Received: 4 October 2018 | Revised: 17 December 2018 | Accepted: 23 January 2019

DOI: 10.1111/jen.12620

ORIGINAL CONTRIBUTION

WILEY JOURNAL OF APPLIED ENTOMOLOGY

# Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura)

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**Funding information**
Project GREEEN; Georgia Agricultural Commodity Commission for Blueberries; Georgia Blueberry Growers Association; Florida Department of Agriculture and Consumer Services; U.S. Department of Agriculture, National Institute of Food and Agriculture, Grant/Award Number: EIP Award # 2014-70006-22533 and OREI Award # 2015-51300-24154

## Abstract

Spotted-wing drosophila, *Drosophila suzukii* Matsumura, is an invasive pest in the United States that causes considerable damage to fruit crops. It is responsible for many millions of dollars of revenue loss. The female *D. suzukii* has a heavily sclerotized ovipositor and can lay eggs in ripening or ripe fruit. The arrival of this invasive species has disrupted existing integrated pest management programmes, and growers rely on repeated insecticide applications to protect fruit. Organic growers have few chemical control options, and their reliance on spinosad increases the risk of developing insecticide resistance. We hypothesized that combining phagostimulants with insecticides would increase insecticide efficacy by prompting flies to spend more time in contact with residues. Therefore, the objective of this study was to evaluate the effectiveness of sucrose and the yeast *Saccharomyces cerevisiae* as phagostimulants in combination with organic biopesticides against *D. suzukii* in blueberries. Adding sucrose with or without yeast did not improve insecticide efficacy in terms of adult fly mortality or fruit infestation. Spinosad was very effective in all experiments, and for this product, there is little room for improvement. The phagostimulants had no effect on residual activity of any insecticide. The addition of sucrose with or without yeast did not improve the effectiveness of organic insecticides for *D. suzukii*. Concentrations of these phagostimulants in our experiments (0.36%) may have been too low to elicit a response. Further research is recommended to test different types and concentrations of phagostimulants.

KEYWORDS
biopesticide, blueberries, organic, sugar, yeast

## 1 | INTRODUCTION

Invasive species carry large environmental and economic costs. They can displace native species and disrupt agroecosystems. About 40% of arthropod pests in agriculture are introduced species (Pimentel, Zuniga, & Morrison, 2005). Invasive arthropod pests cost an estimated $13 billion in crop losses with about $US 500 million spent on pesticides to control them (Pimentel et al., 2005). *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), also known as spotted-wing drosophila, is native to eastern and

  

south-eastern Asia (Walsh et al., 2011). It was first detected in North America and Europe in 2008 and South America in 2013 and has become a pest of global significance (Calabria, Máca, Bächli, Serra, & Pascual, 2012; Deprá, Poppe, Schmitz, De Toni, & Valente, 2014; Gutierrez, Ponti, & Dalton, 2016; Hauser, 2011). *Drosophila suzukii* can reproduce quickly, and its heavily sclerotized ovipositor enables females to cut into the flesh of ripening or ripe fruit to deposit eggs (Attalah, Teixeira, Salazar, Zaragoza, & Kopp, 2014; Hauser, 2011). Most other *Drosophila* can only oviposit in overripe or rotting fruit which are much softer or have split skin. Primary injury from *D. suzukii* is due to larval feeding within fruit. Damage from oviposition can also lead to secondary infection by plant pathogens (Walsh et al., 2011).

*Drosophila suzukii* is highly polyphagous. Cultivated fruit crops susceptible to *D. suzukii* include blackberry, blueberry, cherry, raspberry, strawberry, peach and grape (Bellamy, Sisterson, & Walse, 2013; Burrack, Fernandez, Spivey, & Kraus, 2013; Ioriatti et al., 2015; Lee et al., 2011). Farnsworth et al. (2017) calculated that *D. suzukii* accounted for revenue losses of $39.8 million in the California raspberry industry with $3.43 million (5.74% of realized revenues) coming from organic production. They estimated that chemical purchases for *D. suzukii* management increased annual per-hectare production costs by $US 1,161 and $US 2,933 for conventional and organic producers, respectively (Farnsworth et al., 2017). *Drosophila suzukii* can also infest many wild non-crop hosts (Lee et al., 2015). Other factors that make *D. suzukii* difficult to manage are its short life cycle, high fecundity and the need to protect fruit at harvest which necessitates insecticides with short pre-harvest intervals (Walsh et al., 2011).

The arrival of invasive species is almost always detrimental to existing integrated pest management (IPM) programmes (Hoddle, 2006). Many fruit growers have shifted their pest management programmes in response to *D. suzukii*. Use of selective insecticides applied based on scouting data has been abandoned for prophylactic application of broad-spectrum insecticides applied when fruit is ripe and vulnerable to infestation (Van Timmeren & Isaacs, 2013). Intensive chemical control is not sustainable but can serve as an emergency measure to meet immediate pest management needs for profitable production until integrated management programmes are developed and adopted (Diepenbrock, Hardin, & Burrack, 2017). Challenges of prophylactic pesticide use include complying with maximum residue limits for export and mitigating the development of insecticide resistance (Haviland & Beers, 2012; Van Timmeren, Mota-Sanchez, Wise, & Isaacs, 2018). For certified organic fruit growers, the challenge is greater because there are fewer chemical classes available to them. Spinosad has been shown to be one of the most effective organically approved insecticides for controlling *D. suzukii* (Beers, Van Steenwyk, Shearer, Coates, & Grant, 2011; Bruck et al., 2011; Cahenzli, Strack, & Daniel, 2018; Van Timmeren & Isaacs, 2013). Label restrictions, however, limit the number of applications of spinosad that can be made during a growing season, and reliance on one product will hasten development of resistance. Other biopesticides such as *Chromobacterium subtsugae* and sabadilla alkaloids have some efficacy against *D. suzukii* and could be used as rotation partners with spinosad (Fanning, Grieshop, & Isaacs, 2018).

With limited chemical control options, it is important to make the best use of what products are available (Cowles et al., 2015). One option for enhancing insecticides is the addition of one or more behaviour-modifying chemicals, such as phagostimulants (Foster & Harris, 1997). Phagostimulants can increase exposure to toxins that must be ingested or increase contact with toxins that might be suppressed if pests respond by ceasing to feed (Foster & Harris, 1997). Cowles et al. (2015) showed that *D. suzukii* is sensitive to and able to detect low concentrations of sucrose on surfaces in their environment and that both contact insecticides and those acting through ingestion benefitted from the addition of sucrose. Fanning et al. (2018), however, did not find a benefit of combining corn syrup with select biopesticides in fall red raspberries. Yeasts are also important components in *Drosophila* ecology, affecting physiology and behaviour (Bellutti et al., 2018; Hamby & Becher, 2016). *Drosophila suzukii* has a specific association with the yeast *Hanseniaspora uvarum* (Niehaus) (Hamby, Hernández, Boundy-Mills, & Zalom, 2012). In a laboratory bioassay, Mori et al. (2017) observed that spinosad in combination with *H. uvarum* significantly increased mortality of *D. suzukii* females over spinosad alone. Knight, Basoalto, Yee, Hilton, and Kurtzman (2015) found that the combination of yeasts and sugar can improve efficacy of diamide and spinosyn insecticides. Organic insecticides that are not very effective on their own could be enhanced when combined with phagostimulants, increasing the number of effective chemical classes available for use in *D. suzukii* management programmes and reducing reliance on spinosad. The goal of this study was to determine the effect of phagostimulants on the toxicity and residual activity of insecticides against *D. suzukii* in organic blueberries.

## 2 | MATERIALS AND METHODS

A series of bioassays were performed to evaluate phagostimulant efficacy in combination with pesticides under laboratory and field conditions (Table . For the laboratory bioassays, each participating laboratory employed a different method of exposing *D. suzukii* to pesticide residues: treated glass vials, treated blueberries, treated Petri dishes and direct spray on flies. Semi-field bioassays were conducted in two states to represent different growing regions for blueberries. Trials in Georgia were conducted on southern highbush blueberry (*Vaccinium corymbosum* L. × *V. darrowi* Camp) and rabbiteye blueberry (*V. virgatum* Aiton), and trials in Michigan were conducted on northern highbush blueberry (*V. corymbosum*). Treatment efficacy in laboratory bioassays was determined based on adult fly mortality, and in the case of the fruit dip method, the number of progeny able to develop on treated fruit. In the semi-field trials, efficacy was also assessed based on adult fly mortality and the number of progeny able to develop on treated fruit as well as infestation in fruit collected from treatment plots.

**TABLE 1** OMRI-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.

## 2.1 | 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 anaesthetized using $CO_2$.

## 2.2 | Chemical treatments

Insecticides and rates used in this study are listed in Table 1. All products were listed by the Organic Materials Review Institute (OMRI) as acceptable for use in certified organic production. Phagostimulants were sucrose (white granulated sugar, Great Value™ Pure Sugar, Wal-Mart Stores, Inc., Bentonville, AR) at 3.6 g/L and yeast (active dry yeast, *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, Red Star, Milwaukee, WI) at 3.6 g/L (Knight et al., 2015). Phagostimulant treatments were sucrose with each insecticide, sucrose + yeast with each insecticide and yeast with each insecticide in one of the laboratory assays.

## 2.3 | Laboratory experiments

### 2.3.1 | Glass vial bioassay

A glass vial bioassay was conducted at the University of Florida. The bioassay included six treatments (azadirachtin + pyrethrins, spinosad, *Chromobacterium subtsugae*, hydrogen peroxide + peroxyacetic acid (PAA), *Burkholderia* spp. and sabadilla alkaloids) plus a control (acetone only) with and without phagostimulants.

Pesticides were mixed with acetone to equal a total volume of 1 ml. Spinosad, *C. subtsugae*, *Burkholderia* spp. and any treatment containing sucrose + yeast did not mix well when added directly to acetone, resulting in uneven coverage of the vials. Therefore, these treatments were mixed with 100 µl deionized water before mixing with acetone. Treatment 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 3 day post-exposure.

### 2.3.2 | Fruit dip bioassay

Fruit dip bioassays were conducted at the University of Georgia in 2016 and 2017. The 2016 bioassay included eight pesticide treatments (azadirachtin, azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, hydrogen dioxide + PAA, pyrethrins and *Burkholderia* spp.) with an untreated control with and without phagostimulants in five replicates. The 2017 bioassay included six pesticide

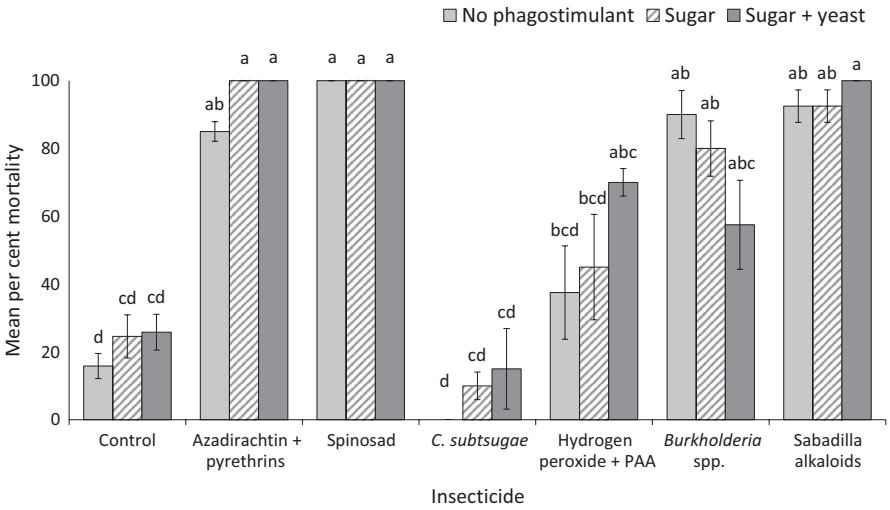


**FIGURE 1** Mean (±*SE*) per cent *Drosophila suzukii* mortality by pesticide treatment in a glass vial laboratory bioassay at 3-day exposure. Bioassays were conducted at the University of Florida. Means within a column followed by the same letter were not significantly different (LSD test, $p < 0.05$)

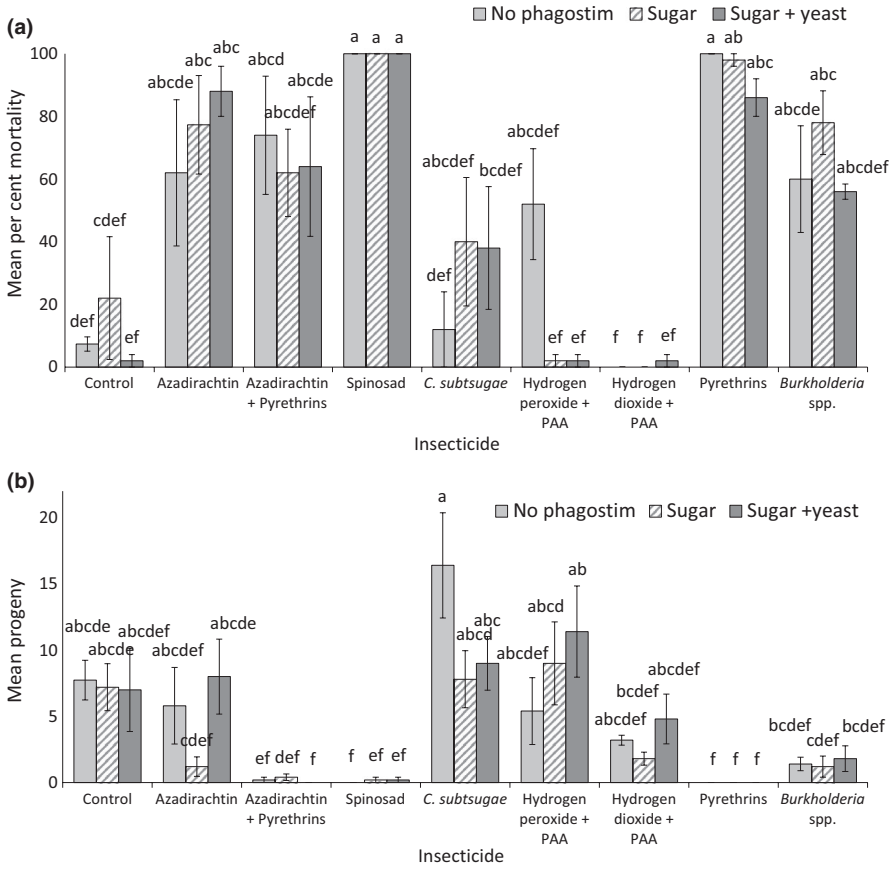


**FIGURE 2** Mean (±*SE*) per cent *Drosophila suzukii* adult mortality at 3-day exposure (a) and mean (±*SE*) number of progeny per five blueberries (b) by pesticide treatment in fruit dip laboratory bioassays. Bioassays were conducted at the University of Georgia in 2016. Means with the same letter were not significantly different (LSD test, $p < 0.05$)

treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, *Burkholderia* spp. and sabadilla alkaloids) with an untreated control with and without phagostimulants in five replicates. Treatments were applied to store-bought organic blueberries. Berries were rinsed two to three times in deionized water to wash off any pesticide 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 (Fabri-Kal Corp., Kalamazoo, MI) 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 chambers at 24°C, 70% RH and a photoperiod of 14:10 [L:D] hr. Mortality was assessed at 3 day post-exposure. After 3 day, the berries were transferred to clean deli cups without flies and were held for 2 weeks to allow for 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. There were two pesticide treatments (spinosad and *C. subtsugae*) plus an untreated control with and without phagostimulants in six replicates. Treatments were sprayed directly onto $CO_2$-anaesthetized *D. suzukii* adults in Petri dishes (100 × 15 mm; Fisher Scientific Company LLC, Pittsburgh, PA) using a Potter Spray Tower (Burkard Scientific, Uxbridge, UK) set at 103.4 kPa with 2 ml of spray solution applied to each replicate (Van Timmeren et al., 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. Mortality was assessed at 3 day 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. This bioassay consisted of six pesticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, *Burkholderia* spp. and sabadilla alkaloids) plus an untreated control with and without phagostimulants in four replicates. Treatments were sprayed onto plastic Petri dishes using a Potter Spray Tower (Burkard Scientific, Uxbridge, UK) set at 103.4 kPa with 2 ml of spray solution applied to each replicate (Van Timmeren et al., 2018). 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 3 day post-application. Petri dishes were maintained in a growth chamber at 25°C, 75% RH and a photoperiod of 16:8 [L:D] hr.

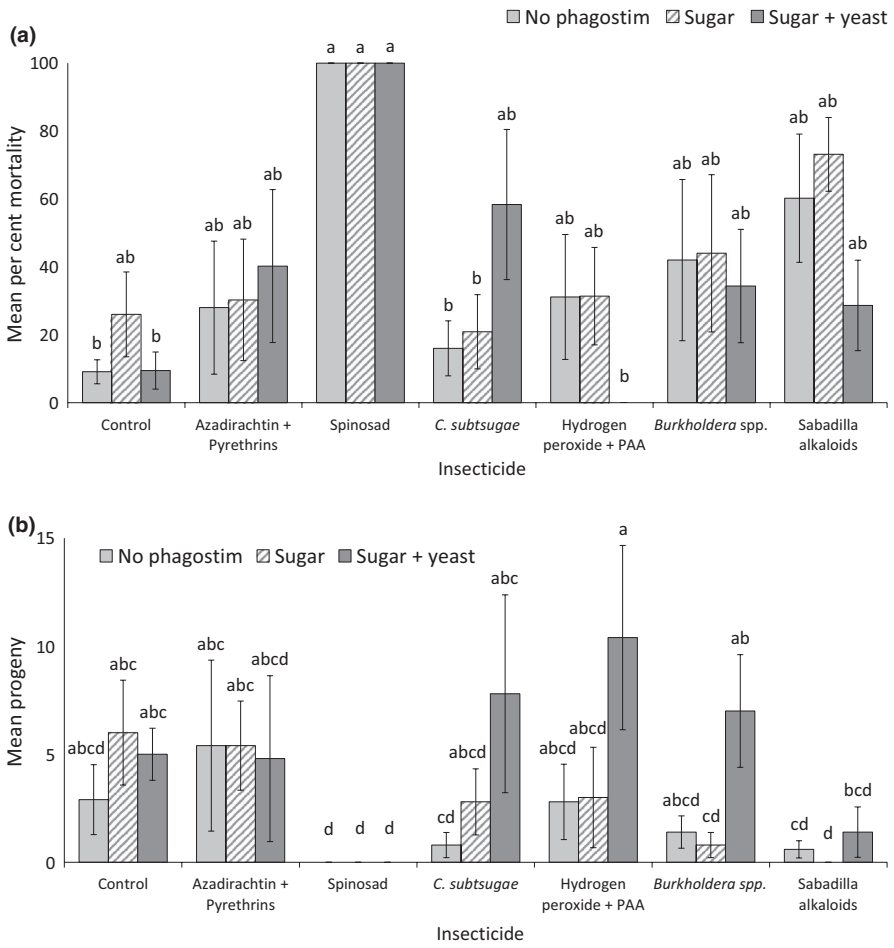


**FIGURE 3** Mean (±*SE*) per cent *Drosophila suzukii* adult mortality at 3-day exposure (a) and mean (±SE) number of progeny per five blueberries (b) by pesticide treatment in fruit dip laboratory bioassays. Bioassays were conducted at the University of Georgia in 2017. Means with the same letter were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

## 2.4 | Semi-field experiments

Semi-field experiments were performed in Georgia and Michigan. Each bioassay sample consisted of a single cut blueberry branch containing five to seven leaves and five ripe berries, all 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 blueberry 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 5 day, and the number of progeny (larvae, pupae and adults) coming out of the berries was counted.

Fruit infestation in the field was determined 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). Samples consisted of 0.12–0.24 L of ripe berries from each plot. Berries were weighed prior to assessment, and infestation was reported as the number of larvae per gram berries.

### 2.4.1 | Georgia

The Georgia semi-field trials were conducted on certified organic blueberry farms in Baxley, GA (Appling County). The 2016 experiment was conducted in rabbiteye blueberry ("Premier" variety) and ran from 7 June to 3 July 2016. Two experiments were conducted in 2017, one in southern highbush blueberry ("Star" variety) from 21 to 26 April 2017 and one in rabbiteye blueberry ("Premier" variety) from 9 to 14 June 2017. All bushes were 6–8 years old and at least 1.5 m in height, planted on 3.66 m row centres and either 0.91 m (southern highbush) or 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). Mortality was assessed at 5 day. After 5 day, the 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, berries were dissected and the numbers of larvae, pupae and adults were recorded.

In the 2016 experiment in rabbiteye blueberries, there were four insecticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and pyrethrins) plus a control with and without phagostimulants 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 pesticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and hydrogen peroxide + PAA) plus an untreated control with and without

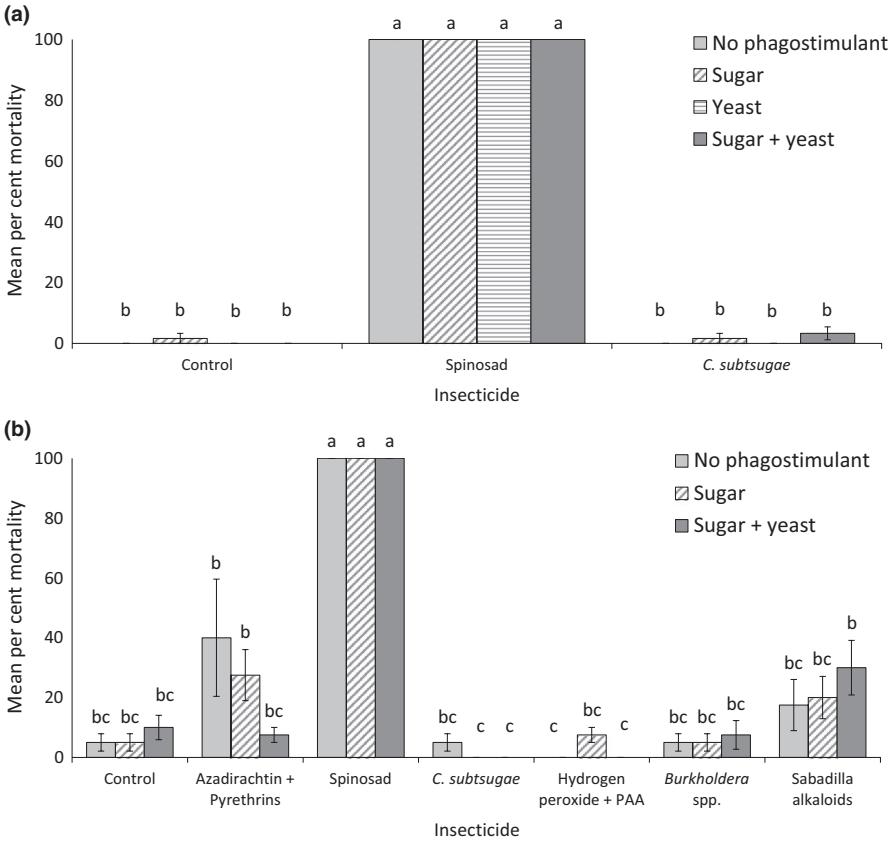


**FIGURE 4** Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment in a topical application laboratory bioassay at 2-day exposure (a) and residual contact laboratory bioassay at 3-day exposure (b). Bioassays were conducted at Michigan State University. Means within each bioassay followed by the same letter were not significantly different (LSD test, $p < 0.05$)

**TABLE 2** Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by pesticide by pesticide treatment and residue age in semi-field bioassays at 5-day exposure and mean (±*SE*) number of *D. suzukii* progeny per five blueberries

| Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|
| 0 DAT | Untreated control | 0.0 ± 0.0 b | 7.7 ± 0.33 bcd |
| | Suc | 13.3 ± 8.82 b | 38.3 ± 2.85 ab |
| | Suc Yst | 10.0 ± 10.00 b | 20.3 ± 2.60 abc |
| | Spinosad | 83.3 ± 8.82 a | 4.0 ± 2.08 cd |
| | Spinosad + Suc | 90.0 ± 0.0 a | 0.7 ± 0.33 d |
| | Spinosad + Suc Yst | 56.7 ± 14.53 ab | 19.0 ± 15.00 abcd |
| | Azadirachtin + pyrethrins | 6.7 ± 3.33 b | 32.0 ± 7.37 ab |
| | Azadirachtin + pyrethrins + Suc | 16.7 ± 8.82 ab | 36.7 ± 4.91 ab |
| | Azadirachtin + pyrethrins + Suc Yst | 33.3 ± 28.48 ab | 39.0 ± 1.53 a |
| | *C. subtsugae* | 0.0 ± 0.0 b | 14.3 ± 5.33 abcd |
| | *C. subtsugae* + Suc | 3.3 ± 3.33 b | 24.3 ± 8.33 abc |
| | *C. subtsugae* + Suc Yst | 36.7 ± 12.02 ab | 24.7 ± 1.20 abc |
| | Pyrethrins | 0.0 ± 0.0 b | 12.0 ± 2.08 abcd |
| | Pyrethrins + Suc | 10.0 ± 5.77 b | 26.0 ± 4.04 abc |
| | Pyrethrins + Suc Yst | 10.0 ± 10.00 b | 32.7 ± 6.12 ab |
| | ANOVA | $F = 6.35$; $df = 14, 28$; $p < 0.001$ | $F = 7.14$; $df = 14, 28$; $p < 0.001$ |
| 3 DAT | Untreated control | 16.7 ± 8.82 ab | 26.67 ± 5.84 |
| | Suc | 10.0 ± 5.77 b | 7.0 ± 1.16 |
| | Suc Yst | 20.0 ± 10.00 ab | 7.3 ± 2.96 |
| | Spinosad | 46.7 ± 12.02 ab | 19.0 ± 6.56 |
| | Spinosad + Suc | 66.7 ± 6.67 a | 5.3 ± 0.67 |
| | Spinosad + Suc Yst | 56.7 ± 18.56 ab | 5.3 ± 2.19 |
| | Azadirachtin + pyrethrins | 13.3 ± 8.82 ab | 13.7 ± 2.96 |
| | Azadirachtin + pyrethrins + Suc | 13.3 ± 6.67 ab | 12.0 ± 2.52 |
| | Azadirachtin + pyrethrins + Suc Yst | 20.0 ± 10.00 ab | 17.7 ± 9.24 |
| | *C. subtsugae* | 13.3 ± 8.82 ab | 7.0 ± 0.58 |
| | *C. subtsugae* + Suc | 23.3 ± 8.82 ab | 9.7 ± 5.70 |
| | *C. subtsugae* + Suc Yst | 26.7 ± 8.82 ab | 15.0 ± 8.39 |
| | Pyrethrins | 20.0 ± 10.00 ab | 7.0 ± 3.00 |
| | Pyrethrins + Suc | 20.0 ± 10.00 ab | 13.0 ± 3.61 |
| | Pyrethrins + Suc Yst | 10.0 ± 0.0 ab | 16.3 ± 3.84 |
| | ANOVA | $F = 2.76$; $df = 14, 28$; $p = 0.01$ | $F = 1.42$; $df = 14, 28$; $p = 0.21$ |
| 7 DAT | Untreated control | 10.0 ± 5.77 | 10.3 ± 2.85 ab |
| | Suc | 3.3 ± 3.33 | 29.3 ± 2.85 a |
| | Suc Yst | 3.3 ± 3.33 | 27.3 ± 3.18 ab |
| | Spinosad | 3.3 ± 3.33 | 6.0 ± 0.58 b |
| | Spinosad + Suc | 10.0 ± 5.77 | 9.3 ± 0.88 ab |
| | Spinosad + Suc Yst | 6.7 ± 6.67 | 5.7 ± 0.88 b |
| | Azadirachtin + pyrethrins | 26.7 ± 17.64 | 8.0 ± 1.53 ab |
| | Azadirachtin + pyrethrins + Suc | 3.3 ± 3.33 | 17.3 ± 6.69 ab |
| | Azadirachtin + pyrethrins + Suc Yst | 6.7 ± 6.67 | 17.7 ± 7.88 ab |
| | *C. subtsugae* | 16.7 ± 3.33 | 7.7 ± 2.19 ab |

*(Continues)*

**TABLE 2** (Continued)

| Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|
| | *C. subtsugae* + Suc | 3.3 ± 3.33 | 21.0 ± 5.51 ab |
| | *C. subtsugae* + Suc Yst | 0.0 ± 0.0 | 17.3 ± 4.10 ab |
| | Pyrethrins | 10.0 ± 10.00 | 13.7 ± 3.84 ab |
| | Pyrethrins + Suc | 10.0 ± 5.77 | 8.7 ± 2.33 ab |
| | Pyrethrins + Suc Yst | 10.0 ± 10.00 | 14.3 ± 0.88 ab |
| | ANOVA | $F = 0.80$; $df = 14, 28$; $p = 0.66$ | $F = 3.31$; $df = 14, 28$; $p < 0.01$ |

*Notes*. DAT: days after treatment; Suc: sucrose; Suc Yst: sucrose and yeast.
The experiment was conducted in rabbiteye blueberries at a blueberry farm in Baxley, GA, in 2016. Means within a column followed by the same letter were not significantly different (LSD test, $p < 0.05$).

phagostimulants in three replications. Samples for bioassays and field infestation were collected at 0, 3 and 5 DAT.

### 2.4.2 | Michigan

The Michigan semi-field trials were conducted at the Trevor Nichols Research Center in Fennville, MI (Allegan County). The 2016 experiment ran from 22 to 29 August 2016, and the 2017 experiment ran from 28 July to 07 August 2017. Each treatment plot consisted of six bushes. 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, 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. Mortality was assessed at 5 day. 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). The numbers of larvae, pupae and adults were recorded.

In the 2016 trial, there were two pesticide treatments (spinosad and *C. subtsugae*) plus an untreated control with and without phagostimulants in four replicates. Insecticides were applied on 22 August, and samples for bioassays were collected at 0 and 3 DAT.

In the 2017 trial, there were four pesticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and hydrogen peroxide + PAA) plus an untreated control with and without phagostimulants in three replicates. Insecticides were applied on 28 July, and samples for bioassays were collected at 0, 3 and 5 DAT. Samples for field infestation were collected on 03 August.

## 2.5 | Statistical analysis

Fly mortality data from all the laboratory bioassays and progeny data from the fruit dip bioassays were analysed using one-way analysis of variance (ANOVA) with insecticide treatment as the main effect and replicate as a random factor (PROC MIXED) (SAS Institute, 2013). Adult mortality data were arcsine square root-transformed, and progeny data were square root-transformed prior to analysis to meet model assumptions. Untransformed means and standard errors are reported in the tables.

Fly mortality data and progeny data from the semi-field bioassays were analysed using two-way ANOVAs with insecticide treatment and residue age (DAT) as main effects with interactions and replicate as a random factor (PROC MIXED). Subsequent analyses were performed for each residue age separately using one-way ANOVAs with insecticide treatment as the main effect and replicate as a random factor. Adult mortality data were arcsine square root-transformed, and progeny data were square root-transformed prior to analysis to meet model assumptions. Untransformed means and standard errors are reported in the tables. Mortalities for both sexes were pooled for analysis. Larvae, pupae and adults were pooled for progeny count analysis.

Field infestation samples from Michigan were collected once at the end of the experiment, so data were analysed using a one-way ANOVA with insecticide treatment as the main effect and replicate as a random factor (PROC MIXED). Field infestation samples from Georgia were collected at four time points over the course of the experiment. These data were analysed using a two-way ANOVA with insecticide treatment and residue age (DAT) as main effects with interactions and replicate as a random factor (PROC MIXED).

For all ANOVAs, model assumptions were tested using Kolmogorov–Smirnov tests for normality and Levene's tests for homogeneity of variance. Where significant differences were observed ($p < 0.05$), means were separated using the Bonferroni adjustment for multiple comparisons (PROC PLM). All analyses were performed using SAS 9.4 (SAS Institute, 2013).

# 3 | RESULTS

## 3.1 | Laboratory experiments

### 3.1.1 | Glass vial bioassay

All treatments containing azadirachtin + pyrethrins, spinosad or sabadilla alkaloids caused significantly higher mortality than the

**TABLE 3** Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment and residue age in semi-field bioassays at 5-day exposure and mean (±*SE*) number of *D. suzukii* progeny per five blueberries

| Blueberry Type | Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|---|
| Highbush | 0 DAT | Untreated control | 45.0 ± 15.00 ab | 22.5 ± 1.50 abc |
| | | Suc | 70.0 ± 5.77 ab | 8.3 ± 8.33 abc |
| | | Suc Yst | 90.0 ± 10.00 ab | 0.3 ± 0.33 bc |
| | | Spinosad | 83.3 ± 3.33 ab | 0.0 ± 0.0 c |
| | | Spinosad + Suc | 100.0 ± 0.0 a | 0.0 ± 0.0 c |
| | | Spinosad + Suc Yst | 90.0 ± 5.77 ab | 0.0 ± 0.0 c |
| | | Azadirachtin + pyrethrins | 46.7 ± 20.28 ab | 26.7 ± 12.12 ab |
| | | Azadirachtin + pyrethrins + Suc | 43.3 ± 20.28 b | 37.7 ± 4.37 a |
| | | Azadirachtin + pyrethrins + Suc Yst | 96.7 ± 3.33 ab | 17.7 ± 5.21 abc |
| | | *C. subtsugae* | 80.0 ± 15.27 ab | 13.3 ± 3.28 abc |
| | | *C. subtsugae* + Suc | 56.7 ± 12.02 ab | 19.3 ± 4.91 abc |
| | | *C. subtsugae* + Suc Yst | 60.0 ± 11.55 ab | 8.3 ± 4.37 abc |
| | | Hydrogen peroxide + PAA | 60.0 ± 10.00 ab | 25.3 ± 2.96 ab |
| | | Hydrogen peroxide + PAA + Suc | 83.3 ± 12.02 ab | 9.3 ± 6.57 abc |
| | | Hydrogen peroxide + PAA + Suc Yst | 80.0 ± 0.0 ab | 5.7 ± 3.18 abc |
| | | ANOVA | $F = 3.15$; $df = 14, 27$; $p < 0.01$ | $F = 5.95$; $df = 14, 27$; $p < 0.001$ |
| | 3 DAT | Untreated control | 16.7 ± 3.33 e | 36.7 ± 5.67 ab |
| | | Suc | 70.0 ± 0.0 abcd | 42.5 ± 12.50 ab |
| | | Suc Yst | 70.0 ± 15.28 ab | 32.0 ± 13.23 ab |
| | | Spinosad | 73.3 ± 12.02 abc | 16.7 ± 3.93 b |
| | | Spinosad + Suc | 76.7 ± 12.02 a | 15.7 ± 3.28 b |
| | | Spinosad + Suc Yst | 76.7 ± 6.67 ab | 31.0 ± 10.41 ab |
| | | Azadirachtin + pyrethrins | 50.0 ± 5.77 abcde | 55.0 ± 13.65 ab |
| | | Azadirachtin + pyrethrins + Suc | 20.0 ± 15.28 e | 63.3 ± 11.47 a |
| | | Azadirachtin + pyrethrins + Suc Yst | 43.3 ± 17.64 abcde | 45.0 ± 7.37 ab |
| | | *C. subtsugae* | 30.0 ± 11.55 de | 38.3 ± 12.25 ab |
| | | *C. subtsugae* + Suc | 46.7 ± 24.04 bcde | 49.7 ± 10.33 ab |
| | | *C. subtsugae* + Suc Yst | 30.0 ± 15.28 de | 53.3 ± 8.95 ab |
| | | Hydrogen peroxide + PAA | 46.7 ± 8.82 abcde | 49.3 ± 8.41 ab |
| | | Hydrogen peroxide + PAA + Suc | 30.0 ± 5.77 cde | 35.3 ± 4.70 ab |
| | | Hydrogen peroxide + PAA + Suc Yst | 33.3 ± 6.67 bcde | 47.7 ± 6.74 ab |
| | | ANOVA | $F = 2.38$; $df = 14, 27$; $p = 0.03$ | $F = 3.13$; $df = 14, 27$; $p = 0.01$ |
| | 5 DAT | Untreated control | 25.0 ± 25.00 | 71.0 ± 40.00 |
| | | Suc | 46.7 ± 3.33 | 32.7 ± 9.96 |
| | | Suc Yst | 46.7 ± 12.02 | 36.7 ± 9.40 |
| | | Spinosad | 46.7 ± 21.86 | 37.7 ± 14.19 |
| | | Spinosad + Suc | 86.7 ± 13.33 | 24.7 ± 14.10 |
| | | Spinosad + Suc Yst | 50.0 ± 5.77 | 25.7 ± 4.06 |
| | | Azadirachtin + pyrethrins | 73.3 ± 13.33 | 36.7 ± 15.17 |
| | | Azadirachtin + pyrethrins + Suc | 10.0 ± 10.00 | 54.7 ± 8.37 |
| | | Azadirachtin + pyrethrins + Suc Yst | 30.0 ± 20.00 | 42.3 ± 6.69 |
| | | *C. subtsugae* | 76.7 ± 8.82 | 21.0 ± 4.04 |
| | | *C. subtsugae* + Suc | 43.3 ± 26.03 | 35.0 ± 7.64 |
| | | *C. subtsugae* + Suc Yst | 26.7 ± 12.02 | 41.7 ± 4.18 |

*(Continues)*

**TABLE 3** (Continued)

| Blueberry Type | Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|---|
| | | Hydrogen peroxide + PAA | 30.0 ± 25.17 | 38.3 ± 15.30 |
| | | Hydrogen peroxide + PAA + Suc | 53.3 ± 21.86 | 52.3 ± 13.86 |
| | | Hydrogen peroxide + PAA + Suc Yst | 23.3 ± 15.53 | 34.7 ± 7.45 |
| | | ANOVA | $F = 1.97$; $df = 14, 27$; $p = 0.06$ | $F = 0.84$; $df = 14, 27$; $p = 0.63$ |
| Rabbiteye | 0 DAT | Untreated control | 13.3 ± 8.82 b | 24.3 ± 8.84 abc |
| | | Suc | 0.0 ± 0.0 b | 47.7 ± 11.10 a |
| | | Suc Yst | 25.8 ± 2.99 b | 43.3 ± 1.67 a |
| | | Spinosad | 100.0 ± 0.0 a | 7.0 ± 2.65 bcd |
| | | Spinosad + Suc | 100.0 ± 0.0 a | 3.7 ± 0.88 cd |
| | | Spinosad + Suc Yst | 100.0 ± 0.0 a | 3.3 ± 3.33 d |
| | | Azadirachtin + pyrethrins | 23.0 ± 9.05 b | 35.0 ± 6.03 ab |
| | | Azadirachtin + pyrethrins + Suc | 13.3 ± 13.33 b | 47.3 ± 2.40 a |
| | | Azadirachtin + pyrethrins + Suc Yst | 13.3 ± 13.33 b | 38.0 ± 9.07 a |
| | | *C. subtsugae* | 16.7 ± 12.02 b | 20.0 ± 2.65 abcd |
| | | *C. subtsugae* + Suc | 15.5 ± 10.85 b | 48.3 ± 12.91 a |
| | | *C. subtsugae* + Suc Yst | 12.4 ± 7.97 b | 39.7 ± 0.88 a |
| | | Hydrogen peroxide + PAA | 36.7 ± 21.86 b | 37.3 ± 10.73 a |
| | | Hydrogen peroxide + PAA + Suc | 26.7 ± 16.67 b | 41.0 ± 9.50 a |
| | | Hydrogen peroxide + PAA + Suc Yst | 47.0 ± 22.16 ab | 33.3 ± 1.45 ab |
| | | ANOVA | $F = 10.3$; $df = 14, 28$; $p < 0.001$ | $F = 9.62$; $df = 14, 28$; $p < 0.001$ |
| | 3 DAT | Untreated control | 20.0 ± 5.77 ab | 60.3 ± 14.38 a |
| | | Suc | 13.0 ± 8.88 ab | 59.7 ± 10.40 a |
| | | Suc Yst | 16.4 ± 12.11 ab | 60.3 ± 8.88 a |
| | | Spinosad | 41.2 ± 12.61 ab | 22.3 ± 1.67 ab |
| | | Spinosad + Suc | 66.1 ± 7.45 a | 14.7 ± 6.36 b |
| | | Spinosad + Suc Yst | 16.7 ± 12.02 ab | 18.3 ± 5.36 ab |
| | | Azadirachtin + pyrethrins | 23.3 ± 6.67 ab | 42.0 ± 11.59 ab |
| | | Azadirachtin + pyrethrins + Suc | 3.3 ± 3.33 b | 50.7 ± 4.18 ab |
| | | Azadirachtin + pyrethrins + Suc Yst | 26.4 ± 12.23 ab | 41.7 ± 1.86 ab |
| | | *C. subtsugae* | 31.1 ± 5.88 ab | 43.3 ± 9.94 ab |
| | | *C. subtsugae* + Suc | 3.0 ± 3.03 b | 49.0 ± 8.51 ab |
| | | *C. subtsugae* + Suc Yst | 23.3 ± 12.02 ab | 50.7 ± 6.17 ab |
| | | Hydrogen peroxide + PAA | 3.3 ± 3.33 b | 42.7 ± 15.59 ab |
| | | Hydrogen peroxide + PAA + Suc | 16.7 ± 3.33 ab | 49.7 ± 7.69 ab |
| | | Hydrogen peroxide + PAA + Suc Yst | 18.8 ± 10.52 ab | 48.0 ± 6.51 ab |
| | | ANOVA | $F = 2.78$; $df = 14, 28$; $p = 0.01$ | $F = 3.10$; $df = 14, 28$; $p = 0.01$ |
| | 5 DAT | Untreated control | 31.5 ± 16.31 | 30.0 ± 4.93 |
| | | Suc | 30.9 ± 21.69 | 36.3 ± 6.98 |
| | | Suc Yst | 10.0 ± 5.77 | 44.3 ± 12.01 |
| | | Spinosad | 6.7 ± 6.67 | 33.3 ± 8.88 |
| | | Spinosad + Suc | 20.0 ± 15.28 | 26.0 ± 4.73 |
| | | Spinosad + Suc Yst | 46.7 ± 20.28 | 30.0 ± 12.77 |
| | | Azadirachtin + pyrethrins | 0.0 ± 0.0 | 28.7 ± 6.57 |
| | | Azadirachtin + pyrethrins + Suc | 3.3 ± 3.33 | 24.7 ± 1.20 |

*(Continues)*

**TABLE 3** (Continued)

| Blueberry Type | Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|---|
| | | Azadirachtin + pyrethrins + Suc Yst | 2.8 ± 2.78 | 47.7 ± 5.90 |
| | | *C. subtsugae* | 13.3 ± 8.82 | 40.7 ± 8.41 |
| | | *C. subtsugae* + Suc | 47.0 ± 23.62 | 39.3 ± 5.78 |
| | | *C. subtsugae* + Suc Yst | 33.3 ± 18.56 | 30.7 ± 9.35 |
| | | Hydrogen peroxide + PAA | 16.1 ± 8.73 | 31.3 ± 7.54 |
| | | Hydrogen peroxide + PAA + Suc | 30.6 ± 30.56 | 23.3 ± 4.67 |
| | | Hydrogen peroxide + PAA + Suc Yst | 26.7 ± 21.86 | 26.3 ± 2.60 |
| | | ANOVA | $F = 1.03$; $df = 14, 28$; $p = 0.45$ | $F = 0.90$; $df = 14, 28$; $p = 0.57$ |

*Notes*. DAT: days after treatment; Suc Yst: sucrose + yeast; Suc: sucrose.
The experiments were conducted at a blueberry farm in Baxley, GA, in 2017. Means within a column followed by the same letter were not significantly different (LSD test, p < 0.05).

controls ($F = 18.04$; $df = 20, 99$; $p < 0.001$; Figure 1). For each individual insecticide, addition of phagostimulants did not have a significant effect on *D. suzukii* mortality. All treatments with spinosad killed 100% of flies.

### 3.1.2 | Fruit dip bioassay

In the 2016 fruit dip bioassay, all treatments with spinosad caused significantly higher mortality than the controls, any treatment with hydrogen dioxide + PAA and hydrogen peroxide + PAA with phagostimulants ($F = 11.40$; $df = 26, 118$; $p < 0.001$; Figure 2a). For each individual insecticide, addition of phagostimulants did not have a significant effect on *D. suzukii* mortality. Treatment had a significant effect on the number of progeny that developed in berries ($F = 8.04$; $df = 26, 118$; $p < 0.001$; Figure 2b). Treatments with azadirachtin + pyrethrins, spinosad or pyrethrins had the fewest progeny. However, like the mortality data, for each individual insecticide, the addition of phagostimulants did not have a significant effect.

In the 2017 fruit dip bioassay, treatment had a significant effect ($F = 4.58$; $df = 20, 93$; $p < 0.001$; Figure 3a). Treatments with spinosad were the only ones that were significantly different from the untreated control. For each individual insecticide, the addition of phagostimulants did not have a significant effect. Treatment had a significant effect on the number of progeny that developed in berries ($F = 1.90$; $df = 20, 99$; $p = 0.02$; Figure 3b). The same pattern as the 2016 fruit dip bioassay was observed here where addition of phagostimulants did not have a significant effect on any given insecticide.

### 3.1.3 | Topical application bioassay

All treatments with spinosad caused 100% mortality, and this was significantly higher than the controls or *C. subtsugae* treatments ($F = 772.55$; $df = 11, 55$; $p < 0.001$) (Figure 4a). Treatments with *C. subtsugae* were not significantly different from the control.

### 3.1.4 | Residual contact bioassay

Treatments with spinosad caused 100% mortality, and this was significantly higher than all other treatments ($F = 27.82$; $df = 20, 63$; $p < 0.001$; Figure 4b). None of the other treatments were significantly different from the control, and addition of phagostimulants did not have a significant effect on any given insecticide.

## 3.2 | Semi-field experiments

### 3.2.1 | Georgia

In the 2016 experiment, insecticide treatment ($F = 5.12$; $df = 14, 90$; $p < 0.001$), residue age ($F = 14.40$; $df = 2, 90$; $p < 0.001$) and their interaction ($F = 2.63$; $df = 28, 90$; $p < 0.001$) were all statistically significant for fly mortality. At 0 DAT, spinosad and spinosad + sucrose were the only treatments that caused significantly higher mortality than the controls (untreated, sucrose and sucrose + yeast; Table 2). At 3 DAT, the only treatments that were significantly different from each other were spinosad + sucrose and sucrose alone. At 7 DAT, there were no significant differences among treatments, and the highest mortality was <27%. For progeny developing in berries, insecticide treatment ($F = 5.34$; $df = 14, 88$; $p < 0.001$), residue age ($F = 12.81$; $df = 2, 88$; $p < 0.001$) and their interaction ($F = 3.20$; $df = 28, 88$; $p < 0.001$) were statistically significant. At 0 DAT, treatments with azadirachtin + pyrethrins had the most progeny, and treatments with spinosad had the fewest. For each insecticide, addition of phagostimulants did not have an effect (Table 2). There were no significant differences in mean progeny at 3 DAT. At 7 DAT, the only significant differences in progeny were between sucrose alone and spinosad alone or spinosad with sucrose + yeast. Field infestation samples did not yield a single *D. suzukii*.

In the 2017 experiment in southern highbush blueberry, insecticide treatment ($F = 4.91$; $df = 14, 85$; $p < 0.001$) and residue age ($F = 18.75$; $df = 2, 85$; $p < 0.001$) had a significant effect on fly mortality, but their interaction was not significant ($F = 1.07$; $df = 28,$

**TABLE 4** Mean (±*SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment and residue age in semi-field bioassays at 5-day exposure and mean (±*SE*) number of *D. suzukii* progeny per five blueberries

| Year | Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|---|
| 2016 | 0 DAT | Untreated control | 6.3 ± 2.08 b | 37.0 ± 10.70 a |
| | | Suc | 2.1 ± 2.08 b | 35.0 ± 6.65 a |
| | | Yst | 0.0 ± 0.0 b | 42.0 ± 7.89 a |
| | | Suc Yst | 2.1 ± 2.08 b | 51.5 ± 9.15 a |
| | | Spinosad | 100.0 ± 0.0 a | 4.5 ± 1.50 bc |
| | | Spinosad + Suc | 100.0 ± 0.0 a | 0.8 ± 0.25 c |
| | | Spinosad + Yst | 100.0 ± 0.0 a | 1.8 ± 1.18 c |
| | | Spinosad + Suc Yst | 100.0 ± 0.0 a | 1.8 ± 1.18 c |
| | | *C. subtsugae* | 2.1 ± 2.08 b | 24.5 ± 3.28 ab |
| | | *C. subtsugae* + Suc | 0.0 ± 0.0 b | 30.3 ± 6.71 a |
| | | *C. subtsugae* + Yst | 16.7 ± 16.67 b | 24.0 ± 5.58 ab |
| | | *C. subtsugae* + Suc Yst | 0.0 ± 0.0 b | 21.5 ± 3.38 ab |
| | | ANOVA | $F = 79.29$; $df = 11, 33$; $p < 0.001$ | $F = 16.83$; $df = 11, 33$; $p < 0.001$ |
| | 3 DAT | Untreated control | 4.2 ± 4.17 b | 17.5 ± 4.41 a |
| | | Suc | 14.6 ± 7.12 b | 18.0 ± 2.89 a |
| | | Yst | 4.2 ± 4.17 b | 14.8 ± 2.75 ab |
| | | Suc Yst | 10.4 ± 3.99 b | 14.0 ± 4.44 abc |
| | | Spinosad | 100.0 ± 0.0 a | 0.8 ± 0.25 d |
| | | Spinosad + Suc | 100.0 ± 0.0 a | 1.5 ± 0.50 cd |
| | | Spinosad + Yst | 100.0 ± 0.0 a | 2.0 ± 0.41 bcd |
| | | Spinosad + Suc Yst | 100.0 ± 0.0 a | 1.5 ± 0.65 cd |
| | | *C. subtsugae* | 4.2 ± 2.41 b | 17.3 ± 8.53 ab |
| | | *C. subtsugae* + Suc | 6.3 ± 3.99 b | 12.8 ± 3.54 abc |
| | | *C. subtsugae* + Yst | 4.2 ± 4.17 b | 5.8 ± 0.25 abcd |
| | | *C. subtsugae* + Suc Yst | 6.3 ± 2.08 b | 13.0 ± 2.94 abc |
| | | ANOVA | $F = 82.39$; $df = 11, 33$; $p < 0.001$ | $F = 7.40$; $df = 11, 33$; $p < 0.001$ |
| 2017 | 0 DAT | Untreated control | 27.8 ± 12.11 | 48.7 ± 9.94 a |
| | | Suc | 16.7 ± 4.81 | 46.3 ± 10.73 a |
| | | Suc Yst | 8.3 ± 4.81 | 50.7 ± 8.29 a |
| | | Spinosad | 25.0 ± 9.62 | 33.7 ± 12.03 ab |
| | | Spinosad + Suc | 80.6 ± 11.11 | 4.0 ± 0.58 b |
| | | Spinosad + Suc Yst | 41.7 ± 20.97 | 29.3 ± 5.55 ab |
| | | Azadirachtin + pyrethrins | 63.9 ± 20.03 | 22.3 ± 4.98 ab |
| | | Azadirachtin + pyrethrins + Suc | 47.2 ± 26.50 | 14.3 ± 7.62 ab |
| | | Azadirachtin + pyrethrins + Suc Yst | 50.0 ± 25.00 | 29.3 ± 14.44 ab |
| | | *C. subtsugae* | 45.8 ± 20.83 | 29.0 ± 9.00 ab |
| | | *C. subtsugae* + Suc | 8.3 ± 4.81 | 40.7 ± 6.69 ab |
| | | *C. subtsugae* + Suc Yst | 47.2 ± 11.11 | 15.7 ± 1.76 ab |
| | | Hydrogen peroxide + PAA | 13.9 ± 10.02 | 35.3 ± 2.73 ab |
| | | Hydrogen peroxide + PAA + Suc | 25.0 ± 8.33 | 45.3 ± 2.67 a |

*(Continues)*

**TABLE 4** (Continued)

| Year | Residue age | Treatment | Mean (±*SE*) per cent adult mortality | Mean (±*SE*) progeny per five berries |
|---|---|---|---|---|
| | | Hydrogen peroxide + PAA + Suc Yst | 13.9 ± 5.56 | 49.3 ± 9.70 a |
| | | ANOVA | $F = 1.97$; $df = 14, 27$; $p = 0.06$ | $F = 3.47$; $df = 14, 27$; $p < 0.01$ |
| | 3 DAT | Untreated control | 16.7 ± 12.73 | 26.0 ± 4.73 |
| | | Suc | 2.8 ± 2.78 | 30.3 ± 15.60 |
| | | Suc Yst | 0.0 ± 0.0 | 55.3 ± 6.17 |
| | | Spinosad | 2.8 ± 2.78 | 26.0 ± 3.06 |
| | | Spinosad + Suc | 41.7 ± 17.35 | 10.7 ± 2.60 |
| | | Spinosad + Suc Yst | 2.8 ± 2.78 | 43.0 ± 3.22 |
| | | Azadirachtin + pyrethrins | 8.3 ± 8.33 | 25.0 ± 6.66 |
| | | Azadirachtin + pyrethrins + Suc | 16.7 ± 9.62 | 21.0 ± 2.31 |
| | | Azadirachtin + pyrethrins + Suc Yst | 38.9 ± 26.50 | 25.3 ± 13.87 |
| | | *C. subtsugae* | 0.0 ± 0.0 | 24.0 ± 3.22 |
| | | *C. subtsugae* + Suc | 8.3 ± 4.81 | 28.7 ± 5.55 |
| | | *C. subtsugae* + Suc Yst | 13.9 ± 13.89 | 31.3 ± 2.40 |
| | | Hydrogen peroxide + PAA | 0.0 ± 0.0 | 41.7 ± 5.84 |
| | | Hydrogen peroxide + PAA + Suc | 5.6 ± 2.78 | 40.7 ± 4.33 |
| | | Hydrogen peroxide + PAA + Suc Yst | 8.3 ± 4.81 | 42.3 ± 13.33 |
| | | ANOVA | $F = 1.80$; $df = 14, 28$; $p = 0.09$ | $F = 2.06$; $df = 14, 28$; $p = 0.05$ |
| | 5 DAT | Untreated control | 5.6 ± 5.56 | 19.7 ± 3.71 |
| | | Suc | 5.6 ± 5.56 | 37.0 ± 3.79 |
| | | Suc Yst | 11.1 ± 2.78 | 31.0 ± 10.44 |
| | | Spinosad | 55.6 ± 28.19 | 10.0 ± 1.53 |
| | | Spinosad + Suc | 38.9 ± 16.90 | 23.3 ± 9.39 |
| | | Spinosad + Suc Yst | 19.4 ± 10.02 | 25.7 ± 2.96 |
| | | Azadirachtin + pyrethrins | 5.6 ± 5.56 | 20.7 ± 5.61 |
| | | Azadirachtin + pyrethrins + Suc | 25.0 ± 12.73 | 19.3 ± 8.97 |
| | | Azadirachtin + pyrethrins + Suc Yst | 11.1 ± 7.35 | 53.0 ± 4.51 |
| | | *C. subtsugae* | 11.1 ± 5.56 | 34.0 ± 10.15 |
| | | *C. subtsugae* + Suc | 8.3 ± 0.0 | 30.3 ± 3.76 |
| | | *C. subtsugae* + Suc Yst | 5.6 ± 2.78 | 25.0 ± 2.65 |
| | | Hydrogen peroxide + PAA | 0.0 ± 0.0 | 35.7 ± 7.69 |
| | | Hydrogen peroxide + PAA + Suc | 5.6 ± 5.56 | 36.7 ± 3.84 |
| | | Hydrogen peroxide + PAA + Suc Yst | 41.7 ± 26.79 | 36.7 ± 15.98 |
| | | ANOVA | $F = 1.46$; $df = 14, 28$; $p = 0.19$ | $F = 1.81$; $df = 14, 28$; $p = 0.09$ |

*Notes*. DAT: days after treatment; Suc Yst: sucrose + yeast; Suc: sucrose; Yst: yeast.
The experiments were conducted on blueberries at a research station in Fennville, MI. Means within a column followed by the same letter were not significantly different (LSD test, $p < 0.05$).

85; $p = 0.40$). The only treatments that were significantly different at 0 DAT were spinosad with sucrose and azadirachtin + pyrethrins with sucrose (Table 3). At 3 DAT, all treatments with spinosad caused significantly higher mortality than the untreated control, but all treatments with azadirachtin + pyrethrins, all treatments with *C. substsugae* and all treatments with hydrogen peroxide + PAA were

not different from the untreated control. There were no significant treatment effects at 5 DAT. For progeny developing in berries, insecticide treatment ($F = 6.05$; $df = 14, 85$; $p < 0.001$) and residue age ($F = 74.46$; $df = 2, 85$; $p < 0.001$) were statistically significant, but their interaction was not significant ($F = 1.45$; $df = 28, 85$; $p = 0.10$). At 0 DAT, no progeny developed in berries from any treatment containing spinosad. At 3 DAT, the only significant differences in mean progeny were between azadirachtin + pyrethrins with sucrose and spinosad alone or spinosad with sucrose (Table 3). There was no significant effect of treatments on mean progeny at 5 DAT. Field infestation samples yielded only one *D. suzukii*.

In the 2017 experiment in rabbiteye blueberry, insecticide treatment ($F = 5.10$; $df = 14, 90$; $p < 0.001$), residue age ($F = 8.45$; $df = 2, 85$; $p < 0.001$) and their interaction were statistically significant ($F = 3.32$; $df = 28, 90$; $p < 0.001$). At 0 DAT, treatments containing spinosad caused significantly higher mortality than all other treatments except hydrogen peroxide + PAA with sucrose and yeast (Table 3). At 3 DAT, spinosad with sucrose caused the highest mortality and this was significantly greater than azadirachtin + pyrethrins with sucrose, *C. subtsugae* with sucrose and hydrogen peroxide + PAA, but not significantly different from the control. At 5 DAT, there was no significant effect of treatment on adult mortality. For progeny developing in berries, insecticide treatment ($F = 8.23$; $df = 14, 90$; $p < 0.001$), residue age ($F = 12.63$; $df = 2, 90$; $p < 0.001$) and their interaction ($F = 2.27$; $df = 28, 90$; $p = 0.001$) were statistically significant. At 0 DAT, only spinosad with sucrose + yeast had significantly fewer progeny than the untreated control (Table 3). At 3 DAT, spinosad with sucrose had significantly fewer progeny than the controls (untreated, sucrose alone and sucrose + yeast). There was no significant effect of treatment on progeny at 5 DAT. The only significant model effect for field infestation samples was residue age ($F = 3.22$; $df = 3, 118$; $p = 0.03$); insecticide treatment ($F = 1.17$; $df = 14, 118$; $p = 0.31$) and treatment by residue age interaction ($F = 1.10$; $df = 42, 118$; $p = 0.34$) were not significant. There were no significant differences among treatments when analysed separately by residue age (0 DAT: $F = 0.73$; $df = 14, 28$; $p = 0.73$, 3 DAT: $F = 0.82$; $df = 14, 28$; $p = 0.65$, 5 DAT: $F = 1.30$; $df = 14, 28$; $p = 0.27$, 7 DAT: $F = 0.95$; $df = 14, 28$; $p = 0.52$). The number of *D. suzukii* larvae and pupae collected from berries was low, averaging <4/100 g of berries.

### 3.2.2 | Michigan

In the 2016 experiment, insecticide treatment ($F = 158.94$; $df = 11, 69$; $p < 0.001$) and residue age ($F = 4.20$; $df = 1, 69$; $p = 0.04$) had a significant effect on fly mortality, but their interaction was not significant ($F = 1.34$; $df = 11, 69$; $p = 0.22$). All treatments containing spinosad killed 100% of the flies at 0 and 3 DAT (Table 4). All other treatments caused <10% mortality at 0 DAT except for *C. subtsugae* with yeast which caused <17% mortality. For progeny developing in berries, insecticide treatment ($F = 24.09$; $df = 11, 72$; $p < 0.001$), residue age ($F = 42.04$; $df = 1, 72$; $p < 0.001$) and their interaction ($F = 2.90$; $df = 11, 72$; $p = 0.003$) were statistically significant. At 0 and 3 DAT, all treatments containing spinosad had the fewest progeny, but addition of phagostimulants did not have an effect.

In the 2017 experiment, insecticide treatment ($F = 3.06$; $df = 14, 89$; $p < 0.001$) and residue age ($F = 17.18$; $df = 2, 89$; $p < 0.001$) had a significant effect on fly mortality, but their interaction was not significant ($F = 1.18$; $df = 28, 89$; $p = 0.28$). There were no significant treatment effects on mean mortality at any residue age (Table 4). For progeny developing in berries, insecticide treatment was statistically significant ($F = 4.77$; $df = 14, 89$; $p < 0.001$), but residue age ($F = 0.42$; $df = 2, 89$; $p = 0.66$) and treatment by residue age interaction ($F = 1.46$; $df = 28, 89$; $p = 0.09$) were not significant. At 0 DAT, spinosad with sucrose was the only treatment that was significantly different from any of the controls (untreated, sucrose alone or sucrose with yeast; Table 4).There were no significant differences among treatments in field infestation samples ($F = 1.19$; $df = 14, 28$; $p = 0.33$). Mean infestation was <13 flies per 100 g of blueberries.

## 4 | DISCUSSION

In this study, we tested sucrose and yeast in combination with various organically approved pesticides to determine whether these phagostimulants can increase *D. suzukii* adult mortality and reduce fruit infestation. Spinosad has proven effective in multiple studies and is one of the main products used in organic *D. suzukii* chemical control programmes (Beers et al., 2011; Bruck et al., 2011; Cahenzli et al., 2018; Van Timmeren & Isaacs, 2013). Other biopesticides that showed efficacy in some of our experiments and in other studies included azadirachtin, *C. subtsugae*, pyrethrins and sabadilla alkaloids (Fanning et al., 2018; Iglesias & Liburd, 2017; Shawer, Tonina, Tirello, Duso, & Mori, 2018). However, these other studies also noted the short residual activity of these products and recommended using them in rotation with spinosad.

Biopesticide efficacy varied depending on laboratory methods or regions in the case of semi-field experiments. Differences in the semi-field bioassay could be due to differences in *D. suzukii* populations between regions (each laboratory used their own colony established using wild-caught flies from their region) in addition to differences between blueberry cultivars. What was consistent was that all treatments with spinosad killed all adult flies regardless of laboratory method. Adding phagostimulants to insecticides did not improve insecticide efficacy in terms of increasing adult fly mortality or reducing progeny developing in fruit. Some work has been done investigating the association of yeast and *D. suzukii*, and it is possible that specific strains could be effective as phagostimulants (Hamby et al., 2012; Knight et al., 2015; Mori et al., 2017). These strains would have to be commercially available and affordable to be of any practical use in spray programmes. We used *S. cerevisiae*, the most common commercially available yeast, and not *H. uvarum*, the yeast specifically associated with *D. suzukii*. However, *S. cerevisiae* has been used effectively as bait in *D. suzukii* monitoring traps (Iglesias, Nyoike, & Liburd, 2014), and Knight et al. (2015) used

*S. cerevisiae* in their experiments and observed significantly higher fly mortality when combined with spinosad and dark brown cane sugar. It does not appear that species of yeast explains our lack of phagostimulant effects. The response of *D. suzukii* to fermentation-based substances is influenced by physiological status (Wong, Wallingford, Loeb, & Lee, 2018). Unmated females and females with few eggs show preference for fermentation odours and transition to fruit odours after mating and as their egg loads increase (Wong et al., 2018). Knight et al. (2015) used flies that were 3–4 day old, while we used flies that were 4–10 day old and were all likely mated and ready to lay eggs. Future studies would be necessary to elucidate the relationship between fly age, mating status and efficacy of yeast-based phagostimulants with insecticides.

Adding phagostimulants to hydrogen peroxide + PAA reduced mortality in the 2016 fruit dip bioassay, and this makes sense because hydrogen peroxide + PAA is a sanitizer and is expected to be incompatible when mixed with yeast. The mechanism of hydrogen peroxide + PAA is thought to be removal of yeasts that are important to *D. suzukii*, thereby disrupting the pest's biology. Adding yeast to the tank mix would be counterproductive. In organic berry production, sanitizers such as hydrogen peroxide + PAA are typically used in tank mixes with insecticides or in rotation with insecticides. Future research would need to be done to see whether tank mixes with insecticides, sanitizers and phagostimulants enhance *D. suzukii* control.

Cowles et al. (2015) found that *D. suzukii* responds to sucrose at low concentrations. However, since spinosyns were very effective on their own, there was little room for improvement adding sucrose to insecticides from this class. The addition of sucrose enhanced mortality when combined with neonicotinoids and malathion (Cowles et al., 2015). For organic insecticides which are not as effective as spinosad, a higher concentration of sucrose may be needed, and more work is needed testing *D. suzukii* response to different rates.

Non-nutritive sugars and sugar alcohols are toxic to *Drosophila* (Choi et al., 2017). These products are used as food additives and sugar alternatives for human consumption. They provide no nutritional benefit to the flies and cause abnormally high osmotic pressure in the haemolymph because flies cannot excrete these substances. Erythritol was effective at killing immature stages of *D. suzukii* in laboratory feeding assays and in small plot field trials in blueberries and blackberries (Sampson, Marshall et al., 2017; Sampson, Werle, Stringer, & Adamczyk, 2017). However, high concentrations would be required to kill larvae in fruit and the cost might be prohibitive (Sampson, Werle et al., 2017). There is potential to use these non-nutritive sugars at lower concentrations as phagostimulants in combination with insecticides (Sampson, Werle et al., 2017).

Current reliance on insecticides is not sustainable for long-term management of *D. suzukii*. The situation is even more problematic in organic agriculture where the number of approved insecticide classes is limited. Adding behaviour-modifying compounds such as phagostimulants has been shown to improve insecticide efficacy against *D. suzukii*, potentially expanding the list of effective products, but this study did not show a benefit of combining low concentrations of sucrose and/or yeast with new OMRI-listed insecticides. More research is needed to determine the types of phagostimulants and concentrations that are effective against *D. suzukii* in organic berry production.

## ACKNOWLEDGEMENTS

This research was partially supported by grants from the U.S. Department of Agriculture, National Institute of Food and Agriculture under Organic Agriculture Research and Extension Initiative (Award # 2015-51300-24154), Extension Implementation Program (Award # 2014-70006-22533), Florida Department of Agriculture and Consumer Services, Georgia Blueberry Growers Association, Georgia Agricultural Commodity Commission for Blueberries and Project GREEEN.

## AUTHORS' CONTRIBUTIONS

AAS, OEL and RI conceived the study. CRR, BKG, PDF, SVT, JS, SC and BAL conducted the experiments. CRR, PDF and JS analysed the data and wrote the manuscript. All authors read and approved the manuscript.

## DATA ACCESSIBILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

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**How to cite this article:** Roubos CR, Gautam BK, Fanning PD, et al. Impact of phagostimulants on effectiveness of OMRI-listed insecticides used for control of spotted-wing drosophila (*Drosophila suzukii* Matsumura). *J Appl Entomol*. 2019;143:609–625. https://doi.org/10.1111/jen.12620