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Influence of *Meloidogyne incognita* on the Water Relations of Cotton Grown in Microplots[1]

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Abstract: The effects of *Meloidogyne incognita* on the growth and water relations of cotton were evaluated in a 2-year field study. Microplots containing methyl bromide-fumigated fine sandy loam soil were infested with the nematode and planted to cotton (*Gossypium hirsutum* L.). Treatments included addition of nematodes alone, addition of nematodes plus the insecticide-nematicide aldicarb (1.7 kg/ha), and an untreated control. *Meloidogyne incognita* population densities reached high levels in both treatments where nematodes were included. Root galling, plant height at harvest, and seed cotton yield were decreased by nematode infection. In older plants (89 days after planting [DAP]), leaf transpiration rates and stomatal conductance were reduced, and leaf temperature was increased by nematode infection. Nematode infection did not affect ($P = 0.05$) leaf water potential in either young or older plants but lowered the osmotic potential. The maximum rate and cumulative amount of water flowing through intact plants during a 24-hour period were lower, on both a whole-plant and per-unit-leaf-area basis, in infected plants than in control plants. Application of aldicarb moderated some of the nematode effects but did not eliminate them.

Key words: aldicarb, cotton, *Gossypium hirsutum, Meloidogyne incognita,* nematicide, nematode, plant water relations, root-knot nematode, stomatal resistance, transpiration, water flux, water potential.

Infection of cotton (*Gossypium hirsutum* L.) roots by *Meloidogyne incognita* (Kofoid & White) Chitwood results in disruption of the xylem, epidermis, and cortical tissue in response to giant-cell development and gall formation (9). Cotton plants infected by *M. incognita* often show symptoms of water-deficit stress, particularly under field conditions (8). Information on the effects of *M. incognita* on the water status of infected host plants is limited (12), and effects of nematode infection on the water relations of field-grown cotton have not been quantified.

In growth chamber studies, *M. incognita* suppressed water flow through intact roots in a nematode-susceptible cotton cultivar (Stoneville 506) but did not affect water flow through the roots of the highly resistant breeding line Auburn 634 (4). In this study, nematode infection did not alter stomatal conductance, transpiration, or the components of leaf-water potential relative to uninfected control plants. However, in both tomato and bean seedlings, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood suppressed leaf water potentials and root hydraulic conductivity (5,11), and *M. javanica* suppressed stomatal conductance in tomato seedlings (5). Water consumption of cotton infected by *M. incognita* was as great over a 10-week period as in uninfected plants when soil moisture was maintained near field capacity but was significantly lower where soil moisture was not always maintained at field capacity (8).

A partial explanation of the paucity of information on nematodes and plant-water relations may be the difficulty encountered in conducting studies of this nature. This is particularly true in studies of root flux where unavoidable wounding or excision of roots during analysis may obscure nematode effects (12). A more thorough understanding of the role of nematodes in host water status is needed. Our objective was to study the effects of *M. incognita* on the plant-water relations of cotton grown full season in microplots.

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Materials and Methods

Microplots fashioned from concrete drainage tiles (76 cm-d, buried to a depth of 80 cm) were filled with Smithdale fine sandy loam (fine loamy siliceous, thermic Typic Paleudult) in the fall of 1991 and fumigated with methyl bromide (100 g/m^2). On 27 May 1992 all plots except the controls were infested with *M. incognita* host race 3 that originally had been collected from a cotton field in Arkansas, maintained on cotton in a greenhouse, and increased on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) to provide inoculum for the plots. Inoculum consisted of finely chopped, infected tomato roots and infested soil. Each plot was infested with approximately 8.5×10^5 eggs and juveniles (J2)/plot contained in 3.5 liters of the soil-root mixture. The inoculum was incorporated by thoroughly mixing it into the upper 20 cm of soil in each plot using a shovel and a rake. Control plots received an equal volume of soil containing finely chopped, uninfected tomato roots. Twenty acid-delinted cotton seeds of the *M. incognita*-susceptible cultivar Stoneville 825 were planted into each plot immediately after incorporation of the inoculum. All except six randomly spaced plants per plot were removed 2 weeks after emergence.

Treatments included *M. incognita* alone; *M. incognita* plus aldicarb (Temik 15 G, Rhone-Poulenc Ag Co., Research Triangle Park, NC), 1.7 kg/ha applied at planting; and a control with no *M. incognita* or aldicarb. Aldicarb was applied to appropriate plots immediately following incorporation of inoculum and before planting by broadcasting the granular material over the surface of the plot and incorporating it to a depth of 5 cm with a rake. The experiment was arranged in a completely random design with 16 replications of each treatment. Following planting, one tensiometer was installed in the center of each microplot at a depth of 20 cm. Tensiometers were read three times each week during the study using a tensiocorder (Soil Measurements Systems, Las Cruces, NM) to determine the soil water potential. Microplots were watered thoroughly when soil moisture dropped below −30 kPa.

Microplots were fertilized prior to nematode infestation and planting based on soil nutrient recommendations of the Arkansas Cooperative Extension Service for cotton in southern Arkansas (2). A side dressing of NH_4NO_3 (224 kg/ha) was incorporated into the soil of each plot on 1 July and again on 28 July 1992. Seven insecticide applications, based on populations and species found during inspection of the plots, were made according to Arkansas Cooperative Extension Service recommendations (2). Frost on 4 November killed the plants prematurely, and many bolls failed to open. Final plant height was recorded on 17 November, but accurate seed cotton yield data were not available for 1992.

The microplots were fumigated again in March 1993, and the experiment was repeated during the 1993 season. Experimental design, methodology, and the nematode population used for inoculum were the same as in 1992. In 1993, plots were infested and planted on 3 May. Preplant fertilization was based on preplant soil assay. Additional NH_4NO_3 (224 kg/ha) was incorporated into each plot on 15 June, 16 July, and 1 August. Insect control was based on scouting and performed according to Arkansas Cooperative Extension Service suggestions (eight applications). To aid boll opening and to defoliate the plants, all plots were treated with a combination of merphos (Def 6, Bayer, Inc., Kansas City, MO) at 2.3 liters/ha plus ethephon (Prep, Rhone-Poulenc Ag Co., Research Triangle Park, NC) at the rate of 1.2 liters/ha on 1 October with a CO_2 backpack sprayer. Seed cotton was harvested by hand from all plots on 25 October 1993.

Nematode population densities were measured in each microplot in 1992 at 51 days after planting (DAP) and at harvest on 17 November (174 DAP). In 1993, populations were quantified at 93 DAP and at harvest on 25 October (175 DAP). Composite soil samples (250 cm^3) collected

from each plot with a 2.5 cm-d soil probe were processed by gravity-screening and centrifugal-flotation (1) and the J2 counted. The root fraction collected during the screening process for each sample was processed using NaOCl (3) for 4 minutes, and the eggs were counted. Following harvest, all root systems were inspected for galling and rated using a scale of 0–100 where 0 = no galls and 100 = 100% of the root system galled. Plant height was calculated as the distance from the cotyledonary node to the tip of the main stem terminal and was recorded for all plants at 85 DAP in 1992 and 86 DAP in 1993. Final plant height was recorded at harvest both years.

Leaf water potential was measured from one randomly selected plant in each of 10 replications of each treatment on 24 August (89 DAP) in 1992 and 1 July (58 DAP) and 19 August (108 DAP) in 1993. Samples were all collected between the hours of 11 a.m. and 1 p.m. End-window thermocouple psychrometers (Model 84-2VC, JRD Merrill Specialty Equipment, Logan, UT) that used a single 0.9-cm disk cut from the uppermost fully expanded leaf with a leaf-disc sampler were used for these measurements (7). Also at 89 DAP (1992) and 58 and 108 DAP (1993), stomatal conductance to water vapor, transpiration, and leaf temperature were measured from one plant in each of the same plots using a LI-1600 steady-state porometer (LICOR Inc., Lincoln, NE).

Stem water flux through intact plants was measured using the Dynagage Flow 32 sap flow system (Dynamax, Houston, TX), utilizing the stem heat balance and the 5-mm sensor method (10) at 89 DAP in 1992 and at 58 and 108 DAP in 1993. These measurements are a direct indication of the water consumption of whole, intact plants. Due to limited equipment, only three nematode-infected and three control plants were measured at each measurement time. No measurements were taken on plants treated with aldicarb.

To correct for differences in plant size, stem water flux was expressed on a leaf area basis. Total leaf area of the plants was estimated by measuring leaf length from base to tip of the blade. Leaf area was calculated using the equation: Leaf area = $-64.7 + 16.76 \cdot$ leaf length (Ball and Oosterhuis, unpublished). Using a leaf area basis for water flow biases somewhat against larger plants because these plants absorb less radiation per unit leaf area, and larger plants would be expected to transpire less water on a leaf area basis. Because the control plants were generally larger than the nematode-infected plants, this method would be expected to bias against the control plants. However, leaf area allows a better comparison between treatments than the use of uncorrected values, which would be influenced greatly by plant size. Statistical comparisons were made for maximum stem water fluxes observed during a sampling period and for the cumulative stem water fluxes for the 24-hour period.

Analysis of variance was conducted using SAS (SAS Institute, Raleigh, NC) to evaluate the effects of treatments on plant responses. Means were compared using Fisher's protected LSD. Because environmental conditions were dramatically different during the 1992 and 1993 growing seasons and cotton growth patterns were considerably different each year, data are presented by individual years.

Results

Nematode population densities increased on cotton in both 1992 and 1993 (Table 1). Application of aldicarb did not decrease either the number of *M. incognita* J2 found in soil or the number of eggs recovered from roots at mid-season or at harvest in either year. Midseason juvenile and egg densities were much higher in 1993 when samples were collected at 93 DAP rather than at 51 DAP, as was the case in 1992. Severe root galling was found at harvest in both 1992 and 1993 (data not shown), and final soil densities of eggs and J2 were comparable both years. No nematodes were found in the noninfested control plots.

TABLE 1. Densities of *Meloidogyne incognita* (MI) juveniles (J2) and eggs at 51 and 74 days after planting (DAP) in 1992 and at 93 and 175 DAP in 1993 in microplots infested with *M. incognita*.

	1992				1993			
	J2 (DAP)		Eggs (DAP)		J2 (DAP)		Eggs (DAP)	
Treatment[a]	51	174	51	174	93	175	93	175
MI	180 a	2,304 a	4,452 a	10,180 a	227 a	5,495 a	18,064 a	7,163 a
MI + Aldicarb	127 a	2,407 a	7,568 a	6,500 a	178 a	4,480 a	14,047 a	5,524 a
No MI	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b

Data are means of 16 replications. Means within columns followed by the same letter do not differ at $P = 0.05$ by Fisher's PLSD.

[a] Treatments were: 1) *M. incognita* eggs and juveniles (8.5×10^5) added to microplots; 2) *M. incognita* eggs and juveniles (8.5×10^5) + 1.7 kg/ha aldicarb added to microplots; and 3) control with no nematodes or aldicarb.

Plants were taller in aldicarb-treated plots both at mid-season and at harvest in 1992 but not in 1993 ($P = 0.05$) (Table 2). In 1992, control plants and those in plots infested with *M. incognita* without aldicarb were comparable in height at mid-season, although nematode infection resulted in shorter plants at the end of the season. The negative effect of *M. incognita* on final plant height was more pronounced in 1993, and the beneficial effect of aldicarb was not as great.

Although reliable yield data were not available for the 1992 season, seed cotton yield in 1993 was lowest where *M. incognita* was not subjected to aldicarb ($P = 0.05$) (Table 2). Application of the nematode resulted in greater seed cotton yield (334 g/plot) but did not result in yields that were comparable to the uninfested control plots (480 g/plot).

At 89 DAP in 1992, the stomatal conductance and leaf transpiration rate of nematode-infected plants were lower than that of uninfected plants, while the leaf temperature was higher in nematode-infected plants than control plants (Table 3). The application of aldicarb to infested plots did not affect stomatal conductance, transpiration, or leaf temperature in 1992.

Nematode effect on cotton leaf conductance and transpiration was not apparent early in the 1993 growing season. Stomatal conductance, transpiration, and leaf temperature were similar in all three treatments at 58 DAP (Table 3). However, by 108 DAP, plants infected with nematodes had lower stomatal conductance and leaf transpiration rates and a higher leaf temperature than the control plants. Aldicarb tended to decrease, but did not eliminate, the effects of the nematodes.

Leaf water potential and pressure potential were not significantly affected by any of the treatments in either year. Infection did result in a decrease in osmotic po-

TABLE 2. Cotton plant height (cm) in 1992 and 1993 at mid-season (Hm) and harvest (Hf) and 1993 seed cotton yield (g/plot) in microplots infested with *Meloidogyne incognita* (MI).

	1992		1993		
Treatment[a]	Hm	Hf	Hm	Hf	Yield (g/plot)
MI	71.1 b	80.5 c	38.6 c	59.4 c	38.4 c
MI + Aldicarb	93.5 a	105.9 a	68.3 b	88.6 b	333.7 b
No MI	69.1 b	99.3 b	78.4 a	98.5 a	480.4 a

Data are means of 16 replications. Means within columns followed by the same letter do not differ at $P = 0.05$ by Fisher's PLSD.

[a] Treatments were: 1) *M. incognita* eggs and juveniles (8.5×10^5) added to microplots; 2) *M. incognita* eggs and juveniles (8.5×10^5) + 1.7 kg/ha aldicarb added to microplots; and 3) control with no nematodes or aldicarb. Hm = height in cm 85 days after planting (DAP) in 1992 and 86 DAP in 1993; Hf = height in cm 174 DAP in 1992 and 175 DAP in 1993. Yield was not recorded in 1992.

TABLE 3. *Meloidogyne incognita* (MI) and water relations of cotton in microplots at different days after planting (DAP).

Treatment[a]	Stomatal conductance (sec · cm^{-1})	Transpiration rate (μg · cm^{-2} · sec^{-1})	Leaf temperature (C)	Leaf potential		
				Water	Osmotic (Mpa)	Pressure
		89 DAP (1992)				
MI	1.43 b	17.21 b	32.1 a	−1.14 a	−1.65 b	0.53 a
MI + Aldicarb	1.48 b	18.41 b	32.1 a	−1.14 a	−1.61 b	0.49 a
No MI	2.13 a	22.96 a	31.1 b	−0.91 a	−1.44 a	0.54 a
		58 DAP (1993)				
MI	0.70 a	10.11 a	32.8 a	−1.03 a	−1.35 b	0.31 a
MI + Aldicarb	0.85 a	11.23 a	32.2 a	−0.98 a	−1.23 a	0.25 a
No MI	1.02 a	12.52 a	31.9 a	−1.00 a	−1.24 a	0.25 a
		108 DAP (1993)				
MI	0.43 b	10.10 b	37.8 a	−1.49 a	−1.75 a	0.26 a
MI + Aldicarb	0.61 b	13.26 ab	37.0 ab	−1.19 a	−1.53 a	0.34 a
No MI	0.87 a	16.82 a	36.1 b	−1.19 a	−1.52 a	0.33 a

Data are means of 16 replications. Means for the same DAP within columns do not differ at $P = 0.05$ by Fisher's PLSD.
[a] Treatments were: 1) *M. incognita* eggs and juveniles (8.5×10^5) added to microplots; 2) *M. incognita* eggs and juveniles (8.5×10^5) + 1.7 kg/ha aldicarb added to microplots; and 3) control with no nematodes or aldicarb.

tential. The application of aldicarb negated the nematode effects on osmotic potential in 1993 but not in 1992 (Table 3).

In general, the stem water flux through intact plants per unit leaf area was lower in nematode infected plants than in controls in both 1992 and 1993 (Table 4, Fig. 1). Differences between treatments were especially obvious during the period of high evaporative demand. The fluctuations in stem water flux were closely correlated to changes in light intensity (data not shown). Because of the limited number of replications, 1992 treatment effects on the cumulative stem water flux were significant only at $P = 0.10$, whereas the maximum stem water fluxes were not significantly different. In 1993 only the treatment effects found at 58 DAP were significant.

DISCUSSION

Symptoms of *M. incognita* infection in cotton may include stunting and a tendency for the plants to wilt temporarily during the heat of the day when evaporative demand is at a maximum. In greenhouse studies, when soil water was maintained at field capacity, *M. incognita acrita*-infected cv. Pima S-2 cotton seedlings grew at approximately the same rate as uninfected plants, and water consumption was similar (8). However, when soil water was allowed to fall below field capacity for periods during the study, plant growth was slowed, and water consumption was lower in infected plants than in uninfected controls. In our study, soil moisture was allowed to decrease to approximately −30

TABLE 4. Maximum stem water flux and cumulative stem water flow per unit leaf area over an entire day through intact cotton plants in microplots at different days after planting (DAP) in 1993 as influenced by *Meloidogyne incognita* (MI).

Treatment	Maximum stem water flux[a]		Cumulative stem water flux	
	58 DAP	108 DAP	58 DAP	108 DAP
MI	0.23 b	0.34 a	2.16 b	1.86 a
No MI	0.54 a	0.41 a	3.99 a	3.22 a

Data are means of 16 replications. Means for the same DAP within a column do not differ at $P = 0.05$ by Fisher's PLSD.
[a] Stem water flux is measured in liter/hour/meter^2. Cumulative stem water flux is measured in liter/meter^2.

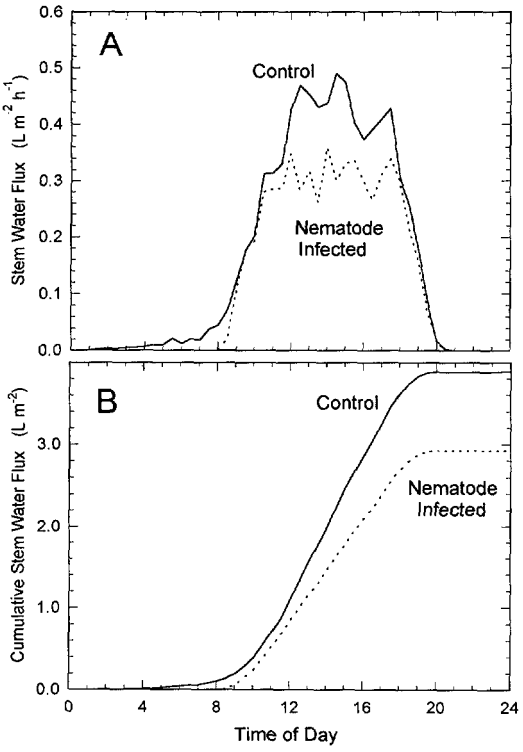

FIG. 1. Stem water flux (A) and cumulative water flux (B) through *Meloidogyne incognita*-infected and control plants at 89 days after planting in 1992. The data are expressed on a leaf-area basis to correct for differences in plant size.

kPa before irrigation was applied to return the plots to field capacity. This approximates the situation that would be expected in the field where soil moisture levels fluctuate according to rain or irrigation events. Our results appear to be consistent with those of earlier studies: Nematode infection resulted in more pronounced symptoms of drought stress than in uninfected control plants, particularly during the latter part of the growing season.

The 1992 and 1993 growing seasons in southwestern Arkansas were considerably different in terms of ambient temperature and rainfall. Average ambient air temperature in 1992 (June–September) was approximately 24.4 C, and rainfall during this period was relatively abundant (50.44 cm). In contrast, the 1993 average ambient temperature during June–September was 25.9 C, and total accumulated rainfall was 21.23 cm. The substantially lower midseason and harvest heights for cotton plants in 1993 likely reflect the impact of severe water and temperature stress in addition to nematode effects on plant growth.

Nematode infection decreased stomatal conductance, which resulted in decreased leaf transpiration rates. Because of the lower leaf transpiration rates, evaporative cooling of the leaves decreased with a concomitant increase in leaf temperature of infected plants. Because of increased leaf temperatures, infected plants may have experienced greater environmental stress than control plants during the growing season, particularly during the hot summer of 1993.

The decrease in leaf transpiration rate also was reflected in stem water flux. The lower stem water flux of *M. incognita*-infected plants during periods of high evaporative demand suggests that the hydraulic resistance was substantially greater in infected plants than in control plants. A decrease in root hydraulic conductance as a result of nematode infection has been reported in tomato (5) and bean (11). This would be a logical effect of the disruption of the xylem in nematode-infected roots (9) and corroborates our previous findings that nematode infection can decrease the water flow through intact root systems (4). Decreased leaf osmotic potential in nematode-infected plants may have been a result of water deficits in the leaves. Cotton has been shown to adapt to water deficits by accumulating solutes (6) to help plants maintain turgor.

Many of the effects seen in this study tended to be more severe in plants during the latter part of the growing season. Increased demand for water during fruiting, as well as greater environmental stress, appear to amplify the effects of *M. incognita.* Greater root infection because of increasing nematode populations during the growing season may have enhanced root damage and subsequent effects. *Meloidogyne incognita* greatly influences the water relations of cotton and results in greater

plant stress in addition to direct damage to the root system. This stress is reflected in the growth and yield of the plant and should be considered in both irrigated and nonirrigated cotton production systems.

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