Sampling Procedures for Determining Severity of Alfalfa Leaf Spot Diseases

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ABSTRACT

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Alfalfa fields in three North Carolina counties were sampled during spring and summer, 1982, to estimate leaf spot severity. Three stems per quadrat were selected from 1- × 1-m quadrats arranged in grids of 64 or 256. Two lower and two upper leaves were chosen randomly from each stem and percent diseased leaf area was estimated for each leaf. *Leptosphaerulina briosiana* was the most commonly isolated leaf-spotting organism. Variance components were estimated to partition total variation into variation among leaves within a stem, stems within a quadrat, and quadrats within a field. Variance components were used to determine optimum sampling rates based on linear cost functions. Three to four leaves per stem half was optimum for most samples. Spatial autocorrelations were estimated for quadrat mean disease severity and these were used to estimate optimum quadrat size and shape based on a cost matrix. Autocorrelations among neighboring quadrats were generally low, indicating that several quadrats should be grouped into rectangular-shaped sampling units.

Leaf spot diseases are common throughout the alfalfa (*Medicago sativa* L.) growing regions of the world (4). Leaf-spotting pathogens on alfalfa include *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell, *Pseudopeziza medicaginis* (Lib.) Sacc., and *Stemphylium botryosum* Wallr., which mainly attack the leaves, and *Cercospora medicaginis* Ell. & Ev., and *Phoma medicaginis* Boerema, which attack the leaves and stem.

The effects of foliar diseases on alfalfa yield and quality can be significant. Yield increases of 16 and 18% have been attained for the first harvest with chemical control of foliar diseases (16,17). Yield increases were attributed mainly to a reduction in defoliation caused by Pseudopeziza leaf spot. Results from growth chamber and field experiments indicated that Pseudopeziza leaf spot can limit dry matter production by more than 40% (13). Increased production of compounds potentially toxic to livestock has also been associated with foliar disease on alfalfa (2,5,7).

Efficient and reliable sampling methods are needed to survey the extent and severity of leaf spot diseases and to study the effect of leaf spots on alfalfa yield. Sampling procedures for alfalfa leaf spot have often employed measures of disease incidence rather than disease severity (1,10); however, results from such studies may not apply when severity is used as a measure of disease. This is especially true when leaf spots are present on most plants in the field as was often the case in fields we have observed.

The objective of the present research was to determine the important sources of variation and optimal allocation of resources when sampling for alfalfa leaf spot severity. Two methods were used in the analysis. Variance component estimates were used to optimize sampling rates for leaves within a stem and stems within a 1- × 1-m quadrat. Spatial correlation analysis was used for optimizing the number of quadrats in a sampling unit.

MATERIALS AND METHODS

Collection of samples. Two alfalfa fields in each of Rowan and Wake counties and one field in Forsyth County, NC, were sampled during 1982. Sampling dates and the size of grid sampled varied for each location (Table 1). Samples in March through May were collected on 16× 16 grids of contiguous 1-m-square quadrats. An 8 × 8 grid was used in subsequent samples because of time considerations. Quadrats were identified with x and y coordinates where the y-direction corresponded to the direction of harvesting. Three stems were selected from each quadrat by reaching to the base of the canopy at an arbitrary location within a quadrat and removing the first stem encountered. Samples were placed in plastic bags on ice and stored at 4 C until rated. The same sampling procedure was used for both the 8 × 8 (Fig. 1) and 16 × 16 grids. Estimates of stem density within sampled grids were obtained starting on 15 April, by counting stems in a 30-cm square located in

TABLE 1. Location, date, grid size, and host characteristics of contiguous quadrat samples selected to determine alfalfa leaf spot severity at fields in four North Carolina counties during 1982

County	Field	Plot	Date	Grid[a] size	Cultivar	Growth[b] stage	Plant[c] density
Wake	1	A	3/22	16	Arc	12	–
			4/02	16	Arc	13	–
			4/22	16	Arc	12	319
			5/28	16	Arc	13	586
			6/28	8	Arc	12	333
			7/15	8	Arc	23	300
			8/02	8	Arc	12	274
			8/10	8	Arc	13	214
			8/27	8	Arc	32-33	175
		B	3/26	16	Arc	12	–
			4/15	16	Arc	13	308
			6/03	8	Arc	21	536
	2	A	8/27	8	Arc	31-32	456
Rowan	1	A	4/16	16	Cimmaron	12	1,214
		B	5/25	16	Cimmaron	13	1,164
Rowan	2	A	4/16	16	Cimmaron	12	567
		B	5/25	16	Cimmaron	13	689
Forsyth	1	A	7/22	8	Classic	13	281
		B	7/22	8	Classic	13	453

[a] Number of quadrats along one side of a square grid of contiguous quadrats.
[b] Growth stage using criteria of James (1971).
[c] Estimated number of stems per square meter within grid. Dash indicates that density was not estimated.

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each of four quadrants of the grid. Table 1 summarizes host characteristics for the 19 samples.

Rating plant material for disease. Preliminary samples collected in 1981 indicated that use of percent diseased leaflets per stem did not give an adequate estimate of disease severity because most leaves contained some lesions and diseased leaflets varied greatly in severity. Simply designating each leaflet as diseased or not diseased gave too much emphasis to the many leaves that contained only a few lesions. Therefore, the 1982 samples were evaluated by visually estimating percent diseased leaf area (PDLA) on selected leaves. Sampled stems were divided into a lower and upper half and two leaves were chosen randomly without replacement from each stem half (four leaves were selected per half in samples collected 27 August 1982). The selected leaves were compared with a visual rating key (Fig. 2) to estimate PDLA. The key followed a logarithmic scale similar to the Horsfall–Barratt scale (8) and was based on drawings by James (9). For all analyses, the original log scale disease values were used in order to stabilize variances, improve normality of error values and reduce interactions.

Isolations. Samples of five or 10 stems were collected on each sampling date for isolation of leaf-spotting pathogens. Infected leaves were placed on moistened filter paper in lids of inverted petri plates containing water agar. Necrotic tissue or ejected spores on the water agar were transferred to modified V-8 juice agar after 1–2 days of sporulation. Plates were incubated at room temperature (23–25 C) for several days, and fungal colonies then identified. Fungi isolated from each sample were recorded to give an estimate of the relative occurrence of each pathogen.

Cost functions. The time required to collect and rate diseased plant material was recorded for several of the samples. The costs at two stages of the sampling procedure were examined: 1) T_s = the time to select a stem within the quadrat, and 2) T_l = time to randomly select, rate, and record the disease level of a single leaf. These estimates were used in linear cost functions to estimate optimum sampling rates (18). A function of the form

$$C_1 = sT_{st} + 2snT_l \quad (1)$$

describes the cost of rating a quadrat with a variable number of leaves per stem and stems per quadrat. C_1 represents the cost of rating a quadrat with s stems and n leaves per stem half. A second cost function

$$C_2 = qC_1 \quad (2)$$

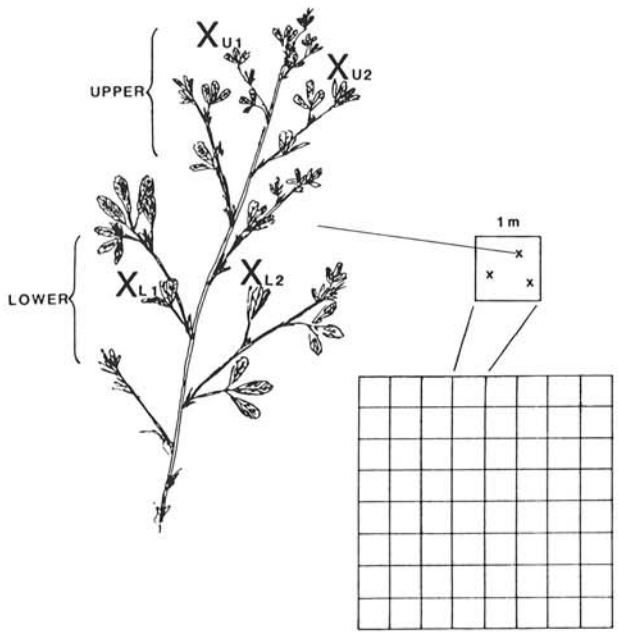

Fig. 1. Selection of samples from an 8 × 8 grid of contiguous quadrats. Three stems were selected from each quadrat (X's).

describes the cost to sample a cluster of quadrats, each containing s stems with n leaves rated per stem half.

Estimation of variance components. The model

$$Y_{ijk} = u + Q_i + S_{(i)j} + L_k + e_{(ij)k} \quad (3)$$

describes the severity of disease on an individual leaf. u is the overall mean, Q_i is the quadrat effect, $S_{(i)j}$ is the effect of an individual stem within a quadrat, L_k is the fixed effect of the two plant halves sampled and $e_{(ij)k}$ is random error due to variation between leaves within a stem half. The interaction terms of L_k with quadrat and stem were generally negligible and were thus pooled with the error term to simplify the model. The expected mean squares for a sample are those for a straight nested design with stems nested within quadrat and leaves (error) nested within stems. Variance components were estimated using the Statistical Analysis System (SAS) procedure VARCOMP (14). All factors are random except level (L), which is fixed. Variance of a quadrat mean can be expressed in terms of the variance components as

$$V(Y_{i...}) = \sigma_Q^2/s + \sigma^2/2sn \quad (4)$$

where s is the number of stems sampled per quadrat, and n is the number of leaves sampled per stem half. Variance of the overall mean would not properly estimate variances expected from random quadrats in a field, because sampled quadrats were contiguous and thus are likely to have correlated disease levels. The relationship among quadrats is considered below using spatial correlation analysis.

The effect of changing stem and leaf sampling rates can be estimated by varying values for s and n in equation 4 (15). Optimum sampling rates were estimated by minimizing $V(Yi...)$ under the constraint of a fixed total cost, C_1 (18). The resulting formulae for optimum sampling rates are

$$n_{opt} = \sqrt{T_s/T_l\,(s/s_{s(Q)})} \quad (5)$$

and

$$S_{opt} = C_1/(T_s + 2n_{opt}\,T_l). \quad (6)$$

The estimate of n_{opt} should generally be adjusted to take account of the finite number of leaves in a level of stem. This modified estimate, n'_{opt} is obtained by multiplying n_{opt} by a finite population correction factor, $(N-n_{opt})/N$, which reduces the estimate of n_{opt}. It can be seen from equation 5 that a high cost of selecting a stem (T_s) and low cost of selecting and rating individual leaves on a stem (T_l) cause n_{opt} to increase. s_{opt} is determined by n_{opt}, and by the amount of time available to sample the quadrat, C_1.

Gain due to stratification. The reduction in variance due to stratification of stems into two halves can be estimated by comparing the variance in the stratified sample with an estimate of

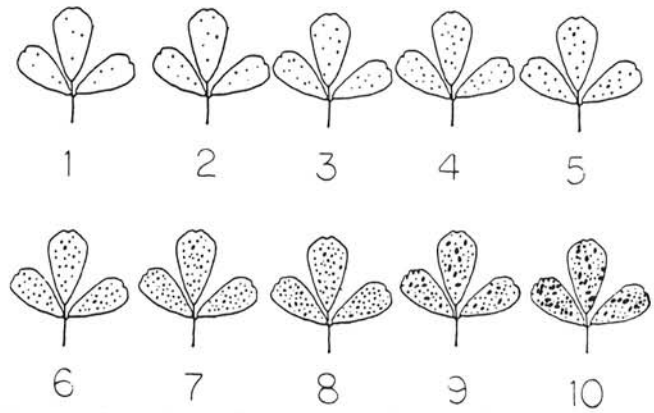

Fig. 2. Rating scale used for estimation of percent diseased leaf area on alfalfa (1 = 1.0%, 2 = 1.5%, 3 = 2.2%, 4 = 3.2%, 5 = 4.8%, 6 = 7.1%, 7 = 10.5%, 8 = 15.5%, 9 = 22.9%, 10 = 33.8%).

what the variance would be if a simple random sample had been used (3). The gain due to stratification can be expressed as

$$\text{gain} = (V_{ran} - V_{st})/V_{ran} \quad (7)$$

where V_{ran} is the estimate of the variance in a simple random sample, and V_{st} is the variance in the stratified sample. This value will be positive if stratification decreases the variance of the sample, and zero or negative if it does not improve the variance.

Spatial correlation analysis. Mean disease values in each quadrat were used to estimate a matrix of autocorrelations between quadrats at different distances in both the x and y directions (12). $\rho(l,k)$ is the autocorrelation between a quadrat and the quadrat with distance l quadrats in the x-direction and k quadrats in the y-direction. Correlations were estimated based on the following definitions:

$$\rho(l,k) = \text{cov}(l,k)/\text{cov}(0,0) \quad (8)$$

$$\text{cov}^{+}(l,k) = E(Y_{ij}\, Y_{i+l,j+k}) - E(Y_{ij})\, E(Y_{i+l,j+k}) \quad (9)$$

$$\text{cov}^{-}(l,k) = E(Y_{i,j-k}\, Y_{i+l,j}) - E(Y_{i,j-k})\, E(Y_{i+l,j}) \quad (10)$$

where E indicates expected value (14). Correlations were calculated for distances up to $m/2+1$, where m is the dimension of the grid sampled (either 8 or 16 for our samples). A matrix of mean square errors (MSE) was calculated for estimating variance of sampling units with dimensions ranging from 1×1 to $(m/2+1) \times (m/2+1)$ quadrats. MSE is based on the variance of a sum of quadrats,

$$\text{MSE}\,(a \times c) = \text{Var}\left[\sum_{i=l}^{a} \sum_{j=j}^{c} y_{ij}\right], \quad (11)$$

which incorporates the covariances among quadrats within a plot. A standardized MSE matrix (STMSE) was then calculated using elementwise division of the MSE matrix by the variance among unit plots, Cov(0,0).

A cost matrix, K, was estimated based on the cost function for C_2 and containing costs for plots of different quadrat sizes. Cost per unit information (C/I) was then computed as

$$\text{C/I} = (K\#/\text{MSE})\,\#/N \quad (12)$$

where # indicates elementwise division or multiplication and N is a matrix containing the number of quadrats per plot corresponding to each value in the cost (C) and MSE matrices.

RESULTS

Isolations. *L. briosiana* was isolated most frequently in each of the samples (Table 2). *P. medicaginis* and *S. botryosum* also were isolated from many of the samples. *P. medicaginis* was most common in the 2 August sample from Wake County. In some cases, more than one fungus was isolated from leaves on a single stem. *Pseudopeziza* sp. was not isolated from any of the samples and Cercospora leaf spot, although present in a few samples, was not evaluated.

Variance component estimates. Estimated variance components are presented in Table 3. The error variance, which represents variation among leaves within a stem half, was the greatest source of variation in all samples. This component accounted for 63–89% of the variation after removal of the fixed effect, level (L). Stems accounted for up to 26% of the variation. PDLA in these samples ranged from 2.1% for the 26 March sample in Wake County to 11.9% for the 25 May sample from Rowan County, field 1.

Gain due to stratification. Table 3 also contains the estimated gain due to stratification. This value ranged from near zero for the 22 July samples in Forsyth County to 0.17 for the 27 August sample in Wake County, field 1. Although the gain was always positive, it was often quite small. The gain did not increase with disease level and was not large enough to recommend stratification of stems as a general practice.

Optimum sampling rates using variance components. Sampling costs were estimated as: 1) time to select a single stem, $T_q = 1/3$ minutes, and 2) time to select and rate a single leaf within a stem, $T_s = 1/4$ minutes. Optimum values for the number of leaves per stem section, n_{opt}, were computed for each sample, based on equation 5 and are given in Table 3. Values for n'_{opt} are also given for $N = 20$, which is a reasonable estimate of the number of leaves in a level of stem for a young plant. The two estimates, n and n', will be similar for larger, more mature plants because these plants have a large numbers of leaves, which results in a correction factor close to 1. The average values for n_{opt} and n'_{opt} over all samples were approximately four and three leaves per stem level respectively. The optimum number of stems per quadrat, s_{opt} depends on the value of n and on the total allotted cost per quadrat, C_1. It is computed from equation 6 above.

Spatial correlation analysis. Results of the spatial correlation analysis are summarized in Table 4. Autocorrelations for lags (1,0),

TABLE 2. Frequency of isolation of three leaf spot fungi

County	Field	Plot	Date	Grid[a] size	Stems sampled	Times isolated (no.) *Leptosphaerulina briosiana*	*Phoma medicaginis*	*Stemphylium botryosum*
Wake	1	A	3/22	16	10	9	1	2
			4/02	16	10	8	2	3
			4/22	16	10	8	0	2
			5/28	16	10	10	0	2
			6/28	8	5	5	1	1
			7/15	8	5	5	0	0
			8/02	8	5	5	3	1
			8/10	8	5	5	0	1
			8/27	8	5	5	0	2
	1	B	3/26	16	10	10	0	1
			4/15	16	10	10	1	0
			6/03	8	5	5	1	1
Wake	2	A	8/27	8	5	5	0	1
Rowan	1	A	4/16	16	10	10	1	0
		B	5/25	16	10	8	0	2
	2	A	4/16	16	10	10	2	2
		B	5/25	16	10	7	0	2
			7/22	8	5	5	0	2
			7/22	8	5	5	1	1

[a] Number of quadrats along one side of a square grid of contiguous quadrats.

(0,1), and (1,1) are given, where the first number of a pair is the number of quadrats away in the x-direction, and the second number is the distance in the y-direction. Correlations generally dropped off with distance and in some cases became negative. Lag 1 correlations were generally positive but were never greater than 0.5, and were usually less than 0.2. In most cases correlations were not significant ($P = .05$).

The correlations were used to estimate the relative cost per unit information for plots of different size and shape, also given in Table 4. The costs were estimated based on the cost function for C_2 (Eq. 2). A sampling rate of three leaves per level of stem was used because this was the average value of n'_{opt} given in Table 4. Allotting approximately 5 min per quadrat gives s equal to three stems, which was also used in calculating the cost matrices. Table 4 gives the relative cost per unit information for plots of 1×1, 4×1, 2×2, 1×4, 9×1, 3×3, 1×9, and $a \times a$ quadrats, where a is the maximum plot dimension for which this value was calculated, and is equal to 9 for 16×16 grids and 5 for 8×8 grids. The values for 9×1 and 1×9 plots were only calculated for 16×16 grids. These plot sizes allow comparison of different shapes for plots of four and eight to nine quadrats and also indicate C/I values for the largest and smallest plots calculated. In general, larger plots were more efficient than plots of just a few quadrats.

DISCUSSION

This study attempted to take a statistical approach to sampling for severity of alfalfa leaf spot diseases based on several factors. The relative variation among plants and among leaves within a plant was investigated to determine optimum sampling rates. Spatial pattern was also considered in terms of the spatial correlation structure within an area of a field. The major constraint in most sample surveys, time, was taken into account in determining optimum sampling methods.

The study allowed us to establish some general guidelines for setting up a sampling procedure for leaf spots and to determine where further research would be helpful. This is all that one can hope for when sampling populations are as inherently variable as agricultural systems can be. The fact that populations of plants in agricultural systems are variable should not be viewed as a hinderance in research toward understanding these systems, but should be taken into account when performing research in such systems.

Several generalizations can be derived from our study. First, most of the variability in our samples was among the leaves within the individual stems. Sampling rates of three to four leaves per stem half were optimum for determining disease severity, based on the stated cost functions. Modification of the costs for the different stages in the sampling process is easily done using the given formulae and variance components. Second, there was a consistent improvement in sampling variances for stratified versus random sampling within stems; however, this effect was not as large as we had expected based on our observation of stems. It adds some time to the sampling process, but it leads to only a slight improvement in sampling variance. Thus, the importance of this is questionable. If stratification was not used, a simple random sample of six to eight leaves per stem would appear generally to give only slightly larger variances. Third, spatial correlations were present among quadrats, but were generally low. This suggests the need to use larger groupings of quadrats. For several samples, the most efficient plot size (i.e., lowest C/I) was the largest plot ($a \times a$) for which C/I was calculated, whereas plots of one unit usually had the highest cost. In a few cases, such as the samples on 3/22, 4/16 (field 2), and 5/25 (field 1), large plots performed significantly worse than smaller plots. These samples generally had higher correlations. Plots of eight to nine units generally performed slightly better than plots of four units, but both sizes were generally lower and much more consistent in cost per unit information than either the 1×1 or the $a \times a$ plots. Shape and orientation of plots had little effect on efficiency. In general, one would expect rectangular plots to perform best when correlations among plots are high because this type of plot has a greater average distance between quadrats. Most of our samples showed fairly low correlations, and efficiency was not greatly affected by shape. Intermediate-sized plots (e.g., eight quadrats) would represent a reasonable compromise and would be less affected by situations where correlations were greater.

The present study has provided some broad guidelines for use in surveys of alfalfa leaf spot severity, but several aspects need further study. Our use of contiguous quadrats provided information on the importance of plot size, but at the same time, did not give an indication of variation on a larger scale within the field. More

TABLE 3. Estimated variance components for alfalfa leaf spot samples on 8×8 and 16×16 grids of 1-m-square quadrats in four North Carolina counties during 1982

							Variance components[b]							Optimum # of leaves per stem[d]	
										% of total			Gain[c] due to		
County	Field	Plot	Date	PDLA[a]	sd	cv	Q	S	Error	Q	S	E	stratification	n	n′
Wake	1	A	3/22	2.2	1.6	69	0.37	0.42	2.45	11.4	13.0	75.6	0.05	5.1	4.0
			4/02	2.4	1.5	60	0.04	0.36	2.16	1.6	14.0	84.4	0.13	5.2	4.1
			4/22	2.9	1.5	57	0.17	0.87	2.26	5.1	26.3	68.6	0.16	2.2	2.0
			5/28	5.3	1.4	31	0.22	0.73	1.89	7.9	25.7	66.5	0.10	2.2	2.0
			6/28	7.5	2.4	54	0.23	0.74	5.81	3.4	10.9	85.7	0.00	6.8	5.1
			7/15	4.5	1.5	39	0.43	0.72	2.18	12.9	21.7	65.4	0.15	2.6	2.3
			8/02	2.4	1.5	61	0.17	0.42	2.25	6.2	14.6	79.2	0.07	4.6	3.8
			8/10	2.8	1.9	77	0.27	0.84	3.55	5.8	18.0	76.2	0.09	3.7	3.1
			8/27	6.4	1.8	38	0.12	0.83	3.09	3.0	20.5	76.5	0.17	3.2	2.8
		B	3/26	2.1	1.4	60	0.20	0.34	1.88	8.1	14.2	77.7	0.04	4.8	3.9
			4/15	2.8	1.4	54	0.12	0.69	2.10	4.1	23.7	72.2	0.14	2.6	2.3
			6/03	9.3	1.8	32	0.12	1.17	3.29	2.6	25.6	71.9	0.10	2.4	2.2
	2	A	8/27	6.6	1.6	33	0.53	0.56	2.60	14.2	15.3	70.5	0.10	4.0	3.3
Rowan	1	A	4/16	2.2	1.2	52	0.21	0.57	1.50	9.1	24.9	66.0	0.16	2.3	2.0
		B	5/25	6.3	1.4	30	0.59	0.68	2.08	17.6	20.4	62.0	0.09	2.6	2.3
	2	A	4/16	2.9	1.8	70	0.76	0.41	3.19	17.4	9.5	73.1	0.09	6.7	5.0
		B	5/25	11.9	1.3	19	0.33	0.66	1.66	12.5	25.0	62.5	0.06	2.2	2.0
Forsyth	1	A	7/22	4.9	1.8	45	0.32	0.58	3.34	7.5	13.7	78.8	0.01	5.0	4.0
		B	7/22	5.5	2.2	53	0.02	0.59	4.71	0.4	11.1	88.5	0.05	6.9	5.1
													Average	4.0	3.2

[a] Percent diseased leaf area (PDLA).
[b] Variance components due to Quadrat (Q), stems within a quadrat (S), and leaves within a stem (Error).
[c] Gain = (V random − V stratified)/V random.
[d] n′ = optimum number of leaves per stem level adjusted for finite population.

TABLE 4. Lag I correlations and cost/unit information for selected plot sizes and shapes from samples to determine alfalfa leaf spot severity in fields in four North Carolina counties during 1982

County	Field	Plot	Date	Correlations[a] c00 c01 c10 c11	Cost per unit information 1×1	4×1	2×2	1×4	4×2	3×3	2×4	a×a
Wake	1	A	3/22	1.00 .18* .20* .23*	12	9	10	9	12	12	11	44
			4/02	1.00 .07 0.9 .00	6	4	4	3	3	3	3	2
			4/22	1.0 .05 −.01 −.04	12	7	6	6	6	5	5	5
			5/28	1.00 .07 .12 .08	11	9	7	7	9	8	6	6
			6/28	1.00 −.19 .15 −.04	16	7	8	8	6	5	6	2
			7/15	1.00 .04 .13 .01	15	9	9	6	8	7	6	4
			8/02	1.00 .16 .17 −.09	9	8	6	6	7	7	6	9
			8/10	1.00 .06 .18 .18	13	8	9	6	9	9	7	6
			8/27	1.00 −.06 .31* .17	10	4	8	8	5	7	8	7
		B	3/26	1.00 .33* .22* .14*	8	8	7	5	9	8	6	6
			4/15	1.00 .09 .05 .03	10	6	6	5	5	5	5	8
			6/03	1.00 −.09 −.07 −.09	14	6	5	7	4	3	5	4
	2	A	8/27	1.00 .14 .10 .04	14	11	9	8	10	9	9	14
Rowan	1	A	4/16	1.00 .04 .04 −.06	10	6	5	6	5	4	4	5
		B	5/25	1.00 .10 .12 .02	16	10	11	11	11	11	11	25
	2	A	4/16	1.00 .50* .45* .43*	19	25	24	19	36	35	28	56
		B	5/25	1.00 −.03 −.04 −.06	15	8	7	7	6	5	5	2
Forsyth	1	A	7/22	1.00 .15 .17 .08	15	9	11	10	11	12	10	16
		B	7/22	1.00 .24 −.08 −.10	11	8	6	6	6	6	6	7

[a] Estimated lag (0,0), lag (1,0), lag (0,1), and lag (1,1) correlations–designated c00, c10, c01, c11. * = correlation coefficient significant at $P = 0.05$.
[b] Relative cost per unit information (time/standard deviation) for plots of different size and shape based on cost function with $Tq = 10$ and $Cl = 5$. The $a \times a$ plot size refers to a 5×5 plot for samples from 8×8 grids (3/22 to 5/28) and a 9×9 plot for sample from 16×16 grids (6/3 to 8/17).

information is needed on this aspect, and this could be obtained using the recommended procedures from the present study. Although we have addressed the subject of cost functions, we have not looked at and tested these in detail. These values should be estimated and verified for each sampling situation because they may differ widely from one survey to another.

Finally, methods of reducing the error variance, due to variation from leaf to leaf, should be investigated further. This type of variability is avoided in many disease surveys by rating entire plants. Although this approach reduces the sampling variation within a plant to zero, it introduces an estimation error of unknown magnitude and unknown distribution (6). The methods suggested in this should allow easy adaptation of newer technologies such as computerized video image analysis (11).

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