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ORIGINAL CONTRIBUTION

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Evaluation of adjuvants to improve control of spotted-wing drosophila in organic fruit production

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Abstract

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is a key pest of berry crops in the United States. It is managed intensively using insecticides, but organic fruit growers have few effective chemical control options. Spinosad is the most effective organically approved product for control of *D. suzukii*, while other organic options have not shown high levels of control. Adjuvants are products added to pesticides to improve effectiveness, and these may function as stickers, spreaders or surfactants improving the spray coverage of insecticides on surfaces and thereby increasing the likelihood that pests will contact residues. We conducted experiments evaluating organically approved biopesticides in combination with three adjuvants including poly-1-p-menthene, alcohol ethoxylate and polyether-polymethylsiloxane-copolymer polyether to determine whether addition of adjuvants improved efficacy and residual activity of these products. Alcohol ethoxylate and poly-1-p-menthene showed some inherent insecticidal activity against *D. suzukii* in laboratory assays. Adjuvants increased mortality of some insecticides but not to a level that would provide adequate fruit protection. Poly-1-p-menthene had a negative effect when combined with hydrogen peroxide + PAA and sabadilla alkaloids. Mortality in semi-field bioassays was quite low except for spinosad. Polyether-polymethylsiloxane-copolymer polyether had a negative effect on the efficacy of spinosad. The adjuvants did not extend residual activity of the insecticides. Adjuvants did not provide the expected benefits of increased performance against *D. suzukii* when combined with organic biopesticides. Other methods for enhancing these insecticides will need to be explored to provide organic growers with more effective chemical control options for this invasive pest.

KEYWORDS
bioassay, blueberries, efficacy, insecticides, spinosad

1 | INTRODUCTION

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura), is a vinegar fly that is native to South-East Asia. It is an invasive species that was first detected in the continental United States and Europe in 2008. Since then, it has spread to all fruit growing regions in North America and Europe and has also been found in South America (Calabria, Máca, Bächli, Serra, & Pascual, 2012; Deprá, Poppe, Schmitz, De Toni, & Valente, 2014; Hauser, 2011; Walsh et al., 2011). *Drosophila suzukii* has become a critical insect pest of

berries (blueberry, blackberry, raspberry, strawberry) and cherries in its new geographical range (Asplen et al., 2015; Lee et al., 2011). Female *D. suzukii* can lay eggs in ripe or ripening soft-skinned fruits, and larval feeding degrades the fruit making it unmarketable.

Pesticides typically play a minor role in organic agriculture, used as a last resort (Zehnder et al., 2007). However, they can be key for keeping organic production profitable when invasive pests arrive, providing control until sustainable methods (biological and cultural control) are developed and adopted (Diepenbrock, Rosensteel, Hardin, Sial, & Burrack, 2016; Leskey, Short, & Lee, 2014). A zero-threshold for *D. suzukii* contamination has prompted growers to spray insecticides throughout the harvest period when fruit is most vulnerable (Van Timmeren & Isaacs, 2013). On both conventional and organic farms, insecticide use has increased since the arrival of this pest (Diepenbrock et al., 2016; Van Timmeren & Isaacs, 2013). Three classes of pesticides show good insecticidal activity against *D. suzukii*: organophosphates, pyrethroids and spinosyns (Beers, Van Steenwyk, Shearer, Coates, & Grant, 2011; Bruck et al., 2011; Gautam et al., 2016; Van Timmeren & Isaacs, 2013). Organic growers have few pesticide options for control of *D. suzukii* compared to conventional growers, and spinosad is the most effective organically approved product for its control (Beers et al., 2011; Bruck et al., 2011; Cahenzli, Strack, & Daniel, 2018; Van Timmeren & Isaacs, 2013). Reliance on a single insecticide like spinosad is neither practical nor sustainable. Label restrictions limit the number of applications that can be made in a season, and no more than two consecutive applications are allowed, requiring rotation to an alternative insecticide. Of greater concern is development of insecticide resistance in this species. A reduction in sensitivity to a spinosyn class insecticide has already been reported (Gress & Zalom, 2018; Van Timmeren, Mota-Sanchez, Wise, & Isaacs, 2018). Several National Organic Program (NOP)—compliant insecticides have recently been evaluated in a series of laboratory and semi-field experiments (Fanning, Grieshop, & Isaacs, 2018; Guédot & Perry, 2015; Iglesias & Liburd, 2017; Van Timmeren & Isaacs, 2013; Wise, VanWoerkom, & Isaacs, 2017). These products included biopesticides representing various modes of action.

Agricultural sanitizers may impact naturally occurring yeasts on fruit. Yeasts are an integral part of *Drosophila* ecology, affecting physiology and behaviour (Hamby & Becher, 2016). Disrupting yeasts could have a detrimental effect on the ability of *D. suzukii* to oviposit on and develop in fruit. With limited pesticide options for *D. suzukii* management in organic production, it is important to optimize use of the products that are available (Haye et al., 2016).

One approach for optimizing the performance of insecticides is the addition of adjuvants. Adjuvants are additives that are part of a formulated pesticide or added along with the formulated pesticide in the spray tank prior to spraying, which aid or modify the action of the active ingredient(s) (Foy, 1996). There are many types of adjuvants. Some important adjuvant functions include improving deposition and coverage, reducing run-off, improving rainfastness, reducing drift, protection from environmental degradation and increased plant penetration (Foy, 1996). Some adjuvants increase pesticide toxicity or are themselves toxic to insects (Acheampong & Stark, 2004; Stark & Walthall, 2003).

The objective of this study was to determine the effect of organically acceptable adjuvants on the toxicity and residual activity of organic insecticides against *D. suzukii*. The three adjuvants used in this study include poly-1-p-menthene (P1M), alcohol ethoxylate (AE) and polyether-polymethylsiloxane-copolymer polyether (PEPMS). These adjuvants were selected based on grower feedback. They are products commonly used and available for organic growers in the United States. Gautam et al. (2016) and Wise et al. (2017) used P1M in their experiments, but most insecticide studies targeting *D. suzukii* have not included adjuvants. Fanning et al. (2018) used eight adjuvants including PEPMS in their study, but they only tested these in combination with a single product. Effective adjuvants could expand the number of useful insecticide options for *D. suzukii* management in organic berry crops.

2 | MATERIALS AND METHODS

A series of bioassays were performed to evaluate adjuvant efficacy in combination with pesticides under laboratory and field conditions (Supporting Information Table S1). Semi-field bioassays were conducted in three states with each state evaluating a different adjuvant. Trials in Florida and 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 the laboratory bioassays was determined based on adult fly mortality and number of progeny able to develop in treated fruit, in the case of the fruit dip bioassays. In the semi-field trials, efficacy was also assessed based on adult fly mortality and the number of progeny able to develop in treated fruit as well as infestation in fruit collected from the treatment plots.

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) and a different adjuvant. The glass vial method was used because it is based on a commonly used method for evaluating insecticide resistance (Aïzoun et al., 2013; Denlinger, Lozano-Fuentes, Lawyer, Black, & Bernhardt, 2015). Treating Petri dishes using a spray tower was used to test flies contacting a treated substrate. Fruits were also used to test adult flies contacting a treated natural substrate, making it possible to evaluate the treatments in terms of the number of progeny produced. The direct spray bioassay was used to test adult flies coming in direct contact with the pesticide.

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

TABLE 1 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 per 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 in the USA.

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. The treatment list also includes two oxidizing agents (sanitizers) registered as fungicides and algaecides.

The adjuvants and rates used in this study are listed in Table 2. PEPMS and P1M are listed by OMRI. AE is not ORMI-listed but is registered by the Washington State Department of Agriculture for use in organic agriculture.

2.3 | Semi-field experiments

Semi-field experiments were conducted in Florida, Georgia and Michigan. Each bioassay sample consisted of a single cut blueberry branch containing 5–7 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. Only blueberries that did not have signs of *D. suzukii* larval infestation were selected. Soft, split or mouldy berries were not used. The branches with leaves and berries were exposed to *D. suzukii* adults reared in the laboratory, mortality was assessed after 5 days, and the number of progeny (larvae, pupae and adults) coming out of the berries was counted.

Field infestation samples were collected at the end of each experiment (6 or 7 DAT) to determine treatment effects over a one-week period. Growers have been applying insecticides at one-week intervals. Field infestation samples consisted of 0.12–0.24 L of ripe berries collected from each plot. Larvae were extracted using the filter salt method as described in Van Timmeren, Diepenbrock, Bertone, Burrack, and Isaacs (2017). Berries were weighed prior to assessment, and infestation was reported as the number of larvae per g berries.

2.3.1 | Florida

The Florida semi-field trial was conducted on an organic blueberry farm in Island Grove, FL (Alachua County), from 25 April to 2 May 2017. Plots consisted of two cultivars of southern highbush: "Farthing"

TABLE 2 Adjuvants and rates used in laboratory and semi-field bioassays

Trade name	Active ingredient	Manufacturer	Rate (AI/ha)[a]
Nu Film P®	Poly-1-p-menthene (100%)	Miller Chemical & Fertilizer LLC, Hanover, PA	440 ml
Oroboost™	Alcohol ethoxylate (13.58%)	Oro Agri Inc., Fresno, CA	496 ml
Leaf Life®	Polyether-polymethylsiloxane-copolymer, polyether (100%)	Loveland Products Inc., Greeley, CO	290 ml

[a]Rate of formulated product applied at the equivalent of 467.5 L water per ha.

and "Meadowlark." 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 (3.7 m). There were four pesticide treatments (spinosad rotated with pyrethrins, azadirachtin + pyrethrins, *C. subtsugae* and hydrogen peroxide + PAA) with and without AE. Insecticide treatments were compared to an AE treatment and a control (water only) for a total of 10 treatments. Treatments were applied on 25 April using a hand-held 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 DAT. Each bioassay chamber received 10 *D. suzukii* adults (five males and five females). Mortality was assessed at 5 days. Berries were kept for seven days after the final mortality assessment 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. Samples for field infestation were collected on 2 May.

2.3.2 | 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 to 14 June. Two experiments were conducted in 2017, one in southern highbush blueberry ("Star" variety) from 21 to 26 April and one in rabbiteye blueberry ("Premier" variety) from 9 to 16 June. 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 hand-held CO_2 sprayers with an output equivalent to 467.5 L/ha at 241.3 kPa. Each bioassay chamber received 10 *D. suzukii* adults (five males and five females). Mortality was assessed at 5 days. After 5 days, 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, there were four insecticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and pyrethrins) plus a control with and without P1M in three replicates. Samples for bioassays and field infestations were collected at 0, 3 and 7 DAT.

In both semi-field experiments in 2017, there were four pesticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae* and hydrogen peroxide + PAA) plus an untreated control with and without P1M in three replications. Insecticides were applied on 21 April in the experiment in southern highbush blueberry and on 9 June in the experiment in rabbiteye blueberry. Samples for bioassays were collected at 0, 3 and 5 DAT. Field infestation samples were collected in the experiment in rabbiteye on 16 June.

2.3.3 | Michigan

The Michigan semi-field trials were conducted at the Trevor Nichols Research Center in Fennville, MI (Allegan County). The 2016 experiment ran from 3 to 10 August, and the 2017 experiment ran from 28 July to 3 August. 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 treatment plot consisted of six bushes. Each bioassay chamber received 12 *D. suzukii* adults (six males and six females), and chambers were kept at 25°C, 75% RH and 16:8 [L:D] hr. Mortality was assessed at 5 days. After the mortality assessment, berries were left in the chambers for seven 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 eight pesticide treatments (azadirachtin, azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, hydrogen dioxide + PAA, pyrethrins and *Burkholderia* spp.) plus an untreated control with and without PEPMS in four replicates. Insecticides were applied on 3 August, and samples for bioassays were collected at 0, 3, 5 and 7 DAT. Samples for field infestation were collected on 9 August.

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 PEPMS 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 3 August.

2.4 | Laboratory experiments

2.4.1 | Glass vial bioassay

A glass vial bioassay was conducted at the University of Florida in 2017. The bioassay included six treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, *Burkholderia* spp., and sabadilla alkaloids) plus a control (acetone only) with and without AE.

Pesticides were mixed with acetone to equal a total volume of 1 ml. Spinosad, *C. subtsugae* and *Burkholderia* spp. 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. Pesticide 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 (five males and five 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 days post-exposure.

TABLE 3 Analysis of variance results (main effects and interactions) from semi-field bioassays. Adult *Drosophila suzukii* (females and males pooled) mortality at 120 hr of exposure to insecticide residues and progeny from blueberries

				Adult mortality			Progeny		
State	Year	Trial	Factor	*df*	*F*	*p*	*df*	*F*	*p*
Florida	2017	SHB	Insecticide	**4, 30**	**8.49**	**<0.001**	**4, 30**	**13.04**	**<0.001**
			Adjuvant	1, 30	0.96	0.334	1, 30	1.21	0.280
			Insecticide × Adjuvant	**4, 30**	**0.68**	**<0.001**	4, 30	1.89	0.139
			DAT	2, 59	22.44	0.609	**2, 59**	**3.91**	**0.025**
			Insecticide × Adjuvant × DAT	18, 59	0.93	0.548	18, 59	0.65	0.841
Georgia	2016	RE	Insecticide	**4, 20**	**21.51**	**<0.001**	**4, 20**	**5.65**	**0.003**
			Adjuvant	1, 20	2.80	0.110	1, 20	2.41	0.136
			Insecticide × Adjuvant	4. 20	1.01	0.424	4, 20	1.36	0.284
			DAT	2, 40	2.64	0.084	**2, 40**	**9.81**	**<0.001**
			Insecticide × Adjuvant × DAT	**18, 40**	**3.48**	**0.001**	**18, 40**	**3.45**	**0.001**
	2017	SHB	Insecticide	4, 20	1.82	0.165	4, 20	2.77	0.055
			Adjuvant	1, 20	0.07	0.801	1, 20	0.35	0.562
			Insecticide × Adjuvant	4, 20	2.60	0.067	4, 20	1.56	0.223
			DAT	**2, 37**	**5.85**	**0.006**	**2, 37**	**20.63**	**<0.001**
			Insecticide × Adjuvant × DAT	18. 37	1.08	0.406	18, 37	1.75	0.074
	2017	RE	Insecticide	**4, 20**	**7.18**	**0.001**	**4, 20**	**4.94**	**0.006**
			Adjuvant	1, 20	0.13	0.726	1, 20	2.95	0.101
			Insecticide × Adjuvant	4, 20	1.14	0.368	4, 20	0.18	0.948
			DAT	**2, 40**	**16.36**	**<0.001**	**2, 40**	**5.87**	**0.006**
			Insecticide × Adjuvant × DAT	**18, 40**	**4.38**	**<0.001**	18, 40	1.56	0.118
Michigan	2016	NHB	Insecticide	**3, 24**	**16.46**	**<0.001**	**3, 24**	**17.47**	**<0.001**
			Adjuvant	1, 24	4.09	0.054	**1, 24**	**9.48**	**0.005**
			Insecticide × Adjuvant	**3, 24**	**4.02**	**0.019**	3, 24	2.69	0.069
			DAT	**3, 71**	**39.25**	**<0.001**	**3, 72**	**10.82**	**<0.001**
			Insecticide × Adjuvant × DAT	21, 71	0.82	0.682	21, 72	1.05	0.416
	2017	NHB	Insecticide	4, 20	1.34	0.289	4, 20	1.26	0.317
			Adjuvant	1, 20	0.24	0.627	1, 20	0.73	0.404
			Insecticide × Adjuvant	4, 20	0.75	0.570	4, 20	0.93	0.469
			DAT	**2, 39**	**11.36**	**<0.001**	**2, 39**	**4.47**	**0.018**
			Insecticide × Adjuvant × DAT	18, 39	1.80	0.061	**18, 39**	**1.92**	**0.044**

Note. NHB: northern highbush blueberry; RE: rabbiteye blueberry; SHB: southern highbush blueberry.
[a]Values in bold were statistically significant ($p < 0.05$).

TABLE 4 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment and residue age in a semi-field bioassay at 120 hr of exposure and mean (± *SE*) number of *D. suzukii* progeny per five blueberries. The adjuvant used in this experiment was alcohol ethoxylate. The trial was conducted at a blueberry farm in Island Grove, FL, in 2017. *p*-Values are from planned contrasts comparing each pesticide with and without the adjuvant. Values in bold were significantly different between treatments ($p < 0.05$)

		Mean (± *SE*) per cent adult mortality			Mean (± *SE*) progeny per five berries		
Residue age	Treatment	Without adjuvant	With adjuvant	*p*-Value	Without adjuvant	With adjuvant	*p*-Value
0 days	Control	32.5 ± 6.29	45.0 ± 21.02	0.460	**56.0 ± 14.6**	**30.0 ± 8.26**	**0.028**
	Spinosad	75.0 ± 12.58	90.0 ± 4.08	0.376	7.0 ± 4.14	2.5 ± 2.18	0.692
	Azadirachtin + pyrethrins	72.5 ± 6.29	72.5 ± 4.79	1.000	17.5 ± 4.17	20.0 ± 1.47	0.826
	C. subtsugae	45.0 ± 12.58	43.3 ± 3.33	0.927	26.0 ± 13.64	27.7 ± 5.04	0.892
	Hydrogen peroxide + PAA	52.5 ± 13.77	57.5 ± 16.52	0.767	17.3 ± 4.13	24.0 ± 8.63	0.554
3 days	Control	15.0 ± 6.46	32.5 ± 8.54	0.157	**48.0 ± 4.06**	**31.8 ± 7.23**	**0.043**
	Spinosad	65.0 ± 12.58	45.0 ± 2.89	0.108	18.8 ± 3.86	18.3 ± 3.15	0.949
	Azadirachtin + pyrethrins	35.0 ± 6.46	45.0 ± 6.46	0.414	30.3 ± 5.50	30.5 ± 9.79	0.974
	C. subtsugae	32.5 ± 9.47	35.0 ± 6.46	0.837	23.3 ± 4.94	35.5 ± 5.56	0.122
	Hydrogen peroxide + PAA	25.0 ± 12.58	27.5 ± 8.54	0.837	32.5 ± 3.23	31.5 ± 3.23	0.897
5 days	Control	20.0 ± 4.08	20.0 ± 4.08	1.000	48.5 ± 11.52	44.3 ± 9.24	0.698
	Spinosad	45.0 ± 6.46	32.5 ± 12.50	0.335	21.5 ± 6.98	13.3 ± 5.82	0.453
	Azadirachtin + pyrethrins	17.5 ± 2.50	37.5 ± 13.15	0.127	38.8 ± 9.20	31.3 ± 10.05	0.495
	C. subtsugae	32.5 ± 8.54	37.5 ± 11.09	0.698	21.3 ± 4.79	25.3 ± 5.36	0.715
	Hydrogen peroxide + PAA	32.5 ± 9.47	30.0 ± 10.80	0.846	34.3 ± 6.87	32.0 ± 2.04	0.837

2.4.2 | 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 P1M in five replicates. The 2017 bioassay included six pesticide treatments (azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, *Burkholderia* spp., and sabadilla alkaloids) with an untreated control with and without P1M in five replicates.

Treatments were applied to store-bought organic blueberries. Berries were rinsed 2–3 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 (five males and five 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 3 days post-exposure. After 3 days, the berries were transferred to clean deli cups without flies and were held for two weeks to allow for progeny to develop. Berries were then dissected, and the numbers of larvae, pupae and adults were recorded.

2.4.3 | Topical application bioassay

A topical application bioassay was performed at Michigan State University in 2016. There were eight pesticide treatments (azadirachtin, azadirachtin + pyrethrins, spinosad, *C. subtsugae*, hydrogen peroxide + PAA, hydrogen dioxide + PAA, pyrethrins and *Burkholderia* spp.) plus an untreated control with and without PEPMS in six replicates. Treatments were sprayed directly onto CO_2-anaesthetized *D. suzukii* adults in Petri dishes (100 × 15 mm, Fisher Scientific Company LLC) 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 days post-application. Petri dishes were maintained in a growth chamber at 25°C, 75% RH and a photoperiod of 16:8 [L:D] hr.

TABLE 5 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment and residue age in semi-field bioassays at 120 hr of exposure and mean (± *SE*) number of *D. suzukii* progeny per five berries. The adjuvant used in these experiments was poly-1-p-menthene. The experiment was conducted in rabbiteye blueberries at a blueberry farm in Baxley, GA, in 2016. *p*-Values are from planned contrasts comparing each pesticide with and without the adjuvant. Values in bold were significantly different between treatments ($p < 0.05$)

		Mean (± *SE*) per cent adult mortality			Mean (± *SE*) progeny per five berries		
Residue age	Treatment	Without adjuvant	With adjuvant	*p*-Value	Without adjuvant	With adjuvant	*p*-Value
0 days	Control	0.0 ± 0.0	20.0 ± 20.00	0.065	7.7 ± 0.33	16.3 ± 6.06	0.178
	Spinosad	83.3 ± 8.82	100.0 ± 0.0	0.119	4.0 ± 2.08	3.7 ± 0.88	0.958
	Azadirachtin + pyrethrins	6.7 ± 3.33	6.7 ± 3.33	1.000	32.0 ± 7.37	31.7 ± 2.67	0.958
	C. subtsugae	0.0 ± 0.0	3.3 ± 3.33	0.748	14.3 ± 5.33	22.3 ± 7.17	0.213
	Pyrethrins	0.0 ± 0.0	6.7 ± 3.33	0.522	12.0 ± 2.08	17.0 ± 2.31	0.430
3 days	Control	16.7 ± 8.82	13.3 ± 13.33	0.844	26.7 ± 5.84	13.7 ± 0.88	0.051
	Spinosad	46.7 ± 12.02	63.3 ± 14.53	0.329	19.0 ± 6.56	9.7 ± 3.48	0.152
	Azadirachtin + pyrethrins	13.3 ± 8.82	20.0 ± 15.28	0.693	13.7 ± 2.96	17.3 ± 1.67	0.565
	C. subtsugae	13.3 ± 8.82	26.7 ± 3.33	0.433	**7.0 ± 0.58**	**21.3 ± 2.03**	**0.033**
	Pyrethrins	20.0 ± 10.00	16.7 ± 16.67	0.844	**7.0 ± 3.00**	**23.0 ± 9.02**	**0.019**
7 days	Control	10.0 ± 5.77	0.0 ± 0.0	0.526	10.3 ± 2.85	15.0 ± 4.16	0.245
	Spinosad	3.3 ± 3.33	33.3 ± 17.64	0.067	6.0 ± 0.58	7.3 ± 2.85	0.736
	Azadirachtin + pyrethrins	26.7 ± 17.64	30.0 ± 20.00	0.832	8.0 ± 1.53	11.7 ± 3.28	0.358
	C. subtsugae	16.7 ± 3.33	3.3 ± 3.33	0.400	7.7 ± 2.19	6.0 ± 0.0	0.673
	Pyrethrins	10.0 ± 10.00	16.7 ± 3.33	0.672	13.7 ± 3.84	7.0 ± 3.06	0.103

2.4.4 | 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 PEPMS 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 days post-application.

2.5 | Statistical analysis

Mortality and progeny data from the semi-field bioassays were analysed using generalized linear mixed models in PROC GLIMMIX with insecticide, adjuvant, insecticide × adjuvant, residue age (DAT) and insecticide × adjuvant × DAT as fixed effects. The effect of adjuvants on the efficacy of each insecticide at each residue age was measured using linear contrasts. There were no significant differences between female and male mortality for any treatment (Wilcoxon rank-sum test, PROC NPAR1WAY), so both sexes were pooled for analysis. Larvae, pupae and adults were pooled for progeny count analysis. Field infestation data from berries collected in the field were analysed using generalized linear mixed models in PROC GLIMMIX with insecticide, adjuvant and insecticide × adjuvant as fixed effects.

Mortality data from all the laboratory bioassays and progeny data from the fruit dip laboratory bioassays were analysed using generalized linear mixed models in PROC GLIMMIX (SAS 9.4, SAS Institute, 2013) with insecticide, adjuvant and insecticide × adjuvant as fixed effects. The effect of adjuvants on the efficacy of each insecticide was measured using linear contrasts. There were no significant differences between female and male mortality for any treatment (Wilcoxon rank-sum test, PROC NPAR1WAY), so both sexes were pooled for analysis.

3 | RESULTS

3.1 | Semi-field experiments

3.1.1 | Florida

The only factors in the model that had a significant effect on *D. suzukii* mortality were insecticide and DAT. Addition of AE did not have a significant effect on *D. suzukii* mortality for any insecticide at any residue age (Table 3). In the progeny analysis, the only significant factors were insecticide and DAT (Table 3). Addition of AE had an effect on mean progeny in the controls at 0 and 3 DAT but not for any of the other treatments (Table 4). For the field infestation analysis, none of the effects were statistically significant ($p > 0.05$), but this may be because the number of *D. suzukii* larvae

TABLE 6 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment and residue age in semi-field bioassays at 120 hr of exposure and mean (± *SE*) number of *D. suzukii* progeny per five berries. The adjuvant used in these experiments was poly-1-p-menthene. The experiments were conducted at a blueberry farm in Baxley, GA, in 2017. *p*-Values are from planned contrasts comparing each pesticide with and without the adjuvant. Values in bold were significantly different between treatments ($p < 0.05$)

			Mean (± *SE*) per cent adult mortality			Mean (± *SE*) progeny per five berries		
Blueberry type	Residue age	Treatment	Without adjuvant	With adjuvant	*p*-Value	Without adjuvant	With adjuvant	*p*-Value
Highbush	0 days	Control	45.0 ± 15.00	73.3 ± 17.64	0.238	22.5 ± 1.50	13.7 ± 8.09	0.402
		Spinosad	83.3 ± 3.33	76.7 ± 14.53	0.752	0.0 ± 0.0	2.3 ± 2.33	0.803
		Azadirachtin + pyrethrins	46.7 ± 20.28	75.0 ± 25.00	0.238	26.7 ± 12.12	36.0 ± 17.00	0.377
		C. subtsugae	80.0 ± 15.27	63.3 ± 18.56	0.433	13.3 ± 3.28	7.3 ± 3.38	0.523
		Hydrogen peroxide + PAA	60.0 ± 10.00	50.0 ± 5.77	0.636	25.3 ± 2.96	18.0 ± 6.00	0.436
	3 days	Control	16.7 ± 3.33	53.3 ± 23.33	0.090	36.7 ± 5.67	38.7 ± 11.05	0.896
		Spinosad	73.3 ± 12.02	46.7 ± 8.82	0.209	16.7 ± 3.93	42.0 ± 8.72	0.110
		Azadirachtin + pyrethrins	50.0 ± 5.77	50.0 ± 23.09	1.000	55.0 ± 13.65	51.3 ± 17.53	0.811
		C. subtsugae	30.0 ± 11.55	43.3 ± 16.67	0.524	38.3 ± 12.25	50.0 ± 12.86	0.450
		Hydrogen peroxide + PAA	46.7 ± 8.82	43.3 ± 16.67	0.873	49.3 ± 8.41	26.0 ± 4.36	0.139
	5 days	Control	25.0 ± 25.00	66.7 ± 17.64	0.119	**71.0 ± 40.00**	**24.7 ± 5.46**	**0.035**
		Spinosad	46.7 ± 21.86	60.0 ± 5.77	0.566	37.7 ± 14.19	32.7 ± 2.96	0.788
		Azadirachtin + pyrethrins	**73.3 ± 13.33**	**10.0 ± 0.0**	**0.012**	36.7 ± 15.17	44.7 ± 4.98	0.667
		C. subtsugae	**76.7 ± 8.82**	**26.7 ± 14.53**	**0.041**	**21.0 ± 4.04**	**59.7 ± 14.17**	**0.048**
		Hydrogen peroxide + PAA	30.0 ± 25.17	23.3 ± 18.56	0.773	38.3 ± 15.30	76.3 ± 10.53	0.052
Rabbiteye	0 days	Control	13.3 ± 8.82	10.0 ± 5.77	0.834	**24.3 ± 8.84**	**53.3 ± 11.67**	**0.012**
		Spinosad	100.0 ± 0.0	90.0 ± 5.77	0.532	7.0 ± 2.65	10.3 ± 1.45	0.755
		Azadirachtin + pyrethrins	23.0 ± 9.05	18.8 ± 10.52	0.790	35.0 ± 6.03	52.3 ± 5.33	0.116
		C. subtsugae	16.7 ± 12.02	33.3 ± 12.02	0.302	20.0 ± 2.65	29.0 ± 11.85	0.403
		Hydrogen peroxide + PAA	36.7 ± 21.86	30.0 ± 11.55	0.676	37.3 ± 10.73	27.0 ± 2.08	0.338
	3 days	Control	13.3 ± 8.82	3.3 ± 3.33	0.406	60.3 ± 14.38	32.7 ± 14.86	0.110
		Spinosad	**41.2 ± 12.61**	**9.1 ± 9.09**	**0.013**	22.3 ± 1.67	25.0 ± 9.50	0.874
		Azadirachtin + pyrethrins	23.3 ± 6.67	20.0 ± 11.55	0.780	42.0 ± 11.59	46.0 ± 14.15	0.811
		C. subtsugae	31.1 ± 5.88	29.4 ± 10.32	0.886	43.3 ± 9.94	54.3 ± 4.37	0.513
		Hydrogen peroxide + PAA	3.3 ± 3.33	10.0 ± 5.77	0.578	42.7 ± 15.59	66.7 ± 12.24	0.162
	5 days	Control	12.4 ± 7.97	20.0 ± 5.77	0.616	30.0 ± 4.93	44.3 ± 4.67	0.207
		Spinosad	6.7 ± 6.67	0.0 ± 0.0	0.659	33.3 ± 8.88	29.7 ± 7.22	0.742
		Azadirachtin + pyrethrins	0.0 ± 0.0	19.7 ± 10.16	0.201	28.7 ± 6.57	34.3 ± 3.93	0.612
		C. subtsugae	13.3 ± 8.82	6.7 ± 6.67	0.659	40.7 ± 8.41	44.0 ± 12.90	0.765
		Hydrogen peroxide + PAA	16.1 ± 8.73	30.6 ± 25.77	0.340	31.3 ± 7.54	35.7 ± 8.65	0.698

and pupae collected from berries was low, averaging <1 fly per 100 g of blueberries.

3.1.2 | Georgia

In the 2016 experiment, the only factors in the model that had a significant effect on *D. suzukii* mortality were insecticide and insecticide × adjuvant × DAT (Table 3). Addition of P1M did not have a significant effect on *D. suzukii* mortality for any insecticide at any residue age (Table 5). In the progeny analysis, the only significant factors were insecticide, DAT and insecticide × adjuvant × DAT (Table 3). Addition of P1M affected mean progeny only for *C. subtsugae* and pyrethrins at three DAT (Table 5). In both cases, berries treated with P1M had more larvae and berries treated with

TABLE 7 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment and residue age in semi-field bioassays at 120 hr of exposure and mean (± *SE*) number of *D. suzukii* progeny per five berries. The adjuvant used in these experiments was polyether-polymethylsiloxane-copolymer, polyether. The experiments were conducted on blueberries at a research station in Fennville, MI. *p*-Values are from planned contrasts comparing each pesticide with and without the adjuvant. Values in bold were significantly different between treatments ($p < 0.05$)

			Mean (± *SE*) per cent adult mortality			Mean (± *SE*) progeny per five berries		
Year	Residue age	Treatment	Without adjuvant	With adjuvant	*p*-Value	Without adjuvant	With adjuvant	*p*-Value
2016	0 days	Control	51.3 ± 6.28	66.7 ± 20.41	0.501	29.8 ± 2.25	30.8 ± 2.69	0.852
		Spinosad	100.0 ± 0.0	100.0 ± 0.0	1.000	2.5 ± 1.26	5.0 ± 1.41	0.642
		Azadirachtin + pyrethrins	64.6 ± 18.12	56.3 ± 25.32	0.715	**12.8 ± 5.22**	**27.0 ± 5.61**	**0.013**
		C. subtsugae	72.9 ± 16.45	89.6 ± 10.42	0.467	15.8 ± 2.63	17.5 ± 5.56	0.744
	3 days	Control	27.1 ± 13.77	45.8 ± 15.40	0.434	23.3 ± 4.68	20.5 ± 4.21	0.596
		Spinosad	87.5 ± 12.50	75.0 ± 19.84	0.600	3.5 ± 1.19	11.8 ± 2.96	0.120
		Azadirachtin + pyrethrins	43.8 ± 20.80	31.3 ± 7.89	0.600	17.3 ± 2.93	20.5 ± 5.68	0.532
		C. subtsugae	58.3 ± 19.84	56.3 ± 18.75	0.930	13.8 ± 0.75	18.8 ± 3.71	0.339
	5 days	Control	6.3 ± 2.99	4.2 ± 2.41	0.833	28.8 ± 3.12	21.8 ± 5.79	0.437
		Spinosad	**77.1 ± 10.96**	**10.4 ± 2.08**	**<0.001**	7.3 ± 3.90	21.0 ± 5.05	0.134
		Azadirachtin + pyrethrins	8.3 ± 8.33	6.3 ± 3.99	0.833	19.3 ± 6.66	30.3 ± 9.11	0.226
		C. subtsugae	**27.1 ± 11.97**	**4.2 ± 2.41**	**0.027**	14.8 ± 4.79	30.8 ± 8.93	0.084
	7 days	Control	8.3 ± 5.89	8.3 ± 4.81	1.000	14.0 ± 2.86	12.3 ± 2.14	0.612
		Spinosad	**70.8 ± 14.63**	**14.6 ± 5.24**	**<0.001**	3.0 ± 1.78	8.8 ± 1.60	0.104
		Azadirachtin + pyrethrins	20.8 ± 7.98	6.3 ± 3.99	0.172	10.5 ± 2.60	13.5 ± 4.25	0.386
		C. subtsugae	6.3 ± 3.99	10.4 ± 5.24	0.691	8.3 ± 1.32	8.3 ± 1.11	1.000
2017	0 days	Control	27.8 ± 12.11	8.3 ± 8.33	0.336	48.7 ± 9.94	42.0 ± 4.04	0.518
		Spinosad	25.0 ± 9.62	50.0 ± 25.00	0.220	33.7 ± 12.03	17.3 ± 4.33	0.123
		Azadirachtin + pyrethrins	63.9 ± 20.03	38.9 ± 15.47	0.220	22.3 ± 4.98	25.0 ± 8.00	0.795
		C. subtsugae	45.8 ± 20.83	16.7 ± 4.81	0.201	**29.0 ± 9.00**	**53.0 ± 7.64**	**0.047**
		Hydrogen peroxide + PAA	13.9 ± 10.02	16.7 ± 0.0	0.889	35.3 ± 2.73	38.3 ± 5.04	0.770
	3 days	Control	16.7 ± 12.73	2.8 ± 2.78	0.096	26.0 ± 4.73	36.0 ± 4.04	0.413
		Spinosad	2.8 ± 2.78	8.3 ± 4.81	0.493	26.0 ± 3.06	34.0 ± 16.52	0.511
		Azadirachtin + pyrethrins	8.3 ± 8.33	2.8 ± 2.78	0.493	25.0 ± 6.66	34.0 ± 9.54	0.461
		C. subtsugae	0.0 ± 0.0	2.8 ± 2.78	0.731	24.0 ± 3.22	25.7 ± 6.67	0.891
		Hydrogen peroxide + PAA	0.0 ± 0.0	5.6 ± 2.56	0.493	41.7 ± 5.84	37.0 ± 13.05	0.701
	5 days	Control	5.6 ± 5.56	38.9 ± 22.74	0.131	19.7 ± 3.71	16.3 ± 6.49	0.745
		Spinosad	55.6 ± 28.19	13.9 ± 5.56	0.063	10.0 ± 1.53	24.3 ± 10.14	0.172
		Azadirachtin + pyrethrins	5.6 ± 5.56	8.3 ± 4.81	0.897	**20.7 ± 5.61**	**43.3 ± 8.51**	**0.037**
		C. subtsugae	11.1 ± 5.56	5.6 ± 2.78	0.796	34.0 ± 10.15	26.0 ± 3.79	0.438
		Hydrogen peroxide + PAA	0.0 ± 0.0	27.8 ± 27.78	0.204	35.7 ± 7.69	18.3 ± 8.37	0.102

the insecticides alone. The field infestation samples did not yield a single *D. suzukii*.

In the 2017 experiment in southern highbush blueberry, the only factor in the model that had a significant effect on *D. suzukii* mortality was DAT (Table 3). Addition of P1M significantly reduced *D. suzukii* mortality at five DAT when added to azadirachtin + pyrethrins and *C. subtsugae* (Table 6). In the progeny analysis, the only significant factor was DAT (Table 3). P1M alone had fewer progeny compared to the control at five DAT, and addition of P1M significantly increased the number of progeny at five DAT when added to *C. subtsugae* (Table 6). Field infestation samples yielded only one *D. suzukii*.

In the 2017 experiment in rabbiteye blueberry, the only factors in the model that had a significant effect on *D. suzukii* mortality were insecticide, DAT and insecticide × adjuvant × DAT (Table 3). Addition of P1M significantly reduced *D. suzukii* mortality at five DAT when added to spinosad at three DAT (Table 6). In the progeny

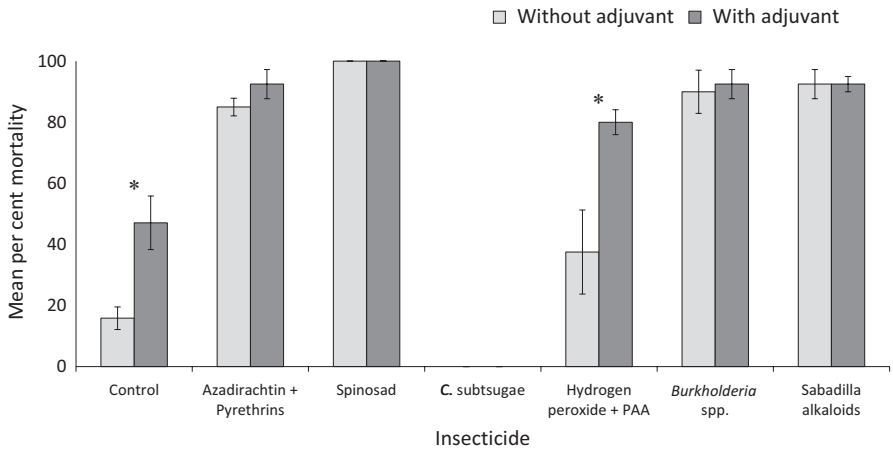


FIGURE 1 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment in glass vial laboratory bioassays at 72 hr of exposure. The adjuvant was alcohol ethoxylate. Bioassays were conducted at the University of Florida in 2017. Significant differences between pesticides with and without the adjuvant are denoted with an asterisk (*) ($p < 0.05$)

analysis, the only significant factors were insecticide and DAT (Table 3). Berries treated with P1M alone had significantly more progeny than the control at 0 DAT (Table 6). For the field infestation analysis, none of the effects were statistically significant ($p > 0.05$). The number of *D. suzukii* larvae and pupae collected from berries was low, averaging less than four flies per 100 g of blueberries.

3.1.3 | Michigan

In the 2016 experiment, a significant effect on *D. suzukii* mortality was found for insecticide, insecticide × adjuvant and DAT (Table 3). Addition of PEPMS reduced *D. suzukii* mortality at five and seven DAT when added to spinosad and at five DAT when added to *C. subtsugae* (Table 7). In the progeny analysis, the only significant factors were insecticide, adjuvant and DAT (Table 3). Berries treated with azadirachtin + pyrethrins with PEPMS had more larvae than berries treated with the insecticide alone (Table 7). For the field infestation analysis, there was no significant effect of the treatments on *D. suzukii* infestation ($p > 0.05$). The number of *D. suzukii* larvae and pupae collected from berries was low, averaging less than three flies per 100 g of blueberries.

In the 2017 experiment, the only factor in the model that had a significant effect on *D. suzukii* mortality was DAT (Table 3). Addition of PMPMS did not have a significant effect on *D. suzukii* mortality for any insecticide at any residue age (Table 7). In the progeny analysis, the only significant factors were DAT and insecticide × adjuvant × DAT (Table 3). The only significant adjuvant effects on mean number of progeny were for *C. subtsugae* at 0 DAT and azadirachtin + pyrethrins at five DAT (Table 7). For the field infestation analysis, none of the effects were statistically significant ($p > 0.05$). The number of *D. suzukii* larvae and pupae collected from berries was low, averaging less than 10 flies per 100 g of blueberries.

3.2 | Laboratory experiments

3.2.1 | Glass vial bioassay

In the glass vial bioassay, the effect of insecticide was statistically significant ($F = 24.46$; $df = 6, 82$; $p < 0.001$), but the effects of adjuvant ($F = 3.44$; $df = 1, 82$; $p = 0.067$) and insecticide × adjuvant interaction ($F = 1.48$; $df = 6, 82$; $p = 0.196$) were not significant. AE alone caused significantly higher mortality than the control, and addition of AE resulted in a statistically significant increase in mortality when added to hydrogen peroxide + PAA but no other products (Figure 1).

3.2.2 | Fruit dip bioassay

In the 2016 bioassay, the effects of insecticide ($F = 11.86$; $df = 8, 72$; $p < 0.001$), adjuvant ($F = 6.69$; $df = 1, 72$; $p = 0.012$) and insecticide × adjuvant interaction ($F = 4.78$; $df = 8, 72$; $p < 0.001$) were all statistically significant. Addition of the adjuvant P1M had a significant effect on *D. suzukii* mortality when added to azadirachtin, hydrogen peroxide + PAA and hydrogen dioxide + PAA (Figure 2a). With hydrogen peroxide + PAA, however, the adjuvant reduced mortality. Hydrogen dioxide + PAA alone did not kill any flies, but with P1M it caused 100% mortality. Mortality from P1M alone was not statistically different from the untreated control; however, mean progeny in P1M-treated berries was statistically lower than control (Figure 2b). For the 2016 progeny data, the effect of insecticide was statistically significant ($F = 8.18$; $df = 8, 72$; $p < 0.001$), but adjuvant ($F = 0.25$; $df = 1, 72$; $p = 0.619$) and insecticide × adjuvant interaction ($F = 1.80$; $df = 8, 72$; $p = 0.091$) were not significant. The only statistically significant effect of P1M on mean progeny was seen with *C. subtsugae* (Figure 2b). Adding P1M to hydrogen peroxide + PAA significantly reduced mortality compared to hydrogen peroxide + PAA alone, and the mean number of progeny developing in berries was higher but not statistically significant.

In the 2017 bioassay, the effect of insecticide was statistically significant ($F = 16.10$; $df = 6, 55$; $p < 0.001$), but the effects of adjuvant ($F = 2.88$; $df = 1, 55$; $p = 0.096$) and insecticide × adjuvant interaction ($F = 0.51$; $df = 6, 55$; $p = 0.797$) were not significant. There were no significant differences in mean fly mortality between any of the products alone and with P1M (Figure 3a). Adding P1M to hydrogen dioxide + PAA resulted in lower mortality, but the difference was not statistically significant. P1M alone did not show toxicity compared to the control. For the 2017 progeny data, the effect of insecticide was statistically significant

(F = 4.27; df = 6, 56; p = 0.001), but adjuvant (F = 2.45; df = 1, 56; p = 0.123) and insecticide × adjuvant interaction (F = 2.06; df = 6, 56; p = 0.072) were not significant. The one significant difference in mean progeny was with hydrogen peroxide + PAA where berries treated with hydrogen peroxide + PAA and P1M had more larvae (Figure 3b).

3.2.3 | Topical application bioassay

In the topical application bioassay, the effect of insecticide was statistically significant (F = 43.06; df = 8, 89; p < 0.001), but the effects of adjuvant (F = 3.66; df = 1, 89; p = 0.059) and insecticide × adjuvant interaction (F = 1.57; df = 8, 89; p = 0.146) were not significant. PEPMS alone did not show toxicity compared with the untreated control (Figure 4a). Mean mortality was significantly higher when PEPMS was added to azadirachtin + pyrethrins compared to azadirachtin + pyrethrins alone, but the increase was less than 50% (Figure 4a). In all other treatments, there was no effect on mortality due to the addition of the adjuvant.

3.2.4 | Residual contact bioassay

In the residual contact bioassay, the effect of insecticide was statistically significant (F = 33.36; df = 6, 42; p < 0.001), but the effects of adjuvant (F = 0.49; df = 1, 42; p = 0.489) and insecticide × adjuvant interaction (F = 0.47; df = 6, 42; p = 0.825) were not significant. PEPMS did not show toxicity compared to the untreated control (Figure 4b). There were no statistically significant differences between any of the products with and without the adjuvant.

4 | DISCUSSION

The results from this study were mixed. We defined insecticide activity improvement as increasing adult fly mortality, reducing the number of larvae infesting fruit or extending residual activity of the insecticide. In some instances, the selected adjuvants did improve insecticide activity while in other instances, adjuvants had a negative effect on insecticide activity. In most cases, however, adding an adjuvant had no effect on insecticide activity. The assessments based on fruit infestation from bushes in the treated field plots did not show any treatment effects due to adjuvant. The numbers of *D. suzukii* larvae collected were too low to resolve any treatment differences.

The semi-field bioassays were used to test field-applied and field-aged insecticide residues under controlled conditions with a known number of *D. suzukii* adults. Exposure to treated leaves and fruit provided an opportunity to understand the plant–chemical interactions and the impact of the adjuvants on insecticide activity. We did not observe a treatment effect of AE on adult mortality or progeny. The adjuvant P1M had significant effects on adult mortality

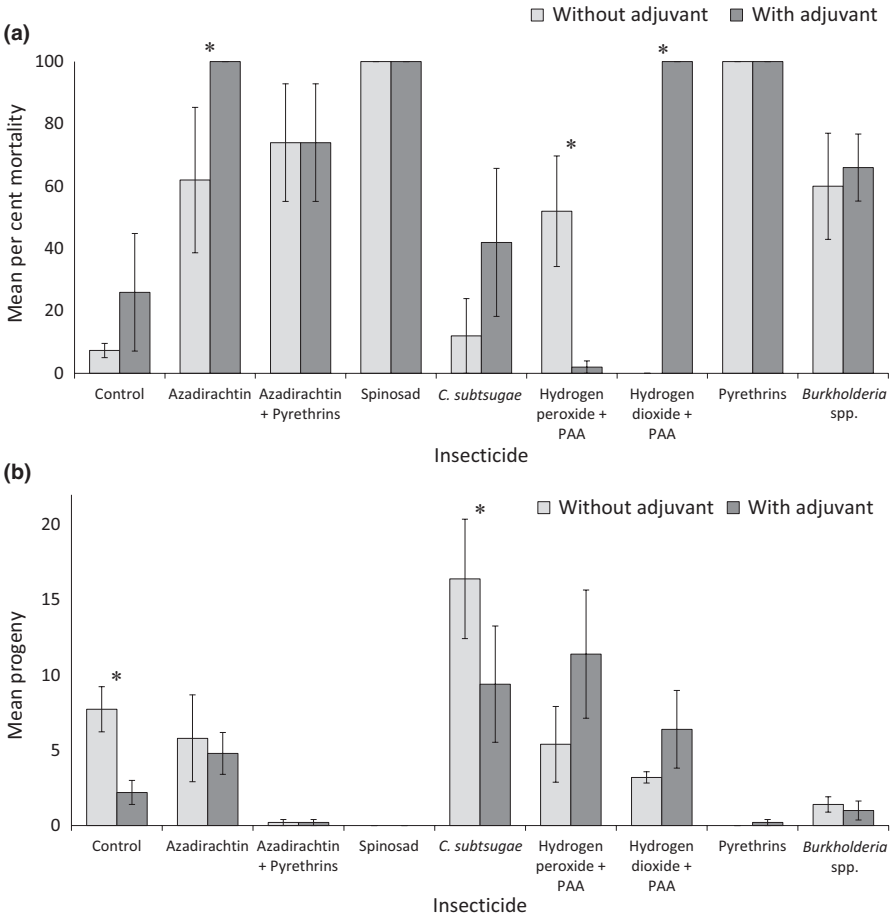
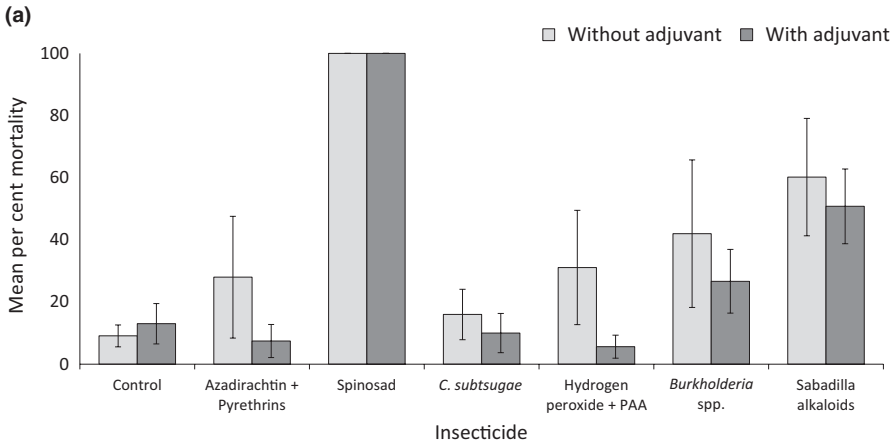


FIGURE 2 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality at 72 hr of exposure (a) and mean (± *SE*) number of progeny per five blueberries (b) by pesticide treatment in fruit dip laboratory bioassays. The adjuvant was poly-1-p-menthene. Bioassays were conducted at the University of Georgia in 2016. Significant differences between pesticides with and without the adjuvant are denoted with an asterisk (*) (p < 0.05)



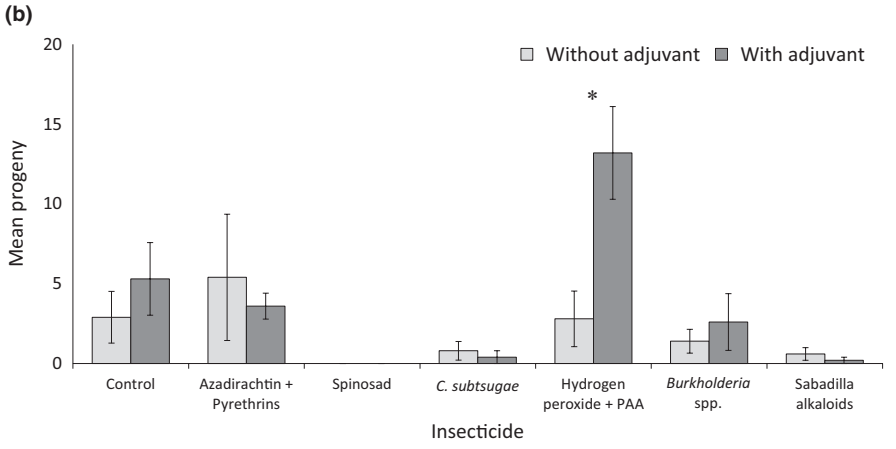
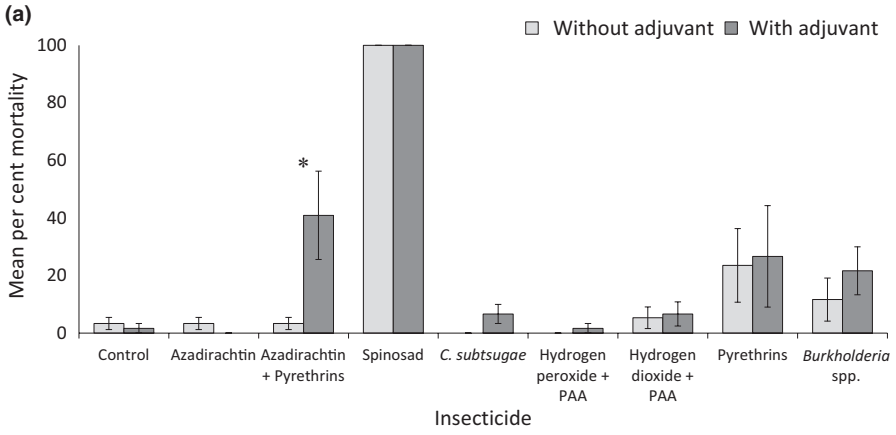


FIGURE 3 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality at 72 hr of exposure (a) and mean (± *SE*) number of progeny per five blueberries (b) by pesticide treatment in fruit dip laboratory bioassays. The adjuvant was poly-1-p-menthene. Bioassays were conducted at the University of Georgia in 2017. Significant differences between pesticides with and without the adjuvant are denoted with an asterisk (*) ($p < 0.05$)



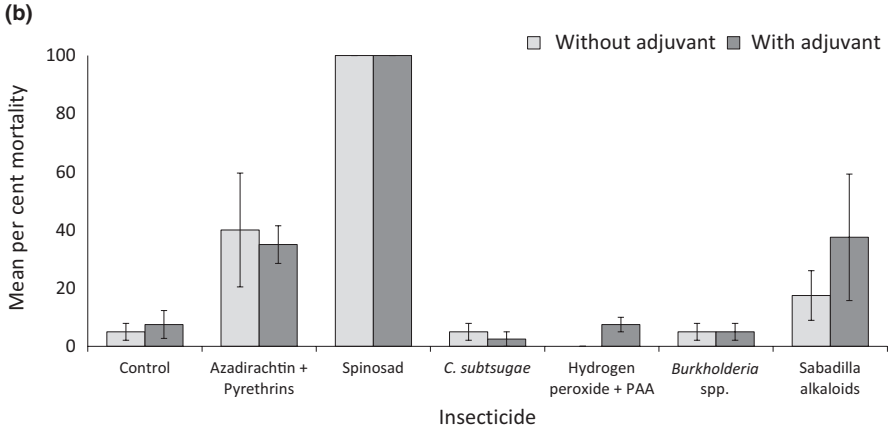


FIGURE 4 Mean (± *SE*) per cent *Drosophila suzukii* adult mortality by pesticide treatment in a topical application laboratory bioassay at 48 hr of exposure (a) and residual contact laboratory bioassay at 72 hr of exposure (b). The adjuvant was polyether-polymethylsiloxane-copolymer, polyether. Bioassays were conducted at Michigan State University. Significant differences between pesticides with and without the adjuvant are denoted with an asterisk (*) ($p < 0.05$)

with azadirachtin + pyrethrins and spinosad and on progeny with *C. subtsugae* and pyrethrins. These effects, however, were not improvements in insecticide activity. Addition of P1M decreased adult mortality and increased the number of larvae in berries. Wise et al. (2017) evaluated the efficacy of several rates and timings of various insecticides, organic and conventional, against *D. suzukii*. Among the organic products tested, they found that *C. subtsugae* + P1M and *C. subtsugae* + P1M + pyrethrins significantly reduced *D. suzukii* infestation in fruit. We did not, however, see a benefit of *C. subtsugae* + P1M in our semi-field bioassays. In some of the samples, we collected more larvae from berries treated with azadirachtin + pyrethrins or *C. subtsugae* than the untreated control. Insecticides could affect the number of fly progeny developing in berries in several ways including lethal effects (females die before laying eggs, eggs die before hatching), sublethal effects (number of eggs laid, larval development rate) or behavioural effects (attraction or repellency). Spinosad did not show long residual activity in our trials, and at 3 DAT, it was already losing effectiveness. In a rain fastness study, Gautam et al. (2016) observed that the effectiveness of insecticides, including spinosad, declines rapidly in the field even in the absence of rainfall. The addition of P1M did not improve residual activity (Gautam et al., 2016). The adjuvant PEPMS had significant effects on adult mortality with *C. subtsugae* and spinosad and on progeny with azadirachtin + pyrethrins and *C. subtsugae*. The effects were the same as those observed with P1M, addition of the adjuvant decreased adult mortality and increased the number of larvae in berries.

Laboratory bioassays included all the insecticides used in the field experiments plus additional products. Results from the treated glass vials, treated Petri dishes and direct spray bioassays were used to determine how adjuvants impacted insecticide toxicity via direct exposure. The fruit dip bioassays allowed for some testing of the plant–chemical interactions. This method was only used with the adjuvant P1M.

In the glass vial bioassays, AE showed toxicity to *D. suzukii* with mean mortality of 47.1%, and it significantly improved the effectiveness of hydrogen peroxide + PAA. Mortality in the laboratory assays was much higher than in the semi-field assay. This discrepancy is likely due to interaction of spray residues with the plant tissue and penetration of the chemical. Bioassay chambers used in the semi-field experiments were larger, and interior surfaces were not treated with insecticides so that it was possible for flies to avoid treated surfaces.

In the fruit dip bioassays, mean mortality in the P1M control was higher in 2016 compared to 2017. In 2017, we implemented a method improvement where the cotton plugging the deli containers was remoistened more frequently. This would have provided a more consistent water source for the flies compared to the 2016 bioassay. Gautam et al. (2016) found no difference between their water control and water with either di-1-p-menthene or P1M. Gardner, Seaman, and Hoffmann (2018) tested organic insecticides P1M for control of striped cucumber beetle, *Acalymma vittatum* (Fabricius). Their untreated check, which consisted of P1M in water, did not cause any beetle mortality (Gardner et al., 2018). The greatest effect we saw was when P1M was combined with the sanitizers, hydrogen peroxide + PAA and hydrogen dioxide + PAA. The effect of P1M on hydrogen peroxide + PAA, however, was negative resulting in lower adult mortality. It was not clear why hydrogen peroxide + PAA had lower mortality than hydrogen peroxide + PAA alone, but it is also not understood exactly how hydrogen peroxide + PAA affects *D. suzukii*. One hypothesis is that hydrogen peroxide + PAA functions by removing naturally occurring yeasts from the fruit surface which could affect physiology and behaviour of the flies (Hamby & Becher, 2016). Future studies are needed to investigate the mechanisms of how these sanitizers affect *D. suzukii*. Hydrogen dioxide + PAA by itself did not kill any flies, but hydrogen dioxide + PAA with P1M caused 100% mortality. The reason for this difference is not clear. It does not match the magnitude of the effect of P1M alone and hydrogen dioxide + PAA alone did not kill any flies.

In the topical application experiment, adding PEPMS to azadirachtin + pyrethrins increased adult mortality, but the change was modest, less than 45%. This would not be adequate for controlling *D. suzukii* in the field. Azadirachtin + pyrethrins with PEPMS was more toxic when sprayed directly onto the flies than when flies were exposed to the residues on Petri dishes. PEPMS is a silicone surfactant, and the mode of action of silicone adjuvants against insects is thought to be suffocation (Purcell & Schroeder, 1996). Spraying flies directly with an insecticide + PEPMS mixture was the effective route of exposure and not contact with dry residues.

The safety data sheets (SDS) for AE and P1M list strong oxidizing agents as incompatible materials. The varied effect of combining these products with the sanitizers hydrogen peroxide + PAA and hydrogen dioxide + PAA, both strong oxidizing agents, could be due to the reactions of these chemicals. In the case of AE plus oxidizers, we generally observed an increase in toxicity to *D. suzukii*, while with P1M plus oxidizers, we observed a decrease in toxicity. PEPMS is an organosilicone, and the SDS does not list any incompatible materials.

Drosophila suzukii has disrupted integrated pest management in organic as well as conventional fruit production systems. Intensive insecticide use targeting *D. suzukii* has become the norm in berry crop systems to maintain production standards. This practice will likely continue until other effective non-chemical management methods can be developed and adopted. While some studies have shown positive impacts of adjuvants (Cocco & Hoy, 2008; Demkovich, Siegel, Walse, & Berenbaum, 2018; Seal, Ciomperlik, Richards, & Klassen, 2006), most of those studies included conventional insecticides. Our results, however, did not show significant benefits of using adjuvants with the selected organic insecticides. We observed positive effects of adjuvants in a few instances, but the effects were not consistent enough or strong enough to justify the use of adjuvants in the field to improve efficacy of these organic insecticides to control *D. suzukii*.

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AUTHORS' CONTRIBUTIONS

AS, OL, and RI conceived the study. CR, BG, PF, SVT, JS, BL and SC conducted the experiments. CR, PF and JS analysed the data and wrote the manuscript. All authors read and approved the manuscript.

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SUPPORTING INFORMATION

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