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Potential Alternatives to Spinosad as the Killing Agent Mixed With Two Attractant Products in Attract-and-Kill Formulations Used to Manage the Spotted-Wing Drosophila, *Drosophila suzukii* (Diptera: Drosophilidae)

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Abstract

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a key pest of many berry and fruit crops worldwide. The primary method of controlling this pest is the application of insecticides. Attract-and-kill is a management tactic that may reduce the number of insecticide applications needed to manage *D. suzukii*. ACTTRA SWD OR1 and ACTTRA SWD TD, developed by ISCA Technologies Inc., combine *D. suzukii* attractants with a gel matrix. Growers add an insecticide as a killing agent. The only USDA National Organic Program approved organic insecticide that has been shown to be effective as a killing agent is spinosad. This study aimed to determine the efficacy of other USDA National Organic Program approved organic insecticides, including Grandevo 30 WDG (*Chromobacterium subtsugae* strain PRAA4-1 30%), MBI-203 SC2 (*C. subtsugae* strain PRAA4-1 98%), Venerate XC (*Burkholderia* spp. Strain A396 94.45%), MBI-306 SC1 (*B. rinojensis* Strain A396 94.45%), Azera (azadirachtin 1.2% + pyrethrins 1.4%), and PyGanic (pyrethrins 1.4%), when used as the killing agent with the two ACTTRA SWD products. Lab and cage bioassays were conducted. Entrust (spinosad 22.5%) and PyGanic were the only compounds that showed some efficacy when used with ACTTRA SWD OR1 and ACTTRA SWD TD.

Key words: attract-and-kill, ACTTRA OR1, ACTTRA TD, blueberries, spotted-wing drosophila

Spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is a major pest of berry and thin-skinned fruit crops in Asia, where it is native, and in Europe, the Americas, and Africa (Hauser 2011; Lee et al. 2011a,b; Walsh et al., 2011; Calabria et al. 2012; Cini et al. 2012; Deprá et al. 2014; Boughdad et al. 2021). They infest ripening and ripe fruit using the female's serrated, heavily sclerotized ovipositor (Hauser 2011). A single larva detected in a fruit can cause an entire shipment of fruit to be rejected.

Growers rely heavily on insecticide applications to manage *D. suzukii* populations (Haviland and Beers 2012, Timmeren and Isaacs 2013, Diepenbrock et al. 2016). Conventional growers have many effective options, while organic growers are limited to USDA National Organic Program approved organic spinosad products and a few others (Liburd and Rhodes 2020). Concerns about resistance development (Gress and Zalom 2019, Timmeren et al. 2019, Isaacs et al. 2022), pesticide residues, and secondary pest outbreaks highlight the need for alternative tactics to manage *D. suzukii*. One such alternative tactic is the attract-and-kill technique.

In attract-and-kill techniques, an attractant, usually a pheromone or food bait, is used to bring large numbers of a pest to a specific site where the pests are exposed to an insecticide or other killing agent (Vargas et al. 2008). Attract-and-kill tactics can be deployed as treated spheres, bait stations, and liquid gels that partially solidify once applied (Vargas et al. 2008, Rice et al. 2017). Attractants need to be specific to the target pest to be effective and avoid nontarget effects (Gregg et al. 2018). The best insecticides for use as killing agents are quick acting and not repellent to the target pest (Gregg et al. 2018).

A commonly used attract-and-kill tactic against Tephritid fruit flies is the use of insecticide treated spheres (Liburd et al. 2003, Wright et al. 2012, Morrison et al. 2016). The attractant component combines a visual attractant (shape and color of the sphere) with a food bait (sucrose). The killing agent is an insecticide impregnated into the spheres. Rice et al. (2017) tested insecticide treated spheres against *D. suzukii* and found that they reduced fruit infestation in the field. Fruit infestation was further reduced with the addition of insecticide applications.

ISCA Technologies Inc. uses a gel matrix called SPLAT (Specialized Pheromone and Lure Application Technology) to carry attractants and killing agents for many pests. They initially developed a product to manage *D. suzukii* called HOOK SWD, which used food-based odors and red color as the attractant and spinosad as the killing agent. Results of field trials have shown efficacy but have been variable (Disi and Sial 2019, Klick et al. 2019). For example, Disi and Sial (2019) found no differences in *D. suzukii* trap catch comparing a grower's standard treatment to HOOK SWD plus grower's standard. In contrast, Klick et al. (2019) found a 2–8 times lower fruit infestation in blueberry crops and a 2–5 times lower fruit infestation in raspberry crops treated with HOOK SWD compared with untreated controls. Laboratory and field cage studies have shown that both fruit and *D. suzukii* density affect the efficacy of HOOK SWD (Urbaneja-Bernat et al. 2022).

ISCA has recently developed two products containing only a blend of semiochemical attractants, sugar as a phagostimulant, and the red dye mixed in the SPLAT matrix called ACTTRA SWD OR1 (OR1) and ACTTRA SWD TD (TD). OR1 contains the same 4-component attractant blend used in the HOOK SWD product. TD uses an 8-component attractant blend. Omitting spinosad allows for the use of various insecticides as the killing agent. Babu et al. (2021) found that several conventional products mixed with TD were effective in reducing *D. suzukii* numbers in laboratory studies. The two organic products that showed some efficacy when mixed with TD were Entrust 2SC and Azera 0.21SL.

The purpose of this study was to determine the efficacy of OR1 and TD mixed with various organic insecticides against *D. suzukii* in laboratory and field cage assays. Products tested included azadirachtin + pyrethrins, pyrethrins, two formulations of *Chromobacterium subtsugae* strain PRAA4-1 (Neisseriales: Neisseriaceae), two formulations of *Burkholderia rinojensis* strain A396 (Burkholderiales: Burkholderiaceae), and spinosad. Both adult mortality and progeny emergence from the fruit were measured.

Materials and Methods

Colonies

Colonies of *D. suzukii* were established and maintained by each collaborating university (University of Florida, and University of Georgia). Flies were reared on a standard diet of either cornmeal-molasses-yeast or cane sugar-yeast medium (Jaramillo et al. 2015, Gautam et al. 2016). Adults 4–10 d old were used in the bioassays and were starved for ~ 2 hr before use. Individuals were removed from colony rearing containers by aspiration or anesthetized using CO_2.

Lab Studies

Blueberry leaf terminals used in the assays were collected from the University of Florida's Plant Science Research and Education Unit (PSREU) in Citra, FL., and the University of Georgia Blueberry Research and Demonstration Farm in Alma, GA. and were from the varieties 'Farthing' and 'Star' highbush blueberries, respectively. Organic fruit was purchased from two grocery stores, Publix (Florida) and Kroger (Georgia). Fruit purchased in Florida was originally from Peru and distributed by Wish Farms (Plant City, FL), while fruit purchased in Georgia was from Simple Truth Organic (Kroger Co. Cincinnati, OH).

Highbush blueberry leaf terminals with 3–4 leaves were clipped from the field as described above and were placed in 50 ml centrifuge tubes cut down to 30 ml containing tap water or inserted into a water pick (DL 3805, Diamond Line, Akron, OH), filled with distilled water, which was then fitted through a circular hole in the bottom center of the arena. Each terminal received a single 0.2 ml drop of one ACTTRA treatment, applied to a leaf using a 1 ml disposable syringe. Each treatment was replicated 5 times. One treated terminal was placed in each assay container, consisting of a 946 ml deli container. Each assay container had 13–15 loose un-infested blueberry fruit placed in the bottom. Each chamber also contained a water source for the flies that was composed of a plastic test tube filled with DI water with a dental wick inserted in the top.

Treatments tested in Florida included six organic insecticides (Table 1) mixed with ACTTRA SWD TD (8 component attractant blend, Batch B406, 15 Oct. 2020, ISCA Technologies, Riverside, CA): Grandevo WDG, MBI-203 SC2, Venerate XC, MBI-306 SC1, Azera, and PyGanic, which were compared to the commercially produced HOOK SWD (4 component attractant blend + 0.25% spinosad, Batch 2458781, ISCA Technologies, Riverside, CA), a control treatment consisting of the TD product with no insecticide added, and an untreated control treatment. Treatments tested in Georgia also included six organic insecticides (Azera, Entrust, Grandevo WDG, MBI-203 SC2, PyGanic, and Venerate XC) mixed with ACTTRA SWD OR1 (4 component attractant blend, Batch B408, 14 Oct. 2020, ISCA Technologies, Riverside, CA), OR1 with no insecticide added, and an untreated control. Treatment insecticides were incorporated into ACTTRA at a uniform rate of 0.25% A.I. (percent active ingredient) to match the A.I. of spinosad in the commercial

Table 1. Insecticides used in the lab and cage assays

Insecticide Trade Names	Active ingredient(s)	Company information
Azera[a]	Azadirachtin (1.2%) + pyrethrins (1.4%)	Valent, Walnut Creek, CA
Grandevo 30 WDG[a]	*Chromobacterium subtsugae* strain PRAA4-1 (30%)	Marrone Bio Innovations, Davis, CA
MBI-203 SC2[b]	*Chromobacterium subtsugae* strain PRAA4-1 (98%)	Marrone Bio Innovations, Davis, CA
Entrust 2 SC[a]	Spinosad (22.5%)	Dow AgroSciences, Indianapolis, IN
PyGanic 1.4 EC[a]	Pyrethrins (1.4%)	MGK, Minneapolis, MN
Venerate XC[a]	*Burkholderia* spp. strain A396 (94.45%)	Marrone Bio Innovations, Davis, CA
MBI-306 SC1[b]	*Burkholderia rinojensis* strain A396 (94.45%)	Marrone Bio Innovations, Davis, CA

[a]USDA National Organic Program approved.
[b]Organic insecticides under development

HOOK SWD product. DI water was added to all treatments to dilute the ACTTRA in each treatment down to the concentration found in the treatment with the insecticide that had the lowest % A.I.

Flies were added to the assay containers within 1–2 hr after terminals were treated. For each bioassay chamber, a total of 10 adult flies (5 females and 5 males) were removed from the *D. suzukii* colony and kept in a clean 50 ml centrifuge tube before being released into an assay container. Flies were 5–7 d old (sexually mature) and were starved for ~2 hr before release. After *D. suzukii* flies were added to the assay containers, the containers were capped and placed on a bench in the lab in a completely randomized design under a 14:10 (L:D) photoperiod and at ambient temperature (~25°C) during the observation period of 6 d.

Adult fly mortality data were collected at 1, 3, and 6 d after exposure to treated foliage, and percent mortality was calculated. Berries were removed from assay containers on day 6–7, placed in ventilated 237 ml deli containers, and incubated in an environmental chamber (Percival Scientific Inc., Perry, IA, USA) at 23 °C and 65% RH under a 14:10 (L:D) hr photoperiod.

Small Cage Assays

The experiment was conducted in 1.8 × 1.8 × 1.8 m field cages (0.5 mm mesh) in shaded, grassy areas on the University of Florida Entomology and Nematology Department grounds from 12 October 2021 through 17 November 2021 and on the University of Georgia Entomology Department grounds from 27 October 2021 through 18 November 2021. Cages were spaced at least 1 m apart. A 2-yr-old potted southern highbush var 'Farthing' (Florida) and 'Star' (Georgia) blueberry plant was placed in the center of each mesh cage and surrounded by a tomato cage.

The experiment was a randomized complete block design with the blocking factor being week. Each of the 6 treatments was replicated 4 times, once each week. In Florida, the 6 treatments included untreated (no TD), TD with no insecticide added, TD + Entrust, TD + Azera, TD + PyGanic, and TD + MBI-306 SC1. The treatments in Georgia were similar, except that instead of TD, OR1 was the attractant matrix choice. Treatments were randomly assigned to cages and treatment location was re-randomized each week.

Twenty-four hours before the start of each block, 2 ml of each ACTTRA treatment was applied to a strip of clear plastic per cage. After the 24 hr drying period, the clear plastic strip was placed on the stem of each plant so that the bottom of the strip was just above the soil level in the plant pots in each cage. No strip was hung in the untreated control.

After the placement of the ACTTRA treatments, 5 blueberry clusters (cluster = 5 fruit) were hung from the tomato cages and blueberry branches for each treatment. In Florida, for block 1 and 2, USDA-certified organic blueberries were purchased the day before the experiment (Block. 1: Driscoll's Inc., Watsonville, CA; Block. 2: Wish Farms Inc., Plant City, FL). For block 3 and 4, var 'Farthing' blueberries were collected from the research farm at the Citra PSREU one day before the experiment. In Georgia, DIVINO organic blueberries (Peoagro S. A., Lima, Peru) were used in the experiment. Berries were washed with Fit Fruit & Vegetable Wash (HealthPro Brands, Inc., Cincinnati, OH) using the directions on the label and then air dried on a paper towel for about 10 min. Clusters of 5 fruit were then placed in mesh bags (3 mm diameter openings; Jo-Ann Stires, LLC, Hudson, OH) tied closed with twist-ties. These bagged fruit clusters were kept in the refrigerator overnight. Additionally, a sample of 20 fruit was also taken and placed in a Percival environmental chamber (Percival Scientific Inc., Perry, IA, USA) at 23°C and 65% RH under a 14:10 (L:D) hr photoperiod for 2.5–3 wk to determine natural infestation. No *D. suzukii* emerged from any of these samples.

Next, 50 female *D. suzukii* per treatment were removed from colony bottles and placed into individual 50 ml centrifuge tubes. Flies were 5–7 d old and starved for at least an hour before being released into each cage. Flies were released after 4 pm EDT.

After 24 hr, berry clusters and ACTTRA strips were removed from the cages. A single red sticky trap baited with a Trece lure (Trece Inc., West Adair, OK) was then placed in each cage to capture any surviving *D. suzukii*. These traps were collected after another 24 hr had passed, and the number of *D. suzukii* on them was counted and recorded. In Georgia, any live female *D. suzukii* observed inside the cage, which were not captured by the sticky trap, were hand collected by inspecting each cage for 5 minutes and were added to the trap capture count before analysis.

Each fruit cluster was placed into a 30 ml plastic portion cup. The tops of the containers were punctured with small holes to allow air exchange. The containers were placed in the same environmental chamber indicated above for 2.5–3 wk. After this time, emerged adult *D. suzukii* were counted and sexed.

Data Analysis

For the lab studies, cumulative percent mortality was arcsin transformed to normalize the data and then analyzed in proc glm using a repeated measures analysis of variance (ANOVA) with treatment and sex as factors, and means were separated by Tukey's multiple comparison tests (SAS 2016). Total *D. suzukii* per fruit was square root transformed to normalize the data and analyzed in proc glm using one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests.

For the small cage studies, mean *D. suzukii* per trap and per fruit were square root transformed to normalize the data and then analyzed in proc glm using a two-way analysis of variance (ANOVA) with treatment and sex as factors and Tukey's multiple comparison tests (SAS 2016).

Results

Lab Studies

In the laboratory TD study done in Florida (Fig. 1a), there were significant differences among treatments ($F = 34.3$; df = 8, 68; $P < 0.0001$), but not between the sexes ($F = 1.7$; df = 1, 68; $P = 0.20$) nor was there a trt*sex interaction ($F = 0.46$; df = 8, 68; $P = 0.44$). There was a significant interaction between day and treatment ($F = 3.01$; df = 16, 136; $P = 0.0003$), so data from each day is shown. The other interactions with day were not significant (day*sex: $F = 0.33$; df = 2, 136; $P = 0.68$; day*trt*sex: $F = 0.61$; df = 16, 136; $P = 0.87$). The HOOK SWD treatment caused significantly higher mortality compared with all other treatments on 1, 3, and 6-d post-treatment, except compared with TD + PyGanic 6-days post-treatment. On 3 and 6-days post-treatment, TD + PyGanic caused higher mortality compared with all the other treatments except HOOK SWD on both days and TD + MBI-306 SC1 3 d post-treatment. There was no difference in mean emergence among treatments ($F = 0.75$; df = 8, 44; $P = 0.65$). Mean progeny emergence ranged from 13 ± 4 (mean ± SEM) *D. suzukii* per sample in the TD + Grandevo WDG treatment to 26 ± 11 *D. suzukii* per sample in the untreated control. Means of 14 ± 5, 14 ± 5, 23 ± 6, 15 ± 8, 14 ± 3, 17 ± 9, and 19 ± 6 progeny emerged from the TD + Pyganic, TD + Azera, TD + MBI-306 SC1, TD + Venerate XC, TD + MBI-203 SC2, HOOK, and TD treatments respectively.

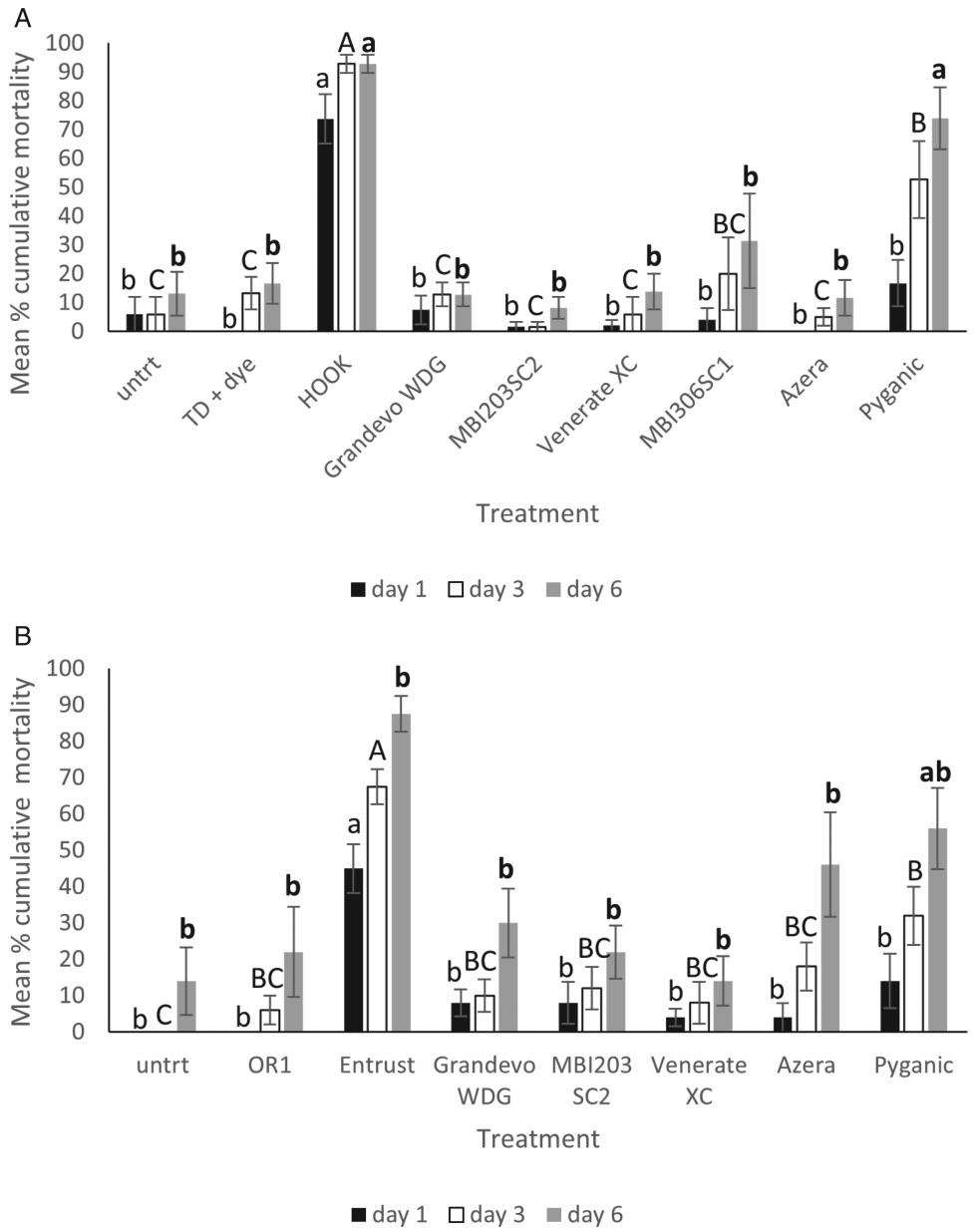

Fig. 1. Mean % mortality at 1-, 3-, and 6-d post-treatment in the a) Florida ACTTRA SWD TD lab study and b) Georgia ACTTRA SWD OR1 lab study. Treatments with the same letter on the same sampling day (day 1 = lower case, day 3 = upper case, day 6 = lower case bold) are not statically different at $P = 0.05$. Error bars represent the standard error of the mean.

Results from the Georgia OR1 lab study were similar (Fig. 1b). There were significant differences among treatments ($F = 22.6$; df = 7, 60; $P < 0.0001$), but not between the sexes ($F = 2.6$; df = 1, 60; $P = 0.11$) nor was there a trt*sex interaction ($F = 0.81$; df = 7, 60; $P = 0.59$). There was a significant interaction between day and treatment ($F = 3.84$; df = 14, 120; $P < 0.0001$), so data from each day is shown. The other interactions with day were not significant (day*sex: $F = 0.02$; df = 2, 120; $P = 0.98$; day*trt*sex: $F = 1.08$; df = 14, 120; $P = 0.38$). The OR1 + Entrust treatment caused significantly higher mortality compared to all other treatments 1 and 3-days post-treatment and compared with all other treatments except OR1 + PyGanic 6-days post-treatment ($F = 6.92$; df = 7, 39; $P < 0.0001$). The OR1 + PyGanic treatment had significantly higher mortality compared with the untreated control 3-d post-treatment. There was no difference in mean progeny emergence among treatments ($F = 1.0$; df = 7, 39; $P = 0.45$). Mean emergence ranged from 44 ± 10 *D. suzukii* per sample in the OR1 + Entrust treatment to 72 ± 4 *D. suzukii* per sample in the OR1 + Venerate treatment. Means of 51 ± 10, 51 ± 3, 50 ± 7, 57 ± 8, 62 ± 15, and 66 ± 14 progeny emerged from the OR1 + Pyganic, OR1 + Azera, OR1 + MBI-203 SC2, OR1 + Grandevo, OR1, and untreated control treatments respectively.

Small Cage Trials

In the small cage trial with TD in Florida (Fig. 2a), there were significantly higher numbers of *D. suzukii* per trap in the TD + Venerate treatment compared with the TD + Pyganic treatment ($F = 2.69$; df = 5, 47; $P = 0.04$). There was no difference between males and females per trap ($F = 0.16$; df = 1, 47; $P = 0.70$) nor was there a significant interaction between treatment and sex ($F = 0.83$; df = 5, 47; $P = 0.54$). There were no significant differences in *D. suzukii* emergence among treatments ($F = 1.42$; df = 5, 47; $P = 0.24$), between males and females ($F = 1.76$; df = 1, 47; $P = 0.19$), nor was there a significant interaction between treatment and sex ($F = 0.40$; df = 5, 47; $P = 0.84$).

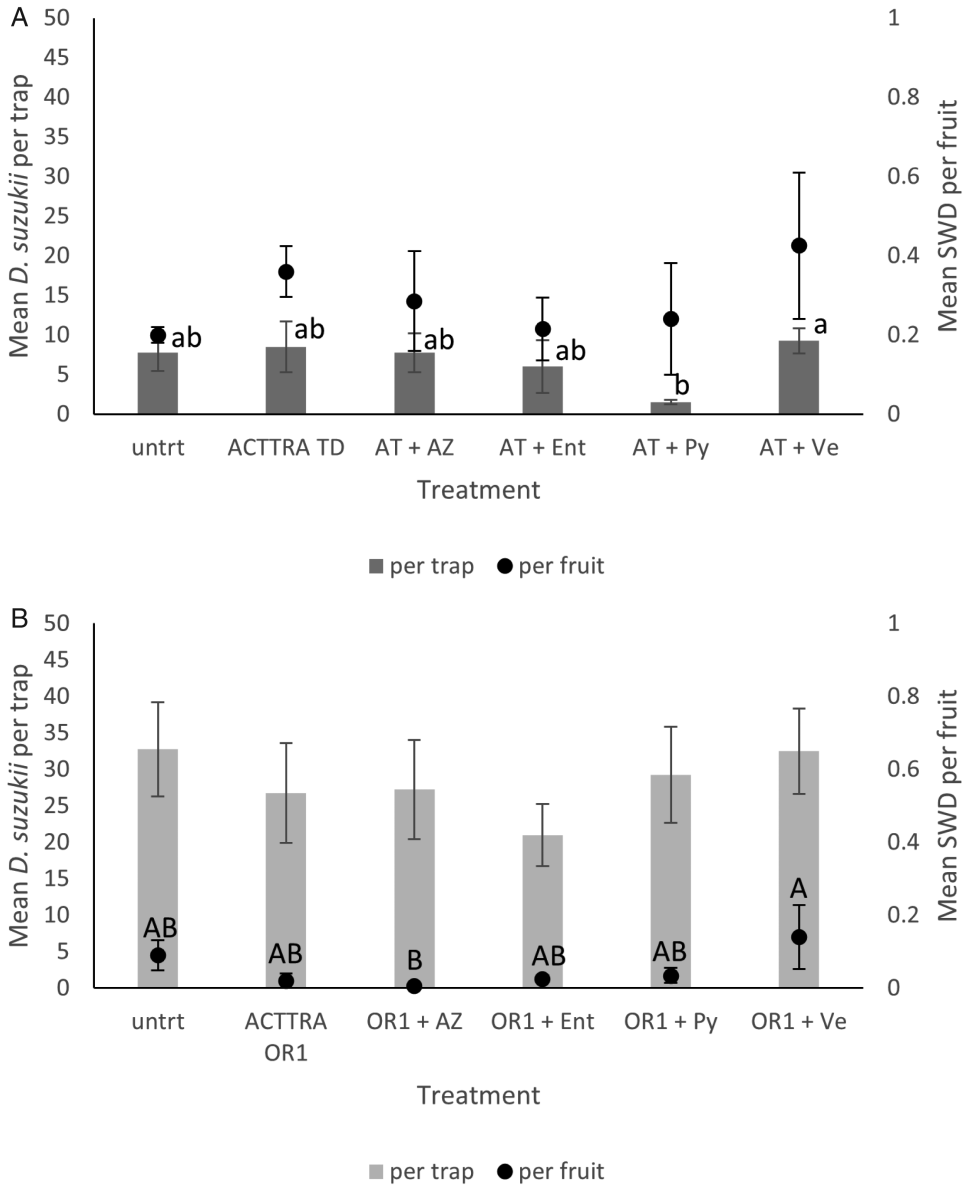

Fig. 2. Mean *D. suzukii* per trap (bars) and adult progeny emergence per fruit (points) in each treatment (untrt = untreated, ACTTRA TD = ACTTRA SWD TD, AT + AZ = ACTTRA SWD TD + Azera, AT + ENT = ACTTRA SWD TD + Entrust, AT + Py = ACTTRA SWD TD + Pyganic, and AT + Ve = ACTTRA SWD TD + Venerate) from the a) Florida ACTTRA SWD TD and b) Georgia ACTTRA SWD OR1 small cage trials. Error bars represent SEMs. Treatments with the same letter are not statistically different at $P = 0.05$.

In the small cage trial with OR1 in Georgia (Fig. 2b), there were no significant differences in *D. suzukii* per trap among treatments ($F = 2.18$; df = 5, 47; $P = 0.08$), between males and females ($F = 0.78$; df = 1, 47; $P = 0.38$), nor was there a significant interaction between treatment and sex ($F = 1.18$; df = 5, 47; $P = 0.34$). Significantly higher numbers of *D. suzukii* emerged from the OR1 + Venerate treatment compared with the OR1 + Azera treatment ($F = 3.57$; df = 5, 47; $P = 0.01$). There was no significant difference in emergence between males and females ($F = 0$; df = 1, 47; $P = 0.96$) nor was there a significant interaction between treatment and sex ($F = 0.77$; df = 5, 47; $P = 0.58$).

Discussion

In the laboratory bioassays, Entrust caused high *D. suzukii* mortality when mixed with OR1 and spinosad, the active ingredient in Entrust, caused high mortality as the killing agent in the HOOK SWD product. This is not surprising since Entrust applied as a spray is a highly effective *D. suzukii* management tool (Timmeren and Isaacs 2013), and HOOK SWD has been shown to reduce *D. suzukii* numbers in the field (Klick et al. 2019). The only other insecticide that caused some mortality in the laboratory when mixed with TD and OR1 was PyGanic. Generally, synthetic pyrethroids are highly effective against *D. suzukii* (Timmeren and Isaacs 2013). However, when PyGanic, a pyrethrins-based organic insecticide, is used as a spray, it has shown minimal efficacy (Timmeren and Isaacs 2013). Babu et al. (2022a) found that, among the four organic insecticides tested, Entrust was the only effective organic insecticide in combination with OR1 and TD in reducing *D. suzukii* numbers and emergence. In our study, in contrast, there were no differences in *D. suzukii* progeny emergence among treatments. One reason for this

could be the design of the experimental arenas. When the flies are introduced into the arenas, they fall to the bottom where the fruit is located. The females may lay eggs before flying up to the ACTTRA dollop located on one of the leaves of the blueberry plant terminals. This is especially true for mated females, which are more attracted to blueberry fruit than to either TD or OR1 (Babu et al. 2022b).

There were no differences in *D. suzukii* trap captures or emergence in the small cage bioassays. Given the results from the laboratory bioassays, it is not surprising that most of the tested organic insecticides were ineffective. The failure of Entrust, however, was surprising. Numerically, *D. suzukii* emergence was lower in the TD + Entrust and TD + PyGanic treatments compared with TD alone. Numerically, TD + PyGanic had the lowest *D. suzukii* trap catch, while OR1 + Entrust had the lowest trap catch in the OR1 bioassay. Variability caused by replicating the experiment over time was a likely factor in the nonsignificant results. In contrast, both the number of *D. suzukii* (50) and the number of berries (25) were within the optimal ranges (20–60 SWD and 25 berries) for HOOK SWD to be effective in small cage bioassays (Urbaneja-Bernat et al. 2022), so it is unlikely this played a role in the nonsignificant results.

Our results indicate that even if other effective organic insecticides can be found to use as the killing agent along with the TD and OR1 attractants, it is unlikely this attract-and-kill tool will be a stand-alone treatment. Rice et al. (2017) found that insecticide-treated spheres did not reduce *D. suzukii* numbers as well as insecticide sprays. HOOK SWD plus a grower's standard spray application was effective in reducing *D. suzukii* numbers in the field (Disi and Sial 2019, Klick et al. 2019). Therefore, the use of ACTTRA based attract-and-kill technologies may reduce the number of insecticide applications needed but is unlikely to eliminate the need for spraying.

Entrust and PyGanic were the only organic insecticides tested in these trials that showed some efficacy against *D. suzukii* when used as the killing agent in combination with OR1 and TD. This and other studies cited in this discussion indicate that neither treatment could be used to effectively manage *D. suzukii* on its own. Further research is needed to determine field efficacy. For example, the optimum amount of ACTTRA to apply per bush and the number of bushes in a given area that need to be treated for maximum efficacy both need to be determined. It is also important to determine what other tactics work well in combination with the ACTTRA-based attract-and-kill tactic.

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