Spatial Patterns of *Verticillium dahliae* Propagules in Potato Field Soils of Oregon's Columbia Basin

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ABSTRACT

Johnson, K. B., Apple, J. D., and Powelson, M. L. 1988. Spatial patterns of *Verticillium dahliae* propagules in potato field soils of Oregon's Columbia Basin. Plant Disease 72:484-488.

Frequency distributions of propagules of *Verticillium dahliae* were determined in three potato fields. Eighty or 100 soil cores were analyzed from each of eight 10× 10 m sites. Taylor's power law, an empirical measure of aggregation, indicated clumping of propagules when it was applied to the site means and variances (Taylor's $b = 2.51$, $R^2 = 0.87$). The variance-to-mean ratio and Lloyd's index of patchiness also indicated aggregation of propagules at each site. The Poisson distribution model did not fit the observed population frequencies, and conclusions about aggregation derived from the negative binomial distribution model provided little additional information beyond that obtained from the other indices. Moran's I statistic indicated a random pattern of spatial autocorrelation at each site. For all sites, means and variances of the samples were greatly influenced by a small number of extremely high propagule counts. The relative precision of confidence intervals for both the mean and the median were compared for each site. In some situations, the median may be a better estimator of central tendency than the mean. Two subsamples per soil core adequately described the number of propagules in a single soil sample.

Additional keywords: index of dispersion, potato early dying disease

Potato production in the Columbia Basin of Oregon and Washington is characterized by intensively managed center pivot-irrigated fields located on very coarse, textured sandy soils. Yields of Russet Burbank, the most widely grown cultivar, have exceeded 85 metric tons/ha on ground not previously cropped to potatoes. However, after a field has been cropped to potatoes several times, yield declines of up to 30% have been reported by some Oregon growers. Much of this yield reduction is attributed to the early dying disease (18). Recent studies (10,12,17) have implicated *Verticillium dahliae* Kleb. as a causal agent of this disease.

Reports have indicated that *V. dahliae* can persist in fields cropped to nonhosts for 4 (9) to 8 years (26). This long survival time reduces the practicality of crop rotation as a disease management practice, especially in fields with high populations of this pathogen (9). Increasingly, growers have turned to soil fumigation for control. At the present time, the decision to fumigate is based on the amount of disease in the previous potato crop, the number of years a field has been cropped to potatoes, and the number of years since the last potato crop. Economic thresholds based on propagule populations for this area are unknown.

Potato yield loss has been correlated to soil populations of *V. dahliae* by Nnodu and Harrison (16) in Colorado. DeVay et al (6), however, reported difficulties in determining inoculum density-disease relationships for Verticillium wilt of cotton under field conditions. They concluded that propagules of *V. dahliae* were not uniformly distributed in the soil. Distribution of propagules of *V. dahliae* in an Ohio potato field soil has been reported to be aggregated and described by the negative binomial (20). Similar results have been reported for other soilborne pathogens (7,8,13,14,19,23).

The purpose of this study was first, to examine the spatial patterns of propagules of *V. dahliae* in three irrigated fields in northeastern Oregon, and second, to evaluate the precision of statistical methods for estimating soil populations of *V. dahliae*.

MATERIALS AND METHODS

Field sampling. Three 48-ha fields located in Morrow County, OR were sampled in early December of 1982. Each field had potatoes cropped in them at least once in the last three years. Within

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Technical paper 8327 of the Oregon Agricultural Experiment Station, Corvallis.

Accepted for publication 21 January 1988 (submitted for electronic processing).

each field, two or three 10 × 10 m sites were selected. Each site was divided into 100 contiguous quadrats, each 1 m on a side. At each site, a soil sample was taken from the center of each quadrat with a standard 2.54-cm soil probe inserted to a depth of 15 cm. The probe was rinsed with water between cores to prevent cross contamination. Each soil sample was placed into individual paper bags and returned to the laboratory for further analysis. A total of 100 samples was taken at each site. At site A-2, 20 of the samples were not assayed.

Soil assay. Soil samples were air-dried in paper bags at 22–24 C for at least 4 wk, and then stored until assayed under similar conditions. Each sample was individually broken up by hand and then thoroughly mixed. Five 0.1-g subsamples from each soil core were individually plated on a selective medium described by Butterfield and DeVay (2) with an Andersen Air Sampler (Andersen Air Samplers and Consulting Service, Provo, UT 84601). Plates were incubated for 2 wk at 22–24 C. After incubation, the surface of each plate was gently washed under running tap water and then examined for colonies of *V. dahliae*. The plating began in early January of 1983 and was completed in early May. A check soil, plated periodically during this period, did not have a significant reduction in recovery of propagules of *V. dahliae*.

Statistical analyses. Frequency distributions using the number of propagules per gram of air-dried soil as classes were constructed for each site within each field. The observed distributions from each site were tested against the Poisson and negative binomial probability distribution models with the chi-square goodness-of-fit test. In testing the frequency distributions, data in tail classes were combined when expected frequencies were less than one (22). The method outlined by Steele and Torrie (22) was used for the Poisson distribution and the method of moments and the maximum likelihood estimates described by Bliss and Fisher (1) were used for the negative binomial distribution.

Lloyd's index of patchiness (25), variance-to-mean ratios, and Taylor's power law (21) were used as indicators to evaluate aggregation of propagules among soil cores. The parameters a and b of Taylor's power law were estimated using least squares regression of logarithmically transformed data. Moran's I statistic, describing spatial autocorrelation, was calculated for each site (3). Taylor's power law was also applied to data from the individual subsamples of 78 randomly chosen soil cores, representing 10% of the total cores taken for this study, to determine if there was aggregation of propagules in the individual soil samples.

Sampling precision. Ninety-five percent confidence intervals for both the mean and median were computed for the original site data and for smaller random samples of 30 cores taken from each site. Confidence intervals for the median were computed using an adaptation of the nonparametric sign test (11). The data were ranked and the limits of the confidence interval set at rank positions that would lead to rejection of the hypothesis: ratio of rank positions greater than the critical rank to rank positions less than the critical rank equals 1.0. The value of the data at the two critical ranks gives the confidence interval, and the interval can be asymmetric. The relative precision of each confidence interval was calculated by dividing the width of the interval by twice the respective mean or median.

Subsets of core subsamples were randomly sampled from the original core data to determine if fewer than five subsamples per core could adequately estimate the mean. A subset is defined as a sample of one, two, three, or four of the original five subsamples in a core. Random samples were taken for each subset size from data for site A-2. For each individual core, the percent difference (D) between the mean based on five subsamples and means based on fewer subsamples was calculated as $D = [(\bar{X}_5 - \bar{X}_y)/\bar{X}_5]\,100$, in which $\bar{X}_5$ is the mean based on five subsamples per core and $\bar{X}_y$ is the subset mean based on y number of subsamples per core, where y = 1, 2, 3, or 4. The site mean and median of each subset were compared with the site mean based on five subsamples per core.

RESULTS

Propagule distribution. The number of propagules per gram of air-dried soil ranged from 0 to 414. Overall field means were 43.8, 20.4, and 20.1 and median values were 34.0, 16.0, and 16.0 propagules per gram of air-dried soil for fields A, B, and C, respectively (Table 1). Student's t tests among sites within the same field indicated that there were significant differences ($P = 0.05$) in mean estimates.

The variance-to-mean ratio and Lloyd's index of patchiness, two measures of nonuniformity in a distribution, indicated clumping or aggregation of propagules (Table 1). All variance-to-mean ratios were significantly greater than one, the expected value given a random distribution. In all but a single case (site C-2), Lloyd's index of patchiness values also were greater than one, which indicates clumping of propagules (25). The degree of aggregation, as reflected by these indicators, varied by site within a field.

The Poisson distribution model was rejected as a choice for a model fitting observed frequency data for all sites (Table 2). A goodness-of-fit test of the negative binomial distribution model was dependent, in some cases, on the method of estimating the dispersion parameter, k. The method of moments estimate of the dispersion parameter (k_1) resulted in values of k ranging from 0.46 to 4.04 and of "fitting" the negative binomial distribution model at sites B-2, B-3, and C-2 (Table 2). In contrast, the maximum likelihood estimate of the dispersion parameter (k_3) resulted in larger values of k and in "fitting" the negative binomial distribution model only at site C-2 (Table 2).

None of the sites showed significant spatial autocorrelation based on Moran's

Table 1. Descriptive statistics for the number of *Verticillium dahliae* propagules per gram of air-dried soil from three fields in Morrow County, OR

Field-site	Number of soil samples	Range	Mean $\bar{x}$	Median	Variance s^2	Indices[a] $s^2/\bar{x}$	Lloyd's index of patchiness	Moran's I[b]
A-1	100	0–348	51.8 *[c]	35.0	2,791.2	53.9	1.99	0.008
A-2	80	0–168	33.8	30.0	580.8	17.2	1.44	0.006
B-1	100	0–286	34.8 *	28.0	1,028.8	29.6	1.78	−0.005
B-2	100	0–58	14.2	12.0	135.2	9.5	1.52	0.067
B-3	100	0–44	12.2	10.0	76.0	6.2	1.34	−0.034
C-1	100	0–76	16.2	12.0	131.2	8.1	1.38	−0.008
C-2	100	0–54	17.2	16.0	108.0	6.3	1.24	0.035
C-3	100	6–414	27.0 *	21.0	1,652.8	61.2	3.17	0.011

[a] Determined from mean and s^2 of propagules/0.5 g of air-dried soil.

[b] Values obtained were not significantly different from zero at $P = 0.05$.

[c] Indicates mean number of propagules at a site is significantly different ($P \leqslant 0.05$) from the mean number of propagules at other sites in the same field.

I statistic (Table 1). A spatial frequency diagram of the propagule counts per core shows the lack of spatial autocorrelation among cores within a site (Fig. 1).

Taylor's power law, $s^2 = a\bar{x}b$ where s^2 and $\bar{x}$ are the variance and sample mean, respectively, and *a* and *b* are constants, was applied to the site data and core data.

Regression of the $\log_{10} s^2$ on $\log_{10} \bar{X}$ for each site resulted in Taylor's $b = 2.51 \pm 0.57$. The slope of this regression was significantly greater ($P \leqslant 0.05$) than 1.0, indicating aggregated distributions between soil cores (Fig. 2A). Within a thoroughly mixed core, regression of $\log_{10} s$ on $\log_{10} \bar{X}$ for the five 0.1-g subsamples from 78 randomly chosen cores indicated a random distribution of propagules (Fig. 2B), with Taylor's $b = 1.14 \pm 0.19$. The slope of this regression did not differ from 1.0 ($P > 0.05$).

Sampling precision. For sample sizes of 80–100, the relative precision of 95% confidence intervals for the mean and median ranged from 12 to 29% and 13 to 25%, respectively (Table 3). Absolute widths of median intervals were smaller than or equal to five of eight corresponding intervals for the mean.

When random samples of 30 soil cores were drawn from each site, the range in relative precision increased to 15–36% and 20–54% for the mean and median, respectively (Table 3). Widths of median intervals were smaller than the corresponding interval for the mean in three of eight samples.

A comparison of means for 80 cores from site A-2 indicated that two subsamples per core would result in similar mean and median estimates, as would five subsamples per core (Table 4). However, the average percent difference

Table 2. Fit of statistical models to observed distributions of propagules of *Verticillium dahliae* in three fields in Morrow County, OR[a]

Field-site	Number of soil samples	Number of classes	Poisson distribution (P^b)	Negative binomial distribution			
				k_1[c]	P^b	k_3[d]	P^b
A-1	100	48	<0.001	1.00	<0.01	2.94	<0.01
A-2	80	34	<0.001	2.23	<0.01	4.03	<0.05
B-1	100	30	<0.001	1.27	<0.01	8.16	<0.01
B-2	100	26	<0.001	1.92	0.55	2.12	<0.01
B-3	100	23	<0.001	2.92	0.10	3.68	<0.05
C-1	100	30	<0.001	2.62	<0.01	4.18	<0.01
C-2	100	30	<0.001	4.04	0.71	4.00	<0.80
C-3	100	30	<0.001	0.46	not defined	7.10	<0.01

[a] Distributions were based on number of propagules per 0.5 g/air-dried soil.
[b] Probability of exceeding tabulated chi-square value by chance alone.
[c] Method of moments estimate.
[d] Maximum likelihood estimate.

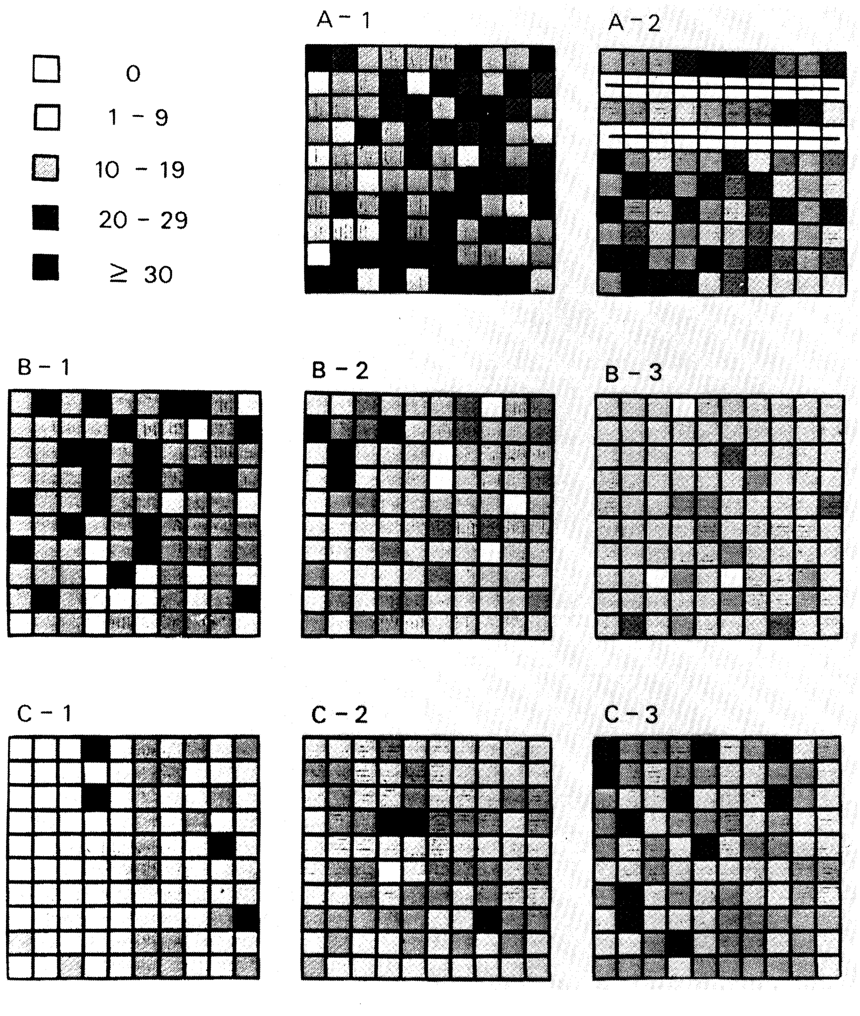

Fig. 1. Spatial distribution of propagules of *Verticillium dahliae* in three fields located in Morrow County, OR. Small squares represent sampling quadrats from which individual soil core samples were taken. Shadings represent the indicated range of propagules per 0.5 g of air-dried soil in each core. Horizontal lines in site A-2 represent missing data.

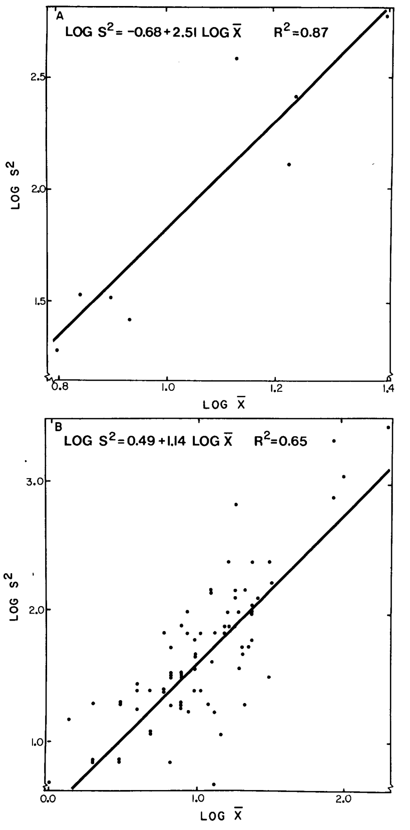

Fig. 2. Application of Taylor's power law to means and variances of propagules of *Verticillium dahliae* obtained from **(A)** soil cores within sites and **(B)** subsamples within cores. For the site data, each mean and variance was computed from a sample size of 80 or 100 soil cores. For the subsample data, each point was derived from the mean and variance of the five subsamples taken from a soil core. Seventy-eight points were regressed, which represented a random sample of 10% of the soil cores taken.

between core means based on one subsample per core and those based on five subsamples per core was large.

DISCUSSION

The number of propagules of *V. dahliae* recovered from field soils previously cropped to potatoes was high compared with reported counts for Ohio potato fields (20), but within the range of those recovered in Colorado potato fields (15), Idaho potato fields (5), and cotton fields in California (6).

Propagules of *V. dahliae* were found to be nonrandomly distributed within the fields. The large variance-to-mean ratios are indicative of the degree of aggregation of propagules within each site. In general, the magnitude of these ratios followed the range in propagule number found at a site and was influenced by the low occurrence of high propagule counts. Lloyd's index of patchiness values also suggested an aggregated distribution. According to Vandermeer (25), Lloyd's index is independent of population density and is not influenced by slight modifications to a particular clumped pattern.

The dispersion parameter (k) of the negative binomial distribution model is also an index of clustering (19), where low k values (<2) indicate clumping (21). In this study, values of k were dependent on method of estimation (k_1 or k_3); goodness-of-fit tests for the negative binomial model were rejected for 12 of 16 sites. Inconsistencies between the methods used to estimate k and failure of the negative binomial to fit some aggregated distributions have been previously noted for biological data (1,15,19,21). We concluded that the negative binomial distribution provided little additional information about inoculum distribution beyond that provided by other aggregation indices.

In a recent article, Nicot et al (15) suggested that Moran's I statistic may be applicable for describing spatial autocorrelation patterns of soilborne plant pathogens. In our study, Moran's I indicated a random pattern of inoculum distribution between 2.5-cm soil cores sampled on a 1-m grid within a 10 × 10 m site. However, the significant differences observed in number of propagules between sites in the same field suggest that there may be spatial aggregation on a scale larger than the quadrat sampled in this study.

Sampling several sites in different fields provided the variation necessary to estimate b of Taylor's power law. Taylor's thesis (24) is that the value of b is constant for a particular species and the value of a depends on the sampling unit chosen. For soil cores taken within a site (Fig. 2A), the value of Taylor's b indicated an aggregated distribution of *V. dahliae* propagules (21). This knowledge is useful in sampling for soilborne plant pathogens because an estimate of sample variance can be made for any expected mean.

Results of the Taylor's power law analysis are also appropriate for understanding a problem associated with the Anderson Air Sampler technique for estimating the number of propagules in each core. In the core subsample data (Fig. 2B), at low propagule counts the variance-to-mean ratio is greater than at high propagule counts. This is indicated by the positive value of the Y intercept (Taylor's $a = 0.69 \pm 0.29$, $P \leq 0.05$). As a result, there is decreased precision at low propagule counts (<2 propagules per gram) and this may be why Smith and Rowe (20) preferred wet sieving to the Andersen Air Sampler in their work when the recovery of *V. dahliae* propagules was low. Also, because this analysis indicates a lack of aggregation or clumping of propagules in subsamples within a thoroughly mixed core, each plate is fairly representative of the core. Cochran (4) states that when subsamples of a similar unit agree closely, greater precision is obtained by increasing the number of whole units and reducing the number of subsamples. Thus, two subsamples per core, that estimated within 26% the mean number of propagules determined with five subsamples, is probably the best choice of the number of subsamples to plate. However, the exact number of subsamples should depend on both the variance and relative cost for each subsample in comparison to variances within sites and the cost of sampling additional sites (4).

The measures of mean, variance, and aggregation were greatly influenced by a low frequency of cores with very high propagule counts. Given this situation, the median may be a better measure of central tendency. The relative precision of confidence intervals for the median were similar to those for the mean in sample sizes of 80–100 (Table 3). Values of the median averaged 80% of those obtained for the mean and may more accurately reflect the inoculum dose to

Table 3. Confidence intervals and relative precision of the interval for the mean and median number of *Verticillium dahliae* propagules per gram of air-dried soil in three fields in Morrow County, OR

		Mean		Median	
Sample size	Field-site	95% Confidence interval	Relative precision[a] (%)	95% Confidence interval	Relative precision (%)
100	A-1	(41.2, 62.4)	20	(30, 46)	23
80	A-2	(28.5, 39.1)	16	(24, 36)	20
100	B-1	(28.5, 41.1)	18	(24, 32)	14
100	B-2	(11.9, 16.5)	16	(8, 14)	25
100	B-3	(10.5, 13.9)	14	(8, 12)	20
100	C-1	(14.0, 18.4)	14	(12, 16)	17
100	C-2	(15.2, 19.2)	12	(14, 18)	13
100	C-3	(19.1, 34.9)	29	(18, 24)	14
30	A-1	(34.8, 75.1)	36	(26, 56)	44
30	A-2	(23.6, 36.0)	20	(22, 34)	24
30	B-1	(25.3, 39.3)	21	(22, 34)	25
30	B-2	(9.1, 16.0)	27	(6, 18)	52
30	B-3	(9.4, 15.6)	24	(6, 16)	45
30	C-1	(10.6, 13.1)	21	(8, 16)	40
30	C-2	(13.1, 20.8)	22	(10, 22)	38
30	C-3	(20.9, 28.4)	15	(18, 28)	20

[a] Relative precision was defined as the width of the confidence interval divided by twice the mean.

Table 4. Site mean, median, and average percent difference from the mean[a] for number of propagules of *Verticillium dahliae* estimated in 80 soil cores using one to five subsamples per core[b]

Number of subsamples per core[c]	Site median[d]	Site mean[d]	Average percent difference from mean
5	30	33.9	···
4	28	34.0	11.1
3	30	35.4	17.1
2	30	32.0	25.7
1	30	34.0	96.2

[a] Difference between core mean based on one to four subsamples and core mean based on five subsamples averaged over 80 cores.

[b] Data from site A-2.

[c] Each subsample represents 0.1 g of air-dried soil deposited on a selective medium with an Anderson Air Sampler.

[d] Number of propagules per gram of air-dried soil.

which roots are exposed. Further research is needed on the frequency of highly aggregated propagules of *V. dahliae* and the corresponding influence on disease incidence and severity.

ACKNOWLEDGMENTS

The authors wish to thank John Orlowsky for his technical assistance. This research was supported in part by a grant from the Oregon Potato Commission.

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