Testing a Novel Attract-and-Kill Strategy for *Drosophila suzukii* (Diptera: Drosophilidae) Management

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Abstract

The invasion of the spotted wing drosophila, *Drosophila suzukii* Matsumura, across the Americas and Europe has led to increased insecticide applications to protect fruit crops. This insecticide usage conflicts with integrated pest management programs, as well as harvest, export, and pollination services in the affected crops. A novel management tool was assessed against *D. suzukii* that may mitigate these conflicts. HOOK SWD, an attract-and-kill (A&K) formulation applied as a sprayable bait, was evaluated for three growing seasons in two berry crops in New Jersey and California. In blueberry crops treated with HOOK SWD, fruit infestations by *D. suzukii* were 2–8 times lower than in untreated crops. In trials in commercial raspberry fields, weekly or biweekly HOOK SWD applications combined with a single grower standard *D. suzukii*-targeted cover spray resulted in nearly 2–5 times fewer fruit infestations compared to the grower standard cover spray alone. Assays of the residual activity of HOOK SWD resulted in more than 78–93% adult *D. suzukii* mortality when exposed to raspberry leaves after the formulation had aged for 35 d in the field under plastic hoop houses. These results suggest that this A&K strategy can be integrated in *D. suzukii* management programs.

Key words: spotted wing drosophila, semiochemical, small fruit, specialized pheromone and lure application technology (SPLAT), HOOK SWD

Since 2008, the spotted wing drosophila, *Drosophila suzukii* Matsumura, has emerged as a major pest of soft and stone fruits in the Americas and Europe (Asplen et al. 2015). Growers of the affected crops have largely reacted to the threat posed by this invasive pest by employing the prophylactic use of broad-spectrum insecticides during the harvest season (Bruck et al. 2011, Van Timmeren and Isaacs 2013). This approach has numerous negative consequences to berry production, including the disruption of integrated pest management (IPM) programs, reduction of harvest efficiency, limitation of marketing and export opportunities, greater risk to insect pollinators such as honey bees (*Apis mellifera* Linnaeus (Hymenoptera: Apidae)), increased production costs, and a broad range of environmental concerns (Farnsworth et al. 2017, Mazzi et al. 2017). To mitigate these negative consequences, more effective strategies to control *D. suzukii* without increasing pesticide inputs are needed. Current examples of such management options include netting, sanitation, fruit harvest frequency, and push-pull strategies using repellents and mass trapping (Leach et al. 2016, 2018; Wallingford et al. 2018). Border spray and alternate-row spray application techniques have been evaluated and shown to have similar efficacy as complete field cover sprays; however, conditions of higher pest pressure and increased risk limit these practices (Klick et al. 2016a).

Another strategy proposed to reduce insecticide inputs is a technique called attract-and-kill (A&K). Rather than applying insecticide in a ubiquitous cover spray, an A&K system aims to attract the pest to a discrete point source, reducing the amount of insecticide applied per unit area. This is achieved by means of a lure component and induces the attracted insect to either contact or consume the formulation (depending on the mechanism of the specific formulation). Examples of A&K formulations include insecticide-treated netting with olfactory attractants (Kuhar et al. 2017), visually attractant spheres with insecticidal wax (Rice et al. 2017), containerized bait stations, and liquid attractants with insecticides (Vargas et al. 2008). A&K products Noctovi (ISCA Technologies, Inc.) and GF-120 (Dow...
ticides were used. The A&K formulations were applied using a CO2-

Vaccinium corymbosum evaluated in small-scale field experiments at two different highbush The HOOK SWD-B and HOOK SWD-C A&K formulations were assessed for their impacts on the target insects. Because this sprayable A&K formulation is operationally easier to deploy, and thus better positioned as a product for successful and rapid adoption by growers while meeting the aforementioned beneficial criteria.

HOOK SWD (ISCA Technologies, Inc.) is an experimental sprayable A&K formulation specifically developed for D. suzukii, containing a blend of proprietary olfactory and feeding attractants, pink coloring for visual attraction, sugars for phagostimulation, and the insecticide, spinosad, incorporated at a concentration of 0.5% by weight. These AIs are formulated with a slow-release matrix SPLAT (Specialized Pheromone and Lure Application Technology, ISCA Technologies, Inc.) and does not require any mixing with water or adjuvants. The SPLAT matrix provides controlled release of labile semiochemicals (Stelinski et al. 2005) and has been used in mating disruption, A&K, and attractant and repellent formulations for insect pests and pollinators (e.g., Vargas et al. 2008, Rodriguez-Saona et al. 2010, Mafra-Neto et al. 2013). The volatile compounds used in the HOOK formulation are known attractants to D. suzukii adults (El Hadi et al. 2013, Kleiber et al. 2014, Aprea et al. 2015), and flies have been observed making direct contact with HOOK in laboratory assays (W. H. Urrutia, unpublished data).

The objective of the studies was to determine the effects of the HOOK SWD A&K formulation on D. suzukii in small- and large-scale blueberry and raspberry field trials, and to conduct a residual activity test in raspberry. We hypothesized that HOOK SWD A&K provides season-long fruit protection (i.e., reduced fruit infestation) against D. suzukii in small fruit crops.

Materials and Methods

A&K Formulations

Drosophila suzukii A&K prototype formulations were evaluated between 2014 and 2016 for impact on fruit infestation levels. They were slightly adjusted and improved three times throughout the trials and are differentiated by HOOK SWD-A, HOOK SWD-B, and HOOK SWD-C. These experimental formulations of HOOK SWD were assessed for their impacts on the target insects. Because this formulation constitutes proprietary technology, the authors are unable to disclose detailed compositions, but experimental formulations are available for further study while regulatory approval is pending.

Small-Scale Field Experiments

The HOOK SWD-B and HOOK SWD-C A&K formulations were evaluated in small-scale field experiments at two different highbush blueberry (Vaccinium corymbosum L.) farms where no other insecticides were used. The A&K formulations were applied using a CO2-powered MeterJet spray gun (Spraying Systems Co., Wheaton, IL). The sprayer was calibrated to deliver 16.1 liters of each formulation per ha at 275 kPa. Each blueberry plant received four spray shots of 1.34 ml HOOK SWD-B. These experiments were conducted in open (i.e., non-protected by plastic, glass, etc.) blueberry fields.

In 2014, three 0.04 ha blocks of blueberries (cv. ‘Elliott’) at the Rutgers P.E. Marucci Center in Chatsworth, NJ (39°43′02.3″N 74°30′53.7″W) were divided into two plots (each 0.02 ha) per block, with one row (1.5 m) buffer in between plots. These plots were randomly assigned to either HOOK SWD-B or untreated control, and each plot was considered a replicate. Treatment applications were made on 27 July and 4 August. At 1900 hours on 5 August, 1,500 D. suzukii adults (3–7 d old, 1:1 female:male) from a laboratory colony were released to increase D. suzukii pest pressure. The colony was initiated in 2013 from individuals collected in Hammon, NJ, and were maintained in a laboratory growth chamber (24°C, 45% RH, and a photoperiod of 16:8 (L:D) h) on solid food diet as described by Dalton et al. (2011). Flies were released about 1 m above ground from 150 vials spread evenly across the experimental area. On 11 August, 7 d after the second HOOK SWD application, 10 fruit samples were collected: 340 g (~150 berries) of newly developed marketable blueberry fruit were harvested along two diagonal transects across the center of each plot. Larval extractions from fruit were made using the salt water extraction method. Fruit was crushed in salt solution of 10 brix for 15 min and all larvae removed and counted (Dreves et al. 2014).

A second field experiment was conducted in 2015 at a commercial blueberry (cv. ‘Bluecrop’) farm in Hammonton, NJ (39°40′11.6″N 74°46′58.1″W). The experimental protocol used in the 2014 study was similar in 2015, with a few exceptions. The A&K formulation applied was experimental prototype HOOK SWD-C rather than HOOK SWD-B, and because this field had higher D. suzukii pressure based on current and historical trapping data, it was not considered necessary to release colony flies into the field. There were two replicated plots per treatment and HOOK SWD applications were made on 29 July and 4 August. On 14 August, 10 d after the second HOOK SWD application, fruit samples were collected following the 2014 protocol; larval extractions from fruit were also the same as in 2014.

Large-Scale Field Experiments

Blueberry Field Experiment

A field experiment was conducted in 2016 to test HOOK SWD in a commercial highbush blueberry farm located in New Lisbon, NJ (39°55′55″N, 74°36′58″W). Eight plots, each ~0.2 ha in size, were selected from an open field planted with cv. ‘Elliot’ blueberries not being subjected to any form of insecticide treatment. Four of these plots were randomly assigned to receive treatment with HOOK SWD-A, while the remaining four plots were left untreated to serve as controls. HOOK SWD-A treatments were applied weekly for four weeks to every fourth bush in each row in treated plots with the same application method as described in the previous section but at a lower rate of 3.7 liters/ha. Each week, six samples were collected in a transect across each plot, 6 d after each application. Each sample consisted of 180 g (~80 berries) of marketable fruit chosen randomly from five bushes at each sampling point. In HOOK plots, each sample included one treated bush and the four neighboring untreated bushes. Fruit samples from treated and untreated bushes were then placed together in individual plastic cups and incubated ~5 d under laboratory conditions (21°C, 53% RH and 12:12 (L:D) h), at which point larval infestations were determined with the saltwater extraction method.

Raspberry Field Experiments

In the spring of 2016, two HOOK SWD formulations were evaluated for their impact on D. suzukii in red raspberry (Rubus idaeus L.) crops, on a field under standard grower management practices.
for this pest. The trial site was established on a farm in Camarillo, CA (34°10.17′N, 119°2.30′W), planted with raspberry cv. ‘#1’ (Driscoll’s proprietary cultivar), and grown under plastic hoop tunnels (each tunnel is typically 4.1 m tall by 91.4–106.8 m long) with open sides and entrances. The site was divided into a series of plots approximately 1–1.5 ha in size, assigned to one of three treatments: 1) grower standard D. suzukii management spray, which was a single cover spray of spinetoram (105.1 g AI/ha, Delegate WG, Dow AgroSciences, Indianapolis, IN) applied to the entire canopy and trial area on week 4 of the harvest season; 2) HOOK SWD-A plus the same grower standard spray; 3) HOOK SWD-B plus the grower standard spray. The HOOK SWD formulations were applied weekly at a rate of 3.7 liters/ha starting at the beginning of harvest through the end of harvest 9 wk later, using a hand-pump backpack sprayer (#190359, Smith 4-gallon tank sprayer, Lowe’s, Mooresville, NC) with an even flat spray nozzle (#TP8002E, TeeJet Technologies, Wheaton, IL) as shown in Fig. 1a. A liquid splatter (Fig. 1b) covering an area of approximately 1 meter was treated every 3–5 m to one side of each row (three rows per tunnel), as illustrated in Fig. 1c. Eight samples per treatment of fifty marketable fruits were collected weekly within 25 m of a plant row. Larvae were extracted from each fruit sample with the salt water extraction method after a 2 d incubation in the laboratory.

Two experiments were conducted in the fall of 2016 to evaluate the effects of HOOK SWD-A formulation on D. suzukii larval counts in fruits of raspberry crops under standard D. suzukii grower management practices. The first experiment was conducted in Oxnard, CA (34°7.26′N, 119°3.4′W). Three 2.4 ha blocks of raspberry fields, planted with cv. ‘#2’ (Driscoll’s proprietary cultivar) grown under plastic hoops, were divided into two plots of 1.2 ha, each randomly assigned to one of the following two treatments: 1) HOOK SWD-A plus grower standard D. suzukii management spray; or 2) standard sprays alone. Each plot had four sampling areas as described in the previous raspberry trial. In plots assigned to HOOK SWD-A treatment, the A&K formulation was applied biweekly (every 2 wk), starting at the beginning of harvest through end of the harvest 7 wk later. The grower standard cover spray, applied to the entire canopy of the trial area, was spinetoram + malathion (2,241.7 g AI/ha, Malathion 8 Aquamul, Loveland Products, Inc., Greeley, CO) on week 1.

The second fall experiment was conducted at a raspberry farm in Watsonville, CA (36°54.42′N, 121°39.53′W), planted with cv. ‘#1’ grown under plastic hoops, and had the same treatments as in Oxnard. Treatment plots were assigned to entire 1.5 ha blocks and each replicated three times. Each plot had fruit sampling areas as described before. HOOK SWD-A formulation was applied weekly starting at the beginning of harvest and ending 9 wk later. The grower standard cover spray was a zeta-cypermethrin (56.0 g AI/ha, Mustang 1.5 EW, FMC Corporation, Philadelphia, PA) application to the entire canopy and trial area 2.5 wk before the end of harvest.

Residual Activity Test
A residual activity assay was done to determine how field-aged A&K formulations influence D. suzukii adult mortality. A raspberry field in Watsonville, CA (36°55.09′N, 121°40.45′W) was selected to evaluate residual activity under plastic hoops. Approximately 200 raspberry leaflets were each treated across 10 plant rows with 0.2 ml dollops of the HOOK SWD-A or B formulations using a syringe on 7 June 2016. Fifteen leaflets with the A formulation, B formulation, and untreated were removed from the same side of a plant canopy on 1, 7, 14, 21, 29, and 35 days after treatment. Each leaflet was placed in a 50 ml conical centrifuge tube (Falcon, Oxnard, CA) filled with water and wrapped in Parafilm (Bemis NA, Neenah, WI), which was adhered inside a 946 ml plastic deli cup with 18 small holes (each 1 mm diameter) on the sides. Ten D. suzukii adults (4- to 8-d-old) of each sex were added to each container. Every container had a wick of 10% sugar water for food. Flies were originally collected from a managed field in Watsonville, CA, in fall 2015 and again in spring 2016 and were reared in a laboratory colony room at 24°C, 50% RH, and a photoperiod of 14:10 (L:D) h on solid food diet of 78 ml mashed potatoes (General Mills, Inc., Minneapolis, MN), 5 ml granulated sugar, 1.25 ml active dry yeast (Lesaffre Yeast Corporation, Milwaukee, WI), and 118 ml water per 946 ml plastic deli cup. Adult D. suzukii mortality was assessed after 24 h of exposure to treatments with laboratory conditions about 21°C and 53% RH and natural day length.

Statistical Analysis
Mean number of larvae per berry was calculated for each plot prior to analysis. Data were analyzed using analysis of variance (ANOVA) if assumptions of normality and homogeneity of variances were met. Welch’s ANOVA was used when the assumption of homogeneity of variances was not met. Post hoc comparisons were made with the Tukey-Kramer HSD test. Analyses were done using Minitab version 16 (Minitab, Inc., State College, PA) for blueberry data and JMP version 13.2.1 (SAS Institute, Inc., Cary, NC) for raspberry data. The alpha was 0.05 in all tests.

Results
Small-Scale Field Experiments
In 2014 field trials, blueberry fruit collected 7 d after the second HOOK SWD application had an average of 0.026 (±0.001 SE) D. suzukii larvae per berry compared to 0.061 larvae (±0.009 SE) per berry in

![Fig. 1.](image-url)
untreated control fruit ($F = 15.29; \text{df} = 1, 5; P = 0.017$). In 2015, blueberry fruit collected 7 d after HOOK SWD application had an average of 0.022 ($\pm 0.008$ SE) larvae per berry compared to 0.169 ($\pm 0.024$ SE) larvae per berry in the control ($F = 33.28; \text{df} = 1, 3; P = 0.029$).

Large-Scale Field Experiments

Blueberry Field Experiment

There was no difference in the number of *D. suzukii* larvae between treatments on week 1, prior to the first application of the A&K formulation in the field ($F = 1.161; \text{df} = 1, 7; P = 0.322$). However, berries collected from HOOK SWD-treated bushes had 5.9, 5.2, and 3.8 times fewer larvae compared to the untreated control bushes at week 2 ($F = 12.71; \text{df} = 1, 7; P = 0.011$), week 3 ($F = 29.92; \text{df} = 1, 7; P = 0.001$), and week 4 ($F = 92.67; \text{df} = 1, 7; P < 0.001$), respectively (Fig. 2).

Raspberry Field Experiments

Pre-trial sampling on the first collection week showed that raspberry fruit from the HOOK SWD-A and grower standard pesticide spray treatment had more larvae than fruit treated with HOOK SWD-B plus grower standard ($P = 0.0153$, Tukey-Kramer) and the grower standard alone ($P = 0.0055$, Tukey-Kramer) (Fig. 3a). However, by week 8, HOOK SWD-A and SWD-B-treated crops had significantly fewer larvae than those treated with the grower standard spray alone ($P = 0.0448$ and $P = 0.0478$, respectively, Tukey-Kramer). These results remained consistent for HOOK SWD-A for the rest of the season (week 9 [$P = 0.0344$, Tukey-Kramer], week 10 [$P < 0.0001$, Tukey-Kramer], and for HOOK SWD-B only on week 10 ($P = 0.0001$, Tukey-Kramer). Averaged across the entire harvest season, crops treated with HOOK SWD-A plus grower standard (0.04 larvae per berry $\pm 0.01$ SE) and with HOOK SWD-B plus grower standard (0.05 larvae per berry $\pm 0.01$ SE) had 82% and 73% fewer larvae, respectively, than crops treated with the grower standard spray alone (0.20 larvae per berry $\pm 0.04$ SE).

In the fall Oxnard raspberry trial, HOOK SWD plus grower standard pesticide spray had significantly fewer larvae in fruits starting on the fourth week of the trial ($F = 5.02; \text{df} = 1, 21; P = 0.0362$) and continuing through the end of harvest (week 5 [$F = 15.82; \text{df} = 1, 17; P = 0.0010$], week 6 [$F = 38.26; \text{df} = 1, 20; P < 0.0001$], and week 7 [$F = 4.90; \text{df} = 1, 20; P = 0.0387$]), as in Fig. 3b. Averaged across the entire season, there were 38% fewer larvae in samples from HOOK SWD-treated crops (0.11 larvae per berry $\pm 0.02$ SE) than in those from crops treated with the grower standard spray alone (0.18 larvae per berry $\pm 0.03$ SE).

![Fig. 2. Average number of *Drosophila suzukii* larvae per berry (±SE) collected from a commercial blueberry field in New Lisbon, NJ treated weekly with HOOK SWD-A and untreated control in 2016. Averages with two stars within a week are significantly different from each other.](image)

![Fig. 3. Average number of *Drosophila suzukii* larvae per raspberry fruit (±SE) of the (a) spring trial in Camarillo, CA, with grower standard *D. suzukii* spray alone, weekly HOOK SWD-A + standard, and weekly HOOK SWD-B + standard. The grower standard sprays are indicated by an arrow: spinetoram on week 4. Averages with different letters within a week are significantly different from each other; (b) fall trial in Oxnard, CA, with grower standard alone and biweekly HOOK SWD-A + standard, and spinetoram + malathion spray on week 1 indicated by arrow. Averages with two stars within a week are significantly different from each other; (c) fall trial in Watsonville, CA, of grower standard alone and weekly HOOK SWD-A + standard, and zeta-cypermethrin spray on week 7 indicated by arrow. Averages with two stars within a week are significantly different from each other.](image)
In the fall Watsonville trial, HOOK SWD plus grower standard pesticide spray had significantly fewer larvae in fruits starting on the fourth week \((F = 13.89; \text{df} = 1, 19; P = 0.0014)\) of the trial through the end of harvest (week 5 \([F = 14.06; \text{df} = 1, 17; P = 0.0016]\), week 6 \([F = 11.82; \text{df} = 1, 16; P = 0.0034]\), week 7 \([F = 9.96; \text{df} = 1, 11; P = 0.0088]\), week 8 \([F = 18.40; \text{df} = 1, 12; P = 0.0010]\), and week 9 \([F = 16.75; \text{df} = 1, 13; P = 0.0012]\), as in Fig. 3c. Averaged across the entire season, there were 76% fewer larvae in samples from HOOK SWD-treated crops (0.18 larvae per berry ± 0.02 SE) than from those treated with the grower standard spray alone (0.74 larvae per berry ± 0.11 SE).

**Residual Activity Test**

Both HOOK SWD formulations (A and B) induced significantly higher percent mortality in *D. suzukii* adults exposed to field-aged product on raspberry leaves compared to untreated leaves on all days after application (all Ps < 0.001, Tukey-Kramer) (Table 1).

**Discussion**

In blueberry crops treated with HOOK SWD, fruit infestations by *D. suzukii* were 2–8 times lower than in untreated crops. Similarly, in trials in commercial raspberry fields in southern California, weekly or biweekly HOOK SWD applications plus a single grower standard *D. suzukii*-targeted cover spray resulted in nearly 2–5 times fewer fruit infestations compared to the grower standard alone. This study provides the first evidence of an A&K strategy at large commercial scale that can be implemented in small fruit IPM programs to manage this invasive pest. Moreover, our study indicates that HOOK SWD is equally effective under open blueberry field conditions and in semi-open (i.e., not completely enclosed plastic hoops) raspberries where movement of *D. suzukii* flies from outside into the crop was nothampered. Thus, we expect HOOK SWD to perform equally well or better in crops growing under closed conditions (i.e., use of exclusion nets for blueberries or where plastic hoops have netted ends for raspberries; Cormier et al. 2015, Leach et al. 2016, Rogers et al. 2016) where movement of flies from outside is much reduced.

The residual assays of flies exposed to field-aged HOOK SWD had kill rates above 78% when the formulations had aged up to 35 d. However, the protocol used for residual analysis will likely not translate to a real-world field environment. In the lab, competitive forces are removed (e.g., alternative sources of food or odor), and high humidity within the deli containers rehydrate and revitalize the product. Also, our residue assays were conducted in a raspberry field under (not completely closed) plastic hoops, which could have somewhat protected HOOK SWD from environmental breakdown. In fact, the tested formulations of HOOK SWD are not rainfast, somewhat protected HOOK SWD from environmental breakdown.

**Table 1. Percent mortality of *D. suzukii* adults in raspberry residual activity tests**

| Treatment          | 1 DAT | 7 DAT | 14 DAT | 21 DAT | 29 DAT | 35 DAT |
|--------------------|-------|-------|--------|--------|--------|--------|
| Untreated control  | 0.3b  | 0.2   | 8.1b   | 1.2    | 4.8b   | 0.7    |
| HOOK SWD-A         | 88.6a | 2.6   | 87.0a  | 2.2    | 90.4a  | 1.6    |
| HOOK SWD-B         | 87.1a | 1.9   | 91.1a  | 1.7    | 92.6a  | 1.5    |

*Numbers with different letters within a column are significantly different.
DAT, days after treatment.*
tactic to broad-spectrum chemical control for managing D. suzukii in small fruit operations. From 2009 to 2014, D. suzukii was found to be responsible for nearly US$ 40 million in revenue losses in California raspberry (Farnsworth et al. 2017), despite the prophylactic use of broad-spectrum insecticide sprays. When HOOK SWD was combined with grower standard complete cover sprays in raspberry field trials, very little fruit infestation was detectable shortly after, suggesting that significant losses could be avoided when a single broad-spectrum spray is combined with a preventative program such as HOOK SWD. Further studies are required to evaluate optimal HOOK SWD spray frequency, longevity compared to other pesticides, efficacy on other drosophila pests, nontarget impacts, and effect on D. suzukii populations in other production systems (e.g., open versus closed fields, organic versus conventional farming, etc.) and regions.

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