# Identification of Effective Toxicants for Inclusion in Attracticidal Spheres for Management of *Drosophila suzukii* (Matsumura)

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## Project Background

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is an invasive species recently introduced into the U.S. Unlike many other fruit fly species, SWD will readily oviposit into ripening berries, brambles, grapes and stone fruits leading to potential economic losses in quality and yield. Once the eggs are laid and the maggots hatch, the fruit likely cannot be recovered and thus control efforts must focus on the adult SWD. To properly mitigate injury to the crop, chemical control measures must be timed to kill adults prior to oviposition. However, commercial growers still do not have tools that allow them to monitor at regular, short intervals to permit a properly timed spray and thus invasion into plots may occur before treatment can be applied.

Fig. 1. Attracticidal sphere for apple

Current monitoring traps require growers to positively identify the small fruit fly and repeatedly replace the liquid attractant, so as to ascertain when new populations develop. Given that SWD has a very short developmental period, growers need to constantly monitor and reapply insecticides through harvest. A potential solution to this problem is a behaviorally based management strategy: an attract-and-kill system that is very attractive to SWD, pulling them away from the host plants where a lethal dose of toxicant can be delivered to the adult fly. A project previously funded through the NABG Research Foundation showed that the presence of spheres could reduce the infestation rates of raspberries by as much as 50%. However, the toxicants previously tested (bifenthrin, lambda-cyhalothrin, spinosad and spinetoram) all had significant weaknesses that warranted further investigation of other potential materials for inclusion into our attracticidal spheres. Here we present the results of those studies aimed at evaluating the lethality and durability of alternative toxicants for management of SWD.

## Objectives and Methods

### *1. Evaluate alternative toxicants for lethality against SWD in the laboratory.*

We evaluated nine different active ingredients and some materials in combination. Additionally, lambda-cyhalothrin was tested as both a liquid and dry formulation. Table 1 (see below) summarizes the materials and rates tested. All materials were formulated into attracticidal sphere caps as reported in Wright et al. (2012). Attracticidal spheres were suspended inside 45.7 $cm^3$ acrylic boxes. Sphere caps were misted with water to activate the release of both toxicant and feeding stimulant (sugar) onto the surface of the sphere. Spheres formulated with only the sugar feeding stimulant and no toxicant served as controls. Individual flies were placed at the equator of the sphere and permitted to forage freely for up to 5 min. Subsequently, flies were removed and placed individually into 1 oz portion cups with sugar water for 48 h. Fly condition was assessed after 24 and 48 h. Flies were recorded as alive, moribund or dead. Moribund and dead flies were combined to calculate percent mortality as no flies recovered from the moribund state. Percent mortality and mean residence and foraging time were calculated. Percent mortality was compared among treatments with analysis of variance (ANOVA) and means separated using Tukey's HSD test. Residency and foraging time was compared among treatments with analysis of variance and mean's separated from the control using Dunnett's multiple comparison test.

***2. Evaluate the field durability of selected toxicants in attracticidal spheres.***

Based on results from Objective 1, we selected 1% spinetoram, 1% dinotefuran, 0.5% dinotefuran, 0.1% spinetoram + 0.1% dinotefuran and control to be subjected to field durability trials. Twenty attracticidal spheres were formulated with each of the aforementioned materials and exposed to natural abiotic conditions in the field at Appalachian Fruit Research Station in Kearneysville, WV. Spheres were hung in the field on trellis wire and exposed to natural conditions for a period of either 0, 3, 6, 9 or 12 weeks. The trial was conducted from 23 June through 15 September, 2014. At time 0, spheres were weighed and color readings were taken using a Minolta Colorimeter and hue angle was recorded. Hue angle is computed as how much a color varies from red, green, blue or yellow on a continuum from 0-360. The hue angle for red is reported as 0, so the lower the hue angle the closer to red. Four color readings per sphere were recorded, averaged and analyzed. Then after 3, 6, 9 or 12 weeks weights and color readings were recorded to assess field degradation. Additionally, at each time interval SWD adults were exposed to spheres as conducted in Objective 1 and percent mortality was recorded after 24 and 48 h. The optimal color parameters are defined from our control spheres at time 0, so the color reading changes over time are assessed in comparison with the control. Mean change in weight was compared using analysis of variance with means compared against control spheres using Dunnett's multiple comparison test. Differences in total color were compared within an active ingredient across duration of time in the field by analysis of variance with means separated by Tukey's HSD test. Differences in total color were compared among active ingredients within individual time periods by analysis of variance with means separated by Tukey's HSD test.

## Results

***1. Evaluate alternative toxicants for lethality against SWD in the laboratory.***

**Table 1. List of toxicants and concentrations evaluated for SWD lethality**

| **Trade Name** | **Active Ingredient (a.i.)** | **Concentration (%)** | **N** |
|---|---|---|---|
| Acephate 97UP | acephate | 1.0 | 40 |
| | | 0.5 | 40 |
| | | 0.1 | 40 |
| Boric Acid | hydrogen borate | 10.0 | 20 |
| | | 0.1 | 19 |
| Conserve | spinosad | 1.0 | 20 |
| Delegate | spinetoram | 1.0 | 40 |
| Grandevo (30%) | *Chromobacterium subtsugae* | 10.0 | 20 |
| Kaiso | lambda-cyhalothrin | 1.0 | 40 |
| Perm-Up | permethrin | 1.0 | 20 |
| Venom | dinotefuran | 1.0 | 40 |
| | | 0.5 | 40 |
| | | 0.1 | 40 |
| Warrior II | lambda-cyhalothrin | 1.0 | 39 |
| Delegate + Kaiso | spinetoram + lambda-cyhalothrin | 0.1 + 0.1 | 40 |
| Delegate + Venom | spinetoram + dinotefuran | 0.1 + 0.1 | 40 |
| Control | none | N/A | 50 |

There was no significant difference in the mean percent mortality between 24 h and 48 h ($t = 0.557211$; $df = 1173.468$; $p = 0.5775$), so only the 24 h data are presented below. There was a significant effect of treatment on SWD lethality ($F = 68.2266$; $df = 16, 571$; $p < 0.0001$) (Table 2). In terms of mean lethality, only the 1% concentration of six insecticides killed 100% of the tested flies within 24 h. Those insecticides were dinotefuran, permethrin, spinosad, spinetoram and both formulations of lambda-cyhalothrin. While laboratory assays do reflect the relative lethality of insecticide performance under field conditions, they do also tend to overestimate the true effect. Because of this, we chose to select only those materials that yielded the highest rates of SWD mortality in laboratory tests as candidates for further field-based evaluations. From that subset, 1% Venom, 0.5% Venom, 1% Delegate, and 0.1% Delegate + 0.1% Venom were chosen for evaluation in Objective 2. The other materials that yielded that high rates of SWD mortality were excluded for the following reasons: 1) Conserve leeched rapidly from the cap and left a greasy, sticky residue on our formulation equipment, 2) Perm-Up leeched rapidly from the cap, and 3) Kaiso was discontinued by the registrant. 0.1% Delegate + 0.1% Venom was included as a treatment in Objective 2 to test the potential of an additive or synergistic effect of a combination treatment while still reducing costs of materials.

**Table 2. Mean Percent Mortality for SWD Exposed to Attracticidal Spheres in Laboratory Assays**

| Active Ingredient (a.i.) | Conc. (%) | Mean ± SEM Percent Mortality @ 24 h |
|---|---|---|
| acephate | 1.0 | 90.0 ± 4.8 ab |
| | 0.5 | 95.0 ± 3.5 a |
| | 0.1 | 77.5 ± 6.7 b |
| hydrogen borate | 10.0 | 5.0 ± 5.0 c |
| | 0.1 | 21.1 ± 9.6 c |
| spinosad | 1.0 | 100.0 ± 0.0 a |
| spinetoram | 1.0 | 100.0 ± 0.0 a |
| *Chromobacterium subtsugae* | 10.0 | 10.0 ± 6.9 c |
| lambda-cyhalothrin (Kaiso) | 1.0 | 100.0 ± 0.0 a |
| permethrin | 1.0 | 100.0 ± 0.0 a |
| dinotefuran | 1.0 | 100.0 ± 0.0 a |
| | 0.5 | 92.5 ± 4.2 ab |
| | 0.1 | 70.0 ± 7.3 b |
| lambda-cyhalothrin (Warrior II) | 1.0 | 100.0 ± 0.0 a |
| spinetoram + lambda-cyhalothrin | 0.1 + 0.1 | 92.5 ± 4.2 ab |
| spinetoram + dinotefuran | 0.1 + 0.1 | 92.5 ± 4.2 ab |
| Control | N/A | 2.0 ± 2.0 c |

*Means followed by different letters within a column are significantly different ($p < 0.05$)

There was a significant treatment effect on residency ($F = 61.1659$; df = 16, 571, $p < 0.0001$) and foraging ($F = 30.0753$; df = 16, 571; $p < 0.0001$) time. Treatments were only separated in comparison with the control which served as a baseline for expected outcomes of residency and foraging time. Results of the Dunnett's test are shown in Table 3, where an asterisk indicates a significant difference from the control. Residency time was not significantly reduced in the hydrogen borate, spinetoram, *C. subtsugae* and spinetoram + lambda-cyhalothrin treatments compared with the control. Additionally, only spinetoram was not significantly different than the control for time spent foraging by SWD on spheres.

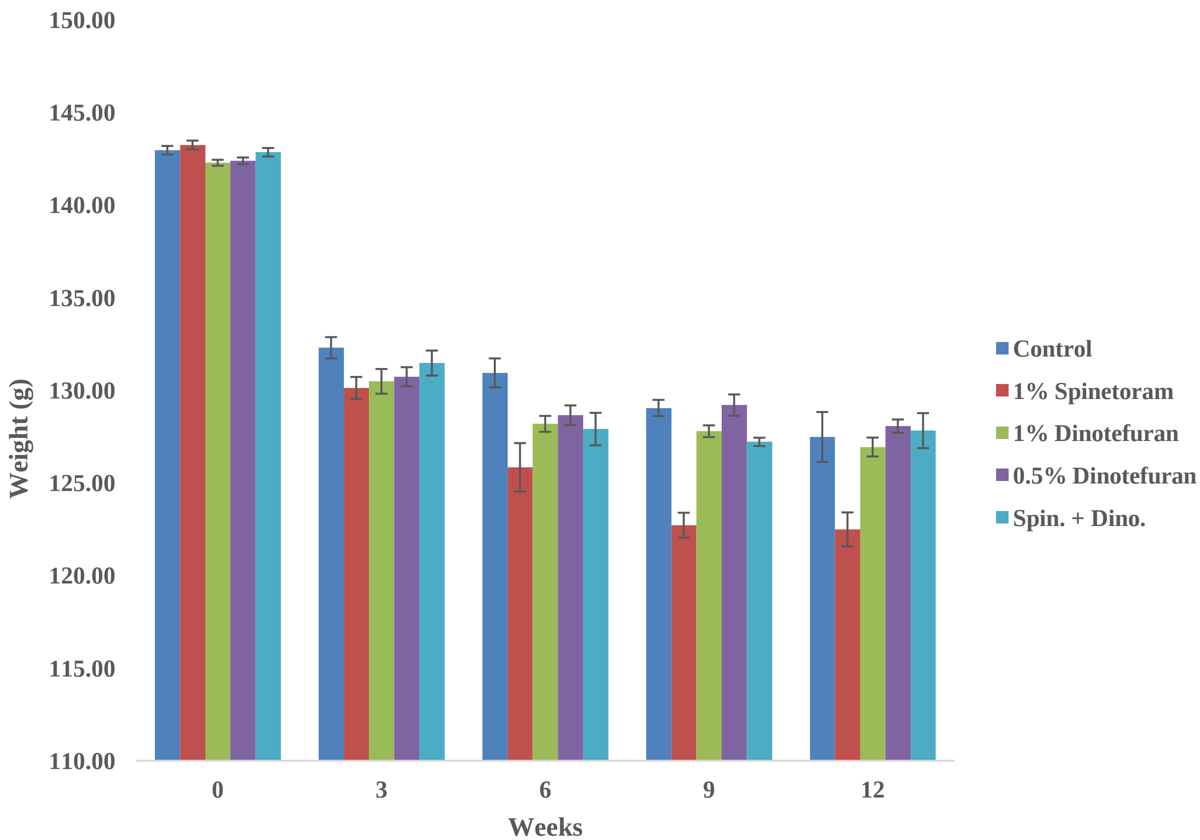
**Table 3. Mean Residency and Foraging Time of SWD Exposed to Attracticidal Spheres in Laboratory Assays**

| **Active Ingredient (a.i.)** | **Conc. (%)** | **Mean ± SEM Res. Time (s)** | **Mean ± SEM Foraging Time (s)** |
|---|---|---|---|
| acephate | 1.0 | 211.3 ± 15.2* | 89.1 ± 8.9* |
| | 0.5 | 193.7 ± 16.7* | 62.5 ± 6.8* |
| | 0.1 | 169.2 ± 14.7* | 57.5 ± 6.6* |
| hydrogen borate | 10.0 | 300.0 ± 0.0 | 128.6 ± 16.6* |
| | 0.1 | 300.0 ± 0.0 | 100.3 ± 17.8* |
| spinosad | 1.0 | 190.0 ± 22.6* | 61.5 ± 9.2* |
| spinetoram | 1.0 | 298.0 ± 2.0 | 191.5 ± 12.9 |
| *Chromobacterium subtsugae* | 10.0 | 300.0 ± 0.0 | 116.9 ± 16.9* |
| lambda-cyhalothrin (Kaiso) | 1.0 | 170.2 ± 14.7* | 55.6 ± 5.8* |
| permethrin | 1.0 | 204.3 ± 20.4* | 79.5 ± 9.2* |
| dinotefuran | 1.0 | 44.8 ± 5.4* | 21.2 ± 1.8* |
| | 0.5 | 39.6 ± 4.2* | 19.3 ± 1.4* |
| | 0.1 | 118.4 ± 13.1* | 45.1 ± 3.6* |
| lambda-cyhalothrin (Warrior II) | 1.0 | 243.0 ± 13.5* | 83.7 ± 8.1* |
| spinetoram + lambda-cyhalothrin | 0.1 + 0.1 | 282.6 ± 7.2 | 116.4 ± 12.4* |
| spinetoram + dinotefuran | 0.1 + 0.1 | 81.3 ± 11.9* | 34.1 ± 7.2* |
| Control | N/A | 300.0 ± 0.0 | 175.0 ± 13.4 |

***2. Evaluate the field durability of selected toxicants in attracticidal spheres.***

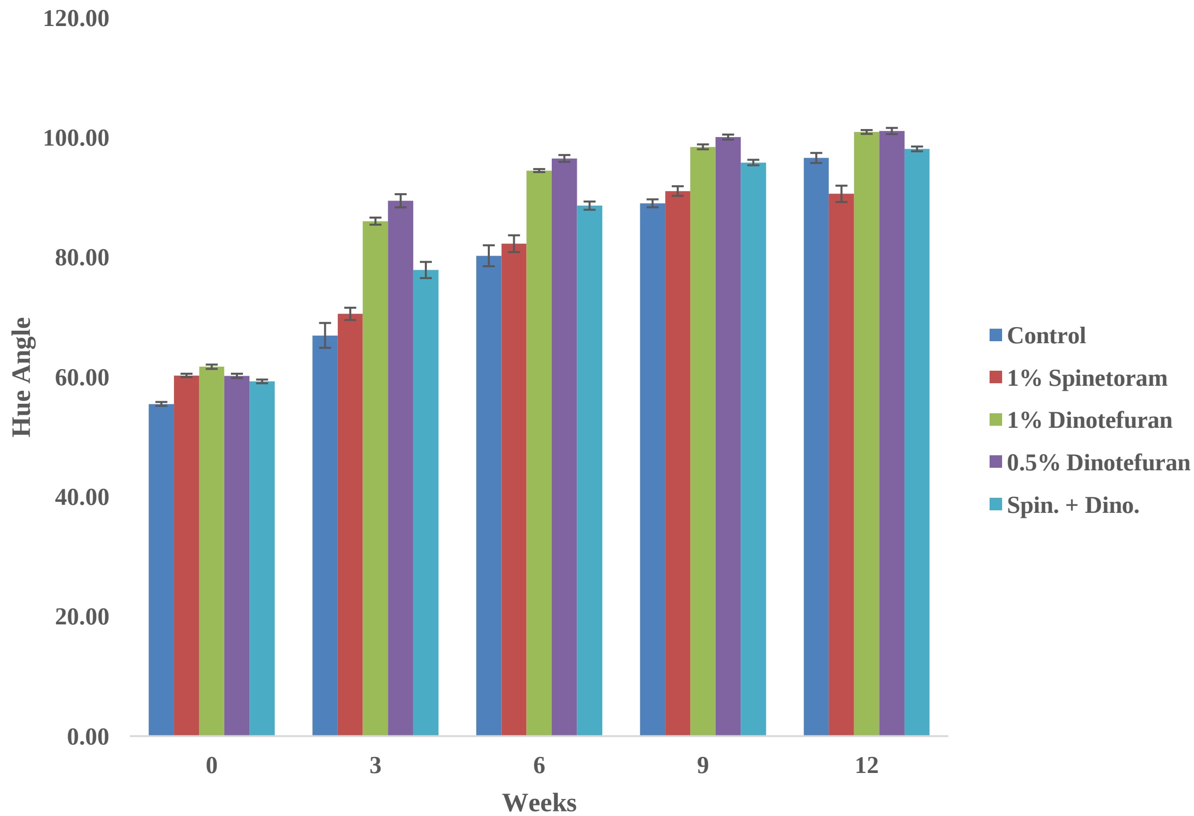
Mean weights of attracticidal spheres throughout the test are shown below in Figure 1. There was no significant difference in sphere weight among the control and any of the other 4 treatments until week 6. At week 6, the weight of 1% spinetoram was significantly lower than the control ($p < 0.05$). In week 9, both 1% spinetoram and 0.1% spinetoram + 0.1% dinotefuran spheres weighed significantly less than the control ($p < 0.05$). At week 12, only the weight of the 1% spinetoram sphere was significantly lower than the control sphere ($p < 0.05$). As expected, attracticidal sphere weights significantly decreased from 0 to 12 weeks as dye, the feeding stimulant and toxicant are released from the cap; however, the change between week 0 and 3 was greater than later in the season.

**Figure 1. Mean ± SEM Weight (g) of Attracticidal Spheres after 0, 3, 6, 9 and 12 Weeks of Field Aging.**



There was a significant difference in hue angle among attracticidal spheres before they were even deployed to the field (0 wk; $F = 49.2502$; $df = 4, 95$; $p < 0.0001$). At wk 0, all spheres with toxicant were significantly different than the hue of the control sphere. After spheres were hung in the field, all treatments except 1% spinetoram differed in hue angle from the control until week 12 ($p < 0.05$). By week 12, 1% spinetoram was the only treatment with a significantly smaller hue angle than the control (the lower the hue angle the closer to red) ($p < 0.05$).

**Figure 2. Mean ± SEM Hue Angle of Attracticidal Spheres after 0, 3, 6, 9 and 12 Weeks of Field Aging.**




There was no significant difference in mean lethality at 24 or 48 h after treatment so only the 24 h data are shown below in Table 4 ($t = 0.312012$; $df = 3942.567$; $p = 0.7550$). There was no significant difference in mean lethality for SWD exposed to control spheres across all aged spheres ($F = 0.4794$; $df = 4,395$; $p = 0.7509$). However, there was a significant effect of aging the spheres on SWD lethality for all treatments with a toxicant ($p < 0.0001$). 1% dinotefuran and 0.1% spinetoram + 0.1% dinotefuran maintained significantly equal levels of lethality on SWD after 3 weeks of aging as they did before exposure to field conditions ($p > 0.05$); however, fly survivorship increased significantly thereafter.

**Table 4. Mean ± SEM Percent Mortality for SWD on Attracticidal Spheres Aged in the Field for 0, 3, 6, 9 or 12 Weeks**

| Insecticide (n=50) | Treatment Week | Mean ± SEM Percent Mortality @ 24 h* |
|---|---|---|
| 1% spinetoram | 0 | 96.0 ± 1.4 a |
| | 3 | 64.0 ± 6.9 b |
| | 6 | 60.0 ± 7.0 b |
| | 9 | 70.0 ± 6.5 b |
| | 12 | 56.0 ± 7.1 b |
| 1% dinotefuran | 0 | 87.0 ± 2.4 a |
| | 3 | 96.0 ± 2.4 a |
| | 6 | 48.0 ± 7.1 c |
| | 9 | 62.0 ± 6.9 bc |
| | 12 | 82.0 ± 5.5 ab |
| 0.5% dinotefuran | 0 | 94.0 ± 1.7 a |
| | 3 | 58.0 ± 7.1 b |
| | 6 | 72.5 ± 7.1 b |
| | 9 | 64.0 ± 6.9 b |
| | 12 | 70.0 ± 6.5 b |
| 0.1% spinetoram + 0.1% dinotefuran | 0 | 96.1 ± 1.4 a |
| | 3 | 98.0 ± 2.0 a |
| | 6 | 14.0 ± 5.0 b |
| | 9 | 80.0 ± 6.0 b |
| | 12 | 58.0 ± 7.1 b |
| Control | 0 | 2.5 ± 1.1 a |
| | 3 | 0.0 ± 0.0 a |
| | 6 | 2.0 ± 2.0 a |
| | 9 | 2.0 ± 2.0 a |
| | 12 | 4.0 ± 2.8 a |

*Means followed by different letters within a treatment are significantly different ($p < 0.05$)

## Conclusions

The most effective materials that we tested against SWD were spinosad, spinetoram, dinotefuran, permethrin and lambda-cyhalothrin. In order to maintain sufficiently high levels of control, a concentration of at least 1% is needed. However, each of these materials presents some level of challenge from a formulation perspective. As shown in the durability studies, there is a significant amount of breakdown in the integrity of attracticidal sphere cap. The integrity of the cap is crucial for management, such that the loss of color can reduce fly response which has been shown to be greatest to red and black spheres. Additionally, reductions in weight may reflect excess loss of toxicant or feeding stimulant which subsequently impacts overall lethality. None of the spheres maintained high levels of lethality after exposure to natural abiotic conditions over more than 3 weeks. This is not a reflection of loss of cap color as flies were evaluated under laboratory conditions and purposely introduced onto spheres. However, the combination of reduced visual attractiveness and toxicity poses a significant challenge for field management.

Spinetoram spheres had lower levels of color loss than other tested formulations, but also had higher levels of weight loss. We expect that the release of sugar would be somewhat similar across all caps, so it is possible that this significant amount of weight loss may be attributable more rapid loss of toxicant. Because spinetoram (sold as Delegate) is manufactured at only 25% a.i., we had to incorporate more total product into a sphere cap to obtain a 1% concentration than would be necessary in a material like dinotefuran (sold as Venom) which is produced at 70% active. The impact of this additional level of inert ingredients in the Delegate formulation may have been a causal agent in the increased weight loss. SWD exposed to dinotefuran spheres resided and forage for a shorter period of time than on control or other formulations. This reduction in residency was because flies were rapidly intoxicated by the dinotefuran and fell off of spheres and subsequently died. The expediency at which dinotefuran acts on a foraging fly is a very desirable trait in an attract-and-kill system as fly residency time in a field situation may be significantly shorter than observed under laboratory conditions. Additionally, if flies are rapidly incapacitated then they are unable to mate, oviposit or cause injury to the protected crop prior to death.

Unfortunately, our results showed that dinotefuran spheres hue changed rapidly and effects of lethality diminished significantly after 3 weeks in the field. While it would be possible to exchange out attracticidal sphere caps after 3 weeks, optimally we endeavor for this product to require no grower maintenance over each growing season. Moving forward, we will continue to search for organically based materials for incorporation into attracticidal sphere caps as well as modifications to our formulation chemistry to reduce losses of color and toxicity.