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## Insecticide Resistance and Resistance Management

# Standardized Field Trials in Cotton and Bioassays to Evaluate Resistance of Tobacco Thrips (Thysanoptera: Thripidae) to Insecticides in the Southern United States

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### Abstract

Foliar-applied insecticide treatments may be necessary to manage thrips in cotton (*Gossypium hirsutum* L.) under severe infestations or when at-planting insecticide seed treatments do not provide satisfactory protection. The most common foliar-applied insecticide is acephate. Field observations in Tennessee suggest that the performance of acephate has declined. Thus, the first objective was to perform leaf-dip bioassays to assess if tobacco thrips, *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae), in cotton production regions have evolved resistance to foliar-applied insecticides. A second objective was to assess the performance of commonly applied foliar insecticides for managing thrips in standardized field trials in Arkansas, Tennessee, Mississippi, and Texas. For both objectives, several insecticides were evaluated including acephate, dicrotophos, dimethoate, lambda-cyhalothrin, imidacloprid, and spinetoram. Field trials and bioassays were completed from 2018 to 2021. Dose-response bioassays with acephate were performed on tobacco thrips field populations and a susceptible laboratory population. Bioassay results suggest that tobacco thrips have developed resistance to acephate and other organophosphate insecticides; however, this resistance seems to be most severe in Arkansas, Tennessee, and the Delta region of Mississippi. Resistance to other classes of insecticides were perhaps even more evident in these bioassays. The performance of these insecticides in field trials was variable, with tobacco thrips only showing consistent signs of resistance to lambda-cyhalothrin. However, it is evident that many populations of tobacco thrips are resistant to multiple classes of insecticides. Further research is needed to determine heritability and resistance mechanism(s).

**Key words:** *Frankliniella fusca*, resistance, bioassay, field trial

Upland cotton, *Gossypium hirsutum* L., is a major commodity grown in the southern U.S., and thrips can be found infesting cotton throughout this region. The most common species found in cotton include tobacco thrips, *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae); flower thrips, *Frankliniella tritici* (Fitch) (Thysanoptera: Thripidae); western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae); onion thrips (Thysanoptera: Thripidae), *Thrips tabaci* (Lindeman); and soybean thrips, *Neohydatothrips variabilis* (Beach) (Thysanoptera: Thripidae) (Cook et al. 2003, Albeldaño et al. 2008, Stewart et al. 2013). Of these species, tobacco thrips is the most abundant in seedling cotton in the Mid-South (Arkansas, Tennessee, Missouri, Mississippi, Louisiana) and the majority of the southeast (Virginia, North Carolina, South Carolina, Georgia, Alabama, Florida) (Cook et al. 2003, Stewart et al. 2013).

Thrips feeding injury can cause plant mortality, stunted growth, delayed maturity, and yield loss (Gaines 1934, Dunham and Clark 1937, Watts 1937, Bourland et al. 1992, Faircloth et al. 1999, Cook et al. 2013). Seedlings are most susceptible to thrips injury from emergence to about the fourth true leaf stage (Stewart and Lentz 2010). Because thrips are early-season, consistent pests, most growers use a proactive approach to control them using at-planting insecticides which can be applied as a seed or in-furrow (liquid or granular) treatment (Cook et al. 2011). The insecticide seed treatments (IST) available include acephate (Orthene 97; AMVAC Chemical Corporation, Los Angeles, CA), imidacloprid (Gaucho 600, Bayer CropScience, Research Triangle Park, NC), thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Greensboro, NC), and a pre-pack formulation that combines imidacloprid and thiodicarb (Aeris, Bayer CropScience). Liquid in-furrow applications of acephate or imidacloprid are also options. Acephate is in the organophosphate (IRAC MoA group 1B) insecticide class. Imidacloprid and thiamethoxam are neonicotinoids (IRAC MoA group 4A).

Growers extensively adopted neonicotinoid seed treatments in the early 2000s. The primary at-planting insecticide used to manage thrips before the introduction of neonicotinoids was aldicarb (Temik, Bayer CropScience), a granular in-furrow carbamate insecticide (IRAC MoA group 1A). In 2010, aldicarb was voluntarily removed from the market because of its high toxicity to wildlife and humans and concerns about contamination of groundwater, but neonicotinoid seed treatments were already the most widely used at-planting thrips treatment. The primary seed treatments used were imidacloprid (Gaucho) and thiamethoxam (Cruiser) (Cook et al. 2020). After more than a decade of neonicotinoids being the primary thrips control method, decreased performance of this class of insecticide, particularly thiamethoxam, was observed in several Tennessee cotton fields during 2011 and 2012 (S. D. Stewart, personal observation). The efficacy of neonicotinoid seed treatments in Tennessee was assessed by Vineyard et al. (2017) in 2013 and 2014. Imidacloprid and aldicarb provided the greatest level of control in this study, while thiamethoxam was not different from the untreated control in terms of effects on plant vigor, crop maturity, or yield. Due to the limited protection provided by thiamethoxam, university Extension programs stopped recommending thiamethoxam for thrips control (Gore et al. 2014, Stewart 2014, Bogren et al. 2015, Hollis 2015). Researchers confirmed that the reduced efficacy of neonicotinoid seed treatments was due to tobacco thrips resistance to this insecticide class (Huseth et al. 2016, 2018; Darnell-Crumpton et al. 2018).

Thrips resistance to neonicotinoids has caused growers to change their management strategies. A control tactic some growers have utilized is supplementing imidacloprid seed treatments with acephate as a seed treatment or liquid, in-furrow treatment (Cook et al. 2020). Aldicarb (AgLogicTM 15G, AgLogic Chemical LLC, Chapel Hill,

NC) was recently reintroduced into the market as an alternative thrips insecticide, especially where nematodes are also an issue.

At-planting insecticide treatments may fail to provide adequate thrips control because of resistance to neonicotinoids, severe thrips pressure, or unfavorable growing conditions, so foliar-applied insecticide applications may be needed for optimal plant protection. Foliar applications have become more common because of resistance to neonicotinoid seed treatments (Cook et al. 2020). Recommended foliar-applied insecticide options include spinosyns (IRAC MoA group 5) and organophosphates such as acephate, dicrotophos, and dimethoate (Stewart et al. 2022). Multiple studies have evaluated the efficacy of foliar-applied insecticides for thrips control (Toews et al. 2012; Williams et al. 2014; Siebert et al. 2016; Huseth et al. 2017; D'Ambrosio et al. 2018; Cook et al. 2020, 2022). Pyrethroids (IRAC MoA group 3A) provide poor thrips control (Toews et al. 2012, Cook et al. 2020), and university Extension programs do not recommend this class of chemistry (Carchot 2020, Greene 2020). Where resistance to neonicotinoid seed treatments has been confirmed, it is expected foliar treatments made with this same mode of action will not provide adequate control. Indeed, research to confirm resistance to neonicotinoid insecticides was done with traditional dose response bioassays using treated leaf disks (Huseth et al. 2017).

For many years, acephate has been the primary foliar insecticide option to manage thrips due to its effectiveness and low cost (Stewart et al. 2020). However, a decline in the efficacy of acephate in Tennessee has been observed. From 2005 to 2019, thrips control with acephate declined, with greater than 90% control in 2005 to less than 40% control by 2018 (Stewart et al. 2020). In contrast, the performance of spinetoram did not change, albeit over a shorter period of time. The response of thrips populations to acephate has not been uniformly studied across the Mid-South, Southeast, and Texas cotton production regions. In this study, our goal was to better understand patterns of acephate resistance throughout the southern U.S. To do this, we combined laboratory-based bioassays with field screening studies to document differences in insecticide susceptibility across a broad geography. To estimate resistance status, we performed dose-response bioassays using acephate and other common insecticides on tobacco thrips populations collected from the Mid-South, Southeast, and Texas. The second objective was to evaluate the efficacy of foliar-applied insecticides through standardized field trials. Together, these results provide important context for changing sensitivity of tobacco thrips populations to common foliar insecticides used for early season management in cotton.

## Materials and Methods

### Thrips Collections

From 2018 through 2021, adult tobacco thrips populations were collected across a large geography in the southern U.S. to perform insecticide bioassays (Table 1). Field collected tobacco adult thrips were gathered from wild host plants, peanut (*Arachis hypogaea* L.), wheat (*Triticum aestivum* L.), or cotton (Table 1). Thrips were either collected with a sweep net or by gently beating plants into a white bucket or a white surface and aspirating them into a 1.5-ml microcentrifuge tube (No. 111558; Globe Scientific, Mahwah, NJ). The aspirators were made based on the design described by Darnell-Crumpton et al. (2018).

For comparison purposes, a tobacco thrips population from North Carolina State University, with known susceptibility to imidacloprid, thiamethoxam, acephate, (Huseth et al. 2016, 2017; Darnell-Crumpton et al. 2018), and presumably other insecticides, was included in the assays. This colony has been maintained

**Table 1.** The collection location, host, generation tested, bioassay date, and percent water-check mortality for tobacco thrips populations tested in discriminating dose and dose response curve bioassays

Population	Location	Host	Generation <sup>a</sup>	Bioassay Date	%Check Mortality
AL1	Tallassee, AL	Cotton	F1	30 June 2020	8.9
LA1	St. Joseph, LA	Cotton	F1	23 June 2020	17.9
AR1	Marianna, AR	Cotton	F0	16 June 2018	4.0
AR2	Marianna, AR	Cotton	F1	23 June 2020	–
AR3	Tillar, AR	Cotton	F1	30 June 2020	0.0
AR4	Marianna, AR	Cotton	F1	21 June 2021	31.5
MS1	Starkville, MS	Cotton	F0	6 June 2019	5.3
MS2	Stoneville, MS	Cotton	F0	19 May 2019	0.0
MS3	Stoneville, MS	Cotton	F1	23 June 2020	21.9
MS4	Starkville, MS	Cotton	F1	8 July 2020	6.3
MS5	Stoneville, MS	Cotton	F0	21 June 2021	32.3
MSLab1	Lab Colony, MSU	Cabbage	>F10	23 June 2018	1.4
MSLab2	Lab Colony, MSU	Cabbage	>F10	10 June 2019	2.2
NC1	Lees Mill Township, NC	Wheat	F0	22 May 2019	9.6
NC2	Oconeechee Township, NC	Wheat	F0	22 May 2019	10.7
NC3	LaGrange, NC	Wild hosts	F1	16 June 2020	21.3
NC4	Fountain, NC	Wild hosts	F1	16 June 2020	17.8
NC5	Seaboard, NC	Wild hosts	F1	16 June 2020	19.4
NC6	Township 6-Upper Fishing Creek, NC	Wheat	F0	25 May 2021	0.0
NC7	North Whitakers Township, NC	Wheat	F0	25 May 2021	0.0
NC8	Plymouth, NC	Cotton	F1	17 July 2021	2.6
NCLab1	Lab Colony, NCSU	Cabbage	>F10	12 June 2020	2.6
NCLab2	Lab Colony, NCSU	Cabbage	>F10	12 Aug. 2021	0.0
NCLab3	Lab Colony, NCSU	Cabbage	>F10	21 Dec. 2021	13.2
TN1	Jackson, TN	Wild hosts	F0	6 June 2018	---
TN2	Jackson, TN	Cotton	F0	16 June 2018	4.1
TN3	Jackson, TN	Wild hosts	F0	30 May 2019	1.4
TN4	Jackson, TN	Cotton	F0	4 June 2019	4.6
TN5	Milan, TN	Cotton	F0	6 June 2019	7.0
TN6S	Milan, TN	Cotton	>F1	26 June 2019	8.6
TN7U	Milan, TN	Cotton	>F1	26 June 2019	0.0
TN8	Milan, TN	Wild hosts	>F2	17 July 2021	3.6
TN9	Milan, TN	Wild hosts	>F2	21 July 2021	2.3
TN10	Milan, TN	Wild hosts	>F2	31 July 2021	2.7
TN11	Jackson, TN	Cotton	F0	4 June 2021	6.2
TN12	Milan, TN	Cotton	F0	4 June 2021	5.6
TN13	Jackson, TN	Cotton	F0	10 June 2021	2.9
TX1	Snook, TX	Cotton	F0	6 June 2019	10.5
TX2	Snook, TX	Wild hosts	F0	31 May 2021	17.3
VA1	Suffolk, VA	Peanut	F0	6 June 2019	1.3
VA2	Suffolk, VA	Peanut	F0	31 May 2021	14.5

<sup>a</sup>F0 = Submitted field population, F=generation of rearing in lab.

on insecticide-free cabbage since 2012 at North Carolina State University. Mississippi State University also maintained a susceptible laboratory population that was originally sourced from the North Carolina State University colony. From 2018 to 2019, the susceptible colony maintained at Mississippi State University was tested in bioassays, and from 2020 to 2021, the susceptible colony maintained at North Carolina State University was tested.

Except for the Tennessee populations, thrips populations were prepared for shipping after collection by placing them in a sealable container with a fresh cabbage leaf or on the host from which the thrips were collected. A paper towel was sometimes placed in the container to reduce condensation. The thrips were shipped overnight to the West Tennessee Research and Education Center

in Jackson, Tennessee in insulated coolers with ice packs within one to two days after collection. Upon arrival, tobacco thrips were transferred into sealable rearing containers (T808160B and L808 Berry Plastics, Evansville, Indiana). Holes were cut in the lids and bottom of the rearing buckets and covered with thrips-proof gauze (100- $\mu$ m nylon mesh screen, Midwest Filter Corp., Highwood, IL) to allow ventilation. Populations were maintained on fresh cabbage leaves in an incubator at 27–29°C, 60–70% relative humidity, and 14:10 L:D (hours of Light:Dark). Before the cabbage leaves were placed in the bucket, they were sterilized by soaking them in a one percent sodium hypochlorite solution for one minute. Then the leaves were rinsed with water and patted dry with a paper towel.

### Discriminating Dose Bioassays

Generally, bioassays were performed on the submitted field populations in 2018, 2019, and 2021. In 2020, field-collected tobacco thrips were reared to the F1 generation. Rearing procedures were based on methods described by Darnell-Crumpton *et al.* (2018). Fresh cabbage leaves were placed in the buckets twice a week as a food source and oviposition substrate. Each time a new cabbage leaf was placed in the bucket, the old leaf was removed and placed in a new bucket. All the thrips were gently shaken off the leaf before being placed in the new bucket. The old buckets were discarded when only a few adults remained. When immature thrips hatched from the leaves, the leaves were replaced with a fresh leaf and allowed to grow to adults. Bioassays were done on the thrips approximately three days after adult eclosion. For the bioassays performed on the submitted field populations, thrips were kept in the incubator for three days after arrival before assays were performed to account for potential negative effects of shipping. The collection location, host, bioassay date, percent mortality in the water-check treatment, and generation the bioassays were done on for each population can be found in Table 1.

Five insecticides representing four classes were tested in the bioassays (Table 2), closely following the methods used by Huseeth *et al.* 2017 to document tobacco thrips resistance to neonicotinoids. Bioassay procedures are displayed in Fig. 1. The bioassay diet from 2018 to 2020 consisted of leaf disks made from field-collected cotton leaves that had not been treated with insecticide. The cotton leaves were rinsed with water and patted dry before leaf disks were made. In 2021, the leaf disks were made from fresh cabbage leaves and washed and dried similarly. A size five cork borer (Humboldt, Elgin, IL) was used to make the leaf disks that were the same diameter as the 1.5-ml microcentrifuge tube. The disks were treated with the appropriate insecticide by dipping them in the solution for one second and then allowed to dry. Fresh insecticide dilutions were mixed each day that bioassays were performed at a concentration to simulate a foliar application volume of 93.5 liter/ha (Table 2). No adjuvants were used, and a water-only check was included in each bioassay.

Eight thrips were aspirated into a microcentrifuge tube with a treated leaf disk, although this number occasionally varied between 6 and 10. Ten microcentrifuge tubes were used for each treatment, giving a total of approximately 80 thrips per insecticide dose. However, the total number of thrips tested varied depending upon the number of adults available. Also, some collections had too few individuals to test all insecticides, so only selected insecticides were tested. After 24 hr, the percent mortality was evaluated using a stereomicroscope. Thrips were classified as alive, dead, or moribund. Adults were considered moribund if they did not move greater than one body length when gently prodded. Moribund insects were considered as dead in the analysis. Abbott's formula was used to

correct the mortality for each insecticide treatment relative to the untreated control (Abbott 1925).

### Dose-Response Bioassays with Acephate

Dose-response bioassays were done with acephate (Orthene 97) for three unselected Tennessee field populations (TN4, TN7U, and TN9), another Tennessee field population that was selected with acephate (TN6S), and the NC laboratory colony (NCLab3). The selected (TN6S) and unselected (TN7U) thrips were from the same original collection. The selected Tennessee population was adults exposed for 24 hr to a cabbage leaf that was dipped into an acephate solution having 1.5 g of active ingredient/liter of water. After the exposure period, the surviving thrips were given a fresh untreated cabbage leaf and reared to the next generation so that a dose-response bioassay could be performed on the selected generation. This pre-selection was done to eliminate susceptible individuals from the field population to compare the estimated resistance level of a selected population to an unselected population.

For each bioassay, a stock solution was made with acephate (Orthene 97) and diluted to obtain the desired concentration. Three to five concentrations between 0.7 and 23.4 g of active ingredient/liter were tested on each field-collected population, and six concentrations ranging from 0.05 to 5.8 g/liter were tested on the North Carolina laboratory population (NCLab3) (Fig. 2). No adjuvants were used, and a water-only check was included in each bioassay. The procedures for making and treating the leaf disks, aspirating thrips into microcentrifuge tubes, and evaluating the percent mortality were the same as described for the discriminating dose bioassays. Abbott's formula was also used to correct each rate based on the check mortality (Abbott 1925).

### Standardized Foliar-Applied Insecticide Field Trials

From 2019 to 2021, standardized, replicated field trials were done to evaluate the efficacy of multiple insecticides on thrips and plant injury. The locations for the tests included Tillar and Marianna, AR; Stoneville (Delta Region) and Starkville (Hill Region), MS; Jackson and Milan, TN; and Snook, TX. All tests were arranged in a randomized complete block design with four-row plots (0.97–1.02 m row centers, 9.4–12.2 m long) and four replicates. Fungicide only treated seed was used. Foliar insecticide applications were made at the first true leaf stage. In a few tests, thrips pressure was so severe that a second application was made, allowing treatment effects to be more evident. Ratings of thrips injury and thrips density were made by cooperating researchers, and we selected data from the last ratings date to evaluate treatment effects because this was generally when treatment differences were most evident (Table 3). Treatments consisted of several classes of insecticides (Table 4). Application

**Table 2.** Insecticides used in discriminating dose bioassays, showing concentrations of product and active ingredient used in bioassays, and the equivalent rate of product used per hectare

Trade Name	Formulated Insecticide/liter	g Active/liter	Product/ha	IRAC Class <sup>a</sup>	Manufacturer
Orthene 97	Acephate, 3.00 g	2.92	0.280 kg	1B, Organophosphate	AMVAC (Los Angeles, CA)
Radiant	Spinetoram, 0.586 ml	0.070	0.055 liter	5, Spinosyn	Corteva Agriscience (Indianapolis, IN)
Bidrin	Dicrotophos, 1.56 ml	1.5	0.146 liter	1B, Organophosphate	AMVAC (Los Angeles, CA)
Warrior II	Lambda-cyhalothrin, 0.780 ml	0.195	0.073 liter	3A, Pyrethroid	Syngenta Crop Protection (Greensboro, NC)
Admire Pro	Imidacloprid, 0.980 ml	0.54	0.091 liter	4A, Neonicotinoid	Bayer CropScience (Raleigh, NC)

<sup>a</sup>Insecticide Resistance Action Committee (IRAC). <https://irac-online.org/>.

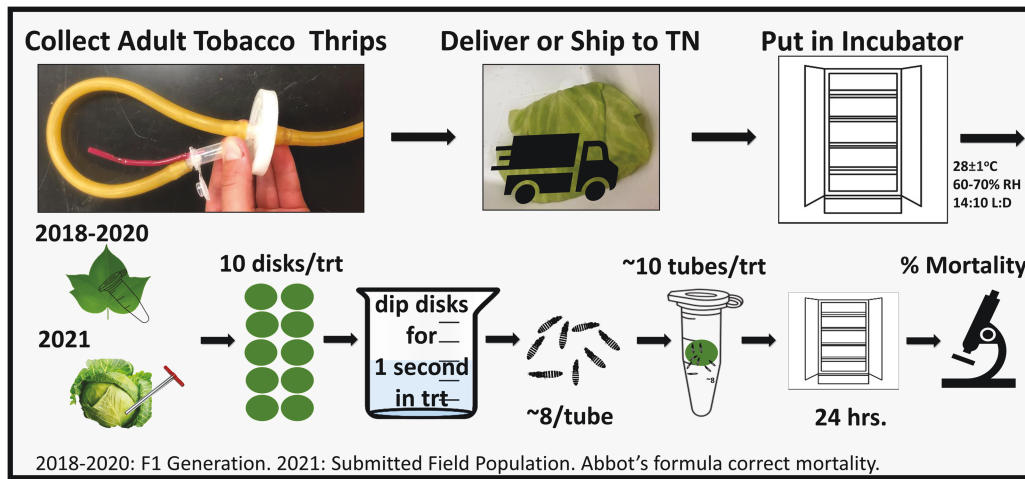


Fig. 1. Procedures used for bioassays.

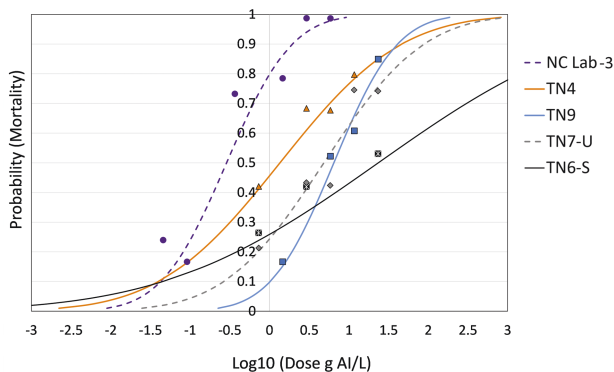


Fig. 2. Predicted 24-hr acephate (Orthene 97) mortality curves from probit analyses in grams of active ingredient (AI) per liter of three Tennessee tobacco thrips populations, an acephate-selected Tennessee population (TN6-S), and the North Carolina laboratory susceptible population. Uncorrected mean mortality data are represented by point markers.

parameters, such as nozzle type and spacing, varied slightly across test locations, but all applications were applied at a volume of 75–94 liter/ha. The percent reduction in thrips injury from insecticide treatment was calculated relative to the injury observed in untreated plots. Thrips injury from feeding on the epidermal and mesophyll cells can lead to tearing and twisting of the leaves, death of the apical meristem or whole plant, and cause the leaves to have a silvery appearance. Visual thrips injury ratings were made based on the condition of the plants in the whole plot, the plants in the plot were rated on a 0–5 scale, with 0 representing no injury and 5 representing extreme injury where almost all plants are dead (Kerns et al. 2019).

Five plants per plot were sampled, and an alcohol wash technique was used to estimate thrips numbers in these tests (Burris et al. 1989, Graham and Stewart 2018). The plants were cut below the cotyledons and placed in a container to be taken back to the laboratory. An alcohol wash solution was used to dislodge thrips from the plant, and a sieve (150  $\mu\text{m}$ ) was used to separate thrips from plant material. The sample was then placed under a stereomicroscope so thrips could be counted. The thrips were identified as either adults or immatures.

### Statistical Analysis

Data for the discriminating dose bioassays were analyzed using GLIMMIX procedures in SAS version 9.4 (SAS Institute 2021), to

perform a generalized linear mixed model analysis of variance. Each insecticide was analyzed separately across locations. Thrips population was treated as the fixed effect and year was random. Degrees of freedom were estimated using the containment method (SAS/STAT USER'S Guide 2019). Means were estimated using LSMEANS and separated using Tukey's significant difference test ( $\alpha = 0.05$ ). Thrips populations with a water-check mortality greater than 20% were excluded from the analysis. Dose-response bioassay data were analyzed in SAS using PROC PROBIT procedures with a log10 transformation to obtain  $LC_{50}$  values and 95% fiducial limits. Resistance ratios were calculated by dividing the  $LC_{50}$  of the field population by the laboratory susceptible colony from North Carolina. For the standardized field trials, the percent reduction in total thrips numbers, which was based on alcohol wash techniques, and injury was calculated for insecticide treatments relative to sample values in control plots that were not treated with insecticide. These data were analyzed using GLIMMIX procedures in SAS version 9.4 (SAS Institute 2021), to perform a generalized linear mixed model analysis of variance. The fixed effect was insecticide treatment, and the location was random. Degrees of freedom were estimated using the Satterthwaite's formula (Satterthwaite 1946). Means were estimated using LSMEANS and separated using Tukey's significant difference test ( $\alpha = 0.05$ ).

## Results

### Discriminating Dose Bioassays

The percent mortality of tobacco thrips for acephate differed significantly by population ( $F = 9.56$ ;  $df = 27, 237$ ;  $P < 0.001$ ; Table 5). Assays done on tobacco thrips from the susceptible lab colony, two Texas, one Virginia, and four North Carolina populations had mortalities over 90%. One Virginia, Louisiana, Alabama, Mississippi-Hill, and two North Carolina populations had mortalities that ranged from 82 to 89%. One Mississippi-Hill population had a lower mortality at 76%. Populations from Arkansas, Tennessee, and one Mississippi-Delta population had mortalities that ranged from 46 to 68%, with one Tennessee population having a higher mortality of 76%.

The mortalities differed for bioassays done with dicrotophos on four Tennessee populations (48–74%), one Virginia population (83%), and the susceptible lab colony (92%) ( $F = 5.13$ ;  $df = 5, 54$ ;  $P < 0.001$ ; Table 5). Significant differences across locations were also found in assays with imidacloprid ( $F = 22.67$ ;  $df = 6, 61$ ;  $P < 0.001$ ; Table 5). The Tennessee population had the lowest mortalities

ranging from 27 to 43%. One bioassay was done on a Virginia population with a mortality of 57%, and the susceptible laboratory colony thrips had mortalities of 94% and greater.

Percent mortality also varied among populations for bioassays with lambda-cyhalothrin ( $F = 217.09$ ;  $df = 8, 77$ ;  $P < 0.001$ ; Table 5). All the field populations had less than 5% mortality, except the Texas population (50%). The susceptible laboratory colony had mortalities of 78% and greater. Although there were differences across locations in the bioassays with spinetoram ( $F = 2.08$ ;  $df = 16, 140$ ;  $P = 0.013$ ; Table 5) mortality for all populations was greater than 96%.

### Dose-Response Bioassays with Acephate

$LC_{50}$  values, 95% fiducial limits, statistical parameters from the Probit analyses, and resistance ratios for acephate were determined for three Tennessee field populations and the North Carolina laboratory colony (Table 6). Dose-response curves and the raw means to create the curves are shown in Fig. 2. The North Carolina laboratory colony had the lowest  $LC_{50}$  (0.29 g of active ingredient/liter). The Pearson goodness-of-fit statistic was poor for this analysis ( $P < 0.001$ ); however, the raw data points show close agreement with the dose-response regression curve (Fig. 2). For the adults of the Tennessee population (TN6S) which were selected with acephate the generation before testing, the percent mortality observed at the highest dose tested (53% at 23.4 g of active ingredient/liter) was 80.7-fold greater than the North Carolina laboratory colony. The 2019 Jackson (TN4), 2019 Milan (TN7U), and 2021 Milan (TN9)

populations had  $LC_{50}$ 's that were 4.7, 16.7, and 22.3-fold greater than the  $LC_{50}$  of the North Carolina laboratory colony, respectively.

### Standardized Foliar-Applied Insecticide Field Trials

The percent reduction in thrips injury and thrips numbers were calculated for insecticide treatments relative to sample values in plots that did not receive an insecticide treatment. The percent reduction in thrips injury and thrips numbers provided by foliar insecticides, averaged across years, is shown for individual test locations, and the distribution of these averages across all locations is shown as box-and-whisker plots in Fig. 3. There were significant differences between treatments for thrips injury ( $P < 0.004$ ). The average percent decrease in thrips injury across all locations provided by foliar applications of acephate, dicotophos, dimethoate, and spinetoram ranged from 26 to 35% (Fig. 3). There was a greater suppression of thrips injury with acephate in one Starkville, MS test (61%). Lambda-cyhalothrin reduced thrips injury on average by less than 13%, but a greater suppression of thrips injury was observed in three Texas tests (42, 43, and 61%). The average reduction in thrips numbers from acephate, dicotophos, dimethoate, and spinetoram ranged from 47 to 66% (Fig. 3). On average, plots with lambda-cyhalothrin had significantly more thrips (11%) compared to the plots that did not receive an insecticide treatment ( $P < 0.001$ ). Spinetoram provided an average of 15–18% more control than the organophosphate treatments; however, differences among these treatments were not different.

**Table 3.** The field location, year, and the number of days after treatment (DAT) that thrips numbers and injury ratings were collected after an insecticide application

Location	Year	Thrips Numbers DAT-(number of applications)	Thrips Injury Ratings DAT-(number of applications)
Jackson, TN	2019	6-(1)	6-(1)
Jackson, TN	2020	3-(2)	9-(2)
Jackson, TN	2021	7-(1)	7-(1)
Milan, TN	2020	4-(2)	7-(2)
Milan, TN	2021	6-(2)	6-(2)
Snook, TX	2019	14-(1)	14-(1)
Snook, TX	2020	3-(2)	6-(2)
Snook, TX	2021	7-(2)	7-(2)
Starkville, MS	2019	6-(1)	14-(1)
Starkville, MS	2020	7-(2)	7-(2)
Starkville, MS	2021	6-(1)	6-(1)
Stoneville, MS	2019	14-(1)	14-(1)
Stoneville, MS	2021	7-(1)	7-(1)
Stoneville, MS	2020	13-(1)	13-(1)
Tillar, AR	2020	10-(1)	10-(1)
Tillar, AR	2021	13-(1)	13-(1)
Marrianna, AR	2020	13-(1)	13-(1)
Marrianna, AR	2021	13-(1)	13-(1)

### Discussion

Overall, the objective of this research was to use laboratory-based insecticide bioassays in conjunction with field trials to evaluate the efficacy of currently available foliar insecticides across different cotton production regions and assess insecticide resistance in populations of tobacco thrips. There was considerable interest in assessing the efficacy of acephate because it is the most common foliar insecticide for managing thrips, and the performance of acephate has declined in Tennessee (Stewart et al. 2020). Limitations in time and with the number and size of submitted tobacco thrips populations did not allow screening of populations against all insecticides. Most bioassays were done on field-collected populations of tobacco thrips, so it can be presumed that populations were potentially heterogeneous mixtures of susceptible and resistant thrips. Also, the 'health' of populations was potentially affected by the age of adults tested, the hosts from which they were collected, and handling, and this almost certainly introduced additional variability into the results for the assays done on the submitted field populations that were not kept in the colony. However, there was clear evidence that insecticide resistance has developed in some populations of tobacco thrips, particularly in the upper Mid-South.

When averaging mortality from the discriminating dose bioassays for locations across years, there was reduced efficacy for acephate in field populations collected from Arkansas, Tennessee, and

**Table 4.** Insecticide treatments for regional thrips foliar-applied insecticide field trials

Trade Name	Formulated Insecticide/ha	kg Active/ha	IRAC Class	Manufacturer
Orthene 97	Acephate, 0.235 kg	0.228	1B, Organophosphate	AMVAC (Los Angeles, CA)
Radiant	Spinetoram, 0.110 liter	0.013	5, Spinosyn	Corteva Agriscience (Indianapolis, IN)
Bidrin 8E	Dicotophos, 0.234 liter	0.224	1B, Organophosphate	AMVAC (Los Angeles, CA)
Karate Z	Lambda-cyhalothrin, 0.094 liter	0.023	3A, Pyrethroid	Syngenta Crop Protection (Greensboro, NC)
Dimethoate 4EC	Dimethoate, 0.468 liter	0.224	1B, Organophosphate	Drexel Chemical Company (Memphis, TN)

**Table 5.** Effect of location on percent mortality for discriminating dose bioassays. See Table 2 for insecticides and rates used

Population <sup>b</sup>	Percent Average Mortality ± SE <sup>a</sup> in Leaf-Dip Bioassays				
	Acephate	Dicrotophos	Imidacloprid	Lambda-cyhalothrin	Spinetoram
MSLab1	97.3 ± 5.3a	.	.	.	.
MSLab2	94.0 ± 5.9ab	.	.	.	.
NCLab1	92.4 ± 5.3ab	92.1 ± 7.3a	98.7 ± 5.9a	98.7 ± 2.6a	100.0 ± 0.81ab
NCLab2	90.7 ± 5.3ab	.	93.8 ± 6.2a	78.0 ± 2.6b	.
TX1	100.0 ± 6.3a	.	.	.	.
TX2	93.2 ± 5.3ab	.	.	49.6 ± 3.7c	100.0 ± 0.81ab
NC6	96.7 ± 5.9ab	.	.	.	100.0 ± 1.04ab
NC4	96.5 ± 5.3ab	.	.	.	.
NC8	94.0 ± 5.0ab	.	.	4.3 ± 2.5d	100.0 ± 0.77a
NC7	92.6 ± 5.5ab	.	.	.	.
NC2	83.5 ± 6.3a-d	.	.	.	100.0 ± 0.96ab
NC1	83.3 ± 6.3a-d	.	.	.	100.0 ± 0.96a
VA1	96.6 ± 5.3ab	.	.	.	100.0 ± 0.81ab
VA2	82.3 ± 3.7a-d	82.7 ± 7.3ab	56.6 ± 5.9b	4.7 ± 2.6d	100.0 ± 0.81a
LA1	89.4 ± 6.3abc	.	.	.	.
AL1	87.7 ± 6.8a-d	.	.	.	.
MS1	83.3 ± 5.3a-d	.	.	.	100.0 ± 0.81ab
MS4	75.7 ± 5.9a-f	.	.	.	.
MS2	68.3 ± 5.3b-f	.	.	.	100.0 ± 0.81a
AR3	68.1 ± 5.5b-f	.	.	.	.
AR1	57.1 ± 5.3def	.	.	.	.
TN2	75.9 ± 5.3a-e	.	.	.	95.5 ± 0.85b
TN12	67.5 ± 5.3b-f	64.1 ± 7.3abc	42.7 ± 5.9bc	1.8 ± 2.6d	100.0 ± 0.81ab
TN5	59.0 ± 5.3c-f	.	.	.	98.7 ± 0.81ab
TN3	58.9 ± 5.3c-f	.	.	.	98.3 ± 0.81ab
TN11	51.0 ± 5.3ef	47.8 ± 7.3c	26.9 ± 6.2c	0.00 ± 2.6d	100.0 ± 0.81ab
TN13	47.0 ± 5.3f	74.1 ± 7.3abc	42.5 ± 5.9bc	2.8 ± 2.6d	100.0 ± 0.81ab
TN8	46.0 ± 5.9f	.	.	.	100.0 ± 0.96ab
TN10	.	.	34.5 ± 5.9bc	0.00 ± 2.6d	.
TN4	.	56.2 ± 7.3bc	.	.	.

<sup>a</sup>Standard error of the mean for pooled treatment effects.

<sup>b</sup>The collection location, host, bioassay date, percent mortality in the water-check treatment, and generation the bioassays were done on for each population can be found in Table 1.

Means within a column followed by a common letter are not significantly different (Tukey's significant difference test  $\alpha = 0.05$ ).

**Table 6.** LC<sub>50</sub> values (g active ingredient/liter) for acephate with 95% fiducial limits, slope, X<sup>2</sup> goodness of fit for the probit lines, and resistance ratios from three Tennessee field populations, a Tennessee population that was pre-selected with acephate, and the North Carolina laboratory susceptible population

Population	Assay Date	N <sup>a</sup>	Slope (Log10 Dose)	LC <sub>50</sub> (95% FL) <sup>b</sup>	X <sup>2c</sup>	df	P > X <sup>2d</sup>	RR (95% FL) <sup>e</sup>
NCLab3	21 Dec. 2021	462	1.54 ± 0.17	0.29 (0.20, 0.40)	128.6	58	<0.001	–
TN4	4 June 2019	258	0.84 ± 0.18	1.36 (0.59, 2.17)	49.28	38	0.104	4.69 (3.92, 4.73)
TN7U	26 June 2019	293	1.01 ± 0.16	4.85(3.37, 6.91)	40.81	38	0.348	16.72 (15.24, 17.30)
TN9	21 July 2021	279	1.59 ± 0.24	6.47 (4.70, 8.55)	48.69	36	0.077	22.31 (20.54, 22.62)
TN6S <sup>f</sup>	26 June 2019	152	0.47 ± 0.28	23.50*	50.83	19	<0.001	81.03 <sup>f</sup>

<sup>a</sup>Total number of tobacco thrips assayed.

<sup>b</sup>LC<sub>50</sub> reported in grams of product per liter. Mortality was calculated based on the number of dead and moribund thrips.

<sup>c</sup>Pearson Chi-Square Goodness-of-fit Statistic.

<sup>d</sup>Pearson Chi-Square Goodness-of-fit Statistic  $P > X^2$  (poor fit with  $P < 0.10$ ).

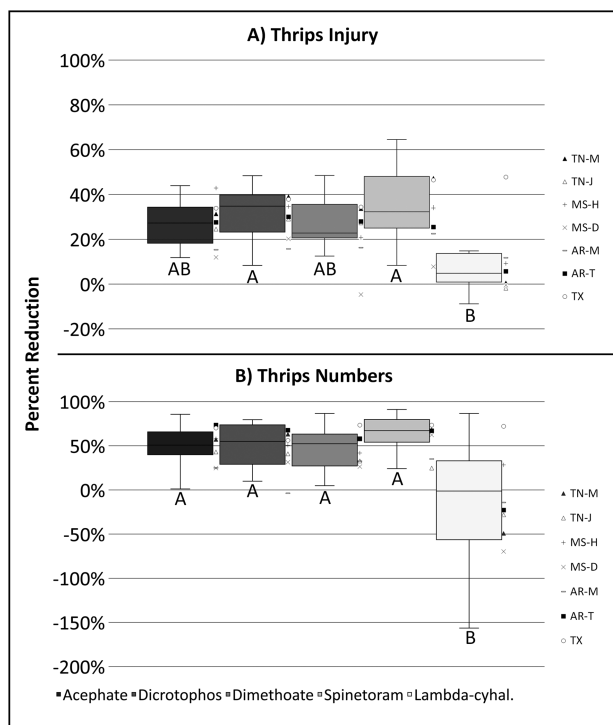
<sup>e</sup>Resistance ratios were calculated by dividing the LC<sub>50</sub> for each field colony by the NC laboratory colony.

<sup>f</sup>Tennessee population selected at a rate of 1.5 g of active ingredient/liter for 24 hr. Probit fit was not significant.

Mississippi (Stoneville) ( $\leq 76\%$  mortality), and in some populations, less than 50% mortality was observed (Table 5). In comparison, field populations from Alabama, Louisiana, Virginia, and North Carolina were more susceptible ( $> 82\%$  mortality). Resistance ratios for acephate for three representative populations from Tennessee, relative to a known susceptible population, ranged from 4.7 to 22.3 (Table 6). A higher resistance ratio ( $\approx 80$ ) was indicated for the Tennessee population that had been selected with acephate, indicating that this resistance is heritable.

In bioassays, there was a trend of lower mortality with dicrotophos when acephate also caused low mortality. Across locations, the reduction in thrips injury and thrips density caused by dimethoate in field trials were the same to those caused by acephate and dicrotophos. These observations suggest that tobacco thrips have developed cross resistance among organophosphate insecticides (Fig. 3). However, linear regression of mortality for the five populations that were tested with both acephate and dicrotophos did not show a significant correlation ( $F = 4.05$ ;  $df = 1, 4$ ;  $P = 0.138$ ;  $R^2 = 0.575$ ).





**Fig. 3.** Distributions (box and whisker plots) of the average percent reduction of foliar insecticides based on thrips injury ratings (A) and total numbers of thrips (B) observed in all foliar insecticide tests. The boxes show the inclusive interquartile range, with the top and bottom of the box representing the upper and lower quartiles. The whiskers at the end show the highest and lowest values. Common letters above plots indicate mean values are not different (Tukey's significant difference test,  $\alpha = 0.05$ ). The points represent the average percent reduction in thrips injury (top) and percent reduction of thrips numbers (bottom) observed in foliar insecticides tests by location. The percent reduction in thrips injury and thrips numbers were calculated for insecticide treatments relative to sample values in plots that did not receive an insecticide treatment. Foliar insecticide test locations included: TN-M and TN-J (2 Trials Milan and 3 trials Jackson), MS-H and MS-D [2 trials each in Hills (Starkville) and Delta (Stoneville)], AR-M and AR-T (2 trials each in Marianna and Tillar), and TX (3 trials). See Table 3 for insecticides and rates used.

Further testing is needed to validate the heritability of acephate or organophosphate resistance. Additionally, research is necessary to identify the mechanism of resistance. We speculate this resistance is likely metabolic in nature, partly because this resistance appears to be moderate and also because resistance to diazinon, an organophosphate, has been documented in western flower thrips (Zhao et al. 1994, 1995), and the mechanism of this resistance was either by metabolic detoxification or by a combination of metabolic detoxification and alteration of the acetylcholinesterase target site.

Our discriminating dose assay results showed reduced efficacy to imidacloprid in tobacco thrips populations relative to the susceptible population from North Carolina (Table 5). These results are congruent with findings by Huseth et al. (2016, 2018) and Darnell-Crumpton et al. (2018), which document the widespread occurrence of tobacco thrips resistance to neonicotinoids. The agreement of imidacloprid resistance between these studies validates the assay method, which supports the above conclusion that resistance to acephate has also developed.

Lambda-cyhalothrin provides poor thrips control (Toews et al. 2012, Cook et al. 2020), and pyrethroid insecticides are not recommended to manage tobacco thrips in cotton (Catchot 2020, Greene 2020). Therefore, the low mortalities in bioassays of field

populations from Tennessee, Mississippi (Stoneville), and Virginia were expected. The Texas population was more susceptible to lambda-cyhalothrin (Table 5), and this is consistent with the better control provided lambda-cyhalothrin in the field study (Fig. 3) and also consistent with previous observations in that geography (D. Kerns, personal observation). The mortality for the North Carolina laboratory colony was substantially higher than the field-collected populations. This suggests that tobacco thrips have evolved resistance to pyrethroid insecticides over a wide geography in the South, and presumably may be the first insecticide class to which tobacco thrips developed resistance. In fact, past field trials in the south in cotton showed that pyrethroids significantly reduced thrips numbers compared to untreated plots (Micinski 1984, Fitt and Teetes 1986, Ratchford et al. 1987, Reed and Grant 1987).

All thrips populations experienced high mortality in the bioassays with spinetoram (Table 5). Indeed, spinetoram was considered a positive control that was included, in part, to validate the quality of our assays. Tobacco thrips resistance to this class of insecticides was not expected because this class of insecticides, and spinetoram in particular, is relatively new and historically has not been used widely in field crops or for thrips control. Bioassay results would have predicted that field control with spinetoram would be markedly better than the other insecticides. Although spinetoram numerically reduced thrips numbers and injury more than other insecticide treatments, differences among insecticides were not statistically significant with the exception of lambda-cyhalothrin (Fig. 3). In general, when compared to bioassay results, there was less variation in the performance of foliar insecticide in the field trials performed from 2018 to 2021. The notable exception, as referenced above, was the better performance of lambda-cyhalothrin at the Texas location. Also, for all insecticide applications, the reduction of thrips injury, in particular, but also thrips density was not dramatic following the insecticide applications (Fig. 3). This is partly due to the migratory nature of tobacco thrips where re-infestation often occurs quickly (Layton and Reed 2014). Under continuous pest pressure, even more efficacious insecticides may not perform obviously better unless they provide longer residual control. Secondly, because preventative insecticide treatments were not used, the injury observed after foliar applications was almost certainly affected by thrips feeding that occurred before the applications.

Even when foliar insecticides were applied, substantial levels of thrips injury were still observed in some tests. While plants often compensate for early-season thrips injury, crop maturity can be delayed when growing conditions are not optimal and/or thrips densities are high, and yield may be negatively impacted (Cook et al. 2011). Delayed crop maturity can lead to the need for additional inputs such as insecticides and harvest aids, which increases production costs (Freeland et al. 2004, Parvin et al. 2005, Cook et al. 2011). Thus, an at-planting insecticide treatment for thrips is typically recommended in most regions of the southern U.S., with recommendations for making supplemental foliar applications in some circumstances. Reduced insecticide efficacy affects these recommendations.

Collectively, this study documented that resistance to acephate, and likely other organophosphate insecticides, has developed in tobacco thrips. This resistance appears primarily localized to the upper Mid-South, including West Tennessee and parts of the Delta cotton-growing areas of northern Mississippi and Arkansas (Table 5). Recent bioassays conducted on tobacco thrips populations collected in 2022 indicate that acephate and dicrotophos resistance has become more widely apparent in the Mid-South (S. Brown, unpublished data). Where resistance occurs, rotation to alternative

chemistries such as spinetoram may be justified. However, more monitoring is needed to better define the geographic variability of resistance. The need for foliar-applied insecticide applications for thrips management is expected to be minimal with the introduction of ThryvOn (Bayer CropScience) because this new Bt trait provides substantial protection against thrips (Bachman et al. 2017, Graham and Stewart 2018, Akbar et al. 2019). However, it will be several years before cotton varieties with ThryvOn become widely planted, and the rate of adoption will be influenced by the cost of the technology. Thus, continued awareness and management of insecticide resistance in tobacco thrips are important.

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