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Use of baited attract and kill stations as IPM alternative to control invasive *Drosophila suzukii* Matsumura (Spotted Wing Drosophila)

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Abstract: The newly invasive *Drosophila suzukii* Matsumura (Spotted Wing Drosophila, SWD) is an economically significant pest of berries and other small fruit in the Northeast United States. Methods of controlling this insect typically consist of frequent sprays of conventional insecticide, often with long pre-harvest intervals. This experiment is intended to determine the efficacy of a unique Attract and Kill (AtK) design using super-absorbent gelatinous media saturated with highly attractive SWD bait adhered to durable netting, sprayed regularly with a range of insecticides. Our lab and field studies determined that presence of AtK stations treated with insecticides placed in vicinity of raspberries caused a drop in *D. suzukii* oviposition into the fruit.

Introduction: *Drosophila suzukii* is a newly invasive insect to the Northeast United States, with a native range in Southeast Asia, where it is predominantly considered a pest of cherry. Due to their high fecundity, lack of native natural enemies, rapid rate of infestation and ability to infest unripe fruit via a specialized ovipositor (Atallah et al., 2014); *D. suzukii* is considered a serious pest of small fruit such as Raspberries, Blackberries, and Blueberries (Lee et al., 2011). They are also occasional/opportunistic pests of Cherry, Plum, and Peach. First establishing in the Northeast region in 2011, *D. suzukii* infestations reached approximately 90-100% fruit loss in some orchards by 2013 (Walsh et al., 2011) (Lee et al., 2015).

While conventional orchards have been able to obtain some measure of control in the past several years, generally by utilizing relatively intense spray regimens, organic growers were left with only a fraction of these options (Van Timmeren & Isaacs, 2013). These growers are receptive to IPM-based methods of control, including cultural control and trapping. Trapping of this insect has been studied by multiple sources, and attractants have been exhaustively tested (Landolt et al. 2012). Acting on the needs of these growers, we attempted to develop an attract-and-kill (AtK) control system that uses

push-pull mechanics to alleviate insect pressure on crops, while reducing populations and oviposition with no added sprays.

The proposed AtK system consists of a gelatinous media, attached to a core of strong, flexible netting. The media is a mixture of several attractants, (apple cider vinegar, raspberry concentrate, and dry active yeast) and gelatinous substrates, (super-absorbent polymer and unflavored gelatin). Materials in these disks were based on both our own laboratory testing and on external research (Landolt & Rogg, 2012) (Lee et al., 2013). The resulting trap design is that of a flexible disk, punctured with grommets on each end to allow for simple placement by hanging in the crop, typically by attaching to guide wires). The disk is highly water retentive, able to absorb moisture from the air during periods of high humidity, allowing disks to regain attractiveness after periods of dew, high humidity, or rain.

Disks can be sprayed with any material labeled for fruit flies. The attractiveness of the disks was found to not only cause flies to alight upon their surfaces, but to elicit a phagostimulant response. This type of response has been shown by research into sucrose as an insecticide tank mix by Cowles et al. (2015) and in the use of yeasts by Knight et al. (2015) to increase the efficacy of insecticides sprayed with this stimulant present. Disks can be treated even with household insect controls such as Boric acid and demonstrate good control. In our laboratory trials, we tested 10 different materials (including an untreated control), and in the field, we tested 1 material (boric acid) at 2 different spacings and 2 different spray schedules. Boric acid was chosen due to its unique (although not entirely understood) mode of action. Research by Habes et al. (2006) attempted to better document the effects of this material on their target species of *Blattella germanica* inducing glutathione S-transferases (GSTs), and reducing the activity of acetylcholinesterase (AChE), essentially causing starvation via alterations of the midgut. The compound was also found to present a neurotoxic action. In addition to its efficacy, boric acid has never been linked to the development of insecticide resistance in its target species.

Materials and Methods

Attract and Kill Discs:

These trials tested the efficacy of a disk of absorbent bait, designed to attract and elicit a phagostimulant response in *D. suzukii* adults. This response has been garnered *D. suzukii* adults through the use of sucrose to increase the efficacy of insecticides (Cowles et al. 2015). These Attract and Kill disks (AtK) were newly developed materials, designed by Cornell University's Hudson Valley research laboratory's department of Entomology. AtK disks were designed to be tough, absorbent, and attractive to *D. suzukii* over an entire field season.

After several iterations, final version of field-tested disks were completed in early 2015. Final disk design utilizes tough neoprene netting, cut into a 90mm diameter circle, coated in a mixture of gelatin, super absorbent polymer (SAP), water, and AtK bait (fig 1.). The bait is comprised of 250ml raspberry concentrate, 750ml apple cider vinegar, and 5g dissolved active dry yeast. This bait is also used as spray when combined with 1% active ingredient of the chosen insecticide. This spray is applied at a rate of 2mL/disk during testing.

Figure 1: Completed AtK disk

Disk Production

The following steps were used to build the type of disks used in both laboratory and fieldwork.

1. Place a waxed 9 cm petri dish onto a calibrated digital scale.
2. Distribute 0.5 g of coarse super-absorbent polymer (SAP) evenly over the surface of the dish.
3. When producing multiple disks, continue with the large particles for each disk, moving the disk on and off the scale, until all dishes have coarse SAP particles evenly distributed over the surface.
4. Place dish with coarse SAP onto the scale and distribute 0.5 g of fine SAP particles evenly. Repeat with the remaining dishes.
5. Place 9 cm mesh disks onto half of the SAP prepared dishes. These dishes serve as the "bottom" mold of the disks while the dishes without netting will become the "top" of the mold.
6. Apply 10 mL of AtK solution to each dish in a circular motion to ensure an even distribution.
7. Leave dishes out to set (approximately one hour at room temperature).
8. When the disks are firm enough to be peeled out of the petri dishes, apply 10 mL of AtK solution to the bottom dishes.
9. Carefully remove the disks without netting from the petri dish and place them face down onto the petri dishes containing netting and the additional AtK solution.
10. Transfer the combined plates to the refrigerator using a tray and allow them to set (~30 minutes).

Lab Methods:

Prior to field-testing, laboratory evaluations of different materials were conducted. These tests were performed as timed bioassays, wherein 25 adult (12 male/13 female) lab-reared *D. suzukii* were exposed to an AtK disk, a water source, and 5 ripe raspberries over a 48-hour period. At intervals of 18, 24, and 48 hours, counts of adult

mortality and oviposition into the berries were taken, and an eggs/larvae per gram ratio was obtained for data analysis. Assays took place in Bioquip BugDorm rearing and observation cages, set up in "testing configuration" (See fig. 2). Dorms were placed in climate controlled testing environment, at a constant 65-70% RH, and 21°C.

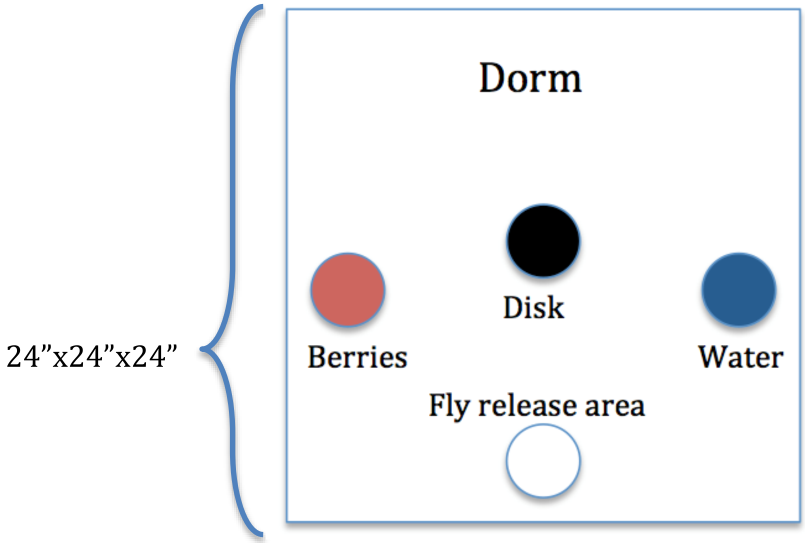

Figure 2: Testing arena configuration

Prior to testing, flies were chosen at random from among 24 hour-old laboratory-reared adults, cooled to 3.5°C to reduce movement, and placed in holding vials via aspirator. Vials were placed in closed BugDorm and uncapped, allowing movement of flies into arena.

AtK disks were sprayed with a 2ml mixture of 99% AtK bait to 1% active ingredient of whichever insecticide was tested (1ml per side of disk), and allowed 1 hour to dry under running fume hood, placed on wire mesh, 1" above work surface. Using these methods, materials were tested with the intention of choosing an effective yet safe insecticide for use in field trials. Prior research by Bruck et al. (2011) demonstrated that materials that demonstrate efficacy in laboratory bioassays, show similar levels of efficacy in the field, making laboratory screenings of insecticides a valuable method of determining a material's worth.

We tested the following treatments using the previously listed protocols:

Chemical Name	Trade Name	Manufacturer	Rate (AI/AtK Bait)
Acetamiprid	Assail® 30SG	United Phosphorous, Inc., King of Prussia, PA	0.01/0.99
Azadiracchtin	AzaGuard®	BioSafe Systems LLC 22 Meadow Street, East Hartford, CT 06108	0.01/0.99
Boric Acid	Boric Acid	Duda Energy LLC, 111 Brooks St. Decatur, AL 35601	0.01/0.99
Cyantraniliprole	Exirel™	E. I. du Pont de Nemours and Company 1007 Market Street Wilmington, Delaware 19898	0.01/0.99
Zeta-cypermethrin	MustangMax™ 0.8 EC	FMC Corporation, Philadelphia, PA	0.01/0.99
Pyrethrin	Pyganic® 1.4EC	McLaughlin Gormley King Co., Minneapolis, MN	0.01/0.99
Spinosad	Entrust® 80WP	Dow AgroSciences LLC, Indianapolis, IN	0.01/0.99
Carbaryl	Sevin® XLR	Bayer CropScience, Research Triangle Park, NC	0.01/0.99
Unsprayed Disk	NA	NA	NA
Untreated Control	NA	NA	NA

With the exception of boric acid, these insecticides were chosen due to previous testing as potential materials to use against *D. suzukii* (Van Timmeren & Isaacs, 2013). Boric acid was chosen as a potential material due to its ease of procurement and demonstrably good results as an insecticide for other target organisms (Habes et al., 2006).

Field Methods:

Experimental field design using 3 fall-bearing raspberry (primarily Caroline) plantings on 3 farms in 2 NY counties was implemented 14th June 2016. Each site received an identical replicated block design, with 3 replicates of the following treatments (see fig. 3a). Disks were hung at 2 heights (low and high) in a "zigzag" pattern across the rows (see fig 3b) to better obtain a spread of disk coverage across the surface area of the bramble row. Low disks were hung at approximately 18", while high disks were hung at approximately 48."

Sprayed 1x weekly:

- 1% Boric acid treated disks spaced at 1.5’ (120) Disks/ side = 240 disks/ row
- 1% Boric acid treated disks spaced at 3’ (60) Disks/ side = 120 disks/ row
- Untreated disks spaced at 3’ (60) Disks/ side = 120 disks/ row

Sprayed 2x weekly:

- 1% Boric acid treated disks spaced at 1.5’ (120) Disks/ side = 240 disks/ row
- 1% Boric acid treated disks spaced at 3’ (60) Disks/ side = 120 disks/ row
- Untreated disks spaced at 3’ (60) Disks/ side = 120 disks/ row

Disks were sprayed using a calibrated spray bottle, which released an atomized spray of 1ml of mixture to each side of the disk. Weekly collections consisting of berries across a range of ripeness from all 3 sites, approximately 25 fruit per replicate, which were inspected for eggs/larvae per gram in the same manner as in the lab trials. Representative samples of berries were kept for up to 14 days (or until adult eclosion) to confirm identity of *D. suzukii*.

Additionally, disks were placed in each study site in rows corresponding to phenological events in the *D. suzukii* life cycle. Row A was placed at the 1st capture of *D. suzukii* in New York State, (14th June). Row B was placed after the 1st incidence of *D. suzukii* capture at any one test site, (19th June), and Row C was placed after 1st incidence of *D. suzukii* oviposition in fruit at any test site (25th June).

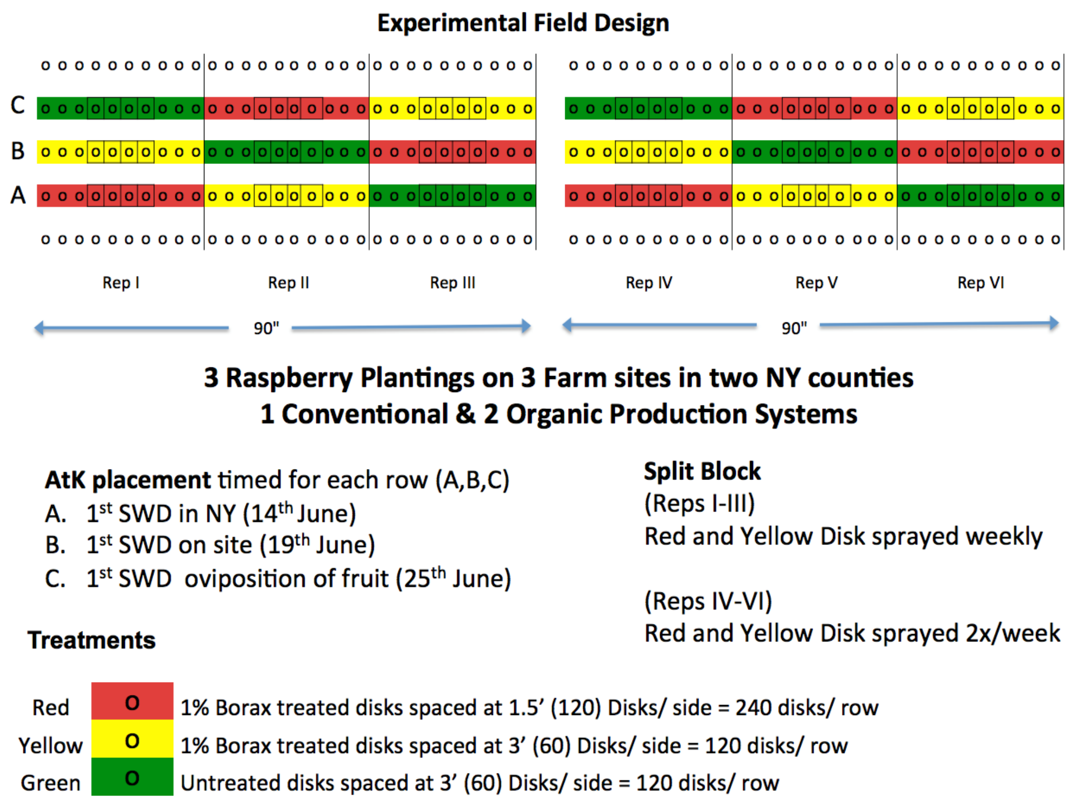

Figure 3a: Replicated block design

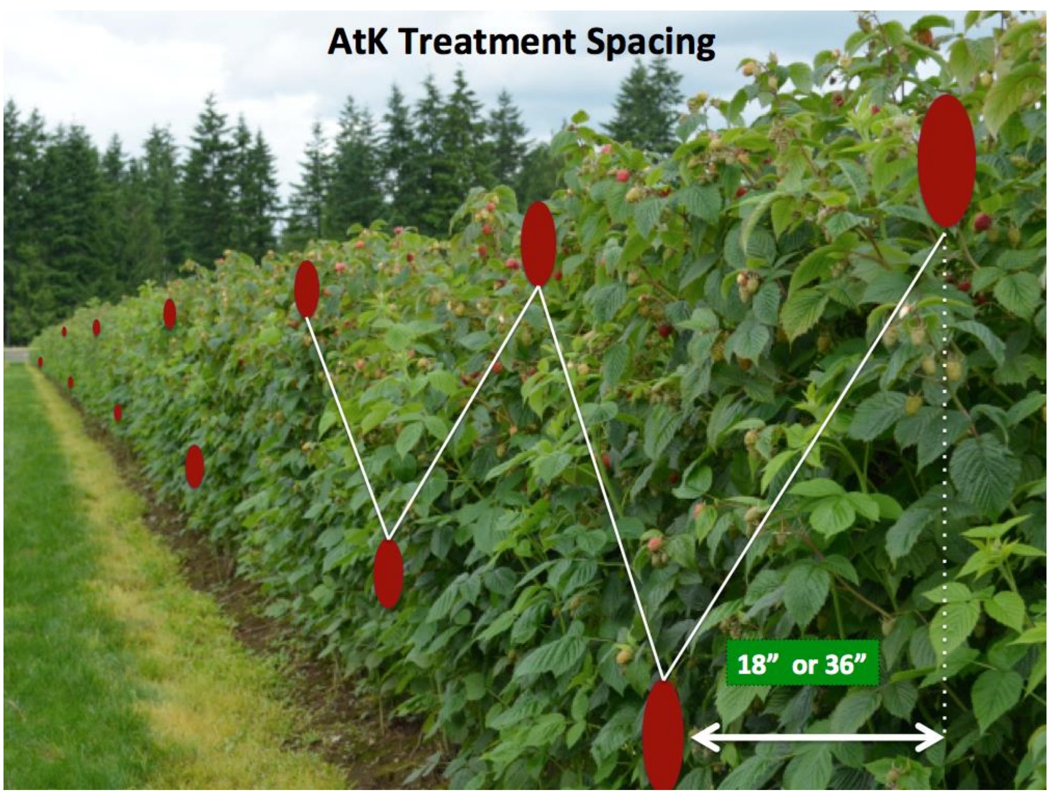

Figure 3b: AtK Disk placement in field

Results:

In both field and laboratory trials, the presence of AtK disks in proximity to berries reduced oviposition, regardless of the material (or lack thereof) sprayed on the disks. Again, both lab and field trials demonstrated that disks sprayed with an insecticidal material (in this case Boric Acid), prevented oviposition more effectively than unsprayed disks. However, unsprayed disks reduced oviposition to a degree, which was relatively consistent between lab and field. In the field, we observed a 51.4% reduction in oviposition in berries located in sections treated with boric acid AtK disks when spaced at 18", compared to sections with no disks at all. We also observed a smaller reduction of 17.1% in sections treated with unsprayed disks. There was no measurable difference between rows A, B, and C, in spite of deployment timing. Analysis of Variance (ANOVA) was performed using SuperANOVA. Data were transformed using log10(x+1) using Fishers Protected LSD ($P \leq 0.05$). Treatment means followed by the same letter are not significantly different.

Lab Results:

In controlled laboratory settings, we observed significant reductions in oviposition in tents with disks present. Disks treated with boric acid were as effective as conventional insecticide treatments, displaying statistically identical control in both mortality and ovipositional decrease (see fig 4a-5). Additionally, we noted that untreated disks reduced oviposition at levels similar to treated disks, although it facilitated lower levels of mortality than treated disks. Significance factors in laboratory trials were strong, with significant P values observed in both oviposition and mortality data.

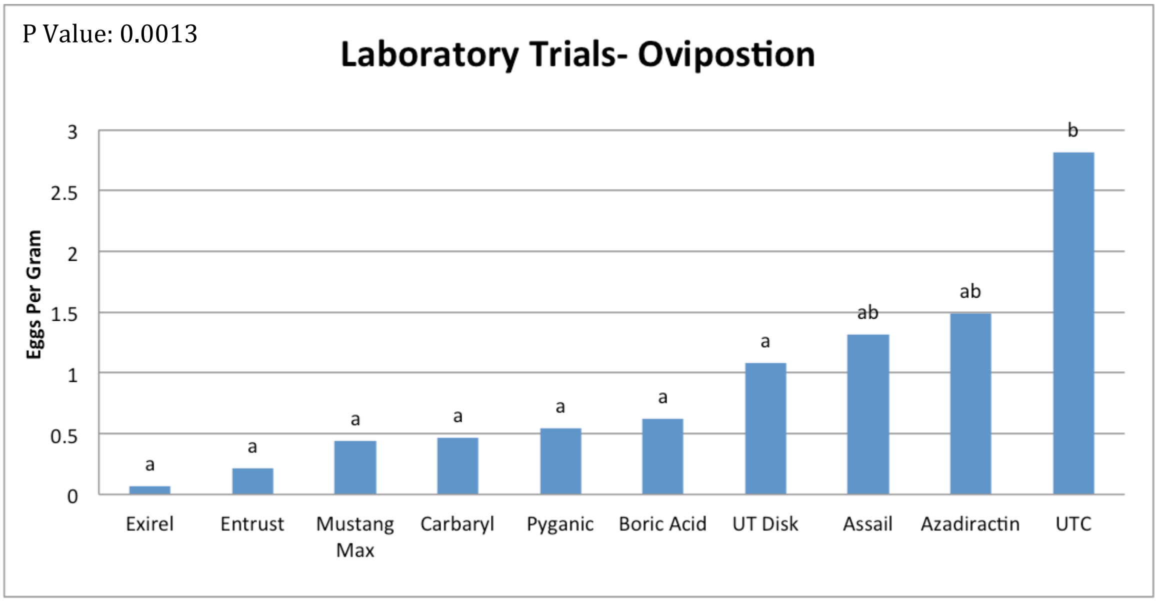

Figure 4a: Eggs/Larvae per gram at 48 hours post introduction

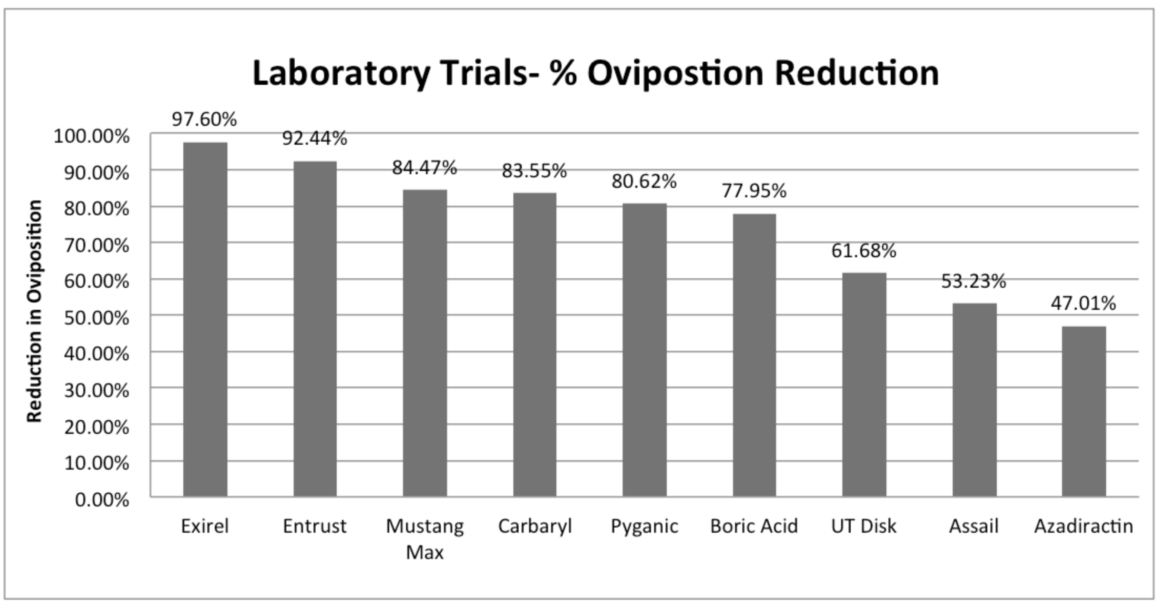

Figure 4b: Percentage reduction in oviposition at 48 hours post introduction

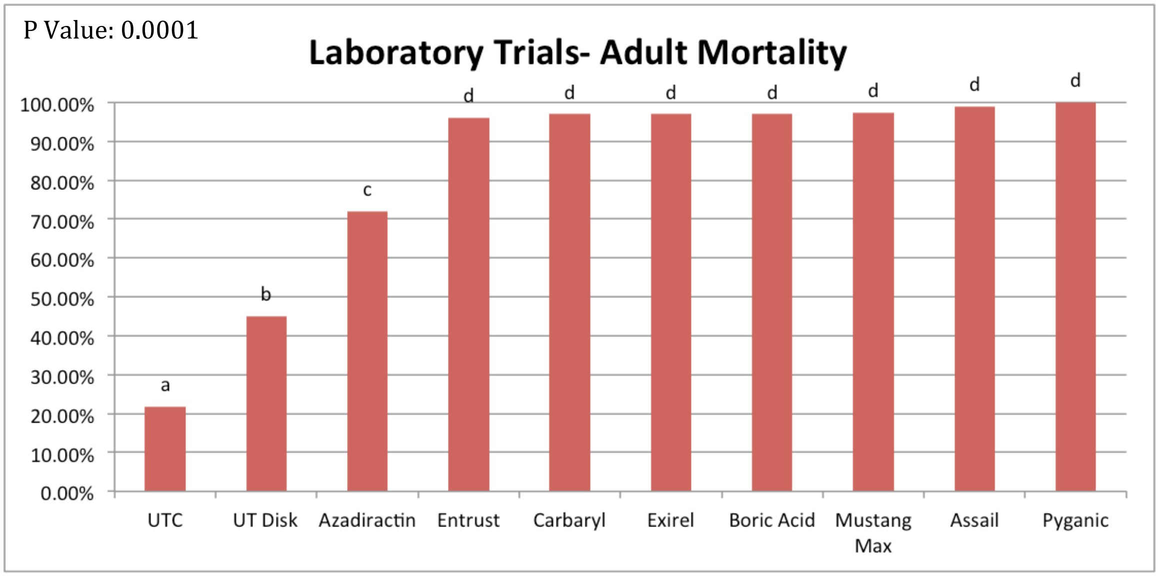

Figure 5: Adult *D. suzukii* mortality at 48 hours post introduction

Field Results:

In field trials, berries taken from sections treated with AtK disks displayed a decrease in oviposition by *D. suzukii*. Additionally, sections treated with unsprayed disks (UT Disks) displayed lower levels of oviposition, although not as dramatically as in laboratory trials (see fig 6-7). Recorded data is the mean of all weekly collections throughout the 2016 field season, June 21st-September 16th. All results include larvae numbers.

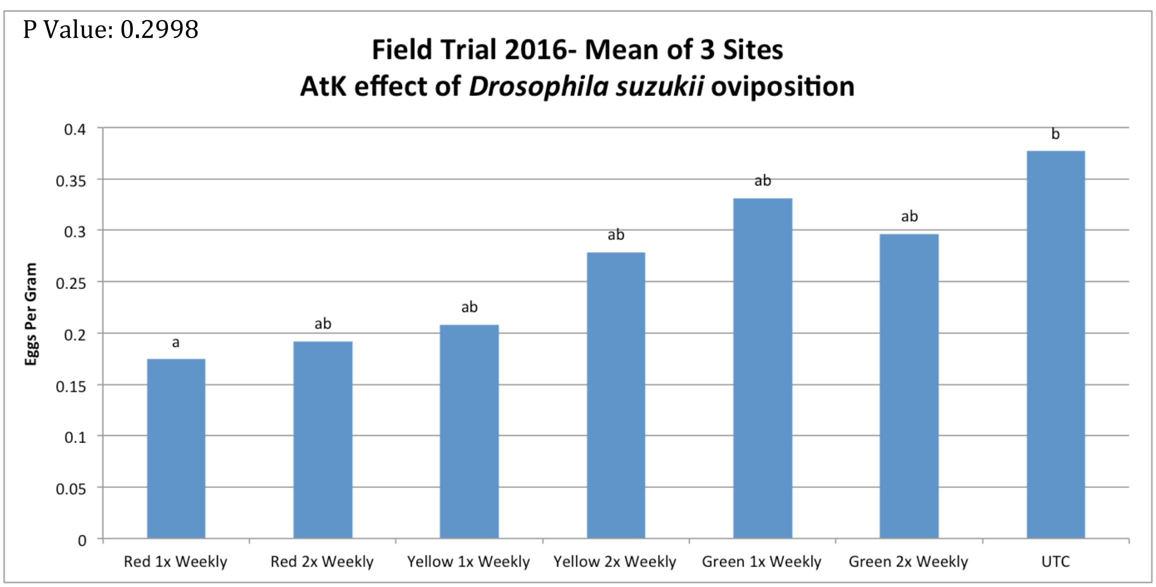

Figure 6: Oviposition. Note that "Green 2x Weekly" was not sprayed; it was simply adjacent to Red 2x and Yellow 2x Weekly

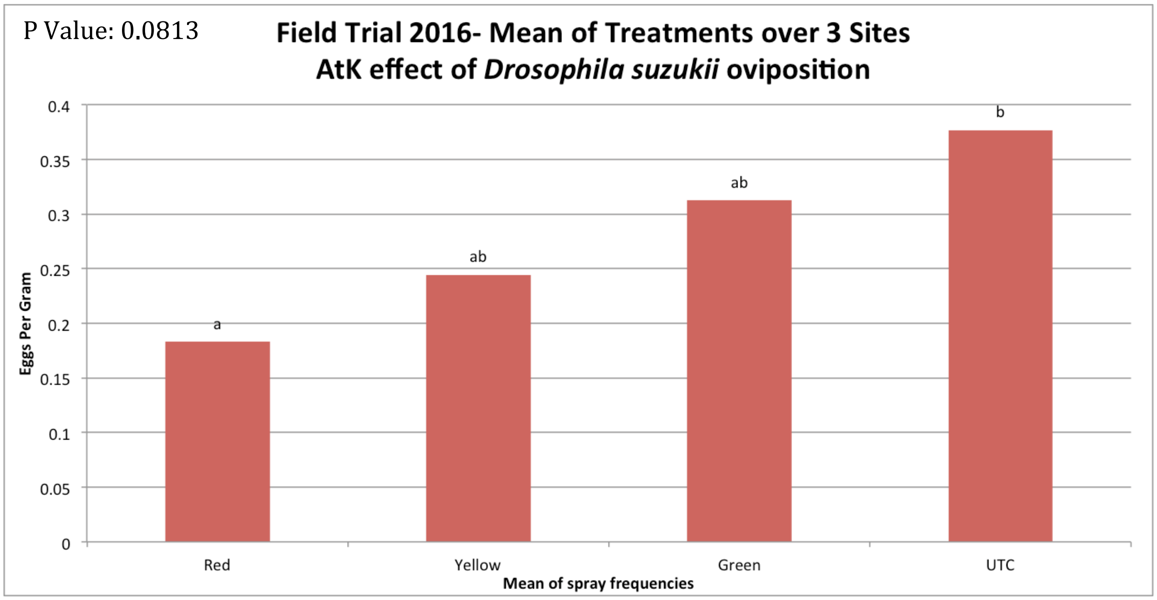

Figure 7: Oviposition in field. Spray frequencies averaged

In field trials, spraying 2x weekly did not result in significantly different control compared to spraying 1x weekly, although spacing did have an effect. Disks spaced 18" resulting in a net egg/larvae per gram reduction of 51.4% compared to a 35.2% reduction in disks spaced at 36." Additionally, we observed a 17.1% reduction in oviposition in sections treated with unsprayed disks (see fig 8).

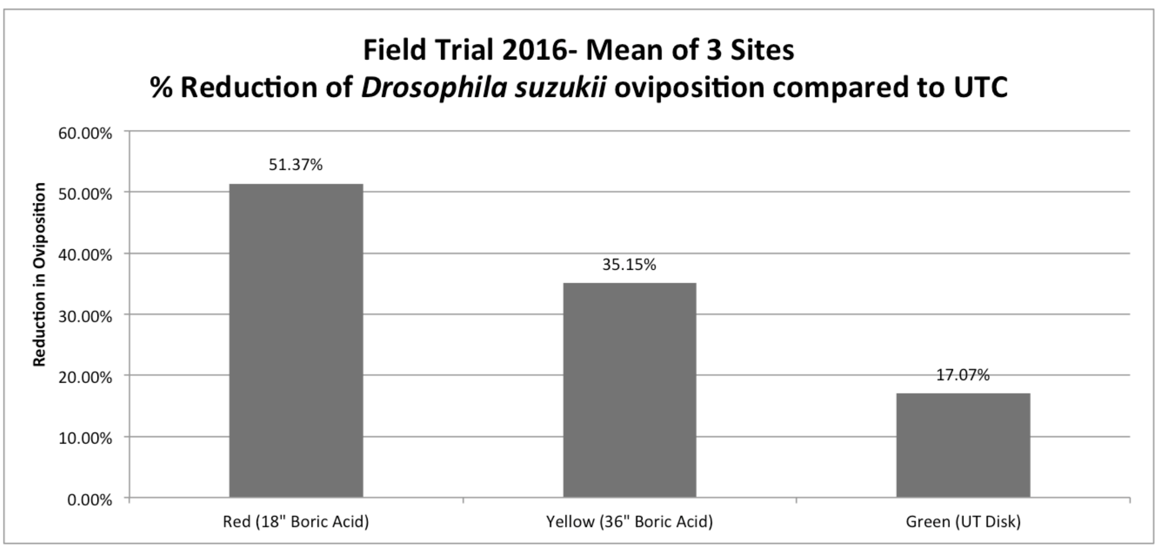

Figure 8: Percentage reduction in oviposition.

Reduction in oviposition was less significant in field trials compared to controlled laboratory environments, however, similar trends were observed. Boric acid treated disks

outperformed untreated disks by 26.2% in the field (Mean of 18" and 36"), and by 16.3% in the lab.

Discussion and future research

We concluded that while the use of AtK disks reduced the oviposition of *D. suzukii* in the field, the levels of oviposition were too high to consider this method a panacea. However, nothing the field of IPM is such, and any material that allows for a safe and relatively simple control measure should be considered as a possibility.

Overall, our findings indicate that in field conditions, spraying the disks more frequently didn't have an effect on the efficacy of them. However, spacing the disks more closely, and therefore using more disks per section did have a significant ovipositional dampening effect.

While it's hard to say whether or not the AtK disks alone caused the decrease, the effects of each treatment were similar in each study site, and it was observed that untreated disks provided a measureable amount of control, despite lacking a killing agent. This fact leads us to believe that the AtK disks affect the behavior of *D. suzukii* adults in some way that inhibits oviposition beyond simply causing mortality. Further research will be necessary to ascertain the actual effect they have, although early direct observations in laboratory testing indicate that the insects may be simply distracted by the presence of the highly attractive AtK disks, leaving them less time to come in contact with the berries. That said, laboratory studies of the effects of untreated disks did demonstrate higher mortality compared to control, indicating that simply feeding on the disks themselves might have had a small toxic effect (albeit a smaller effect than was observed in insecticide trials). Boric acid treated disks outperformed untreated disks by 26.2% in the field (Mean of 18" and 36"), and by 16.3% in the lab. However, both treated and untreated disks were approximately 30% more effective at reducing oviposition in laboratory trials compared to field trials.

These data indicate that untreated disks were more effective relative to treated disks when tested in the smaller, confined space of a bug dorm chamber. In the field, it's likely that the open space and lack of confinement allow *D. suzukii* adults more room to escape from potentially distracting effects of untreated disks.

While certain insecticides produced a slightly higher rate in mortality or reduction in oviposition, boric acid was eventually chosen for exclusive use in field trials. The reasoning for this decision was that boric acid's control was statistically equivalent to the other materials, and allowed for a much simpler procurement and application process. To elaborate; we believe that the use of a common household material would provide fewer hurdles for growers with small operations or even home gardeners to treat these disks themselves (in a hypothetical situation where they were disseminated for individual use), as boric acid can be readily attained from any gardening supply store.

Additionally, boric acid is not implicated in the development of insecticide resistance in any literature related to its use as an insecticide. Resistance management is a constant concern for any pest management program (particularly in an Integrated Pest Management context), and materials that are less likely to contribute to insecticide resistance should be given high priority. The unique mode of action for this material allows for repeated, season long use over multiple generations of *D. suzukii* without a drop in efficacy.

We hope to expand this work in the future, by testing not only disks of different sizes, but used in conjunction with unique materials such as insecticide-laden netting. The prevailing belief amongst researchers such as Tochen et al. (2015) is that *D. suzukii* exhibits increased fecundity and longevity as relative humidity increases. It's been observed that this preference translates to a preference to oviposit more frequently on berries growing in the lower strata of a bramble row (which usually exhibits temperature and RH levels more suitable to *D. suzukii* flight and oviposition). As such, targeted deployment of disks and insecticide laden netting along the shaded lower strata of plants may result in efficient control with fewer disks and sprays required.

Works Cited:

Asplen, M. K., Anfora, G., Biondi, A., Choi, D. S., Chu, D., Daane, K. M., ... & Isaacs, R. (2015). Invasion biology of spotted wing Drosophila (Drosophila suzukii): a global perspective and future priorities. *Journal of pest science*, *88*(3), 469-494.

Atallah, J., Teixeira, L., Salazar, R., Zaragoza, G., & Kopp, A. (2014). The making of a pest: the evolution of a fruit-penetrating ovipositor in Drosophila suzukii and related species. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1781), 20132840.

Cowles, R. S., Rodriguez-Saona, C., Holdcraft, R., Loeb, G. M., Elsensohn, J. E., & Hesler, S. P. (2015). Sucrose improves insecticide activity against Drosophila suzukii (Diptera: Drosophilidae). *Journal of economic entomology*, 108(2), 640-653.

Habes, D., Morakchi, S., Aribi, N., Farine, J. P., & Soltani, N. (2006). Boric acid toxicity to the German cockroach, Blattella germanica: Alterations in midgut structure, and acetylcholinesterase and glutathione S-transferase activity. *Pesticide Biochemistry and Physiology*, *84*(1), 17-24.

Hamby, K. A., Hernández, A., Boundy-Mills, K., & Zalom, F. G. (2012). Associations of yeasts with spotted-wing Drosophila (Drosophila suzukii; Diptera: Drosophilidae) in cherries and raspberries. *Applied and environmental microbiology*, *78*(14), 4869-4873.\

Kilani-Morakchi, S., Aribi, N., & Soltani, N. (2009). Activity of boric acid on German cockroaches: Analysis of residues and effects on reproduction. *African Journal of Biotechnology*, 8(4).

Knight, A. L., Basoalto, E., Yee, W., Hilton, R., & Kurtzman, C. P. (2015). Adding yeasts with sugar to increase the number of effective insecticide classes to manage Drosophila suzukii (Matsumura)(Diptera: Drosophilidae) in cherry. *Pest management science.*

Landolt, P. J., Adams, T., & Rogg, H. (2012). Trapping spotted wing drosophila, Drosophila suzukii (Matsumura)(Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. *Journal of applied entomology*, *136*(1‐2), 148-154.

Lee, J. C., Dreves, A. J., Cave, A. M., Kawai, S., Isaacs, R., Miller, J. C., ... & Bruck, D. J. (2015). Infestation of wild and ornamental noncrop fruits by Drosophila suzukii (Diptera: Drosophilidae). *Annals of the Entomological Society of America*, sau014.

Lee, J. C., Bruck, D. J., Curry, H., Edwards, D., Haviland, D. R., Van Steenwyk, R. A., & Yorgey, B. M. (2011). The susceptibility of small fruits and cherries to the spotted- wing drosophila, Drosophila suzukii. *Pest management science*, *67*(11), 1358-1367.

Lee, J. C., Shearer, P. W., Barrantes, L. D., Beers, E. H., Burrack, H. J., Dalton, D. T., ... & Isaacs, R. (2013). Trap designs for monitoring Drosophila suzukii (Diptera: Drosophilidae). *Environmental entomology*, *42*(6), 1348-1355.

Tochen, S., Dalton, D. T., Wiman, N., Hamm, C., Shearer, P. W., & Walton, V. M. (2014). Temperature-related development and population parameters for Drosophila suzukii (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology*, 43(2), 501-510.

Van Timmeren, S., & Isaacs, R. (2013). Control of spotted wing drosophila, Drosophila suzukii, by specific insecticides and by conventional and organic crop protection programs. *Crop Protection*, *54*, 126-133.

Walsh, D. B., Bolda, M. P., Goodhue, R. E., Dreves, A. J., Lee, J., Bruck, D. J., ... & Zalom, F. G. (2011). Drosophila suzukii (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management*, *2*(1), G1-G7.