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Physiological Effects of *Meloidogyne incognita* Infection on Cotton Genotypes with Differing Levels of Resistance in the Greenhouse

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Abstract: Greenhouse tests were conducted to evaluate (i) the effect of *Meloidogyne incognita* infection in cotton on plant growth and physiology including the height-to-node ratio, chlorophyll content, dark-adapted quantum yield of photosystem II, and leaf area; and (ii) the extent to which moderate or high levels of resistance to *M. incognita* influenced these effects. Cultivars FiberMax 960 BR (susceptible to *M. incognita*) and Stoneville 5599 BR (moderately resistant) were tested together in three trials, and PD94042 (germplasm, susceptible) and 120R1B1 (breeding line genetically similar to PD94042, but highly resistant) were paired in two additional trials. Inoculation with *M. incognita* generally resulted in increases in root gall ratings and egg counts per gram of root compared with the noninoculated control, as well as reductions in plant dry weight, root weight, leaf area, boll number, and boll dry weight, thereby confirming that growth of our greenhouse-grown plants was reduced in the same ways that would be expected in field-grown plants. In all trials, *M. incognita* caused reductions in height-to-node ratios. Nematode infection consistently reduced the area under the height-to-node ratio curves for all genotypes, and these reductions were similar for resistant and susceptible genotypes (no significant genotype × inoculation interaction). Our study is the first to show that infection by *M. incognita* is associated with reduced chlorophyll content in cotton leaves, and the reduction in the resistant genotypes was similar to that in the susceptible genotypes (no interaction). The susceptible PD94042 tended to have increased leaf temperature compared with the genetically similar but highly resistant 120R1B1 ($P < 0.08$), likely attributable to increased water stress associated with *M. incognita* infection.

Key words: chlorophyll, cotton, *Gossypium hirsutum*, height-to-node ratio, host-parasitic relationship, *Meloidogyne incognita*, photosynthesis, physiological stress, southern root-knot nematode.

Cotton is grown in more than 70 countries and is the single most-important fiber crop worldwide, and it has the greatest potential for value-added processing of any crop (Basra, 1999). The United States is the third-largest producer of cotton in the world, producing about 20% of the world's annual supply in recent years (Mitchell and Robinson, 2009).

The southern root knot nematode (*Meloidogyne incognita* [Kofoid & White] Chitwood) is found in all cotton production regions in the United States, is the most widely distributed nematode parasite of economic importance to the crop (Thomas and Kirkpatrick, 2001), and causes greater yield loss in cotton than any other nematode (Koenning et al., 2004). The estimated yield loss in cotton caused by *M. incognita* in the United States was 2.5% in 2012, which was greater than for any other cotton disease, resulting in a loss of more than 139,000,000 kg of lint (Blasingame and Patel, 2013). In Georgia in 2012, *M. incognita* caused an estimated 10% reduction in yield resulting in a loss of 75,000,000 kg of lint (Blasingame and Patel, 2013).

Meloidogyne incognita–induced galls on the tap root and lateral roots can disrupt the normal flow of water and nutrients to the leaves and developing bolls thereby reducing cotton growth and yield (Bird, 1970; Bird and Loveys, 1975; McClure, 1977; Kirkpatrick et al., 1991). Above-ground symptoms of *M. incognita* infection include suppressed plant growth (stunting), nutritional deficiency (chlorosis), and temporary wilting during the heat of the day (Thomas and Kirkpatrick, 2001). Cotton growth and leaf expansion can be reduced by infection with *M. incognita* (Kirkpatrick et al., 1995), as can the number and size of cotton bolls and plant dry weight (Walker et al., 1998).

Infection by *M. incognita* can reduce photosynthetic rates in some plants. Within 2 d of *M. incognita* infection, the photosynthetic rate in inoculated tomato plants was less than in noninoculated plants (Loveys and Bird, 1973). During early stages of infection, photosynthesis expressed on the basis of fresh weight, leaf area, or total chlorophyll content was significantly reduced (Loveys and Bird, 1973). Infection of henbane (*Hyoscyamus niger*) by *M. incognita* reduced plant growth, yield, chlorophyll content, photosynthetic rate, and nutrient concentrations; and reductions were greatest at the highest nematode populations (Haseeb et al., 1990). The effect of *M. incognita* infection on the chlorophyll content and photosynthetic rate in cotton has not been documented. In soybean, changes in nutrient concentration following infection by *M. incognita* can alter host metabolism and contribute to premature leaf abscission and to chlorosis, which is presumed to affect chlorophyll content and photosynthesis (Melakeberhan et al., 1987; Carneiro et al., 2002).

The overall goal of this research was to further our understanding of the changes that occur in a cotton plant when it is parasitized by *M. incognita*. Our specific objective was to evaluate the effect of *M. incognita* infection in cotton on plant growth and physiology including the height-to-node ratio, leaf area, chlorophyll content, and dark-adapted quantum yield of photosystem

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II based on chlorophyll fluorescence. An additional objective was to evaluate the extent to which a moderate or high level of resistance to *M. incognita* influenced the effect on those variables.

Materials and Methods

FiberMax 960 BR (susceptible to *M. incognita*) and Stoneville 5599 BR (moderately resistant to *M. incognita* [Barfield, 2003]) were grown in three greenhouse trials in Athens, GA, in spring 2008, fall 2008, and spring 2009 (trials 1, 2, and 3, respectively) with each trial lasting 75 to 80 d from planting to destructive sampling. Two addition trials (trials 4 and 5) were conducted simultaneously with trials 2 and 3 and included the germplasm PD94042 and the unregistered breeding line 120R1B1 from the University of Georgia cotton breeding program, which was derived from a cross between PD94042 and M-120 RNR. Although the two genotypes are genetically similar, PD94042 is susceptible to *M. incognita* whereas 120R1B1 is highly resistant.

All seeds for a given trial were planted into sterilized soil (87.6% sand, 8.4% silt, and 4% clay) in 15.2-cm-diam. clay plots on a single bench in the greenhouse resulting in one plant per pot. Plants were inoculated with *M. incognita* eggs 10 d after planting at 0, 6,000, or 20,000 eggs per pot. Eggs used for inoculation were extracted from *M. incognita*–infected roots of eggplants with NaOCl (Hussey and Barker, 1973). A split-plot design was used with inoculum level as the main plot and cultivar as the subplot. Trial 1 had 11 replications of each treatment (inoculum × genotype combination) and trials 2 through 5 had 10 replications. Insect and mite control and fertilization were the same for all plants within a trial.

Shoot heights, number of nodes, height-to-node ratios, and chlorophyll-related measurements were recorded weekly. Shoot height was measured from the surface of the soil to the terminal bud. The number of nodes was determined by counting all nodes on the main stem except the cotyledonary node, as long as the leaf associated with the node was greater than 2.5-cm width. The height-to-node ratio was calculated as the shoot height divided by number of nodes. Chlorophyll content was measured on the uppermost fully expanded leaf using a Minolta SPAD-502 chlorophyll meter (Konica Minolta, Ramsey, NJ); three measurements were taken and the mean was recorded. Chlorophyll content was measured on the uppermost fully expanded leaves because they are typically the most photosynthetically active leaves on the plants and will consistently be of similar age in sampled plants. Chlorophyll fluorescence was measured on the uppermost fully expanded leaf using a pulse-amplitude modulation fluorometer (mini-PAM, Heinz Walz GmbH, Effeltrich, Germany) in the evenings after the plants had been in the dark for at least 30 min. Fluorescence measurements were used to calculate the dark-adapted quantum yield of photosystem II, a measure of any potential damage to the plant's photosynthetic system.

The height-to-node ratio, chlorophyll content, and dark-adapted quantum yield were transformed into areas under the variable progress curves using the trapezoidal method (Shaner and Finney, 1977). The areas under the progress curves for each treatment were then analyzed by analysis of variance for a split-plot design, followed by mean separation by least significant difference (LSD) tests using the general linear models (GLM) procedure in SAS 9.2 software (SAS Institute, Cary, NC).

Photosynthetic rate, stomatal conductance, leaf temperature, and substomatal CO_2 concentration were measured on the uppermost fully expanded leaf using a CIRAS-1 portable photosynthesis measuring system (PP Systems, Amesbury, MA) immediately before terminating a trial at 75 to 80 d after planting. After collecting the photosynthetic rate measurement, the plant tops were cut off at the soil line and their fresh weight was measured. Leaves were removed from the plants and total leaf area was measured using an LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE). Cotton squares and bolls were collected and counted together as the number of bolls, and their fresh weight and dry weight were also determined. Shoots were placed into paper bags, dried in an oven at 60°C for 3 d, and then dry weight was measured. Cotton roots were washed to remove soil and the fresh weight was then measured. The galling severity caused by *M. incognita* for each root system was rated on a 0 to 5 scale where 0 = no galling, 1 = trace infection with a few small galls, 2 = galling evident on < 25% of the roots, 3 = 25% to 50%, 4 = 50% to 75%, and 5 = > 75% of the roots galled (Kinloch, 1990). Nematode eggs produced on the roots were extracted with 0.625% NaOCl for 3 min (Hussey and Barker, 1973) and counted.

Results

Inoculation with *M. incognita* resulted in significant increases in root gall ratings and egg counts per gram of root compared with the noninoculated control. However, the difference between mid (6,000 eggs) and high (20,000 eggs) nematode inoculation levels was usually not significant (Tables 1,2). There was a significant cotton genotype effect on gall ratings and egg counts with FiberMax 960BR having greater gall ratings and egg counts than Stoneville 5599BR in all three trials (Table 1). FiberMax 960BR typically had approximately twice the gall rating and egg counts of Stoneville 5599BR. The resistant breeding line 120R1B1 had root gall ratings that were approximately half of those in germplasm line PD94042 (Table 2); however, the egg counts per gram of root in 120R1B1 were only approximately 1/13 of that observed in PD94042 in trial 4 and about 1/23 of that in PD94042 in trial 5.

TABLE 1. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (cultivar) on gall rating and egg counts.

Trial	Nematode level[a]	Gall rating[b] Pooled data[c]	Egg counts/g root Pooled data
1	Medium	4.2	2,100.0
	High	4.1	3,004.4
	P value	0.8682	0.2822
2	Medium	3.2	6,282
	High	3.4	10,102
	P value	0.4254	0.1883
3	Medium	2.9	5,056.0
	High	3.3	6,330.3
	P value	0.0610	0.1923
Genotype effect[d]		FM ST	FM ST
	Average	4.6 3.7	3,443.3 1,777.9
Trial 1	*P* value	0.0503	0.0376
Trial 2	Average	4.2 2.4	11,693.0 4,691.0
	P value	<0.0001	0.0182
Trial 3	Average	3.8 2.4	7,197.8 4,188.5
	P value	<0.0001	0.0039

[a] Nematode inoculation level: medium = 6,000 eggs/pot, high = 20,000 eggs/pot (noninoculated not shown).
[b] Gall rating was based on a 0 to 5 index: 0 = no galling, 1 = trace infection with a few small galls, 2 = galling evident on < 25% of the roots, 3 = 25% to 50%, 4 = 50% to 75%, and 5 = > 75% of the roots galled.
[c] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[d] Cotton cultivar: FM = FiberMax 960BR, ST = Stoneville 5599BR.

TABLE 2. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (germplasm) on gall rating and egg counts.

Trial	Nematode level[a]	Gall rating[b] Pooled data[c]	Egg counts/g root Pooled data
4	Medium	2.3 a[d]	1,650.7
	High	2.8 b	2,955.8
	P value	0.0202	0.0797
5	Medium	3.2	3,317.5
	High	3.0	3,575.4
	P value	0.1995	0.7021
Genotype effect[e]		PD 120	PD 120
Trial 4	Average	3.5 1.6	4,228.0 318.0
	P value	<0.0001	<0.0001
Trial 5	Average	4.2 2.0	6,609.8 283.0
	P value	<0.0001	<0.0001

[a] Nematode inoculation level: medium = 6,000 eggs/pot, high = 20,000 eggs/pot (noninoculated not shown).
[b] Gall rating was based on a 0 to 5 index: 0 = no galling, 1 = trace infection with a few small galls, 2 = galling evident on < 25% of the roots, 3 = 25% to 50%, 4 = 50% to 75%, and 5 = > 75% of the roots galled.
[c] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[d] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[e] Cotton genotype: PD = PD94042, 120 = 120R1B1.

The area under the height-to-node ratio curve was reduced with increased nematode inoculum levels (Tables 3,4). The area under the chlorophyll content progress curve was also reduced by *M. incognita* inoculation, and inoculation with 20,000 eggs resulted in reduced chlorophyll content compared with inoculation with 6,000 eggs in trials 1, 3, and 5, but not in trials 2 or 4. Inoculation with *M. incognita* did not affect the areas under the quantum yield progress curve in trails 1, 2, 4, or 5, but it did cause a reduction in trial 3.

Stoneville 5599BR consistently had a greater area under the height-to-node ratio progress curve than FiberMax 960BR but had a smaller area under the chlorophyll content progress curve (Table 3). The difference between cultivars in area under the quantum yield progress curve was usually not significant and was not consistent among trials. Areas under the height-to-node ratio and the chlorophyll content progress curves were higher in PD94042 than 120R1B1 in only one trial (Table 4). There were no interactions between cotton genotype and nematode inoculation level for any of these variables.

Cotton biomass as measured by shoot fresh weight (data not presented), shoot dry weight, root weight, and total leaf area at the end of each trial was reduced by *M. incognita* in one or more trials (Tables 5,6). For the two commercial cultivars, the biomass was lower in plants inoculated with 20,000 eggs than in plants inoculated with 6,000 eggs with the exception of root weight in trial 3. There were no statistical interactions between cotton genotype and nematode inoculation level on any of these variables, which indicated that reductions in biomass caused in the two cultivars by *M. incognita* was similar. Root weight was not reduced in 120R1B1 and was reduced in PD94042 only in trial 5 (Table 6). Dry weight of above-ground plant parts was reduced by *M. incognita* in PD94042 and 120R1B1 in both trials. Leaf area was reduced for PD94042 in both trials and for 120R1B1 in trial 4. Cotton boll number and boll dry weight were reduced in all genotypes when soil was infested with *M. incognita*. Stoneville 5599BR had greater leaf area, boll number, and boll weight than FiberMax 960BR in all trials, and greater root weight in trials 2 and 3. Above-ground dry weight differed between the cultivars in trials 1 and 2, but the difference was inconsistent. There were no differences between PD94042 and 120R1B1 in dry weight, root weight, or leaf area in trial 4, or boll number and boll weight in trial 5. In trial 5, 120R1B1 had greater dry weight, root weight, and leaf area than PD94042.

Photosynthetic rate was reduced by *M. incognita* on FiberMax 960BR and Stoneville 5599BR in trials 1 and 2 and for PD94042 in trial 5 (Tables 7,8). An interaction between cotton genotype and nematode inoculation level occurred in trial 5 with PD94042 suffering a reduction in photosynthetic rate at the highest inoculum level of *M. incognita*, whereas 120R1B1 did not. Transpiration rate, stomatal conductance, leaf temperature, and substomatal CO_2 concentration were not affected by *M. incognita* except for transpiration rate in trials 2

TABLE 3. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (cultivar) on area under height-to-node ratio, chlorophyll content, and quantum yield progress curves.

Trial	Nematode level[a]	AUHNRPC[b] Pooled data[c]	AUCCPC Pooled data	AUQYPC Pooled data
1	None	243.3	1,979.2 a[d]	40.4
	Medium	232.2	1,880.5 b	40.2
	High	223.0	1,774.4 c	30.0
	P value	0.1889	<0.0001	0.0531
2	None	156.0 a	1,623.7 a	33.9
	Medium	154.4 a	1,530.7 b	33.9
	High	142.4 b	1,484.9 b	33.8
	P value	0.0048	0.0001	0.9085
3	None	165.4 a	1,703.7 a	40.1 a
	Medium	153.0 b	1,570.2 b	39.6 b
	High	141.1 c	1,473.6 c	39.8 b
	P value	<0.0001	<0.0001	0.0216

	Genotype effect[e]	FM	ST	FM	ST	FM	ST
Trial 1	Average	220.3	246.6	1,944.8	1803.7	40.2	40.0
	P value	0.0003		<0.0001		0.1354	
Trial 2	Average	135.1	167.2	1,594.2	1498.7	33.8	33.9
	P value	<0.0001		<0.0001		0.6949	
Trial 3	Average	134.3	172.0	1,639.0	1526.0	39.9	39.7
	P value	<0.0001		<0.0001		0.0452	

[a] Nematode inoculation level: none = 0 eggs/pot, medium = 6,000 eggs/pot, high = 20,000 eggs/pot.
[b] AUHNRPC = area under height-to-node ratio progress curve; AUCCPC = area under chlorophyll content progress curve; AUQYPC = area under quantum yield progress curve. All areas under the curved based on weekly measurements.
[c] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[d] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[e] Cotton cultivar: FM = FiberMax 960BR, ST = Stoneville 5599BR.

TABLE 4. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (germplasm) on area under height-to-node ratio, chlorophyll content, and quantum yield progress curves.

Trial	Nematode level[a]	AUHNRPC[b] Pooled data[c]	AUCCPC Pooled data	AUQYPC Pooled data
4	None	146.1 a[d]	1,615.9 a	34.0
	Medium	135.3 b	1,504.9 b	34.0
	High	130.7 b	1,441.5 b	34.0
	P value	0.0055	<0.0001	0.9886
5	None	157.6 a	1,665.9 a	40.2
	Medium	145.0 b	1,540.1 b	39.8
	High	134.0 c	1,489.7 c	40.2
	P value	0.0002	<0.0001	0.0808

	Genotype effect[e]	PD	120	PD	120	PD	120
Trial 4	Medium	143.9	130.3	1,513.4	1,528.1	34.1	33.8
	P value	0.0037		0.6342		0.0053	
Trial 5	Medium	147.8	143.3	1,550.4	1,580.0	40.0	40.1
	P value	0.1827		0.0474		0.4970	

[a] Nematode inoculation level: none = 0 eggs/pot, medium = 6,000 eggs/pot, high = 20,000 eggs/pot.
[b] AUHNRPC = area under height-to-node ratio progress curve; AUCCPC = area under chlorophyll content progress curve; AUQYPC = area under quantum yield progress curve. All areas under the curved based on weekly measurements.
[c] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[d] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[e] Cotton genotype: PD = PD94042, 120 = 120R1B1.

and 5 and substomatal CO_2 concentration in trial 1. There were no significant differences between FiberMax 960BR and Stoneville 5599BR in any trial for photosynthetic rate, transpiration rate, stomatal conductance, or substomatal CO_2 concentration, but 120R1B1 had a higher transpiration rate, stomatal conductance, and substomatal CO_2 concentration than PD94042 in trial 4. The susceptible PD94042 had increased leaf temperature compared with the genetically similar but highly resistant 120R1B1 in trials 4 and 5 ($P < 0.08$ and 0.06, respectively)

DISCUSSION

This study was intended to identify changes that occur in cotton plants when they are damaged by *M. incognita,* so it was necessary to document that our inoculations with *M. incognita* eggs resulted in damage to the plant. As intended, nematode infection of cotton plants resulted in significant levels of root galling and nematode reproduction compared with the noninoculated check, although the differences in plant measurements between inoculum levels of 6,000 and 20,000 eggs/pot were not significant for many variables, which likely indicates that the carrying capacity was often reached with the lower inoculum level. The egg count data confirmed that Stoneville 5599BR has moderate resistance to *M. incognita* and that 120R1B1 was highly resistant, thereby providing the data needed to evaluate the influence of resistance on the growth and physiological factors measured in this study. Infection by *M. incognita* caused reductions in plant dry weight, root weight, leaf area, boll number, and boll dry weight, thereby confirming that growth of our greenhouse-grown plants was reduced in the same ways that would be expected in field-grown plants.

The development of nodes on a cotton plant is not influenced by stress before boll set, but plant height is highly influenced by various stresses (Albers, 1993). Therefore, the height-to-node ratio in cotton is an indicator of the amount of stress that a cotton plant has encountered with greater height-to-node ratios indicating that less stress occurred. In all greenhouse trials in our study, *M. incognita* caused measureable stress, reflected as reductions in height-to-node ratios. Moderate levels of resistance to *M. incognita* in cotton have been shown to result in reduced damage (Davis and May, 2003). Stoneville 5599BR, which has moderate resistance to *M. incognita*, had greater height-to-node ratios than FiberMax 960BR in all three trials; however, there was no statistical interaction between cotton genotype and nematode inoculation for area under the

TABLE 5. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (cultivar) on plant dry weight, root weight, leaf area, boll number, and boll dry weight.

Trial	Nematode level[a]	Shoot dry weight (g)	Root weight (g)	Leaf area (cm^2)	Boll number	Boll dry weight (g)	
		Pooled data[b]	Pooled data	Pooled data	Pooled data	Pooled data	
1	None	21.53 a[c]	47.68 b	1,496.2 a	6.6 a	8.8 a	
	Medium	17.89 b	59.54 a	1,485.7 a	4.7 b	7.7 ab	
	High	14.64 c	51.23 b	1,320.1 b	3.4 b	2.5 b	
	P value	<0.0001	0.0307	0.0201	<0.0001	0.0017	
		Pooled data	Pooled data	Pooled data	Pooled data	FM	ST
2	None	15.74 a	29.36 a	1,654.0 a	5.8 a	0.5 a	1.4 a
	Medium	11.61 b	26.73 a	1,481.9 a	4.0 b	0.2 b	0.7 b
	High	8.72 c	18.41 b	1,231.1 b	1.9 c	0.0 b	0.3 c
	P value	<0.0001	<0.0001	0.0002	<0.0001	0.0001	<0.0001
		Pooled data	Pooled data	Pooled data	Pooled data	Pooled data	
	None	17.20 a	50.79 a	1,034.7 a	3.7 a	3.2 a	
3	Medium	14.19 b	40.22 b	926.6 b	1.9 b	1.6 b	
	High	9.88 c	37.21 b	770.2 c	0.9 c	0.5 c	
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Genotype effect[d]		FM ST	FM ST	FM ST	FM ST	FM	ST
Trial 1	Average	18.23 17.72	51.43 53.87	1,357.0 1,501.6	4.2 5.5	4.5	7.9
	P value	<0.0001	0.3099	0.0127	0.0006	0.0198	
Trial 2	Average	10.42 13.63	21.02 28.65	1,251.6 1,659.8	2.4 5.4	0.2	0.8
	P value	0.0008	0.0011	0.0007	<0.0001	<0.0001	
Trial 3	Average	13.60 13.80	39.68 49.80	855.2 969.5	1.8 2.5	0.6	3.0
	P value	0.2521	0.0086	0.0026	0.0338	<0.0001	

[a] Nematode inoculation level: none = 0 eggs/pot, medium = 6,000 eggs/pot, high = 20,000 eggs/pot.
[b] Data for two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[c] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[d] Cotton cultivar: FM = FiberMax 960BR, ST = Stoneville 5599BR.

height-to-node ratio curve meaning that *M. incognita* affected the ratio the same in the two genotypes. Similarly, there was no interaction between cotton genotype and nematode inoculation for area under the height-to-node ratio curve for the highly resistant 120R1B1 and the susceptible PD94042. Nematode infection consistently reduced the area under the height-to-node ratio curves for all genotypes, but even though resistant plants (Stoneville 5599BR or 120R1B1) consistently suffered less galling than susceptible plants (FiberMax 960BR or PD94042), the reductions in height-to-node ratio caused by *M. incognita* were similar between resistant and susceptible genotypes (no interaction). It appears that galling and nematode reproduction are not necessary to cause reductions in a cotton plant's height-to-node ratio. Perhaps the stress reactions that reduce the height-to-node ratio are triggered in the plant by initial *M. incognita* penetration, which occurs even in highly resistant plants.

Leaf chlorophyll content provides a measure of photosynthetic capacity and is related to the nitrogen concentration in the plant (Evans, 1989), which *M. incognita* can influence by interfering with water and nutrient transport (Melakeberhan et al., 1987; Kirkpatrick et al., 1991; Carneiro et al., 2002). Therefore, because chlorophyll content is affected by nitrogen concentration, it can be an indicator of the damage caused to the plant by *M. incognita*. Previous studies have shown that infection of plants by *M. incognita* can result in reduced chlorophyll content and photosynthesis (Loveys and Bird, 1973; Haseeb et al., 1990). Our study is the first to show that infection by *M. incognita* is associated with reduced chlorophyll content in cotton leaves. Such physiological response may have resulted from a reduced supply of root-derived photosynthesis-regulating factors. For example, both cytokinins and gibberellins in tomato root tissue and xylem exudates can be decreased in plants infected with *M. incognita* compared with noninfected plants (Brueske and Bergeson, 1972).

Dark-adapted quantum yield is a measure of the maximum efficiency by which plants use absorbed light energy to drive electron transport through the electron transport chain in the thylakoid membranes of chloroplasts. Since photosystem II is the rate limiting step in this electron transport chain, dark-adapted quantum yield is a measure of the health status of photosystem II (Maxwell and Johnson, 2000). In our study, dark-adapted quantum yield was only reduced in one of the five trials, which indicates that *M. incognita* does not consistently cause damage to photosystem II.

The response of both chlorophyll content and, when affected, chlorophyll fluoresce to *M. incognita* inoculation

TABLE 6. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (cultivar) on plant dry weight, root weight, leaf area, boll number, and boll dry weight.

Trial	Nematode level[a]	Shoot dry weight (g)		Root weight (g)		Leaf area (cm^2)		Boll number		Boll dry weight (g)	
		Pooled data[b]		Pooled data		Pooled data		Pooled data		Pooled data	
4	None	15.74 a[c]		34.67		1,949.4 a		5.0 a		0.6 a	
	Medium	13.18 b		36.59		1,782.3 ab		3.0 b		0.3 b	
	High	11.22 c		30.62		1,680.0 b		2.3 b		0.1 b	
	P value	<0.0001		0.1693		0.0135		0.0001		<0.0001	
		PD	120	PD	120	PD	120	Pooled data		PD	120
5	None	19.6 a	20.9 a	66.6 a	70.1	1,218.6 a	1,339.1	3.3 a		3.6 a	1.6 a
	Medium	12.7 b	18.4 b	41.7 b	78.6	949.8 b	1,315.6	1.2 b		0.8 b	0.5 b
	High	11.3 b	15.60 c	50.2 b	69.9	931.8 b	1,216.9	0.7 b		0.0 b	0.1 b
	P value	<0.0001	0.0002	<0.0001	0.1653	0.0007	0.0750	<0.0001		0.0010	0.0257
Genotype effect[d]		PD	120	PD	120	PD	120	PD	120	PD	120
Trial 4	Average	13.74	13.02	34.33	33.60	1,825.8	1,782.0	0.3	0.3	3.2	3.6
	P value	0.3067		0.5025		0.5052		0.5557		0.5005	
Trial 5	Average	14.51	18.39	52.84	72.86	1,033.4	1,292.3	1.6	1.9	1.5	0.8
	P value	0.001		<0.0001		0.0002		0.4575		0.1145	

[a] Nematode inoculation level: none = 0 eggs/pot, medium = 6,000 eggs/pot, high = 20,000 eggs/pot.
[b] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[c] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[d] Cotton genotype: PD = PD94042, 120 = 120R1B1.

was similar between the paired genotypes in our tests. Stoneville 5599BR is moderately resistant to *M. incognita*, and 120R1B21 is highly resistant, but the reduction in chlorophyll content in the resistant lines was similar to the reduction in the susceptible genotypes (no genotype × inoculation interaction). The consistently greater chlorophyll content in FiberMax 960BR than in Stoneville 5599BR was probably attributable to inherent

TABLE 7. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (cultivar) on photosynthesis-related measurements.

Trial	Nematode level[a]	Transp.[b] ($mmol \cdot m^{-2} \cdot s^{-1}$)	GS ($mmol \cdot m^{-2} \cdot s^{-1}$)	TL (°C)	PN ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol \cdot mol^{-1}$)
		Pooled data[c]	Pooled data	Pooled data	Pooled data	Pooled data
1	None	3.42	173.41	24.63	14.91 a	212.82 b
	Medium	3.28	166.48	24.74	12.16 b	241.24 a
	High	3.09	147.96	25.57	9.97 b	254.87 a
	P value	0.1819	0.5005	0.1582	0.0047	0.0021
2	None	3.73 a[d]	519.45	21.70	10.20 a	341.32
	Medium	3.22 b	420.32	21.78	8.46 b	340.32
	High	3.30 b	427.41	22.13	8.71 b	336.18
	P value	0.0451	0.1311	0.4888	0.0016	0.3738
3	None	4.56	459.65	25.99	11.47	318.75
	Medium	4.32	417.30	26.10	12.04	310.35
	High	4.21	409.35	26.28	11.57	316.85
	P value	0.4275	0.3464	0.0839	0.8151	0.4869
Genotype effect[e]		FM ST	FM ST	FM ST	FM ST	FM ST
Trial 1	Average	3.49 3.05	177.28 148.26	25.28 24.72	12.78 11.87	240.47 232.79
	P value	0.9919	0.1989	0.1586	0.4951	0.3240
Trial 2	Average	3.56 3.27	466.45 445.00	22.15 21.59	9.07 9.18	339.70 338.85
	P value	0.1551	0.6449	0.1463	0.7239	0.8302
Trial 3	Average	4.52 4.21	445.37 412.17	26.13 26.11	12.35 11.03	314.03 316.60
	P value	0.3012	0.5558	0.8123	0.2021	0.5653

[a] Nematode inoculation level: none = 0 eggs/pot, medium = 6,000 eggs/pot, high = 20,000 eggs/pot.
[b] Transp. = transpiration rate; GS = stomatal conductance; TL = leaf temperature; PN = photosynthetic rate; Ci = substomatal CO_2 concentration.
[c] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[d] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[e] Cotton cultivar: FM = FiberMax 960BR, ST = Stoneville 5599BR.

TABLE 8. Effect of *Meloidogyne incognita* inoculation level and cotton genotype (germplasm) on photosynthesis-related measurements.

		Transp.[b]	GS	TL	PN		Ci	
		($mmol \cdot m^{-2} \cdot s^{-1}$)	($mmol \cdot m^{-2} \cdot s^{-1}$)	(°C)	($\mu mol \cdot m^{-2} \cdot s^{-1}$)		($\mu mol \cdot mol^{-1}$)	
Trial	Nematode level[a]	Pooled data[c]	Pooled data	Pooled data	Pooled data		Pooled data	
4	None	3.76	440.18	22.84	10.30		329.95	
	Medium	3.83	458.32	23.03	10.17		331.64	
	High	3.80	455.05	22.95	9.50		334.23	
	P value	0.9323	0.9004	0.8714	0.0849		0.5129	
Genotype effect[c]		Pooled data	Pooled data	Pooled data	PD	120	PD	120
5	None	3.47 a[d]	1,709.9	24.97	15.13 a	16.06	311.5	318.7
	Medium	2.92 b	1,992.3	24.72	16.74 a	14.29	301.7	317.3
	High	3.03 b	1,142.4	25.28	9.99 b	14.56	326.9	306.5
	P value	0.0286	0.5985	0.5334	0.0040	0.7032	0.1034	0.4904
Genotype effect[c]		PD 120	PD 120	PD 120	PD	120	PD	120
Trial 4	Average	3.62 3.97	390.82 511.55	23.14 22.74	9.92	10.06	327.03	336.85
	P value	0.0643	0.0126	0.0758	0.6577		0.0076	
Trial 5	Average	3.20 3.08	1,510.3 1,719.4	25.26 24.71	13.95	14.97	313.37	314.17
	P value	0.4696	0.6732	0.0586	0.4505		0.8791	

[a] Nematode inoculation level, none = 0 eggs/pot, medium = 6,000 eggs/pot, high = 20,000 eggs/pot.
[b] Transp. = transpiration rate; GS = stomatal conductance; TL = leaf temperature; PN = photosynthetic rate; Ci = substomatal CO_2 concentration.
[c] Data for the two genotypes were pooled when no significant cultivar × inoculation level interaction occurred.
[d] $LSD_{(0.05)}$ comparisons among nematode inoculation levels within a trial. Means in a column within a trial followed by the same letter are not significantly different.
[e] Cotton genotype: PD = PD94042, 120 = 120R1B1.

genetic differences. PD94042 was the recurrent parent of 120R1B1 in a back-cross sequence, so the two genotypes were very similar genetically except for nematode resistance, and chlorophyll content did not differ significantly between them despite *M. incognita* infection, which suggests that this character is strongly influenced by genetics. As with the height-to-node ratio, resistance to *M. incognita* does not appear to protect plants from suffering reduced chlorophyll content.

Photosynthetic rate was reduced by *M. incognita* infection in some trials but not in others. Our finding that photosynthetic rate was reduced even though substomatal CO_2 concentration was increased (trial 1) or not affected (trials 2 and 5, genotype PD94042) indicates that the reductions in photosynthesis were not caused by reduced diffusion of CO_2 into the leaves. Thus, these reductions in photosynthesis must have been caused by nonstomatal factors (Jones, 1985), which include the diffusion of CO_2 to the chloroplasts, photosynthetic light reactions, and Calvin cycle biochemistry. This is consistent with the reduction in chlorophyll content in inoculated plants. Although not statistically different in any trial, leaf temperature in all trails was numerically greater for plants inoculated with 20,000 eggs than for noninoculated plants. The susceptible PD94042 had increased ($P = 0.076$ in trial 4, and 0.059 in trial 5) leaf temperature compared with the genetically similar but highly resistant 120R1B1, likely because of increased water stress associated with *M. incognita* infection.

The physiological effects of *M. incognita* infection documented in this study may affect cotton fiber quality, although this was not examined. For example, micronaire, a fiber quality measurement based on the air permeability of a specified plug of cotton fibers, is influenced by carbohydrates that are produced through photosynthesis and are deposited on the interior walls of the hollow fiber, which increases micronaire values (Silvertooth, 1999). Infection by *M. incognita* may result in increased micronaire because infection reduces the number of bolls on a cotton plant resulting in reduced competition among bolls for nutrients so that more carbohydrates are deposited in each boll even if the plant is producing less total carbohydrates. Fiber length, another important fiber quality characteristic, is determined as the fibers elongate, which requires the deposition of carbohydrate polymers (DeLanghe, 1986), and water pressure inside the developing fiber influences fiber elongation by regulating the deposition of carbohydrate polymers (Bradow and Davidonis, 2000). Therefore, if *M. incognita* infection reduces the production of carbohydrate polymers by reducing chlorophyll content and photosynthesis, as our study showed it can, or if *M. incognita* reduces water pressure in the developing fibers by inhibiting water translocation in the plant, then *M. incognita* infection could potentially reduce fiber length. However, the effect on fiber length may be mitigated if the number of bolls on the plant is reduced as it was in our study. Fiber strength and uniformity might also be influenced by these same stresses (Bradow and Davidonis, 2000). Increased cotton micronaire following infection by *M. incognita* was recently reported, but reductions in fiber length, strength, or uniformity have not been documented (Davis et al., 2014). Research is currently underway to determine the relationships between nematode numbers, host physiology, and cotton fiber quality in more detail.

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