





# Field, semi-field and greenhouse testing of HOOK SWD, a SPLAT-based attract-and-kill formulation to manage spotted-wing drosophila

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## Abstract

Native to Southeast Asia, the spotted-wing drosophila (*Drosophila suzukii*) has become a major pest of small fruit crops in the Americas and Europe because females can oviposit in ripening fruit. Currently, *D. suzukii* management relies heavily on chemical control; thus, more sustainable approaches like attract-and-kill need to be evaluated to reduce insecticide use. In previous studies, HOOK SWD, an attract-and-kill product that combines attractants, phagostimulants and an insecticide (spinosad), showed promise for *D. suzukii* control; still, little is known about its performance under field conditions and under varying pest and host-fruit densities, as well as its residual activity. Here, we evaluated the efficacy of HOOK SWD when applied in commercial blueberry farms, when deployed in semifield cages under different *D. suzukii* adult and fruit densities and when aged for 30 days in the greenhouse. Results from field experiments showed that HOOK SWD could reduce fruit infestation in blueberry farms; however, its efficacy varied in space and time. Semifield cage studies revealed that relatively high densities of both *D. suzukii* adults and fruit can reduce the effectiveness of HOOK SWD, which might explain the variation in its efficacy in the field. Ageing HOOK SWD in a greenhouse for at least 30 days yielded no significant decrease in insecticidal activity. Altogether, these studies show that HOOK SWD can potentially suppress *D. suzukii* populations in the field, but it should be used as part of an integrated pest management program instead of a stand-alone control tool, especially under high pest and fruit densities.

## KEYWORDS

behavioural control, *Drosophila suzukii*, integrated pest management, *Vaccinium corymbosum*

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## 1 | INTRODUCTION

Native to Southeast Asia, the spotted-wing drosophila, *Drosophila suzukii* (Matsumura; Diptera: Drosophilidae), has become a major pest of soft- and thin-skinned fruit crops, including raspberries, strawberries, blueberries, blackberries and cherries, resulting in significant economic losses (Walsh et al., 2011). This frugivorous pest has rapidly expanded its geographical range across multiple countries in Asia, the Americas, Europe and recently Africa (Arnó et al., 2016; Asplen et al., 2015; Boughdad et al., 2021; Hauser, 2011). In the continental United States, *D. suzukii* was first detected in the Western states (California) in 2008 and quickly spread to several other states including most Northeastern states by 2011 (Asplen et al., 2015; Hauser, 2011). Current management programs for *D. suzukii* rely heavily on insecticides, particularly cover sprays using organophosphates, pyrethroids and spinosyns (Beers et al., 2011; Diepenbrock et al., 2016; Shower et al., 2018). The overuse of these insecticides can result in the onset of resistant populations (Disi & Sial, 2021; Ganjisaffar et al., 2022; Gress & Zalom, 2019) and could have negative effects on nontarget species (Roubos et al., 2014; Sarkar et al., 2020). For this reason, there is a need to develop more sustainable control practices to manage *D. suzukii*.

Recently, Tait et al. (2021) reviewed the most promising Integrated Pest Management (IPM)-based practices developed in the last 10 years to manage *D. suzukii* and considered behaviour-based management strategies a suitable option to control this pest and reduce insecticide applications. Particularly, one of these strategies that have gained the most attention is attract-and-kill, a strategy that combines an attractive stimulus either visual or chemical (e.g., fruit, yeast or other related odours), phagostimulants (e.g., sugars) and a killing agent (e.g., an insecticide) (Cloonan et al., 2018). For example, red attracticidal spheres, that combine a phagostimulant (e.g., sugar), a visual stimulus (e.g., red colour) and an insecticide, can reduce *D. suzukii* infestation in raspberry and blueberry (Nixon et al., 2022; Rice et al., 2017). In a recent study, Rehmann et al. (2022) showed that the yeast *Hanseniaspora uvarum* (Niehaus), the predominant yeast species found in the midgut of *D. suzukii* larvae and adults and known to attract the adults (Hamby et al., 2016; Knight et al., 2016; Mori et al., 2017), can be combined with the insecticide spinosad as an attract-and-kill formulation to reduce fruit infestation and insecticide residues on the fruit. Other promising behaviour-modifying approaches that combine an attractant, a phagostimulant, and/or an insecticide include a 'food-grade-gum', a novel gum matrix derived from a dry multi-ingredient plant-based powder that acts as an oviposition substrate for *D. suzukii* and thus reduces fruit infestation but does not contain an insecticide (Rossi Stacconi et al., 2020; Tait et al., 2018). Noble et al. (2021) recently tested various insecticides combined with three baits, Combi-protec® Insect Bait (a proprietary mixture of plant extracts, proteins and sugars; Andermatt UK), a suspension of *H. uvarum* and molasses for efficacy against *D. suzukii*. They found that low rates of insecticides (e.g., cyantraniliprole and spinosad) mixed with Combi-protec or molasses baits were equally

effective in *D. suzukii* control compared with the full rates of insecticides, which resulted in lower insecticide residues on fruit.

HOOK SWD™ (ISCA Technologies, Inc.) is a sprayable attract-and-kill formulation developed for *D. suzukii* based on the slow-release matrix SPLAT (Specialized Pheromone and Lure Application Technology; Mafra-Neto et al., 2014) that contains a blend of proprietary attractants, pink colouring for visual attraction, sugars as phagostimulants and the insecticide spinosad, incorporated at a concentration of 0.5% by weight. Klick et al. (2019) tested HOOK SWD in raspberries and blueberries and found nearly 2–5 times fewer larvae in fruit compared with a grower standard cover spray under field conditions. However, a field study by Disi and Sial (2019) conducted in blueberries mostly showed no significant differences in the number of flies captured from traps between a grower standard and HOOK SWD treatments applied at 7- or 14-day intervals; except for the last week of the experiment, where they showed fewer flies captured in traps from HOOK SWD applied at a 7-day interval compared with the grower standard. These previous studies indicate that the effects of HOOK SWD under field conditions can be variable. In fact, using laboratory bioassays, Babu et al. (2022) showed that the presence of competitive fruit odours can affect the efficacy of HOOK SWD, and of a new product named ACTTRA SWD (a formulation of HOOK SWD that lacks the insecticide; ISCA Technologies, Inc.) mixed with spinosad, which could explain the variable results observed in field experiments.

In this study, we sought to expand previous findings by examining the efficacy of HOOK SWD in reducing *D. suzukii* fruit infestation in large commercial highbush blueberry (*Vaccinium corymbosum* L., Ericaceae) farms. In addition, to address the issue of variability in effectiveness, we conducted semi-field (cage) studies to test the efficacy of HOOK SWD under variable *D. suzukii* fly and host-fruit densities. We hypothesized that the efficacy of HOOK SWD breaks down with increasing fly and fruit densities, which may explain its loss of efficacy under certain field conditions. Lastly, we tested the residual activity of HOOK SWD on *D. suzukii* survival and fruit infestation under greenhouse conditions.

## 2 | MATERIAL AND METHODS

### 2.1 | Field experiments

#### 2.1.1 | Field sites and treatments

Field studies were conducted from July through September of 2018 and 2019 in two commercial highbush blueberry farms located in Hammonton, Atlantic County (New Jersey, USA) and separated by 9.3 km (Farm A: Latitude: 39°38'41.27"N, Longitude: 74°40'55.52"W; Farm B: Latitude: 39°42'31.28"N, Longitude: 74°45'13.97"W). Within farms, a total of six (in 2018) and eight (in 2019) highbush blueberry (*V. corymbosum* var. 'Elliott') plots were used. In 2018, three treatments were evaluated: (1) weekly applications of HOOK SWD (7-day interval; a total of six applications on

21 July, 29 July, 06 August, 14 August, 22 August and 30 August), (2) applications of HOOK SWD every 2 weeks (bi-weekly, 14-day interval; a total of three applications on 23 July, 06 August and 22 August) and (3) control that received no HOOK SWD application (i.e. grower's standard insecticide control). Each treatment was replicated twice (blocks) in each farm for a total of four replicates per treatment, in a randomized complete block design.

In 2019, to maximize the effect of HOOK SWD and because there was no clear difference in fruit infestation when applied weekly and bi-weekly (see Results), two treatments were evaluated: (1) weekly applications of HOOK SWD (7-day interval; total of six applications on 21 July, 27 July, 02 August, 09 August, 16 August and 23 August) and (2) control without HOOK SWD (i.e. grower's standard insecticide control). Each treatment was replicated four times at each farm for a total of 8 replicates (blocks) per treatment, in a randomized complete block design.

In both years, each individual treatment was applied to one of the plots, and the first HOOK SWD application was made on 21 July. All HOOK SWD applications contained the insecticide spinosad, incorporated at a concentration of 0.5% by weight (Klick et al., 2019). Plots were ~1 ha each, and plots within blocks were separated at least 10 m while blocks were separated at least 100m from each other. Although all HOOK SWD and control treatments were initiated while the growers were applying insecticides (see section 2.1.3 below for details) and continued after the applications had stopped,

most HOOK SWD applications and evaluations were carried out after insecticide applications were completed.

## 2.1.2 | HOOK SWD application method

In 2018, HOOK SWD applications were made with a custom-made sprayer using compressed CO<sub>2</sub> mounted on a 4-wheel-drive All-Terrain Vehicle (ATV; Yamaha Motor Corporation, U.S.A.; Figure 1a). The sprayer was calibrated to deliver 3.75 L/ha of HOOK SWD at 35 psi using two D3 orifice nozzles, one on each side of the ATV. The flow was controlled by two 12 VDC electric solenoids (model USS2-00051; U.S. Solid®), each one connected to a programmable 12 VDC digital cycle delay timer switch (model 870901; ICStation, IS brand, Amazon.com). The switches were programmed to apply the HOOK SWD intermittently down the row by opening for 1 s followed by a 3-s closure, with the right and left sides offset to alternate the open side. This intermittent spray pattern resulted in 2.5 treated bushes followed by 7.5 untreated bushes within rows. The ATV was driven at ~3 m/sec (6 mph) according to a Speed-o-meter LR™ (Micro-Trak® Systems Inc.).

In 2019, the HOOK SWD application method was modified to increase spray coverage from an intermittent application to a steady stream. The sprayer was calibrated to deliver 6.25 L/ha of HOOK SWD at ~40 psi using two D2 orifice nozzles (Figure 1b), as a steady



**FIGURE 1** Field applications of HOOK SWD in commercial blueberry farms were made using a customized 4-wheel-drive ATV (a, c), with type D2 or D3 orifice nozzles (b) to achieve a consistent spray flow. The sprayer was calibrated to deliver 3.75 L/ha at 35 psi (2018) or 6.25 L/ha at 40 psi (2019) of HOOK SWD. HOOK SWD applications were aimed at the lower third of blueberry bushes to avoid contamination of harvestable fruit (d). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]



stream, with the ATV driven at a speed of 2.4 m/s (5.5 mph). The line pressure was provided by an electric diaphragm pump (1.0 GPM, 60 psi, 12 VDC, Pentair SHURflo, model 8009-541-236) attached to a 25-gallon poly tank (Figure 1c). A 50-mesh in-line strainer was added to the pressure line between the pump and nozzles to avoid clogging the smaller D2 orifice nozzles. This application method eliminated the use of control timers but required an increase in HOOK SWD volume per hectare, which in turn necessitated a larger poly tank and diaphragm pump system.

In both years, HOOK SWD applications were aimed at the lower third of bushes to avoid contamination of harvestable fruit (Figure 1d). All HOOK SWD applications were carried out in the afternoon (12:00–18:00 hours) under sunny and dry conditions.

### 2.1.3 | Insecticide spray regime

The following insecticides were applied by growers to all three treatments between 10 July and 4 September 2018 (the period when the study was conducted) to manage *D. suzukii*: Farm A (3 applications, the last two made during the HOOK SWD trial) = Malathion (active ingredient [a.i.] = malathion) at 2.34 L/ha on 07 July, Mustang Maxx (a.i. = zeta-cypermethrin) at 0.29 L/ha on 29–30 July and Delegate WG (a.i. = spinetoram) at 0.42 kg/ha on 08 August; Farm B (5 applications, last two made during the HOOK SWD trial) = Delegate WG at 0.42 kg/ha on 09 July, Imidan 70W (a.i. = phosmet) at 1.49 kg/ha on 13 July, Bifenture 10DF (a.i. = bifenthrin) at 1.17 L/ha on 20 July and 29 July, and Imidan 70W at 1.49 kg/ha on 08 August.

The following insecticides were applied to the two treatments by growers between 01 July and 01 September 2019 (the period when the study was conducted) to manage *D. suzukii*: Farm A (4 applications, the last two made during the HOOK SWD trial) = Lannate LV (a.i. = methomyl) at 3.51 L/ha on 06 July, Delegate WG at 0.42 kg/ha on 13 July, Brigade 2EC (a.i. = bifenthrin) at 0.47 L/ha on 22 July and Malathion 8 at 2.92 L/ha on 29–31 July; Farm B (5 applications, last two made during the HOOK SWD trial) = Delegate WG at 0.42 kg/ha on 12 July, Brigade WSB at 1.12 kg/ha on 09 July, Lannate LV at 2.34 L/ha on 15 July, Delegate WG at 0.42 kg/ha on 24 July and Lannate LV at 2.34 L/ha on 29 July.

### 2.1.4 | Adult trapping

To monitor adult *D. suzukii* abundance among treatments, a trap was placed in the centre of the middle row of each field (treatment) and left in the field for 6–7 days before collecting and counting the captured *D. suzukii* flies. Traps consisted of 1-L deli containers with two (~2 cm) holes placed approximately halfway on the sides of the containers and covered by a mesh netting with ~2.5 mm openings. Traps were baited with a commercial Scentry™ lure (Scentry Biologicals Inc.) and had 150 ml of soapy water added to the traps to drown any flies captured. Unscented soap (Free & Clear dish liquid; Seventh Generation Inc.) was mixed at 3 ml per 4 L of water. In 2018, traps

were installed on 26 July, and checked and re-installed six times on 02 August, 09 August, 16 August, 23 August, 29 August and 04 September. In 2019, traps were installed on 26 July and checked and re-installed five times on 01 August, 08 August, 15 August, 22 August and 28 August. Because multiple drosophila species were captured in the traps, all flies were checked individually using a stereomicroscope to determine whether they were *D. suzukii* or 'other' drosophila species. Data were recorded for the total *D. suzukii* flies (combined males and females) per trap.

### 2.1.5 | Fruit infestation

To determine whether applications of HOOK SWD reduced fruit infestation by *D. suzukii*, fruit samples were collected weekly for eight consecutive weeks on 19 July, 26 July, 02 August, 09 August, 16 August, 23 August, 29 August and 04 September in 2018, and seven consecutive weeks on 19 July, 26 July, 01 August, 08 August, 15 August, 22 August and 28 August in 2019. In both years, the last four samples were collected at least a week after all insecticide sprays had stopped. On each sampling date, a total of 20 samples of 15 and 25 berries each were collected for each field (treatment) in 2018 and 2019, respectively (i.e. total of 300 and 500 berries per field in 2018 and 2019, respectively). Within each field, samples were collected from four random rows spaced ~15 m from each other, and five samples were taken within each row spaced ~10 m between each sampling site.

In 2018, fruit samples were placed in 3.8 L Ziplock plastic bags, taken to the laboratory and incubated on a laboratory bench (approx. 22°C, 14L:10D photoperiod) for 3–4 days before the fruits were submerged in salt water (92.5 g of salt/L of water) to extract the larvae (Cowles et al., 2015; Van Timmeren et al., 2017). Samples were left in the salt water overnight, lightly crushed, and then, all larvae and pupae caught by a 30-mesh sieve were counted and recorded. In 2019, field-collected fruit samples were stored individually in 30 ml plastic cups with ventilated lids and left under a laboratory bench for 15 days. Cups were checked for adult emergence every 3–4 days and removed to prevent eggs from being laid in the fruit. The fruit processing method was modified in 2019 because, although it takes longer to process, incubating berries to allow flies to emerge can be more sensitive than the salt solution method for evaluating *D. suzukii* fruit infestation (Van Timmeren & Isaacs, 2013). The total number of immature stages (larvae and pupae; 2018) and adults (2019) present in the samples was used to calculate the number of *D. suzukii* per fruit as a proxy of fruit infestation.

## 2.2 | Semi-field experiments

Semi-field experiments were conducted in outdoor cages (1.83 m × 1.83 m × 1.83 m, Outdoor Cage Mesh Enclosures; BioQuip Products) to evaluate the effect of *D. suzukii* adult and host-fruit density on the efficacy of HOOK SWD.

## 2.2.1 | Insect colonies

*Drosophila suzukii* adults used in these experiments were maintained at the Rutgers P.E. Marucci Center (Chatsworth, New Jersey, USA) and the University of Florida Small Fruit and Vegetable IPM Laboratory (Gainesville, Florida, USA). The colonies were maintained on a standard *Drosophila* artificial diet (Formula 4-24® Instant *Drosophila* Media; Carolina Biological Supply Company) in 50 ml polystyrene vials (Fisher Scientific) with approximately 15 ml of diet and closed with BuzzPlugs (Fisher Scientific Dalton et al., 2011; Jaramillo et al., 2015). Colonies were kept in an incubator (Percival Scientific, Perry, IA, USA) at 25°C, 55% relative humidity (RH) and a 16:8 light:dark (L:D) cycle. Female flies, 3–7 days old (i.e. sexually matured flies; Revadi et al., 2015), were removed from the colony 2–3 h before being used in the experiments.

## 2.2.2 | HOOK SWD treatments

Three treatments were tested: (1) HOOK SWD applied to the foliage, (2) HOOK SWD applied to the bark and (3) no HOOK SWD application (control treatment). For the treatments containing HOOK SWD, 24 h before the onset of the experiments, 0.4 ml of HOOK SWD was applied using a syringe to strips of clear tape and five strips were placed per cage, for a total of 2 ml of HOOK SWD per cage. After a 24 h drying period, the strips were placed randomly either on the bark of different stems or on leaves, depending on the treatment, to blueberry bushes inside cages (one bush per cage, see below). For each experiment, new HOOK SWD strips were prepared. No other treatments, i.e. insecticides, were applied to bushes.

## 2.2.3 | Adult density

This study tested the efficacy of HOOK SWD under variable *D. suzukii* densities. The study was conducted from June through July of 2019 (mean temperature = 23.6°C ± 0.5 SE) in an empty field at the Rutgers P.E. Marucci Center and consisted of the three treatments described above (under 'HOOK SWD treatments') at four *D. suzukii* densities, 0, 20, 40 and 80 flies (obtained from laboratory colonies, see above), for a total of 12 treatments in a completely randomized design. Each treatment was replicated five times using eight outdoor cages (described above); thus, the experiment was repeated eight times across four consecutive weeks. Outdoor cages were spaced at least 1 m apart. One (2-year-old) potted highbush blueberry (*V. corymbosum* var. 'Bluecrop') plant was placed inside each cage, and a (36.5 cm square × 99 cm tall) steel wire tomato wire cage (Gardener's Supply Company) was placed around the potted plant, where five blueberry fruit clusters were hung at random locations around it. Each berry cluster had five fruits, for a total of 25 fruits per cage.

The berry clusters were collected from a highbush blueberry field (var. 'Bluecrop'), located at the Rutgers P.E. Marucci Center; branches were bagged with 6 L (50 × 61 × 19 cm) Super-Aire fibre

plant sleeves (A-ROO Company) on 30 May (at the green fruit stage) and harvested in June–July to ensure that fruits used in the study were not previously infested by *D. suzukii*. Prior to the study, all berries were checked for infestation and found to be clear of *D. suzukii*. To maintain their turgor, immediately after harvest, berry clusters were placed into a floral water pick (10.7 cm long) filled with water. After berry clusters were placed inside the cages, *D. suzukii* females were released in the evening (4–5 pm), when the temperatures were cool. After 24 h, clusters were taken to the laboratory and placed individually in 30 ml plastic cups with ventilated lids. The number of eggs laid in fruits was counted under a stereomicroscope by observing the oviposition hole and two white breathing filaments protruding out of the egg (Lee et al., 2011). Fruits were then incubated for 15 days on a bench in the laboratory (21°C, 14L:10D photoperiod) to assess adult emergence.

## 2.2.4 | Fruit density

This study was similar to the one described above for adult density but instead tested the efficacy of HOOK SWD under variable fruit densities. The study was conducted from November 2019 to February 2020 (mean temperature = 15.4°C ± 0.2 SE) in an empty field at the University of Florida (Gainesville, FL, USA) and consisted of the three treatments described above (under 'HOOK SWD treatments') at 5 (25 fruits), 10 (50 fruits) and 20 (100 fruits) fruit (blueberry) clusters, for a total of 9 treatments in a completely randomized design. Each treatment was replicated five times, and treatments were assigned randomly to one of eight field cages (as described above); cages were spaced at least 1 m apart. The study was repeated in time until all replicates were completed for all treatments. Because blueberries were not in season at the time of this study, USDA-certified organic blueberries (unknown variety) were purchased the day before the start of the experiment, washed with Fit Fruit Vegetable Wash (HealthPro Brands Inc.) and air dried on a paper towel for about 10 min. Following, clusters of five fruit were placed in mesh bags (3 mm diameter; Jo-Ann Stores, LLC) tied closed with twist ties, kept in the refrigerator overnight and then hung at random locations around the bushes inside the cages. Forty *D. suzukii* females were collected from a laboratory colony (described above) and released inside each cage. After 24 h of exposure to flies, berries from each cage were collected, taken to the laboratory (21°C, 14L:10D photoperiod) and incubated for 15 days to assess adult emergence.

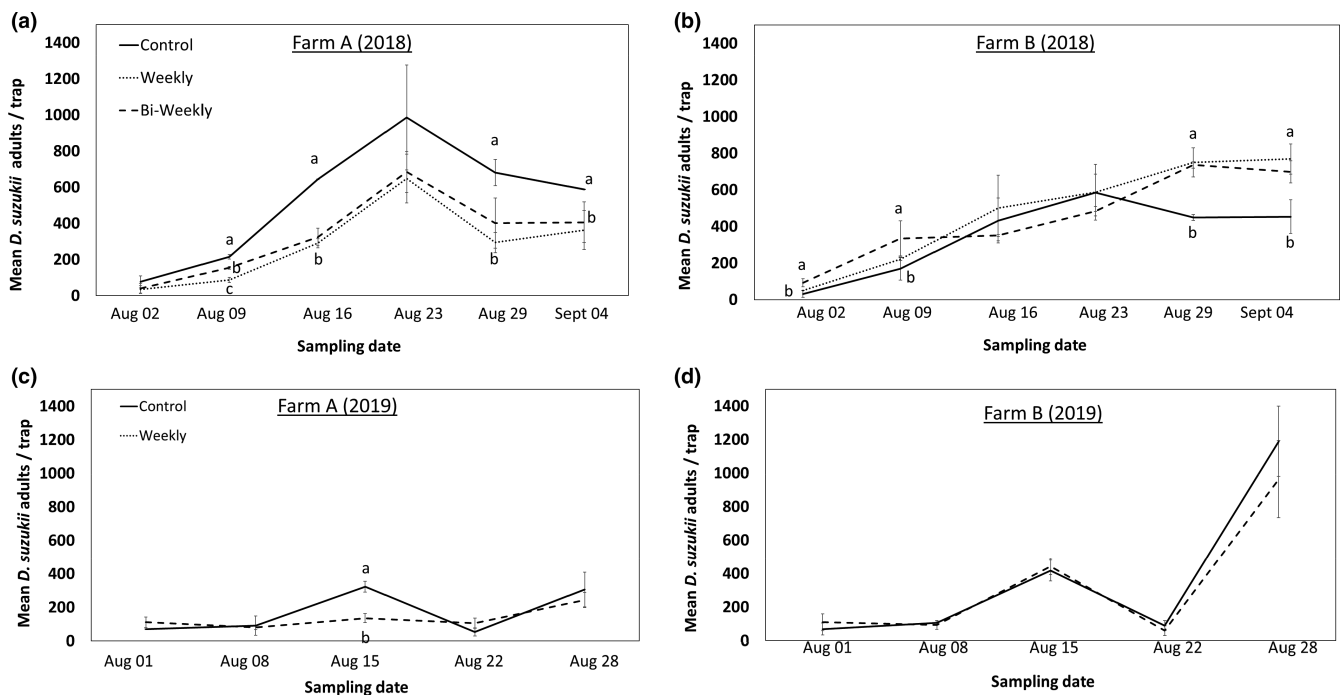
## 2.3 | Greenhouse experiment

To evaluate the residual activity of HOOK SWD on *D. suzukii* adult mortality and fruit infestation, an experiment was conducted in a greenhouse (26°C, 50% RH and a photoperiod of 14:10 (L:D) h) in October–November 2016 at the Rutgers P.E. Marucci Center (Chatsworth, New Jersey, USA) using potted blueberry bushes (var.

**TABLE 1** Results from generalized linear mixed models (GLMM) for the effects of 'Treatment' (control, HOOK SWD applied weekly and HOOK SWD applied bi-weekly), date of sampling ('Date') and their interaction on the number of *Drosophila suzukii* adults captured in traps in Farms A and B during the 2018 and 2019 field seasons

Farm ID	Source of variance	Adult trapping					
		2018			2019		
		F	df	<i>p</i> <sup>a</sup>	F	df	<i>p</i> <sup>a</sup>
Farm A	Model	7.65	17	<b>&lt;0.001</b>	5.59	9	<b>&lt;0.001</b>
	Treatment	10.58	2	<b>0.001</b>	0.30	1	0.586
	Date	20.52	5	<b>&lt;0.001</b>	9.84	4	<b>&lt;0.001</b>
	Treatment × Date	0.662	10	0.777	2.68	4	<b>0.05</b>
Farm B	Model	516.16	17	<b>&lt;0.001</b>	19.43	9	<b>&lt;0.001</b>
	Treatment	130.71	2	<b>&lt;0.001</b>	0.41	1	0.528
	Date	156.63	5	<b>&lt;0.001</b>	42.91	4	<b>&lt;0.001</b>
	Treatment × Date	47.57	10	<b>&lt;0.001</b>	0.71	4	0.594

<sup>a</sup>Values in bold are significant ( $p \leq 0.05$ ).



**FIGURE 2** Effect of treatment (control, HOOK SWD applied weekly and HOOK SWD applied every 2 weeks [bi-weekly]) on the number (mean  $\pm$  SE) of *Drosophila suzukii* adults captured in traps during the 2018 (a and b) and 2019 (c and d) field seasons in commercial highbush blueberry Farms A (a and c) and B (b and d), respectively. In 2018, all three treatments were tested while, in 2019, we tested only two of the treatments (control and HOOK SWD applied week).

'Elliott'). Treatments consisted of bushes either treated with HOOK SWD dollops (0.5 ml) applied directly with a syringe to one randomly selected leaf in each bush ( $n = 22$  bushes treated with HOOK SWD) or untreated bushes (control 'clean' plants without HOOK SWD;  $n = 9$  control bushes). Following, leaf terminals ( $n = 4-5$ ) containing the HOOK SWD dollop were collected on the day of treatment (day 0), and 7, 14, 21 and 30 days after application; leaf terminals ( $n = 9$ ) from the control bushes were also collected. Each leaf terminal was considered a replicate. Leaf terminals were then transferred to the laboratory and placed individually in 1-L deli cup containers with a mesh lid. The containers had a hole cut in the bottom in which a florist's water pick fitted tightly, and the terminal's stems were inserted through the water pick. Ten store-bought organic blueberry fruits

were placed inside the containers. Subsequently, ten adult *D. suzukii* flies (five males and five females) from the laboratory colony (see above) were released inside each container, and mortality was recorded after 24 h. Fruit infestation was assessed using the salt extraction method, as described above, and the number of immature stages (i.e. the sum of larvae and pupae) per fruit was calculated.

## 2.4 | Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 23.0 (IBM Corp.). Data on *D. suzukii* trap captures and fruit infestation (i.e. number of larvae per fruit or number of adults emerged

Farm ID	Source of variance	Fruit infestation					
		2018			2019		
		<i>F</i>	<i>df</i>	<i>p</i> <sup>a</sup>	<i>F</i>	<i>df</i>	<i>p</i> <sup>a</sup>
Farm A	Model	63.54	11	<0.001	50.21	5	<0.001
	Treatment	17.63	2	<0.001	115.07	2	<0.001
	Date	198.99	3	<0.001	0.85	1	<0.001
	Treatment × Date	11.11	6	<0.001	10.03	2	<b>0.001</b>
Farm B	Model	25.05	11	<0.001	155.04	5	<0.001
	Treatment	2.61	2	0.075	355.98	2	<0.001
	Date	85.97	3	<0.001	53.35	1	0.357
	Treatment × Date	2.07	6	0.056	7.07	2	<0.001

<sup>a</sup>Values in bold are significant ( $p \leq 0.05$ ).

TABLE 2 Results from generalized linear mixed models (GLMM) for the effects of 'Treatment' (control, HOOK SWD applied weekly and HOOK SWD applied bi-weekly), sampling date ('Date') and their interaction on the number of *Drosophila suzukii* larvae (2018) or adults emerged (2019) per fruit in Farms A and B

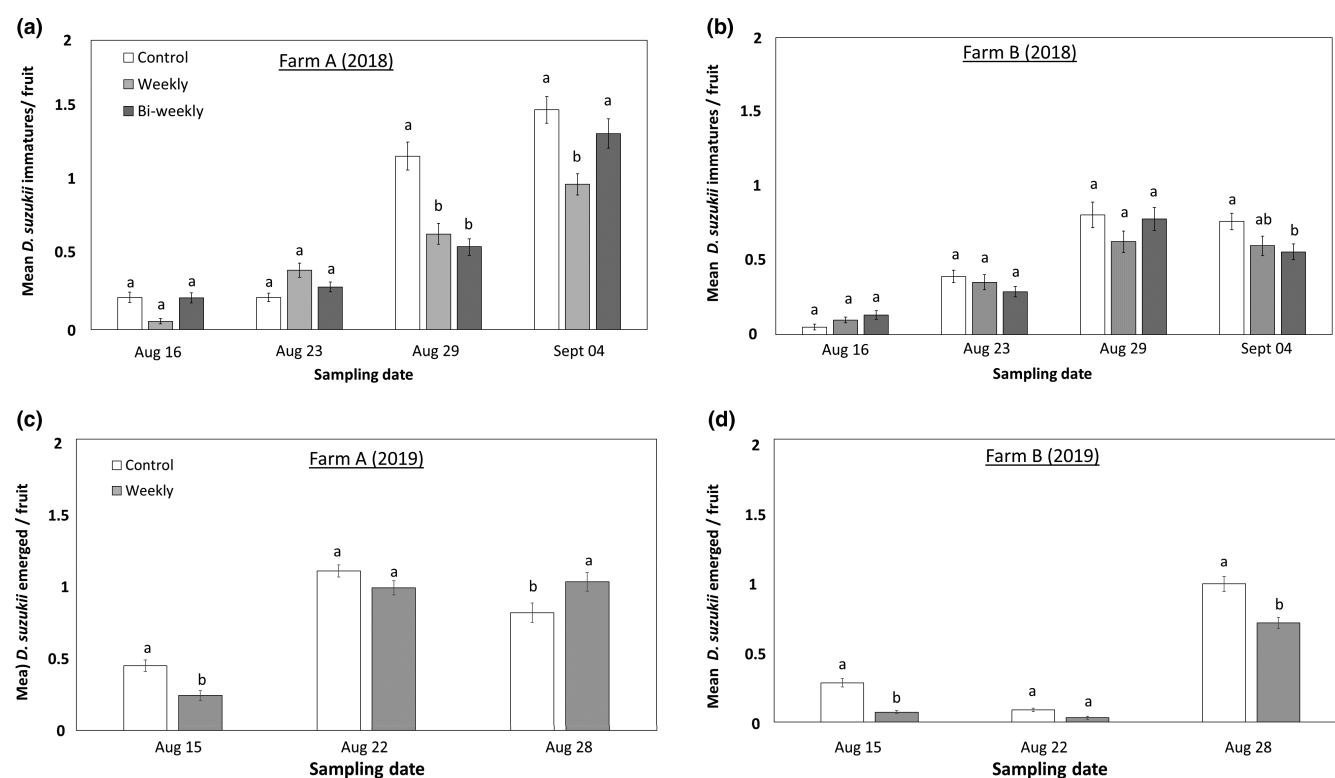


FIGURE 3 Effect of treatment (control, HOOK SWD applied weekly and HOOK SWD applied every 2 weeks [bi-weekly]) on the number (mean  $\pm$  SE) of *Drosophila suzukii* immature stages (larvae + pupae) per fruit (a and b) in 2018 and the number (mean  $\pm$  SE) of adults emerged per fruit (c and d) in 2019 in commercial highbush blueberry Farms A (a and c) and B (b and d), respectively. In 2018, all three treatments were tested while, in 2019, we tested only two of the treatments (control and HOOK SWD applied weekly).

per fruit) from field experiments were analysed separately for each year (2018 and 2019) because of the differences in HOOK SWD application methods. In a preliminary analysis using a generalized linear mixed model (GLMM) with a normal distribution of error and logit link function, with 'Treatment' (HOOK SWD versus control), 'Farm' and their interaction ('Treatment'  $\times$  'Farm') as fixed factors, we found that farm either alone or together with treatment significantly affected *D. suzukii* trap counts and fruit infestation (Table S1), indicating considerable variation in fly density and

fruit injury between farms. Thus, in our final analyses, data for each farm were analysed separately using GLMM with 'Treatment', 'Date' (i.e. date of sampling) and their interaction as fixed factors; replicate (block) was used as a random factor. Pairwise comparisons between treatments at each sampling date were performed using Bonferroni post hoc tests ( $p < 0.05$ ). For the data on adult emergence, we only included the last 4 weeks of sampling in 2018 (02 August, 16 August, 23 August, 29 August and 04 September) and the last 3 weeks in 2019 (15 August, 22 August and 28 August)

because adult *D. suzukii* emergence from berries during the first weeks of the study was almost zero in all treatments.

In the semi-field experiments, we analysed the data on the number of eggs laid per fruit (from the 'Adult density' study) and the number of *D. suzukii* emerged per fruit (from the 'Adult density' and 'Fruit density' studies) using GLMM. The model included 'Treatment' (HOOK SWD applied to the bark or leaf and control), 'Density' (number of adult flies or fruit) and their interaction as fixed factors; replication was included as a random factor. Pairwise comparisons among treatments at each fly or fruit density were performed using Bonferroni post hoc tests ( $p < 0.05$ ).

Lastly, to compare the effects of HOOK SWD residues (i.e. dates after application) versus the control treatment (without HOOK SWD) on percent adult *D. suzukii* survival (i.e. number of flies alive after 24 h) and fruit infestation (i.e. number of immature stages in fruit), we used a generalized linear model (GLM) with a normal distribution of the error and logit link function.

## 3 | RESULTS

### 3.1 | Field experiments

#### 3.1.1 | Adult trapping

In 2018, the application of HOOK SWD had a significant effect on the number of *D. suzukii* adults captured in traps on both farms (Farms A and B; [Table 1](#)). This treatment effect was influenced by sampling date (i.e. significant Treatment-by-Date interaction, [Table 1](#)) in Farm B but not in Farm A. In Farm A, the number of *D. suzukii* adult captures was significantly lower in plots treated with HOOK SWD than in control plots ([Figure 2a](#)). By contrast, in Farm B, the number of *D. suzukii* captured on three samplings dates (02 August, 29 August and 04 September) was significantly higher in plots treated with HOOK SWD than in control plots ([Figure 2b](#)).

In 2019, the number of *D. suzukii* adults captured in traps was not significantly different between treatments in both farms ([Table 1](#); [Figure 2c,d](#)), except on 15 August in Farm A, where plots treated with HOOK SWD had lower *D. suzukii* adult captures than the control plots (significant Treatment-by-Date interaction, [Table 1](#); [Figure 2c](#)).

#### 3.1.2 | Fruit infestation

In 2018, the application of HOOK SWD had a significant effect on *D. suzukii* fruit infestation in Farm A but not in Farm B ([Table 2](#)). In Farm A, HOOK SWD application reduced *D. suzukii* fruit infestation; however, this effect was influenced by sampling date (significant Treatment-by-Date interaction, [Table 2](#)). On 4 of 6 sampling dates (08 August, 16 August, 29 August and 04 September), HOOK SWD (either applied weekly or bi-weekly) reduced *D. suzukii* larvae per fruit ([Figure 3a](#)). Although there was no significant treatment effect

in Farm B, the interaction effect between treatment and sampling date was marginal ( $p = 0.056$ ; [Table 2](#)). HOOK SWD applied bi-weekly reduced larval infestation in fruit compared with the control on 04 September (the last sampling week; [Figure 3b](#)).

In 2019, there were significant differences between treatments (HOOK SWD versus control) on fruit infestation in both farms; however, this effect was influenced by sampling date (significant Treatment-by-Date interaction, [Table 2](#)). In Farm A, HOOK SWD reduced fruit infestation on only one of the sampling dates (15 August; [Figure 3c](#)). Although, in the last week of sampling (04 September), fruit infestation was higher in HOOK SWD-treated plots than in the control plots. In Farm B, HOOK SWD reduced the number of *D. suzukii* adult emergence on 15 August and 28 August compared with the control ([Figure 3d](#)).

## 3.2 | Semi-field experiments

### 3.2.1 | Adult density

The number of *D. suzukii* eggs laid per fruit was influenced by treatment (HOOK SWD applied to bark, HOOK SWD applied to foliage and control;  $F = 17$ ;  $df = 2, 11$ ;  $p < 0.001$ ), adult fly density ( $F = 32$ ;  $df = 3, 11$ ;  $p < 0.001$ ) and their interactions ( $F = 3$ ;  $df = 6, 11$ ;  $p = 0.023$ ). HOOK SWD, either applied on the foliage or the bark, reduced the number of *D. suzukii* eggs laid per fruit at densities of 40 and 60 flies per cage compared with the control treatment but not at low (20 flies per cage) or high densities (80 flies per cage; [Figure 4a](#)).

The number of *D. suzukii* adults that emerged per fruit was also affected by treatment ( $F = 33.67$ ;  $df = 2, 11$ ;  $p < 0.001$ ) and adult fly density ( $F = 10.65$ ;  $df = 3, 11$ ;  $p < 0.001$ ) but not by their interaction ( $F = 0.69$ ;  $df = 6, 11$ ;  $p = 0.651$ ). Although GLMM results showed that, in general, HOOK SWD applied on the foliage or bark reduced the number of adults that emerged from fruit compared with the control at all fly densities, post hoc comparison tests showed no differences among treatments at the lowest fly density ([Figure 4b](#)).

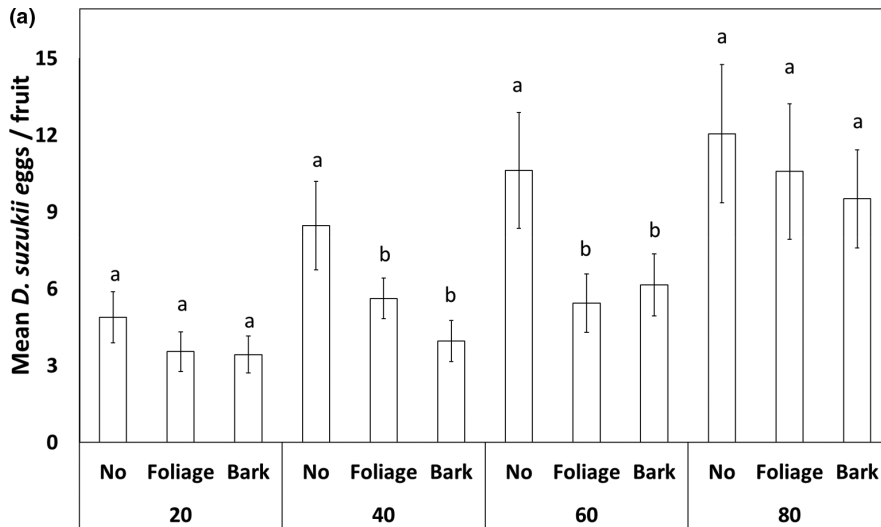
### 3.2.2 | Fruit density

Although there were no significant effects of treatment ( $F = 0.133$ ;  $df = 2, 11$ ;  $p = 0.876$ ) and fruit density ( $F = 1.895$ ;  $df = 2, 11$ ;  $p = 1.65$ ) alone, these factors interacted to affect the number of *D. suzukii* adults that emerged per fruit ( $F = 3.842$ ;  $df = 4, 11$ ;  $p = 0.011$ ). HOOK SWD applied on the foliage or bark reduced fruit infestation only at low fruit densities (25 fruits; [Figure 5](#)).

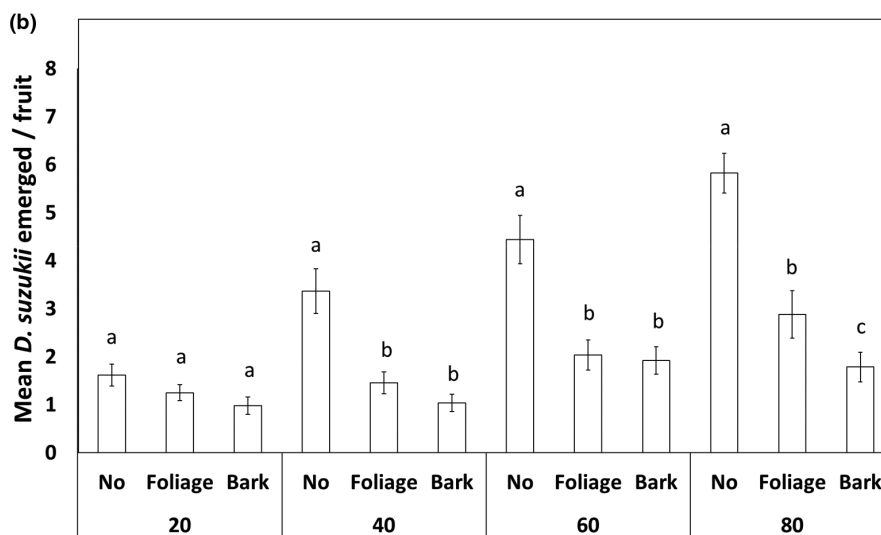
## 3.3 | Greenhouse experiment

Based on our residue study, the residual activity of HOOK SWD can persist for at least 30 days under greenhouse conditions ([Figure 6](#)).

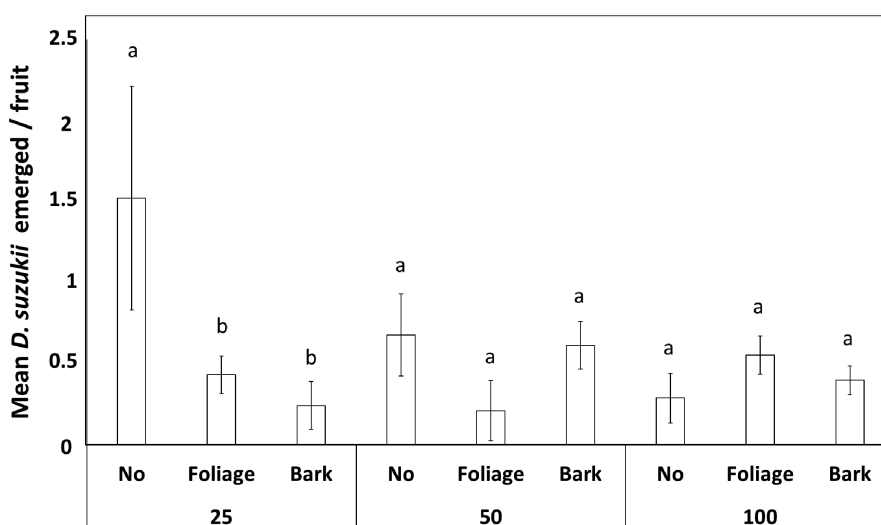




**FIGURE 4** Effect of treatment (control, HOOK SWD applied on the foliage and HOOK SWD applied on the bark) and different *Drosophila suzukii* adult densities (20, 40, 60 and 80 individuals per cage) on the number (mean  $\pm$  SE) of eggs laid per fruit (a) and the number ( $\pm$  SE) of adults emerged per fruit (b). The number of blueberry fruits per cage remained constant (25 fruits).  $n = 8$  per treatment.



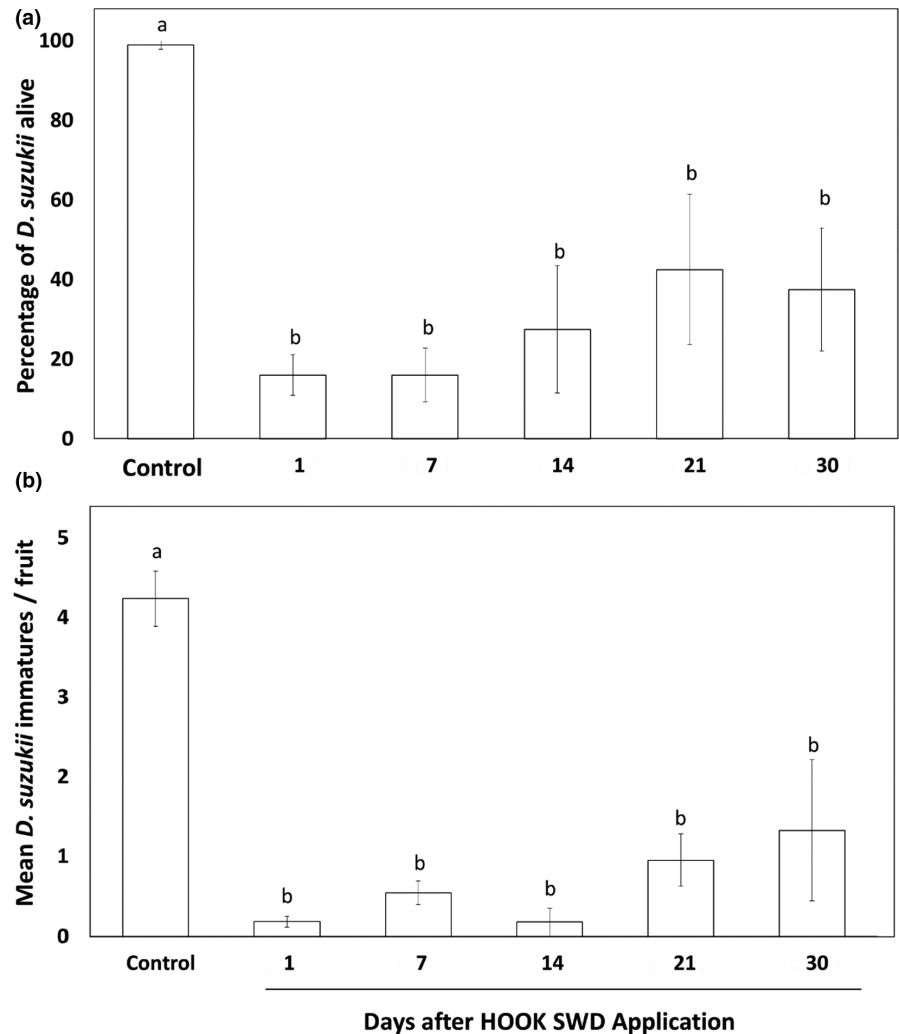
**FIGURE 5** Effect of treatment (control, HOOK SWD applied on the foliage and HOOK SWD applied on the bark) and different blueberry fruit densities (25, 50 and 100 fruits per cage) on the number (mean  $\pm$  SE) of *Drosophila suzukii* adults emerged per fruit. The number of *D. suzukii* flies per cage remained constant (40 females).  $n = 5$ .



Although there was a tendency of reduced activity in time, adult *D. suzukii* survival ( $F = 57.532$ ;  $df = 5, 31$ ;  $p < 0.001$ ; Figure 6a) and the number of larvae/pupae in fruit ( $F = 21.718$ ;  $df = 5, 31$ ;  $p < 0.001$ ;

Figure 6b) were significantly reduced when exposed to HOOK SWD, at 1, 7, 14, 21 and 30 days after application, compared with when not exposed to HOOK SWD.

**FIGURE 6** Residual activity of HOOK SWD on percent (mean  $\pm$  SE) adult *Drosophila suzukii* mortality (a) and number (mean  $\pm$  SE) of immature stages (larvae + pupae) per fruit (b) after 1, 7, 14, 21 and 30 days of application.  $n = 4$ –5 for HOOK SWD residual treatments;  $n = 9$  for control treatment.



## 4 | DISCUSSION

This study aimed to better understand the efficacy of HOOK SWD on *D. suzukii* in blueberry farms and how fly and fruit density and exposure to environmental conditions affect its efficacy. Our results provide three key findings. First, in commercial highbush blueberry farms, we showed that HOOK SWD could suppress fruit infestation; however, its efficacy was variable in time and space. Second, in semi-field cage studies, we demonstrated that HOOK SWD suppressed fruit infestation, but this suppression breaks down under low and high *D. suzukii* adult densities and high fruit densities. Third, in greenhouse studies, HOOK SWD residual activity lasted at least 30 days after application.

In commercial blueberry farms, the efficacy of HOOK SWD was spatially and temporally variable. At one of the farms (Farm A), a reduction in *D. suzukii* fly trap captures was observed on 4 out of 6 (67%) sampling dates in 2018 but only on one sampling date in 2019. By contrast, HOOK SWD did not decrease *D. suzukii* adult populations in farm B, where trap captures were even higher in HOOK SWD-treated plots in 2018. However, trap captures of adult *D. suzukii* are not always a good predictor of larval fruit infestation (Leach et al., 2019; but see Drummond et al., 2019; Rodriguez-Saona et al., 2020). In both years, out of 14 sampling dates, 6 dates (43%) showed ~40% lower fruit infestation in HOOK SWD-treated plots, while infestation in these

plots was higher in only one late-season date (7%), compared with the untreated plots. Similarly, a study by Disi and Sial (2019) conducted in blueberry fields showed that HOOK SWD treatments reduced the number of *D. suzukii* adults in traps but only later in the season, and no differences were observed in fruit infestation between the HOOK SWD and control treatments. Klick et al. (2019) also found that fruit infestations by *D. suzukii* were 2–8 times lower in small (0.2 ha) blueberry fields treated with HOOK SWD than in untreated fields. In addition, these authors showed that, in commercial raspberry fields, weekly or bi-weekly HOOK SWD applications plus a single grower standard *D. suzukii*-targeted cover spray resulted in nearly 2–5 times lower fruit infestations compared with a grower standard treatment alone. Altogether, our study and the above-mentioned previous studies show that HOOK SWD can reduce fruit infestation in small fruit farms, but its efficacy varies in space and time.

The efficacy of HOOK SWD can be context-dependent (Babu et al., 2022). As such, many factors could explain its variable efficacy in the field, including field size and location, as well as the method and amount of HOOK SWD application. For instance, the proximity of fields to non-crop habitats can affect *D. suzukii* population size because these habitats often contain alternative host plants and thus serve as a source for flies moving into nearby crops (Kenis et al., 2016; Klick et al., 2016; Leach et al., 2019; Santoiemma

et al., 2018; Tait et al., 2021; Urbaneja-Bernat et al., 2020). Within fields, resource (fruit) availability for feeding and oviposition can also affect *D. suzukii* populations. Since *D. suzukii* females can utilize both healthy and fermented fruit for oviposition (Kienzle et al., 2020), the amount of dropped fruit on the soil in fields could affect the efficacy of behavioural control strategies such as HOOK SWD; for example, through adult attraction to volatiles from fallen fruits. This led us to investigate the potential effects of adult fly and host-fruit density on the efficacy of HOOK SWD.

We found that both *D. suzukii* fly and fruit density can influence the efficacy of HOOK SWD. According to our results, we failed to detect differences in fruit infestation between HOOK SWD and control treatments when fly: fruit ratios were either <1:1 or >3:1. It is likely that, under low fly densities, we were unable to detect differences between the two treatments because the probability of flies encountering the HOOK SWD droplets was low. However, as fly density increases, intraspecific competition among flies for oviposition sites also increases (Bezerra Da Silva et al., 2019), which may increase their exposure to HOOK SWD. In fact, there is recent evidence of a host marking pheromone in *D. suzukii* (Elsensohn et al., 2021). When the fly pressure is too high, however, HOOK SWD becomes less effective at controlling *D. suzukii*, which has been observed for other semiochemical control approaches targeting insect pests such as mating disruption (Barclay & Judd, 1995) when the suppressive effect of the sex pheromone treatment is competitive or population-density dependent (Miller et al., 2006). For instance, comparable results were observed in similar semi-field cage studies with attracticidal spheres where high relative densities of *D. suzukii* flies resulted in greater emergence of adult flies from raspberry and blueberry fruit than at moderate or low relative densities (Nixon et al., 2022).

Interestingly, although HOOK SWD had no effect on the number of eggs laid per fruit at high *D. suzukii* densities, the number of flies that emerged from these fruits was reduced in the HOOK SWD treatment compared with the control. It is possible that some eggs laid in the HOOK SWD treatment, particularly at high *D. suzukii* densities, were infertile since similar formulations like ACTTRA SWD are known to especially increase the mortality of *D. suzukii* males (Babu et al., 2022). Further studies will be needed to investigate any effects of HOOK SWD and other similar formulations on *D. suzukii* egg fertility. Our results also showed that HOOK SWD treatments reduce the emergence of *D. suzukii* per fruit at relatively low, but not at higher, densities of blueberry fruits. Babu et al. (2022) reported that odours from fruits, including blueberries, were more attractive to *D. suzukii* than HOOK SWD, suggesting that, as fruit density increases, fruit odours will likely outcompete HOOK SWD for adult attraction. Also, as expected, fewer number of *D. suzukii* adults emerged per fruit as the density of fruit increased, likely because females laid eggs more evenly among fruits when more fruits were available.

Our residue studies showed that HOOK SWD efficacy can persist for at least 30 days on blueberry leaves, with a 60%–85% adult mortality under greenhouse conditions and no rainfall. These results are in accordance with Klick et al. (2019), who showed a residual activity of 35 days for HOOK SWD on raspberry leaves, with a 78%–93% adult mortality under plastic hoop houses. UV exposure and

rainfall might, however, reduce the longevity of HOOK SWD under field conditions. In our 2018 study, HOOK SWD sprayed weekly or bi-weekly yielded similar results, indicating that its activity could last at least 2 weeks under open field conditions.

In conclusion, our findings indicate that HOOK SWD could be used as an attract-and-kill tool for behavioural manipulation of *D. suzukii* in small fruit crops such as blueberries. However, HOOK SWD is a semiochemical tool that should be integrated into an IPM program. Indeed, because its efficacy was variable in space and time, HOOK SWD is not likely to work as a stand-alone management tool, especially under high *D. suzukii* adult and fruit densities. Therefore, this and other semiochemical attract-and-kill approaches are likely to work best when combined with other management methods to reduce *D. suzukii* adult and fruit densities inside and outside the crops, such as cultural, chemical and biological control methods. In fact, cultural control tactics, such as harvest frequency and sanitation (i.e. removal of fallen fruit; Schöneberg et al., 2021), could reduce fruit density in fields and thus improve HOOK SWD efficacy. Field sanitation is a strategy commonly employed for managing fruit flies to support synergies with other components of IPM, such as the use of attract-and-kill baits and biological control (Vargas et al., 2015). The removal and disposal of infested or uninfested (cull) fruit either prevents oviposition and fruit fly larvae from developing or sequesters young emerging adult flies so that they cannot return to the crop to reproduce (Liquido, 1991; Liquido, 1993).

An Asian parasitoid, *Ganaspis brasiliensis* (von Ihering), has recently been approved for release in parts of North America and Europe (Abram et al., 2022). Future studies need to determine the compatibility of HOOK SWD with biological control efforts and how this technology can be implemented into current IPM programs to manage *D. suzukii*. Because HOOK SWD is applied as point sources in spot applications, it drastically reduces the area of the crop covered by an insecticide compared with the conventional blanket insecticide sprays, thus reducing the likelihood of nontarget exposure of beneficial insects to the insecticide. In addition, HOOK SWD could reduce the frequency of blanket insecticide applications by either replacing cover sprays or by lengthening spray intervals and thus could reduce the number of insecticide applications in susceptible crops. This should make crops treated with HOOK SWD more amenable for the establishment and growth of *G. brasiliensis* populations and other biological control agents.

#### AUTHOR CONTRIBUTIONS

P.U-B, C.R-S., E.M.R., O.E.L., A.A.S. and A.M-N. designed the experiments. P.U-B., R.H., J.H-C. and E.M.R. did the experiments. P.U-B. analysed the data. P.U-B. and C.R-S. wrote the first draft of the manuscript. All the authors approved the manuscript.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.


## DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available from an online repository accessed via the following link: <https://doi.org/10.5281/zenodo.6536686>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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