The Influence of Several Crop Sequences on the Incidence of Verticillium Wilt of Cotton and on the Population of Verticillium dahliae in Field Soil

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The authors thank the California Planting Cotton Seed Distributors, Bakersfield, for their financial support of this research and J. S. Pullman for technical assistance.

Accepted for publication 27 February 1978.

ABSTRACT

BUTTERFIELD, E. J., J. E. DE VAY, and R. H. GARBER. 1978. The influence of several crop sequences on the incidence of Verticillium wilt of cotton and on the populations of Verticillium dahliae in field soil. Phytopathology 68: 1217-1220.

Since *Verticillium dahliae* was not detectable by soil assay or by subsequent infection of cotton plants, it was considered to be eradicated from most field test plots following a 1-yr rotation between cotton and paddy rice (continuous flooding). In contrast, populations of *V. dahliae* per gram of soil were increased with rotations to safflower or with continuous cotton. Keeping soil wet but not flooded for 6 wk and/or rotations with grain sorghum or dry fallow did not change the concentration of *V. dahliae* propagules in soil. Significant ($P = 0.05$) decreases in disease incidence and increases in cotton lint yields resulted from rotations with paddy rice, perennial ryegrass, and in some fields from safflower, grain sorghum, or frequent soil irrigation followed by grain sorghum.

Verticillium wilt of cotton, incited by *Verticillium dahliae* Kleb., is the major disease problem in the production of cotton in the USA. In California, the disease is now partially managed by the use of wilt-tolerant Acala cultivars (*Gossypium hirsutum* L.) but strains of *V. dahliae* highly virulent to those cultivars are prevalent (12) and cause significant losses in yield. Tarping and deep placement of fumigants have reduced the incidence of Verticillium wilt in cotton (3, 15) but have not eradicated the fungus from field soils. Erratic plant responses and high costs, however, have presently eliminated soil fumigation for control of Verticillium wilt in cotton. Systemic fungicides and growth-regulating compounds have shown promise for control of Verticillium wilt but their use is not economically feasible at present (4). Improvements in cotton yields and reduction of disease severity have been reported in numerous studies (11) involving the interruption of continuous cotton culture with other crops or with fallowing. The use of cultural practices, including crop rotations, for management of Verticillium wilt has been reviewed by Ranney (11). Attrition of propagules of *V. dahliae* under nonhost crops is slow (6) and evidence indicates that even long rotations of planting to nonsusceptible crops do not eliminate *V. dahliae* from the soil (13). The development of a dry soil assay technique using an Anderson Sampler (2, 5) has provided a reliable method to estimate the numbers of propagules of *V. dahliae* in field soil. A further modification of that technique (1) was used in the present study.

Numbers of soilborne propagules of *V. dahliae* in soil have been reduced by periods of flooding. Menzies (8) found that 6 wk of open field flooding reduced the concentration of *V. dahliae* to undetectable concentrations, as indicated by soil assay or by baiting with susceptible tomato plants. Disappearance of propagules was hastened by amendment of soil with 1% alfalfa meal or 0.1% sucrose. Using artificially infested soil in laboratory studies, Nadakavukaren (10) obtained similar results. All infectivity and all detectable propagules were eliminated within 2 mo in soils held at field capacity at temperatures of 10 C or higher. At 30 C all propagules were eliminated within 14 days. In California, cotton growers in Fresno County who practice rotation with rice have observed that this rotation is effective in reducing Verticillium wilt in following cotton crops.

The objectives of this study were to compare the effects of continuous flooding vs. frequent soil irrigation, and paddy rice vs. other rotation crops on the survival of *V. dahliae*, on the incidence of Verticillium wilt in cotton, and on cotton lint yields.

MATERIALS AND METHODS

Crop rotation studies.—Field experiments consisted of 2-yr crop rotations at Five Points, Shafter, and Davis, California. Two separate experiments were conducted at the Five Points location where the area used for the experiments had been cropped to cotton and foliar symptoms of Verticillium wilt had appeared in 15 to 30% of the plants. Soil assays for *V. dahliae* indicated an

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average population of about 20 propagules per gram of soil with no field areas of significant variation observed. The experimental plot consisted of six replications of five treatments. Each individual treatment plot was approximately 8 × 40 m and was separated from adjoining plots by a 1.5-m levee which remained in place throughout the experiment. The first treatment was a dry fallow maintained weed free by hand-hoeing with no additional cultivation or irrigation. The second treatment (wet-sorghum) consisted of a three-times-weekly application of 4-6 cm of water from 20 April through 7 June (105 cm total). Surface water remained no more than 12 hr after the end of each application. Grain sorghum [*Sorghum bicolor* (L.) Moench 'NK 125'] then was planted on 15 June in double rows on raised beds, 102 cm from center to center. The third treatment (dry-sorghum) consisted of grain sorghum planted 15 June after the plot had received the normal single preirrigation of 15 cm. In both sorghum treatments the grain was removed at maturity and the plant debris was incorporated into the soil. The fourth treatment was planted with Verticillium wilt-susceptible safflower (*Carthamus tinctorius* L.). The fifth treatment was planted to cotton cultivar Acala SJ-1.

A following study at the same location utilized 15 wk of soil flooding with rice (*Oryza sativa* L. 'Colusa 1600'). The water level within the plots was maintained at a depth of 15-30 cm by the continuous addition of fresh water. No outlet was provided for surface water circulation. Other treatments in this same test included dry fallow, cotton, and two cultivars of safflower. One safflower cultivar was Gila which is susceptible to Verticillium wilt and the other was an experimental cultivar which is resistant to Verticillium wilt. All plots were planted to Acala SJ-2 cotton in the second year of the tests. Plot sizes were similar to those of the previous study and were similarly separated by permanent levees. Yield data in all cotton plots were obtained by machine harvesting the center six of eight 40 m rows and weighing the seed cotton immediately. No significant variations in moisture content were found.

Field experiments at Shafter were conducted in an area in which the preceding cotton crop had a moderate incidence of foliar symptoms of Verticillium wilt (30-60%). Soil assays indicated an average inoculum density of about five propagules per gram of soil. One field area with a much greater inoculum density was detected and data from it were not used in the tabulation of results.

Eight replications of four treatments were placed in a randomized block design. Each plot was 8 × 20 m and was separated from adjoining plots by a 1.5-m-wide levee. The first treatment was dry-fallow as previously described. The second treatment (wet-sorghum) received a weekly application of 15 cm of water from 20 April through 7 June (105 cm total). Grain sorghum was planted on 15 June. The third and fourth treatments were grain sorghum (dry-sorghum) and cotton as previously described. All plots were planted to Acala SJ-1 cotton in the 2nd yr of the study.

The third location at Davis is 160 km north of most commercial cotton fields in California. In the year before these experiments, 80 to 100% of the cotton plants grown at this location had foliar symptoms of Verticillium wilt. The average inoculum density of *V. dahliae* was measured at 120 propagules per gram of soil. In 1971, alternate 8.1-m-wide strips of the plot were planted to perennial ryegrass (*Lolium perenne* L.) sod. The sod was maintained during 1972 when other rotations were planted in the nonsoded areas. The rotation treatments were the same as those described for the Shafter test except deletion of the dry fallow. Each treatment was replicated four times and the plot sizes were 8 × 8 m. In 1973, the sod was plowed under and the entire plot was planted to Acala SJ-1 cotton.

Soil assays for Verticillium dahliae.—The inoculum densities of *V. dahliae* in the test plots were analyzed with an Anderson Sampler using the technique of Butterfield and DeVay (1). The standard error by this method was 10-12%. A subplot of approximately 60 m^2 was established within each treatment replicate. These subplots were sampled at least four times during each year of the test by taking twelve 30 × 2.5-cm cores from each. The cores from each subplot were bulked, air dried, subsampled three to five times, and analyzed to determine the number of *V. dahliae* propagules per gram of air-dried (6-wk) soil.

Rating of root vigor and disease.—Following the cotton harvest at Shafter, the plots were irrigated and 30 cotton plants from each plot were removed at random. Each root system was visually rated for vigor and the presence of root disease lesions.

Isolation of Thielaviopsis basicola from field soil.—Soil samples from the test plot at Shafter were baited with fresh carrot disks to determine the presence of *T. basicola* (Berk. & Br.) Ferr. For each subplot, 10 fresh carrot disks were treated with 5 g of soil, moistened with 50 μg/ml streptomycin sulfate in water, and incubated for 3 days. Then the soil was washed away and the disks were incubated for an addition 7 days after which each disk was scored for the presence or absence of *T. basicola*

RESULTS

Soil flooding, as practiced with rice culture in the 1974 trial at Five Points, was the most effective treatment for reducing the population of *V. dahliae* in soil (Table 1). The average reduction in inoculum density was approximately 95% in 1 yr. The fungus was not detectable by soil assay and it did not cause infection of the subsequent cotton crop in four of the six replications. The only other treatment that reduced the initial population of *V. dahliae* was the rotation to perennial ryegrass sod which caused an 80% reduction in inoculum density.

In contrast to the continuous flooding treatment with paddy rice, frequent soil irrigation for 6 wk at Shafter, Five Points (1972), and at Davis (wet-sorghum) resulted in no significant decreases in inoculum densities. Similarly, 1-yr rotations to either dry fallow or grain sorghum caused little or no change from the inoculum densities of *V. dahliae* found in 1972. Inoculum densities increased under continuous cotton at Shafter and Five Points and rotation to safflower (another host crop) caused increases in inoculum densities in both tests at Five Points. Inoculum density of the continuous cotton treatments at Davis did not increase during the period of the test.

A strong association existed between inoculum density and foliar and vascular wilt symptoms at Shafter. An association also existed between reduced cotton lint yields and higher inoculum densities as well as higher incidences of foliar and vascular wilt symptoms at Shafter. At Five Points in 1973, the percentage of cotton plants with foliar symptoms of wilt was reduced following fallow, wet-sorghum, or safflower compared to continuous cotton or sorghum. Similarly at Davis, cotton following ryegrass was more vigorous and had fewer plants with foliar and vascular wilt symptoms than did cotton following other treatments.

Root systems of cotton plants grown at Shafter following dry fallow, wet-sorghum, or sorghum were strikingly more vigorous and free from root discoloration than were the roots of plants from the continuous cotton plots (Table 2). Isolations from the root lesions were not attempted but *T. basicola* was isolated readily from the soil samples taken from the test plots by using the carrot disk assay. There was a strong relationship between the number of disks colonized and the amount of root discoloration observed (Table 2).

DISCUSSION

Continuous soil flooding associated with culture of lowland rice was highly effective for the eradication of soilborne propagules of *V. dahliae*, reduction of Verticillium wilt incidence, and increasing cotton lint yields. In view of the work of Menzies (8) and Nadakavukaren (10), the importance of the rice plants for the eradication of *V. dahliae* is unknown but income from that crop does make it more economically attractive. We observed no decline in numbers of propagules in plots that received frequent irrigation. This would sustain the conclusions that soil saturation is required for rapid elimination of propagules of *V. dahliae* (8, 10). Moore (9) and Stoner and Moore (14) made similar observations in their studies of *Sclerotinia sclerotiorum* in Florida. They observed that decomposition of sclerotia occurred much more rapidly under flooded and rice paddy conditions than under conditions of frequent water application.

Apparently the decreases in inoculum density and disease incidence are directly related to the effects of flooding on the propagules of *V. dahliae*. The observed increases in lint yield are due also (at least in part) to the reduced incidence of Verticillium wilt. Other less well recognized soil pathogens also may be affected by the flooding and thus affect yields. Kelman and Cook (7) indicated that the low incidence of soilborne plant diseases in certain parts of the People's Republic of China may be due to the extensive culture of paddy rice. The

TABLE 1. Effect of various cultural practices on the population of propagules of *Verticillium dahliae* in soil, the incidence of Verticillium wilt, and lint yields of Acala SJ-1 cotton at two California locations

Location	Rotation treatment		Initial prop./g soil	Prop./g soil	Plants with:		Yield of lint/hectare[e]
	1972	1973	(May 72)[a] (no.)	(May 73) (no.)	Foliar symptoms[b] (%)	Vascular symptoms[c] (%)	(kg)
Shafter	Fallow	Cotton	2 x	1 x	0.8 x	14.6 x	1,185 x
	Wet-sorghum	Cotton	3 x	4 x	1.8 x	36.7 x	1,320 x
	Dry-sorghum	Cotton	5 x	4 x	0.8 x	24.6 x	1,181 x
	Cotton	Cotton	5 x	18 y	7.8 y	78.8 y	998 y
Five Points	Fallow	Cotton	23 x	15 x	36.0 x	n.c.[d]	1,283 x
	Wet-sorghum	Cotton	23 x	18 x	43.7 x	n.c.	1,068 y
	Dry-sorghum	Cotton	12 x	20 x	52.1 y	n.c.	1,218 x
	Cotton	Cotton	21 x	38 y	59.8 y	n.c.	1,112 y
	Safflower (susceptible)	Cotton	28 x	40 y	40.6 x	n.c.	1,299 x
							(cm)
Davis	Dry-sorghum	Cotton	140 x	131 x	85.0 x	99.7 x	88.5 x
	Wet-sorghum	Cotton	128 x	124 x	85.1 x	98.0 x	97.7 x
	Cotton	Cotton	122 x	129 x	96.2 x	99.3 x	91.6 x
	Ryegrass	Cotton	60 y	24 y	47.7 y	76.7 y	112.9 y
	1974	1975	(May 74)	(May 75)			(kg)
Five Points	Fallow	Cotton	63 x	61 x	80.8 x	97.5 x	1,058 x
	Paddy rice	Cotton	51 x	3 y	2.3 y	3.3 y	1,711 y
	Safflower (resistant)	Cotton	40 x	52 x	77.5 x	95.7 x	1,086 x
	Cotton	Cotton	45 x	57 x	95.5 x	100.0 x	1,004 x
	Safflower (susceptible)	Cotton	42 x	66 x	73.4 x	93.8 x	1,148 x

[a]Numbers followed by different letters differ significantly ($P = 0.05$) within each test plot location.
[b]The percentage of plants with foliar symptoms of Verticillium wilt immediately before defoliation for harvest, second year of study.
[c]The percentage of plants with vascular discoloration of the main stem immediately following harvest, second year of study.
[d]The abbreviation n.c. = not counted.
[e]At the Davis location, lint yields were unavailable. Figures represent plant height (cm).

TABLE 2. Effect of various cultural practices on root vigor and disease development in cotton roots and the relative soil populations of *Thielaviopsis basicola*

Treatment	Diseased roots[a] (no.)	Diseased roots (%)	Root vigor rating[b]	Colonization of carrot disks by *T. basicola*[c] (%)
Fallow	0.9 x	2.9	2.3	1 x
Wet-sorghum	3.3 x	10.8	2.4	7 x
Dry-sorghum	3.1 x	10.4	2.3	6 x
Cotton	12.4 y	41.3	1.8	30 y

[a]Average number of diseased roots out of 30 roots per replication for eight replications. Numbers followed by the same letter are not significantly different ($P = 0.05$).

[b]Average rating of 30 root systems per replication for eight replications. Scale: 0 = tap root blackened with few or no lateral roots; 3 = vigorous and apparently healthy root.

[c]Percent of carrot disks colonized from soil overlays. Ten disks per 5 g soil per replication, eight replications.

chemical and physical changes in the soil caused by prolonged flooding also may be involved in the yield increase.

Of the other rotations studied, only perennial ryegrass was effective in reducing the inoculum density of *V. dahliae*. Sewell and Wilson (13) observed that populations of *V. albo-atrum* were greatly reduced in soil under a ryegrass sod but disease potential from *V. dahliae* was still present after 4 yr of sod. They were not able to monitor the population of *V. dahliae*, however, so the effect of the sod on the total population is unknown. It does indicate that this treatment may not eradicate *V. dahliae*.

The other rotations examined in this study did not result in reduction in numbers of *V. dahliae* propagules and the responses of the following cotton crop were variable. Huisman and Ashworth (6) also observed the lack of rapid attrition of propagules in the presence of nonhost crops such as wheat and corn. Our results also indicate that the potential of crop rotation for effective management of Verticillium wilt is limited in fields with high infestations of *V. dahliae*. In areas where the culture of lowland rice or perennial ryegrass is feasible, rotations with these crops could be important in the management of Verticillium wilt of cotton and other susceptible crops.

The information presented here on the effect of rotations on cotton root rot incidence and *T. basicola* populations is not extensive enough to support sound conclusions. Nevertheless, it does illustrate that the plant responses observed in this test may not be attributable solely to suppression of Verticillium wilt.

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