

PLANT PATHOLOGY AND NEMATODOLOGY

New Sources of Resistance to the Reniform (*Rotylenchulus reniformis* Linford and Oliveira) and Root-Knot (*Meloidogyne incognita* (Kofoid & White) Chitwood) Nematode in Upland (*Gossypium hirsutum* L.) and Sea Island (*G. barbadense* L.) Cotton

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ABSTRACT

The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) is an important problem in U.S. cotton, and all cultivars support high *R. reniformis* populations. The objectives of this research were to find better sources of resistance to *R. reniformis* than are known within *G. hirsutum* L. and *G. barbadense* L. and to determine if any of these sources are also resistant to the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. A two-tiered study evaluated 1866 primitive accessions of *G. hirsutum* and 907 of *G. barbadense*. To quickly eliminate highly susceptible genotypes, tier one compared one plant per accession with six plants of susceptible ‘Deltapine 16’ and six of moderately resistant *G. barbadense* ‘TX-1348’ in the greenhouse for resistance to the reniform nematode. Tier two used fully replicated experiments in growth chambers to test promising accessions from tier one experiments against *R. reniformis* and *M. incognita* separately. Plants were inoculated 2 wk after planting in 500-cm³ pots and nematodes extracted from soil 7 wk later. Most accessions were highly susceptible, and only 5% of *G. hirsutum* and 12% of *G. barbadense* accessions had fewer *R. reniformis* than TX-1348. In growth chambers, 34 of 78 accessions (44%) suppressed *R. reniformis* ($P \leq 0.05$) compared with Deltapine 16. *G. hirsutum* accessions TX-2469, TX-1586, TX-748, TX-25, TX-1828, and TX-1860; and *G. barbadense* accessions GB-127, GB-1083, GB-1141, GB-1143, TX-110, GB-1147, GB-207, GB-833, GB-210, GB-212, GB-126, GB-581, GB-1113, GB-1081, TX-502, GB-485, GB-536, and GB-262 had > 10% and < 34% of the *R. reniformis* on Deltapine 16 and were classified moderately resistant. TX-1828,

TX-25, and TX-1860 were root-knot nematode resistant. *G. barbadense* accessions GB-49, GB-13, GB-264, GB-171, and GB-713 had < 11% of the *R. reniformis* of Deltapine 16 ($P \leq 0.01$) and were classified resistant. *G. barbadense* GB-713 had 3% of the *R. reniformis* of Deltapine 16 in three experiments.

The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveria) is an important problem in Upland cotton (*Gossypium hirsutum* L.) production in the mid-south and southeastern United States, and resistant cultivars are lacking (Jones et al., 1959; Robinson, 2002; Robinson et al., 1999). The *G. hirsutum* germplasm lines La. 434-1031, La. RN 4-4, La. RN 909, La. RN 910, and La. RN 1032 (Jones et al., 1988) supported statistically lower levels of reproduction in pots than the highly susceptible cultivar Deltapine 41 (now obsolete), and have been used to develop several additional germplasm lines with field tolerance to *R. reniformis* (Cook et al., 1997a; 1997b). These and other tolerant genotypes (Cook et al., 2001; 2002; 2003) yield well under high nematode pressure when grown under favorable growing conditions; however, in general, available tolerant genotypes sustain nematode populations at high enough levels to expect substantial yield losses when growing conditions are suboptimal or intolerant cultivars are planted.

Searches for nematode resistance in cotton have been more extensive for the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood than for *R. reniformis*. Almost all contemporary and obsolete commercial cultivars of Upland cotton are susceptible to *M. incognita* and *R. reniformis* (Jenkins et al., 1993; Robinson et al., 1999; Koenning et al., 2001). Resistance to *M. incognita* within primitive accessions of *G. hirsutum* is not rare, and at least 27 (5%) of 517 primitive accessions that have been tested are resistant (Shepherd, 1983; Robinson and Percival, 1997). Resistance in primitive accessions is perhaps not surprising, since only a limited amount of the primitive Upland cotton is represented within

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cultivated material (May et al., 1995). Of 110 primitive accessions of *G. hirsutum* tested so far against *R. reniformis*, only TX-874, TX-893, and TX-903 had significantly fewer nematodes than the susceptible control Deltapine 16 in one study (Yik and Birchfield, 1984), and none were different from Deltapine 16 when retested in another study (Robinson and Percival, 1997). The observed low frequency or absence of resistance to *R. reniformis* within *G. hirsutum* emphasizes the need to look for improved sources of resistance in other *Gossypium* species.

Yik and Birchfield (1984) defined a resistant genotype as one which can suppress *R. reniformis* populations by 75% relative to cv. Deltapine 16. Twenty-four genotypes with this level of resistance were observed within the diploid species *G. anomalum* Wawra & Peyritsch, *G. arboreum* L., *G. herbaceum* L., *G. longicalyx* J. B. Hutchinson & Lee, *G. raimondii*, *G. somalense* (Gurke) J. B. Hutchinson, *G. stocksii* Masters, and *G. thurberi* Todaro. None of these species hybridize with tetraploid *G. hirsutum*. Within tetraploid *G. barbadense* L., which can cross with *G. hirsutum* to yield fertile progeny (Percival et al., 1999), resistance has been reported and confirmed repeatedly in TX-110, TX-1347, and TX-1348 (Robinson and Percival, 1997; Yik and Birchfield, 1984), and additional accessions with greater levels of resistance may await discovery among ca. 1300 untested accessions of *G. barbadense* within the U.S. Cotton Germplasm Collection (Percival et al., 1999).

Combined resistance to both *R. reniformis* and *M. incognita* has not been reported in any *Gossypium* species, so resistance in known sources of resistance to the two nematodes appears controlled by different genes. Since these are the two most common nematodes damaging cotton in the United States, discovery of a single gene or a set of closely linked genes conferring resistance to both nematodes would greatly simplify development and use of nematode resistant cultivars in cotton.

The objective of this research was to examine previously untested accessions from the U.S. Cotton Germplasm Collection to identify more and better sources of resistance to *R. reniformis* than are currently known within *G. hirsutum* and *G. barbadense*, and to ascertain whether genotypes with the most resistance to *R. reniformis* also exhibit resistance to *M. incognita*.

MATERIALS AND METHODS

All genotypes selected for testing came from the U.S. Cotton Germplasm Collection and included

1866 primitive accessions of *G. hirsutum* and 907 of *G. barbadense*. A two-tiered approach (Luzzi et al., 1987) with tier one in a greenhouse and tier two in a growth chamber was taken to evaluate resistance. Tier-one assays compared one plant per accession with six plants of the susceptible control Deltapine 16 and six plants of the moderately resistant *G. barbadense* TX-1348 (Robinson and Percival, 1997). Most greenhouse experiments included 60 to 70 accessions of *G. hirsutum* and 40 to 45 accessions of *G. barbadense* selected sequentially from the collection by accession number.

The single-plant initial screen was predicated on phenotypic uniformity of plants within accessions of the U.S. Cotton Germplasm Collection observed during seed increases (A.E. Percival, personal communication), an expected low incidence of resistant accessions within *G. hirsutum* and *G. barbadense*, and an expected low level of variability in nematode reproduction on a resistant accession relative to the level of reproduction on the susceptible control (Robinson and Percival, 1997; Robinson et al., 1999). In both tiers, three seed per pot were planted in 500-cm³ pots equipped with drainage holes that contained a 6:1 mixture of fine sand (<400 μm particle size) and vermiculite supplemented with 5 g/kg pelletized limestone. Pots were retained in plastic flats with drainage holes and each flat had one Deltapine 16, one TX-1348, and 18 test pots arranged randomly. Growth chamber lamps were set for 14 h of light daily with 383 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of mixed fluorescent and incandescent light during the middle 12 hours and reduced light during the first and last hour. Temperature in the greenhouse was maintained at 30 \pm 12 $^{\circ}\text{C}$ and in the growth chamber was controlled with a 1-hour hold at 26 $^{\circ}\text{C}$ beginning at first light, followed by a linear 4-h ramp to 30 $^{\circ}\text{C}$, a 6-h hold, a 3-h ramp down to 28.5 $^{\circ}\text{C}$, and a final 10-h ramp back to 26 $^{\circ}\text{C}$ that ended at first light. Growth chamber relative humidity was maintained above 50%. Plants were watered daily and supplemented weekly with 50 ml of liquid fertilizer containing 100 mg dissolved nutrients (15N:16P:17K:1.0Mg:0.2Fe:0.1Zn).

Upon seedling emergence, plants were thinned to one per pot. Ten to 12 d after planting, each pot was infested with 2000 vermiform *R. reniformis* or 1000 J2 of *M. incognita* by injecting 2 to 5 ml of nematode suspension 1 to 5 cm deep at three points 2 to 3 cm from the plant stem. Pots with *R. reniformis* received a second aliquot of 2000 nematodes 1 wk later to enhance uniformity of root infection

(Robinson, 2002). Mixed vermiform stages of *R. reniformis* were obtained by Baermann funnel extraction of infested soil the night before inoculation, and second-stage juveniles (J2) of *M. incognita* were obtained by hatching eggs extracted from tomato roots 3 d before inoculation. Both species were at least 95% motile when applied to pots. Inocula of *Meloidogyne incognita* were used only in growth chamber experiments.

Seven weeks after the first inoculation, plant heights were measured, unusual plant attributes (such as flower morphology and color, leaf shape, and growth habit) were noted, roots were removed, washed, and weighed, and soil from pots with *R. reniformis* was thoroughly mixed to provide a 100 ± 35 -g sample that was weighed and subjected to Baermann funnel extraction (Robinson and Heald, 1991). Roots from pots inoculated with *M. incognita* were washed and given a 0 to 5 gall rating where 0 = no galls and 1, 2, 3, 4, and 5 indicate < 10% and ca. 25, 50, 75, and 100% of the root system galled, respectively. For each plant, a final population density of *R. reniformis* was calculated as the number of vermiform nematodes per gram soil per gram root fresh weight, expressed as a percentage of the corresponding mean value for the susceptible control Deltapine 16 in that test. Since all plants were in pots of the same size, this statistic essentially was a measure of the number of nematodes per gram of root tissue, so it provided a direct measure of the intrinsic ability of root tissue to support nematode development and reproduction independent of the quantity of roots present. Furthermore, it was recognized that low counts from pots containing weak plants with exceptionally small root systems were not a reliable measure of resistance, because part of the root weight came from the base of the stem on which nematodes cannot feed. Therefore, plants with roots below 1.0 g were rejected as unclassifiable unless final *R. reniformis* population density in soil exceeded 20 nematodes/g soil, in which case they were classified as “probably susceptible” in spite of the low root weight. A population density exceeding twenty nematodes per gram was selected as the default criterion for rejecting a plant as possibly resistant because it represents a concentration universally considered damaging to cotton. Thresholds for *R. reniformis* damage to cotton recommended by the Cooperative Agricultural Extension Service vary 10-fold across times of year and states where *R. reniformis* occurs. The highest threshold, expressed for farmers’ con-

venience in Louisiana as 10,000 nematodes per pint of soil at harvest (personal communication, Charles Overstreet, Louisiana Agricultural Extension Service, Baton Rouge, LA), is equal to 20 nematodes/g for soil with a bulk density of 1.06.

For each greenhouse experiment, coefficients of variation for the nematode population density on controls were calculated, and Z values were used to estimate the likelihood of erroneously rejecting resistant accessions as susceptible for various resistance levels. Accessions were classified as “susceptible” if nematode populations were greater than on Deltapine 16, “probably susceptible” if less than Deltapine 16 but greater than TX-1348, and “possibly resistant” if less than TX-1348. The second tier of the study included four fully replicated growth chamber experiments in a completely randomized design with six replications of the most promising accessions from the possibly resistant category. Deltapine 16 and TX-1348 were included as controls considered to be susceptible and moderately resistant, respectively, to *R. reniformis*, and Auburn 623 was included as a control resistant to *M. incognita*. Experiments 1, 2, and 3, respectively, included 16, 10, and 18 accessions of *G. hirsutum* and *G. barbadense* combined with six replicates of plants inoculated with *R. reniformis* and six with *M. incognita*. The fourth experiment also had six replications and included 34 accessions of *G. hirsutum*, no *G. barbadense* accessions, 12 replications each of the two standard controls, and 12 of the *G. barbadense* accession GB-713, but without the *M. incognita* treatments. In the fourth experiment, plants were saved at the end of the experiment for inheritance studies, and nematode populations were evaluated by collecting and compositing three 1.3-cm-diameter cores of soil from the top to bottom of each pot, and extracting nematodes by Baermann funnel. Plants were assumed to have equal root weights because they were all accessions of *G. hirsutum* and plant heights indicated robust root systems. Means in growth chamber experiments were separated from the susceptible control Deltapine 16 by Dunnett’s test. Accessions with nematodes/g soil/g root significantly < 10% and < 33% of that for Deltapine 16 were considered “resistant” and “moderately resistant”, respectively.

RESULTS AND DISCUSSION

The mean plant height for Deltapine 16 in the greenhouse was 36 cm and in the growth chamber was

44 cm. Deltapine 16 plants began to flower and often had set one or two bolls by the end of experiments. TX-1348 plants were 57% taller than Deltapine 16 in both environments and did not flower in most experiments. The mean fresh root weight for Deltapine 16 inoculated with *R. reniformis* was 4.52 and 3.17 g in greenhouse and growth chamber assays, respectively, and roots of TX-1348 were 31 and 67% heavier than those of Deltapine 16 in the greenhouse and growth chamber, respectively. Roots of other *G. barbadense* accessions also were typically heavier than Deltapine 16 and other *G. hirsutum* genotypes.

In the greenhouse, *G. barbadense* was generally less susceptible than *G. hirsutum*; however, most accessions from both species supported prolific reproduction, and only 5% of *G. hirsutum* and 12% of *G. barbadense* plants had less reproduction by *R. reniformis* than TX-1348 (Fig. 1). The mean number of nematodes/g soil/g root on TX-1348 was 28.6% of Deltapine 16, and ranged from 8 to 49% among the 23 experiments. Deltapine 16 supported 146% greater ($P = 0.006$ by *t*-test) and TX-1348 113% greater ($P = 0.001$ by *t*-test) nematode reproduction in the summer (Julian dates 135-260) than in other times of year (Fig. 2).

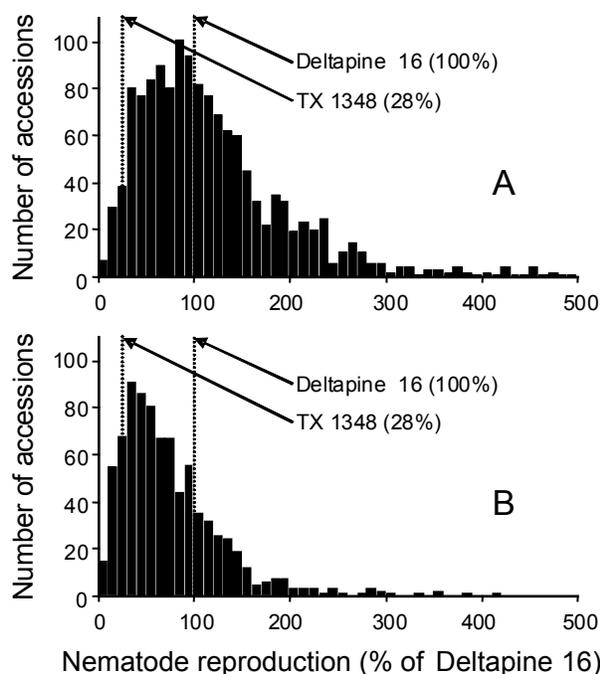


Fig. 1. Distribution of reproduction levels for the reniform nematode (*Rotylenchulus reniformis*) observed for single plants of primitive accessions of *Gossypium hirsutum* and *G. barbadense* from the U.S. Cotton Germplasm Collection that were contrasted with mean values observed for 141 entries each of (A) susceptible *G. hirsutum* Deltapine 16 and (B) moderately resistant *G. barbadense* TX-1348.

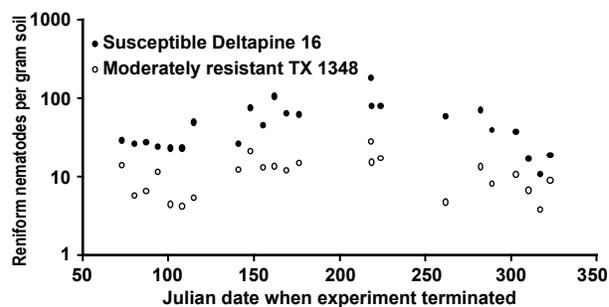


Fig. 2. Population density of the reniform nematode (*Rotylenchulus reniformis*) in soil from 500-cm³ pots 7 weeks after inoculation of 10-day-old plants of susceptible *Gossypium hirsutum* Deltapine 16 and moderately resistant *G. barbadense* TX-1348 with mixed vermiform stages of the nematode for greenhouse experiments initiated at various times during the year. Each dot is the mean of six measurements and data for 3 years are compiled by Julian date.

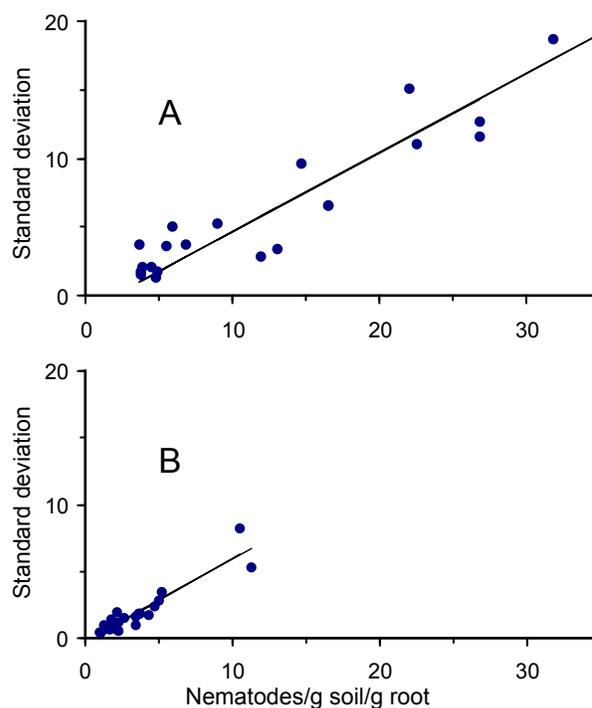


Fig. 3. Relationship between standard deviation and mean for population density of the reniform nematode (*Rotylenchulus reniformis*) on experimental controls, (A) Deltapine 16 and (B) TX-1348, from greenhouse experiments conducted at various times during the year over a 3-year period. Deltapine 16 is a highly susceptible *Gossypium hirsutum* cultivar, and TX-1348 is a moderately resistant primitive accession of *G. barbadense*.

In greenhouse assays (tier one), standard deviations for each control were directly related to means across experiments (Fig. 3), and coefficients of variation (CV) of resistant and susceptible controls

(TX-1348 and Deltapine 16) were essentially identical (51 and 52%). Assuming that all accessions had the same average CV as the controls (51.5%), the expected standard deviation for highly resistant accessions would be much smaller than for susceptible accessions. Using values of $Z = (Y - \mu)/\sigma$ from Table A.4 of Steel and Torrie (1980), the predicted chance of misclassifying a borderline resistant accession (i.e. one that would average 10% the nematodes of Del-

tapine 16, if numerous replications were included) as probably susceptible in the greenhouse was 1 in 5000. Conversely, the chances of misclassifying a borderline susceptible accession (one averaging 34% the nematodes of Deltapine 16 over numerous replications) were 38 in 100. The observed overall percentage of possibly resistant accessions that were reclassified as susceptible in subsequent growth chamber experiments was 44% (Table 1).

Table 1. Growth chamber evaluations of resistance to *Rotylenchulus reniformis* (Rr) and *Meloidogyne incognita* (Mi) in accessions of *Gossypium hirsutum* and *G. barbadense* from the U.S. Cotton Germplasm Collection that had supported poor reproduction by *R. reniformis* in previous greenhouse assays

Experiment I			Experiment II			Experiment III			Experiment IV			
Accession	Rr density GH GC (% of DP16) ^{wx}	Mi gall rating ^{xy}	Accession	Rr density GH GC (% of DP16) ^{wx}	Mi gall rating ^{xy}	Accession	Rr density GH GC (% of DP16) ^{wx}	Mi gall rating ^{xy}	Accession	Rr density GH GC (% of DP16) ^{wx}	Accession	Rr density GH GC (% of DP16) ^{wx}
<i>G. hirsutum</i>												
TX-71	13 85	2.0	TX-1167	16 137	1.5	TX-25	10 30**	0.2**	TX-8	30 83	TX-1849	27 57
TX-79	16 39	3.7	TX-1403	1 93	3.4	TX-1414	8 48**	2.1	TX-9	25 75	TX-1854	22 78
TX-112	8 88	2.8				TX-1828	10 28**	0.8**	TX-11	28 146	TX-1856	27 88
TX-390	16 175	3.1				TX-1860	10 26**	0.5**	TX-244	31 125	TX-1857	19 111
TX-408	23 138	3.0				TX-1960	15 63**	3.8	TX-289	23 112	TX-1861	32 63
TX-459	11 90	1.6*							TX-464	18 82	TX-1864	19 78
<i>G. barbadense</i>												
GB-13	15 9**	2.8	GB-459	6 56	1.9	GB-126	10 24**	2.9	TX-748	29 32**	TX-1868	15 88
GB-49	12 10**	3.3	GB-485	12 19**	2.8	GB-127	17 30**	4.4	TX-768	33 143	TX-1873	19 49
GB-207	34 27*	2.3	GB-536	8 17**	3.3	GB-171	14 8**	2.2	TX-1421	12 48	TX-1884	32 80
GB-208	9 37	3.3	GB-581	10 24**	2.5	GB-212	14 26**	2.9	TX-1536	17 57	TX-2051	32 70
GB-210	18 26*	3.8	GB-681	0 65	3.4	GB-833	4 27**	2.7	TX-1585	19 103	TX-2086	22 80
GB-211	12 41	4.1	GB-706	1 60	2.8	GB-1045	14 35**	2.2	TX-1586	13 33**	TX-2161	12 117
GB-214	9 39	2.6	GB-713	3 3**	3.2	GB-1081	4 23**	4.1	TX-1666	20 76	TX-2408	17 107
GB-262	6 13**	3.1	TX-110 ^z	6 29*	2.8	GB-1083	14 30**	2.8	TX-1736	26 58	TX-2459	18 68
GB-264	6 9**	3.3				GB-1113	10 24**	3.9	TX-1787	25 40*	TX-2468	17 53
TX-502 ^z	12 22*	2.5				GB-1141	7 30**	2.5	TX-1810	32 108	TX-2469	2 34**
						GB-1143	10 30**	3.7				
						GB-1145	14 34**	3.4				
						GB-1147	17 28**	3.3				
Susceptible control												
DP-16	100	3.2	DP-16	100	3.0	DP-16	100	2.8			DP-16	100
<i>Mi</i> -resistant control												
Aub-623	—	1.1**	Aub-623	98	0.0**	Aub-623	—	0.7**			None	—
<i>Rr</i> -resistant controls												
TX-1348	13**	—	TX-1348	20**	3.5	TX-1348	13**	—			TX-1348	27**
											GB-713	3**

^w*Rotylenchulus reniformis* per gram soil per gram root 7 wk after inoculation with mixed vermiform stages, where values on left and right sides of vertical line (|) indicate, respectively, the value obtained in the previous greenhouse (GH) assay and the value obtained in the growth chamber (GC) experiment indicated.

^xValues for controls of Experiment IV are means of 12 replications, and all other values are means of six replications, with six plants tested against *R. reniformis* and six against *M. incognita*. Asterisks (*, **) indicate *R. reniformis* mean different from Deltapine 16 (DP-16) and *M. incognita* different from Auburn 623 (Aub-623) at $P \leq 0.05$ and $P \leq 0.01$ according to Dunnett's test for comparisons with a standard control.

^y*Meloidogyne incognita* gall ratings of 0, 1, 2, 3, 4, and 5 were assigned to root systems with 0, 10, 25, 50, 75, and 100% of root tissue galled, respectively.

^zTX-502 and TX-110 are *G. barbadense* phenotypes from the *G. hirsutum* collection.

Of the 1866 accessions of *G. hirsutum* planted in the greenhouse tests, 447 were not classified due to roots lighter than 1.0 g (179 accessions) or no germination (55 accessions), or were rejected for other reasons related to weather, insect pressure, or mechanical damage (213 accessions). Of the 1419 that were classified, 703 were rated susceptible, 650 probably susceptible, and 66 possibly resistant. The corresponding numbers for the 907 accessions of *G. barbadense* were 57 not classified, 201 susceptible, 544 probably susceptible, and 105 possibly resistant. Since only 47 and 31 of the *G. hirsutum* and *G. barbadense* accessions, respectively, that were classified as possibly resistant in greenhouse assays were tested in replicated growth chamber experiments, it is likely that additional resistant and moderately resistant accessions remain among the untested 20 and 74 accessions of the two species. From a germplasm improvement standpoint resistant accessions of *G. hirsutum* would be the most valuable, and since the most promising 70% of possibly resistant accessions of *G. hirsutum* were tested, it is unlikely that more than one or two moderately resistant accessions remain. Untested accessions of *G. hirsutum* include TX- 21, 22, 48, 150, 228, 241, 245, 249, 502, 1816, 1820, 1914, 1965, 2009, 2013, 2022, 2028, 2058, 2060, and 2061.

In growth chamber experiments, 34 of the 78 (44%) accessions tested suppressed nematode reproduction significantly ($P \leq 0.05$ or 0.01) compared with Deltapine 16 (Table 1). Of these, 28 were classified as resistant or moderately resistant. Accessions within each group in increasing order of resistance are as follows: *G. hirsutum* TX-2469, TX-1586, TX-748, TX-25, TX-1828, and TX-1860; and *G. barbadense* GB-127, GB-1083, GB-1141, GB-1143, TX-110, GB-1147, GB-207, GB-833, GB-210, GB-212, GB-126, GB-581, GB-1113, GB-1081, TX-502, GB-485, GB-536, and GB-262 had more than 10% but less than 34% of the *R. reniformis* observed for Deltapine 16 and were classified moderately resistant. TX-1828, TX-25, and TX-1860 were also root-knot nematode resistant (gall rating ≤ 0.8 on a 0 to 5 scale; $P \leq 0.01$). TX-110 and TX-502 are *G. barbadense* phenotypes from the *G. hirsutum* 'TX' collection. *G. barbadense* GB-49, GB-13, GB-264, GB-171, and GB-713 had less than 11% of the *R. reniformis* observed for Deltapine 16 ($P \leq 0.01$ in all cases) and were classified resistant. *Gossypium barbadense* GB-713 had the highest resistance to *R. reniformis* observed, with only 3% of the nematodes observed for Deltapine 16 in three experiments. *Gossypium hirsutum* lacked

highly resistant accessions; however, the moderately resistant accessions identified have diverse geographical origins, suggesting different genes that might be combined to enhance resistance. The origins of these accessions are Chiapas, Mexico (TX-25); Michoacán, Mexico (TX-748); Haiti (TX-1586, TX-1828); Guadeloupe (TX-1860); and Ceara, Brazil (TX-2469). The accession TX-25 was previously reported root-knot nematode resistant (Shepherd, 1983), but supported 51% and 105% of the *R. reniformis* egg production of a Deltapine control in two pot experiments (Beasley and Jones, 1985).

In conclusion, the *G. barbadense* accessions TX-1348 and TX-110 were reconfirmed to be moderately resistant (nematode population $< 34\%$ that on Deltapine 16) to *R. reniformis* and 17 additional moderately resistant accessions of *G. barbadense* were identified. Five additional resistant accessions were identified that supported $< 10\%$ the population observed on Deltapine 16, and one of these (GB-713) was consistently more resistant than the other four, with 3% of the nematodes per gram soil per gram root observed on Deltapine 16 in three experiments. None of the *G. barbadense* accessions resistant to *R. reniformis* was also resistant to the root-knot nematode *M. incognita*. Six *G. hirsutum* accessions were classified as moderately resistant to *R. reniformis* under the conditions tested, and three of these, TX-25, TX-1828, and TX-1860, were also root-knot nematode resistant. Based on the incidence of resistance observed in growth chamber experiments, there probably are at least one or two additional moderately resistant accessions of *G. hirsutum* in the collection, as well as 40 moderately resistant and 10 or 12 resistant *G. barbadense* accessions. For a list of the 200 most promising accessions see <http://www.ars-grin.gov/cgi-bin/npgs/html/desclist>.

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