# Plant Pathology and Nematology

## Root-Knot Nematode Reproduction and Root Galling Severity on Related Conventional and Transgenic Cotton Cultivars

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### INTERPRETIVE SUMMARY

The root-knot nematode is a serious pest of cotton that reduces yield and delays maturity, but it can be managed through the use of fumigant and non-fumigant nematicides, crop rotation, and resistant cultivars. Nematicides are effective but do not provide season-long protection, and their future availability is uncertain due to environmental concerns. Crop rotation is not widely practiced because many potential rotation crops have relatively low market value, or they are also hosts for the nematode. Although high levels of resistance are available in breeding lines, there are only three commercial cotton cultivars that exhibit moderate resistance to the root-knot nematode. Several genetically modified cotton cultivars that have genes for resistance to the tobacco budworm, to glyphosate herbicide (e.g., Roundup), or to both the budworm and the herbicide have been released recently for production. There is an assumption that these cultivars are similar or even identical to the original cultivar except for the expression of the introduced insect and/or herbicide resistance, but many of the transgenic cultivars have not been evaluated for susceptibility to the root-knot nematode.

Experiments were conducted in a naturally infested field and in artificially infested pots in a growth room to compare root-knot nematode resistance in selected cotton cultivars and their transgenic progeny. Susceptibility of the cultivars was based on the severity of root galling and level of nematode reproduction. Results of the field and growth-room experiments were similar, indicating that some transgenic cultivars have greater susceptibility to the root-knot nematode than their nontransgenic parents do. These data indicate the importance of evaluating transgenic cultivars for resistance to pests other than the species targeted by the genetic modification before such cultivars are recommended for planting.

### ABSTRACT

**The root-knot nematode (*Meloidogyne incognita* Kofoid & White), a widespread and serious pest of cotton (*Gossypium hirsutum* L.) throughout the Cotton Belt, is managed in many areas in part through cultivar resistance. Recently, commercial cotton cultivars modified with genes for resistance to the tobacco budworm (*Heliothis virescens* F.), to glyphosate herbicide (e.g., Roundup, Monsanto, St. Louis, MO), or in some cases to both the budworm and the herbicide have been released. The objective of this study was to compare the root-knot nematode resistance or susceptibility of several transgenic cotton cultivars with that of their unmodified parent cultivars. The cultivars were evaluated in a field naturally infested with the root-knot nematode and in a growth room in pots infested with the nematode. A dramatic increase in root-knot nematode susceptibility was seen in the transgenic cultivar, Paymaster 1560 BG, compared with its nontransgenic parent, Paymaster 1560. Although only a limited number of cultivars were studied, the data demonstrate that differences in susceptibility to the root-knot nematode exist between some transgenic cultivars and their nontransgenic parents. These data indicate the importance of screening transgenic cultivars for resistance to pests other than the particular pest species targeted by the genetic modification before the transgenic cultivars are recommended for planting.**

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The root-knot nematode *Meloidogyne incognita* is a widespread and serious pest of cotton throughout the Cotton Belt (Kinlock and Sprenkel,

1994; Martin et al., 1994; Baird et al., 1996; Bateman et al., 2000). Yield suppression in cotton is due primarily to root damage and extensive galling that are produced by nematode infection and result in the inhibition of translocation of water and nutrients to the plant (Bridge, 1992). In addition to direct damage, such as a reduction in the number and size of bolls, nematode infection also may delay maturity (Walker et al., 1998).

Moderate to high levels of resistance to *M. incognita* have been reported in upland cotton (Shepherd, 1974; Jones et al., 1988, 1991). Although breeding lines with this resistance have been developed (Kirkpatrick and Shepherd, 1989; Cook et al., 1997; Robinson and Percival, 1997), only three commercial cultivars exhibit moderate resistance: Stoneville LA 887, Paymaster 1560, and CPCSD Acala Nem-X (Robinson and Percival, 1997). While these cultivars have been effective in lowering yield losses due to the nematode in some regions, limitations in both cultivar adaptation and seed availability have prevented their widespread use across the Cotton Belt.

Several genetically modified cotton cultivars are commercially available, including Paymaster 1560, which contain genes that provide resistance to the tobacco budworm, to glyphosate herbicide, or in some cases to both the budworm and the herbicide. In many cases, these cultivars are genetically modified selections from existing conventional cultivars. Many cotton producers have assumed that phenotypic and genotypic characteristics of the transgenic cultivar are similar or identical to the parent, except for the expression of the introduced trait. The objective of this study was to compare the root-knot nematode resistance or susceptibility of several transgenic cotton cultivars with that of their unmodified parent cultivars.

## MATERIALS AND METHODS

### Field Experiments

Field experiments were conducted at the Red River Research Station in Bossier City, Louisiana. The soil was a Caplis very fine sandy loam (coarse-silty over clayey, mixed, superactive, calcareous, thermic Oxyaquic Udifluvents) with a pH of 6.8 and 0.2% organic matter. The average

particle-size distribution was 58% sand, 37% silt, and 5% clay. Historically, cotton planted at this location has experienced severe root galling caused by *M. incognita*. The experimental design was a randomized complete block with four replications in 1997 and eight replications in 1998. Plots consisted of single rows in 1997 and four rows in 1998 on a 101-cm spacing by 13.7 m in length.

The cotton cultivars evaluated were Paymaster 1560 (root-knot nematode resistant conventional cultivar), Paymaster 1560 BG (a transgenic cultivar selected from Paymaster 1560 containing the *Bacillus thuringiensis* [Bt] gene that confers tobacco budworm resistance), Deltapine 50 (a root-knot susceptible conventional cultivar), Deltapine 50 B (a transgenic budworm-resistant selection of Deltapine 50), Deltapine 5415 (a root-knot susceptible conventional cultivar), and Deltapine 5415 RR (a transgenic, glyphosate-resistant cultivar selected from Deltapine 5415). The conventional cultivar Stoneville LA 887 was included in the test as the nematode-resistant control. All plots were treated in the planting furrow with DiSyston 15 G (Bayer Corp., Kansas City, MO) disulfoton {*O,O*-iethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate} at 1.11 kg a.i. ha^{-1} for early-season insect control and Terraclor Super X PCNB (Uniroyal Chemical Co., Middlebury, CT) (10% pentachloronitrobenzene + 2.5% etridiazol) at 1.12 kg PCNB plus 0.28 kg etridiazol ha^{-1} for suppression of seedling diseases. Cotton was planted in both 1997 and 1998 on 20 May. Fertilization, weed, and insect control were performed according to Louisiana Cooperative Extension Service guidelines.

Nematode population density (second-stage juveniles, J2) was determined from each plot at crop maturity in 1998, but not in 1997. Twenty soil cores were collected from the two center rows of each plot to a depth of 20 cm with a 2.5-cm-diameter sampling tube and bulked. Nematodes were extracted from a 500-cm$^3$ subsample using a semi-automatic elutriator (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964). The density of J2 in each plot was recorded and transformed [log (J2 + 1)] for statistical analysis, but actual numbers are reported. The severity of root galling was rated by sampling 10 plants per plot at crop maturity. Galling severity per plant was rated according to the following scale: 0 = no galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to

30 galls; 4 = 31 to 100 galls; 5 = >100 galls per root system.

Data were subjected to analysis of variance with PC/SAS software (SAS Institute, Cary, NC). Means were separated using Fischer's least significant differences test ($P \leq 0.05$).

### Growth-Room Experiments

The cultivars Paymaster 1560 and Deltapine 50 and their genetically modified insect-resistant selections (Paymaster 1560 BG and Deltapine 50 B) were evaluated for nematode resistance in 10-cm-diameter clay pots in a growth room. Stoneville 474 and Stoneville LA 887 were included in these tests as the nematode-susceptible and nematode-resistant standards, respectively. Pots were filled with a 1:1 (v:v) mixture of methyl bromide-fumigated Smithdale fine sandy loam soil (fine-loamy, siliceous, subactive, thermic Typic Hapludults) and fine builder's sand. The soil in each pot was infested with 5000 *M. incognita* eggs that had been collected from stock cultures of *M. incognita* host race 3 that had been maintained on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Inoculum was prepared by washing the infected tomato roots and chopping them into 1- to 2-cm segments. The galled root segments were processed in 0.05% NaOCl (Hussey and Barker, 1973) for 4 min using a wrist-action shaker to free eggs from egg masses. Eggs were then rinsed thoroughly and placed in a water suspension. Ten milliliters of the egg suspension containing the appropriate number of eggs was pipetted onto the soil surface in each pot and incorporated into the soil with a small hand trowel. Uninfested pots served as controls.

Two seeds of the appropriate cultivar were planted into each pot immediately after infestation of the soil with the nematodes. After planting, the pots were watered and transferred to the growth room that was maintained at 30 ± 2°C with a 12-h diurnal cycle. Treatments were replicated six times, and pots were arranged in a randomized complete block. Seven days after planting, pots were thinned to one seedling per pot. The experiment was terminated after 40 d, all plants were removed from the pots, and the roots were washed gently to remove soil and then rated for galling using the same rating scale as in the field trials. The entire root system from each

plant was processed for 4 min in 0.05% NaOCl as described above to recover *M. incognita* eggs from the egg masses for counting. For statistical analysis, eggs per root system were transformed using log (eggs + 1), but actual numbers are reported in the tables and below. The experiment was repeated once using the same inoculum levels, growth-room conditions, duration, and procedures as indicated for the first test, but with 10 replications of each treatment.

Data were subjected to analysis of variance with PC/SAS software (SAS Institute, Cary, NC). Means were separated using Fischer's least significant differences test ($P \leq 0.05$).

### RESULTS

### Field Experiments

Root gall ratings differed among cultivars in both years (Table 1). Deltapine 50 was not included in the test in 1997. In both years, Paymaster 1560 and Stoneville LA 887 exhibited less severe galling than the other cultivars tested. In 1997, Paymaster 1560 BG was more severely galled than its parent cultivar, Paymaster 1560, while Deltapine 5415 RR was comparable in root galling with its parent cultivar (Table 1). In 1998, all cultivars except Stoneville LA 887 and Paymaster 1560 exhibited severe root galling. Galling was more severe on Paymaster 1560 BG and Deltapine 50 B than on their respective parent cultivars, Paymaster 1560 and

**Table 1. Nematode population density and root gall ratings for selected cotton cultivars planted in a *Meloidogyne incognita*-infested field nursery in 1997 and 1998.**

| Cultivar | J2/500cm³ soil† 1998 | Root gall rating‡ 1997 | Root gall rating‡ 1998 |
|---|---|---|---|
| Stoneville LA 887 | 114 | 1.2 | 1.5 |
| Paymaster 1560 | 71 | 1.5 | 1.8 |
| Paymaster 1560 BG | 298 | 3.1 | 4.9 |
| Deltapine 50 | 341 | -- | 3.3 |
| Deltapine 50 B | 298 | 2.5 | 4.0 |
| Deltapine 5415 | 440 | 4.0 | 4.7 |
| Deltapine 5415 RR | 270 | 3.2 | 4.4 |
| LSD (*P* ≤ 0.05) | NS | 0.9 | 0.5 |

† J2, second stage nematode juveniles. Nematode populations data were transformed using log (juveniles +1) for statistical analysis; nematode populations were not measured in 1997.

‡ Root gall rating on a scale of 0 (none) to 5 (severe) root galling.

**Table 2. Root gall ratings and nematode eggs per plant from two growth-room trials.**

| Cultivar | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | Eggs/plant† | Root gall rating‡ | Eggs/plant | Root gall rating |
| Stoneville LA 887 | 2 534 | 1.6 | 6 909 | 2.6 |
| Paymaster 1560 | 1 650 | 2.7 | 10 118 | 2.8 |
| Paymaster 1560 BG | 48 665 | 4.8 | 146 795 | 5.0 |
| Deltapine 50 | 24 904 | 4.9 | 86 316 | 4.8 |
| Deltapine 50 B | 11 562 | 4.1 | 64 919 | 4.7 |
| Stoneville 474 | 23 025 | 4.3 | 52 860 | 4.7 |
| LSD ($P \leq 0.05$) | 17 183 | 0.7 | 36 559 | 0.6 |

† Eggs per plant data were transformed using log (eggs + 1) for statistical analysis.
‡ Root gall rating on a scale of 0 (none) to 5 (severe) root galling.

Deltapine 50. Galling of the transgenic cultivar Deltapine 5415 RR was not different from its nontransgenic parent. Root-knot nematode populations in the soil at crop maturity in 1998 were low in all plots, likely due to the extremely dry conditions at the time the samples were taken. Root-knot nematode J2 population densities at crop maturity were not significantly different among the cultivars (Table 1).

## Growth-Room Experiments

Results from the two growth-room trials were similar (Table 2). In both trials, Stoneville LA 887 and Paymaster 1560 had the lowest root gall ratings, while Paymaster 1560 BG, Deltapine 50, and Deltapine 50 B had gall ratings that were similar to the susceptible standard cultivar Stoneville 474. The greatest nematode reproduction occurred on the cultivar Paymaster 1560 BG, while reproduction on Paymaster 1560 and Stoneville LA 887 was similar and was significantly lower than on the other cultivars, except for Deltapine 50 B in the first trial. In both trials, root gall rating and number of eggs per plant were higher for Paymaster 1560 BG than for its nontransgenic parent, Paymaster 1560. Nematode reproduction and root galling severity were similar for Deltapine 50 B and its nontransgenic parent Deltapine 50, except that root galling severity was lower for Deltapine 50 B in the first trial.

## DISCUSSION

Although a limited number of cultivars were studied, the data demonstrate that differences in susceptibility to the root-knot nematode exist between some transgenic cultivars and their nontransgenic parents. A dramatic difference in root-knot nematode susceptibility was observed between Paymaster 1560 and its genetically modified, insect-resistant counterpart, Paymaster 1560 BG. On the other hand, both Deltapine 50 and its genetically modified counterpart Deltapine 50 B exhibited similar levels of root-galling and supported similar levels of nematode reproduction. It is beyond the scope of this study to identify the reasons for differences in nematode resistance between transgenic selections and their parent cultivars, because information on the mechanism that was used to transfer the genes conferring insect resistance to the transgenic cultivars was not available to these authors. If the Bt toxin genes that confer tobacco budworm resistance were introgressed into Paymaster 1560 BG by genetic transformation, then it is possible that a transformation event altered the genes that confer the nematode resistance seen in Paymaster 1560. In this case, the probability of identifying root-knot resistant progeny through screening would be low. However, if the insect resistance was introduced into Paymaster 1560 by classical breeding techniques, it is likely that the progeny from this cross simply were not screened and selected for expression of nematode resistance as rigorously as they were screened for insect resistance.

These data indicate the importance of screening transgenic cultivars for resistance to pests other than those targeted by the genetic modification before the transgenic cultivars are recommended to producers. The utility and effectiveness of cultivars genetically modified for insect and herbicide resistance could be improved substantially if the cultivars also were resistant to nematodes or to other important diseases,

such as *Fusarium* or *Verticillium* wilt. Information on the susceptibility of transgenic cultivars to other diseases is important for a comprehensive disease management program.

## ACKNOWLEDGMENT

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