# DEPTH AND TEXTURE OF SOIL GREATLY ALTER THE EXPRESSION OF RENIFORM NEMATODE RESISTANCE IN KEY EXOTIC COTTONS

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## Abstract

Greenhouse pot studies conducted previously by several laboratories identified certain primitive accessions of exotic cottons that show potential as sources of reniform nematode resistance for breeding programs aimed at developing reniform nematode resistant cultivars. In 2001, the first field experiment was conducted to directly examine the ability of the most promising of these accessions to reduce reniform nematode populations in the field. Accessions were planted in a field with a sandy clay loam soil 7 km north of Weslaco, Texas, in a randomized complete block design on 102-cm-wide beds during the first week of March, 2001, and soil cores for analysis of nematodes, roots, soil moisture, and texture were taken to a depth of 122 cm in March as well as during the first week of July, just prior to harvest of agronomic genotypes in the same field. The mean reniform nematode population density at planting, when averaged from the surface to 120 cm, was 3 Baermann funnel-extractable nematodes/g soil (estimated equivalent to 3,690 nematodes/pint by sugar flotation). In July, nematode populations at 15-cm increments from the soil surface to the 120-cm depth revealed that reniform nematode populations under *G. arboreum* A2-87, *G. herbaceum* A1-17, and the *G. barbadense* accessions GB-13, GB-49 and TX-110 had increased about 4-fold, did not differ significantly from the susceptible control, and thus did not exhibit appreciable resistance to the nematode. Statistically significant ($P = 0.05$) nematode population suppression was achieved only for GB-264, and the level of suppression measured (48%) was not considered agronomically useful. Follow-up experiments conducted under microplot and growth chamber conditions confirmed that these accessions were able to exhibit moderate to high levels of resistance in a sandy loam soil and in a sand-based potting mix but not in soil taken directly from the Weslaco field site. Loss of resistance expression was unrelated to nematode genotype, moisture, nematode extraction technique, ambient temperature or light quality. Possible remaining explanations included downward movement of nematodes in the field, potent antagonists in the upper soil profile in the field, and abnormal root-growth in pot and microplot experiments.

## Introduction

The reniform nematode (*Rotylenchulus reniformis*) is believed to cause major yield losses on many farms in Mississippi and Louisiana, northern Alabama and the Lower Rio Grande Valley of Texas. Resistance to the nematode is not apparent in Upland cotton (*Gossypium hirsutum*) but pot studies have shown varying levels of resistance in many primitive accessions of *G. arboreum*, *G. barbadense*, and *G. herbaceum* (Carter, 1981; Robinson and Percival, 1997; Robinson et al., 1999; Yik and Birchfield, 1984). An important step in developing breeding programs to transfer resistance from these species into agronomic cultivars is to confirm that the levels of resistance observed in pots will hold up when they are grown in the field. The objective of this research was to evaluate the level of resistance under field conditions for six of the most promising accessions identified to date. Additional growth chamber and microplot experiments were then conducted to find out why levels of resistance observed in the field differed greatly from those observed in previous studies in pots.

## Materials and Methods

### Field Experiment

The location was the USDA North Farm, ca. 5 miles north of Weslaco, Texas. Entries included *G. arboreum* A2-87; *G. herbaceum* A1-17; *G. barbadense* GB-13, GB-49, GB-264, and TX-110; *G. hirsutum* Suregrow 501 (SG 501) and Fibermax 832 (FM 832) (control). The soil was a sandy clay loam, 0-120 cm. The experimental design was a randomized complete block with four blocks and eight treatments on a 102-cm bed, one row per plot. Soil from representative spots was sampled at planting, and every plot was sampled at the end of season (first of July) with a 120-cm soil core extractor. Each core was

divided into 15-cm lengths to analyze vertical distributions. Nematodes were extracted from soil by Baermann funnel and by sugar flotation. Roots were extracted by wet sieving and total cm root per sample measured with MacRhizo root analyzer. Moisture and texture were also determined at each depth. The experiment was planted and sampled first week of March 2001. Plant heights and final soil samples were taken the first week of July.

**Microplot Experiment**

The location was College Station, Texas. Entries were identical to those in the in field study, above. The experimental design was a randomized complete block with six blocks and eight treatments in 40-liter microplots 30 cm deep. The soil was a loamy sand. Each microplot was inoculated with 10,000 vermiform nematodes after seedling emergence. Soil samples were composites of three 2-cm-diam probes from the surface to the bottom of each microplot container. Cotton was planted the first week of June. Soil samples were taken on October 1 for nematode analysis only and on November 15 for both root and nematode analysis. Most large roots from the upper central part of microplots were removed with plants just before the Nov 15 sampling.

**Growth Chamber Experiment #1**

The chamber conditions consisted of a 14-hour photoperiod with 26 C night/30 C day and relative humidity held above 55%. Entries included the *G. barbadense* accessions GB-459, GB-485, GB-536, GB-581, GB-706, GB-713, and TX-110, and the *G. hirsutum* accessions TX-1167, TX-839, and TX-1403, plus Auburn 623 RNR as a root-knot nematode-resistant control, *G. barbadense* TX-1348 as a reniform nematode-resistant control, and Delta and Pineland 16 as a control susceptible to both nematodes. The experimental design was a randomized complete block with six replications and 14 treatments of plants in individual 0.5-liter pots. The soil was a sand-based potting mix. Inoculum consisted of 1,000 root-knot nematode eggs or 4,000 vermiform reniform nematodes injected into the soil. Pots were planted on day 1, inoculated on day 14, and harvested on day 63.

**Growth Chamber Experiment #2**

The soil, chamber conditions, and chronology were the same as in Growth Chamber Experiment #1, above. The entries were the same as in the field and microplot experiments above except A1-17 was omitted and *G. barbadense* GB-536 and GB-713 were substituted for GB-13 and GB-49. The experimental design was a randomized complete block with six blocks and seven treatments in 0.5-liter pots. Inoculum consisted of 4,000 vermiform reniform nematodes injected into the soil. Root lengths on day 63 were estimated from wet root weights based on an assumed density of 1.0 g/cm$^3$ and the length/volume ratio of 1,415 cm/g averaged for 300 previous samples.

## Results and Discussion

**Field Experiment**

All accessions outgrew Fibermax 832 in the same field (Fig. 1). Accessions were not resistant in the field experiment when the total number of nematodes in the soil profile was considered (Fig. 3). The number of nematodes per cm of root 3 to 4 feet deep in the soil, however, consistently showed less reproduction on the exotic accessions than on resistant controls. Overall root growth patterns were similar with some subtle differences. The number of nematodes per cm root increased as much as 100-fold between the surface and 120 cm, possibly due to antagonists or differences in root age. Vertical differences in nematode numbers were not attributable to soil moisture or extraction technique.

**Microplot and Growth Chamber Experiments**

Microplot results showed differences in levels of resistance consistent with previous pot studies, indicating that ambient sunlight and temperature were not significant factors influencing resistance expression (Tables 1-4). Growth chamber results showed lower root growth, lower nematode reproduction, and almost no resistance expression to occur the sandy clay loam field soil, similar to results in the field study; this was in striking contrast to high levels of resistance expression measured in the sand mix (Tables 1-5). In the sand mix the general pattern of resistance expression to the two nematode populations was similar, with subtle differences.

## Conclusion

In the context of classical notions about nematode resistance, these results indicate that resistance in the *G. barbadense*, *G. arboreum* and *G. herbaceum* accessions tested in the field was broken by unknown factors associated with soil texture and depth, but not associated with moisture, extraction technique, or geographic origin of the nematode. Alternatively it can be argued that resistance was in fact expressed but was masked by much more important factors. Possible factors contributing to differences between levels of resistance observed in field and pot studies include downward movement of nematodes in the field, potent antagonists in the upper soil profile in the field, and abnormal root-growth in pot and microplot experiments.

## References


Carter, W. W. 1981. Resistance and resistant reaction of *Gossypium arboreum* to the reniform nematode, *Rotylenchulus reniformis*. J. Nematol. 13:368-374.

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.

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. J. Nematol. 29:746-755.

Yik, C.-P., and Birchfield, W. 1984. Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus rniformis*. J. Nematol. 16:146-153.


Table 1. Average number of reniform nematodes per gram of soil.

| | Texas population | | | Louisiana population | | |
|---|---|---|---|---|---|---|
| | Sandy clay loam | | Sand | Sand | Sandy loam | |
| Genotype | Field | G. chamber | G. chamber | G. chamber | Microplot Oct 1 | Microplot Nov 15 |
| A1-17 | 7 | - | - | - | 2 ** | 72 * |
| A2-87 | 7 | 16 | 87 ** | 104 ** | 1 ** | 5 ** |
| TX-110 | 9 | 19 | 39 ** | 190 ** | 12 ** | 54 * |
| GB-13 | 7 | - | - | - | 12 ** | 85 |
| GB-49 | 7 | - | - | - | 17 ** | 83 |
| GB-536 | - | 14 | 76 ** | 124 ** | - | - |
| GB-713 | - | 18 | 27 ** | 48 ** | - | - |
| GB-264 | 5 | 19 | 91 ** | 206 ** | 11 ** | 42 ** |
| SG 501 | 12 | 22 | 923 | 496 | 47 | 177 |
| FM 832 | 10 | 25 | 899 | 654 | 77 | 218 |

*, ** Significantly different from FM 832 at the 0.05 and 0.01 level of probability, respectively, based on Dunnett's test.

Table 2. Reniform nematodes per gram of soil, as percentage of FM 832.

| | Texas population | | | Louisiana population | | |
|---|---|---|---|---|---|---|
| | Sandy clay loam | | Sand | Sand | Sandy loam | |
| Genotype | Field | G. chamber | G. chamber | G. chamber | Microplot Oct 1 | Microplot Nov 15 |
| A1-17 | 72 | - | - | - | 3 ** | 33 * |
| A2-87 | 73 | 64 | 10 ** | 16 ** | 1 ** | 2 ** |
| TX-110 | 90 | 74 | 4 ** | 29 ** | 15 ** | 25 * |
| GB-13 | 64 | - | - | - | 16 ** | 39 |
| GB-49 | 64 | - | - | - | 22 ** | 38 |
| GB-536 | - | 54 | 8 ** | 19 ** | - | - |
| GB-713 | - | 74 | 3 ** | 7 ** | - | - |
| GB-264 | 52 | 74 | 10 ** | 31 ** | 14 ** | 19 ** |
| SG 501 | 113 | 86 | 103 | 76 | 61 | 81 |
| FM 832 | 100 | 100 | 100 | 100 | 100 | 100 |

*, ** Significantly different from FM 832 at the 0.05 and 0.01 level of probability, respectively, based on Dunnett's test.

Table 3. Average number of reniform nematodes per cm root.

| | Texas population | | | | | Louisiana population | |
|---|---|---|---|---|---|---|---|
| | Sandy clay loam | | | | Sand | Sand | Sandy loam |
| | Field | | | | | | Microplot |
| Genotype | 0-30 cm | 90-120 cm | 0-120 cm | G. chamber | G. chamber | G. chamber | (Nov 15) |
| A1-17 | 9 | 58 | 40 * | - | - | - | 15 ** |
| A2-87 | 7 | 435 | 128 | 9 | 6 ** | 9 ** | 3 ** |
| TX-110 | 10 | 461 | 189 | 9 | 10 * | 3 ** | 10 ** |
| GB-13 | 16 | 351 | 111 | - | - | - | 19 * |
| GB-49 | 10 | 642 | 212 | - | - | - | 16 ** |
| GB-536 | - | - | - | 4 | 7 ** | 4 ** | - |
| GB-713 | - | - | - | 5 ^ | 2 ** | 1 ** | - |
| GB-264 | 8 | 219 | 112 | 6 | 9 ** | 6 ** | 8 ** |
| SG 501 | 16 | 807 | 376 | 11 | 36 | 40 ** | 40 |
| FM 832 | 7 | 1301 | 389 | 11 | 49 | 61 | 63 |

*, ** Significantly different from FM 832 at the 0.05 and 0.01 level of probability, respectively, based on Dunnett's test.

Table 4. Reniform nematodes per cm root, as percentage of FM 832.

| | Texas population | | | | | Louisiana population | |
|---|---|---|---|---|---|---|---|
| | Sandy clay loam | | | | Sand | Sand | Sandy loam |
| | Field | | | | | | Microplot |
| Genotype | 0-30 cm | 90-120 cm | 0-120 cm | G. chamber | G. chamber | G. chamber | (Nov 15) |
| A1-17 | 129 | 4 | 10 * | - | - | - | 23 ** |
| A2-87 | 100 | 33 | 33 | 85 | 12 ** | 14 ** | 5 ** |
| TX-110 | 143 | 35 | 49 | 82 | 20 * | 5 ** | 17 ** |
| GB-13 | 229 | 27 | 28 | - | | | 31 * |
| GB-49 | 143 | 49 | 54 | - | | | 26 ** |
| GB-536 | - | - | - | 34 | 14 ** | 6 ** | - |
| GB-713 | - | - | - | 47 ^ | 4 ** | 2 ** | - |
| GB-264 | 114 | 17 | 29 | 51 | 18 ** | 10 ** | 12 ** |
| SG 501 | 229 | 62 | 97 | 101 | 73 | 65 ** | 65 |
| FM 832 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

*, ** Significantly different from FM 832 at the 0.05 and 0.01 level of probability, respectively, based on Dunnett's test.

Table 5. Reniform nematode reproduction on most promising of 750 accessions of the USDA National Cotton Germplasm Collection (September 2001).

| | Average | Reniform nematodes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | root-knot | Number per sample | | | | | | | No. per | Mult. | % of |
| Entry | rating (0-5) | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Mean | g soil | factor | DPL 16 |
| TX-110 (GB) | 2.8 | 32 | 61 | 93 | 42 | 35 | 142 | 67.5 | 135 | 21.3 | 43.8% |
| TX-839 | 4.3 | 37 | 223 | 158 | 173 | 309 | 69 | 161.5 | 323 | 50.9 | 104.8% |
| TX-1167 | 1.5 | 174 | 300 | 285 | 376 | 289 | 309 | 242.2 | 484 | 76.3 | 157.1% |
| TX-1403 | 3.4 | 246 | 285 | 180 | 180 | 386 | 26 | 217.2 | 434 | 68.4 | 140.9% |
| GB-459 | 1.9 | 75 | 205 | 100 | 165 | 467 | 419 | 238.5 | 477 | 75.1 | 154.7% |
| GB-485 | 2.8 | 18 | 127 | 112 | 71 | 49 | 57 | 72.3 | 145 | 22.8 | 46.9% |
| GB-536 | 3.3 | 30 | 23 | 38 | 20 | 35 | 46 | 32.0 | 64 | 10.1 | 20.8% * |
| GB-581 | 2.5 | 50 | 156 | 68 | 65 | 27 | 67 | 72.2 | 144 | 22.7 | 46.8% |
| GB-681 | 3.4 | 82 | 235 | 185 | 197 | 344 | 114 | 192.8 | 386 | 60.7 | 125.1% |
| GB-706 | 2.8 | 15 | 191 | 172 | 128 | 174 | 163 | 140.5 | 281 | 44.3 | 91.1% |
| GB-713 | 3.2 | 11 | 11 | 13 | 20 | 10 | 12 | 12.8 | 26 | 4.0 | 8.3% ** |
| A-623 | 0.0 | 42 | 83 | 73 | 126 | 156 | 79 | 93.2 | 186 | 29.3 | 60.4% |
| TX-1348 | 3.5 | 50 | 63 | 56 | 47 | 67 | 51 | 55.7 | 111 | 17.5 | 36.1% * |
| DPL 16 | 3.0 | 139 | 251 | 84 | 209 | 119 | 123 | 154.2 | 308 | 48.6 | 100.0% |

*, ** Significantly different from FM 832 at the 0.05 and 0.01 level of probability, respectively, based on Dunnett's test.

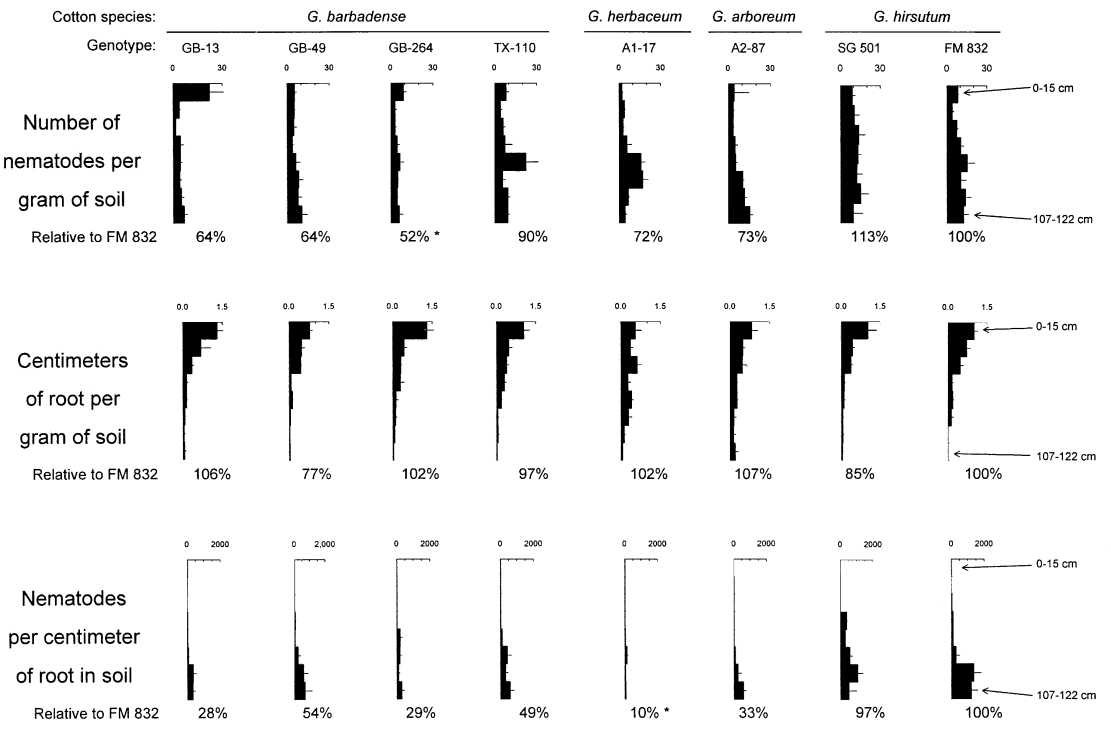


Figure 1. Roots and reniform nematodes in soil at end of season for exotic cotton species at North Farm, 2001. SG 501=Suregrow 501. FM 832=Fibermax 832. Each value based on 4 replicate soil samples. Fine lines emerging from bars are standard errors. Asterix (*) indicates significantly different from FM 832 at $P$=0.05 or lower based on Dunnett's test.