

**REPRODUCTION BY ROOT-KNOT AND
RENIFORM NEMATODES ON TRANSGENIC
COTTON CULTIVARS
GROWN COMMERCIALY IN 1998**

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Abstract

Twenty-one transgenic genotypes of Upland cotton, including the 15 most widely planted transgenic cultivars in 1998 and their nontransgenic parents, were evaluated under growth chamber conditions for resistance to the nematodes *Meloidogyne incognita* (root-knot nematode) and *Rotylenchulus reniformis* (reniform nematode). The highly *M. incognita*-resistant breeding line Auburn 623 RNR, the *M. incognita*-resistant cultivar Stoneville LA887, and the susceptible obsolete cultivar Deltapine 16 were included as controls. No important decrease in nematode reproduction was attributable to any transgene in any cultivar. Some other differences in nematode reproduction, however, were apparent. *R. reniformis* reproduction was prolific on all cultivars and on eight significantly exceeded Deltapine 16. In contrast, 10 cultivars exhibited *M. incognita* gall ratings and/or egg densities on roots significantly lower than on Deltapine 16. Six Stoneville breeding lines (experimental transgenics) had levels of *M. incognita* root galling and reproduction comparable to Stoneville LA887.

Introduction

Nematodes are an important factor limiting cotton production throughout the U.S. cotton belt (Blasingame, 1998). The most important nematodes of cotton are the cotton root-knot nematode, *Meloidogyne incognita*, and the reniform nematode, *Rotylenchulus reniformis* (Robinson, 1999a). Three cultivars (Stoneville LA887, Paymaster 1560 and CPCSD Acala Nem-X) with appreciable resistance to *M. incognita* are available (Robinson et al., 1999b). These cultivars lack the high resistance in Auburn 623 RNR and derived lines. Apparently no breeding line or cultivar has a useful level of resistance to *R. reniformis*.

This year cotton cultivars with transgenes for insect or herbicide resistance were widely planted in the United States. These transgenic cultivars were not designed for nematode resistance. However, nematodes typically trigger the expression of a wide range of genes in tissue where normally unexpressed, and the way that nematode resistance works at the molecular level is not understood in any plant. (Bird, 1996). Some of the morphological and biochemical changes that root-knot and reniform

nematodes usually induce are specific to the cells on which they feed while others are general and appear plant hormone mediated. Obviously, transgenes could alter nematode resistance in completely unexpected ways. Our objective was to measure reproduction of the two major nematodes of Upland cotton, *R. reniformis* and *M. incognita*, on the transgenic cultivars available in 1998.

Materials and Methods

Two experiments were conducted. The experimental design for each was a randomized complete block with six replications of 20 plant genotypes infested with either *M. incognita* or *R. reniformis*. The nontransgenic parent cultivar was included for comparison for each transgenic cultivar tested in each experiment. Several transgenic breeding lines also were tested. The experimental controls were Deltapine 16, Stoneville LA887 and Auburn 623 RNR, which are considered respectively to be susceptible, resistant and highly resistant to *M. incognita*. Altogether, 21 transgenic genotypes with insect and/or herbicide resistance transgenes were evaluated.

Plants were grown individually in 500-cm³ pots containing a 6:1 mixture of fine sand and vermiculite supplemented with 5 g/kg pelletized limestone. Each pot was infested with 4,000 vermiform *R. reniformis* or 1,000 second-stage juveniles (J2) of *M. incognita* race 3 by injecting nematode suspension 1-5 cm deep at several points 2-3 cm from the plant stem 10 days after planting. Plants were exposed to a 14-hour photoperiod with day and night temperatures of 30 and 26 °C, respectively, watered daily and fertilized weekly.

Seven weeks after infesting pots with nematodes, foliar plant heights and weights were taken, main stem nodes and fruiting structures were counted, and roots were weighed. Vermiform *R. reniformis* were extracted from 100 g soil per pot by Baermann funnel. Root systems of plants in pots inoculated with *M. incognita* were assigned a 1-5 root gall rating in increments of 0.5, where 0 = no galls found, 2 = 25%, 3 = 50%, 4 = 75% and 5 = 100% of roots galled. Nematode eggs were extracted from each entire root system in both nematode treatments with dilute NaOCl, then concentrated by sieving and centrifugal flotation (Jenkins, 1964), and counted.

Counts of nematodes and nematode eggs were transformed with $\log(x + 1)$ prior to analysis of variance and treatment means were separated from means of controls with Fisher's protected least significant difference.

Results and Discussion

The average plant measurements for plants inoculated with *M. incognita* were 50 cm plant height, 11.1 main stem nodes, 2.4 flowers, squares and bolls, 10.7 g fresh root weight, and 91.4% root moisture. The corresponding

values for plants inoculated with *R. reniformis* were 52, 11.1, 2.2, 6.6 and 88.8. Thus the two nematode treatments were similar except for the obviously heavier roots of plants inoculated with *M. incognita*. Roots galled by *M. incognita* are typically heavy (Franklin, 1978). Part of the root weight difference, however, may have come from damage caused by *R. reniformis* because roots of the highly resistant control, Auburn 623 RNR, that were inoculated with *M. incognita* appeared healthy and did not gall, yet were nearly twice as heavy as those inoculated with *R. reniformis*.

All genotypes supported prolific reproduction by *R. reniformis*, with population multiplication factors ranging from 65% to 208% of that on the highly susceptible Deltapine 16 (Tables 1,2).

No large suppression of reproduction of either nematode was caused by any transgene in any commercial cultivar (Tables 1,2,3,4). Some patterns apparently unrelated to transgenes, however, were apparent. Cultivars derived from Delta and Pine Land (DP) 20, DP 90, DP 5690, Paymaster HS 26 and Paymaster 183 tended to have lower gall ratings, somewhat lower egg production by *M. incognita* and somewhat higher egg production by *R. reniformis* than the susceptible control Deltapine 16. These differences were small compared with those that typify contrasts between Deltapine 16 and the resistant control Auburn 623 RNR.

The experimental transgenic breeding lines, Stoneville 023 through 028, exhibited resistance to *M. incognita* comparable to that in the resistant control Stoneville LA887 (Table 4). Information regarding the transgenes and parent material for these lines is not available.

References

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Table 1: Parameters of nematode resistance for plants inoculated with *Rotylenchulus reniformis* in first of two growth chamber experiments evaluating transgenic cotton cultivars.

Genotype	Eggs per plant	Eggs per gram dry root	Multiplication factor(%) [^]	Vermiform per plant
DP 50	60,000**	113,000**	138	315,000
DP 50B	73,000**	104,000*	134	294,000
DP 90	93,000*	139,000**	161*	346,000
DP 90B	81,000*	127,000**	137	292,000
DP 5415	49,000	110,000*	92	211,000
DP NU 33B	46,000	77,000	109	250,000
PM HS 26	123,000*	161,000***	190**	395,000
PM 2326 RR	68,000**	96,000*	165*	382,000
PM 183	82,000*	138,000**	163*	377,000
PM 2183 BG	43,000	58,000	129	308,000
PM 1215	67,000**	84,000	131	292,000
PM 1215 BG	35,000	53,000	119	290,000
PM 1220	51,000	107,000	142	338,000
PM1220BG/RR	45,000	67,000	104	247,000
STV 474	51,000*	88,000	111	268,000
STV 4740 BG	45,000	85,000	126	312,000
STVBXN47	48,000	86,000	120	278,000
Controls				
AUB 623 RNR	37,000	57,000	90	216,000
STV LA887	54,000*	90,000	208**	514,000*
Deltapine 16	32,000	59,000	100	241,000

[^] Expressed as a percentage of nematode population increase on DP 16. ^{^^} 0-5 rating where 0 = no galls and 5 = 100% galled.

^{^^^} Experimental transgenic breeding lines.

DP = Delta and Pine Land. PM = Paymaster. STV = Stoneville. AUB = Auburn. AUB 623 RNR = Control highly resistant to *M. incognita*. STV LA887 = Control resistant to *M. incognita*. DP 16 = Control susceptible to *M. incognita*.

Asterisks (*, **, ***) indicate values different by 1 LSD from DP 16 at 0.05, 0.01, and 0.001 levels, respectively.

Table 2: Parameters of nematode resistance parameters for plants inoculated with *Rotylenchulus reniformis* in the second of two growth chamber experiments evaluating transgenic cotton cultivars.

Genotype	Eggs per Pplant	Eggs per gram dry root	Multiplication factor (%)^	Vermiform per plant
DP 20	27,000	37,000	141	71,000
DP 20B	15,000	29,000	120	69,000
DP 5415	22,000	39,000	164	92,000
DP 5415 RR	15,000	31,000	96	52,000
DP 5690	15,000	24,000	133	78,000
DP 5690 RR	21,000	32,000	121	63,000
DP 32B	12,000	17,000	96	55,000
PM HS 200	22,000	32,000	138	74,000
PM 2200 RR	18,000	35,000	116	63,000
PM 145	23,000	40,000	121	62,000
PM 2145 RR	16,000	25,000	125	72,000
STV 023^^	4,000	6,000	93	61,000
STV 024	16,000	23,000	105	57,000
STV 025	12,000	22,000	65	33,000
STV 026	8,000	12,000	83	50,000
STV 027	7,000	12,000	133	85,000
STV 028	15,000	27,000	92	49,000
Controls				
AUB 623RNR	13,000	18,000	111	65,000
STV LA887	12,000	21,000	84	47,000
Deltapine 16	7,000	12,000	100	62,000

^Expressed as a percentage of nematode population increase on DP^^Experimental transgenic breeding lines.

DP = Delta and Pine Land. PM = Paymaster. STV = Stoneville. AUB = Auburn. AUB 623 RNR = Control highly resistant to *M. incognita*. STV LA887 = Control resistant to *M. incognita*. DP 16 = Control susceptible to *M. incognita*.

Asterisk (*) indicates value different by 1 LSD from DP 16 at the 0.05 level.

Table 3. Parameters of nematode resistance for plants inoculated with *Meloidogyne incognita* race 3 in the first of two growth chamber experiments evaluating transgenic cotton cultivars.

Genotype	Eggs per plant	Eggs per gram dry root	Multiplication factor (%)^	Gall rating (0-5)^^
DP 50	49,000*	56,000	45*	3.08
DP 50B	62,000	75,000	57	2.58
DP 90	36,000**	43,000**	33**	2.42*
DP 90B	39,000*	40,000**	36**	2.67
DP 5415	52,000	64,000	48*	2.92
DP NU33B	70,000	78,000	65	3.08
PM HS26	37,000**	41,000***	34**	2.58
PM 2326RR	52,000*	56,000	48*	3.25
PM 183	13,000***	20,000***	12***	1.58***
PM 2183BG	90,000	91,000	83	2.42*
M 1215	271,000*	223,000***	250*	3.33
PM 1215BG	178,000	169,000*	164	3.58
PM 1220	173,000	163,000	160	3.67
PM1220BG/RR	115,000	119,000	106	3.83
STV 474	55,000	59,000	50	3.42
STV 4740BG	97,000	114,000	89	3.17
STV BXN47	63,000	65,000	58	3.25
Controls				
AUB 623 RNR	3,000***	3,000***	3***	0.25***
STV LA887	10,000***	11,000***	9***	1.00***
Deltapine 16	108,000	117,000	100	3.50

^Expressed as a percentage of nematode population increase on DP 16. ^^0-5 rating where 0 = no galls and 5 = 100% galled.

DP = Delta and Pine Land. PM = Paymaster. STV = Stoneville. AUB = Auburn. AUB 623 RNR = Control highly resistant to *M. incognita*. STV LA887 = Control resistant to *M. incognita*. Deltapine 16 = Control susceptible to *M. incognita*.

Asterisks (*, **, ***) indicate values different by 1 LSD from DP 16 at 0.05, 0.01, and 0.001 levels, respectively.

Table 4. Parameters of nematode resistance for plants inoculated with *Meloidogyne incognita* in the second of two growth chamber experiments evaluating transgenic cotton cultivars.

Genotype	Eggs per plant	Eggs per gram dry root	Multiplication factor (%)^	Gall rating (0-5)^^
DP 20	49,000	52,000	58	3.33
DP 20B	20,000**	22,000 *	23**	2.92
DP 5415	48,000	53,000	57	3.50
DP 5415 RR	29,000	36,000	34	3.08
DP 5690	28,000*	32,000	33*	3.17
DP 5690 RR	27,000*	35,000	31*	3.08
DP 32B	30,000	29,000	35	2.67
PM HS 200	87,000	104,000	103	4.17
PM 2200 RR	79,000	99,000	92	3.83
PM 145	61,000	79,000	71	4.25
PM 2145 RR	67,000	83,000	79	4.08
Controls				
STV 023^^^	10,000***	13,000 **	12***	1.75***
STV 024	6,000***	7,000 ***	7***	1.92***
STV 025	5,000***	6,000 ***	6***	1.75***
STV 026	3,000***	5,000 ***	4***	1.58***
STV 027	3,000***	4,000 ***	3***	1.83***
STV 028	12,000**	13,000 **	13***	2.00**
Controls				
AUB 623 RNR	1,000***	500 ***	0***	0.50***
STV LA887	11,000**	18,000 **	13***	1.75***
Deltapine 16	85,000	83,000	100	3.42

^Expressed as a percentage of nematode population increase on DP 16. ^^0-5 rating where 0 = no galls and 5 = 100% galled.

^^^Experimental transgenic breeding lines.

DP = Delta and Pine Land. PM = Paymaster. STV = Stoneville. AUB = Auburn. AUB 623 RNR = Control highly resistant to *M. incognita*. STV LA887 = Control resistant to *M. incognita*. DP 16 = Control susceptible to *M. incognita*.

Asterisks (*, **, ***) indicate values different by 1 LSD from DP 16 at 0.05, 0.01, and 0.001 levels, respectively.