



## Field screening of wild cotton, *Gossypium hirsutum*, landraces for resistance to thrips (Thysanoptera: Thripidae)

Sophia R. Conzemius<sup>a</sup>, Francis P.F. Reay-Jones<sup>b,\*</sup>, Jeremy K. Greene<sup>a</sup>, B. Todd Campbell<sup>c</sup>, Dominic D. Reisig<sup>d</sup>, Hehe Wang<sup>a</sup>, William C. Bridges<sup>e</sup>

<sup>a</sup> Department of Plant and Environmental Sciences, Clemson University, Edisto Research and Education Center, Blackville, SC, USA

<sup>b</sup> Department of Plant and Environmental Sciences, Clemson University, Pee Dee Research and Education Center, Florence, SC, USA

<sup>c</sup> Coastal Plain Soil, Water and Plant Conservation Research, USDA-ARS, Florence, SC, USA

<sup>d</sup> Department of Entomology and Plant Pathology, North Carolina State University, Vernon G. James Research and Extension Center, Plymouth, NC, USA

<sup>e</sup> Department of Experimental Statistics, Clemson University, Clemson, SC, USA

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### ABSTRACT

Thrips (Thysanoptera) are the most prevalent early season pests of cotton and are often detrimental to the establishing crop. Heavy reliance on prophylactic insecticides, primarily used as seed treatments, has led to insecticide resistance in populations of *Frankliniella fusca* Hinds. Sustainable, alternative management tactics are needed to protect seedling cotton from thrips. We evaluated day-neutral, exotic landraces (N = 164) of Upland cotton (*Gossypium hirsutum* L.) for thrips-resistance traits in three field trials using insecticide-treated and -untreated plots. The cotton landraces were chosen for their diverse backgrounds, unique genotypes, and day-neutrality, allowing them to easily cross with USA breeding lines. The trials were conducted at the North Carolina State University Sandhills Research Station in Jackson Springs, NC (NC2018), and the Clemson University Pee Dee Research and Education Center in Florence, SC (SC2018 and SC2020). A selection index was created to quantify thrips resistance based on densities of thrips and ratings of injury by thrips at the first and third true-leaf stages in untreated plots, and genotypic differences in above-ground dry biomass between treated and untreated plots at 42 days after planting. Subsamples of adult thrips were collected for species identification. Dominant thrips species were *F. fusca* in NC2018 and SC2020 and *Frankliniella tritici* in SC2018. Eight putatively thrips-resistant genotypes (TX-1109, TX-1975, TX-2320, TX-2383, TX-101, TX-2347, TX-2362, TX-251) and four putatively thrips-susceptible genotypes (TX-203, TX-1212, TX-1094, TX-2403-2) were identified in the field trials. Further investigation of the putatively thrips-resistant and -susceptible genotypes should focus on mechanisms of thrips resistance to enhance future breeding efforts.

Thrips (Thysanoptera: Thripidae) are consistent early season pests of Upland cotton (*Gossypium hirsutum* L.) throughout the southeastern USA. Injury symptoms from thrips include a silvery appearance of emptied plant cells, malformation of leaves, stunting, delayed maturity, or death of seedlings (Gaines 1934; Telford and Hopkins 1957; Reed and Reincke 1990). The complex of thrips that feeds on cotton is comprised of five main species in the USA: *Frankliniella fusca* Hinds (tobacco thrips), *Frankliniella occidentalis* Pergande (western flower thrips), *Frankliniella tritici* Fitch (flower thrips), *Thrips tabaci* Lindeman (onion thrips), and *Neohydatothrips variabilis* Beach (soybean thrips) (Wang et al., 2018). In the southeastern USA, *F. fusca* is the dominant thrips species (85%) on

seedling cotton (Reay-Jones et al., 2017).

Upland cotton is the main *Gossypium* species grown in the USA, accounting for 97% of production (USDA ERS, 2020). *Gossypium barbadense* L. accounts for the remainder of USA cotton production. An estimated 56–96% of the total Upland cotton acreage in the USA is infested with thrips annually (Cook et al., 2011; Allen et al., 2018; Cook and Cutts 2019; Cook and Threet 2020). In 2020, management and yield loss from thrips cost USA cotton producers \$70.7 million (Cook and Threet 2021). Upland cotton is generally considered highly susceptible to thrips (Zhang et al., 2013). Currently, no commercially grown cotton cultivars are resistant to thrips (Cook et al., 2011; Zhang et al., 2013;

\* Corresponding author. Department of Plant and Environmental Sciences, Clemson University, Pee Dee Research and Education Center, 2200 Pocket Road, Florence, SC, 29506-9727, USA.

E-mail address: [freayjo@clemson.edu](mailto:freayjo@clemson.edu) (F.P.F. Reay-Jones).

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Bachman et al., 2017, USDA AMS 2020). The *Bacillus thuringiensis* (Bt) toxin Cry51Aa2.834\_16, expressed in transgenic cotton with event MON 88702, has demonstrated some thrips resistance (Akbar et al., 2018; Graham et al., 2019; D'Ambrosio et al., 2020a) and is pending foreign regulatory approvals for global commercialization (Bayer 2021). Common strategies for managing thrips in cotton currently include using prophylactic insecticides delivered as seed treatments or separate in-furrow applications (Jones et al., 2017a, b), reactive sprays of post-emergence insecticides when systemic, at-plant materials lose efficacy, and targeting control strategies using prediction models with phenological and site-specific weather data (i.e., Thrips Infestation Predictor for Cotton; Kennedy et al., 2019; Chappell et al., 2020). Heavy reliance on seed treatments has led to the development of neonicotinoid-resistant populations of the key seedling pest, *F. fusca*, throughout the mid-southern and southeastern USA (Huseth et al., 2016). Alternate control tactics are needed to reduce reliance on insecticides, particularly given the decline in efficacy of neonicotinoid insecticides on *F. fusca*. Cultural control, in the form of adjusting planting date (Kerns et al., 2019) to avoid peak risk of injury from thrips using the aforementioned online tool, could be useful, as would any identified host plant resistance incorporated into commercial varieties.

Heritable plant characteristics which influence thrips damage on cotton are classified as host plant resistance traits. Much of the progress to describe resistance in cotton to thrips has been made for *T. tabaci* (e.g., Abdel-Gawaad et al., 1973; Sadras and Wilson 1998; Arif et al., 2006; Khalil et al., 2015) and *F. occidentalis* (e.g., Chen et al., 2006; Zhang et al., 2014a; Wann et al., 2017a; Abdelraheem et al., 2021). Morpho-physical traits, such as high density of leaf hairs (Ramey 1962; Rummel and Quisenberry 1979; Zareh 1985; Naveed et al., 2011), low density of leaf hairs (Leigh 1995), or thicker cuticle (Wann et al., 2017b), have been associated with reduced numbers of thrips. Chemical traits, including cotton with (Bourland and Benson, 2002) and without gossypol glands (Zareh 1985; Zhang et al., 2014a), have also been associated with lower numbers of thrips. Genes *Thr* (Zhang et al., 2013) and *Thr2* (Wann et al., 2017a) from *G. barbadense* are thought to confer thrips resistance. Other cotton species thought to be more resistant to thrips include *G. barbadense*, *G. darwinii* Watt, *G. mustelinum* Miers, *G. tomentosum* Seem, *G. arboreum* L., *G. thurberi* Tod., and *G. trilobum* Sessé & Moc. (Zhang et al., 2013; Miyazaki et al., 2017; Wann et al., 2017a).

Although studies continue to reveal the complexity of thrips resistance in *Gossypium*, little is known if such traits are effective with *F. fusca*. Moreover, introgressing specific traits such as thrips resistance from other *Gossypium* species into Upland cotton can be difficult (Saha et al., 2012; Zhang et al., 2014b). Photoperiod sensitivity occurs in many *Gossypium* species and poses significant challenges for breeders, often requiring remote nurseries with conducive photoperiods for floral initiation to cross wild species with day-neutral breeding lines (Kushanov et al., 2017; McCarty et al., 2018). Even after successful crosses, offspring may exhibit adverse effects from wild parents or yield drag (Ganesh Ram et al., 2008; Trapero et al., 2016; Campbell et al., 2018). Campbell et al. (2019) identified naturally occurring, day-neutral, exotic landraces of *G. hirsutum* that could provide broad genetic diversity while maintaining easier crosses with breeding lines. Further exploration within Upland cotton diversity could provide the thrips resistance needed for accelerated breeding efforts, reducing injury to the crop while complementing and preserving other plant protectants. Thus, the objective of this study was to evaluate wild Upland cotton landraces as potential sources of thrips resistance for accelerated cotton breeding efforts.

## 1. Materials and methods

**Cotton Genotypes.** Previously, Campbell et al. (2019) identified approximately 200 day-neutral, wild landrace accessions of *G. hirsutum* originally obtained from the USDA Cotton Germplasm Collection in

College Station, TX. The landraces were purified, hereafter referred to as genotypes, through 2–3 generations of self-pollination and seed increase. Based upon their geographical origins across five continents, the day-neutral genotypes represent a wide range of geographical diversity (Fig. 1). In this study, 164 of the 200 day-neutral genotypes with sufficient seed amounts were evaluated for thrips resistance in the field. As a basis of comparison, field trials also contained two commercial cultivars, ST 4946GLB2 and ST 6182GLT (BASF, Research Triangle Park, NC), and two breeding lines with partial thrips resistance, CA 4005 and CA 4006 (Texas A&M University, College Station, TX; Wann et al., 2017b).

**Field Experiments.** Field evaluations were performed at the North Carolina State University Sandhills Research Station in Jackson Springs, NC, in 2018 (i.e., 'NC2018') and the Clemson University Pee Dee Research and Education Center in Florence, SC, in 2018 and 2020 (i.e., 'SC2018' and 'SC2020'), for a total of three field trials. Planting dates were adjusted to reflect the predicted maximum relative risk of injury by thrips with the recommended planting window for cotton according to the Thrips Infestation Predictor for Cotton (Kennedy et al., 2019). Cotton was planted on 2 May for NC2018, 9 May for SC2018, and 28 April for SC2020. Experimental design was an alpha-lattice split-plot design, with the main plot as treatment (insecticide-treated or -untreated) and the subplot as plant genotype. There were three replications, with each replication consisting of 14 incomplete blocks of size 12 used to account for variation within the replicate. This resulted in 168 plots per replicate and a total of 1,008 plots in each field trial experiment. Genotypes were planted at a rate of three seeds per 0.30 m in single-row plots 10.7 m in length and a row spacing of 0.71 m. All seeds were treated with fungicides; in the insecticide-treated plots, seeds were additionally coated with 0.375 mg AI imidacloprid and 0.375 mg AI thiodicarb per seed (Aeris®, Bayer CropScience LP, St. Louis, MO) before planting, and seedlings were foliar sprayed with 210 g ha<sup>-1</sup> of acephate (Orthene® 97, AMVAC® Chemical Corp., Los Angeles, CA) on 15 May for NC2018, 24 May for SC2018, and 12 May for SC2020 between the cotyledon and first true-leaf stage (TLS) before data collection.

**Data Collection.** Seven variables (i.e., combinations of measurements and sampling dates) were measured in the field-trials to assess each genotype's response to natural populations of thrips. Ratings of injury by thrips and counts of adult and immature thrips were evaluated in the untreated plots at both the 1st TLS (~21 days after planting, DAP) and 3rd TLS (~28 DAP). Above-ground dry biomass was measured in both the untreated and treated plots at 42 DAP in order to use the difference in biomass between treated and untreated plots as a measure of tolerance. A total of 21 variables were measured over the sampling date and field trial combinations.

Plots were visually rated on a per plot basis for thrips-related plant injury (Kerns et al., 2019). For counts of thrips, five seedlings from each plot were randomly sampled at the 1st and 3rd TLS. Seedlings were pulled by hand, submerged shoot side down into 1-L jars with 70% isopropyl alcohol, and vigorously shaken to dislodge thrips. Methods for counting the dislodged thrips were conducted in the laboratory based on Kerns et al. (2019). Briefly, the jars of solution were poured over filter paper into a Buchner funnel attached to filtration flasks and an electric pump. After the solution filtered, numbers of adult and immature thrips on the filter paper were recorded using a dissecting microscope, and a subsample of up to 20 adult thrips from these untreated samples for each genotype was retained to determine composition of species (see Thrips Species Identification section). At 42 DAP, above-ground dry biomass data were collected from five randomly selected plants per treated and untreated plot, cutting the main stems at ground level, drying the five-plant sample in ovens at 60 °C for at least four days, and weighing the composite sample.

**Thrips Species Identification.** Thrips species were identified using a dichotomous key for 2018 collections (Palmer et al., 1992) combined over leaf stages. In 2020, species composition was determined for collections of thrips at both leaf stages using probe-based qPCR. With species-specific primers and probes, the qPCR procedure, described by



**Fig. 1.** Origins of the exotic, naturally day-neutral landraces of *G. hirsutum* (164 unique genotypes) used in our field study to screen for host plant resistance traits to thrips. The genotypes were maintained at the U.S. National Plant Germplasm System. Numbers of genotypes identified as putatively resistant (R) or susceptible (S) are indicated in parenthesis. Gray-scale used to indicate relative number of genotypes from a particular country of origin.

Wang et al. (2018), was used to identify the five most common species of thrips in the field trials. The positive results from the qPCR assay were used to represent the species composition in the whole population of thrips. Samples that were not resolved by qPCR were run in 2% agarose gel to examine the presence or absence of PCR products.

**Data Analyses.** Each variable [i.e., ratings of thrips-related plant injury (1), counts of immature (2) and adult (3) thrips at the 1st TLS; ratings of thrips injury (4), counts of immature (5) and adult (6) thrips at the 3rd TLS; and above-ground biomass (7) at 42 DAP] was analyzed separately for each of the three field trials. To assess thrips resistance (e.g., tolerance), biomass from a genotype's untreated (U) plot was subtracted from the biomass of its respective treated (T) plot (i.e., biomass T - biomass U). The plots with a negative value, i.e., failure of insecticidal management or lack of an impact of insecticide on biomass, were given a value of '0' before analysis. A statistical model was developed for each field trial (NC2018, SC2018, SC2020) that included the fixed effect of genotype and the random effects of replicate and incomplete block nested within replicate. The model was used to estimate the least squares mean of each genotype, for all variables, for each field trial. Using K-means clustering, three clusters of genotypes were created based on the least square means. The basic purpose of the clustering was to create groups or clusters of genotypes to maximize differences in least square means among clusters and minimize differences within clusters. All statistical calculations were performed using JMP Pro 16.0.0 (SAS Institute Inc.® 2021).

A selection index was created to quantify each genotypes' relative vulnerability to thrips to determine putative thrips resistance that combined information across all variables and field trials. Within each variable and field trial combination, scores of '+1' (resistant), '0' (intermediate), and '-1' (susceptible) were assigned to genotypes based on the clustering. The NC2018 1st TLS rating of injury by thrips will be used as an example to illustrate how the scores were assigned. The overall mean rating of injury for genotypes in each cluster was calculated (Table 1). Genotypes in the cluster with the smallest mean rating of injury (i.e., the desirable outcome) were assigned a score of '+1'. Genotypes in the cluster with the greatest mean rating of injury (i.e., undesirable outcome) were assigned a score of '-1'. Genotypes in the

cluster with the intermediate mean rating of injury were assigned a score of '0'. This score assignment process was repeated for all 21 combinations of variables and field trials. Following, the scores for the 21 variables were summed to create an index value for each genotype that could range from '+21' to '-21' to represent the genotypic variation across our trials and were used to determine putatively resistant (higher scoring) and susceptible (lower scoring) genotypes.

## 2. Results

**Thrips Species Composition.** In NC2018, thrips species (N = 2,947) were comprised of *F. fusca* (75.5%), *T. tabaci* (21.8%), *N. variabilis* (1.7%), *F. tritici* (0.9%), and *F. occidentalis* (0.1%) (Fig. 2A). In SC2018 (N = 1,154), *F. tritici* (58.9%) was the dominant species, followed by *F. fusca* (38.4%), *N. variabilis* (1.3%), *T. tabaci* (1.2%), and other species (0.2%). In 2020, of the 6,101 samples assessed using the probe-based qPCR assay, a single species was assigned to 5,524 of the samples (Fig. 2B; Supp. Table S1). Results of the remaining 577 samples were inconclusive and were therefore not included in the results. At the 1st TLS in SC2020, thrips (N = 2,659) consisted of *F. fusca* (96.9%), *F. tritici* (1.3%), *N. variabilis* (0.8%), *T. tabaci* (0.8%), and *F. occidentalis* (0.2%). At the 3rd TLS in SC2020, thrips (N = 2,865) consisted of *F. fusca* (93.8%), *F. tritici* (5.4%), *T. tabaci* (0.4%), *N. variabilis* (0.3%), and *F. occidentalis* (0.1%).

**Thrips Injury.** Ratings of injury by thrips at the 1st TLS among untreated genotypes ranged from  $2.36 \pm 0.16$  (least square mean  $\pm$  standard error) (TX-1870) to  $3.40 \pm 0.16$  (TX-2402) in NC2018,  $2.00 \pm 0.03$  (all other genotypes) to  $2.33 \pm 0.03$  (TX-206) in SC2018, and  $0.71 \pm 0.24$  (TX-1300-1 and TX-1115) to  $1.86 \pm 0.24$  (TX-2308) in SC2020 (Fig. 3A). Ratings of injury by thrips at the 3rd TLS among untreated genotypes ranged from  $2.67 \pm 0.10$  (TX-1870) to  $3.31 \pm 0.10$  (TX-1425-1) in NC2018,  $2.18 \pm 0.29$  (TX-2352) to  $3.64 \pm 0.29$  (TX-1311) in SC2018, and  $2.98 \pm 0.26$  (TX-1233) to  $4.21 \pm 0.26$  (TX-691-1) in SC2020 (Fig. 3A).

**Thrips Counts.** Counts of immature thrips per five untreated plants at the 1st TLS ranged among genotypes from  $17.09 \pm 14.28$  (TX-202) to  $122.15 \pm 14.31$  (TX-1147) in NC2018,  $0.73 \pm 5.22$  (TX-2144) to  $32.61$

**Table 1**

Selection index scoring based on three clusters of genotypes per response variable based on least squares means. Genotypes were grouped into one of three resistance categories and scored accordingly. The groups are shown below with K-cluster means  $\pm$  standard deviations (n). Genotypes with the lowest (i.e., desirable outcome) means were labeled as resistant for the respective variable and received a score of +1, intermediate scored 0, and genotypes with the highest means (i.e., undesirable outcomes) were labeled susceptible (-1).

| Trial                  | Variable               | K-cluster Means $\pm$ SD |                          |                           |                          |
|------------------------|------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
|                        |                        | Resistant                | Intermediate             | Susceptible               |                          |
|                        |                        | "+1"                     | "0"                      | "-1"                      |                          |
| NC2018                 | Adults 1 TLS           | 4.18 $\pm$ 0.82<br>(50)  | 7.06 $\pm$ 0.95<br>(85)  | 10.60 $\pm$ 1.46<br>(33)  |                          |
|                        | Adults 3 TLS           | 2.57 $\pm$ 0.87<br>(49)  | 5.03 $\pm$ 0.77<br>(68)  | 8.74 $\pm$ 1.41<br>(51)   |                          |
|                        | Immatures 1<br>TLS     | 40.11 $\pm$ 6.69<br>(80) | 59.28 $\pm$ 5.77<br>(69) | 83.23 $\pm$ 11.28<br>(19) |                          |
|                        | Immatures 3<br>TLS     | 22.43 $\pm$ 4.64<br>(52) | 33.88 $\pm$ 3.59<br>(71) | 47.29 $\pm$ 4.73<br>(45)  |                          |
|                        | Thrips Injury 1<br>TLS | 2.67 $\pm$ 0.10<br>(30)  | 2.98 $\pm$ 0.06<br>(107) | 3.20 $\pm$ 0.06<br>(31)   |                          |
|                        | Thrips Injury 3<br>TLS | 2.88 $\pm$ 0.06<br>(50)  | 3.02 $\pm$ 0.03<br>(95)  | 3.18 $\pm$ 0.05<br>(23)   |                          |
|                        | Biomass<br>Tolerance   | 5.35 $\pm$ 2.37<br>(1)   | 11.56 $\pm$ 1.93<br>(83) | 19.47 $\pm$ 3.29<br>(84)  |                          |
|                        | SC2018                 | Adults 1 TLS             | 0.14 $\pm$ 0.17<br>(134) | 0.86 $\pm$ 0.29<br>(33)   | 3.02 $\pm$ 0.00 (1)      |
|                        |                        | Adults 3 TLS             | 0.81 $\pm$ 0.47<br>(63)  | 2.42 $\pm$ 0.62<br>(87)   | 5.31 $\pm$ 0.78<br>(18)  |
|                        |                        | Immatures 1<br>TLS       | 6.05 $\pm$ 2.07<br>(99)  | 13.21 $\pm$ 2.56<br>(61)  | 25.47 $\pm$ 4.57<br>(8)  |
|                        |                        | Immatures 3<br>TLS       | 1.64 $\pm$ 0.67<br>(64)  | 3.41 $\pm$ 0.60<br>(82)   | 6.13 $\pm$ 1.24<br>(22)  |
|                        |                        | Thrips Injury 1<br>TLS   | 2.00 $\pm$ 0.00<br>(167) | N/A                       | 2.33 $\pm$ 0.00 (1)      |
| Thrips Injury 3<br>TLS |                        | 2.55 $\pm$ 0.12<br>(55)  | 2.82 $\pm$ 0.08<br>(74)  | 3.12 $\pm$ 0.14<br>(39)   |                          |
| Biomass<br>Tolerance   |                        | 2.38 $\pm$ 1.92<br>(25)  | 9.01 $\pm$ 1.86<br>(73)  | 16.58 $\pm$ 2.36<br>(70)  |                          |
| SC2020                 |                        | Adults 1 TLS             | 7.35 $\pm$ 1.66<br>(46)  | 11.91 $\pm$ 1.26<br>(79)  | 16.86 $\pm$ 2.60<br>(43) |
|                        |                        | Adults 3 TLS             | 4.64 $\pm$ 0.74<br>(45)  | 7.12 $\pm$ 0.72<br>(76)   | 9.77 $\pm$ 1.22<br>(47)  |
|                        |                        | Immatures 1<br>TLS       | 15.72 $\pm$ 5.26<br>(89) | 31.05 $\pm$ 5.10<br>(69)  | 53.01 $\pm$ 7.45<br>(10) |
|                        |                        | Immatures 3<br>TLS       | 12.96 $\pm$ 3.17<br>(86) | 22.09 $\pm$ 3.11<br>(68)  | 35.56 $\pm$ 5.28<br>(14) |
|                        |                        | Thrips Injury 1<br>TLS   | 0.93 $\pm$ 0.10<br>(46)  | 1.20 $\pm$ 0.08<br>(82)   | 1.50 $\pm$ 0.13<br>(40)  |
|                        | Thrips Injury 3<br>TLS | 3.33 $\pm$ 0.12<br>(35)  | 3.66 $\pm$ 0.07<br>(77)  | 3.98 $\pm$ 0.08<br>(56)   |                          |
|                        | Biomass<br>Tolerance   | 0.47 $\pm$ 0.32<br>(7)   | 1.42 $\pm$ 0.26<br>(77)  | 2.71 $\pm$ 0.59<br>(84)   |                          |

$\pm$  5.22 (TX-251) in SC2018, and 0.00  $\pm$  14.54 (TX-2139) to 72.46  $\pm$  14.54 (TX-2382) in SC2020 (Fig. 3B). Counts of immature thrips per five untreated plants at the 3rd TLS ranged among genotypes from 8.74  $\pm$  10.66 (TX-2328) to 62.35  $\pm$  10.67 (TX-1283-2) in NC2018, 0.04  $\pm$  1.64 (TX-2383 and ST 4946GLB2) to 9.47  $\pm$  1.64 (TX-749-2) in SC2018, and 3.09  $\pm$  8.14 (TX-932) to 46.18  $\pm$  8.13 (TX-1212) in SC2020. Counts of adult thrips per five untreated plants at the 1st TLS ranged among genotypes from 1.57  $\pm$  2.63 (TX-1192) to 14.57  $\pm$  2.63 (TX-1212) in NC2018, 0.00  $\pm$  0.37 (TX-64) to 3.02  $\pm$  0.37 (TX-1326) in SC2018, and 2.38  $\pm$  4.33 (TX-1042-1) to 26.52  $\pm$  4.31 (TX-1403) in SC2020 (Fig. 3C). Counts of adult thrips per five untreated plants at the 3rd TLS ranged among genotypes from 0.40  $\pm$  2.22 (TX-202) to 12.64  $\pm$  2.23 (TX-1326) in NC2018, 0.00  $\pm$  1.45 (TX-240) to 7.03  $\pm$  1.45 (CA 4005) in SC2018, and 3.13  $\pm$  2.35 (TX-1122) to 13.88  $\pm$  2.35 (TX-1364-4) in SC2020.

**Biomass.** Differences in above-ground biomass at 42 DAP (per five plants) between insecticide treated and untreated plots ranged from

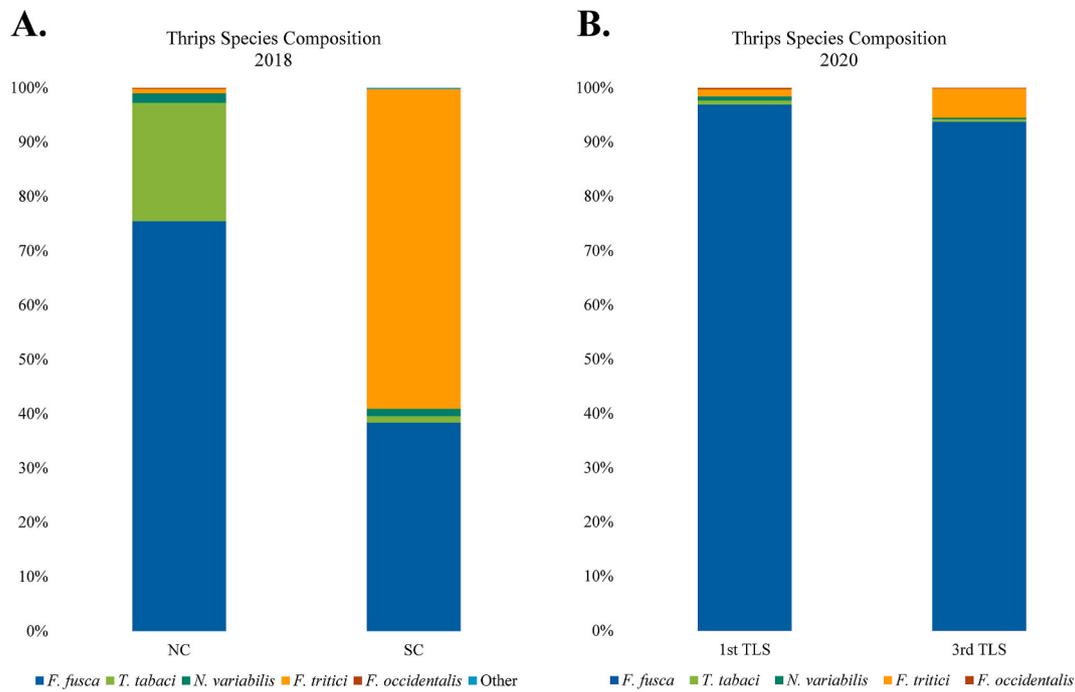
0.00  $\pm$  4.99 g (TX-40 and TX-183-2) to 26.04  $\pm$  4.99 g (TX-665-1) in NC2018, 0.00  $\pm$  5.39 g (TX-0984, TX-85, TX-101, TX-251, TX-621-2, TX-763, TX-931-1, TX-1094, TX-1115, TX-1192, TX-1194-1, TX-1425-1, TX-1462-3, TX-1718, TX-2316) to 22.67  $\pm$  5.39 g (TX-2361) in SC2018, and 0.00  $\pm$  0.83 g (TX-206, TX-226, TX-488, TX-1197, TX-1211, TX-1307, TX-1322, TX-1718, TX-2318) to 4.23  $\pm$  0.83 g (TX-0984) in SC2020 (Fig. 3D).

**Selection Index.** Three clusters of genotypes (i.e., resistant, intermediate, and susceptible) were created based on their least squares means for each variable (Fig. 3) and genotypes were scored accordingly (Table 1); the scores were summed to create an index value for each genotype. Genotypes classified by the thrips resistance selection index ranged in scores from +14 out of 21 to -4 out of -21 (Fig. 4; Supp. Table S2), where the higher scored genotypes were considered more putatively thrips-resistant. The two commercial cultivars ST 4946GLB2 and ST 6182GLT were scored +7 and +1, respectively. The two breeding lines 'CA 4005' and 'CA 4006', with partial thrips resistance, were scored +7 and +6, respectively. Eight genotypes from diverse geographical origins were considered putatively thrips-resistant due to their relatively high number of variables exhibiting desirable outcomes, i.e., index sums of +14 to +11, namely TX-1109 (Mexico), TX-1975 (Mexico), TX-2320 (Russia), TX-2383 (Uzbekistan), TX-101 (Guatemala), TX-2347 (China), TX-2362 (Paraguay), and TX-251 (Guatemala). The four putatively thrips-susceptible genotypes TX-203 (Mexico), TX-1212 (Brazil), TX-1094 (Guatemala), and TX-2403-2 (Brazil), were defined by a score of -2 to -4.

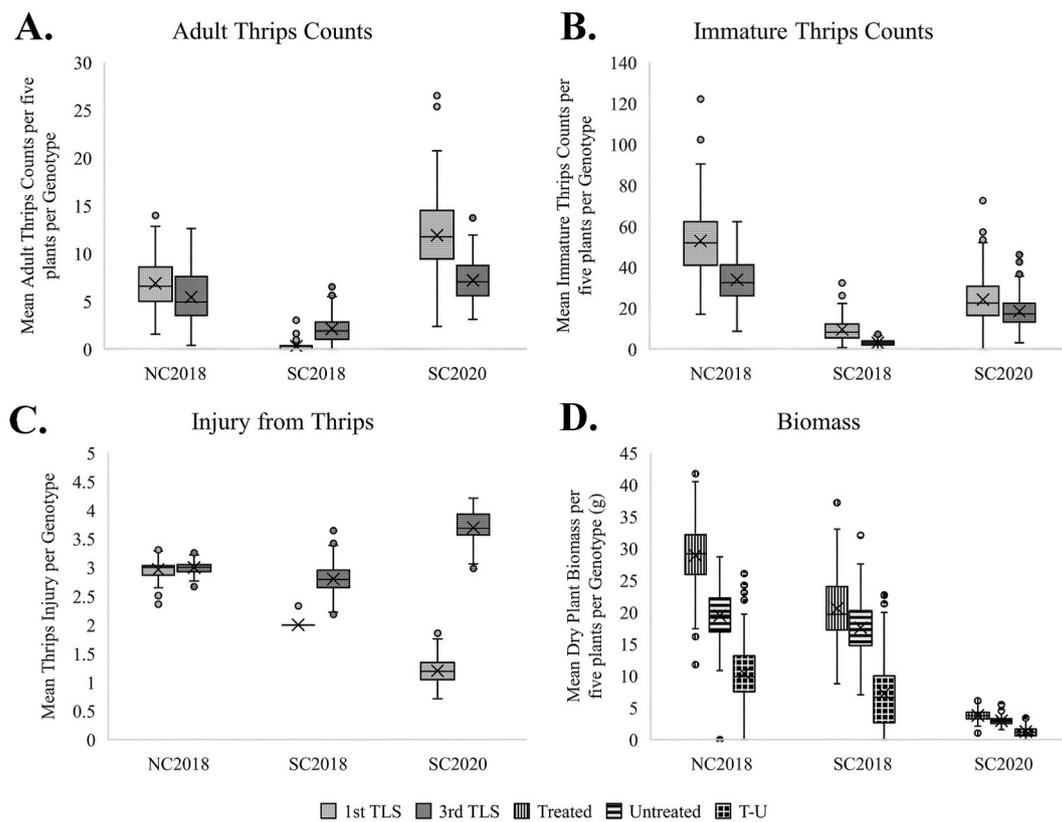
### 3. Discussion

Our research investigated the relatively unexplored diversity of exotic, day-neutral genotypes of *G. hirsutum* for their resistance to thrips in the southeastern USA. A challenge of conducting field trials with a large number of genotypes is the variability in trends between trials and years. A key to identifying resistant genotypes is finding sufficient consistency in trends across trials. Reduced injury from thrips across all field trials at the 1st TLS was observed in putatively thrips-resistant genotypes TX-2320 and TX-251. Reduced counts of adult thrips across field trials at the 1st TLS were documented for TX-2347, TX-101, and TX-251. Reduced counts of immature thrips in the three field trials at the 3rd TLS were found in TX-1975 and TX-2347. Host plant resistance trials quantify tolerance as a mechanism by comparing yields from plants with and without the presence of insect herbivores (Strauss and Agrawal 1999; Bernaola and Stout 2021). Since thrips are economic pests only at the seedling stage in cotton, biomass at the seedling stage is likely a better proxy for tolerance than end-of-season yield. Based on the difference in biomass between insecticide treated and untreated plots, tolerance was found in TX-101 and TX-2362 in all field trials. Collectively, these genotypes display at least partial resistance to thrips and should be selected as breeding parents to develop resistant cotton cultivars.

Putatively thrips-resistant genotypes were found from several countries, namely China (TX-2347), Guatemala (TX-101, TX-251), Mexico (TX-1109, TX-1975), Paraguay (TX-2362), Russia (TX-2320), and Uzbekistan (TX-2383). Both putatively resistant genotypes from Guatemala had low counts of adult thrips at the 1st TLS across all field trials. Two countries had both putatively thrips-resistant and -susceptible genotypes, namely Guatemala and Mexico. For the Guatemala genotypes, high tolerance was observed in two and three of the trials for the putatively thrips-resistant genotypes and low tolerance in biomass was observed in two of the fields for the putatively susceptible genotype. The most notable difference between resistant and susceptible genotypes from Mexico was their injury from thrips ratings at the 3rd TLS, with reduced injury in two of the fields for the putatively thrips-resistant genotypes and high injury ratings in two of the fields for the putatively susceptible genotype. These susceptible genotypes may serve as useful comparisons for future breeding efforts to develop cultivars with



**Fig. 2.** Thrips species composition from a subsample of  $\leq 20$  adult thrips from the untreated samples of each exotic cotton landrace ( $N = 168$ ) in NC and SC thrips resistance field trials. A) In 2018, thrips ( $N = 2947$ ) were identified using a dichotomous key and grouped for both collection dates: at the first (1st TLS) and third true-leaf stages (3rd TLS). B) In 2020, a probe-based qPCR assay was used to identify the thrips collected from the 1st ( $N = 2659$ ) and 3rd TLS ( $N = 2865$ ).



**Fig. 3.** Box-and-whisker plots of least square means of cotton genotypes ( $N = 168$ ) across three thrips resistance field trials conducted in Jackson Springs, NC, in 2018 (NC2018) and Florence, SC, in 2018 (SC2018) and 2020 (SC2020). A) Ratings of injury by thrips, B) counts of immature and C) adult thrips were measured at the 1st true-leaf stage (i.e., light gray boxes) and 3rd TLS (i.e., dark gray boxes). D) Above-ground dry biomass was taken at 42 days after planting from treated (i.e., boxes with vertical lines) and untreated plots (i.e., boxes with horizontal lines); differences in treated and untreated biomass (i.e., checkered boxes) were used to determine selection index resistance groups. Counts of thrips and dry biomass were measured by five plants per genotype.

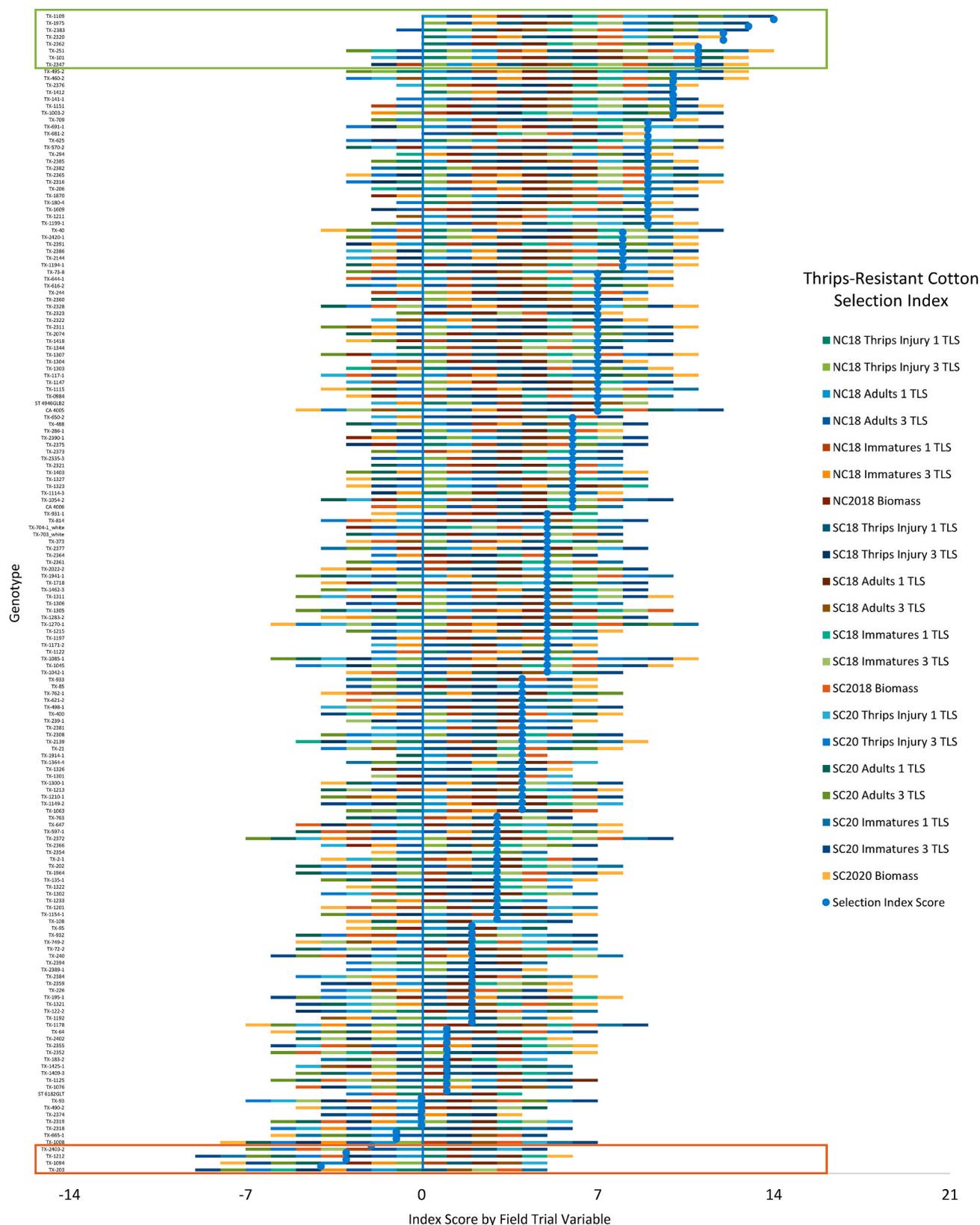


Fig. 4. Visual representation of our Thrips Selection Index. Cotton genotypes separated based on number of variables (i.e., colored boxes) that grouped with desirable (“+1”) and undesirable (“-1”) mean outcomes. If a response variable for a given genotype was clustered in the intermediate group, the genotype was assigned a score of “0” and was not added to the figure. Blue dots indicate the summed selection index score per genotype (variables given equal weight to create an index value for each genotype that could range from ‘+21’ to ‘-21’), where the higher scored genotypes were considered more putatively thrips-resistant. Genotypes within the red box indicates those that were determined putatively thrips susceptible. Genotypes within the blue box indicates those that were determined putatively thrips resistant.

resistance to thrips.

Biomass differed greatly between SC2018 and SC2020 which was likely due to the unusually cool, wet weather in Florence, SC, in 2020, which slowed growth and led to lower biomass at 42 DAP. Average temperature and total rainfall in May were 22.8 °C and 45.97 mm, respectively, for NC2018, 23.4 °C and 136.91 mm, respectively, for SC2018, and 19.7 °C and 245.62 mm, respectively, for SC2020. Densities of adult thrips were also highly variable across trials. Total numbers of adult thrips collected from untreated plots at the field trials were 6,193 in NC2018, 1,223 adults in SC2018, and 9,536 adults in SC2020. Total numbers of immature thrips collected at the field trials were 43,581 in NC2018, 6,384 in SC2018, and 21,210 in SC2020. Despite this variability, the range of selection index values suggested that a number of genotypes showed a greater overall level of resistance than other, more susceptible genotypes. Compared to the moderately-resistant breeding lines CA 4005 and CA 4006, resistance to thrips was greater for 37 and 58 genotypes, respectively. Compared to the commercial cultivars ST 4946GLB2 and ST 6182GLT, resistance to thrips was greater for 37 and 147 genotypes, respectively.

Ideally, host plant resistance for thrips in cotton would prevent either pest densities or injury from reaching economic threshold to preclude the need to use insecticides. While economic thresholds vary among states in the southeastern and southern USA, recommendations are consistent in that control is not needed beyond the 4th or 5th TLS (Cook et al., 2011), which is why we did not sample for thrips beyond seedling stages. The economic threshold for thrips in seedling cotton in South Carolina is two or more thrips per plant with injury present (Jones et al., 2019) and two immature thrips per plant or an average of one immature thrips per true-leaf in North Carolina (Reisig and Huseuth 2021). At the 1st TLS, the number of plots reaching or exceeding the economic thresholds in the untreated plots were 496 (98%) in NC2018, 233 (46%) in SC2018, and 401 (81%) in SC2020. At the 3rd TLS, the number of plots reaching or exceeding the economic thresholds in the untreated plots were 436 (87%) in NC2018, 111 (22%) SC2018, and 453 (99%) in SC2020. In addition to thresholds based on density of thrips, a rating of injury by thrips of '3' using the scale we used in our study has been suggested as a threshold (Graham and Smith 2021). Using this injury threshold, the number of untreated plots reaching or exceeding the threshold at the 1st TLS was 454 (90%) in NC2018, one (0.2%) in SC2018, and three (0.6%) in SC2020. At the 3rd TLS, the number of plots reaching or exceeding this injury threshold in untreated plots was 454 (90%) in NC2018, 385 (77%) in SC2018, and 504 (100%) in SC2020. None of the genotypes were rated consistently low or high for ratings of injury by thrips, likely due to the variability in densities of thrips and growing conditions across field trials.

The dominant thrips species present in our field trials was *F. fusca* in NC2018 (75.5%) and SC2020 (96.9%) and *F. tritici* in SC2018 (58.9%). Studies in the region have similarly found *F. fusca* to be the dominant early season thrips pest of cotton (Stewart et al., 2013; Reay-Jones et al., 2017). *Frankliniella tritici* is not known to be a dominant pest of cotton at the seedling stage, and conclusions from the combined field data may be confounded by the unusual thrips composition we experienced in SC2018. *Frankliniella tritici* can often be found in greater numbers in cotton later in the season, especially on flowers (Cook et al., 2003; Mailhot et al., 2007; Reay-Jones et al., 2017). In Texas, *F. occidentalis* and *T. tabaci* are the dominant thrips species (Arnold et al., 2012; Wann et al., 2017a; Vyavhare et al., 2021). In New Mexico and California, *F. occidentalis* is the dominant thrips species of cotton seedlings (Pickett et al., 1988; Zhang et al., 2014a). In Greece, *Frankliniella intonsa* Trybom was found to be the dominant early season thrips species of cotton (Deligeorgidis et al., 2002). In India, *Scirtothrips dorsalis* Hood (chilli thrips) is a major thrips pest of early season cotton (Uppar and Nandihalli 2014). In many other countries, such as Australia (Sadras and Wilson 1998; Miyazaki et al., 2017), China (Fang et al., 1995), Egypt (Abdel-Gawaad et al., 1973), Iran (Zareh 1985), and Pakistan (Arif et al., 2006; Khalil et al., 2015), *T. tabaci* is the dominant thrips species of

seedling cotton. Because *F. fusca* and *F. tritici* were the dominant species in our trials, our findings may not be applicable in regions where other species are dominant on cotton.

Studies of host plant resistance for thrips in cotton have often used untreated field trials, using natural thrips populations, to assess injury by thrips (e.g., Zhang et al., 2014a), unique physical traits (e.g., Arif et al., 2006), chemical composition (e.g., Saleem et al., 2013), and counts of thrips (Miyazaki et al., 2017) to identify resistant genotypes. Many of these were small-scale trials which evaluated five (Rummel and Quisenberry 1979) to 32 (Zhang et al., 2014a) cotton genotypes. In Australia, Miyazaki et al. (2017) found *G. hirsutum* to have high ratings of injury and counts of thrips (predominantly *T. tabaci*) compared with other cotton species and concluded diploid cotton was more resistant to thrips. In Egypt, cotton varieties with a thicker lower epidermis were found to be more resistant to *T. tabaci* (Abdel-Gawaad et al., 1973). In Iran, hairy and glandless *G. hirsutum* cultivars were found with fewer *T. tabaci* (Zareh 1985). In Pakistan, reduced populations of *T. tabaci* have been found on *G. hirsutum* with gossypol glands and long hairs on leaf veins, lamina, and upper leaf midribs (Arif et al., 2006; Saleem et al., 2013; Khalil et al., 2015). Additionally, high density of leaf hairs on midribs and veins was found to be negatively correlated with *T. tabaci* on cotton genotypes (Arif et al., 2006), though the same characters were found to be positively correlated with *T. tabaci* on Bt lines (Saleem et al., 2013). In Arizona, populations of *F. occidentalis* were higher on sticky cards near okra-leaf cotton than normal-leaf cotton (Chen et al., 2006). In New Mexico, glandless cotton lines were found to have lower ratings of thrips injury from *F. occidentalis* than glanded commercial and breeding lines (Zhang et al., 2014a). In Texas, hairy-leafed genotypes were found to have lower ratings of injury and greater yields regardless of foliar sprays (Rummel and Quisenberry 1979). In North Carolina, Kaur et al. (2018) screened 391 *G. hirsutum* accessions for resistance to thrips, predominantly *F. fusca*, by rating injury by thrips for all accessions and assessing counts of thrips and dry biomass for a select few. The cotton accessions with resistance to thrips were correlated with faster relative rate of growth and higher density of trichomes in greenhouse trials but were not found to have significant differences in plant heights or leaf area compared with the susceptible accessions (Kaur et al., 2018). In five southeastern USA states, commercial varieties with larger seed size, greater dry plant biomass, and taller seedlings were found to have fewer thrips (Kerns 2018). Across trials, ST 6182GLT had lower counts of immature thrips but was not considered resistant to thrips; ST 4946GLB2 had low ratings of injury and was characterized with large, heavy seeds (Kerns 2018). Our data support the findings from Kerns (2018); ST 4946GLB2 had low ratings of injury at both leaf stages in both of our 2018 field trials.

Thrips are the most prevalent early season pest of cotton and the main reason for prophylactic insecticide use (Allen et al., 2018). Indeed, our results confirmed that a majority of cotton genotypes in most locations and years (two out of three in this study) will exceed thresholds for thrips. Neonicotinoid-resistant populations of *F. fusca* pose major challenges for pest management in seedling cotton (Huseuth et al., 2016). *Aphis gossypii* Glover (cotton aphid) is an occasional seedling pest of cotton and has been characterized with a neonicotinoid-resistant biotype (Gore et al., 2013). Neonicotinoid seed treatments have also been shown to exacerbate occasional pests of seedling cotton, such as *Tetranychus urticae* Koch (twospotted spider mite) (Smith et al., 2013). Because of such issues related to the use of neonicotinoid seed treatments, other control options are urgently needed for thrips in cotton. In addition to a need for breeding programs to develop resistant varieties, one promising management tool is the soon-to-be commercialized cotton with Bt toxin Cry51Aa2.834\_16, demonstrating resistance to *F. fusca* (Akbar et al., 2018; Graham et al., 2019; D'Ambrosio et al., 2020a). *Frankliniella fusca* was found to establish, probe, ingest, and oviposit fewer times on the Bt line than on similar non-Bt cotton (i.e., anti-xenosis), though adult and larval mortality between the cotton lines were statistically similar (Graham et al., 2019; Huseuth et al., 2019, 2020;

D'Ambrosio et al., 2020b). *Frankliniella occidentalis* is also affected by the toxin with greater larval and adult mortality, slower larval development, and decreased oviposition on the Bt line compared with non-Bt cotton (i.e., antibiosis and antixenosis) (Huseth et al., 2019; D'Ambrosio et al., 2020a). With these exciting advances in thrips resistance in Bt cotton, a long-term resistance management strategy is essential. Cultivars expressing a single Bt protein to a pest are especially vulnerable for resistance development (Trapero et al., 2016). Use of an insecticide (e.g., neonicotinoid seed treatment) has been suggested as a means to reduce selection pressure against the new Bt trait, but it is not considered a long-term strategy (D'Ambrosio et al., 2020a; Huseth et al., 2020). Whether resistance is obtained from transgenic or conventional breeding techniques, revised economic thresholds will likely be needed, since current thresholds were developed for susceptible cotton varieties. Sustainable management strategies for thrips in cotton should be based within an IPM approach that incorporates a range of tactics, including cotton varieties with resistance to *F. fusca* developed from the data reported in our study.

Host plant resistance traits can play a fundamental role in crop protection and have shown to provide synergistic effects with Bt traits for chewing insects in other crops (e.g., Coombs and Douches 2002; Walker et al., 2002; Zhu et al., 2008; Ortega et al., 2016). For the cotton pest *Pectinophora gossypiella* Saunders (pink bollworm), the addition of gossypol to their diets was shown to delay insect resistance to Cry proteins in cotton (Carriere et al., 2004; Williams et al., 2011); high gossypol cotton could be a host plant resistance tactic for cotton to protect Bt traits (Trapero et al., 2016). Host plant resistance traits for thrips in cotton could reduce the need for prophylactic insecticides, while complementing and safeguarding other plant-incorporated protectants. By assessing cotton seedlings across several variables, we aimed to identify genotypes with multiple types of thrips resistance (e.g., tolerance, antibiosis, antixenosis). To further explore thrips resistance in the genotypes, we will continue this research in choice and no-choice laboratory tests to identify types of resistance. Plants with confirmed thrips resistance attributes will be further measured for unique morpho-physical traits and metabolite compounds to explain the cause of resistance and serve as biomarkers to accelerate future breeding efforts.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2022.106113>.

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