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Histological Observations of *Rotylenchulus reniformis* on *Gossypium longicalyx* and Interspecific Cotton Hybrids[1]

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Abstract: Observations on the development of reniform nematode (*Rotylenchulus reniformis*) on roots of *Gossypium longicalyx, G. hirsutum,* and two interspecific hybrids derived from them were made by light microscopy. *Gossypium longicalyx* is reported to be immune to reniform nematode, but the mechanism(s) for resistance are unknown. Penetration of *G. longicalyx* roots by female nematodes was confirmed, and incipient swelling of the females, indicating initiation of maturation of the reproductive system, was observed. Female maturation occurred up to the formation of a single embryo inside the female body but not beyond this point. In both hybrids, development was inhibited but progressed further than in the immune parent. Reactions ranged from highly compatible, with the formation of active syncytia and full development of females, to incompatible with little or no development of the female. Compatible plants showed characteristic hypertrophied cells, enlarged nuclei, dense cytoplasm, and partial dissolution of cell walls, whereas incompatible plant reactions included lignification of the cells adjacent to the nematode head, or the complete collapse and necrosis of the cells involved. The need to characterize reactions and to carefully select among the plants descended from the hybrids during the introgression process, as well as the importance of combining the results of reproduction tests with histological observation of the plant-nematode interactions, is discussed.

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

The reniform nematode (*Rotylenchulus reniformis*) is an economically important parasite of upland cotton (*Gossypium hirsutum*) (Koenning et al., 2004). Useful resistance to reniform nematode in *G. hirsutum* appears to be limited (Robinson and Percival, 1997; Robinson et al., 1999; Yik and Birchfield, 1984), so there has been increased interest in the introgression of resistance to this nematode from *G. longicalyx* and other diploid *Gossypium* species into cultivated genotypes (Robinson et al., 2004; Stewart and Robbins, 1996). Among the primitive species and races of *Gossypium* that have been tested against reniform nematode, only *G. longicalyx* is reported to be immune (Yik and Birchfield, 1984). However, the mechanisms for its resistance are not known, and no observations have been published on the cellular changes induced by the nematode in *G. longicalyx* or *G. longicalyx* hybrids that are being used to introgress resistance into agronomic cotton (Robinson et al., 2004).

Parasitism by reniform nematode in a wide range of plant hosts, including cotton, involves the formation of syncytia to provide nutrition for the developing female. The events that occur at this feeding site may determine the degree of susceptibility of cotton plants to the nematode (Agudelo et al., 2005; Rebois et al., 1975). The objective of this work was to make histological observations of reniform nematode penetration and development on roots of *G. longicalyx, G. hirsutum,* and interspecific *Gossypium* hybrids.

Materials and Methods

Plants of the triple-species hybrids *G. hirsutum* × *G. longicalyx* × *G. arboreum* (HLA), and *G. hirsutum* × *G. herbaceum* × *G. longicalyx* (HHL) (Bell and Robinson, 2004) were propagated by rooting stem cuttings in tap water and planting the rooted cuttings in 500-cm^3 plastic pots for 8 to 12 weeks. Eleven cuttings of each hybrid and 15 seedlings each of *G. hirsutum* cv. Tamcot CAMD-E and *G. longicalyx* accession F1-1 were maintained separately in 500-cm^3 plastic pots for 8 to 12 weeks. The pot-bound root ball of each plant was removed, slipped into a pot-shaped container made from 2-mm-mesh nylon screen, and transplanted into a 4-liter pot containing sand that was uniformly infested with 8 *R. reniformis*/cm^3 soil by mixing 1 part artificially infested silt soil with 20 parts sand. Roots that grew out into the surrounding sand from the original root ball were collected from five plants of each genotype 6, 14, and 22 days after transplanting. The only exception occurred on day 6, when roots from only one plant of *G. longicalyx* were collected. Collected roots were fixed in FAA (parts by volume of water, ethanol, formalin, acetic acid = 40:20:6:1). Fifty centimeters of root length from each plant was examined microscopically at 200 X. The nematodes protruding from roots in each of the following categories were counted: vermiform and not swollen; vermiform but starting to swell; 50% swollen; fully swollen but no gelatinous egg matrix formed; fully swollen with egg matrix but no eggs; fully swollen with eggs in egg matrix. Roots from each plant were then shipped to the University of Arkansas (Fayetteville, AR), transferred to Karnovsky's fixative, and dehydrated in an ethanol series. Short lengths of roots with observable female nematodes attached were further dehydrated with 100% propylene oxide and embedded in Spurr's

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TABLE 1. Penetration, development, and feeding site establishment by *Rotylenchulus reniformis* on *G. longicalyx, G. hirsutum,* and *Gossypium* triple species hybrids HLA and HHL 6, 14, and 22 days after transplanting pot-bound plants into nematode-infested sand.

		Reniform nematodes on roots									
		Separate developmental stages (% of total number)						All stages combined			
			Developing		Fully saccate						
Days after inoculation	Genotype	Vermiform	Some	50%	Exposed	With matrix	With eggs	Total number examined	% density on *G. hirsutum*	Number per cm root	Feeding sites with normal syncytia
Day 6	*G. hirsutum*	86	13	1	0	0	0	495	100	1.98	1/5
	HLA hybrid	100	>0	0	0	0	0	395	80	1.58	0/5
	HHL hybrid	94	6	0	0	0	0	404	82	1.62	0/5
	G. longicalyx	99	1	0	0	0	0	74	75	1.48	0/5
Day 14	*G. hirsutum*	23	10	10	22	19	17	212	100	0.85	5/5
	HLA hybrid	91	6	2	0	1	0	90	42	0.36	6/10
	HHL hybrid	98	2	0	0	0	0	133	62	0.53	7/10
	G. longicalyx	100	0	0	0	0	0	130	61	0.52	0/5
Day 22	*G. hirsutum*	16	9	20	20	14	22	148	100	0.59	5/5
	HLA hybrid	96	4	0	0	0	0	46	31	0.18	7/10
	HHL hybrid	85	12	0	0	2	0	82	56	0.33	8/10
	G. longicalyx	100	0	0	0	0	0	95	64	0.38	0/5

G. hirsutum is cv. TAMCOT CAMD-E; each nematode count value is the mean of 5 plants, 50 cm root/plant.

FIG. 1. Incipient swelling of female observed on resistant *Gossypium longicalyx* (F1-1) roots 14 days after inoculation, with abnormal development of single embryo.

epoxy resin. Thick sections (1 μm) were stained with 1% toluidine blue and examined using a phase contrast light microscope.

RESULTS AND DISCUSSION

Root growth progressed across the 3 weeks of root collection, and sufficient roots were present to collect 50 cm of root from every plant at each time. Most (86% to 100%) of the nematodes protruding from newly grown roots 6 days after transplanting were vermiform with no swelling (Table 1). The average densities of nematodes on the four genotypes were similar, ranging from 1.48 to 1.98 nematodes/cm, indicating that resistance had no influence on the number of nematodes invading root tissue. Between days 6 and 22, the density of nematodes found on *G. hirsutum* roots diminished from 1.98 to 0.59 nematodes/cm of root, and proportional decreases were observed in densities on *G. longicalyx* and the hybrids, indicating that the number of nematodes present in roots of all genotypes diminished similarly over time. On days 14 and 22, nematodes at all stages of development were common on roots of *G. hirsutum,* but no indication of development beyond the

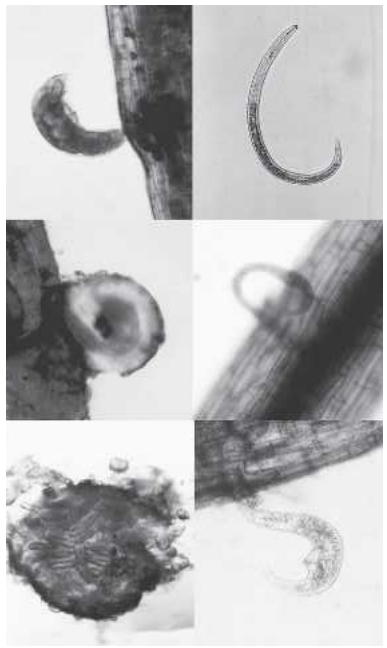

FIG. 2. Progression of normal female maturation on a susceptible cotton plant, from infective vermiform female to the development of the gelatinous matrix containing the eggs.

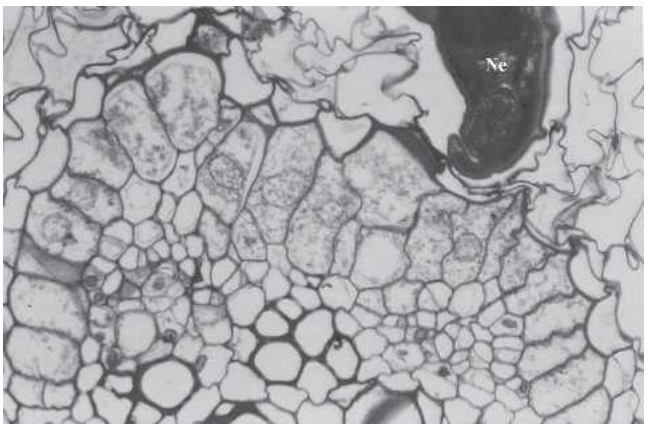

FIG. 3. Active syncytium observed on cross section of susceptible Tamcot CAMD-E roots, 14 days after inoculation. (Ne: nematode).

vermiform stage was noted on *G. longicalyx,* and only limited development (incipient swelling and gonad maturation) was apparent for nematodes on the hybrids (less than 15% of the total).

In the *G. longicalyx* feeding sites examined histologically, incipient swelling of the females, indicating initiation of maturation of the reproductive system, was observed (Fig. 1). Maturation up to the formation of a single embryo inside the female body occurred in some instances. No maturation beyond this point was observed. In susceptible plants, the female reproductive system matured before production of the gelatinous matrix and eggs, and embryos developed outside the female body (Fig. 2). In the hybrids, most females did not develop and diverse responses were observed among feeding sites. Reactions ranged from incompatible (resistant) with no apparent development of the female to highly compatible (susceptible) (Fig. 3), with the formation of active syncytia and full development of females (Fig. 4–6). Compatible reactions showed characteristic hypertrophied cells, enlarged nuclei, dense cytoplasm, and partial dissolution of cell walls (Figs. 3,4a). Incompatible plant reactions included lignification of the cells adjacent to the nematode head, or the complete collapse and necrosis of the cells involved (Fig. 5).

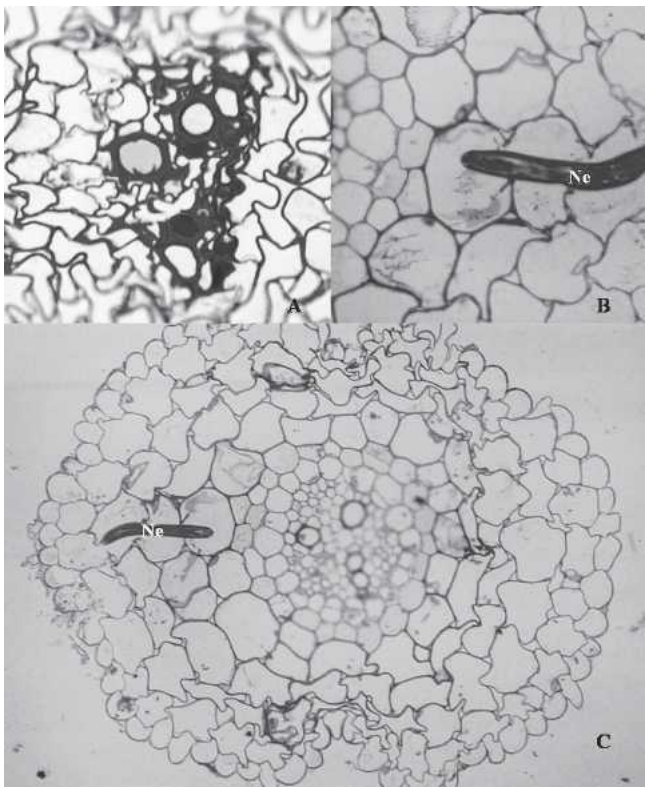

FIG. 5. Genotype ADDF, 6 days after inoculation. A) Incompatible interaction; necrosis and collapse of pericycle and phloem cells, indicated by the arrows. B) Detail of nematode anterior portion; no collapse of cells. C) Plant with apparent compatible reaction but no observable modification of host cells. (Ne: nematode).

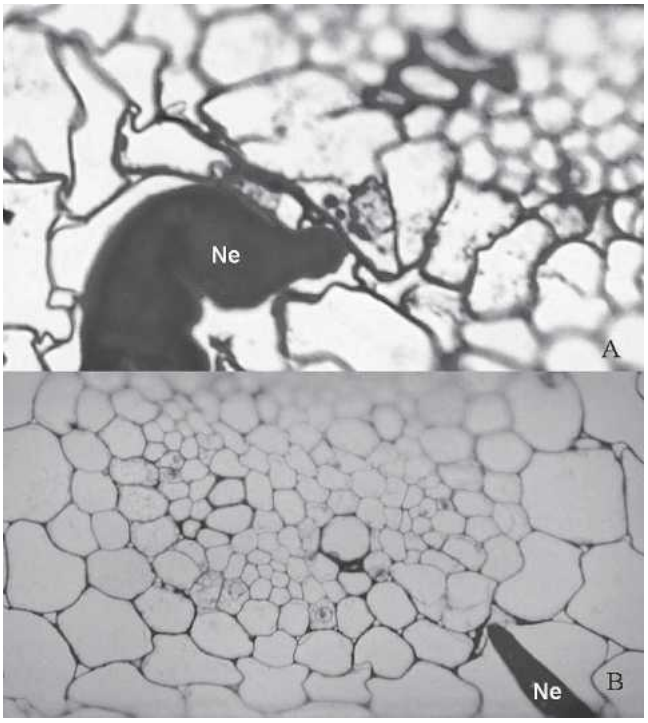

FIG. 4. Sections of roots of genotype AADF, 14 days after inoculation. A) Compatible interaction; swollen female and normal syncytium. B) Absence of syncytium and moderate swelling of female. (Ne: nematode).

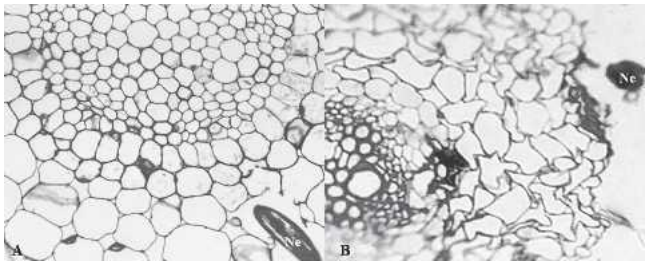

FIG. 6. Cross sections of roots of resistant genotype F1-1, 6 days after inoculation. A) No resistant reaction has initiated. B) Characteristic resistant reaction observed. (Ne: nematode).

These results indicate that the resistance found in *G. longicalyx* is incompletely dominant because the mechanism(s) of resistance was expressed in hybrid combinations but differed among feeding sites in contrast to the response of *G. longicalyx,* where maturation beyond a slightly swollen female with only one egg apparently did not occur. Some female nematodes did develop on the hybrids, and in those cases a compatible plant response was observed. However, the number of females reaching reproductive maturity on the hybrids overall was low. This result is consistent with a recent greenhouse experiment (Robinson and Bell, unpubl.), in which 28 replicate plants of the susceptible *G. hirsutum* cv. Deltapine 16 averaged 724 ± 129 (± confidence interval at $P = 0.05$) nematodes/g soil 3 months after inoculation, in contrast with 1 ± 0.5 nematodes/g soil for 27 replicate plants of the HLA hybrid.

Lignification of cells in the area of penetration indicate that a physical barrier to the nematode was erected. Cell collapse and necrosis in the area where a syncytium would normally form suggests that the plant undergoes a hypersensitive reaction that denies the female nematode a feeding site in the plant. Once the female has penetrated the root, she cannot move, and if she does not receive sufficient nourishment she cannot reproduce; therefore, the nematode population does not increase.

The relationship between plant/nematode compatibility reactions and reniform nematode reproduction needs to be examined in greater detail. Different levels of resistance among plants of segregating populations descended from the *G. longicalyx* hybrids indicate that the resistance is heritable and can probably be transferred to upland cotton. It will be instructive to characterize resistant reactions as the introgression project progresses, in relation to overall rates of reniform nematode reproduction. Identification of candidate molecular markers for the resistance trait will facilitate introgression of resistance into *G. hirsutum* genotypes.

Literature Cited

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

Bell, A. A., and A. F. Robinson. 2004. Development and characteristics of triple species hybrids used to transfer reniform nematode resistance from *Gossypium longicalyx* to *Gossypium hirsutum*. Proceedings of the Beltwide Cotton Conference 1:422–426.

Koenning, S. R., T. L. Kirkpatrick, J. L. Starr, J. A. Wrather, N. R. Walker, and J. D. Mueller. 2004. Plant-parasitic nematodes attacking cotton in the United States: Old and emerging production challenges. Plant Disease 88:100–113.

Rebois, R. V., P. A. Madden, and B. J. Eldridge. 1975. Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by *Rotylenchulus reniformis*. Journal of Nematology 7:122–139.

Robinson, A. F., A. A. Bell, N. Dighe, and D. Stelly. 2004. Status report on introgression of reniform nematode resistance from *Gossypium longicalyx*. Proceedings of the Beltwide Cotton Conference 1: 119 (Abstr.).

Robinson, A. F., C. G. Cook, and A. E. Percival. 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.

Stewart, J. M., and R. T. Robbins. 1996. Enhancement of cotton germplasm resistant to reniform nematode. Special Report, Agricultural Experiment Station, Division of Agriculture, University of Arkansas No. 178:20–22.

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