Research Article

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Baseline susceptibility of spotted wing Drosophila (*Drosophila suzukii*) to four key insecticide classes

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Abstract

BACKGROUND: The invasive drosophilid pest, *Drosophila suzukii* Matsumura, is affecting berry production in most fruit-producing regions of the world. Chemical control is the dominant management approach, creating concern for insecticide resistance in this pest. We compared the insecticide susceptibility of *D. suzukii* populations collected from conventional, organic or insecticide-free blueberry sites.

RESULTS: The sensitivity of *D. suzukii* to malathion and spinetoram declined slightly across the 3 years of monitoring, whereas it was more consistent for methomyl and zeta-cypermethrin. The sensitivity of *D. suzukii* to all four insecticides (LC_{50} and LC_{90} values) did not differ significantly among the blueberry fields using different management practices.

CONCLUSIONS: The baseline sensitivity of *D. suzukii* has been characterized, allowing future comparisons if field failures of chemical control are reported. The concentration achieving high control indicates that effective levels of control can still be achieved with field rates of these four insecticides. However, declining susceptibility of some populations of *D. suzukii* to some key insecticides highlights the need for resistance monitoring.

Keywords: methomyl; malathion; zeta-cypermethrin; spinetoram; monitoring

1 INTRODUCTION

The spotted wing Drosophila, *Drosophila suzukii* Matsumura, has spread quickly since being discovered in California in 2008[1] and is now found globally in many regions of fruit production.[2] Achieving control of this pest has been challenging in a number of cropping systems including strawberries, cherries, caneberries and blueberries.[3–6] Integrated pest management (IPM) practices that previously relied on targeted insecticide sprays applied in response to pest monitoring have been replaced with prophylactic applications initiated when this fly is present and fruit begin to ripen.[2] This has resulted in a major increase in the number of insecticide sprays applied in these crops.[5,7]

The combination of higher insecticide use and the short generation time of *D. suzukii* (~ 13 days) has led to concerns regarding the development of insecticide resistance in this species.[2,8] Previous research on the closely related *D. melanogaster* has discovered multiple pathways for insecticide resistance development and detected resistance in field-collected populations,[9] as well as in laboratory-selected populations.[10] In this same species, resistance was detected to multiple chemical classes including pyrethroids,[11] organophosphates and carbamates,[12] and spinosyns.[13] To date, limited research has been published investigating the potential for resistance development in *D. suzukii* or surveying populations of *D. suzukii* to determine baseline sensitivity. These approaches will be important for future testing of whether resistance is developing in field populations. Smirle *et al.*[14] tested several insecticides and determined LC_{50} and LC_{90} values for male and female *D. suzukii* collected from British Columbia, Canada. In addition, they attempted to accelerate resistance to malathion in the laboratory through selection pressure, but no resistance was evident after 30 generations. Hamby *et al.*[15] collected *D. suzukii* in California and reported differential toxicity of malathion depending on the time of day, and found that this was correlated with the level of detoxification gene activity. Neither of these studies reported evidence of resistance to insecticides in the tested populations.

Commercial blueberry production in many regions involves regular insecticide applications from the beginning of fruit ripening in late June or early July through to final harvest in mid to late September.[5,7,16,17] Insecticides used to control *D. suzukii* are dominated by broad-spectrum insecticides that provide the necessary level of control and are also cost-effective. This includes pyrethroids such as zeta-cypermethrin and bifenthrin, and the organophosphates malathion and phosmet. Additional insecticide classes including carbamates such as methomyl, and spinosyns such as spinetoram (or in organic production, spinosad) may also be represented depending on other considerations such as certification, target export market, and pre-harvest interval restrictions. Growers will typically rotate insecticide classes during the season to delay the development of resistance, but there is

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widespread concern regarding the potential for resistance development in this species due to the short generation time, high fecundity and repeated exposure to insecticides.[2]

Monitoring field populations of *D. suzukii* for their susceptibility to the most used insecticide classes is crucial for early detection of resistance development.[18] Detecting these early warning signs can help in counteracting and delaying widespread resistance. Intense use of organophosphate insecticides in apple orchards in New Zealand in the mid-1900s led to concerns of resistance development in several pest species, including *Epiphyas postvittana* (light brown apple moth) and *Planotortrix octo* (greenheaded leafroller).[19,20] Faced with the prospect of control failure, as well as public concern regarding insecticide residues on fruit, growers instituted resistance monitoring and adopted a more diverse suite of control techniques including the use of reduced-risk insecticides, biological control and mating disruption.[21] Similar approaches have been successful in Arizona to reduce dependence on insecticides for whitefly management.[22–24] Although a broad range of approaches for sustainable management of *D. suzukii* are being explored, it is important that sensitivity to effective insecticides is maintained and that any loss of this sensitivity is detected before it leads to field failures.

We conducted baseline susceptibility monitoring of *D. suzukii* populations in highbush blueberry fields in Michigan through three field seasons that constituted the third, fourth, and fifth year of its arrival in this region. Adult *D. suzukii* collected from farms with different management histories were tested for susceptibility to insecticides from the four main insecticide classes used for its control, including malathion (organophosphate), methomyl (carbamate), spinetoram (spinosyn) and zeta-cypermethrin (pyrethroid). We addressed the following questions: (1) at what concentrations do these insecticides kill 50% and 90% of *D. suzukii* adults; (2) does sensitivity of *D. suzukii* to insecticides vary between farms receiving high and low insecticide exposure; and (3) has the sensitivity of *D. suzukii* to insecticides changed over time?

2 MATERIALS AND METHODS

2.1 Colony collection and preparation.

Colonies of *D. suzukii* were established in 2013, 2014 and 2015 by collecting infested fruit from highbush blueberry, *Vaccinium corymbosum* L., fields in Allegan, Ottawa and Van Buren counties in southwest Michigan. Ripe fruit samples were collected in August and September of each year from six fields in 2013, 18 fields in 2014, and 19 fields in 2015. These blueberry fields were located across a broad geographic region with 105 km between the farthest fields; the closest fields were 1.5 km apart. Collection fields were classified into one of five categories based on the level of insecticide exposure expected from the grower's typical insecticide use. Conventional fields (4 in 2013, 11 in 2014, and 12 in 2015) were managed using conventional insecticides and typically received between 6 and 10 *D. suzukii*-targeted insecticide applications each season. Malathion, methomyl, phosmet and zeta-cypermethrin were among the most commonly applied insecticides for *D. suzukii* control at these sites. Fields designated in the light conventional category (none in 2013, two in 2014, and two in 2015) were also managed using conventional insecticides but received far fewer insecticides (two to three sprays per season) and included reduced-risk chemistries such as spinetoram. Organic fields (two in 2015) were managed using organic insecticides only (spinosad, pyrethrins). Minimally managed fields (one in 2013, one in 2014, none in 2015) received only isolated insecticide sprays, usually in small sections of the field and were not managed for commercial blueberry production. Unsprayed fields (one in 2013, three in 2014, and three in 2015) received no insecticide sprays during the year and were not managed for commercial production.

Collections of fruit took place as soon as infested berries became plentiful, within 1–2 weeks of the final harvest of the season at fields receiving insecticide sprays or at peak ripe fruit timing at sites that received no or few sprays. One of the unsprayed fields where collections were made each year (Site 1) was designated as the standard susceptible site. This field is located in Allegan County, Michigan and is isolated from areas of commercial blueberry production in counties to the south and north of the field location. The field has received no insecticide sprays for at least 25 years and is located at least 4 km from the nearest commercial fruit field.

The collected fruit was placed in 436 ml clear plastic deli cups (Gordon Food Service®, Wyoming, MI, USA) and adult flies were aspirated out of containers upon emergence. Ten female isolines were established for each site from flies emerging from collected fruit. Fly colonies were reared on a cornmeal diet (Drosophila Stock Center, San Diego, CA, USA) and after one to two generations, 10 isolines from each site were subsequently blended to create one representative colony for each site.

2.2 Insecticides

The formulated insecticides used in this study were malathion (Malathion 8F, Gowan Company LLC, Yuma, AZ, USA), methomyl (Lannate® 90SP, DuPont de Nemours & Company, Wilmington, DE, USA), spinetoram (Delegate™ 25WG, Dow AgroSciences LLC, Indianapolis, IN, USA) and zeta-cypermethrin (Mustang® Maxx 0.8EC, FMC Corporation, Philadelphia, PA, USA). Malathion, methomyl and spinetoram were tested on *D. suzukii* colonies established from collections in each of 3 years (2013, 2014 and 2015). Zeta-cypermethrin was also tested in all 3 years, but the manufacturer's change from the Mustang® Max to the Mustang® Maxx formulation between 2013 and 2014 made it impossible to compare across all years. As a result, only the 2014 and 2015 data from tests of the new formulation are presented here. All insecticides used in experiments were never more than a year old and new product was used in each year of the study.

2.3 Bioassays

All insecticide bioassays were conducted on adult female *D. suzukii* using a Potter spray tower (Burkard Scientific, Uxbridge, UK). All adult flies used in experiments were 2–5 days post eclosion at the time of experiments and were anesthetized using a CO_2 gun inserted into the rearing tubes, and then sorted on a CO_2 fly pad (Genesee Scientific, San Diego, CA, USA) immediately prior to being treated in the Potter spray tower. To reduce any negative effects of CO_2, flies were never left on the fly pad for more than 5 min. Preliminary fly pad mortality experiments with adult females show no adult mortality up to 20 min on the fly pad (Van Timmeren S, unpublished). Ten flies were transferred from the fly pad to the center of a 100 × 15 mm Petri dish using a superconductive fly-pushing brush (Genesee Scientific) and then sprayed with 2 ml of solution at 103.4 kPa using the Potter spray tower. Seven to eight concentrations of each insecticide plus a distilled water control were tested against flies from two to three colonies in each application session. Ten (2013) or four (2014, 2015) replicates of each colony were sprayed and insecticides were tested sequentially for each colony. Generations of flies tested ranged from F3 to F11, with an average of F8.4 ± 0.3 across all years.

The range of concentrations tested was different for each insecticide, because concentrations were selected to achieve optimal representation along a mortality curve from 0% to 100% mortality (malathion, 0–150 parts per million [ppm]; methomyl, 0–400 ppm; spinetoram, 0–75 ppm; zeta-cypermethrin, 0–15 ppm). Immediately after being treated, each group of flies was transferred to a clean 100 × 15 mm Petri dish that contained a portion of cornmeal diet and was placed in an environmental chamber set at 20 °C, 70% relative humidity (RH), and on a 16: 8 h light/dark cycle. To ensure low control mortality, diet portions were placed in the Petri dish by pipetting 5 ml of liquid diet (reheated from frozen) into silicone ice cube trays (Arctic Chill, Newberry, SC, USA), allowing the diet to cool and solidify, and slicing each diet cube into thirds such that a 2.5 × 2.5 × 0.7 cm cube was added to each Petri dish. Fly health was assessed after 24 h at which point flies were classified as alive, moribund or dead. Alive flies were those that were moving normally and appeared healthy, while moribund flies were ones that were clearly showing signs of toxicity such as twitching legs or slow uneven movements. Both moribund and dead individuals were included in subsequent probit analysis.

2.4 Statistical analysis

Mortality data for *D. suzukii* adults were corrected using Abbott's formula[25] relative to the untreated groups of flies. Probit analysis on these data was conducted using SAS version 9.4[26] to determine the LC_{50} and LC_{90} values and the 95% fiducial limits. The slopes of the probit lines and standard errors were also determined for each insecticide for each tested colony. Origin® (OriginLab, Northampton, MA, USA) was used to plot the mortality data for the four insecticides when tested against the susceptible population collected from the untreated site (Site 1). Resistance ratios (RR_{50} and RR_{90}) were calculated for the LC_{50} and LC_{90} levels as a ratio of the mortality of each collection relative to the untreated standard site (Site 1). Comparisons across the 3 years were made using analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test for post-hoc comparisons. Data were tested for normality using a Shapiro–Wilk test for normality and tested for homogeneity of variances using a Levene's test. Non-normal data were log $(X + 1)$ transformed to achieve normality and heteroscedastic data were $1/X$ transformed to achieve homogeneous variances. For the comparison between 2 years for zeta-cypermethrin, LC_{50} and LC_{90} data were log $(X + 1)$ transformed and analyzed using a two-sample *t*-test. A comparison of spray intensity was conducted by categorizing sites into one of two groups, high or no/low spray. Sites classified as conventional were considered high spray and all other sites (light conventional, organic, minimally managed and unsprayed) were considered no/low spray. The LC_{50} and LC_{90} data in the two categories were log $(X + 1)$ transformed where appropriate and compared between the two groups using two-sample *t*-tests.

3 RESULTS

The toxicity of insecticides to the *D. suzukii* population collected from the untreated blueberry site was highest for zeta-cypermethrin, followed by spinetoram, malathion, and then methomyl (Fig. 1). We also found that control mortality was consistently low across all experiments and years (2013, 0.1 ± 0.1%; 2014, 0.5 ± 0.2%; 2015, 1.0 ± 0.3%).

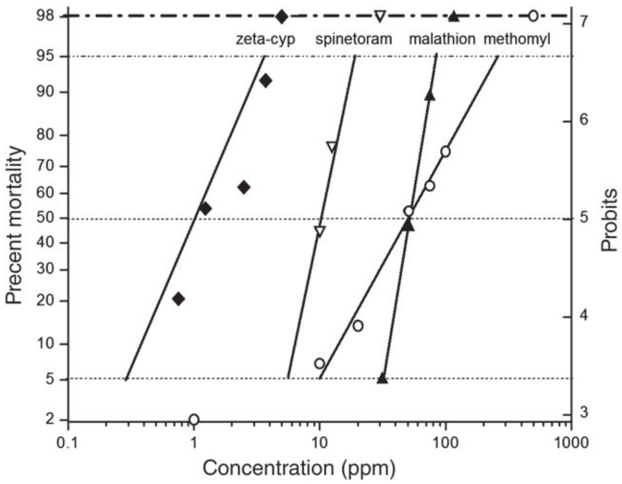

Figure 1. Insecticide log dose–mortality probit plots for adult female *Drosophila suzukii* reared from fruit collected from an untreated blueberry field (Site 1) exposed to four insecticides using a Potter spray tower. Malathion, methomyl and spinetoram were tested on flies collected in 2013 and zeta-cypermethrin was tested on flies collected in 2014.

In the malathion experiments the average LC_{50} values were 60.6 ± 2.3 ppm (2013), 53.6 ± 1.9 ppm (2014), and 67.5 ± 3.3 ppm (2015), while the average LC_{90} values were 81.8 ± 2.5 ppm (2013), 82.2 ± 3.0 ppm (2014), and 105.3 ± 4.4 ppm (2015) (Table 1). The LC_{50} values in 2015 were significantly higher than those in 2014 ($F = 4.85$, $df = 2, 33$, $P = 0.014$) and the LC_{90} values in 2015 were significantly higher than those in 2013 and 2014 ($F = 11.28$, $df = 2, 33$, $P < 0.0001$).

For methomyl, the susceptibility was less stable among the years, with the average LC_{50} values at 105.2 ± 15.1 ppm in 2013, 78.9 ± 4.2 ppm in 2014, and 100.1 ± 6.6 ppm in 2015. For this same insecticide, the LC_{90} values were 298.8 ± 32.4 ppm in 2013, 211.2 ± 19.6 ppm in 2014, and 269.3 ± 21.8 ppm in 2015 (Table 2). The LC_{50} values were significantly higher in 2015 than 2014 ($F = 3.40$, $df = 2, 40$, $P = 0.043$) and the LC_{90} values in 2014 were significantly lower than both 2013 and 2015 ($F = 3.73$, $df = 2, 40$, $P = 0.033$).

In the tests with spinetoram, the sensitivity of the flies declined during the 3 years of the project. The average LC_{50} values were 13.7 ± 1.5 ppm (2013), 21.1 ± 1.2 ppm (2014), and 25.9 ± 1.0 ppm (2015), while the average LC_{90} values were 25.1 ± 2.7 ppm (2013), 40.0 ± 1.9 (2014), and 55.7 ± 3.2 (2015) (Table 3). There were statistically significant differences among years for both the LC_{50} and LC_{90} values (LC_{50}: $F = 18.14$, $df = 2, 38$, $P < 0.0001$; LC_{90}: $F = 20.72$, $df = 2, 38$, $P < 0.0001$).

Fly mortality in zeta-cypermethrin experiments was fairly consistent in the 2 years of experiments (Table 4). The average LC_{50} values were the lowest of the four insecticides, at 3.7 ± 0.6 ppm (2014) and 3.4 ± 0.2 ppm (2015), while the average LC_{90} values were 11.0 ± 2.3 ppm (2014) and 10.5 ± 0.7 ppm (2015). There were no significant differences in the LC_{50} or LC_{90} values between the two years (LC_{50}: $t = -0.58$, $df = 1, 25$, $P = 0.57$; LC_{90}: $t = -1.09$, $df = 1, 25$, $P = 0.30$).

When the individual site values were compared, we observed variability in susceptibility to insecticides among sites within each year, with at least some sites showing separation based on non-overlapping fiducial limits.[27] In comparison to the susceptible strain, we found resistance ratios for the four insecticides were ≤2 for most fields for both the LC_{50} and LC_{90} values, although there were individual fields where resistance ratios were >2. For malathion, three fields in 2015 (two unsprayed, one conventional)

Table 1. Results of adult direct contact bioassays of malathion insecticide. *Drosophila suzukii* populations were tested for susceptibility in 2013, 2014 and 2015. Mortality was recorded at 24 h after treatment

Site	Field management	*n*	Slope (± SE)	LC_{50} (mg L^{-1})	95% Fiducial limits	LC_{90} (mg L^{-1})	95% Fiducial limits	χ^2	RR50	RR90
2013										
1	Unsprayed	600	7.8 ± 1.0	51.7	(47.8, 54.9)	75.3	(70.1, 83.9)	0.636	1.0	1.0
20	Minimally managed	500	8.6 ± 2.7	63.7	(0.2, 114.5)	89.7	(67.5, 2.6 x 10^9)	< 0.0001	1.2	1.2
13	Conventional	500	7.6 ± 0.6	57.7	(54.4, 60.8)	85.2	(80.0, 92.2)	0.461	1.1	1.1
18	Conventional	800	12.9 ± 92.4	59.1	*	74.3	*	< 0.0001	1.1	1.0
21	Conventional	799	12.1 ± 11.6	64.0	*	81.7	*	< 0.0001	1.2	1.1
22	Conventional	800	12.9 ± 74.5	67.4	*	84.6	*	< 0.0001	1.3	1.1
2014										
1	Unsprayed	290	7.1 ± 1.8	55.9	(37.1, 78.0)	84.7	(64.5, 224.3)	< 0.0001	1.0	1.0
2	Unsprayed	250	6.5 ± 1.0	55.4	(49.0, 60.5)	87.0	(78.7, 101.6)	0.812	1.0	1.0
3	Unsprayed	280	6.7 ± 0.8	44.1	(40.6, 48.0)	68.6	(61.5, 79.9)	0.114	0.8	0.8
20	Minimally managed	240	7.5 ± 0.9	52.8	(48.5, 57.2)	78.1	(70.6, 90.1)	0.449	0.9	0.9
7	Light conventional	210	6.4 ± 1.7	55.4	(33.7, 83.0)	88.2	(64.6, 338.8)	0.001	1.0	1.0
8	Light conventional		Not tested							
9	Conventional	280	8.3 ± 1.6	49.4	(39.9, 61.0)	70.5	(57.9, 116.3)	0.022	0.9	0.8
4	Conventional	210	5.6 ± 1.7	46.3	(11.5, 222.1)	78.5	(51.7, 7.5 × 10^7)	0.001	0.8	0.9
10	Conventional		Not tested							
11	Conventional	340	8.2 ± 0.8	60.5	(56.6, 64.8)	86.7	(79.6, 97.1)	0.138	1.1	1.0
12	Conventional		Not tested							
13	Conventional	280	8.4 ± 1.0	49.4	(45.8, 53.3)	70.1	(63.6, 80.4)	0.434	0.9	0.8
14	Conventional		Not tested							
15	Conventional		Not tested							
16	Conventional	350	5.0 ± 0.9	53.9	(36.1, 69.9)	96.8	(74.3, 168.3)	0.003	1.0	1.1
17	Conventional		Not tested							
18	Conventional	339	8.1 ± 0.9	66.1	(61.7, 70.7)	95.3	(87.3, 107.4)	0.287	1.2	1.3
19	Conventional		Not tested							
2015										
1	Unsprayed	320	4.9 ± 1.0	40.1	(27.2, 50.7)	86.7	(64.7, 211.2)	0.013	1.0	1.0
2	Unsprayed	270	7.8 ± 0.9	91.0	(84.8, 98.0)	132.9	(120.5, 153.2)	0.201	2.3	1.5
3	Unsprayed	239	5.9 ± 1.0	53.8	(43.0, 64.8)	88.7	(72.3, 134.0)	0.033	1.2	1.0
4	Unsprayed	267	6.7 ± 1.5	91.9	(71.6, 118.5)	143.0	(112.8, 312.7)	0.010	2.3	1.6
5	Organic	162	8.9 ± 1.4	68.6	(63.0, 74.4)	95.7	(86.4, 113.0)	0.359	1.7	1.1
6	Organic	318	5.6 ± 1.2	65.2	(51.7, 82.5)	127.9	(95.9, 290.4)	0.002	1.6	1.5
7	Light conventional	240	9.2 ± 1.9	64.6	(52.9, 76.2)	89.0	(75.6, 140.4)	0.039	1.6	1.0
8	Light conventional	320	8.6 ± 1.0	63.9	(60.0, 67.8)	99.4	(90.7, 113.8)	0.831	1.6	1.1
9	Conventional	189	7.6 ± 1.1	82.2	(75.3, 89.7)	121.3	(108.4, 144.4)	0.616	2.0	1.4
10	Conventional	216	10.3 ± 1.4	84.2	(78.6, 90.4)	112.2	(102.8, 128.3)	0.934	2.1	1.3
11	Conventional	319	7.4 ± 1.9	59.5	(36.2, 81.4)	99.4	(75.6, 1006.7)	0.006	1.5	1.1
12	Conventional	190	9.7 ± 1.6	57.9	(53.2, 92.3)	78.6	(71.9, 91.0)	0.609	1.4	0.9
13	Conventional	320	6.1 ± 0.9	41.1	(33.4, 48.6)	76.8	(62.0, 120.2)	0.077	1.0	0.9
14	Conventional	240	7.2 ± 0.9	71.4	(66.1, 77.1)	107.5	(96.8, 126.1)	0.882	1.8	1.2
15	Conventional	237	6.5 ± 1.5	73.5	(66.5, 88.9)	115.6	(93.6, 198.5)	0.225	1.8	1.3
16	Conventional	191	8.3 ± 1.2	62.2	(57.1, 67.3)	88.8	(80.4, 104.0)	0.949	1.6	1.0
17	Conventional	318	9.0 ± 1.8	63.0	(52.0, 73.3)	95.7	(80.1, 161.8)	0.020	1.6	1.1
18	Conventional	320	8.0 ± 0.9	71.0	(66.7, 75.5)	113.9	(103.2, 131.6)	0.455	1.8	1.3
19	Conventional	187	5.9 ± 0.8	77.9	(70.7, 85.7)	128.5	(112.2, 159.4)	0.405	1.9	1.5

*Fiducial limits could not be calculated.

had RR_{50} values that were >2 (sites 2, 4 and 10), but none had a RR_{90} value >2. For methomyl, there were three conventional fields in 2013 with RR_{50} values >2 (sites 13, 21 and 22), and one of these fields also had an RR_{90} value >2 (site 22). In 2014, one conventional light field also had a higher RR_{90} value (site 8) and in 2015 there was one organic field with a RR_{90} value >2 (site 6). For spinetoram, there were no populations in which RR_{50} or RR_{90} values were >2. The response of different *D. suzukii* populations to zeta-cypermethrin, showed that four conventional fields in 2014 had RR_{50} values >2 (sites 9, 13, 15, and 19) and all seven conventional fields had RR_{90} values >2 (sites 9, 11, 12, 13, 15, 16 and 19). Of these, site 15 had a RR_{50} value of 3.8 and a RR_{90} value of 4.0. There were no significant differences between high-spray and no/low-spray sites for either LC_{50} or LC_{90} values for any of the insecticides in either

Table 2. Results of adult direct contact bioassays of methomyl insecticide. *Drosophila suzukii* populations were tested for susceptibility in 2013, 2014 and 2015. Mortality was recorded at 24 h after treatment

Site	Field management	*n*	Slope (± SE)	LC_{50} (mg L^{-1})	95% Fiducial limits	LC_{90} (mg L^{-1})	95% Fiducial limits	χ^2	RR50	RR90
2013										
1	Unsprayed	700	2.3 ± 0.2	50.2	(44.5, 56.8)	177.4	(145.2, 229.6)	0.316	1.0	1.0
20	Minimally managed	700	2.1 ± 0.3	83.4	(58.8, 120.6)	346.5	(211.5, 898.3)	0.001	1.7	2.0
13	Conventional	699	3.6 ± 0.7	143.9	(91.9, 200.6)	330.3	(231.1, 747.8)	< 0.0001	2.9	1.9
18	Conventional	700	3.1 ± 0.3	88.4	(69.5, 107.4)	228.3	(182.8, 314.4)	0.046	1.8	1.3
21	Conventional	700	3.5 ± 0.7	137.7	(83.7, 201.5)	321.4	(216.7, 813.1)	< 0.0001	2.7	1.8
22	Conventional	698	2.6 ± 0.3	127.3	(99.7, 170.7)	388.6	(263.9, 771.8)	0.010	2.5	2.2
2014										
1	Unsprayed	340	4.5 ± 0.5	92.6	(83.4, 102.8)	178.8	(153.8, 222.0)	0.632	1.0	1.0
2	Unsprayed	240	2.6 ± 0.3	43.6	(34.5, 52.8)	134.0	(106.6, 185.7)	0.625	0.5	0.7
3	Unsprayed	317	3.4 ± 0.8	106.7	(63.5, 215.0)	251.1	(148.3, 2218.0)	0.000	1.2	1.4
20	Min. Man.	210	2.7 ± 0.4	76.6	59.2, 93.1)	233.1	(181.9, 346.7)	0.106	0.8	1.3
7	Light conventional	203	2.7 ± 0.4	71.9	(58.7, 87.7)	218.5	(162.0, 360.0)	0.547	0.8	1.2
8	Light conventional	231	1.9 ± 0.3	99.6	(78.6, 129.7)	472.4	(296.5, 1152.0)	0.575	1.1	2.6
9	Conventional	269	2.8 ± 0.5	63.9	(29.4, 99.7)	180.6	(113.4, 648.5)	0.047	0.7	1.0
4	Conventional	330	3.4 ± 0.5	119.1	(92.1, 158.8)	285.1	(201.6, 576.9)	0.090	1.3	1.6
10	Conventional	250	3.6 ± 0.5	66.1	(55.8, 76.3)	148.8	(124.5, 192.5)	0.625	0.7	0.8
11	Conventional	240	3.8 ± 0.5	77.8	(21.7, 224.5)	178.7	(102.0, 212416)	0.112	0.8	1.0
12	Conventional	240	3.5 ± 1.0	81.5	(70.8, 93.0)	192.6	(156.8, 266.5)	0.001	0.9	1.1
13	Conventional	339	3.0 ± 0.3	79.7	(67.8, 91.3)	210.3	(175.6, 271.6)	0.449	0.9	1.2
14	Conventional	250	4.5 ± 0.7	75.1	(63.8, 84.8)	144.0	(123.6, 184.7)	0.695	0.8	0.8
15	Conventional	263	3.0 ± 0.4	92.7	(79.5, 108.8)	245.0	(193.2, 349.6)	0.564	1.0	1.4
16	Conventional	260	3.6 ± 0.4	65.1	(54.6, 75.4)	149.4	(125.4, 191.7)	0.164	0.7	0.8
17	Conventional	243	4.2 ± 0.6	63.2	(53.2, 72.4)	128.5	(109.8, 161.5)	0.856	0.7	0.7
18	Conventional	210	3.8 ± 0.8	67.6	(54.7, 79.1)	148.4	(118.1, 238.6)	0.168	0.7	0.8
19	Conventional	203	2.2 ± 0.4	77.3	(61.1, 101.9)	301.9	(195.3, 692.6)	0.360	0.8	1.7
2015										
1	Unsprayed	215	2.6 ± 0.7	74.0	(20.6, 108.3)	229.5	(148.4, 1799.0)	0.038	1.0	1.0
2	Unsprayed	271	3.3 ± 0.9	145.5	(101.6, 251.1)	351.6	(218.8, 2862.0)	0.004	2.0	1.5
3	Unsprayed	238	3.0 ± 0.4	65.7	(54.1, 77.4)	176.3	(143.7, 236.2)	0.250	0.9	0.8
4	Unsprayed	271	3.1 ± 0.6	136.2	(105.8, 185.3)	358.3	(241.5, 944.5)	0.068	1.8	1.6
5	Organic	269	3.2 ± 0.6	115.4	(88.1, 151.3)	287.7	(202.8, 644.6)	0.048	1.6	1.3
6	Organic	222	2.5 ± 0.4	148.5	(123.4, 186.8)	481.6	(333.5, 937.0)	0.312	2.0	2.1
7	Light conventional	161	2.8 ± 0.4	116.0	(95.0, 142.7)	328.5	(242.2, 555.7)	0.594	1.6	1.4
8	Light conventional	162	3.1 ± 0.7	117.1	(78.4, 178.6)	302.3	(192.9, 1138.0)	0.064	1.6	1.3
9	Conventional	230	2.7 ± 0.4	103.7	(86.9, 123.4)	314.3	(239.8, 481.4)	0.665	1.4	1.4
10	Conventional	270	2.2 ± 0.3	117.1	(97.4, 142.2)	442.8	(314.8, 781.2)	0.309	1.6	1.9
11	Conventional	238	3.6 ± 0.7	66.6	(43.4, 90.3)	150.0	(107.1, 339.4)	0.082	0.9	0.7
12	Conventional	191	3.2 ± 0.5	89.3	(73.6, 108.2)	227.1	(174.4, 348.7)	0.315	1.2	1.0
13	Conventional	240	3.6 ± 0.5	63.8	(53.8, 73.9)	143.5	(119.0, 189.4)	0.275	0.9	0.6
14	Conventional	270	3.3 ± 0.4	94.0	(81.0, 107.3)	230.7	(190.5, 307.3)	0.868	1.3	1.0
15	Conventional	192	3.6 ± 0.5	70.3	(57.8, 82.5)	160.2	(131.8, 214.7)	0.247	0.9	0.7
16	Conventional	188	2.9 ± 0.4	62.8	(49.8, 75.8)	171.5	(135.7, 245.7)	0.744	0.8	0.7
17	Conventional	240	2.8 ± 0.8	88.0	(41.3, 146.7)	250.6	(149.4, 2170.0)	0.001	1.2	1.1
18	Conventional	167	4.2 ± 0.6	133.3	(114.7, 154.5)	267.8	(220.0, 364.8)	0.569	1.8	1.2
19	Conventional	161	3.1 ± 0.5	95.2	(78.2, 114.3)	243.4	(189.3, 364.8)	0.464	1.3	1.1

2014 (malathion LC_{50}, $t = 0.39$, $df = 1, 9$, $P = 0.71$; malathion LC_{90}, $t = 0.27$, $df = 1, 9$, $P = 0.80$; methomyl LC_{50}, $t = -0.48$, $df = 1, 16$, $P = 0.64$; methomyl LC_{90}, $t = -1.27$, $df = 1, 16$, $P = 0.22$; spinetoram LC_{50}: $t = -1.24$, $df = 1, 14$, $P = 0.24$; spinetoram LC_{90}, $t = -1.83$, $df = 1, 14$, $P = 0.089$) or 2015 (malathion LC_{50}, $t = 0.035$, $df = 1, 17$, $P = 0.97$; malathion LC_{90}, $t = -0.49$, $df = 1, 17$, $P = 0.63$; methomyl LC_{50}, $t = -2.07$, $df = 1, 17$, $P = 0.054$; methomyl LC_{90}, $t = -1.88$, $df = 1, 17$, $P = 0.077$; spinetoram LC_{50}, $t = -1.41$, df $= 1, 17$, $P = 0.18$; spinetoram LC_{90}, $t = -1.33$, $df = 1, 17$, $P = 0.2$; zeta-cypermethrin LC_{50}, $t = -0.35$, $df = 1, 17$, $P = 0.73$; zeta-cypermethrin LC_{90}, $t = 0.035$, $df = 1, 17$, $P = 0.97$).

4 DISCUSSION

During the 3 years of this study, the concentration–response relationships of *D. suzukii* populations collected across southwest

Table 3. Results of adult direct contact bioassays of spinetoram insecticide. *Drosophila suzukii* populations were tested for susceptibility in 2013, 2014 and 2015. Mortality was recorded at 24 h after treatment

Site	Field management	*n*	Slope (± SE)	LC_{50} (mg L^{-1})	95% Fiducial limits	LC_{90} (mg L^{-1})	95% Fiducial limits	χ^2	RR50	RR90
2013										
1	Unsprayed	799	6.1 ± 0.8	10.1	(9.2, 10.8)	16.3	(14.9, 18.7)	0.429	1.0	1.0
20	Minimally managed	802	4.1 ± 0.3	14.3	(13.2, 15.4)	29.1	(26.3, 32.9)	0.344	1.4	1.8
13	Conventional	799	4.2 ± 0.7	15.1	(11.2, 19.3)	30.6	(23.3, 50.5)	< 0.0001	1.5	1.9
18	Conventional	800	5.9 ± 0.4	19.8	(18.6, 21.0)	32.6	(30.1, 35.9)	0.549	2.0	2.0
21	Conventional	804	6.1 ± 0.5	13.3	(12.5, 14.2)	21.6	(19.9, 24.0)	0.199	1.3	1.3
22	Conventional	798	3.9 ± 0.6	9.5	(7.0, 12.1)	20.5	(15.6, 34.0)	0.004	0.9	1.3
2014										
1	Unsprayed	349	4.0 ± 0.6	25.8	(20.5, 33.1)	54.4	(40.4, 101.0)	0.082	1.0	1.0
2	Unsprayed	250	3.5 ± 0.6	18.2	(14.7, 21.1)	42.4	(35.6, 56.8)	0.137	0.7	0.8
3	Unsprayed	298	4.6 ± 1.0	22.0	(14.1, 29.5)	41.9	(30.8, 114.9)	0.002	0.9	0.8
20	Minimally managed		Not tested							
7	Light conventional	250	5.1 ± 0.9	25.2	(17.7, 34.4)	45.0	(33.3, 104.1)	0.039	1.0	0.8
8	Light conventional	263	5.9 ± 0.7	24.6	(22.6, 26.7)	40.4	(35.9, 47.7)	0.914	1.0	0.7
9	Conventional	280	4.9 ± 0.5	23.6	(21.4, 25.8)	43.1	(38.0, 51.5)	0.481	0.9	0.8
4	Conventional	281	4.0 ± 0.5	24.4	(21.9, 27.2)	51.2	(43.4, 65.5)	0.364	0.9	0.9
10	Conventional	217	5.1 ± 0.8	16.6	(14.1, 18.6)	29.6	(26.0, 36.3)	0.108	0.6	0.5
11	Conventional	330	5.4 ± 0.5	20.7	(18.7, 22.7)	35.7	(31.7, 41.7)	0.213	0.8	0.7
12	Conventional	210	4.9 ± 0.8	16.2	(13.2, 18.6)	29.6	(25.8, 36.7)	0.642	0.6	0.5
13	Conventional	669	4.7 ± 0.5	22.8	(19.7, 26.1)	42.9	(36.0, 57.2)	0.051	0.9	0.8
14	Conventional	218	3.5 ± 0.7	13.4	(8.8, 16.5)	31.0	(26.3, 40.9)	0.461	0.5	0.6
15	Conventional	250	6.1 ± 0.7	29.8	(27.4, 32.4)	48.4	(43.2, 57.2)	0.158	1.2	0.9
16	Conventional	143	4.0 ± 0.9	15.3	(10.6, 18.5)	32.2	(26.8, 45.1)	0.233	0.6	0.6
17	Conventional	250	3.9 ± 0.6	15.9	(12.6, 18.4)	33.8	(29.1, 42.9)	0.285	0.6	0.6
18	Conventional	210	5.5 ± 1.4	22.5	(14.2, 31.2)	38.6	(28.6, 120.7)	0.003	0.9	0.7
19	Conventional		Not tested							
2015										
1	Unsprayed	191	4.2 ± 0.6	22.7	(19.5, 25.8)	45.8	(38.7, 59.0)	0.404	1.0	1.0
2	Unsprayed	191	3.0 ± 0.7	32.3	(21.4, 52.7)	87.7	(53.4, 624.0)	0.029	1.4	1.9
3	Unsprayed	238	4.6 ± 0.6	27.1	(24.2, 30.2)	51.5	(44.2, 64.8)	0.256	1.1	1.1
4	Unsprayed	170	3.8 ± 0.6	22.8	(19.2, 26.4)	49.3	(40.6, 67.0)	0.117	0.9	1.1
5	Organic	191	3.8 ± 0.5	28.8	(25.0, 33.2)	62.7	(51.1, 87.2)	0.322	0.9	1.4
6	Organic	189	4.2 ± 0.6	37.5	(33.1, 43.3)	75.2	(61.1, 106.2)	0.942	1.7	1.6
7	Light conventional	237	3.0 ± 0.4	23.6	(19.9, 27.5)	64.1	(51.0, 92.6)	0.274	0.7	1.4
8	Light conventional	238	4.6 ± 1.0	25.4	(18.4, 34.2)	48.3	(35.4, 124.6)	0.035	1.1	1.1
9	Conventional	238	3.4 ± 0.7	24.8	(17.7, 33.2)	58.4	(41.2, 141.6)	0.035	0.8	1.3
10	Conventional	190	3.9 ± 0.5	25.3	(21.8, 29.1)	54.4	(45.0, 73.1)	0.939	0.9	1.2
11	Conventional	240	4.8 ± 0.6	21.6	(19.1, 24.1)	39.9	(34.9, 48.2)	0.388	1.1	0.9
12	Conventional	188	3.8 ± 0.5	25.3	(21.7, 29.0)	54.7	(45.2, 73.8)	0.726	0.9	1.2
13	Conventional	240	4.5 ± 0.5	22.1	(19.5, 24.7)	42.4	(36.7, 51.9)	0.113	1.0	0.9
14	Conventional	243	3.3 ± 0.4	27.7	(24.0, 31.7)	67.7	(54.8, 94.4)	0.53.8	1.2	1.5
15	Conventional	240	3.9 ± 0.5	23.8	(20.8, 26.9)	51.1	(43.1, 65.5)	0.497	0.9	1.1
16	Conventional	234	3.5 ± 0.5	33.7	(29.6, 38.9)	77.9	(62.3, 112.0)	0.810	1.5	1.7
17	Conventional	240	4.1 ± 0.5	20.5	(15.2, 25.7)	37.2	(29.0, 66.5)	0.127	1.0	0.8
18	Conventional	238	5.2 ± 0.6	22.6	(20.2, 25.0)	39.8	(35.1, 47.7)	0.578	1.2	0.9
19	Conventional	237	4.1 ± 0.5	24.4	(21.5, 27.5)	50.4	(42.8, 64.0)	0.454	1.0	1.1

Michigan were characterized for malathion, methomyl, spinetoram, and zeta-cypermethrin. Our results provide a baseline for future comparisons of the sensitivity of this invasive fruit pest to four insecticides that represent the primary classes being used to protect fruit from *D. suzukii*, each with a different mode of action. The range of concentrations across which these populations responded, including the reference collection depicted in Fig. 1, will allow baseline sensitivity studies in other regions to quickly test across the active range of response for these insecticides.

Finding low resistance ratios provides confidence that this species has retained a general level of susceptibility to insecticides, despite repeated applications. It is notable that the LC_{90} values for the tested insecticides were much lower than the concentrations being used in sprays against *D. suzukii*, making it unlikely that any reports of infestation by this pest in commercial farms in this region

Table 4. Results of adult direct contact bioassays of zeta-cypermethrin insecticide. *Drosophila suzukii* populations were tested for susceptibility in 2013, 2014 and 2015. Mortality was recorded at 24 h after treatment

Site	Field management	*n*	Slope (± SE)	LC_{50} (mg L^{-1})	95% Fiducial limits	LC_{90} (mg L^{-1})	95% Fiducial limits	χ^2	RR50	RR90
2014										
1	Unsprayed	349	3.0 ± 0.7	1.4	(0.5, 2.3)	3.7	(2.3, 30.2)	0.009	1.0	1.0
2	Unsprayed		Not tested							
3	Unsprayed		Not tested							
20	Minimally managed		Not tested							
7	Light conventional		Not tested							
8	Light conventional		Not tested							
9	Conventional	280	2.9 ± 0.6	3.8	(2.4, 5.7)	10.6	(6.8, 33.1)	0.020	2.8	2.8
4	Conventional		Not tested							
10	Conventional		Not tested							
11	Conventional	340	2.6 ± 0.3	2.7	(2.3, 3.2)	8.3	(6.8, 11.2)	0.294	2.0	2.2
12	Conventional	340	2.8 ± 0.5	2.8	(1.9, 3.8)	8.0	(5.5, 18.4)	0.059	2.0	2.2
13	Conventional	280	2.2 ± 0.3	3.0	(2.4, 3.5)	9.2	(7.3, 12.9)	0.983	2.1	2.5
14	Conventional		Not tested							
15	Conventional	280	2.8 ± 0.3	5.3	(4.5, 6.2)	14.9	(11.7, 21.3)	0.429	3.8	4.0
16	Conventional	259	2.4 ± 0.5	2.7	(1.3, 4.0)	8.9	(5.5, 39.8)	0.031	1.9	2.4
17	Conventional		Not tested							
18	Conventional		Not tested							
19	Conventional	179	4.6 ± 0.7	4.4	(3.9, 5.1)	8.4	(7.0, 11.4)	0.429	3.2	2.3
2015										
1	Unsprayed	193	3.2 ± 0.4	3.2	(2.7, 3.8)	8.0	(6.3, 11.5)	0.755	1.0	1.0
2	Unsprayed	299	3.0 ± 0.6	4.4	(3.0, 6.7)	15.3	(9.1, 58.7)	0.003	1.4	1.9
3	Unsprayed	234	2.9 ± 0.3	3.2	(2.7, 3.8)	8.7	(6.8, 12.2)	0.609	1.0	1.1
4	Unsprayed	192	2.8 ± 0.5	5.0	(3.3, 7.9)	14.3	(8.7, 50.2)	0.066	1.6	1.8
5	Organic	193	3.0 ± 0.4	2.6	(2.1, 3.1)	6.9	(5.4, 10.0)	0.872	0.8	0.9
6	Organic	192	2.1 ± 0.3	3.6	(2.8, 4.7)	15.1	(10.4, 27.8)	0.676	1.1	1.9
7	Light conventional	239	2.9 ± 0.3	3.5	(2.9, 4.2)	9.6	(7.6, 13.6)	0.338	1.1	1.2
8	Light conventional	229	2.7 ± 0.3	2.7	(2.2, 3.2)	8.0	(6.2, 11.4)	0.494	0.8	1.0
9	Conventional	240	2.3 ± 0.5	4.4	(2.8, 7.4)	15.6	(8.8, 70.4)	0.026	1.4	2.0
10	Conventional	234	2.3 ± 0.3	3.1	(2.5, 3.7)	11.2	(8.4, 17.3)	0.289	1.0	1.4
11	Conventional	239	3.0 ± 0.4	4.9	(4.1, 5.8)	12.8	(10.0, 18.5)	0.364	1.5	1.6
12	Conventional	228	2.5 ± 0.3	2.7	(2.2, 3.3)	8.6	(6.5, 12.9)	0.895	0.8	1.1
13	Conventional	239	2.7 ± 0.3	4.0	(3.3, 4.8)	11.9	(9.1, 17.6)	0.693	1.2	1.5
14	Conventional	240	2.6 ± 0.4	2.8	(1.9, 4.0)	8.7	(5.6, 22.1)	0.073	0.9	1.1
15	Conventional	191	3.7 ± 0.5	3.0	(2.5, 3.6)	6.6	(5.3, 9.5)	0.756	0.9	0.8
16	Conventional	190	2.6 ± 0.3	3.6	(2.9, 4.5)	11.4	(8.4, 18.2)	0.259	1.1	1.4
17	Conventional	239	3.3 ± 0.5	2.9	(2.0, 4.1)	7.2	(5.0, 15.4)	0.050	0.9	0.9
18	Conventional	239	2.4 ± 0.3	3.4	(2.8, 4.1)	11.6	(8.7, 17.7)	0.282	1.1	1.4
19	Conventional	240	3.0 ± 0.4	2.6	(2.2, 3.1)	6.8	(5.4, 9.8)	0.378	0.8	0.9

are caused by resistance to insecticides. Although their effectiveness is expected to be maintained in the near future, our data also provide an early indication of a shift in sensitivity to a spinosyn class insecticide, highlighting the need for monitoring populations in regions where flies are exposed to repeated insecticide exposure.

The fly colonies tested in this study were established from infested fruit collected soon after the final harvest of the season. This is typically when *D. suzukii* populations are increasing across the region and fly movement in and out of crop fields is possible.[28] Resistance development may be less likely in fields with high levels of fly immigration from unsprayed habitat than fields with low levels of immigration. Future studies should compare *D. suzukii* populations collected from within the crop field to those in adjacent unsprayed habitats, particularly at sites where there is evidence for declining susceptibility. Fly populations should also be tested at several times over the course of the summer instead of only later in the season. These types of future tests could help elucidate the causes of variability in insecticide susceptibility found among different blueberry fields. It is also important to recognize that these bioassays were performed using a treatment applied directly over the flies, similar to a direct exposure of flies during an application. The long-term efficacy of pesticide applications will also rely on residues on and in plant tissues. Any resistant individuals are more likely to survive after the residue levels decline, so we consider these tests to be relatively conservative. Development of assay methods for this species using dry residues of pesticides should be pursued for future screening of populations as part of resistance management programs.[29] It is also important to highlight that the colonies took an average of eight generations to have sufficient flies to complete these tests, and during that time the sensitivity to insecticides may have returned to pre-exposure levels. Resistance can decline in the absence of insecticide exposure[30] and may not be stable if there are associated fitness costs[31] so it will be important to collect large numbers of flies in the field to establish colonies for rapid testing in the first generation post collection.

Although a range of susceptibility was exhibited by the different populations of *D. suzukii* tested, in general, the susceptibility was found to be relatively high and with low resistance ratio values across most of the tested populations. An exception to this pattern was seen at two of the conventional fields where populations exhibited decreased susceptibility to zeta-cypermethrin in 2014. We do not know what levels of resistance ratio will result in practical resistance[30,31] for this species. In the case of codling moth, low resistance levels to pyrethroids and organophosphates (less than sixfold) resulted in significant damage in apples.[32] Given the very low threshold of damage set by the blueberry processing industry it is likely that *D. suzukii* will not need very high levels of resistance to cause greater economic damage.

The overall similarity in susceptibility of *D. suzukii* to insecticides in populations collected from fields receiving minimal insecticide input and those that received an intensive chemical management program to combat this pest suggests that populations in this system are maintaining susceptibility despite selection pressure from insecticides. This may reflect the landscape context of the fields sampled in this study. In this region, there is abundant natural habitat containing alternative host plants[33] near to the crop fields, with populations of *D. suzukii* actively using this untreated habitat[34] and with movement between the habitats expected to occur regularly.[28] Recent research on non-crop hosts of *D. suzukii* has focused on the negative effects of these hosts as a source for flies that can invade the field.[28,33,35–37] However, these non-crop host areas may also serve as vital refuges for susceptible flies in the population. Such refuges have been successfully used for resistance management of other insect pests,[38,39] and may prove important for *D. suzukii*. Although promoting early season fruiting non-crop hosts such as *Lonicera* and *Rubus* species would be inadvisable as they could increase *D. suzukii* populations during harvest,[28] encouraging later-season non-crop hosts such as *Phytolacca americana* may not pose as great of a risk to the commercial crop.[36] This would also suggest that regions of production without extensive wild host habitat would be at greater risk of resistance development,[40] however, this prediction remains untested for this species.

We detected a steady increase in the LC_{50} and LC_{90} values for spinetoram over the 3 years that monitoring was conducted, with an 89% increase in the LC_{50} and a 122% increase in the LC_{90} from 2013 to 2015. These results are somewhat surprising because there is relatively limited application of this specific insecticide against *D. suzukii* in blueberries due to its higher cost than alternatives such as zeta-cypermethrin. Cross-resistance to insecticides has been widely reported in insects[41,42] and can occur between insecticides used for the target pest and those used for other pests.[43] Because the resistance ratio levels remain low in our tested populations it is premature to investigate cross-resistance in *D. suzukii*, but this may be a fruitful topic for future studies if there is more significant loss of sensitivity.

Variation in the susceptibility of *D. suzukii* populations collected from fields with high levels of insecticide exposure and with low exposure further highlights that *D. suzukii* have retained susceptibility to insecticides at some locations even after multiple years of spraying. Resistance often develops in spatially variable patterns, as seen in mosquitoes,[44] aphids,[45] and beetles[46] among others. Consequently, the comparison of overall susceptibility among these two groups can mask the site-specific changes that may occur, particularly at the start of resistance development. Our field -specific results suggest this may be happening as individual populations become less sensitive: a greater proportion of the conventional fields exhibited high resistance ratio values, such as the seven conventional sites exhibiting RR_{90} values greater than 2 for zeta-cypermethrin in 2014. However, when these sites were retested in 2015 only one of these sites had a RR_{90} value of 2.0, highlighting the need for multi-year testing and transient nature of apparent tolerance to insecticide.

The invasion of *D. suzukii* into new regions has threatened many successful IPM programs and brought about the necessity to identify and implement new IPM practices. Insecticide resistance management (IRM) is a crucial component of a successful IPM program and needs to be a part of this rebuilding process.[47] Preventative approaches need to be identified and implemented instead of relying on a reactive approach. In certain instances, such as organic production, preventative approaches are even more important as viable reactive approaches are lacking given that organic producers have only a few insecticide classes that provide consistent control against *D. suzukii*.[16] In addition to rotating insecticide classes to delay resistance development, we suggest that growers stop applying insecticides as soon after the final harvest as possible. Late season reproduction of *D. suzukii* in unsprayed fields may reduce the prevalence of recessive resistance alleles in the population. In temperate climates these flies are more likely to survive the winter than late summer-emerging flies due to physiological adaptions such as increased melanization and larger body size.[48,49] Subsequently, resistant flies may not survive the winter increasing the chance of susceptible flies reinvading the crop fields the following spring. Comparing susceptibility of winter and summer morphs of *D. suzukii* would provide some important insights into this prediction.[50,51]

As part of the endeavor to fill in the knowledge gaps, more robust resistance monitoring programs need to be implemented for *D. suzukii* in different cropping systems and different geographic locations. Although the susceptibility monitoring we conducted in this study using a Potter spray tower was highly effective, it was also labor intensive and impractical to implement on a wider scale. Ideally, for monitoring programs to be as effective as possible they should be easy to use and efficient to operate. Development of a monitoring system that could be utilized by local crop scouts, consultants, and extension agents has the potential to be highly effective at providing a broad view of the state of resistance in *D. suzukii* populations across cropping systems and regions.

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