Variables Associated with Intensity
of Alternaria Leaf Spot in Pima Cotton

D. Shtienberg

Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

ABSTRACT

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The effect of variables associated with the intensity of Alternaria leaf spot (caused by *Alternaria macrospora*) in Pima cotton (*Gossypium barbadense*) was investigated over three years in two growing regions in Israel. Alternaria leaf spot intensity was recorded at the initiation of flowering and at advanced stages of boll development. Variables characterizing the field and its surroundings (seven variables), the crop (seven variables), and plant protection actions (five variables) were recorded. The data were analyzed with a general linear model (GLM), and the relative contribution of each variable to disease incidence or disease-induced defoliation was determined. The first GLM explained 72% of

the variance in disease incidence at the initiation of flowering ($P \le 0.0001$). Of the variables examined, only four significantly contributed to the explanation of variance in disease incidence: the previous crop (4.4% of the explained variance, 3.2% of the total variance), the growing season (6.1 and 4.4%), the farm (42.4 and 30.5%), and the interaction between farm and soil type (47.1 and 33.9%). The second GLM explained 62% of the variance of disease-induced defoliation at advanced stages of boll development ($P \le 0.0001$). Of the variables examined, only four had a significant effect: disease incidence at flowering (9.9 and 6.2%), type of irrigation system (10.1 and 6.3%), region (37.4 and 23.2%), and farm (42.6 and 26.4%).

Additional keywords: integrated pest management, survey.

Alternaria leaf spot in Pima cotton (*Gossypium barbadense* L.), caused by the fungus *Alternaria macrospora* A. Zimmerm., occurs in most cotton-growing areas in the world (3). In Israel, *A. macrospora* first appeared soon after the introduction of cotton in the 1950s, and by the 1970s, the disease had begun to cause economic losses (2). The pathogen produces symptoms on cotyledons, leaves, twigs, stems, and bolls. The lesions appear as small, brown, circular spots, sometimes with a purple margin. Fungicides are used to suppress the disease, with up to 12 applications per growing season. Despite intensive management, however, disease may develop in commercial fields and lead to economic losses (14).

In the field, inoculum may be introduced by seeds, originate in debris from the previous year, or be transported by wind from neighboring fields. Seedborne transmission of *A. macrospora* has been reported in many locations (14). In Israel, up to 70% of mechanically delinted seeds from infected bolls were found to carry the pathogen on the seed coat; chemical delinting reduced the degree of infestation but left some infections at the chalazal end of the seed (5). In the field, however, debris rather than seeds is the main source of initial inoculum. Dead leaves carry the pathogen over the winter but tend to disintegrate easily; stem debris and branch debris are more persistent (11,13,14).

Susceptibility of cotton to *A. macrospora* is highest in seedlings, decreases in young plants, and then increases again with age to a second peak in senescent plants (1,16,19,21,22). Consequently, epidemics develop in three phases, which correspond closely to the emergence, vegetative, and reproductive

growth stages of the host. The first phase is at the seedling stage; the cotyledons are five to eight times more susceptible to infections than the first true leaves and support the initial stages of the epidemic (1,22). The second phase extends from the seedling stage to the initiation of flowering. During this period, night temperatures are low and the canopy is open and thus does not create a microclimate favorable for disease development. In addition, the host is relatively resistant to infection at this time (21). Accordingly, *Alternaria* infections are scattered, and the progress of the epidemic is associated mainly with an increase in the number of infected plants. During the third phase of the epidemic, the development of a dense canopy and higher night temperatures create a more favorable microclimate. Concurrently, the host becomes progressively more susceptible, and the amount of damaged foliage increases rapidly (21). *Alternaria*-infected leaves senesce and drop prematurely. Leaves are shed when lesions cover an average of 1 to 3% (or more) of the leaf surface (1).

Data recorded in comprehensive surveys, if analyzed properly, may provide information about the significance and relative importance of variables associated with disease epidemics. Variables may describe weather parameters, such as temperature and duration of leaf wetness (e.g., 6,18), as well as cultural or crop parameters, such as host resistance and planting date (e.g., 7,24). The knowledge gained from such studies may provide useful information for directing disease management actions.

In the 1990, 1991, and 1992 growing seasons, a study was conducted in Pima cotton fields in Israel. Alternaria leaf spot intensity was recorded twice in the season, once at the initiation of flowering and again at advanced stages of boll development. In addition, details characterizing the field and its surroundings, the crop, and plant protection actions were recorded. The data were analyzed with the objective of identifying the importance and relative contributions of the various variables to Alternaria leaf spot intensity.

Corresponding author: D. Shtienberg; E-mail address: dani@agri.huji.ac.tl

MATERIALS AND METHODS

The effect of variables associated with intensity of *A. macrospora* infection in Pima cotton was ascertained over three years (1990, 1991, and 1992) in two growing regions (Hadera and Rehovot-Lakhish) in Israel. In 1990, the cultivated area of Pima cotton was relatively large (about 5,000 ha), and about 50% of the area was sampled. A list of all farms growing Pima cotton was prepared with the assistance of the regional extension officers (Y. Sachs and J. Dreishpoun), and the farms to be sampled in each region were selected at random from the list. Because of a sharp decrease in the price of Pima cotton on the world market, the area under cultivation was reduced markedly in 1991 and 1992 (about

1,000 ha each year); in these years, all farms growing Pima cotton were inspected. On each of the surveyed farms, all Pima cotton fields were inspected, for a total of 117 fields in 1990, 26 fields in 1991, and 13 fields in 1992.

Each field was inspected twice in the growing season, once at the initiation of flowering in early to mid-June and again during advanced stages of boll development in mid-August to early September. At both dates, disease was assessed in each field at six to 12 sampling sites. The number of sampling sites in a particular field was determined according to the field's size and uniformity. In large (>40 ha) or nonuniform fields, more samples were taken than in smaller or uniform fields. Plant height, crop density, and development stage were considered in determining uniformity. In cases where soil type, type of irrigation system, the previous crop, or the cultivar varied within a field, the field was divided into several subunits, and each subunit was then considered as a separate "field" for data collection and analyses.

Within each field, the exact location of sampling sites was determined with a field map, in a way such that most of the field area was included. In a rectangular field, for example, six sampling sites were designated, one at each of the four sides plus two in the middle of the field. Sampling sites were located at least 30 m from the edge of the field. This particular sampling procedure has been used previously in disease surveys (e.g., 23). *A. macrospora* infections are distributed randomly within Pima cotton fields (D. Shtienberg, *unpublished*).

At each sampling site, disease was assessed as follows. On the first disease assessment date, 20 plants were chosen arbitrarily in

TABLE 1. Analysis of variance for a general linear model of incidence of Alternaria leaf spot at the initiation of flowering of Pima cotton grown on farms in Israel during 1990 to 1992

Source	df	SS		F	P	R^2
Model	62	43,036	(100.0)	3.77	0.0001	0.72
Error	91	16,734	(0.0)			
Total	153	59,770	(0.0)			
Previous crop	2	1,903	(4.4)[a]	5.18	0.0074	
Year	2	2,628	(6.1)	7.15	0.0013	
Farm	37	18,245	(42.4)	2.62	0.0001	
Farm × soil type	21	20,260	(47.1)	5.25	0.0001	

[a] Percentage of the total sum of squares (SS) of each component relative to the total SS explained by the model.
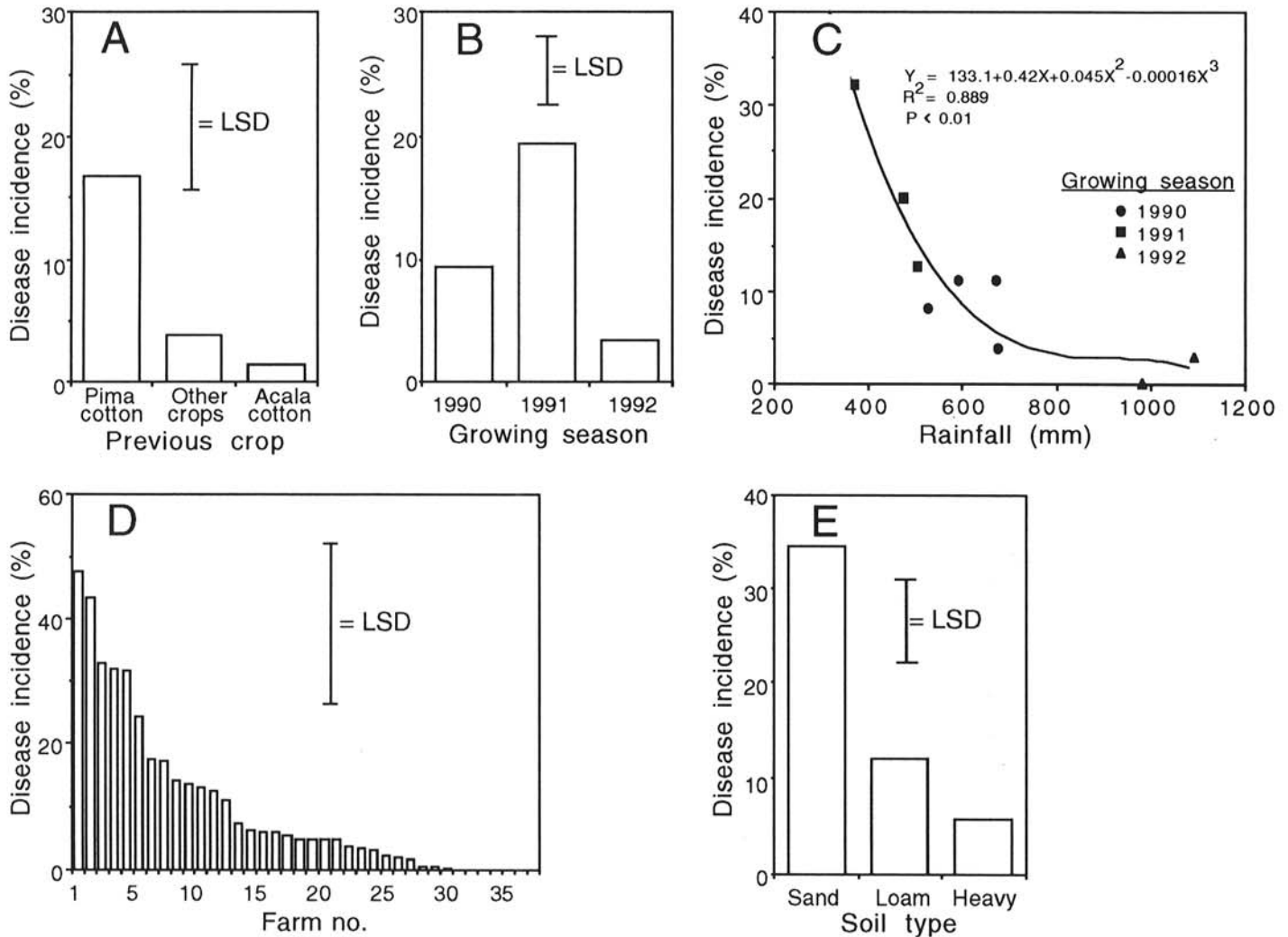

Fig. 1. Variables associated with the incidence of Alternaria leaf spot on Pima cotton at the initiation of flowering: **A,** previous crop; **B,** growing season; **C,** amount of rain in the preceding winter; **D,** farm; and **E,** soil type. Analysis of variance is presented in Table 1. LSD = least significant difference at $P \leq 0.05$.

In figure C: $Y_2 = 133.1 + 0.42X + 0.045X^2 - 0.00016X^3$, $R^2 = 0.889$, $P < 0.01$.

an area of approximately 4×10 m. Each plant was inspected, and the number of infected plants was recorded. These data were used to calculate disease incidence (the frequency of infected plants) for each sampling site, from which the field average was calculated.

On the second disease assessment date, *Alternaria*-induced defoliation was assessed on 10 plants at each sampling site (not necessarily those inspected in the first assessment), as described by Shtienberg and Dreishpoun (20). Disease severity on attached leaves and proportion of shed leaves were assessed separately for the lower (<30 to 50 cm), middle (30 to 50 to 70 to 90 cm), and upper (>70 to 90 cm) levels of the canopy. Disease severity was rated with the aid of a disease assessment scale (4). Leaf abscission sites on the main stem and branches were easily distinguished, and leaf shedding was determined with the aid of an assessment key (20). Defoliation induced by Alternaria leaf spot was estimated on the basis of these assessments (calculated as a weighted sum of the disease severity on attached leaves and the proportion of shed leaves [20]).

During the growing season, several variables related to the sampled fields were recorded. The data characterized each field with respect to its location and the surrounding area, the crop, and plant protection actions. The data were then analyzed by means of a general linear model (GLM) procedure of SAS (release 6.03; SAS Institute, Cary, NC). A GLM is defined as an equation of random variables, parameters, and mathematical (nonstochastic) variables (8); it is not restricted to a linear response surface. The term "linear" refers to the fact that the model is linear in the parameters, and not to the shape of the response surface. GLMs can handle classification variables that have discrete levels as well as continuous variables that measure quantities (9).

Two basic models were used in this study. For the first disease assessment date, the model was as follows:

$$I = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 \\ + \beta_8 Z_1 + \beta_9 Z_2 + \beta_{10} Z_3 + \varepsilon .$$

For the second disease assessment date, the model was as follows:

$$F = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_8 + \beta_8 X_9 \\ + \beta_9 Z_1 + \beta_{10} Z_2 + \beta_{11} Z_3 + \beta_{12} Z_4 + \beta_{13} Z_5 + \beta_{14} Z_6 + \beta_{15} Z_7 \\ + \beta_{16} Z_8 + \beta_{17} Z_9 + \beta_{18} Z_{10} + \varepsilon ,$$

where I is disease incidence (%) at the initiation of flowering, F is disease-induced defoliation (%) at advanced stages of boll development, X_1 to X_9 are discrete variables, Z_1 to Z_{10} are continuous variables, β_1 to β_{18} are parameters, and ε is random error. The analyses also included interaction terms among discrete variables and covariance effects among continuous and discrete variables, but these were excluded from the equations for simplicity.

Of the nine discrete variables, X_1 was year (1990, 1991, or 1992), and X_2 was region. The two growing regions surveyed were Hadera (central Mediterranean seashore) and Rehovot-Lakhish (southern Mediterranean seashore and the inland plains). Each region was divided into three subregions according to the specific climatic conditions prevailing in the area at the time of concern (June to September). The subdivision was made after consultation with Z. Gat, an agrometeorologist of the Israel National Weather Forecast Service. Subregions for Hadera were Ha'Sharon, Pardess Hanna, and the Carmel seashore; subregions for Rehovot-Lakhish were the southern seashore, the inland plains, and Mevo'ot Jerusalem.

The variable X_3 represents the farms, which were all collective villages (kibbutzim); X_4 was the relief of the area surrounding the field (flat, hilly, mountainous, low, or slope land); X_5 was the soil type (sandy, loam, or heavy); X_6 was the cultivar of Pima cotton (S-5, F-177, F-27, BF-19, or 362); X_7 was the previous crop (Pima cotton, Acala cotton [*G. hirsutum* L.], or a crop other than cotton);

X_8 was the type of irrigation system (sprinkler or drip); and X_9 was crop density and uniformity (dense, intermediate, or well spaced).

Of the 10 continuous variables, Z_1 was altitude (meters above sea level), Z_2 was distance from the Mediterranean Sea (kilometers), and Z_3 was amount of rainfall in the preceding winter, as recorded at weather stations of the National Weather Forecast Service. Z_4 was crop height, determined on the second assessment date on five plants. Z_5 was the total number of fungicidal sprays, and Z_6, Z_7, Z_8, and Z_9 were the number of sprays applied in June, July, August, and September, respectively. Disease incidence at the initiation of flowering was the last continuous variable (Z_{10}).

At the end of the 1990 and 1991 growing seasons, a questionnaire was sent to all the growers, asking them to specify all the plant protection actions applied to their fields. Requested details included the time, product, rate, and mode of application (ground or aerial) of fungicidal sprays.

The correlation among continuous variables was examined to avoid multicollinearity. Because a significant correlation was found between variable Z_5 and variables Z_6 to Z_9, they were not included in the same GLM analysis.

Several models were computed. They were evaluated on the basis of the significance of the estimated parameters, normality of the residuals, and coefficients of determination. In this report, only two models are presented, one for each sampling date; the models include only the independent variables that significantly affected the dependent variables.

The GLM was also used to quantify the relative contribution of each independent variable to the variance of the dependent variables. The relative contribution of a variable was calculated as the product of the sum of squares due to the variable and the total sum of squares explained by the GLM analysis. The relative contribution of a variable was expressed as a percentage, with 100% as the total sum of squares explained by the model.

RESULTS

Variables associated with disease incidence at flowering. Of all variables examined for their influence on disease incidence, only four were significant: previous crop, year, farm, and soil type; effects of soil type and farm interacted. The first GLM explained 72% of the variability in disease incidence at flowering (Table 1). None of the other variables listed previously and none of the other interactions among two or three variables had a significant effect on disease incidence at flowering.

The previous crop had a relatively small yet significant influence on disease incidence at flowering: 4.4% of the variance explained by the model (3.2% of the total variance) was attributed to this variable. Pima plants grown in a field in which the previous crop also had been Pima cotton had a significantly higher incidence of infection than those grown in fields in which other crops had been cultivated previously (Fig. 1A). There were no differences among other crops (including Acala cotton) with respect to their effect on disease incidence.

TABLE 2. Analysis of variance for a general linear model of defoliation induced by Alternaria leaf spot at advanced stages of boll development of Pima cotton grown on farms in Israel during 1990 to 1992

Source	df	SS		F	P	R^2
Model	41	5,894	(100.0)	3.7	0.0001	0.62
Error	94	3,619				
Total	135	9,513				
Disease incidence	1	587	(9.9)[a]	15.2	0.0002	
Irrigation system	2	595	(10.1)	15.4	0.0002	
Subregion	5	2,204	(37.4)	11.4	0.0001	
Farm	33	2,508	(42.6)	1.97	0.0058	

[a] Percentage of total sum of squares (SS) of each component relative to the total SS explained by the model.

Disease incidence varied significantly among the three years of study. Disease was most severe in 1991, moderate in 1990, and relatively mild in 1992 (Fig. 1B). Year had a relatively slight effect on the overall variability of disease incidence, accounting for 6.1% of the explained variance (4.4% of total variance) (Table 1).
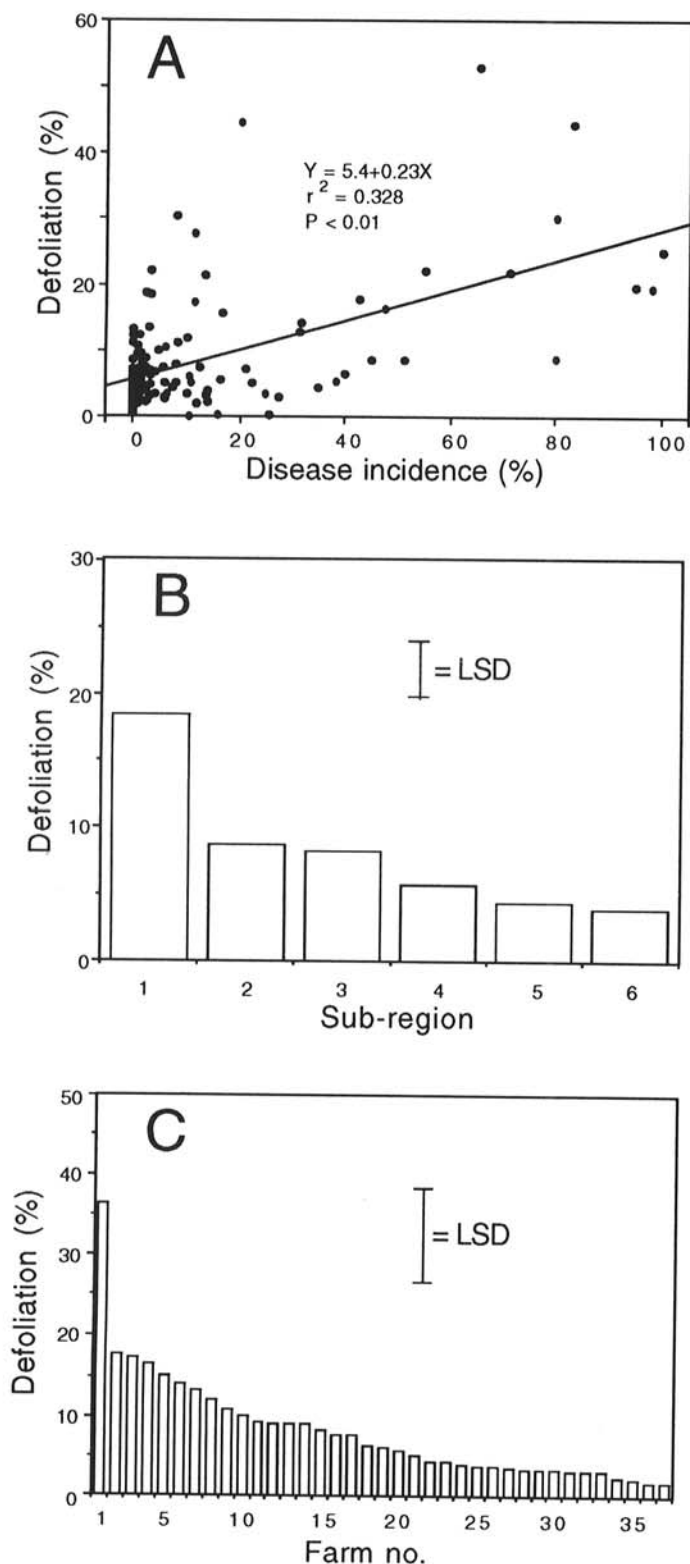

Fig. 2. Variables associated with *Alternaria*-induced defoliation of Pima cotton at advanced stages of boll development: **A,** disease incidence at the flowering stage; **B,** subregion (1 = Pardess Hanna; 2 = Ha'Sharon; 3 = inland plains; 4 = Carmel seashore; 5 = Mevo'ot Jerusalem; 6 = southern seashore); **C,** farm. Analysis of variance is presented in Table 2. LSD = least significant difference at $P \leq 0.05$.

Subregional averages of disease incidence at flowering were significantly related to the amount of rainfall in the previous winter: disease incidence decreased with increasing rainfall (Fig. 1C). The 1991-92 winter was exceptionally rainy, and disease in the following 1992 season was relatively mild; the 1990-91 winter was dry and was followed by severe Alternaria leaf spot during the 1991 growing season.

A substantial part of the variability in disease incidence (42.4% of the explained variance, 30.5% of total variance) was attributable to the farm variable. In some cases, farms located very close to each other within a subregion had significantly different disease incidence. For example, two farm pairs (farms 2 and 15 and farms 1 and 32) located less than 10 km apart within one subregion had markedly different disease incidence values (Fig. 1D).

Soil type was another fundamental variable, and the effect of this variable interacted significantly with that of the farm variable. The interaction term accounted for 47.1% of the explained variance (33.9% of total variance). Disease incidence was significantly higher in plants grown in sandy soils than in those grown in loam or heavy soils (Fig. 1E).

Variables associated with disease-induced defoliation during boll development. Of all the variables examined, only four had significant effects on disease-induced defoliation at advanced stages of boll development: disease incidence at flowering, type of irrigation system, region, and farm. Although the GLM was highly significant ($P \leq 0.0001$), it explained only 62% of the variability in disease-induced defoliation (Table 2).

Disease incidence at the initiation of flowering significantly affected disease-induced defoliation at advanced stages of boll development (Fig. 2A). When only these two variables were considered, the correlation between them was low ($r^2 = 0.328$), although significant ($P \leq 0.01$). As part of the GLM, this correlation was more significant ($P \leq 0.0002$). Nevertheless, only 9.9% of the explained variance in disease-induced defoliation (6.2% of the total variance) was attributable to disease incidence at the initiation of flowering (Table 2).

Cotton is grown in Israel during the summer (the dry season) and thus is always irrigated. The type of irrigation system, either overhead sprinklers or drip, had an intermediate effect on disease-induced defoliation, accounting for 10.1% of the explained variance (6.3% of total variance). The average defoliation in fields irrigated by overhead sprinklers was 18.1%, significantly higher than the 6.5% average defoliation in fields irrigated by a drip system.

Disease-induced defoliation varied significantly among the six subregions; the subregion accounted for 37.4% of the explained variance (23.2% of total variance) (Table 2, Fig. 2B). Within each subregion, the farm variable had a significant effect as well, contributing 42.6% of the explained variance (26.4% of total variance), and was, therefore, the most influential variable. As in the first GLM analysis (Table 1, Fig. 1D), there were cases in which defoliation in nearby farms differed significantly (e.g., in farms 2 and 29) (Fig. 2C).

Disease incidence at the initiation of flowering had a relatively limited effect on the number of fungicidal sprays applied against *A. macrospora*. When disease incidence at flowering was low (<20%), the number of fungicidal sprays applied ranged widely (from two to 10). In fields where disease incidence at the initiation of flowering exceeded 20%, fungicides were applied at weekly intervals (on average), for a total of nine to 12 sprays (Fig. 3A). Disease-induced defoliation at advanced stages of boll development was not directly related to the number of sprays applied in the growing season. Fields sprayed eight or nine times in a growing season had significantly more disease than fields sprayed fewer than eight or more than nine times (Fig. 3B). The average number of fungicide sprays was 6.2 per season. The most common fungicides were maneb and tin (triphenyltin-hydroxide and triphenyltin-acetate) products.

DISCUSSION

The amount of initial inoculum in a field is the result of two processes: the amount of inoculum left at the end of the previous growing season, and the survival of the inoculum. Therefore, in fields in which crops other than Pima cotton preceded the current crop, disease incidence at flowering would be expected to be lower than in fields where Pima cotton was the preceding crop. *A. macrospora* does not infect the crops grown in rotation with Pima cotton (e.g., wheat, legumes, corn, or sunflower) and is rare in Acala cotton (*G. hirsutum*) (14,17). Thus, the contribution of previous crop to the variance of disease incidence at the initiation of flowering in the first GLM model was surprisingly low (4.4% of the explained variance) (Table 1). One explanation for this result is that disease intensity at the end of the previous growing season, which probably varied among fields, was not considered. Another explanation may be that conditions for successful over-wintering of inoculum among growing seasons were variable.

A study (13) of edaphic influences on the survival of *A. macrospora* in cotton debris revealed that the fungus survived better in sterilized compared to nonsterilized soils, in dry compared to wet soils, and in light compared to heavy soils. It survived better at low than at high temperatures, in fields left uncultivated in winter compared to those cropped with wheat in winter, and in debris on the soil surface compared to debris buried in the soil. The survival of a pathogen in debris is conditioned by survival of the debris itself. In general, the destruction of debris in soil is affected by processes that decompose organic materials, mainly moisture, oxygen, and microbial activity. Rainy winters and heavy soils are associated with increased activity of micro-organisms that degrade infected plant residues (10,12–14). The results of this study seem to confirm these observations. Disease incidence at flowering, which is a direct outcome of and an indication of the amount of overwintering inoculum, was higher in seasons following dry rather than rainy winters (Fig. 1C) and in crops grown in sandy rather than heavy soils (Fig. 1E). These two variables contributed markedly to the variance of disease incidence (Table 1).

Disease-induced defoliation at advanced stages of boll development was affected by disease incidence at the initiation of flowering (an indication of the amount of initial inoculum) and by variables affecting the rate of disease development. Our results indicate that the latter component was more important than the former (Table 2, Fig. 2A). In general, *A. macrospora* infects cotton under a wide range of conditions. The minimum, optimum, and maximum temperatures for growth are <10°C, 20 to 30°C, and 35°C, respectively (1). Epidemics may be associated with periods of wet and overcast weather, or high humidity, or nightly dew periods of 8 to 12 h (1,14,15). The microclimatic conditions within the crop canopy are affected by the type of irrigation system used. In contrast to drip irrigation, overhead irrigation wets the foliage, which produces conditions favorable for infection (14). Another variable affecting crop microclimate is the region. The direction and intensity of the prevailing winds, relative humidity, and temperature vary slightly among the subregions and may have affected the microclimate within the crop canopy. Consequently, disease-induced defoliation varied significantly among subregions (Fig. 2B). The irrigation and region variables accounted for approximately 50% of the explained variance in disease-induced defoliation (Table 2).

Individual farms had the greatest influence in GLMs that accounted for variation in disease incidence at the initiation of flowering and in disease-induced defoliation at advanced stages of boll development (Tables 1 and 2). This variable does not necessarily reflect regional effects on disease, because in some cases farms located very close to one another had significantly different disease intensity. Unfortunately, the analysis did not allow more precise definition of this variable. It most likely has several components, such as the professionalism and experience of the grower and the type and quality of the cultural equipment.

The importance of the farm variable was reported in another study that examined variables associated with wheat yields (25). This finding should focus attention on the fact that accurate and well-defined guidelines for disease management will not necessarily result in adequate disease suppression. Control efficacy may vary substantially among farms. Determination of the components of the farm variable is important and deserves a separate study. To yield comprehensive conclusions, such a study should be conducted by a multidisciplinary team of researchers including plant pathologists, extension personnel, agricultural education specialists, and growers' representatives.

Fig. 3. A, Relationship between Alternaria leaf spot incidence at the initiation of flowering and the number of fungicidal sprays applied to Pima cotton during the growing season. **B,** Relationship between the number of sprays applied during the growing season and *Alternaria*-induced defoliation at advanced stages of boll development. Bars represent standard error.

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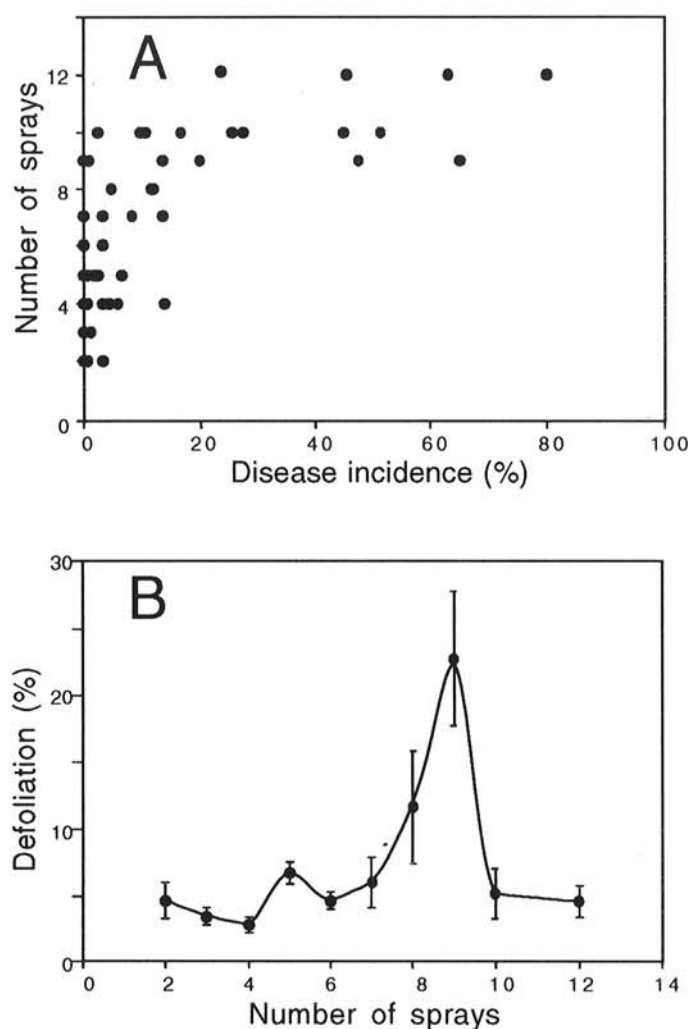
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