Resistance and Resistant Reaction of Gossypium arboreum to the Reniform Nematode, Rotylenchulus reniformis

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Abstract: *Gossypium arboreum* 'Nanking CB 1402' possessed a high level of resistance to *Rotylenchulus reniformis*. Within 16 h, the nematode penetrated roots of resistant and susceptible cottons equally. After 36 h, significantly fewer nematodes were found in resistant roots. Larvae fed in either an endodermal or pericyclic cell and had no specificity for root tissue of a particular age. In roots of resistant *G. arboreum* '1402,' wall breakdown of pericyclic cells was evident after 3 d, endodermal and cortical cells collapsed, and the hypertrophied pericyclic cells disintegrated within 12 d. Cell walls immediately adjacent to the nematode's head were thickened and more safranin positive in resistant than in susceptible cotton cultivars. Several other cultivars of *G. arboreum* were also resistant to *R. reniformis*, based on nematode fecundity and percent egg reduction. *Key words:* cotton, histopathology, nematode reproduction.

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, is an important pest of cotton in subtropical and tropical areas (7). Although cotton cultivars vary in susceptibility to *R. reniformis* (3,12), resistance has not been reported for any cultivar of the important commercial cottons, *Gossypium hirsutum* L. and *G. barbadense* L. R. V. Rebois (unpublished data) and Carter (4), however, found that *G. arboreum* L. 'Nanking CB 1402' appeared to be resistant to *R. reniformis*.

The responses of root tissues of various plants to *R. reniformis* are well documented (1,5,6,8,10,13,14,15,16,17). Birchfield (1) originally reported the feeding site of *R. reniformis* on cotton *(G. hirsutum)* to be the phloem tissues of the root. Studies on other plant species, however, led him to conclude that *R. reniformis* fed primarily in the pericycle (2). Cohn (5,6) observed a consistent pattern of parasitism by *R. reniformis* in several species of plants, including cotton *(G. hirsutum)*. He observed that the reniform nematode "comes to rest" in the endodermis and induces a synctium composed primarily of pericycle cells extending around the root to either side of the initial feeding cell. Oteifa (12,13) reported hypertrophy of pericycle cells in roots of seedlings and of periderm cells in 4- to 5-wk-old *G. barbadense* plants.

Previous studies with cotton described only susceptible reactions to *R. reniformis*. However, Rebois et al. (14,15) described a resistant reaction to the reniform nematode in soybeans, *Glycine max* L. Merr. They found that the initial stages of infection appeared similar to those in susceptible plants and described two subsequent types of resistant reactions.

This study describes a resistant response to, and the penetration and development of, *R. reniformis* in *G. arboreum* Nanking CB 1402. In addition, several other cultivars of *G. arboreum* were evaluated for resistance to *R. reniformis*.

METHODS AND MATERIALS

Histology: Seed of *G. arboreum* CB 1402, resistant to *R. reniformis*, and *G. arboreum* '16' and *G. hirsutum* 'Deltapine 16' (DP16), susceptible to *R. reniformis*, were germinated in closed-end 5.5-cm plastic containers filled with a steam-sterilized sandy clay loam. The plants were grown in growth chambers at 29 C ± 1 C and 24,800 lux with 14-h days and 10-h nights. Larvae of *R. reniformis* were collected from roots of cowpea, *Vigna unguiculata* (L.) Walp., and from soil using a Baermann funnel containing aqueous 8-quinolinol sulfate (20 mg/liter). The suspension of larvae collected after 24 h was centrifugated at 1,000 × *g* for 3 min, and the pellet was washed three times by resuspension in sterile distilled water and centrifugation.

Water suspensions of 2,500 larvae/seedling were introduced with a syringe 4 cm into the soil next to each of 70 10-d-old seedlings. Plants were carefully removed from the soil 48 h after inoculation with nematodes and the roots washed in tap water to remove larvae not embedded in root tissue.

Received for publication 12 January 1981.

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The seedlings were then transplanted to containers of fresh, sterile soil.

Ten plants of each cultivar were harvested 1, 2, 3, 4, 7, 12, and 21 d after inoculation and their roots washed free of soil. The roots were fixed in FAA (formalin-acetic acid-alcohol) or CRAF (chromic acid-acetic acid-formalin) under a slight vacuum for at least 48 h, dehydrated in a tertiary butyl alcohol series, embedded in Paraplast (melting point, 56–57 C), sectioned at 12 μm, stained with safranin and fast green (9), mounted in Permount, and examined microscopically.

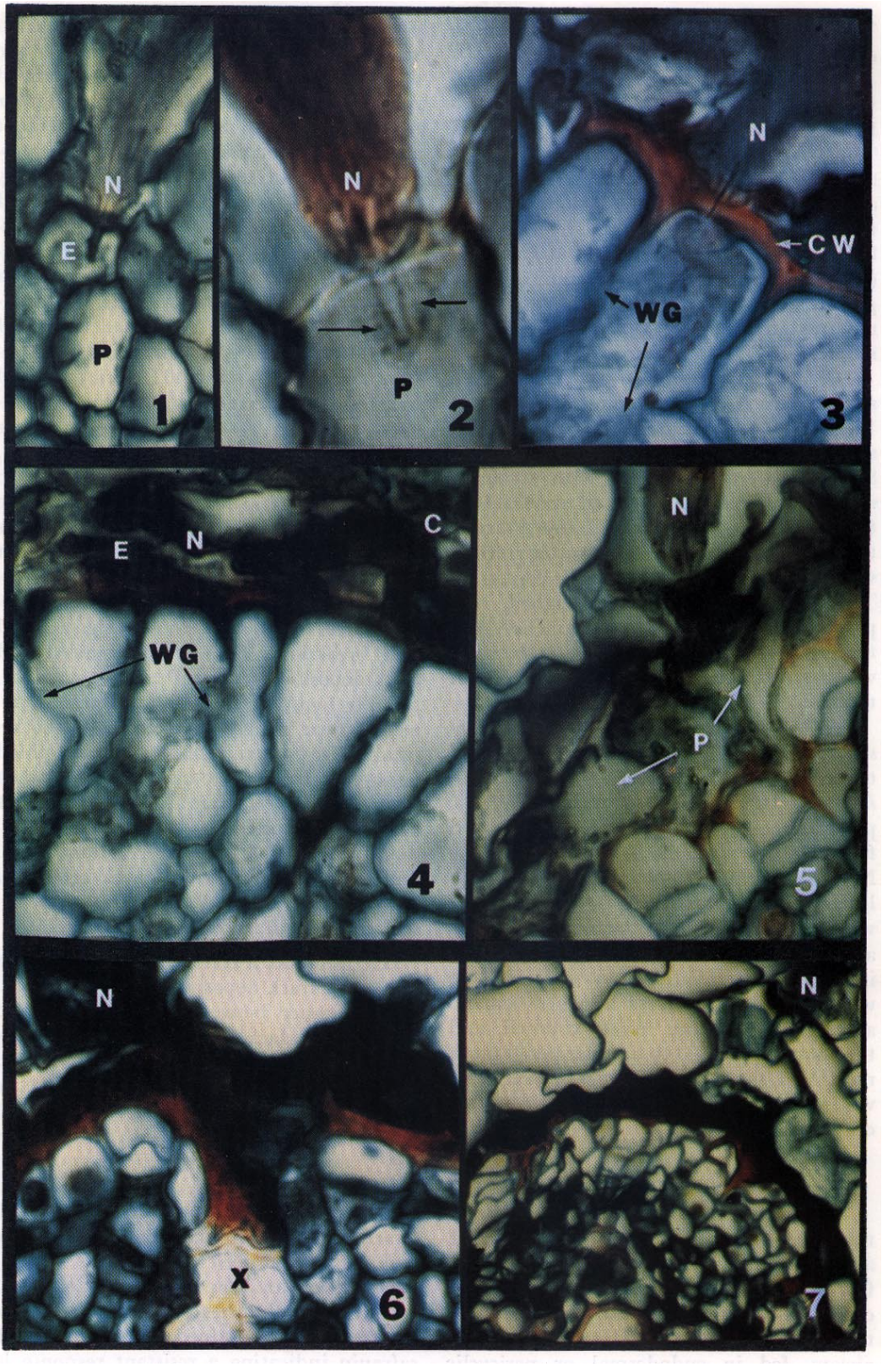
Larval penetration of roots: Eighteen seedlings each of *G. arboreum* 16 and CB 1402 and *G. hirsutum* DP16 were grown singly in 20-ml closed-end plastic containers. Seedlings were inoculated with 1,000 *R. reniformis* larvae as described above. At 4, 8, 16, and 36 h, and 4 and 7 d after inoculation, three seedlings of each cotton cultivar were carefully washed from their container. The roots were removed, weighed, and placed in hot acid fuchsin-lactophenol. The total number of larvae penetrating the roots was counted. The experiment was repeated twice.

Nematode development and G. arboreum susceptibility: Fifteen seedlings each of twelve cultivars of *G. arboreum* and *G. hirsutum* DP16 were grown in 15-cm plastic pots, five seedlings/pot. Seed was obtained from the *G. arboreum* collection of the National Cotton Pathology Laboratory, College Station, Texas. Each pot was infested with 5,000 *R. reniformis* larvae as described above. All plants were harvested 30 d after inoculation. Individual root systems were weighed, the number of attached females and egg bearing egg masses counted, and the counts converted to number per gram of root. Also, the number of eggs per egg mass was counted. The experiment was repeated once.

RESULTS

Histology: Larvae of *R. reniformis* penetrated roots of resistant *G. arboreum* 1402 and susceptible cotton cultivars at 24 h and fed in either endodermal (Fig. 1) or pericyclic cells (Figs. 2, 3). Larvae penetrated the cortex to the stele and usually, but not always, fed in endodermal or pericyclic cells near a protoxylem pole (Fig. 10). No specificity for root tissue of a particular age was observed. Cytoplasmic agglutination of the initial feeding cell (prosyncyte) similar to that reported by Rebois (15) was evident around the stylet, particularly when the prosyncyte was initiated in the pericycle (Fig. 2). Within 3 d, pericycle cells hypertrophied near feeding sites in both resistant and susceptible roots (Figs. 8, 9). A "plug" or indentation of densely-stained material often formed around the stylet whether the nematode was feeding in an endodermal cell (Fig. 8) or a pericyclic cell (Fig. 3). In resistant *G. arboreum* 1402 pericycle wall breakdown was evident at 3 d (Fig. 9); similar breakdown was not observed in either susceptible *G. arboreum* 16 (Fig. 8) or *G. hirsutum* DP16. Nematodes appeared to develop equally in resistant and susceptible cultivars for 7 d after penetration; further development of nematodes in the resistant cultivar, however, was restricted. Endodermal and cortical cells collapsed and disintegrated in resistant roots (Fig. 4). Cell wall breakdown became pronounced and thick deposits of safranin positive material, possibly lignin or lignin-like substance, appeared near nematodes' heads. In the resistant *G. arboreum* 1402, the hypertrophied pericycle cells began to disintegrate between 7 and 12 d after penetration (Fig. 5); this eventually resulted in death of the nematodes. Compatible cellular reactions to *R. reniformis* in the susceptible *G. arboreum* 16 and *G. hirsutum* DP16 confirms those described in detail in cotton by Cohn (5,6).

Occasionally a resistant reaction characterized by thick deposits of a safranin positive substance between the pericycle and cortex and a lack of pericycle hypertrophy was observed (Fig. 6). This deposit extended approximately one-half the circumference of the stele (Fig. 7). The development of the nematode associated with the deposit was retarded compared with nematodes in susceptible roots; the gelatinous matrix and eggs were absent in roots of resistant cultivars. Occasionally larvae penetrate the endodermis and pericycle and feed in a cell near the protoxylem (Fig. 11). Cell walls surrounding the head of the nematode thickened and stained deep red with safranin indicating a resistant response in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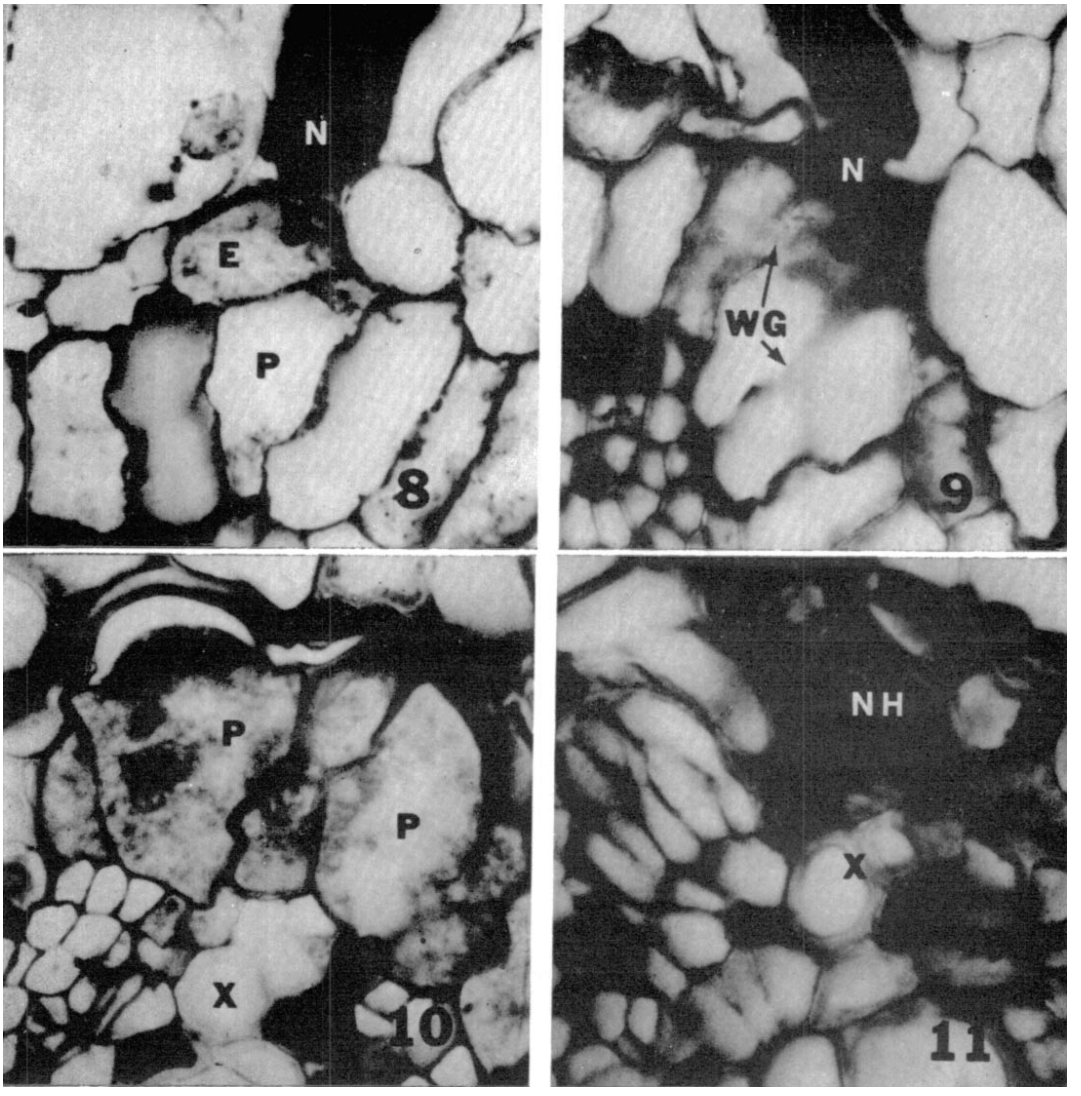

Fig. 8–11. Comparison of susceptible and resistant responses of *Gossypium arboreum* to *Rotylenchulus reniformis*. 8) Susceptible '16' 3 d after inoculation. Nematode (N) feeding in an endodermal cell (E); note hypertrophy of pericyclic cells (P) and dense area around the stylet (ca. ×1000). 9) Resistant 'CB1402' 3 d after inoculation. Nematode (N) feeding in pericyclic cell. Cell wall breakdown (WG) is evident near the initial feeding cell (×900). 10) Susceptible '16' 21 d after inoculation. Hypertrophied pericyclic cells (P). The syncytium is located near the protoxylem (X) (×900). 11) Resistant 'CB1402' 21 d after inoculation. Nematode feeding in cells near a protoxylem (X) element. Cell walls near the nematode head (NH) are thickened (×900).

Fig. 1–7. The resistant response of *Gossypium arboreum* to *Rotylenchulus reniformis*. 1) Cross section of resistant root showing a nematode (N) with stylet in an endodermal cell (E) 24 h after inoculation (P = pericycle) (×900). 2) Nematode (N) penetrating pericyclic (P) cell 24 h after inoculation. Note wedge of denser material (→) immediately surrounding the stylet (ca. ×2000). 3) Deeply stained, thickened cell walls (CW) near nematode head (N) and wall gaps (WG) in pericyclic cells (ca. ×1750). 4) Disintegrated endodermal (E) and cortical (C) cells near the nematode head (N), 7 d after inoculation; cell wall gaps (WG) are visible in the pericyclic cells (ca. ×1750). 5) Lysis of pericyclic cells (P) and collapse of the syncytium, 12 d after inoculation (N = nematode) (×1000). 6) Resistant reaction 21 d after inoculation is characterized by a lack of pericycle hypertrophy and by the thickened cells between the stele and cortex extending to the protoxylem (X). The pericycle is not identifiable (N = nematode) (×900). 7) Resistant reaction showing a thick, lignin-like deposite around about one-half the circumference of the stele 21 d after inoculation; note lack of hypertrophied pericyclic cells (N = nematode) (×400).

these cells. Hypertrophy of the pericycle was absent in these infections.

Larval pentration of roots: R. reniformis larvae entered roots of each cultivar equally between 8 and 16 h after inoculation; however, after 36 h and 4 and 7 d, significantly ($P = 0.01$) fewer larvae were counted in roots of resistant *G. arboreum* 1402 (Table 1). No larvae were found in any roots at 4 and 8 h after inoculation.

Nematode development and G. arboreum susceptibility: Significant differences in *R. reniformis* development were found among cultivars of *G. arboreum* (Table 2). CB 1402 appeared to be the most incompatible cultivar when the number of attached fe-

Table 1. The number* of *Rotylenchulus reniformis larvae* detected in resistant and susceptible cottons at various times after inoculation.

Gossypium sp. and cultivar	Host response†	Time after inoculation					
		Hours				Days	
		4	8	16	36	4	7
G. arboreum:							
CB 1402	R	0	0	3.1 a‡	6.4 a	10.5 a	9.4 a
16	S	0	0	6.4 a	20.6 b	35.2 b	36.2 b
G. hirsutum:							
DP 16	S	0	0	5.2 a	22.4 b	41.6 b	37.8 b

*Average per gram root tissue.
†R = resistant, S = susceptible.
‡Values in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple-range test.

Table 2. Resistance indices of various cotton cultivars, based on the number of attached *Rotylenchulus reniformis* females and egg masses per gram of root, eggs per egg mass, fecundity, and nematode reproduction.

Gossypium sp. and cultivar	Attached ♀ ♀* gram of root	Egg masses/ gram of root	Eggs/ egg mass†	Fecundity‡ index	% egg§ reduction
G. arboreum:					
CB 1402	2.3 a‖	2.3 a	4.2 a	4.2 a	99.2 a
27	8.1 b	7.4 ab	5.5 a	5.0 a	96.6 a
32	8.2 b	7.1 ab	12.8 b	11.1 ab	92.5 a
41	12.4 bc	9.8 b	6.2 a	4.9 a	95.0 a
20	16.6 c	11.3 b	11.7 ab	7.9 a	89.1 a
36	19.6 cd	16.2 bc	21.4 c	17.7 b	71.5 b
44	23.4 d	19.3 c	24.2 c	20.0 b	61.5 bc
30	23.9 d	19.4 c	27.1 c	22.0 b	56.7 c
42	24.2 d	19.2 c	28.6 c	22.7 b	54.8 c
28	29.1 d	25.9 c	30.8 c	27.4 b	34.3 d
47	41.7 e	35.8 d	31.2 c	26.8 b	8.0 e
16	48.3 e	44.2 e	42.1 d	38.5 c	0 e
G. hirsutum:					
DP 16	44.6 e	38.8 d	31.3 c	27.2 b	...

*Number of both immature and mature (with egg masses) females.
†Eggs were counted from 10 randomly selected egg masses.
‡Fecundity index $= \frac{\text{(egg masses/g root)} \times \text{(eggs/egg mass)}}{\text{♀ ♀ /g root}}$.
§% egg reduction (compared to DP16) $= \frac{\text{(egg masses} \times \text{eggs, susceptible)} - \text{egg masses} \times \text{eggs, test plant)} \times 100}{\text{(egg masses} \times \text{eggs, susceptible)}}$.
‖Values in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple-range test.

males was the resistance factor. Cultivars CB 1402, 27, 32, 41, and 20 were significantly more incompatible than other cultivars based on a fecundity index and percentage of egg reduction (Table 2).

DISCUSSION

Several studies with a number of different susceptible hosts have revealed a consistent plant response to parasitism by *R. reniformis*. Depending on the host, the nematode feeds in either an endodermal or pericyclic cell. In all cultivars, a syncytium is then induced and is composed of uninucleated or multinucleated, hypertrophied pericycle cells extending to either side of the initial feeding cell. Observations in this study did not indicate the number of nuclei within an individual cell. The susceptible reactions of *G. arboreum* and *G. hirsutum* to *R. reniformis* in this study confirm the observations of Cohn (5,6) for cotton.

The development of syncytia in resistant and susceptible *G. arboreum* is initially similar, although partial dissolution and separation of cell walls did occur earlier in resistant *G. arboreum* 1402 than in susceptible 16. In resistant CB 1402, the formation of cell wall gaps proceeded into cell lysis and eventually cell necrosis. The sequence of syncytial development in resistant and susceptible soybeans (15) appears to be similar to resistant and susceptible cotton, respectively. The rate of wall lysis proceeded faster in resistant 'Peking' soybeans and 1402 cotton than in the susceptible cultivars. The resistant reaction in soybeans was described as relatively uncontrolled lysis which ultimately denied the parasite a suitable energy source for development (15).

Originally we did not observe cell wall dissolution or breakdown in either resistant or susceptible cottons (4). Observations in this study, based on a much larger sample size, indicated that cell wall lysis between adjoining hypertrophied cells of the pericycle is a common phenomenon. The more rapid (earlier) formation of cell wall gaps is characteristic of the resistant reaction. Cohn (5) also showed cell wall breakdown in cells close to the feeding site in a susceptible cotton *(G. hirsutum)*.

In susceptible *G. arboreum* 16, a resistant reaction was not associated with development of *R. reniformis* away from the protoxylem as observed with soybeans (14).

The development of thickened, safranin positive cell walls near the nematode feeding site was common to both susceptible and resistant reactions. The thickening was, however, much more extensive and pronounced in resistant 1402 and may contribute to a walling-off of the parasite. Cell walls also thickened when nematodes developed at the xylem, suggesting an incompatible reaction.

The resistance of *G. arboreum* 1402 appears to be physiological rather than morphological. This is based on the following observations: numbers of nematodes penetrating resistant and susceptible roots is similar; syncytia are initiated in resistant roots and initially appears similar to susceptible reactions; and *R. reniformis* larvae occasionally mature and produce eggs in resistant roots.

G. arboreum as a species had considerably more resistance to *R. reniformis* than did *G. hirsutum;* 5 of 12 cultivars tested were classified as resistant. The value of this genetic resistance to the development of commercially acceptable cotton cultivars is not known.

The use of resistance ratings, based on egg production, is potentially useful in categorizing resistance to *R. reniformis*. The fecundity index, in particular, measures the actual egg production per attached female nematode on a gram of root and is, therefore, a direct measure of not only numbers of attached nematodes but also reproduction potential. Lim and Castillo (11) developed a resistance rating from an average of several parameters. They found highly significant positive linear correlations among all resistant indices; egg masses per gram of root was the least efficient. Egg production was based on eggs per egg mass and did not reflect the relative degree of nematode infection.

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