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Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs

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ABSTRACT

Spotted wing drosophila, *Drosophila suzukii*, is an invasive insect pest that has spread into many fruit production regions of the world. Strategies to protect fruit from infestation by this insect are currently dominated by insecticide applications, so producers need information on relative efficacy and residual activity of insecticides to be able to select effective treatments. Semi-field bioassays in which highbush blueberry shoots with berries were treated then exposed to adult flies at different times after application revealed that fresh residues of organophosphate, pyrethroid, and spinosyn insecticides have strong initial activity on flies, with varying levels of residual protection against fruit infestation. An organic pyrethrum insecticide was not effective, whereas the neonicotinoid insecticide acetamiprid was found to have activity for up to five days. Rainfall after application greatly reduced the level of control achieved by some insecticides. Field-scale evaluation of conventional and organic spray programs initiated in response to capture of *D. suzukii* flies in monitoring traps indicated that both types of management provide significant fruit protection compared to untreated fields, with less larval detection in the conventionally-produced berries.

1. Introduction

The arrival and spread of spotted wing drosophila, *Drosophila suzukii* Matsumura, into major fruit production regions of the northern hemisphere (Hauser, 2011; Cini et al., 2012) has caused significant economic and sociological impacts (Goodhue et al., 2011; Walsh et al., 2011). In susceptible crops such as soft-skinned berries, millions of dollars of fruit have been put at risk of infestation by this pest (Bolda et al., 2010; Pfeiffer et al., 2012). In response, many growers have recently changed part of their pest management programs from the use of selective insecticides applied in response to pest monitoring and scouting to calendar spray programs dominated by use of broad-spectrum insecticides.

The zero tolerance of the fresh and processed berry markets for insect infestation of fruit, coupled with the high populations of this pest found in and around berry crop fields, have resulted in growers taking a very proactive approach to protecting their crops from *D. suzukii*. Given that fruit with thin skins and soft flesh become susceptible to egg laying by *D. suzukii* once they start to ripen (Lee et al., 2011; Burrack et al., 2013), detection of adult flies of this species in monitoring traps is being used as the trigger to initiate repeated applications of crop protectants to ripening or ripe fields until harvest is complete. An additional five to eight insecticide applications may be required to cover this period depending on the temperature-driven speed of ripening and the level of rainfall during the ripening period of the crop.

Evaluations of insecticides for control of *D. suzukii* have been initiated in most major regions of its distribution. These include laboratory bioassays that compared the mortality of flies treated and evaluated in Petri dishes, coupled with field evaluations where treated plots were sampled for infestation and compared for their control of adult flies. These methods provided important early guidance on the direction of research on control of *D. suzukii* (e.g. Bruck et al., 2011). However, to make effective management decisions about which insecticide to use, it is essential to understand the relative ability of the available options to prevent larval infestation by *D. suzukii* and to know how much residual control is provided before additional protection is required. These parameters are expected to be dependent on the conditions under which the trials are conducted, so it is important for these trials to be conducted in each of the main production regions or across a range of environmental conditions. Bruck et al. (2011) and Beers et al.

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Table 1
Insecticides and rates used in small plot trials for control of *Drosophila suzukii* in 2012 and in program comparisons at commercial blueberry farms in 2011 and 2012.

Chemical name	Trade name	Manufacturer	Rate (g AI ha^{-1})
Residual semi-field bioassays			
Acetamiprid	Assail® 30SG	United Phosphorous, Inc., King of Prussia, PA	111.4
Bifenthrin	Bifenture™ 10DF	United Phosphorous, Inc., King of Prussia, PA	56.0, 112.1
Carbaryl	Sevin® XLR	Bayer CropScience, Research Triangle Park, NC	1977.2
Malathion	Malathion 8F	Gowan Company LLC, Yuma, AZ	1113.8, 1782.2, 2227.7
Methomyl	Lannate® 90SP	DuPont de Nemours & Co., Wilmington, DE	1008.8
Phosmet	Imidan® 70WP	Gowan Company LLC, Yuma, AZ	1043.5
Pyrethrin	Pyganic® 1.4EC	McLaughlin Gormley King Co., Minneapolis, MN	62.8
Spinetoram	Delegate™ 25WG	Dow AgroSciences LLC, Indianapolis, IN	52.5, 78.8, 105.1
Spinosad	Entrust® SC	Dow AgroSciences LLC, Indianapolis, IN	252.0
Zeta-cypermethrin	MustangMax™ 0.8 EC	FMC Corporation, Philadelphia, PA	26.9
On-farm program comparisons			
Acetamiprid	Assail® 30SG	United Phosphorous, Inc., King of Prussia, PA	105.1
Azadirachtin	Aza-Direct®	Gowan Company LLC, Yuma, AZ	10.5
B.t. kurstaki	Dipel® DF	Valent U.S.A. Corporation, Walnut Creek, CA	302.6, 605.3, 907.9
Bifenthrin/zeta-cypermethrin	Hero™	FMC Corporation, Philadelphia, PA	42.0
Fenpropathrin	Danitol® 2.4 EC	Valent U.S.A. Corporation, Walnut Creek, CA	303.1
Imidacloprid	Provado® 1.6EC	Bayer CropScience, Research Triangle Park, NC	48.8
Methoxyfenozide	Intrepid 2F	Dow AgroSciences LLC, Indianapolis, IN	190.0
Phosmet	Imidan® 70WP	Gowan Company LLC, Yuma, AZ	1043.5
Pyrethrin	Pyganic® 1.4EC	McLaughlin Gormley King Co., Minneapolis, MN	31.4, 62.8
Pyrethrin	Pyganic® 5.0EC	McLaughlin Gormley King Co., Minneapolis, MN	28.0
Spinosad	Entrust® 80WP	Dow AgroSciences LLC, Indianapolis, IN	56.0, 70.1, 112.1
Zeta-cypermethrin	Mustang Max™ 0.8 EC	FMC Corporation, Philadelphia, PA	26.9

(2011) conducted laboratory and field trials in raspberries (*Rubus idaeus* L.), highbush blueberries (*Vaccinium corymbosum* L.), strawberries (*Fragaria* × *ananassa* Duchesne), sweet cherries (*Prunus avium* L.) and grapes (*Vitis* spp.) in Washington, Oregon, and California, but there is no current information from the humid climate of the major blueberry production region in the Great Lakes region of the United States, and insecticide performance can be affected by humidity (e.g. Barson, 1983). Within this region, current insect control programs in highbush blueberry already focus on prevention of fruit infestation by lepidopteran, dipeteran, and coleoptean pests such as the cranberry fruitworm (*Acrobasis vaccinii* Riley), blueberry maggot (*Rhagoletis mendax* Curran), and Japanese beetle (*Popillia japonica* Newman), respectively. It is important to understand which of the products registered for control of these pests might also provide control of *D. suzukii*, and for how long. Additionally, while malathion is a commonly-used insecticide for control of *R. mendax*, recent regulatory reductions of the malathion rate allowed in blueberry prompted interest in testing the former rate, the current rate, and an intermediate rate. In addition, there has been little work conducted on the effect that rainfall and the resulting wash-off of insecticides has on survival and reproduction of *D. suzukii*. Recent research by Hulbert et al. (2012) found that adult *P. japonica* beetles fed significantly more on leaves exposed to higher amounts of simulated rainfall, and a similar result would be expected for *D. suzukii*.

Blueberries are produced using both conventional and organic methods, and there is concern about the level of control that can be achieved against *D. suzukii*, especially within the restrictions for certified organic production (Grieshop et al., 2012). Currently, spinosyn and pyrethrum are two organic insecticides registered for use in blueberry and it will be important to determine the relative performance of these products when evaluated in small plots and when used at the full commercial field scale.

The objectives of this study were to compare mortality of *D. suzukii* flies and fruit infestation in semi-field bioassays that employed fruit with field-aged residues in laboratory bioassays. These assays were conducted with insecticides approved for application on blueberry, with and without exposure to rainfall. We also compared grower-applied insecticide programs at commercial highbush blueberry farms during two growing seasons to determine whether conventional or organic insecticide-based management programs provide significant control of *D. suzukii*.

2. Material and methods

2.1. General insecticide trial methods

Insecticide trials were conducted at a two-year old blueberry planting (*V. corymbosum*, cv. 'Aurora') at the Trevor Nichols Research Complex in Fennville, Michigan during July and August, 2012. Insecticides (Table 1) were applied to two-bush plots within the planting using a CO_2-powered backpack sprayer operating at 2.3 kPa (50 psi) in a volume of water equivalent to 1122 L per hectare and equipped with a single head boom and a TeeJet® 8003VS spray nozzle. Other bushes were left unsprayed as controls. At different numbers of days after treatment (DAT), shoots containing 10 leaves and 5 ripe berries were cut off the bushes and placed in water picks (10 cm long single anchor water pick, AquaPic brand, Syndicate Sales, Inc., Kokomo, IN) inside 0.95 L clear plastic containers (Gordon Food Service®, Wyoming, MI). The water picks were inserted through a hole in the bottom of the container such that the lip of the water pick was even with the bottom of the cup.

Ten adult *D. suzukii* (5 male, 5 female) that were between 2 and 5 days old were gently removed from a laboratory colony, anesthetized with CO_2, and added to the cups (4 replicates per treatment). To limit fly mortality, a 4 cm long piece of dental wicking moistened with distilled water was placed in the cup. A small plastic container cut from a soufflé cup lid (19 mm diameter, 3 mm deep, Gordon Food Service®) was filled with drosophila diet (cornmeal recipe, Drosophila Species Stock Center, San Diego, CA) and placed in each cup to provide food for the flies. To minimize moisture build up, lids had a 5 cm diameter hole cut in them and fine mesh (150 μm, The Cary Company, Addison, IL) affixed over the hole using hot glue. Cups with collected blueberry fruit and flies were placed in an environmental chamber at 25 °C, 75% RH, and a 16:8 L:D cycle. After 24 h the number of dead, moribund, or alive flies was recorded. Flies were left in the cups for seven days at which point the fruit was taken out and aged for an additional two

Table 2
Insecticides applied to blueberry fields managed using conventional or organic spray programs in 2011, including compounds and rates applied (g AI ha^{-1}).

Week of	Conventional	Organic		
	Sites 1, 2, and 3	Site 1	Site 2	Site 3
May 8	Methoxyfenozide 190.0[b]			
May 29	Methoxyfenozide 190.0[c]			
June 5	Phosmet 1043.5	*B.t. kurstaki* 907.1[d]	*B.t. kurstaki* 605.3	*B.t. kurstaki* 605.3
June 12	Phosmet 1043.5, imidacloprid 48.8[a]	*B.t. kurstaki* 907.1[d]	*B.t. kurstaki* 605.3[c] *B.t. kurstaki* 605.3[c]	*B.t. kurstaki* 605.3
June 19	Phosmet 1043.5, imidacloprid 48.8[a]			*B.t. kurstaki* 605.3
June 26	Acetamiprid 105.1	*B.t. kurstaki* 907.1[d]	*B.t. kurstaki* 302.6[c]	
July 3		Spinosad 112.1[d]		
July 10	Zeta-cypermethrin 26.9			
July 17	Fenpropathrin 303.1	Pyrethrin 62.8	Spinosad 56.0	Spinosad 112.1
July 24		Spinosad 112.1, pyrethrin 62.8[d]	Spinosad 112.1, azadirachtin 10.5	Spinosad 112.1
July 31				Spinosad 112.1
August 7			Spinosad 112.1	
August 14	Fenpropathrin 303.1		Spinosad 112.1, pyrethrin 31.4	
August 21		Spinosad 112.1		
Final Harvest	September 8	August 25	August 21	August 10

[a] Nu-Film-17® sticker-spreader added at 121.1 g AI ha^{-1}.
[b] Nu-Film-17® sticker-spreader added at 322.8 g AI ha^{-1}.
[c] Nu-Film-P® sticker-spreader added at 33.6 g AI ha^{-1}.
[d] Nu-Film-P® sticker-spreader added at 807.0 g AI ha^{-1}.

days before being assessed for the presence of drosophila larvae and pupae using a boil method for larval detection. To conduct the boil test, fruit were covered with water in a 473 ml plastic container (Gordon Food Service®) and heated in a 900 W microwave so that they boiled for 1 min. Fruit were then lightly mashed over a hardware cloth screen (0.64 cm hole size) over a dark colored tray, and rinsed with 500 ml of cold water poured over the crushed berries. The hardware cloth was then taken off the tray and the number of drosophila larvae on the tray was counted. An additional negative control sample was taken for each of the insecticide trials to determine if any drosophila larvae were in the berries at the time when trials were conducted, but no drosophila emerged from these controls during any of the experiments.

2.2. Semi-field comparison of insecticide performance 1

The first insecticide trial conducted was to compare some of the more commonly used insecticides in commercial blueberries, including malathion (1782.2 g AI ha^{-1}), methomyl (1008.8 g AI ha^{-1}), phosmet (1043.5 g AI ha^{-1}), pyrethrin (62.8 g AI ha^{-1}), spinetoram (78.8 g AI ha^{-1}), spinosad (252.0 g AI ha^{-1}), and zeta-cypermethrin (26.9 g AI ha^{-1}). Foliage and fruit were collected at 1, 3, 5, 7, and 10 DAT.

All statistical analyses were performed using Systat 13 (Systat Software, Inc., Chicago, IL). Adult percent mortality data were arcsine transformed before being subjected to analysis of variance (ANOVA) followed by Fisher's least significant difference test (LSD) for means separation. The data on the number of larvae and pupae per container did not fit the assumptions of normality and were analyzed using a Kruskal–Wallis test followed by a Conover–Inman test for post-hoc comparisons (Conover, 1999). Untransformed percentages are presented ± standard error, and an alpha value of 0.05 was used for all experiments.

2.3. Semi-field comparison of insecticides 2

The second set of insecticide trials tested three rates of one of the commonly used organophosphate insecticides used in commercial blueberry production, malathion (1113.8 g AI ha^{-1}, 1782.2 g AI ha^{-1}, and 2227.7 g AI ha^{-1}), compared to three additional insecticides (acetamiprid at 111.4 g AI ha^{-1}; bifenthrin at 112.1 g AI ha^{-1}; carbaryl at 1977.2 g AI ha^{-1}). Assessments were conducted at 3, 5, and 7 DAT. Statistical analyses performed on adult percent mortality and number of larvae and pupae per container were the same as in the first insecticide trial.

2.4. Semi-field assessment of the effects of rain on insecticide efficacy

The third set of insecticide trials was designed to test effectiveness of insecticides after exposure to a rain event in the field. Six chemicals were tested: acetamiprid (111.4 g AI ha^{-1}), malathion (1782.2 g AI ha^{-1}), methomyl (1008.8 g AI ha^{-1}), phosmet (1043.5 g AI ha^{-1}), spinetoram (78.8 g AI ha^{-1}), and zeta-cypermethrin (26.9 g AI ha^{-1}). Insecticides were applied to bushes prior to a natural rain event on 8 August 2012 where 2.06 cm of rain fell from 12 to 44 h after insecticide application. Half of the bushes were covered using a frame and a tarpaulin (white in color on top, brown bottom) to prevent residue wash off by rain (No Rain treatment), and half were left exposed during the rain event (Rain treatment). Larval assessments were made at 3, 5 and 7 DAT and adult mortality data were taken at 3 and 7 DAT.

Statistical tests on adult and larval data were performed in the same manner as the first two insecticide trials, using ANOVA or Kruskal–Wallis tests. Comparisons of the average number of larvae and pupae between Rain and No Rain treatments were conducted using either a *t*-test for the arcsine transformed percent mortality data or a Mann–Whitney *U* test for the data on the number of larvae and pupae. Due to unequal variances, data were log ($X + 1$) transformed before analysis.

2.5. On-farm evaluation of field performance of insecticides

Highbush blueberry fields sampled for this study were located in Allegan and Ottawa Counties in southwest Michigan. Fields were managed using one of three management practices: conventional, organic, or minimally managed (Tables 2 and 3). Conventional fields (three in 2011, four in 2012) received sprays early in the growing

Table 3
Insecticide spray programs applied to blueberry fields managed using conventional or organic spray programs in 2012, including compounds and rates applied (g AI ha^{-1}).

Week of	Conventional	Organic			
	Sites 1, 2, 3, and 4	Site 1	Site 2	Site 4	Site 5
April 22	Methoxyfenozide 190.0				
May 6				*B.t. kurstaki* 302.6	*B.t. kurstaki* 302.6
May 13				*B.t. kurstaki* 302.6	*B.t. kurstaki* 302.6
May 20	Phosmet 1043.5		*B.t. kurstaki* 605.3	*B.t. kurstaki* 302.6	*B.t. kurstaki* 302.6
May 27				*B.t. kurstaki* 302.6	*B.t. kurstaki* 302.6
June 3	Phosmet 1043.5, imidacloprid 48.8	*B.t. kurstaki* 907.9			
June 10				*B.t. kurstaki* 302.6	*B.t. kurstaki* 302.6
June 17	Bifenthrin/zeta-cypermethrin 84.1			Spinosad 70.1	
June 24				Pyrethrin 28.0 Spinosad 70.1	Spinosad 70.1
July 1			Spinosad 70.1[b]	Pyrethrin 28.0	
July 8	Phosmet 1043.5			Spinosad 70.1	Spinosad 70.1
July 15	Zeta-cypermethrin 26.9[a]		Spinosad 70.1[b]	Spinosad 70.1	Pyrethrin 28.0 Spinosad 70.1
July 22		Spinosad 112.1	Spinosad 70.1[b]		
July 29	Phosmet 1043.5		Spinosad 70.1[b]	Pyrethrin 28.0 Pyrethrin 28.0	Pyrethrin 28.0 Pyrethrin 28.0
August 5	Zeta-cypermethrin 26.9[a]	Spinosad 112.1	Spinosad 70.1[b]	Spinosad 70.1	Spinosad 70.1
August 12	Bifenthrin/zeta-cypermethrin 42.0[c]				
August 19				Spinosad 70.1, pyrethrin 28.0	Spinosad 70.1, pyrethrin 28.0
August 26				Pyrethrin 28.0	Pyrethrin 28.0
Final Harvest	August 8	August 9	August 9	September 3	September 3

[a] Nu-Film 17 sticker-spreader added at 322.8 g AI ha^{-1}.
[b] Nu-Film-P sticker-spreader added at 807.0 g AI ha^{-1}.
[c] Post-harvest insecticide application.

season for *Grapholita packardii*, *Acrobasis vaccinii*, and *Illinoia pepperi*, including methoxyfenozide and imidacloprid. Mid-season and late-season sprays targeting *P. japonica*, *R. mendax*, and *D. suzukii* included acetamiprid, imidacloprid, zeta-cypermethrin, fenpropathrin, and phosmet. Organic fields (three in 2011, four in 2012) were managed using organic insecticides, including *Bacillus thuringensis kurstaki* for *G. packardii* and *A. vaccinii*, pyrethrin and azadirachtin for *P. japonica*, and spinosad for *R. mendax* and *D. suzukii*. Minimally managed fields (three in 2011, three in 2012) received no insecticide inputs yet received regular upkeep (mowing, pruning, etc.). Overall, nine fields were used in 2011 and eleven were used in 2012, with eight of the nine fields used in 2011 used again in 2012.

Monitoring traps for *D. suzukii* were deployed at each of the blueberry fields in May and June of each year. Within each field, one trap was placed at the field border and another trap was placed near the field border trap, 3–6 m into an adjacent woodlot. Traps used were 0.95 L clear plastic containers (Gordon Food Service®, Wyoming, MI) with holes in the sides, and containing a small yellow sticky insert (7.6 cm by 8.9 cm, Great Lakes IPM, Inc., Vestaburg, MI) hung from the inside lid of the trap. The traps were baited with a mix of yeast (Red Star® active dry yeast, Lesaffre Yeast Corporation, Milwaukee, WI) plus white pure cane granulated sugar (Meijer® Inc., Grand Rapids, MI) at a ratio of 6.2 ml yeast, 24.6 ml sugar, and 150 ml tap water per trap. Baits were replaced and traps were checked for adult *D. suzukii* each week for the duration of this study.

Blueberries were collected weekly from six of the nine fields in 2011 (only one of the minimally managed sites) and from all 10 fields in 2012. Sampling began at first ripening (7 July 2011 and 17 June 2012), continuing until the middle of September (2011) and late August (2012). Fruit were collected at four locations along a wooded border of each field, sampling 473 ml (approximately 560 berries) at the beginning of the season, declining as harvest progressed down to final samples of 29.6 ml. Fruit were held for 15 days at 24 ± 0.2 °C until adult flies emerged and these were either aspirated out of containers (2011) or collected from a sticky card in each container (2012) weekly after fruit collection. Flies were placed in 70% ethanol for later identification as *D. suzukii* males, females, or other *Drosophila* species. To reduce the potential for contamination, only flies that emerged in the first 15 days after fruit collection were counted.

Additional blueberry samples were collected at the sampling locations at each of the fields in 2012 to test fruit for the presence of larvae at the time of collection. The amount of fruit collected was the same as that collected for adult rearing samples, and each sample was placed in a 3.8 L Ziplock bag with enough salt water to cover the berries. Salt water was made by mixing 236.6 ml (1 cup) of table salt (Cargill brand Top-Flo® granulated salt, Cargill Salt, Minneapolis, MN) into 3.79 L of tap water. Berries were lightly crushed (just enough to break the berry skin) and samples were left for a minimum of 1 h before placing the bag against a dark background and counting the total number of drosophila larvae floating in the liquid.

The average number of *D. suzukii* adults caught per trap per week and the number of *D. suzukii* adults reared per liter of fruit were analyzed for the three week period before the last insecticide spray prior to the final harvest at each site. Since fruit were not commercially harvested at the minimally managed sites, the week of the most common final spray for the other sites was used for the minimally managed sites (week of 21 August 2011 and 5 August 2012). Adult flies caught in traps in both years were analyzed using ANOVA while the number of adult SWD reared from fruit, or the number of Drosophila larvae detected in the salt samples, were analyzed using a *t*-test for 2011 data and ANOVA followed by Fisher's LSD test for post-hoc comparisons in 2012.

3. Results

3.1. Semi-field comparison of insecticide performance 1

In the first insecticide trial, all of the insecticides except pyrethrin caused adult mortality significantly higher than the untreated at 1 DAT, with greatest activity from malathion and methomyl (Table 4). At 3 DAT, adult mortality remained above 50% in the phosmet, methomyl, spinetoram, and spinosad treatments.

Table 4
Percent mortality of *Drosophila suzukii* adults after exposure for 24 h to foliage and fruit sprayed with each of seven insecticides, and the average number of *Drosophila suzukii* larvae and pupae in blueberries treated with insecticides. Larvae and pupae were extracted from berries (five per treatment replicate) using a boil test. Berries were collected from bushes at certain times after treatment and exposed to adult *Drosophila suzukii* (five males, five females) for seven days. Values in a group followed by the same letter are not significantly different ($P > 0.05$).

Treatment	g AI ha^{-1}	Days after treatment				
		1 DAT	3 DAT	5 DAT	7 DAT	10 DAT
Percent adult mortality						
Untreated		7.5 ± 4.8 d	7.5 ± 4.8 cd	13.3 ± 3.3 cd	16.7 ± 8.8 bc	5.0 ± 2.9 de
Malathion	1782.2	85.0 ± 8.5 ab	32.5 ± 7.5 bc	27.5 ± 7.5 bcd	3.3 ± 3.3 c	15.0 ± 8.7 de
Methomyl	1008.8	92.5 ± 4.8 a	52.5 ± 17.0 ab	60.0 ± 12.9 ab	46.7 ± 20.3 ab	20.0 ± 10.0 cd
Phosmet	1043.5	67.5 ± 4.8 bc	70.0 ± 16.0 b	67.5 ± 11.8 a	73.3 ± 6.7 a	72.5 ± 10.3 a
Pyrethrin	62.8	2.5 ± 2.5 d	5.0 ± 5.0 d	5.0 ± 5.0 d	10.0 ± 10.0 bc	2.5 ± 2.5 e
Spinetoram	78.8	45.0 ± 11.1 c	52.5 ± 2.5 b	45.0 ± 18.5 abc	36.7 ± 21.9 ab	12.5 ± 6.3 de
Spinosad	252.0	40.0 ± 4.1 c	77.5 ± 6.3 a	70.0 ± 10.8 a	30.0 ± 5.8 abc	40.0 ± 10.8 bc
Zeta-cypermethrin	26.9	42.5 ± 7.1 bc	45 ± 6.5 b	42.5 ± 12.5 abc	40.0 ± 10.0 ab	57.5 ± 7.5 ab
F		20.7	7.7	4.4	3.5	9.5
df		7, 24	7, 24	7, 23	7, 16	7, 24
P		<0.0001	<0.0001	0.003	0.017	<0.0001
Average no. of larvae and pupae						
Untreated		18.5 ± 3.4 a	23.0 ± 6.6 a	15.8 ± 6.5 a	8.3 ± 3.3 a	9.5 ± 2.5 a
Malathion	1782.2	0 ± 0 b	7.5 ± 5.7 ab	2.3 ± 1.9 bc	14.0 ± 2.6 a	8.5 ± 2.7 a
Methomyl	1008.8	0 ± 0 b	0.3 ± 0.3 bc	0 ± 0 c	0 ± 0 a	0 ± 0 b
Phosmet	1043.5	0.5 ± 0.3 b	0 ± 0 c	0.5 ± 0.3 bc	1.0 ± 1.0 a	0 ± 0 b
Pyrethrin	62.8	13.5 ± 2.3 a	24.8 ± 6.2 a	18 ± 1.9 a	10.3 ± 4.5 a	4.3 ± 0.6 a
Spinetoram	78.8	0.8 ± 0.5 b	1.0 ± 0.6 bc	4.0 ± 3.0 b	3.7 ± 2.8 a	0.3 ± 0.3 b
Spinosad	252.0	0.3 ± 0.3 b	0.5 ± 0.3 bc	0.3 ± 0.3 c	4.0 ± 2.1 a	0.8 ± 0.8 b
Zeta-cypermethrin	26.9	0.8 ± 0.5 b	0.8 ± 0.8 bc	0.3 ± 0.3 bc	1.3 ± 0.7 a	0.5 ± 0.5 b
H		23.2	20.6	21.1	13.4	25.1
df		7, 24	7, 24	7, 23	7, 16	7, 24
P		0.002	0.004	0.004	0.062	0.001

Malathion and zeta-cypermethrin caused some mortality, while pyrethrin was not significantly different from the untreated throughout the series of bioassays. Adult mortality at 5 DAT was similar to the 3 DAT trials, except that methomyl, phosmet, and spinosad retained the highest levels of activity. By 7 DAT, only the phosmet treatment was significantly different from the untreated control, and this was the only treatment providing high levels of mortality at 10 DAT.

Table 5
Percent mortality of *Drosophila suzukii* adults after exposure for 24 h to foliage and fruit sprayed with three rates of the insecticide malathion and three other insecticides and the average number of *Drosophila suzukii* larvae and pupae in the blueberries. Larvae and pupae were extracted from berries (five per treatment replicate) using a boil test. Berries were collected from bushes at certain times after treatment and exposed to adult *Drosophila suzukii* (five males, five females) for seven days. Values in a group followed by the same letter are not significantly different ($P > 0.05$).

Treatment	g AI ha^{-1}	Days after treatment		
		3	5	7
Percent adult mortality				
Untreated		7.5 ± 4.8 c	0 ± 0 c	0 ± 0 c
Malathion	1113.8	75.0 ± 7.5 a	52.5 ± 25.0 ab	7.5 ± 4.8 bc
Malathion	178.2	80.0 ± 6.3 a	55.0 ± 11.9 ab	30.0 ± 17.8 ab
Malathion	2227.7	87.5 ± 2.5 a	77.5 ± 10.3 a	35.0 ± 17.1 ab
Acetamiprid	111.4	20.0 ± 0 b	27.5 ± 11.1 bc	10.0 ± 7.1 bc
Bifenthrin	112.1	70.0 ± 8.7 a	50.0 ± 12.9 ab	55.0 ± 2.9 a
Carbaryl	1977.2	2.5 ± 2.5 c	15.0 ± 6.5 bc	7.5 ± 4.8 bc
F (6,21)		34.4	4.3	3.7
P		<0.0001	0.005	0.012
Average no. of larvae and pupae				
Untreated		5.5 ± 2.1 a	13.8 ± 2.9 a	12.0 ± 5.5 a
Malathion	1113.8	1.0 ± 1.0 bc	2.3 ± 1.3 b	9.3 ± 5.0 a
Malathion	178.2	0 ± 0 c	0.3 ± 0.3 b	8.8 ± 4.0 a
Malathion	2227.7	0 ± 0 c	0 ± 0 b	5.0 ± 3.2 a
Acetamiprid	111.4	0 ± 0 c	0.8 ± 0.8 b	1.3 ± 1.5 a
Bifenthrin	112.1	0 ± 0 c	0.5 ± 0.5 b	5.3 ± 0.3 a
Carbaryl	1977.2	3.3 ± 2.4 ab	0.5 ± 0.5 b	0.3 ± 2.5 a
H (6,21)		17.2	15.2	10.7
P		<0.0001	0.019	0.1

With regards to the average number of larvae and pupae per treatment replicate, at 1 DAT all of the insecticides had significantly fewer larvae and pupae than the untreated except for the pyrethrin treatment (Table 4). At 3 DAT, larvae and pupae in fruit in the insecticide treatments remained significantly lower than the untreated, except for the malathion and pyrethrin treatments, which were not significantly different from the untreated. From 5 DAT onwards, the number of larvae and pupae in the pyrethrin treatment was not significantly different from the untreated controls. At this time, the malathion and spinetoram treatments had some larvae in the fruit, but at levels that were lower than the untreated. While several of the treatments had larvae in the fruit at 7 DAT, the results were not significant ($P = 0.062$). All insecticide treatments except malathion and pyrethrin continued to have a significantly lower number of larvae in fruit.

3.2. Semi-field comparison of insecticide performance 2

In the second insecticide trial, adult mortality at 3 DAT was significantly higher than untreated in all insecticide treatments except the carbaryl treatment (Table 5). While mortality was lower in most treatments at 5 DAT, all the insecticide treatments except acetamiprid and carbaryl had significantly higher mortality than the untreated. At 7 DAT, only the bifenthrin treatment had adult mortality above 50% and malathion 1113.8 g, acetamiprid, and carbaryl were not significantly different from the untreated. All the insecticide treatments had significantly fewer larvae and pupae than the untreated at 3 DAT and 5 DAT, except the carbaryl treatment at 3 DAT. At 7 DAT, there were more larvae in the malathion treatments than the other chemical treatments, although none of the treatments was significantly better than the untreated control.

3.3. Semi-field assessment of the effects of rain on insecticide efficacy

In the third set of trials, while there were trends in adult mortality among treatments, none of those trends were significant except for

Table 6
Percent mortality of *Drosophila suzukii* adults after exposure for 24 h to foliage and fruit sprayed with six insecticides and either exposed to rain or not and the average number of *Drosophila suzukii* larvae and pupae in the blueberries. Larvae and pupae were extracted from berries (five per treatment replicate) using a boil test. Berries were collected from bushes at certain times after treatment and exposed to adult *Drosophila suzukii* (five males, five females) for seven days. Values in a group followed by the same letter are not significantly different ($P > 0.05$).

Treatment	g AI ha^{-1}	Rain			No rain		
		Days after treatment			Days after treatment		
		3	5	7	3	5	7
Percent adult mortality							
Untreated		6.7 ± 3.3 a		5.0 ± 5.0 c			
Acetamiprid	111.4	6.7 ± 3.3 a		2.5 ± 2.5 c	32.5 ± 10.3 a		55.0 ± 9.6 a
Malathion	1782.2	20.0 ± 11.5 a		2.5 ± 2.5 c	80.0 ± 7.1 a		82.5 ± 22.5 a
Methomyl	1008.8	40.0 ± 15.3 a		10.0 ± 4.1 bc	72.5 ± 14.9 a		82.5±10.3 a
Phosmet	1043.5	56.7 ± 20.3 a		30.0 ± 7.1 b	65.0 ± 15.5 a		65.0±13.2 a
Spinetoram	78.8	16.7 ± 12.0 a		10.0 ± 5.8 c	70.0 ± 4.1 a		67.5 ± 4.8 a
Zeta-cypermethrin	26.9	23.3 ± 14.5 a		62.5 ± 14.7 a	65.0 ± 9.6 a		47.5 ± 2.5 a
F		1.7		7.8	2.2		0.9
df		6, 14		6, 21	5, 18		5, 18
P		0.19		<0.0001	0.095		0.49
Average no. of larvae and pupae							
Untreated		6.3 ± 2.8 a	10.0 ± 2.3 a	10.5 ± 3.1 a			
Acetamiprid	111.4	14.8 ± 3.5 a	11.0 ± 4.0 a	10.5 ± 2.4 a	0.5 ± 0.3 a	0.5 ± 0.5 a	0.3 ± 0.3 a
Malathion	1782.2	21.8 ± 9.3 a	19.0 ± 7.2 a	8.8 ± 2.6 a	0 ± 0 a	0.3 ± 0.3 a	7.0 ± 0 a
Methomyl	1008.8	0 ± 0 a	8.0 ± 2.5 a	0.3 ± 0.3 b	0 ± 0 a	0 ± 0 a	0 ± 0 a
Phosmet	1043.5	0.3 ± 0.3 a	5.0 ± 2.0 a	1.5 ± 1.0 b	0 ± 0 a	0 ± 0 a	0 ± 0 a
Spinetoram	78.8	4.5 ± 2.2 a	12.3 ± 1.9 a	9.3 ± 3.5 a	0.3 ± 0.3 a	0 ± 0 a	0.3 ± 0.3 a
Zeta-cypermethrin	26.9	0.8 ± 0.8 a	2.0 ± 0.5 a	0.5 ± 0.5 b	0 ± 0 a	0 ± 0 a	0 ± 0 a
H		12.4	11.4	19.4	7.7	4.2	3.3
df		6, 14	6, 14	6, 21	5, 18	5, 18	5, 18
P		0.054	0.078	0.004	0.18	0.52	0.66

higher mortality in the zeta-cypermethrin and phosmet treatments in the Rain 7 DAT trials (Table 6). Likewise, there were no significant differences in the number of larvae and pupae among treatments except the Rain 7 DAT trials, where the methomyl, phosmet, and zeta-cypermethrin treatments had significantly fewer larvae and pupae than the other treatments. A direct comparison of Rain versus No Rain treatments for each chemical indicate significantly higher adult mortality at 3 DAT in No Rain acetamiprid ($t = 3.2$, df = 1, 4, $P = 0.034$), malathion ($t = 3.2$, df = 1, 4, $P = 0.034$), and spinetoram ($t = 3.4$, df = 1, 4, $P = 0.026$) treatments than in the Rain treatment equivalents. At 7 DAT there was significantly higher adult mortality in the No Rain acetamiprid ($t = 5.9$, df = 1, 6, $P = 0.001$), methomyl ($t = 4.7$, df = 1, 6, $P = 0.003$), and spinetoram ($t = 5.1$, df = 1, 6, $P = 0.002$). There were no cases where adult mortality was higher in one of the Rain treatments on either 3 or 7 DAT. A direct comparison of the average number of larvae and pupae at 3 DAT show a significantly higher number of larvae and pupae in Rain acetamiprid ($U = 0$, df = 1, 4, $P = 0.046$) and spinetoram ($U = 0$, df = 1, 4, $P = 0.046$) treatments than in the No Rain treatment equivalents. The direct comparison of Rain and No Rain treatments at 5 DAT shows a significantly higher number of larvae and pupae in all of the Rain insecticide treatments compared to their No Rain equivalents ($U = 0$, df = 1, 4, $P < 0.03$), while at 7 DAT the acetamiprid ($U = 0$, df = 1, 6, $P = 0.018$) and spinetoram treatments ($U = 0$, df = 1, 6, $P = 0.018$) were the only insecticides which had significantly more larvae and pupae in the Rain treatment.

3.4. On-farm evaluation of field performance of insecticides

Captures of *D. suzukii* flies were much higher in 2012 than in 2011. However, in both years there were no significant differences among the three management practices in the average total *D. suzukii* trap captures in the three weeks preceding the final insecticide application prior to harvest (2011: $F = 3.7$, df = 2, 6, $P = 0.09$; Conventional: 0.2 ± 0.2; Organic: 0.4 ± 0.2; Minimally Managed: 9.6 ± 5.2; and 2012: $F = 0.7$, df = 2, 8, $P = 0.51$; Conventional: 58.4 ± 16.2; Organic: 90.5 ± 30.9; Minimally Managed: 104.7 ± 34.7).

When the number of *D. suzukii* adults per liter reared out of fruit collected during the three weeks prior to the final insecticide application before harvest were analyzed, there were no significant differences between the organic and conventional programs in 2011 ($t = -0.96$, df = 1, 4, $P = 0.39$; Conventional: 0.30 ± 1.15; Organic: 1.49 ± 1.15). During 2012, significantly fewer flies were reared out of fruit collected from conventionally-managed fields (5.2 ± 3.8) than the minimally-managed fields (103.7 ± 39.3), with the organic fields being intermediate (34.7 ± 23.6) ($F = 5.7$, df = 2, 7, $P = 0.035$). When fruit collected during this harvest period were sampled for SWD infestation, the number of larvae detected was significantly lower ($F = 9.2$, df = 2, 7, $P = 0.011$) in both the conventional (1.4 ± 0.8) and organic (1.1 ± 0.7) fields compared to the minimally managed fields (16.9 ± 7.8).

4. Discussion

This study shows that the currently available insecticides for control of *D. suzukii* provide protection against infestation by this pest, in both conventional and organic blueberry production. We used a semi-field residual bioassay with somewhat different methods than the studies reported by Bruck et al. (2011) to measure both adult control and larval infestation levels with several insecticides. These laboratory bioassays on field-aged residues supported the earlier reports that pyrethroid, organophosphate, and spinosyn insecticides provide from 5 to 14 days of residual control against *D. suzukii*, as measured by percent adult mortality. Beers et al. (2011) conducted several laboratory bioassays in sweet cherries by testing field-aged residues on foliage and fruit, including one trial that examined eggs laid and adult emergence after exposure of flies to foliage and fruit 24 h after treatment. The resulting adult mortality was high (>90%) for many of the insecticides tested, including malathion, spinetoram, and spinosad. Even with this high adult mortality, flies were able to lay eggs in fruit which were then able to develop to adults, albeit at lower levels than the controls. In our current study, high adult mortality was also observed for many of the compounds tested, especially at 1 DAT, but most treatments exhibited lower residual control from that point onwards, even in the contained setup of this bioassay method.

The corresponding larval data show that for some insecticides, such as malathion, the reduction in adult mortality over the period of these assays corresponded to an increase in the number of *D. suzukii* larvae in the berries. Other compounds, such as methomyl and acetamiprid, continued to cause high larval control in the fruit even with corresponding low adult mortality. These differences between effects on adult mortality and on larval infestation of the fruit highlight the difficulties in assessing how well insecticides are able to protect fruit from *D. suzukii* in the field, unless larval infestation measurements are taken. Further research is needed on insecticide performance against the main life stages to elucidate whether low larval infestation (despite high adult survival) is due to lack of egg laying, eggs not hatching, or young larvae dying in the fruit as suggested by some recent laboratory studies (J. Wise, unpublished data). It will also be essential to determine how these results from controlled semi-field assays in which bushes were treated with a high degree of coverage translate into real world settings where coverage may not be as complete and surviving flies can choose where they fly and lay eggs.

The effectiveness of malathion at the two lowest rates dropped quickly over time, such that after 3 days, low levels of larvae were present in the fruit. Malathion is sensitive to breakdown from exposure to ultraviolet light (Awad et al., 1967) which likely contributed to this decline in effectiveness. However, increasing the rate of malathion to 2227.7 g AI ha^{-1} extended the effectiveness to at least 5 DAT, indicating that increasing the rate can help mitigate the effects of environmental degradation. This higher rate is now permitted in blueberry production in some regions to control this pest through special registration by the US Environmental Protection Agency.

Efficacy of most treatments was reduced greatly after exposure to just over 2 cm of rain, such that by one week after treatment adult mortality was not significantly different from the untreated controls for most insecticides that had been exposed to rain. Likewise, larval infestation was significantly higher in many of the treatments, although there were some compounds such as methomyl, phosmet, and zeta-cypermethrin that were able to provide some larval control even after exposure to rain. An additional consideration besides the amount of rain that falls is the total time of a rain event. The rain that fell in this experiment did so over a 32 h period. During that time there were several extended periods of light rain, a few periods of heavier rain, and a few periods in between with no rain at all. In a farm setting this kind of rain event could provide opportunity for *D. suzukii* adults to lay eggs on unprotected fruit before a post-rain re-application, although it is not known how oviposition activity is affected by rainfall. The effectiveness of these insecticides could possibly be enhanced by the addition of adjuvants and/or spreader-stickers to reduce loss due to rain, if berry protection is needed but rain is forecast.

The higher variability in the experiment after rainfall reflects the effects of rain redistributing some insecticide residues on the plants, resulting in leaves and fruit on different parts of the bush having higher or lower concentrations of the insecticide. This has implications where bushes are larger, with more dense foliage, and where spray deposition can vary considerably depending the sprayer type, spray configuration, and amount of water used to apply insecticides (Hanson et al., 2000). Maintaining coverage of fruit clusters will be essential for effective protection against *D. suzukii*.

Growers managing the blueberry fields used in this study experienced a high degree of variability in the level of *D. suzukii* activity from one year to the next. Timing was later and activity of *D. suzukii* was lower in 2011, based on capture of adults in traps and adults reared out of fruit, while pressure in the following year was earlier and much higher. Winter temperature conditions are expected to limit *D. suzukii* populations (Dalton et al., 2011), and the observed annual variation may have been a result of the unusually mild 2011–2012 winter characterized by limited periods of very cold temperatures. For example, the winter before this study (2010–2011) the minimum winter air temperature recorded at the TNRC was −24.7 °C compared to −15 °C in the 2011–2012 winter. Additionally, at this site the two winters had twelve vs. four days of −10 °C or less, respectively. The observed changes in fly populations from year to year present difficulties in preparing management plans for this pest, especially when efficient and cost-effective IPM tools are lacking, but winter conditions may ultimately be used to provide a risk ranking for the coming season's level of pest pressure from *D. suzukii*. In higher pressure years where *D. suzukii* populations increase earlier in the season, effective control will require more sprays and tighter spray intervals than years where population increases are delayed. In addition, the amount and timing of rainfall will impact the effectiveness of insecticides and the need for re-application to keep fruit protected. The development of reliable trapping (Lee et al., 2013) and larval detection techniques will aid growers, scouts, and extension agents in determining the timing and magnitude of the pest population, and the performance of control programs.

Our results from the fruit sampling at commercial fields indicate that the method of holding berries to allow flies to emerge provides a more sensitive method than the salt solution for evaluation of fruit infestation by Drosophila, although the incubation period means that it takes longer to get the results. Our detection of statistical differences in the number of adult flies emerging from conventional and organic programs, but not from the direct fruit sampling, coupled with the higher number of flies in the reared samples further supports our recommendation that the rearing method be used to provide an accurate indication of the level of fruit infestation. Despite the better accuracy of the rearing method, and the ability to confirm the insect's identity, the salt solution may provide a more accurate indication of the level of infestation that is likely to be detected by the casual observer since eggs and young larvae are small and challenging to see.

Some *D. suzukii* were detected in the fruit in commercial fields prior to the final harvest, but none of the fields that were harvested experienced any fruit rejections or downgrades. Since the presence of *D. suzukii* in fruit in this study was measured by rearing adult flies out of the fruit, it is possible that the flies present in the fruit were at a life stage (eggs or young larvae) not detectable through standard sampling methods. Even with the spray programs in place at the fields sampled for this study, the presence of some larvae in the fruit near harvest underscores the need for growers to be vigilant until final harvest. It also highlights the unsustainability of current management practices and the necessity of non-chemical control measures for this pest, especially for organic growers where the limited insecticide options make for a greater chance of resistance developing over the years.

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