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Development of a rapid assessment method for detecting insecticide resistance in spotted wing Drosophila (*Drosophila suzukii* Matsumura)

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ABSTRACT

BACKGROUND: Spotted wing Drosophila is an invasive pest of fruit crops in most production regions globally, and insecticides are commonly used for its control. The biology of this pest combined with repeated pesticide exposure increases the risk of resistance to insecticides. We tested malathion, methomyl, spinetoram, spinosad, and zeta-cypermethrin against multiple colonies from each state using a contact bioassay method to determine diagnostic doses for assessment of insecticide susceptibility in this species. These were used to test populations collected in Michigan and Georgia, USA.

RESULTS: LC_{50} and LC_{90} values were calculated for the tested populations, and male mortality consistently occurred at lower concentrations than females. Fly mortality did not vary significantly among populations collected from unmanaged, organic, and conventional fields. Similar results were found using the diagnostic concentrations applied to glass jars.

CONCLUSIONS: Using this method, samples of *D. suzukii* that are freshly caught or reared from fruit can be tested within one day for their mortality in response to discriminating doses of five key insecticides. This method can be used to inform proactive resistance management strategies within Integrated Pest Management programs.

Keywords: bioassay, monitoring, IPM, resistance management

1 INTRODUCTION

After spotted wing Drosophila, *Drosophila suzukii* Matsumura, was first detected in North America in 2008 and rapidly spread across the continent, there was a rapid response to determine how to control this new pest.[1] As *D. suzukii* has transitioned from a new invader to an established pest, most affected growers have implemented more intensive use of insecticides to maintain marketable fruit.[2] While significant progress has been made towards developing and implementing effective biological tools for control, most pest management programs for this pest continue to rely on repeated insecticide applications.[2-4]

Drosophila suzukii is estimated to have seven to 13 generations per year,[5,6] so flies in western portions of North America have already gone through 70 to 130 generations since first being detected in 2008. Repeated applications of insecticide sprays in commercial fruit fields each year[3,7] have raised concerns about the potential for development of resistance in *D. suzukii* populations, and research on this topic should be a priority.[1,8] However, surveys of *D. suzukii* populations to assess their susceptibility to insecticides have so far been limited.[8-10] Hamby et

al.[9] investigated toxicity of malathion and fenpropathrin to *D. suzukii* at different times of day and under different day length and temperature regimes. More recently, Smirle et al.[8] tested insecticide susceptibility of *D. suzukii* based on fly age and sex. Several insecticides were tested as well as a test for accelerated resistance to malathion over multiple generations in the laboratory. Both of these studies tested residual activity of insecticides using glass vial bioassays, however, in both cases testing was limited to only one[9] or two[8] *D. suzukii* populations and no resistance was detected. Van Timmeren et al.[11] conducted a more comprehensive survey of insecticide susceptibility of *D. suzukii* populations in southwest Michigan using direct contact bioassays on adult female flies over three years. This revealed some variation among populations in their susceptibility, and again no evidence of resistance was found. This study used a Potter spray tower[12] to apply insecticides to adult flies, a method that is effective but extremely labor intensive. In addition, direct contact bioassays may not accurately represent how flies are exposed to insecticides in the field. Since flies are active primarily around dawn and dusk,[13,14] they may not be directly exposed to insecticides if applications are made during daytime hours. Finally, since this survey tested only female flies in one region, an extensive survey of male *D. suzukii* has yet to be conducted.

Resistance monitoring programs are often challenging to implement due to the time and money required. Using a diagnostic dose approach can provide rapid and reliable monitoring to support widespread implementation of resistance monitoring.[15] For example, Stanley[15] describes a resistance monitoring program that used a two-dose diagnostic system to monitor for

cypermethrin resistance in *Helicoverpa armigera* Hubner in West Africa. Vials were treated at a central location in France, shipped with a pictorial instruction card to investigators in Africa, and datasheets were subsequently returned for data analysis. This allowed for extensive sampling of *H. armigera* populations in rural areas in a cost effective and efficient manner, and identified some areas with lower efficacy.[15]

Currently no diagnostic doses have been identified for *D. suzukii*, yet there is a need for more comprehensive surveys of populations of this pest, particularly in regions with more intensive agricultural production. An efficient and economical method to detect resistant populations would facilitate monitoring to reduce the risk that resistance is not detected until it has become widespread within a population. Development of such a method could also provide confidence in whether *D. suzukii* populations maintain their susceptibility.

Glass vial bioassays have been used successfully for other insect pest species, with psyllids, stink bugs, and mosquitoes among the pests that have been exposed to insecticide residues coated on the insides of glass vials.[16-20] This method was also used earlier in toxicity studies on *D. suzukii*[8,9] and is a flexible method that can be adapted for efficient monitoring of susceptibility to insecticides. However, the earlier studies used a rolling device to coat the inside of the vials with insecticide active ingredients, requiring specialized equipment and access to these chemicals. For a rapid assessment assay, use of formulated product that is more available, and is what *D. suzukii* flies are being exposed to, should further improve the chances of adoption.

The goal of this study was to develop and test a resistance monitoring system that can be implemented easily and efficiently, to inform management of *D. suzukii*. The first objective was to develop the glass vial bioassay method using insecticides from the most common classes, by determining the appropriate concentrations of malathion, methomyl, spinetoram, spinosad, and zeta-cypermethrin to use in these tests. The second objective was to test the susceptibility of *D. suzukii* to these four insecticides using multiple populations collected in Michigan and Georgia.

2 EXPERIMENTAL METHODS

2.1 Colony Establishment.

In Michigan, *Drosophila suzukii* colonies were established from flies collected at 14 sites (2016), 11 sites (2017), and 15 sites (2018) in late August-September from locations in Allegan, Ottawa, and Van Buren counties. The majority of sites (12 in 2016, 10 in 2017, 13 in 2018) were from fields of highbush blueberry, *Vaccinium corymbosum* L., one site was a homeowner raspberry field (Site 4), one was a raspberry planting in an urban community garden (Site 19), and two sites were local parks where wild honeysuckle, *Lonicera* sp., was the primary fruit present (Site 3 and Site 18). In Georgia, *D. suzukii* colonies were established from flies collected at 6 sites (2016) and 5 sites (2017) in late July-September from locations in Appling, Bacon, Clarke, Clinch, Pierce, and Ware counties. The majority of sites (4 in 2016, 5 in 2017) were from fields of rabbiteye blueberry, *Vaccinium ashei* L. Fly colonies were established by collecting infested fruit samples and rearing adults, or by using live traps when ripe fruit was no longer present.

Live traps were baited with washed organic blueberries or raspberries and placed at sites for 1-3 days after which they were brought back to the laboratory and adult flies were aspirated out of traps and *D. suzukii* adults sorted and used to establish colonies.

Collection sites were classified into three categories based on insecticide exposure history as described in Van Timmeren et al.[11] The three categories were conventional, organic, and unsprayed, reflecting the types of insecticides and management approach used at the sites. In 2018, populations were only established from conventional and unsprayed sites. In Michigan, one of the unsprayed sites (Site 1) was designated as the standard susceptible site, as it is a highbush blueberry field that has received no insecticide sprays for at least 25 years and is at least 4 km from the nearest commercial farm. For one bioassay (zeta-cypermethrin, males, 2016) control mortality for Site 1 exceeded 20%, so Site 2 was used as the standard susceptible site in this instance. Site 2 is a highbush blueberry field that has received no insecticide sprays for at least 10 years. In Georgia, one of the unsprayed sites (Site 1) was designated as the standard susceptible site, since the parent flies for this colony were collected from a homeowner backyard with no commercial fruit farms within 10 km of the site.

Log dose-morality plots were created separately for males and females for each insecticide for both states (Fig. 1 and Fig. 2). Only the data from the unsprayed standard sites (Site 1) were used to create these graphs, except for two instances where control mortality exceeded 20% (Michigan spinosad and zeta-cypermethrin in 2016). In these cases, data from Site 2 were used instead.

2.2 Insecticides Tested

Five formulated insecticides were tested in this study including 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), spinosad (Entrust® 22.5SC, Dow AgroSciences LLC, Indianapolis, IN, USA), and zeta-cypermethrin (Mustang® Maxx 0.8EC, FMC Corporation, Philadelphia, PA, USA). Recently-produced insecticides less than one year old were used in all bioassays. In Michigan, all five insecticides were tested in 2016-2018 except for methomyl which was not included in 2016. In Georgia, malathion, spinetoram, and zeta-cypermethrin were tested in 2016 and malathion, spinosad, and zeta-cypermethrin were tested in 2017.

2.3 Bioassays

Insecticide bioassays were conducted using 20 ml glass vials with a screw top opening (Fisher Scientific, Pittsburgh, PA). Stock solutions of the formulated insecticides were prepared using either acetone (for malathion, methomyl, and zeta-cypermethrin) or distilled water (for spinetoram and spinosad). Insecticides mixed in distilled water were added with an adjuvant (1266.6 µl per liter) to ensure even coating inside glass vials. The adjuvant used was a mixture of alkyl aryl polyoxylkane ethers and free fatty acids (Induce® brand, Helena Chemical Company, Collierville, TN). Serial dilutions were used to create 7-8 concentrations of each insecticide that

were tested against the flies. Specific concentrations of active ingredient were selected to provide maximum representation along a mortality curve from 0 to 100% mortality (malathion: 0-30 parts per million [ppm]; methomyl: 0-4.2 ppm; spinetoram: 0-900 ppm; spinosad: 0-1000 ppm; zeta-cypermethrin: 0-2 ppm). For each concentration, vials were treated by pipetting 1.0 ml of insecticide solution into a vial, placing the cap on the vial, and gently turning the vial on its side and upside down until all internal surfaces were evenly coated with insecticide. Vial caps were then removed, excess liquid was poured into a waste collector, and vials were gently tapped upside down five times to remove remaining liquid from the vial. This tapping process was conducted twice for vials treated with the aqueous solutions to ensure the excess liquid was removed. Vials and lids were then placed upright at an angle of 30 degrees in a fume hood and allowed to dry for 20 hours before use in bioassays.

Adult flies used in experiments were 3-5 days post eclosion and were added to vials by either anesthetizing them using a CO_2 gun and fly pad (Genesee Scientific, San Diego, CA, USA) (Michigan) or using an aspirator (Georgia). Anesthetized flies were exposed to CO_2 for less than 5 minutes which is well below the duration when flies start to exhibit any negative effects from CO_2.[11] In 2016 and 2017 we used five male and five female flies in each vial, and in 2018 the assays were conducted with ten female flies. Vials were kept on their side for the duration of the experiment, placed in an environmental chamber at 23 ± 2 °C and 75 ± 10% relative humidity. Fly condition was assessed 6 hours after flies were added to vials, except for spinosad experiments in 2017 where this was also assessed after 8 hours. At these assessments,

male and female flies were classified as alive, moribund, or dead. Alive flies were ones that were standing and walking around normally, while moribund flies were those that were clearly suffering the effects of the insecticides including twitching legs, the inability to right themselves when flipped on their back, or slow, uneven movements. Moribund and dead individuals were combined for inclusion in the probit analyses.

In 2016, flies used in Michigan bioassays were from F2-F6 generations, with the majority of bioassays conducted using F2 flies, while Georgia bioassays were conducted using F2-F5 generations. In 2017, all bioassays in Michigan were conducted using F1 flies, and with F3-F6 flies in Georgia. In 2018 most bioassays were conducted using F1 flies, a few bioassays were conducted using F2 flies, and only one colony (Site 18) had a few bioassays conducted using F3 flies.

In 2018, additional bioassays were conducted on three of the colonies (from Sites 18, 21, and 22) to test the repeatability of this method for monitoring susceptibility of *D. suzukii*. Three replicates of each of these colonies were tested for each of the insecticides on three separate dates in Fall 2018. An average of 169.7 ± 5.9 flies were tested on each date, such that probit analysis could be conducted for each date.

Bioassays were also conducted in 2018 to determine whether the bioassay method would work using a commonly-available size of glass jar. Clear jars with metal screw tops (240 ml volume, 98.8 mm height, 71.4 mm diameter, O.Berk Company, LLC, Union, NJ) were treated using the same methods described previously. Bioassays were conducted for zeta-cypermethrin

and spinosad representing a test of both the acetone-based method and the distilled water+adjuvant method. Five concentrations of each of the insecticides were tested using two colonies, including flies from the Site 18 colony as well as a laboratory colony established in 2018 from a blend of ten individual site colonies. Flies used in bioassays were from generations F3-F5.

2.4 Statistical Analysis

Mortality data were corrected using Abbott's formula[21] relative to the untreated flies, and bioassays where control mortality exceeded 20% were excluded from analyses. Probit analysis was conducted using SAS version 9.4[22] to calculate the concentrations required to reach 50% (LC_{50}) and 90% mortality (LC_{90}) as well as the slope (± S.E.) of the log dose-probit line and the 95% fiducial limits. Resistance ratios were calculated for the LC_{50} (RR_{50}) and LC_{90} (RR_{90}) levels as a ratio of the mortality of each site collection relative to the untreated standard site in each state (Site 1 for both states, except where stated earlier). For comparisons among years and between sexes and states, the LC_{50} and LC_{90} values were analyzed using analysis of variance (ANOVA) or two-sample t-tests. Data were tested for normality using a Shapiro-Wilk test for normality; data that did not meet the assumptions of normality were log (X+1) transformed or square-root transformed prior to analysis. Post-hoc comparisons in ANOVA tests were conducted using Tukey's honestly significant difference test. In a few instances data did not meet the assumptions of normality after transformation and were analyzed using a Mann-Whitney U

test. The effect of management strategies on male and female LC_{50} and LC_{90} values were determined using ANOVA followed by Tukey's honestly significant difference test for post-hoc comparisons (2016 and 2017), or by using two-sample t-tests (2018).

3 RESULTS

Bioassays with each of the five insecticides resulted in a typical log-dose relationship to mortality of *D. suzukii*. This allowed probit analysis of their responses in Michigan (Figure S1) and Georgia (Figure S2), with similar response to the insecticides in both states. Male mortality was achieved at lower concentrations than females in all assays from all sites in both states (Table 1). Control mortality for female flies in the bioassays was low for all insecticides tested in both states in all three years (Michigan 2016: 7.1 ± 1.6, Michigan 2017: 1.1 ± 0.7, Michigan 2018: 2.3 ± 0.5, Georgia 2016: 6.3 ± 3.3, Georgia 2017: 3.9 ± 0.9). However, control mortality for males was consistently higher in the bioassays and was also more variable than that of females during the two years that testing on males took place (Michigan 2016: 19.2 ± 2.4, Michigan 2017: 10.1 ± 4.3, Georgia 2016: 12.6 ± 3.9, Georgia 2017: 35.1 ± 6.8). Due to higher and more variable control mortality, results for males for specific insecticides can be found in the supporting information (Tables S1-S6) and only female results are presented here.

For malathion tested against female *D. suzukii* flies there were significant differences among years for LC_{50} and LC_{90} values (Table 1 and Table 3), with 2018 having the lowest LC_{50}

values and 2017 the highest values ($F = 57.54$, $df = 2, 36$, $P < 0.0001$). For the LC_{90} values, those in 2018 were significantly lower than the 2017 values ($F = 5.13$, $df = 2, 36$, $P = 0.011$). In Georgia there were no significant differences in LC_{50} or LC_{90} values between the two years (Table 2) (LC_{50}: $t = -0.3$, $df = 1, 7$, $P = 0.78$; LC_{90}: $t = -0.052$, $df = 1, 7$, $P = 0.96$). When the LC_{50} and the LC_{90} values for Michigan and Georgia were compared they were very similar in 2016 (LC_{50}: $t = -0.27$, $df = 1, 17$, $P = 0.80$; LC_{90}: $t = 0.91$, $df = 1, 17$, $P = 0.38$) and in 2017 the LC_{50} values were significantly higher in Michigan than in Georgia ($t = -3.21$, $df = 1, 12$; $P = 0.008$). However, there were no significant differences between states for the LC_{90} values in 2017 ($t = 0.076$, $df = 1, 12$, $P = 0.94$).

Methomyl was tested in 2017 and 2018 and LC_{50} and LC_{90} values declined significantly from 2017 to 2018 (Table 1 and Table 4) (LC_{50}: $H = 17.31$, $df = 1, 23$, $P < 0.0001$; LC_{90}: $H = 17.31$, $df = 1, 23$, $P < 0.0001$).

For spinetoram, there were significant differences among years for LC_{50} and LC_{90} values (Table 1 and Table 5). Each of the three years were significantly different for LC_{50} values, with values increasing from 2016 to 2018 ($F = 88.79$, $df = 2, 34$, $P < 0.0001$). The LC_{90} values in 2018 were also significantly higher than those in 2016 and 2017 ($F = 45.67$, $df = 2, 34$, $P < 0.0001$). When 2016 LC_{50} and LC_{90} values were compared between Michigan and Georgia values for females were significantly higher in Michigan than in Georgia (LC_{50}: $t = -4.93$, $df = 1, 15$, $P = 0.004$; LC_{90}: $t = -2.39$, $df = 1, 15$, $P = 0.03$).

In the spinosad experiments, the LC_{50} and LC_{90} values for Michigan colonies assessed after 6 hours in 2017 were highly variable with a few extremely high values (LC_{50}: 262.6 ± 118.6; LC_{90}: 21,376.0 ± 15,492.7). This contrasted with much lower and more consistent results in 2016 (Table 1 and Table 6). The majority of mortality in the 2017 experiments were flies classified as moribund (80 ± 2.1%), giving an indication that flies were beginning to die but had not yet fully succumbed to the slower-acting spinosad insecticide. Because of this, an 8 hour assessment was also conducted for all 2017 and 2018 experiments. In these 8 hour assessments the percentage of affected flies that were moribund was lower (54.8 ± 1.9%) than at the 6 hour assessment and most of these moribund flies were highly moribund exhibiting very little movement. For Georgia, the LC_{50} and LC_{90} values at 6 hours were higher in certain instances, but did not reach levels as high as in the Michigan experiments (6 hour: LC_{50}: 93.1 ± 41.6; LC_{90}: 715.8 ± 285.4).

There were significant differences between years for the LC_{50} and LC_{90} values (Table 1 and Table 6), with values in 2018 significantly higher than those in 2016 (LC_{50}: $F = 10.47$, $df = 2, 35$, $P < 0.0001$; LC_{90}: $F = 10.66$, $df = 2, 35$, $P < 0.0001$). Flies from Michigan and Georgia showed no significant difference in their LC_{50} or LC_{90} values (LC_{50}: $t = 2.79$, $df = 1, 11$, $P = 0.10$; LC_{90}: $t = 1.50$, $df = 1, 11$, $P = 0.16$).

Zeta-cypermethrin LC_{50} values in 2018 were significantly lower than 2016 ($F = 34.53$, $df = 2, 37$, $P < 0.0001$) and LC_{90} values in 2018 were significantly lower than 2016 and 2017 ($F = 20.54$, $df = 2, 37$, $P < 0.0001$). The LC_{50} and LC_{90} values for *D. suzukii* from Georgia were

significantly higher in 2017 than in 2016 (Table 1 and Table 2) (LC_{50}: t = -3.31, df = 1, 6, P = 0.041; LC_{90}: t = -4.10, df = 1, 6, P = 0.006). LC_{50} and LC_{90} values in 2016 were higher in Michigan than they were in Georgia, although this result was only significant for the LC_{50} values (LC_{50}: t = -2.69, df = 1, 16, P = 0.016; LC_{90}: t = 1.67, df = 1, 16, P = 0.18). In 2017, the LC_{50} and LC_{90} values were both significantly higher for Georgia colonies than for Michigan colonies (U = 44.0, df = 1, 13, P = 0.004).

When the three pest management categories were compared, we found no significant differences among them in susceptibility of *D. suzukii* collected within Michigan for any of the tested insecticides (malathion 2016: F < 1.77, P > 0.21; malathion 2017: F < 1.44, P > 0.29; malathion 2018: t < 1.02, P > 0.32; methomyl 2017: F < 1.78, P > 0.23, methomyl 2018: t < 0.70, P > 0.50; spinosad 2016: F < 0.95, P > 0.42; spinosad 2017: F < 0.36, P > 0.71; spinosad 2018: t < 1.38, P > 0.18; zeta-cypermethrin 2016: F < 0.56, P > 0.0.60; zeta-cypermethrin 2017: F < 1.65, P > 0.1; zeta-cypermethrin 2018: t < 0.69, P > 0.49). This was the case for boththe LC_{50} and LC_{90} values. For spinetoram, *D. suzukii* from conventional sites had significantly lower LC_{90} values than unsprayed sites in 2016 (F = 4.68, df = 2, 10, P = 0.037), but significantly higher LC_{90} values than unsprayed sites in 2017 (F = 9.55, df = 2, 6, P = 0.014). In 2018 there were no significant differences for either the LC_{50} values (t = 1.57, df = 1, 13, P = 0.14) or the LC_{90} values (t = -0.27, df = 1, 13, P = 0.79).

In experiments testing the repeatability of this susceptibility monitoring method, LC_{50} and LC_{90} values were consistent across the three sets of bioassays for each site (Table 8). Of the

thirty sets of assays run to explore repeatability, only spinosad tested on flies from Site 18 were found to have fiducial limits that did not overlap across all three sets.

Testing *D. suzukii* susceptibility to spinosad and zeta-cypermethrin in the larger glass jars resulted in very similar levels of mortality to those found in the smaller scintillation vial bioassays. Average mortality values were within the fiducial limits for colonies tested using the scintillation vials, shown in Table 1. The LC_{50} and LC_{90} values and the corresponding fiducial limits for the two colonies tested were similar for spinosad (Lab colony LC_{50} = 19.5 [12.0, 30.1], Site 18 LC_{50} = 16.0 [no fiducial limits], Lab colony LC_{90} = 100.6 [60.7, 215.7], Site 18 LC_{90} = 105.6 [no fiducial limits]) and zeta-cypermethrin (Lab colony LC_{50} = 0.17 [0.14, 0.20], Site 18 LC_{50} = 0.17 [0.14, 0.20], Lab colony LC_{90} = 0.34 [0.28, 0.45], Site 18 LC_{90} = 0.31 [0.26, 0.40]).

4 DISCUSSION

In this study we determined dose-mortality relationships of *D. suzukii* to five insecticides from four chemical classes that are most commonly used for the control of this pest, enabling determination of the LC_{50} and LC_{90} values. As expected due to their smaller body size, male flies were killed at lower concentrations than females, as in other insect species such as *Drosophila melanogaster* Meigen[23], *Rhagoletis pomonella* Walsh[24], and many other species.[25] The extent of this difference depended on the insecticide, with greater sex-based differences for malathion and zeta-cypermethrin than spinetoram and spinosad. Comparing the Michigan and Georgia colonies,

dose-response relationships were similar and significant differences were generally small in magnitude and may reflect the natural variability in populations.

For most colonies and most insecticides there was little difference in susceptibility between populations collected from managed farms and those collected from unmanaged sites, indicating that populations of *D. suzukii* in these two regions have retained susceptibility to insecticides. Although exposed to insecticides during crop protection, there is a relatively high amount of unmanaged land surrounding most farms in these regions,[26,27] providing opportunities for exchange of susceptible genes. Farms within contiguous production of susceptible fruit and without adjacent wild hosts are therefore expected to have the highest likelihood of developing resistance, as found recently for spinosad susceptibility in an area of intensive berry production[28].

In the Michigan populations of *D. suzukii* tested in this study, the LC_{50} and LC_{90} values increased for spinetoram between 2016 and 2018, with resistance ratios as high as 4.1 (Table 5). This change was particularly evident in populations from Sites 12, 15, 18, and 20 suggesting that those sites should be a focus of future monitoring using the diagnostic doses. This insecticide is the slowest acting of those we tested, and we found wider fiducial limits from the probit analyses, so while these results highlight the value of long-term resistance monitoring they also should not be seen as indicating failure of this insecticide to control *D. suzukii* without confirmation from more detailed follow up testing.

Any successful resistance monitoring program developed for *D. suzukii* will need to be easy and efficient to use in order to be adapted to multiple monitoring situations. Resistance monitoring should ideally be deployed broadly to detect resistance in populations before it becomes widespread.[15] This can only be accomplished through the investment of significant monetary resources, or by using a less expensive method for quick initial screening of populations. We developed and tested a monitoring method that meets these easier to use criteria. The bioassays in this study (20 hour vial drying time and fly health assessments at 6 or 8 hours after adding flies to vials) were designed to maximize efficiency and ease of use by 1-2 people. The glass container can be treated in the afternoon, left to dry overnight, then flies can be added the next morning and mortality assessments completed that same day. Previous studies that have used a glass vial bioassay technique for *D. suzukii* used a hot dog roller to dry residues in the vials.[8,9] While this ensures an even coating of insecticide residue inside the vials it also necessitates purchase of specialized equipment and the vials take time to dry. By using spreading the solution inside the vial and tapping out excess, we eliminated this barrier. Also, in Georgia all experimental flies were sorted using a low-cost mouth aspirator rather than with a CO_2 gun and pad that require a CO_2 tank, further simplifying the method and keeping the cost down. Having an easy method with inexpensive equipment makes it more likely to be adopted by crop scouts, extension agents, and growers to conduct resistance testing. As our last experiment showed, this bioassay method is also robust enough to be run in commonly-available glass jars, making it even more accessible to those without access to specialized glass vials.

This bioassay method is much more efficient to implement than the Potter spray tower method reported by Van Timmeren et al.[11] As a comparison, all bioassays in Michigan in 2017 were conducted on F1 flies within two weeks of field collection, whereas the Potter spray tower bioassays required 3-11 generations to successfully complete testing due to the labor intensive method of testing the flies. This allows for testing soon after parent flies are collected from the field, minimizing the risk of resistant populations not being detected due to loss of selection pressure while multiple generations are reared in the laboratory.[29] While this method is quicker to implement, there is a risk of variability in results within and among populations. Any populations where potential resistance is suspected will need follow up testing with detailed laboratory experiments, including against established laboratory colonies, to better quantify susceptibility to insecticides. Additionally, selecting for increased survival against insecticides could provide definitive proof of resistance, as found recently by Gress and Zalom for *D. suzukii* collected in central coast California.[28]

Mortality assessments for most of this study were conducted at 6 hours after flies were added to the vials. This timing allows for vials to be loaded and assessed on the same day, thus eliminating the need for extra steps to reduce control mortality that are common in bioassays conducted over longer periods of time. Having a method that can be conducted in one day using a simple glass vial should also increase the chance of adoption. In this study there were instances where male control mortality exceeded 20%, however, control mortality for females remained consistently low for both states in all three years (<7%). Thus, this method can be utilized for

females without concern for control mortality affecting the results, and for males with an awareness of the risk of control mortality. If monitoring of males is desired, small portions of fly diet or moistened dental wicking could be added to vials to reduce male mortality (Van Timmeren, unpublished data).

The 6 hour assessment time was consistent for all insecticides except spinosad. The slower acting nature of spinosad[30] meant that the 6 hour point was at the threshold where flies start exhibiting symptoms of toxicity, and so an 8 hour timing was used and is recommended to ensure the full effect of the insecticide is reflected in the results.

The use of diagnostic doses is common in resistance monitoring programs and there are established methods for selecting these levels.[15] Testing one or two diagnostic concentrations allows for quicker initial testing of multiple populations, and then suspect populations can subsequently be tested in more detail to determine whether there is resistance in a population. Our study provides diagnostic doses for use in widespread resistance monitoring programs for *D. suzukii* by employing this easy to use method. Based on the results of this study, two diagnostic doses were used to test *D. suzukii* in multiple states in 2017.[31] These tests resulted in the first detection of insecticide resistant *D. suzukii* from California,[28] validating this approach as an effective method for monitoring populations of this species for resistance.

The method developed and tested in this study provides a simple, effective, and reliable means for monitoring the susceptibility of *D. suzukii* to different insecticides to support insecticide resistance management (IRM) programs.[32,33] Where resistance has not been detected,

IRM programs can be proactive in nature, including monitoring for early detection of resistance and implementation of practices that can reduce the chance that resistance develops in populations. Where resistance has been detected, this bioassay could be used to monitor populations to determine the severity, distribution, and stability of resistance, as well as implementing practices to reduce the presence of resistance alleles in populations. Additional research is needed to determine how best to prevent and react to resistance in *D. suzukii* populations. This is extremely important if currently used insecticides are to remain effective components of *D. suzukii* management programs.

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Table 1. Average LC50 and LC90 values for glass vial residue bioassays conducted with five insecticides. *Drosophila suzukii* populations from Michigan and Georgia were tested for susceptibility in 2016, 2017, and 2018. Adult male and female flies were tested and mortality was assessed at 6 hours after exposure to residues or 8 hours for spinosad in 2017 and 2018.

	Georgia				Michigan			
	Males		Females		Males		Females	
Insecticide	LC50 (mg L^{-1})	LC90 (mg L^{-1})	LC50 (mg L^{-1})	LC90 (mg L^{-1})	LC50 (mg L^{-1})	LC90 (mg L^{-1})	LC50 (mg L^{-1})	LC90 (mg L^{-1})
2016								
malathion	3.4 ± 0.6	8.4 ± 2.0	5.2 ± 1.2	14.3 ± 3.7	3.1 ± 0.1	7.8 ± 1.0	5.3 ± 0.2	11.3 ± 1.5
methomyl								
spinetoram	4.0 ± 3.0	26.4 ± 10.0	4.5 ± 1.5	36.3 ± 14.2	12.5 ± 1.7	77.6 ± 15.9	16.2 ± 1.7	104.2 ± 20.2

spinosad					10.0 ± 1.4	100.4 ± 14.0	16.3 ± 1.9	103.8 ± 15.8
zeta-cypermethrin	0.1 ± 0.02	0.4 ± 0.2	0.1 ± 0.05	0.5 ± 0.2	0.1 ± 0.007	0.4 ± 0.04	0.2 ± 0.01	0.9 ± 0.06
2017								
malathion	2.9	5.0	5.1 ± 0.3	14.6 ± 2.1	3.8 ± 0.4	10.1 ± 1.7	7.1 ± 0.4	14.4 ± 1.2
methomyl					0.4 ± 0.02	1.5 ± 0.2	0.6 ± 0.03	2.1 ± 0.3
spinetoram					18.5 ± 2.3	164.6 ± 45.6	24.1 ± 1.8	108.7 ± 12.5
spinosad	128.0	5981.0	83.0 ± 44.2	806.8 ± 459.2	7.2 ± 1.4	810.5 ± 454.2	13.1 ± 1.3	291.5 ± 90.3
zeta-cypermethrin	0.3 ± 0.04	1.5 ± 0.9	1.5 ± 0.06	4.2 ± 1.5	0.07 ± 0.01	0.5 ± 0.1	0.1 ± 0.01	0.8 ± 0.1
2018								
malathion							3.4 ± 0.1	9.1 ± 0.6
methomyl							0.2 ± 0.01	0.5 ± 0.04
spinetoram							75.0 ± 4.9	669.3 ± 97.6
spinosad							40.1 ± 4.8	476.2 ± 110.2
zeta-cypermethrin							0.1 ± 0.01	0.4 ± 0.04

Table 2. Results of adult female residue bioassays of four insecticides including malathion, spinosad, spinosad, and zeta-cypermethrin. *Drosophila suzukii* populations from Georgia were tested for susceptibility in 2016 and 2017. Mortality was recorded at 6 hours after exposure to insecticide residues. Site 1 is used as the standard susceptible site for resistance ratio calculations for all insecticides in both years.

Site	Field management	n	Slope ± S.E.	LC_{50} (mg L^{-1})	95% fiducial limits	LC_{90} (mg L^{-1})	95% fiducial limits	X^2	RR50	RR90
Malathion 2016										
1	Unsprayed	153	2.6 ± 1.4	5.3	†	16.1	†	<0.0001	1.0	1.0
2	Unsprayed									
3	Organic	96	5.8 ± 17.2	4.2	†	7.0	†	<0.0001	0.8	0.4
4	Organic	90	2.3 ± 0.5	3.1	(2.0, 4.4)	11.3	(7.3, 28.0)	0.874	0.6	0.7
5	Conv	92	2.8 ± 0.6	9.9	(7.2, 14.9)	27.8	(17.6, 74.6)	0.646	1.9	1.7
6	Conv	90	2.8 ± 1.4	3.3	†	9.4	†	0.0003	0.6	0.6
Malathion 2017										
1	Unsprayed	227	1.3 ± 0.4	3.8	(1.1, 1177)	39.1	(5.9, 6.4 x 10^7)	0.003	1.0	1.0
4	Organic	108	3.0 ± 0.6	3.2	(1.9, 5.8)	8.6	(4.9, 24.3)	0.923	0.8	0.2
7	Conv	98	2.1 ± 0.7	0.8	†	3.2	†	0.014	0.2	0.1
8	Conv	95	2.4 ± 0.6	0.6	(0.5, 1.3)	2.6	(1.4, 11.6)	0.987	0.2	0.1
Spinetoram 2016										
1	Unsprayed	60	1.5 ± 0.4	7.9	(4.1, 19.7)	53.7	(21.0, 611.6)	0.292	1.0	1.0
2	Unsprayed									
3	Organic	57	2.3 ± 0.6	1.0	(0.5, 1.8)	3.7	(2.0, 17.0)	0.941	0.1	0.1
4	Organic									
5	Conv	58	2.2 ± 0.6	6.0	(3.3, 11.1)	22.5	(12.0, 97.1)	0.385	0.8	0.4
6	Conv	60	1.0 ± 0.6	3.1	†	65.4	†	0.005	0.4	1.2
Spinosad 2017										
1	Unsprayed	92	1.0 ± 0.4	25.6	†	568.8	†	0.012	1.0	1.0
5	Conv	86	2.7 ± 0.5	53.5	(35.3, 78.0)	157.7	(103.5, 339.2)	0.649	2.1	0.3
8	Conv	181	1.3 ± 0.7	169.9	†	1694.0	†	<0.0001	6.6	3.0
Zeta-cypermethrin 2016										
1	Unsprayed	151	1.9 ± 0.3	0.3	(0.2, 0.4)	1.2	(0.7, 2.8)	0.358	1.0	1.0
2	Unsprayed	61	3.1 ± 0.8	0.1	(0.1, 0.2)	0.4	(0.2, 1.4)	0.896	0.5	0.3
3	Organic		Not tested							
4	Organic	63	3.1 ± 0.8	0.1	(0.1, 0.2)	0.4	(0.2, 1.3)	0.943	0.5	0.3
5	Conv		Not tested							
6	Conv	60	1.9 ± 1.0	0.03	†	0.1	†	0.646	0.1	0.1
Zeta-cypermethrin 2017										
1	Unsprayed	120	3.98 ± 4.6	1.1	†	2.3	†	<0.0001	1.0	1.0
4	Organic	108	2.96 ± 0.6	3.2	(1.9, 5.8)	8.6	(4.9, 24.3)	0.923	2.9	3.7
7	Conv	98	2.06 ± 0.7	0.8	†	3.2	†	0.014	0.7	1.4
8	Conv	95	2.41 ± 0.6	0.8	(0.5, 1.3)	2.6	(1.4, 11.6)	0.987	0.7	1.1

† Fiducial limits could not be calculated.

Table 3. Results of adult female residue bioassays of malathion insecticide. *Drosophila suzukii* populations from Michigan were tested for susceptibility in 2016, 2017, and 2018. Mortality was recorded at 6 hours after exposure to insecticide residues. Site 1 is used as the standard susceptible site for resistance ratio calculations in each of the three years.

Site	Field management	n	Slope ± S.E.	LC_{50} (mg L^{-1})	95% fiducial limits	LC_{90} (mg L^{-1})	95% fiducial limits	X^2	RR50	RR90
2016										
1	Unsprayed	80	4.2 ± 1.1	5.4	(4.2, 7.0)	10.9	(8.1, 24.1)	0.748	1.0	1.0
2	Unsprayed	80	5.7 ± 2.7	4.2	†	7.1	†	0.065	0.8	0.7
3	Unsprayed	120	1.7 ± 087	5.1	†	27.6	†	0.008	0.9	2.5
4	Unsprayed	121	4.4 ± 0.9	4.6	(3.7, 5.5)	9.0	(7.2, 14.0)	0.109	0.9	0.8
5	Organic	120	4.4 ± 0.9	6.8	(5.7, 8.5)	13.2	(10.0, 24.3)	0.847	1.3	1.2
6	Organic	120	3.9 ± 0.8	4.9	(3.9, 5.9)	10.3	(8.0, 17.8)	0.666	0.9	0.9
7	Organic	120	3.9 ± 1.3	4.3	(1.0, 9.2)	9.2	(5.6, 6703)	0.031	0.8	0.8
8	Conv	120	8.9 ± 2.8	4.6	(0.002, 12.1)	6.4	(4.9, 1.3×10^{26})	0.072	0.9	0.6
9	Conv	120	5.5 ± 1.1	5.5	(4.7, 6.5)	9.4	(7.7, 14.6)	0.380	1.0	0.9
10	Conv	120	7.0 ± 1.4	5.6	(4.9, 6.5)	8.6	(7.3, 11.8)	0.377	1.0	0.8
11	Conv	120	9.3 ± 2.1	6.1	(5.4, 6.9)	8.4	(7.3, 11.2)	0.525	1.1	0.8
12	Conv	120	3.0 ± 1.0	7.0	(3.5, 40.1)	18.6	(9.4, 26387)	0.008	1.3	1.7
13	Conv	120	4.0 ± 2.4	5.1	†	10.7	†	<0.0001	0.9	1.0
14	Conv	120	3.9 ± 1.5	4.4	†	9.3	†	0.007	0.8	0.9
2017										
1	Unsprayed	139	4.7 ± 1.5	5.7	(3.5, 13.6)	10.7	(7.1, 853.8)	0.033	1.0	1.0
2	Unsprayed	162	5.0 ± 4.6	7.7	†	13.8	†	<0.0001	1.3	1.3
15	Unsprayed	189	6.1 ± 0.9	6.7	(6.0, 7.5)	10.8	(9.2, 14.2)	0.937	1.2	1.0
4	Unsprayed	165	4.5 ± 2.2	8.4	†	16.2	†	<.0001	1.5	1.5
5	Organic	157	3.3 ± 1.3	6.7	(2.8, 1.1×10^{7})	16.6	(8.5, 2.9×10^{37})	0.0003	1.2	1.6
6	Organic		Not tested							
7	Organic	154	3.9 ± 2.0	5.3	†	11.4	†	<0.0001	0.9	1.1
8	Conv	151	3.2 ± 2.5	8.5	†	21.6	†	0.056	1.5	2.0
16	Conv	143	6.4 ± 1.2	7.6	(6.7, 9.1)	12.0	(9.9, 17.5)	0.158	1.3	1.1
17	Conv	201	4.4 ± 1.4	6.0	(4.0, 13.0)	11.7	(7.7, 357.1)	0.003	1.1	1.1
12	Conv	78	3.5 ± 1.0	8.2	(6.2, 15.2)	19.1	(11.7, 107.6)	0.305	1.4	1.8
2018										
1	Unsprayed	251	2.6 ± 0.5	3.2	(2.2, 5.4)	10.0	(5.8, 41.7)	0.023	1.0	1.0
5	Unsprayed	175	3.7 ± 0.7	2.3	(1.8, 2.8)	5.1	(4.0, 7.9)	0.176	0.7	0.5
15	Unsprayed	227	4.8 ± 0.7	4.0	(3.5, 4.6)	7.4	(6.2, 10.0)	0.204	1.2	0.7
18	Unsprayed	628	2.5 ± 0.5	3.6	(2.4, 5.3)	11.7	(7.2, 39.5)	<0.0001	1.1	1.2
19	Unsprayed	348	2.4 ± 0.5	3.0	(1.9, 4.8)	10.4	(6.0, 42.6)	0.007	0.9	1.0
8	Conv	168	2.5 ± 0.6	3.6	(2.2, 6.8)	11.7	(6.3, 95.8)	0.072	1.1	1.2
12	Conv	262	2.3 ± 0.5	3.1	(1.8, 4.9)	11.2	(6.6, 43.4)	0.050	1.0	1.1
16	Conv	223	3.0 ± 0.8	3.4	(1.6, 6.9)	8.9	(5.0, 126.8)	0.001	1.0	0.9
17	Conv	222	2.8 ± 0.4	3.6	(2.9, 4.4)	10.2	(7.6, 16.5)	0.843	1.1	1.0
20	Conv	254	5.4 ± 1.5	4.4	(2.5, 6.7)	7.6	(5.5, 44.2)	0.007	1.4	0.8
21	Conv	654	2.4 ±0.3	2.9	(2.2, 3.8)	10.0	(6.7, 21.8)	0.060	0.9	1.1
22	Conv	755	2.3 ± 0.5	3.2	(2.0, 5.6)	11.4	(6.2, 82.2)	0.0007	1.0	1.1

23	Conv	190	4.2 ± 4.3	3.4	†	6.9	†	<0.0001	1.1	0.7
24	Conv	199	5.2 ± 0.8	3.9	(3.5, 4.5)	6.9	(5.8, 9.4)	0.173	1.2	0.7
25	Conv	252	4.4 ± 0.6	3.5	(3.0, 4.0)	6.8	(5.7, 8.9)	0.808	1.1	0.7

† Fiducial limits could not be calculated.

Table 4. Results of adult female residue bioassays of methomyl insecticide. *Drosophila suzukii* populations from Michigan were tested for susceptibility in 2017 and 2018. Mortality was recorded at 6 hours after exposure to insecticide residues. Site 1 is used as the standard susceptible site for resistance ratio calculations in both years.

Site	Field management	n	Slope ± S.E.	LC_{50} (mg L^{-1})	95% fiducial limits	LC_{90} (mg L^{-1})	95% fiducial limits	X^2	RR50	RR90
2017										
1	Unsprayed	141	2.6 ± 0.6	0.5	(0.3, 1.0)	1.6	(0.8, 14.1)	0.090	1.0	1.0
2	Unsprayed	155	3.4 ± 0.6	0.8	(0.7, 1.1)	1.9	(1.4, 3.8)	0.223	1.6	1.2
15	Unsprayed	156	3.3 ± 3.5	0.6	†	1.4	†	<0.0001	1.1	0.9
4	Unsprayed	152	1.8 ± 1.0	0.8	†	4.0	†	<0.0001	1.5	2.6
5	Organic	150	1.6 ± 0.7	0.6	†	4.0	†	0.0001	1.3	2.5
6	Organic	58	3.1 ± 0.9	0.6	(0.5, 1.4)	1.7	(0.9, 13.1)	0.924	1.3	1.1
7	Organic	139	3.3 ± 1.1	0.6	(0.3, 3.3)	1.5	(0.8, 575.3)	0.003	1.2	1.0
8	Conv	154	2.9 ± 0.4	0.7	(0.6, 0.9)	1.9	(1.4, 3.4)	0.944	1.4	1.2
16	Conv	149	2.4 ± 0.7	0.6	(0.2, 3.3)	2.0	(0.9, 17201)	0.041	1.2	1.3
17	Conv	109	4.1 ± 0.9	0.7	(0.5, 0.9)	1.3	(0.9, 3.2)	0.200	1.3	0.9
12	Conv		Not tested							
2018										
1	Unsprayed	162	3.0 ± 0.4	0.2	(0.1, 0.2)	0.5	(0.4, 0.7)	0.123	1.0	1.0
5	Unsprayed	171	3.0 ± 0.9	0.2	(0.03, 1.1)	0.6	(0.3, 13375.0)	0.023	1.3	1.3
15	Unsprayed	204	3.0 ± 0.4	0.2	(0.1, 0.2)	0.5	(0.4, 0.7)	0.428	1.0	1.1
18	Unsprayed	783	2.7 ± 0.3	0.1	(0.1, 0.2)	0.4	(0.3, 0.7)	0.015	0.8	0.9
19	Unsprayed	318	2.7 ± 0.6	0.1	0.05, 0.2)	0.3	(0.2, 2.1)	0.044	0.6	0.7
8	Conv	292	2.8 ± 0.3	0.2	(0.1, 0.2)	0.5	(0.4, 0.7)	0.188	1.0	1.1
12	Conv	295	3.9 ± 1.9	0.2	†	0.5	†	0.013	1.3	1.0
16	Conv	154	3.5 ± 0.6	0.1	(0.1, 0.2)	0.3	(0.3, 0.5)	0.483	0.8	0.7
17	Conv	158	2.1 ± 0.5	0.1	(0.04, 0.1)	0.3	(0.2, 0.8)	0.235	0.5	0.7
20	Conv	357	2.7 ± 0.6	0.2	(0.1, 0.4)	0.6	(0.4, 2.3)	0.0001	1.2	1.3
21	Conv	733	1.8 ± 0.4	0.2	(0.1, 0.4)	0.9	(0.4, 15.6)	0.002	1.0	1.8
22	Conv	657	2.0 ± 0.4	0.1	(0.05, 0.2)	0.5	(0.3, 1.9)	0.0008	0.6	1.0
23	Conv	116	4.4 ± 1.1	0.2	(0.1, 0.3)	0.5	(0.3, 0.7)	0.890	1.3	1.0
24	Conv	198	5.3 ± 3.1	0.3	†	0.5	†	0.0002	1.5	1.0
25	Conv	188	4.1 ± 2.6	0.2	†	0.5	†	0.123	1.4	1.0

† Fiducial limits could not be calculated.

Table 5. Results of adult female residue bioassays of spinetoram insecticide. *Drosophila suzukii* populations from Michigan were tested for susceptibility in 2016, 2017, and 2018. Mortality was recorded at 6 hours after exposure to insecticide residues. Site 1 is used as the standard susceptible site for resistance ratio calculations in each of the three years.

Site	Field management	n	Slope ± S.E.	LC_{50} (mg L^{-1})	95% fiducial limits	LC_{90} (mg L^{-1})	95% fiducial limits	X^2	RR50	RR90
2016										
1	Unsprayed	80	1.8 ± 0.4	24.5	(14.7, 44.9)	129.7	(64.9, 504.0)	0.820	1.0	1.0
2	Unsprayed	120	1.7 ± 0.5	21.5	(6.0, 3054.0)	123.0	(32.4, 9.7 x 10^9)	0.020	0.9	1.0
3	Unsprayed	120	1.2 ± 0.2	24.3	(14.4, 44.3)	260.3	(114.8, 1208.0)	0.724	1.0	2.0
4	Unsprayed	120	1.0 ± 0.2	13.4	(7.0, 25.7)	241.7	(92.4, 1712.0)	0.387	0.5	1.9
5	Organic	120	1.7 ± 0.3	23.4	(14.9, 37.3)	136.5	(74.8, 400.6)	0.178	1.0	1.0
6	Organic	120	1.7 ± 0.3	10.4	(6.8, 16.8)	57.0	(30.9, 186.0)	0.671	0.4	0.4
7	Organic	120	2.7 ± 0.5	11.8	(8.6, 17.4)	35.6	(22.7, 85.3)	0.204	0.4	0.4
8	Conv	120	3.3 ± 0.7	12.8	(9.6, 18.2)	31.2	(21.1, 71.4)	0.409	0.5	0.2
9	Conv	120	1.6 ± 0.3	6.6	(4.0, 10.3)	41.6	(23.7, 113.3)	0.215	0.3	0.3
10	Conv	121	3.0 ± 0.6	11.4	(8.5, 16.3)	30.7	(20.4, 69.3)	0.321	0.5	0.2
11	Conv	119	1.5 ± 0.3	17.3	(10.9, 28.8)	123.3	(63.3, 405.6)	0.241	0.7	1.0
12	Conv	120	1.7 ± 0.3	25.6	(16.8, 41.3)	140.5	(77.2, 394.6)	0.709	1.1	1.1
13	Conv	120	2.1 ± 0.4	9.5	(6.5, 14.2)	39.1	(23.5, 124.2)	0.910	0.4	0.3
14	Conv	120	‡							
2017										
1	Unsprayed	160	2.2 ± 0.5	16.6	(12.1, 24.6)	62.2	(37.5, 180.2)	0.190	1.0	1.0
2	Unsprayed	183	2.3 ± 0.4	18.5	(13.9, 26.6)	68.6	(42.4, 171.5)	0.275	1.1	1.1
15	Unsprayed	156	2.9 ± 0.6	26.4	(19.8, 40.1)	74.0	(46.7, 193.7)	0.142	1.6	1.2
4	Unsprayed	155	2.9 ± 0.5	33.8	(25.1, 48.8)	94.4	(62.2, 195.0)	0.658	2.0	1.5
5	Organic	125	1.9 ± 0.3	22.1	(14.8, 33.4)	104.7	(61.1, 283.4)	0.860	1.3	1.7
6	Organic		Not tested							
7	Organic	125	1.9 ± 0.3	30.6	(20.4, 48.6)	150.4	(85.1, 400.3)	0.738	1.8	2.4
8	Conv	194	1.5 ± 0.4	22.2	(8.1, 115.0)	159.8	(47.9, 24950)	0.003	1.3	2.4
16	Conv	137	1.9 ± 0.3	24.9	(17.2, 38.5)	115.7	(67.1, 294.3)	0.300	1.5	1.9
17	Conv	184	1.5 ± 0.2	21.4	(14.9, 31.2)	149.1	(88.5, 332.0)	0.160	1.3	2.4
12	Conv		Not tested							
2018										
1	Unsprayed	265	1.9 ± 0.5	74.3	(26.6, 211.1)	348.7	(140.5, 5746.0)	<0.0001	1.0	1.0
5	Unsprayed	180	1.6 ± 0.4	61.0	(18.4, 250.5)	366.9	(120.7, 23087.0)	0.0002	0.8	1.1
15	Unsprayed	182	1.1 ± 0.2	81.9	(33.9, 232.8)	1103.0	(340.0, 28028.0)	0.049	1.1	3.2
18	Unsprayed	746	1.0 ± 0.1	59.6	(36.3, 110.8)	1055.0	(409.9, 6174.0)	0.025	0.8	3.0
19	Unsprayed	245	1.1 ± 0.2	46.1	(18.9, 141.7)	616.7	(181.6, 26555.0)	0.035	0.6	1.8
8	Conv	230	1.9 ± 0.3	88.9	(52.4, 156.1)	417.1	(221.8, 1335.0)	0.097	1.2	1.2
12	Conv	166	1.1 ± 0.4	108.0	(25.3, 2388.0)	1421.0	(271.2, 2.8 x 10^8)	0.0007	1.5	4.1
16	Conv	175	1.7 ± 0.3	80.1	(38.1, 189.0)	453.0	(191.3, 3646.0)	0.032	1.1	1.3
17	Conv	263	1.6 ± 0.4	93.3	(40.4, 262.2)	575.2	(218.3, 8680.0)	0.0003	1.3	1.7
20	Conv	516	1.1 ± 0.2	91.7	(46.4, 216.8)	1379.0	(468.3, 13898.0)	0.0003	1.2	4.0
21	Conv	497	1.5 ± 0.2	70.4	(42.8, 124.2)	487.3	(240.1, 1773.0)	0.002	1.0	1.4
22	Conv	402	1.4 ± 0.1	54.0	(41.7, 70.6)	433.9	(288.9, 748.9)	0.572	0.7	1.2

23	Conv	184	1.5 ± 0.2	59.4	(40.0, 92.1)	406.2	(222.5, 1123.0)	0.154	0.8	1.2
24	Conv	186	1.6 ± 0.3	54.1	(28.7, 103.4)	323.7	(155.1, 1423.0)	0.086	0.7	0.9
25	Conv	403	1.6 ± 0.2	102.0	(64.9, 170.9)	653.2	(341.4, 1974.0)	0.043	1.4	1.9

† Fiducial limits could not be calculated.

‡ Control mortality exceeded 20%.

Table 6. Results of adult female residue bioassays of spinosad insecticide. *Drosophila suzukii* populations from Michigan were tested for susceptibility in 2016, 2017, and 2018. Mortality was recorded at 6 hours (2016) or 8 hours (2017 and 2018) after exposure to insecticide residues. Site 1 is used as the standard susceptible site for resistance ratio calculations in each of the three years.

Site	Field management	n	Slope ± S.E.	LC_{50} (mg L^{-1})	95% fiducial limits	LC_{90} (mg L^{-1})	95% fiducial limits	X^2	RR50	RR90
2016										
1	Unsprayed	80	1.6 ± 0.3	30.7	(16.3, 55.2)	194.5	(98.6, 662.2)	0.753	1.0	1.0
2	Unsprayed	70	1.0 ± 0.4	11.9	†	223.4	†	0.030	0.4	1.2
3	Unsprayed	120	1.6 ± 0.2	11.3	(6.6, 18.4)	73.6	(41.0, 185.2)	0.143	0.4	0.4
4	Unsprayed	120	2.7 ± 0.6	14.5	(9.5, 21.7)	43.2	(27.5, 109.7)	0.575	0.5	0.2
5	Organic	120	2.0 ± 0.5	8.3	(2.0, 27.1)	36.0	(13.8, 1267.0)	0.066	0.3	0.2
6	Organic	119	1.6 ± 0.3	17.5	(10.4, 28.8)	105.9	(57.8, 299.2)	0.448	0.6	0.5
7	Organic	120	1.6 ± 0.3	10.7	(6.2, 17.6)	66.8	(36.7, 180.5)	0.317	0.4	0.3
8	Conv	121	1.5 ± 0.3	24.7	(14.7 ,41.6)	169.3	(88.0, 541.7)	0.242	0.8	0.9
9	Conv	137	2.0 ± 0.3	25.0	(15.6, 37.4)	109.0	(68.0, 361.8)	0.274	0.8	0.6
10	Conv	120	1.8 ± 0.3	15.0	(9.2, 24.0)	77.4	(44.4, 196.1)	0.730	0.5	0.4
11	Conv	120	1.7 ± 0.3	23.6	(13.7 ,38.4)	134.8	(74.0, 413.3)	0.173	0.8	0.7
12	Conv	119	1.5 ± 0.2	16.1	(9.5, 26.5)	113.9	(62.3, 291.4)	0.162	0.5	0.6
13	Conv	120	2.1 ± 0.6	7.6	(1.5, 27.1)	31.4	(11.8, 100.5)	0.047	0.3	0.2
14	Conv	119	‡							
2017										
1	Unsprayed	229	0.6 ± 0.2	5.1	(0.3, 13.6)	480.6	(159.3, 13888.0)	0.482	1.0	1.0
2	Unsprayed	239	1.3 ± 0.4	17.1	(0, 95.5)	179.3	(46.5,1.0 x 10^{26})	0.0001	3.4	0.4
15	Unsprayed	192	2.3 ± 1.0	9.0	†	33.3	†	0.070	1.8	0.1
4	Unsprayed	290	1.2 ± 0.2	17.0	(10.3, 26.1)	202.9	(107.6, 611.5)	0.241	3.4	0.4
5	Organic	279	1.3 ± 0.3	16.8	(3.7, 46.4)	162.4	(55.5, 10177.0)	0.002	3.3	0.3
6	Organic	149	0.7 ± 0.2	16.5	(3.1, 35.7)	973.4	(256.4, 101124)	0.479	3.3	2.0
7	Organic	157	1.2 ± 0.2	9.9	(4.4, 17.0)	111.9	(59.7, 336.2)	0.683	1.9	0.2
8	Conv	258	1.1 ± 0.2	15.0	(4.0, 33.4)	200.7	(81.8, 1406.0)	0.015	3.0	0.4
16	Conv	327	1.5 ± 0.3	10.1	(0.9, 26.5)	74.9	(28.1, 7411.1)	0.035	2.0	0.2
17	Conv	179	0.8 ± 0.2	14.4	(1.2, 40.0)	495.5	(151.9, 18391.0)	0.070	2.8	1.0
12	Conv		Not tested							
2018										
1	Unsprayed	286	1.3 ± 0.3	46.0	(13.3, 121.0)	451.8	(159.8, 8112.0)	0.002	1.0	1.0
5	Unsprayed	208	1.1 ± 0.2	37.2	(20.1, 66.5)	507.1	(226.6, 2245.0)	0.159	0.8	1.1
15	Unsprayed	245	1.6 ± 0.3	32.7	(13.7, 61.9)	212.2	(106.7, 719.4)	0.038	0.7	0.5
18	Unsprayed	817	1.7 ± 0.3	27.9	(14.5, 47.3)	165.4	(91.7, 439.9)	<0.0001	0.6	0.4
19	Unsprayed	440	1.2 ± 0.2	11.0	(2.7, 24.8)	120.6	(51.9, 622.6)	0.001	0.2	0.3
8	Conv	279	1.6 ± 0.2	28.3	(18.8 ,40.0)	175.9	(113.9 ,343.0)	0.292	0.6	0.4
12	Conv	226	1.4 ± 0.2	23.3	(14.9, 34.5)	187.7	(112.7, 407.2)	0.138	0.5	0.4
16	Conv	278	1.5 ± 0.2	31.5	(21.0, 44.6)	214.5	(140.9, 385.9)	0.169	0.7	0.5
17	Conv	251	1.4 ± 0.3	34.0	(10.3, 69.8)	293.7	(131.1, 2017.0)	0.060	0.7	0.7
20	Conv	419	1.0 ± 0.2	68.5	(27.1, 139.4)	1386.0	(514.7, 13151.0)	0.005	1.5	3.1
21	Conv	551	1.3 ± 0.1	24.5	(14.8, 37.2)	237.1	(146.4, 462.9)	0.060	0.5	0.5
22	Conv	676	1.4 ± 0.1	60.5	(45.9, 77.6)	536.0	(386.7, 810.6)	0.450	1.3	1.2

23	Conv	185	0.8 ± 0.6	40.9	†	1433.0	†	<0.0001	0.9	3.2
24	Conv	317	1.6 ± 0.2	56.5	(40.1, 76.3)	347.1	(241.9, 559.8)	0.774	1.2	0.8
25	Conv	260	1.2 ± 0.1	79.4	(53.8, 114.2)	875.1	(521.6, 1852.0)	0.255	1.7	1.9

† Fiducial limits could not be calculated.

‡ Control mortality exceeded 20%.

Table 7. Results of adult female residue bioassays of zeta-cypermethrin insecticide. *Drosophila suzukii* populations from Michigan were tested for susceptibility in 2016, 2017, and 2018. Mortality was recorded at 6 hours after exposure to insecticide residues. Site 1 is used as the standard susceptible site for resistance ratio calculations in each of the three years.

Site	Field management	n	Slope ± S.E.	LC_{50} (mg L^{-1})	95% fiducial limits	LC_{90} (mg L^{-1})	95% fiducial limits	X^2	RR50	RR90
2016										
1	Unsprayed	120	2.3 ± 0.6	0.3	(0.1, 1.1)	1.3	(0.5, 83.1)	0.037	1.0	1.0
2	Unsprayed	80	2.1 ± 0.7	0.2	(0, 0.7)	0.7	(0.3, 4.3 x 10^5)	0.091	0.6	0.6
3	Unsprayed	121	2.4 ± 0.4	0.2	(0.2, 0.3)	0.7	(0.5, 1.4)	0.244	0.7	0.6
4	Unsprayed	120	2.1 ± 0.4	0.2	(0.1, 0.3)	0.7	(0.5, 1.5)	0.656	0.6	0.6
5	Organic	160	1.9 ± 0.3	0.3	(0.2, 0.3)	1.1	(0.7, 2.3)	0.691	0.7	0.9
6	Organic	120	1.9 ± 0.4	0.2	(0.1, 0.2)	0.7	(0.4, 1.9)	0.382	0.4	0.5
7	Organic	160	2.3 ± 0.3	0.3	(0.2, 0.3)	0.9	(0.6, 1.6)	0.389	0.7	0.7
8	Conv	120	2.3 ± 0.4	0.2	(0.2, 0.3)	0.9	(0.6, 1.8)	0.386	0.7	0.7
9	Conv	160	2.0 ± 0.3	0.2	(0.2, 0.3)	1.0	(0.6, 1.9)	0.397	0.7	0.8
10	Conv	120	2.1 ± 0.3	0.3	(0.2, 0.4)	1.0	(0.6, 2.3)	0.300	0.8	0.9
11	Conv	120	2.7 ± 0.4	0.3	(0.2, 0.4)	0.8	(0.5, 1.6)	0.394	0.8	0.6
12	Conv	120	1.8 ± 0.3	0.3	(0.2, 0.4)	1.3	(0.8, 3.2)	0.160	0.9	1.1
13	Conv	120	2.5 ± 0.4	0.	(0.2, 0.4)	0.9	(0.6, 1.8)	0.313	0.9	0.8
14	Conv	120	2.4 ± 0.4	0.2	(0.1, 0.3)	0.7	(0.4, 1.5)	0.189	0.6	0.5
2017										
1	Unsprayed	225	1.0 ± 0.3	0.07	(0.02, 0.3)	1.2	(0.3, 252.8)	0.0002	1.0	1.0
2	Unsprayed	240	1.8 ± 0.4	0.08	(0.04, 0.2)	0.5	(0.2, 3.0)	0.009	1.2	0.4
15	Unsprayed	221	1.4 ± 0.2	0.1	(0.09, 0.2)	0.9	(0.5, 2.0)	0.474	1.9	0.7
4	Unsprayed	280	2.1 ± 0.7	0.2	(0.06, 3.7)	0.7	(0.2, 9503.0)	<0.0001	2.7	0.6
5	Organic	266	1.7 ± 0.3	0.1	(0.06, 0.3)	0.7	(0.3, 7.5)	0.0121	1.8	0.6
6	Organic	126	1.7 ± 0.2	0.06	(0.04, 0.09)	0.3	(0.2, 0.8)	0.330	0.9	0.3
7	Organic	156	1.8 ± 0.2	0.1	(0.08, 0.2)	0.6	(0.4, 1.4)	0.149	1.7	0.5
8	Conv	161	1.6 ± 0.4	0.09	(0.06, 0.1)	0.5	(0.3, 1.2)	0.169	1.3	0.4
16	Conv	213	1.9 ± 0.4	0.2	(0.1, 0.5)	0.9	(0.4, 6.2)	0.088	3.0	0.8
17	Conv	239	1.3 ± 0.4	0.2	(0.05, 1.5)	1.6	(0.4, 1069.0)	<0.0001	2.4	1.4
12	Conv	208	1.5 ± 0.2	0.1	(0.1, 0.2)	1.0	(0.6, 2.6)	0.908	2.0	0.8
2018										
1	Unsprayed	216	1.9 ± 0.7	0.04	†	0.2	†	0.032	1.0	1.0
5	Unsprayed	161	2.7 ± 0.4	0.1	(0.08, 0.1)	0.3	(0.2, 0.6)	0.482	2.6	1.7
15	Unsprayed	105	2.9 ± 0.6	0.09	(0.06, 0.1)	0.3	(0.2, 0.5)	0.106	2.2	1.3
18	Unsprayed	440	2.6 ± 0.7	0.1	(0.05, 0.3)	0.3	(0.2, 44.4)	0.012	2.5	1.6
19	Unsprayed	264	1.7 ± 0.5	0.1	(0.02, 0.2)	0.6	(0.3, 120.6)	0.0005	2.6	3.0
8	Conv	179	2.6 ± 0.5	0.07	(0.05, 0.1)	0.2	(0.2, 0.4)	0.464	1.8	1.2
12	Conv	207	5.5 ± 0.9	0.1	(0.1, 0.1)	0.2	(0.2, 0.3)	0.111	2.6	0.9
16	Conv	245	2.6 ± 0.4	0.2	(0.1, 0.3)	0.6	(0.4, 1.8)	0.061	4.5	2.9
17	Conv	236	3.2 ± 0.9	0.09	(0.02, 0.2)	0.2	(0.1, 97.1)	0.077	2.0	1.1
20	Conv	409	1.9 ± 0.2	0.1	(0.1, 0.2)	0.6	(0.4, 0.9)	0.711	3.0	2.9
21	Conv	482	2.3 ± 0.2	0.1	(0.1, 0.2)	0.5	(0.4, 0.7)	0.627	3.2	2.5
22	Conv	593	2.7 ± 0.3	0.09	(0.07, 0.1)	0.2	(0.2, 0.3)	0.912	2.0	1.2

23	Conv	137	2.6 ± 0.5	0.2	(0.1, 0.2)	0.5	(0.3, 1.0)	0.184	3.6	2.3
24	Conv	216	1.6 ± 0.3	0.05	(0.03, 0.08)	0.3	(0.2, 0.8)	0.863	1.3	1.7
25	Conv	243	2.4 ± 0.4	0.06	(0.04, 0.08)	0.2	(0.2, 0.3)	0.230	1.5	1.1

† Fiducial limits could not be calculated.

Table 8. Results of adult female residue bioassays of five insecticides for three populations of *Drosophila suzukii* populations from Michigan, collected in 2018. Bioassays were conducted three times for each colony. Mortality was recorded at 6 hours after exposure to insecticide residues (8 hours for spinosad). LC50 and LC90 values are listed with 95% fiducial limits listed in parentheses after LC values.

	malathion		methomyl		spinetoram		spinosad		zeta-cypermethrin	
Bioassay number	LC50	LC90	LC50	LC90	LC50	LC90	LC50	LC90	LC50	LC90
Site 18										
1	4.6 (†)	8.4 (†)	0.2 (0.1, 0.2)	0.3 (0.2, 0.5)	56.8 (42.4, 77.3)	213.3 (142.4, 406.4)	61.0 (25.8, 115.6)	399.5 (195.4, 1728)	0.1 (0.1, 0.1)	0.3 (0.2, 0.5)
2	4.0 (1.8, 22.4)	15.0 (6.3, 18030)	0.2 (0.1, 0.3)	0.5 (0.4, 0.9)	173.7 (†)	2695.0 (†)	9.1 (4.8, 14.4)	57.2 (33.7, 146.3)	0.2 (0.1, 0.3)	0.8 (0.5, 2.9)
3	1.7 (1.1, 2.3)	3.7 (2.6, 8.5)	0.1 (0.04, 0.1)	0.4 (0.3, 1.0)	52.8 (34.6, 80.7)	431.5 (240.3, 1088)	16.7 (5.8, 32.7)	278.3 (133.2, 1002)	0.2 (0.1, 0.4)	0.6 (0.3, 13.4)
Site 21										
1	3.2 (2.5, 3.7)	5.5 (4.6, 7.5)	0.1 (0.1, 0.2)	0.4 (0.3, 0.7)	72.6 (†)	422.8 (†)	40.3 (24.2, 60.5)	307.2 (194.2, 590.8)	0.1 (0.1, 0.2)	0.3 (0.2, 0.5)
2	2.3 (1.4, 3.3)	4.8 (3.3, 13.4)	0.1 (†)	0.4 (†)	77.4 (50.9, 120.1)	478.5 (271.0, 1204)	41.0 (23.8, 63.5)	305.0 (185.0, 638.4)	0.1 (0.1, 0.1)	0.3 (0.2, 0.8)
3	1.8 (1.4, 2.2)	5.1 (4.0, 7.4)	0.05 (†)	0.2 (†)	34.2 (21.4, 52.2)	379.1 (210.5, 946.7)	16.7 (8.1, 28.1)	199.0 (111.9, 481.6)	0.1 (0.1, 0.1)	0.2 (0.2, 0.4)
Site 22										
1	4.9 (2.2, 8.5)	10.0 (6.6, 281.7)	0.1 (0.07, 0.2)	0.5 (0.3, 1.0)	96.1 (15.2, 1057)	795.0 (206.9, 1.9 x 10^8)	30.0 (4.2, 88.0)	246.9 (84.9, 13507)	0.1 (0.1, 0.1)	0.3 (0.2, 0.6)
2	2.7 (2.2, 3.3)	6.1 (4.8, 9.0)	0.1 (0.07, 0.2)	0.4 (0.3, 0.7)	41.4 (9.4, 166.1)	982.1 (218.1, 392034)	30.2 (16.9, 47.1)	196.4 (119.9, 414.1)	0.1 (0.03, 0.1)	0.2 (0.2, 0.8)
3	3.4 (1.8, 8.3)	16.3 (7.1, 602.8)	0.1 (0.06, 0.1)	0.3 (0.2, 0.5)	9.4 (2.4, 19.7)	464.5 (156.5, 6937)	13.0 (7.1, 21.2)	78.0 (42.1, 294.6)	0.1 (0.1, 0.2)	0.4 (0.2, 0.8)

† Fiducial limits could not be calculated.

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