Incidence of Verticillium Wilt and Yield Losses of Cotton Cultivars (*Gossypium hirsutum*) Based on Soil Inoculum Density of *Verticillium dahliae*

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ABSTRACT

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Inoculum density (propagules per gram of soil) of *Verticillium dahliae* at planting time, when plotted against the disease incidence (percent foliar symptoms) for two cotton cultivars for three successive years, closely followed the negative exponential curve $Y = 57.09*(1 - e^{-0.351*X})$. Correlation coefficients for each of the 3 yr separately and all 3 yr cumulatively were 0.71, 0.96, 0.84, and 0.85, respectively. The highest correlation between soilborne populations of the pathogen and ratios of lint yields of a Verticillium wilt-tolerant cultivar (Acala GC-510) over a susceptible cultivar (Acala SJ-2) was expressed by the power equation $Y = 97.08*X^{0.038}$. Correlation coefficients for the 3 yr separately and for all 3 yr together were 0.88, 0.95, 0.86, and 0.87, respectively. The fitted line predicts that with about three propagules per gram of soil both cultivars should produce equal yields; at higher inoculum density, tolerant cultivars, such as Acala GC-510, should have higher lint yields than susceptible cultivars, such as Acala SJ-2.

Verticillium wilt of cotton (*Gossypium hirsutum* L.) causes economic losses in California that are often greater than losses caused by any other disease or pest (15). The causal agent, *Verticillium dahliae* Kleb, is a soilborne fungus capable of infecting plant roots throughout the growing season (12). The pathogen persists in the soil as microsclerotia that may remain viable for more than 20 yr (17).

The disease is managed by the use of wilt-tolerant cultivars, crop rotation, and cultural practices (5,8,13,14). Among the main factors recognized in the epidemiology of the disease are pathotypes of *V. dahliae*, their inoculum density in the soil, air temperature, soil water status, plant density, and potassium and nitrogen availability to the growing plants.

Because Verticillium wilt is a single-cycle disease, inoculum levels of *V. dahliae* in the soil at planting time play a critical role in disease development and are directly reflected in lint-yield losses. Disease incidence is usually assessed as percent foliar symptoms or vascular discoloration. Although vascular discoloration is a positive indicator of plant infection in cotton, it has little or no correlation with lint yields (4,12). In contrast, the incidence of foliar symptoms of Verticillium wilt is directly correlated to lint and seed losses (7,12).

A model simulating Verticillium wilt in relation to cotton growth and development has been constructed. The model is based on a mathematical model for cotton growth and development (16) but also considers the compensation effects derived from plant density interactions and is coupled to the effects of pathotypes and the inoculum density of *V. dahliae* (6,7). Although the model gives good predictive values, it is in a form not readily used by growers and extension personnel.

Previous work on Verticillium wilt has emphasized the direct relationship of vascular discoloration of cotton stems to the inoculum density of the pathogen in the soil (1,2). However, the relationship between inoculum density of the pathogen and disease progression (foliar symptoms) is more variable and depends on environmental fluctuations (3,4). To reduce the variability due to crop history and differences in the densities of different pathotypes of *V. dahliae* in cotton fields, Pullman and DeVay (12) determined the relationship between inoculum density at planting time and foliar symptom development using a single-field site for seven successive years. This approach limited their study to local scale and one cotton cultivar.

The objective of the present study was to investigate the relationship between soil inoculum levels and disease incidence under broader cultural and environmental conditions than previously studied and to develop a simple equation to guide growers in choosing the best yielding cotton cultivars, based on wilt tolerance and soil infestation levels of *V. dahliae*.

MATERIALS AND METHODS

Soil sampling. During the years 1987–1989, the San Joaquin Valley Acala Cotton Board planted over 73 ha throughout the San Joaquin Valley of California for cultivar field trials. Different cotton cultivars and lines were tested each year for disease resistance and lint yields under field conditions. Because Verticillium wilt may be a limiting factor in cotton production, the susceptibility of these cultivars to *V. dahliae* was emphasized. Soilborne populations of the pathogen were determined just before the planting of seven of the cultivar trial fields. These field sites were at Hanford, Madera, Maricopa, Merced, Porterville, Wasco, and West Side Field Station. At each location the varieties were replicated four times, and in each replication, four soil samples were taken in a Z pattern (11).

Soil assays. Except for the West Side Field Station, where four samples were taken, each data point represents the mean of 160 numbers, since 16 samples were taken from each field and duplicates of five plates each were analyzed. Each soil sample was a composite of 12 cores. Samples were air-dried in the laboratory for 3 wk. After drying, the soil was pulverized and mixed well. Subsequently, 10 g of soil from each sample was placed into a snap-cap vial and mixed with 15 mg of dl-methionine in 2 ml of distilled water to break the dormancy of some microsclerotia (9). The samples were then incubated at 33 C for 1 wk. After incubation, vials were opened, and the soil was dried for 5 days. Samples were then pulverized using a mortar and pestle and dry plated on sodium-polypectate medium using the modified Anderson air sampler method (2). Two subsamples per soil sample were plated using a total of 0.5 g of dried soil per subsample. Five plates with 100 mg per plate were used for each subsample. Plates were incubated in the dark at 22–24 C for 3 wk. After incubation, plates were washed with tap water so that soil and any microbial growth on the agar surface were removed. This facilitated the counting of colonies of the pathogen under a stereoscope. Colonies of *Verticillium tricorpus*, a nonpathogenic fungus to cotton, may be confused with *V. dahliae*; however, they are distinguished from the pathogen based on

TABLE 1. Inoculum density of *Verticillium dahliae*, disease incidence for the cultivar Acala SJ-2 and yield ratio between cultivars Acala GC-510 and Acala SJ-2[a]

Field location	Inoculum density (propagules/g soil)			Disease incidence[b] (% foliar symptoms)			Yield ratio × 100 (GC-510/SJ-2)		
	1987	1988	1989	1987	1988	1989	1987	1988	1989
Hanford	1.2 ± 0.65[c]	4.1 ± 0.79	25.3 ± 3.77	42 ± 1.08	46 ± 2.62	50 ± 3.95	97	106	107
Madera	1.4 ± 0.57	0.1 ± 0.08	8.8 ± 3.08	28 ± 2.53	10 ± 1.00	23 ± 6.36	100	93	109
Maricopa	2.6 ± 1.13	0.9 ± 0.25	1.5 ± 0.45	15 ± 1.00	11 ± 1.00	17 ± 1.00	96	100	96
Merced	0.4 ± 0.27	0.1 ± 0.08	1.6 ± 0.32	10 ± 1.00	10 ± 1.00	10 ± 1.00	96	87	89
Porterville	26.9 ± 3.56	17.4 ± 2.43	5.4 ± 1.03	69 ± 1.15	61 ± 1.14	60 ± 0.89	124	109	105
Wasco	4.3 ± 0.80	0.3 ± 0.14	2.6 ± 0.62	30 ± 2.65	11 ± 1.00	53 ± 0.43	103	90	116
West Side Field Station	24.0 ± 7.84	3.3 ± 0.96	15.0 ± 5.62	20 ± 3.19	37 ± 3.13	55 ± 1.02	108	106	109

[a]Soil assays for 1987–1989 at seven different field locations in the San Joaquin Valley of California.
[b]Disease incidence was assessed in mid-September.
[c]Standard error of a mean.

TABLE 2. Cotton lint yields (kg/ha) for cultivars Acala SJ-2 and Acala GC-510

Field location	Acala SJ-2			Acala GC-510		
	1987	1988	1989	1987	1988	1989
Hanford	300 ± 0.77[a]	225 ± 1.01	218 ± 4.87	289 ± 1.54	238 ± 9.02	234 ± 4.98
Madera	219 ± 6.41	264 ± 4.34	256 ± 6.92	220 ± 4.92	247 ± 3.09	279 ± 1.63
Maricopa	301 ± 7.32	204 ± 4.59	276 ± 9.94	289 ± 8.09	102 ± 1.45	264 ± 7.87
Merced	280 ± 16.04	299 ± 4.46	203 ± 14.74	270 ± 13.02	260 ± 4.84	181 ± 13.70
Porterville	161 ± 6.91	167 ± 4.09	279 ± 9.05	199 ± 6.43	182 ± 3.20	292 ± 3.42
Wasco	282 ± 0.85	156 ± 10.12	259 ± 3.63	289 ± 10.16	152 ± 6.50	300 ± 3.19
West Side Field Station	262 ± 15.92	212 ± 4.22	265 ± 4.66	285 ± 8.11	224 ± 2.29	290 ± 5.03

[a]Standard error of a mean.

microsclerotia size, morphology, and pattern of formation (10).

Disease incidence and yields. In mid-September, disease incidence was evaluated from 0 to 100% in the overall populations of plants in each cultivar plot. With increased incidence of Verticillium wilt, there was increased severity ranging from little or none to complete defoliation of plants. Only the plants showing foliar symptoms were counted. Each cultivar plot consisted of about 400 plants.

The present study is confined to two of the cultivars in the trials. One, Acala SJ-2, is susceptible to Verticillium wilt and is representative of other susceptible cultivars; the other is Acala GC-510, a cultivar with field tolerance to wilt and representative of an advanced series of wilt-tolerant cultivars.

Data processing. Data were analyzed using the nonlinear regression procedure of the SAS (Statistical Analysis System, SAS Institute Inc., Raleigh, NC) package for a PC microcomputer. Disease incidence was recorded as the percentage of foliar wilt symptoms on cv. Acala SJ-2, whereas lint production was analyzed as the yield ratio of cv. Acala GC-510/cv. Acala SJ-2. Data analyses for both disease incidence and lint yield ratio for each of the three cropping seasons (1987–1989) were made, separately as well as cumulatively. For all fitted lines, plots of the residuals of the observed values were used for identifying obvious outliers.

RESULTS

Soil populations of *V. dahliae* and cotton yields. The average number of propagules of *V. dahliae* per gram of soil in each of the seven locations for each of the 3 yr of sampling (1987–1989) are summarized in Table 1. Inoculum density of *V. dahliae* varied between 0.1 and 26.9 propagules per gram of soil, whereas disease incidence ranged from 10 to 69%. Yield ratios of Acala GC-510/Acala SJ-2 were less variable and ranged from 0.87 to 1.24. There was a high correlation between propagules per gram of soil and disease incidence for all 3 yr of the study except at one location (Madera, 1989). At Madera, there was a high inoculum density and low disease incidence. The percentage of foliar symptoms on cv. Acala SJ-2 and the yield ratios between cv. Acala GC-510 (tolerant) and cv. Acala SJ-2 (susceptible) also are presented in Table 1. Cotton lint yields for Acala SJ-2 and Acala GC-510 for 1987–1989 are given in Table 2.

Inoculum density and disease incidence. Inoculum density at planting time was plotted against the percent foliar symptoms on cv. Acala SJ-2 (Fig. 1). For each of the 3 yr, the data closely followed the negative exponential curve. Correlation coefficients (R) for 1987, 1988, and 1989 were 0.71, 0.96, and 0.84, respectively. Data for the 3 yr were then combined (Fig. 1), and a negative exponential curve was fitted [$(Y = 57.09*(1 - e^{0.351*X})$] with $R = 0.85$.

A regression analysis of disease incidence versus inoculum density of *V. dahliae* also was made for the wilt-tolerant cotton cultivar Acala GC-510 (Fig. 2). The correlation coefficient was $R = 0.15$, which indicated that no clear relationship was apparent between inoculum density and foliar-disease development for a wilt-tolerant cultivar.

Inoculum density and cotton yields. Soilborne populations of *V. dahliae* for each year as well as for all 3 yr cumulatively were plotted against the ratios of cotton lint yields of cv. Acala GC-510 over cv. Acala SJ-2. Data followed the power curve with correlation coefficients of 0.88, 0.95, 0.86, and 0.87 for each year separately and all 3 yr together, respectively (Fig. 3). Readings at Porterville in 1987 and Wasco in 1989 were excluded from the analyses because a plot of the residuals revealed them as outliers. A line parallel to the X axis at a yield ratio equal to 100 intercepted the predicted line ($Y = 97.08*X^{0.038}$) at 2.2 propagules per gram of soil.

DISCUSSION

During 1983, a model for Verticillium wilt in relation to cotton growth and development was developed (7). Although the model simulates very well the relationships of cotton phenology, lint yields, and disease development, it is complex and difficult to use on a routine basis for prediction of yield losses due to Verticillium wilt. Data from earlier studies used for the development of this physiological model revealed a relationship between inoculum density and disease incidence. However, a single-field site was monitored for 7 yr to circumvent the variability found

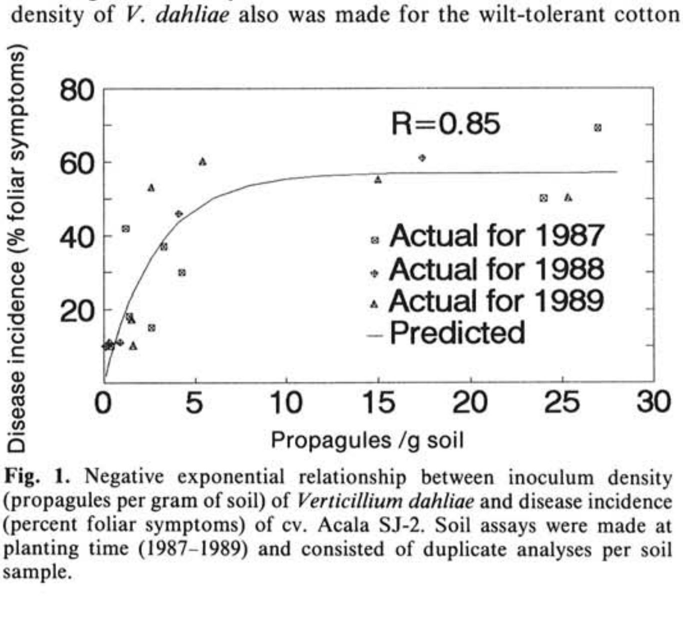

Fig. 1. Negative exponential relationship between inoculum density (propagules per gram of soil) of *Verticillium dahliae* and disease incidence (percent foliar symptoms) of cv. Acala SJ-2. Soil assays were made at planting time (1987–1989) and consisted of duplicate analyses per soil sample.

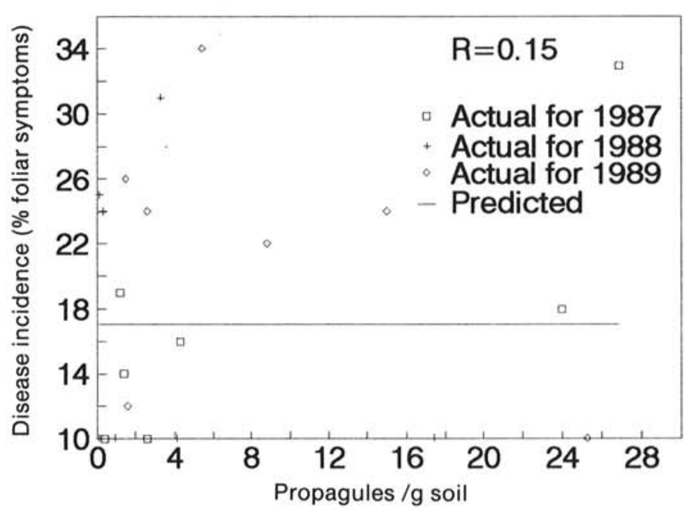

Fig. 2. Regression analysis between inoculum density (propagules per gram of soil) of *Verticillium dahliae* and disease incidence (percent foliar symptoms) of cv. Acala GC-510. Soil assays were made at planting time (1987–1989) and consisted of duplicate analyses per soil sample.

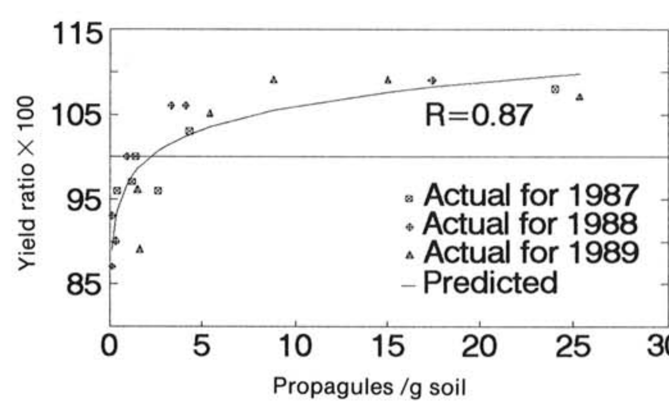

Fig. 3. Power equation relationship between inoculum density (propagules per gram of soil) of *Verticillium dahliae* and yield ratio of cv. Acala GC-510 over Acala SJ-2. Soil assays were made at planting time (1987–1989) and consisted of duplicate analyses per soil sample. The line parallel to the abscissa at the 100 point denotes equal yields for both cultivars.

in earlier studies (3,12).

We found a significant correlation between inoculum density at planting time and disease incidence at the end of the cropping season for different field locations. In a previous study (3) including multiple fields of cotton, a low correlation was found between disease incidence and propagule density of *V. dahliae*. Later, in a 7-yr study at a single-field site (12), a high correlation was found using a quadratic equation for a nonlinear regression of the data. However, in a second-order polynomial, as the X factor increased beyond a certain point, Y decreased. Therefore, in a field situation, the equation will eventually predict lower levels of disease at higher numbers of propagules of the pathogen per gram of soil. In the present study, we fitted a negative exponential curve on the data that more realistically described the disease incidence. Final disease incidence increased steeply as the inoculum in the soil increased from zero to about five propagules per gram of soil. Subsequently, the line progressively plateaued (Fig. 1) because of the decrease in the number of plants available for infection and the high incidence of symptoms observed. The curve fit ($R = 0.85$) provided a reliable prediction of the incidence of Verticillium wilt of Acala SJ-2 cotton in relation to soilborne populations of *V. dahliae*.

The ultimate goal of this research was to develop a simple model that, based on the agronomic traits and the disease responses of the host to the pathogen, would allow selection of the best cotton cultivar for planting. For that purpose, the inoculum density was plotted against the yield ratio of the wilt-tolerant cv. Acala GC-510 over the less tolerant cv. Acala SJ-2 (Fig. 3). The resulting power line ($Y = 97.08*X^{0.038}$) showed a high correlation ($R = 0.87$) between inoculum density and yield ratio. The graph suggests that yield responses are dependent on the inoculum density of the pathogen as well as on the disease tolerance of the cotton cultivars. When inoculum density was between 0 and ~3 propagules per gram of soil, Acala SJ-2 gave better yields than the tolerant Acala GC-510. Above these levels of soil infestation, the incidence of wilt for Acala SJ-2 reaches about 40%, and its yield decreases relative to that of Acala GC-510. This is an important factor to consider when selecting a cotton cultivar for planting because Acala SJ-2 is a popular cultivar among California growers. Based on the present data, a soil assay for inoculum density of *V. dahliae* at planting time would assist a grower in selecting the best cotton cultivar for maximum production.

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