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Root Galling and Reproduction of *Meloidogyne incognita* Isolates from Texas on Resistant Cotton Genotypes[1]

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Abstract: Several cotton genotypes with resistance to *Meloidogyne incognita* have been released in recent years. To estimate the durability of this resistance, galling severity on these resistant genotypes by *M. incognita* was measured. Nematode isolates (115 total) were collected from cotton fields in 14 Texas counties in August and September 1996 and 1997. Four additional isolates from Maryland, Mississippi, and North Carolina were also tested. The isolates were evaluated in 12 greenhouse experiments for their ability to gall roots of the resistant cotton genotypes M315, Acala NemX, and Stoneville LA887 and the susceptible cultivar Deltapine 90. Numbers of galls on each genotype by each isolate were counted 60 days after inoculation with 10,000 eggs/plant. M315 consistently had the fewest galls for each nematode isolate, whereas Deltapine 90 had the greatest number of galls. Numbers of galls on NemX and LA887 were usually intermediate and more variable. For each separate experiment, analysis of variance indicated that the effects of nematode isolates, cotton genotypes, and isolate-genotype interaction were significant ($P < 0.05$). In two of the experiments, nematode reproduction was also measured and galling was positively correlated ($r = 0.68$ and 0.86) with egg production by *M. incognita.* Nematode isolates from one field exhibited higher root galling and reproduction ($P < 0.05$) on resistant genotypes than other isolates, suggesting a need for gene deployment systems that will enhance the durability of resistance.

Key words: cotton, durable resistance, *Gossypium hirsutum,* host resistance, *Meloidogyne incognita,* nematode, root knot.

Root-knot nematodes (*Meloidogyne incognita* races 3 and 4) are economically important pathogens of cotton (*Gossypium hirustum* L.) that are found in nearly all of the cotton production regions in the United States. This nematode species is especially important in the Southern High Plains of Texas where more than 50% of the cotton fields are infested in some counties (Starr et al., 1993). Recently, two cotton cultivars with resistance to root-knot nematodes, Stoneville LA887 and Acala NemX, were released (Jones et al., 1990; Garber and Oakley, 1996). These cultivars exhibit less severe root galling and support lower nematode reproduction than do cultivars lacking the resistance. Data are not available on the effectiveness of these resistant cultivars on nematode isolates from widely separated geographic regions of cotton production.

The resistance to *M. incognita* in the cotton breeding line M315 is inherited as two major genes, presumably one each from the Clevewilt 6 and Wild Mexico Jack Jones (McPherson et al., 1995). Clevewilt 6 is also believed to be the source of resistance for LA 887 (Jones et al., 1990); thus, LA 887 may have at least one resistance gene in common with M315 (Robinson et al., 1997). The source of resistance in NemX is uncertain. Resistance based on a few genes, as may be the case in these cotton genotypes, may impose a selection pressure on nematode isolates for the development of virulence (Janssen et al., 1990). Roberts et al. (1995) reported significant variation in reproduction among isolates of *M. incognita* on cowpea genotypes with the Rk resistance gene. In tomato, the Mi gene provides resistance to three species of root-knot nematodes (*M. incognita, M. javanica,* and *M. arenaria*), but isolates of *M. incognita* having high levels of reproduction on tomato cultivars with the Mi gene have been detected following its widespread deployment (Kaloshian et al., 1996). Selection for increased reproduction of *M. incognita* on resistant cotton also may have occurred in California (Ogallo et al., 1997), where isolates with the highest level of reproduction on the resistant cultivar NemX were found in fields previously

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planted to this source of resistance. Therefore, cotton cultivars with few resistance genes may be vulnerable to attack by isolates of *M. incognita* that are capable of overcoming that resistance.

If isolates of *M. incognita* that can attack resistant cultivars are widely distributed, then resistance-gene deployment strategies should be developed to enhance the durability of the resistance. The objective of this research was to determine the range and frequency of galling severity and reproduction of *Meloidogyne incognita* isolates from Texas on resistant cotton genotypes.

Materials and Methods

During August and September 1996 and 1997, a total of 115 isolates of root-knot nematodes from 70 infested cotton fields were collected. Among these isolates, 106 were collected from 59 cotton fields in 12 counties in the cotton production region of the Southern High Plains of Texas. Fields in each county were selected randomly using a line intersect method (Gates, 1979). Three composite samples (20 subsamples/sample) were collected from each field, with each sample being collected from a 1-ha area of the field. Additionally, nine isolates were collected from seven fields in Brazos and Robertson counties in central Texas in 1997. One isolate each from Mississippi and Maryland, and 2 isolates from North Carolina, also were sent to us for analysis in this study. All isolates were cultured on tomato (*Lycopersicon esculentum* Mill 'Rutgers') for 2 to 3 months to provide inoculum for each experiment.

Root galling was measured in greenhouse experiments on the *M. incognita*-resistant cotton genotypes M315, Acala NemX, and Stoneville LA887, and on the susceptible Deltapine 90 (DP90). A total of 12 individual experiments were conducted, with 8 to 11 isolates included in each experiment. A laboratory isolate of *M. incognita* (#82-2) was included in each experiment as an internal standard. Seeds of each cotton genotype were planted directly into a sand/peat soil mix (6:1, v/v) in 14-cm-diam. pots. At the appearance of the first true leaves, plants of each cotton genotype were inoculated separately, with 10,000 eggs of the nematode isolate being experimented. Inoculum was prepared using 0.05% NaOCl (Hussey and Barker, 1973). Plants were harvested 60 days after inoculation, and the numbers of galls were counted. Triplicate plants of each cotton genotype were used for each nematode isolate. The effects of nematode isolate, cotton genotype, and the isolate × genotype interaction were determined by analysis of variance using the SAS (SAS Institute Inc., Cary, NC) general linear model procedure.

Reproduction of nematode isolates was measured in two of the 12 experiments—experiments 7 and 8. In these two experiments, after counting the number of galls per root system, 5 g of roots from each plant was treated with 1.0% NaOCl (Hussey and Barker, 1973) to extract eggs of *M. Incognita* from egg masses adhering to the roots. Because egg viability was not a concern, the higher concentration of NaOCl was used for increased extraction efficiency. Data on numbers of eggs per g of roots were subjected to analysis of variance using SAS to determine if nematode isolates differed in reproduction on different cotton genotypes. The relationship between root galling and nematode reproduction was examined using Pearson's correlation coefficient.

To confirm the high level of galling and reproduction observed for two isolates in the initial experiments, additional isolates were collected from the same field where the original isolates were collected. This was done to avoid the potential for a loss of aggressiveness with repeated culture on susceptible tomato. In the second experiment, conducted as described above, both the number of galls per root system and the number of eggs per g of roots were measured.

Results

All isolates galled DP90 and induced more galls on DP90 than on any of the three resistant genotypes ($P \leq 0.05$) (Table 1). For each nematode isolate, M315 consis-

TABLE 1. Mean number of galls per root system on cotton genotypes resistant (R) and susceptible (S) to *Meloidogyne incognita.*

Experiment	Number of isolates	M315 R	NemX R	LA887 R	DP90 S	F value		
						Isolate	Genotype	Isolate × genotype
				Number of galls/root system				
1	10	3	24	22	122	5.33**	61.50**	1.65*
2	10	12	77	48	373	3.56**	62.84**	3.12**
3	11	4	20	19	65	3.65**	67.20**	3.90**
4	10	3	16	15	68	2.16*	75.96**	2.51**
5	10	10	30	28	88	6.49**	104.48**	3.00**
6	10	13	35	35	105	3.05**	87.69**	2.80**
7	10	9	26	22	83	5.08**	38.67**	1.65*
8	11	7	25	21	94	5.61**	74.57**	1.46**
9	11	3	14	9	34	7.61**	67.48**	6.06**
10	12	4	18	10	79	3.21*	26.74**	1.54*
11	9	4	25	9	78	9.74**	50.73**	3.70**
12	11	11	53	72	306	11.59**	117.86**	5.09**

*, ** = significance at 0.05 and 0.01, respectively. Values are mean values for all isolates of each experiment.

tently had the fewest galls ($P \leq 0.05$) (Fig. 1). The mean number of galls on M315 for all isolates in each experiment was generally less than 11% of that on DP90 (Table 1). Galling on NemX and LA887 by each isolate was intermediate between DP90 and M315 and more variable. In each experiment, mean galling on NemX and LA887 for all isolates ranged from 12% to 41% of that on DP90 (Table 1). However, two isolates produced higher numbers of galls on the resistant cotton genotypes ($P < 0.05$). Galling of resistant genotypes by isolate 104b ranged from 40% of DP90 on M315 to 88% of DP90 on LA 887 (Table 2). Additionally, galling of NemX by isolate 104c, which was collected from the same field as 104b, was 79% of the galling on DP90.

In experiments 7 and 8, the mean number of eggs per g of roots was the highest on DP90 and lowest on M315 ($P \leq 0.05$) (Table 3). Reproduction on NemX and LA887 was intermediate. In these two experiments, the mean number of eggs per g of roots produced by all nematode isolates on M315 was <4% of that on DP90, and the mean number of eggs per g of roots produced by all nematode isolates on NemX and LA887 ranged from 5% to 20% of that on DP90. There was a positive correlation between numbers of root galls and eggs per g of roots in experiment 7 ($r = 0.86$) and experiment 8 ($r = 0.68$).

Analysis of variance indicated that for each of the separate experiments the effects of isolate, genotype, and the isolate × genotype interaction on the number of galls were significant at $P \leq 0.05$ (Table 1). The effects of isolate, genotype, and the isolate × genotype interaction were also significant for nematode reproduction measured as eggs per g of roots in experiment 8 (Table 3). In experiment 1 (Fig. 1), which was typical of all of the experiments, there were five isolates for which no difference ($P > 0.05$) in gall number among the nematode-resistant and susceptible genotypes was observed. For each of these isolates there was one DP90 plant that had a low number of galls, although the mean number of galls was highest for DP90 in comparison to the other genotypes. When DP90 was removed from the analysis, then six isolates did not differ ($P > 0.05$) in gall number on the resistant genotypes, and four nematode isolates differed ($P < 0.05$) with respect to gall number. Of these latter four isolates, NemX had more galls ($P \leq 0.05$) than LA 887 and M315 for two isolates and gall number was higher ($P \leq 0.05$) for both NemX and LA 887 than for M315 for the other two isolates.

Similarly, in experiment 2 (Fig. 1), four of 14 isolates did not differ ($P > 0.05$) in gall number among nematode-resistant and susceptible genotypes. Again, this lack of interaction between cultivar and genotype was

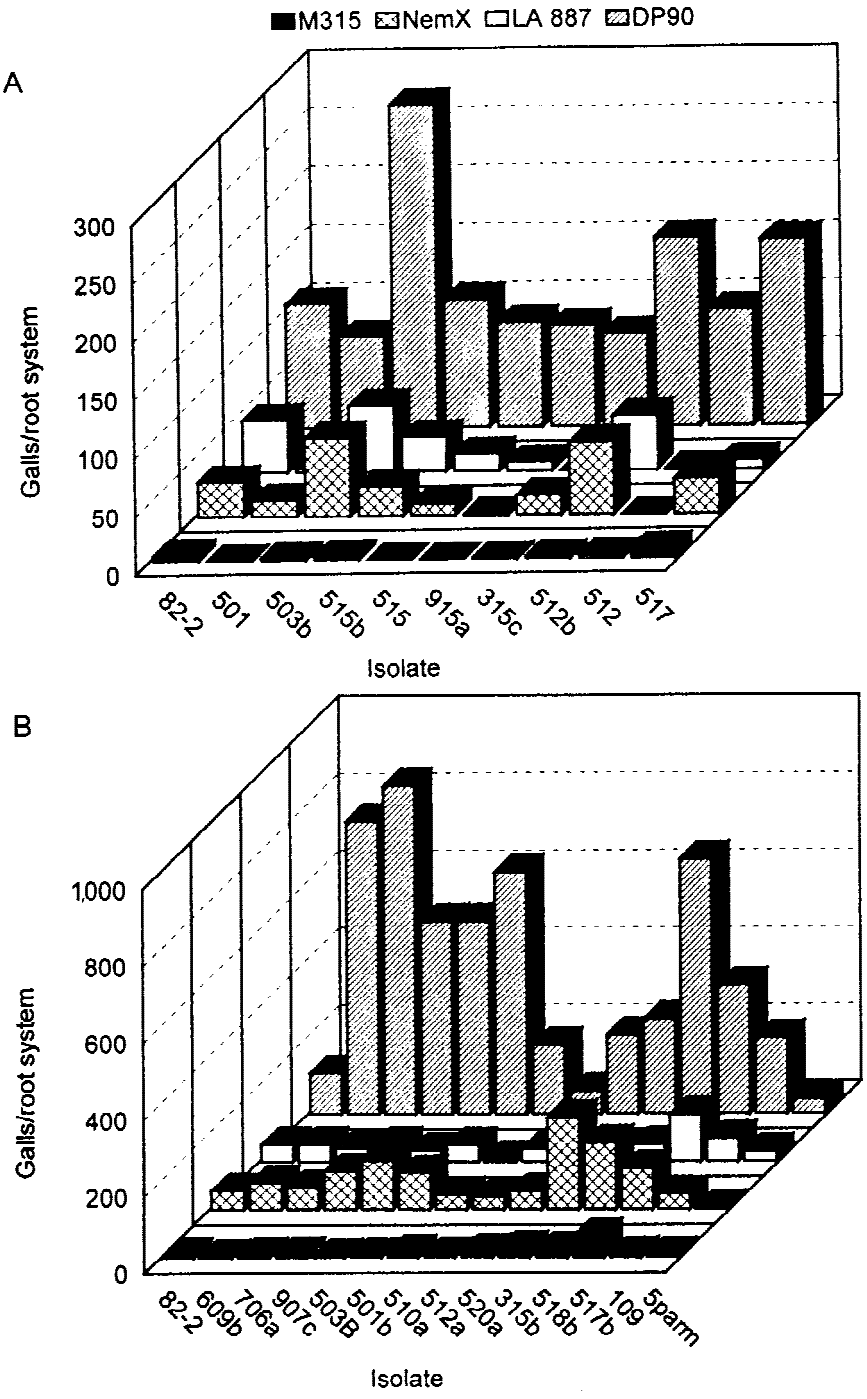

FIG. 1. Variation in numbers of galls per root system caused by isolates of *Meloidogyne incognita* collected from cotton fields in Texas on the resistant cotton genotypes M315, Acala NemX, and Stoneville LA 887 and the susceptible Deltapine 90 (DP90). A) Experiment 1. B) Experiment 2.

due to low gall number for one DP90 plant. If DP90 was removed from the analysis, then NemX was more susceptible ($P \leq 0.05$) than LA 887 and M315 for four isolates, NemX and LA 887 were more susceptible ($P \leq 0.05$) than M315 for two isolates, and

TABLE 2. Mean number of galls per root system and eggs per g of roots on resistant (R) and susceptible (S) cotton genotypes by isolates of *Meloidogyne incognita.*

Isolate	M315 R	NemX R	LA 887 R	DP90 S	F-value
First experiment—galls/root system					
104b	47	70	100	113	0.86 ns
104c	13	85	12	107	3.07*
Second experiment—galls/root system					
104d	50	70	88	122	0.45 ns
104e	19	56	22	116	1.78 ns
First experiment—eggs/g root					
104b	202	372	831	997	1.56 ns
104c	59	893	206	1404	1.01 ns
Second experiment—eggs/g root					
104d	165	605	1198	2013	1.06 ns
104e	42	384	129	1317	3.69*

* $P < 0.10$.
ns = not significant.

there were no differences among nematode-resistant cultivars for 10 isolates.

In experiment 7, two of 12 isolates were not different with respect to galls or eggs per g of roots between resistant and susceptible genotypes (Table 3). When DP90 was removed from the analysis, then NemX was more susceptible ($P \leq 0.05$) than LA 887 and M315 for one isolate with respect to both gall number and eggs, NemX and LA 887 were more susceptible ($P \leq 0.05$) than M315 for two other isolates with respect to gall number, and eight isolates with respect to eggs per g roots. LA 887 was more susceptible ($P \leq 0.05$) than NemX or M315 for one isolate with respect to number of galls.

Because of the relatively high level of galling and reproduction observed for isolates 104b and 104c on the resistant genotypes, two additional samples (isolates 104d and 104e) were collected from the same field in 1998. Root galling by isolate 104d on M315 was 21% of the galling on DP90, galling on NemX was 58% of DP90, and galling on LA887 was 73% that on DP90 (Table 2). Isolate 104e produced 17%, 49%, and 19% of the galls on M315, NemX, and LA887, respectively, as on DP90. Additionally, 104d produced 30% of the eggs on NemX as it did on DP90 and 59% of the eggs on LA887 as on DP90. Isolate 104e produced 29% and 10% of the eggs on NemX and LA887, respectively, as on DP90 (Table 2). Analysis of variance in the first experiment and the second experiment indicated that the effect of cotton genotype on the number of galls and the number of eggs per g of roots was not significant ($P > 0.1$) except for isolate 104e in the second experiment, in which the effect of genotype on egg production was significant ($P \leq 0.1$).

DISCUSSION

Except for four isolates from one field in Texas, the isolates of *M. incognita* collected in this study did not cause severe galling or reproduce at high rates on resistant cotton

TABLE 3. Mean number of eggs per g roots produced on cotton genotypes resistant (R) and susceptible (S) to *Meloidogyne incognita* by isolates of the root-know nematode collected from cotton fields in Texas.

Experiment	Number of isolates	M315 R	NemX R	LA887 R	DP90 S	F value		
						Isolate	Genotype	Isolate × genotype
7	10	29	163	145	802	1.40	14.02**	0.52
8	11	6	141	481	2,818	2.36**	38.12**	2.21**

*, ** = significance at 0.05 and 0.01, respectively. Values are mean values for all isolates of each experiment.

genotypes. Because none of the resistant genotypes used in this study are adapted to the environment of west Texas, it is unlikely that any have been planted in the field from which the more aggressive isolates were collected. In a similar study in South Carolina, Elliot et al. (1998) also reported that the resistance in these same cotton cultivars was effective against most South Carolina isolates of *M. incognita* but a few isolates with higher levels of root galling were identified. Collectively, these data suggest that the resistance in LA 887, NemX, and M315 will have broad applicability, but it will not be uniformly effective in all fields. Ogallo et al. (1997) reported that reproduction on NemX for isolates of *M. incognita* collected from a field in California where NemX was grown repeatedly was greater than that of isolates not previously exposed to NemX. This observation, along with those of Elliot et al. (1998) and from this study, suggests that it will be important to monitor the performance of these resistant cultivars because widespread use of these cultivars over long periods of time may result in the selection of isolates of *M. incognita* that can overcome the resistance.

Because M315 and LA887 may have one resistance gene in common (Jones et al., 1990; Robinson et al., 1997), rotating resistance derived from M315 with that of LA 887 or other cultivars with the same source of resistance will not reduce selection pressure for development of increased galling and reproduction on these sources of resistance. Additionally, the genetic basis for resistance to *M. incognita* in NemX and other cotton accessions (Robinson and Percival, 1997; Shepherd, 1983) needs to be determined. The durability of resistance to *M. incognita* in cotton may be dependent on the development of resistance gene deployment systems that reduce the selection pressure for increased levels of aggressiveness in the nematode isolate.

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