Influence of Inoculum Density of Defoliating and Nondefoliating Pathotypes of *Verticillium dahliae* on Epidemics of Verticillium Wilt of Cotton in Southern Spain

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ABSTRACT

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Linear regression analysis of data transformed according to several mathematical models showed no significant correlation between the inoculum density of *Verticillium dahliae* determined at sowing time in randomly chosen cotton fields and the incidence of plants with vascular discoloration, the incidence of plants with foliar symptoms, or a disease intensity index for Verticillium wilt of cotton calculated at the end of the crop season. Epidemic development of Verticillium wilt in cotton was investigated in field plots naturally infested with different inoculum densities of the defoliating and nondefoliating pathotypes of *V. dahliae* in 1986 and 1987. The straight line model adequately described the increase in the incidence of plants showing foliar symptoms and the disease intensity index over the accumulated physiological time from sowing. For similar levels of initial inoculum, the defoliating pathotype of *V. dahliae* caused an earlier epidemic onset, a higher rate of increase in the incidence of foliar symptoms and the disease intensity index over physiological time, and a higher final amount of both disease measurements than the nondefoliating pathotype. Also, for the two disease measurements, the rate of progress increased with the initial inoculum density of the defoliating pathotype up to a threshold of 24 to 44 CFU/g of dry soil in 1986 and 44 to 75 CFU/g of dry soil in 1987. Differences in severity among Verticillium wilt epidemics were described more appropriately by the descriptive parameters of the increase in the disease intensity index over physiological time than by the corresponding parameters of the increase in the incidence of foliar symptoms.

Additional keywords: epidemiology, *Gossypium hirsutum*.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major constraint to cotton (*Gossypium hirsutum* L.) production in almost all countries in which *G. hirsutum* is cultivated (10). A mean annual loss of fiber of 2.37% due to the disease was reported in the USA in the period from 1952 to 1981 (21), but in some particular fields and years the losses exceeded 75% (29). In Spain, Verticillium wilt of cotton was first reported in 1954 (2) and is now the most important disease of the crop. The disease occurs in 74 to 82% of the fields throughout the Guadalquivir Valley of southern Spain (12), an area in which an average of 83,000 ha of cotton is grown annually (3). Disease incidence and severity are highest in the lower Guadalquivir Valley and occurrence is associated with the high inoculum densities of a cotton-defoliating pathotype of *V. dahliae* widespread in that area (12).

Research in California shows that the percentage of cotton plants infected (showing a dark brown discoloration of vascular tissues) by *V. dahliae* at the end of the crop season is directly related to the inoculum density of the pathogen in soil (6). Vascular discoloration is a good indicator of infection by *V. dahliae*, but it appears to have little influence on lint yield. Losses of lint yield depend mainly on the developmental stage of plants when foliar

symptoms appear (18,26). Development of foliar symptoms in infected plants is influenced by several factors, of which the most important are the susceptibility of cotton cultivars, the environmental conditions, and the pathotypes of *V. dahliae* (5,11,23,25). Pathotypes of *V. dahliae* infecting cotton are differentiated mainly by whether or not they defoliate the plant (30). The defoliating pathotype has a more pronounced effect than the nondefoliating pathotype on growth parameters of the affected plants and reduction of lint yield (18). As a consequence of the multiple factors involved in the expression of foliar symptoms, the relationship between foliar symptoms development and inoculum density may be of little value for disease prediction (16). However, when studies were done in a limited number of fields for several consecutive years, thus reducing experimental variability, it was found that the rate of increase in the incidence of foliar symptoms in Acala SJ-2 over physiological time and the final disease incidence were significantly related to the inoculum density of *V. dahliae* in soil at the time of sowing (23,25).

Little is known about the epidemiology of Verticillium wilt of cotton under conditions prevailing in southern Spain, and few comparative studies have been done on the influence of different *V. dahliae* pathotypes on the epidemiology of the disease. The objectives of this work were to determine the influence of initial inoculum density of the defoliating and nondefoliating pathotypes of *V. dahliae* on the epidemic development of Verticillium wilt of cotton, and the relationship between the initial inoculum of the pathogen and the final amount of the disease.

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MATERIALS AND METHODS

Initial inoculum density of *V. dahliae* and final disease. Systematic disease surveys were carried out in 57 cotton fields at the Guadalquivir Valley in May 1985. Most of the surveyed fields were sown to cotton cultivar Coker 310, which is highly susceptible to *V. dahliae* (13). The population of *V. dahliae* in those fields was assessed from soil samples taken 15 to 30 days after sowing. Thirty 200-g soil samples were collected from each field with a cylindrical auger (2.5 × 20 cm) to a depth of 20 cm after removing 3 to 5 cm of surface soil. A three-diagonal path sampling pattern (15) was used that covered a 40 × 40-m sampling plot, one sampling plot per field. Soil samples from each plot were bulked, thoroughly mixed, and air-dried for 5 to 6 months at a temperature and relative humidity that ranged from 22 to 25°C and 30 to 35%, respectively. The dry soil was broken up by hand and with a wooden hammer, milled in a rotating drum with steel cylinders for 20 min, and screened through a sieve with a 0.8-mm mesh. Five 100-mg aliquots of dry soil were processed per plot. Each subsample was plated onto a semiselective sodium-polypectate agar (SPA) medium (14) by means of a modified Andersen air sampler (Andersen Samplers, Inc., Atlanta), following the procedure described by DeVay et al. (16). Plates were incubated at 23 to 25°C in darkness for 14 days, after which the SPA surface was washed free of soil with tap water. Fungal colonies that grew on the medium were observed with a stereoscope at 15×. Colonies of *V. dahliae* were identified on the basis of microsclerotia formed in the SPA (14) and the colonies were counted. For each plot, the inoculum density was expressed as the number of colony forming unit per gram of dry soil (CFU/g).

Verticillium wilt in each sampled plot was assessed on 11 to 27 September, 1985, at the time the crop was at an early stage of maturation. The incidence of foliar symptoms (proportion of plants with foliar symptoms) (29) and disease severity were determined in six groups of 20 randomly chosen, consecutive plants. Disease severity was assessed for each plant on a 0 to 4 rating scale according to the percentage of foliage affected by acropetal chlorosis, necrosis, wilt, and/or defoliation (0 = healthy plant, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 99%, and 4 = dead plant). The incidence (proportion) of infected plants was then determined. Infection in a plant was based on the occurrence of vascular discoloration in the stem at 4 to 6 cm above soil level. Incidence of foliar symptoms and severity data were used to calculate a disease intensity index (D_I) (1) by the following equation: $D_I = (I \times S) \div M$, in which I and S were incidence (%) and mean severity of foliar symptoms, respectively, and M was the maximum, 4, severity rating. Thus, D_I expressed the mean value of disease intensity at any given moment as a percentage of the maximum possible disease.

The relationship between the inoculum density of *V. dahliae* in soil at the time of sowing (initial inoculum) and the incidence of plants showing foliar symptoms at the end of the cultivation period was examined by means of linear regression analysis using several mathematical models that have different biological implications: (i) untransformed data (I versus inoculum density); (ii) semilogarithmic, or multiple infection transformation ($\ln(1 - I)^{-1}$ versus inoculum density) (20); (iii) Baker's transformation ($\log[\ln(1 - I)^{-1}]$ versus log inoculum density) (7); (iv) direct log-log transformation (log I versus log inoculum density) (6); and (v) logit transformation ($\ln[I/(1 - I)]$ versus log inoculum density) (32). In addition to I, models also were analyzed for the incidence of vascular discoloration, or D_I. Data close to 100% or inoculum density equal to zero were not used in these transformations, thus, regression analyses were limited to the logarithmic phase of the corresponding growth curves (7,8,9). Consequently, for analyses concerning the incidence of vascular discoloration, the incidence of foliar symptoms, or D_I, only data from 18, 17, and 17 fields, respectively, were used. The appropriateness of the

regression models to describe the disease/inoculum density relationships was evaluated by the coefficient of determination (r^2), the significance level of the relationship, and the pattern of residuals (15,17).

Defoliating and nondefoliating pathotypes of *V. dahliae*. Pathotypes of *V. dahliae* were characterized in 18 of 57 fields surveyed in 1985, as well as in five field plots (one 30 × 30-m plot/field) designated A to E. Fields A to E were not included in the surveys and were selected for further experiments because of their range of inoculum density of *V. dahliae* in soil and the severity of defoliation observed in previous years.

In mid-September 1985, 50 diseased plants were collected at random in each of the field plots A to E, as well as in fields in which the relationship between inoculum density and final disease was studied and isolations were made from their petioles and stems. Tissues were washed in running tap water, cut into pieces 5 to 10-mm long, surface-disinfested in 0.5% NaOCl for 1 to 2 min, and dried with sterile filter paper. The disinfested pieces were plated onto water-aureomycin agar (WAA) (1 liter of distilled water, 20 g of agar, and 30 mg of aureomycin) and incubated in darkness at 22 to 24°C for 2 weeks. Monoconidial isolates obtained from the *V. dahliae* colonies that developed were grown on plum-extract agar (PEA) (900 ml of distilled water, 20 g of agar, 100 ml of concentrated plum extract, 1 g of yeast extract, 5 g of lactose, and pH 5.6 to 6.0) and stored under autoclaved (twice for 70 min at 121°C) liquid paraffin (E. Merck AG, Darmstadt, Germany) in darkness at 5 to 10°C.

Monoconidial isolates of *V. dahliae* were characterized by the morphology of microsclerotia, the growth rates on sanguinarine-amended potato-dextrose agar (PDA), and the pathogenicity to cotton cultivar Acala SJ-2 and line PI 70-110. Microsclerotia morphology was observed in 28- to 35-day-old water agar (WA) cultures incubated at 24°C in darkness (28). Isolates were cultured on PDA supplemented with 10^{-3} M sanguinarine nitrate at 24°C in darkness for 7 to 14 days, and then observed for fluorescence when exposed to UV light (360 nm) (24,28). Controls in these experiments were *V. dahliae* isolates V-4 and V-117, which had been characterized as mildly virulent and nondefoliating, and highly virulent and defoliating, respectively, in previous work carried out in our laboratory (12).

The pathogenicity of 15 monoconidial isolates of *V. dahliae* from field A, 15 from B, 13 from C, 12 from D, and eight from E were tested on line PI 70-110 (very susceptible to Verticillium wilt) and cultivar Acala SJ-2 (moderately susceptible to the disease) (4,5,12). Seeds of the two genotypes were provided by J. E. DeVay (Department of Plant Pathology, University of California, Davis). Plants were grown from surface-disinfested seeds (1% NaOCl for 2.5 min) in 12-cm-diameter plastic pots (one plant per pot) containing about 1 liter of a nonsterile sand-lime-peat mixture (2:1:1, vol/vol/vol). Plants were held in the greenhouse at 24 ± 5/17 ± 3°C (day/night) and a 12-h photoperiod in which sunlight was supplemented with fluorescent light at 180 to 216 $\mu Em^{-2}s^{-1}$. Plants were fertilized every 2 weeks with a water soluble fertilizer (20-10-20, N-P-K). Inocula consisted of conidial suspensions from 7-day-old PDA cultures incubated in darkness at 24°C. Conidial suspensions were prepared by adding sterile distilled water, rubbing the culture surface gently with a bent glass rod, and filtering through three layers of cheesecloth. Concentration in the suspension was adjusted to 3 × 10^6 conidia/ml with sterile distilled water. Plants, 6- to 7-weeks-old, with two pairs of well-developed leaves were inoculated with the conidial suspensions by puncturing two points on the bases of the first and second internodes and placing a 5-μl drop of inoculum in each hole. Control plants were either inoculated with the reference isolates V-4 or V-117, or with 5-μl drops of sterile distilled water. Inoculated and control plants were transferred to the greenhouse and grown as described above for 5 weeks. Plants were observed periodically for symptom development. Disease severity was re-

corded at 2, 3, 4, and 5 weeks after inoculation by means of the 0 to 4 rating scale used for the disease surveys. The experiments had a factorial design with six randomized, complete blocks (replications of single plants) for each isolate-cultivar combination. A total of five experiments were carried out, each of which had isolates V-4 and V-117 in common but different isolates from each field. Analysis of variance was performed on the data from the disease assessment 5 weeks after inoculation. Similarity among common isolates across experiments was tested by analysis of variance using a split-plot design, in which experiments were main plots and the isolate-cultivar combinations were subplots (19). Data were analyzed using Statistix (Analytical Software, Roseville, MN). Treatment means were compared using ortogonal contrasts or Fisher's protected least significant difference (LSD) at $P = 0.05$.

Initial inoculum density of *V. dahliae* and development of Verticillium wilt. Experiments were carried out in selected field plots at several locations of the Guadalquivir Valley in 1986 and 1987. Soil in these fields was naturally infested among locations with either the defoliating or nondefoliating pathotypes of *V. dahliae*, as indicated by pathogenicity tests with a sample of monoconidial isolates of the pathogen from affected cotton performed in the previous year.

In 1986, experimental plots were established in five fields (one 30 × 30-m plot/field) designated A to E. They were selected to represent the several levels of inoculum density of the two pathotypes observed during disease surveys in 1985. Fields A, B, C, and E (vertic soil, about 62% clay, 0.9 to 1.4% organic matter, and pH 7.8 to 8.8) were in the lower Valley (Sevilla province), while field D (loamy soil, 27% clay, 1.1% organic matter, and pH 8.2) was in the higher Valley (Jaén province). In 1987, three 7.5 × 10-m experimental plots were established in field A, three 4 × 10-m plots in field B, and one 20 × 15-m plot in field D. All the 1987 plots were located as near as possible to the experimental sites in 1986. In both years, plots were sown to susceptible cultivar Coker 310 between mid-April and the beginning of May, in rows 0.95-m apart (except 0.75-m apart for field D) with a plant stand adjusted to 100 to 120 × 10³ plants/ha. Seedbed preparation, fertilization, and furrow irrigation were performed according to farmer's practices (27).

In each plot, 200-g soil samples were collected with an Edelman auger (Eijkelkamp, Giesbeek, Netherlands) to a depth of 20 cm in a three-diagonal path (15) within 1 month after sowing. Numbers of samples varied with the size of the plot. In 1986, 30 samples were collected from each of the plots; while in 1987, nine, six, and twenty samples were collected from the plots in fields A, B, and D, respectively. The soil samples from each plot were bulked, thoroughly mixed, air-dried for 4 to 6 weeks at room temperature, and then milled and screened as described above. Six 500-mg aliquots of soil were assayed per plot. Each aliquot was divided into five 100-mg subsamples that were plated onto SPA (14), incubated, and examined as described previously.

To calculate the physiological time maximum and minimum, air temperatures were recorded in a meteorological station near the experimental plots. Celsius degree-days accumulated in each 12-h interval were calculated from the area enclosed between 11.9°C (threshold temperature for cotton growth) and the line that connects the daily maximum and minimum temperatures among consecutive days, using a triangulation method (31). Physiological time was determined as the cumulative number of degree-days from the time of sowing.

Epidemics of Verticillium wilt in the plots were characterized by the increase in the incidence of foliar symptoms and the increase of D_I over physiological time accumulated from the time of sowing. The increase in the incidence of foliar symptoms (total cumulative number of affected plants per total plant number examined per plot) over time was determined for each plot by recording plants with foliar symptoms at 7- to 14-day intervals

from mid-June to mid-September, or until the incidence of foliar symptoms was 90 to 95% (15,32). At each recording date, the disease severity was assessed for each plant in a plot using the 0 to 4 rating scale as for the disease surveys, and the increase of D_I over time was determined. For D_I, the area under the D_I progress curve (AUDPC) was calculated by trapezoidal integration between 0 and 1,400 degree-days and expressed as a percentage of the maximum possible area for this period (15). When the 1,400-degree-days period was completed (on average at about August 22 in all the experimental plots), the crop was at the phenological stage of bolls fully developed or, in some cases, the first bolls had opened. A total of 300 plants were examined per plot each year. In 1986, 50 consecutive plants in each of six rows 5-m apart were inspected. In 1987, 75 consecutive plants in each of four adjacent rows and 100 consecutive plants in each of three adjacent rows were examined for each of the three plots of fields A and B, respectively. For the plot in field D in 1987, 100 consecutive plants in each of three rows 6-m apart were examined.

Ordinary least squares regression analyses were performed with nontransformed data (straight line and second-order polynomial models) and with data transformed appropriately by the linearized versions of the Gompertz, logistic, log-logistic, and monomolecular models. The coefficient of determination (r^2), the standard error and significance of the estimated parameters, the mean error square, and the pattern of residuals were used to indicate the goodness-of-fit of data to the models and to choose the best regression model (15,17). The parameters derived from the models to describe the relationship between disease and physiological time were the rate of disease increase (estimated by the slope of the regression line), the physiological time of the disease onset (determined by the intersection of the regression line with the axis of physiological time), and the final value of D_I and the incidence of foliar symptoms.

RESULTS

Initial inoculum density of *V. dahliae* and final disease. The incidence of plants with vascular discoloration, plants with foliar symptoms, and D_I at the end of the crop season as related to the inoculum density of *V. dahliae* at the time of sowing are shown in Figure 1. Isolates of *V. dahliae* sampled from the 18 surveyed fields were all characterized as nondefoliating, except for two fields in which only the defoliating pathotype occurred. None of the transformations for the several models used (untransformed data, semilogarithmic, Baker's, log-log, and logit) gave significant linear correlations ($P = 0.05$) between the inoculum density of the pathogen in soil and the final indices of foliar symptoms or vascular discoloration, or the final D_I. Correlation coefficients were positive, but r^2 values were low (i.e., 0.07 to 0.17 for the incidence of vascular discoloration, 0.10 to 0.15 for the incidence of foliar symptoms, and 0.08 to 0.09 for D_I).

Defoliating and nondefoliating pathotypes of *V. dahliae*. All the isolates of *V. dahliae* from fields A, B, and C behaved similarly to isolate V-117, a known defoliating isolate used as a control in artificial inoculation experiments. These isolates caused defoliation and severe foliar symptoms in line PI 70-110 and cultivar Acala SJ-2. Mean severity values of isolates from fields A, B, and C were 3.5, 3.3, and 3.1 in PI 70-110; and 2.7, 2.3, and 1.9 in Acala SJ-2, respectively. Consequently, these isolates were classified as highly virulent and defoliating. On the other hand, all the isolates from field D and seven of the eight isolates from field E behaved similarly to isolate V-4, a known nondefoliating isolate used as control, giving rise to moderately severe symptoms without defoliation in PI 70-110 and Acala SJ-2. Mean disease severity values of those isolates from fields D and E were 1.9 and 1.6 in PI 70-110, and 1.5 and 1.0 in Acala SJ-2, respectively. One of the eight isolates from field E was classified as defoliating and caused severe symptoms on PI 70-110 (4.5) and Acala SJ-2 (2.8).

All defoliating isolates formed elongated and rounded microsclerotia in WA, grew without inhibition on sanguinarine-amended PDA forming colonies which were creamy-white underneath, and fluoresced under UV light. The nondefoliating isolates formed only rounded microsclerotia in WA, their growth was markedly inhibited on sanguinarine-amended PDA, and they did not fluoresce under UV light (24,28).

Initial inoculum density of *V. dahliae* and development of Verticillium wilt. Regression analyses of data indicated that the Gompertz, straight line, logistic, log-logistic, and monomolecular models were all equally appropriate to describe the increase in the incidence of plants with foliar symptoms and in D_I over physiological time accumulated from sowing. Also, these models explained a high percentage of the temporal variation in the incidence of foliar symptoms and D_I values in experimental plots. Differences between maximum and minimum values of r^2 obtained with the several models varied among the experimental plots, ranging from 0.02 (0.97 to 0.95) to 0.13 (0.98 to 0.85) for the incidence of foliar symptoms and from 0.03 (0.99 to 0.96) to 0.11 (0.96 to 0.85) for D_I. Adding a quadratic term to the straight line model did not improve the goodness-of-fit, thus, the second-order polynomial model was discounted. Consequently, we chose the straight line model for analyses of disease increase curves over physiological time because, of those considered, it was the simplest and easiest to use.

The incidence of plants with foliar symptoms increased over physiological time accumulated from sowing in 1986 and 1987 (Fig. 2A and B). In both years, disease increase showed a positive and significant linear relationship ($P < 0.05$) with physiological time, with r^2 values ranging between 0.91 and 0.99 (Table 1). The linear relationship was significantly influenced by the nature of the pathotype and the initial inoculum density of *V. dahliae* in soil (Table 1). In 1986, epidemic onset was earlier in fields with high inoculum density levels of the defoliating pathotype (fields A and B) than in field D with a high inoculum density of the nondefoliating pathotype. Onset in field D occurred earlier than in fields with low inoculum densities of either of the pathotype (fields C and E). In 1987, this relationship between the outbreak of the epidemic and the level of inoculum density was clear in the three plots sown in field B, in which the higher the inoculum density the earlier the epidemic onset. This relationship was also noticed for field A in 1987; although the epidemics started at similar dates in plots A-3 and A-4, which had very high inoculum densities (55.5 to 75.5 CFU/g) of the defoliating pathotype (Table 1).

In the 2 years of this study, within individual fields, the rate of increase in the incidence of foliar symptoms over physiological time increased with the inoculum density in plots infested with either of the pathotypes of *V. dahliae*. This correlation, however, did not hold with the high inoculum densities of the defoliating pathotype (i.e., 24.0 to 44.0 CFU/g in 1986 and 44.5 to 75.5 CFU/g in 1987), for which disease increased at similarly high rates (Table 1). Independent of the inoculum density, the rates of disease increase in plots containing the defoliating pathotype were always higher than those in plots with the nondefoliating strain. For example, in 1986 the rate of disease increase in fields B and C (defoliating pathotype) were 1.6 and 1.7 times higher, respectively, than in fields D and E (nondefoliating pathotype), although all those fields had a similar inoculum density (Table 1). Also, while the final incidence of foliar symptoms approached 100% in all plots infested with the defoliating pathotype, except for those with low inoculum density (2.0 to 5.5 CFU/g), a similar amount of final disease was reached only with high inoculum density of the nondefoliating pathotype (34.0 CFU/g) (Table 1). It is noteworthy that inoculum densities of 2.0 to 8.0 CFU/g of the defoliating pathotype gave rise to rates of disease increase and final incidences of foliar symptoms similar or higher than those in plots with inoculum densities between 27.5 to 34.0 CFU/g of the nondefoliating pathotype (Table 1).

In both 1986 and 1987, D_I showed a positive and significant ($P < 0.05$) linear relationship with physiological time, with r^2 ranging from 0.84 to 0.99 (Table 2) (Fig. 2C and D). In general, the time of epidemic onset, calculated as the intersect of the linear regression of D_I against physiological time with the time axis (Table 2), occurred slightly later than that obtained for the inci-
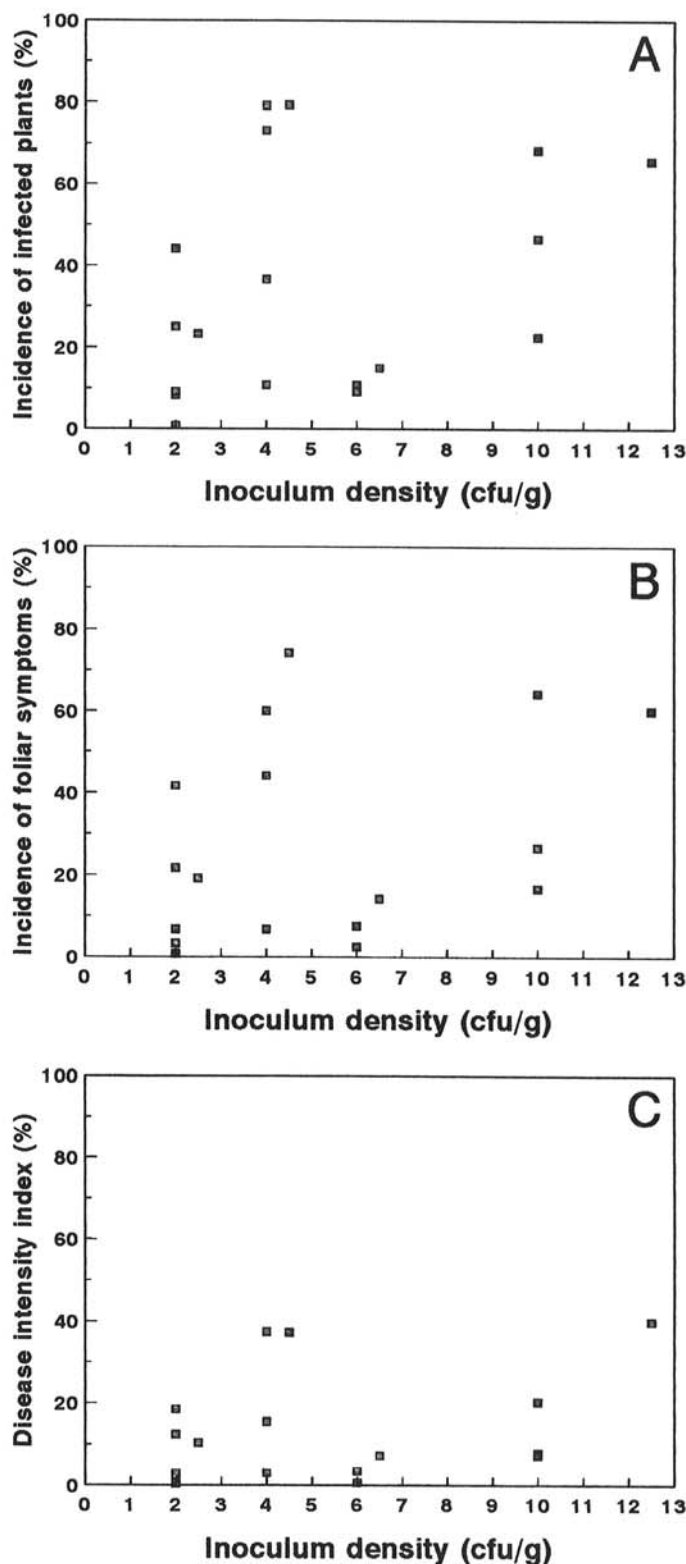

Fig. 1. Relationship between the amount of Verticillium wilt at an early crop maturation stage and the inoculum density of *Verticillium dahliae* in cotton fields in southern Spain. **A,** Incidence of infected plants; **B,** incidence of foliar symptoms; and **C,** disease intensity index.

dence of foliar symptoms (Table 1). The rate of D_I increase in plots infested with the defoliating pathotype increased with the inoculum density up to, what appeared to be, threshold values around 24.0 to 44.0 CFU/g in 1986 and 44.5 to 75.5 CFU/g in 1987 (Table 2). For all inoculum densities, the rate of D_I increase in plots infested mainly with the defoliating pathotype was higher than in plots infested mainly with the nondefoliating pathotype. For example, in 1986, the rate of D_I increase in fields B and C (defoliating) was, respectively, 3.3 and 2.5 times higher than in fields D and E (nondefoliating) (Table 2).

In fields infested with the defoliating pathotype, the final values of D_I ranged from 56.9 to 61.4% for low inoculum densities (2.0 to 5.5 CFU/g), and 70.1 to 87.0% for higher inoculum densities (8.0 to 75.5 CFU/g). On the other hand, fields infested with the nondefoliating pathotype had a much lower final value of D_I, that ranged from 25.3 to 31.0% for inoculum densities ranging from 9.0 to 34.0 CFU/g (Table 2).

The AUDPC of D_I over physiological time was also positively correlated with both the inoculum density and the V. dahliae pathotype. Thus, in 1986, the maximum values of AUDPC (32.9 to 36.4%) occurred in fields with the higher inoculum density of the defoliating pathotype, whereas, minimum values (3.9 to 6.9%) occurred in fields with low or high levels of the nondefoliating pathotype. Similarly, in 1987, the maximum AUDPC (39.0%) occurred in plots with 55.5 to 75.5 CFU/g of the defoliating pathotype and a minimum value of 5.5% occurred in plots infested with 27.5 CFU/g of the nondefoliating pathotype (Table 2). Overall, disease development assessed by D_I was strongly influenced by the V. dahliae pathotype. Thus, even the lowest inoculum density of the defoliating pathotype (2.0 to 5.5 CFU/g) appeared to determine average values for the rate of increase of D_I, the AUDPC, and the final D_I which were respectively 2.4, 2.2, and 2.0 times larger than the corresponding values in plots with the highest inoculum density (27.5 to 34.0 CFU/g) of the nondefoliating pathotype (Table 2).

The rate of D_I increase in plots at the same experimental fields infested with a similar inoculum density of the defoliating pathotype was larger in 1987 than in 1986 (Table 2). Thus, in 1986, when the inoculum density was 44.0 CFU/g in field A the rate of D_I increase was 0.072 and, in 1987, with a inoculum density of 44.5 CFU/g in plot A-2 the rate of D_I increase was 0.118. Such an increase of the rate was less marked in plots infested with the nondefoliating pathotype than in those with the defoliating pathotype (Table 2).

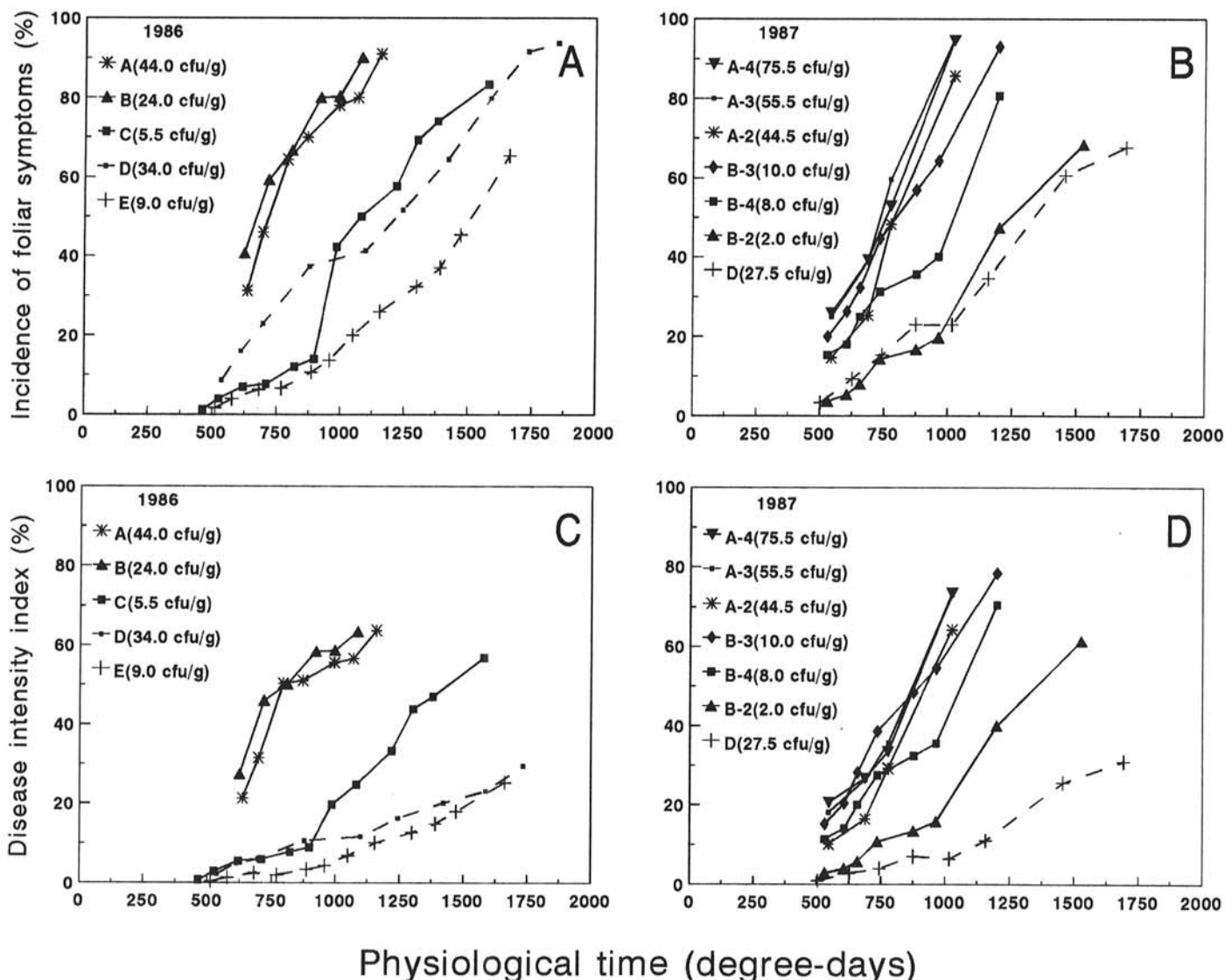
Fig. 2. Increase of Verticillium wilt foliar symptoms (%) (A and B) and disease intensity index (%) (C and D) over cumulative physiological time from sowing in five cotton fields in southern Spain with different inoculum densities (CFU/g) of the defoliating (solid) and nondefoliating (dashed) pathotypes of Verticillium dahliae in 1986 (A and C) and 1987 (B and D).

DISCUSSION

This work was carried out to determine the relationship between the inoculum density of *V. dahliae* in soil at the time of sowing and the final incidence of plants with foliar symptoms, the final incidence of vascular discoloration, and the final D_I in cotton fields in southern Spain. It also aimed to determine the influence of the initial inoculum density of defoliating and nondefoliating pathotypes of *V. dahliae* on the epidemic development of Verticillium wilt of cotton. However, our results in extensive disease

surveys indicated that a very low, nonsignificant ($P = 0.05$) correlation existed between any of the variables studied. These results agreed with those of DeVay et al. (16), who concluded that the inoculum density of the pathogen in soil is not the only determinant of the final incidence of foliar symptoms and, consequently, that the inoculum density is not a unique parameter indicator of the potential development of Verticillium wilt in cotton fields. On the other hand, our results differed from those of Ashworth et al. (6), who established a numerical relationship between the inoculum density and the percentage of cotton plants infected by *V.*

TABLE 1. Linear regression analysis of the incidence (%) of plants with foliar symptoms of Verticillium wilt over cumulative physiological time from sowing in five cotton fields in southern Spain with different inoculum densities of the defoliating and nondefoliating pathotypes of *Verticillium dahliae*

Year	Pathotype and field-plot	Initial inoculum density[a] (CFU/g)	Regression equation[b]	r^{2}[b]	Error degree of freedom	P[b]	Cumulative physiological time (degree-days) to		Date of		Final disease incidence[e] (%)
							Epidemic onset[c]	Final disease assessment[d]	Epidemic onset[c]	Final disease assessment[d]	
1986	Defoliating										
	A-1	44.0	$y = 0.103x - 25.3$	0.92	5	0.0000	247	1,473	5/10	8/26	99.3
	B-1	24.0	$y = 0.100x - 16.6$	0.95	4	0.0000	165	1,491	5/20	9/3	97.0
	C-1	5.5	$y = 0.084x - 45.8$	0.94	10	0.0000	545	1,578	6/27	9/18	83.3
	Nondefoliating										
	D-1	34.0	$y = 0.064x - 24.1$	0.99	8	0.0000	376	1,853	6/7	9/19	93.7
	E-1	9.0	$y = 0.051x - 30.5$	0.94	10	0.0000	596	1,661	6/27	9/18	65.3
1987	Defoliating										
	A-4	75.5	$y = 0.147x - 58.0$	0.98	2	0.0078	396	1,338	6/6	8/20	100
	A-3	55.5	$y = 0.150x - 59.2$	0.99	2	0.0064	394	1,338	6/6	8/20	100
	A-2	44.5	$y = 0.154x - 73.5$	0.98	2	0.0124	476	1,596	6/17	9/9	100
	B-3	10.0	$y = 0.108x - 38.1$	0.99	5	0.0000	351	1,526	5/8	8/20	98.7
	B-4	8.0	$y = 0.090x - 36.2$	0.91	5	0.0000	404	1,526	5/18	8/20	96.0
	B-2	2.0	$y = 0.067x - 36.2$	0.96	6	0.0000	543	1,526	6/3	8/20	68.4
	Nondefoliating										
	D-1	27.5	$y = 0.056x - 27.2$	0.97	6	0.0000	484	1,692	6/12	9/4	67.7

[a] Determined by the method of Butterfield and DeVay (14). Mean of six replications of five 100-mg aliquots each.
[b] y = Incidence of foliar symptoms (%). x = Physiological time accumulated from sowing (determined as the cumulative number of degree-days on a threshold of 11.9°C). r^2 = Coefficient of determination for each regression line. P = Probability value for F statistic.
[c] Time of epidemic onset determined by the point where the calculated regression of % plants with foliar symptoms on physiological time crossed the x-axis.
[d] Time of final disease assessment determined at 118 to 158 days after sowing.
[e] Percentage of plants with foliar symptoms at final disease assessment date.

TABLE 2. Linear regression analysis of Verticillium wilt intensity index (%) over cumulative physiological time from sowing in five cotton fields in southern Spain with different inoculum densities of the defoliating and nondefoliating pathotypes of *Verticillium dahliae*

Year	Pathotype and field-plot	Initial inoculum density[a] (CFU/g)	Regression equation[b]	r^{2}[b]	Error degree of freedom	P[b]	Cumulative physiological time (degree-days) to		Date of		Final disease incidence[e] (%)	AUDPC[f] (%)
							Epidemic onset[c]	Final disease assessment[d]	Epidemic onset[c]	Final disease assessment[d]		
1986	Defoliating											
	A-1	44.0	$y = 0.072x - 16.5$	0.84	5	0.0035	229	1,473	5/8	8/26	72.8	32.9
	B-1	24.0	$y = 0.070x - 9.7$	0.88	4	0.0060	138	1,491	5/18	9/3	70.1	36.4
	C-1	5.5	$y = 0.053x - 29.8$	0.94	10	0.0000	562	1,578	6/29	9/18	56.9	12.5
	Nondefoliating											
	D-1	34.0	$y = 0.021x - 8.7$	0.98	7	0.0000	421	1,734	6/11	9/9	29.5	6.9
	E-1	9.0	$y = 0.021x - 12.7$	0.93	10	0.0000	619	1,661	6/29	9/18	25.3	3.9
1987	Defoliating											
	A-4	75.5	$y = 0.114x - 48.0$	0.93	2	0.0372	420	1,025	6/9	7/28	73.6	39.0
	A-3	55.5	$y = 0.118x - 51.2$	0.96	2	0.0202	434	1,025	6/11	7/28	73.2	39.0
	A-2	44.5	$y = 0.118x - 59.2$	0.96	2	0.0200	503	1,338	6/19	8/20	75.1	31.2
	B-3	10.0	$y = 0.094x - 33.9$	0.99	5	0.0000	362	1,199	5/10	7/27	78.4	36.0
	B-4	8.0	$y = 0.083x - 35.3$	0.93	5	0.0000	428	1,526	5/21	8/20	86.7	28.0
	B-2	2.0	$y = 0.060x - 34.0$	0.95	6	0.0000	566	1,526	6/4	8/20	61.4	14.3
	Nondefoliating											
	D-1	27.5	$y = 0.026x - 15.2$	0.93	6	0.0000	583	1,692	6/22	9/4	31.0	5.5

[a] Determined by the method of Butterfield and DeVay (14). Mean of six replications of five 100-mg aliquots each.
[b] y = Disease intensity index (%). x = Physiological time accumulated from sowing (determined as the cumulative number of degree-days on a threshold of 11.9°C). r^2 = Coefficient of determination for each regression line. P = Probability value for F statistic.
[c] Time of epidemic onset determined by the point in which the calculated regression of disease intensity index (%) on physiological time crossed the x-axis.
[d] Time of final disease assessment determined at 118 to 158 days after sowing.
[e] Disease intensity index (%) at final disease assessment date.
[f] Area under the curve of disease intensity index increase between 0 and 1,400 degree-days expressed as percentage of the maximum possible area for this period.

dahliae. However, later it was shown that the influence of inoculum density on the amount of infection or foliar symptoms may vary with the tolerance of the cultivar (5,23), and also among years for a given cultivar (5). For this reason, the lack of correlation found in our study could have resulted from a high variability among the sampled fields concerning factors which influence the development of the disease, including cultural practices, soil type, environmental conditions, host cultivar, and, particularly, virulence of *V. dahliae* isolates prevailing in the soil.

For epidemic analyses and comparisons in selected fields, curves of the temporal relationship between foliar symptoms and D_I over cumulative physiological time were linearized by means of the Gompertz, logistic, log-logistic, and monomolecular models (15,32), as well as by linear regression of nontransformed data. Whether disease increase was measured as the incidence of foliar symptoms or D_I, all epidemiological models considered were similarly appropriate to describe disease increase over time. However, we chose the simple straight line regression as the most convenient for this work because of its simplicity for comparison of epidemics.

When the incidence of foliar symptoms and D_I in cultivar Coker 310 were expressed as a linear function against physiological time, the parameters of the curves of disease increase were related to both the initial inoculum density in soil and the nature of the *V. dahliae* pathotype. This was similar to that described for the indice of foliar symptoms in cultivar Acala SJ-2 grown in fields in California infested mainly with nondefoliating isolates of *V. dahliae* of intermediate virulence (25), and also in fields infested with the defoliating pathotype (22). The physiological time accumulated from the time of sowing to the epidemic onset, determined by the intersect of the regression equation with the time axis, decreased as both the inoculum density and the virulence of the pathotype increased. The theoretically determined dates of the epidemic's onset agreed well with our observations of dead plants at the stages of cotyledons to one or two pairs of true leaves in cotton fields severely affected by Verticillium wilt (J. Bejarano-Alcázar et al., *unpublished data*). The rate of increase in the incidence of plants with foliar symptoms over physiological time increased with the inoculum density of the defoliating pathotype of *V. dahliae*, until it reached an apparent threshold level of 24 to 44 CFU/g in 1986 and 44 to 75 CFU/g in 1987. This result was similar to that found for the rate of D_I increase, suggesting that, for the defoliating pathotype, the inoculum density-disease level relationship in susceptible cultivars likely reached the saturation phase (7).

Comparisons of the rates of disease increase and the final incidences of foliar symptoms and D_I among plots with a range of inoculum densities of either pathotype (Tables 1 and 2) showed that, under natural conditions, *V. dahliae* pathotypes differing in virulence required different levels of inoculum density in soil to cause similar amounts of the disease. Within the range of inoculum density studied in this work, the rate of disease increase over physiological time was higher in plots infested mainly with the defoliating pathotype than in plots infested with the nondefoliating pathotype. Schnathorst and Mathre (30) demonstrated such a relationship in artificial inoculations of resistant, tolerant, and highly susceptible cotton cultivars with defoliating and nondefoliating isolates of *V. dahliae*. Thus, our results supported suggestions made above that several factors may influence the incidence of the disease, and stressed the need of better techniques to quantitatively and qualitatively determine the inoculum of *V. dahliae* in soil. We have shown that the low inoculum density of the defoliating pathotype was sufficient to cause severe epidemics in cotton crops. Consequently, when selecting and applying control measures against the disease, considerations should be given to the amount and virulence of the existing inoculum, as well as to the efficacy of the control measure, so that the residual inoculum after the control measure is low enough to avoid severe crop losses.

On the other hand, comparisons of the rates of D_I increase over physiological time in plots with similar inoculum density levels of either of the pathotypes showed that the disease in plots infested with the defoliating pathotype increased at rates of 2.5 to 3.3 times higher than those in plots infested with the nondefoliating pathotype. These magnitudes of disease increase were much higher than those found when comparisons were done for the rates of increase in the incidence of foliar symptoms (1.6 to 1.7), which illustrated the faster development of severe foliar symptoms in plots infested with the defoliating pathotype (Tables 1 and 2). Similar large differences occurred between the AUDPC of D_I in plots infested with different pathotypes; the AUDPC values in plots infested with the defoliating pathotype were 3.2 to 5.3 times higher than those in plots infested with the nondefoliating one, and the corresponding final D_I values were 2.3 to 2.4 times higher. Consequently, D_I would appear to be a more appropriate parameter to indicate the extent of Verticillium wilt attacks than the incidence of foliar symptoms, as it expresses the combined effect of the incidence and mean severity of foliar symptoms. In this way, losses of lint yield caused by Verticillium wilt epidemics might be explained better by the descriptive parameters of the D_I increase curve over physiological time than by parameters of the curve of increase in the incidence of foliar symptoms (J. Bejarano-Alcázar et al., *unpublished data*).

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