JOURNAL OF NEMATOLOGY

VOLUME 48 DECEMBER 2016 NUMBER 4

Journal of Nematology 48(4):223–230. 2016.

Gossypium arboreum Accessions Resistant to *Rotylenchulus reniformis*

SALLIANA R. STETINA AND JOHN E. ERPELDING

Abstract: In the southeastern United States, reniform nematode (*Rotylenchulus reniformis*) is a serious pest of upland cotton (*Gossypium hirsutum*), a species which has no naturally occurring resistance against this nematode. To identify sources of reniform nematode resistance in species closely related to upland cotton, 222 *G. arboreum* accessions from the U.S. germplasm collection were evaluated in repeated growth chamber experiments. In initial screenings, root infection was measured 4 wks after inoculation. The 15 accessions supporting the fewest infections (PI 529992, PI 615755, PI 615766, PI 615788, PI 615848, PI 615856, PI 615950, PI 615977, PI 615991, PI 616008, PI 616016, PI 616062, PI 616126, PI 616159, and A2 553) were evaluated again in confirmation tests lasting 8 wk. The combined totals of nematodes extracted from soil and eggs extracted from roots were analyzed. All 15 accessions tested supported significantly smaller reniform nematode populations than the susceptible controls (*G. hirsutum* cultivar Deltapine 16 and *G. arboreum* accession PI 529729). Nine accessions (PI 529992, PI 615755, PI 615766, PI 615788, PI 615856, PI 615950, PI 615991, PI 616008, and PI 616159) supported reniform nematode populations comparable to the resistant control (*G. arboreum* accession PI 615699), and accession PI 615848 had significantly fewer reniform nematodes than the resistant control. Cotton breeders would benefit from introgressing the newly identified resistance from these accessions into their upland cotton improvement programs.
Key words: cotton, *Gossypium hirsutum*, reniform nematode, resistance

Cotton (*Gossypium hirsutum* L.) farmers from Texas to the Atlantic seaboard experience yield losses as a result of the reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) on an annual basis. Losses to reniform nematode for the 2013, 2014, and 2015 growing seasons averaged 3.3%, 6.1%, and 4.0% for cotton in Louisiana, Mississippi, and Alabama, respectively (Lawrence et al., 2014, 2015, 2016). A number of factors including lack of resistance within commercially available cultivars (Robinson et al., 1999; Usery et al., 2005; Starr et al., 2007), loss of effective soil-applied fumigants and nematicides from the market (Starr et al., 2007; Mueller, 2011), and grower preference for cotton monoculture over crop rotation (Robinson, 2007; Starr et al., 2007) allow nematode survival and reproduction resulting in population densities at or above damaging thresholds at planting and throughout the cropping season.

Host plant resistance would be highly advantageous to cotton growers because it is cost effective, environmentally friendly, simple to deploy, and it persists throughout the entire growing season. The primary reason for the lack of reniform nematode resistant cultivars is the lack of high levels of resistance to this nematode in *G. hirsutum*. Robinson et al. (2004) surveyed more than 1,800 primitive *G. hirsutum* accessions obtained from the U.S. National Plant Germplasm System (NPGS) cotton collection and found only six that were moderately resistant.

Germplasm lines have been released with resistance to reniform nematode derived from relatives of *G. hirsutum*. The tetraploid species *Gossypium barbadense* L. is the source of resistance in several germplasm lines released within the past decade. In 2010, two breeding lines of cotton, TAM RKRNR-9 (PI 662039) and TAM RKRNR-12 (PI 662040), with reniform nematode resistance derived from *G. barbadense* TX 110 (PI 163608) were released (Starr et al., 2011). *Gossypium barbadense* accession GB 713 (PI 608139) was the source of reniform nematode resistance in four other germplasm lines released in 2012. Three lines, M713 Ren1 (PI 665928), M713 Ren2 (PI 665929), and M713 Ren5 (PI 665930), were developed from a cross between *G. barbadense* GB 713 and the *G. hirsutum* cultivar SureGrow 747 (McCarty et al., 2013). The fourth germplasm line, BARBREN-713 (PI 671965), was developed by crossing *G. barbadense* GB 713 with the cultivar Acala NemX, followed by several backcrosses to *G. hirsutum* lines (Bell et al., 2015); this line has resistance to *Meloidogyne incognita* (Kofoid and White) Chitwood in addition to reniform nematode resistance. To date, no commercial cultivars have been released that have these germplasm lines in their pedigrees.

A greater research challenge is the exploitation of the reniform nematode resistance found in diploid *Gossypium* species. Transferring resistance from diploid *Gossypium* species into tetraploid cotton is difficult. Barriers to hybridization between the different species include mechanisms that prevent fertilization or inhibit development of viable seed from successful fertilizations

Received for publication September 1, 2016.
Research Plant Pathologist and Research Geneticist (Plants), USDA ARS Crop Genetics Research Unit, PO Box 345, Stoneville, MS 38776.
Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer. Financial support for this project was provided through USDA ARS project 6066-22000-074-00D. Technical assistance provided by K. Jordan is appreciated.
The authors thank Drs. Nancy Kokalis-Burelle and Linghe Zeng for helpful suggestions during manuscript preparation.
E-mail: sally.stetina@ars.usda.gov.
This paper was edited by Jim Lamondia.

(Brubaker et al., 1999; Mehetre et al., 2003; Mehetre and Aher, 2004; Ganesh Ram et al., 2008). Techniques such as bridging lines (Brubaker et al., 1999; Romano et al., 2009), induced polyploidy (Mehetre et al., 2003), in vitro interspecific fertilization (Liu et al., 1992), protoplast fusion (Sun et al., 2006), and ovule culture (Stewart and Hsu, 1977, 1978; Gill and Bajaj, 1984, 1987) have been used to overcome these breeding limitations.

Immunity to reniform nematode in *G. longicalyx* Hutch. & Lee (Yik and Birchfield, 1984; Stewart and Robbins, 1996); resistance in *G. arboreum* L. (Carter, 1981; Stewart and Robbins, 1995; Sacks and Robinson, 2009), *G. somalense* (Gurke) Hutch. (Yik and Birchfield, 1984), and *G. stocksii* Mast. Ex. Hook. (Yik and Birchfield, 1984); and moderate levels of resistance in *G. aridum* (Sacks and Robinson, 2009), *G. herbaceum* (Yik and Birchfield, 1984), and *G. raimondii* Ulbr. (Yik and Birchfield, 1984), have been reported. With the exception of *G. longicalyx*, in which all accessions tested to date have exhibited immunity, variability in resistance to reniform nematode exists within the diploid *Gossypium* species.

To date, the only germplasm lines released with resistance from a diploid species are LONREN-1 and LONREN-2, with resistance that had been introgressed from *G. longicalyx* (Bell et al., 2014). However, this resistance has been linked to intolerance (Sikkens et al., 2011; Weaver et al., 2013), with plants exhibiting stunting when challenged with high inoculum levels of the nematode. Because of this problem, nearly all breeding programs have stopped using this source of resistance. *Gossypium hirsutum* lines with reniform nematode resistance introgressed from *G. arboreum* accession A2-190 (PI 615699) (Sacks and Robinson, 2009) and *G. arboreum* accession A2-19 (PI 129723) (Avila et al., 2005) have been developed, though no germplasm lines from these programs have been released to date.

Because reniform nematode resistance has just recently become available in upland cotton, no data are available with respect to the durability of any one source of resistance. Variability within reniform nematode has been well documented on a genetic, morphological, and physiological basis (Dasgupta and Seshadri, 1971; Nakasono, 2004; Agudelo et al., 2005b; Arias et al., 2009; McGawley et al., 2010; Leach et al., 2012). Over time, reniform nematode may adapt to one or more resistance sources, as has been documented with development of races in pathogens such as *Phytophthora infestans* (Mont.) de Bary and *Heterodera glycines* Ichinohe. Use of a single source of resistance over time may result in development of nematode biotypes that can reproduce on the resistant cultivar (Young, 1998), so rotation among different resistance sources may be necessary to reduce selection pressure on the nematodes (Starr and Roberts, 2004). If different resistance genes can be identified, they could be combined ("pyramided") into the same plant to make resistance more durable.

The objectives of this research were to evaluate a selection of *Gossypium arboreum* accessions for their reaction to the reniform nematode, and to identify sources of host plant resistance that could be introgressed into upland cotton and used to manage this pathogen.

Materials and Methods

Identification of resistant lines: A total of 222 *G. arboreum* accessions were evaluated in growth chamber tests for resistance to infection by reniform nematode. The specific accessions tested are listed in Tables 1, 2, and 3. Seeds not already in the authors' research collections were obtained from the NPGS (College Station, TX).

Accessions were arbitrarily divided into three screening tests of approximately 75 entries each due to growth chamber space limitations. The susceptible controls *Gossypium hirsutum* cultivar Deltapine 16 (Yik and Birchfield, 1984; Robinson and Percival, 1997) and *G. arboreum* accession PI 529729 (Sacks and Robinson, 2009; Erpelding and Stetina, 2013), and the resistant control *G. arboreum* accession PI 615699 (Sacks and Robinson, 2009) were included in each test. The experimental design for each screening was a completely randomized design with three replications, and each test was repeated. The growth chamber temperature was maintained at 28°C and the daylength was set at 16 hours. Soil moisture was maintained using an automated watering system, with the timing adjusted periodically during the experiment to supply additional water as plants grew.

Screening test protocols were similar to those described by Stetina et al. (2014). Briefly, single plants of each accession were established in conical plastic pots (Ray Leach SL-10 Cone-tainer, Stuewe & Sons, Inc., Tangent, OR) containing 120 cm^3 of a steam-sterilized soil mixture consisting of one part sandy loam soil mixed with two parts sand. Approximately 7 days after planting, soil in each pot was infested with 1,000 reniform nematodes (mixed vermiform life stages) suspended in 1 ml water. Mississippi reniform nematode population MSRR04 (Arias et al., 2009), originally isolated from upland cotton and maintained in a greenhouse on tomato (*Solanum lycopersicon* cultivar Rutgers), was used for all experiments. Plants were harvested 4 wk after inoculation. Shoots were removed at the soil line and discarded. Roots were separated from soil, stained with red food coloring using standard protocols (Thies et al., 2002), and the number of swollen females attached to the roots were counted at ×50 magnification. After counting, roots were allowed to drain briefly on paper towels to remove excess water and fresh weights were recorded. Counts were expressed as females per gram of fresh root tissue to compensate for differences in root sizes.

In addition to statistically comparing root infection levels, accessions within each test were classified based on a nematode index, following that described by Schmitt and Shannon (1992) for soybean cyst nematode.

TABLE 1. Infection of *Gossypium* roots by *Rotylenchulus reniformis* females 4 wk after inoculation in growth chamber Test 1. All accessions are *Gossypium arboreum* except for susceptible control *Gossypium hirsutum* cultivar Deltapine 16.

Accession	Count[a]	Index[b]	Rating[c]
PI 183202	107.1 a	140.3	S
PI 129742	90.8 ab	119.0	S
PI 408772	80.1 ab	105.0	S
PI 529806	76.8 ab	100.7	S
G. hirsutum 'Deltapine 16' (S control)	76.3 ab	100.0	S
PI 529729 (S control)	62.1 ab	81.4	S
PI 615786	51.4 bc	67.4	S
PI 529716	48.6 bcd	63.7	S
PI 408756	47.3 b-e	62.0	S
PI 615753	42.4 b-f	55.6	MS
PI 529750	39.6 b-g	51.9	MS
PI 529719	38.7 b-h	50.7	MS
PI 529720	38.2 b-h	50.1	MS
PI 529712	37.4 b-h	49.0	MS
PI 615745	36.2 b-i	47.4	MS
PI 615757	35.6 c-i	46.6	MS
PI 615756	35.0 c-i	45.9	MS
PI 180244	33.8 c-i	44.3	MS
PI 615752	33.7 c-i	44.1	MS
PI 529787	33.4 c-i	43.8	MS
PI 175033	31.5 c-j	41.2	MS
PI 185786	30.9 c-j	40.5	MS
PI 615761	30.7 c-k	40.2	MS
PI 152088	30.6 c-k	40.1	MS
PI 615739	29.4 c-k	38.5	MS
PI 615797	28.9 c-l	37.9	MS
PI 408755	28.8 c-l	37.7	MS
PI 615785	28.8 c-l	37.7	MS
PI 529722	28.7 c-l	37.6	MS
PI 615763	28.7 c-l	37.6	MS
PI 529762	28.4 c-l	37.2	MS
PI 529802	28.2 c-l	37.0	MS
PI 615772	28.0 c-l	36.6	MS
PI 615765	27.8 c-l	36.4	MS
PI 529794	27.7 c-l	36.3	MS
PI 179607	27.7 c-l	36.3	MS
PI 529759	27.4 c-l	35.9	MS
PI 529754	26.9 c-l	35.3	MS
PI 615771	26.9 c-l	35.3	MS
PI 529764	25.8 c-l	33.9	MS
PI 615700	25.0 d-l	32.8	MS
PI 615795	24.5 d-l	32.2	MS
PI 615751	23.6 e-l	30.9	MS
PI 129723	23.3 f-l	30.5	MS
PI 529756	22.7 f-l	29.8	MR
PI 408764	22.4 f-l	29.3	MR
PI 529714	22.2 f-m	29.1	MR
PI 529751	22.2 f-m	29.1	MR
PI 529780	21.5 f-n	28.2	MR
PI 529784	21.3 f-n	27.9	MR
PI 615782	20.8 f-n	27.2	MR
PI 529774	20.4 f-n	26.8	MR
PI 529713	19.8 g-n	26.0	MR
PI 529788	19.6 g-n	25.7	MR
PI 615767	19.5 g-n	25.6	MR
PI 615783	19.5 g-n	25.6	MR
PI 183168	19.4 g-n	25.5	MR
PI 615787	19.1 g-n	25.1	MR
PI 615743	18.5 h-n	24.3	MR
PI 529708	18.4 h-n	24.1	MR
PI 529749	18.2 i-n	23.9	MR

(*Continued*)

TABLE 1. Continued.

Accession	Count[a]	Index[b]	Rating[c]
PI 180245	17.8 i-n	23.3	MR
PI 442919	16.8 i-n	22.0	MR
PI 529744	16.3 i-n	21.4	MR
PI 615769	15.7 j-n	20.5	MR
PI 615734	14.5 k-o	19.0	MR
PI 615781	14.4 k-o	18.9	MR
PI 615789	14.2 l-o	18.6	MR
PI 529731	14.2 l-o	18.6	MR
PI 615779	13.8 l-o	18.0	MR
PI 615788	10.7 mno	14.1	MR
PI 615755	10.1 no	13.2	MR
PI 615766	7.0 op	9.2	R
PI 615699 (R control)	4.2 p	5.5	R
	$F = 4.08$		
	$P < 0.0001$		

Values are backtransformed means of six replications in two trials combined; means followed by the same letter are not significantly different based on differences of least squares means ($P \leq 0.05$).

[a] Number of females per g of fresh root tissue.

[b] Nematode index; females per g of fresh root tissue expressed as a percentage of the average number observed on the susceptible upland cotton cultivar Deltapine 16.

[c] Rating follows the index described by Schmitt and Shannon (1992) for soybean cyst nematode, where an index <10% is resistant (R), 10% to 30% is moderately resistant (MR), 31% to 60% is moderately susceptible (MS) and >60% is susceptible (S).

Infection on an accession is expressed as a percentage of the average number of females that developed on susceptible *G. hirsutum* cultivar Deltapine 16. Based on the nematode index, accessions were classified as resistant (nematode index <10%), moderately resistant (10% to 30%), moderately susceptible (31% to 60%), or susceptible (>60%).

Confirmation of reaction to reniform nematode: A subset consisting of 15 of the most resistant accessions identified in the initial screening tests was further evaluated in a longer-duration test that measured reniform nematode reproduction. As in the screening tests, the susceptible controls *Gossypium hirsutum* cultivar Deltapine 16 and *G. arboreum* accession PI 529729, and the resistant control *G. arboreum* accession PI 615699 were included. To monitor survival of the nematode with no roots present, a fallow treatment also was included.

Test establishment and inoculation procedures were the same as described for the initial screenings. The experimental design was a completely randomized design with five replications, and the test was repeated. The test duration was extended to 8 wk. At the end of the test, standard elutriation (Byrd et al., 1976) and sucrose centrifugation (Jenkins, 1964) protocols were used to extract vermiform stages of nematodes from all of the soil in each pot. In addition, eggs were extracted from the root system by cutting the roots into 2.5-cm segments, stirring for 10 min in a 0.6% NaOCl solution (Hussey and Barker, 1973), and collecting eggs on a standard 25-µm-pore sieve. Egg and vermiform counts were added together, and total numbers were analyzed.

TABLE 2. Infection of *Gossypium* roots by *Rotylenchulus reniformis* females 4 wk after inoculation in growth chamber Test 2. All accessions are *Gossypium arboreum* except for susceptible control *Gossypium hirsutum* cultivar Deltapine 16.

Accession	Count[a]	Index[b]	Rating[c]
PI 529729 (S control)	53.5 a	123.6	S
G. hirsutum 'Deltapine 16' (S control)	43.3 ab	100.0	S
PI 615902	39.6 abc	91.4	S
PI 615898	38.4 a-d	88.6	S
PI 615877	38.1 a-e	88.0	S
PI 615890	36.0 a-f	83.2	S
PI 615895	31.9 a-g	73.7	S
PI 615879	30.7 a-h	71.0	S
PI 615853	30.2 a-h	69.7	S
PI 615826	27.4 a-i	63.3	S
PI 615824	26.7 a-i	61.8	S
PI 615894	26.5 a-i	61.3	S
PI 615876	25.9 a-j	59.8	MS
PI 615866	25.9 a-j	59.7	MS
PI 615911	24.9 b-k	57.6	MS
PI 615838	24.7 b-k	57.1	MS
PI 615886	24.5 b-k	56.6	MS
PI 615860	24.2 b-k	55.8	MS
PI 615800	23.9 b-k	55.2	MS
PI 615920	23.7 b-l	54.7	MS
PI 615903	23.6 b-l	54.4	MS
PI 615924	23.1 b-m	53.3	MS
PI 615875	22.6 b-m	52.2	MS
PI 615798	22.4 b-n	51.7	MS
PI 615814	22.4 b-n	51.7	MS
PI 615812	22.2 b-n	51.2	MS
PI 615872	21.9 b-n	50.7	MS
PI 615806	21.2 b-o	49.0	MS
PI 615807	20.8 b-o	47.9	MS
PI 615865	20.1 c-o	46.5	MS
PI 615884	19.4 c-o	44.7	MS
PI 615873	19.3 c-o	44.5	MS
PI 615867	18.8 c-o	43.4	MS
PI 615809	18.6 d-o	42.9	MS
PI 615845	18.3 d-o	42.3	MS
PI 615912	18.3 d-o	42.3	MS
PI 615834	17.8 e-o	41.2	MS
PI 615802	17.5 e-p	40.5	MS
PI 615822	17.2 f-q	39.8	MS
PI 615846	16.9 g-q	39.1	MS
PI 615849	16.9 g-q	39.1	MS
PI 615870	16.6 g-r	38.3	MS
PI 615881	16.3 g-r	37.6	MS
PI 615926	16.2 g-s	37.6	MS
PI 615843	16.2 g-s	37.3	MS
PI 615893	16.0 g-s	37.0	MS
PI 615819	15.9 g-s	36.8	MS
PI 615909	15.9 g-s	36.8	MS
PI 615878	15.2 g-s	35.0	MS
PI 615815	14.8 h-s	34.1	MS
PI 615839	14.1 i-s	32.6	MS
PI 615821	14.0 i-s	32.3	MS
PI 615699 (R control)	13.3 i-s	30.6	MS
PI 615836	12.9 i-s	29.8	MR
PI 615851	12.7 i-s	29.3	MR
PI 615811	12.3 j-s	28.4	MR
PI 615818	12.2 j-s	28.3	MR
PI 615810	12.1 k-s	27.9	MR
PI 615816	12.1 k-s	27.9	MR
PI 615852	11.0 l-s	25.4	MR
PI 615854	11.0 l-s	25.4	MR
PI 615817	10.9 l-s	25.2	MR
PI 615801	10.9 l-s	25.2	MR
PI 615871	10.7 m-s	24.7	MR
PI 615907	10.7 m-s	24.7	MR
PI 615891	10.6 m-s	24.5	MR
PI 615888	10.5 n-s	24.2	MR
PI 615844	10.4 n-s	24.1	MR
PI 615858	10.1 o-s	23.4	MR
PI 615805	10.0 o-s	23.2	MR
PI 615889	9.3 o-s	21.5	MR
PI 615830	8.8 o-s	20.3	MR
PI 615804	8.0 p-s	18.6	MR
PI 615823	7.9 qrs	18.3	MR
PI 615914	7.7 rs	17.7	MR
PI 615813	7.3 rs	16.8	MR
PI 615885	6.5 s	15.1	MR
PI 615848	6.4 s	14.8	MR
PI 615856	6.2 s	14.3	MR
	$F = 2.90$		
	$P < 0.0001$		

(*Continued*)

TABLE 2. Continued.

Values are backtransformed means of six replications in two trials combined; means followed by the same letter are not significantly different based on differences of least squares means ($P \leq 0.05$).

[a] Number of females per g of fresh root tissue.

[b] Nematode index; females per g of fresh root tissue expressed as a percentage of the average number observed on the susceptible upland cotton cultivar Deltapine 16.

[c] Rating follows the index described by Schmitt and Shannon (1992) for soybean cyst nematode, where an index <10% is resistant (R), 10% to 30% is moderately resistant (MR), 31% to 60% is moderately susceptible (MS) and >60% is susceptible (S).

In addition to statistically comparing reniform nematode population sizes, a reproduction factor was determined for each of the accessions. The reproduction factor is calculated by dividing the number of nematodes per pot at the end of test by the initial inoculum level of 1,000 nematodes. Reproduction factor values of 1.0 or more indicate that the plant is a good host for the nematode; poor hosts have values smaller than 1.0 (Walters et al., 1996).

Statistical analysis: Prior to analysis of variance (ANOVA), nematode counts were subjected to $\log_{10}(x+1)$ transformation to normalize data; backtransformed means are presented. Initial data analyses identified no significant differences between trials, and no significant interactions between trial and accession. Therefore, data from both trials of each identification and confirmation test were combined for final analysis, and trials and their interactions were modeled as random effects. Where significant differences among genotypes were found using ANOVA, differences of least squares means ($P \leq 0.05$) were used to compare means. SAS statistical software (PROC MIXED; SAS Institute, Cary, NC) was used for analysis.

RESULTS

The reactions to reniform nematode for all 222 *G. arboreum* accessions evaluated are presented in Tables 1, 2, and 3. The susceptible controls were significantly

TABLE 3. Infection of *Gossypium* roots by *Rotylenchulus reniformis* females 4 wk after inoculation in growth chamber Test 3. All accessions are *Gossypium arboreum* except for susceptible control *Gossypium hirsutum* cultivar Deltapine 16.

Accession	Count[a]	Index[b]	Rating[c]
PI 616101	64.9 a	110.4	S
PI 529729 (S control)	62.9 a	107.0	S
G. hirsutum 'Deltapine 16' (S control)	58.8 a	100.0	S
PI 529983	36.5 ab	62.1	S
A2 545[d]	34.4 ab	58.5	MS
PI 615949	32.6 ab	55.5	MS
PI 529980	32.4 ab	55.2	MS
PI 616078	32.3 ab	54.9	MS
PI 616157	31.9 abc	54.3	MS
PI 616086	30.2 a-d	51.3	MS
PI 616025	30.2 a-d	51.3	MS
PI 615969	29.9 a-d	50.8	MS
PI 616097	28.0 a-e	47.7	MS
PI 616076	26.5 a-f	45.1	MS
PI 616104	26.0 a-f	44.3	MS
PI 616107	25.0 a-f	42.5	MS
PI 616010	24.6 a-f	41.8	MS
PI 615967	24.2 a-f	41.2	MS
PI 616154	23.3 a-g	39.5	MS
PI 616156	21.8 a-h	37.1	MS
PI 616160	20.9 a-i	35.5	MS
PI 616132	20.0 a-j	34.0	MS
PI 529986	19.5 b-j	33.2	MS
PI 529979	18.6 b-j	31.7	MS
A2 543[d]	18.1 b-k	30.8	MS
PI 615978	17.6 b-k	29.9	MR
PI 615942	17.4 b-k	29.6	MR
PI 616069	17.3 b-k	29.5	MR
PI 615927	17.2 b-k	29.2	MR
PI 616083	16.9 b-k	28.8	MR
PI 616113	16.8 b-k	28.6	MR
PI 615971	16.7 b-k	28.5	MR
PI 615968	16.3 b-k	27.7	MR
PI 529985	16.2 b-k	27.6	MR
PI 616144	16.1 b-k	27.4	MR
PI 616134	16.0 b-k	27.2	MR
PI 616005	15.5 b-l	26.4	MR
PI 615933	15.4 b-l	26.2	MR
PI 616085	15.1 b-l	25.6	MR
PI 615931	14.5 b-l	24.7	MR
PI 616021	14.4 b-l	24.4	MR
PI 615970	14.1 b-l	24.0	MR
PI 616109	13.9 b-m	23.7	MR
PI 616072	13.7 b-m	23.4	MR
PI 615986	13.3 b-m	22.6	MR
PI 616023	12.9 b-m	21.9	MR
PI 616098	12.5 b-m	21.3	MR
PI 616118	12.5 b-m	21.3	MR
PI 615932	12.1 c-n	20.6	MR
PI 616057	12.1 c-n	20.6	MR
PI 616077	12.0 c-n	20.4	MR
PI 616080	11.6 d-n	19.7	MR
PI 616007	11.2 e-n	19.1	MR
PI 616111	11.0 e-n	18.7	MR
PI 615995	10.9 e-n	18.5	MR
PI 615972	10.8 e-n	18.4	MR
PI 616121	10.7 e-n	18.2	MR
PI 615960	10.7 e-n	18.2	MR
PI 616015	10.4 f-n	17.7	MR
PI 616158	9.5 f-n	16.1	MR
PI 615983	9.1 f-n	15.5	MR
PI 616151	9.1 f-n	15.5	MR
PI 616004	9.0 f-n	15.2	MR
PI 616068	8.6 g-n	14.7	MR
PI 616084	8.5 h-n	14.4	MR
PI 616108	7.9 i-n	13.5	MR
PI 529989	7.8 i-n	13.3	MR
PI 616126	7.2 j-n	12.3	MR
PI 616159	6.9 j-n	11.8	MR
PI 616062	6.8 k-n	11.5	MR
PI 616016	6.6 k-o	11.3	MR
A2 553[d]	5.6 l-o	9.5	R
PI 615991	5.6 l-o	9.5	R
PI 615699 (R control)	4.9 mno	8.4	R
PI 616008	4.3 no	7.4	R
PI 615950	3.6 no	6.0	R
PI 529992	3.2 no	5.5	R
PI 615977	1.9 o	3.3	R
	$F = 2.90$		
	$P < 0.0001$		

(*Continued*)

TABLE 3. Continued.

Values are backtransformed means of six replications in two trials combined; means followed by the same letter are not significantly different based on differences of least squares means ($P \leq 0.05$).

[a] Number of females per g of fresh root tissue.

[b] Nematode index; females per g of fresh root tissue expressed as a percentage of the average number observed on the susceptible upland cotton cultivar Deltapine 16.

[c] Rating follows the index described by Schmitt and Shannon (1992) for soybean cyst nematode, where an index <10% is resistant (R), 10% to 30% is moderately resistant (MR), 31% to 60% is moderately susceptible (MS) and >60% is susceptible (S).

[d] Site identifier; no current PI designation in the U.S. National Plant Germplasm System.

different from the resistant control in each of the three tests based on the number of females infecting the roots, although the number of infections on the resistant control was higher than expected in Test 2. These initial screening experiments identified 19 susceptible, 96 moderately susceptible, 100 moderately resistant, and 7 resistant accessions in total.

Though not statistically distinguishable from the control, four accessions classified as resistant had lower infection indices than the resistant control: PI 529992, PI 615950, PI 615977, and PI 616008 (Table 3). At the other end of the spectrum, five accessions classified as susceptible had higher infection indices than the susceptible controls: PI 183202, PI 129742, PI 408772, PI 529806 (Table 1); and PI 616101 (Table 3).

The 15 most resistant accessions identified in the initial screenings were tested again in longer experiments to confirm their reaction to the reniform nematode (Table 4). All accessions tested reduced reniform nematode populations compared to the susceptible controls. Nine accessions were comparable to the resistant control with respect to final population sizes, and accession PI 615848 supported significantly smaller reniform nematode populations than the resistant control. However, none of the accessions suppressed the populations to the same level as the fallow treatment. A comparison of the reproduction factors showed that 14 accessions and the

TABLE 4. Comparison of reniform nematode population development on 17 *Gossypium arboreum* accessions, the susceptible control *Gossypium hirsutum* cultivar Deltapine 16, and one fallow treatment in a growth chamber.

Treatment	Nematodes per container[a]	Reproduction factor[b]
G. hirsutum 'Deltapine 16' (S control)	85,999 a	89.6 a
PI 529729 (S control)	46,902 a	51.0 b
A2 553[c]	10,883 b	14.8 c
PI 616062	8,326 bc	9.2 cd
PI 616016	4,308 cd	5.3 de
PI 615977	3,657 de	4.0 de
PI 616126	3,257 def	4.3 de
PI 615991	2,954 d-g	3.5 de
PI 615766	2,909 d-g	3.2 de
PI 615788	2,896 d-g	3.2 de
PI 615950	2,002 e-h	2.9 de
PI 616159	1,790 f-i	2.1 de
PI 615699 (R control)	1,665 ghi	2.2 de
PI 529992	1,426 hi	1.7 de
PI 615755	1,085 hij	1.5 e
PI 615856	1,057 hij	1.3 e
PI 616008	954 ij	1.0 e
PI 615848	707 j	0.8 e
fallow	136 k	0.2 e
	$F = 40.04$ $P < 0.0001$	$F = 65.75$ $P < 0.0001$

Values are backtransformed means of 10 replications in two trials combined; means followed by the same letter are not significantly different based on differences of least squares means ($P \leq 0.05$).

[a] Vermiform stages in 120 cm^3 soil plus root-associated eggs extracted 8 wk after inoculation with 1,000 reniform nematodes.

[b] Reproduction factor is calculated by dividing the number of nematodes per pot at the end of the test by the initial inoculum level of 1,000 nematodes.

[c] Site identifier; no current PI designation in the U.S. National Plant Germplasm System.

fallow treatment were comparable to the resistant control, though only PI 615848 and the fallow treatment had reproduction factors less than 1.0, indicative of poor host status.

DISCUSSION

Ten *G. arboreum* accessions were identified as resistant to reniform nematode in both initial screening and subsequent confirmation tests. This conclusion was based on the number of females infecting the roots and on the nematode population development in growth chamber tests as compared to the resistant control *G. arboreum* accession PI 615699. The nine accessions that were comparable to the resistant control in supporting reniform nematode population development were PI 529992, PI 615755, PI 615766, PI 615788, PI 615856, PI 615950, PI 615991, PI 616008, and PI 616159. One accession, PI 615848, was more effective than the resistant control at suppressing reniform nematode population development, and had a reproduction factor of 0.8, indicative of poor host status. All of these sources supported 3% or less of the reniform nematode population development that was observed on the susceptible *G. hirsutum* control cultivar Deltapine 16. As such, any of them would be excellent candidates for inclusion in a germplasm improvement program.

Results from this study indicate that a reduced number of infections and smaller population sizes are associated with the 10 resistant accessions identified. However, specific mechanisms governing the successful establishment and maintenance of a feeding site, the rate of nematode development, and the number of eggs produced by each female were not evaluated (Agudelo et al., 2005a; Starr et al., 2011; Stetina, 2015), though any or all of these factors could be contributing to the observed resistance. Discerning the mechanism(s) behind the observed reniform nematode population suppression could be the subject of future research.

Within the subset of 222 accessions that were tested from the *G. arboreum* germplasm collection, the plants were divided fairly evenly between the resistant and susceptible ends of the reniform nematode resistance spectrum. Most of the accessions tested were classified as either moderately resistant or moderately susceptible based on root infection levels, with only a few lines initially identified as resistant. The subset of accessions tested represents only about 12% of the *G. arboreum* collection. A significant time investment will have to be made to screen the remainder of the accessions using the methods employed in this study. To facilitate discovery of new sources of resistance in this germplasm collection, molecular markers associated with the resistance already documented are needed. The markers could be used to rapidly evaluate the remaining accessions to identify accessions having similar DNA banding patterns as resistant accessions so that future screening efforts could be directed toward identifying putatively unique types of resistance.

In the screening experiments, 19 accessions susceptible to the reniform nematode were identified. Of these, PI 129742, PI 183202, PI 408772, PI 529806, and PI 616101 had higher female indices than the susceptible controls. While these accessions are not useful for developing cultivars resistant to reniform nematode, they do have utility in understanding how resistance is controlled. Populations from crosses between the susceptible and resistant accessions can be studied to determine how resistance is inherited, to identify molecular markers for resistance, and to map the location of the gene(s) conferring resistance.

A limitation of this study is that the accessions were screened using a single isolate of reniform nematode. There are reports in the literature documenting cotton (Agudelo et al., 2005b; Arias et al., 2009; McGawley et al., 2010) and soybean (Agudelo et al., 2005b; McGawley et al., 2011) lines responding differently to unique geographic populations of reniform nematode. Therefore, the accessions identified as resistant in this study could show a different level of resistance if challenged with different populations of the nematode.

In summary, this research provides new phenotypic information on 222 *G. arboreum* accessions, including the identification of 10 accessions with useful levels of reniform nematode resistance. Public and private cotton breeding programs could benefit from using these resistant accessions as parents, although there may be challenges related to the introgression of the resistance that were not evaluated in this study.

Literature Cited

Agudelo, P. A., Robbins, R. T., Kim, K. S., and Stewart, J. M. 2005a. Histological changes in *Gossypium hirsutum* associated with reduced reproduction of *Rotylenchulus reniformis*. Journal of Nematology 37:185–189.

Agudelo, P. A., Robbins, R. T., Stewart, J. M., and Szalanski, A. L. 2005b. Intraspecific variability of *Rotylenchulus reniformis* from cotton-growing regions in the United States. Journal of Nematology 37:105–114.

Arias, R. S., Stetina, S. R., Tonos, J. L., Scheffler, J. A., and Scheffler, B. A. 2009. Microsatellites reveal genetic diversity in *Rotylenchulus reniformis* populations. Journal of Nematology 41:146–156.

Avila, C. A., Stewart, J. M., and Robbins, R. T. 2005. Transfer of reniform nematode resistance from diploid cotton species to tetraploid cultivated cotton. P. 182 *in* Proceedings of the Beltwide Cotton Conferences, New Orleans, LA. Cordova: National Cotton Council. (Abstr.).

Bell, A. A., Robinson, A. F., Quintana, J., Dighe, N. D., Menz, M. A., Stelly, D. M., Zheng, X., Jones, J. E., Overstreet, C., Burris, E., Cantrell, R. G., and Nichols, R. L. 2014. Registration of LONREN-1 and LONREN-2 germplasm lines of Upland cotton resistant to reniform nematode. Journal of Plant Registrations 8:187–190.

Bell, A. A., Robinson, A. F., Quintana, J., Duke, S. E., Starr, J. L., Stelly, D. M., Zheng, X., Prom, S., Saladino, V., Gutiérrez, O. A., Stetina, S. R., and Nichols, R. L. 2015. Registration of BARBREN-713 germplasm line of Upland cotton resistant to reniform and root-knot nematodes. Journal of Plant Registrations 9:89–93.

Brubaker, C. L., Brown, A. D. H., Stewart, J. M., Kilby, M. J., and Grace, J. P. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. Euphytica 108:199–213.

Byrd, D. W., Jr., Barker, K. R., Ferris, H., Nusbaum, C. J., Griffin, W. E., Small, R. H., and Stone, C. A. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206–212.

Carter, W. W. 1981. Resistance and resistant reaction of *Gossypium arboreum* to the reniform nematode, *Rotylenchulus reniformis*. Journal of Nematology 13:368–374.

Dasgupta, D. R., and Seshadri, A. R. 1971. Reproduction, hybridization, and host adaptation in physiological races of the reniform nematode, *Rotylenchulus reniformis*. Indian Journal of Nematology 1:128–144.

Erpelding, J. E., and Stetina, S. R. 2013. Genetics of reniform nematode resistance in *Gossypium arboreum* germplasm line PI 529728. World Journal of Agricultural Research 1:48–53.

Ganesh Ram, S., Hari Ramakrishnan, S., Thiruvengadam, V., and Kannan Bapu, J. R. 2008. Prefertilization barriers to interspecific hybridization involving *Gossypium hirsutum* and four diploid wild species. Plant Breeding 127:295–300.

Gill, M. S., and Bajaj, Y. P. S. 1984. Interspecific hybridization in the genus *Gossypium* through embryo culture. Euphytica 33:305–311.

Gill, M. S., and Bajaj, Y. P. S. 1987. Hybridization between diploid (*Gossypium arboreum*) and tetraploid (*Gossypium hirsutum*) cotton through ovule culture. Euphytica 36:625–630.

Hussey, R. S., and Barker, K. R. 1973. A comparison of methods for collecting inocula for *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Lawrence, K., Hagan, A., Olsen, M., Faske, T., Hutmacher, R., Mueller, J., Wright, D., Kemerait, B., Overstreet, C., Price, P., Lawrence, G., Allen, T., Atwell, S., Thomas, S., Goldberg, N., Edmisten, K., Boman, R., Young, H., Woodward, J., and Mehl, H. 2016. Cotton disease loss estimate committee report, 2015. Pp. 113–115 *in* Proceedings of the 2016 Beltwide Cotton Conferences, New Orleans, LA. Cordova: National Cotton Council.

Lawrence, K., Olsen, M., Faske, T., Hutmacher, R., Muller, J., Mario, J., Kemerait, R., Overstreet, C., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R., Young, H., Woodward, J., and Mehl, H. 2014. Cotton disease loss estimate committee report, 2013. Pp. 247–248 *in* Proceedings of the 2014 Beltwide Cotton Conferences, New Orleans, LA. Cordova: National Cotton Council.

Lawrence, K. S., Olsen, M., Faske, T., Hutmacher, R., Mueller, J., Mario, J., Kemerait, B., Overstreet, C., Price, P., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R., Young, H., Woodward, J., and Mehl, H. 2015. Cotton disease loss estimate committee report, 2014. Pp. 188–190 *in* Proceedings of the 2015 Beltwide Cotton Conferences, San Antonio, TX. Cordova: National Cotton Council.

Leach, M., Agudelo, P., and Lawton-Rauh, A. 2012. Genetic variability of *Rotylenchulus reniformis*. Plant Disease 96:30–36.

Liu, C., Shun, J., and Liu, J. 1992. In vitro interspecific fertilization, embryo development and formation of hybrid seedlings between *Gossypium hirsutum* and *G. arboreum*. Euphytica 60:79–88.

McCarty, J. C., Jr., Jenkins, J. N., Wubben, M. J., Gutiérrez, O. A., Hayes, R. W., Callahan, F. E., and Deng, D. 2013. Registration of three germplasm lines of cotton derived from *Gossypium barbadense* accession GB713 with resistance to the reniform nematode. Journal of Plant Registrations 7:220–223.

McGawley, E. C., Overstreet, C., and Pontif, M. J. 2011. Variation in reproduction and pathogenicity of geographic isolates of *Rotylenchulus reniformis* on soybean. Nematropica 41:12–22.

McGawley, E. C., Pontif, M. J., and Overstreet, C. 2010. Variation in reproduction and pathogenicity of geographic isolates of *Rotylenchulus reniformis* on cotton. Nematropica 40:275–288.

Mehetre, S. S., and Aher, A. R. 2004. Embryo rescue: a tool to overcome incompatible interspecific hybridization in *Gossypium* Linn.—a review. Indian Journal of Biotechnology 3:29–36.

Mehetre, S. S., Aher, A. R., Gawande, V. L., Patil, V. R., and Mokate, A. S. 2003. Induced polyploidy in *Gossypium*: A tool to overcome interspecific incompatibility of cultivated tetraploid and diploid cottons. Current Science 84:1510–1512.

Mueller, J. D. 2011. The use of Temik® 15G on cotton and soybean in the southeast. Pp. 208–214 *in* Proceedings of the 2011 Beltwide Cotton Conferences, Atlanta, GA. Cordova: National Cotton Council.

Nakasono, K. 2004. Studies on morphological and physio-ecological variations of the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940 with an emphasis on differential geographic distribution of amphimictic and parthenogenetic populations in Japan. Journal of Nematology 36:356–420.

Robinson, A. F. 2007. Reniform in U.S. cotton: where, when why and some remedies. Annual Review of Phytopathology 45:11.1–11.25.

Robinson, A. F., Bridges, A. C., and Percival, A. E. 2004. New sources of resistance to the reniform (*Rotylenchulus reniformis*) and root-knot (*Meloidogyne incognita*) nematode in upland (*Gossypium hirsutum*) and sea island (*G. barbadense*) cotton. Journal of Cotton Science 8:191–197.

Robinson, A. F., Cook, C. G., and Percival, A. E. 1999. Resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 in the major cotton cultivars planted since 1950. Crop Science 39:850–858.

Robinson, A. F., and Percival, A. E. 1997. Resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico. Supplement to the Journal of Nematology 29:746–755.

Romano, G. B., Sacks, E. J., Stetina, S. R., Robinson, A. F., Fang, D. D., Gutiérrez, O. A., and Scheffler, J. A. 2009. Identification and genomic location of a reniform nematode (*Rotylenchulus reniformis*) resistance locus (*Ren*^{ari}) introgressed from *Gossypium aridum* into upland cotton (*G. hirsutum*). Theoretical and Applied Genetics 120:139–150.

Sacks, E. J., and Robinson, A. F. 2009. Introgression of resistance to reniform nematode (*Rotylenchulus reniformis*) into upland cotton (*Gossypium hirsutum*) from *Gossypium arboreum* and a *G. hirsutum/ Gossypium aridum* bridging line. Field Crops Research 112:1–6.

Schmitt, D. P., and Shannon, G. 1992. Differentiating soybean responses to *Heterodera glycines* races. Crop Science 32:275–277.

Sikkens, R. B., Weaver, D. B., Lawrence, K. S., Moore, S. R., and van Santen, E. 2011. LONREN Upland cotton germplasm response to *Rotylenchulus reniformis* inoculum level. Nematropica 41:68–74.

Starr, J. L., Koenning, S. R., Kirkpatrick, T. L., Robinson, A. F., Roberts, P. A., and Nichols, R. L. 2007. The future of nematode management in cotton. Journal of Nematology 39:283–294.

Starr, J. L., and Roberts, P. A. 2004. Resistance to plant-parasitic nematodes. Pp. 879–907 *in* Z. X. Chen, S. Y. Chen, and D. W. Dickson, eds. Nematology Advances and Perspectives, Volume 2: Nematode Management and Utilization. Wallingford: CABI Publishing.

Starr, J. L., Smith, C. W., Ripple, K., Zhou, E., Nichols, R. L., and Faske, T. R. 2011. Registration of TAM RKRNR-9 and TAM RKRNR-12 germplasm lines of upland cotton resistant to reniform and root-knot nematodes. Journal of Plant Registrations 5:393–396.

Stetina, S. R. 2015. Postinfection development of *Rotylenchulus reniformis* on resistant *Gossypium barbadense* accessions. Journal of Nematology 47:302–309.

Stetina, S. R., Smith, J. R., and Ray, J. D. 2014. Identification of *Rotylenchulus reniformis* resistant *Glycine* lines. Journal of Nematology 46:1–7.

Stewart, J. McD., and Hsu, C. L. 1977. In-ovulo embryo culture and seedling development of cotton *(Gossypium hirsutum* L.). Planta 137:113–117.

Stewart, J. McD., and Hsu, C. L. 1978. Hybridization of diploid and tetraploid cottons through in-ovulo embryo culture. Journal of Heredity 69:404–408.

Stewart, J. M., and Robbins, R. T. 1995. Evaluation of Asiatic cottons for resistance to reniform nematode. Pp. 165–168 *in* D. M. Oosterhuis, ed. Proceedings of the 1994 Cotton Research Meeting and 1994 Summaries of Cotton Research in Progress. Special Report 166. Arkansas Agricultural Experiment Station, Fayetteville, AR.

Stewart, J. M., and Robbins, R. T. 1996. Identification and enhancement of resistance to reniform nematode in cotton germplasm. P. 225 *in* Proceedings of the Beltwide Cotton Conferences, Nashville, TN. Cordova: National Cotton Council.

Sun, Y., Nie, Y., Guo, X., Huang, C., and Zhang, X. 2006. Somatic hybrids between *Gossypium hirsutum* L. (4x) and *G. davidsonii* Kellog (2x) produced by protoplast fusion. Euphytica 151:393–400.

Thies, J. A., Merrill, S. B., and Corley, E. L. 2002. Red food coloring stain: new safer procedures for staining nematodes in roots and egg masses on root surfaces. Journal of Nematology 34:121–133.

Usery, S. R., Jr., Lawrence, K. S., Lawrence, G. W., and Burmester, C. H. 2005. Evaluation of cotton cultivars for resistance and tolerance to *Rotylenchulus reniformis*. Nematropica 35:121–133.

Walters, S. A., Wehner, T. C., and Barker, K. R. 1996. NC-42 and NC-43: Root-knot nematode-resistant cucumber germplasm. HortScience 31:1246–1247.

Weaver, D. B., Sikkens, R. B., Lawrence, K. S., Sürmelioğlu, Ç., van Santen, E., and Nichols, R. L. 2013. *REN*^{lon} and its effects on agronomic and fiber traits in Upland cotton. Crop Science 53:913–920.

Yik, C.-P., and Birchfield, W. 1984. Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus reniformis*. Journal of Nematology 16:146–153.

Young, L. D. 1998. Breeding for nematode resistance. Pp. 187–207 *in* K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant Nematode Interactions. Madison: American Society of Agronomy.