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Research

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Special Collection: Research Advances in Spotted-Wing Drosophila suzukii Management

Monitoring of Spotted-Wing Drosophila (Diptera: Drosophilidae) Resistance Status Using a RAPID Method for Assessing Insecticide Sensitivity Across the United States

Rufus Isaacs,[1,10] Steven Van Timmeren,[1] Brian E. Gress,[2] Frank G. Zalom,[2] Fatemeh Ganjisaffar,[2] Kelly A. Hamby,[3] Margaret T. Lewis,[3] Oscar E. Liburd,[4] Nupur Sarkar,[4] Cesar Rodriguez-Saona,[5] Robert Holdcraft,[5] Hannah J. Burrack,[6] Aurora Toennisson,[6] Francis Drummond,[7] Nathan Spaulding,[8] Srinivas Lanka,[8,9] and Ashfaq Sial[8]

[1]Department of Entomology, Michigan State University, East Lansing, MI 48824, USA, [2]Department of Entomology and Nematology, University of California, Davis, CA 95616, USA, [3]Department of Entomology, University of Maryland, College Park, MD 20742, USA, [4]Entomology and Nematology Department, University of Florida, Gainesville, FL 32606, USA, [5]P.E. Marucci Center, Department of Entomology, Rutgers University, Chatsworth, NJ 08019, USA, [6]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA, [7]School of Biology and Ecology, University of Maine, Orono, ME 04469, USA, [8]Department of Entomology, University of Georgia, Athens, GA 30602, USA, [9]School of Plant, Environmental and Soil Sciences, Louisiana State University, Baton Rouge, LA 70803, USA, and [10]Corresponding author, email: isaacsr@msu.edu

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Abstract

Drosophila suzukii (Matsumura) has spread rapidly, challenging berry and cherry crop production due to its ability to lay eggs into ripening fruit. To prevent infestation by this pest, insecticides are applied during fruit ripening and harvest. We field-tested the Rapid Assessment Protocol for IDentification of resistance in *D. suzukii* (RAPID) on seventy-eight populations collected across eight U.S. states in 2017 and 2018. Exposure to LC_{50} rates of malathion, methomyl, spinetoram, spinosad, and zeta-cypermethrin led to average female fly mortality of 25.0% in 2017, and after adjusting concentrations the average was 39.9% in 2018. Using $LC_{99} \times 2$ discriminating concentrations in 2017 and $LC_{90} \times 8$ rates in 2018, average female mortalities were 93.3% and 98.5%, respectively, indicating high overall susceptibility. However, using these high concentrations we found 32.0% of assays with survival of some female flies in 2017 and 27.8% in 2018. The adjustment in discriminating dose from 2017 to 2018 also reduced the proportion of assays with <90% survival from 17.6 to 2.9%. Populations with low mortality when exposed to spinosad were identified using this assay, triggering more detailed follow-up bioassays that identified resistant populations collected in California coastal region berry crops. Widespread evaluations of this method and subsequent validation in California, Michigan, and Georgia in 2019–2021 show that it provides a quick and low-cost method to identify populations of *D. suzukii* that warrant more detailed testing. Our results also provide evidence that important insecticide classes remain effective in most U.S. regions of fruit production.

Keywords: *Drosophila suzukii*, invasive, bioassay, screening, susceptibility

Insecticide resistance is an increasing barrier to effective pest management in multiple settings around the world (Denholm et al. 2002, Whalon et al. 2008, Onstad 2014a). Repeated exposure to toxicants provides selection pressure that can result in greater survival of resistant individuals, and these survivors can proliferate resulting in economic loss from the combination of reduced yield and increased control costs (e.g., Grafius 1997). Insect species with high reproductive rates and short generation durations, such as vinegar flies (*Drosophila* spp.), have increased potential for resistance development, due to the greater opportunity for mutations leading to a selective advantage (Daborn et al. 2007, Mutero et al. 1994, Sun et al. 2019). This potential in their life histories requires a strong selection pressure for resistance to be realized under field conditions, and *Drosophila* have been used extensively to explore resistance mechanisms and strategies for its mitigation (e.g., Daborn et al. 2002).

As spotted-wing drosophila, *Drosophila suzukii* (Matsumura) has moved into the world's main fruit production regions from its native range in Asia (Asplen et al. 2015), it has caused significant economic losses for producers of susceptible crops (Farnsworth et al. 2017). Management of this pest is dominated by insecticide applications in organic and conventional agriculture, increasing the chance that insecticide resistance will develop. Dependence on a few chemical classes and their repeated use for fruit protection against this pest (Beers et al. 2011, Van Timmeren and Isaacs 2013, Diepenbrock et al. 2016) highlights the need for active resistance management to prevent the development of *D. suzukii* populations that are not controlled by key insecticides. The sensitivity of adult *D. suzukii* to insecticides has been reported for populations collected from berry plantings in California (Gress and Zalom 2019), Michigan (Van Timmeren et al. 2018), and Brazil (Morais et al. 2021), and from cherry orchards in Washington (Whitener and Beers 2015), British Columbia (Smirle et al. 2017), and Italy (Civolani et al. 2021). These investigators treated flies with varying concentrations of insecticides to determine dose-response relationships and to calculate LC_{50} and LC_{90} values, revealing some populations with elevated LC_{50} values compared to the susceptible populations, or increasing LC values over time. Many of these studies found no evidence for resistance in *D. suzukii* populations in these regions, whereas a few identified field-collected populations showing resistance to spinosad (Gress and Zalom 2019), malathion (Gress and Zalom in review), and to cyantraniliprole and deltamethrin (Civolani et al. 2021). Studies to select populations for resistance have also provided varying outcomes. Smirle et al. (2017) found no significant increase in susceptibility in a population collected in a British Columbia (Canada) cherry orchard after 30 generations of selection with malathion. In contrast, Disi and Sial (2021) were able to select populations from blueberry fields in Georgia (USA) for reduced susceptibility to malathion and spinosad indicating a potential for selection of resistant populations. The patterns emerging indicate varying susceptibility to insecticides currently used to control *D. suzukii*, and also varying potential for resistance in the populations tested.

The above studies required time-consuming bioassays using specialized equipment to quantify the relationship between insecticide concentration and fly mortality, and this can be a barrier to widespread monitoring for resistance. Monitoring insecticide resistance is a critical component of integrated pest management (IPM) programs in agricultural systems to ensure that growers are using effective chemical controls against key pests (Stanley 2014). Recently, Van Timmeren et al. (2019) developed a method to quickly test *D. suzukii* flies for their susceptibility to insecticides (named the Rapid Assessment Protocol for IDentification of resistance or RAPID test), which was used to assess flies collected from Michigan and Georgia. This method was tested with residues of malathion, methomyl, spinetoram, spinosad, and zeta-cypermethrin coated on the inside of glass vials. In this assay, flies collected from the field can be assayed within one day using the discriminating concentrations that are expected to kill susceptible flies. If there are any survivors of exposure to these discriminating concentrations (Robertson et al. 2017), further testing is warranted and detailed follow-up studies can be conducted.

For a widespread monitoring program, a method to identify resistant populations must be easy to conduct, rapid to assess, accurate, and inexpensive. Sample sizes in monitoring programs should also be large to allow for early detections of resistance (Roush and Miller 1986). Fortunately, *Drosophila* flies are relatively easy to collect and to rear large numbers of individuals. After the initial report of insecticide resistance from California's Central Coast region (Gress and Zalom 2019), this current study was developed to test multiple insecticides at a national scale across the United States. Here we report on two years of resistance monitoring using the RAPID method at sites across the United States to address the following objectives: 1) determine the current susceptibility of *D. suzukii* to insecticides representing the main classes of insecticide used for this pest; 2) identify potentially resistant populations of *D. suzukii*; and 3) determine the utility of RAPID-SWD for monitoring insecticide sensitivity in this pest.

Methods

Insects for Testing

Drosophila suzukii were collected from strawberry, blueberry, blackberry, and raspberry farms in eight U.S. states (California, Florida, Georgia, Maine, Maryland, Michigan, New Jersey, and North Carolina) near the end of harvest in 2017 and 2018, with the timing relevant for each crop and region. This timing was selected to ensure that insect populations would have experienced exposure to insecticide applications around the time of fruit harvest. Flies were either collected from infested fruit samples and reared out to adults, or live traps were used when ripe fruit was no longer present to trap adults. Live traps baited with washed organic berries or with synthetic attractants were placed at sites for 1–3 d, after which they were brought back to the laboratory. Adult *D. suzukii* flies were subsequently aspirated out of traps and used for assays.

Assay Methods

In each state, 20 ml scintillation vials (Fisher Scientific, Pittsburg, PA) were treated with 1 ml of an insecticide solution using rates listed in Table 1. Five formulated insecticides were tested in this study, including malathion (Malathion 8F, Gowan Company, Yuma, AZ), methomyl (Lannate 2.4LV, DuPont de Nemours & Company, Wilmington, DE), spinetoram (Delegate 25WG, Corteva, Indianapolis, IN), spinosad (Entrust 22.5SC, Corteva, Indianapolis, IN), and zeta-cypermethrin (Mustang Maxx 0.8EC, FMC Corporation, Philadelphia, PA). Recently-produced insecticides less than one-year-old were used in all bioassays. For each insecticide, we tested the LC_{50} of each insecticide (Table 1) based on the earlier research of Van Timmeren et al. (2019).

To prepare for treating the vials, insecticides listed in Table 1 were dissolved either in acetone for malathion, methomyl, and zeta-cypermethrin, or if they did not dissolve in this solvent we used water with 1% v/v Induce spray adjuvant (Helena Chemical Company, Collierville, TN) for spinetoram and spinosad. Each vial received 1 ml of the insecticide in a fume hood and then the cap was tightly closed. The vial was then shaken gently to distribute the solution across all interior surfaces. Any excess solution was then poured out into a waste container and the vials and lids were left upright in the fume hood at an angle of 30 degrees and allowed to dry overnight for 20 hours before use in bioassays.

Table 1. Insecticides tested against male and female *Drosophila suzukii* flies, and the concentrations[a] used in the residual surface assays in 2017 and 2018

		Concentration of active ingredient (ppm)			
Active ingredient	Insecticide	LC_{50} (2017)	LC_{50} (2018)	LC_{99} × 2 (2017)	LC_{90} × 8 (2018)
zeta-cypermethrin	Mustang Maxx 0.8EC	0.21	0.18	5.9	6.9
methomyl	Lannate 2.4LV	0.36	0.64	4.2	16.6
malathion	Malathion 8F	4.98	6.16	32.5	102.8
spinetoram	Delegate WG	11.73	20.20	895.6	861.9
spinosad	Entrust SC	12.54	16.70	928.7	847.7

[a] Concentrations at the LC_{50} level were updated in 2018 based on additional information from 2017. In 2018, LC_{90} × 8 was used to provide greater confidence in the concentration.

The next morning, 10 adult *D. suzukii* flies that were 3–5 d old (2017: 5 males and 5 females; 2018: 10 females) from a single population were placed in each vial and re-sealed with the cap. Wherever possible flies were loaded in a humid environment, ideally >50% relative humidity, to reduce mortality. After 6 h in the vial (8 hours for spinosad), we counted the number of flies that were alive, moribund, or dead. Alive flies were those standing and walking around normally, while moribund flies were those that were clearly suffering the effects of the insecticides including twitching legs, inability to right themselves when flipped on their back, or slow, uneven movements. The number of moribund and dead individuals were combined for calculation of mortality.

Nationwide Bioassays

In 2017, we tested discriminating concentrations of insecticides (Table 1) that were twice the LC_{99} value determined from untreated populations of *D. suzukii* in the assays of Van Timmeren et al. (2017). These LC_{99} × 2 concentrations were used to assay the susceptibility of *D. suzukii* in eight states (California, Florida, Georgia, Maine, Maryland, Michigan, New Jersey, and North Carolina) for a total of 39 different populations. In 2018, flies were exposed to eight times the LC_{90} concentration (Table 1) to improve the accuracy of the discriminating concentration calculation, tested against new collections of this pest (Table 2). These LC_{90} × 8 concentrations were used to test in seven states (Florida, Georgia, Maine, Maryland, Michigan, New Jersey, and North Carolina), also against 39 different populations (Table 3). As shown in Table 1, the LC_{99} × 2 and LC_{90} × 8 values were within 1–81 ppm of each other, depending on the insecticide.

Regional Validation

To validate the results developed in 2017–2018, further testing with the RAPID method bioassays was conducted in 2019–2021 in California, Georgia, and Michigan. In these three states representing major regions of production of fruit susceptible to *D. suzukii*, larvae were collected in infested strawberry (California) and blueberry (Michigan and Georgia) fields at commercial farms at the end of harvest. Adult flies were reared from berries and assays were conducted on F1 or F2 adult female *D. suzukii*. All three states conducted bioassays with spinosad and malathion, with two states testing spinetoram, methomyl, and zeta-cypermethrin. In these assays, the populations tested were all from unique commercial fruit farms with new collections each year.

Data Analyses

The average, standard deviation, standard error, and coefficient of variation for mortality of flies were determined for each insecticide at the LC levels tested and for each population tested in each year. We also calculated the percentage of assays each year with any survivors, and with <90% survival. To compare sensitivity of *D. suzukii* to insecticides among the U.S. states where validation assays were conducted in 2019–2021, we used Mann–Whitney U tests when there were two states and Kruskal–Wallis tests when there were three states to compare. All statistical analyses were conducted using Systat 13 (Systat Software, Inc., Chicago, IL).

Results

Nationwide Bioassays

In 2017, eight participating U.S. states from California to North Carolina tested two to five of the insecticides against two to eleven populations of *D. suzukii*, resulting in 18–36 separate assays for each insecticide tested at the LC_{50} and LC_{99} × 2. Average mortality of the flies tested with LC_{50} values ranged from 12.7 to 40.9% with some assays having zero mortality (Table 2). The results from testing LC_{50} rates of each insecticide in 2017 were characterized by wide variability among states, with coefficients of variation across all assays ranging from 70% for zeta-cypermethrin (average 40.9% mortality) to 140% for malathion (average 22.4% mortality). In 2018 with the higher LC_{50} values used for most insecticides (Table 1), the average overall percent mortality ranged from 26.4% for spinosad to 50.3% for malathion (Table 3). In Georgia and New Jersey, some assays recorded zero mortality. The assays with LC_{50} remained highly variable, with coefficients of variation of 80-90%.

Discriminating concentrations of the different insecticides are expected to yield 100% mortality if the flies are susceptible, and to have some survivors if resistant individuals are present. Using the LC_{99} × 2 rates in 2017, we found most assays resulted in all flies being dead at 6- or 8-hour assessment times, with an overall mortality level of 93.3% from 125 bioassays. There were, however, 22.0% of the assays with some survivors. This was most apparent with spinosad in California, Maryland, Michigan, and New Jersey populations. Coefficients of variation were much lower in these assays, with the lowest being 0.7% for spinetoram and the highest being 21.5% for malathion. In most cases, these populations were re-tested using the same populations and generation, revealing higher mortality. However, in a few California sites the retesting and subsequent dose response curve assays by Gress and Zalom (2019) demonstrated that populations were resistant to spinosad. In contrast, the same bioassays with a range of spinosad concentrations using Michigan populations did not indicate any evidence of resistant populations to this insecticide. Assessment of male flies was included only in the 2017 studies, and those results are presented in Supp Table 1 (online only) indicating very similar results to the assays with females. Based

Table 2. Average percent mortality of female *Drosophila suzukii* adults exposed to LC_{50} or $LC_{99} \times 2$ residues of insecticides in scintillation vial bioassays conducted in 2017

		LC_{50}					$LC_{99} \times 2$				
State	Site	malathion	methomyl	spinetoram	spinosad	zeta-cypermethrin	malathion	methomyl	spinetoram	spinosad	zeta-cypermethrin
California	1	0.0			5.0		43.2			85.0	
	2	0.0			13.3					80.0	
	3	0.0			5.0		53.2			96.7	
	4	0.0			20.0		73.2			96.7	
	5	0.0			10.0					83.1	
	6	26.0			35.0		88.0			100	
Florida	1	44.0		52.0	40.0		100		100	100	
	2	48.0		56.0	52.0		100		100	100	
Georgia	1	6.7	0.0	16.7	0.0	26.7	83.3	93.3	100	100	100
	2	0.0	0.0	0.0	0.0	23.3	53.3	96.7	100	100	100
	3	0.0	0.0	10.0	0.0	3.3	100	96.7	100	100	100
	4	0.0	0.0	3.3	3.3	23.3	100	100.0	100	100	100
	5	0.0	0.0	0.0	3.3	22.7	76.7	83.3	100	100	100
Maine	1	100					100				
	2	100					100				
	3	95.0					100				
Maryland	1				37.5	46.7				93.8	93.3
	2				80.0	75.0				93.8	100
	3				65.0	71.3				100	100
	4				75.0	62.5				100	100
Michigan	1	40.0	40.0	25.0	20.9	46.7	100	100	100	90.9	100
	2	60.0	49.5	14.5	13.1	53.3	100	100	100	96.7	100
	3		20.0	36.7	11.2		100	100	100	96.4	
	4		13.3	22.2	21.3		100	100	100	100	
	5		16.7	36.0	10.3		100	100	100	84.0	
	6		8.0	26.7	23.3		100	100	100	81.4	
	7	10.0	8.0	13.3	28.6		100	100	100	94.3	
	8		16.7	38.3	5.6		100	100	96.9	45.7	
	9	15.6	6.7	45.7	10.0		100	100	100	36.7	
	10	15.8	14.7	16.7	16.7		100	100	100	100	
	11		18.0	80.0	24.0		100	100	100	60.0	
New Jersey	1	2.2	0.0		35.0	2.2	40.0	62.2		98.8	91.1
	2	8.9	21.1		31.3	4.4	63.0	73.3		91.3	97.8
	3	0.0	8.9		35.0	2.2	62.2	42.2		100	97.8
North Carolina	1	12.0			60.0	48.0	100			100	100
	2	20.0			20.0	92.0	100			100	100
	3	32.0			12.0	24.0	100			100	100
	4	5.0			24.2	65.0	100			100	100
	5	8.0			16.0	84.0	100			100	100
Average		**22.4**	**12.7**	**27.4**	**24.0**	**40.9**	**89.0**	**92.0**	**99.8**	**91.8**	**98.9**

Table 3. Average percent mortality of female *Drosophila suzukii* adults exposed to LC_{50} or $LC_{90} \times 8$ residues of insecticides in scintillation vial bioassays conducted in 2018

		LC_{50}					$LC_{90} \times 8$				
State	Site	malathion	methomyl	spinetoram	spinosad[a]	zeta-cypermethrin	malathion	methomyl	spinetoram	spinosad[a]	zeta-cypermethrin
Florida	1	54.0		42.0	66.0		100		100	100	
	2	38.0		28.0	56.0		100		100	100	
	3	56.0		46.0	44.0		100		100	100	
Georgia	1	0.0	0.0	71.0	7.0	3.0	100	100	93.0	100	100
	2	0.0	0.0	36.0	0.0	0.0	100	100	100	93.0	93.0
	3	0.0	0.0	4.0	0.0	0.0	100	100	90.0	100	97.0
	4	0.0	0.0	50.0	0.0	0.0	100	100	97.0	97.0	97.0
	5	0.0	8.0	63.0	0.0	0.0	100	100	100	100	97.0
	6	0.0	27.0	0.0	4.0	0.0	100	100	94.0	96.0	100
	7	69.0	22.0	3.0	0.0	0.0	100	100	100	100	100
	8	0.0	23.0	31.0	0.0	0.0	97	100	96.0	100	100
	9	60.0	0.0	0.0	0.0	0.0	100	100	100	100	97.0
	10	0.0	15.0	0.0	4.0	0.0	100	100	97.0	100	97.0
Maine	1	61.7			24.4		98.3			97.5	
	2	68.6			17.5		94.3			100	
Maryland	1				62.2	31.7				100	100
	2				45.6	36.6				100	100
Michigan	1	85.0	95.9	6.0	25.2	95.0	100	95.9	97.1	100	100
	2	100	90.0	20.0	30.0	43.7	100	90.0	100	100	100
	3	76.0	100	30.0	23.3	81.1	100	100	100	95	100
	4	100	100	13.3	39.2	85.0	100	100	100	93.3	100
	5	84.1	100	22.5	22.5	49.4	100	100	100	100	100
	6	82.2	88.9	5.4	16.2	83.3	100	88.9	100	100	100
	7	96.7	98.0	20.0	37.8	40.0	100	98.0	100	100	100
	8	84.4	100	11.4	17.7	72.5	100	100	100	97.1	100
	9	85.7	100	16.2	43.3	88.9	100	100	100	100	100
	10	86.2	93.6	14.4	36.8	62.5	100	93.6	97.8	85.4	100
	11	86.3	100	23.8	20.3	75.0	100	100	100	97.1	100
	12	85.3	100	17.1	18.5	85.2	100	100	97.2	88.1	100
	13	84.2	91.5	17.6	42.3	56.1	100	91.5	100	100	100
	14	87.8	95.0	22.8	35.3	76.3	100	95.0	100	94.4	100
New Jersey	1	3.3	30.0		43.3	35.0	86.7	100		100	97.5
	2	3.3	20.0		6.7	40.0	100	93.3		96.7	87.5
	3	3.3	20.0		20.0	23.6	100	100		96.7	95.0
	4	0.0	56.7		10.0	15.0	100	100		96.7	100
	5	3.3	43.3		47.5	15.0	100	100		96.7	91.8
	6	16.7	23.3		20.0	37.5	100	100		96.7	97.5
North Carolina	1	100		96.0	80.0	80.0	100		100	100	100
	2	100		96.0	64.1	93.8	100		100	100	100
Average		**50.3**	**54.7**	**27.8**	**26.4**	**41.3**	**99.4**	**98.2**	**98.6**	**97.9**	**98.5**

[a] Assessments of mortality for spinosad were conducted at 8 h after exposure.

on these results and because females infest fruit, subsequent testing focused only on female *D. suzukii*.

In 2018 when LC_{90} × 8 rates were used, mortality increased to 98.5% overall from the 169 bioassays run across the same five insecticides tested in seven U.S. states, with only forty-seven bioassays (5%) having some survivors and only five (2.9%) having survival below 90%. Coefficients of variation were only 2–3% for the LC_{90} × 8 assays in 2018. Results from the LC_{50} assays ranged from 0 (populations in Georgia and New Jersey) to 100% mortality (populations in Michigan and North Carolina). For the tests using the LC_{90} × 8 concentrations, populations from Florida, Maryland, and North Carolina all had 100% mortality, whereas those from Georgia, Maine, Michigan, and New Jersey had some survivors with the lowest mortality rate being 85.4% in a population from Michigan.

Regional Validation

The more recent validation bioassays conducted in Georgia (2019), Michigan (2020–2021), and California (2019–2021) using the LC_{90} × 8 discriminating concentrations indicated a similar pattern to the original assays, with a general susceptibility to insecticides found in a much greater proportion of the assays from the eastern states of Georgia and Michigan and a significant number of surviving flies in the populations collected in California (Fig. 1). In Georgia, we found 100% mortality in assays using methomyl, malathion, and spinetoram, with 99.5% mortality with spinosad, from a total of seven bioassays for each insecticide. Similar assays in Michigan found 100% mortality for all five tested insecticides across the fifteen different populations collected. In contrast, the California populations collected from the same region where insecticide resistance was detected previously by Gress and Zalom (2019) exhibited low mortality when exposed to the LC_{90} × 8 discriminating concentrations of spinosad (63.3%), malathion (12.0%) and zeta-cypermethrin (9.4%). The patterns of mortality in RAPID bioassays conducted across the three states are shown in Fig. 1, highlighting the significantly lower mortality in California populations of *D. suzukii* than the populations in Georgia and Michigan.

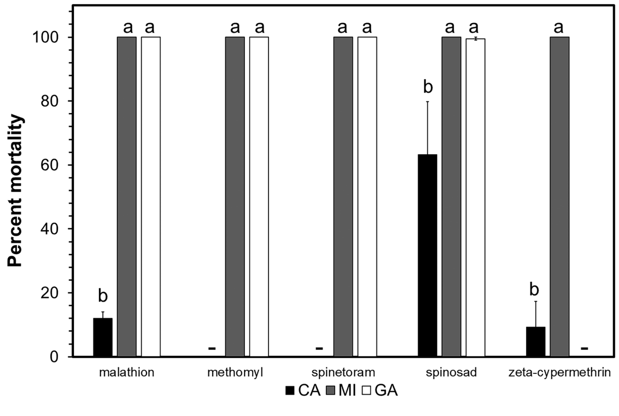

Fig. 1. Mortality of *D. suzukii* (average ± S.E.) when exposed to discriminating concentrations of insecticides in RAPID bioassays on populations collected in California (2019–2021), Georgia (2019), and Michigan (2020-2021) using five insecticides. Populations were collected in commercial strawberry farms in California (10 sites for spinosad, seven for zeta-cypermethrin, and two for malathion) and at commercial blueberry farms in Georgia (seven farms for all insecticides) and Michigan (15 farms for each insecticide). Bars within an insecticide treatment with different letters are significantly different ($P < 0.05$, Kruskal–Wallis or Mann–Whitney tests) between or among states. "–" indicates that insecticide was not tested in that state.

Discussion

Our widespread testing of the RAPID protocol for assessing *D. suzukii* susceptibility to insecticides indicates that the majority of populations in these states are still susceptible to the main insecticide classes used for control of this pest in the United States. This result aligns with reports from cropping systems across the United States (e.g., Beers et al. 2011, Haviland and Beers 2012, Van Timmeren et al. 2013, Diepenbrock et al. 2016, and Andika et al. 2020) that show high levels of control using currently registered insecticides. We also identified some populations of concern that have since been tested with full dose–response assays, verifying their status as being resistant to specific insecticides (Gress and Zalom 2019, Gress and Zalom in review, Ganjisaffar et al. 2022). This study also provided widespread experience on how to effectively run these assays in multiple laboratories.

A key goal of this project was to survey the current status of insecticide susceptibility in this pest across the United States. Resistance monitoring can benefit greatly from the use of a discriminating concentration approach, since it provides a level of efficiency that can facilitate adoption by people with limited time, expertise, and financial resources (Knight et al. 1990). We found that most of the populations tested were highly susceptible to the discriminating concentrations used, similar to the recent reports from Brazil (Morais et al. 2021). There were also survivors in some of our assays for some populations suggesting that the populations may have lost some susceptibility to specific insecticide classes. However, in almost all cases the re-testing of these populations found high susceptibility, which shows the potential for false positive results using this method. This is an important point regarding the use of the RAPID test. It provides a quick and low-cost approach for a first screening of populations without requiring detailed dose-response analysis. By design, this procedure is a less rigorous assessment of susceptibility than conducting a dose–response analysis. The goals are to identify populations that require further, more detailed investigation and to quickly exclude populations that show high susceptibility.

It is apparent from comparing results within and among the U.S. states over two years, that the LC_{50} is too variable to use as a discriminating concentration for these insecticides in tests with *D. suzukii*. While some states had populations with close to 50% mortality there was also wide variation. Testing flies using the LC_{90} × 8 provided a more consistent result, and the experience in California indicates that using this approach (with a confirmatory repeat of the test if some survivors are found) is effective for identifying potentially resistant populations.

This RAPID method is considered a first screening method to identify populations that require further investigations using detailed bioassay methods. This is in contrast to the detailed study of *D. suzukii* resistance assay optimization by Blouquy et al. (2021) that showed how best to conduct bioassays for reliable results with this species. We expect that eventually the RAPID assay would be used in a distributed network of extension agents, crop consultants, and others interfacing with farmers who would use it to identify populations of concern that would subsequently be tested in a laboratory setting using the optimal methods described by Blouquy et al. (2021). We also expect these methods to change as more experience is gained. For example, Halliday and Burnhaw (1990) analyzed bioassay data, concluding that using slightly lower concentrations that do not kill an extremely high proportion of susceptible subjects is preferable to a very high dose strategy for identifying populations developing tolerance. Re-evaluation of the optimal diagnostic concentration for resistance monitoring will be an important component to consider as this program develops, and should be reviewed annually as new information is gathered about population susceptibility (Rahim et al. 2016).

Based on the RAPID assays conducted in 2017, follow up dose–response bioassays in California identified populations that have subsequently been found to be resistant to spinosad (Gress and Zalom 2019), whereas other populations were found not to be resistant after further assays (S. Van Timmeren, unpublished). In Georgia, deployment of this method has also been used to identify populations that could be subsequently selected for resistance under laboratory conditions, with 10 and 11 generations of selection leading to 7.5- and 2.2-fold resistance to spinosad and malathion, respectively (Disi and Sial 2021). There is also a recent confirmation of resistance to zeta-cypermethrin in populations of *D. suzukii* collected in California, based on conducting follow-up dose-response assays with populations of concern that were first identified using this RAPID screening method (Ganjisaffar et al. in revision). These results from U.S. populations of *D. suzukii* reflect the emerging global pattern, with most tested populations showing continued susceptibility to insecticides (Smirle et al. 2017, Morais et al. 2021), and with a small proportion of trials revealing reduced susceptibility (e.g., Civolani et al. 2021).

If resistance to insecticides continues to develop in this pest across different geographic regions, we expect this monitoring approach to be adopted to inform spray decisions by growers and their consultants, similar to the program develop for tobacco budworm, *Heliothis virescens* (F.) and bollworm, *Helicoverpa zea* (Boddie) by McCutchen et al. (1989) in cotton. Vials treated with discriminating concentrations of key insecticide classes can be shipped from a central location to people wanting to assay their *D. suzukii* populations using the RAPID method (Van Timmeren et al. 2019). Disi et al. (2020) found that with appropriate treatment and shipment methods, vials treated with malathion or methomyl can provide reliable assessment of insecticide susceptibility for this species for up to 28 d, facilitating transportation and distribution to sites for use, whereas zeta-cypermethrin, phosmet, and spinosyns were not reliable. Use of resistance monitoring within IPM programs can help manage the response to resistance detection by guiding the selection of effective pest management tactics, while also providing a mechanism to track whether progress is being made towards regaining susceptibility. Onstad (2014b) describes five pest species where resistance management was incorporated into IPM programs, and reviews a series of guidelines for managing insecticide resistance within an IPM framework.

The goals of our study were to demonstrate the utility of the RAPID method for early resistance screening, and to refine the method so it provides consistent results. We found that the discriminating concentration assays had an increasing proportion of the assays with 100% mortality, and that the average mortality across all assays increased over the years of testing. The 2019–2021 assays were conducted by staff with multiple years of experience, suggesting that training and very clear protocols will be needed for implementation of this method. To address this, we have posted our protocols for the RAPID method at www.swdmanagement.org. This survey of *D. suzukii* susceptibility to the main insecticide classes provides a synopsis of populations in key production areas across the United States. Most of our sampling locations were in eastern regions of the country where farms generally have smaller plantings of fruit crops and greater landscape diversity than the major fruit production regions in western United States. These factors coupled with the mobility of this pest are likely to support a large susceptible population of *D. suzukii* in these settings and make it less likely that resistant genotypes persist, as found for other crop pests (Huseth et al. 2015). In contrast, the range of fruit crops grown and long period of harvesting in coastal California berry farms creates settings that require protection from *D. suzukii* infestation for many months in landscapes with limited unmanaged habitat. In both types of settings, regular monitoring for susceptibility would be advisable with this method to determine whether *D. suzukii* remains susceptible to registered insecticides or requires further testing to verify resistance and to explore resistance mechanisms.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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