Influence of Soil Characteristics and Assay Techniques on Quantification of *Verticillium dahliae* in Ohio Soils

T. A. WHEELER, Texas Agricultural Experiment Station and Texas Tech University, Lubbock 79401, and R. C. ROWE, Department of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

Wheeler, T. A., and Rowe, R. C. 1995. Influence of soil characteristics and assay techniques on quantification of *Verticillium dahliae* in Ohio soils. Plant Dis. 79:29-34.

Samples of soil from 45 commercial potato production fields in Ohio were analyzed for physical characteristics. Subsamples from 21 soils were either infested with two levels of *Verticillium dahliae* or left uninfested, then dried for 0, 2, 4, or 6 wk and assayed for colony-forming units of *V. dahliae* per cubic centimeter of soil. Recovery efficiency from naturally infested fine-textured soils was similar for all four drying periods, while recovery in peat and coarse-textured soils was higher when soil was dried for 2 or 4 wk. Recovery from artificially infested soils was highly variable among samples, even within similar textural groupings. Recovery from coarse-textured soils was variable across all drying times. Recovery was highest at 2 and 4 wk of drying for peat soils and least variable at all drying times for fine-textured soils. Organic matter was not generally associated with low recovery of *V. dahliae* propagules, while gravel content and pH were inversely associated with recovery efficiency. The influence on recovery of *V. dahliae* of glass vs. plastic petri plates, soil concentration in a direct-plate assay, and the total soil sample volume were also examined. Recovery of *V. dahliae* was higher with glass than with plastic petri plates in two of three soils tested. Soil dilution had no effect on colony-forming units per cubic centimeter of soil. Increasing the number of soil cores that made up a composite soil (up to 20) from which a single subsample was assayed resulted in less deviation between observed and expected colony-forming units per cubic centimeter.

Additional keywords: soil texture

Verticillium dahliae (Kleb.) is a causal agent of many economically important wilt diseases (13,15,20,22,24). The fungus survives in the absence of host plants as microsclerotia associated with plant debris or free in soil (12,16). Positive relationships between the density of microsclerotia in soil and subsequent disease in a susceptible crop have been reported (18,20,21,23,27). To use these relationships as a predictor of potential disease development prior to planting, the density of microsclerotia in soil must be determined. Therefore, any quantitative assay for *V. dahliae* microsclerotia must be consistent over a range of soils, and the effects of different soil characteristics on the recovery of *V. dahliae* should be known.

Methods to recover microsclerotia of *V. dahliae* include wet sieving (10,11,19), Anderson sampler (5,19), and direct soil assays (16,19). Nicot and Rouse (19) compared these techniques and found that wet-sieving was the most precise but also was the most biased and time-consuming and provided the lowest recovery of microsclerotia. Direct plating was the least biased (with nearly 100% recovery), the least time-consuming, and moderately variable.

Recovery of microsclerotia may also be affected by soil microbial activity and sample drying time. Since microsclerotia of *V. dahliae* are sensitive to soil fungistasis (4,7), the wet-sieving and direct-plating methods, which require some washing of soil, may reduce fungistasis (12). Competition from other fungi and bacteria may also limit germination of *V. dahliae* microsclerotia. Soil samples are often air-dried for various time periods before being assayed for *V. dahliae* microsclerotia, although some decrease in viability does occur (16). Air-drying facilitates mixing a soil sample and may reduce populations of fungi and bacteria that compete with *V. dahliae* on agar plates. The optimal drying period for soils of different textural groupings has not been determined.

The objectives of this study were to determine if recovery of *V. dahliae* microsclerotia from various soils was affected by texture (i.e., percent sand, silt, clay, gravel, and organic matter), pH, and/or length of drying time of a sample. In addition, we tested the effects on recovery of *V. dahliae* of: 1) glass vs. plastic petri plates, 2) the soil dilution used in a direct-plate assay procedure, and 3) the total volume of soil from which a single subsample was assayed.

Manuscript 232-93.

Accepted for publication 22 September 1994.

© 1995 The American Phytopathological Society

MATERIALS AND METHODS

Soil sampling and characterization. Samples were collected from commercial production fields in Ohio (45 locations) in 1992 that were in a 2-yr rotation with potatoes and either wheat or onion or in a 3-yr rotation with potatoes, corn, and wheat. Soil was collected (30–45 cm deep) and bulked (10–12 kg total) from each corner and the center of a 10 × 10 m quadrat in each field.

A buoyancy particle-size analysis was done on an air-dried subsample (passed through a 2-mm sieve) from each of the 45 test soils, at 0.5, 1, 5, 15, 30, 60, 90, and 1,440 min in a 20 C incubator. Sample sizes were 100 g for soils with high sand content and 50 g for all other soils. Temperature was monitored at each time, and the buoyancy was read with an ASTM No. 152 H hydrometer. Particle size at each time was calculated by the method of Gee and Bauder (9). The percentages of soil at 50 μm (representing the sand-silt boundary) and at 2 μm (representing the silt-clay boundary) were estimated by taking the natural logarithm of the two closest particle sizes to the 50- or 2-μm size and then using interpolation based on the known percentages of soil that had been found at those closest readings (6). Organic matter contents were determined by ignition of the soils at high temperature (17). Soil pH was determined from 10 cm^3 of soil added to 20 ml of distilled water and then testing the suspension with a pH meter after 30 min. Gravel content of each soil was estimated by adding 100–150 g of soil to a 2-mm sieve and calculating the proportion that failed to pass through the sieve.

Soil texture classes determined for 21 soils tested included five silt loams, five loamy sands, four peat soils, three sandy loams, two loams, a clay loam, and a silty clay loam (Table 1). The organic matter content of these soils ranged from 0.7 to 92%, and the pH ranged from 5.1 to 7.4 (Table 1). The percentage of gravel (soil particles > 2 mm in diameter) ranged from 7 to 44% (Table 1).

Inoculum production, soil infestation, and assay. All soil samples were assayed for *V. dahliae* microsclerotia (ms) by the direct-plating method described below. From these results, 13 soils with <5 ms/cm^3 of soil and representing a range of textures were selected. The experiment was repeated with eight additional soils with 0 to high (15 ms/cm^3 of soil) densities of *V. dahliae*. Soils were air-dried for 2 days, crushed with a rolling pin,

and then divided into nine 1-L aliquots. Aliquots were infested with microsclerotia of *V. dahliae* to achieve three inoculum densities (0, 10, or 40 ms/cm^3 of soil for the first experiment and 0, 40, or 100 ms/cm^3 of soil for the second). Three replicate infested aliquots per density level were mixed for 3.5 min in a twin-shell mixer.

Microsclerotia of *V. dahliae* were produced by inoculating minimal medium plates (11), overlaid with cellophane, with conidia from two isolates of *V. dahliae* and incubating the plates in the dark at approximately 25 C for 6 wk. Preparation of microsclerotia and determination of density have been described by Francl et al (8). After mixing microsclerotia inoculum with air-dried test soils, the soils were rehydrated by adding 100 ml of tap water to each liter of infested soil. Each liter of infested soil was subdivided into four aliquots. One aliquot was placed at 2 C immediately after rehydration for the first assay and after a 1-day drying time when the experiment was repeated. The remaining aliquots were air-dried in closed cupboards in an air-conditioned room for 2, 4, or 6 wk. All samples were then stored at 2 C and assayed within 2–3 wk.

Assays for *V. dahliae* were conducted by suspending two 20-cm^3 subsamples in 80 ml of water in a 125-ml flask, agitating the suspension vigorously on a magnetic stirer for 1 min, and plating 1-ml aliquots on each of five plastic petri plates containing NPX medium (3). Plates were left uncovered until the surface had dried (usually 1–2 hr) and then covered and incubated in the dark for 13 days. Following incubation, plates were washed to remove soil, and colonies of *V. dahliae* were counted within 2 days. The number of colony-forming units per cubic centimeter of soil (recovery density = RV) for the two subsamples were averaged. A total of 40 cm^3 soil was used for each assay unless otherwise stated.

Influence of soil drying time. The influence of soil drying time (0, 2, 4, or 6 wk) on recovery of *V. dahliae* was examined by regression analysis using only uninfested soils. The regression models tested for each set of soil samples were: 1) $RV = a + b(\text{Time})$, 2) $RV = a + b(\text{Time}) + c(\text{Time}^2)$, 3) $RV = a + b(\text{Time}^{0.5})$, and 4) $\text{Log}(RV + 1) = a + b\text{Log}(\text{Time} + 1)$, where *a, b*, and *c* were parameters to be estimated. A regression model was considered further if the overall F test was significant at $P < 0.10$. If more than one model was significant, then the model with the highest F value was chosen. For soil samples where the same type of model could be fitted, t tests were used to determine differences between rate parameters (b and/or c) of different soil samples at $P = 0.05$. The soil samples from each run of the experiment then were grouped together as fine-textured, coarse-textured, and peat soils, and regression analysis was performed with the same four models as above. Comparisons between estimated slope parameters ($P = 0.05$) were performed if the same model could be fitted to different textural groupings.

Influence of soil texture. The effect of soil texture on the recovery of *V. dahliae* from artificially infested soils was studied by regression analysis. Since soils could contain natural inoculum of *V. dahliae*, recovered microsclerotia at each infested density was less important than the slope of the lines (recovery efficiency). Each soil sample was modeled separately, and then soils from each experiment were combined into fine, coarse, and peat soil groupings and analyzed as groups. A t test ($P < 0.05$) was used to determine which estimated slopes were greater than 0 for individual and groups of soil samples.

To investigate whether soil physical characteristics were a factor in predicting the recovery of *V. dahliae* propagules, a stepwise regression procedure was used with colony-forming units per cubic centimeter values as the dependent variable (RV) and the percentage of sand, silt, clay, gravel, and organic matter, the pH, and the infested density of *V. dahliae* as independent variables. The two runs of the experiment were analyzed separately and combined for each drying time (0, 2, 4, and 6 wk). The stepwise regression was done with both a backward elimination and stepwise algorithim (25), and models were selected where the overall model was significant at $P = 0.05$ and each variable was significant at $P = 0.10$; then, R^2 (coefficient of determination) and C_p (bias measurement) were used as indicators of the best model. Estimated coefficients of variables that were included at more than one drying time were then compared between drying times with a t test.

Comparisons of glass and plastic petri plates. A silt loam soil was air-dried for 4 wk and infested with 0, 12.5, 15, 20, 30, 40, 60, 80, or 100 ms/cm^3 of *V. dahliae* as described previously. There were six replications of each density level. The soil was assayed as described above, except two sets of five 1-ml aliquots were taken from each flask. One set was added to five glass plates containing NPX medium and the other was added to five plastic plates of NPX medium. The experiment was repeated with a different silt loam soil and a loam soil that had been infested with 0, 10, and 40 ms/cm^3 of soil. There were three replications per density of *V. dahliae* for the second run of the experiment. The recovery efficiency of *V. dahliae* on plastic and glass plates was estimated by linear regression, with RV as the dependent variable and infested density as the independent variable. The estimated rate parameters were then compared between glass and plasic plates with a t test ($P = 0.05$).

Effect of soil dilution on the direct assay procedure. Silt loam soil (10 cm^3) was added to 40, 65, 90, 115, and 190 ml of distilled water in either a 125- or a 250-ml flask. There were three replications of each dilution. Each suspension was stirred as described previously, and five 1-ml aliquots were pipetted onto plastic plates of NPX medium. The experiment was repeated with a slightly different procedure. Sandy loam, silt loam, and loamy sand soils that had been infested with 40 ms/cm^3 of soil were added in samples of 5, 10, 15, or 20 cm^3

Table 1. Physical characteristics of 21 Ohio soils used to determine recovery efficiency of population densities of *Verticillium dahliae*

Soil identification number	Percentage of			pH	Gravel[a] (%)	Organic matter (%)	Soil texture designation[b]
	Sand	Silt	Clay				
1	12	68	21	7.0	35	1.8	Silt loam
7	20	65	16	6.7	37	2.3	Silt loam
21	22	61	17	7.4	28	2.6	Silt loam
22	22	59	19	6.2	33	2.6	Silt loam
23	19	58	23	6.8	28	2.9	Silt loam
24	16	54	30	6.8	41	4.8	Silty clay loam
39	22	45	33	6.8	26	2.9	Clay loam
35	45	36	19	7.1	39	3.4	Loam
40	38	47	15	6.8	29	2.1	Loam
10	80	14	7	6.8	7	1.4	Loamy sand
12	79	14	6	6.9	24	2.1	Loamy sand
28	85	11	4	6.9	7	0.7	Loamy sand
29	79	14	7	6.6	7	2.0	Loamy sand
33	81	13	6	7.0	13	1.3	Loamy sand
13	72	19	8	6.4	7	2.1	Sandy loam
27	68	16	16	6.9	22	2.7	Sandy loam
34	62	31	8	7.0	7	1.2	Sandy loam
14	30	43	27	6.0	12	67.0	Peat
15	29	42	29	5.4	42	73.0	Peat
16	22	33	45	5.9	19	92.0	Peat
18	28	40	32	5.1	44	76.0	Peat

[a]Soil particles >2 mm in diameter.

[b]Determined from a textural triangle, with the exception of soils composed primarily of organic matter, which were designated as peat soils.

to 95, 90, 85, or 80 ml of distilled water, respectively. There were three replications of each dilution. A linear regression model was fitted to recovery of *V. dahliae* ms/cm^3 of soil, with the concentration of soil as the independent variable and the slope tested with a t test ($P \leq 0.05$). The hypothesis test was whether the slope (recovery efficiency) differed from 0, which would mean that soil concentration affected the recovery of *V. dahliae*.

Effect of total soil volume on observed and predicted population densities of *V. dahliae* from a subsample. Single soil cores (2.5 cm in diameter) were collected to a depth of 15–20 cm before potatoes were planted in two commercial fields with low (average uncorrected density of 1 cfu/cm^3 of soil) and high (average uncorrected density of 11 cfu/cm^3 of soil) populations of *V. dahliae*. A total of 212 soil cores were collected (88 from the high-density field and 124 from the low-density field). The soil from each core was mixed and air-dried for 4 wk and then weighed. A single subsample was taken from each core and mixed with water (20 cm^3 of soil + 80 ml of water). Five 1-ml aliquots were then plated onto NPX medium in glass plates. The remaining soil from two assayed single cores was then mixed in a plastic bag and weighed, and a 20-cm^3 subsample was again assayed in the same manner. This was repeated for 4, 6, 8, 12, 16, and 20 cores, with 10 replications of each combination.

The recovered *V. dahliae* population density from each single core and the relative weights of each core that contributed to the mixed samples were used to calculate an expected density for each combination. A measure of the error between expected and observed values was developed: A = (expected − observed)^2/expected. ANOVA was used to test for significant ($P = 0.05$) effects of the number of combined cores on the errors. The experiment was repeated with some variations. Cores were weighed and combined into composite samples containing 2, 4, 6, 8, and 12 cores with nine replications of each combination. Data were analyzed as previously described. Major changes in the experiment were as follows: 1) All soil cores were collected from a single commercial potato field in 1990, 2) individual cores were air-dried for 6 wk and a wet-sieving procedure (11) was used to recover microsclerotia, 3) soil to be assayed (15 g) was suspended with 200 ml of water + 0.01% Tergitol for 30 sec in a Waring blender at a low speed (40–50), 4) the solution was poured through a 140-μm mesh sieve and then through a 25-μm mesh sieve, and 5) the residue retained on the 25-μm mesh sieve (approximately 30 ml) was distributed onto 15 plastic plates of NPX medium and the plates were left uncovered until visually dried.

RESULTS

Influence of soil drying time. Recovery of *V. dahliae* could be described as a function of drying time in nine of the 21 soil samples (Table 2). Drying time did not have a consistent effect over the different soils. The soils fitted to the quadratic and power models—two sandy loams and two peat soils, respectively (Table 2)—were characterized by higher *V. dahliae* recovery after a 0- or 1-day drying period, i.e., the peak recovery for soils fitted to a quadratic model (sandy loams) was between 17 and 25 days and that for soils fitted to the power models (peat soils) was 6 wk. However, soil samples fitted by a square root transformation—two loamy sands, two peat soils, and a silty clay loam (Table 2)—showed a reduction of *V. dahliae* recovery with increased drying time. Of the nine fine-textured soils examined, only

Table 2. Regression models that describe the recovery of *Verticillium dahliae* (RV) as a function of drying time (DT) for individual samples taken from Ohio soils

Sample no.	Regression model	R^2
10	$RV = 59.3 - 5.0(DT^{1/2})$ [SE = 1.4]	0.56
15	$RV = 22.5 - 2.3(DT^{1/2})$ [SE = 0.8]	0.42
24	$RV = 2.2 - 0.4(DT^{1/2})$ [SE = 0.1]	0.60
12	$RV = 2.7 - 0.3(DT^{1/2})$ [SE = 0.15]	0.31
16	$RV = 0.6 - 0.1(DT^{1/2})$ [SE = 0.04]	0.30
13	$RV = 3.1 + 1.5(DT) - 0.04(DT^2)$ [$SE_{DT} = 0.2$, $SE_{DT2} = 0.004$]	0.90
27	$RV = 29.3 + 1.6(DT) - 0.04(DT^2)$ [$SE_{DT} = 0.6$, $SE_{DT2} = 0.01$]	0.51
14	$\log(RV + 1) = 0.07 + 0.3(\log(DT + 1))$ [SE = 0.1]	0.45
18	$\log(RV + 1) = 0.2 + 0.2(\log(DT + 1))$ [SE = 0.08]	0.27

Table 3. Recovery efficiency (RE)[a] of *Verticillium dahliae* from artificially infested soils of different texture and air-dried for different times

		Drying time (wk)							
		0		2		4		6	
Soil texture[b]	Soil sample	RE	R^2[c]	RE	R^2	RE	R^2	RE	R^2
F	1	20 (5.4)[d]	0.67	11 (5.7)	0.34	0		0	
F	7	33 (10.4)	0.62	13 (3.3)	0.72	27 (1.9)	0.97	0	
C	10	0		52 (12.0)	0.73	0		40 (16.6)	0.45
C	12	6 (2.5)	0.42	0		5 (1.4)	0.64	5 (1.8)	0.49
C	13	57 (24.6)	0.47	0		114 (14.8)	0.91	0	
P	14	0		205 (47.6)	0.79	40 (15.0)	0.58	0	
P	15	0		0		0		14 (5.8)	0.44
P	16	17 (3.9)	0.72	10 (3.0)	0.62	16 (6.3)		17 (4.1)	
P	18	31 (3.0)	0.94	125 (42.0)	0.56	143 (33.8)	0.72	8 (4.1)	0.36
F	21	0		0		27 (6.9)	0.74	9 (1.7)	0.81
F	22	0		23 (3.3)	0.89	20 (5.5)	0.71	0	
F	23	59 (25.9)	0.46	14 (7.1)	0.44	19 (8.4)	0.45	0	
F	24	45 (8.3)	0.81	14 (3.7)	0.67	16 (2.8)	0.81	0	
C	27	0		0		0		60 (27.2)	0.41
C	28	45 (12.9)	0.64	0		99 (50.5)	0.36	15 (6.4)	0.44
C	29	23 (9.6)	0.46	0		0		47 (18.1)	
C	33	0		0		0		0	
C	34	84 (16.5)	0.81	20 (8.9)	0.47	55 (5.3)	0.95	0	
F	35	23 (8.2)	0.56	15 (3.0)	0.81	8 (2.3)	0.54	0	
F	39	0		11 (3.6)	0.55	4 (1.6)	0.46	11 (2.0)	0.80
F	40	43 (8.6)	0.78	22 (7.5)	0.56	0		0	

[a]Slope of the linearized model relating recovered *V. dahliae* propagules to infested *V. dahliae* microsclerotia.
[b]F = fine-textured soil, C = coarse-textured soil, and P = peat soil.
[c]Coefficient of determination.
[d]Standard error of the estimated slope.

Table 4. Regression equations for recovery of *Verticillium dahliae* microsclerotia per cubic centimeter of soil (*RV*) as a function of artificial infestation level (*VD*) and percentage of sand, silt, clay, gravel, organic matter (*OM*), and pH

Data	Equation	R^2	R^{2a}
All data			
$DT^b = 0$	$RV = 4.6 + 0.13\,(VD)\,[0.04] + 18.2\,(silt)\,[5.5] - 22.3\,(gravel)\,[9.2]$	0.10	0.04
$DT = 2$	$RV = 43.5 + 0.17\,(VD)\,[0.04] - 8.1\,(sand)\,[4.8] - 5.0\,(\text{pH})\,[1.9] - 25.7\,(gravel)\,[10.4]$	0.19	0.11
$DT = 4$	$RV = 42.3 + 0.10\,(VD)\,[0.03] - 5.4\,(\text{pH})\,[1.6] - 15.7\,(gravel)\,[7.3]$	0.12	0.06
$DT = 6$	$RV = 2.5 + 0.19\,(VD)\,[0.03]$	0.24	0.24
Run 1			
$DT = 0$	$RV = -25.9 + 0.42\,(VD)\,[0.08] + 4.4\,(\text{pH})\,[2.3]$	0.23	0.21
$DT = 2$	$RV = -4.7 + 0.38\,(VD)\,[0.07] + 28.1\,(OM)\,[4.8]$	0.37	0.17
$DT = 4$	$RV = 45.8 + 0.45\,(VD)\,[0.07] + 12.4\,(sand)\,[4.7] - 7.7\,(\text{pH})\,[2.0]$	0.37	0.27
$DT = 6$	$RV = 0.26 + 0.04\,(VD)\,[0.01] - 1.1\,(sand)\,[0.7]$	0.13	0.11
Run 2			
$DT = 0$	$RV = 0.2 + 0.10\,(VD)\,[0.04]$	0.10	0.10
$DT = 2$	$RV = 5.9 + 0.13\,(VD)\,[0.03] - 25.0\,(gravel)\,[9.3]$	0.26	0.18
$DT = 4$	$RV = 32.2 + 0.06\,(VD)\,[0.03] - 14.1\,(silt)\,[4.6] - 4.1\,(\text{pH})\,[1.8]$	0.19	0.06
$DT = 6$	$RV = 0.7 + 0.25\,(VD)\,[0.05] - 13.9\,(silt)\,[8.0]$	0.27	0.25

[a] Partial R^2 for *V. dahliae*.
[b] DT = drying time (wk) for soil before being stored at 2 C; standard error in brackets.

Table 5. Association and significance (*P*) between physical factors for 21 soils using Spearman's correlation coefficient

	Silt (*P*)	Clay (*P*)	pH (*P*)	Gravel (*P*)	OM (*P*)
Sand	−0.22 (0.01)	−0.36 (0.01)	0.49 (0.01)	−0.64 (0.01)	−0.79 (0.01)
Silt		0.82 (0.01)	0.33 (0.01)	0.44 (0.01)	−0.03 (0.64)
Clay			0.19 (0.01)	0.53 (0.01)	0.32 (0.01)
pH				−0.08 (0.27)	−0.51 (0.01)
Gravel					0.56 (0.01)

one showed a response of *V. dahliae* propagule recovery to drying time (Table 2).

When soils were pooled on the basis of texture, there was a significant quadratic response of drying time (DT) to recovery of *V. dahliae* in coarse-textured soils for run 1 of the experiment ($RV = 3.95 + 0.52(DT) - 0.01(DT^2)$), and a power model was fitted to *V. dahliae* recovery in peat soils ($RV = 1.13(DT^{0.21})$). No model could be fitted to combined fine-textured soils in run 1 or 2 or to combined peat and coarse-textured soils in run 2 of the experiment.

Recovery efficiency from artificially infested soils. Linear regression models for recovered colony-forming units per cubic centimeter of soil as a function of infested colony-forming units were fitted to the combined soil textural groupings of coarse, fine, and peat soils for each drying time in run 1 of the experiment (Table 3). In the fine-textured soils, there was generally low but significant recovery of microsclerotia in 56–67% of the samples during 0-, 2-, and 4-wk drying time (Table 3). At 6-wk drying time, only 22% of the fine-textured samples showed significant recovery of microsclerotia (Table 3). In the coarse-textured samples, recovery of microsclerotia was highly unpredictable. In the most extreme case (sample 13), recovery ranged from 0 at drying times of 0, 2, and 6 wk to 114% at 4 wk (Table 3). In the peat soils, recovery of microsclerotia was generally highest at the 2- and 4-wk drying times (Table 3).

Multiple regression was used to relate the physical characteristics of the soils and the artificially infested densities of *V. dahliae* to recovery of the fungus. When data from both experiments were combined, at each of the drying times, the rate of recovery of *V. dahliae* as a function of infested density ranged from 10% at 4-wk drying time to 19% at 6-wk drying time (Table 4). Although significantly less *V. dahliae* was recovered at 4 wk than at 6 wk, the overall recovery was relatively low. Percent gravel was negatively related to RV at 0-, 2-, and 4-wk drying times, and the parameter estimates were similar between these three drying times (Table 4). Soil pH was negatively related to RV at 2- and 4-wk drying, and the parameter estimates were not significantly different (Table 4). Because the R^2 and partial $R^2_{(V.\ dahliae)}$ values were low, the two runs of the experiment were also analyzed separately. The recovery efficiency of *V. dahliae* in run 1 ranged from 4% at 6-wk drying to 45% at 4-wk drying. Soil organic matter had a strong positive relation to RV at 2-wk drying (Table 2). In run 2 at 0- and 6-wk drying time, infested microsclerotia density was almost the only factor related to RV (Table 4). The most striking difference was the higher rate of recovery of infested propagules in run 1 than in run 2. Whether this was due to different environmental conditions in the drying area (humidity, temperature) or to storage time of the soils at 4 C before infestation with *V. dahliae*, which may have resulted in differences within the microflora of the soils, is unknown.

Correlations between soil factors are presented in Table 5 because the predictor variables were not independent. There was a positive association between pH and percent sand and a negative association between sand and all the other factors (silt, clay, gravel, and organic matter).

Glass vs. plastic petri plates. In run 1 of the experiment, recovered microsclerotia densities were regressed on artificially infested densities of *V. dahliae* for eight soils. There was a significant linear relationship between infested and recovered densities ($P = 0.05$). There was also a significant interaction between *V. dahliae* density and type of petri plates, indicated by different slopes of the two linear equations. The line fitted to glass plates was $RV = 1.68 + 0.11(VD)$ and the line fitted to plastic plates was $RV = -0.31 + 0.04(VD)$. The standard error for the rate parameter (slope) estimate and the R^2 for glass plates were 0.026 and 0.34, respectively, and those for plastic plates were 0.011 and 0.29, respectively.

The experiment was repeated with a silt loam and a loam soil, each at three initial microsclerotia densities. In the silt loam soil, the rate of recovery was not significantly different from 0 when either glass or plastic plates were used. With the loam soil, the linear equation describing recovery of *V. dahliae* with glass plates was $RV = 3.58 + 0.23(VD)$, with a standard error of 0.07 for the rate parameter and an R^2 of 0.62. With plastic plates, the rate parameter for the linear equation was not significantly different from 0. Thus, in two of three soils tested, we found a higher rate of recovery of *V. dahliae* propagules with glass plates than with plastic. In the third soil, recovery of the fungus was poor with both types of plates.

Effect of soil dilution on direct assay procedure. In both runs of the experiment, the recovery of *V. dahliae* did not differ between soil concentrations tested ($P = 0.05$), i.e., slopes of the equation RV = soil dilution did not differ from 0.

Table 6. Comparisons of the error in recovered population densities of *Verticillium dahliae* in composite samples made by combining two to 20 soil cores

Direct-plating procedure[a]					Wet-sieving procedure[b]				
No. of cores combined	OBS[c]	EXP[d]	$\frac{(OBS - EXP^2)}{EXP}$	Total soil weight (g)	No. of cores combined	OBS	EXP	$\frac{(OBS - EXP^2)}{EXP}$	Total soil weight (g)
2	17.0	36.2	25.2 (26.6)[e]	76	2	41.0	24.0	33.2 (27.8)[f]	161
4	40.8	25.5	21.5 (28.9)	98	4	5.9	15.3	7.8 (10.4)	294
6	26.9	20.4	8.0 (12.7)	155	6	9.3	11.7	4.8 (5.1)	471
8	10.3	17.1	7.3 (7.9)	207	8	5.9	13.3	5.6 (4.8)	569
12	11.0	11.1	5.4 (6.4)	373	10	5.5	8.3	1.6 (1.6)	726
16	2.7	7.9	4.2 (3.6)	532	12	8.0	11.6	1.7 (2.4)	866
20	4.2	6.9	3.4 (2.9)	723					

[a] In the direct-plating procedure, 20 cm^3 of soil was mixed with 80 ml of distilled water, and five 1-ml aliquots were then plated onto five glass plates of *Verticillium*-selective media.
[b] In the wet-sieving procedure, 15 g of soil was blended in 200 ml of 0.1% Tergitol and washed through a 26-μm sieve, and the soil on the sieve was plated onto 15 glass plates of *Verticillium*-selective media.
[c] Observed mean value (cfu/cm^3).
[d] Expected mean value based on average densities of individual cores and their relative weights.
[e] Standard deviation with 10 samples per core.
[f] Standard deviation with 10 samples for 2, 4, 6, and 8 cores, six samples for 10 cores, and nine samples for 12 cores.

Therefore, recovery of microsclerotia was equally effective at soil dilutions ranging from 5 to 20% (actual soil plated out ranged from 0.05 to 0.2 cm^3 of soil per plate).

Effect of total soil volume on expected and observed population densities. The mean of the observed and expected population density for each sample declined as the total volume of soil increased (Table 6) because the few cores with exceptionally high densities were diluted out as more cores with lower densities of *V. dahliae* were added. We took this into account by using an error term (A) which was divided by the expected RV. Linear regression analyses were performed on calculated error with core number or combined soil weight as the independent variable. With both the direct-plating and wet-seiving procedures, there was a decrease in error as core number and weight increased (Table 7). Therefore, combining soil cores (up to 20) resulted in a reduction in the error of predicting population densities of *V. dahliae* compared with assaying a composite of fewer cores.

Table 7. Estimates of the slope parameters for the linear relationship between measurement error[a] of recovered *Verticillium dahliae* microsclerotia and combined number or weight of soil cores

Assay procedure[b]	Parameter estimate	SE	No. of samples	$P > t$	R^2
DP-w	−0.026	0.009	70	0.01	0.12
WS-w	−0.034	0.008	55	0.01	0.25
DP-c	−1.106	0.319	70	0.01	0.15
WS-c	−2.550	0.578	55	0.01	0.27

[a] (Observed − expected cfu)^2/expected cfu.
[b] DP-w = direct-plating assay procedure where regression model is based on core weight as the independent term; WS-w = wet-sieving assay procedure with core weight as the independent term; DP-c = direct-plating assay procedure with core number as the independent term; and WS-c = wet-sieving assay procedure with core number as the independent term.

DISCUSSION

Quantitative soil assays have potential as tools for use in management decisions with diseases caused by *V. dahliae*. In the potato early dying syndrome, when the lesion nematode (*Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven) is present, the addition of 10, 30, and 100 ms/cm^3 of soil of *V. dahliae* in microplots resulted in predicted tuber weight losses of 16, 35, and 56%, respectively, using a nonlinear model (27). In cotton, a nonlinear function was also used to describe yield losses that occurred when a susceptible cotton cultivar vs. a tolerant cultivar was planted in *V. dahliae*-infested soil (21). In both cases, the type of yield loss curve was concave, indicating that the rate of yield loss decreased as the density of the fungus increased. Therefore, detection of low densities of *V. dahliae* is important in disease management, since control strategies may change with small changes in inoculum.

In this study, recovery of *V. dahliae* from different soils was found to be variable at all drying times, even within a similar soil textural group. Similarly, high variability in estimation of microsclerotia densities occurred from sample to sample in soils taken from naturally infested strawberry fields (10). The clustering of *V. dahliae* microsclerotia within soil aggregates (14) may affect the ability to recover microsclerotia with reproducibility, even when soil samples are well mixed. Sample drying time influenced the recovery of *V. dahliae* in our study, particularly with coarse-textured soils or those with high organic matter. However, it was difficult to predict the most appropriate drying time for any particular soil to achieve maximum or consistent recovery of microsclerotia.

Among the soils tested, we included some with high organic matter content, which may also have higher microbial activity. We wanted to test the theory that higher microbial activity may lower the recovery efficiency of *V. dahliae*. However, this was not demonstrated in our experiments, since the recovery of *V. dahliae* in peat soils was not significantly less than that in soils with considerably less organic matter. Drying times of 2–4 wk were critical to recovery of *V. dahliae* in most peat soils. High gravel content and high pH were most consistently associated with low recovery of *V. dahliae*. Harris et al (10) found that sieving soils to assay the 20- to 160-μm fraction gave higher recovery of *V. dahliae* than unsieved soils. The sieving would have eliminated the clay particles, part of the silt particles, and all of the sand particles and gravel. In our study, the elimination of gravel was the single most important factor that led to higher recovery of *V. dahliae*.

Recovery of *V. dahliae* was higher with glass than with plastic petri dishes in most but not all soils tested. This factor can be important in soils with a low recovery efficiency, since the number of plates necessary to detect low but damaging densities of *V. dahliae* may be two to three times higher with use of plastic petri plates. Recovery of *V. dahliae* was not appreciably affected by the amount of soil (up to 20%, v/v) used with a direct-plating assay. The amount of soil affects two parts of the assay: 1)

the amount of soil that settles to the bottom of the flask when mixing soil and water prior to taking aliquots (also a function of percent sand in the sample) and 2) the actual amount of soil that is plated onto the agar surface. Harris et al (10) found that with a wet-sieving procedure, the number of colonies increased as more soil was plated but, in contrast to our study, that the relationship was not proportional to the amount of soil added to each plate. They described the relationship with an exponential model that assumes a maximum number of colonies per plate (10). These differences may be due to the assay procedure (wet-sieving vs. direct-plating) and to the amount of soil added per plate (0.05–0.2 cm^3 in our study vs. 0.125–2 g in theirs [10]).

Combining soil cores to obtain a composite sample is generally done when sampling for soilborne plant pathogens (1,2). The assumption is that the soil can be mixed adequately so that one or more subsamples would describe the entire sample. In a study involving plant-parasitic nematodes (26), combining 40–80 soil cores resulted in a coefficient of variation significantly lower than when 10–20 soil cores were combined. We were concerned that increasing the number of combined cores in a composite sample would result in a poorer estimate because of the small amount of soil actually assayed compared with that for nematodes (500 cm^3). We found that combining up to 20 soil cores (the maximum tested) gave an adequate representation of the overall soil sample.

When soil assays are used to predict Verticillium wilt, limitations of the technique must be recognized. Assayed soil population values are only indicators of disease potential, since many other factors also influence severity of subsequent disease expression. Accuracy of soil population estimates is also problematic, and our study indicates that this is compromised by many factors. Our data would suggest that a well-mixed composite soil sample of 20 soil cores should be used. Soil samples should be air-dried 2–4 wk prior to assay to minimize growth of competing microorganisms on assay plates. Glass rather than plastic petri plates should be used for field assays, but where precision is critical, plastic plates are recommended. When direct plating is used, up to 20% of the mixture can be soil, and two subsamples are recommended.

The use of standards composed of soils with known propagule densities is important when comparing microsclerotia densities from different fields. Internal controls subjected to the same conditions as test samples can aid in calculation of actual recovery efficiencies. An alternative to including standards for each field is to collect samples over many years so that the history of population densities and corresponding yield losses is established. With proper controls and user experience, current assay techniques for soil populations of *V. dahliae* can be used reliably by growers, agricultural consultants, or researchers. Improved techniques not biased by other microorganisms present in soil may soon lead to greater reliability.

ACKNOWLEDGMENT

Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

LITERATURE CITED

1. Barker, K. R. 1985. Sampling nematode communities. Pages 3-17 in: An Advance Treatise on Meloidogyne. Vol. II, Methodology. K. R. Barker, C. C. Carter, and J. N. Sasser, eds. North Carolina State University Graphics, Raleigh.
2. Bruehl, G. W. 1987. Soilborne Plant Pathogens. Macmillan, New York.
3. Butterfield, E. J., DeVay, J. E., and Garber, R. H. 1978. The influence of several crop sequences on the incidence of Verticillium wilt of cotton and on the populations of *Verticillium dahliae* in field soil. Phytopathology 68:1217-1220.
4. Chinn, S. H. F. 1967. Differences in fungistasis in some Saskatchewan soils with special reference to *Cochliobolus sativus*. Phytopathology 57:224-226.
5. Davis, J. R., Pavek, J. J., and Corsini, D. L. 1983. A sensitive method for quantifying *Verticillium dahliae* colonization in plant tissue and evaluating resistance among potato genotypes. Phytopathology 73:1009-1014.
6. Day, P. R. 1965. Particle fractionation and particle-size analysis. Pages 545-567 in: Methods of Soil Analysis, Part I. C. A. Black, ed. Agronomy No. 9, Madison, WI.
7. Emmatty, D. A., and Green, R. J., Jr. 1969. Fungistasis and the behaviour of the microsclerotia of *Verticillium albo-atrum* in soil. Phytopathology 59:1590-1595.
8. Francl, L. J., Rowe, R. C., Riedel, R. M., and Madden, L. V. 1988. Effects of three soil types of potato early dying disease and associated yield reductions. Phytopathology 78:159-166.
9. Gee, G. W., and Bauder, J. W. 1986. Particle-size analysis. Pages 383-411 in: Methods of Soil Analysis, Part I. 2nd ed. A. Klute, ed. Agronomy No. 9, Madison, WI.
10. Harris, D. C., Yang, J. R., and Ridout, M. S. 1993. The detection and estimation of *Verticillium dahliae* in naturally infested soil. Plant Pathol. 42:238-250.
11. Huisman, O. C., and Ashworth, L. J., Jr. 1974. Quantitative assessment of *Verticillium albo-atrum* in field soils: Procedural and substrate improvements. Phytopathology 64:1043-1044.
12. Isaac, I., Fletcher, P., and Harrison, J. A. C. 1971. Quantitative isolation of *Verticillium* spp. from soil and moribund potato haulm. Ann. Appl. Biol. 67:177-183.
13. Isaac, I., and Harrison, J. A. C. 1968. The symptoms and causal agents of early-dying disease (Verticillium wilt) of potatoes. Ann. Appl. Biol. 61:231-244.
14. Jordan, V. W. L. 1974. Verticillium wilt of strawberry: Cultivar reaction and effect on runner health and production. Plant Pathol. 23:8-13.
15. Menzies, J. D., and Griebel, G. E. 1967. Survival and saprophytic growth of *Verticillium dahliae* in uncropped soil. Phytopathology 57:703-709.
16. Nadakavukaren, M. J., and Horner, C. E. 1959. An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. Phytopathology 49:527-528.
17. Nelson, D. W., and Sommers, L. E. 1975. A rapid and accurate procedure for estimation of organic carbon in soil. Proc. Indiana Acad. Sci. 84:456-462.
18. Nicot, P. C., and Rouse, D. I. 1987. Precision and bias of three quantitative soil assays for *Verticillium dahliae*. Phytopathology 77:875-881.
19. Nicot, P. C., and Rouse, D. I. 1987. Relationship between soil inoculum density of *Verticillium dahliae* and systemic colonization of potato stems in commercial fields over time. Phytopathology 77:1346-1355.
20. Nnudo, E. C., and Harrison, M. D. 1979. The relationship between *Verticillium albo-atrum* inoculum density and potato yield. Am. Potato J. 56:11-25.
21. Paplomatas, E. J., Bassett, D. M., Broome, J. C., and DeVay, J. E. 1992. Incidence of Verticillium wilt and yield losses of cotton cultivars (*Gossypium hirsutum*) based on soil inoculum density of *Verticillium dahliae*. Phytopathology 82:1417-1420.
22. Pegg, G. F. 1974. *Verticillium* diseases. Rev. Plant Pathol. 53:157-182.
23. Pullman, G. S., and DeVay, J. E. 1982. Epidemiology of Verticillium wilt of cotton: A relationship between inoculum density and disease progression. Phytopathology 72:549-554.
24. Rowe, R. C., Davis, J. R., Powelson, M. L., and Rouse, D. I. 1987. Potato early dying: Causal agents and management strategies. Plant Dis. 71:482-489.
25. SAS Institute. 1986. SAS User's Guide: Statistics. Version 6 ed. SAS Institute, Cary, NC.
26. Schmitt, D. P., Barker, K. R., Noe, J. P., and Koenning, S. R. 1990. Repeated sampling to determine the precision of estimating nematode population densities. J. Nematol. 22:552-559.
27. Wheeler, T. A., Madden, L. V., Rowe, R. C., and Riedel, R. M. 1992. Modeling of yield loss in potato early dying caused by *Pratylenchus penetrans* and *Verticillium dahliae*. J Nematol 24:99-102.