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Postinfection Development of *Rotylenchulus reniformis* on Resistant *Gossypium barbadense* Accessions

Salliana R. Stetina

Abstract: The reniform nematode (*Rotylenchulus reniformis*) causes significant cotton (*Gossypium hirsutum*) losses in the southeastern United States. The research objective was to describe the effects of two resistant *G. barbadense* lines (cultivar TX 110 and accession GB 713) on development and fecundity of reniform nematode. Nematode development and fecundity were evaluated on the resistant lines and susceptible *G. hirsutum* cultivar Deltapine 16 in three repeated growth chamber experiments. Nematode development on roots early and late in the infection cycle was measured at set intervals from 1 to 25 d after inoculation (DAI) and genotypes were compared based on the number of nematodes in four developmental stages (vermiform, swelling, reniform, and gravid). At 15, 20, and 25 DAI, egg production by individual females parasitizing each genotype was measured. Unique reniform nematode developmental patterns were noted on each of the cotton genotypes. During the early stages of infection, infection and development occurred 1 d faster on susceptible cotton than on the resistant genotypes. Later, progression to the reniform and gravid stages of development occurred first on the susceptible genotype, followed by *G. barbadense* cultivar TX 110, and finally *G. barbadense* accession GB 713. Egg production by individual nematodes infecting the three genotypes was similar. This study corroborates delayed development previously reported on *G. barbadense* cultivar TX 110 and is the first report of delayed infection and development associated with *G. barbadense* accession GB 713. The different developmental patterns in the resistant genotypes suggest that unique or additional loci may confer resistance in these two lines.

Key words: cotton, *Gossypium*, reniform nematode, resistance, *Rotylenchulus reniformis*.

The reniform nematode (*R. reniformis*) is found throughout the southern United States, from Texas to the east coast. This nematode parasitizes more than 300 plant species including cotton (*G. hirsutum*), a major crop in the region (Robinson et al., 1997). Reniform nematode causes the greatest cotton losses in the states of Alabama, Louisiana, and Mississippi where losses in 2012 and 2013 averaged 4.5%, 4.0%, and 5.3%, respectively (Blasingame and Patel, 2013; Lawrence et al., 2014). Plants infected with reniform nematode have fewer, smaller bolls than noninfected plants (Jones et al., 1959) and lint percentage (the ratio of lint to seed in the harvested cotton) also is reduced (Jones et al., 1959; Cook and Namken, 1994).

Though there are no commercially available cotton cultivars with resistance to reniform nematode (Robinson et al., 1999; Robinson, 2007), research to develop germplasm with resistance is underway in several laboratories (Koenning et al., 2004; Robinson, 2007; Starr et al., 2007). In 1987, four germplasm lines with moderate resistance to reniform nematode (La. RN 4-4, La. RN 909, La. RN 910, and La. RN 1032) derived from *G. hirsutum* were developed by researchers at Louisiana State University in Baton Rouge, and these breeding lines represented the first upland cotton lines with any reported resistance to reniform nematode (Jones et al., 1988). Almost a decade later, these original releases were credited as the reniform nematode resistance source for four more improved germplasm lines (N220-1-91, N222-1-91, N320-2-91, and N419-1-91) released by USDA Agricultural Research Service (ARS) scientists in Texas (Cook et al., 1997). However, it appears that these lines never were used to successfully develop a marketable resistant cotton cultivar. The 2011 release of three more upland cotton lines (MT2468 Ren1, MT2468 Ren2, and MT2468 Ren3) derived from *G. hirsutum* primitive race accession T2468 by researchers at the USDA ARS and the Mississippi Agricultural and Forestry Experiment Station (McCarty et al., 2012) is the latest attempt to make the moderate level of resistance found in *G. hirsutum* available to cotton breeders.

Because there is little resistance available in upland cotton and because what resistance exists is moderate at best, researchers are working to introgress resistance from related species including *Gossypium arboreum* (Sacks and Robinson, 2009), *Gossypium aridum* (Romano et al., 2009), *Gossypium longicalyx* (Robinson et al., 2007; Bell et al., 2014), and *G. barbadense* (Starr et al., 2011; McCarty et al., 2013; Bell et al., 2015). Currently, there have been no germplasm releases with resistance from either *G. arboreum* or *G. aridum*.

LONREN-1 and LONREN-2, released in 2007 (Bell et al., 2014), were the first upland cotton germplasm lines released with introgressed reniform nematode resistance; the resistance was derived from *G. longicalyx* (Robinson et al., 2007). Unfortunately, reduced root growth and associated stunting of these lines and progeny derived from them under high reniform nematode pressure (Sikkens et al., 2011) has limited their utility. To date, no commercial cultivars have been released that utilize this source of resistance.

Robinson et al. (2004) reported moderate resistance in *G. barbadense* cultivar TX 110 (PI 163608) and resistance

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Research Plant Pathologist, USDA ARS Crop Genetics Research Unit, PO Box 345, Stoneville, MS 38776.

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E-mail: Sally.Stetina@ars.usda.gov

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in *G. barbadense* accession GB 713 (PI 608139). These two lines are the source of reniform nematode resistance in recent germplasm releases. Scientists at Texas AgriLife Research released two germplasm lines (TAM RKRNR-9 and TAM RKRNR-12) derived from *G. barbadense* cultivar TX 110 in 2011 (Starr et al., 2011). These lines were selected for resistance to reniform nematodes based primarily on nematode reproduction measured under controlled conditions in greenhouse experiments. Scientists at the USDA ARS and the Mississippi Agricultural and Forestry Experiment Station utilized *G. barbadense* accession GB 713 as the source of reniform nematode resistance for three germplasm lines (M713 Ren1, M713 Ren2, and M713 Ren5) released in 2011 (McCarty et al., 2013). Marker-assisted selection using simple sequence repeat markers for three quantitative trait loci (QTLs) identified in *G. barbadense* accession GB 713 (Gutiérrez et al., 2011) were used to develop these lines, along with egg mass scores and egg counts from greenhouse experiments. Plants are reported to be similar in stature and agronomic characteristics to their adapted parent, cultivar SureGrow 747 (McCarty et al., 2013). In 2012, the germplasm line BARBREN-713 was released by scientists at the USDA ARS, Mississippi Agricultural and Forestry Experiment Station, Texas AgriLife Research, and Cotton Incorporated (Bell et al., 2015). Selection of individual plants was based primarily on soil populations of reniform nematode supported under both controlled environment and field conditions. Reniform nematode resistance in this line is conferred by two loci, Ren^{barb2} on chromosome 21 and Ren^{barb3} on chromosome 18 (Gutierrez et al., 2011). BARBREN-713 does not have the stunting and associated yield reductions noted in the LONREN lines (Sikkens et al., 2012). Because the germplasm lines derived from *G. barbadense* are agronomically superior to the LONREN lines, they are expected to be more widely integrated into cotton improvement programs as a source of reniform nematode resistance, and the original *G. barbadense* sources of resistance were chosen as the subject of this investigation.

All of the germplasm releases with reniform nematode resistance were based on a reduction in the reniform nematode population; however, the life stage(s) assessed varied from one breeding effort to the next. Though smaller nematode populations were supported on resistant lines, the mechanism or mechanisms contributing to that reduction are not well defined. Failure to establish an infection may be one reason for the smaller reniform nematode populations observed on resistant plants. Studies that described the histopathology of the reniform nematode on various crops focused on changes taking place in the plant root as the nematode establishes a feeding site (Birchfield, 1962; Heald, 1975; Rebois et al., 1975; Robinson and Orr, 1980; Carter, 1981; Agudelo et al., 2012), with degeneration of syncytia or failure of syncytial cells to enlarge commonly reported in resistant plants (Rebois et al., 1975; Carter, 1981; Agudelo et al., 2005). However, information on the rate of development and subsequent egg production of reniform nematodes that successfully establish and maintain feeding sites, especially on resistant plants, is limited. Agudelo et al. (2005) identified a single-plant selection of *G. hirsutum* cultivar Deltapine 50 that supported significantly smaller reniform nematode populations than other plants of this susceptible cultivar. The production of fewer eggs per egg mass was associated with the smaller nematode population, though infection and rate of development of the nematodes was not different between the susceptible and resistant variants of the host plant (Agudelo et al., 2005).

It is possible that, in addition to delayed infection, mechanisms such as delayed development or reduced fecundity could contribute to the overall reduction in the reniform nematode population associated with plants expressing resistance from *G. barbadense*. Therefore, the objective of this research was to describe the effects of two resistant *G. barbadense* lines (cultivar TX 110 and accession GB 713) on development and fecundity of reniform nematode.

Materials and Methods

Development and fecundity of reniform nematode were evaluated on three cotton genotypes in three growth chamber experiments. In each experiment, the temperature was held constant at 28°C and the d length was set at 16 hr. Adequate soil moisture was maintained throughout each experiment with an automated watering system, with the watering interval increased as needed during the experiment to supply additional water as plants grew. Mississippi reniform nematode population MSRR04 (Arias et al., 2009), derived from a single egg mass collected from cotton in 2003 and maintained on tomato (*Solanum lycopersicum* cv. Rutgers), was used for all experiments.

Assessment of reniform nematode development early in the infection cycle: Fifty plants each of susceptible *G. hirsutum* cultivar Deltapine 16, resistant *G. barbadense* cultivar TX 110, and resistant *G. barbadense* accession GB 713 were established in containers (Ray Leach SL-10 Cone-tainer; Stuewe & Sons, Inc., Tangent, OR) filled with 120 cm^3 of a steam-pasteurized soil mixture consisting of one part sandy loam soil mixed with two parts sand. Approximately 6 d after planting, 500 reniform nematodes (mixed vermiform life stages) suspended in 1 ml water were added to the soil in each container.

Beginning 1 DAI, root infection was measured on 10 individual plants of each genotype daily for 5 d. Plant roots were separated from plant shoots at the soil line. The shoots were discarded and the roots were separated from the soil and stained with red food coloring using published protocols (Thies et al., 2002). Root-associated nematodes in each of four developmental stages were counted at ×200 magnification. Nematodes were

classified as either vermiform (attached but not yet beginning to swell), swelling (enlargement of body but not yet assuming the kidney-shape characteristic of this species), reniform (kidney-shaped female with no egg mass), or gravid (kidney-shaped female with associated egg mass). After counting, fresh weights were recorded after roots were drained briefly on paper towels to remove excess water. Counts were expressed as females per gram of fresh root tissue to compensate for slight differences in root sizes.

Assessment of reniform nematode development late in the infection cycle: Fifty plants each of susceptible *G. hirsutum* cultivar Deltapine 16, resistant *G. barbadense* cultivar TX 110, and resistant *G. barbadense* accession GB 713 were evaluated using the same test establishment, inoculation, and root infection measurements as described for assessment of the early stages of reniform nematode development. Beginning 5 DAI, root infection was measured on 10 individual plants of each genotype at 5-d intervals through day 25.

Assessment of reniform nematode fecundity: Thirty plants each of susceptible *G. hirsutum* cultivar Deltapine 16, resistant *G. barbadense* cultivar TX 110, and resistant *G. barbadense* accession GB 713 were evaluated using the same test establishment and inoculation protocols as described for assessment of the early stages of reniform nematode development. Beginning 15 DAI, egg production by individual females was measured at 5-d intervals through day 25. At each sampling interval, roots of 10 individual plants of each genotype were separated from soil and 10 randomly selected gravid females were removed from the roots of each plant. The gelatinous matrix of a single egg mass was dissolved in a 0.6% NaOCl solution (Hussey and Barker, 1973), and the number of eggs per female was determined.

Experimental design and statistical analysis: The design for each experiment was a completely randomized design with 10 replications at each sampling date. Each experiment was conducted twice. Preliminary analyses (data not shown) showed no significant differences between trials and no significant trial by genotype interactions, so data from both trials of each experiment were combined for final analysis. Trials and their interactions were modeled as random effects in the final analysis. In each experiment, data from each sampling date were analyzed independently. Nematode counts were transformed ($\log_{10}[x + 1]$) prior to analysis of variance (ANOVA) to normalize data. Geometric (backtransformed) means are presented. Although ANOVA indicated significant differences among genotypes, differences of least squares means ($P \leq 0.05$) were used to compare means for the cotton genotypes. SAS statistical software (PROC MIXED of version 9.3; SAS Institute, Cary, NC) was used for analysis.

In addition to analysis of count data, patterns of nematode development were examined based on developmental cohorts. The percentage of the nematode population representing each developmental stage was calculated based on the number per gram of fresh root tissue and graphed to visualize the progression of nematode development on each genotype over time.

Results

Reniform nematodes were not seen in association with roots on the first DAI for any genotype. The total number of reniform nematodes infecting roots at daily intervals from 2 to 5 DAI are summarized in Table 1. As soon as 4 DAI, the susceptible genotype could be distinguished from resistant genotype *G. barbadense* GB 713; by 5 DAI, the difference between the susceptible and both resistant genotypes was distinct. *Gossypium barbadense* cultivar TX 110 and accession GB 713 were similar to each other with respect to the total number of infections during the early stages of disease development.

Reniform nematodes that successfully infected roots began to progress through the developmental stages immediately. Both vermiform and swelling nematodes were observed 2 DAI on susceptible *G. hirsutum* cultivar Deltapine 16; by 3 DAI, these developmental stages were observed on both of the resistant genotypes (Fig. 1, Table 2). However, the proportion of the nematode population in each developmental cohort on the first day nematodes were observed on the roots (day 2 for *G. hirsutum* cultivar Deltapine 16 and day 3 for the *G. barbadense* lines) differed between the susceptible and resistant genotypes. On susceptible *G. hirsutum* cultivar Deltapine 16, about 67% of the root-associated nematodes were swelling, as compared to 29% and 17% on resistant *G. barbadense* cultivar TX 110 and accession GB 713, respectively (Fig. 1). Individual nematodes that developed the full reniform shape were first seen 4 and 5 DAI on susceptible and resistant genotypes, respectively. No gravid females were seen on any genotype during the first 5 DAI. During the early stages of

Table 1. Total number of reniform nematodes (*Rotylenchulus reniformis*) per gram of fresh root on *Gossypium hirsutum* cultivar Deltapine 16 (susceptible), *Gossypium barbadense* cultivar TX 110 (resistant), and *G. barbadense* accession GB 713 (resistant) at daily intervals 2 to 5 d after inoculation (DAI) in growth chamber tests.

	DAI			
Genotype	2	3	4	5
Deltapine 16	0.3	1.7	6.5 a	29.3 a
TX 110	0.0	2.1	2.6 ab	5.9 b
GB 713	0.0	0.6	1.3 b	3.6 b
F value	2.96	1.41	4.10*	16.30***

Data are geometric (backtransformed) means of 20 observations from two tests combined.

F values followed by * and *** are significant at $P \leq 0.05$ and 0.001, respectively.

For each sampling interval, means followed by the same letter are not significantly different from each other based differences of least squares means ($P \leq 0.05$) of $\log_{10}(x + 1)$ transformed data.

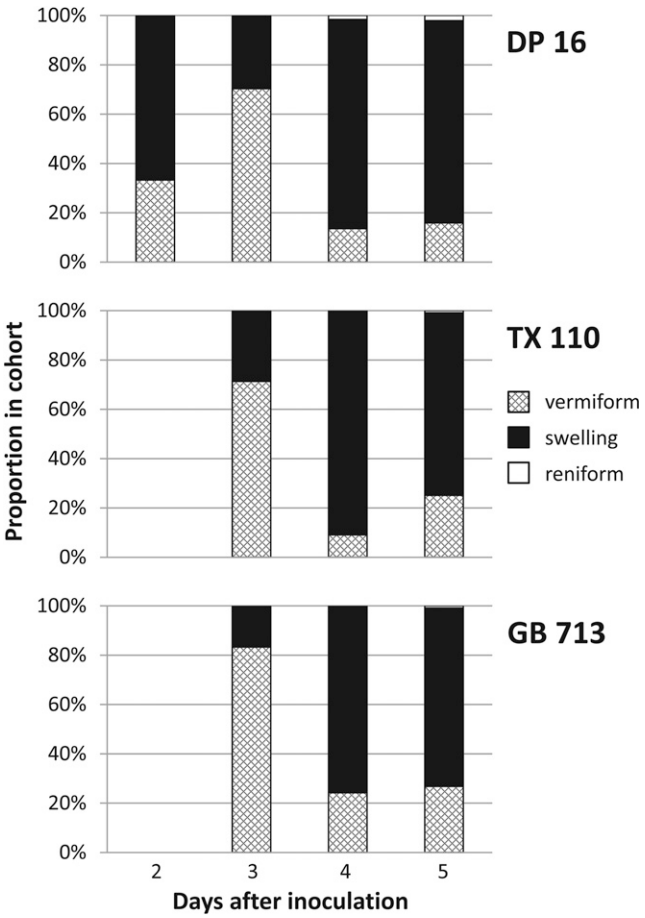

FIG. 1. Proportion of the reniform nematode population in each developmental cohort at 1-d intervals from 2 to 5 d after inoculation on susceptible *Gossypium hirsutum* cultivar Deltapine 16 (DP 16), moderately resistant *Gossypium barbadense* cultivar TX 110 (TX 110), and resistant *G. barbadense* accession GB 713 (GB 713).

infection, differences between the susceptible and one or both of resistant genotypes were evident for most developmental stage and DAI combinations (Table 2). In all cases, the susceptible genotype was in the group that supported the most individuals. Though fewer nematodes were found on roots of resistant genotypes, the proportion of individuals in each developmental cohort was similar to that observed for susceptible *G. hirsutum* cultivar Deltapine 16 at 3, 4, and 5 DAI (Fig. 1); most of the nematodes on each genotype were swelling by 5 DAI.

The total number of infections seen during the later stages of the infection cycle is summarized in Table 3. Throughout the later stages of the infection cycle, susceptible *G. hirsutum* cultivar Deltapine 16 had the highest number of infections at each evaluation interval, followed by *G. barbadense* cultivar TX 110 and then *G. barbadense* accession GB 713. *Gossypium barbadense* cultivar TX 110 supported only about 30% of the nematodes that developed on *G. hirsutum* cultivar Deltapine 16, and infection levels on *G. barbadense* accession GB 713 were 12% or less of that on the susceptible genotype. The maximum infection levels on *G. hirsutum* cultivar Deltapine 16 ($F = 13.19$, $P < 0.0001$)

TABLE 2. Number of reniform nematodes (*Rotylenchulus reniformis*) per gram of fresh root in three stages of development on *Gossypium hirsutum* cultivar Deltapine 16 (susceptible), *Gossypium barbadense* cultivar TX 110 (resistant), and *G. barbadense* accession GB 713 (resistant) at daily intervals from 2 to 5 d after inoculation (DAI) in growth chamber tests.

Stage of development	Genotype	DAI 2	DAI 3	DAI 4	DAI 5
Vermiform	Deltapine 16	0.2 a	1.2 a	0.6 a	3.3 a
	TX 110	0.0 b	1.5 a	0.1 b	1.0 b
	GB 713	0.0 b	0.5 b	0.4 a	1.0 b
	F value	7.32***	8.50***	9.47***	32.28***
Swelling	Deltapine 16	0.2 a	0.8 a	5.2 a	22.6 a
	TX 110	0.0 b	0.6 a	2.3 b	3.9 b
	GB 713	0.0 b	0.1 b	1.0 c	2.5 c
	F value	11.64***	9.04**	19.60***	97.96***
Reniform	Deltapine 16	0.0	0.0	0.1 a	0.4 a
	TX 110	0.0	0.0	0.0 b	0.1 b
	GB 713	0.0	0.0	0.0 b	0.1 b
	F value	-	-	4.33*	5.52**

Data are geometric (backtransformed) means of 20 observations from two tests combined.

F values followed by *, **, and *** are significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

For each sampling interval and developmental stage, means followed by the same letter are not significantly different from each other based differences of least squares means ($P \leq 0.05$) of $\log_{10}(x + 1)$ transformed data.

and *G. barbadense* accession GB 713 ($F = 6.26$, $P < 0.0001$) were reached 10 DAI; the maximum infection level on *G. barbadense* cultivar TX 110 ($F = 27.24$, $P < 0.0001$) was reached 15 DAI. Within each genotype, there were no significant differences between the mean number of infections on these dates and infection levels on later sampling dates.

Less than one vermiform nematode per gram of root was found for any genotype on any sampling date during the late infection cycle experiment (data not shown). Swelling, reniform, and gravid developmental stages were seen on all genotypes (Fig. 2, Table 4). Mean separations within each developmental stage and interval typically revealed the same pattern as seen with respect to total number of nematodes; in all cases, the susceptible genotype supported the most individuals (Table 4). Though fewer nematodes were found on roots of resistant genotypes, the proportion of individuals in each developmental cohort was similar to that that observed for susceptible *G. hirsutum* cultivar Deltapine 16 at 5 DAI (Fig. 2), with most of the nematodes on each genotype in the swelling stage of development. Different developmental patterns between the genotypes were noted starting at 10 DAI, when *G. hirsutum* cultivar Deltapine 16 had about twice as many gravid individuals in the population than either of the resistant genotypes (Fig. 2). Further, most of the nematodes on *G. barbadense* cultivar TX 110 had developed to the reniform stage at 10 DAI, whereas the nongravid individuals on *G. barbadense* accession GB 713 were equally divided between the swelling and reniform stages of development. By 15 DAI, the distribution of individuals among the developmental stages on

TABLE 3. Total number of reniform nematodes (*Rotylenchulus reniformis*) per gram of fresh root on *Gossypium hirsutum* cultivar Deltapine 16 (susceptible), *Gossypium barbadense* cultivar TX 110 (resistant), and *G. barbadense* accession GB 713 (resistant) at 5-d intervals following inoculation in growth chamber tests.

	DAI				
Genotype	5	10	15	20	25
Deltapine 16	27.3 a	46.1 a	48.3 a	54.0 a	46.2 a
TX 110	8.7 b	11.6 b	14.3 b	16.2 b	13.5 b
GB 713	1.1 c	5.8 c	2.5 c	5.8 c	2.8 c
F value	48.05***	33.77***	92.74***	54.08***	70.29***

DAI = days after inoculation.

Data are geometric (backtransformed) means of 20 observations from two tests combined.

F values followed by *** are significant at $P \leq 0.001$.

For each sampling interval, means followed by the same letter are not significantly different from each other based differences of least squares means ($P \leq 0.05$) of $\log_{10}(x+1)$ transformed data.

G. hirsutum cultivar Deltapine 16 and *G. barbadense* cultivar TX 110 were similar, with more than 60% of the individuals in the gravid stage of development. However, on *G. barbadense* accession GB 713, only about 50% of the individuals had begun producing eggs and about 9% were still swelling. Development on *G. barbadense* accession GB 713 continued to lag slightly at 20 and 25 DAI, with about one third of the individuals still in the swelling or reniform stages of development at the end of the experiment.

Reniform nematode fecundity did not differ among the genotypes at either 15 or 20 DAI (Table 5). Slightly more eggs per female were recovered from *G. barbadense* accession GB 713 than from the other two genotypes for plants sampled 25 DAI. However, on all sampling dates, fewer than 10 eggs were recovered from each egg mass on average. Ranges for the number of eggs per female were 0 to 74 for *G. hirsutum* cultivar Deltapine 16, 0 to 60 for *G. barbadense* cultivar TX 110, and 0 to 77 for *G. barbadense* accession GB 713 across all sampling dates.

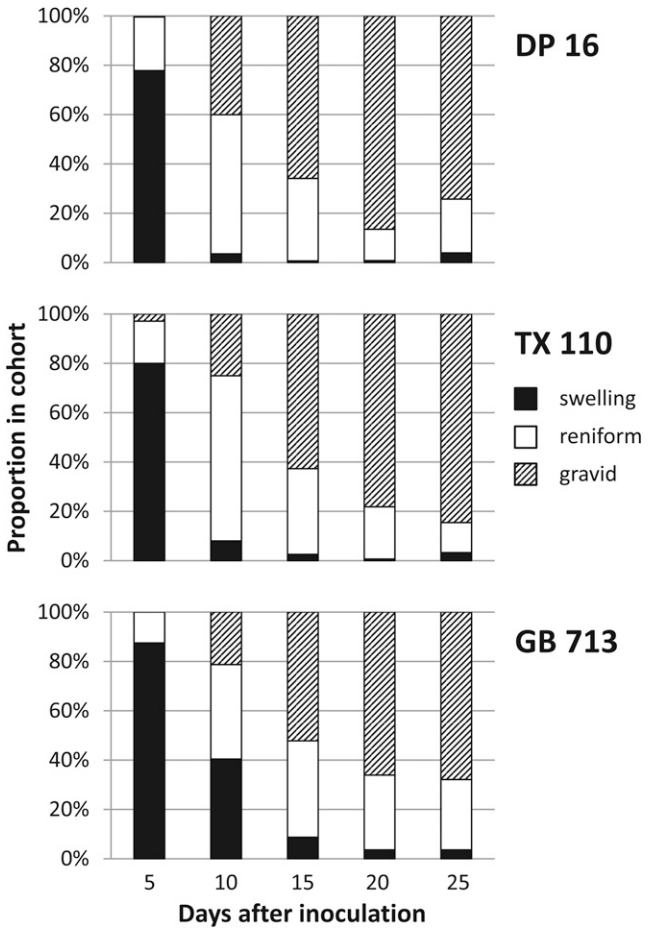

FIG. 2. Proportion of the reniform nematode population in each developmental cohort at 5-d intervals from 5 to 25 d after inoculation on susceptible *Gossypium hirsutum* cultivar Deltapine 16 (DP 16), moderately resistant *Gossypium barbadense* cultivar TX 110 (TX 110), and resistant *G. barbadense* accession GB 713 (GB 713).

DISCUSSION

Unique reniform nematode developmental patterns were noted on each of the three cotton genotypes examined. For all three developmental stages observed during the early stages of infection (i.e., vermiform, swelling, and reniform females), development of the nematodes occurred approximately 1 d faster on susceptible *G. hirsutum* cultivar Deltapine 16 than on the resistant genotypes. The 1-d delay in successful establishment of a feeding site could explain the corresponding 1-d delay in the appearance of reniform females on the resistant genotypes. However, differences in the proportion of swelling nematodes on the day on which infections were first observed suggest that development on the susceptible genotype is proceeding at a slightly faster pace, which may also contribute to the differences in developmental cohort composition observed later in the infection cycle. Approximately 40% of the nematodes on *G. hirsutum* cultivar Deltapine 16 have developed to the gravid stage by 10 DAI, whereas only about 20% of the nematodes on the two resistant genotypes were gravid on this sampling date. At the same time, 40% of the nematodes on *G. barbadense* accession GB 713 were swelling, as compared to less than 10% on the other two genotypes. At 15 DAI, fewer gravid nematodes and more swelling nematodes were associated with *G. barbadense* accession GB 713 than with the other two genotypes. Though delayed development of reniform nematode populations on *G. barbadense* cultivar TX 110 has been reported as contributing to resistance in this genotype (Starr et al., 2011), the current study is the first report of the delayed infection and population development associated with *G. barbadense* accession GB 713 and the first to identify that developmental patterns in these two resistant *G. barbadense* genotypes are different from each other.

Delayed development of reniform nematode on resistant genotypes is not unique to cotton. Lim and Castillo (1978) compared development on susceptible, moderately susceptible, and resistant soybean (*Glycine max*)

TABLE 4. Number of reniform nematodes (*Rotylenchulus reniformis*) per gram of fresh root in three stages of development on *Gossypium hirsutum* cultivar Deltapine 16 (susceptible), *Gossypium barbadense* cultivar TX 110 (resistant), and *G. barbadense* accession GB 713 (resistant) at 5-d intervals following inoculation in growth chamber tests.

Stage of development	Genotype	DAI				
		5	10	15	20	25
Swelling	Deltapine 16	18.6 a	1.4 a	0.3	0.4 a	1.6 a
	TX 110	2.8 b	0.8 b	0.3	0.1 b	0.4 b
	GB 713	0.7 c	1.9 a	0.2	0.2 b	0.1 c
	F value	215.30***	6.67**	2.43	7.32***	46.82***
Reniform	Deltapine 16	5.2 a	22.4 a	15.5 a	6.3 a	8.9 a
	TX 110	0.6 b	6.7 b	4.1 b	3.2 b	1.5 b
	GB 713	0.1 c	1.8 c	0.9 c	1.7 c	0.8 c
	F value	72.13***	126.97***	194.18***	29.53***	120.69***
Gravid	Deltapine 16	<0.1	15.9 a	30.5 a	43.0 a	30.3 a
	TX 110	0.1	2.5 b	7.4 b	11.8 b	10.4 b
	GB 713	0.0	1.0 c	1.2 c	3.7 c	1.9 c
	F value	1.31	76.72***	215.09***	123.79***	173.73***

DAI = days after inoculation.

Data are geometric (backtransformed) means of 20 observations from two tests combined.

F values followed by ** and *** are significant at $P \leq 0.01$ and 0.001, respectively.

For each sampling interval and developmental stage, means followed by the same letter are not significantly different from each other based differences of least squares means ($P \leq 0.05$) of $\log_{10}(x + 1)$ transformed data.

genotypes; on the resistant genotype, penetration of the root was delayed by 1 d and development to the gravid stage was delayed by 5 to 8 d. Rebois (1973) sampled resistant and susceptible soybean roots at 6-d intervals after inoculation and found gravid females at 6 DAI only on roots of susceptible soybean; at 27 DAI, 215 gravid females had developed on susceptible soybean roots, whereas only 25 were found on resistant soybean.

Similarities exist between responses of reniform nematode to resistance in cotton and reactions of other economically important, sedentary nematodes to host plant resistance. Examination of the effects of host plant resistance on development of southern root-knot nematode (*Meloidogyne incognita*) documented slower development to the adult stage in cotton with resistance derived from two different sources, the cultivar Clevewilt-6-3-5 (McClure et al., 1974) and the cultivar Auburn 623 (Jenkins et al., 1995). Lim and Castillo (1978) reported delayed penetration of roots, development of galls, and development to the adult stage in southern root-knot nematode on resistant soybean cultivar L 113 as compared to susceptible soybean cultivar Clark 63. Slower post-penetration development also was reported for peanut root-knot nematode (*Meloidogyne arenaria*) races 1 and 2 on resistant soybean (Pedrosa et al., 1996) and *M. arenaria* race 1 on moderately susceptible *Arachis duranenis* (a wild relative of commercial peanut, *A. hypogaea*); on the highly resistant wild species *A. stenosperma*, development to the adult stage was prevented completely (Proite et al., 2008).

Egg production per gravid female generally did not differ among the nematodes infecting the three *Gossypium* genotypes tested in this study, though reduced egg production was associated with the resistant response noted in a variant of *G. hirsutum* cultivar Deltapine 50 (Agudelo et al., 2005). Although the maximum number of eggs per egg mass for each genotype examined in this study was consistent with other reports in the literature (Lawrence and McLean, 2001; Agudelo et al., 2005), averages were lower than expected. Reasons for this might include reduced production of photosynthates to support egg production due to the plants being grown under artificial lights, slower production of eggs due to ambient temperatures at the lower end of the optimal range for this nematode species (Lawrence and McLean, 2001), or inclusion of a disproportionate number of newly gravid females in the random sample of egg masses collected. Combining unique resistance mechanisms through plant breeding could result in resistant cotton expressing more than one type of resistance, but it does not appear that reduced fecundity can be obtained from either of these *G. barbadense* lines. Reduced reniform nematode fecundity also was noted on resistant soybean cultivars (Rebois, 1973; Lim and Castillo, 1978) and southern

TABLE 5. Number of reniform nematode (*Rotylenchulus reniformis*) eggs per female on *Gossypium hirsutum* cultivar Deltapine 16 (susceptible), *Gossypium barbadense* cultivar TX 110 (resistant), and *G. barbadense* accession GB 713 (resistant) at 5-d intervals beginning 15 d after inoculation (DAI) in growth chamber tests.

Genotype	DAI		
	15	20	25
Deltapine 16	3.7	3.7	3.7 b
TX 110	4.5	5.3	3.4 b
GB 713	5.8	5.5	8.1 a
F value	0.09	1.06	7.12**

Data are geometric (backtransformed) means of up to 200 observations from two tests combined.

F values followed by ** are significant at $P \leq 0.01$.

For each sampling interval, means followed by the same letter are not significantly different from each other based on differences of least squares means ($P \leq 0.05$) of $\log_{10}(x + 1)$ transformed data.

root-knot nematodes on resistant cotton produced fewer eggs per egg mass than their counterparts infecting susceptible cotton (Creech et al., 1995).

Previous research by Gutiérrez et al. (2011) identified three QTLs linked to resistance in *G. barbadense* accession GB 713, but there are no published reports on the number or location of loci linked to the resistance in *G. barbadense* cultivar TX 110. Given the differences in both number of infections (fewer on *G. barbadense* accession GB 713) and rate of development (delayed more on *G. barbadense* accession GB 713) documented in this study, it is possible that *G. barbadense* cultivar TX 110 is lacking one or more of the loci found in *G. barbadense* accession GB 713, or that different loci confer resistance in these two lines. Neither the current study nor previous reports address the question of whether the resistance mechanisms are always "on" or if they are triggered only when the plant is challenged by reniform nematode. The ability to distinguish between the susceptible and resistant genotypes as soon as 5 DAI based on total numbers of nematodes infecting the roots indicates that at least one mechanism conferring resistance is active early in the disease cycle. Additional molecular studies such as genotyping by sequencing and gene expression profiling based on RNA sequencing should be considered to further define how resistance is controlled and when it is expressed.

By 10 to 15 DAI, the total number of nematodes infecting the roots had plateaued, suggesting that separation of susceptible and resistant genotypes might be possible in a shorter period of time. If plants could be reliably evaluated just 10 to 15 DAI, the number of individual plants tested in a year could be doubled compared to methods used in previous screenings that were based on a 4-wk interval to allow completion of the reniform nematode life cycle (Romano et al., 2009; Stetina et al., 2014). However, before a recommendation is made to shorten the screening interval, additional testing is needed to determine if peak infection timing applies to a broader range of cotton genotypes and across other host species for reniform nematode.

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