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Resistance as a Tactic for Management of *Meloidogyne incognita* on Cotton in North Carolina[1]

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Abstract: Selected cotton cultivars were evaluated for resistance to the southern root-knot nematode, *Meloidogyne incognita,* in greenhouse and field experiments. Cotton cultivars LA 887, Auburn 634, and NemX cotton were highly resistant to three North Carolina populations of root-knot nematode in greenhouse experiments compared to susceptible cultivars. The relative susceptibility of cultivars tested in the greenhouse from most to least susceptible were Deltapine 16 > Deltapine 50 > LA 887 or NemX > Auburn 634. The yields of resistant and susceptible cotton cultivars were increased by fumigation in fields infested with root-knot nematode. Reproduction of *M. incognita* in field plots on NemX, Paymaster H 1560, and Stoneville LA 887 was less than on susceptible cultivars. Diminished reproduction of the nematode on resistant cultivars may reduce the need for nematode control tactics in subsequent years.
Key words: crop loss, host-plant resistance, nematode, southern root-knot nematode.

The southern root-knot nematode, *Meloidogyne incognita* Kofoid and White (Chitwood), is a major pathogen of cotton, *Gossypium hirsutum* L., in most cotton production areas of the United States (Blasingame, 1993; Starr, 1998). Yield losses associated with root-knot nematode ranged from 0 to 7% across the cotton belt in 1994 (Koenning et al., 1999). In addition, several diseases of cotton result from the interaction of root-knot nematode and fungal pathogens (Blasingame, 1993). Suppression of the population densities of root-knot nematode in cotton production systems is accomplished through rotation, chemical control, and to a limited extent through host-plant resistance, but nematode management has been categorized as highly dependent on nematicides (Starr, 1998). Rotation is rarely considered as a management tactic for suppression of *M. incognita* population densities for several reasons. Many potential rotational crops either are of relatively low value compared to cotton or are fair to excellent hosts for the southern root-knot nematode. Furthermore, the area for production of some of the crops that would be good alternatives to cotton, such as peanut, are restricted by government programs. Fumigant and nonfumigant nematicides are registered for use on cotton, but their availability in the future is in question (Thomason, 1987).

Cotton genotypes Auburn 623 and 634 with high levels of resistance to *M. incognita* were developed but not grown commercially because of inferior agronomic performance (Elliott, 1999; Roberts et al., 1998; Robinson and Percival, 1997; Shepherd, 1974, 1979, 1982a, 1982b). Resistance and (or) tolerance to root-knot and reniform nematodes in advanced cotton breeding lines and germplasm have been identified but have not yet been incorporated into cultivars (Cook et al., 1997; Robinson and Percival, 1997; Shepherd, 1983; Shepherd et al., 1996). Many cotton cultivars are considered to be tolerant to root-knot nematode, but this tolerance is distinct from resistance (Barker, 1993). Resistant cultivars can serve a secondary role in limiting the population density of this pathogen in subsequent years (Shepherd, 1982b). The only available cultivars with moderate resistance to the root-knot nematode are Stoneville LA 887, Paymaster (formerly Hartz) H 1560, and Acala NemX (Elliott, 1999; Jones et al., 1991; Ogallo et al., 1997; Robinson and Percival, 1997).

The incidence and damage potential of *M. incognita* on cotton in North Carolina have increased with the tripling in cotton production area in the past 10 years. The North Carolina Department of Agriculture, through its Nematode Advisory Service, detected these nematodes in 45% of the cotton fields assayed in 1991 and more than 55% in 1995 (J. L. Imbriani, pers. comm.). Root-knot nematode is most damaging to cotton in coarse textured soils that are common in the coastal plains of the southeastern United States (Blasingame, 1993; Koenning et al., 1996). The majority of cotton production in North Carolina is concentrated on these soils, and cotton yield suppression due to root-knot nematode is a common problem. The resurgence in cotton production in parts of the Southeast makes this pathogen a major concern.

The virulence and (or) aggressiveness of populations of root-knot nematode on cotton vary considerably (Elliott, 1999; Kirkpatrick and Sasser, 1983; Zhou et al., 1998). Herein, virulence is used when high levels of reproduction are observed on a resistant host, whereas aggressiveness is used in relating the relative reproduction and (or) level of galling of different populations of nematode on susceptible hosts. Host races 3 and 4 of *M. incognita* are classified on the basis of their ability to parasitize cotton. Race 4 is separated from race 3 by its

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ability to reproduce on root-knot resistant tobacco cultivars (Hartman and Sasser, 1985). Some controversy about the virulence and management of various populations of *M. incognita* exists (Kirkpatrick and Sasser, 1983; Veech and Starr, 1986).

Research was initiated in 1996 to investigate the potential for using resistant cultivars to manage root-knot nematode on cotton. The objectives of this research were to evaluate the reproductive capacity of several populations of *M. incognita* on resistant and susceptible cotton cultivars, measure cotton yield suppression in the presence of this nematode in the field, and determine the population density of this nematode following the culture of resistant vs. susceptible cotton cultivars to determine the feasibility of using root-knot resistant cotton cultivars to suppress the population density of this pathogen.

Materials and Methods

The greenhouse experiments involved evaluation of selected root-knot nematode resistant and susceptible cotton cultivars to several North Carolina populations of *M. incognita.* The experiment was a 3 × 5 factorial with three nematode populations and five cultivars. Cotton cultivars used in the greenhouse experiments included susceptible Deltapine 16 and Deltapine 50, and resistant Stoneville LA 887, Acala NemX, and Auburn 634. Nematode populations were categorized as to race according to the North Carolina host differential test (Hartman and Sasser, 1985). Specific populations of *M. incognita* from North Carolina included a race 3 population maintained in the greenhouse for several years (designated Race 3a); a population from Robeson, County, North Carolina, recently isolated from cotton designated Race 3b; and a race 4 population isolated from root-knot nematode resistant tobacco in Martin County, North Carolina, in 1986. Cotton seedlings (4 to 5 days old) were transplanted to 15-cm-diam. clay pots containing a mixture of steam pasteurized washed river sand and sandy loam soil. Nematode inoculum consisted of 5,000 eggs of each nematode population, extracted with sodium hypochlorite (Hussey and Barker, 1973), added to the pot and covered with soil. Inoculated cotton plants were grown for 12 weeks in the greenhouse, and then evaluated for nematode resistance (Barker et al. 1986; Hussey and Barker, 1973; Roberts et al., 1998). Plants were removed from soil, roots weighed, the root system rated for galling (0–100 scale), and eggs extracted from a 5-g sub-sample of the root system with sodium hypochlorite. There were eight replicates for each combination of cultivar and nematode population. The experiment was conducted twice.

Three field experiments were conducted in 1997 and 1998. Field trials in 1997 were in Hoke and Scotland Counties, North Carolina, in fields that had been planted with cotton previously. The soil types were a Marlboro sandy loam (clayey, kaolinitic, thermictypic, paleudult; 69% sand, 26% silt, 5% clay; 1% organic matter) and a Marlboro loamy sand (85% sand, 11% silt, 4% clay, < 1% organic matter). A third experiment conducted in 1998 was located in Wayne County, North Carolina, in a field previously planted to cotton. The soil type was a Kenansville loamy sand (fine loamy, siliceous, themic, arenic hapludult; 85% sand, 15% silt, 5% clay, < 1% organic matter). Mean pre-fumigant population densities of *M. incognita* in the field sites were 5 ± 8, 42 ± 60, and 14 ± 21 per 500 cm^3 soil, respectively. The experimental design was a split-plot with six replicates. Sub plots were treated with 56 liters/ha of 1,3-dichloropropene (Telone II, Dow AgroSciences, Indianapolis, IN) vs. nontreated. Whole plots were resistant cultivars Stoneville LA 887, Paymaster H 1560 (formerly Hartz 1560), and Acala NemX and susceptible cultivars Suregrow 125, Deltapine 90 (moderately tolerant), and Deltapine 50 (moderately susceptible). Selected plots were fumigated in April or May and planted in mid-May. Plots were two rows, 12.8 m long with 1.01-m row spacing and 3.7-m alleys. Aldicarb (Temik 15G, Aventis CropScience Inc., Research Triangle Park, NC) was applied to all plots in-furrow (0.5 kg a.i./ha) for insect control. Standard management practices for North Carolina were used for all cotton plots (Anonymous, 2000). Cotton yield was determined with a commercial cotton picker in mid-October. Samples for nematode assays were collected prior to fumigation, at mid-season (mid- to late-July), and at cotton harvest from each plot. Each soil sample consisted of 8 to 10 cores (2.5-cm-diam.) composited. A 500-cm^3 sub-sample was processed by elutriation and centrifugation to extract second-stage juveniles, and roots were processed to extract eggs (Barker et al., 1986; Hussey and Barker, 1973).

Data analysis consisted of analysis of variance (ANOVA) for the appropriate statistical design using PC/SAS software (SAS Institute, Cary, NC). The data from the two greenhouse tests did not differ between trials; therefore, the data were pooled. Trials were considered to be random effects, thus the trial × cultivar × population effect was used as the error term. Similarly, locations for the field tests were considered to be random effects so the cultivar × location, fumigation × location, and fumigation × cultivar × location effects were used to test differences for cultivar, fumigation, and cultivar × fumigation effects, respectively, for combined analysis over years. Significant data for each field location is presented separately because of first-order interactions with location by cultivar, or location by fumigation. Second-order statistical interactions were not significant. Orthogonal contrasts, LSD (least significant difference), and the Waller Duncan k-ratio *t*-test were used to separate means. Nematode numbers were transformed ($\log_{10}$ [χ+ 1]) to normalize the variance. Gall indices were transformed using a square-root arcsin transformation. Untransformed data are presented in tables for clarity.

Results

The *Meloidogyne* populations used in this research differed ($P = 0.10$) in their ability to reproduce on the resistant or susceptible cotton cultivars evaluated in the greenhouse experiments. The race 3a population had lower reproduction and gall indices on all cultivars than the race 4 or race 3b nematode populations (Table 1). The first-order cultivar × nematode population interaction, however, was not significant ($P = 0.10$). There were greater numbers of nematodes per pot, per gram of root, and a higher gall index on Deltapine 16 compared to Deltapine 50 (Table 2). All measures of resistance were consistent within each cultivar tested. The susceptibility of the cultivars to root-knot nematode from highest to lowest was Deltapine 16 > Deltapine 50 > Acala NemX or LA 887 > Auburn 634 (Table 2). Shoot weights were negatively correlated, and gall indices were positively correlated with numbers of eggs ($P = 0.01$; $r = -0.09$ and 0.42, respectively).

Mid-season population densities of *M. incognita* were suppressed by fumigation in field experiments ($P < 0.10$). Highest mid-season numbers of root-knot nematode were found at the Hoke County location in 1997 (Table 3). Nematode densities were higher on susceptible cultivars than on resistant cultivars at all locations. Resistant cultivars did not generally differ at mid-season in their ability to support reproduction of *M. incognita.* The plots planted to the resistant cultivar LA 887 tended to have greater numbers of *M. incognita* than did either NemX or H 1560, although this difference was not significant. Root-knot nematode population densities at cotton harvest were generally greater than those at mid-season (Tables 3, 4). Numbers of root-knot nematode at harvest were lower ($P < 0.10$) in fumigated plots compared to nonfumigated plots. Resistant cultivars had lower numbers of *M. incognita* than susceptible cultivars at all locations ($P < 0.10$). Harvest population densities on Deltapine 90 were low at the Wayne County site, which was also noted for low mid-season numbers.

Table 1. Reproduction and gall indices (0–100) of three populations of root-knot nematode, *Meloidogyne incognita,* on three resistant and two susceptible cotton cultivars.

Population[a]	Eggs per pot[b] ($\times 10^3$)	Eggs per gram of root ($\times 10^3$)	Gall index
M. incognita R3a	55.3a	2.2a	22.5a
M. incognita R3b	106.3b	3.7b	27.6b
M. incognita R4	149.6b	6.0b	29.1b

[a] Races 3a [R3a] greenhouse culture, 3b [R3b] isolated from cotton in Robeson County, North Carolina, in 1992 and reared on tomato, and Race 4 [R4] isolated from resistant tobacco in Martin County, North Carolina, in 1986.

[b] Data are the means of two experiments on five cultivars with eight replications. Means followed by the same letter do not differ according to the Waller-Duncan k-ratio *t*-test (k-ratio = 50).

Table 2. Influence of cotton cultivar on mean numbers of eggs and gall indices of three *Meloidogyne incognita* populations in greenhouse experiments.

Cultivar	Eggs per pot ($\times 10^3$)[a]	Eggs per gram of root ($\times 10^3$)	Gall index (0–100)
Deltapine 16	312.0A	11.9A	58.6A
Deltapine 50	176.7B	6.8B	49.6B
Mean Deltapine 16 and Deltapine 50	244.4a	9.4a	54.1a
Acala NemX	8.6C	0.5C	9.2C
Stoneville LA 887	11.7C	0.4C	11.6C
Mean NemX and LA 887	10.2b	0.5b	10.4b
Auburn 634	2.3Dc	0.1Dc	2.9Dc

[a] Cultivar means are based on two trials with three nematode populations and eight replications. First- and second-order interactions were not significant ($P = 0.10$). Cultivar means followed by the same uppercase letter do not differ according to the Waller-Duncan k-ratio *t*-test (k-ratio = 50). Means followed by the same lowercase letter do not differ according to orthogonal contrasts: Deltapine 16 and 50 vs. Auburn 634; Deltapine 16 and 50 vs. LA 887 and Acala Nemx; and LA 887 and Acala Nemx vs. Auburn 634 ($P = 0.10$).

Fumigation increased cotton lint yield based on the combined analyses and on individual location analyses (Table 5) ($P < 0.10$). The cultivar × location interaction was the only significant interaction ($P < 0.05$). Fumigation increased cotton lint yield on both resistant and susceptible cultivars, although the yield of Deltapine 90 was not affected at the Wayne County site. The yield of the resistant cultivar Acala NemX was lower than all other cultivars ($P < 0.10$, orthogonal contrasts). The mean yield of resistant cultivars LA 887 and H 1560 did not differ from the mean yield of the three susceptible cultivars based on contrasts ($P < 0.10$).

Discussion

The nematode populations used in this research differed in their ability to parasitize the cultivars evaluated in the greenhouse. The cultivar × population interaction, however, was not significant, indicating that virulence on the resistant cultivars was not related to population effects. Considering the small number of populations evaluated in our experiments, the likelihood of detecting a population × cultivar interaction was small. Recent research, however, indicates that populations of *M. incognita* naturally virulent on resistant cotton cultivars do occur (Elliott, 1999).

The aggressiveness and parasitic fitness of races 3 and 4 of *M. incognita* on cotton have been studied in some detail. Researchers in Texas found that the damage potential and reproduction of the two races on cotton were similar and that the race of the nematode need not be considered in management decisions (Veech and Starr, 1986). Other research, however, showed that host race 3 populations had greater reproductive potential on cotton compared to race 4, although there

TABLE 3. Mid-season numbers of *Meloidogyne incognita* per 500 cm^3 soil as affected by fumigation with 56 liters/ha 1,3-dichloropropene and cotton cultivar at two North Carolina locations (Hoke and Scotland Counties) in 1997 and one location (Wayne County) in 1998 using a split-plot design with cultivars as whole plots and fumigation as sub plots.

	Hoke County[a]		Scotland County		Wayne County		Mean	
Cultivar	−fumigant[b]	+fumigant	−fumigant	+fumigant	−fumigant	+fumigant	−fumigant	+fumigant
Deltapine 50	1,074	199	312	17	323	9	570	75
Deltapine 90	2,050	226	1,285	2	111	32	1,148	17
Suregrow 125 (SG 125)	686	140	540	31	158	159	462	145
Mean Dp 50, 90 and SG 125	1,270a	188a	712a	17a	197a	67a	726a	79a
Stoneville LA 887	505	50	600	0	3	0	369	17
Paymaster H 1560	398	60	197	0	0	0	198	21
Acala NemX	301	53	226	0	0	0	176	18
Mean LA 887, H 1560 and NemX	401b	54a	341b	0a	1b	0b	248b	17b
LSD (P = 0.10)	759	247	865	34	354	250	375	117

[a] Data for each cultivar within a county and fumigation treatment is the mean of six replicates.
[b] Fumigation suppressed mid-season (July) numbers of root-knot nematode ($P < 0.10$). The fumigant by location interaction was significant ($P = 0.01$). Means of resistant and susceptible cotton cultivars within a column followed by the same lowercase letter do not differ according to orthogonal contrasts ($P = 0.10$). The LSD (least significant difference) is for comparison of cultivars within columns.

was considerable variation within race 3 populations in their ability to parasitize cotton (Kirkpatrick and Sasser, 1983). Recent research in Texas showed that much variation exists among populations of *M. incognita* in their ability to induce galling on susceptible cotton genotypes (Zhou et al., 1998). The two race 3 *M. incognita* populations used in our research differed in their parasitic fitness in the greenhouse experiments. The race 3a population had lower reproduction and gall indices than did the race 3b or race 4 populations. The race 3b and race 4 populations did not differ in their aggressiveness. Based on the earlier work, and the current research data, host race is probably a poor predictor of population aggressiveness on cotton. Other work with these populations in microplots indicates that although this race 4 population is highly aggressive on cotton, its overwinter survival in the absence of a host is lower than the race 3b population (Barker and Koenning, 1997). An additional difference between race 3 and race 4 populations of *M. incognita* is in their ability to parasitize small grain crops. Races 1 and 4 of this nematode reproduced at higher rates on small grains than races 2 and 3 (Barker et al., 1998). This race 4 population may be able to persist, in spite of poor winter survival, on small grains that are planted as a grain or cover crop in much of the southeastern United States.

The higher level of susceptibility of Deltapine 16 compared to Deltapine 50 was not anticipated in this research. Deltapine 16 is an obsolete cultivar but was used in this research because it is the standard for the North Carolina host differential test used to classify host races of *M. incognita.* This result agrees with recently published data indicating that some limited progress has been made in incorporating higher levels of resistance to *M. incognita* in cotton (Robinson and Bridges, 1999; Robinson et al., 1999). Deltapine 50 is a cultivar still commonly grown and, based on our results, should be classified as moderately susceptible. The lower susceptibility of Deltapine 50 compared to

TABLE 4. Harvest population densities of *Meloidogyne incognita* per 500 cm^3 soil as affected by fumigation with 56 liters/ha 1,3-dichloropropene and cotton cultivar at two North Carolina locations (Hoke and Scotland Counties) in 1997 and one location (Wayne County) in 1998 using a split-plot design with cultivars as whole plots and fumigation as sub plots.

	Hoke[a] County		Scotland County		Wayne County		Mean	
Cultivar	−fumigant[b]	+fumigant	−fumigant	+fumigant	−fumigant	+fumigant	−fumigant	+fumigant
Deltapine 50	2,413	721	165	30	1,432	500	1,274	417
Deltapine 90	2,239	458	440	5	1,112	165	1,264	209
Suregrow 125	1,583	80	188	37	926	451	899	189
Mean Dp 50, 90 and SG125	2,078a	420a	264a	24a	1,156a	372a	1,166a	272a
Stoneville LA 887	997	246	140	8	65	23	401	97
Paymaster H 1560	562	120	117	4	57	37	245	57
Acala NemX	441	394	68	0	34	33	188	142
Mean LA 887, H 1560 and NemX	667b	253a	108b	4a	52b	31b	278b	97b
LSD (P = 0.10)	2,397	492	1,235	64	181	350	591	200

[a] Data for each cultivar within a county and fumigation treatment are the mean of six replicates.
[b] Fumigation suppressed mid-season (July) numbers of root-knot nematode ($P < 0.10$). The fumigation × location interaction was significant ($P = 0.01$). Means of resistant and susceptible cotton cultivars within a column followed by the same lowercase letter do not differ according to orthogonal contrasts ($P = 0.10$). The LSD (least significant difference) is for comparison of cultivars within a column.

TABLE 5. Influence of cultivar and fumigation with 56 liters/ha 1,3-dichloropropene on cotton lint yield (kg/ha) at two North Carolina locations (Hoke and Scotland Counties) in 1997 and one location (Wayne County) in 1998 using a split-plot design with cultivars as whole plots and fumigation as sub plots.

	Hoke County[a]		Scotland County		Wayne County		Mean	
Cultivar	−fumigant[b]	+fumigant	−fumigant	+fumigant	−fumigant	+fumigant	−fumigant	+fumigant
Deltapine 50	1,152	1,213	1,061	1,082	866	928	1,026	1,074
Deltapine 90	1,222	1,302	1,154	1,100	781	865	1,052	1,089
Suregrow 125	1,162	1,171	1,084	1,254	848	938	1,032	1,121
Mean Dp 50, 90 and SG125	1,179a	1,229a	1,100a	1,145a	832a	910a	1,037a	1,095a
Stoneville LA 887	1,124	1,158	1,015	1,083	773	873	971	1,038
Paymaster H 1560	1,231	1,268	1,025	1,058	863	968	1,040	1,098
NemX	863	765	788	952	480	534	711	751
Mean LA 887, H 1560 and NemX	1,073b	1,064b	943b	1,028b	705b	792b	907b	962b
Mean LA 887 and H 1560	1,178a	1,213a	1,020a	1,071a	818a	921a	1,005A	1,068a
LSD (P = 0.10)	120	103	123	129	92	90	67	64

[a] Data for each cultivar within a county and fumigation treatment is the mean of six replicates.
[b] Fumigation suppressed mid-season (July) numbers of root-knot nematode ($P < 0.10$). The fumigation × location interaction was significant ($P = 0.01$). Mean of resistant and susceptible cotton cultivars within a column followed by the same lowercase letter do not differ according to orthogonal contrasts ($P = 0.10$). The LSD (least significant difference) is for comparison of cultivars within a column.

Deltapine 16 may be the result of quantitative genes for resistance rather than a major qualitative gene. Stoneville LA 887, Paymaster H 1560, and Acala NemX probably should be considered moderately resistant, whereas Auburn 634 was highly resistant. Research data suggest that Auburn 634 has two genes for resistance to root-knot nematode (Shepherd, 1979; Shepherd, 1983; Shepherd et al., 1996), while the other resistant cultivars have a single gene for resistance (McPherson et al., 1995). While the pedigrees of Paymaster H 1560 and LA 887 would suggest that these two sister lines probably have the same gene for root-knot resistance derived from Auburn 634, the origin of the resistance gene in Acala NemX is uncertain at this time. The resistance of F_1 and F_2 progeny of crosses of LA 887 and Acala Nemx suggests that the two cultivars share the same gene for resistance to *M. incognita* (Zhou et al., 2000).

This research demonstrates that useable levels of resistance to the root-knot nematode are available in cultivars with adequate yield potential suitable to the southeastern United States. Although the yield of Acala NemX was low in our experiment, this result was anticipated because this cultivar was developed for irrigated agriculture in California. The yield of Acala NemX was slightly lower than the root-knot susceptible cultivar Acala Maxxa at low densities of root-knot nematode, but superior to Acala Maxxa at higher nematode densities in research conducted in California (Ogallo et al., 1997). The yields of Paymaster H 1560 and LA 887 were not different from the standard susceptible cultivars used in this research with respect to yield, and thus should be deployed in areas where *M. incognita* is a yield-limiting factor. Although these cultivars are moderately resistant to *M. incognita,* they apparently are not more tolerant than susceptible cultivars and the use of a nematicide or other management tactic should be considered when population densities of this nematode are above the damage threshold. Other research demonstrated that the yield of LA 887 was increased by nematicidal rates of aldicarb in the presence of *M. incognita* (Colyer et al., 1997). The utility of using these resistant cultivars is that they will limit the population density below the damage threshold for cotton, and possibly other root-knot susceptible crops in North Carolina (Koenning et al., 1996; Ogallo et al., 1997; Shepherd, 1982b). The preplant densities of M. incognita in these field experiments were relatively low, but any detectable level of this nematode in the spring in North Carolina is considered to be potentially damaging to cotton.

Host-plant resistance is the preferred tactic for nematode management because it reduces environmental concerns and increases agricultural sustainability. Still, the progress in developing root-knot resistant cotton cultivars has been limited. Currently, Paymaster H 1560 is no longer available and Stoneville LA 887 will likely be obsolete in the next few years, largely because of the popularity of transgenic cotton cultivars with herbicide and (or) insect resistance. A herbicide resistant version of Paymaster 1560 was released but did not have the same nematode resistance as the non-herbicide resistant variety when compared in one test (S. R. Koenning, unpubl. data), and a later version of Paymaster 1560 did not have the Fusarium wilt resistance present in the parent cultivar (Colyer et al., 1999). More emphasis on developing nematode-resistant cotton cultivars is needed to reduce the need for nematicides and improve agricultural sustainability.

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