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Resistance to *Meloidogyne incognita* Race 3 and *Rotylenchulus reniformis* in Wild Accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico[1]

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Abstract: Forty-six accessions of *G. hirsutum* and two of *G. barbadense* were examined for resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in environmental growth chamber experiments, with the objective of finding new sources of resistance. Only the *G. barbadense* accessions, TX-1347 and TX-1348, supported significantly less reproduction by *R. reniformis* than the susceptible control, Deltapine 16 (USDA accession SA-1186). However, they were highly susceptible to *M. incognita* race 3. The *G. hirsutum* accessions TX-1174, TX-1440, TX-2076, TX-2079, and TX-2107 had levels of resistance to *M. incognita* race 3 as great as or greater than those of Clevewilt 6 and Wild Mexican Jack Jones, which are the primary sources of resistance to *M. incognita* race 3 in the most resistant breeding lines. No accession was as resistant as the highly resistant line Auburn 623 RNR (SA-1492). Resistant accessions were from the Mexican coastal states of Campeche, Quintana Roo, Tabasco, Veracruz, and Yucatan. Populations of *R. reniformis* from Alabama, Mississippi, Louisiana, and Texas, and of *M. incognita* race 3 from Mississippi, Texas, and California, had similar reproductive rates on resistant genotypes. Thus, new sources of resistance to *M. incognita* race 3 but not to *R. reniformis* were identified in wild accessions of *G. hirsutum* from southern Mexico.

Key words: cotton, *Gossypium barbadense, Gossypium hirsutum, Meloidogyne incognita,* nematode, reniform nematode, resistance, root-knot nematode, *Rotylenchulus reniformis.*

The major nematode pests of upland cotton (*Gossypium hirsutum* L.) are *Rotylenchulus reniformis* Linford and Oliveira and race 3 of *Meloidogyne incognita* (Kofoid and White) Chitwood. No genotype of *G. hirsutum* has been found to have more than a moderate level of resistance to *R. reniformis* (Jones et al., 1988). Incorporation of resistance to *R. reniformis* from related, resistant species of cotton, such as *G. longicalyx* (Yik and Birchfield, 1984) and *G. arboreum* (Carter, 1981), has been unsuccessful due to chromosome and ploidy incompatibilities (Wilson, 1968). Significant progress, however, has been made toward developing genotypes with resistance to *M. incognita* race 3 (Shepherd, 1982). Three cultivars, Stoneville LA 887, Hartz 1560, and CPCSD Nem-X, have a moderate level of resistance, and several breeding lines are almost immune. The primary sources of resistance of *M. incognita* race 3 are from an obsolete cultivar, Clevewilt6, and a wild accession of *G. hirsutum* collected from an unknown site in Mexico in the late 1940s. The latter is maintained in the USDA Cotton Germplasm Collection as accession TX-2516 under the name Wild Mexican Jack Jones. Mexico and Central America comprise the geographical origin of *G. hirsutum* and are the region of its greatest genetic variability (Percival and Kohel, 1991).

In the early 1980s, two extensive screening studies were conducted to find additional sources of resistance. Shepherd (1983) tested 471 wild accessions of *G. hirsutum* from the USDA Texas Race Stock Collection for resistance to *M. incognita* race 3; of these, 231 were from Mexico (Fig. 1) and 18 had lower root gall ratings than Clevewilt 6.

Yik and Birchfield (1984) examined reproduction by *R. reniformis* on 30 *G. hirsutum* cultivars, 33 wild accessions of *G. hirsutum* from Mexico (Fig. 1), 34 wild accessions from other regions, 32 entries of 19 wild *Gossypium* spp. from several continents, and more than 20 plant species in seven related

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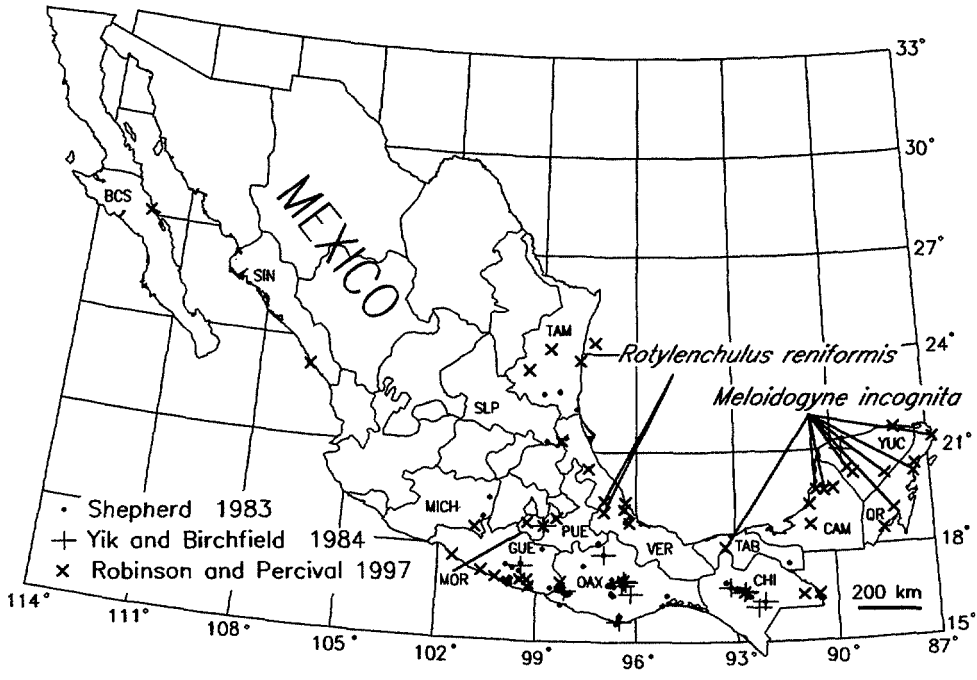

FIG. 1. Origins of accessions of *Gossypium hirsutum* from Mexico examined for resistance to *Meloidogyne incognita* race 3 by Shepherd (1983), to *Rotylenchulus reniformis* by Yik and Birchfield (1984), and to both nematodes in the present study. Accessions showing resistance to each nematode in the present study are indicated. The two accessions with resistance to *R. reniformis* had *G. barbadense* rather than *G. hirsutum* phenotypes. Abbreviated names of Mexican states include Baja California Sur (BCS), Campeche (CAM), Chiapas (CHI), Guerrero (GUE), Michoacán (MICH), Morelos (MOR), Oaxaca (OAX), Puebla (PUE), Quintana Roo (QR), San Luís Potosí (SLP), Sinaloa (SIN), Tabasco (TAB), Tamaulipas (TAM), Veracruz (VER), and Yucatán (YUC).

genera. Several wild species of *Gossypium* had appreciable levels of resistance, but 96% of the wild accessions of *G. hirsutum* tested were susceptible. One accession of *G. hirsutum* from Haiti, USDA TX-893, was considered resistant to *R. reniformis,* and six others had less nematode egg production than the susceptible control, Deltapine 16. Several breeding lines are tolerant to *R. reniformis* (Cook et al., 1997).

Since 1984, more than 1,000 wild accessions of *G. hirsutum* have been added to the USDA Cotton Germplasm Collection, and many of these are from previously unrepresented biotopes of Mexico in northern Tamaulipas or in the southern states of Tabasco, Campeche, Yucatan, and Quintana Roo (Fig. 1). Several phenotypic races of *G. hirsutum* occur in these areas, and their suitability as hosts for nematodes is unknown. The objective of this research was to examine reproduction by *R. reniformis* and *M. incognita* race 3 on previously untested accessions of *G. hirsutum* from Mexico in an effort to identify new sources of resistance to both nematodes.

MATERIALS AND METHODS

Inoculum: Populations of *R. reniformis* from Leighton, Alabama (LAL); Webb, Mississippi (WMS); Baton Rouge, Louisiana (BRLA); West Carroll Parish, Lousiana (WCPLA); and Weslaco, Texas (WTX) were propagated on tomato (*Lycopersicon esculentum* cv. Rutgers) in the greenhouse. Inoculum consisted of mixed vermiform stages obtained overnight by Baermann funnel from soil around tomato plants the day before inoculation. Populations of *M. incognita* race 3 from Minter City, Mississippi (MCMS); Seminole, Texas (STX); an unknown site on the Texas High Plains (HPTX); and the southern San Joaquin Valley of California (SJCA) were propagated on Rutgers tomato.

Inoculum consisted of second-stage juveniles (J2) hatched from eggs that were extracted from tomato roots with 1% NaOCl (Hussey and Barker, 1973) followed by centrifugal flotation in 1 M sucrose solution 3 to 5 days before inoculation. Nematodes of both species appeared unstarved, and more than 95% were motile when used for inoculations.

General protocol: Two seeds of a cotton entry were planted in a 500-cm^3 plastic pot filled with a 6:1 (v:v) mixture of sand (<400-µm particle size) and vermiculite, supplemented with 5 g/kg pelletized limestone. Twenty pots were prepared for each entry. Pots were maintained in a greenhouse at 23 to 32 °C. When seedlings were at the first true leaf stage (10–14 days after planting), they were culled to one per pot. Remaining plants were transplanted to 15-cm-diam. pots and grown to maturity in the greenhouse to confirm plant species identification. On the same day that plants were culled, the planting medium in each pot was infested with nematodes by injecting a nematode suspension 1 to 5 cm deep at two points 2 cm from the stem. Each pot received a single inoculation with 1,000 J2 of *M. incognita* race 3 or two inoculations 7 days apart with 2,000 mixed vermiform stages of *R. reniformis* applied each time. Pots were transferred to a 137-cm × 244-cm growth chamber programmed for a 14-hour light and 10-hour dark period with air temperatures of 30 °C and 26 °C, respectively. Plants were watered daily and fertilized weekly (15:16:17:1.0:0.21:0.1, N:P:K:Mg:Fe:Zn).

Seven weeks after the second inoculation with *R. reniformis,* plants were removed and plant height, number of main-stem nodes, number of fruiting structures, fresh and dry foliar weight, fresh and dry root weight, and taproot length were determined. Eggs were extracted with NaOCl followed by centrifugal flotation in 1 M sucrose solution. A 100-cm^3 aliquot of soil from each pot infested with *R. reniformis* was weighed, and vermiform stages were extracted overnight in Baermann funnels to estimate the total number of vermiform nematodes per pot (Robinson and Heald, 1991). Root systems of plants inoculated with *M. incognita* race 3 were rated for severity of galling using a scale of 0 = no galls detected, 1 = galls detected on <5% of the root system, 2 = approximately 25% of the root system galled, 3 = 50% galled, 4 = 75% galled, and 5 = more than 95% of the root system galled. The reproduction of *R. reniformis* was calculated from the sum of eggs and vermiform stages extracted (Pf) divided by 4,000 (Pi), and that of *M. incognita* from the number of eggs extracted (Pf) divided by 1,000 (Pi).

Experimental design and data analysis: Five experiments were conducted. Each consisted of six replications of pots inoculated with *R. reniformis* and six replications of pots inoculated with *M. incognita* race 3 in a randomized complete-block design. Plants of each entry were blocked by size at the time of inoculation and by position within the growth chamber. In all experiments, root systems from all pots inoculated with *M. incognita* race 3 were given gall ratings, and nematodes from all pots inoculated with *R. reniformis* were extracted by Baermann funnel. In all but the third experiment, nematode eggs were extracted from all root systems. In the third experiment, eggs were extracted only from the controls and from 18 wild accessions considered to be of greatest interest based on gall ratings or numbers of vermiform *R. reniformis* extracted by Baermann funnel. In each experiment, data for each nematode species were analyzed separately with a two-way analysis of variance. Fisher's protected LSD procedure was used to compare all entries with Deltapine 16, the susceptible control included in all experiments. Nematode-resistant controls also were included but differed among experiments. All data on nematode population densities were transformed with log $(x + 1)$ prior to analyses.

First experiment—accessions suspected to have resistance to R. reniformis: This test examined seven wild accessions of *G. hirsutum* from Mexico, Central America, and the Caribbean that had shown possible resistance to *R. reniformis* previously (Yik and Birchfield, 1984). The breeding lines La RN 1032 and M-315 RNR were included in this experiment as controls with resistance to *M. incognita* race 3.

Second experiment—first evaluation of strategically selected Mexican accessions: This experiment included 18 previously untested accessions from localities that were carefully selected to represent a wide range of latitude, precipitation, mean temperature, and elevation in Mexico (Fig. 1; Table 1). Auburn 634 RNR, resistant to *M. incognita* race 3, was included as a control.

Third experiment—second evaluation of Mexican accessions: Forty entries were tested, including seven accessions identified as particularly promising with regard to nematode resistance in the second experiment and 28 additional accessions from localities close to those where the first seven had been collected. Auburn 623 RNR (USDA accession SA-1492), Clevewilt6 (SA-245), Wild Mexican Jack Jones, and Stoneville LA 887 were included as controls with resistance to *M. incognita* race 3. TX-1180 and TX-2309 were tested only with *R. reniformis;* TX-1099 and Clevewilt6 were tested only with *M. incognita* race 3. All other entries were tested with both species.

The third experiment required an additional growth chamber; it was the same size as the first but differed in light, temperature, and relative humidity control. Three replications were maintained in the first chamber and three in the second. The first chamber provided 278 and 24 µmol photons/(m^2 second) of fluorescent and incandescent illumination, respectively, with 50% reduced fluorescent illumination during the first and last hours of daylight, stepwise temperature change, and no relative humidity control. The second chamber provided 340 and 43 µmol photons/(m^2 second) of fluorescent and incandescent illumination, respectively, plus maintenance of relative humidity above 50%, and ramp-and-soak rather than stepwise temperature control. The temperature regimen used was a 1-hour hold at 26 °C beginning at first light, followed by linear 4-hour ramp to 30 °C, a 6-hour hold, a 3-hour ramp down to 28.5 °C, and a final 10-hour ramp back to 26 °C, ending at first light. Lamps in this second chamber were programmed to provide 21 µmol photons/(m^2 second) of incandescent light during the first and last half hour of light, 191 µmol photons/(m^2 second) mixed fluorescent and incandescent light during the second and second-to-last half hour, and 383 µmol photons/(m^2 second) mixed light during the remaining 12 hours.

Fourth experiment—comparative increases of R. reniformis populations on TX-1347: Plants were inoculated separately with each of the

TABLE 1. Geographic data for the first set of Mexican accessions of *Gossypium hirsutum* evaluated for resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 under growth chamber conditions.

Accession number	Locality	State	Elevation (m)	Latitude	Mean temperature (°C)	Precipitation (mm)
TX-254	Tlacolula de Matamoros	Oaxaca	1,620	17°N	18.1	542
TX-751	El Rodeo	Morelos	1,060	19°N	24.3	960
TX-770	Lacantú River Basin	Chiapas	145	16°N	25.5	3,121
TX-1045	Huétamo	Michoacán	300	19°N	29.1	802
TX-1114	Mazatlán	Sinaloa	Sea level	23°N	24.1	812
TX-1182	San Lucas	Baja California Sur	Sea level	27°N	21.5	161
TX-1219	José Cardel	Veracruz	Sea level	19°N	25.4	1,244
TX-1348	Puerto Rico	Veracruz	1,509	19°N	18.0	1,427
TX-1529	El Papayo	Guerrero	Sea level	17°N	26.2	1,142
TX-1967	Tecoanapa	Guerrero	360	18°N	26.4	1,579
TX-1972	20 km W of Chetumal	Quintana Roo	Sea level	19°N	25.5	1,242
TX-1983	Las Coloradas	Yucatán	Sea level	22°N	25.7	550
TX-2076	Cárdenas	Tabasco	21	18°N	26.2	2,320
TX-2079	Chiná	Campeche	<100	20°N	26.8	1,028
TX-2089	Tekax	Yucatán	30	20°N	26.7	1,072
TX-2105	Tulum Ruins	Quintana Roo	Sea level	20°N	25.7	1,518
TX-2309	S. of Bahiá de Algodones	Tamaulipas	Sea level	24°N	22.8	1,354
TX-2310	Jaumave	Tamaulipas	750	23°N	21.8	500

five *R. reniformis* populations. Auburn 623 RNR and Deltapine 16 were included as controls. Plants were grown in the second growth chamber. Pf/Pi values were compared statistically with that obtained for the BRLA population on Deltapine 16, which was selected as the control.

Fifth experiment—comparative increases of M. incognita populations on TX-2079: Plants were inoculated separately with each of the four *M. incognita* populations except for HPTX. Auburn 623 RNR and Deltapine 16 were included as controls. Plants were grown in the second growth chamber. Pf/Pi values were compared statistically with that obtained for the STX population on Deltapine 16, which was selected as the control.

Results

Plant development in growth chambers: Depending on the experiment, the average plant at harvest was 36 to 62 cm tall (CV = 10 to 13%) with 10 or 11 main stem nodes (CV = 7 to 8%) and a dry foliar weight of 3.7 to 5.6 g (CV = 11 to 15%). Most Mexican accessions were slightly taller, heavier plants with one or two more nodes than both the resistant and susceptible control. None were smaller than the controls. Average root weights of Mexican accessions were intermediate between those of the controls. The average taproot length of Mexican accessions (21 cm) was slightly shorter than that of the Deltapine 16 control (24 cm), but only three accessions, TX-1983, TX-2089, and TX-2309, had significantly shorter taproots than Deltapine 16 at $P \leq 0.05$.

First experiment—accessions suspected to have resistance to R. reniformis: All wild accessions supported prolific reproduction by *R. reniformis,* with Pf/Pi values between 17 and 64 (Table 2). The Pf/Pi values for the *M. incognita*-resistant controls, LA RN 1032 and M-315 RNR, were 44 and 25, respectively, indicating them to be highly suitable hosts for *R. reniformis.* All wild accessions were also good hosts for *M. incognita* race 3, with Pf/Pi values between 9 and 52 (Table 3). The Pf/Pi values for *M. incognita* race 3 on resistant controls were less than 1.0, indicating a high level of resistance.

Second experiment—first evaluation of strategically selected Mexican accessions: The Pf/Pi value for *R. reniformis* on Deltapine 16 was 15.8. Auburn 634 RNR and seven wild accessions had lower ($P \leq 0.05$) levels of reproduction by *R. reniformis* than Deltapine 16 did, with Pf/Pi values between 1.8 and 9.7

Table 2. Number of *Rotylenchulus reniformis* collected from accessions of *Gossypium hirsutum* from various localities in Central America and the Caribbean, which previously had been reported to be resistant to *R. reniformis.*

Entry	Eggs per plant	Eggs per g dry root	Vermiform nematodes per pot	Pf/Pi[a]
Mexican accession				
TX-20	65,600**	68,000*	58,500	63.5
TX-69	33,800	32,300	25,000	30.3
TX-709	24,500	20,700	27,500	26.5
TX-834	68,100**	56,800*	47,000	58.8
TX-874	19,000	16,600	26,200	23.0
TX-893	16,200	16,400	22,700	20.0
TX-903	16,800	18,100	15,700	16.7
Resistant control				
La RN1032	53,100*	50,900	31,700	43.5
M-315 RNR	23,900	24,300	25,000	25.0
Susceptible control				
Deltapine 16	26,000	27,900	26,400	26.8

Plants were harvested 7 weeks after inoculation with 4,000 vermiform nematodes per plant. Each value is the mean of six replications; arithmetic means are presented, but data were transformed with log $(x+1)$ prior to analysis. All results were compared to those for Deltapine 16 with Fisher's protected LSD.

[a] Pf = final population (sum of eggs and vermiform nematodes extracted per pot); Pi = initial population (4,000 mixed vermiform stages per pot).

TABLE 3. Root gall ratings and reproduction parameters for *Meloidogyne incognita* race 3 on accessions of *Gossypium hirsutum* from various localities in Central America and the Caribbean, which previously had been reported to be resistant to *Rotylenchulus reniformis*.

Entry	Eggs per plant	Eggs per g dry root	Gall rating (0–5)	Pf/Pi[a]
Mexican accession				
TX-20	39,800	28,600	3.83	40.7
TX-69	24,100	25,400	3.33	25.3
TX-709	8,800	7,300	3.50	9.3*
TX-834	51,000	41,000	4.33	52.0
TX-874	23,000	20,900	3.33	25.0
TX-893	27,800	45,100	3.67	30.2
TX-903	28,600	23,800	3.67	30.2
Resistant control				
La RN1032	400***	292***	1.17***	0.4***
M-315 RNR	880***	493***	1.50***	0.9***
Susceptible control				
Deltapine 16	22,400	20,800	3.67	24.3

Plants were harvested 7 weeks after inoculation with 1,000 second-stage juveniles of *M. incognita* race 3 per plant. Each value is the mean of six replications; arithmetic means are presented, but data were transformed with log $(x+1)$ prior to analysis. All results were compared to those for Deltapine 16 with Fisher's protected LSD.

[a] Pf = final population (eggs plus juveniles extracted per plant); Pi = initial population (1,000 second-stage juveniles per plant).

(Table 4). The three entries with the lowest Pf/Pi values for *R. reniformis* were TX-1348, TX-751, and TX-2309.

The Pf/Pi value for *M. incognita* race 3 on Deltapine 16 was 11.7. Auburn 634 RNR, TX-2079, TX-2105, and TX-2076 had Pf/Pi values for *M. incognita* race 3 of 0.4, 0.2, 0.8, and 0.5, respectively, indicating a high level of resistance; corresponding gall ratings were 0.33, 0.50, 1.83, and 1.00 (Table 4). TX-751 had the second-to-lowest Pf/Pi value for *R. reniformis* and the fourth-to-lowest Pf/Pi value for *M. incognita* race 3.

Third experiment—second evaluation of Mexican accessions: Ratings of root galls induced by *M. incognita,* numbers of vermiform *R. reniformis* extracted from soil, and numbers of eggs of *R. reniformis* extracted from roots did not differ significantly between the two growth chambers (Table 5). All four resistant controls and 14 of the 32 wild accessions tested had lower ($P \leq 0.05$) gall ratings than Deltapine 16 (Table 5). Vermiform *R. reniformis* counts ranged from 22,000 to 150,000 (Table 5). The vermiform *R. reniformis* count for TX-1347 (22,000) was the only one lower ($P = 0.001$) than that of Deltapine 16 (50,000). The second lowest count (29,000) was for TX-1348, the same accession that supported the least reproduction by *R. reniformis* in the second experiment (Table 4). The Pf/Pi values for *R. reniformis* on TX-1347 and TX-1348 also were lower ($P \leq 0.05$) than that on Deltapine 16, while the Pf/Pi values for *R. reniformis* on all three *M. incognita*-resistant controls were numerically greater than on Deltapine 16. Mature plants of TX-1347 and TX-1348 in the greenhouse were identified as *G. barbadense* rather than *G. hirsutum* based on flower, boll, and leaf morphology.

The Pf/Pi values for *M. incognita* race 3, which were based on numbers of eggs extracted from roots, were higher on all entries in the second than in the first growth chamber, and there was a strong entry × chamber interaction (Table 6). Both values for Auburn 623 RNR (0.3 and 1.0), for example, were low, and both values for Deltapine 16 (15.0 and 29.0) were relatively high, while values for TX-2102 and TX-2103 were about 20 times greater in the second than in the first chamber. In both chambers, the Pf/Pi value for Clevewilt6 was about half that of Deltapine 16; Stoneville LA 887 and Wild Mexican Jack Jones were intermediate between Clevewilt6 and Auburn 623. Wild accessions that had Pf/Pi values both ≤ 1.0

TABLE 4. Nematode resistance parameters for the first set of Mexican accessions of *Gossypium hirsutum* evaluated for resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in a growth chamber.

	Meloidogyne incognita			*Rotylenchulus reniformis*		
Entry	Gall rating (0–5)	Eggs per g dry root	Pf/Pi[a]	Vermiform nematodes per pot	Eggs per g dry root	Pf/Pi
Mexican accession						
TX-254	3.00	24,700	21.9	13,400	12,300	10.8
TX-751	1.83	8,100	4.4	7,900***	6,000	5.3***
TX-770	2.00	13,400	12.7	13,600	6,700	9.8
TX-1045	3.00	13,800	14.4	18,000	15,400	16.2
TX-1114	4.17*	42,200	42.0	14,000	10,300	11.8
TX-1182	3.67	26,500	3.0	18,300	15,000	17.7
TX-1219	3.67	27,300	23.6	17,200	14,000	13.5
TX-1348[b]	2.00	27,800	22.7	1,500***	3,000***	1.8***
TX-1529	2.83	16,300	18.0	8,900**	4,500	7.2**
TX-1967	3.17	18,600	21.8	17,000	11,500	15.5
TX-1972	3.17	41,800	26.3	11,400*	14,000	10.3
TX-1983	4.50**	10,500	7.6	17,900	21,800*	15.7
TX-2076	1.00**	500***	0.5***	10,100*	13,600	9.2*
TX-2079	0.50***	200***	0.2***	12,700	9,900	9.7*
TX-2089	2.83	21,700	16.5	9,100**	11,500	8.5**
TX-2105	1.83	1,200***	0.8***	8,600**	18,000	10.2
TX-2309	3.33	53,700*	37.0	6,700***	8,300	5.8***
TX-2310	3.00	24,800	23.4	12,300*	11,200	10.8
Resistant control						
Auburn 634 RNR	0.33***	400***	0.4***	12,700	6,600	8.7*
Susceptible control						
Deltapine 16	2.83	11,700	11.7	23,700	9,500	15.8

Plants were harvested 7 weeks after inoculation with 1,000 second-stage juveniles of *M. incognita* race 3 or 4,000 vermiform *R. reniformis*. Each value is the mean of six replications; all data except gall ratings were transformed to log $(x + 1)$ prior to analysis, but arithmetic means are presented. All results were compared to those for Deltapine 16 with Fisher's protected LSD.

[a] Pf = final population; Pi = initial population. For *M. incognita,* Pf = eggs extracted per plant; Pi = 1,000. For *R. reniformis,* Pf = sum of eggs and vermiform stages extracted per plant; Pi = 4,000.

[b] TX-1348 was subsequently identified as *G. barbadense* based on flower, boll, and leaf morphology.

and lower ($P \leq 0.001$) than Deltapine 16 in the first chamber included TX-1174, TX-1440, and TX-2079 (Table 6). TX-2076 and TX-2079 had *M. incognita* race 3 Pf/Pi values less than 1.0 in the previous experiment (Table 4). The average Pf/Pi values for these four accessions and TX-2107 in the two growth chambers ranged from 2.9 to 7.7 and were the only ones lower than that for Clevewilt 6 (11.0) (Table 6). By comparison, the average Pf/Pi value obtained for Wild Mexican Jack Jones and Stoneville LA 887 were 5.2 and 5.4, respectively. TX-2079 was the only accession with a multiplication factor as low as that of Auburn 623 RNR in either chamber.

Fourth experiment—comparative increases of R. reniformis populations on TX-1347: The LAL, WMS, WCPLA, and WTX populations of *R. reniformis* were similar to the BRLA population in having a Pf/Pi on TX-1347 in a range (5.7–12.6) that was lower than on Deltapine 16 (35.0) or Auburn 623 RNR (30.2) but greater than 1.0 (Table 7). In this sense, TX-1347 had a similar level of partial resistance to all populations.

Fifth experiment—comparative increases of M. incognita populations on TX-2079: Gall ratings and Pf/Pi values for all three populations of *M. incognita* race 3 were intermediate between the corresponding values for the resistant Auburn 623 RNR and susceptible Deltapine 16 (Table 8).

DISCUSSION

No appreciable resistance to *R. reniformis* was found in any accession tested except TX-1347 and TX-1348. Although these accessions were originally lodged in the USDA

TABLE 5. Root gall ratings and vermiform *Rotylenchulus reniformis* extracted from soil for the second set of Mexican accessions of *Gossypium hirsutum* evaluated for nematode resistance in a growth chamber.

Entry	*Mi* gall rating	Vermiform *Rr* per pot	Entry	*Mi* gall rating	Vermiform *Rr* per pot
Mexican accession			Mexican accession		
TX-6	3.75	94,000	TX-2006	3.00*	150,000*
TX-30	3.17*	98,000	TX-2008	4.33	72,000
TX-329	5.00	117,000	TX-2076	2.33***	82,000
TX-373	3.92	123,000	TX-2078	3.67	96,000
TX-703	4.42	122,000	TX-2079	2.00***	76,000
TX-746	4.25	105,000	TX-2082	3.92	99,000
TX-751	3.17*	58,000	TX-2083	4.42	78,000
TX-757	2.67**	53,000	TX-2102	2.83**	85,000
TX-1099	4.08	—[a]	TX-2103	2.50**	92,000
TX-1134	4.58	108,000	TX-2104	4.00	79,000
TX-1174	2.58**	110,000	TX-2105	3.00*	100,000
TX-1180	—	112,000	TX-2107	2.92*	105,000
TX-1220	4.58	81,000	TX-2309	—	112,000
TX-1347[b]	3.08*	22,000***	Resistant control		
TX-1348[b]	3.17*	29,000	Auburn 623 RNR	1.67***	73,000
TX-1427	4.50	85,000	Wild Mex. Jack Jones	1.83***	32,000
TX-1440	2.58**	97,000	Clevewilt 6	3.08*	—
TX-1457	4.67	61,000	Stoneville LA 887	2.75**	77,000
TX-1529	4.58	112,000			
TX-1963	4.50	146,000*	Susceptible control		
TX-1966	4.17	84,000	Deltapine 16	4.17	50,000

Plants were harvested 7 weeks after inoculation with 1,000 second-stage juveniles of *Meloidogyne incognita* race 3 (*Mi*) or 4,000 vermiform *R. reniformis* (*Rr*). Each value is the mean of six replications; vermiform *R. reniformis* data were transformed with log (x + 1) prior to analysis but arithmetic means are presented. All results were compared to those for Deltapine 16 with Fisher's protected LSD.

[a] Not tested.

[b] Subsequently identified as *G. barbadense* based on flower, boll, and leaf morphology.

collection as *G. hirsutum,* we identified them based on morphological characters as *G. barbadense,* or possibly *G. hirsutum* genotypes that are highly introgressed with *G. barbadense.* TX-1347 and TX-1348 would probably be of greatest value to breeders of Egyptian and Pima cotton. The two accessions were collected about 25 km from each other, and mature plants in our greenhouse were phenotypically similar. The *G. barbadense* accession TX-110 also is resistant to *R. reniformis* (Yik and Birchfield, 1984). Among the five populations of *R. reniformis* tested, there was no indication of important differences in the ability of populations to reproduce on TX-1347 or TX-1348.

Results obtained for *M. incognita* race 3 egg production in the third experiment suggest that subtle differences between environmental conditions in the two growth chambers strongly influenced the expression of nematode resistance. A direct effect of environment on nematode survival or development seems an inadequate explanation because Deltapine 16 was a highly suitable host in both chambers while Auburn 623 RNR was highly resistant. Also, egg production by *R. reniformis* was not measurably different in the two chambers.

No wild accession or commercial cultivar has been found in this or any previous screen to have a level of resistance to *M. incognita* race 3 equal to that of Auburn 623 RNR, or to that of the nematode-resistant genotypes, such as Auburn 634 RNR and M-315 RNR, developed from it. A distinctly weaker level of resistance in resistant wild accessions of *G. hirsutum* than in Auburn 634 RNR was observed for our four populations of *M. incognita* and for that used by Shepherd (1983). Auburn 623 RNR is a selection from a cross between Clevewilt 6 and Wild Mexican Jack Jones (Shepherd, 1982). Resistance in Auburn 623 RNR is thought to

TABLE 6. Relative nematode reproduction (Pf/Pi) on the second set of Mexican accessions of *Gossypium hirsutum* evaluated for nematode resistance in two growth chambers.

	Pf/Pi[a]		
	M. incognita		*R. reniformis*
Entry	First growth chamber	Second growth chamber	Mean of both chambers
Mexican accession			
TX-30	24.0	29.8	—[b]
TX-751	12.2	37.0	37.8
TX-757	9.0*	77.8	—
TX-1174	0.2***	5.8**	—
TX-1347	20.2	61.2	20.5*
TX-1348	15.0	71.8	21.7
TX-1440	1.0***	16.2	—
TX-2006	2.0**	23.2	—
TX-2076	1.6***	16.2	—
TX-2079	0.5***	14.0	—
TX-2102	3.4**	26.0	—
TX-2103	1.5***	58.0	—
TX-2105	2.5**	14.2	—
TX-2107	2.0***	21.2	—
Resistant control			
Auburn 623	0.8***	1.5***	40.0
Wild Mexican Jack Jones	1.0***	18.0	45.8
Clevewilt 6	7.0	18.4	—
Stoneville LA 887	2.3**	7.8*	48.8
Susceptible control			
Deltapine 16	23.8	24.0	33.2

Plants were harvested 7 weeks after inoculation with 1,000 second-stage juveniles of *Meloidogyne incognita* race 3 or 4,000 vermiform *Rotylenchulus reniformis*.

Each value is the mean of six replications; arithmetic means are presented, but data were transformed with log $(x + 1)$ prior to analysis. All results were compared to those for Deltapine 16 with Fisher's protected LSD.

[a] For *M. incognita*, Pf = eggs extracted per plant; Pi = 1,000. For *R. reniformis*, Pf = eggs plus vermiform stages extracted per plant; Pi = 4,000.

[b] Not tested with *R. reniformis*.

derive from at least two genes, one major and one additive (Robinson et al., 1996). A hypothesis consistent with this theory and with our observations of apparent resistance regulation in wild accessions of *G. hirsutum* is that the major gene, which is essential for resistance, is environmentally modulated unless the second gene is also present. If so, perhaps only the major gene is present in the photoperiodically sensitive wild accessions of *G. hirsutum* that we and that Shepherd (1983) found.

In the third experiment, 14 accessions had root gall ratings lower ($P \leq 0.05$) than

TABLE 7. Reproduction (Pf/Pi) of *Rotylenchulus reniformis* populations from Leighton, Alabama (LAL); Webb, Mississippi (WMS); Baton Rouge, Louisiana (BRLA); West Carroll Parish, Louisiana (WCPLA); and Weslaco, Texas (WTX) in a growth chamber on three genotypes of *Gossypium hirsutum*.

	Pf/Pi[a]				
Entry	LAL	WMS	BRLA	WCPLA	WTX
TX-1347	9.0***	12.6**	7.3***	5.7***	7.9***
Auburn 623 RNR	14.2**	17.5*	30.2	10.3***	4.8***
Deltapine 16	19.0	15.5*	35.0	19.8	6.9***

Plants were harvested 7 weeks after inoculation with 4,000 vermiform *R. reniformis* per plant.

Each value is the mean of six replications; arithmetic means are presented, but data were transformed with log $(x + a)$ prior to analysis. All results were compared to those for the BRLA population on Deltapine 16 with Fisher's protected LSD.

[a] Pf = final population (sum of eggs and vermiform nematodes extracted per plant); Pi = initial population (4,000).

TABLE 8. Nematode resistance parameters for populations of *Meloidogyne incognita* race 3 from Minter City, Mississippi (MCMS); Seminole, Texas (STX); and the southern San Joaquin Valley of California (SJCA) in a growth chamber.

Entry	Pf/Pi[a]			Gall rating (0–5)		
	MCMS	STX	SJCA	MCMS	STX	SJCA
TX-2079	12***	14**	18**	3.00*	3.00*	2.83**
Auburn 623 RNR	4***	9***	2***	1.50***	1.00***	0.17***
Deltapine 16	34	50	32	4.33	4.33	3.67

Plants were harvested 7 weeks after inoculation with 1,000 second-stage juveniles per plant.

Each value is the mean of six replications; arithmetic means are presented, but data were transformed with log $(x + a)$ prior to analysis. All results were compared to those for the STX population on Deltapine 16 with Fisher's protected LSD.

[a] Pf = final population (eggs extracted per plant); Pi = initial population (1,000).

Deltapine 16. Eight of these also had lower ($P \leq 0.01$) multiplication factors for *M. incognita* race 3 than Deltapine 16 did in at least one growth chamber and were from the same general geographic region (Fig. 1). Seven were from the Yucatan platform (the peninsular states of Quintana Roo, Yucatán, and Campeche), and the other was from the neighboring coastal state of Tabasco. Five accessions, TX-1174, TX-1440, TX-2076, TX-2079, and TX-2107, had average multiplication factors lower than that of Clevewilt 6. Most of the accessions of *G. hirsutum* with lower gall ratings than Clevewilt 6 in Shepherd's study (Shepherd, 1983) were from the Chiapas highlands directly south of Tabasco, one was from Yucatán, and the remainder were from Guatemala, directly south of Chiapas and Campeche.

At least three of the accessions we identified as resistant to *M. incognita* race 3 were collected from so-called dooryard plantings, small numbers of ornamental perennial plants near homes whose lint is collected periodically by residents for various household uses. Seeds for dooryard paintings in southern Mexico often are exchanged by relatives and friends visiting neighboring villages. Resistant accessions were from areas occupied by the ancient Mayan Indians, who cultivated *G. hirsutum* for fiber during the first millennium C.E. Perhaps these accessions possess the same gene(s) for resistance to *M. incognita* race 3. We noted, however, that a number of other accessions from this same general region supported high levels of reproduction by *M. incognita,* as did most accessions from central, western, and northern Mexico.

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