

ARTHROPOD MANAGEMENT

Resistance to Cotton Fleahopper Feeding in Primitive and Converted Race Stocks of Cotton, *Gossypium hirsutum*

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ABSTRACT

The cotton fleahopper, *Pseudatomoscelis seriatus* Reuter, is a widespread and important insect pest of cotton in Texas and Oklahoma. This plant bug feeds on small floral buds, which results in bud abscission, delayed fruiting, and subsequent crop loss. In central and southeastern Texas, two to four insecticide applications are typically applied for cotton fleahopper management. Primitive race stocks of cotton have been identified as an important source of resistance to a wide range of insect pests, but they have not been evaluated for resistance to cotton fleahopper. The objective of this study was to evaluate selected groups of primitive race stocks of *Gossypium hirsutum* L. for resistance to cotton fleahopper. Resistance was identified by caging cotton fleahoppers on cotton plants in a no-choice feeding trial and comparing the mean number of damaged squares per plant to a standard susceptible genotype. Four primitive race stocks, TX706, TX188, TX1530, and TX1156, were identified as resistant to cotton fleahopper in a collection of 65 primitive race stocks representing 18 genetic groups and collected throughout Mexico and Central America. No resistance was found in a collection of 11 accessions previously identified as resistant to *Lygus* spp. and no resistance was identified in a collection of 78 primitive accessions converted to day-neutrality. The possibility that some genetic resistance in these race stocks to the cotton fleahopper might have been lost as a result of the conversion to day-neutrality is discussed.

Transgenic cotton expressing the insecticidal proteins derived from *Bacillus thuringiensis*, Bt, has provided effective control of the major lepidopteran pests of cotton including *Helicoverpa zea* Hübner, bollworm, and *Heliothis virescens* F., tobacco budworm, in the U.S. (Jackson et al., 2003; Tabashnik et al., 2013). Widespread adoption of transgenic Bt cotton and the eradication of *Anthonomus grandis grandis* Boheman, boll weevil, from most of the cotton growing regions of the U.S. have reduced foliar insecticide applications on cotton (Allen, 2008; Head et al., 2005; Williams, 2011). However, plant bugs, including *Lygus* spp. and *Pseudatomoscelis seriatus* (Reuter), cotton fleahopper, remain important cotton pests and in some systems are now the key pest targeted with foliar insecticide applications. Currently, transgenic cotton with resistance to plant bugs has not been deployed in commercial production, although there is research progress in this area (Baum et al., 2012).

Genetic resistance to insect pests of cotton received considerable attention from 1968 to 1986 as investigators searched for sources of resistance to boll weevil, lepidopteran pests, and plant bugs (Jenkins and Wilson, 1996; Niles, 1980). Collections of race stocks, breeding lines, and cultivars of cotton were screened for resistance to *Lygus lineolaris* (Palisot de Beauvois), tarnished plant bug, in the southeastern U.S. (Benedict et al., 1981; Jenkins and Wilson, 1996; Jenkins et al., 1977; Meredith, 1998; Meredith and Schuster, 1979), *Lygus hesperus* Knight, western tarnished plant bug, in the western U.S. (Tingey et al., 1975), and to cotton fleahopper in Texas (Knutson et al., 2013; Lidell et al., 1986; Lukefahr et al., 1968, 1970, 1976; Walker et al., 1974). Although some potential sources of resistance to *Lygus* and cotton fleahopper were identified in these studies, breeding for resistance to plant bugs has received little attention.

Cotton fleahopper is the most important plant bug pest of cotton in Texas and Oklahoma, states that produced 46% of the annual cotton lint in the U.S. in 2010 (USDA, 2011). During 2007, more cotton acres were treated with foliar-applied insecticides for cotton fleahopper than any other single insect pest

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in Texas and Oklahoma (Williams, 2011). Losses due to cotton fleahopper are variable in the Texas Plains region. However, it is a key pest in central and southeastern Texas, where two to four applications of insecticide typically are applied annually (Parker et al., 2009; Williams, 2011).

Adult and immature cotton fleahoppers feed primarily on small cotton buds, termed squares, approximately 2 mm in diameter or less. Feeding results in bud abscission, delayed fruiting, increased risk of exposure to late season pests and inclement weather, and subsequent crop loss (Parker et al., 2009; Reinhard, 1926).

The importance of cotton fleahopper as a cotton pest and limited management options suggest the need for renewed efforts to identify sources of genetic resistance to this pest. A survey of cotton genotypes representing the germplasm pools and breeding lines available to cotton breeders in the U.S. found only moderate levels of tolerance to cotton fleahopper (Knutson et al., 2013). McCarty and Percy (2001) reported that insect resistance is relatively common in the primitive accessions of cotton and that more than 200 accessions have been identified as sources of resistance to a wide range of insects and mites. However, these primitive accessions have not been evaluated for resistance to cotton fleahopper.

Mexico and northern Central America are presumed to be the center of origin of *Gossypium hirsutum* L. (Brubaker and Wendel, 2001) and more than 600 primitive race stocks or landraces of *G. hirsutum* were collected from Mexico and Guatemala during 1946 to 1948 (McCarty and Percy, 2001). This collection and additional primitive race stocks, now more than 2,200 accessions, are maintained in the U.S. National Cotton Germplasm Collection at College Station, TX, and is part of the National Plant Germplasm System (Anonymous, 1997; McCarty and Percy, 2001; Percival, 1987; USDA, 2014). Brubaker and Wendel (2001) concluded that the upland cotton gene pool experienced a stringent genetic bottleneck as germplasm introductions from the Caribbean, Mexico and Central America were selected for day length and other adaptations to local conditions in the southern U.S. The primitive race stocks thus represent a diverse genetic pool that could include pest resistance to cotton fleahopper (McCarty and Percy, 2001).

Many accessions from Mexico and Central America are photoperiodic and require short days to initiate flowering (McCarty and Jenkins, 1992). To overcome this requirement, 95 primitive ac-

cessions were converted by McCarty et al. (1979) and McCarty and Jenkins (1992, 1993, 2002) to day-neutrality through a series of backcrosses to 'DeltaPine 16' (Bowman et al., 2006), a day-neutral donor line. The race stock was crossed as the male parent to DeltaPine 16 and F₂ progeny with the day-neutral flowering habit was selected. These progenies were then backcrossed four times to their respective race stock parent and selected for day-neutrality in the F₂ generation following each backcross. Genetic variability was maintained by selecting only for the day-neutral flowering habit.

The objectives of this study were to evaluate selected groups within the collection of primitive race stocks of *G. hirsutum* for resistance to cotton fleahopper and identify potential sources of resistance that could be used in a cotton breeding program.

MATERIALS AND METHODS

The large number of cotton race stock accessions in the U.S. National Cotton Germplasm Collection dictated that only a small portion of this collection could be evaluated due to limits of time and space. Three groups of *G. hirsutum*, named the race stock group, *Lygus*-resistant group, and converted race stock group, were selected for evaluation. Together, these three groups included 154 accessions. Seeds of all of the accessions were provided by the USDA-ARS Cotton Germplasm Collection at College Station, TX. The primitive race stock accessions were originally assigned a number with a "TX" prefix and this designation is most frequently used to refer to these accessions whereas the converted race stocks are designated with the prefix "M" (McCarty and Percy, 2001). The TX or M number and plant inventory number for each accession are reported herein.

Race Stock Group. Brubaker and Wendel (2001) identified 18 genetic groups within a collection of 65 primitive race stocks of *G. hirsutum* collected in Mexico and Central America. We evaluated this collection of 65 accessions, termed herein the race stock group, as they represent a significant proportion of the genetic diversity present in this larger collection of primitive race stocks and they were collected from throughout the presumed center of origin of *G. hirsutum* (Brubaker and Wendel, 2001). Six plants (each plant a replicate) of each genotype were evaluated for resistance to cotton fleahopper. The cultivar DeltaPine 50 (Bowman et al., 2006) was included as a susceptible standard in this study (Knutson et al., 2013).

Lygus-Resistant Group. The cotton fleahopper and *L. lineolaris* feed on floral buds and because they share similar feeding habits and mechanisms, cotton genotypes with resistance to *L. lineolaris* also might be resistant to cotton fleahopper. To test this hypothesis, we evaluated resistance to cotton fleahopper in 11 accessions from the primitive race collection identified as resistant to plant bugs (Jenkins and Wilson, 1996; Jenkins et al., 1977). Seventeen to 19 plants (each plant a replicate) of each accession were evaluated for resistance to cotton fleahopper and DeltaPine 50 was included as a susceptible standard.

Converted Race Stock Group. This collection included 78 primitive race stocks converted to day-neutrality (McCarty and Jenkins, 1993). The cultivar Acala Maxxa (PVP 9000168) was included as a susceptible check and each entry was replicated four times. Following this initial screening, different plants representing the six converted race stocks with the least mean square damage were again screened for resistance to cotton fleahopper. Eighteen plants of each of these six entries were evaluated and Acala Maxxa was included as a susceptible standard. Three of these converted race stocks from this trial were then compared to their corresponding unconverted race stock and DeltaPine 16, the day-neutral donor parent used in the conversions. In this third trial, each genotype was replicated four to 15 times and Acala Maxxa was again included as the susceptible standard.

No-Choice Screening Procedure. Accessions were evaluated for resistance to cotton fleahopper feeding using a no-choice screening procedure, which caged adults on individual cotton plants (Knutson et al., 2013). Seeds of each accession were planted in 7.5-l pots, thinned to one plant per pot, and grown in a greenhouse at the Texas A&M AgriLife Research and Extension Center, Dallas, Texas. Once plants were in the second to third week of squaring, they supported an abundance of floral buds that were 1 to 2 mm in diameter and most susceptible to feeding by cotton fleahopper (Knutson et al., 2013). Plants were then exposed to cotton fleahoppers by caging insects on the terminal portion of each plant. Cages were constructed of a cylinder of nylon organdy 30 cm in length and 18 cm in diameter; the cage was glued to the bottom of a clear acetate cylinder 15 cm in height and 18 cm in diameter. The top of the cylindrical cage was sealed by a lid made of nylon organdy. The cage was placed over the plant terminal and the bottom of the cage was closed by gathering the nylon organdy around the main stem of the cotton plant with a fine wire.

Cotton fleahoppers were obtained from a laboratory colony maintained on green beans, *Phaseolus vulgaris* L., (Breene et al., 1989). One- to five-day-old unsexed adults were removed from the culture and held in a glass vial closed with a cotton plug overnight at 28° C in an environmental chamber. A piece of paper towel was moistened with water and placed in the vial to provide humidity and a resting site. The following morning, six adults were introduced into each cage through a 1-cm diameter hole in the plastic cylinder that was then closed with a cork. The caged plants were held in an insectary at 30° C, ca. 50% relative humidity, and a 14:10 (L:D) photoperiod. After 72 h, the cages were removed and each square ≤ 3 mm in diameter was examined with a stereomicroscope. Squares were dissected to determine the presence of internal feeding injury characteristic of cotton fleahopper (Williams and Tugwell, 2000). Cotton fleahopper resistance was quantified as the mean number of damaged squares per plant relative to the control.

We chose to evaluate damage based on the number of damaged squares, rather than percent damage, because the number of floral buds susceptible to cotton fleahopper feeding varied among accessions due to differences in when they begin fruiting and their fruiting rate. Accessions were not evaluated until at least the second week of bud initiation at which time 10 or more floral buds susceptible to cotton fleahopper were present on each plant. Preliminary trials (unpublished) demonstrated that a maximum of five to six floral buds would be injured using this screening procedure. Cotton fleahoppers feed only until they are satiated, regardless of the number of susceptible squares available to them. Thus, if damage was scored as a percentage, accessions with large numbers of floral buds would exhibit less percent square damage than accessions with fewer floral buds even though both were equally susceptible to feeding injury. Under field conditions, this could be viewed as a tolerance mechanism but given the short period of time, 3 d, that the genotypes were exposed to cotton fleahopper in these studies, compensation could not be measured. Assessing resistance using the number of damaged squares directly measured cotton fleahopper injury, independent of square number, and avoided assigning resistance to genotypes with large numbers of squares.

Statistical Analyses. Data on the number of damaged squares per plant for the three groups were subjected to ANOVA using PROC GLM and the means for each accession was compared to the

standard susceptible genotype using the CONTRAST option (SAS Institute, 2008). Data on the number of damaged squares for three converted race stocks with the least square damage and their corresponding unconverted form were subjected to ANOVA using PROC GLM and means were separated using Tukey's multiple comparison test. The CONTRAST option was used to compare the mean for the three converted race stocks as a group to the mean for the three corresponding unconverted race stocks as a group.

RESULTS

Race Stock Group. Three accessions, TX21, TX98, and TX99, within the race stock group, did not yield a sufficient number of plants for evaluation due to poor seed germination. As a result, 62 accessions and the standard, DeltaPine 50, were evaluated. The mean number of squares injured by cotton fleahopper was different among these entries ($p < 0.05$) (Table 1). The mean square damage for TX706, TX188, TX1530, and TX1156 was significantly less ($p < 0.05$) than the mean square damage for the standard, DeltaPine 50. Two of these accessions were collected from Guatemala, one from Mexico and one from Belize. Additionally, mean square damage for TX242 and TX1046 was significantly less than the standard at $p < 0.10$.

Lygus-Resistant Group. Two accessions, TX156 and TX195, in the *Lygus*-resistant group, did not yield a sufficient number of plants for evaluation due to poor seed germination and were excluded from the study. The mean number of squares damaged by cotton fleahopper was significantly different among the 10 entries ($p < 0.05$) (Table 2); however, mean square damage was not less than the standard for any of the accessions in this group at $p < 0.05$.

Converted Race Stock Group. Mean square damage among the 78 accessions in the converted race stock group ranged from 3.3 to 12.3 per plant and mean square damage in Acala Maxxa, the susceptible standard, was 7.5 ± 5.8 (data not shown). Mean square damage was not significantly different among these entries ($p < 0.05$). When the six converted race stocks with the least mean number of damaged squares (M-8844-0076, M-8844-0120, M-9044-0017, M-9044-0032, M-9044-0072, and M-9044-0162) were screened again, there were no differences in mean square damage and the susceptible standard Acala Maxxa ($p < 0.05$) (data not shown). Three of these converted race stocks, M-9044-0072, M-9044-0017, and M-9044-0032, were then compared to their

corresponding unconverted race stock TX72, TX17, and TX32 and DeltaPine 16, the day-neutral donor parent. In this evaluation, mean square damage was different among the converted race stocks, their corresponding unconverted race stock, and DeltaPine 16 ($p < 0.05$) (Table 3). Mean square damage in two of the converted race stocks, M-9044-0072 and M-9044-0017, was significantly greater than square damage in their corresponding unconverted form TX72 and TX17. Also, the mean square damage for the three converted race stocks as a group was greater than that for the mean of the three unconverted race stocks as a group ($p = 0.0014$).

DISCUSSION

Recognizing that the small sample size ($n = 3$) limits broad conclusions, the comparison of the converted race stock and their corresponding unconverted form suggests that some genetic resistance in these race stocks to the cotton fleahopper was lost as a result of the conversion to day-neutrality. Using SSR markers, Liu et al. (2000) reported that in some families the primitive photoperiodic parent was largely recovered, whereas in others there was extensive linkage drag. Zhong et al. (2002) found similar results using AFLP markers. Both studies suggested the use of markers to ensure maximum diversity and integrity of primitive accession donor germplasm. This study also suggests that future conversion projects should add a whole genome genotyping component in selecting each succeeding backcross generation donor parent plants where possible to increase the probability that the trait of interest is not lost.

Wendell et al. (1992) determined that within the indigenous range of *G. hirsutum*, there are two centers of genetic diversity, one in the Caribbean and northern South America and the second in Mesoamerica. The cotton fleahopper is widely distributed in Mexico and has been collected from many of the Caribbean islands and from Venezuela (Henry, 1991). The distribution of cotton fleahopper throughout much of the indigenous range of *G. hirsutum* suggests an evolutionary relationship, which could have resulted in the selection of cotton genotypes with resistance to this insect. Thus, the identification of primitive race stocks collected from central Mexico, Guatemala, and Belize with resistance to cotton fleahopper, as reported herein, is not unexpected.

Table 1. Mean number of squares damaged per plant by cotton fleahopper among the accessions evaluated in the race stock group.

Group	Plant Inventory Number	Texas Accession Number	LS Mean Square Damage	P-value ^z	Collection Location
1	PI 529950	TX1166	5.00	0.1742	Michoacan, Mexico
	PI 154048	TX34	3.50	0.7745	Chiapas, Mexico
	PI 154093	TX60	2.67	0.1843	Chiapas, Mexico
	PI 154091	TX58	2.25	0.1306	Chiapas, Mexico
	PI 163607	TX242	2.20	0.0836	Huetenango, Guatemala
2	PI 163654	TX93	5.83	0.0247	Jutiapa, Guatemala
	PI 163665	TX96	4.60	0.3954	Jutiapa, Guatemala
	PI 163634	TX168	4.00	0.8421	Jutiapa, Guatemala
	PI 163612	TX97	3.00	0.3513	Jutiapa, Guatemala
3	PI 163712	TX106	4.83	0.2396	Chiquimula, Guatemala
	PI 163692	TX142	4.50	0.4207	Chiquimula, Guatemala
4	PI 163742	TX180	4.17	0.6685	Santa Rosa, Guatemala
	PI 163602	TX116	2.83	0.2586	Guatemala
5	PI 158563	TX493	5.50	0.0579	Yucatan, Mexico
	PI 163732	TX188	1.17	0.0026	Baja Verapaz, Guatemala
6	PI 153988	TX6	3.00	0.3513	Puebla, Mexico
	PI 165342	TX109	2.67	0.1843	Oaxaca, Mexico
7	PI 163645	TX119	4.67	0.4625	Jutiapa, Guatemala
	PI 163638	TX235	3.33	0.5904	El Salvador
8	PI 165352	TX303	4.33	0.5369	Oaxaca, Mexico
	PI 153998	TX9	4.00	0.8611	Mexico
	PI 153981	TX1	3.00	0.3899	Mexico
	PI 165372	TX322	2.67	0.1843	Guerrero, Mexico
	PI 530161	TX1530	1.83	0.0212	Michoacan, Mexico
9	PI 325839	TX1045	5.50	0.0579	Honduras
	PI 154071	TX51	5.33	0.0856	Chiapas, Mexico
	PI 501496	TX2089	5.17	0.1236	Mocacha, Mexico
	PI 501490	TX2083	3.60	0.8348	Campeche, Mexico
10	PI 163716	TX230	5.20	0.1435	Zacapa, Guatemala
	PI 163718	TX114	4.40	0.5211	Zacapa, Guatemala
	PI 163727	TX115	4.33	0.5369	Zacapa, Guatemala
	PI 163722	TX94	3.75	0.9658	Zacapa, Guatemala
11	PI 163642	TX184	4.83	0.2396	Jutiapa, Guatemala
	PI 163639	TX111	4.40	0.5211	Jutiapa, Guatemala
	PI 163640	TX141	3.83	0.9638	Jutiapa, Guatemala
12	PI 154012	TX461	6.67	0.002	Oaxaca, Mexico
	PI 529877	TX959	5.50	0.0579	Veracruz, Mexico
	PI 165256	TX192	5.17	0.1236	Oaxaca, Mexico
	PI 189534	TX746	4.40	0.5211	Puebla, Mexico
13	PI 163603	TX379	5.20	0.1435	Santa Rosa, Guatemala
	PI 163747	TX367	4.00	0.8123	Santa Rosa, Guatemala
	PI 163012	TX1009	3.25	0.5951	Matapan, El Salvador
	PI 163751	TX372	3.00	0.5743	Santa Rosa, Guatemala
14	PI 154055	TX44	4.50	0.4207	Chiapas, Mexico
	PI 529936	TX1102	3.60	0.8348	Chiapas, Mexico
15	PI 158527	TX481	4.75	0.3612	Yucatan, Mexico
	PI 501501	TX2094	4.20	0.6661	Yucatan, Mexico
	PI 158506	TX656	4.00	0.8261	Peten, Guatemala.
	PI 158547	TX488	4.00	0.8261	Yucatan, Mexico
	PI 189482	TX745	3.20	0.5210	Yucatan, Mexico
16	PI 341876	TX1046	2.33	0.0850	Yucatan, Mexico
	PI 234325	TX1091	4.25	0.6604	Managua, Nicaragua
	PI 265134	TX691	3.50	0.7319	Choluteca, Honduras
	PI 265143	TX706	2.17	0.0490	Belize
17	PI 265158	TX724	4.83	0.2396	Belize
	PI 265159	TX725	4.67	0.3215	Belize
	PI 201602	TX766	4.17	0.6685	Belize
	PI 265127	TX794	3.50	0.7319	Belize
18	PI 304768	TX1163	4.83	0.2396	Alta Verapaz, Guatemala
	PI 163724	TX210	4.50	0.4207	Zacapa, Guatemala
	PI 163730	TX166	4.00	0.8123	Zacapa, Guatemala
	PI 304760	TX1156	1.25	0.0154	Alta Verapaz, Guatemala

^z P-value is the probability that the mean square damage is different than the mean square damage for DeltaPine 50, the standard reference, as determined by PROC GLM and the CONTRAST option. Mean damage for DeltaPine 50 = 3.8 damaged squares per plant.

Table 2. Mean number of damaged squares per plant among the race stocks in the *Lygus* bug group.

Genotype	PI Number	Mean \pm S. D.	P-value ^z
TX113	PI 163704	5.9 \pm 2.4	0.0007
TX158	PI 163714	3.8 \pm 2.6	0.7782
TX247	PI 163631	4.5 \pm 1.4	0.1737
TX254	PI 165231	4.2 \pm 2.0	0.3973
TX481	PI 158527	4.7 \pm 2.2	0.1292
TX655	PI 158505	5.0 \pm 2.1	0.0522
TX658	PI 158508	4.4 \pm 2.1	0.2661
TX682	PI 163663	2.9 \pm 1.9	0.1942
TX701	PI 165329	3.9 \pm 3.0	0.7008
DeltaPine 50	PI 529566	3.7 \pm 2.5	-----

^z P-value is the probability that the mean square damage is different than mean square damage for DeltaPine 50, the standard reference, as determined by PROC GLM and the CONTRAST option.

Table 3. Mean number of squares per plant damaged by cotton fleahopper among three primitive race stocks, designated by TX and their corresponding converted race stock, designated by M, the day-neutral donor parent DeltaPine 16 and Acala Maxxa, a standard susceptible.

Entry	PI or PVP Number	Mean ^z
M-9044-0072	PI 561971	6.0 a
M-9044-0017	PI 561951	5.1 ab
M-9044-0032	PI 561955	3.6 abc
Delta Pine 16	PI 529251	3.3 abc
Acala Maxxa	PVP 9000168	4.3 abc
TX72	PI 153966	2.7 bc
TX32	PI 154046	2.0 c
TX17	PI 154022	1.4 c

^z Means within columns not followed by a common letter are different. Tukey's Test ($p < 0.05$, $df = 8$).

In the U.S., the cotton fleahopper has been reported from 169 plant species representing 35 families (Esquivel and Esquivel, 2009). This wide host range suggests that cotton fleahopper has overcome a wide range of host plant defenses and therefore host plant resistance could be uncommon. However, cotton is less preferred by cotton fleahopper for oviposition relative to three wild hosts (*Monarda punctata* L., *Oenothera laciniata* Hill, and *Croton capitatus* A. Michaux), which are all widely distributed in central Texas (Holtzer and Sterling, 1980). Also, cotton fleahopper colonizes cotton primarily after preferred weedy hosts have senesced in the late spring and before fall hosts appear (Almand et al., 1976). These factors suggest that cotton is not a preferred host relative to its many weedy hosts

and might have physical or chemical attributes that discourage colonization by cotton fleahopper.

American upland cotton cultivars have a narrow genetic base (Brubaker and Wendel, 2001), thus there is interest among breeders to broaden this genetic base by incorporating exotic germplasm, especially for improved fiber quality and pest resistance. McCarty and Percy (2001) reported that more than 200 primitive accessions have been reported to carry resistance to boll weevil, tobacco budworm, bollworm, *Lygus* spp., *Tetranychus* spp., spider mites, and *Pectinophora gossypiella* (Saunders), pink bollworm. Results reported herein suggest that these primitive accessions are also potential sources of resistance to cotton fleahopper.

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