Effects of Soil Temperatures and Inoculum Levels of *Meloidogyne incognita* and *Rhizoctonia solani* on Seedling Disease of Cotton

WILLIAM W. CARTER[1]

Abstract: Soreshin of cotton was more severe from combined infections of *Rhizoctonia solani* and *Meloidogyne incognita* than from either organism alone, when both critical soil temperature and inoculum concentrations were present. Optimum soil temperatures for disease development from combined infections were 18 and 21 C. Either 2,500 or 5,000 *M. incognita* larvae per plant, combined with *R. solani*, increased seedling disease severity over that caused by *R. solani* alone. When 100 or 500 larvae per plant were added with *R. solani*, disease severity did not change. Disease severity increased with the highest level of *R. solani* inoculum either alone or combined with *M. incognita*. *Key Words*: interaction, nematode, fungus, *Gossypium hirsutum*.

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[1]Nematologist, Agricultural Research Service, United States Department of Agriculture, National Cotton Pathology Research Laboratory, P. O. Drawer JF, College Station, Texas 77840. I thank Santos Nieto, Jr., for technical assistance.

The importance of nematodes in soreshin of cotton seedlings was first indicated when Reynolds and Hanson (17) observed that *Rhizoctonia solani* Kühn caused minor

damage to cotton seedlings in fields fumigated for control of *Meloidogyne incognita* (Kofoid and White) Chitwood. Also, a high percentage of plants was infected by *R. solani* when *M. incognita* was added to soil simultaneously or after the fungus was added. Cauquil and Shepherd (4) showed that the disease caused by *M. incognita* and *R. solani* was more severe than that from either pathogen alone. Disease was severe from high inoculum levels of *R. solani*, even without nematodes. The presence of other nematodes, *M. arenaria* Chitwood, *M. hapla* Chitwood, *Rotylenchulus reniformis* Linford and Oliveria, or *Hoplolaimus tylenchiformis* Daday, also increased the severity of *R. solani* on cotton (2). Other cotton diseases caused by *Pythium debaryanum* Hesse (2, 12), *Fusarium oxysporum* (Atk.) Syn. and Hans. (12), or *Thielaviopsis basicola* (Berk. and Br.) Ferr. (18) were increased when *M. incognita* was also present.

Previous studies of the seedling disease caused by *M. incognita* and *R. solani* did not explain the interactions of soil temperature, texture and moisture, and nematode numbers with fungal inoculum concentration. Norton (12) noted that temperature was important for full expression of symptoms, but he did not determine if nematode numbers were critical.

This paper examines the interactions of temperature and inoculum concentrations of *M. incognita* and *R. solani* in soreshin of cotton. An abstract of part of this study has been published (3).

MATERIALS AND METHODS

Organisms: *Gossypium hirsutum* L. 'Deltapine 16,' susceptible to both *M. incognita* and *R. solani*, was used as the host. A single-basidiospore isolate of *R. solani* [*Thanatephorus cucumeris* (Frank) Donk], with intermediate virulence to the cotton, was obtained by the method of Flentje and Stretton (7) and maintained on potato-dextrose yeast extract agar. The fungus was grown 7-9 days on steam-sterilized grain sorghum, *Sorghum bicolor* (L.) Moench, and an infected kernel of sorghum constituted one unit of inoculum (2).

M. incognita, isolated from infected cotton at Maricopa, Arizona, was maintained in the greenhouse on *Sesbania exaltata* (Raf.) Cory or *Lycopersicon esculentum* Mill. Nematodes were harvested by the method of Lownsbery and Viglierchio (11). Collected at 24-h intervals, larvae were surface sterilized in an aqueous solution of 133 μg/ml ethoxyethyl-mercuric chloride and 150μg/ml dihydro-streptomycin sulfate for 1 h (13). The larval suspension was centrifuged at 1,000 *g* for 3 minutes, and the pellet was washed three times by resuspension in sterile distilled water and centrifugation.

Soil mix: Soil from the University of Arizona Cotton Research Center, Phoenix, was adjusted to 87% sand content with 246-μm (60-mesh) silica sand. Analyses of the soil mix by Day's modification of the Bouyoucos method (6) showed 87% sand, 3% coarse silt, 2.5% silt, and 7.5% clay. Soil moisture-holding capacity (MHC) was 8.7%, determined by the method of Olmstead (14). Other physical properties were bulk density, 1.46 g/cc; percentage of pore space, 41.56; and particle density, 2.51 g/cc. The soil was leached with distilled water and air-dried at 80 C for 10-12 h before use. Final pH of the soil ranged from 7.2 to 7.6.

Inoculation techniques: Closed-bottom plastic containers, 5.5-cm in diam and 20 cm deep, were filled to within 4 cm of the top with dry soil and weighed. Four units of fungal inoculum were placed one at each of the apices of a centered 4-cm equilateral triangle and one unit in the center, all 2 cm below the soil surface in each container. Five units were placed the same as three units, with the two extra units in the center. Soils were adjusted be weight to 90% MHC with half-strength Hoagland's nutrient solution (8). Containers then were placed in constant-temperature water tanks at designated temperatures $\pm$ 1 C for 96 h, to allow the fungus to infest the soil before the cottonseeds were planted. Containers which did not receive the fungus were otherwise treated the same. To keep soil moisture at 90% MHC, containers were weighed daily and distilled water was added as needed.

Seeds were immersed in water at 80 C for 60 sec, soaked in 0.5% $NaHClO_3$ for 20 min, and rinsed three times with sterile distilled water. They germinated on moist, sterile filter paper in petri dishes at 28 C. Seeds whose radicles had just emerged from the micropyle after 18 h incubation were selected from dishes visibly free of contaminating microorganisms. Three seeds were placed in each soil container in a triangle, 25 mm below the soil surface between three units of fungal inoculum, when these were present. Water suspensions of 0, 100,

500, or 1,000 larvae of *M. incognita*/ml were prepared, and 5 ml were pipetted into the soil around each newly germinated seed and covered with soil.

Disease evaluation: Evaluation criteria were rate of seedling emergence, numbers of emerged and surviving seedlings, seedling fresh weight, *R. solani*-disease index, and relative abundance of nematode galls on roots of 21-day-old seedlings. The *R. solani* disease index values were 0 = no infection; 1 = yellowing of hypocotyl; 2 = lesion(s), but no girdling of hypocotyl; 3 = lesion(s), complete girdling; and 4 = dead plant. To confirm the presence of the fungus in lesions, bits of this tissue were washed in sterile distilled water and plated on water agar. All treatments were replicated eight times, and each experiment was repeated three times. Combined results were analyzed by Duncan's multiple-range test ($P = 0.01$, or 0.05).

RESULTS

The optimum soil temperature for emergence of cotton seedlings was 27 C. At 15, 18, 21, 24, 27, and 30 C emergence was maximum by 13, 9, 8, 6, 5, and 6 days, respectively. The rate of seedling emergence was not ($P = 0.05$) affected by *M. incognita*, *R. solani*, or the two combined.

Survival of cotton seedlings was ($P = 0.01$) lower when 2,500 or 5,000 larvae of *M. incognita* were combined with three units of *R. solani* at 18 or 21 C, but not when 100 or 500 larvae were applied. Results varied at 15 C. Nematodes had no synergistic effect at 24, 27, or 30 C (Table 1). All levels of *R. solani* combined with 2,500 *M. incognita* per seedling ($P = 0.01$) reduced survival at 21 C, but five units of fungus caused slightly more disease than one or three units (Table 2). *M. incognita* or *R. solani* alone did not affect cotton seedling survival, but 2,500 or 5,000 nematode larvae per plant stunted seedlings at 21 C. *R. solani* (three units) plus *M. incognita* (2,500 or 5,000 larvae/plant) also ($P = 0.05$) stunted surviving cotton seedlings at soil temperatures of 18 and 21 C (Table 3). No

TABLE 1. Effects of soil temperature and inoculum levels of *Meloidogyne incognita* on survival of cotton seedlings grown for 21 days in *Rhizoctonia solani*-infested soil.

Inoculation treatments[y]	Seedlings surviving/24 planted/ soil temperature (C)[z]			
	15	18	21	24
Noninoculated	24e	24e	24e	24e
R. solani	24e	22cde	22cde	24e
R. solani + *M. incognita* 100	20abc	20abc	21bcd	24e
R. solani + *M. incognita* 500	23de	21bcd	21bcd	24e
R. solani + *M. incognita* 2,500	21bcd	18a	19ab	22cde
R. solani + *M. incognita* 5,000	22cde	19ab	19ab	23de

[y]Number of *M. incognita* larvae per seedling is indicated; three units of *R. solani* were used for fungal treatments.
[z]Values followed by the same letter do not differ at $P = 0.01$, refers to all values in table.

TABLE 2. Effects of inoculum levels of *Rhizoctonia solani* and *Meloidogyne incognita* on disease incidence in and survival of cotton seedlings.

Inoculation treatments	Seedlings survived/ 24 planted	*R. solani* disease index[w]	*M. incognita* galling index[x]
Noninoculated control	24c[y]	...	...
R. solani, 1 unit	24c	1.4a[y]	...
R. solani, 3 units	23c	1.4a	...
R. solani, 5 units	24c	1.7b	...
M. incognita[z]	24c	...	3.8a[y]
R. solani, 1 unit + *M. incognita*	20ab	2.5c	3.6a
R. solani, 3 units + *M. incognita*	21b	2.5c	3.8a
R. solani, 5 units + *M. incognita*	19a	2.7d	3.5a

[w]Based on a rating of 0 = no infection to 4 = dead plant.
[x]Based on a rating of 0 = no infection to 4 = heavy galling.
[y]Values followed by the same letter do not differ at $P = 0.01$.
[z]Nematodes at 2,500/seedling were used for all *M. incognita* treatments.

TABLE 3. Effects of soil temperature and inoculum concentration of *Meloidogyne incognita* on the growth of surviving cotton seedlings in soil infested with *Rhizoctonia solani*.

Inoculation treatments[y]	Soil temperature (C)			
	15	18	21	24
	grams/seedling:[z]			
Noninoculated	0.59ab	1.00def	2.16ijk	2.36k-n
M. incognita, 100	0.59ab	1.12ef	2.27i-l	2.32i-m
M. incognita, 500	0.64ab	1.21f	2.10i	2.46l-q
M. incognita, 2,500	0.67ab	0.97de	1.43g	2.24i-l
M. incognita, 5,000	0.67ab	0.93cde	1.47g	2.17ijk
R. solani	0.60ab	1.00def	1.70h	2.23i-l
R. solani + *M. incognita*, 100	0.54a	0.98de	1.80h	2.34k-m
R. solani + *M. incognita*, 500	0.60ab	1.06ef	2.13ij	2.35k-n
R. solani + *M. incognita*, 2,500	0.56a	0.75abc	1.09ef	2.33j-m
R. solani + *M. incognita*, 5,000	0.58ab	0.80bcd	1.02def	2.27i-l

[y]Number of *M. incognita* larvae per seedling is indicated; three units of *R. solani* were used for fungal treatments. Seedlings grown for 21 days.
[z]Values followed by the same letter do not differ at $P = 0.05$, refers to all values in table.

TABLE 4. Effects of soil temperature and inoculum concentration of *Meloidogyne incognita* on the *Rhizoctonia solani* disease indices of cotton seedlings grown for 21 days in *R. solani*-infested soil.

Inoculation treatments[y]	Soil temperature (C)					
	15	18	21	24	27	30
	disease index:[z]					
R. solani	0.67ab	1.17bcd	1.08a-d	1.00a-d	0.83abc	0.58a
R. solani + *M. incognita*, 100	1.14bcd	1.29cde	1.04a-d	1.08a-d	1.04a-d	0.83abc
R. solani + *M. incognita*, 500	1.13bcd	1.29cde	1.42def	1.33c-f	1.29cde	1.04a-d
R. solani + *M. incognita*, 2,500	1.86fgh	2.25ghi	2.46i	2.09ghi	1.79e-h	1.36c-f
R. solani + *M. incognita*, 5,000	1.75efg	2.30hi	2.42i	2.00ghi	1.80e-h	1.27cde

[y]Number of *M. incognita* larvae per seedling is indicated; three units of *R. solani* were used for fungal treatments.
[z]Based on a rating of 0 = no infection to 4 = dead plant; values followed by the same letter do not differ at $P = 0.01$, refers to all values in table.

effect was noted at 24, 27, or 30 C except for increasing weight with an increase in temperature.

R. solani disease indices were increased at all soil temperatures when three units of the fungus were combined with 2,500 or 5,000 *M. incognita* larvae per seedling, compared to the fungus alone (Table 4). Disease was most severe at soil temperatures 18 and 21 C. Lesions caused by *R. solani* usually remained localized in the lower hypocotyl area. Seedlings surviving after 21 days generally had extensive root systems, except at 15 C, and fungal development and damage appeared largely arrested. Five units of *R. solani* resulted in greater incidence of disease than one or three units, with or without *M. incognita* (Table 2).

Galls were present on roots of all seedlings which received larvae of *M. incognita*. However, galls were fewer and smaller at 15 C than at higher soil temperatures.

DISCUSSION

Since field populations of *R. solani* vary in temperature requirements for disease development (9), soil temperatures optimal for the isolate in these experiments may not be optimal for other fungal isolates. However, my data compare favorably with those of Norton (12). Growth of *R. solani* and penetration and maturation of *M. incognita* appeared to be restricted at 15 C since disease incidence was low, and the number and size of galls were reduced. Apparently, neither pathogen is favored by this temperature.

The 2,500 nematodes required to increase disease severity from combined infections or to stunt seedlings from the nematodes alone are similar to the numbers of *Heterodera schachtii* Schm. larvae (10,000) that Polychronopoulos found necessary to increase damping-off of sugar beets by *R. solani* (16). Also, Khoury and Alcorn (10)

observed that minimum inoculum densities of *M. incognita* were required to induce an increased Verticillium wilt of cotton, depending on the variety of cotton. Larvae of *M. incognita* probably do not provide infection sites for *R. solani* since the nematode attacks the roots, and the fungus is generally confined to the hypocotyl. *M. incognita* can cause physiological alterations within plants (15). These alterations might require a certain level of infection by the nematode to increase the susceptibility of cotton seedlings to *R. solani*.

R. solani is an aggressive fungus that can develop significant levels of inoculum rapidly (1). Chester (5) found that cotton seedlings infected with *R. solani* constituted a threat to the health of adjoining plants; seedlings infected with other pathogens did not. In these experiments, no differences in disease severity were observed between one and three units. The effect of fungal inoculum level was independent of *M. incognita*. The 96-h incubation before seeding may have allowed one unit to colonize the soil as thoroughly as three units.

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