

## Aggressiveness of Random and Selected Isolates of *Verticillium dahliae* from Cotton and the Quantitative Relationship of Internal Inoculum to Defoliation

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### ABSTRACT

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Isolates of *Verticillium dahliae* from the Verticillium wilt-tolerant cotton cultivar Acala SJ-5 were considerably more aggressive than isolates from the less tolerant cultivars, 70-110 and Acala SJ-2, toward four differentially tolerant cultivars of cotton. Likewise, isolates from plants defoliated by Verticillium wilt were more aggressive than isolates from randomly selected, infected plants, whether isolates came from a highly susceptible cultivar (70-110) or a tolerant cultivar (Acala SJ-5). Isolates that uniformly caused defoliation did so independently relative to the Verticillium wilt tolerance of cotton cultivars 70-110, Acala SJ-2, Acala SJ-4, and Acala

SJ-5 in the field. Also, isolates that caused uniform defoliation of cotton cultivars were as apt to be isolated from defoliated plants of the least tolerant and the most tolerant cultivars tested. Although the internal inoculum density (ID) of *V. dahliae* conidia of petiole tissue of nondehiscent leaves was greater for plants of cultivar Acala SJ-2 than for plants of Acala SJ-5, the ID of *V. dahliae* conidia in petioles of both cultivars were similar at dehiscence. Data reported here support the concept that the pathogenic aggressiveness of strains of soilborne *V. dahliae* occurs as a highly diverse continuum.

*Additional key words:* pathogenic strains.

Ashworth et al (1) studied the influence of inoculum density of *Verticillium dahliae* Kleb. on disease severity and yield of four differentially tolerant cotton cultivars. These were: 70-110, an Acala type cotton used locally as a susceptible comparison in breeding research; Acala SJ-2, a moderately tolerant cultivar; and two more tolerant cultivars, Acala SJ-4 and Acala SJ-5. The cotton cultivars were tested initially at four inoculum densities (ID), 2, 4, 15, and 21 microsclerotia (MS)/g soil. They observed in 1976 that the percentage of defoliated plants of cultivars 70-110 and Acala SJ-2, near harvest, increased with each increase in ID, but that little defoliation of cultivars Acala SJ-4 and Acala SJ-5 occurred, regardless of ID (1). This observation conflicted with the concept of a single defoliating strain of *V. dahliae*, as suggested for strain T-1 described by Schnathorst and Mathre (11). According to this concept, T-1 was present in blocks planted to cultivar 70-110 and Acala SJ-2, but not in those planted to cultivar Acala SJ-4 and Acala SJ-5 in our tests (1). This was not the case since inoculum for the test came from the same source, infested tomato stem tissue.

The purpose of the experiments reported here was to determine the relative aggressiveness of isolates of *V. dahliae* from randomly selected defoliated and nondefoliated plants with Verticillium wilt toward differentially tolerant cotton cultivars. Tests also were made to determine the nature of inoculum in infected lamina and petiole tissue and to determine whether internal ID of *V. dahliae* was related to leaf dehiscence.

### MATERIALS AND METHODS

Single-spore isolates of *V. dahliae* from cotton cultivars differentially tolerant of Verticillium wilt were tested for disease producing potential (pathogenic aggressiveness) toward four cotton cultivars differentially tolerant of wilt. Late in the growing season, isolations were made from stem tissue (taken about 15 cm above the soil line) of cultivar 70-110, a highly susceptible Acala type cotton, cultivar Acala SJ-2, a moderately tolerant cultivar, and cultivar Acala SJ-5 which is more tolerant of Verticillium wilt than either 70-110 or Acala SJ-2. As reported earlier (1), the tolerance of cultivar Acala SJ-5 is similar to that of cultivar Acala SJ-4.

Isolations were made on 20 October 1979 from plants defoliated by *V. dahliae*, a severe symptom of Verticillium wilt that is closely correlated with yield reduction of cotton (2). These plants are referred to as defoliated plants in this paper. Isolations were made on 1 November 1979 from other infected plants of the same planting following chemically induced defoliation of all plants. Diagnosis of infection of these plants was based upon the vascular necrosis symptom which is not closely correlated with disease severity (2). These plants are referred to in this paper as randomly selected, infected plants. The randomly selected group of infected plants probably included defoliated and nondefoliated plants infected by *V. dahliae* since 30-75% of the plants of the cotton cultivars in the test were defoliated by disease before all plants were chemically defoliated.

A total of 54 isolates, nine each selected from random infected and defoliated infected plants of each of the three cultivars, were tested in the greenhouse. These isolates were compared with three

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isolates of *V. dahliae* furnished by W. C. Schnathorst. Strains T-1 and SS-4 were described, respectively, as a highly aggressive, defoliating strain and as a mild, interveinal chlorosis inducing strain by Schnathorst and Mathre (11). The other isolate was a strain of intermediate aggressiveness described by Schnathorst and Fogle (10).

Plants were grown singly in 15-cm-diameter pots at ~25–28 C during December 1980–June 1981. Plants were inoculated by injection into the elongating internode immediately below the apical meristem with a syringe fitted with a 0.89-mm-diameter (20-gauge) needle. Aqueous spore suspensions used for inoculation were adjusted to  $\sim 10^6$  conidia per milliliter, based upon hemacytometer counts. There were either four or five plants for each treatment. Disease ratings were made 4 wk after plants were inoculated. Disease ratings used were 0 = no symptoms, 1 = interveinal chlorosis but with little leaf necrosis or stunting, 2 = moderate to severe leaf necrosis, stunted growth, and moderate defoliation, and 3 = essential defoliation accompanied by pronounced stunting.

The procedure of Erwin et al (3) for determining concentration of *V. dahliae* propagules in infected cotton plants was used in these tests on naturally infected field-grown and greenhouse-grown plants. Symptomless leaves, leaves showing only interveinal chlorosis, and fully chlorotic dehiscent leaves (all from infected plants) were tested for internal propagules of *V. dahliae*. Leaves that fell from plants as a result of gentle shaking were considered to be dehiscent.

Leaves were harvested, sealed in small polyethylene bags, and held on ice for transport to the laboratory. There they were washed to remove dust, blotted dry with paper towelling, then disks were cut from leaves and sections of petioles by using a 20-mm-diameter (No. 12) cork borer. Five such disks or sections, each from separate leaves, were bulked to make a sample. Fresh weight of samples were determined as soon as a sample was complete. Fresh weight of samples of lamina tissue of greenhouse-grown plants weighed less, an average of 0.19 g (range = 0.17–0.21 g) than those of field-grown plants which had visibly thicker leaves, an average of 0.54 g (range = 0.47–0.65 g). Samples of petiole tissue were similar, 0.49 g and 0.54 g (9% difference), ranging from 0.47–0.55 g for greenhouse-grown plants and from 0.40 to 0.82 g for field-grown plants.

A VirTis model 45 high-speed homogenizer (The Virtis Co., Gardiner, NY 12525) was used to homogenize plant tissues. Leaf and petiole samples were homogenized at 45,000 rpm in 50 ml of deionized water for three 20 sec intervals, depending on the toughness of tissue. Homogenates were filtered through eight layers of grade-80 cheesecloth. Following straining, in initial tests, the homogenates were filtered again through Millipore filters fitted with disks having 8  $\mu$ m pores. Twenty comparisons were made of the numbers of propagules in homogenates of infected chlorotic leaf tissue that had either been strained through cheesecloth or were filtered following straining through cheesecloth.

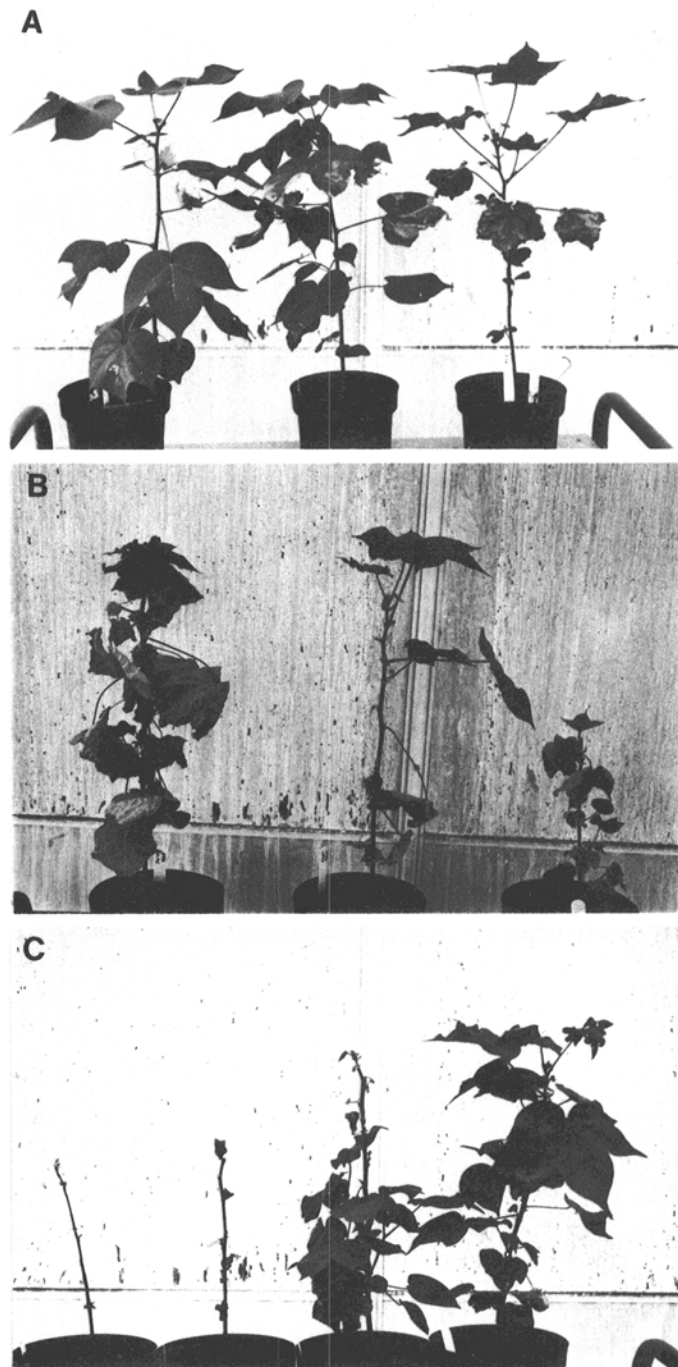
Strained or strained and filtered homogenates were diluted 1:10 and 1:100 with deionized water then 1-ml portions from each dilution were distributed on the surface of five pectate agar (7) plates. The agar substrate contained double amounts of streptomycin sulfate and Penicillin-G antibiotics used in soil assay tests for *V. dahliae* (7). Counts of *V. dahliae* colonies were made following incubation at 26 C for 5–7 days. Internal inoculum densities of the fungus were expressed as number of propagules per gram of fresh tissue.

## RESULTS

**Aggressiveness of isolates of *V. dahliae* from randomly selected infected plants and from defoliated plants of differentially tolerant cotton cultivars.** The range of Verticillium wilt symptoms of disease index classes observed in these tests are listed in Fig. 1. Greatest variability was in class 2—moderate-to-severe leaf necrosis and stunting. Some defoliation also occurred in class 2 plants, but apices were never killed and new leaves continued to emerge and expand during the course of experiments (Fig. 1B). Some plants of class 3 became defoliated and died while others exhibited weak

growth from nodes following the initial defoliation. In still others, regrowth occurred from lower nodes following initial defoliation (Fig. 1C).

Isolate T-1 caused complete defoliation of all four cultivars in three tests while the isolate of intermediate aggressiveness and isolate SS-4 were considerably less aggressive, having disease indices of ~1.4–1.8. The least significant difference (LSD,  $P=0.05$ ) for data of this test (Fig. 2A) was 0.3 disease index units. Four of 54 other isolates tested, two each from defoliated plants of cultivars 70-110 and Acala SJ-5 were comparable to T-1 in aggressiveness, inducing essentially complete defoliation of plants regardless of relative tolerance to Verticillium wilt under field conditions (1).



**Fig. 1.** Range of symptoms associated with Verticillium wilt disease index; **A**, healthy class 0 plant (left) and class 1 plants with interveinal chlorosis and slight leaf necrosis (right); **B**, class 2 plants with severe leaf necrosis and moderate defoliation with and without stunting; **C**, class 3 plants with essential defoliation, leftmost plants, a defoliated plant showing regrowth from lower nodes (second from right) and a healthy, class 0 plant (right).

The other 50 isolates of *V. dahliae* were less aggressive than T-1.

Among the isolates of *V. dahliae* collected from randomly selected infected plants, those from cultivar Acala SJ-5 were more aggressive than were isolates from cultivars 70-110 and Acala SJ-2, regardless of the plant cultivars used to test them. Isolates from cultivars 70-110 and Acala SJ-2 were similar, with regard to aggressiveness, toward the four cultivars upon which they were tested (Fig. 2B). Cultivars Acala SJ-4 and Acala SJ-5 were more tolerant and had lower disease indexes than either cultivars Acala SJ-2 or 70-110; Acala SJ-2 was more tolerant than 70-110 (Fig. 2B).

Isolates of *V. dahliae* from defoliated plants were considerably more aggressive toward all cultivars than isolates from randomly selected, infected plants (Fig. 2B and C); the LSD ( $P = 0.05$ ) for these data was 0.2 disease index units. Among isolates from defoliated plants, all were more aggressive toward cultivars 70-110 and Acala SJ-2 than toward Acala SJ-4 and Acala SJ-5, which reacted similarly (Fig. 2C); the LSD ( $P = 0.05$ ) for these data was 0.2 disease index units. Isolates from cultivar Acala SJ-5 were more

aggressive on cultivars tested, except on cultivar 70-110, than those from cultivars 70-110 and Acala SJ-2, and isolates from cultivar 70-110 were more aggressive than those from cultivar Acala SJ-2 (Fig. 2C).

**The relationship between inoculum density of *V. dahliae* in cotton leaves and symptoms of Verticillium wilt.** Numbers of colonies of *V. dahliae* that developed on pectate agar plates, seeded with homogenates of disks cut from infected leaves having interveinal chlorosis, were the same whether homogenates were strained through eight layers of cheesecloth or were strained then filtered to exclude propagules with minimum dimension  $>8 \mu\text{m}$ . Therefore, we concluded that the propagules were mainly conidia, rather than mycelial fragments. Following 20 initial comparisons, homogenates of lamina and petiole tissues were only strained before being diluted and cultured.

The inoculum densities (ID) of nondehiscent fully green leaves, nondehiscent leaves with interveinal chlorosis, and fully chlorotic dehiscent leaves were determined for cultivars Acala SJ-2 and

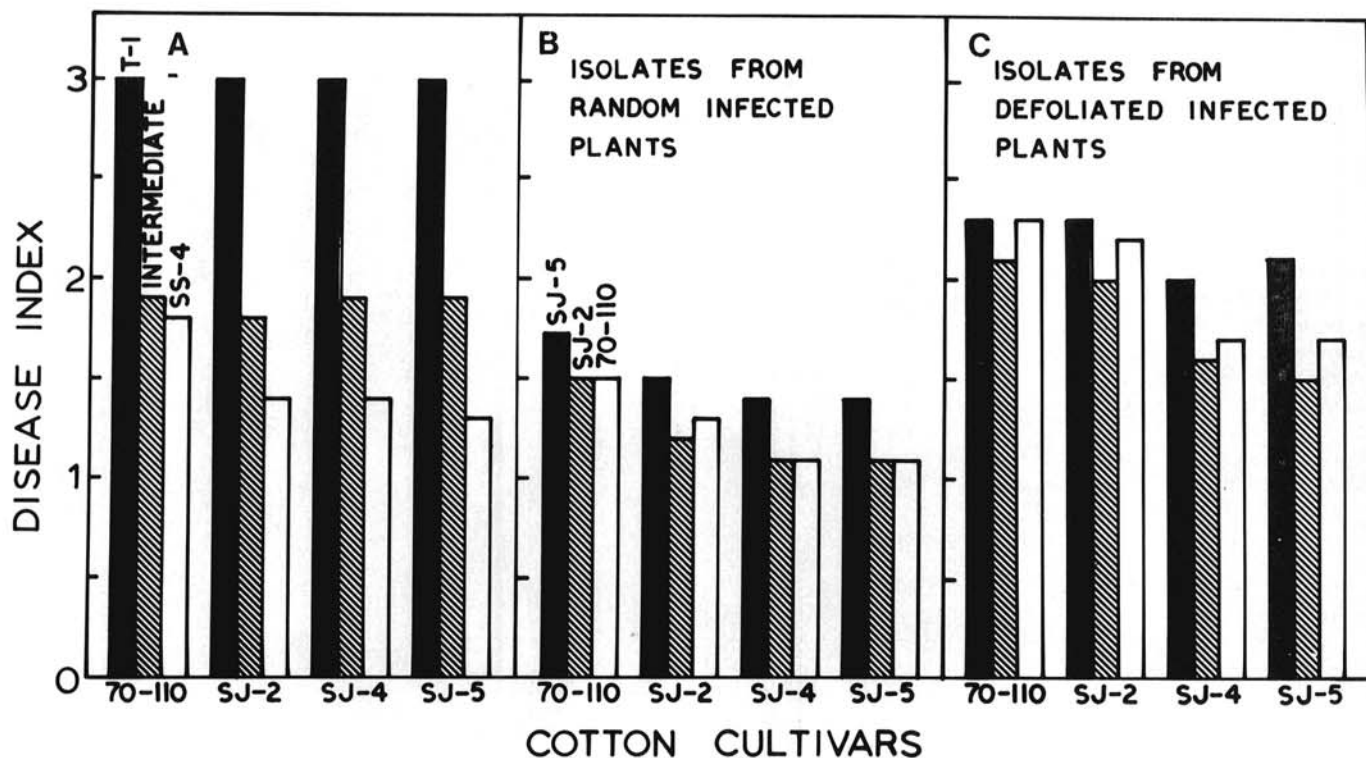


Fig. 2. The relative aggressiveness of isolates of *Verticillium dahliae* to four cotton cultivars (disease index: 0 = no symptoms, 1 = mild symptoms, 2 = severe leaf necrosis and incomplete defoliation with or without stunting, and 3 = all plants completely defoliated). A, the mean reaction of cotton cultivars to strains T-1, intermediate, and SS-4 supplied by W. C. Schnathorst; B, the mean reaction of four cotton cultivars to isolates from randomly selected, infected, field-grown plants of cultivars Acala SJ-5, Acala SJ-2, and 70-110; C, the mean reaction of cotton cultivars to isolates from the cultivars Acala SJ-5, Acala SJ-2, and 70-110, defoliated by *Verticillium* wilt.

TABLE 1. Inoculum density of *Verticillium dahliae* in leaves of infected plants of two cotton cultivars under greenhouse (inoculated) and field (naturally infected) conditions

Leaf symptoms	Acala SJ-2			Acala SJ-5		
	Replicates (no.)	Lamina (ppg)	Petiole (ppg)	Replicates (no.)	Lamina (ppg)	Petiole (ppg)
<b>Greenhouse<sup>a</sup></b>						
Chlorosis absent	10	1,400	1,900	10	1,400	900
Chlorotic, nondehiscent	27	33,000	61,000	23	29,000	46,000
Chlorotic, dehiscent	18	62,000	218,000	17	80,000	122,000
<b>Field</b>						
Chlorosis absent	10	80	10	...	...	...
Chlorotic, nondehiscent	10	18,000	313,000	10	21,000	138,000
Chlorotic, dehiscent	10	66,000	815,000	10	29,000	1,100,000

<sup>a</sup>T-1, isolated from cotton and described as the defoliating strain of *V. dahliae* by Schnathorst and Mathre (11).



Acala SJ-5. Both inoculated greenhouse-grown plants and naturally infected field-grown plants were tested. Except in lamina tissue, leaves of field-grown plants had greater ID than leaves of greenhouse-grown plants. Normal-appearing leaves had the lowest ID followed by chlorotic nondehiscent and fully chlorotic dehiscent leaves (Table 1).

The IDs of both lamina and petiole tissue of greenhouse-grown plants was more variable than those ID of similar tissues of field-grown plants. Thus, while tissues of cultivar Acala SJ-5, the more tolerant of the two cultivars, generally had lower IDs than similar tissues of Acala SJ-2, there were reversals even though tests were replicated 18–27 times (Table 1). Fewer tests of field-grown plants were made, but the results were quite uniform. Very few conidia were detected in normal-appearing leaves. Acala SJ-5 petiole, but not lamina, tissues of chlorotic nondehiscent leaves had lower IDs than similar tissues of Acala SJ-2, the more susceptible cultivar. Differences between cultivars occurred in lamina tissue of fully chlorotic dehiscent leaves. However, the ID of petiole tissue of fully chlorotic dehiscent leaves of both cultivars was similar— $0.8 \times 10^6$ – $1.1 \times 10^6$  conidia per gram of fresh tissue (Table 1).

## DISCUSSION

Isolates of *V. dahliae* that uniformly defoliated cotton plants in these greenhouse tests did so without regard to the differential tolerances known for the cultivars under field conditions (1). This also was true for strain T-1 described by Schnathorst and Mathre (11) and four others, similar to T-1, discovered here. Two uniformly defoliating strains were isolated from defoliated plants of cultivars 70-110 and Acala SJ-5, the least and most tolerant cultivars, respectively, from which isolations were made. No uniformly defoliating isolates were found among those isolated from randomly selected, infected plants. However, this may have been due to chance, since only 27 such isolates were tested. Sixteen of 54 isolates of *V. dahliae* tested defoliated some, but not all, plants of the less-tolerant cultivars 70-110 and Acala SJ-2 while they induced no defoliation of the more tolerant cultivars Acala SJ-4 and Acala SJ-5. Thus, while a small population of isolates was tested, considerable heterogeneity in pathogenic aggressiveness was observed. Also, the isolates from the cultivars most tolerant of Verticillium wilt generally were more aggressive than isolates from the least tolerant cultivars, whether they were isolated from randomly selected, infected plants or from plants defoliated by Verticillium wilt. Further, isolates from defoliated plants, without regard to the cultivars from which they came, were more aggressive than isolates taken from randomly selected, infected plants.

Results of determinations of the internal ID (conidia) of *V. dahliae* in cultivars Acala SJ-2 and Acala SJ-5 indicate that tolerance is due to the ability of tolerant cultivars to inhibit the rate of development of the fungus within plants (Table 1). These results agree with anatomic pathology studies made by Garber and Houston (4). They observed abundant fungal development within vascular tissues of the susceptible cultivar Delta Pine 15, but scant development within the then tolerant cultivar Acala 4-42. They also proposed the concept that symptom expression in leaves results from germination of conidia at the site of the symptom. Thus, disease severity would be a function of (number of conidia)  $\times$  (leaf sites)  $\times$  (germination percentage). Observations made here support that concept. A similar observation was made by Melouk and Horner (8) on mints differentially susceptible to Verticillium wilt.

Data on internal ID of *V. dahliae* from tests made on field-grown plants were less variable than data on greenhouse-grown plants (Table 1). The ID of *V. dahliae* in leaves with interveinal chlorosis only had fewer conidia than fully chlorotic dehiscent leaves. In field-grown plants, internal ID of *V. dahliae* in lamina tissue of chlorotic dehiscent leaves of the more tolerant cultivar, Acala SJ-5, were lower than those of the less tolerant cultivar, Acala SJ-2. However, the ID of *V. dahliae* in petiole tissue of dehiscent leaves was essentially the same for both cultivars. The data indicate, therefore, that dehiscence, occurring later in more tolerant cultivars than in more susceptible cultivars, occurs when a critical

ID is attained, regardless of cultivar.

The data reported here suggest a continuum of pathogenic aggressiveness among isolates of *V. dahliae* from cotton which is influenced by selection pressure applied by differentially tolerant cultivars. These observations agree with observations made on Verticillium wilt of tomato by Grogan et al (5).

Data reported here contradict the concept of a severe (T-1) and mild strain (SS-4) affecting cotton to explain the demise of the once-tolerant cotton cultivar, Acala 4-42, in San Joaquin Valley (SJV) cotton industry (10,11). This concept, proposed by Schnathorst and Mathre (11), was based in part upon the assumption that cotton was not defoliated by Verticillium wilt before about 1960 (11); in fact, defoliation was reported much earlier to be a normal part of the disease syndrome observed in plants of susceptible cultivars. According to Harrison (6), J. A. Denny observed 90% defoliation of plants of the cultivar Durango near Fresno on 20 September 1918, although identity of the pathogen was not established until 1930 when Shapovalov and Rudolf (12) reported severe damage of cotton in Kern County by Verticillium wilt. Likewise, in 1950, Presley (9) illustrated complete defoliation of susceptible cultivars by *V. dahliae*. He also pointed out that certain cultivars were resistant to Verticillium wilt (did not defoliate) in Arizona, but defoliated in response to Verticillium wilt in Mississippi. Secondly, isolates of the fungus that have the ability to cause uniform defoliation of cotton plants do so in the greenhouse without regard to tolerance (Table 1) observed in the field (1). Significantly, two of four such isolates discussed here were from a highly susceptible cotton cultivar, 70-110. Other isolates of the fungus reported here defoliated some, but not all, plants within cultivars, while others never caused defoliation. Inoculated greenhouse-grown plants appeared to express a more severe disease syndrome than generally occurs in the field, although differences in tolerance toward less aggressive isolates of *V. dahliae* were detected in such tests (Fig. 1, Table 1).

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