Influence of Initial Density and Distribution of Inoculum on the Epidemiology of Tobacco Black Shank

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ABSTRACT

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Residual inoculum of *Phytophthora parasitica* var. *nicotianae* was aggregated in field soil at the time tobacco transplants were set. The negative binomial dispersion parameter (k) ranged from 0.02 to 2.03 in the upper 10 cm of soil among individual plots. Aggregation of inoculum increased with depth in the soil profile. Mean inoculum densities were generally greatest in the upper 10 cm of soil and ranged from 0.01 to 1.31 propagules per gram of soil among individual plots. Mortality in the resistant cultivar, Speight G-28, was related directly to initial mean inoculum density throughout the epidemic but not to the aggregation of inoculum. Mortality in the susceptible cultivar, Hicks, was not related to initial mean inoculum density, but late in the epidemic it was related to the aggregation of inoculum (measured by Lloyd's index of patchiness, $1 + 1/k$) and to the interaction between inoculum density and aggregation. For equivalent initial mean inoculum densities, more extreme aggregation of inoculum delayed disease development in Hicks. The combination of both low initial mean density and extreme aggregation of inoculum further delayed disease development and resulted in a reduction in the final percentage of mortality attained.

Additional key words: *Nicotiana tabacum*, Poisson probability distribution.

The relationship between inoculum density and disease is a fundamental component of quantitative epidemiological investigations involving root-infecting pathogens. Once established, this relationship can be used to evaluate the effectiveness of a variety of biological, chemical, or cultural control practices (15). However, this quantitative relationship is valid only under the conditions of environment, host growth stage, and phase of epidemic development for which it was described (1).

The theory relating inoculum density to disease is based on the assumption that initial inoculum is dispersed randomly among potential host plants (8,30). However, inoculum, assessed either directly by sampling or indirectly by the occurrence of disease, is not dispersed truly at random in most pathosystems examined, but rather is aggregated or clumped (2–4, 9–11,14,18–21,24,26–30). The clumping of infections of *Cylindrocladium crotalariae* observed on peanut roots (10) was suggested to result from the clumping of inoculum in soil that had been observed previously (28). Other studies have attempted to associate the nonrandomness of inoculum with the nonrandomness of disease (4,14,21).

Waggoner and Rich (30) provided the basis for modification of this theory when inoculum is aggregated and can be described by the negative binomial probability distribution. Based on a random or Poisson distribution of inoculum, the estimated disease proportion, Y_P, is given by

$$Y_P = 1 - e^{-ID} \quad (1)$$

where ID is the inoculum density per plant and e is the base of the natural logarithm. Alternatively, based on a nonrandom or negative binomial distribution of inoculum, the estimated disease proportion, Y_{NB}, is given by

$$Y_{NB} = 1 - [\,(ID/k) + 1\,]^{-k} \quad (2)$$

where k is the negative binomial dispersion parameter. Equations relating the observed disease proportion to linear functions of ID are obtained by use of the transformations, $\ln[1/(1 - Y)]$ and $k[(1 - Y)^{-1/k} - 1]$, for the Poisson and negative binomial models, respectively. This theory does not relate the nonrandomness of inoculum to the nonrandomness of disease per se; it relates the nonrandomness of inoculum to the level of disease attained. Aggregation of inoculum reduces the estimated disease proportion from that predicted for a random distribution of inoculum and increases the inoculum density required to attain a given level of disease (Fig. 1).

Inoculum density-mortality relationships have been established for the susceptible tobacco (*Nicotiana tabacum* L.) cultivar, Hicks, under controlled conditions with randomly dispersed chlamydospores of *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker (12). The inoculum density required for 50% mortality within 50 days was 0.13 chlamydospores per gram of soil (8.6 chlamydospores per plant). However, soil water matric potential was found to alter this relationship substantially through its influence on the increase of inoculum by the formation of sporangia and the release of zoospores (23). Inoculum density-mortality relationships in this pathosystem have not been examined critically under field conditions, nor have they been examined with regard to initial inoculum distribution or time of disease assessment.

The objectives of this study were 1) to examine the initial density and distribution of inoculum of *P. parasitica* var. *nicotianae* in field soil at the time tobacco transplants were set, and 2) to examine the influence of initial density and distribution of inoculum on subsequent disease development in two tobacco cultivars.

MATERIALS AND METHODS

Studies on black shank development were conducted in a field infested with *P. parasitica* var. *nicotianae*. This field had been planted to tobacco annually since 1967 and served as a black shank disease nursery. The soil was predominantly a well-drained, Arredondo loamy sand (to a depth of 1 m) containing an area of Kendrick loamy sand (to a depth of 0.5 m). The soil of the Ap horizon had a bulk density of approximately 1.45 g/cm^3.

Eighteen, six, and eight pairs of plots of the susceptible cultivar, Hicks, and the resistant cultivar, Speight G-28, were established in 1982, 1983, and 1984, respectively. Plots were 3.75 × 12.5 m and

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contained four rows of 15–22 plants each. Ten-week-old tobacco plants were transplanted on 25 March 1982, 14 April 1983, and 6 and 10 April 1984. Plants were spaced 55–65 cm apart with averages of 90, 82, and 73 plants per plot in 1982, 1983, and 1984, respectively. Plot designs consisted of a modified split-block design with three strips of each cultivar established across the blocks in 1982, a randomized complete block design in 1983, and a completely randomized design in 1984.

A preliminary survey of the initial density and distribution of inoculum of *P. parasitica* var. *nicotianae* in the field was conducted on 26 March 1982. Three of the six plots of each cultivar in a strip were chosen randomly for sampling. A 2.7-m transect was established across the center of each plot, and 10 soil samples were taken 30 cm apart along each transect for a total of 180 samples. Samples were taken with a 2.5-cm-diameter core sampler to a depth of 10 cm in all plots and to a depth of 20 cm in six plots (one randomly chosen plot from each cultivar strip). These 20-cm cores were divided into two 10-cm segments (approximately 49 cm^3 of soil) that were assayed individually.

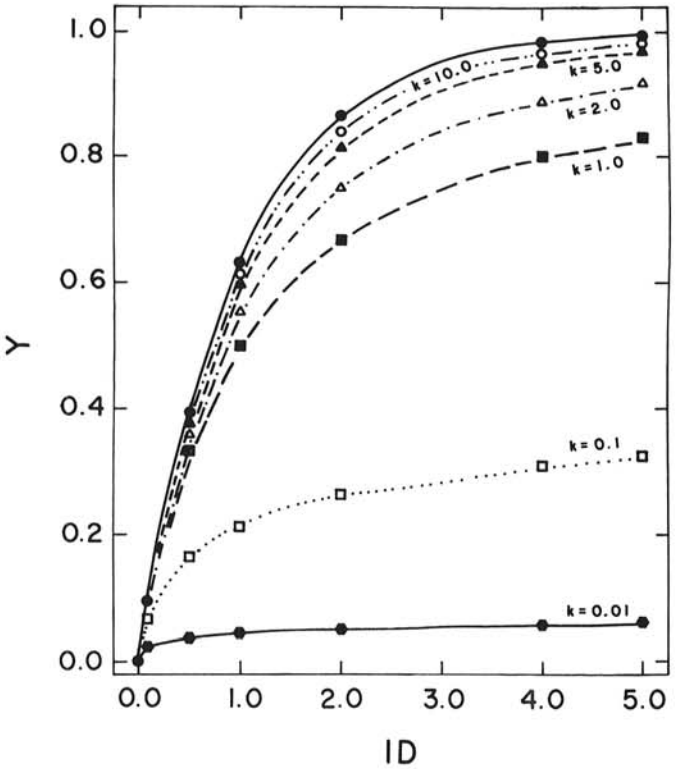

Fig. 1. Theoretical relationships of inoculum density (*ID*) to disease (*Y*) based on a random or Poisson distribution of inoculum calculated by $1 - e^{-ID}$ (●——●) or based on a nonrandom or negative binomial distribution of inoculum calculated by $1 - [(ID/k) + 1]^{-k}$ for *k* values of 0.01, 0.1, 1.0, 2.0, 5.0, and 10.0.

Intensive sampling of individual plots was conducted for one, six, and eight plots to be planted with Hicks in 1982, 1983, and 1984, respectively; eight plots to be planted with Speight G-28 also were sampled intensively in 1984. Sampling within plots was confined to an area of 2 × 10 m established over the center two rows of each plot (Fig. 2). This sample area was divided into ten 1- × 2-m subplots, five of which were chosen randomly for sampling. Samples were taken 25 cm apart along transects established across the center of each subplot for a total of nine samples per transect and 45 samples per depth per plot. Samples were taken to a depth of 30 cm in 1982 and 1983, and each 30-cm core was divided into three 10-cm segments that were assayed individually. Only the upper 10 cm of soil was sampled in 1984. Sampling was conducted on 2 April 1982, 13–15 April 1983, and 2–5 April 1984. All samples were assayed on the day they were collected.

Each sample was shaken before use to ensure thorough mixing of the soil. For the preliminary sample in 1982, 25 and 35 g of soil (dry weight) were used from each sample from depths of 0–10 and 10–20 cm, respectively. The soil was suspended in 100 and 35 ml of dilute water agar (0.25% Difco Bacto-agar) for the respective depths. For samples from individual plots, 35 g of soil per sample were used. This soil was placed in a 250-ml beaker with a magnetic stir bar and 50 ml of dilute water agar (0.25%) amended with 10 mg of rifampicin (Sigma Chemical Co., St. Louis, MO), 10 mg of pimaricin (Gist-Brocades N.V., Delft, Holland), 250 mg of ampicillin (Bristol-Myers Co., Syracuse, NY), and 50 mg of hymexazol (Sankyo Co., Ltd., Tokyo, Japan) per liter. The soil suspensions were stirred on a magnetic stir plate, and 1-ml subsamples were transferred to each of 10 petri plates containing the PARP medium of Kannwischer and Mitchell (12), supplemented with 50 mg of hymexazol per liter of medium. Additionally, 1-ml subsamples were pipetted into aluminum weigh pans, were oven dried, and the average weight of soil introduced onto each plate was determined.

Soil plates were incubated in the dark at 25 C for 48 or 60–72 hr; the soil was rinsed from the plates which then were examined for colonies of *P. parasitica* var. *nicotianae*. All plates were reexamined 24 hr later. Identification of the pathogen was done either macroscopically based on its distinct colony morphology or

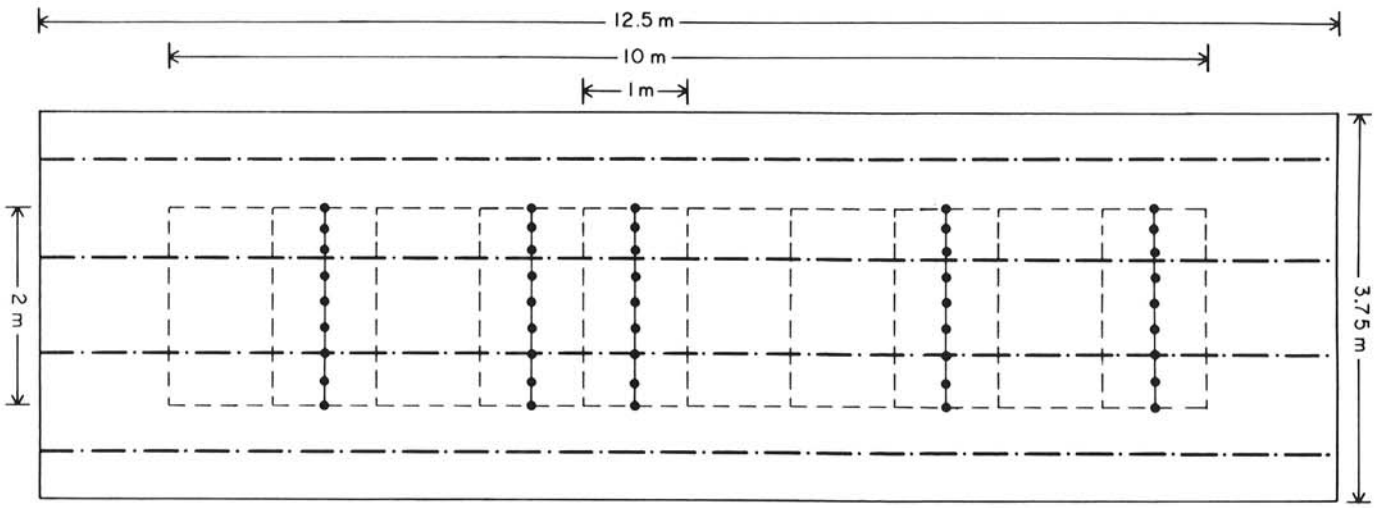

Fig. 2. Diagrammatic representation of the strategy for the preplant sampling of individual tobacco plots to determine the initial density and distribution of inoculum of *Phytophthora parasitica* var. *nicotianae*: row centers (— · —), sample area divided into 10 subplots (---), and transects with sample locations within each of five randomly chosen subplots (—●—).

microscopically based on hyphal characteristics. The total number of colonies obtained on 10 plates per sample was determined. The data, organized into frequency class tables by plot and depth, were tested for goodness-of-fit by the Poisson and negative binomial discrete frequency distributions (17,25) by a chi-square test using the FORTRAN program of Gates and Ethridge (6). This program also provided maximum likelihood estimates of k. A second measure of aggregation, Lloyd's index of patchiness (13), was calculated as $1 + 1/k$. Mean inoculum densities per plot were calculated by dividing the total number of colonies obtained per 10 plates by the average weight of soil introduced onto the plates.

Plant stands were determined once the transplants had become established (after 6–10 days in 1982 and 1984, and after 25 days in 1983). Disease was assessed periodically (approximately every 4–8 days) as plant mortality indicated by the first signs of irreversible wilting of the plants generally followed by the blackening of the lower stems. Periodically, sections were removed from the advance edges of these stem lesions and were plated onto the PARP medium supplemented with 50 mg of hymexazol per liter of medium to confirm the presence of the pathogen.

Analyses of the inoculum density-mortality data were performed using the procedures of the Statistical Analysis System (SAS Institute Inc., Cary, NC). All linear correlation coefficients were calculated with the Correlation Procedure; all linear and polynomial regressions were calculated with the General Linear Models Procedure.

RESULTS

Residual inoculum of *P. parasitica* var. *nicotianae* was aggregated within the field at the time tobacco transplants were set (Table 1). This conclusion was based on a variance-to-mean ratio greater than one, a lack of fit of population data by the Poisson probability distribution, and fit of the data by the negative binomial distribution with a dispersion parameter k of 0.44 in 1982. Aggregation of inoculum increased with depth in the soil profile as indicated by a decrease in k from 0.41 to 0.12 for depths of 0–10 and 10–20 cm, respectively. Mean inoculum density was greater at 10–20 cm. Aggregation of inoculum also increased with depth in 1983; the k parameter decreased from 0.54 to 0.17 and 0.07 for depths of 0–10, 10–20, and 20–30 cm, respectively. However, in contrast to 1982, mean inoculum density decreased with depth in 1983; mean inoculum density decreased from 0.50 to 0.25 and 0.09 propagules per gram of soil (ppg) for successive depths. In 1984, plots were established in an area of the field where inoculum was known to be less dense and more highly aggregated than the areas of the field used in previous years, and samples were taken only from the upper 10 cm of soil. The resulting k parameter was 0.16, and the initial mean inoculum density was 0.17 ppg.

Inoculum within individual plots, compared with the entire field, also was aggregated at the time transplants were set. Initial mean densities and aggregation indices for inoculum in the upper 10 cm of soil from individual plots sampled in 1983 and 1984 are presented (Table 2). The Poisson distribution adequately fit the data ($P \leq 0.05$) for only 3 of 18 data sets in 1983, whereas the negative binomial distribution adequately fit the data for 16 of 18 data sets. The k parameters ranged from 0.15 to 2.03, 0.05 to 0.44, and 0.03 to 0.39 for depths of 0–10, 10–20, and 20–30 cm, respectively, and were always greatest in the upper 10 cm of soil. Mean inoculum densities ranged from 0.11 to 1.31, 0.03 to 0.53, and 0.005 to 0.32 ppg for successive depths. Inoculum density generally decreased with depth in the soil profile and was greatest at a depth of 10–20 cm for only one of six plots. Sample data for the upper 10 cm of soil within individual plots in 1984 were fit adequately by the Poisson distribution for only 3 of 16 plots compared with 13 of 16 plots fit by the negative binomial distribution. The k parameters ranged from 0.02 to 1.77, and mean inoculum densities ranged from 0.01 to 0.43 ppg.

The disease progress curves for the individual plots of Speight G-28 and Hicks in 1984 are presented (Fig. 3). Appreciable mortality generally was not observed in plots of Speight G-28 until day 70, and the final levels of mortality ranged from 2.0 to 42.5%. The final levels of mortality ranged from 33.3 to 100.0% for Hicks. For plots of Hicks with comparable initial mean inoculum densities, a delay in epidemic development was observed for the plot with the greater degree of inoculum aggregation.

Simple linear correlation coefficients were calculated between the observed disease proportions based on mortality (Y) and those

TABLE 1. Summary statistics for the density and distribution of inoculum of *Phytophthora parasitica* var. *nicotianae* in tobacco field soil at the time transplants were set

Year	Depth (cm)	Samples (no.)	V/M[a]	Probability of exceeding chi-square: P[b]	NB[c]	k[d]	ID[e]
1982	0–10	180	2.56	0.000	0.898	0.44	0.25
	0–10	60	3.12	0.003	0.522	0.41	0.27
	10–20	60	27.26	0.000	0.540	0.12	0.50
1983	0–10	270	7.03	0.000	0.886	0.54	0.50
	10–20	270	11.84	0.000	0.602	0.17	0.25
	20–30	270	15.55	0.000	0.406	0.07	0.09
1984	0–10	720	12.00	0.000	0.005	0.16	0.17
	0–10H[f]	360	12.98	0.000	0.298	0.15	0.15
	0–10S[f]	360	13.91	0.000	0.006	0.19	0.19

[a] Variance-to-mean ratio.
[b] Poisson probability distribution.
[c] Negative binomial probability distribution.
[d] Negative binomial dispersion parameter.
[e] Inoculum density (propagules per gram of soil) calculated as the total number of colonies per sample divided by 2.25 and 6.75 g of soil for depths of 0–10 and 10–20 cm, respectively, in 1982, and divided by 4.5 g of soil for all depths in 1983 and 1984.
[f] Plots to be planted with the black shank susceptible cultivar, Hicks (H), or the resistant cultivar, Speight G-28 (S).

TABLE 2. Summary statistics for the density, distribution, and aggregation of inoculum of *Phytophthora parasitica* var. *nicotianae* from the upper 10 cm of soil within individual plots at the time tobacco transplants were set

Year	Plot	Variance-to-mean ratio	Inoculum density[a]	k[b]	Lloyd's index of patchiness[c]
1983	1H[d]	8.49	1.31	1.48	1.68
	2H	3.67	0.40	...[e]	...
	3H	2.85	0.67	1.82	1.55
	4H	1.81	0.30	2.03	1.49
	5H	6.72	0.22	0.21	5.76
	6H	5.17	0.11	0.15	7.67
1984	1H	1.32	0.02	...	...
	2H	5.00	0.02	0.02	51.00
	3H	12.97	0.19	0.08	13.50
	4H	33.98	0.34	0.05	21.00
	5H	1.40	0.19	1.77	1.56
	6H	0.95	0.01	...	...
	7H	5.38	0.29	0.30	4.33
	8H	10.43	0.36	0.51	2.96
	1S[d]	1.64	0.01	...	...
	2S	2.59	0.07	0.20	6.00
	3S	1.52	0.05	0.46	3.17
	4S	5.28	0.14	0.15	7.67
	5S	3.78	0.34	0.65	2.54
	6S	3.23	0.09	0.26	4.85
	7S	27.68	0.43	0.20	6.00
	8S	3.26	0.14	0.29	4.45

[a] Propagules per gram of soil calculated as the total number of colonies per sample divided by 4.5 g of soil.
[b] Negative binomial dispersion parameter.
[c] Calculated as $1 + 1/k$.
[d] Plots to be planted with the black shank susceptible cultivar, Hicks (H), or the resistant cultivar, Speight G-28 (S).
[e] Data could not be fit by the negative binomial distribution and were not used in the analyses.

predicted for the random (Y_P) and nonrandom (Y_{NB}) distributions of inoculum (Table 3). For Speight G-28, significant correlations ($P \leq 0.05$) were observed between Y and Y_P throughout the epidemic and between Y and Y_{NB} only from day 70 through the end of the epidemic. For Hicks, no significant correlations were obtained between Y and Y_P or Y_{NB} on any assessment date in 1983. Significant correlations of Y with Y_P were observed only from day 70 through the end of the epidemic in 1984. In contrast, the correlations of Y with Y_{NB} were significant only through day 70.

Significant linear regressions ($P \leq 0.05$) were observed for mortality (Y) in Speight G-28 as a function of inoculum density (ID) throughout the epidemic in 1984 (Table 4). Significant linear regressions were observed also for Y as a function of ID for the linearized forms of the Poisson and negative binomial models. No distinct patterns of the residual error terms were observed for any of these models. The slopes of the inoculum density-mortality relationships generally increased as the epidemic progressed. No significant linear relations were observed between Y and the aggregation indices, k, or Lloyd's index of patchiness ($1 + 1/k$).

In contrast to Speight G-28, significant linear regressions were observed for Y as a function of ID for Hicks only from day 70 through the end of the epidemic in 1984, but distinct patterns of the residuals were observed. Significant linear regressions were not observed for the linearized forms of the Poisson or negative binomial models. From day 45 through the end of the epidemic, significant negative linear regressions were observed for Y as a function of $(1 + 1/k)$, but distinct patterns of the residuals again

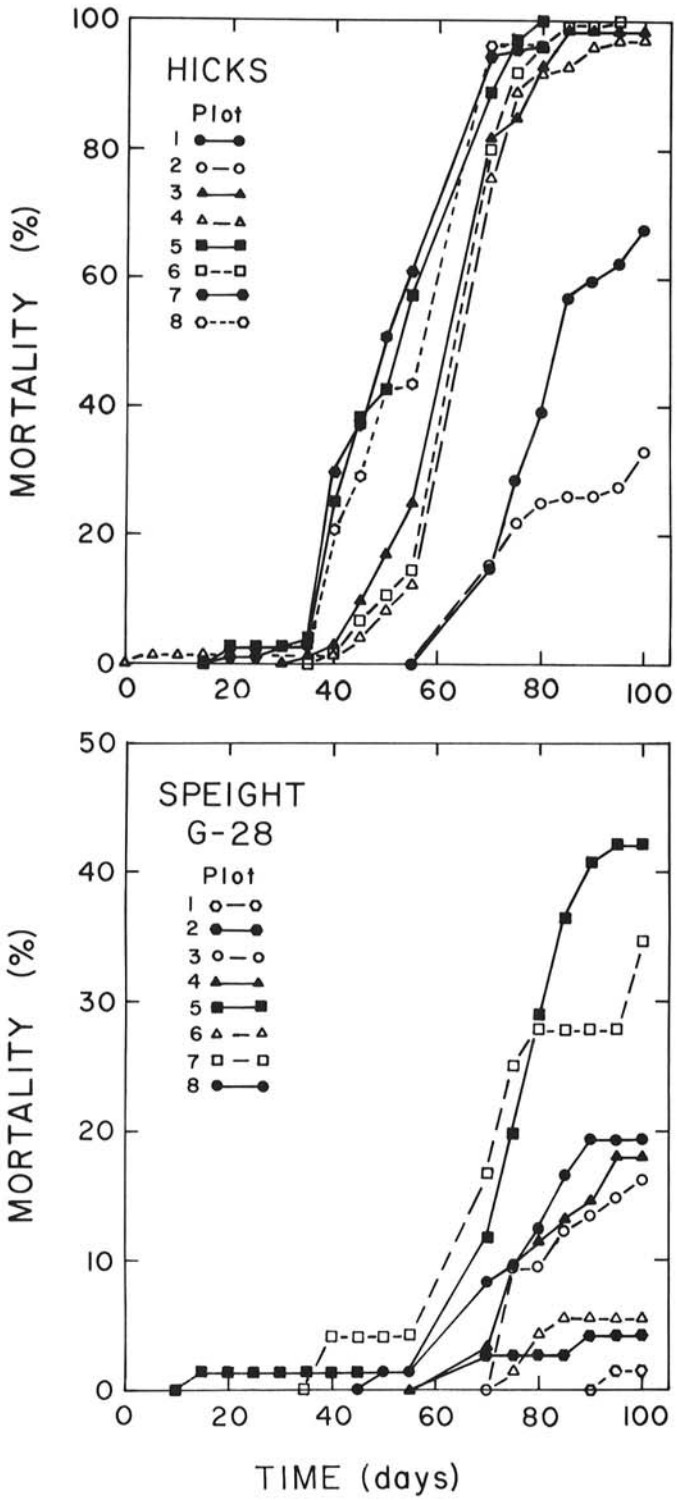

Fig. 3. Disease progress curves for the individual plots of the black shank susceptible tobacco cultivar, Hicks, and the resistant cultivar, Speight G-28, in 1984.

TABLE 3. Mean disease proportions based on mortality and linear correlation coefficients calculated between the observed disease proportions and the values predicted for the random and nonrandom distributions of inoculum of *Phytophthora parasitica* var. *nicotianae* for the black shank resistant tobacco cultivar, Speight G-28, and the susceptible cultivar, Hicks, over time in 1984

	Speight G-28			Hicks		
		r[a]			r	
Day	Y[b]	Y_P[c]	Y_{NB}[d]	Y	Y_P	Y_{NB}
20	...[e]	...	...	0.013	0.593	0.824*
30	...	...	...	0.016	0.637	0.881*
35	...	...	...	0.020	0.487	0.803
40	0.008	0.892**[f]	0.742	0.133	0.424	0.836*
45	0.008	0.892**	0.742	0.198	0.422	0.841*
50	0.010	0.874*[g]	0.736	0.270	0.526	0.896*
55	0.010	0.874*	0.736	0.332	0.475	0.839*
70	0.061	0.949**	0.898**	0.754	0.842*	0.863*
75	0.111	0.925**	0.872*	0.808	0.857*	0.811
80	0.139	0.945**	0.955**	0.842	0.842*	0.788
85	0.164	0.880**	0.945**	0.860	0.829*	0.760
90	0.180	0.842*	0.930**	0.865	0.840*	0.748
95	0.188	0.820*	0.911**	0.870	0.844*	0.742
100	0.200	0.887**	0.930**	0.879	0.844*	0.743

[a] Linear correlation coefficient.
[b] Mean disease proportion based on mortality.
[c] Estimated disease proportion based on the random or Poisson distribution of initial inoculum.
[d] Estimated disease proportion based on the nonrandom or negative binomial distribution of initial inoculum.
[e] No mortality.
[f] ** = Significant at $P \leq 0.01$.
[g] * = Significant at $P \leq 0.05$.

TABLE 4. Summary statistics for the linear functions relating mortality in the black shank resistant cultivar, Speight G-28, to initial density of inoculum of *Phytophthora parasitica* var. *nicotianae* over time in 1984

	Y[a]		$\ln[1/(1-Y)]$[b]		$k[(1-Y)^{-1/k}-1]$[c]	
Day	b_1[d]	R^2[e]	b_1	R^2	b_1	R^2
40	0.098	0.826	0.099	0.823	0.109	0.804
50	0.093	0.782	0.095	0.780	0.105	0.769
70	0.413	0.898	0.451	0.906	0.685	0.882
75	0.543	0.863	0.636	0.882	1.345	0.832
80	0.678	0.888	0.826	0.899	1.871	0.934
85	0.721	0.760	0.910	0.754	2.051	0.965
90	0.732	0.691	0.947	0.676	2.150	0.932
95	0.724	0.653	0.946	0.640	2.151	0.888
100	0.846	0.775	1.127	0.786	3.430	0.935

[a] Observed mortality.
[b] Transformation to linearize the Poisson model.
[c] Transformation to linearize the negative binomial model.
[d] Slope parameter; the y-intercepts (b_0) were not significantly different than zero ($P \leq 0.05$).
[e] Coefficient of determination; all regressions were significant at $P \leq 0.05$.

indicated the inappropriateness of this relationship. Polynomial regression was used to examine further the influence of initial density and aggregation of inoculum as well as the interaction between these two parameters on disease development in Hicks (Table 5). A polynomial model of the form $Y = ID + (1 + 1/k) + ID*(1 + 1/k)$ was significant over the final 30 days of the epidemic and revealed no distinct pattern of the residuals. Lloyd's index of patchiness was a significant term in the model at each assessment date, whereas inoculum density was not significant at any assessment date. However, the interaction between these terms was generally significant over the final 25 days of the epidemic.

DISCUSSION

Residual inoculum of *P. parasitica* var. *nicotianae* was aggregated in field soil at the time tobacco transplants were set. This was true when the population data were analyzed for the entire field or for individual plots. Aggregation of inoculum increased with depth in the soil profile, whereas inoculum density was generally greatest in the upper 10 cm. Both initial density and distribution of inoculum influenced black shank development under field conditions. However, the two cultivars used in this study differed in their response to these parameters.

Initial mean inoculum density had a greater influence on subsequent mortality in the resistant cultivar, Speight G-28, than did the initial distribution of inoculum. Increase in mortality in Speight G-28 was essentially monocyclic in 1984 and was restricted to the period after flowering (5). This time constraint may have restricted disease development to localized areas of high inoculum, which may have been dispersed randomly. This would explain the lack of significant relationships between mortality and the aggregation indices examined. That aggregation of inoculum did have some influence on the latter stages of disease development was supported by the nonrandom occurrence of plant mortality observed at the end of the epidemic (5).

In contrast to Speight G-28, the initial nonrandom distribution of inoculum had a greater influence on mortality for the susceptible cultivar, Hicks, than did the initial mean inoculum density. As the increase in mortality in Hicks was multicyclic in 1984 (5), the influence of initial density and aggregation of inoculum should have been overcome, at least partially, by multiple cycles of inoculum production and dispersal from previously infected plants. That the density and distribution of inoculum of *P. parasitica* var. *nicotianae* changed during the epidemic was revealed by soil samples taken in early July of the single plot sampled intensively in early April of 1982. The results of this sampling indicated that the mean inoculum density had increased and the distribution of inoculum had become more random (Ferrin and Mitchell, *unpublished*). Thus, under the conditions of this study, the influence of more extreme initial aggregation of inoculum for Hicks was observed principally as a delay in epidemic development when initial mean inoculum densities were similar. The combined influence of low initial mean density and extreme aggregation of inoculum was observed also as a delay in epidemic development to the extent that a lower final level of disease was attained.

Other studies have not demonstrated the influence of aggregation of inoculum on subsequent disease development. Martin et al (14) found that even though inoculum of *Rhizoctonia* spp. was considered to be aggregated based on k values of 4.87 and 1.10, disease was uniform to random ($s^2/\bar{x} \leq 1$) and random to aggregated ($s^2/\bar{x} \geq 1$), respectively. Shew et al (21) suggested that inoculum of *Sclerotium rolfsii* assessed in June was dispersed randomly based on the low number of sclerotia encountered, whereas lesions assessed in late September or October were aggregated as indicated by k parameters that ranged from 0.87 to 6.49. Dillard and Grogan (4) concluded that sclerotia of *Sclerotinia minor* were aggregated within plots at the time lettuce was seeded or transplanted. However, infected plants occurred randomly within the plots. No significant correlation was observed between disease incidence and inoculum aggregation measured by k, but a significant correlation was observed between inoculum density and disease incidence. No differences were observed between correlation coefficients calculated between the number of samples with seven or more sclerotia and disease incidence using expected frequency classes for the Poisson and negative binomial distributions ($r = 0.94$ and 0.92, respectively).

The theory relating inoculum density to disease is based on the distribution of inoculum described by the Poisson or negative binomial probability distributions (8,30). Difficulty in relating the distribution of initial inoculum to the subsequent distribution of disease results from the difference in spatial scales and methods employed to determine the nonrandomness of inoculum as compared with the nonrandomness of disease. Fit of population data by statistical distributions to determine the nonrandomness of inoculum cannot be equated to use of ordinary runs analysis (7) or spatial autocorrelation (16) to determine the nonrandomness of diseased plants. Similar methodologies and sampling intensities employed on similar spatial scales are required. However, the theory relates initial density and distribution of inoculum to the disease level attained and not to the distribution of disease itself.

Significant linear correlations were observed between mortality in Speight G-28 and that predicted by both the Poisson and negative binomial models. Significant correlations were observed between mortality in Hicks and that predicted by the negative binomial model early in the epidemic only, whereas significant correlations were observed between mortality and that predicted by the Poisson model late in the epidemic only. However, fit of the data to the linearized forms of these models indicated that the theory was acceptable only for Speight G-28. This may be explained both in terms of established inoculum density-mortality relationships and the extent to which the dispersal of secondary inoculum occurs in these cultivars. For Hicks under growth room conditions, an ID_{50} value of 0.13 chlamydospores per gram of soil was established (12), a value exceeded by the initial mean inoculum density for all but one of the plots used in the analyses in our study. Although similar information is not available for Speight G-28, a greater ID_{50} value would be expected than was observed for Hicks. Furthermore, the spread of *P. parasitica* var. *nicotianae* between plants was quite extensive in Hicks but was severely restricted in resistant cultivars (22). As the theory relates inoculum density to disease based on monocyclic disease development, the

TABLE 5. Summary statistics for polynomial regression to assess the influence of initial density and aggregation of inoculum of *Phytophthora parasitica* var. *nicotianae* on mortality in the black shank susceptible tobacco cultivar, Hicks, over time in 1984

Day	R^{2a}	Term[b]	F	$P > F$[c]
70	0.992	*ID*	0.75	0.478
		LIP	56.06	0.017*
		*ID*LIP*	1.41	0.357
75	0.999	*ID*	0.26	0.659
		LIP	1,175.30	0.001*
		*ID*LIP*	168.89	0.006*
80	0.996	*ID*	0.88	0.446
		LIP	137.10	0.007*
		*ID*LIP*	23.93	0.030*
85	0.988	*ID*	0.91	0.441
		LIP	52.80	0.018*
		*ID*LIP*	10.83	0.081
90	0.990	*ID*	1.05	0.413
		LIP	64.14	0.015*
		*ID*LIP*	15.63	0.058
95	0.991	*ID*	1.13	0.398
		LIP	71.23	0.014*
		*ID*LIP*	18.76	0.049*
100	0.991	*ID*	1.13	0.400
		LIP	71.37	0.014*
		*ID*LIP*	18.57	0.050*

[a] Coefficient of determination.

[b] Parameters for the regression model, $Y = ID + LIP + ID * LIP$, where ID is the initial inoculum density (propagules per gram of soil) and LIP is Lloyd's index of patchiness ($1 + 1/k$).

[c] Probability of exceeding F for testing the significance of the individual terms of the regression model based on partial sums of squares.

acceptability of the theory for Speight G-28 but not for Hicks may be related to the observation that the increase in mortality was essentially monocyclic in Speight G-28, whereas multipe cycles in the increase in mortality were observed in Hicks (5). Lastly, as both the Poisson and negative binomial models were acceptable for Speight G-28, it appears that the model based on a random or Poisson distribution of inoculum is sufficiently robust to justify its use without correcting for the observed nonrandom distribution of inoculum. However, based on fit of the linearized forms of the models, a greater amount of the variation was explained by the negative binomial model late in the season (R^2 values ranged from 0.64 to 0.79 for the Poisson model compared with 0.89 to 0.96 for the negative binomial model).

In this study, it was demonstrated that disease assessments should be made repeatedly over the course of the epidemic because initial density and aggregation of inoculum may influence disease development at times other than at the end of the epidemic. The time during the epidemic at which the influence of initial density and aggregation of inoculum will be expressed will depend largely upon initial inoculum levels, degree of inoculum aggregation, level of host resistance, and the basis for disease assessments. Furthermore, as most pathogens exhibit some capacity for the multiplication and spread of secondary inoculum, the rates of increase and spread of inoculum will determine how readily the effects of initial inoculum density and aggregation are exhibited or overcome. Repeated assessments of the density and distribution of inoculum throughout the epidemic would provide useful information with respect to this problem.

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