Characteristics and Distribution of Propagules of *Verticillium dahliae* in Ohio Potato Field Soils and Assessment of Two Assay Methods

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ABSTRACT

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Soils from 82 Ohio potato fields were assayed for numbers of viable propagules of *Verticillium dahliae* by using a wet-sieving technique. About 75% of the samples had ≤25 propagules per 10 g of air-dried soil, although as many as 172 per 10 g were found. Propagule size distribution was similar among 24 fields assayed in a second study, with the majority of propagules 38–75 μm in diameter. The distribution of propagules in one naturally infested field was found to be nonrandom. Numbers of viable propagules in air-dried field soil declined with increasing time of storage at room temperature. The wet-sieving technique and the Anderson Air Sampler were compared for effectiveness of propagule recovery from soils containing low numbers of propagules. There were no significant differences in propagule recovery between the two techniques, but results with wet-sieving were less variable.

Recent studies conducted in field microplots in Ohio have confirmed the synergistic interaction of *Verticillium dahliae* Kleb. with *Pratylenchus penetrans* (Cobb) Filipjev & Schuurm.-Steckh. in the early dying disease of potato (11). In an effort to improve quantification of microsclerotial inoculum and to develop a system for predicting disease development, additional information on the characteristics of naturally occurring propagules of *V. dahliae* in Ohio potato fields was needed.

Numbers of propagules of this pathogen vary widely in different agricultural systems. In California, microsclerotial populations as high as 300–400 propagules per gram of air-dried soil were associated with Verticillium wilt of cotton (1). With potatoes, reported population levels are generally lower (4,9,12). In addition to numbers, propagule size may also be important in assessing inoculum potential. Naturally occurring microsclerotia of *V. dahliae* are irregularly shaped and range in diameter from 11 to 225 μm, although it is not known if these propagules are all infective (5,9).

Soil sampling methods and assay techniques may significantly affect estimates of numbers of propagules in soil. Numbers of microsclerotia of *Cylindrocladium crotalariae* in a peanut field were estimated to within 5% by taking 16 or 32 samples along diagonal paths (7). Although methods of field sampling for *V. dahliae* have not been studied in detail, two soil assay procedures are in common use; wet-sieving (8,11) and the Anderson Air Sampler (4). There is disagreement, however, regarding the relative efficiency of these two techniques for recovering propagules of *V. dahliae* from soil (4). Longevity of propagules of *V. dahliae* in air-dried soil samples is also critical in these assay systems. The viability of propagules in air-dried soil has been reported to vary from 6 mo to 12 yr (9,14).

The objectives of this study were to characterize naturally occurring propagules (assumed to be microsclerotia) of *V. dahliae* in Ohio potato fields. Information on propagule numbers and size distribution, longevity of propagules in air-dried soil, and comparisons of two soil assay methods is presented.

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MATERIALS AND METHODS

Naturally occurring populations of *V. dahliae*. Eighty-two commercial potato fields in 22 Ohio counties were sampled during the summer of 1981. Fields chosen were those currently in potato production with the Verticillium-susceptible cultivar Superior. Information on cropping history, observations of early dying disease, potato cultivars used, rotational crops, and soil type was obtained from growers at the time of sampling. Soil type was confirmed from published soil surveys. A portion of each field (~1.6 ha) was sampled with a standard 2.5-cm-diameter soil auger by taking 20 cores to a depth of 10–15 cm. Samples were collected from within the rows at 15–20 m intervals following a zig-zag sampling pattern up and back across each sampled area. The 20 cores were then bulked as a single sample. Samples were spread onto paper plates, air-dried for 4 wk on a greenhouse bench at temperatures not exceeding 32 C, and then sifted through an 850-μm pore-size sieve to remove stones, clods, and organic debris. Soil that passed the sieve was assayed by using a previously described wet-sieving technique and sodium polypectate agar (SPA) (11).

Propagule size distribution. Twenty-four fields currently cropped to potato were selected for more intensive sampling in 1982. Fields chosen were the same as or adjacent to those sampled in 1981. All had intermediate to high populations of *V. dahliae* and fairly uniform soil types within the fields. Samples were taken monthly from May through October and samples were air-dried as before. A 10-g subsample of soil passing the 850-μm sieve was washed through nested 250-, 125-, 75-, and 38-μm sieves, and the filtrate passing the 38-μm sieve was collected and vacuum-filtered on Whatman No. 1 filter paper. Residues remaining on sieves were assayed separately as before (11). Residues from the filter paper were washed into a beaker, and the suspension was centrifuged at 3,500 *g* for 1 min. The resulting pellet was resuspended and assayed (11). To ensure that propagules passing the 38-μm sieve were not

being entrapped within the filter paper, one-half of the filtrate from one set of samples was not vacuum-filtered, but instead was centrifuged at 3,500 *g* for 10 min and then assayed; the other half was filtered, centrifuged, and assayed as before.

Distribution of propagules within a heavily infested field and evaluation of sampling pattern. A field (located in Portage County, OH) with a history of continuous potatoes and a high population of *V. dahliae* was selected for intensive sampling in September 1982. A portion of the field, 320 × 137 m (~4.7 ha), was divided into 189 contiguous quadrats, each 15.2 m (50 ft) on a side. A total of 202 samples was collected, each comprised of three bulked, adjacent soil cores taken to a depth of 10–15 cm from the corners of each quadrat. These samples were dried in paper bags at 22 C for 4 wk and sifted through an 850-μm sieve. A 10-g subsample of each was washed through nested 75- and 38-μm sieves and residues were assayed as before.

Five probability distribution models were tested for goodness-of-fit to the microsclerotial distribution data from this study. Models included were the Poisson, Poisson with zeros (both describing random distributions), negative binomial, Thomas double Poisson, and Neyman type A (which describe clustered distributions). Goodness-of-fit was determined by chi-square analysis (13).

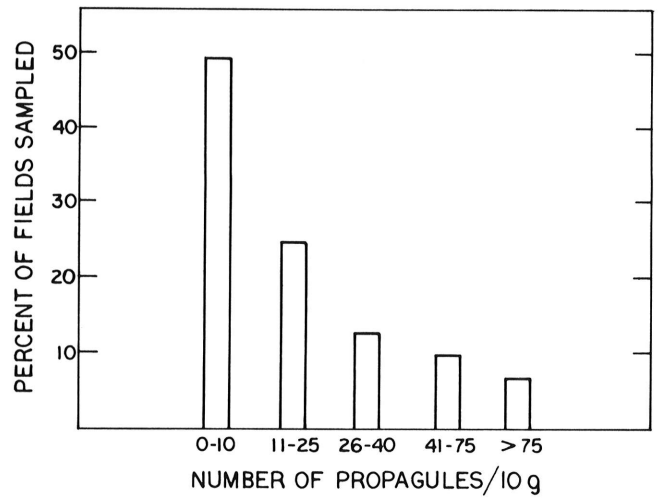

Fig. 1. Population ranges of propagules of *Verticillium dahliae* found in soils collected from 82 Ohio potato fields in 1981.

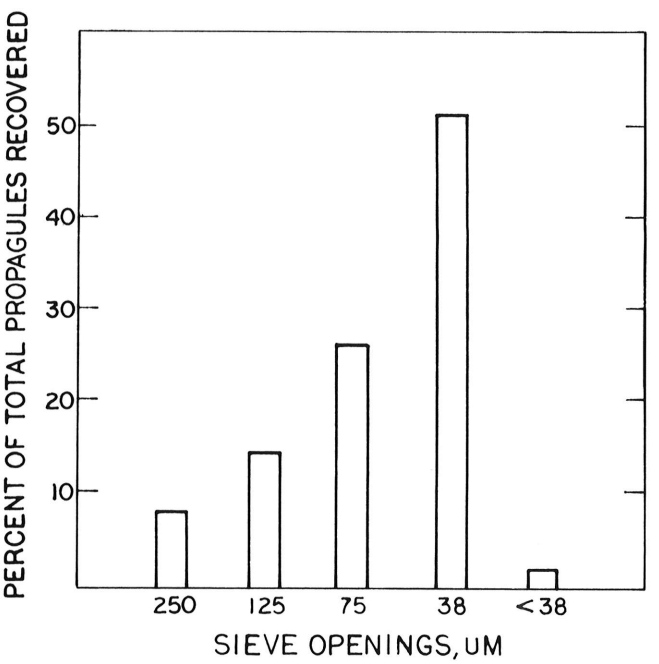

Fig. 2. Percent of propagules of *Verticillium dahliae* retained on sieves of various mesh sizes after wet-sieving six monthly soil samples each from 24 Ohio potato fields in 1982.

Longevity of propagules in air-dried field soil. Soil samples collected from June through August 1982 were stored in paper bags in the laboratory (~22 C) following 4 wk of air drying. Samples that were previously determined to contain high populations of *V. dahliae* were sifted through an 850-μm sieve and stored in capped plastic bottles. Each month, from December 1982 to April 1983, a 10-g subsample from each bottle was washed through nested 75- and 38-μm sieves and assayed as before to determine the percentage of the initial population remaining viable. Because of differences in collection dates, samples varied in the length of time each had been stored.

Comparison of wet-sieving and Anderson Air Sampler techniques. The efficiency of wet-sieving was compared with that of a two-tiered Anderson Air Sampler (Anderson Air Samplers and Consulting Service, Provo, UT 84601) in assaying viable propagules of *V. dahliae* in air-dried soils. Soil samples used were selected from those collected during the 1982 field study and represented an array of propagule numbers. To determine the optimal amount of soil for use with the Anderson Air Sampler, 10 replicates of each of 100-, 200-, or 300-mg subsamples were distributed onto 10 SPA plates (11) by using the Anderson Air Sampler (4). For comparison, 10-g subsamples of the same soils were wet-sieved, using nested 125- and 38-μm sieves and the residues from the 38-μm sieve were assayed as before. Data were tested for differences in propagule recovery by using an LSD test ($P = 0.05$) and one-way analysis of variance.

RESULTS

Field studies. Populations of *V. dahliae* in the 82 sampled fields ranged from 0 to 172 propagules per 10 g of air-dried soil. Forty-nine percent of the fields had counts of 10 or less, 24% had counts of 11 to 25, and the remaining 27% had between 26 and 172 (Fig. 1). No correlations were detected ($P = 0.05$) between propagule numbers and soil type, the last year that potatoes were grown, rotational crops, incidence of early dying as observed by the grower, or prior plantings of the highly Verticillium-susceptible cultivar Kennebec.

Size distribution of propagules of *V. dahliae* determined by wet-sieving was similar for the 24 fields sampled in 1982. Fifty-three percent were retained on the 38-μm sieve, indicating that they were between 75- and 38-μm in diameter. Less than 2% of the propagules passed the 38-μm sieve (Fig. 2). In the samples in which materials passing the 38-μm sieve were split and assayed following vacuum-filtration or centrifugation, the recovery rate was five times higher with the former technique.

Population distribution within a heavily infested field and evaluation of sampling pattern. Among the 202 samples, propagule numbers ranged from 0 to 75 per 10 g air-dried soil (Fig. 3). The ratio of the variance to the mean (V/m) of these samples equalled 12.7, suggesting that the progagules were not randomly distributed throughout the field (13). Distribution models tested by chi-square analysis also indicated a nonrandom distribution of propagules (Table 1). In no case was $P > 0.05$, and in only the negative binomial was $P = 0.05$.

Longevity of propagules in air-dried soil. The mean number of viable propagules of *V. dahliae* in air-dried soil stored at room temperature declined steadily with increasing time of storage. For samples collected in June, the number of propagules declined to 59% of the original following 6 mo of storage (Table 2). For samples collected in July, the percent detected fell to 11% after 6 mo and to 5% after 9 mo. With August samples, the percent detected declined to 3% after 6 mo and none were detected after 9 mo. Overall, the number of propagules detected fell to <10% of the original after 6 mo in storage. In addition, propagules in soil collected in June appeared to have survived longer than those collected in July or August.

Comparison of techniques for recovering propagules of *V.*

***dahliae* from soil.** No significant differences were detected in propagule recovery by the wet-sieving or Anderson Air Sampler methods (Table 3). Wet-sieving had the lowest standard error of the mean and coefficient of variation, indicating that this method was less variable than the Anderson Air Sampler.

DISCUSSION

Numbers of propagules of *V. dahliae* detected in Ohio potato soils were lower than those reported by others in fields cropped to cotton (1) and potato (3,4,9,12). In spite of these relatively low populations, potato early dying can be significant because of the interaction of *V. dahliae* with *P. penetrans* in the disease complex (11). Although no correlations were found between propagule numbers and soil type or crop history, correlations may exist which were not detected due to variable sampling times and because the number of fields with common characteristics was not large enough to make statistically significant correlations.

Size-range analysis of propagules of *V. dahliae* from Ohio potato fields indicated that most propagules persisting after air-drying were 38–75 μm in diameter, and propagules smaller than 38 μm were virtually absent. This size range is similar to that detected in California cotton fields where the majority of the propagules were retained on either 37- or 53-μm sieves, with very few passing the 37-μm sieve (2).

Propagules of *V. dahliae* were found to be nonrandomly distributed within a naturally infested field. The ratio of the variance to the mean (V/m) of these samples equalled 12.7, indicating a clustered distribution of propagules. When tested for goodness-of-fit by chi-square analysis, the Negative binomial model fit the observed distribution of propagules. This model is described by a P value and a k value, the dispersion parameter, which is a measure of the amount of clumping. Values of $k < 2$ indicate clustering (13); in this study $k = 1.31$. The potential

TABLE 1. Statistical models tested for goodness-of-fit to observed data on the distribution of propagules of *Verticillium dahliae* in a heavily infested potato field in Portage County, OH

Model	Chi-square	P[a]	k[b]
Poisson	2,819.9	0.00	
Poisson with zeros	11,475.0	0.00	
Negative binomial	73.0	0.05	1.31
Thomas double Poisson	···	0.00	
Neyman type A	224.5	0.00	

[a] Significance level for rejecting a distribution based on the chi-square statistic.
[b] Dispersion parameter for the negative binomial distribution. Values <2 indicate clustering.

TABLE 2. Decline in viability of propagules of *Verticillium dahliae* in air-dried potato field soils following storage

Month of sampling[x]	Propagule numbers after months of storage[z]						
	0	4	5	6	7	8	9
June	31[y]	ND	ND	18	2	1	1
July	56	ND	20	6	3	3	ND
August	67	30	2	2	1	ND	ND

[x] Samples were collected in June, July, and August 1982.
[y] Soil samples were air-dried 4 wk after collection and stored at room temperature in capped plastic bottles. Propagule numbers per 10 g of soil were determined by wet-sieving.
[z] Figures represent the means of six samples for June and July, and three for August. ND = not determined.

TABLE 3. Recovery of propagules of *Verticillium dahliae* from air-dried soil by wet-sieving and with an Anderson Air Sampler

Assay method[v]	Average number of propagules recovered[w]	Standard error[x]	Coefficient of variation[y]
Wet-sieving	4.6	1.34	90.16
Anderson Sampler			
100 mg soil	4.0[z]	2.21	174.75
200 mg soil	7.5[z]	3.60	152.00
300 mg soil	3.0[z]	1.35	143.29

[v] Samples were air-dried 4 wk prior to assay.
[w] LSD = 6.75.
[x] Variation over 10 replicates.
[y] Expressed as a percentage.
[z] Data were adjusted to 10-g basis from 100, 200, and 300 mg.

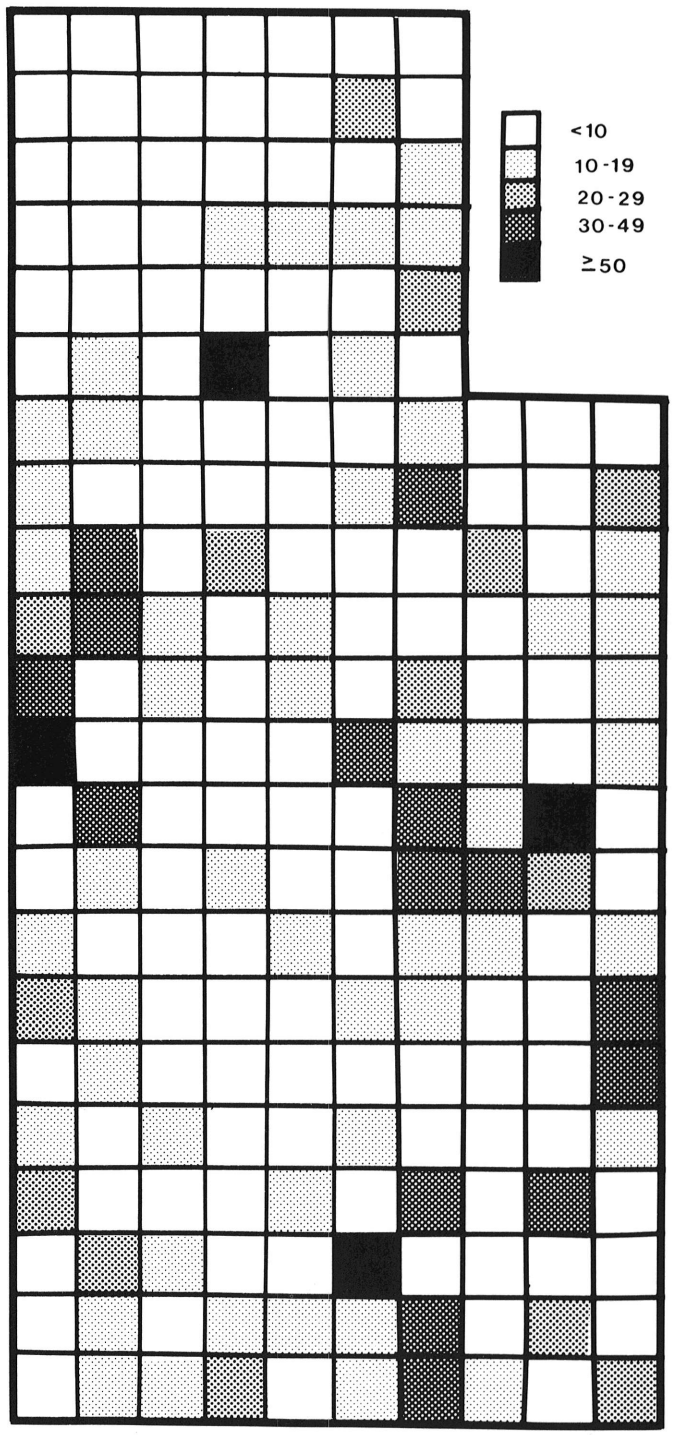

Fig. 3. Distribution of propagules of *Verticillium dahliae* in a heavily infested potato field located in Portage County, OH in 1982 (propagules per 10 g of air-dried soil). Squares represent individual soil samples collected on a 15.2-m grid pattern. Shadings indicate relative numbers of propagules detected in each sample.

sampling error with 20 core samples, calculated according to Southwood (13), was 22%. To reduce this to 10%, 107 core samples would have to be taken from a field with the same variance of propagule numbers. Since doubling the number of cores to 40 would only reduce the error to 16%, a sample size of 20 cores appears to be a reasonable compromise between practicality and precision.

The method of sampling may also influence estimates of numbers of propagules of soilborne pathogens. In a study of *C. crotalariae*, an estimate within 5% error of microsclerotial numbers within a nonrandomly infested peanut field was made by taking 32 samples along a three-diagonal or diamond-shaped path (7). The zig-zag sampling pattern used in this study gave reasonable estimates of the population mean, but not to within 5–10%. Since propagules of *V. dahliae* are similar in size and also nonrandomly distributed in soil, accuracy of sampling may be increased by using a three-diagonal or diamond-shaped path instead of a zig-zag pattern.

Numbers of propagules of *V. dahliae* in air-dried soil at room temperature declined steadily with increasing time of storage. Survival may be influenced by differences in age or size of the propagules and whether they are embedded in organic debris. Green (6) observed an increase in the number of viable microsclerotia in soil during the first few months of storage at various temperature and moisture regimes. He attributed this increase to microbial decay of plant tissues and subsequent release of microsclerotia rather than to saprophytic growth of the fungus. The long-term survival of microsclerotia was poor under all conditions tested; it was lowest under conditions of high moisture and only slightly greater under very dry conditions. In general, no viable microsclerotia were detected after 60 mo of storage (6). Other work has shown that with increasing age of microsclerotia, there is a decrease in germination (10). In our studies, viability declined rapidly in storage. If propagule counts from soil are to be used to predict disease, assays should be completed as quickly as possible after a standard drying interval under standardized conditions to avoid low estimates of the number of viable propagules actually present. Although temperature extremes should be avoided, other studies have shown that drying for 4 wk at temperatures from 20 to 35 C did not affect recovery (*unpublished*).

When comparing recovery of propagules of *V. dahliae* by the two assay techniques, there were no significant differences ($P = 0.05$) between wet-sieving and use of an Anderson Air Sampler with varying amounts of soil. The lower recovery at 300 mg with the Anderson Air Sampler may have been due to some soil particles not being in contact with the agar surface, resulting in any associated propagules failing to germinate. The standard error of the mean for wet-sieving was lower, indicating that wet-sieving would probably give more consistent estimates of propagule numbers than using 200 mg in the Anderson Air Sampler. In another study, comparisons between wet-sieving and use of an Anderson Air Sampler indicated that 30–50% of the propagules passed the 38-μm sieve and were lost from the assay (4). Size-range analysis presented here, and elsewhere (2), do not support this. Most propagules were arrested on the 38-μm sieve, and $<2\%$ passed the sieve and were potentially lost from the assay.

Both methods of assay tested here have advantages and disadvantages. Wet-sieving of soil samples is a slow process, but it can be done with relatively inexpensive equipment available in most soil laboratories. It offers high sensitivity and low variation in estimates of propagule numbers, due to the large sample size used (usually 10–15 g). Overlap of colonies, however, may create difficulties when assaying soils with high propagule counts. Research in California has shown that wet-sieving allows quantitative recovery of microsclerotia from cotton field soil (8). Use of the Anderson Air Sampler is easier and may be more effective for assaying heavily infested soils. The small sample size, usually 100 to 200 mg, reduces the problem of colony overlap and the lower sensitivity is less important when counts exceed 30 per gram (4). However, nearly all fields sampled in the present study had <10 propagules per gram. In these soils, wet-sieving would be the preferred technique to estimate propagule numbers.

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