Effects of Quadrat Size and Time of Year for Sampling of *Verticillium dahliae* and Lesion Nematodes in Potato Fields

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ABSTRACT

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Six potato fields were randomly sampled using quadrat areas ranging from 10 m² to 4,000 m². A composite soil sample consisting of 20 soil cores was taken from each of 20 quadrats of each unit area. All samples were assayed for *Verticillium dahliae* and nematodes. The area in which a composite sample was taken had little effect on estimated mean and variance of *V. dahliae* until quadrat size was $\geq 1,000$ m². Then variances dramatically increased with size. With *Pratylenchus* spp., there was no systematic relationship between quadrat size and variance. Seven additional fields were divided into 2,000 or 4,000 m² grids, and each grid was sampled in both spring and fall for *V. dahliae* and plant-parasitic nematodes (*Pratylenchus* spp. and *Meloidogyne hapla*). Density of *V. dahliae* was lower, and in some cases, degree of aggregation was higher, in the spring than in the fall. Sampling for *V. dahliae* in the fall required 2 to 7 composite samples to estimate mean density within 25% of the true mean, while in the spring, 10 to 19 samples were required. Assays of *Pratylenchus* spp. required a similar number of samples in spring and fall surveys, while *M. hapla* required fewer samples with spring than with fall sampling when estimating densities >20 second-stage juveniles per 100 cm³ of soil.

Verticillium dahliae Kleb. can cause premature senescence and lower yields in potato, cotton, and many other crops (7,18,19). When the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuur., and *V. dahliae* are both present, plant disease symptoms (senescence) and yield losses are more severe than with *V. dahliae* alone (12,14). Root-knot nematode (*Meloidogyne hapla* Chitwood) may be involved in similar disease complexes (10,28). Microsclerotia in soil are the primary infective propagules of *V. dahliae* during early crop development (19,22). These propagules can be recovered from soil and enumerated with semiselective media (16). Considerable efforts have been made to devise sampling procedures for microsclerotia to reflect more accurately the preplant propagule density for use in determining appropriate management strategies (16,28,30).

To maximize benefits from sampling soil, estimates of mean and variance of pathogen density (and hence aggregation) are important. Various measurements of aggregation or heterogeneity have been applied to sampling data for *V. dahliae*

(5,11,25,28). In general, propagules of *V. dahliae* have been found to be aggregated in soil and often can be described by the negative binomial distribution. Thus, many samples are required to achieve a coefficient of variation (*cv*) of less than 10 to 25% (28). The area over which a composite soil sample is taken may affect the estimate of mean density for an organism. In previous studies, population density of *V. dahliae* was measured from a single-sized, consecutive quadrat in fields; then density determinations for single quadrats were combined (mathematically, not experimentally) to estimate larger quadrat areas (28). The objectives of this study were: (i) to test the influence of quadrat size on estimates of mean and variance, and hence aggregation, of *V. dahliae* and *Pratylenchus* spp. in commercial potato fields; and (ii) to compare fall versus spring sampling for aggregation of *V. dahliae*, *Pratylenchus* spp., and *M. hapla*.

The information we gained was then combined with information from other sampling studies (3,5,11,16,25,28,30) to define a sampling plan. The sampling plan was combined with a curvilinear yield-loss model (29) to define the number of samples necessary to predict yield loss and a subsequent management strategy.

MATERIALS AND METHODS

Quadrat size. Six commercial potato fields in Ohio were sampled repeatedly to

determine the effects of quadrat size on estimated mean and variance for soil populations of *V. dahliae* and *Pratylenchus* spp. Field 1 (14 ha) was a silt-loam soil that had been in a potato-wheat rotation for at least 50 years. Field 2 (5 ha) was a silt-loam soil that had been in a potato-wheat rotation for at least 12 years. Fields 3 to 6 were coarse-textured soils that had been in potato-grain crop rotations for <10 years. Fields 3 (24 ha), 4 (7 ha), 5 (19 ha), and 6 (15 ha) were located in northwestern Ohio. Fields 1 and 2 were located in northeastern Ohio. Each of these six fields was divided into contiguous quadrats of 10, 50, 100, 250, and 1,000 m², and fields 1, 3, 5, and 6 (because of their larger size) were also divided into quadrats of 4,000 m². The quadrats were all assigned consecutive identifying numbers; for example, in a 15-ha field there would be 15,000 consecutive numbers for the 10 m² quadrat area, 3,000 for the 50 m² quadrat area, 1,500 for the 100 m² quadrat area, etc. Twenty randomly selected quadrats of each size were sampled for each of the six fields. The position of each quadrat was determined by using a random number generator from the procedure PLAN in SAS (SAS Institute, Cary, NC). A seed number is used in this procedure to generate each set of random numbers, and the seed numbers were selected without pattern. The locations of the selected areas (100 to 120 per field, depending on whether there were 4,000 m² quadrats) were marked with flags, and 20 soil cores per quadrat (2.5 cm diameter, 20 cm deep) were taken using a zigzag pattern that covered the entire area of the quadrat. Cores from each quadrat were mixed in a bucket, and a subsample of 1,000 cm³ was removed and placed in a plastic bag for assays. All samples from a field were collected over a 1- to 2-day period.

To assay for *V. dahliae*, soil samples were dried for 2 weeks at room temperature ($\sim 25^{\circ}\text{C}$), and then each sample was smoothed with a rolling pin, bagged, and kept at 10°C until tested (<6 months). For each sample, a dilution plate assay was conducted twice. This consisted of taking 20 cm³ of soil from each sample, placing in 80 ml of deionized water, stirring for approximately 30 s, and then plating five 1-ml aliquots on semiselective sodium polypectate media

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(16) in sterilized glass petri plates. After incubating plates in the dark for 13 days at 25°C, soil on plates was removed by washing, and *V. dahliae* colonies in the agar were identified and counted under a dissecting microscope.

To assay for *Pratylenchus* spp., 200 cm³ of soil from each sample was placed in a pie-pan apparatus (<1 week after sampling), and nematodes were collected after 36 to 48 h (27). Plant-parasitic nematodes were identified and counted, and samples containing lesion nematodes were treated with an equal volume of boiling water, concentrated to 5 to 10 ml with a sieve of 25-µm pore size, and then treated with an equal volume of 10% formaldehyde. Representative adults were mounted on microscope slides, and species were determined (8).

Analyses. For each set of quadrat sizes, the mean and variance among the 20 quadrats of that size were determined for *V. dahliae* and *Pratylenchus* spp., and homogeneity of variances was compared among quadrat areas by two tests, Box's and Levene's ($P = 0.05$) (15). Both tests were used because little is known about how these tests may differ in determining how population variance of potato early dying pathogens may be similar or different. In addition, a statistical model was developed by using an expansion of Taylor's power

law (3,26) for all fields and quadrat sizes. The full model was

$$\log(v)_{ij} = \log(a) + b\log(m) + F_i + b_i\log(m) + S_j + b_j\log(m) + \text{error} \quad (1)$$

in which v is the variance, m is the mean density, $\log(a)$ is the intercept (predicted $\log(v)$ when $\log(m) = 0$), b is a slope, F_i is a factor for field ($i = 1, \dots, 6$), S_j is a factor for quadrat size ($j = 1, \dots, 6$), and b_i and b_j are slope components of $\log(v)$ in relation to $\log(m)$ for the six quadrat sizes and six fields, respectively. The factors F_i and S_j are grouping or class variables, not continuous variables, with 5 df each. There are also 5 df for both b_i and b_j , because there are six levels of quadrat size and field. The error term in the equation is considered to be equivalent to the sum of F_iS_j and three-way interaction term. If quadrat size and field had no effect on the magnitude of v or the rate at which v increased with m , then equation 1 reduces to

$$\log(v) = \log(a) + b\log(m) + \text{error} \quad (2)$$

which is the standard power law (3,26). The reason for using equation 1 is that a linear relationship between $\log(v)$ and $\log(m)$ is expected for density of many organisms (3,26), including *V. dahliae* (28), and equation 1 allows testing for additional sources of variation in $\log(v)$ after correcting for $\log(m)$. Significance of

the terms in equation 1 was tested with reduced versus full model F tests.

Time of sampling. A comparison was made between soil samples collected in the fall versus spring for accuracy of pathogen density estimates. Seven fields, different from the six fields in the previous study, were selected. Four of the fields were of a coarse-textured soil located in northwest Ohio, and three fields were finer-textured (silt loam) soils located in northeastern Ohio. Soil samples were collected in the spring (April to May) and fall (September to October). Field sizes ranged from 2.4 to 14.4 ha. Each field was divided entirely into 4,000 or 2,000 m² quadrats (depending on the size of the field), and 20 soil cores were collected from each of the quadrats in a zigzag pattern, mixed, and a 1,000 cm³ subsample was assayed for *V. dahliae* and plant-parasitic nematodes. Assay procedures were as described previously. The mean and variance of *V. dahliae*, *Pratylenchus* spp., and *Meloidogyne* populations from each field were used in regression analysis with an expanded power law similar to equation 1, but with the factor T_j ($j = 1, 2$) for season (time) instead of S_j . The number of samples required to estimate the population density of each organism within 25% of the true mean (i.e., with a cv of 0.25) was calculated over the density range of 10 to 100 (units depend on

Table 1. Estimated mean density, variance (VAR), and aggregation index (k) of *Verticillium dahliae* microsclerotia from sampling a range of quadrat unit sizes in commercial potato fields in Ohio

Field	Quadrat sampling size (m ²)																	
	10			50			100			250			1,000			4,000		
	Mean	VAR	k^a	Mean	VAR	k	Mean	VAR	k	Mean	VAR	k	Mean	VAR	k	Mean	VAR	k
1	6.4	18	3.5	3.6	10	2.0	77	38	1.9	7.0	25	2.7	5.9	24	1.9	7.2	13	9.5
2	1.9	5	1.1	1.5	5	0.5	1.6	3	2.2	1.8	4	1.7	2.3	4	2.8	...	...	...
3 ^b	8.3	99	0.7	5.0	38	0.7	4.8	43	0.5	6.9	37	1.5	35.9	1×10 ⁴	0.0	12.2	55	3.4
4	9.8	109	0.9	12.5	230	0.7	11.8	236	0.6	11.5	99	1.5	11.7	117	1.2	...	...	...
5 ^b	6.0	147	0.2	14.1	588	0.3	8.4	99	0.7	2.6	15	0.5	5.8	39	1.0	120.6	2×10 ⁵	0.0
6	7.0	36	1.6	10.8	160	0.7	7.2	46	1.3	10.3	80	1.5	12.6	149	1.1	11.5	59	2.7

^a The aggregation parameter k was calculated as $k = [(\text{mean}^2 - \text{VAR}/n)/(\text{VAR} - \text{mean})]$, where n was the number of samples collected for a sampling unit size, $n = 20$.

^b These fields had significant differences among variances in quadrat areas according to Levene's test, but variances were homogeneous according to Box's test (15).

Table 2. Estimated mean density, variance (VAR), and aggregation index (k) of *Pratylenchus* spp.^a vermiforms from sampling a range of quadrat sizes in commercial potato fields in Ohio

Field	Quadrat sampling size (m ²)																	
	10			50			100			250			1,000			4,000		
	Mean	VAR	k^b	Mean	VAR	k	Mean	VAR	k	Mean	VAR	k	Mean	VAR	k	Mean	VAR	k
1 ^c	40.2	1,165	1.4	37.2	1,012	1.4	26.1	462	1.5	31.1	435	2.3	39.3	1,167	1.3	21.8	491	1.0
2 ^{c,d}	50.0	858	3.0	83.3	3,255	2.1	74.2	1,450	3.9	63.9	1,169	3.6	78.1	1,453	4.3	...	...	...
3 ^c	96.5	17,487	0.5	46.0	2,836	0.7	19.5	921	0.4	53.0	7,632	0.3	67.2	4,151	1.1	52.6	3,454	0.8
4 ^c	11.5	371	0.3	22.0	1,427	0.3	8.5	203	0.3	17.0	759	0.3	10.5	152	0.7	...	...	...
5	8.5	119	0.6	6.8	212	0.2	13.2	189	0.9	14.5	342	0.6	14.7	249	0.9	13.9	237	0.8
6	7.5	136	0.4	7.0	212	0.2	9.5	216	0.4	15.8	648	0.3	14.7	293	0.7	6.5	66	0.7

^a Mean percent *Pratylenchus penetrans* ranges from 1 to 10% in field 1, 1 to 5% in field 2, 26 to 48% in field 3, 53 to 97% in field 4, 54 to 78% in field 5, and 60 to 100% in field 6.

^b The aggregation parameter k was calculated as $k = [(\text{mean}^2 - \text{VAR}/n)/(\text{VAR} - \text{mean})]$, where n was the number of samples taken in a sampling unit area.

^c Variances were significantly different among quadrat areas in these fields according to Levene's test (15).

^d Variances were significantly different among quadrat areas in this field according to Box's test (15).

organism) by the equation 13.7 given in Campbell and Madden (3).

RESULTS

Quadrat size. The estimated mean density of *V. dahliae* in commercial potato fields was not affected by the different quadrat sizes except for field 3 (1,000 m²) and field 5 (4,000 m²) (Table 1). The estimated mean density of the population of *Pratylenchus* spp. (which consisted of a mixture of *P. penetrans*, *P. crenatus*, and *P. scribneri*) differed widely among quadrat sizes in a field, but there was no trend of higher mean or variance associated with any particular quadrat size (Table 2). The two variance-testing methods gave somewhat different results. With Box's test, homogeneity of variances for *V. dahliae* was found in all fields, while for *Pratylenchus* spp., in all but one field variances were homogeneous across the different quadrat sizes (Tables 1 and 2). However, with Levene's test, heterogeneity of variances between quadrat sizes for *V. dahliae* was found in two of six fields (Table 1) and for *Pratylenchus* spp. in four of six fields (Table 2). Large quadrat sizes (1,000 or 4,000 m²) were associated with larger variances for *V. dahliae* in two fields (fields 3 and 5). In both cases, a single sample was responsible for the higher than normal mean and variance within the 20 samples assayed (data not presented). With *Pratylenchus* spp., there was no apparent relationship between variance and quadrat size.

In fitting the expanded power model (equation 1) to the data for *V. dahliae*, the

$\log(m)$ term was highly significant ($P < 0.01$). The factor $b_i \log(m)$ (interaction of field size [F_i] and mean density) was not significant ($P > 0.20$), and F_i was borderline ($P = 0.045$). The other terms were highly significant ($P < 0.01$), and thus the reduced model can be written as

$$\log(v)_j = \log(a) + b \log(m) + S_j + b_j \log(m) + \text{error} \quad (3)$$

which means that the intercept [$\log(a) + S_j$] and slope ($b + b_j$) from the reduced model for the relationship between $\log(v)$ and $\log(m)$ were dependent on quadrat size. The slopes and intercepts for each quadrat size (but across all fields), were obtained from separate regressions (Table 3). These estimated parameters were compared with t tests ($P = 0.05$), which showed that the largest quadrat size (4,000 m²) had a significantly higher slope and lower intercept than all but the next largest size (1,000 m²). The smaller quadrat sizes had similar slopes and intercepts and were not different from each other. The general trend was for increasing slope and decreasing intercept of the relation between $\log(v)$ and $\log(m)$ with increasing quadrat size, starting at 250 m². The significant effect of F_i (not shown in equation 3) indicates that the intercepts (but not slopes) can vary with field (intercept equal to $\log(a) + S_j + F_i$). The intercept for any given field can be obtained by adding the following value to the appropriate quadrat-size intercept (Table 3): 0 for field 1, 0.48 for field 2, 0.27 for field 3, 0.09 for field 4, 0.48 for field 5, and -0.04 for field 6.

For the lesion nematodes, neither quadrat size nor any interaction had significant effects ($P > 0.20$). However, $\log(m)$ was highly significant ($P < 0.01$), giving the equation

$$\log(v) = 1.02(\text{SE} = 0.176) + 1.31(\text{SE} = 0.125)\log(m) \quad (4)$$

which had an R^2 of 0.78. The field parameter was significant ($P = 0.04$), indicating that the intercept depended on field. The intercept for a given field is obtained by adding the following number to 1.02 of equation 4: 0 for field 1, -0.22 for field 2, 0.37 for field 3, 0.37 for field 4, 0.17 for field 5, and 0.27 for field 6.

Time of sampling. Population means and variances for *V. dahliae*, *Pratylenchus* spp., and *M. hapla* populations were estimated for soil samples taken in the spring and the fall (Table 4). Estimated mean density values for *V. dahliae* from fall samples ranged from 2 to 79 microsclerotia per cm³ soil, and from spring samples ranged from 1 to 31 microsclerotia per cm³ soil. In all cases, the mean densities assayed for each field were lower in the spring than in the fall (Table 4). Variances increased with increasing density of *V. dahliae*. The aggregation index, k , was lower in spring than in fall samples (i.e., increased aggregation) in three (fields b, c, f) of seven cases (Table 4). In three cases (fields a, d, h), mean equaled the variance in spring samples, leading to a decrease in aggregation (because k is equal to infinity when $m = v$) compared with fall sampling (Table 4). In field g, aggregation was similar between spring and fall sampling, although both mean and variance were smaller in the spring than in the fall (Table 4).

For *Pratylenchus* spp., estimated mean density ranged from 53 to 107 vermiforms per 100 cm³ of soil for fall samples and 40 to 220 vermiforms per 100 cm³ of soil for spring samples (Table 4). Variances for *Pratylenchus* spp. based on spring and fall samples were much greater than the estimated mean densities, indicating a high degree of aggregation. *M. hapla* estimated mean densities ranged from 0 to 183 second-stage juveniles per 100 cm³ of soil for fall samples, and 1 to 18 second-stage ju-

Table 3. The effect of quadrat size on variance-mean relations when applying Taylor's power law^a to population estimates for *Verticillium dahliae*

Quadrat size (m ²)	Intercept (SE ^b)	Slope (SE)	Coefficient of determination	Prob. > t
10	0.52 (1.13)	1.81 (0.62)	0.68	0.04
50	0.47 (0.52)	2.01 (0.26)	0.94	0.01
100	0.05 (0.53)	2.06 (0.28)	0.93	0.01
250	0.74 (0.40)	1.51 (0.22)	0.92	0.01
1,000	-1.48 (0.86)	2.83 (0.37)	0.94	0.01
4,000	-4.54 (0.29)	3.51 (0.09)	1.00	0.01

^a Taylors power law is: $\log(\text{variance}) = \log(a) + b \log(\text{mean})$, in which $\log(a)$ and b are the intercept and slope, respectively (26).

^b SE is the standard error.

Table 4. Estimated means and variance (VAR) for *Verticillium dahliae*, *Pratylenchus* spp., and *Meloidogyne hapla* populations for fall and spring samples collected in seven commercial potato fields in Ohio

Field	<i>V. dahliae</i>						<i>Pratylenchus</i> spp.				<i>M. hapla</i>			
	Fall			Spring			Fall		Spring		Fall		Spring	
	Mean	VAR	k^a	Mean	VAR	k	Mean	VAR	Mean	VAR	Mean	VAR	Mean	VAR
a	17	110	3.1	3.1	3.3	48	71	1,962	40	930	14	449	18	1,176
b	79	3,170	2.0	31	1,780	0.5	103	10,384	66	4,369	22	1,954	1	16
c	28	225	3.9	8	48	1.5	68	2,016	41	900	183	68,854	7	408
d	37	104	20	5	5	... ^b	107	3,588	220	7,430	0	1	5	40
f	41	132	18	13	74	2.8	76	1,632	166	18,333	13	292	11	286
g	22	172	3.2	7	20	3.5	53	2,421	150	4,449	3	36	10	159
h	2	3	4.0	1	1	... ^b	98	3,329	56	3,080	10	94	15	734

^a k is an aggregation index estimated by the formula: $\text{mean}^2/(\text{VAR} - \text{mean})$.

^b No aggregation index could be calculated because the mean = variance.

veniles per 100 cm³ of soil for spring samples. Variances for *M. hapla* were also greater than estimated mean densities for both spring and fall samples (Table 4).

With the expanded power law model for the seasonal data, $\log(m)$ was highly significant ($P < 0.01$), season and the interaction of season and $\log(m)$ were significant

for *V. dahliae* and *M. hapla* ($P < 0.05$), but not for *Pratylenchus* spp. ($P > 0.20$). All other terms were not significant ($P > 0.10$). Estimated parameters are given in the legend of Figure 1.

Sample size. For *V. dahliae*, greater sampling intensity was required in the spring than in the fall (Fig. 1A) because of the lower mean density and in some cases higher aggregation in the spring. Quadrat size was kept constant during both spring and fall sampling, so that assessed aggregation was a function of heterogeneity of the organisms and not of quadrat size. Sampling intensity for *V. dahliae* also increased as mean density increased for spring sampling due to power law $b > 2$ (Fig. 1A). In the fall, sampling intensity decreased as mean density increased (Fig. 1A). Two to seven samples were adequate to achieve $cv = 0.25$ for fall sampling for *V. dahliae* over a range of tested fungal densities, while for spring sampling, sample number ranged from 9 to 19 to achieve the same cv . Moreover, at low densities of *V. dahliae* (~10 microsclerotia per cm³ of soil), sampling intensity was similar for both fall and spring sampling (Fig. 1A).

For *Pratylenchus* spp., there was no difference between predicted spring and fall sampling numbers because there was no difference in power-law parameters. Sampling intensity declined as mean density increased because $b < 2$ (Fig. 1B). For *M. hapla*, fewer samples were necessary in the spring than in the fall when density of second-stage juveniles was >20 per 100 cm³ of soil (Fig. 1C). However, more than 20 samples were necessary for all *M. hapla* densities calculated.

DISCUSSION

Risk of yield losses due to potato early dying is a function of preplant population density of *V. dahliae*, the presence or absence of *P. penetrans*, susceptibility of host cultivar, and weather conditions (18,20,28). Severity and incidence of plants with potato early dying increases with density of *V. dahliae* (12,14,29). Yield loss has been approximated (27) with the curvilinear equation $y(VD) = b_0 + (1 - b_0)/[1 + (VD/36.7)]$, where b_0 represents the minimum yield (or $1 - \text{maximum yield loss due to the disease}$) and VD is the density of *V. dahliae*. The minimum yield (b_0) is lowered by the presence of a threshold density of *P. penetrans* (29) (from 0.55 to 0.23 under homogeneous conditions) and is also affected by environment. The severity of potato early dying symptoms is not directly related to *P. penetrans* density when density is ≥ 80 vermiforms per 100 cm³ of soil (21). The mechanism of the interaction between *V. dahliae* and *P. penetrans* has not been determined (2,7). Under conditions favorable for disease (cool and wet early in the season), the minimum yield is lower and disease severity is higher, while under unfavorable disease conditions (hot

Fig. 1. Number of samples required to estimate population densities of *Verticillium dahliae* (A), *Pratylenchus* spp. (B), and *Meloidogyne hapla* (C) during fall and spring sampling within 25% of the true mean. The formula for calculating sampling number was: $n = a(\text{mean}^{(b-2)})/C^2$, where n is the sampling number, a and b were parameters estimated from Taylor's power law (26), and C is the coefficient of variation set arbitrarily at 0.25. Estimated a and b parameters for lines are: (A) spring $a = 0.30$, $b = 2.30$; fall $a = 1.47$, $b = 1.46$; (B) $a = 6.30$, $b = 1.41$; (C) spring $a = 7.17$, $b = 1.63$; fall $a = 2.20$, $b = 2.04$. There was no difference in sampling parameters between spring and fall for *Pratylenchus* spp., so only one line is presented.

and dry), minimum yield is higher and disease severity is lower (4). The isolate of *V. dahliae* can also modify estimates of b_0 , so that a more aggressive isolate would have a lower minimum yield than a less aggressive isolate (1).

The yield loss equation above was generated from homogeneous populations of *V. dahliae* and *P. penetrans*. In a heterogeneous environment, yield loss equations should be modified to account for spatial aggregation on the nonlinear response of yield to VD (6,9,13,17,23). One approach is based on use of an expansion of the yield model to obtain mean yield, $y(VD)$:

$$\bar{y}(VD) = y(\bar{VD}) + y''(\bar{VD})v(VD)/2$$

in which $v(VD)$ is the variance of VD in the field, $\bar{VD}$ is mean VD in the field, and $y''(\bar{VD})$ is the second derivative of $y(\bar{VD})$ evaluated at $\bar{VD}$, which equals $2(1 - b_0)/[1,346.89(1 + \bar{VD}/36.7)]^3$. Thus, predicted mean yield is dependent on variability of VD , which is a direct function of aggregation of VD . Aggregation may be a function of the number of years a field has been in a potato rotation, fumigation history, spatial heterogeneity of soil factors such as organic matter, and other factors. A heterogeneous field, in terms of *V. dahliae* and *P. penetrans* densities, results in greater mean yield and hence less loss from disease than a homogeneous case with the same average density of *V. dahliae* (Table 5).

Aggregation of the pathogen plays a second role in assessing risk of early dying. High aggregation (or variance) requires collection of more soil samples than lower aggregation to determine mean density (3). In this study, mean density and variance were examined for sampling at different times during the year and with different quadrat sizes. Sampling in the fall for *V. dahliae* required <10 samples to determine the mean density within 25% of

the true mean. However, sampling in the spring required 10 to 20 samples, with sample number increasing as fungal density increased, to obtain an estimate within 25% of the true mean. Fortunately, sampling in the fall provides sufficient time to process samples and implement a management strategy. Time of the year did not change the sample number required for *Pratylenchus* spp. However, more than 25 samples were required for lesion nematode density near the proposed threshold of 10 vermiforms per 100 cm³ of soil (28). Sample number was reduced to <10 as lesion nematode density increased to 100 vermiforms per 100 cm³ of soil. Sample number for *M. hapla* was high (>20) regardless of time of the year sampled, although required sample number was generally higher (approximately 40 samples at all densities) in the fall than in the spring (20 to 50 samples).

The size of quadrat over which cores were bulked to represent a composite sample did not affect the estimate of *V. dahliae* density and variance until larger ($\geq 1,000$ m²) areas were sampled (Table 1). It is not unusual in current sampling programs to divide a field into quadrats greater than 4,000 m². Because of the positive skewness of the negative binomial distribution (3), greatest errors are expected to result in overprediction rather than underprediction of mean densities of VD , particularly with the larger quadrat sizes. That is, with small numbers of samples, it is more likely that sample mean will be larger than the true population mean. Quadrat size was not a factor in aggregation of the lesion nematode; the mean density, and to a lesser degree, field site, were more important than quadrat size for this nematode.

The effects of pathogen aggregation can be assessed not only in terms of the altered damage (loss) function (as discussed above), but also in terms of variability (i.e., uncertainty) in predicted yield. Based on

large-sample theory and the assumption that the parameters are known (not estimated), variance of predicted yield [$v(y)$] is equal to: $v(VD) \cdot [y'(\bar{VD})]^2$, in which $v(VD)$ is the variance of VD (as before), and $y'(\bar{VD})$ is the first derivative of y with respect to VD , i.e., $y'(VD) = -36.7(1 - b_0)/(36.7 + VD)^2$. Thus, choosing a sample size to obtain an acceptably precise estimate of mean VD is the minimum requirement to precisely assess the risks of yield loss in a field. The minimum yield parameter in the yield loss model (29), b_0 , also impacts the variance of predicted yield because $y'(\bar{VD})$ is a function of b_0 . Thus, $v(y)$ in the presence of *P. penetrans* will be $(1 - 0.23)^2/(1 - 0.55)^2 = 2.9$ times higher than in the absence of *P. penetrans* (where $b_0 = 0.55$ [29]) when mean and variability of *V. dahliae* micro-sclerotia are the same. In sampling terms, this means that as the number of sites with both *V. dahliae* and *P. penetrans* increases in a field, more samples will be required to estimate yield losses adequately. This increase in variance of y could be extended to anything that increases the yield loss reduction per unit increase in pathogen density (y'), such as over-irrigation (4) or isolate aggressiveness (1).

Sampling to determine whether to fumigate soils for potato early dying is a common practice in some potato production areas (18). It is important to recognize those factors that impact potato early dying severity and that can be assessed through sampling. Few soilborne fungi can be assessed accurately and consistently in soils, although protocols have been developed for some pathogens (24), including *V. dahliae* (16). Crop managers who base management decisions on soil population density estimates of *V. dahliae* and *Pratylenchus* spp. could improve their information by maximizing sampling efficiency. This includes collecting sufficient soil samples in the fall after harvest, collecting individual composite samples over relatively small (10 to 250 m²) randomly selected areas, and increasing sample number appropriately for factors that would increase the potential for damage per unit micro-sclerotia of *V. dahliae*, such as the presence of *P. penetrans*. A management program could consist of soil sampling in the fall, with two to seven samples per field, depending on damage threshold (a damage threshold near 10 CFU/cm³ of soil would require seven samples, while one near 100 CFU/cm³ of soil would require two soil samples). The number of samples would increase: if *P. penetrans* was present (by 2.9 times), if the isolate of *V. dahliae* in a particular field was unusually aggressive, or if planning for the most disease-conducive weather. It is essential to estimate or have records for the lowest possible minimum yield experienced in that field due to potato early dying. That value can be substituted into the yield loss model, and the number of extra samples

Table 5. Theoretical yields as a function of selected mean densities of *Verticillium dahliae* (VD), spatial aggregation (k), and presence or absence of *Pratylenchus penetrans* (PP)

$\bar{VD}$	k	PP	$v(VD)^a$	$DER^b \times 10^{-4}$	$y(\bar{VD})^c$	$\bar{y}(VD)^d$
10	0.5	–	210	3.24	0.904	0.938
10	1.5	–	77	3.24	0.904	0.916
10	3.5	–	39	3.24	0.904	0.910
40	0.5	–	3,240	0.73	0.765	0.883
40	1.5	–	1,107	0.73	0.765	0.805
40	3.5	–	497	0.73	0.765	0.783
10	0.5	+	210	5.55	0.835	0.893
10	1.5	+	77	5.55	0.835	0.856
10	3.5	+	39	5.55	0.835	0.845
40	0.5	+	3,240	1.25	0.598	0.800
40	1.5	+	1,107	1.25	0.598	0.667
40	3.5	+	497	1.25	0.598	0.629

^a The formula for estimating variance of VD is $(\bar{VD}) + (\bar{VD})^2/k$ (12).

^b The second derivative of the yield loss model is: $y''(VD) = 2(1 - b_0)/[1,346.89(1 + VD/36.7)]^3$.

^c Yield when spatial aggregation is nonexistent or not considered, $y(VD) = b_0 + (1 - b_0)/[1 + (VD/36.7)]$, where $b_0 = 0.55$ when PP is absent, and 0.23 when PP is present. VD is mean VD .

^d Yield when spatial aggregation is considered: $\bar{y}(VD) = y(VD) + y''(VD)[v(VD)]/2$. That is, mean predicted y is a function of mean VD [i.e., $y(VD)$] plus a function of the product of the second derivative [i.e., $y''(VD)$] evaluated at the mean ($\bar{VD}$) and the variation in VD [i.e., $v(VD)$].

necessary for a given scenario can be calculated. The most difficult part of selecting a minimum sampling number is to know how much loss to attribute to potato early dying.

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LITERATURE CITED

1. Botseas, D. D., and Rowe, R. C. 1994. Development of potato early dying in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans*. *Phytopathology* 84:275-282.
2. Bowers, J. H., Nameth, S. T., Riedel, R. M., and Rowe, R. C. 1996. Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. *Phytopathology* 86:614-621.
3. Campbell, C. L., and Madden, L. V. 1990. *Introduction to Plant Disease Epidemiology*. John Wiley & Sons, New York.
4. Cappaert, M. R., Powelson, M. L., Christensen, N. W., Stevenson, W. R., and Rouse, D. I. 1994. Assessment of irrigation as a method of managing potato early dying. *Phytopathology* 84:792-800.
5. Evans, G., and Gleeson, A. C. 1980. An evaluation of the sampling variation when estimating the population of *Verticillium dahliae* in field soil. *Ann. Appl. Biol.* 95:177-184.
6. Ferrandino, F. J. 1989. A distribution-free method for estimating the effect of aggregated plant damage on crop yield. *Phytopathology* 79:1229-1232.
7. Francl, L. J., and Wheeler, T. A. 1993. Interaction of plant parasitic nematodes with wilt-inducing fungi. Pages 79-103 in: *Nematode Interactions*. M. W. Khan, ed. Chapman & Hall, London, England.
8. Handoo, Z. A., and Golden, A. M. 1989. A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). *J. Nematol.* 21:202-218.
9. Hughes, G. 1990. Characterizing crop responses to patchy pathogen attack. *Plant Pathol.* 39:2-4.
10. Jacobsen, B. J., MacDonald, D. H., and Bissonnette, H. L. 1979. Interaction between *Meloidogyne hapla* and *Verticillium albo-atrum* in the Verticillium wilt disease of potato. *Phytopathology* 69:288-292.
11. 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 Dis.* 72:484-488.
12. MacGuidwin, A. E., and Rouse, D. I. 1990. Role of *Pratylenchus penetrans* in the potato early dying disease of Russet Burbank potato. *Phytopathology* 80:1077-1082.
13. Madden, L. V., and Nutter, F. W., Jr. 1995. Modeling crop losses at the field scale. *Can. J. Plant Pathol.* 17:124-137.
14. Martin, M. J., Riedel, R. M., and Rowe, R. C. 1982. *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72:640-644.
15. Milliken, G. A., and Johnson, D. E. 1984. *Analysis of Messy Data. Vol. I. Designed Experiments*. Lifetime Learning Publications, Belmont, CA.
16. Nicot, P. C., and Rouse, D. I. 1987. Precision and bias of three quantitative soil assays for *Verticillium dahliae*. *Phytopathology* 77:875-881.
17. Noe, J. P., and Barker, K. R. 1985. Overestimation of yield loss of tobacco caused by the aggregated spatial pattern of *Meloidogyne incognita*. *J. Nematol.* 17:245-251.
18. Powelson, M. L., and Rowe, R. C. 1993. Biology and management of early dying of potatoes. *Annu. Rev. Phytopathol.* 31:111-126.
19. 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.
20. 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.
21. Saeed, I. A. M., MacGuidwin, A. E., and Rouse, D. I. 1998. Effect of initial nematode population density on the interaction of *Pratylenchus penetrans* and *Verticillium dahliae* on 'Russet Burbank' potato. *J. Nematol.* 30:100-107.
22. Schreiber, L. R., and Green, R. J. 1962. Comparative survival of mycelium, conidia, and microsclerotia of *Verticillium albo-atrum* in mineral soil. *Phytopathology* 52:288-289.
23. Seinhorst, J. W. 1973. The relation between nematode distribution in a field and loss in yield at different average nematode densities. *Nematologica* 19:421-427.
24. Singleton, L. L., Mihail, J. D., and Rush, C. M., eds. 1993. *Methods for Research on Soil-borne Phytopathogenic Fungi*. American Phytopathological Society, St. Paul, MN.
25. Smith, V. L., and Rowe, R. C. 1984. Characteristics and distribution of propagules of *Verticillium dahliae* in Ohio potato field soils and assessment of two assay methods. *Phytopathology* 74:553-556.
26. Taylor, L. R. 1961. Aggregation, variance and the mean. *Nature* 189:732-735.
27. Thistlethwaite, B. 1970. Reproduction of *Pratylenchus penetrans* (Nematoda: Tylenchida). *J. Nematol.* 2:101-105.
28. Wheeler, T. A., Madden, L. V., Riedel, R. M., and Rowe, R. C. 1994. Distribution and yield-loss relations of *Verticillium dahliae*, *Pratylenchus penetrans*, *P. scribneri*, *P. crenatus*, and *Meloidogyne hapla* in commercial potato fields. *Phytopathology* 84:843-852.
29. 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.
30. 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.