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Tolerance of Selected Cotton Lines to *Rotylenchulus reniformis*[1]

S. R. Koenning,[2] K. R. Barker,[2] and D. T. Bowman[3]

Abstract: The reproductive and damage potential of the reniform nematode, *Rotylenchulus reniformis,* on five cotton breeding lines reported as tolerant to this nematode in Texas were compared with two standard cotton cultivars, Deltapine 50 and Stoneville LA 887, in a North Carolina field naturally infested with *R. reniformis.* Numbers of *R. reniformis* in soil were suppressed at mid-season, and cotton-lint yield was increased by preplant fumigation with 1,3-dichloropropene. Population densities of *R. reniformis* at cotton harvest were unaffected by fumigation in 1998, but were affected in 1999. Some of the putatively tolerant breeding lines supported lower levels of *R. reniformis* and had higher tolerance indices to reniform nematode than the standard cultivars, but the yields of the breeding lines were significantly lower than the standard cultivars. Fumigation resulted in a 100- to 200-kg/ha increase in cotton lint yield for cultivars LA 887 and Deltapine 50.

Key words: cotton, crop loss, *Gossypium hirsutum* host-plant resistance, nematode, plant disease loss, reniform nematode *Rotylenchulus reniformis,* tolerance.

The reniform nematode *Rotylenchulus reniformis* (Linford and Oliveira) is an important pathogen of cotton *Gossypium hirsutum* L. in the southeastern United States (Blasingame, 1993; Heald and Robinson, 1990; Robinson and Percival, 1997; Starr, 1998). Yield losses associated with this nematode were reported from Alabama, Florida, Georgia, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, and Texas in 1994 (Koenning et al., 1999). The most common practices for managing nematodes in cotton include crop rotation, nematicide application, and, to a limited extent, host-plant resistance, where the southern root-knot nematode, *Meloidogyne incognita* is involved. Management of plant-parasitic nematodes in cotton is highly dependent on nematicides (Starr, 1998), although the future availability of fumigant and nonfumigant nematicides for use on cotton is in question (Thomason, 1987). Rotation with crops that are resistant to the reniform nematode is effective in limiting cotton-yield loss caused by *R. reniformis* (Koenning and Barker, 1994), but many rotation crops are of relatively low value, and some crops, such as peanut, are restricted by government programs. Tolerance to *R. reniformis* may be another management option, but information on tolerance to this nematode is limited (Cook et al., 1997). Currently, *R. reniformis* has been found in seven North Carolina counties and its incidence is increasing (J. Imbriani, pers. comm.). Previous research demonstrated that the slopes of the damage functions for reniform nematode and the southern root-knot nematode are equal, but the high reproductive rate and low overwinter mortality of reniform nematode compared to root-knot nematode makes reniform nematode potentially more damaging to cotton than the latter nematode (Koenning et al., 1996). Cotton hectarage has tripled in North Carolina in the past 10 years, and only limited information about the impact of reniform nematodes on cotton in this state is available (Koenning and Barker, 1994; Koenning et al., 1996).

Cotton cultivars have been developed with moderate levels of resistance to the root-knot nematode (Elliot, 1999; Ogallo et al., 1997). However, although Jones et al. (1988) released germplasm resistant to *R. reniformis* and Cook et al. (1997) reported lines tolerant to this nematode, neither re-

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[2] Respectively, Senior Researcher and Emeritus Professor, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.
[3] Professor, Department of Crop Science, North Carolina State University, Raleigh, NC 27695-8604.
E-mail: stephen_koenning@ncsu.edu
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sistance nor tolerance has been incorporated into high-yielding cultivars. Cotton resistance and (or) tolerance to the reniform nematode remains in the developmental stage (Cook et al., 1997; Robinson and Percival, 1997).

The objectives of this research were to: (i) evaluate the reproductive capacity of *R. reniformis* on selected breeding lines potentially tolerant to this nematode compared to standard susceptible cotton cultivars, (ii) measure cotton yield suppression in the presence of this nematode in the field, and (iii) determine if selected breeding lines have levels of tolerance and (or) resistance to *R. reniformis* that would be suitable for North Carolina.

Materials and Methods

Field experiments were conducted in 1998 and 1999 in different areas within the same field located in Hoke County, North Carolina. The soil type was a Marlboro sandy loam (clayey, kaolinitic, thremictypic paleudult; 57% sand, 25% silt, 18% clay; organic matter 1%). The prefumigation population density of reniform nematode vermiform stages per 500 cm^3 soil was 2,810 (SD 1,710) and 2,736 (SD 2,226) in 1998 and 1999, respectively. The experimental design was a split-plot with six replications. Sub-plots consisted of either fumigation with 56 liters/ha of 1,3-dichloropropene (Telone II, Dow AgroSciences, Indianapolis, IN) or no fumigation. Whole plots were cultivars (Stoneville LA 887 and Deltapine 50) or breeding lines (RN 96126-1, RN 96424, RN 96527, RN 96528M, and RN 96625) obtained from Charles Cook (USDA, ARS). Selected plots were fumigated in April and planted in late May. Plots were two rows, 12.8 m long, with 0.96-m row spacing and 3.7-m alleys. Standard management practices for North Carolina were used for all cotton plots including the application of 0.5 kg a.i./ha of aldicarb (Temik 15G, Aventis CropScience, Research Triangle Park, NC) in-furrow for insect control (Anonymous, 1999). Cotton yield was determined with a commercial two-row cotton picker in late October 1998 and early December 1999. Samples for nematode assays were collected prior to fumigation, at midseason (mid-July in 1998 and mid-August in 1999), and at cotton harvest from each plot. Each soil sample consisted of 8 to 10 2.5-cm-diam. cores, which were composited. A 500-cm^3 subsample was processed by elutriation and centrifugation, and roots were processed to extract eggs (Barker et al., 1986).

Data analysis consisted of analysis of variance (ANOVA) for the appropriate statistical design using PC/SAS software (SAS Institute Inc. Cary, NC). The year effect was considered to be random, so the genotype × year, fumigation × year, and fumigation × genotype × year effects were used to test differences for genotype, fumigation, and genotype × fumigation effects, respectively, for combined analysis over years. Significant data for each year are presented separately because of first-order interactions with year × cultivar, or year × fumigation. Second-order statistical interactions were not significant. Orthogonal contrasts LSD (least significant difference) and the Waller Duncan k-ratio *t*-test were used to separate means. Nematode numbers were transformed ($\log_{10}[x + 1]$) to normalize the variance. A tolerance index (TI) was calculated where TI = yield of untreated/yield of treated × 100, and ANOVA was performed for a randomized complete-block design.

Results

Fumigation with 1,3-dichloropropene suppressed ($P \leq 0.05$) mid-season numbers (Pm) of *R. reniformis* both years (Table 1). Average Pm was greater in 1999 than in 1998, resulting in a significant year effect in the pooled analysis. The breeding lines averaged together had a lower reniform Pm than the cultivars used as standards (LA 887 and Deltapine 50) in nontreated plots both years, according to orthogonal contrasts. Nevertheless, one breeding line, RN 96527, had the highest Pm of any cultivar or line in nontreated plots both years.

Final *R. reniformis* population densities (Pf) differed between years ($P \leq 0.01$), with much greater densities in 1998 than in 1999 (Table 2). The fumigation × year interaction

TABLE 1. Mid-season numbers of *Rotylenchulus reniformis* eggs plus juveniles per 500 cm^3 soil as affected by fumigation with 1,3-dichloropropene and cotton genotype in 1998 and 1999 in Hoke County, North Carolina.

Cultivar	1998 –Fumigant[a]	1998 +Fumigant	1999 –Fumigant	1999 +Fumigant
Deltapine 50	2,060	309	37,835	559
LA 887	2,516	588	41,850	802
Mean Deltapine 50 and LA 887	2,288A	449A	39,843A	403A
RN 96126-1	1,710	347	33,820	4,429
RN 96424	1,810	223	14,117	94
RN 96527	2,713	338	45,364	312
RN 96528	1,760	313	36,089	2,238
RN 96625	1,921	395	25,202	923
Mean breeding lines	1,928B	413A	31,062B	1,599A
LSD ($P = 0.10$)[b]	1,690	441	32,757	4,161

Each value is the mean of six replicates.
[a] The fumigant × genotype interaction was not significant, but the genotype × year interaction was. Means of breeding lines do not differ from means of cotton cultivars within a column if followed by the same letter according to orthogonal contrasts ($P \leq 0.05$).
[b] LSD for comparing cultivars within nematicide treatments.

also was significant for Pf ($P \leq 0.01$). The population density of *R. reniformis* in soil at cotton harvest in 1998 was not affected by fumigation, but fumigated plots in 1999 had lower nematode population densities than nonfumigated plots.

The genotype effects and the genotype × year interaction were significant ($P \leq 0.01$) for cotton-lint yield, with lower yields in 1999 than in 1998 (Table 3). The rankings of the two standard cultivars changed between years, with LA 887 yielding highest in 1998 and Deltapine 50 yielding highest in 1999. The mean yield of Deltapine 50 and LA 887 exceeded that of the breeding lines by about 200 kg/ha in 1998 and 100 kg/ha in 1999 ($P \leq 0.05$). Differences in reniform nematode tolerance among genotypes were indicated (orthogonal contrasts, $P \leq 0.05$) (Table 4). The tolerance indices for Deltapine 50 and LA 887 were 85.1 and 80.5, respectively; the tolerance indices for the breeding lines ranged from 87.2 to 97.5.

DISCUSSION

Severe drought in summer 1999, followed by excessive rainfall in September as a result of three hurricanes, was the probable cause of significant interactions between years.

TABLE 2. Influence of genotype and fumigation with 1,3-dichloropropene on numbers of *Rotylenchulus reniformis* eggs plus juveniles per 500 cm^3 soil at cotton harvest in 1998 and 1999 in Hoke County, North Carolina.

Cultivar	1998 –Fumigant[a]	1998 +Fumigant	1999 –Fumigant	1999 +Fumigant
Deltapine 50	14,355	14,391	332	32
LA 887	19,144	13,489	2427	232
Mean Deltapine 50 and LA 887	16,750A	13,940A	1,380A	132A
RN 96126-1	10,463	11,004	1363	26
RN 96424	12,625	6,340	440	27
RN 96527	11,632	8,045	128	52
RN 96528	9,963	7,518	133	32
RN 96625	12,379	3,745	1,542	52
Mean of breeding lines	11,412B	7,330B	721A	39A
LSD ($P = 0.010$)[b]	7,029	6,263	2,297	181

Each value is the mean of six replicates.
[a] The fumigant × cultivar interaction was not significant, but the fumigant × year interaction was. Means of breeding lines do not differ from cotton cultivars within a column if followed by the same letter according to orthogonal contrasts ($P \leq 0.05$).
[b] LSD is for comparison of cultivars within columns.

TABLE 3. Cotton-lint yield (kg/ha) influenced by genotype and fumigation with 1,3-dichloropropene in a field infested with *Rotylenchulus reniformis* in 1998 and 1999 in Hoke County, North Carolina.

	1998		1999	
Cultivar	–Fumigant[a]	+Fumigant	–	+Fumigant
Deltapine 50	811	879	266	365
LA 887	854	1,028	244	321
Mean Deltapine 50 and LA 887	833A	953A	255A	343A
RN 96126-1	670	825	225	211
RN 96424	721	786	168	199
RN 96527	782	878	220	276
RN 96528	727	831	242	234
RN 96625	691	799	224	226
Mean of breeding lines	718B	823B	216B	229B
LSD ($P = 0.10$)[b]	206	137	90	56

Each value is the mean of six replicates.

[a] Fumigation increased cotton-lint yield ($P \leq 0.05$). The genotype × year interaction was significant. Means of cotton breeding lines do not differ from the standard cultivars if followed by the same letter according to orthogonal contrasts ($P < 0.05$).

[b] LSD is for comparison of means within a year and fumigation treatment.

Nevertheless, trends in response of the nematode to genotypes and fumigation remained fairly consistent throughout the experiments. The higher reniform nematode numbers encountered at mid-season in 1999 compared to 1998 were probably an indirect effect of the 1999 summer drought because sampling had to be delayed from mid-July until early August 1999, when moisture was adequate. This may have allowed a longer period of time for reproduction compared to 1998. The delayed sampling in 1999, however, allowed better discrimination between standard cultivars and one of the breeding lines (RN 96424) with regard to host status for reniform nematode. Although there was a trend toward lower *R. reniformis* Pm on breeding lines than on the standard cultivars, the differences were not statistically significant ($P \leq 0.10$).

TABLE 4. Cotton tolerance to *Rotylenchulus reniformis* using pooled data from 1998 and 1999 in Hoke County, North Carolina.

Cultivar	Tolerance index[a]
Deltapine 50	85.1A
LA 887	80.5A
Mean of Deltapine 50 and LA 887	82.8a
RN 96126-1	97.5A
RN 96424	89.0A
RN 96527	87.2A
RN 96528	97.1A
RN 96625	96.6A
Mean of breeding lines	93.5b

Data for each cultivar is the mean of 12 replicates. Neither the cultivar by year interaction nor the cultivar effect was significant ($P = 0.05$).

[a] Tolerance index = (yield nontreated ÷ yield treated) × 100. Means followed by the same uppercase letter do not differ according to the Waller Duncan k-ratio *t*-test (*k*-ratio = 50). Means of cotton cultivars compared to means of the breeding lines are significantly different according to orthogonal contrasts ($P < 0.05$).

Nematode population densities at harvest in 1998 showed that some of the breeding lines supported less *R. reniformis* reproduction than the standard cultivars. The 1998 Pm and 1999 Pf data also support the conclusion that some of the breeding lines are less suitable hosts for *R. reniformis* than the standard cultivars. However, even the lower reproduction on the breeding lines would likely sustain population densities at levels that are still damaging to cotton. Soil samples for nematode assays at cotton harvest were delayed in 1999 because of extremely wet conditions associated with hurricanes and late cotton maturity associated with drought early in the season. Consequently, final population densities were much lower in 1999 than in 1998 and lower than those encountered at mid-season the same year. The 1998 data on reniform nematode Pf, however, indicate that even relatively high rates of fumigant nematicides may be insufficient to limit *R. reniformis* to levels that would not damage subsequent crops.

The yields of both standard cultivars were superior to the yields of all of the breeding lines both years. The relatively high rates of nematicides used in this study resulted in a substantial improvement in lint yield and, depending on the price of cotton, might provide a net economic return on conventional cultivars when reniform nematode populations are above the damage threshold. On the other hand, the relatively small differences in reproduction of *R. reniformis* on the breeding lines compared with the standard cultivars, and their poor yield performance indicate that they will not be effective in reniform nematode management in North Carolina. Although other research has shown that lines with superior yield in the presence of the reniform nematode may be available in some geographic regions (Cook et al., 1997; Robinson and Percival, 1997), considerable improvement in the adaptation and level of resistance or tolerance to the nematode will be needed for our region.

Literature Cited

Anonymous. 1999. 2000 cotton information. AG 417. Raleigh, NC: North Carolina Cooperative Extension Service.

Barker, K. R., J. F. Townshend, G. W. Bird, I. J. Thomason, and D. W. Dickson. 1986. Determining nematode population responses to control agents. Pp. 283–296 *in* K. D. Hickey, ed. Methods for evaluating pesticides for control of plant pathogens. St. Paul, MN: APS Press.

Blasingame, D. 1993. Nematode distribution and density. Pp. 4–6 *in* Cotton nematodes: Your hidden enemies. Memphis, TN: Cotton Foundation.

Cook, C. G., A. F. Robinson, and L. N. Namken. 1997. Tolerance to *Rotylenchulus reniformis* and resistance to *Meloidogyne incognita* race 3 in high-yielding breeding lines of upland cotton. Journal of Nematology 29:322–328.

Elliot, C. S. 1999. Virulence of *Meloidogyne incognita* isolates on resistant cotton genotypes. M.S. thesis, Clemson University, Clemson, SC.

Heald, C. M., and A. F. Robinson. 1990. Survey of current distribution of *Rotylenchulus reniformis* in the United States. Supplement to the Journal of Nematology 22:695–699.

Jones, J. E., J. P. Beasley, J. I. Dickson, and W. D. Caldwell. 1988. Registration of four cotton germplasm lines with resistance to reniform and root-knot nematodes. Crop Science 28:199–200.

Koenning, S. R., and K. R. Barker. 1994. Damage thresholds and rotation systems for managing *Rotylenchulus reniformis* on cotton. Phytopathology 84:1103 (Abstr.).

Koenning, S. R., C. Overstreet, J. W. Noling, P. A. Donald, J. O. Becker, and B. A. Fortnum. 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. Supplement to the Journal of Nematology 31:587–618.

Koenning, S. R., S. A. Walters, and K. R. Barker. 1996. Impact of soil texture on the reproductive and damage potential of *Rotylenchulus reniformis* and *Meloidogyne incognita* on cotton. Journal of Nematology 28:527–536.

Ogallo, J. L., P. B. Goodell, J. Eckert, and P. A. Roberts. 1997. Evaluation of Nem-X, a new cultivar of cotton with high resistance to *Meloidogyne incognita*. Journal of Nematology 29:531–537.

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

Starr, J. L. 1998. Cotton. Pp. 359–380 *in* K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant and nematode interactions. Madison, WI: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Thomason, I. J. 1987. Challenges facing nematology: Environmental risks with nematicides and the need for new approaches. Pp. 469–476 *in* Vistas on nematology. J. A. Veech and D. W. Dickson, eds. Hyattsville, MD: Society of Nematologists, Inc.