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Interaction of *Meloidogyne incognita* and Water Stress in Two Cotton Cultivars[1]

T. L. KIRKPATRICK,[2] D. M. OOSTERHUIS,[3] AND S. D. WULLSCHLEGER[3]

Abstract: A series of controlled-environment experiments were conducted to elucidate the effects of *Meloidogyne incognita* on host physiology and plant–water relations of two cotton (*Gossypium hirsutum*) cultivars that differed in their susceptibility to nematode infection. Inoculation of *M. incognita*-resistant cultivar Auburn 634 did not affect growth, stomatal resistance, or components of plant-water potential relative to uninoculated controls. However, nematode infection of the susceptible cultivar Stoneville 506 greatly suppressed water flow through intact roots. This inhibition exceeded 28% on a root-length basis and was similar to that observed as a consequence of severe water stress in a high evaporative demand environment. Nematodes did not affect the components of leaf water potential, stomatal resistance, transpiration, or leaf temperature. However, these factors were affected by the interaction of *M. incognita* and water stress. Our results indicate that *M. incognita* infection may alter host-plant water balance and may be a significant factor in early-season stress on cotton seedlings.

Key words: cotton, *Gossypium hirsutum*, *Meloidogyne incognita*, nematode, root-knot nematode, water stress.

Root-knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood, are major parasites of cotton (*Gossypium hirsutum* L.) and cause significant yield losses both directly (5) and indirectly through interactions with soilborne fungal pathogens (12). Symptoms of nematode infection include host-plant stunting and yield suppression. Although cotton cultivars differ in their resonse to *M. incognita* (4), only moderate levels of resistance have been incorporated into commercial cultivars (13). The presence of *M. incognita* in cotton fields constitutes a serious limitation to effective crop management and optimum yields.

Anatomical changes in roots infected by *Meloidogyne* spp. include disruption of the xylem, root epidermis, and cortical tissues in response to giant-cell development and gall formation (1,6,14). Root damage and dysfunction due to alterations in root anatomy may affect host-plant water relations and retard plant growth and development (17). Plants infected with *M. incognita* often show symptoms of water deficit stress, particularly under field conditions (8).

Few studies have included measurement of host physiology and water relations of plants infected by *Meloidogyne* spp. Both *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood decreased leaf-water potentials in tomato (6) and bean seedlings (15,16). Stomatal conductance decreased more rapidly during an 8-week period in *M. javanica*-infected tomato plants than in healthy plants (6). In contrast, tobacco infected by either *M. hapla* or *M. incognita* had higher transpiration during the first 8 weeks after inoculation than did uninfected plants (9). Similarly, water consumption by cotton infected by *M. incognita* "*acrita*" was greater than in uninfected plants over a 10-week period (8).

Water flux through roots of infected plants also appears to be suppressed by nematode infection. In both *M. hapla*-infected beans and *M. javanica*-infected tomato plants, root hydraulic conductivity was lower than in healthy plants (6,15). The effects of nematode parasitism on plant–water relations remain unclear. Because nematode infection may damage the water-conducting capacity of young roots, comparison of water movement through infected and uninfected roots should help quantify effects of these pathogens on

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[2] Associate Professor, Department of Plant Pathology, University of Arkansas Southwest Research and Extension Center, Hope, AR 71801.

[3] Associate Professor and Research Assistant, Department of Agronomy, University of Arkansas, Fayetteville, AR 72701.

plant–water relations. The objectives of this study were 1) to quantify the effects of nematode infection on the shoot–water relations and root hydraulic conductivity of seedling cotton and 2) to compare these responses in cotton cultivars resistant and susceptible to root-knot nematodes.

Materials and Methods

Growth conditions: Seeds of cotton cultivars Stoneville 506 (susceptible to *M. incognita*) and Auburn 634 (resistant to *M. incognita*) were sown in 0.25-liter plastic containers filled with steam-pasteurized sand. The containers were placed in a controlled-environment chamber, and seeds were allowed to germinate and grow under conditions maintained at a 14-hour photoperiod with an average day–night temperature of 31–24 ± 2 C and a relative humidity of 40%. Irradiance was supplied by overhead fluorescent tubes and 60-W incandescent bulbs which provided a photosynthetic photon flux of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$, as measured with a LI-190S quantum sensor (Li-COR, Lincoln, NE). The experimental design was a randomized complete block with four replicates. Data were subjected to analysis of variance, and significant means were separated with Fisher's LSD ($P = 0.05$).

Nematode inoculation and analysis: Three weeks after planting, eight seedlings of each cotton cultivar were inoculated by pipetting 10 ml water containing 1.2×10^4 eggs of *M. incognita* onto the soil surface around each seedling. An additional 50 ml water was applied immediately following inoculation to help move the eggs into the soil. The nematode population used in this study was *M. incognita* race 3 originally isolated from cotton in Lafayette County, Arkansas, and increased on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) for 60 days in the greenhouse. Eggs for inoculum were collected from the tomato roots by the NaOCl method (3) and quantified. Control cotton seedlings of each cultivar received 60 ml water without *M. incognita,* applied in the same manner as with inoculated plants.

Two weeks after inoculation, four cotton plants in each treatment were sacrificed to verify the presence of nematodes in roots. The root system of each seedling was washed free of soil, weighed, and cut into 1–2-cm segments. A random sample (0.5 g fresh weight) from each root system was agitated for 4 minutes in 1.5% NaOCl and rinsed in running tap water for approximately 5 minutes. Root samples were then heated to boiling in 30 ml water to which 1 ml acid fuchsin stain (3.5 g acid fuchsin, 250 ml acetic acid, 750 ml distilled water) had been added (2). Samples were allowed to cool to room temperature, and the stain was rinsed from the roots in running water. The samples were then placed in 20 ml glycerin and heated to destain root tissue. After destaining, root pieces were pressed between glass plates, and nematodes were quantified at 40× with a dissecting microscope.

Four weeks after inoculation, following physiological measurements, remaining plants were removed from their containers, and the roots were washed and stained with Phloxine B (0.15 g/liter tap water) for 15 minutes to increase visibility of egg masses. Root systems were rated for egg masses according to the following scale: 0 = no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = > 100 egg masses per root system. Each root system was then cut into 1–2-cm segments, and the segments were comminuted in a Waring blender with 200 ml water for 1 minute. The roots were collected in a 500-ml container and extracted with NaOCl as described. Following extraction *M. incognita* eggs were quantified at 40× with a dissecting microscope.

Physiological measurements: All plants were watered 3 or 4 times daily with half-strength Hoagland's solution to provide adequate soil moisture for vigorous plant growth. At approximately 4 weeks after inoculation, half of the plants of each cultivar were water stressed by withholding water from the pots for 4–5 days until stomatal closure and wilting occurred. The adequately watered control plants were watered with deionized

TABLE 1. Infection and reproduction of *Meloidogyne incognita* in water-stressed and adequately watered cotton plants.

Cultivar	Treatment	Juveniles/g root†	Egg mass rating‡	Eggs/root system§
Stoneville 506	Infected			
	Adequately watered	340	2.3	5,470
	Water-stressed	370	4.0	6,770
	Control	0	0	0
Auburn 634	Infected			
	Adequately watered	250	0	210
	Water-stressed	380	0	290
	Control	0	0	0
LSD cultivar (C)		NS	0.4	1,991
LSD nematode (N)		62	0.4	1,991
LSD C × N		NS	0.5	995
LSD C × water (W)		NS	0.5	NS
LSD C × N × W		NS	0.7	NS
CV (%)		52	44	76

† At 2 weeks after inoculation.
‡ Ratings made 4 weeks after inoculation according to a 0–5 scale where 0 = no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–20, 4 = 31–100, and 5 = > 100 egg masses/root system.
§ At 4 weeks after inoculation.

water during the water-stress period. The severity of the water stress was monitored by measuring leaf water potential, stomatal resistance, transpiration, and leaf temperature. The remaining plants received adequate water to maintain vigorous growth. No water-stress symptoms were observed in these plants. Measurements of plant growth and water relations were recorded at 7 weeks after planting. Measurements of leaf water potential were made with thermocouple psychrometers (Model 84, JDR Merrill Specialty Equipment, Logan, UT) that used a single 0.9-cm disk taken from the uppermost fully expanded leaf (11). Stomatal resistance to water vapor diffusion, transpiration, and leaf temperature were determined with a LI-1600 steady state porometer (LI-COR).

Measurements of root water flux were determined with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR) to apply a 0.2-MPa hydrostatic pressure to detopped root systems (10). Flux measurements represented those of an intact root system in soil that had been saturated to field capacity prior to measurement. After an initial equilibration period of 15 minutes, water flux from the intact root system was determined by collecting the exudate onto filter paper enclosed in tygon tubing for a 5-minute period. A moistened piece of filter paper was placed over the tygon tube at all times to prevent evaporative losses. Following the flux measurements, roots were washed and root length was determined (7). All experiments were repeated once.

RESULTS

Meloidogyne incognita pre-adults were found in roots of all inoculated plants 2 weeks after inoculation (Table 1). At 4 weeks after inoculation, egg masses were present on Stoneville 506 roots, but none were found on Auburn 634 roots. More egg masses were visible in roots of Stoneville 506 plants subjected to water stess than in adequately watered plants, and fewer eggs were recovered from roots of these plants. Very low numbers of eggs were detected on roots of infected Auburn 634 plants in both water treatments.

Cultivar differences in leaf surface area, plant weight, and root length were evident (Table 2). Stoneville 506 produced a greater leaf surface area and had higher plant dry weights than did Auburn 634, whereas the latter exhibited greater root length. However, these differences were not re-

TABLE 2. Effects of nematode infection on the growth of two cultivars of cotton differing in their susceptibility to *Meloidogyne incognita*.

Cultivar	Treatment	Leaf area (cm^2)	Dry weight (g)	Root length (cm)
Stoneville 506	Control			
	Adequately watered	183.9	1.833	1,067
	Stressed	155.8	1.529	1,045
	Infected			
	Adequately watered	187.3	1.903	1,120
	Stressed	204.6	2.036	1,126
Auburn 634	Control			
	Adequately watered	144.4	1.554	1,265
	Stressed	119.8	1.106	1,218
	Infected			
	Adequately watered	138.2	1.398	1,218
	Stressed	117.6	1.357	1,222
LSD Cultivar		24.7	0.178	72
CV (%)		22	15	9

lated to infection by *M. incognita*. Parasitism by *M. incognita* did not affect ($P = 0.05$) any of the growth parameters measured for either cultivar.

Water stress increased ($P = 0.05$) leaf stomatal resistance and leaf temperature and decreased transpiration (Table 3). The interaction of nematodes and water stress affected stomatal resistance but not transpiration or leaf temperature. Water stress and the interactive effects of water stress × cultivar × nematode infection also were associated with restricted ($P = 0.05$) leaf water potential and solute potential. Only

TABLE 3. Influence of nematode infection on the stomatal resistance, transpiration, and leaf temperature of two cultivars of cotton differing in their susceptibility to *Meloidogyne incognita*.

Cultivar	Treatment	Stomatal resistance ($s \cdot cm^{-1}$)	Transpiration ($\mu g \cdot cm^{-2} \cdot s^{-1}$)	Temperature (C)
Stoneville 506	Control			
	Adequately watered	7.5	3.0	28.1
	Stressed	23.0	0.8	28.6
	Infected			
	Adequately watered	7.8	2.6	28.2
	Stressed	28.0	0.7	28.6
Auburn 634	Control			
	Adequately watered	6.6	2.9	27.3
	Stressed	28.0	0.8	28.2
	Infected			
	Adequately watered	4.7	3.8	26.7
	Stressed	31.4	0.7	28.1
LSD cultivar (C)		NS	NS	0.2
LSD nematode (N)		NS	NS	NS
LSD water (W)		2.2	0.3	0.2
LSD C × N		NS	0.7	NS
LSD C × W		4.4	NS	0.4
LSD N × W		4.4	NS	NS
CV (%)		18	24	1

TABLE 4. Effects of nematode infection on the components of leaf water potential and root water flux of two cultivars of cotton differing in their susceptibility to *Meloidogyne incognita*.

Cultivar	Treatment	Leaf water potential (MPa)	Solute potential (MPa)	Pressure potential (MPa)	Flux ($\mu l \cdot min^{-1}$)	Flux density ($\mu l \cdot m^{-1} \cdot min^{-1} \cdot MPa^{-1}$)
Stoneville 506	Control					
	Adequately watered	−1.03	−1.54	0.51	10.6	1.00
	Stressed	−1.77	−2.06	0.30	3.7	0.38
	Infected					
	Adequately watered	−1.12	−1.59	0.47	5.2	0.46
	Stressed	−1.64	−1.99	0.35	4.0	0.35
Auburn 634	Control					
	Adequately watered	−1.07	−1.57	0.51	9.1	0.72
	Stressed	−1.74	−2.04	0.30	5.2	0.42
	Infected					
	Adequately watered	−0.84	−1.34	0.50	9.9	0.82
	Stressed	−1.72	−2.02	0.30	4.8	0.40
LSD cultivar (C)		NS	NS	NS	0.8	NS
LSD nematode (N)		NS	NS	NS	0.8	0.09
LSD water (W)		0.08	0.08	0.8	0.8	0.09
LSD C × N		NS	NS	NS	1.5	0.17
LSD N × W		NS	NS	NS	1.5	0.17
LSD C × N × W		0.17	0.18	NS	2.2	0.24
CV (%)		8	5	27	16	20

water stress decreased leaf pressure potential for both cultivars (Table 4). The decrease in components of water potential was slightly greater in Auburn 634 than in Stoneville 506.

Water stress decreased root water flux and flux density in both cotton cultivars, but *M. incognita* depressed these only in Stoneville 506 (Table 4). The interaction of cultivar × water did not affect flux or flux density, but the interaction of nematodes with both cultivar and water stress as well as the interaction of all three factors was significant for both parameters. Flux and flux density tended to be decreased to a greater degree in Stoneville 506 than in Auburn 634. Nematode infection decreased root flux in the adequately watered Stoneville 506 plants to approximately the same level as that recorded for water-stressed plants. This decrease did not occur in inoculated, adequately watered Auburn 634 plants. Infection of either cultivar did not contribute additional decreases in flux in water-stressed plants.

DISCUSSION

Decreases in water movement from roots through the plant may contribute to seedling water stress and limit nutrient movement in plants. Currently there is little information on the effects of nematode parasitism on water uptake or movement in cotton. Our data indicate that in susceptible cultivars, infection by *M. incognita* may decrease the movement of water from roots to leaves. Under the conditions used in this study, the decrease in root flux due to nematode infection was equivalent to decreases seen following a severe water-deficit stress. With *M. incognita*-resistant Auburn 634, nematode infection did not affect water flux.

The mechanisms by which *M. incognita* alters water movement through the plant have not been determined. Apparently nematode entry into roots does not affect water flux. Roots examined at 2 weeks after inoculation indicated that approximately equal numbers of *M. incognita* ju-

veniles were present in roots of both Stoneville 506 and Auburn 634; however, a much greater proportion of these nematodes in Stoneville 506 developed into mature females and produced eggs. The ability of *M. incognita* juveniles to enter roots and the failure of most individuals to develop into egg-laying adult females have been demonstrated in both Auburn 634 and in its parent line, Auburn 623 (14). Our results indicate that nematode development, probably with resulting vascular disruption in the root due to giant-cell formation, may be required for decreases in water movement within the plant.

Effects of nematode infection on water flux were not apparent in plants subjected to water-deficit stress. In these plants, the severity of the water stress may have masked effects due to nematodes. Evaluation of nematode-induced changes in plant–water relations under conditions where water-deficit stress is less severe but of longer duration may prove instructive. It is difficult, however, to establish these conditions in growth chambers where both space and soil volume for root development are limited. Longer duration studies, either in the field or in microplots, may allow measurement of nematode contribution to overall drought stress under more realistic conditions. Our data indicate that infection of susceptible cotton by *M. incognita* affects water movement in seedlings and young plants. In the field, population densities of this pathogen increase on susceptible cultivars throughout the growing season. The related effects on seedlings should be amplified as numbers of nematodes parasitizing the roots increase. Field studies will be necessary to determine the significance of these effects on mature plants and their relationship to cotton yield and quality.

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