FIELD EVALUATION OF A NEW THRIPS CONTROL AGENT FOR COTTON: A MECHANICAL INSECTICIDE

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Abstract

Tobacco thrips, *Frankliniella fusca* (Hinds), are a serious threat to cotton production in the United States; their host range is not limited to cotton and they have the ability to feed on a variety of other host plants of economic importance. Their small size, cryptic color, and multiple reproductive strategies are among several other factors that make tobacco thrips extremely difficult to detect and manage. They are also a competent vector for Tomato Spotted Wilt Virus (TSWV), a destructive virus that destroys the plant material it infects and contributes to reduced leaf area, reduced plant height, loss of apical dominance, and wilting. While TSWV is not found in cotton, it infects many closely related plant species. *F. fusca* have developed resistance to some of the commonly used and best chemical insecticides implemented for their control. There are also concerns about deregistration of chemical insecticides like neonicotinoids that are currently used in their control. In our current work, the mechanical insecticide Celite® 610 (DEadzone), a 100% natural diatomaceous earth (DE) formulation, was evaluated as a foliar spray in the field on cotton for tobacco thrips control; it was as effective as Orthene®, an organophosphate chemical insecticide commonly referred to as acephate. Celite® 610 (DEadzone) provides an alternative mode of action to control tobacco thrips than currently available chemical insecticides and has a number of additional advantages. It has a non-toxic mode of action, is fast acting and persistent, active at high humidity, has no restrictions for field reentry after an application, and is applicable to organic farming. Our studies also showed Celite® 610 in a tank mix could enhance the insecticidal activity of Orthene®, potentially reducing the amount of chemical needed for thrips control. Celite® 610 as a powder in these studies readily mixed with water and was applied as a mist using standard spray equipment.

Introduction

Tobacco thrips, *Frankliniella fusca* (Hinds), are a major pest of cotton plants, but can feed on a variety of other host plants including watermelon, tomato, soybean, and peanut. They can cause significant economic losses totaling in the hundreds of millions of dollars annually (Jones, 2005; USDA, 2015). *F. fusca* are native to North America and widely distributed across the mid-south and southeast cotton production regions of the United States, but have also been introduced to Martinique, Mexico, Puerto Rico, and the Netherlands (Anon, 2002; Mound, 1996; Nakao et al., 2013). These thrips are dark brown or black, slender, and extremely small with adults typically measuring 1-2 mm. Tobacco thrips can complete an entire life cycle in approximately 18 days and adults typically survive 3-5 weeks, with generation time influenced by temperature (Morse et al., 2006; Morsello et al., 2008). *F. fusca* reproduce either sexually (i.e., mating) or parthenogenically (i.e., unfertilized eggs) as necessary to propagate the species and are a vector of Tomato Spotted Wilt Virus (TSWV), a virus in the *Tospovirus* genera (Jones, 2005). In addition to problems with insecticide resistance, the combination of their small size, cryptic color, short life span, multiple reproduction strategies, and the ability to vector microbes harmful to an array of host plants make tobacco thrips a pest that are extremely difficult to detect and manage.

Thrips adults and larvae feed on plant epidermal cells and tissue damage manifests as silvery or whitish outlines traced around the veins, which is the result of cell fluids being replaced with air (Cook et al., 2011). Excessive feeding can result in delayed maturation, reduced leaf area, reduced plant height, reduced root growth, loss of apical dominance, and yield loss. However, many producers are most concerned with TSWV transmission. While cotton plants are not infected by TSWV, many closely related plant species are affected. Damage from TSWV infection in a host plant produces symptoms common to tospoviruses including chlorosis, mottling, yellowing, and wilting (Riley et al., 2011).

Viral particles can move between various host plant species via tobacco thrips, which can feed on host cotton plants to survive and transmit viral particles to nearby host plant species. The virus can also overwinter in competent wild plant species and infect subsequent generations of thrips the following season (Groves et al., 2001).

The most effective method of control for the tobacco thrips has been the use of chemical pesticides, but many of them are failing due to thrips resistance, and there is currently no commercially available transgenic approach to control thrips available to farmers. The current practices for cotton control typically include seed treatments with acephate or imidacloprid, in-furrow treatments with aldicarb or acephate, and application of the foliar insecticides spinetoram or acephate (Burris et al., 2000; Cook et al., 2011). Neonicotinoids have also been successful in controlling tobacco thrips, but resistance has been observed in the southeastern United States (Gao et al., 2012; Huseth et al., 2016). There are also concerns related to the continued use of neonicotinoids, some of which are being deregistered.

We conducted a replicated, small field plot test to explore the efficacy of the mechanical insecticide Celite® 610 to control tobacco thrips, *Frankliniella fusca*, infesting *Bacillus thuringiensis* (Bt) cotton (Widestrike® II) in early summer. A mechanical insecticide is a mineral that, on the surface of the insect cuticle at low doses, causes rapid insect death (Imerys, 2016). The efficacy of the mineral alone derived from natural diatomaceous earth (DE) was compared in separate applications to acephate (Orthene®) both at two different treatment rates and compared to a tank mix of Celite® 610 with Orthene® and to untreated cotton at different times after application up to six days.

Materials and Methods

Test Location

This study was carried out at the Upper Coastal Plain Research Station in Rocky Mount, NC (Latitude: 35.89295°, Longitude: -77.67996°, Elevation: 88 feet above sea level). This station in Edgecombe County is part of the Tar River basin with a sandy loam soil texture. Small field plots of Bt cotton (Phytogen 499, a Widestrike® II variety, with Acceleron seed treatment, 0.39 hectares) were planted on 5-9-17 (early summer) and grown at this location for the duration of the experiment. The average monthly temperature (°F) and relative humidity (%) at the site were as follows for the duration of the study, respectively: May, 69.1°F, 69%; June, 75.2°F, 71%; July, 79.6°F, 74%; August, 76.4°F, 77%; September, 71.1°F, 78%; October, 63.7°F, 75% (NCCO, 2018).

Treatment Parameters

Upland cotton, *Gossypium hirsutum*, seeds were treated with imidacloprid to facilitate their emergence above ground and appearance of first true leaves (to minimize plant death from tobacco thrips infestation) at the cotyledon stage. The trial consisted of 6 unique conditions (i) untreated control, (ii) Orthene® (Amvac Chemical Corporation, Newport Beach, CA, USA) at 4 oz/acre, (iii) Orthene® at 6 oz/acre, (iv) Celite® 610 (Imerys, Roswell, GA) at 6 lbs/acre, (v) Celite® 610 at 20 lbs/acre, and (vi) a mixture of Orthene® at 4 oz/acre plus Celite® 610 at 6 lbs/acre) replicated four times using a randomized complete block design. Orthene® is the commercial name for acephate, and Celite® 610 is the commercial name for the diatomaceous earth formulation used in this study. Plots consisted of four 36-inch rows, each 40 feet long, and plant samples were only collected from the middle two rows of each plot.

Untreated control plots were not sprayed during the experiment. Orthene® solutions were prepared by adding the appropriate amount of solid white pellets and non-ionic surfactant (Brandt® Ultra 90, Brandt Consolidated, Inc., Springfield, IL) to tap water in a CO_2 backpack sprayer calibrated at 60 psi (2 gallon mix) using a double TeeJet TX-10 Hollow Cone nozzle per row. The solution was mixed initially by shaking before spraying began and applied at a steady rate of approximately 3 mph; there was no need for additional agitation during spraying. Celite® 610 solutions were prepared similarly by adding the appropriate amount of solid white powder and non-ionic surfactant (Brandt® Ultra 90) to water in the same CO_2 backpack sprayer calibrated at 60 psi. The solution was mixed by shaking before spraying, but no further agitation was necessary. The total volume of spray (36.3 gallons per acre) was also the same for all treatments where 2 gallons was made up one time for each treatment condition and used on all four replicates.

Sampling Procedure

At our field site, we randomly collected 5 seedlings from each plot (24 plots total, 6 conditions tested, 4 replicates each) on three separate days post treatment, and these were processed separately for thrips counting per plant. The plants were excised at ground level with a razor blade. On 6-9-17 (2 days post insecticide application) and 6-15-17 (6 days post insecticide application), whole seedlings were collected and submerged in soapy water in a glass Mason jar and vigorously shaken to dislodge thrips. On 6-13-17 (4 days post insecticide treatment), untreated terminals with two expanded leaves at the top of each plant were collected separately from the rest of the plant (i.e., terminals from top of 5 seedlings in one jar and "rest of plant" from those same 5 seedling in a separate jar). This sampling did not prove informative after the thrips count, and the number of thrips per plant from both plant collection sites were added together and reported as the total number of thrips per plant.

Thrips Counting

For each sample the contents of a Mason jar were poured over a fine metal sieve under running water. The plants in the sieve were washed with tap water to dislodge thrips and any other debris and remove any soap from the specimens. Washed cotton seedlings and large pieces of debris were removed from the sieve and discarded, and adult and immature thrips (along with any other fine particulate matter) were decanted into a 50 mL glass vial with 70% ethanol. To count thrips for each treatment, a single vial and its contents were poured into a gridded plastic petri dish and observed under a dissecting stereomicroscope. Adults and immatures were identified to species and were counted separately according to the stage of development for each sample.

Injury Analysis

Thirty-four days (6/13/2017) after the cotton was planted, each plant was visually evaluated for thrips damage and assigned an overall score (Toews et al., 2010). Injury was rated on a scale of 1-5, where a score of 1 represented 0-24% injury caused by thrips, a score of 2 was 25-49% injury, a score of 3 was 50-74% injury, a score of 4 was 75-99% injury, and a score of 5 represented plant death caused by thrips infestation. Damage was assessed by observing the severity of several factors typical of thrips infestation including silvering of the lower leaf surface, deformed or discolored leaves, and terminal loss.

Cotton Yield

The center 2 rows of each plot were harvested with a 2-row John Deere mechanical harvester on 10-16-17. Yields were estimated using a mechanical picker, with lint yield calculated using an estimate of 38% gin turnout.

Statistical Analysis

Thrips per plant were compared among treatments using a single factor analysis of variance (ANOVA) with R software (R Core Team, 2013). The standard error of the mean (SEM) at a 95% confidence interval (CI) (α = 0.05) was used to estimate how close a sample mean was likely to be to the population mean.

Results and Discussion

Testing Location and Plot Arrangement

Upland cotton, *Gossypium hirsutum*, was planted in early summer on May 9, 2017 at the Upper Coastal Plains Research Station in Rocky Mount, NC (Figure 1). The plants emerged in approximately 7 days, the cotyledon stage was approximately 7 days, and the first true leaves appeared in 2.5 to 3 weeks. The chemical seed treatment (imidacloprid) provided adequate thrips control to prevent plant death giving us time to conduct our experimental treatments.

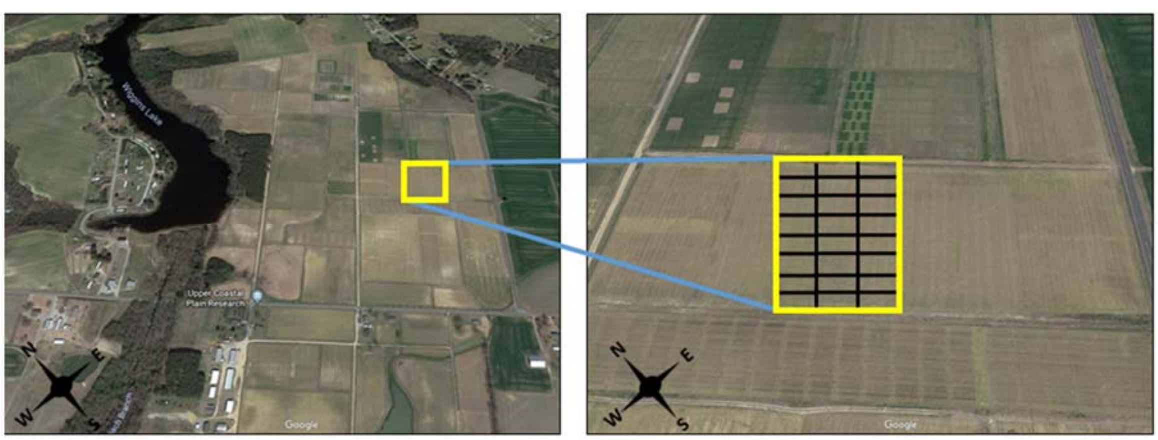

Figure 1. Field site at the Upper Coastal Plain Research Station in Rocky Mount, NC. The left panel is a wide view of the research station and surroundings, and the right panel is a zoomed-in view of the field site including plot organization and orientation. Areas as viewed on Google Earth (Google, 2018).

The treatments we applied to control thrips were acephate (an organophosphate insecticide) and diatomaceous earth (a mechanical insecticide) at varying concentrations. The acephate used in this study is sold under the trade name Orthene® by Amvac Chemical Corporation (Newport Beach, CA, USA), and diatomaceous earth is sold as Celite® 610 by Imerys Filtration Minerals, Inc (Roswell, GA, USA). The treatment categories were as follows: untreated control, Orthene® at 4 oz/acre, Orthene® at 6 oz/acre, Celite® 610 at 6 lbs/acre, Celite® 610 at 20 lbs/acre, and a mixture of Orthene® at 4 oz/acre plus Celite® 610 at 6 lbs/acre. The field plots were arranged in a randomized fashion as shown in Figure 2.

Row	Field Setup (Plots are 12 ft. by 40 ft. each)							
1	**101**	**104**	**201**	**204**	**301**	**304**	**401**	**404**
2								
		Celite®	Celite®	Celite®	Celite®	Celite®	Celite®	
3	Control	610	610	610	610	610	610	Orthene
4		+Orthene	+Orthene	20 lbs/a	20 lbs/a	+Orthene	6 lbs/a	4oz
5	**102**	**105**	**202**	**205**	**302**	**305**	**402**	**405**
6								
	* Celite®						Celite®	
7	610	**Orthene	Orthene	Control	Control	Orthene	610	Control
8	20 lbs/a	4oz	4oz			6oz	20 lbs/a	
9	**103**	**106**	**203**	**206**	**303**	**306**	**403**	**406**
10								
	Celite®			Celite®	Celite®		Celite®	
11	610	Orthene	Orthene	610	610	Orthene	610	Orthene
12	6 lbs/a	6oz	6oz	6 lbs/a	6 lbs/a	4oz	+Orthene	6oz

*Celite®610 (Mechanical insecticide, diatomaceous earth (DE), Imerys).

**Orthene® (Acephate, a.i.); organophosphate insecticide.

Figure 2. Plot layout. White squares represent the control plots (no sprays). Blue squares represent the acephate (Orthene®) treatment at 4 oz. Gray squares represent the acephate treatment at 6 oz. Orange squares represent the Celite® 610 (diatomaceous earth) treatment at 6 lbs/acre. Red squares represent the diatomaceous earth treatment at 20 lbs/acre. Green squares represent the mixture of acephate at 4 oz. with diatomaceous earth at 6 lbs/acre. Abbreviations: lbs = pounds; a = acre. Bold numbers in each square are the plot number.

Insecticide Application

Twenty-nine days after planting, the average cotton plant was approximately 4.5 inches tall with 2 true leaves when we applied our treatments for thrips control. We conducted our testing at a time when the seedlings were most susceptible to thrips injury (Cook et al., 2011). Brandt® Ultra 90, a non-ionic surfactant containing an anti-foaming agent was incorporated in all treatments to improve wetting of foliage and enhance the effectiveness of the spray. A double (per row) TX-10 hollow cone nozzle with a 100 micron filter was sprayed in a bottom-up manner at 60 psi as shown in Figure 3 (for one nozzle) for all treatments containing DE. Orthene® treatments were top down at the same pressure.

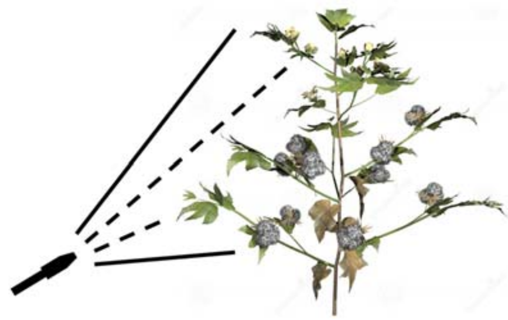

Figure 3. Bottom-up spray technique used to apply diatomaceous earth (DE).

Figure 4 shows visually the coverage and dispersal of the bottom-up spraying method on cotton leaves, including typical droplet sizes and color of the diatomaceous earth on leaves and rows. The residual diatomaceous earth that coats the ground around the plant should also be active but this was not specifically evaluated.

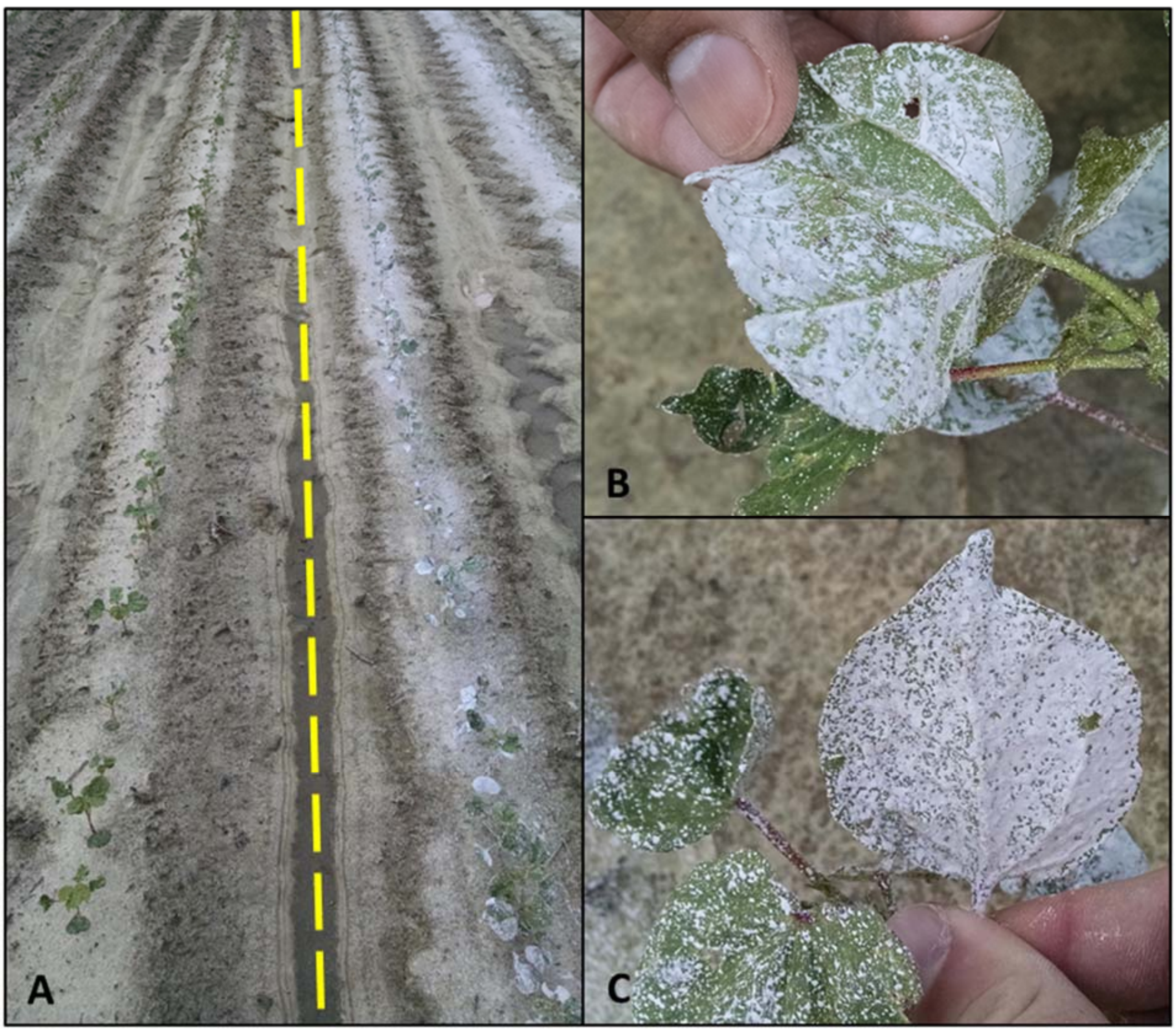

Figure 4. DE treated cotton. In (A) the dotted yellow line separates non-DE treated (left) and DE treated (right) rows. (B) and (C) show close-up of the treated leaf.

Sampling and Thrips Quantification

Whole plant test samples were collected from each plot 48 hours (2 days) after the test applications to monitor the effectiveness of the insecticides. All of the adult thrips in the following results were identified as tobacco thrips, *Frankliniella fusca*, since larvae cannot be accurately identified to species using morphological characteristics. Since 87% of the thrips counted were larvae, the thrips data presented in Figure 5 and other figures that follow are the total thrips count per plant (i.e., adult and larvae counts added together). The Orthene®, Celite® 610, and the tank mix of Orthene® and Celite® 610 treatments all resulted in a reduction in thrips per plant compared to the untreated control (Figure 5). Orthene® at 4 and 6 oz and Celite® 610 at 6 lbs/acre and 20 lbs/acre had similar effectiveness in controlling tobacco thrips. The combination of Celite® 610 at 6 lbs/acre with Orthene® at 4 oz/acre demonstrated greater control than the other treatments on average and based on the SEM except for the Orthene® at 6 oz/acre.

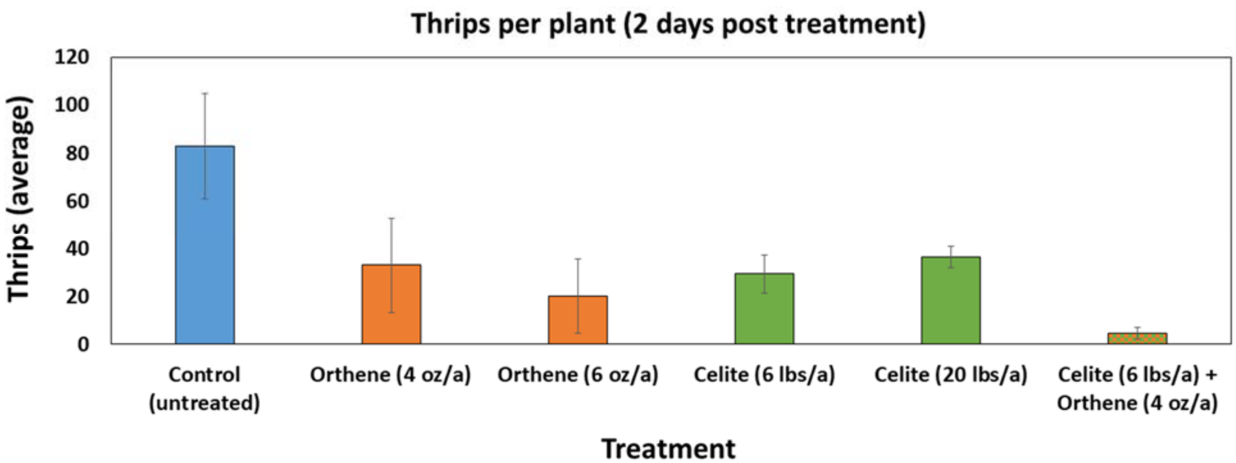

Figure 5. Average number of thrips per plant two days after insecticide application. a = acre; error bars ±1.96 standard errors of the mean (SEM).

The results in Figure 6 represent the total number of thrips per plant (thrips counts from top and bottom of plant combined) 4 days after treatment. See justification for combining data explained in the Materials and Methods section. Four days after application, the trend was that all treatments showed lower thrips per plant than the control. While Celite® 610 at 6 lbs/acre and 20 lbs/acre control was not statistically better than the control based on the SEM (at a 95% CI), the results for Celite® for some reason had greater variability than at the other post-treatment times. This increased variability may have resulted from the change in the plant sampling method (also see 6 days post treatment which confirms findings observed at 2 days post treatment). There was little difference in thrips control between Celite® 610 at 6 and 20 lbs/acre although a trend was found of less control at the higher treatment level; this was similar to what was found at 2 days post treatment (also see results at 6 days which follow). The tank mixing of Orthene® with Celite® 610 produced levels of thrips per plant which on average was lower but statistically was similar to Orthene® at 4 oz/acre and 6 oz/acre (the tank mixing increase in orthene activity alone also seen at 2 and 6 days; discussed before and discussed more later).

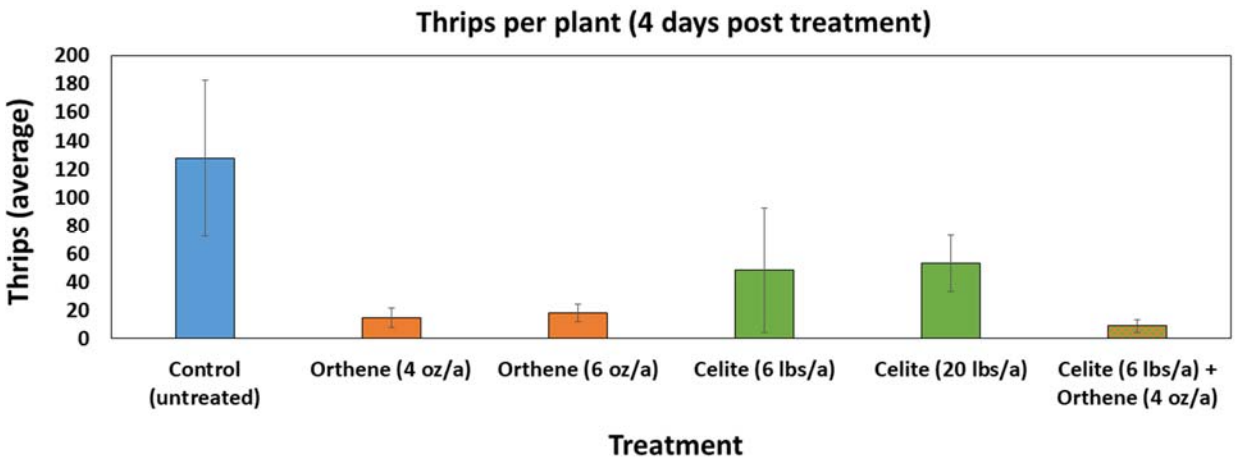

Figure 6. Average number of thrips per plant four days after insecticide application. a = acre; error bars ±1.96 standard errors of the mean (SEM).

Six days after application, whole plants were collected using the same approach used at the 2 day post treatment time point. The six day collection also was after a day of heavy rain at the study site. All treatments resulted in a reduction in thrips per plant compared to the control except for Celite® 610 at 20 lbs/acre which was marginally not statistically significant based on the SEM CI (Fig. 7). At six days post treatment, Orthene® at 4 and 6 oz/acre, Celite® 610 at 6 lbs per acre, and the mixture of Celite® 610 with Orthene® produced the same thrips reduction levels. Like 2 and 4 days, the higher level of Celite® 610 resulted in a higher level of thrips; specifically at 6 days, the thrips levels trended higher at 20 versus 6 lbs/acre. These results suggest that a lower dose of Celite® 610 than 6 lbs per acre may be even more effective for thrips control. Our data also suggest that tank mixing of Orthene® with Celite® 610 may be used to reduce the level of chemistry needed. However, variable concentrations of both products must be tested to support this observation further. Because the mechanisms of action are different between Orthene® and Celite® 610, tank mixing could also be a useful strategy with Orthene® to slow the evolution of insecticide resistance. If this Celite® 610 effect on Orthene® is translated to other compounds like neonicotinoids, Celite® 610 might also be a useful tool for resistance management in general for foliar treatments for thrips.

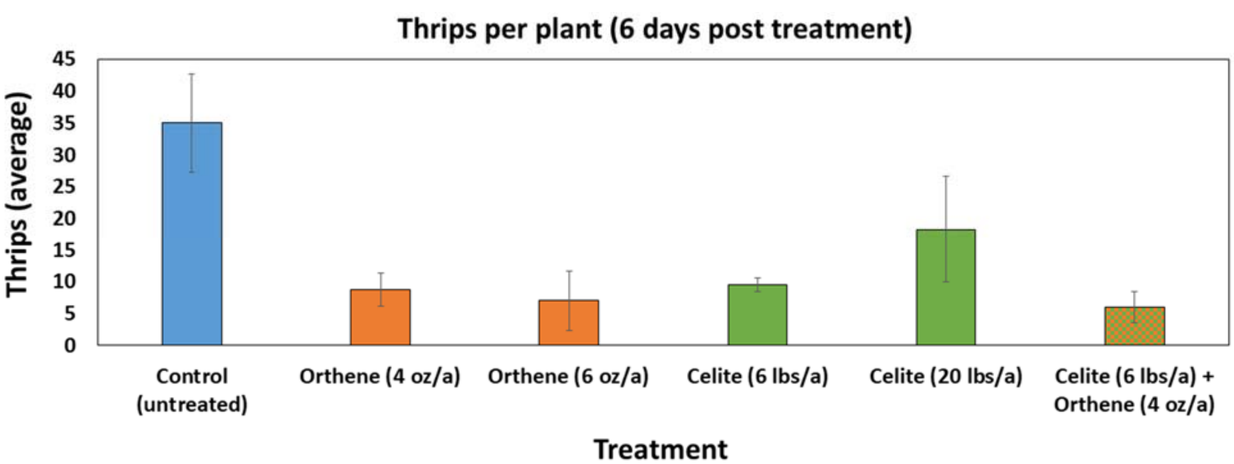

Figure 7. Average number of thrips per plant six days after insecticide application. a = acre; error bars ±1.96 standard errors of the mean (SEM).

Injury Assessment and Cotton Yield

Each plant was visually inspected for thrips damage and scored 34 days (approximately 5 weeks) after the cotton was planted. All treatments reduced thrips plant injury compared to the control with no differences between treatments (Figure 8). The cotton plants were harvested on 10-16-17(163 days, or approximately 23 weeks, after planting) to assess the yield. No differences were found in yield (Figure 9).

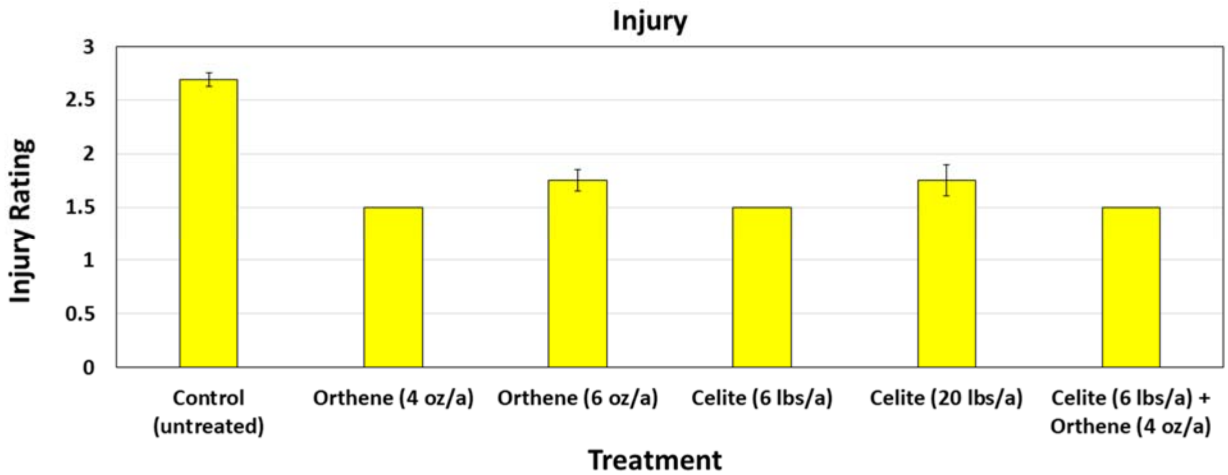

Figure 8. Injury ratings based on visual inspection. Error bars $\pm$1.96 standard errors of the mean.

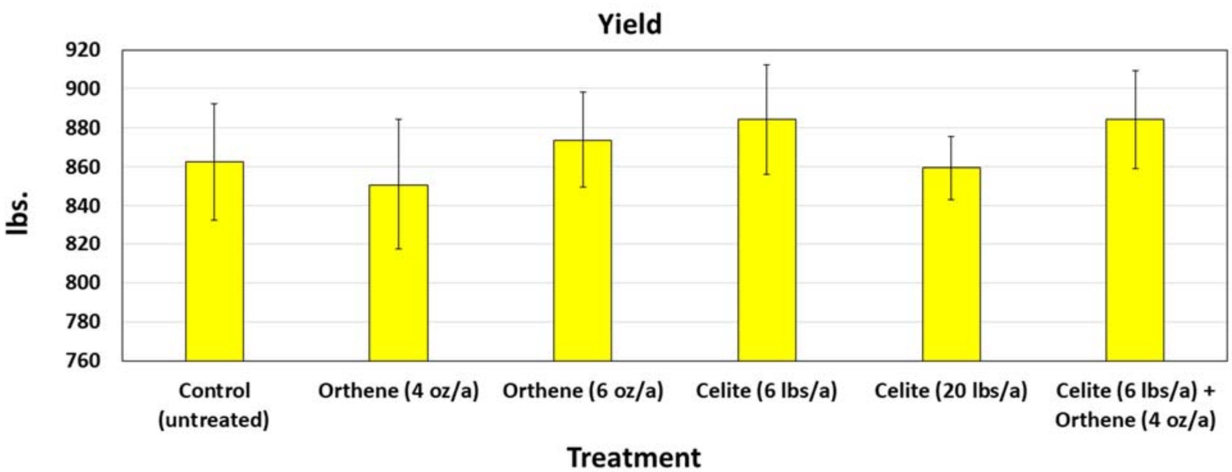

Figure 9. Cotton yield data. Error bars $\pm$1.96 standard errors of the mean; lbs = pounds.

Conclusions and Future Directions

The objective of the work was to determine if a mechanical insecticide, in this case Celite® 610, could be used as an alternative to Orthene® as a foliar spray for tobacco thrips control in cotton production. In the small plot studies conducted at a single field site in North Carolina and during one field season in 2017, we found that Celite® 610 produced similar control to that of Orthene®. Tank mixing of Celite® 610 with Orthene® produced the best control and suggests that this approach could be used to reduce chemical insecticide treatment levels. More research is needed to determine the optimum dose for Celite® 610 as a stand-alone control system for thrips and the optimum parameters for tank mixes for Celite® 610 versus Orthene®. It is also important to investigate the impact of Celite® 610 tank mixing on other chemical foliar sprays for thrips control in cotton. Tank mixing might have favorable applications in insecticide resistance management for thrips.

There are several advantages for the use of mechanical insecticides for thrips control as outlined.

Advantages of the technology
- Produced from nature
- Non-chemical, not-toxic mode of action
- Applicable to organic farming
- Fast acting and persistent
- Safe to animals and plants
- Applied as a wettable powder; has residual activity
- Active at high temperature and humidity
- Can enhance chemical insecticide activity
- Can be used in combination with chemistry to reduce amount of chemical insecticide needed

There are also other practical aspects of the technology as outlined.

Other practical aspects of the technology
- EPA approved and registered in all 50 states in USA
- 100% natural diatomaceous earth
- High potential for absorption from epicuticle lipid layer
- OMRI listed mechanical insecticide
- Food Chemical Codex (FCC) and Generally Recognized as Safe (GRAS)
- Zero Restricted Entry Intervals (REI)
- Physical mode of action which deters chemical resistance development

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