Effect of a Seedling Disease Caused by Rhizoctonia solani on Subsequent Growth and Yield of Cotton

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ABSTRACT

The effect of damage caused by *Rhizoctonia solani* during the seedling stage on subsequent growth and yield of cotton was determined in greenhouse-, growth chamber-, and field tests. Under greenhouse conditions, damage caused by *Rhizoctonia* lesions ranged from no significant growth retardation, to a marked reduction in shoot and root growth, and in boll production. Temperature appeared to be a significant factor in the degree of damage observed in greenhouse studies conducted over a 2-year period. In growth chamber experiments, plants damaged by *R. solani* produced significantly less shoot and root growth than check plants at 19 C, but not at 28 C. Generally, growth differences between infected and check plants were greater in natural field soil than in fumigated field soil. Soil assays of organisms in the natural soil, plus the increased growth and yield of cotton in soil treated with methyl bromide or pentachloronitrobenzene, suggest that soil-borne organisms (particularly *R. solani*) contributed to marked stunting of plants in natural soil. In the field, plants with *Rhizoctonia* lesions generally grew as well as check plants, but they produced significantly less seed cotton.

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Rhizoctonia solani Kuehn is a major cause of seed rot, preemergence damping-off, and especially postemergence damping-off of cotton (*Gossypium hirsutum* L.) throughout much of the Cotton Belt in the USA (1, 5, 9, 15, 16, 19). Plants that survive attack may exhibit various degrees of root rot or hypocotyl lesions near the soil line, the latter condition sometimes being referred to as soreshin (3, 9, 16, 19). Incidence and severity of cotton seedling disease caused by *R. solani* have been correlated, either experimentally or under production conditions, with soil conditions such as temperature, moisture, and pH (3, 5, 7, 9, 15, 19); fungicide (1, 3, 18) and herbicide (17) use; attack by plant parasitic nematodes (4) and insects (16); seed quality (2); exposure of germinating seed to low temperature (13); the isolate of the organism involved (5, 12); and the influence of associated soil-borne organisms (5).

Most research concerned with *R. solani* on cotton has involved the seedling disease phase and resulting seedling losses (1, 5, 7, 9, 18, 19). The limited information on persistence of symptoms past the seedling stage prompted us to conduct a series of studies to compare the growth and yield of *Rhizoctonia*-damaged and healthy plants under various conditions.

MATERIALS AND METHODS.—*Production of lesions.*—Nontreated cotton seed were planted four seeds per pot 3.8-cm deep in fumigated (methyl bromide, 454 g/m^3) soil-sand-vermiculite (3:1:1,v/v) in 5-cm diameter plastic pots placed on a greenhouse bench. The seedlings in each pot were thinned to one plant 10 days after planting. A highly virulent isolate of *R. solani*, obtained from a diseased cotton seedling in Georgia, was used in all studies. Inoculum was grown in potato-dextrose broth (Difco, 100 ml in 250-ml flasks) for 10-12 days in shake culture at room temperature (25-27 C). Resulting mycelial growth was filtered from the broth through cheesecloth, rinsed in sterile distilled water, and fragmented in sterile distilled water in a Waring Blendor for approximately 10 seconds. Concentrations of the mycelial suspension were adjusted to give 50% light transmission (580 nm) on a Bausch and Lomb Spectronic-20 spectrophotometer. Inoculum was pipetted (5 ml/pot) onto the soil surface around 10-day-old cotton seedlings, and the pots were watered thoroughly to spread the inoculum evenly around the hypocotyls. Lesions that occurred near the soil line were allowed to develop for 10 days before the plants (hereafter called "lesion" plants) were used in various studies to determine the effect of lesions on subsequent growth and yield. In all studies, extra plants were inoculated to allow selection of plants with uniform lesion development. Appropriate check plants were also grown simultaneously with the production of lesion plants.

Greenhouse studies.—Five studies were conducted during a 2-year period to determine the effect of *R. solani* lesions on the subsequent growth of cotton. In the first study, Stoneville 213 plants with moderate-to-severe lesions and check plants were transplanted into fumigated (methyl bromide, 490 kg/ha) soil in 4.3 × 4.5-m ground beds during October. Three rows (each with 12 plants) of each treatment were planted alternately with 0.6 m between rows, and 0.3 m between plants within rows. Height measurements and boll counts were taken monthly after transplanting until harvest of seed cotton in March.

A second study was conducted to determine the effect of different degrees of lesion development on subsequent growth and yield. Stoneville 213 and Coker 201 plants were inoculated to produce lesions as described earlier, except that the inoculum concentration was adjusted to give 20, 40, and 60 percent transmission on a Spectronic 20 spectrophotometer. After inoculation and lesion

development, 10 plants with either shallow (necrosis confined to outer layer of cortex), moderately deep (necrosis approximately half-way through cortex), or deep (necrosis extended to stele) lesions, and check plants were transplanted into fumigated soil in 20-liter polyethylene pots and placed on a greenhouse bench. Height measurements were taken weekly and root weights were determined after 90 days.

In the first two studies, growth responses of lesion and check plants were determined in fumigated soil. To determine any interaction with other soil-borne organisms, a third study was conducted in which lesion and check plants were transplanted into natural soil obtained from a cotton field near Waynesboro, Georgia. The soil was a sandy clay loam with a history of vascular wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. and Hans. Prior to planting, the soil was assayed for common soil-borne pathogens and nematodes. Populations of *Pythium* spp. were determined on modified Kerr's medium (6), *Fusarium* spp. on Nash and Snyder's medium (14), *R. solani* with Ko and Hora's selective medium (11), and nematodes were assayed by a modified centrifugal-flotation method (8). The field soil was screened to remove coarse debris, mixed with vermiculite (3:1, v/v), and fertilized and limed for cotton production according to soil test results. Ten lesion and 10 check plants each of the Stoneville 213, Coker 201, and Auburn 56 cotton cultivars (selected for high susceptibility, moderate resistance, and high resistance to *Fusarium oxysporum* f. sp. *vasinfectum*, respectively) were transplanted singly into 20-liter polyethylene pots arranged in a randomized complete block design on a greenhouse bench. Symptoms of Fusarium wilt were recorded as they occurred to determine if *Rhizoctonia* lesions predisposed plants to *F. oxysporum* f. sp. *vasinfectum*. After 164 days, height measurements, and numbers of open and green bolls and squares were recorded. Surface-sterilized (alcohol-flamed) stem sections from each plant were plated on water agar and potato-dextrose agar (Difco) to determine the presence of the wilt pathogen in the vascular system.

In an additional study, the natural soil was removed from the pots, screened to remove roots, mixed thoroughly, and fertilized for optimum growth of cotton. Half the soil was fumigated with methyl bromide and half was kept natural. Fourteen Coker 201 plants with moderately-deep to deep lesions, and 14 check plants, were transplanted singly into the fumigated and natural soils in 20-liter pots arranged in a randomized complete block design in the greenhouse. Height measurements were taken weekly and the incidence of Fusarium wilt was recorded. After 85 days, root weights were determined, and stem sections were plated for isolation of *F. oxysporum* f. sp. *vasinfectum*.

The significant differences in growth of plants in the fumigated versus natural soil prompted us to conduct a final test with broad-spectrum and selective chemicals to determine what organisms might be involved. Thoroughly mixed natural soil was divided into six portions, and five portions were treated with different chemicals. Treatments and rates per kg of soil were: (i) methyl bromide (Dowfume MC-2, 6.73 g); (ii) PCNB (pentachloronitrobenzene, Terraclor 75 WP, 0.41 g); (iii) Terrazole (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole 35 WP, 0.21 g); (iv) benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], Benlate, 0.036 g); and (v) DBCP (1,2-dibromo-3-chloropropane, Nemagon, 0.021 ml). PCNB, Terrazole, and benomyl were mixed in the soil by blending in a cement mixer. Methyl bromide was applied to the soil under a 102-μm (4-mil) polyethylene film. DBCP was applied to layers of soil sealed inside a polyethylene container. Fifteen pots (20-cm diameter) of each soil treatment were arranged in a randomized complete block design on a greenhouse bench. One Coker 201 plant was established in each pot by direct seeding with four seeds, followed by thinning. Pots were placed on inverted clay saucers, and each pot was equipped with a splash guard (cylinder of polyethylene-coated screen wire extending 13 cm above the rim of the pots) to prevent cross-contamination during watering. Height measurements were taken weekly. After 86 days, root weights were also determined and the soils were assayed for microorganisms.

Growth chamber test.—The greenhouse studies were conducted over a 2-year period during which temperature varied greatly with the season. Variable results suggested

TABLE 1. Effect of severity of stem lesions caused by *Rhizoctonia solani* on the growth of two cotton cultivars in methyl bromide-fumigated soil in the greenhouse during the 90-day period after transplanting[a]

Cultivar and lesion severity[b]	Height (cm) days after transplanting					Dry root weight (g)
	15	30	45	60	90	
Coker 201						
No lesion (check)	26.1 x[c]	46.9 x	58.9 x	62.0 xy	76.9 x	32.6 x
Shallow lesion	25.5 x	45.2 xy	56.8 x	61.5 xy	73.9 x	31.1 x
Moderately-deep lesion	22.2 y	41.5 yz	58.0 x	66.0 x	77.8 x	28.4 x
Deep lesion	20.3 y	41.4 yz	53.5 x	57.7 y	68.8 x	27.1 x
Stoneville 213						
No lesion (check)	21.7 x	41.1 x	51.8 x	56.8 x	76.2 x	25.5 x
Shallow lesion	23.7 x	43.8 x	52.3 x	55.5 x	71.8 x	24.3 x
Moderately-deep lesion	23.8 x	43.4 x	52.5 x	57.7 x	75.6 x	26.2 x
Deep lesion	17.6 y	35.3 y	47.3 x	56.9 x	71.1 x	21.9 x

[a]Each value is a mean of 10 replications, each consisting of one plant grown in soil in a 20-liter pot.

[b]Shallow lesion–necrosis restricted to the outer layers of the cortex of the hypocotyl; moderately deep–necrosis approximately half way through the cortex; deep–necrosis extending to near the stele.

[c]Values within each cultivar in a column followed by the same small letter are not significantly different ($P = 0.05$).

the need for tests under controlled temperature conditions. Fourteen Coker 201 plants with moderately-deep to deep lesions and 14 check plants, all transplanted into methyl bromide-fumigated soil in 25-cm diameter pots, were placed in growth chambers maintained at approximately 19 C (range 18-21 C) and 28 C (range 27-30 C). The pots were arranged in a modified complete block design to account for light variation within the chamber. Splash guards, described earlier, were used to prevent contamination. Height measurements and root weights were taken after 56 days.

Field studies.—Greenhouse and growth chamber studies were supplemented with field studies conducted near Athens, Georgia, during 1973 and 1974. After routine land preparation and fertilization, trifluralin (α, α, α-trifluoro-2, 6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, Treflan, 0.56 kg/ha) was applied for weed control. Coker 201 plants with moderately deep lesions and checks were produced in the greenhouse. The lesion and check plants, grown for 5 weeks in the greenhouse, were transplanted into the field in alternate rows with six replications. Rows were 22.5 m in length, spaced 1.0 m apart, with individual plants within the row 0.6 m apart. Transplanting dates were 2 July 1973, and 22 May 1974. Routine cultural and insect control practices were followed. Height measurements were taken periodically throughout the growing season, and yield of seed cotton was determined by hand harvesting in early December of each year.

RESULTS.—*Greenhouse studies.*—The effect of *Rhizoctonia* lesions on the growth of cotton varied considerably in the different greenhouse studies. When Stoneville 213 plants were grown in ground beds from October through March, plants with lesions were significantly smaller than control plants throughout the growth period. At the end of the study, lesion plants were 79 percent as tall and produced 37 percent fewer bolls than control plants. Growth differences were less obvious when Coker 201 and Stoneville 213 plants with different degrees of lesion severity were grown in fumigated soil in pots on a greenhouse bench (Table 1). Although plants of both cultivars with deep lesions were slightly smaller than plants with less severe lesions, and check plants during most of the growth period, no significant differences in height and dry root weights were evident when the study was terminated after 90 days. Plants of both cultivars with shallow and moderately deep lesions generally grew as well as plants without lesions. There were also no

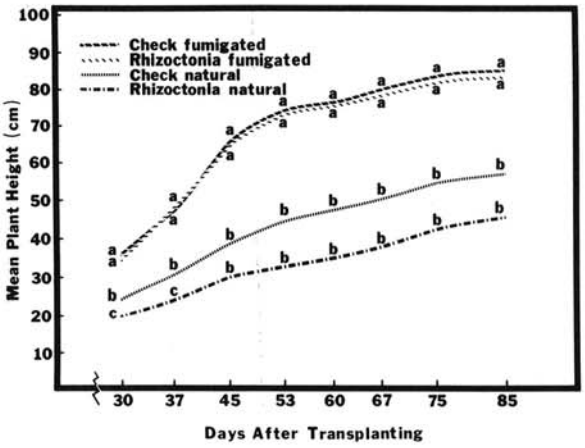

Fig. 1. Growth of Coker 201 cotton plants with and without stem lesions caused by *Rhizoctonia solani* in natural- and methyl bromide-fumigated soil in the greenhouse. Height means for various treatments on a given data are not significantly different ($P = 0.05$) when followed by the same letter.

significant differences in the final shoot height and the number of bolls between lesion and check plants of the Auburn 56, Coker 201, and Stoneville 213 cultivars transplanted into natural soil obtained from a Fusarium wilt problem field. Seventy percent of both lesion and check Stoneville 213 plants showed symptoms of Fusarium wilt. Approximately 30 and 50% of Coker 201 and 40 and 10% of Auburn 56 check and lesion plants, respectively, showed symptoms of wilt. Soil assays showed that the natural soil contained 3.8×10^3, and 6.8 propagules per gram of *Fusarium* spp., and *Pythium* spp., respectively. *R. solani* grew from 2.5 percent of the soil plugs assayed by Ko and Hora's method (11).

When Coker 201 plants were transplanted into the natural soil used previously, and into the same soil fumigated with methyl bromide, both lesion and check plants produced more shoot growth in fumigated than in natural soil (Fig. 1). Lesion plants transplanted into natural soil were smaller than check plants throughout the 85-day growth period. However, differences were generally not significant ($P = 0.05$) except during the first 40 days after transplanting. There were no significant differences in the growth of lesion and check plants in

TABLE 2. Effect of five chemical soil treatments on the growth of Coker 201 cotton in natural soil in the greenhouse during the 75-day period after seeding

Treatment[a]	Height (cm) days after seeding			Dry root weight (g)
	35	55	75	
None (natural soil)	13.4[b]	27.9	39.7	8.9
Methyl bromide (6.73 g)	19.3*[c]	40.6*	54.1*	11.0*
PCNB (Terraclor, 0.41 g)	19.0*	35.1*	49.1*	9.7*
Terrazole (0.21 g)	14.4	26.2	37.5	8.5
Benomyl (Benlate, 0.036 g)	17.1*	25.7	35.1	8.2
DBCP (Nemagon, 0.021 ml)	15.4	25.0	34.5	8.0
LSD ($P = 0.05$)	2.1	3.7	5.4	0.7

[a]Rates are expressed as grams per kilogram of soil for all treatments except DBCP (ml/kg).
[b]Each value is a mean of 15 replications, each replication consisting of one plant in a 20-cm diameter clay pot.
[c]Asterisk indicates the mean is significantly greater than the check (natural soil) as determined by the LSD ($P = 0.05$).

TABLE 3. Growth of Coker 201 cotton plants with and without stem lesions caused by *Rhizoctonia solani* in fumigated soil in growth chambers at two temperatures

	Mean temperature (C)[a]			
	19 C		28 C	
Treatment	Shoot height[b] (cm)	Dry root[b] wt (g)	Shoot height[b] (cm)	Dry root[b] wt (g)
Nonlesion plants (check)	46.2 x[c]	4.21 x	64.6 x	2.48 x
Lesion plants	29.2 y	1.09 y	54.7 x	1.71 x

[a]Temperatures ranged from 18 to 21 C (mean 19 C) and from 27 to 30 C (mean 28 C) in two growth chambers.

[b]Each value is a mean of 14 replications, each consisting of one plant grown 56 days in a 20-cm diameter pot.

[c]Values in a given column followed by the same letter are not significantly different ($P = 0.05$).

fumigated soil. In the natural soil, wilt symptoms were observed in 7 percent and 29 percent of the check and lesion plants, respectively. Isolations indicated the presence of *F. oxysporum* f. sp. *vasinfectum* in 21 percent of the check plants, and 57 percent of the lesion plants, in natural soil. Dry root weights were 5.63, 5.75, 3.07, and 2.13 g for the fumigated-check, fumigated-lesion, natural-check, and natural-lesion treatments, respectively. Root weights were significantly greater ($P= 0.05$) in fumigated than in natural soil, but there were no significant differences between lesion and check plants grown in each soil.

Plants grown in natural soil frequently had poorly developed and necrotic root systems. Isolations from roots consistently failed to associate any one organism with the necrotic tissue. Consequently, a test with broad-spectrum and selective chemicals was conducted in an attempt to determine the cause of root necrosis and stunted growth. Treatment of the soil with methyl bromide and PCNB resulted in significant increases in shoot and root growth of Coker 201 plants compared with plants grown in natural soil (Table 2). Applications of Terrazole, benomyl, or DBCP to the soil failed to give a significant growth response. Initial populations of *Pythium* and *Rhizoctonia* spp. were low or nondetectable in both natural soil, and in soil treated with the chemicals. Populations of *Rhizoctonia* spp. remained low in soil treated with methyl bromide and PCNB, but reached relatively high levels in nontreated soil and soil treated with benomyl and DBCP. Populations of *Pythium* increased in all soils except the one treated with methyl bromide. Populations of *Fusarium* spp. remained fairly stable or increased slightly, except in Terrazole-treated soil, where they increased. Methyl bromide and DBCP were effective in eliminating most nematodes from the soil. Populations of *Meloidogyne* spp. increased in natural soil and in soil treated with PCNB, Terrazole, and benomyl.

Growth chamber study.—Both plants with lesions and check plants grew better at 28 C than at 19 C (Table 3). At 28 C, plants with lesions grew as well as check plants; but at 19 C, plants transplanted with lesions produced less shoot and root growth than plants without lesions.

Field studies.—During 1973, there was a significant difference in the vegetative growth of plants with lesions compared with those without lesions during the first month after transplanting, but differences were not significant during the remainder of the growing season (Table 4). There were no significant differences in vegetative growth of lesion versus check plants during the 1974 growing season. However, plants without lesions yielded 39 and 13 percent more seed cotton during 1973 and 1974, respectively, than lesion plants.

DISCUSSION.—*Rhizoctonia solani* is recognized as an important pathogen of cotton seedlings (1, 5, 9), and evidence of damage that occurs near the soil line during the seedling stage persists on plants in the field throughout much of the growing season. Until the present study, however, little was known about the effect of the early-season damage on subsequent growth and yield of cotton. Our results indicate that cotton plants have a capacity for recovery from even severe stem damage caused by *R. solani* if optimum growing conditions are provided. However, reduced shoot growth, root growth, and yield may occur on plants damaged by *R. solani* when various other stress factors also are present. Results from the various greenhouse tests, conducted over a 2-year period, ranged from no significant damage caused by *Rhizoctonia* lesions, to a marked reduction in shoot and root growth and boll production. We believe the reduced growth of plants with lesions in some tests occurred during periods when greenhouse soil temperatures were below the optimum for growth of cotton. For example, the test with Stoneville 213 plants in fumigated soil in ground beds, where growth differences between lesion and check plants were greatest, was conducted during the winter months when soil temperatures were lower. In the growth chambers, where temperatures were controlled, damage caused by *Rhizoctonia* lesions occurred even in

TABLE 4. Growth and yield of Coker 201 cotton plants with and without stem lesions caused by *Rhizoctonia solani* in field plots near Athens, Georgia, during 1973 and 1974[a]

	1973				1974			
	Plant height (cm) days after transplanting			Yield of seed cotton	Plant height (cm) days after transplanting			Yield of seed cotton
Treatment	31	89	159	(kg)	36	102	193	(kg)
Check plant	24.1 x[b]	63.5 x	90.9 x	1.38 x	26.7 x	77.8 x	126.4 x	3.80 x
Lesion plant	19.8 y	60.2 x	90.9 x	0.99 y	28.1 x	76.9 x	128.4 x	3.35 y

[a]Each value is a mean of six replications, each consisting of one row 22.5 m in length with 35 plants.

[b]Values in the same column followed by the same letter are not significantly different ($P = 0.05$).

fumigated soil at 19 C, but not at 28 C. The results of the growth chamber tests suggested that temperature below the optimum for the growth of cotton may be a stress factor that contributes to long-term damage caused by *Rhizoctonia* lesions. This conclusion is supported by the observation by Neal (15) that damage caused by *R. solani* may occur on cotton as late as the flowering stage during cool, wet springs. Hunter and Guinn (7) reported that higher sugar content of hypocotyls under low temperature conditions increased the severity of damage caused by *R. solani* on cotton seedlings. Stewart and Whitehead (20) also suggested that recovery of cotton plants with root damage caused by *R. solani* and other seedling disease organisms depends on stress factors in the environment, especially soil moisture. The plants in our greenhouse and growth chamber tests had adequate moisture at all times.

Cotton plants grown in natural soil obtained from a Fusarium wilt problem field produced significantly less shoot and root growth than plants in the same soil that had been fumigated with methyl bromide. Although no constant association of any particular organism with the necrotic root systems of plants from the natural soil could be established, observation of *Rhizoctonia* lesions near the soil line, and increased growth resulting from treatment of the soil with PCNB in the chemical test, plus the results of various soil assays, suggest that *R. solani* was probably the major pathogenic organism involved.

Field observations (Brown and McCarter, *unpublished*) have suggested increased incidence of Fusarium wilt among cotton plants in fields heavily damaged by *R. solani*. Khoury and Alcorn (10) reported that *R. solani* increased the susceptibility of cotton plants to *Verticillium albo-atrum*. In the present studies, a high percentage of plants of the wilt-susceptible cultivar Stoneville 213 transplanted into soil naturally infested with *F. oxysporum* f. sp. *vasinfectum* showed wilt symptoms in both the presence and absence of lesions caused by *R. solani*. The higher percentage of stems of Coker 201 with lesions that contained the wilt organism in the vascular system may indicate increased susceptibility of this moderately resistant cultivar. However, additional tests are necessary before such an association can be definitely established.

Cotton plants with and without lesions caused by *R. solani* were grown in field plots during two consecutive years. The plot area had no known soil-borne disease problems, and both seasons were fairly favorable for the growth of cotton. Although there were no great differences in the vegetative growth of the lesion and check plants during either growing season, plants with lesions produced significantly less seed cotton during both years. We believe these yield differences were due to delayed flowering and boll maturity on the lesion plants. The large differences in yield between 1973 and 1974 tests were due to the very late transplanting date in 1973.

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