Effect of Root-knot Nematode-Fungi Combinations on Cotton Seedling Disease

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ABSTRACT

Synergistic effects on the severity of cotton (*Gossypium hirsutum*) seedling diseases were found between root-knot nematodes (*Meloidogyne incognita acrita*) and *Alternaria tenuis, Fusarium oxysporum* f. sp. *vasinfectum, Glomerella gossypii,* and *Rhizoctonia solani.*

Cotton seedling disease was severe in the presence of high inoculum levels of *G. gossypii* and *R. solani* without nematodes. At these inoculum levels, nematode effects were masked. *A. tenuis* and *F. oxysporum* f. sp. *vasinfectum* alone caused slight or no disease, but when combined with nematodes, disease was severe.

Chaetomium spp., *Cladosporium* spp., *Fusarium moniliforme,* and *Xanthomonas malvacearum* caused little or no seedling damage, either alone or combined with nematodes. Phytopathology 60:448-451.

Root-knot nematodes influence the severity of wilt disease in cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum* (3, 5, 6, 9, 10). In Arizona, Reynolds & Hanson (8) observed less *Rhizoctonia solani* damping-off in cotton fields fumigated for root-knot control than in untreated fields. Norton (7) demonstrated in greenhouse tests that seedling diseases caused by *R. solani, Pythium debaryanum,* and *F. oxysporum* f. sp. *vasinfectum* were greater in combination with root-knot nematodes than by either pathogen alone.

White (12) showed a similar interaction between *M. incognita acrita* and two fungi, *Rhizoctonia solani* and *Thielaviopsis basicola,* in nematicide- and fungicide-treated pots of naturally infested soil.

Brodie & Cooper (1) observed prolonged susceptibility to *P. debaryanum* of cotton seedlings grown in soil infested by nematodes; in the case of *R. solani,* prolonged susceptibility also was associated with reduction in seedling growth.

This research was designed to determine the combined effects of root-knot nematodes and certain fungi and bacteria on the severity of cotton seedling disease.

MATERIALS AND METHODS.—Cotton seedlings with damping-off were collected from cotton fields in central and northern Alabama during the spring of 1966. Of numerous fungi isolated from these seedlings, the following occurred most frequently: *Alternaria tenuis* Auct.; *Chaetomium* spp.; *Cladosporium* spp.; *Fusarium moniliforme* Sheldon; *F. oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans.; *Glomerella gossypii* Edg.; and *Rhizoctonia solani* Kühn. The effect on root-knot susceptible and resistant cotton seedlings to *Meloidogyne incognita* var. *acrita* (Kofoid & White) Chitwood, 1949, in combination with each of these fungi and a mixture of *Xanthomonas malvacearum* (E. F. Sm.) Dows. (races 1 and 2), was studied in a series of 12 tests.

The above organisms were cultured in petri dishes at 25 C and used in tests as follows: test 1, *X. malvacearum,* and test 4, *Chaetomium* spp., on potato-carrot-dextrose agar (PCDA); test 2, *F. moniliforme,* and test 5, *F. oxysporum* f. sp. *vasinfectum,* on Czapek's agar; test 3, *Cladosporium* spp.; tests 6 and 7, *A. tenuis*; tests 8 and 9, *R. solani*; and tests 10 through 12, *G. gossypii* on potato-dextrose agar (PDA).

Fungal and bacterial inocula were prepared by mixing 2-week-old cultures in a blender with 350 ml of tap water. The contents of one to six petri dishes were used in tests as follows: one petri dish in tests 11 and 12; three petri dishes in tests 1 through 6 and 8 through 10; and six petri dishes in test 7. The agar medium was included with the mycelium so that the inoculum would not leach from the soil.

Root-knot nematode larvae were reared on tomato plants and extracted from galled roots by misting. Larvae were surface disinfected by exposure to 0.001% 8-quinolinol sulfate for 30 min, followed by return to tap water. Larval suspensions containing 1,000 larvae/ml of water constituted the root-knot inoculum.

Cottonseeds were acid-delinted and soaked in hot water at 80 C for 60 sec. They were then placed in a germinator at 28 C for 12-18 hr. Since germination time varied among cotton entries, times allowed for germination were adjusted so that all entries reached the same stage of germination for planting at the same time. Only healthy seeds with emerging radicles were planted.

Metal flats, 56 cm long × 35 cm wide × 10 cm deep, filled with methyl bromide-fumigated fine sandy loam, were used for all tests. Four rows of six seeds each were planted in each flat. Pregerminated seeds were dropped individually into holes of uniform size 8 cm apart in rows, and a 1-ml aliquot of nematode inoculum or a 2-ml aliquot of fungal or bacterial inoculum or both were injected into each hole with an automatic pipette. Checks received the same quantities of sterile water. Seeds and inoculum were covered with sterile dry soil and lightly watered.

In tests 1 through 11, root-knot tolerant *Gossypium hirsutum* 'Auburn 56' and root-knot susceptible *G. hirsutum* 'M-8' were planted in alternate rows of each flat. Twelve flats planted in this manner were used in each test. The flats were divided into four sets of three

flats/set. At planting, each set was assigned one of four treatments. Treatments for test 1 were: B, bacteria alone; N, nematodes alone; B + N, bacteria + nematodes; C, sterile H_2O control. Treatments for tests 2 through 11 were: F, fungus alone; N, nematodes alone; F + N, fungus + nematodes; C, sterile H_2O control. After planting, flats were arranged with treatments at random, in three replicates.

For test 12, preparation for planting, row dimension, and seed spacings were the same as for previous tests. Cotton entries used for this test were the two previously described, plus a root-knot resistant wild *G. barbadense* and a root-knot resistant wild *G. hirsutum*. Single rows of each of these four cotton entries were planted in each of eight flats. Seeds in four of these flats were inoculated with F, *G. gossypii* alone; and seeds in the remaining four flats were inoculated with F + N, *G. gossypii* + root-knot nematodes. Flats were then arranged in four replicates.

The experimental design for all tests was a split-plot with treatments being whole plots and cotton entries subplots.

Tests were conducted in the greenhouse where temperatures ranged from 25 to 32 C during the day and between 15 and 25 C at night. Test 9 with *R. solani* was conducted in a growth chamber regulated to 14-hr, 28 C days, and 10-hr, 21 C nights. The duration of each test was 3 weeks.

Dead seedlings were counted weekly, and live seedlings were graded for disease severity after 3 weeks. Disease severity grades were as follows: 0 = no external lesions; 1 = light external lesions; 2 = moderate external lesions and light internal discoloration; 3 = heavy external lesions and moderate internal discoloration; 4 = severe external lesions and severe internal discoloration; and 5 = dead.

RESULTS.—Synergism occurred between root-knot nematodes and *A. tenuis, F. oxysporum* f. sp. *vasinfectum, G. gossypii,* and *R. solani* (Fig. 1). Symptoms were similar in F and F + N treatments, but disease severity grades and number of dead seedlings (Table 1) were greater in F + N treatments.

Treatments F, F + N, and N were not significantly different from C in tests with *X. malvacearum, Fusarium moniliforme, Cladosporium* spp., or *Chaetomium* spp. (tests 1, 2, 3, and 4, respectively), and no data are presented. These organisms caused little or no seedling damage, either alone or combined with nematodes.

Seedling mortality and disease grades for C treatment was most frequently 0, and there was not more than 1 dead seedling or 0.1 (disease grade) for any cotton entry in any test. In all tests, all seedlings in N treatment were galled on roots and stems to a degree that varied directly with resistance of the seedlings to root-knot nematodes regardless of the presence or absence of the other organisms. However, N and C treatments did not differ in number of dead seedlings and disease grade. Therefore, the following results concern only F and F + N treatments of each test.

In test 5, *F. vasinfectum* disease developed slowly. In both F and F + N treatments, cotyledons of many

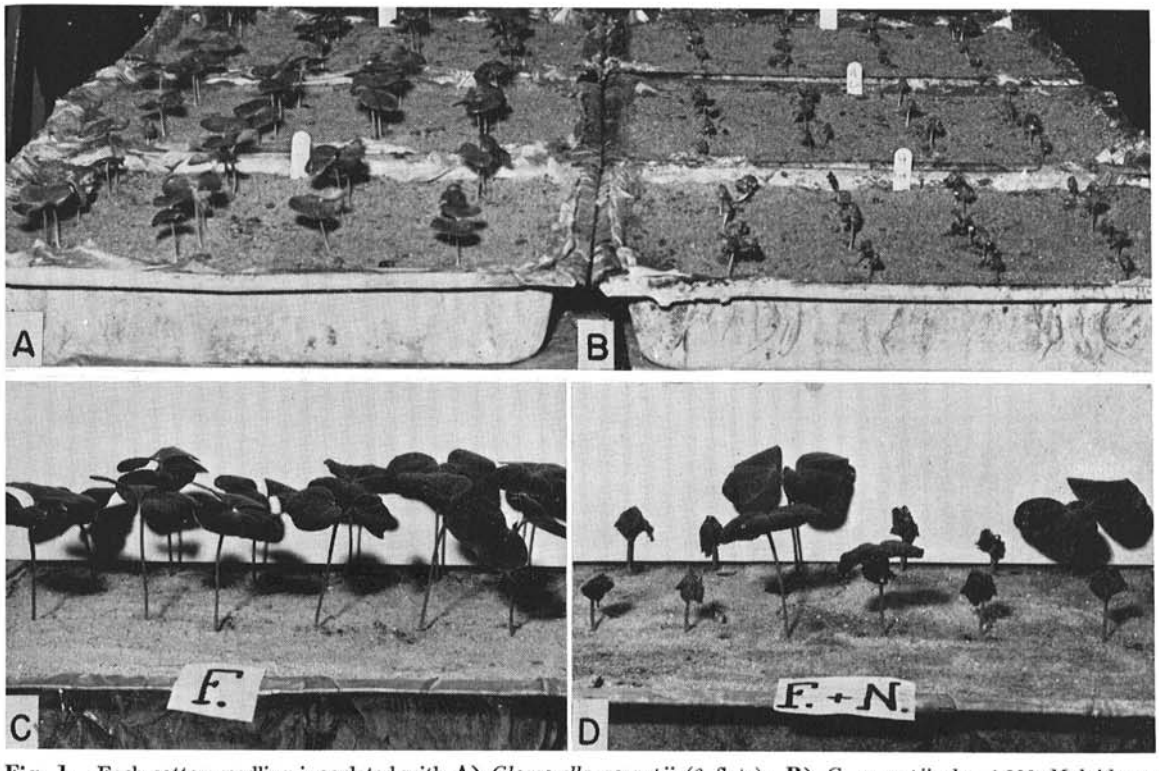

Fig. 1. Each cotton seedling inoculated with **A)** *Glomerella gossypii* (3 flats); **B)** *G. gossypii* plus 1,000 *Meloidogyne incognita acrita* larvae (3 flats); **C)** *Alternaria tenuis*; and **D)** *A. tenuis* plus 1,000 *M. incognita acrita* larvae.

TABLE 1. Mean disease severity grades and percentage of dead cotton seedlings 3 weeks after inoculation with fungi alone and fungi combined with *Meloidogyne incognita acrita*

Test no.	Treatment	Disease severity grade[a]	Dead seedlings %[b]
5	*Fusarium oxysporum* f. sp. *vasinfectum*	0.9**[c]	3**
	Fusarium oxysporum f. sp. *vasinfectum* + nemas	3.8	69.5
6	*Alternaria tenuis*	0.2*	0**
	Alternaria tenuis + nemas	2.9	30.5
7	*Alternaria tenuis* (doubled inoculum)	1.4**	5.5*
	Alternaria tenuis + nemas	3.5	47.5
8	*Rhizoctonia solani*	1.0*	1.5*
	Rhizoctonia solani + nemas	2.1	12.5
9	*Rhizoctonia solani*	2.6*	14*
	Rhizoctonia solani + nemas	4.0	50
11	*Glomerella gossypii* (⅓ inoculum level)	2.9**	41**
	Glomerella gossypii + nemas	5.0	98

[a] Grades scored on basis of 0 (no apparent disease) to 5 (dead); each value is mean of 2 cotton entries, 3 replicates/cotton entry, 12 plants/replicate.

[b] Each value is mean of 2 cotton entries; percentages for each cotton entry obtained from (no. dead in treatment minus no. dead in check/36 × 100) 3 replicates, 12 plants/replicate.

[c] *, ** Within fungi, fungus alone differed from fungus + nematodes at 5% and 1% level of probability, respectively.

seedlings were flaccid after 12 to 15 days, and these seedlings died after 14 to 20 days. These seedings showed wilt disease.

Of the live seedlings after 21 days, 54 and 98% exhibited some degree of disease damage in the F and F + N treatments, respectively; but typical vascular discoloration showed only in the F + N treatment. Live seedlings in the F treatment exhibited only necrotic spots on the taproots. Seedling disease damage and mortality (Table 1) were greatest in the F + N treatment.

In test 6 with *A. tenuis,* we used three petri dishes of fungus in 350 ml of water for inoculum; 16 and 84% of the seedlings showed disease in the F and F + N treatments, respectively. However, with this inoculum rate, disease severity grades were low, and only a few seedlings died (Table 1). Test 7 was conducted with six petri dishes of *A. tenuis* in 350 ml of water. Compared to the previous test, disease was more extensive. Seventy and 96% of seedlings showed some disease in the F and F + N treatments, respectively. Lesions on stems of seedlings in the F treatment were lighter and tissue discoloration was more external than in the F + N treatment, and in the latter treatment more plants died than in test 6 (Table 1, Fig. 1). Many of the plants exhibited severe stem girdling, but no plants died until the 3rd week. Disease from *A. tenuis* developed slower than from *R. solani* or *G. gossypii* in tests 9 and 10, respectively.

R. solani caused less seedling damping-off and lesions on base of stems in greenhouse test 8 than in the growth chamber test 9, perhaps because the greenhouse was more favorable for cotton than for the fungus. In both the greenhouse and growth chamber, disease severity grades and percentage of dead plants (Table 1) were significantly higher in the F + N than in the F treatments in both tests. Most seedlings in the F + N treatments in the growth chamber test were typically girdled at the soil line, and died within 15 days. The few that lived were weakened.

In test 10, *G. gossypii* inoculum at the rate of three petri dishes of fungus in 350 ml of water was extremely virulent. Within 5 days after planting, many seedlings were dead in both the F and F + N treatments; within 2 weeks, all plants were dead.

In test 11, with *G. gossypii* inoculum at the rate of one petri dish in 350 ml of water, the synergistic relationship of the fungus and root-knot nematodes was clearly demonstrated (Table 1, Fig. 1). Within 15 days, 98% were dead in the F + N treatment, compared with 41% dead in the F treatment (Table 1).

In tests 9, 10, and 11, seedling roots in the F + N treatments were too decayed after 3 weeks to be rated for nematode root-galling.

Auburn 56 and M-8 are known to react differently to root-knot nematodes, but in the foregoing tests there was no significant variety × treatment interaction. However, in tests 5, 7, and 9, M-8 had significantly greater disease damage, and in test 7, greater seedling mortality than Auburn 56.

When *G. gossypii* was tested (test 12) against four cotton entries, more Auburn 56 and M-8 seedlings died in the F + N than in F treatment (Table 2).

TABLE 2. Percentages of dead cotton seedlings and disease severity grades 10 days after inoculation with *Glomerella gossypii* alone and combined with *Meloidogyne incognita acrita*

Varieties	Disease severity		Dead seedlings	
	F[a]	F + N	F	F + N
	grade[b]	grade	%[c]	%
Gossypium barbadense (root-knot resistant)	2.3	2.7	40	40
Gossypium hirsutum (root-knot resistant)	4.9	4.8	92	96
Gossypium hirsutum Auburn 56 (root-knot tolerant)	3.2	4.3	21*[d]	58
Gossypium hirsutum M-8 (root-knot susceptible)	3.3	4.1	4*	38

[a] F = fungus alone; F + N = fungus + nematodes.

[b] Grades scored 0 (no infection) through 5 (dead); each value is mean of 4 replicates, 6 plants/replicate.

[c] Percentages obtained from (no. dead in treatment minus no. dead in check/36 × 100) 3 replicates, 12 plants/replicate.

[d] *Within cotton entries, treatments were different at 5% level of probability.

DISCUSSION.—The combined effect of root-knot nematodes and each of four fungi, *A. tenuis, F. oxysporum* f. sp. *vasinfectum, G. gossypii,* and *R. solani,* was greater than either one of the fungi or nematodes alone. Results with the first two fungi are similar to those previously reported by Brodie & Cooper (1) and Minton (4), and results with the latter two fungi are additional evidence that root-knot nematodes are an important factor in the cotton seedling disease complex.

R. solani and *G. gossypii* were strong pathogens, since they infected and killed young seedlings without the aid of nematodes when inoculum level was sufficiently high. In combination with nematodes, these fungi killed or damaged the seedlings faster, and less fungus inoculum was required for infection.

In tests with *F. oxysporum* f. sp. *vasinfectum* and *A. tenuis* alone, infection was slow and very mild on young seedlings. *A. tenuis* is generally considered to be a secondary invader. Recently, Maier (2) called it a "junior partner of the seedling disease complex". However, the present work indicates that this organism can be a relatively strong pathogen when combined with nematodes.

The symptoms caused by each fungus varied only in intensity between the F and F + N treatments. Symptoms were typical of the particular fungus being tested, and were not dependent on fungus-nematode interactions.

No studies were conducted to determine how root-knot nematodes predispose cotton seedlings to disease. However, stress and weakness of seedlings caused by nematode damage was probably the major factor. In addition, nematodes may provide infection courts through which fungi enter the host, as was suggested by Smith (11). Also, exudate from nectorial cells, formed by host roots in response to root-knot nematode attack, may stimulate and attract fungi; and these cells may be more easily parasitized by fungi than normal cells.

Further study is needed to determine the relationship of nematodes and fungi in causing seedling disease.

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