

## Influence of *Rhizoctonia solani* on the Susceptibility of Cotton Plants to *Verticillium albo-atrum* and on Root Carbohydrates

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Based on a portion of a Ph.D. thesis by the senior author and supported in part by the Ford Foundation. Journal Paper No. 1771 Agricultural Experiment Station, University of Arizona.

We are grateful to Robert O. Kuehl, Roger L. Caldwell, and L. S. Stith for their advice on statistical, biochemical, and horticultural procedures, respectively.

Accepted for publication 18 September 1972.

### ABSTRACT

Newly emerged seedlings of *Gossypium hirsutum* 'Deltapine Smooth Leaf' (DSL) and *G. barbadense* 'Pima S-2' (PS-2) grown in the greenhouse were less susceptible to *Verticillium albo-atrum* than were older plants. *Rhizoctonia solani* significantly increased the susceptibility of both cultivars to *V. albo-atrum*; generally PS-2 was as susceptible as DSL. The degree to which *Rhizoctonia* affected susceptibility varied with plant age,

soil temperature, inoculum concentration, and interval between inoculations with the two pathogens. Levels of potassium hydroxide-soluble carbohydrates were significantly higher in roots of DSL at the first-leaf stage and of PS-2 at the cotyledonary and first-leaf stages when these cultivars were exposed to *R. solani*.

Phytopathology 63:352-358

*Additional key words:* breeding, control, inoculum potential.

Certain lines of evidence suggest that elevated carbohydrate (CHO) levels in host roots can enhance their susceptibility to *Verticillium albo-atrum* Reinke & Berth., increase the severity of symptom expression, or both. Roberts (21) found that partial defoliation of tomato plants reduced their susceptibility to *V. albo-atrum*; this change was associated with a decrease in root CHO levels. Selman & Buckley (23) reported that the degree of invasion of tomato roots by *V. albo-atrum* was directly related to the concentration of sucrose in the nutrient solutions in which the plants were grown. More recently, several cultivars of cotton plants with poorly aerated roots were found to have greater concentrations of root CHO and more severe wilt symptoms than did plants with aerated roots (8). Results of experiments on physiological age, shade, temperature, and defoliation experiments also suggest (24) an association between increased susceptibility of young cotton plants and elevated CHO levels in their roots. Such observations led Horsfall & Dimond (12) to postulate that a stem-girdling pathogen, such as *Rhizoctonia solani* Kuehn, should reduce the susceptibility of a host to *V. albo-atrum* by preventing translocation of CHO into the roots. However, there is evidence (6) that *R. solani* can increase the susceptibility to *V. albo-atrum* of the cotton species *Gossypium hirsutum* L. 'Deltapine Smooth Leaf' (DSL) and of *G. barbadense* L. 'Pima S-2' (PS-2), which, respectively, have been considered susceptible and resistant to *V. albo-atrum* (19).

This study was undertaken to determine whether *R. solani* alters the susceptibility of cotton plants to *V. albo-atrum* and the CHO levels in their roots.

**MATERIALS AND METHODS.**—*General.*—Acid-delinted seeds of DSL or PS-2 were planted in 15-cm pots containing a mixture of Mesa loam, sand, and

peat moss (2:2:1) which had been exposed to 121 C for 4 hr. Approximately 5 g of 16-20-0 fertilizer were added to each pot at the time of planting. For experiments on changes in susceptibility, seedlings immediately after emergence were thinned to five/pot. All plants were maintained in the greenhouse at 20 to 29 C.

*V. albo-atrum* isolate DX-2, which produces abundant microsclerotia and defoliates cotton plants, was grown on Difco potato-dextrose agar (PDA) at room temperature (ca. 25 C). *R. solani* was isolated from a naturally infected cotton seedling and also was cultured on PDA at room temperature.

We prepared standard inocula by blending for 1 min in 500 ml of sterile distilled water the contents of five 10-cm petri plates overgrown either with DX-2 or with *R. solani*. Each mixture was diluted to 1 liter and used within 30 min. Inoculation at the time of planting involved our mixing a given fungal suspension with the upper third of the soil in a pot. To minimize root damage when infesting the soil at intervals after planting the seeds, we inserted a bundle of wooden pot labels into the soil at the center of each pot at planting time and these, when removed, left a hole into which the inoculum was poured. Plants were watered immediately after the treatments were applied and thereafter on a daily basis as needed to prevent wilting.

Twenty-eight days after infesting the soil with *V. albo-atrum*, we assayed each seedling for infection by removing a 3- to 5-cm section from the center of each hypocotyl. Each piece was thoroughly washed in running tap water, dried, then placed in an aqueous 0.525% solution of sodium hypochlorite for 5 min. Subsequently, a 1-cm long subsection was removed from near the center of each piece and partially embedded (end down) in agar containing

streptomycin (18, but minus the alcohol) and sterilized barley straw (11). The samples were incubated at room temperature for 3 weeks. A plant was considered infected only when *V. albo-atrum*, indicated by the formation of microsclerotia, was isolated. Since we were concerned with infection *per se*, detailed notes regarding symptoms were not kept.

Plants assayed for *V. albo-atrum* infection also were examined for lesions characteristic of those caused by *R. solani*. In addition, in the first experiment, plants with and without typical lesions were randomly collected from soil infested with *R. solani*. Sections ca. 2-cm long, which included both below- and aboveground portions of the hypocotyls, were removed and surface-sterilized as previously described. Tissues were removed from the margin of a lesion or from the central portion of apparently healthy sections and cultured on PDA at room temperature for 3-4 days. A positive recovery of *R. solani* was indicated by the presence of typical hyphae, devoid of fruiting structures (2).

*Concurrent inoculations with R. solani and V. albo-atrum.*—Plantings of DSL and PS-2 were timed to provide on a given date plants at three stages of development: newly planted seeds; plants with nearly expanded first leaves (18 days old); and plants with nearly expanded third leaves (30 days old). These either were not inoculated or were inoculated singly with *R. solani*, or *V. albo-atrum*, or concurrently with both fungi. The amount of inoculum added per pot was 3, 6, 25, or 50 ml for *R. solani* and 30, 60, or 120 ml for *V. albo-atrum*. Each treatment was replicated 4 times.

The effect of soil temperature on the susceptibility of DSL or PS-2 to *V. albo-atrum* alone and in combination with *R. solani* was determined in another experiment. Three, 25, and 25 ml of *R. solani* inoculum were added, respectively, to nine pots each of newly seeded pots and to first-leaf and to third-leaf plants of both cultivars. Sixty ml of *V. albo-atrum* inoculum then were added immediately to each pot. Equal numbers of comparable plants inoculated singly either with *R. solani* or *V. albo-atrum*, or left noninoculated, served as controls. All pots were placed in metal containers slightly larger than the pots, and these then were distributed equally between controlled temperature tanks maintained at 16, 23, and 30 ± 1 C in a greenhouse. Air temperatures ranged from ca. 22 to 29 C.

*Inoculation with R. solani prior to inoculation with V. albo-atrum.*—Thirty-two pots of soil were planted with DSL or with PS-2, and 3 ml of *R. solani* inoculum were added to each pot; 25 ml of inoculum were similarly added to each of 20 pots of DSL and PS-2 in the first-leaf stage. Sixty ml of *V. albo-atrum* inoculum were added to each of four replicate pots of each of the newly seeded cultivars 0, 4, 8, 12, 16, 20, 24, and 28 days after infestation of the soil with *R. solani* and to an equal number of pots of the *Rhizoctonia*-treated, first-leaf-stage plants of each cultivar at similar intervals, but only through 16 days. Controls for each cultivar and time interval consisted of plants inoculated only with *R. solani* (four pots),

plants inoculated only with *V. albo-atrum* (four pots), and noninoculated plants (four pots).

*The effect of Rhizoctonia on root CHO.*—DSL and PS-2 seeds were planted separately in 130 pots, and 3 ml of *R. solani* suspension were added to the soil in each of 65 pots of each cultivar. For each cultivar, 60 ml of *V. albo-atrum* were added to each of four pots with noninfested soils and to four pots with *Rhizoctonia*-infested soil at 4, 8, 12, 18, or 30 days after the addition of *R. solani*; i.e., when the seedlings were in the pre-emergence, emergence, cotyledonary, first-, and third-leaf stages, respectively.

CHO analyses were made on composite samples of roots of plants from five pots each of noninfested or *Rhizoctonia*-infested soils and collected at the time of each inoculation with *V. albo-atrum*. Plants were thinned to five/pot except for the 4- and 8-day plantings for CHO analyses; these required 10 plants/pot. No *Rhizoctonia* lesions were noted on any plants collected at 4 or 8 days or on any noninoculated plants. The roots of plants collected after 8 days were analyzed only when the plants had *Rhizoctonia* lesions. All root samples analyzed included the uppermost lateral roots.

Roots were washed, dried at 70 C for 24 hr in a forced-air oven, then ground in a Wiley mill to pass through a 40-mesh screen and stored up to 5 days at room temperature. Soluble CHO were extracted and analyzed by the procedures of Takacs (24) and Yemm & Willis (28). Three determinations were made for each sample of roots.

*Statistical analyses.*—The concurrent inoculation tests and the temperature tests were repeated once. Because results for the two trials (14) were similar, only the data from one experiment each are presented here. The experiment that involved inoculations with *R. solani* prior to inoculations with *V. albo-atrum* was repeated in part in the experiment concerned with the effect of *R. solani* on root CHO levels, which was performed once.

Most data were subjected to a multiple factorial analysis by means of an appropriately programmed computer. All results for a given experiment were combined and analyzed for the effects of particular treatments. For example (Table 1), the mean percentage infection (MPI) by *V. albo-atrum* of DSL plants exposed to 3 ml of *R. solani* is based on the combined information for all ages of DSL plants exposed to the several concentrations (0-120 ml) of *V. albo-atrum*. Similarly, the MPI by *V. albo-atrum* of first-leaf-stage plants exposed to 6 ml of *R. solani* is based on the pooled information for all first-leaf-stage plants of both cultivars exposed to the various concentrations of *V. albo-atrum*. Least significant difference (LSD) determinations were made at the 5% level of confidence. The LSD's given in the Tables can be used both for horizontal and vertical statistical comparisons of the means obtained in a particular test.

**RESULTS.**—*Concurrent inoculations of plants with R. solani and V. albo-atrum.*—The over-all incidence of disease among plants of both cotton

TABLE 1. Infection by *Verticillium albo-atrum* of the cotton cultivars Deltapine Smooth Leaf (DSL) and Pima S-2 (PS-2) when concurrently inoculated with *Rhizoctonia solani*<sup>a</sup>

<i>R. solani</i> suspension (ml) 15-cm pot	Mean percentage infection by <i>V. albo-atrum</i>								
	Cultivars inoculated <sup>b</sup>		Growth stage when inoculated <sup>c</sup>			ml <i>V. albo-atrum</i> suspension/pot <sup>d</sup>			
	DSL	PS-2	Seeds <sup>e</sup>	First leaf	Third leaf	0	30	60	120
0	35.4	26.7	0.0	41.9	51.3	0.0	33.3	42.5	48.3
3	38.8	31.7	0.0	53.1	52.5	0.0	41.7	48.3	50.8
6	40.8	39.2	0.0	63.1	56.9	0.0	46.7	54.2	59.2
25	45.8	45.4	0.0 <sup>f</sup>	71.3	65.6	0.0	56.7	61.7	65.0
50	50.0	46.7	0.0 <sup>f</sup>	75.0	70.0	0.0	63.3	64.2	65.0

<sup>a</sup> Isolations of *V. albo-atrum* were made 28 days after infesting the soil with the fungi; treatments, each with five plants, were replicated 4 times. Multiple factorial analyses were made of pooled data for the indicated relationships.

<sup>b</sup> Each value is a mean for 48 sets of plants (three ages × four *Verticillium* concentrations × four replicates); LSD at 5% = 3.9.

<sup>c</sup> Each value is a mean for 32 sets of plants (two cultivars × four *Verticillium* concentrations × four replicates); LSD at 5% = 4.8.

<sup>d</sup> Each value is a mean for 24 sets of plants (two cultivars × three ages × four replicates); LSD at 5% = 5.6.

<sup>e</sup> Soil was infested at time of seeding.

<sup>f</sup> 100% mortality within 10 days of planting caused by *R. solani*.

cultivars exposed only to *V. albo-atrum* varied directly with the volume of inoculum applied (Table 1). *V. albo-atrum* was not isolated from seedlings grown in soil that was infested concurrently with the planting of the seeds.

Of the seedlings exposed only to *V. albo-atrum*, third-leaf-stage plants were significantly more susceptible than first-leaf-stage plants. *R. solani* increased the susceptibility to *V. albo-atrum* of both first- and third-leaf-stage plants; first-leaf-stage plants exposed to the largest amount of *R. solani* were the most susceptible (Table 1). DSL was significantly more susceptible to *V. albo-atrum* than was PS-2 when the amount of *R. solani* inoculum added per pot was 0 or 3 ml (Table 1).

In addition to the information presented in Table 1, the multifactorial analyses of the data from this experiment showed that the MPI of DSL and PS-2 by *V. albo-atrum* were, respectively: 0.0 and 0.0 for plants inoculated at the time of seeding; 60.0 and 61.3 for first-leaf-stage plants; and 66.0 and 52.5 for third-leaf-stage plants (LSD at 5% = 3.0 for all relationships). Furthermore, when data for effects of plant ages and amounts of *R. solani* inocula were pooled, the MPI of DSL and PS-2 by *V. albo-atrum* were, respectively: 50.3 and 46.3 for treatments involving 30 ml of *V. albo-atrum* inoculum/pot; 58.0 and 50.7 for 60 ml of inoculum/pot; and 60.3 and 54.7 for 120 ml of inoculum/pot (LSD at 5% = 3.5 for all relationships).

At the time plants were cultured for *V. albo-atrum* (Table 1), attempts also were made to isolate *R. solani* from 30 randomly collected plants with typical lesions and from 25 plants without lesions from each cultivar. *R. solani* was recovered from all plants of both cultivars that had lesions, but from only 8% of the plants that lacked lesions. The incidence of *Rhizoctonia* lesions on plants infected

with *V. albo-atrum* was related to the amount of inoculum, plant age, and cultivar (Table 2). First-leaf-stage plants of PS-2 exposed to 3 or 6 ml of *R. solani* inoculum, and third-leaf-stage plants exposed to 25 ml of the inoculum, had significantly more stem lesions caused by *R. solani* than did DSL plants that were similarly treated at the same stage of development.

Twenty-five and 50 ml of *R. solani* inoculum added to each pot at planting effected a near 100% mortality of both cultivars within 10 days. Therefore, when the experiment was repeated, the amount of inoculum was reduced to 0, 1, 2, 3, or 6 ml/pot for the treatment that involved the soil when the seeds were planted. The trends observed in the duplicate trial (14) were the same as those presented here (Table 1), except that the mean incidence of

TABLE 2. Mean percentages of *Rhizoctonia solani* lesions on *Verticillium*-infected Deltapine Smooth Leaf (DSL) and Pima S-2 (PS-2) cotton plants following concurrent inoculations with both fungi<sup>a</sup>

Growth stages when inoculated	<i>R. solani</i> suspension (ml)/15-cm pot			
	3	6	25	50
DSL				
First leaf	48.7 <sup>b</sup>	89.7	100.0	100.0
Third leaf	39.3	67.7	92.7	100.0
PS-2				
First leaf	90.7	98.0	100.0	100.0
Third leaf	38.3	69.7	96.0	98.0

<sup>a</sup> Readings were made 28 days after inoculation.

<sup>b</sup> Values are means for plants treated with 30, 60, or 120 ml of *V. albo-atrum*/pot. Each treatment consisted of four pots, each with five plants. LSD at 5% = 3.0. Control plants had no lesions.

Verticillium wilt was 1.3, 8.1, and 9.4% after the addition of 0, 3, and 6 ml, respectively, of *Rhizoctonia* inoculum.

*Effect of soil temperatures.*—Analysis of the combined data resulting from inoculation of plants at three stages of development with *V. albo-atrum* alone, or in combination with *R. solani*, indicated that DSL and PS-2 did not differ significantly in their susceptibility to *V. albo-atrum* at a given temperature. The MPI for DSL were 20.6, 28.9, and 13.9, respectively, at 16, 23, and 30 C (LSD at 5% = 3.1). MPI based on pooled data for plants of both cultivars exposed to *V. albo-atrum* alone or in combination with *R. solani* were, respectively, 15.0 and 27.2 at 16 C, 21.7 and 35.6 at 23 C, and 13.3 and 17.2 at 30 C (LSD at 5% = 3.1 for all relationships). In the same test 7.5%, 10.8%, and 3.3% (LSD at 5% = 3.8) of all plants grown, respectively, at 16, 23, and 30 C became infected with *V. albo-atrum* as a consequence of being exposed at the time of planting to *V. albo-atrum* either alone or in combination with *R. solani*. The MPI by *V. albo-atrum* of first- and third-leaf-stage plants (inoculated either with *V. albo-atrum* or *R. solani* and *V. albo-atrum*), were, respectively, 25.0 and 30.8 at 16 C, 36.7 and 38.3 at 23 C, and 21.7 and 20.8 at 30 C (LSD at 5% = 3.8 for all relationships).

All *Verticillium*-infected plants exposed to *Rhizoctonia* had typical *Rhizoctonia* lesions except those of PS-2 at 23 C and in the first- and third-leaf

TABLE 3. Infection by *Verticillium albo-atrum* of cotton plants inoculated with *Rhizoctonia solani* (RS) at planting time and subsequently inoculated at various intervals with *V. albo-atrum* (VA)<sup>a</sup>

Days between RS and VA inoculations	Growth stage when inoculated with VA	Mean percentage infection by <i>V. albo-atrum</i> <sup>b</sup>	
		Plants inoculated with	
		VA	RS + VA
0	Seed <sup>c</sup>	0.0	17.5
4	Pre-emergence	0.0	17.5
8	Emergence	2.5	20.0
12	Cotyledon	32.5	55.0
16	First leaf	52.5	90.0
20	First to second leaf	52.5	85.0
24	Second to third leaf	60.0	87.0
28	Fourth leaf	57.0	92.0

<sup>a</sup> Readings were made 28 days after inoculation with *V. albo-atrum* (60 ml/pot); *R. solani* inoculum was 3 ml/pot. Treatments each with five plants, were replicated 4 times.

<sup>b</sup> Since there was no significant difference in susceptibility to *V. albo-atrum* between the Deltapine Smooth Leaf (DSL) and Pima S-2 (PS-2) cultivars at any stage of growth, the mean percentage infection for the eight sets of plants (two cultivars × four replicates) for each treatment is presented. LSD at 5% = 6.5. No *Verticillium* wilt occurred in noninoculated plants or in those exposed only to *R. solani*.

<sup>c</sup> Soil was infested at the time of seeding.

TABLE 4. Infection by *Verticillium albo-atrum* of cotton plants inoculated with *Rhizoctonia solani* (RS) in the first-leaf stage and subsequently inoculated at various intervals with *V. albo-atrum* (VA)<sup>a</sup>

Days between RS and VA inoculations	Growth stage when inoculated with VA	Mean percentage infection by <i>V. albo-atrum</i> <sup>b</sup>	
		Plants inoculated with	
		VA	VA + RS
0	First leaf	57.5	95.0
4	First to second leaf	62.5	92.5
8	Second to third leaf	57.5	92.5
12	Third leaf	57.5	82.5
16	Third to fourth leaf	65.0	80.0

<sup>a</sup> Readings were made 28 days after inoculation with *V. albo-atrum* (60 ml/pot); *R. solani* inoculum was 25 ml/pot. Treatments, each with five plants, were replicated 4 times. There was no significant difference in susceptibility to *V. albo-atrum* between the Deltapine Smooth Leaf (DSL) and Pima S-2 (PS-2) cultivars at any stage of development except at the third-leaf stage. Therefore, the data for the two cultivars were pooled for these analyses. At the third-leaf stage, DSL was significantly more susceptible than was PS-2.

<sup>b</sup> Mean percentage of eight sets of plants (two cultivars × four replicates); LSD at 5% = 7.2. No *Verticillium* wilt occurred in noninoculated plants or in those treated only with *R. solani*.

stages of which 92 and 93%, respectively, had lesions; and those of DSL and PS-2 at 30 C and in the third-leaf stage, of which 85 and 75% had lesions.

*Prior inoculations with R. solani.*—Cotyledonary stage and older plants were markedly more susceptible to *V. albo-atrum* alone than were younger plants (Table 3); the most susceptible plants were those in the second- to third-leaf stage. Infesting the soil with *R. solani* at planting time significantly increased the susceptibility of all plants subsequently exposed to *Verticillium*. Maximum infection (85-92%) by *V. albo-atrum* occurred when the addition of this fungus followed that of *R. solani* by 16 to 28 days (Table 3).

There was a significant increase in the susceptibility of third- to fourth-leaf-stage plants over first-leaf-stage plants exposed only to *Verticillium* (Table 4). *R. solani* significantly enhanced the susceptibility of older plants to *V. albo-atrum*, but this effect diminished as the interval between the inoculations with the two fungi increased (Table 4).

All *Verticillium*-infected plants developed typical *Rhizoctonia* lesions, except that only 85 and 94%, respectively, of DSL exposed to *V. albo-atrum* 8 and 16 days after the first-leaf stage had lesions. In all experiments, roots of plants exposed to *R. solani* were darker than nontreated roots, whether or not lesions occurred.

*Effect of R. solani on root CHO.*—CHO levels were significantly greater in the roots of first-leaf-stage DSL plants and cotyledonary and first-leaf-stage PS-2 plants exposed to *R. solani* than

TABLE 5. Root carbohydrate (CHO) levels and mean percentage infection (MPI) by *Verticillium albo-atrum* (VA) of the cotton cultivars Deltapine Smooth Leaf (DSL) and Pima S-2 (PS-2) inoculated with *Rhizoctonia solani* (RS) at planting and subsequently at several intervals inoculated with VA

Days after inoculation with RS	Growth stage when inoculated with VA	MPI by <i>V. albo-atrum</i> <sup>a</sup>				CHO/g dry wt of roots <sup>b</sup>			
		DSL <sup>c</sup> inoculated with		PS-2 <sup>d</sup> inoculated with		DSL <sup>e</sup>		PS-2 <sup>f</sup>	
		VA	RS + VA	VA	RS + VA	Control	RS	Control	RS
		%	%	%	%	mg	mg	mg	mg
4	Pre-emergence	10.0	20.0	15.0	20.0	76.7	77.3	116.7	114.0
8	Emergence	15.0	30.0	15.0	25.0	50.7	61.3	99.3	94.0
12	Cotyledon	45.0	70.0	35.0	60.0	66.0	72.0	47.3	69.3
18	First leaf	50.0	95.0	55.0	100.0	40.7	59.3	58.0	84.7
30	Third leaf	70.0	85.0	60.0	75.0	94.7	57.3	64.7	67.3

<sup>a</sup> Readings were made 28 days after inoculation with VA (60 ml/pot); RS inoculum was 3 ml/pot. Each figure is the mean percentage of four sets of pots each with five plants. No *Verticillium* wilt occurred in noninoculated plants or in those treated only with RS.

<sup>b</sup> Each value is a mean of three analyses of roots collected at the time other plants were inoculated with VA.

<sup>c</sup> LSD at 5% = 10.2.

<sup>d</sup> LSD at 5% = 11.8.

<sup>e</sup> LSD at 5% = 16.2.

<sup>f</sup> LSD at 5% = 13.2.

in roots of comparable control plants. In comparison to control plants, CHO levels were significantly less in roots of third-leaf-stage DSL plants exposed to *R. solani* (Table 5). The amount of CHO in the roots of *Rhizoctonia*-treated and nontreated plants of both cultivars varied with plant age. Through the 18th day after inoculation with *R. solani* (i.e., through the first-leaf stage), changes in CHO concentrations in treated roots approximately paralleled such changes in the nontreated roots of comparable plants of the same cultivar. However, CHO concentrations significantly increased in roots of nontreated, third-leaf-stage plants of DSL, whereas concentrations in treated roots of these plants were slightly less than those in treated roots of first-leaf-stage plants. Nontreated, third-leaf-stage plants of PS-2 had slightly more CHO in their roots than did the nontreated first-leaf-stage plants. However, CHO concentrations in the *Rhizoctonia*-treated roots of third-leaf-stage plants of PS-2 were significantly less than those in treated first-leaf-stage plants (Table 5).

In this test, 100% of the *Verticillium*-infected plants also had *Rhizoctonia* lesions.

**DISCUSSION.**—Although cultivars of *G. barbadense* generally have been considered more resistant to *V. albo-atrum* than are cultivars of *G. hirsutum* (19), under certain conditions PS-2 can be as susceptible as DSL (24). We had 43 individual treatments for which comparisons could be made of the relative susceptibilities of DSL and PS-2 to *V. albo-atrum*. In 10 instances, PS-2 was significantly less susceptible than DSL. However, such differences were not always consistent under different experimental conditions. For example, in tests involving plants at three stages of development and exposed concurrently to different amounts of inocula of *R. solani* and *V. albo-atrum*, MPI by *V. albo-atrum*

of third-leaf-stage plants of PS-2 was significantly less than that of comparable DSL plants. This also was true when first-leaf-stage plants were inoculated first with 25 ml/pot *Rhizoctonia* inoculum, then inoculated at the third-leaf stage with 60 ml of *V. albo-atrum* inoculum (Table 5). However, DSL and PS-2 plants exposed to 3 ml/pot of *R. solani* inoculum at the time of seeding and 60 ml/pot of *V. albo-atrum* at the third-leaf stage did not differ in susceptibility (Table 4).

Our observations confirm those of Takacs (24), who reported that PS-2 has a potential for susceptibility to *V. albo-atrum* equal to that of DSL. We also would agree that PS-2 is more tolerant to *V. albo-atrum* than is DSL in that symptoms consistently either were delayed and/or were milder for PS-2 than for DSL. In addition, our data verify reports (20, 24, 26) that young cotton plants within the age range that we studied are apparently more resistant to *Verticillium* than are older plants.

Evidence has accumulated (8, 21, 23) that during some stages of growth, conditions that enhance the susceptibility of a plant to *V. albo-atrum* also effect an increase in CHO in their roots. Increases in susceptibility to *V. albo-atrum* and in root CHO also have been noted for cotton plants during the period from the first- to fourth-leaf stages (24). We found that CHO levels in healthy roots of DSL decreased between the pre-emergence and first-leaf stages; and in PS-2, between the pre-emergence and cotyledonary stages. Yet the incidence of disease in both cultivars increased most rapidly after emergence. If elevated CHO levels do increase the susceptibility of cotton plants to *V. albo-atrum*, such enhancement would seem to be initiated about the onset of photosynthesis (i.e., at emergence). However, qualitative changes in CHO in the plant also could be

involved as a consequence of the metabolic changes associated with the initiation of photosynthesis.

Because of the possible relationship of CHO to the susceptibility of plants to *V. albo-atrum*, it has been postulated that hosts concurrently infected with a fungus such as *R. solani*, which can effect a girdling lesion of the stem, should be less susceptible to *V. albo-atrum* (12). Yet our tests indicate that the incidence of infection by *V. albo-atrum* (based on the recovery of this fungus from hypocotyls) in both cultivars was usually enhanced by concurrent or prior exposures of particularly the first-leaf plants to *R. solani*. Furthermore, the roots of most assayed plants exposed to *R. solani* contained as much CHO as did the control roots. However, our data indicate that the enhanced susceptibility induced by *R. solani* in young cotton plants does not generally relate to increases in total CHO, as measured by our techniques. There is the suggestion that the effect postulated for girdling by *Rhizoctonia* might have been "operating" in this test by approximately the 18th day after treatments with *R. solani*. After this date, the incidence of infection by *V. albo-atrum* declined among *R. solani*-treated plants; coincidentally, CHO concentrations in treated roots also declined.

*R. solani* could enhance the susceptibility of cotton plants to *V. albo-atrum* by at least several mechanisms. Artificial injury of roots has increased the susceptibility of various plants to *V. albo-atrum* (6, 17, 23). Our findings indicate that even with low levels of *R. solani* inoculum, 38% of the *Verticillium*-infected plants had typical *Rhizoctonia* lesions. It seems possible, therefore, that the type of injury caused by *Rhizoctonia* (5, 16) in the normal areas of *Verticillium* penetration (i.e., the hypocotyls and roots of cotton plants) (7) might also increase plant susceptibility by providing avenues of ingress for the wilt pathogen. Within a plant, degradation of pectic compounds and cellulosic compounds by enzymes secreted by *R. solani* (1, 25, 27) might facilitate penetration of root tissues by *V. albo-atrum* and, consequently, more rapid secondary colonization of upper regions of the vascular system (7). This effect could be particularly important when *V. albo-atrum* populations are low (7). While the influence of *R. solani* may be merely a matter of removing physical barriers to colonization (7), the accompanying loss of integrity of host cells also may result in a reduction in phytoalexin production (3).

The apparent resistance of young cotton plants to *V. albo-atrum* has been related to the presence of intact end-walls of vessels, which inhibit the acropetal movement of conidia in the xylem (20). If *R. solani* infection accelerated maturation of vessel elements, then at some stage of development normal maturation of vessel elements should result in a recovery of *V. albo-atrum* from the hypocotyls of control plants equal to that obtained from *R. solani*-treated plants. However, except for one test that involved third-leaf-stage plants of DSL exposed to *R. solani*, significantly more *Rhizoctonia*-treated plants in the cotyledonary to fourth-leaf stages had *V. albo-atrum* (in the hypocotyls) than did

nontreated plants. Since a greater proportion of vessels should be mature in the hypocotyls of third- or fourth-leaf-stage plants, we conclude that the *Rhizoctonia* effect on susceptibility does not relate to an acceleration of vessel maturation.

Our observations on the influence of *R. solani* on the susceptibility of DSL and PS-2 to *V. albo-atrum* support those of El Khash (6). However, we cannot support his suggestion that *R. solani* enhances the susceptibility of PS-2 more than that of DSL. We did not observe any consistent significant differences in the susceptibility of these cultivars to *R. solani* alone.

The incidence of *Verticillium* wilt of cotton is favored by cool, moist weather (15, 29); such conditions also favor the pathogenicity of *R. solani* (13). *R. solani* infection enhanced the susceptibility to *V. albo-atrum* of cotton plants, particularly through the first-leaf stage and at "low" concentrations of this fungus; the greatest influence was at 23 and 16 C and the least influence at 30 C. This effect also was greatest when treatments with *R. solani* at the time of planting preceded by 16 days subsequent inoculations with *V. albo-atrum*. We, therefore, conclude that those environmental conditions which have been indicated as favoring infection by *V. albo-atrum* may enhance the susceptibility of young cotton plants to this fungus at least in part by facilitating prior infections of the plant by *R. solani*. Hence, planting cotton when soil temperatures are "high" might minimize infection by *R. solani* (22). Such temperatures could provide additional salubrious effects by directly reducing the growth and activity of *V. albo-atrum* (10), reducing the accumulation of CHO in roots (9), and increasing the rate of synthesis of the phytoalexin, gossypol (4).

Should the results of our greenhouse study be corroborated by field experiments, plant breeders will need to be cognizant of the possibility that a cultivar that is resistant to *V. albo-atrum* when grown in soil free of *Rhizoctonia* may not show the same level of resistance when grown in *Rhizoctonia*-infested soil. Thus, soil or seed treatments to control *R. solani* in tests of cotton lines for resistance to *V. albo-atrum* could lead to erroneous interpretations.

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