Verticillium Wilt Disease of Cotton: Influence of Inoculum Density in the Field

L. J. Ashworth, Jr., O. C. Huisman, D. M. Harper,
L. K. Stromberg, and D. M. Bassett

Respectively, plant pathologist, assistant professor, and research associate, University of California, Berkeley 94720; farm advisor, University of California, Cooperative Extension, Fresno 93702; and agronomist, University of California, Shafter 93263.
We thank H. B. Cooper, U.S. Cotton Research Station, Shafter, CA 93263 for use of certain unpublished breeding trial results.
Accepted for publication 22 November 1978.

ABSTRACT

ASHWORTH, L. J., Jr., O. C. HUISMAN, D. M. HARPER, L. K. STROMBERG, and D. M. BASSETT. 1979. Verticillium wilt disease of cotton: Influence of inoculum density in the field. Phytopathology 69:483-489.

These are the first quantitative data on effects of inoculum of *Verticillium dahliae* on infection, disease severity, and yield of a tolerant host under field conditions. Infection rates were greater at high than at low inoculum densities. Degree of infection at harvest varied between genotypes; the most tolerant cultivars (Acala SJ-4 and Acala SJ-5) were more prone to infection than the less tolerant cultivars (70-110 and Acala SJ-2). However, the former showed fewer foliage symptoms and less defoliation than the latter and their yields were unaffected at the maximum inoculum density tested, 21 microsclerotia (MS) per gram of soil. The most sensitive cultivar was quite tolerant of Verticillium wilt disease, based upon comparative yields, although visually it appeared to be severely affected at even the lowest inoculum density, 1.7 MS/g soil. The data suggest that tolerant cultivars withstand defoliation at relatively large inoculum densities. An alternate but untested hypothesis, however, is that the less tolerant genotypes are susceptible to a greater proportion of the native soil population of *V. dahliae* than the most tolerant cultivars.

Additional key words: Verticillium albo-atrum, soilborne pathogens, fungal disease, epidemiology, *Gossypium hirsutum,* disease resistance.

Disease incidence and severity may be synonymous in lethal diseases, but it is possible for damage to be slight within tolerant or resistant populations, even in epidemics. Both extremes occur among hosts of *Verticillium dahliae* Kleb.

The quantitative influence of inoculum of *Verticillium dahliae* Kleb. on wilt of a highly susceptible host, the pistachio nut tree, was recently described (4). Infections by *V. dahliae* usually are lethal to rootstocks of pistachio (*Pistacia atlantica* Desf. and *P. terebinthus* L.) and to scions (cultivars of *P. vera* L.). Therefore, it was possible to relate the percentages of pistachio trees killed in commercial orchards over a 2-yr period with inoculum densities, expressed as the number of microsclerotia (MS)/g of air dry soil (4). We report here the results of field tests of the influence of inoculum density of *V. dahliae* upon disease incidence and disease severity in cotton. (*Gossypium hirsutum* L.) a species much more tolerant of Verticillium wilt than the pistachio nut tree. Similar information for tomato, also a wilt tolerant species, is reported in another paper (2).

MATERIALS AND METHODS

Thirty-seven comparisons were made between two differentially tolerant cotton cultivars in naturally infested commercial fields. Cultivar Acala SJ-2 was less tolerant than Acala SJ-4, but it was considerably more tolerant than cultivars developed for areas of the USA where Verticillium wilt is not a problem (5). Entries in all tests were replicated four times in four-row blocks. Blocks were, depending upon the test, 15 m, 0.5 km, or 0.8 km long. In all cases, the center two rows were harvested by mechanical cotton pickers.

In the comparisons described above, isolations were made near harvest from 10 plants of each cultivar to verify that the vascular necrosis symptom of wilt disease was caused by *V. dahliae.* Isolations were made 15–25 cm above the soil line, and inoculum determinations were made in midsummer.

Field-to-field yield variability in commercial plantings was related to inoculum density and infection percentages in 13 comparisons of Acala SJ-1. Inoculum densities were determined in midsummer and infection was determined at harvest. Yields were taken from records of appropriate cotton gins.

00031-949X/79/000088$03.00/0
©1979 The American Phytopathological Society

Field tests were made at two locations about 50 miles apart in Fresno County of California. These locations were chosen for differences in soils. Soil utilized for experiments at the Kearney Horticultural Field Station (KHFS) was a Hanford sandy loam with a compacted layer at depths 15–60 cm below the soil surface (7). Penetrometer measurements (7) of this dense layer exceeded 18 bars (maximum about 30 bars), of sufficiently high strength to severely limit root growth of cotton and corn. Soil in fields used at the West Side Field Station (WSFS) was a Panoche clay loam and homogeneous to a considerable depth. Penetrometer readings through a depth of about 200 cm did not exceed 12 bars, thus it did not impede root growth of cotton and corn in tests of Grimes, et al (7). Inoculum densities of *V. dahliae*, measured during midsummer as before (9), initially were less than 1 MS/g soil at both locations.

Inoculum for field tests begun in 1974 was produced at WSFS in 1973 by growing tomato (cultivar Red Top) in a 0.5-ha plot of naturally infested soil. Judging from vascular necrosis symptoms, nearly all plants were infected with *V. dahliae* by midsummer. In December, after the plants had been killed by frost and when microsclerotia were abundant in stem tissues, the plants were raked into windrows. When dry, plants were stored under cover until used to infest plots at both WSFS and KHFS.

There were four inoculum treatments, a noninoculated control and three differential infestations, at KHFS and WSFS in 1974. All treatments were replicated five times. The infested tomato vines were broken into short segments by hand and spread, also by hand, over the four-row blocks at rates of approximately 10, 70, and 400 g/m^2, which resulted in inoculum densities of 4.3, 6.3, and 80 MS/g soil at KHFS and in 2.6, 4.7, and 23.9 MS/g soil at WSFS; control blocks at both locations had 1.9 MS/g soil. Blocks at KHFS were 280 m^2 and were 120 m^2 at WSFS. Inoculum was spread on the blocks 31 January to 2 February 1974, the soils were disked, and planting beds (1 m apart) were made immediately. Plots were split in the centers and cotton (cultivar Acala SJ-2) and tomato (cultivar Early Pak-7) were planted in one-half the length of each block.

AT KHFS in 1975, all blocks of the 1974 test were replanted as described above. A new site was established at WSFS in 1975. There, a block of about 1 ha was fumigated with approximately 200 kg of 57% methyl bromide and 43% chloropicrin on 21 November 1974 when soil temperature was about 14 C at injection depth, in order to reduce native inoculum to a low level. The fumigant was

injected 3.2 cm deep from shanks set 4.8 cm apart. The plot was not covered with a polyethylene tarp following injection of the fumigant. The inoculum used to infest this test site was infected tomato vines harvested from the 1974 test at KHFS. Vines were handled as before except that they were passed through a dry bean harvester which reduced them to fragments ranging 1–4 cm in length. Blocks in this test were eight rows wide by 90 m long and there were five replications of four inoculum densities. No vine material was added to control blocks; others were infested with 3.6, 18.2, or 182 kg of vine material per block on 27 February 1975. Blocks were disked and planting beds were made immediately following distribution of inoculum. As before, blocks were split in the center, resulting in 45-m-long blocks each for cotton (cultivar Acala SJ-2) and tomato (cultivar Early Pak-7).

The cotton portion of the 1975 test site described above was used for further tests with cotton in 1976 and 1977. In 1976, each eight-row × 45 m block was divided into four blocks each ~ 11 m in length to accommodate planting of four differentially susceptible cotton cultivars. They were: 70-110, a highly susceptible type; Acala cultivar SJ-2, with moderate tolerance; and cultivars SJ-4 and SJ-5, both more tolerant than SJ-2. The 1977 planting was identical

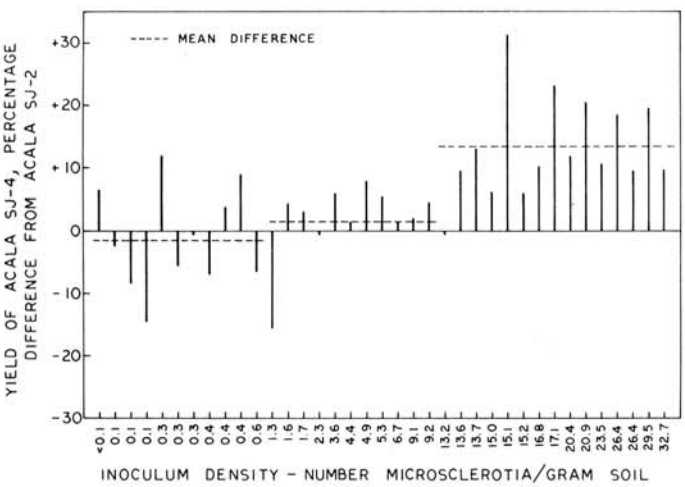

Fig. 1. Comparative yields of cotton cultivars Acala SJ-2 and SJ-4 in 37 soils naturally infested with microsclerotia of *Verticillium dahliae*.

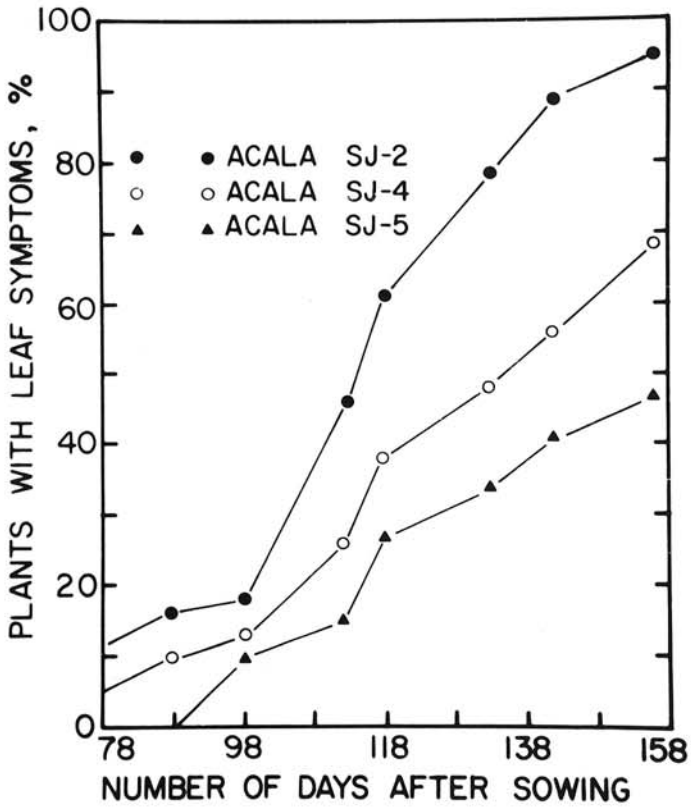

Fig. 2. Differential development of leaf symptoms of Verticillium wilt disease among three cotton cultivars at an *Verticillium dahliae* inoculum density of ~41 MS/g soil.

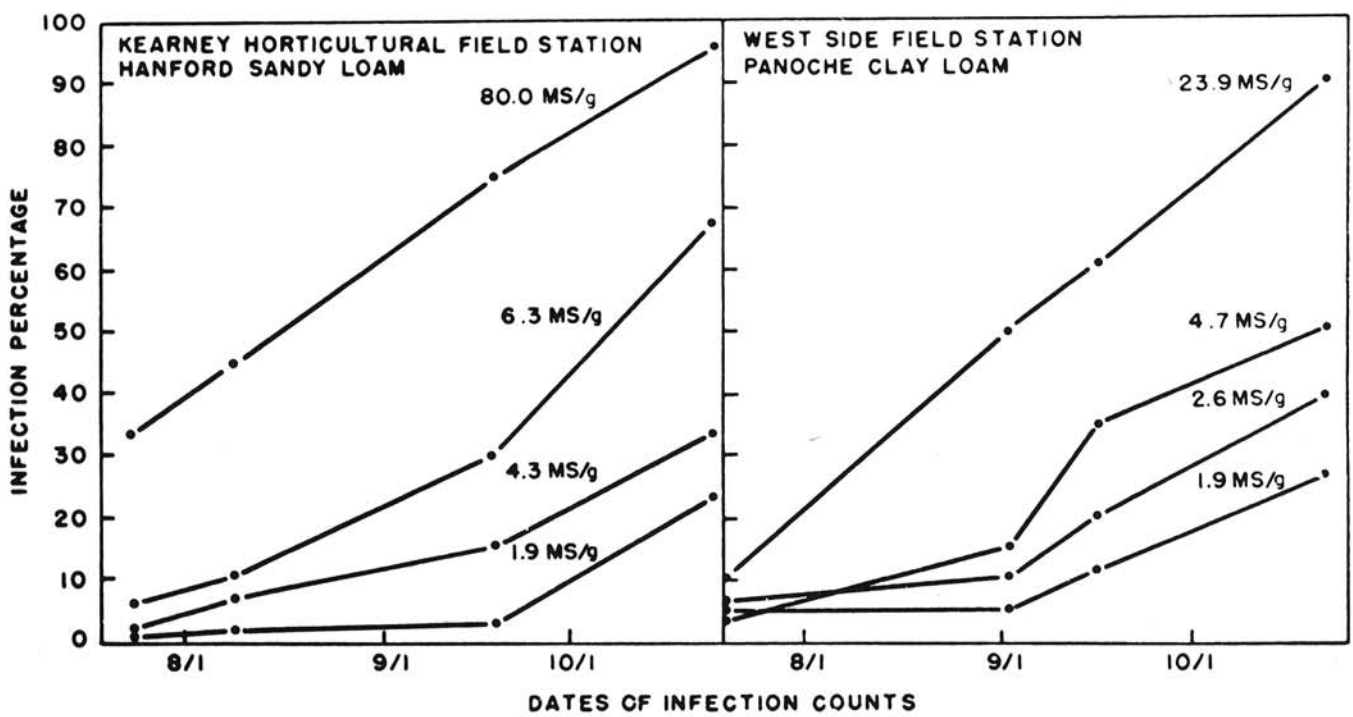

Fig. 3. The relationship between inoculum density of *Verticillium dahliae* and percentage infection of cotton cultivar Acala SJ-2 (a less tolerant cultivar) at two California locations in 1974.

with that of 1976, except that cultivar 70-110 was replaced with Acala SJ-4. This was the 3rd yr these blocks were planted following the initial inoculation and inoculum was variable; the average for the field was 41 MS/g soil.

In tests made at KHFS and WSFS, either the center two or center four rows within the blocks were harvested depending upon whether blocks were four or eight rows wide. All tests were machine harvested.

The amounts of inoculum used in the tests at the field stations were comparable with amounts observed in commercial fields.

RESULTS AND DISCUSSION

Differential responses of cotton cultivars to inoculum. *Internal necrosis symptom.* In 37 comparisons of cultivars Acala SJ-2 and SJ-4 (Fig. 1) *V. dahliae* was isolated from approximately 85% of plants with vascular necrosis at harvest time, indicating that vascular necrosis is a reliable symptom of infection by *V. dahliae*. Acala SJ-4, the more tolerant of the two cultivars was more prone to infection than the less tolerant cultivar, Acala SJ-2; vascular necrosis was detected in 80–96% of Acala SJ-4 plants where

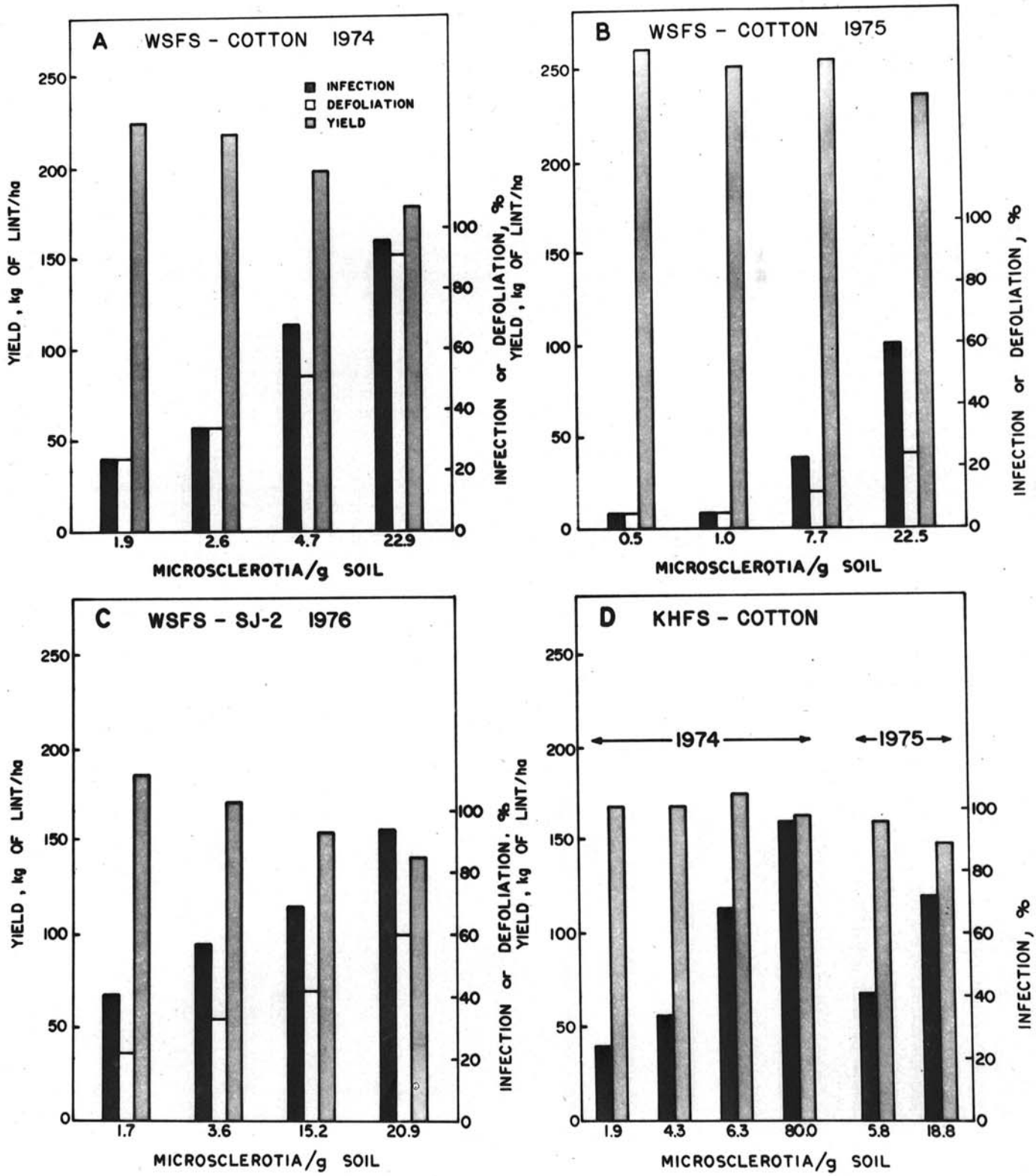

Fig. 4. The relationship between inoculum density of *Verticillium dahliae* and percentage infection, defoliation, and yield of cotton cultivar Acala SJ-2 at the West Side Field Station in California in **A)** 1974, **B)** 1975, **C)** 1976; and at the Kearney Horticultural Field Station in California **D)** in 1974 and 1975.

inoculum density was ≤0.1 MS/g soil while <10% of Acala SJ-2 plants developed vascular necrosis following exposure to similar low inoculum densities. Nevertheless, Acala SJ-4 always outyielded Acala SJ-2 where inoculum density exceeded ~10 MS/g soil (Fig. 1) demonstrating its tolerance to wilt disease.

In another test, cultivars Acala SJ-4 and SJ-5 (the latter also a highly tolerant cultivar) had 70–90% infected plants (with vascular necrosis at harvest time), whether inoculum density was 1.7 or 20.9 MS/g soil (Fig. 5). In the same test, however, each increase in inoculum density resulted in an increase in the percentage of infected plants of less tolerant cultivars 70-110 and Acala SJ-2. The cause for these anomalous results is not known.

Leaf symptoms. Differential development of leaf symptoms was observed among cultivars, as shown by results of a comparison made in 1977 of three cultivars in soil at WSFS infested with 41 MS/g soil. The least tolerant cultivar (Acala SJ-2) had more plants showing leaf symptoms than the more tolerant cultivars (Acala SJ-4 and SJ-5) at each observation, beginning 78 days after planting. Also, the differences increased as the growing season progressed (Fig. 2). The type of leaf symptom observed at WSFS was typical for the disease; extensive interveinal chlorosis followed by necrosis (Fig. 7A), wilting, and abscission.

The relationship between inoculum density and infection rate. The rate of development of infection in the moderately tolerant cultivar, Acala SJ-2, was determined at both WSFS and KHFS in 1974. Percentages of plants infected during 17–24 July, based on isolations made on 20 plants from each of the five replications, were less than 10% except in soils with an inoculum density of 80 MS/g soil, where 30% of plants were infected (Fig. 3). Midseason counts, two at each location, were based on foliage symptoms and involved all plants in the two record rows of each block. The data (Fig. 3) show that an increase in inoculum density generally resulted in an increase in the percentage of infected plants as the growing season progressed. Final infection (vascular necrosis) counts were made in late October by observing 100 plants per replication. As with leaf symptoms, increased inoculum density resulted in increased percentage of plants with vascular necrosis near harvest time.

Infection, defoliation, and yield. A numerical relationship between inoculum density and percentage of infected plants was demonstrated in 1971 (3). Later tests, however, showed that neither inoculum density nor percentage of infected plants at harvest were related to yield of cultivar Acala SJ-1 in commercial fields (Fig. 6). Apparently, interlocation variability of other parameters affecting yield outweighted the influence of *V. dahliae*. These observations led to tests in which inoculum was varied within location, as discussed below. Yield reductions were associated with defoliation at the WSFS (Fig. 4A, C), but at KHFS, where defoliation at harvest was nil, yields were not reduced, Fig. 4D. Differences in early maturation and stunting were obvious between blocks with 1.9 and 80 MS/g soil inoculum densities at KHFS in 1974. However, defoliation essentially was absent at KHFS even though leaf symptoms were abundant (Fig. 4D). At KHFS, leaf symptoms were atypical with interveinal necrosis being the dominant symptom, Fig. 7B. On the other hand, typical spreading chlorosis, followed by necrosis (Fig. 7A) and defoliation (Fig. 7C, D) occurred at WSFS where yields were diminished in 1974 and 1976 as defoliation percentages increased (Fig. 4A, C). Little defoliation occurred at WSFS in 1975 and yields were not significantly reduced, even at an inoculum density of 22.5 MS/g soil (Fig. 4B).

Symptoms observed at KHFS (Fig. 7B) suggested that infections within leaves were severely limited, which may account for the absence of defoliation. This situation is unusual since defoliation is typical of this disease of cotton. Differences in strains of *V. dahliae* at the two locations do not appear to be responsible for the anomalous results described above because, except for background amounts, inoculum at both locations was from the same original

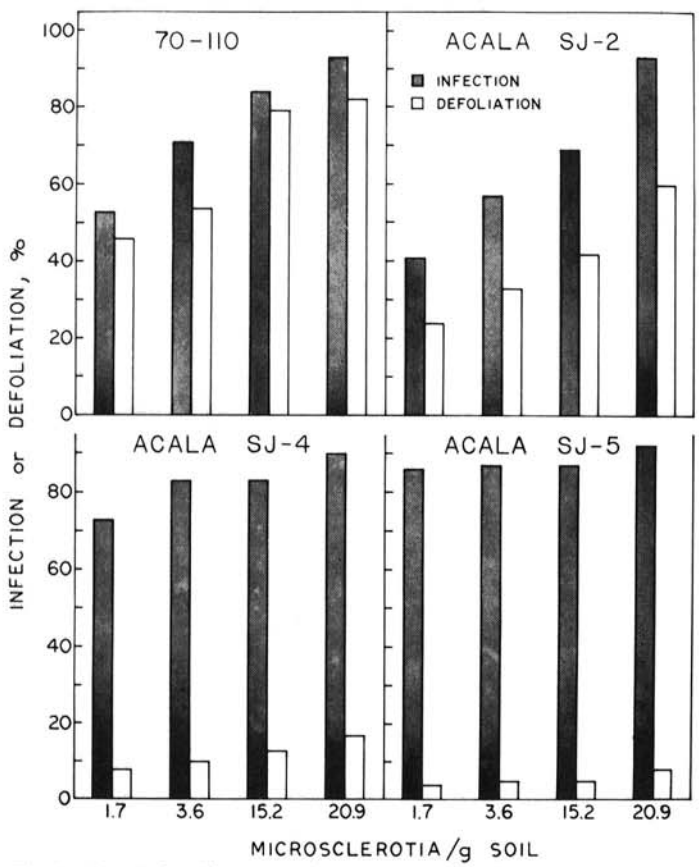

Fig. 5. The relationship between inoculum density of *Verticillium dahliae* and percentage infection and defoliation of four cotton cultivars at the West Side Field Station in California in 1976.

TABLE 1. The relationship between microsclerotial inoculum density of *Verticillium dahliae* and yields of four cotton cultivars at the West Side Field Station in California in 1976.

Cotton cultivars	Yield (kg lint/ha) of cotton grown in soil with inoculum densities of:			
	1.7[a]	3.6	15.2	20.9
70-110	198[b]	170	149	125
Acala SJ-2	202	188	167	155
Acala SJ-4	208	200	177	193
Acala SJ-5	184	182	178	188
LSD ($P = 0.01$)	27			

[a]Number of microsclerotia per gram of air dry soil.

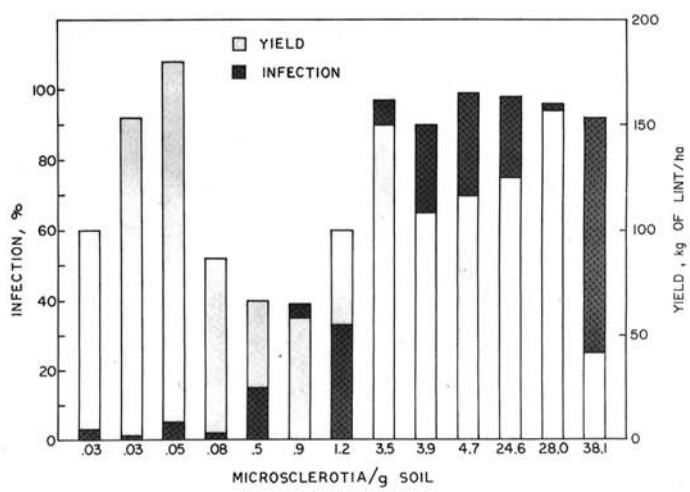

Fig. 6. Percentages of infection by *Verticillium dahliae* (based upon vascular necrosis symptoms at harvest time) and yields of cotton cultivar Acala SJ-1 relative to microsclerotial inoculum concentrations at 13 naturally infested locations.

source. That is, inoculum for the 1975-1976 tests at WSFS came from the 1974 test at KHFS, and inoculum for 1974 tests at both locations came from infected tomato grown at WSFS in 1973 (see Materials and Methods section).

Resistance of cotton plants to defoliation is associated with cultivar tolerance to relatively high *V. dahliae* inoculum densities. Cultivar 70-110 had the greatest amount of defoliation (Fig. 5, 8) and the lowest yields of the four cultivars in a test at WSFS in 1976, in which there were four inoculum densities (Table 1). Cultivar Acala SJ-2 was intermediate in expression of resistance to defoliation (Fig. 5, 8) and in yield (Table 1). Cultivars Acala SJ-4 and Acala SJ-5 had less than 20% defoliation (Fig. 5) and yields were not depressed, regardless of inoculum density (Table 1). The final amount of infection was not correlated with cultivar yield differences (Fig. 5, Table 1).

Cultivar 70-110, although suffering severe defoliation compared

Fig. 7. Symptoms of Verticillium wilt caused in cotton by *Verticillium dahliae* in California. A comparison of **A**) typical leaf symptoms on cotton cultivar Acala SJ-2 with **B**) atypical leaf symptoms observed on that cultivar at the Kearney Horticultural Field Station. (C–D) Relative amounts of defoliation on cotton cultivar Acala SJ-2 by *V. dahliae* at the West Side Field Station in plots infested with **C**) 1.9 and **D**) 22.9 microsclerotia per gram of soil.

to that of cultivars Acala SJ-2, Acala SJ-4, and Acala SJ-5 in these tests, was quite tolerant to wilt disease when only yield was considered. This cultivar yeilded as well as all other cultivars at inoculum densities of 1.7 and 3.6 MS/g soil and as well as cultivar Acala SJ-2 at 15.2 MS/g soil (Table 1).

Seasonal variability in inoculum density and disease severity. The overall average inoculum in cotton soils of the San Joaquin Valley appears to be relatively stable. The mean inoculum density of 40 fields assayed in 1971 was 7.4 MS/g soil. The maximum inoculum density observed was 47 MS/g soil and only ~20% of fields had more than 10 MS/g soil. Similar results were observed when soils of 120 fields were assayed in 1977. The mean inoculum density was 5.3 MS/g soil, the maximum was 73 MS/g soil and, as before, ~20% of fields had more than 10 MS/g soil.

Total infection (amount of infection at harvest time) was positively related to inoculum density in all experiments. However, similar amounts of inoculum did not always induce the same amount of infection. For instance, an inoculum density of ~20 MS/g soil resulted in 95% infection at harvest in 1974 (Fig. 4A) and 1976 (Fig. 4C) but in only 60% infection in 1975 at the same location (Fig. 4B). Fungistasis of microsclerotia (as discussed in an earlier report [1]) was assumed to be an important factor affecting these differences in inoculum efficiency. This is likely to be true because each year infection of cotton by *V. dahliae* generally is favored for a month or more before harvest in the San Joaquin Valley of California (1).

For reasons discussed above, the quantitative effect of inoculum on defoliation and yield varies from year to year. The season-to-season performance of cultivar Acala SJ-2 illustrates this point. Relatively little defoliation occurred in 1975 and yield of SJ-2 in the treatment with 22.5 MS/g soil did not differ significantly from that in the treatment with 0.5 MS/g soil (Fig. 4B). On the other hand, an inoculum density of 22.9 MS/g soil in 1974 caused a significant reduction in yield (49 kg of lint; LSD [P = 0.01] = 20 kg of lint), compared with the lowest inoculum density, 1.9 MS/g soil (Fig. 4A). In 1976, an inoculum density of 15.2 MS/g soil, but not 3.6 MS/g soil, significantly reduced yield, compared with the lowest inoculum density (LSD [$P = 0.0$l] = 26 kg of lint) (Fig. 4C).

Although inoculum densities and environmental differences varied from season to season, the information presented here can be used to make competent management decisions in selecting which cotton cultivars to be grown in specific situations. Information for decision making is based upon the comparative performance of cultivars at 37 locations having various inoculum densities during 4 yr, 1973–1976. Cultivar Acala SJ-2 more often than not outyielded cultivar Acala SJ-4 when exposed to inoculum densities below about one MS per gram of soil. On the other hand, cultivar Acala SJ-4 usually outyielded cultivar Acala SJ-2 at inoculum densities greater than about one MS per gram of soil, although the average difference in yield between about one and 10 Ms per gram of soil was less than 3% (Fig. 1). Above inoculum densities of ~10 MS/g, however, cultivar Acala SJ-4 always outyielded cultivar Acala SJ-2; the difference was as great as 30% but averaged 13% (Fig. 1).

Differences in performance of genotypes in these tests appeared to be related to differential ability of some genotypes to withstand relatively higher inoculum densities of *V. dahliae* than others. We saw no evidence of new pathogenic strains as reported for cotton in Russia by Popov, et al (11). They observed that resistant genotypes introduced between 1940 and 1950 reduced the incidence of Verticillium wilt disease from 80–100% to 14–15%. Within 10 yr, however, their resistant cultivars, C-460 and 108-F, were 90_B–100% affected. These were replaced by cultivars Tashkent-1, -2, and -3 which originated from crosses begun in 1959 (10); but they also failed within 10 yr (11). Parallel with their observations with cotton, was the recent appearance in California of a strain of *V. dahliae* that seriously affects tomato cultivars with the *Ve* gene for resistance (8,12). In addition, cotton cultivar Acala 4-42 now typically exhibits severe defoliation in California (13) although initially it was highly tolerant of *V. dahliae* (6,14).

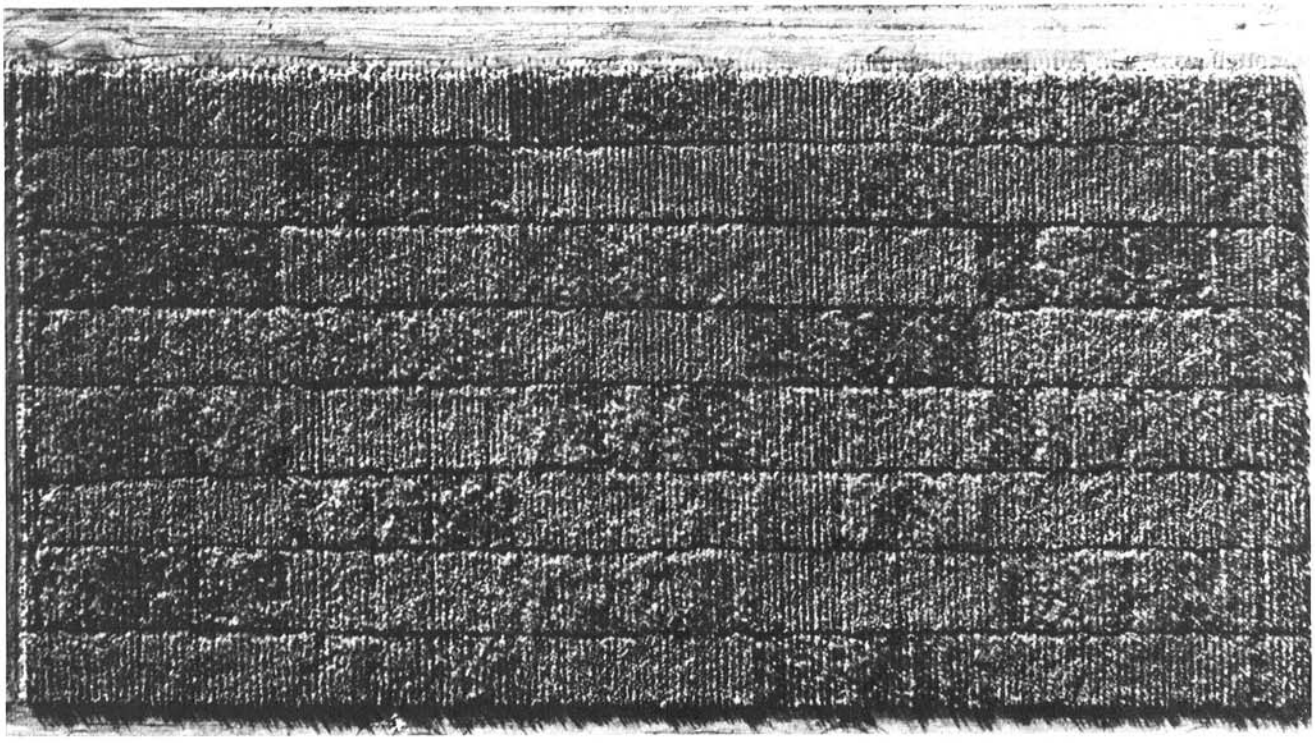

Fig. 8. The relationship between inoculum density of *Verticillium dahliae* and defoliation of cotton cultivars at West Side Field Station, 1976, detected from the infrared film near time of harvest. Cultivars are planted in 32-row blocks with blocks having eight-row sub-blocks infested with 1.7, 3.6, 15.2, and 20.9 microsclerotia per gram of soil. Cultivars Acala SJ-4 and Acala SJ-5 were relatively unaffected (uniformly light colored blocks with dense foliage). Cultivar 70-110 was severely defoliated at all inoculum densities (uniformly darker blocks), and cultivar Acala SJ-2 was severely defoliated at highest inoculum densities (variable blocks).

Considering these observations, another interpretation of our data, might be as follows. Cultivar 70-110 may exhibit more defoliation than cultivar Acala SJ-2 because it is sensitive to a greater number of defoliating strains within the population of *V. dahliae* (13) (Fig. 5). The same may be said for cultivar Acala SJ-2 relative to cultivars Acala SJ-4 and Acala SJ-5. Although fewer than 10% of cultivar Acala SJ-5 plants were defoliated regardless of inoculum density, the percentage of defoliated cultivar Acala SJ-4 plants increased with each increase of inoculum density (Fig. 5). This hypothesis should be tested further; if it is true, the rate of selection of strains highly virulent to cultivar Acala SJ-4 could be reduced by growing cultivar Acala SJ-2 except where inoculum densities require the more tolerant cultivar (Fig. 1). Thus, the useful life span of other tolerant cultivars such as Acala SJ-5 might thus be extended by using this management practice.

LITERATURE CITED

1. ASHWORTH, L. J., Jr., O. C. HUISMAN, R. G. GROGAN, and D. M. HARPER. 1976. Copper induced fungistasis of microsclerotia of Verticillium albo-atrum and its influence on infection of cotton in the field. Phytopathology 66:970-977.
2. ASHWORTH, L. J., Jr., O. C. HUISMAN, D. M. HARPER, and L. K. STROMBERG. 1979. Verticillium wilt of tomato: influence of inoculum density and root extension upon disease severity. Phytopathology 69:490-492.
3. ASHWORTH, L. J., Jr., O. D. McCUTCHEON, and A. GEORGE. 1972. Verticillium albo-atrum: the quantitative relationship between inoculum density and infection of cotton. Phytopathology 62:901-903.
4. ASHWORTH, L. J., Jr., and G. ZIMMERMAN. 1976. Verticillium wilt of the pistachio nut tree: Occurrence in California and control by soil fumigation. Phytopathology 66:1449-1451.
5. BASSETT, D. M. 1971. Performance of cotton varieties on different soils and in different locations in the San Joaquin Valley. Cotton disease research in the San Joaquin Valley, California. Pages 144–149 in Univ. Calif. Div. Agric. Sci, Res. Rep. for 1968-69. 251 pp.
6. GARBER, R. H., and B. R. HOUSTON. 1967. Nature of Verticillium wilt resistance in cotton. Phytopathology 57:885-888.
7. GRIMES, D. W., R. J. MILLER, and P. L. WILEY. 1975. Cotton and corn root development in two field soils of different strength characteristics. Agron. J. 67:519-523.
8. HALL, D. H., and K. A. KIMBLE. 1972. An isolate of Verticillium found pathogenic to wilt-resistant tomatoes. Calif. Agric. 26:3.
9. HUISMAN, O. C., and L. J. ASHWORTH, Jr. 1974. Quantitative assessment of Verticillium albo-atrum in field soils: procedural and substrate improvements. Phytopathology 64:1043-1044.
10. IBRAGIMOV, S. I., and B. P. STRAUMOL. 1971. Selection of high-yield varieties of cotton highly resistant to wilt. Agric. Biol. 6:338-343. (Trans. from Russian by L. A. Hutchison, Muir College, Univ. Calif., San Diego).
11. POPOV, V. I., T. A. TARUNINA, and Z. U. USMANOV. 1972. On the physiological races of Verticillium dahliae Kleb. a causal agent of cotton wilt. Mycol. Phytopathol. 6:500-502. (Trans. from Russian by L. A. Hutchison, Muir College, Univ. Calif., San Diego).
12. SCHAIBLE, L., O. S. CANNON, and V. WADDOUPS. 1951. Inheritance of resistance to Verticillium wilt in a tomato cross. Phytopathology 41:986-990.
13. SCHNATHORST, W. C., and D. E. MATHRE. 1966. Host range and differentiation of a severe form of Verticillium albo-atrum in cotton. Phytopathology 56:1155-1161.
14. TURNER, J. H. 1974. History of Acala cotton varieties bred for San Joaquin Valley, California. U.S. Dep. Agric., ARA W-16. 23 pp.