate analyses were conducted for each residue age in the semi-field bioassay. All analyses were performed using SAS 9.4 (SAS Institute, 2013).

3 | RESULTS

3.1 | Laboratory experiments

3.1.1 | Glass vial bioassay

In the glass vial bioassay, insecticide treatment had a significant effect on *D. suzukii* mortality ($F = 40.26$; $df = 6, 41$; $p < 0.001$) (Figure 1).

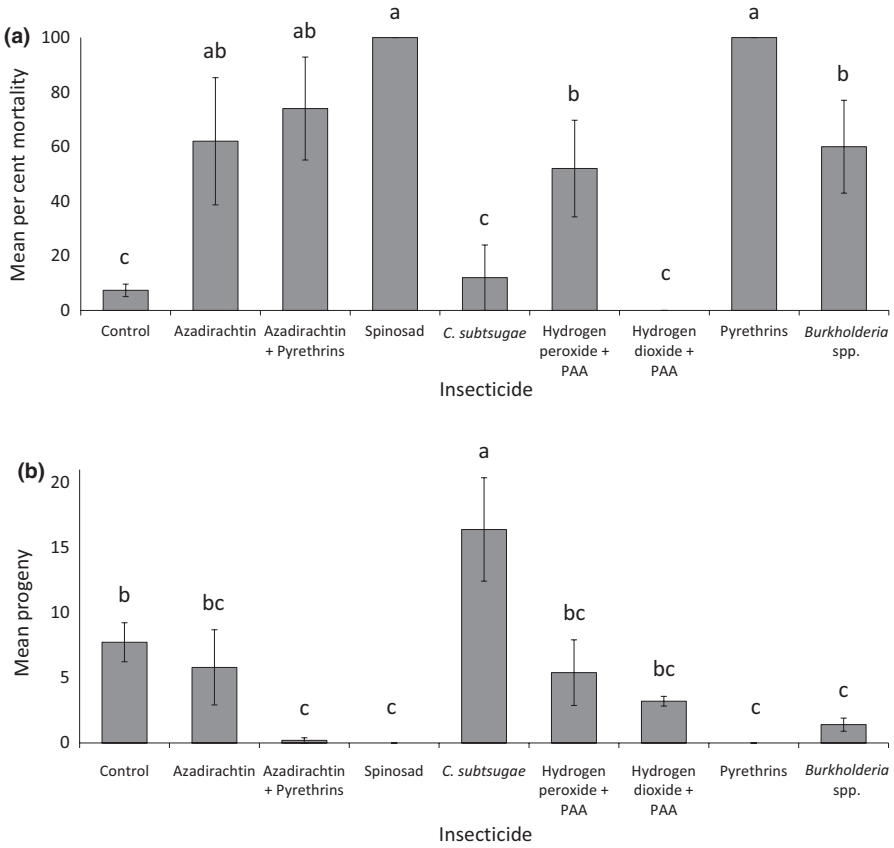


FIGURE 2 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality at 72-hr exposure (a) and mean (±*SE*) number of progeny per blueberry (b) by insecticide treatment in fruit dip laboratory bioassays. Bioassays were conducted at the University of Georgia in 2016. Means with the same letter were not significantly different (LSD test, $p < 0.05$)

Spinosad was the only product to cause 100% mortality. Mortality in the *Burkholderia* spp. and sabadilla alkaloids treatments was 90% or higher. Hydrogen peroxide + PAA caused <40% mortality, and none of the flies died in the *C. subtsugae* treatment.

3.1.2 | Fruit dip bioassay

In the 2016 fruit dip bioassay, insecticide treatment had a significant effect on *D. suzukii* adult mortality ($F = 7.91$; $df = 8, 36$; $p < 0.001$) (Figure 2a). Spinosad and pyrethrins caused 100% mortality. The sanitizer hydrogen dioxide + PAA caused approximately 50% mortality, and hydrogen peroxide + PAA did not kill any adults. Insecticide treatment also had a significant effect on the number of *D. suzukii* progeny developing in the berries ($F = 6.91$; $df = 8, 42$; $p < 0.001$) (Figure 2b). More progeny developed in *C. subtsugae* treated berries than any other treatment. No progeny developed in the spinosad and pyrethrins treated berries.

In the 2017 fruit dip bioassay, insecticide treatment had a significant effect on *D. suzukii* adult mortality ($F = 3.80$; $df = 6, 28$; $p = 0.007$) (Figure 3a). Spinosad caused 100% mortality, and sabadilla alkaloids and *Burkholderia* spp. were the only other treatments that caused significantly higher mortality than the control. There was no significant effect of insecticide treatment on progeny ($F = 0.94$; $df = 6, 33$; $p = 0.481$) (Figure 3b). Mean progeny was very low. No progeny developed in the spinosad treated berries, and the *C. subtsugae* and sabadilla alkaloids treated berries averaged less than one *D. suzukii* per five berries.

3.1.3 | Topical application bioassay

The treatments had a significant effect on *D. suzukii* mortality in the topical application bioassay ($F = 34.11$; $df = 8, 44$; $p < 0.001$) (Figure 4a). All treatments caused low mortality (<25%) except for spinosad which caused 100% mortality. Only spinosad, pyrethrins and *Burkholderia* spp. caused significantly higher mortality than the control.

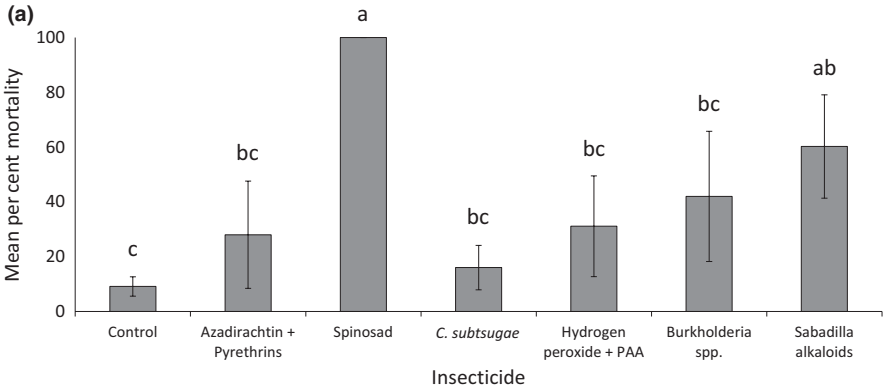
3.1.4 | Residual contact bioassay

In the residual contact bioassay, the treatments had a significant effect on *D. suzukii* mortality ($F = 18.74$; $df = 6, 21$; $p < 0.001$) (Figure 4b). All treatments caused low mortality except for spinosad which caused 100% mortality. Azadirachtin + pyrethrins was the only other product to cause significantly higher mortality than the control. No flies died in the hydrogen peroxide + PAA treatment.

3.2 | Semi-field experiments

3.2.1 | Florida

Insecticide treatment had a significant effect on *D. suzukii* mortality at 0 DAT and 3 DAT but not 5 DAT (Table 2). At 0 DAT, spinosad and azadirachtin + pyrethrins caused significantly higher mortality than the control. At 3 DAT, spinosad caused significantly higher mortality than all other treatments, and there were no differences



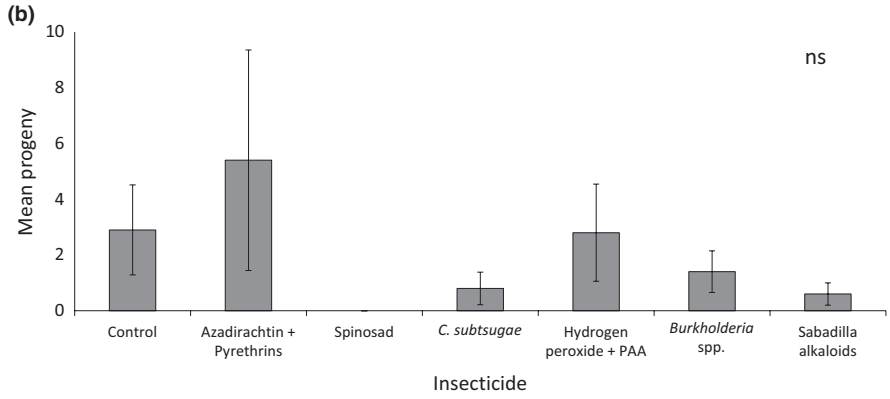
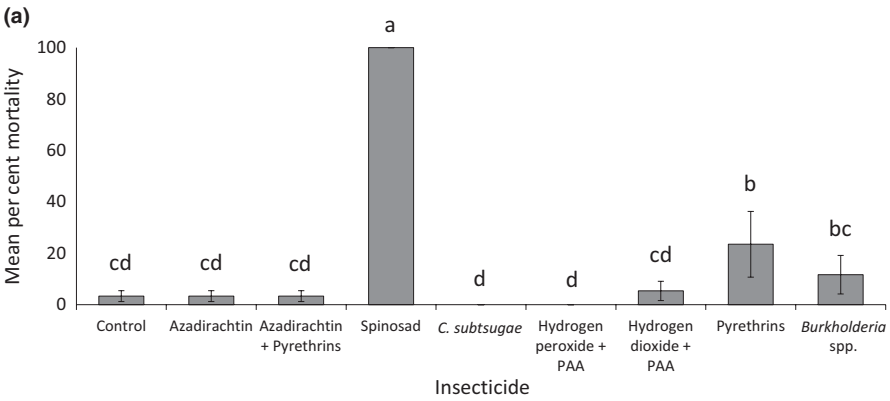


FIGURE 3 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality at 72-hr exposure (a) and mean (±*SE*) number of progeny per blueberry (b) by insecticide treatment in fruit dip laboratory bioassays. Bioassays were conducted at the University of Georgia in 2017. Means with the same letter were not significantly different (LSD test, $p < 0.05$). ns: overall model not significant



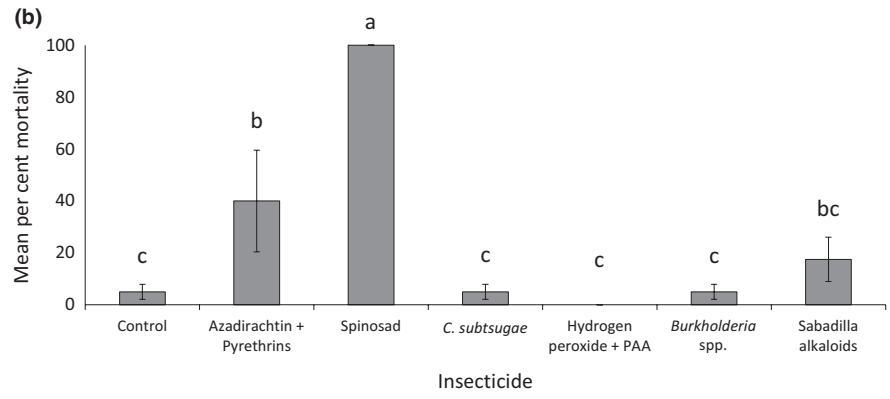


FIGURE 4 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment in a topical application laboratory bioassay at 48-hr exposure (a) and residual contact laboratory bioassay at 72-hr exposure (b). Bioassays were conducted at Michigan State University. Means within each bioassay followed by the same letter were not significantly different (LSD test, $p < 0.05$)

among any of the other insecticides and the control. Treatment had a significant effect on the number of progeny emerging from berries at 0 DAT and 3 DAT but not 5 DAT (Table 2). In the cases with statistical significance, each of the insecticide treatments had significantly fewer progeny than the control. There were no significant differences among treatments for field infestation as determined by

TABLE 2 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment and residue age in a semi-field bioassay at 120-hr exposure and mean (±*SE*) number of *D. suzukii* progeny per 5-berry sample. The trial was conducted at a blueberry farm in Island Grove, FL, in 2017

Residue age	Treatment	Mean (±*SE*) per cent adult mortality	Mean (±*SE*) progeny per 5 berries
0 days	Control	32.5 ± 6.29 c	56.0 ± 14.60 a
	Spinosad	75.0 ± 12.58 a	7.0 ± 4.14 b
	Azadirachtin + pyrethrins	72.5 ± 6.29 ab	17.5 ± 4.17 b
	C. subtsugae	45.0 ± 12.58 c	26.0 ± 13.64 b
	Hydrogen peroxide + PAA	52.5 ± 13.77 bc	17.3 ± 4.13 b
	ANOVA	$F = 6.56$; $df = 4, 12$; $p = 0.005$	$F = 4.15$; $df = 4, 12$; $p = 0.024$
3 days	Control	15.0 ± 6.46 b	48.0 ± 4.06 a
	Spinosad	65.0 ± 12.58 a	18.8 ± 3.86 c
	Azadirachtin + pyrethrins	35.0 ± 6.46 b	30.3 ± 5.50 bc
	C. subtsugae	32.5 ± 9.47 b	23.3 ± 4.94 bc
	Hydrogen peroxide + PAA	25.0 ± 12.58 b	32.5 ± 3.23 b
	ANOVA	$F = 3.59$; $df = 4, 15$; $p = 0.030$	$F = 6.49$; $df = 4, 15$; $p = 0.003$
5 days	Control	20.0 ± 4.08	48.5 ± 11.52
	Spinosad	45.0 ± 6.46	21.5 ± 6.98
	Azadirachtin + pyrethrins	17.5 ± 2.50	38.8 ± 9.20
	C. subtsugae	32.5 ± 8.54	21.3 ± 4.79
	Hydrogen peroxide + PAA	32.5 ± 9.47	34.3 ± 6.87
	ANOVA	$F = 3.03$; $df = 4, 12$; $p = 0.061$	$F = 2.03$; $df = 4, 15$; $p = 0.142$

Note. Means within each column followed by the same letter were not significantly different (LSD test, $p < 0.05$).

filter salt extraction ($F = 1.03$; $df = 4, 12$; $p = 0.432$). The number of *D. suzukii* larvae and pupae from field infestation samples was low, averaging <1 fly per 100 g of blueberries.

3.2.2 | Georgia

In the 2016 experiment in southern highbush blueberry, insecticide treatment had a significant effect on *D. suzukii* mortality only at 0 DAT (Table 3). Mortality at 0 DAT was not significantly different among spinosad, *C. subtsugae*, *Burkholderia* spp. and hydrogen dioxide + PAA. Although adult mortality was not statistically different among insecticides at 3 DAT, there was a significant effect of insecticide on progeny that developed in berries. Spinosad was the only treatment that had significantly fewer progeny than the control. There were no treatment effects at 5 or 7 DAT for adult mortality or progeny. Field infestation samples from the rabbiteye blueberry experiment in 2017 were the only ones that yielded enough *D. suzukii* larvae to analyse, and there were no treatment effects.

In the 2016 experiment in rabbiteye blueberry, insecticide treatment had a significant effect on *D. suzukii* mortality only at 0 DAT (Table 3). Mortality in the spinosad treatment was higher than all other treatments, and all other treatments were not different from the control. Insecticide treatment had a statistically significant effect on progeny at 0 and 3 DAT (Table 3). At 0 DAT, azadirachtin + pyrethrins had higher mean progeny than all other treatments. None of the other treatments were statistically different from the control. At 3 DAT, *C. subtsugae* and pyrethrins had significantly fewer progeny than the control, and spinosad and azadirachtin + pyrethrins were not different from the control. The field infestation samples did not yield a single *D. suzukii*.

In the 2017 experiment in southern highbush blueberry, insecticide treatment had a significant effect on *D. suzukii* mortality only at 3 DAT (Table 4). Spinosad had the highest mortality, but it was not significantly different from azadirachtin + pyrethrins or hydrogen peroxide + PAA. Insecticide treatment also had a significant effect on progeny only at 3 DAT (Table 4). Spinosad had the lowest number of progeny, but this was not significantly different from the control or *C. subtsugae*. Field infestation samples yielded only one *D. suzukii*.

In the 2017 experiment in rabbiteye blueberry, insecticide treatment had a significant effect on mortality at 0 DAT and 3 DAT

TABLE 3 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment and residue age in semi-field bioassays at 120-hr exposure and mean (±*SE*) number of *D. suzukii* progeny per 5-berry sample. The experiments were conducted at a blueberry farm in Baxley, GA, in 2016

Blueberry type	Residue age	Treatment	Mean (±*SE*) per cent adult mortality	Mean (±*SE*) progeny per 5 berries
Highbush	0 days	Control	48.3 ± 16.19 bc	27.0 ± 5.21
		Spinosad	87.1 ± 3.15 a	10.5 ± 3.33
		Azadirachtin	22.2 ± 22.22 cd	26.8 ± 3.17
		Azadirachtin + pyrethrins	12.5 ± 12.50 d	21.0 ± 5.58
		C. subtsugae	89.2 ± 6.39 a	18.5 ± 0.96
		Hydrogen peroxide + PAA	23.6 ± 13.68 cd	27.5 ± 10.12
		Hydrogen dioxide + PAA	62.7 ± 21.46 ab	12.8 ± 1.25
		Burkholderia spp.	69.7 ± 9.12 ab	16.0 ± 1.29
		ANOVA	$F = 7.19$; $df = 7, 21$; $p < 0.001$	$F = 1.92$; $df = 7, 24$; $p = 0.110$
	3 days	Control	25.4 ± 11.20	32.0 ± 3.08 ab
		Spinosad	21.7 ± 15.72	18.0 ± 2.92 c
		Azadirachtin	34.0 ± 12.93	27.3 ± 2.32 bc
		Azadirachtin + pyrethrins	30.5 ± 6.23	28.8 ± 2.43 abc
		C. subtsugae	25.1 ± 16.24	25.0 ± 4.18 bc
		Hydrogen peroxide + PAA	37.7 ± 16.41	39.0 ± 8.01 a
		Hydrogen dioxide + PAA	49.2 ± 13.77	28.0 ± 2.38 abc
		Burkholderia spp.	46.5 ± 5.21	39.5 ± 3.52 a
		ANOVA	$F = 0.62$; $df = 7, 24$; $p = 0.737$	$F = 3.16$; $df = 7, 24$; $p = 0.016$
	5 days	Control	19.4 ± 15.96	72.8 ± 8.82
		Spinosad	25.5 ± 4.04	72.0 ± 14.02
		Azadirachtin	37.8 ± 13.59	63.3 ± 3.86
		Azadirachtin + pyrethrins	28.7 ± 12.58	73.3 ± 3.43
		C. subtsugae	11.2 ± 4.57	72.3 ± 11.08
		Hydrogen peroxide + PAA	25.0 ± 12.32	70.3 ± 3.73
		Hydrogen dioxide + PAA	33.1 ± 14.19	67.5 ± 9.75
		Burkholderia spp.	35.0 ± 12.93	58.3 ± 5.98
		ANOVA	$F = 0.53$; $df = 7, 24$; $p = 0.804$	$F = 0.41$; $df = 7, 24$; $p = 0.887$
	7 days	Control	5.0 ± 5.00	89.8 ± 18.05
		Spinosad	36.6 ± 19.04	44.8 ± 9.69
		Azadirachtin	13.4 ± 6.20	67.8 ± 4.68
		Azadirachtin + pyrethrins	21.1 ± 9.97	69.5 ± 15.48
		C. subtsugae	15.0 ± 6.88	58.0 ± 11.75
		Hydrogen peroxide + PAA	14.2 ± 10.49	48.3 ± 14.38
		Hydrogen dioxide + PAA	16.7 ± 9.62	72.8 ± 9.53
		Burkholderia spp.	13.1 ± 5.10	46.3 ± 6.98
		ANOVA	$F = 0.85$; $df = 7, 21$; $p = 0.557$	$F = 1.87$; $df = 7, 21$; $p = 0.127$

(Continues)

TABLE 3 (Continued)

Blueberry type	Residue age	Treatment	Mean (±*SE*) per cent adult mortality	Mean (±*SE*) progeny per 5 berries
Rabbiteye	0 days	Control	0.0 ± 0.0 b	7.67 ± 0.33 b
		Spinosad	83.3 ± 8.82 a	4.0 ± 2.08 b
		Azadirachtin + pyrethrins	6.7 ± 3.33 b	32.0 ± 7.37 a
		C. subtsugae	0.0 ± 0.0 b	14.3 ± 5.33 b
		Pyrethrins	0.0 ± 0.0 b	12.0 ± 2.08 b
		ANOVA	$F = 77.94$; $df = 4, 8$; $p < 0.001$	$F = 6.39$; $df = 4, 10$; $p = 0.008$
	3 days	Control	16.7 ± 8.82	26.7 ± 5.84 a
		Spinosad	46.7 ± 12.02	19.0 ± 6.56 ab
		Azadirachtin + pyrethrins	13.3 ± 8.82	13.7 ± 2.96 ab
		C. subtsugae	13.3 ± 8.82	7.0 ± 0.58 b
		Pyrethrins	20.0 ± 10.00	7.0 ± 3.00 b
		ANOVA	$F = 2.72$; $df = 4, 8$; $p = 0.107$	$F = 3.69$; $df = 4, 10$; $p = 0.043$
	7 days	Control	10.0 ± 5.77	10.3 ± 2.85
		Spinosad	3.3 ± 3.33	6.0 ± 0.58
		Azadirachtin + pyrethrins	26.7 ± 17.64	8.0 ± 1.53
		C. subtsugae	16.7 ± 3.33	7.67 ± 2.19
		Pyrethrins	10.0 ± 10.00	13.67 ± 3.84
		ANOVA	$F = 1.18$; $df = 4, 8$; $p = 0.390$	$F = 1.50$; $df = 4, 8$; $p = 0.290$

Note. Means within each column followed by the same letter were not significantly different (LSD test, $p < 0.05$).

(Table 4). Spinosad caused 100% mortality at 0 DAT, but the other treatments were not significantly different from the control. At 3 DAT, spinosad, *C. subtsugae* and azadirachtin + pyrethrins were not different from the control. There was no significant effect of insecticides on progeny at any residue age (Table 4). There were no significant differences among treatments in the field infestation samples ($F = 1.30$; $df = 4, 8$; $p = 0.347$). The number of *D. suzukii* larvae and pupae collected from berries was low, averaging <4 flies per 100 g of blueberries.

3.2.3 | Michigan

In the 2016 experiment, insecticide treatment had a significant effect on *D. suzukii* mortality and progeny at 0 DAT but not at 7 DAT (Table 5). At 0 DAT, spinosad caused the highest mortality, and none of the other insecticides were significantly different from the control. Berries in the spinosad treatment had significantly fewer progeny than any of the other insecticides or the control. There were no significant differences among treatments in the field infestation samples ($F = 0.31$; $df = 7, 21$; $p = 0.941$). Mean infestation was <3 flies per 100 g of blueberries.

In the 2017 experiment, there were no significant effects due to insecticide for either *D. suzukii* adult mortality or the number of progeny in berries (Table 5). Mean mortalities for all insecticides were <10% at 3 DAT. Although not significant, spinosad had the highest mortality at 5 DAT, but it was <60%. There were no significant differences among treatments in the field infestation samples ($F = 0.21$; $df = 4, 23$; $p = 0.932$). Mean infestation was less than 10 flies per 100 g blueberries.

3.2.4 | Minnesota

In the 2016 trial, insecticide treatment had a significant effect on *D. suzukii* mortality at 0 and 5 DAT (Table 6). Spinosad caused the highest mortality at 0 DAT. At 5 DAT, mean mortality was very low (≤10%) and none of the insecticide treatments were significantly different from the control.

In the 2017 trial, insecticide treatment had a significant effect on *D. suzukii* mortality only at 0 DAT (Table 6). Spinosad caused the highest mortality, and there was no difference among azadirachtin + pyrethrins, *C. subtsugae* or the control. At 3 DAT, mean mortality from the insecticides was <25%, and at 5 DAT mean mortality was <10%.

4 | DISCUSSION

This study shows that most insecticides currently labelled for use in organic berry production do not, by themselves, provide adequate protection against *D. suzukii*. Spinosad was the only product that consistently caused high mortality of *D. suzukii* in both laboratory and semi-field experiments. This was true across different laboratory methods (vials, fruit dip, spray tower) and in semi-field trials across different regions (FL, GA, MI, MN). Results from the current

TABLE 4 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment and residue age in semi-field bioassays at 120-hr exposure and mean (±*SE*) number of *D. suzukii* progeny per 5-berry sample. The experiments were conducted at a blueberry farm in Baxley, GA, in 2017

Blueberry type	Residue age	Treatment	Mean (±*SE*) per cent adult mortality	Mean (±*SE*) progeny per 5 berries
Highbush	0 days	Control	45.0 ± 15.00	22.5 ± 1.50
		Spinosad	83.3 ± 3.33	0.0 ± 0.0
		Azadirachtin + pyrethrins	46.7 ± 20.28	26.7 ± 12.12
		C. subtsugae	80.0 ± 15.27	13.3 ± 3.28
		Hydrogen peroxide + PAA	60.0 ± 10.00	25.3 ± 2.96
		ANOVA	$F = 2.02$; $df = 4, 7$; $p = 0.194$	$F = 3.27$; $df = 4, 9$; $p = 0.065$
	3 days	Control	16.7 ± 3.33 c	36.7 ± 5.67 ab
		Spinosad	73.3 ± 12.02 a	16.7 ± 3.93 b
		Azadirachtin + pyrethrins	50.0 ± 5.77 ab	55.0 ± 13.65 a
		C. subtsugae	30.0 ± 11.55 bc	38.3 ± 12.25 ab
		Hydrogen peroxide + PAA	46.7 ± 8.82 ab	49.3 ± 8.41 a
		ANOVA	$F = 6.48$; $df = 4, 8$; $p = 0.013$	$F = 4.29$; $df = 4, 8$; $p = 0.038$
	5 days	Control	25.0 ± 25.00	71.0 ± 40.00
		Spinosad	46.7 ± 21.86	37.7 ± 14.19
		Azadirachtin + pyrethrins	73.3 ± 13.33	36.7 ± 15.17
		C. subtsugae	76.7 ± 8.82	21.0 ± 4.04
		Hydrogen peroxide + PAA	30.0 ± 25.17	38.3 ± 15.30
		ANOVA	$F = 1.47$; $df = 4, 9$; $p = 0.288$	$F = 0.94$; $df = 4, 9$; $p = 0.484$
Rabbiteye	0 days	Control	13.3 ± 8.82 b	24.3 ± 8.84
		Spinosad	100.0 ± 0.0 a	7.0 ± 2.65
		Azadirachtin + pyrethrins	23.0 ± 9.05 b	35.0 ± 6.03
		C. subtsugae	16.7 ± 12.02 b	20.0 ± 2.65
		Hydrogen peroxide + PAA	36.7 ± 21.86 b	37.3 ± 10.73
		ANOVA	$F = 8.21$; $df = 4, 10$; $p = 0.003$	$F = 3.09$; $df = 4, 10$; $p = 0.068$
	3 days	Control	20.0 ± 5.77 ab	60.3 ± 14.38
		Spinosad	41.2 ± 12.61 a	22.3 ± 1.67
		Azadirachtin + pyrethrins	23.3 ± 6.67 ab	42.0 ± 11.59
		C. subtsugae	31.1 ± 5.88 a	43.3 ± 9.94
		Hydrogen peroxide + PAA	3.3 ± 3.33 b	42.7 ± 15.59
		ANOVA	$F = 3.50$; $df = 4, 10$; $p = 0.049$	$F = 1.75$; $df = 4, 8$; $p = 0.232$
	5 days	Control	31.5 ± 16.31	30.0 ± 4.93
		Spinosad	6.7 ± 6.67	33.3 ± 8.88
		Azadirachtin + pyrethrins	0.0 ± 0.0	28.7 ± 6.57
		C. subtsugae	13.3 ± 8.82	40.7 ± 8.41
		Hydrogen peroxide + PAA	16.1 ± 8.73	31.3 ± 7.54
		ANOVA	$F = 1.51$; $df = 4, 10$; $p = 0.272$	$F = 0.41$; $df = 4, 10$; $p = 0.799$

Note. Means within each column followed by the same letter were not significantly different (LSD test, $p < 0.05$).

study are consistent with other experiments evaluating organic insecticides. Cahenzli et al. (2018) screened 25 natural crop protection products and found that only spinosad was toxic to *D. suzukii* and reduced oviposition on treated berries. Shawer et al. (2018) found that spinosad was one of the products that caused high *D. suzukii* adult mortality in a fruit dip laboratory bioassay using cherries. In field trials, however, an organic programme using spinosad and pyrethrins did not reduce *D. suzukii* fruit damage (Shawer et al., 2018). The reasons why our field programme involving spinosad did not perform as well as expected are unclear but may be related to abiotic factors that degrade the active ingredient including light, temperature, humidity and rainfall. Some of the controls in our semi-field bioassays

TABLE 5 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment and residue age in semi-field bioassays at 120-hr exposure and mean (±*SE*) number of *D. suzukii* progeny per 5-berry sample. The experiments were conducted on blueberries at a research station in Fennville, MI

Year	Residue age	Treatment	Mean (±*SE*) per cent adult mortality	Mean (±*SE*) progeny per 5 berries
2016	0 days	Control	6.3 ± 3.99 cd	22.8 ± 4.09 a
		Spinosad	87.5 ± 9.92 a	1.0 ± 0.0 b
		Azadirachtin	4.2 ± 2.41 cd	24.8 ± 6.74 a
		Azadirachtin + pyrethrins	0.0 ± 0.0 d	21.8 ± 3.57 a
		C. subtsugae	18.8 ± 9.24 bc	24.0 ± 2.38 a
		Hydrogen peroxide + PAA	8.3 ± 4.81 bcd	21.5 ± 2.40 a
		Hydrogen dioxide + PAA	2.1 ± 2.08 cd	22.3 ± 4.52 a
		Burkholderia spp.	25.0 ± 9.00 b	17.5 ± 0.96 a
		ANOVA	$F = 21.38$; $df = 7, 24$; $p < 0.001$	$F = 4.47$; $df = 7, 24$; $p = 0.003$
	7 days	Control	10.4 ± 10.42	45.5 ± 7.38
		Spinosad	20.8 ± 5.38	23.3 ± 4.84
		Azadirachtin	27.1 ± 7.12	25.8 ± 3.07
		Azadirachtin + pyrethrins	14.6 ± 5.24	36.3 ± 8.34
		C. subtsugae	10.4 ± 3.99	36.3 ± 6.21
		Hydrogen peroxide + PAA	10.4 ± 5.24	31.5 ± 5.20
		Hydrogen dioxide + PAA	20.8 ± 12.96	34.5 ± 9.26
		Burkholderia spp.	14.6 ± 7.12	20.0 ± 1.73
		ANOVA	$F = 0.64$; $df = 7, 24$; $p = 0.720$	$F = 1.78$; $df = 7, 24$; $p = 0.139$
2017	0 days	Control	27.8 ± 12.11	48.7 ± 9.94
		Spinosad	25.0 ± 9.62	33.7 ± 12.03
		Azadirachtin + pyrethrins	63.9 ± 20.03	22.3 ± 4.98
		C. subtsugae	45.8 ± 20.83	29.0 ± 9.00
		Hydrogen peroxide + PAA	13.9 ± 10.02	35.3 ± 2.73
		ANOVA	$F = 1.92$; $df = 4, 9$; $p = 0.192$	$F = 1.46$; $df = 4, 7$; $p = 0.307$
	3 days	Control	16.7 ± 12.73	26.0 ± 4.73
		Spinosad	2.8 ± 2.78	26.0 ± 3.06
		Azadirachtin + pyrethrins	8.3 ± 8.33	25.0 ± 6.66
		C. subtsugae	0.0 ± 0.0	24.0 ± 3.22
		Hydrogen peroxide + PAA	0.0 ± 0.0	41.7 ± 5.84
		ANOVA	$F = 1.05$; $df = 4, 10$; $p = 0.430$	$F = 2.86$; $df = 4, 8$; $p = 0.096$
	5 days	Control	5.6 ± 5.56	19.7 ± 3.71
		Spinosad	55.6 ± 28.19	10.0 ± 1.53
		Azadirachtin + pyrethrins	5.6 ± 5.56	20.7 ± 5.61
		C. subtsugae	11.1 ± 5.56	34.0 ± 10.15
		Hydrogen peroxide + PAA	0.0 ± 0.0	35.7 ± 7.69
		ANOVA	$F = 2.90$; $df = 4, 10$; $p = 0.078$	$F = 2.75$; $df = 4, 10$; $p = 0.088$

Note. Means within each column followed by the same letter were not significantly different (LSD test, $p < 0.05$)

had high mortality. This may represent variation in batches of flies from our laboratory colony; however, all flies used in a given experiment at a given residue age were from the same batch.

In the current study, neither azadirachtin nor pyrethrins alone were effective against *D. suzukii*. Bruck et al. (2011) found that two applications of pyrethrin showed less control than pyrethrin in rotation with spinosad in an organic red raspberry trial. In other studies, spinosad has performed well against other pests such as yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Balusu & Fadamiro, 2012) and brown marmorated stink bug, *Halyomorpha halys* (Stål) (Lee, Short, Nielsen, & Leskey, 2014; Leskey, Short, & Lee, 2014). The efficacy of spinosad may be attributed to its broad-spectrum activity and multiple modes of entry (contact and stomach poison) (Balusu & Fadamiro, 2012). Application method

TABLE 6 Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by insecticide treatment and residue age in semi-field bioassays at 120-hr exposure. The experiments were conducted on raspberries at a farm in Hastings, MN

Year	Residue age	Treatment	Mean (±*SE*) per cent adult mortality
2016	0 days	Control	2.0 ± 2.00 c
		Spinosad	96.0 ± 4.00 a
		Azadirachtin + pyrethrins	28.0 ± 10.20 b
		C. subtsugae	34.0 ± 12.08 b
		ANOVA	$F = 23.51$; $df = 3, 16$; $p < 0.001$
	3 days	Control	0.0 ± 0.0
		Spinosad	14.0 ± 9.27
		Azadirachtin + pyrethrins	2.0 ± 2.00
		C. subtsugae	0.0 ± 0.0
		ANOVA	$F = 2.32$; $df = 3, 12$; $p = 0.127$
	5 days	Control	4.0 ± 2.45 ab
		Spinosad	10.0 ± 4.47 a
		Azadirachtin + pyrethrins	0.0 ± 0.0 b
		C. subtsugae	0.0 ± 0.0 b
		ANOVA	$F = 3.94$; $df = 3, 12$; $p = 0.036$
2017	0 days	Control	12.0 ± 5.83 b
		Spinosad	46.1 ± 10.38 a
		Azadirachtin + pyrethrins	13.1 ± 8.18 b
		C. subtsugae	6.4 ± 2.64 b
		ANOVA	$F = 6.03$; $df = 3, 16$; $p = 0.006$
	3 days	Control	9.5 ± 7.26
		Spinosad	6.7 ± 4.44
		Azadirachtin + pyrethrins	22.7 ± 12.35
		C. subtsugae	22.2 ± 10.54
		ANOVA	$F = 0.84$; $df = 3, 16$; $p = 0.493$
	5 days	Control	33.0 ± 17.29
		Spinosad	6.7 ± 4.44
		Azadirachtin + pyrethrins	9.2 ± 4.60
		C. subtsugae	8.7 ± 2.18
		ANOVA	$F = 1.80$; $df = 3, 16$; $p = 0.188$

Note. Means within each column followed by the same letter were not significantly different (LSD test, $p < 0.05$).

also affects efficacy of these products, and we observed differences in insecticide efficacy depending on the laboratory bioassay method. Cahenzli et al. (2018) used direct contact, indirect contact and treated berries in laboratory bioassays. Spinosad was effective with all three methods; azadirachtin and pyrethrum were effective in the direct and indirect contact assays but not on treated berries (Cahenzli et al., 2018). This difference between the direct and indirect contact assays was because azadirachtin and pyrethrum were formulated in oil and killed by suffocation (Cahenzli et al., 2018). Bruck et al. (2011) found pyrethrins caused intermediate control and azadirachtin caused low control in direct spray bioassays. Azadirachtin also caused low adult mortality in bioassays using different substrates for insecticide screening, but in these bioassays, azadirachtin was not applied directly to the flies (Pavlova, Dahlmann, Hauck, & Reineke, 2017). Conversely, we saw higher mortality due to azadirachtin and pyrethrins in the treated fruit bioassay and lower mortality in the direct contact bioassay.

The insecticides evaluated in this study are biopesticides representing various modes of action. Most of these products are labelled for primarily leaf-feeding insects and insects with sucking mouthparts. This could explain limited efficacy of these products because an insecticide effective against *D. suzukii* would need to have rapid contact activity to kill the adult female flies before they lay eggs. The organic formulations of spinosad, *C. subtsugae*, *Burkholderia* spp. and sabadilla alkaloids are labelled for control of *D. suzukii*, and pyrethrins and azadirachtin + pyrethrins are labelled for vinegar flies, but azadirachtin is not labelled for any drosophilids. In a field trial,

sabadilla alkaloids and *C. subtsugae* both reduced the number of *D. suzukii* larvae in raspberry fruit when used in rotation with spinosad (Fanning et al., 2018). Azadirachtin and *Burkholderia* spp. have some activity as insect growth regulators (IGR) that disrupt moulting. For an IGR to be effective, immature stages should be targeted, while the bioassays in this study focused on adult flies. Immature stages of *D. suzukii* are protected from most insecticides as they feed within fruit. Efficacy of insecticides post-infestation has been shown using conventional insecticides (Wise, Vanderpoppen, Vandervoort, O'Donnell, & Isaacs, 2015), so there is potential for some efficacy through this route of exposure of eggs and larvae.

The sanitizers tested in these trials did not exhibit insecticidal properties. It is a common practice in berry production in the Pacific Northwest to supplement insecticides with a product such as hydrogen peroxide + PAA. This is done in rotation or tank mixing to maintain disease control, but it is possible this provides a crop protection benefit against *D. suzukii*. Additional experiments will be required to determine how these oxidizing agents affect the microbial community on berry crops and how that in turn affects *D. suzukii* (Guedes, Corbett, Rodriguez, Goto, & Walse, 2018; Hamby et al., 2016).

None of the insecticides in this study had long residual activity. Biopesticides approved for use in organic systems are generally less potent and have shorter residual activity than synthetic conventional products (Zehnder et al., 2007). In an evaluation of season-long programmes in blueberries and blackberries, Diepenbrock et al. (2016) and Diepenbrock, Hardin, and Burrack (2017) also observed no residual efficacy of any organic insecticide programme at 7 DAT. The short residual activity of organic insecticides is desirable from the standpoint of short preharvest intervals (Andreazza et al., 2017) and not reaching maximum residue limits (Haviland & Beers, 2012), but it is problematic because it means many applications are needed to keep *D. suzukii* in check through the entire harvest period. Azadirachtin + pyrethrins and sabadilla alkaloids showed promise in some of our experiments and similar findings were reported by Iglesias and Liburd (2017), but results were not consistent across all research sites. With a zero tolerance threshold for *D. suzukii* infestation in fresh fruit, an effective insecticide needs to kill a high percentage of the population (close to 100%) to provide sufficient protection.

Adult insect mortality is only one measure of insecticide efficacy. The primary goal is protecting the fruit from insect damage, so the assays that included fruit provided important information for determining treatment effectiveness in this context. Treatments that killed a high percentage of *D. suzukii* adults tended to have few progeny in the fruit. In one of the fruit dip bioassays, spinosad and pyrethrin killed 100% of adults by the end of the experiment and no progeny were found in the berries. Lack of progeny may be due to adults dying before oviposition, but it is also possible that eggs were subsequently killed before hatching. The mechanism is unknown because we did not count oviposition holes or eggs in the berries after exposure to adults. In the 2016 fruit dip bioassay, more progeny developed in *C. subtsugae* treated berries than any other treatment and the control. This could be an odour response to the product resulting in flies spending more time on the berries and laying more eggs. Low numbers of progeny in the fruit dip bioassays could also be due to characteristics of the berries that reduced susceptibility to oviposition. We used store-bought organic blueberries of unspecified cultivar(s). Blueberry cultivar was shown to influence susceptibility of blueberries to *D. suzukii* oviposition (Kinjo, Kunimi, Ban, & Nakai, 2013).

Field infestation samples did not show treatment differences in most cases. This may be due to low infestation levels, particularly in the southern states. In the 2016 experiments in Georgia, for instance, sampling did not detect any *D. suzukii* immatures in berries. We did not check fruit for oviposition holes or egg filaments, but fruit was held in the laboratory for several days before filter salt extraction, so eggs, if present and viable, had time to hatch. Small plot sizes may also contribute to high variability in the data. Larger plots with larger berry samples may be necessary to resolve treatment differences in future field studies.

Although this study did not identify an insecticide that could stand alone as a substitute for spinosad, some of the products evaluated could still prove useful in a rotation programme with spinosad. Additional trials to evaluate season-long rotation programmes on a larger scale (field or farm) would need to be performed. In addition, trials should include the crop sanitizers in combination with insecticides as recommended by the sanitizer manufacturers (C. R. Roubos, personal communication). Suppressing *D. suzukii* populations in berry crops is critical for profitable production, but continued reliance on control programmes using primarily spinosad is not sustainable due to the risk of selecting for resistance (Gress & Zalom, 2018; Van Timmeren et al., 2018).

The use of insecticides by growers in organic and conventional fruit agriculture has changed rapidly in response to invasive pests such as *D. suzukii*. Maintaining control is challenging and there is an urgent need to find effective and selective control methods for these invasive fruit pests. Insecticides will continue to play an important role in many organic berry farms in the short term while other tactics (cultural and biological control) are developed, optimized and adopted.

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AUTHORS' CONTRIBUTIONS

AAS, MAR, OEL and RI conceived the study. CRR, BKG, PDF, SVT, JS, AP and BAL conducted experiments. CRR analysed the data and

wrote the original manuscript. All authors read and approved the manuscript.

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