Ecology and Epidemiology

Efficiency of Multistage Sampling for Estimation of Intensity of Leaf Spot Diseases of Alfalfa in Field Experiments

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This research supported by CSRS Southern Region IPM Grant 88-34103-3251 and funds provided by the North Carolina Agricultural Research Service.

We thank Charles R. Harper for his assistance.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

Accepted for publication 5 March 1991 (submitted for electronic processing).

ABSTRACT

Duthie, J. A., Campbell, C. L., and Nelson, L. A. 1991. Efficiency of multistage sampling for estimation of intensity of leaf spot diseases of alfalfa in field experiments. Phytopathology 81:959-964.

In each of two field experiments, efficiency of multistage sampling was evaluated for estimation of intensity of leaf spot diseases during 10 periods of alfalfa growth over 2 yr. Disease was assessed visually on sections of the alfalfa canopy with the aid of descriptive rating scales. In experiment A, severity of disease was assessed every 7 days on four canopy sections (0.5 × 0.5 m) per replicate plot (3 × 3 m) with 12 plots per treatment. In experiment B, incidence of diseased leaves and severity of disease on diseased leaves were assessed every 2–4 days on four canopy sections (1.0 × 0.17 m) per subplot (1.0 × 0.68 m) with two subplots per replicate plot (10.0 × 4.1 m) and five plots per treatment. In each experiment, efficiency of sampling was evaluated based on estimates of costs of sampling per unit, measured in units of time, and on estimates of components of error variance. Efficiency of sampling could not be improved by changing the rate of sampling during periods of alfalfa growth because none of the variance components changed consistently over time. When the total time allocated to sampling was constrained to C min per treatment, severity of disease was estimated most efficiently in experiment A by sampling four canopy sections in each of $0.417C$ plots. In experiment B, incidence of diseased leaves was estimated most efficiently with two canopy sections, two subplots, and $0.323C$ plots, and severity of disease on diseased leaves was estimated most efficiently with three canopy sections, two subplots, and $0.270C$ plots.

In field experiments designed to compare effects of treatments on intensity of plant diseases, efficient sampling procedures are needed because resources available for sampling usually are limited. Efficiency of sampling may be measured as the ratio of precision and cost, where precision is the inverse of the error variance of a treatment mean and cost is the total cost of sampling per treatment. Multistage or nested sampling may improve efficiency (5,7). For example, intensity of disease may be measured on subsamples of plant material selected from within replicate plots. The variance of a treatment mean then depends on the number of subsamples per plot and plots per treatment, and on the inherent variation among subsamples and among plots. Similarly, the total cost of sampling per treatment depends on the number of subsamples and plots, the cost per subsample, and the cost per plot. If total cost is fixed at some upper limit, many combinations of numbers of subsamples per plot and plots per treatment may be selected. However, with the optimal combination, variance is minimized and, therefore, efficiency is maximized.

To compare intensity of leaf spot diseases of alfalfa among treatments in field experiments, a number of workers (6,9,11,17, 23) have used a multistage sampling scheme in which disease was measured on two or more sections of the alfalfa (*Medicago sativa* L.) canopy marked within each replicate plot. A canopy section consisted of the leaves and stems within a defined horizontal area. A disease score, which represented the average intensity of disease on all leaves taken together, was assigned to each canopy section with the aid of a descriptive rating scale. With this approach, the cost of measuring disease on individual leaves was avoided and a large number of leaves were examined quickly.

Efficiency of multistage sampling has not been evaluated critically for field experiments in which the procedures described above are used. The contribution to total error variance of variance among canopy sections within plots and variance among plots within treatments is unknown. In addition, because alfalfa is harvested repeatedly during each growing season, leaf spot epidemics occur during periods of rapid host growth (23). During each growth period, magnitudes of components of variance may tend to change. Clustering of disease may increase with an increase in the density of leaves because of effects of leaf density on spore dispersal and/or environmental heterogeneity. Such clustering then may be reflected in an increase in variation among sampling units. Measurement errors also may increase because mean intensity is measured on an increasing number of leaves in each canopy section. As a result, optimal rates of sampling will change during growth periods. However, information on changes in components of variation over time is lacking.

The objective of this study was to evaluate efficiency of sampling in field experiments in which intensity of leaf spot diseases was measured by assigning disease scores to subsamples of the alfalfa canopy within replicate plots. Optimal rates of sampling, estimated assuming a constant rate of sampling during periods of alfalfa growth, were determined for two experimental protocols. In one protocol, severity of disease was measured using two-stage sampling in plots arranged in a completely randomized design. In a second strategy, incidence of diseased leaves and severity of disease on diseased leaves were measured using three-stage sampling in plots arranged in a randomized complete block design. Changes in magnitudes of components of error variance over time were assessed to determine whether efficiency could be improved by varying the rate of sampling during periods of alfalfa growth.

MATERIALS AND METHODS

Sampling procedures were designed to provide information on the efficiency of multistage sampling in two experiments conducted near Raleigh, NC. Experiments have been described previously (6,9), but relevant details are presented here. In experiment A, effects of leaf spot diseases on alfalfa yield and quality were investigated (6). In experiment B (9), relationships among plant debris, growth of alfalfa, and intensity of leaf spot diseases were evaluated. Except for treatments, alfalfa was cultivated using standard practices. Alfalfa was harvested when plants reached the early (10–25%) bloom stage. Five harvests, numbered I to V consecutively, were made in each growing season. Periods of alfalfa growth preceding harvests I to V were designated periods I to V, respectively; period VI followed harvest V. Leaf spots were caused by species of *Leptosphaerulina, Phoma, Stemphylium,* and/or *Cercospora* (6,9). Formulae for calculating optimal numbers of sampling units were derived following the method of Marcuse (16), which also is described by Sokal and Rohlf (20) and Steel and Torrie (21), without correction for the finite sizes of populations of sampling units.

Experiment A. Experiment A (6) was conducted during 1986 and 1987 in a 0.5-ha field of alfalfa cv. Arc. The field was broadcast-planted in September 1984, and managed as a production field in 1984. Twenty-four plots (3 × 3 m), separated by a minimum of 6 m, were established in a 8 × 3 rectangular grid within the field. Treatments consisted of 1) an untreated control and 2) a weekly application (0.13 g a.i./m^2) of chlorothalonil (Bravo 500, Fermenta ACS Corp., Mentor, OH) during each period of alfalfa growth. Experimental plots were arranged in a completely randomized design with 12 plots per treatment.

A two-stage sampling design was used. Four contiguous sections of the alfalfa canopy (0.5 × 0.5 m) were sampled in a 1 × 1 m area at the center of each plot. The severity of disease, i.e., the average percentage of the area of leaves that was covered by leaf spots, was estimated in each canopy section with the aid of the Horsfall-Barratt scale (12). In practice, Horsfall-Barratt scores in the range 0 (0%) to 4 (12–25%) were used. Plots were sampled once each week during growth periods I to V in 1986 and 1987. In each period, sampling was begun when diseased leaves appeared. On each date, sampling was begun at approximately 9:00 a.m. There were 23 sampling dates in 1986 and 25 in 1987.

The severity of disease in a canopy section (Y_{ijk}) on each sampling date was described by

$$Y_{ijk} = \mu + T_i + P_{j(i)} + R_{k(ij)} \quad (1)$$

where μ was the overall mean, T_i was the effect of the ith treatment ($i = 1,2, \ldots t$), $P_{j(i)}$ was the effect of the jth replicate plot ($j = 1,2, \ldots p$) within the ith treatment, and $R_{k(ij)}$ was the effect of the kth section of the canopy ($k = 1,2, \ldots r$) within the jth plot within the ith treatment. The number of treatments, the number of plots per treatment, and the number of canopy sections per plot were t, p, and r, respectively. All effects were assumed to be random with the exception of μ and T_i. Variation among plots (v_p) and variation among canopy sections within plots (v_r) were estimated by analysis of variance (Table 1). From the expectations of the mean squares

$$v_p = (MSP - MSR)/r \quad (2)$$

$$v_r = MSR \quad (3)$$

where MSP and MSR were mean squares due to plots and canopy sections within plots, respectively.

Costs of sampling were assumed to be equivalent to the time required to sample and to be constant over sampling dates. Other costs, such as the cost of travel to research plots, fertilizer and herbicide applications, land, planting, and crop maintenance, were disregarded. On each date, the cost per plot (c_p) was the additional time required if one replicate plot was added to the experiment, omitting the time to sample canopy sections within plots. The cost per canopy section (c_r) was the additional time required if one additional canopy section per plot was sampled. Thus, the time required to walk between plots and the time required to measure and record disease severity in each plot were c_p and c_r, respectively. Values of c_p, c_r, p, and r were assumed to be independent. The optimal number of canopy sections per plot (r^*) was calculated by

$$r^* = [(v_r/v_p)(c_p/c_r)]^{1/2} \quad (4)$$

and optimal number of replicate plots per treatment (p^*) was calculated by

$$p^* = [1/(c_p + r^* c_r)]C \quad (5)$$

where C was the preassigned upper limit of total cost and r^* was rounded to the nearest integer. Given a combination of p and r, the expected overall variance of a treatment mean (V) was calculated by

$$V = v_p/p + v_r/pr, \quad (6)$$

TABLE 1. Analysis of variance for severity of alfalfa leaf spots in experiment A for estimating variance among plots (v_p) and variance among canopy sections (v_r)

Source	df[a]	Mean square[b]	Expected mean square[c]
Treatments	$t - 1 = 1$	MST	$v_r + 4v_p + 48O_t$
Plots	$(p - 1)t = 22$	MSP	$v_r + 4v_p$
Canopy sections	$(r - 1)pt = 72$	MSR	v_r
Total	$rpt - 1 = 95$		

[a] The number of treatments (t), replicate plots per treatment (p), and canopy sections per plot (r) were 2, 12, and 4, respectively.

[b] MSP and MSR were estimated by analysis of variance. Equations 2 and 3 then were used to calculate v_p and v_r in the expected mean squares.

[c] Effect of treatments was assumed to be fixed. All other effects were assumed to be random. O_t was the variance due to treatments.

and an approximate value of the least significant difference (14) between two means, assuming that the value of Student's t statistic at $P = 0.05$ was 2.0, was calculated by

$$\text{LSD} = 2(2V)^{1/2}. \quad (7)$$

Experiment B. Experiment B was conducted during 1987 and 1988 (9). Five treatments were assigned randomly to plots (10.0 × 4.1 m) arranged in five complete blocks (50 × 4.1 m). Blocks consisted of 24 rows of alfalfa cv. Raidor spaced 0.17 m apart. Treatments included an untreated control, removal of infested alfalfa debris from the surface of soil, addition of infested debris to the surface of soil, weekly applications of chlorothalonil during the entire growth period, and weekly applications of chlorothalonil for the first 2 wk of the growth period. Chlorothalonil (1.0 g a.i./L water) was applied until runoff using a backpack sprayer.

A three-stage sampling design was used in experiment B because plots were larger than in experiment A. An area 1.36 m (eight rows) wide at the center of each plot was divided into subplots. In 1987, the area was 4 m long and was divided into eight subplots. In 1988, the area was 6 m long and was divided into 12 subplots. In both years, a subplot measured 0.68 m (four rows) × 1 m and consisted of four canopy sections. Each canopy section consisted of a 1-m row of plants.

Plots were sampled, usually at 2- to 4-day intervals, during growth periods II to VI in 1987 and periods I to V in 1988. In each period, sampling began when new leaves appeared. There was a total of 37 sampling dates during 1987 and 44 during 1988. On each date, sampling began at approximately 9:00 a.m. Incidence of diseased leaves and severity of disease on diseased leaves were measured on each canopy section in each of two subplots selected randomly from each plot. Disease incidence was the percentage of leaves with symptoms of leaf spot. Incidence scores of 0 to 10 corresponded to percentages of diseased leaves of 0, 1, 5, 10, 25, 50, 75, 90, 95, 99, and 100. Severity of disease on diseased leaves was the average percentage of the area of diseased leaves in a canopy section that was covered by spots. Severity scores of 0 to 7 corresponded to percentages of 0, 2, 5, 10, 25, 35, 50, and 70. The diagrams of Broscious et al (4) were used as a rating aid. In practice, incidence scores in the range 0 to 9 and severity scores in the range 0 to 5 were used.

For incidence of diseased leaves or severity of disease on diseased leaves, an observation (Y_{ijkl}) was described by

$$Y_{ijkl} = \mu + B_i + T_j + P_{ij} + S_{k(ij)} + R_{l(kij)} \quad (8)$$

where μ was the overall mean, B_i was the effect of the ith replicate block ($i = 1,2, \ldots p$), T_j was the effect of the jth treatment ($j = 1,2, \ldots t$), P_{ij} was the effect of the plot in the ith block treated with the jth treatment, $S_{k(ij)}$ was the effect of the kth subplot ($k = 1,2, \ldots q$) within the plot in the ith block treated with the jth treatment, and $R_{l(kij)}$ was the effect of the lth canopy section ($l = 1,2, \ldots r$) within the kth subplot in the plot in the ith block treated with the jth treatment. The number of treatments, replicate plots per treatment, subplots per plot, and canopy sections per subplot were t, p, q, and r, respectively. All effects in the model were assumed to be random with the exception of μ and T_j. Variation among plots (v_p), variation among subplots (v_q), and variation among canopy sections (v_r) were estimated by analysis of variance (Table 2). From the expectations of the mean squares,

$$v_p = (MSP - MSQ)/qr \quad (9)$$

$$v_q = (MSQ - MSR)/r \quad (10)$$

$$v_r = MSR \quad (11)$$

where MSP, MSQ, and MSR were mean squares due to plots, subplots within plots, and canopy sections within subplots, respectively.

The cost per plot (c_p) was the time to walk from one plot to the next plot within a block plus one-fifth of the time to walk from the last plot in one block to the first plot in the next block. The cost per subplot (c_q) was the time to randomly select and walk to a subplot within a plot. The cost per canopy section (c_r) was the time to measure and record the incidence of diseased leaves and the severity of disease on diseased leaves in each canopy section. Values of c_p, c_q, c_r, p, q, and r were assumed to be independent. The optimal number of canopy sections per subplot (r^*) was calculated by

$$r^* = [(v_r/v_q)(c_q/c_r)]^{1/2}, \quad (12)$$

the optimal number of subplots per plot (q^*) was calculated by

$$q^* = [(v_q/v_p)(c_p/c_q)]^{1/2}, \quad (13)$$

and the optimal number of plots per treatment (p^*) was calculated by

$$p^* = [1/(c_p + q^*c_q + r^*q^*c_r)]C \quad (14)$$

where C was the preassigned upper limit of total cost and q^* and r^* were rounded to the nearest integer. Given a combination of p, q, and r, the expected overall variance of a treatment mean (V) was calculated by

$$V = (v_p/p) + (v_q/pq) + (v_r/pqr), \quad (15)$$

and approximate value of the least significant difference (15) between two means again was calculated by equation 7.

Analysis of data. In both experiments, analyses of variance were conducted using the procedure GLM of the Statistical Analysis System (19). Untransformed disease scores were analyzed throughout. For each analysis, standardized residual errors were plotted against predicted values to evaluate heterogeneity of variances and to detect outliers. To evaluate efficiency based on a constant rate of sampling during a period of alfalfa growth, optimal numbers of sampling units were calculated using mean values of components of error variance, calculated for each sampling date within the period.

To evaluate changes in magnitudes of components of variance, estimates of each component were regressed against the day of the year during each period of alfalfa growth using procedure REG of the Statistical Analysis System (19). The fit of linear and quadratic polynomial models was assessed using tests of significance of regression coefficients and by inspection of coefficients of determination, plots of residuals versus predicted values, and plots of actual values versus predicted values.

TABLE 2. Analysis of variance table for alfalfa leaf spot incidence and disease severity data in experiment B for estimating variance among plots (v_p), variance among subplots (v_q), and variance among canopy sections (v_r)

Source	df[a]	Mean square[b]	Expected mean square[c]
Blocks	$p - 1 = 4$	MSB	$v_r + 4v_q + 8v_p + 32v_b$
Treatments	$t - 1 = 4$	MST	$v_r + 4v_q + 8v_p + 32O_t$
Plots	$(p - 1)(t - 1) = 16$	MSP	$v_r + 4v_q + 8v_p$
Subplots	$(q - 1)pt = 25$	MSQ	$v_r + 4v_q$
Canopy sections	$(r - 1)qpt = 150$	MSR	v_r
Total	$rqpt - 1 = 199$		

[a] The number of treatments (t), replicate plots (blocks) per treatment (p), subplots per plot (q), and canopy sections per subplot (r), were 5, 5, 2, and 4, respectively.

[b] MSP, MSQ, and MSR were estimated by analysis of variance. Equations 9, 10, and 11 then were used to calculate v_p, v_q, and v_r in the expected mean squares.

[c] Effect of treatments was assumed to be fixed. All other effects were assumed to be random. Variance due to treatments and blocks were O_t and v_b, respectively.

RESULTS

In experiment A (Table 3), 4.22 canopy sections per plot were optimal for measuring disease severity. With four canopy sections and with C min per treatment allocated, $0.417C$ replicate plots per treatment were optimal. Thus, given $C = 20$ min per treatment, the optimal combination (number of canopy sections per plot—number of replicate plots per treatment) was 4–8. With this combination, the overall error variance was 0.0381. Differences between two means of at least 0.33, measured in units of disease severity scores, were significant ($P = 0.05$). If the time allocated was increased to 40 min per treatment, the optimal combination was 4–16, overall variance was reduced to 0.019, and the least significant difference (LSD), in units of disease scores, was reduced to 0.23.

For estimates of incidence of diseased leaves in experiment B (Table 4), optimal combinations (numbers of canopy sections per subplot—subplots per plot—replicate plots per treatment) were 2-2-6 or 2-2-12 when 20 or 40 min per treatment, respectively, was allocated for sampling. With these combinations, overall error variances were 0.0381 or 0.0190, respectively, and LSDs, in units of incidence scores, were 0.55 or 0.39, respectively. Severity of disease on diseased leaves was estimated more precisely than incidence of diseased leaves, because variances among plots and subplots were smaller for severity of disease on diseased leaves (Table 4). Variance among canopy sections was similar for the two variables. Optimal combinations for the two variables also were similar. Sampling to estimate severity was slightly more efficient with fewer replicate plots per treatment and with more canopy sections per subplot than for incidence because plot and subplot variances were smaller for severity. Thus, when 20 or 40 min per treatment was allocated for sampling, optimal combinations were 3-2-5 or 3-2-10, respectively, overall error variances were 0.0228 or 0.0114, respectively, and LSDs, in units of severity scores, were 0.43 or 0.30, respectively. With the suboptimal combinations 2-2-6 and 2-2-12, overall variances increased to only 0.0231 or 0.0116, respectively, and, therefore, LSDs were similar.

Magnitudes of components of error variance did not vary consistently over time in either experiment A or experiment B. Therefore, efficiency of sampling could not have been improved by varying the number of sampling units on successive sampling dates during periods of alfalfa growth. For example, in experiment A, estimates of variance among plots and variance among canopy sections in 1986 ranged from −0.01 to 0.43 and from 0.01 to 0.38, respectively (Fig. 1). (Note that the estimate of variance among plots but not the true value was negative if the estimated mean square for canopy sections was greater than the estimated mean square for plots; Table 1.) During growth periods, changes in magnitudes of components of variance did not follow a regular pattern. Variance among plots changed linearly with time only during period III. Variance among canopy sections increased linearly during period I but decreased linearly during period II. Results were similar in 1987 for experiment A and for both years in experiment B. In no case was there evidence of a quadratic effect of time on any component of variance.

TABLE 3. Estimates of costs of sampling, components of error variance, and optimal numbers of replicate plots per treatment and canopy sections per plot for severity of alfalfa leaf spots in experiment A

Sampling unit	Cost of sampling (min)	Variance[a]	Optimal number
Plots	1.6	0.680	$0.417C$[b]
Canopy sections	0.2	0.152	4.22

[a] Values of variance among replicate plots and variance among canopy sections within plots were mean values for 48 sampling dates.
[b] The optimal number of replicate plots per treatment is calculated given a constraint on the total cost of sampling per treatment (C).

TABLE 4. Estimates of costs of sampling, components of error variance, and optimal numbers of replicate plots per treatment, subplots per plots, and canopy sections per subplot for incidence of leaves with alfalfa leaf spots and severity of disease on diseased leaves in experiment B

Sampling unit	Cost of sampling (min)	Incidence		Severity	
		Mean variance[a]	Optimal number	Mean variance	Optimal number
Plots	1.1	0.099	$0.323C$[b]	0.039	$0.270C$[b]
Subplots	0.4	0.110	1.74	0.050	1.86
Canopy sections	0.3	0.298	1.90	0.301	2.85

[a] Values of variance among plots, variance among subplots within plots, and variance among canopy sections within subplots were mean values for 81 sampling dates.
[b] The optimal number of replicate plots per treatment is calculated given a constraint on the total cost of sampling per treatment (C).

DISCUSSION

Guidelines for multistage sampling to compare effects of treatments on intensity of leaf spot diseases of alfalfa have been developed from our analyses. Similar guidelines, based on components of error variance, were established to compare the abundance of three pathogens on potato (2) and severity of Cytospora canker on peach (1), although costs of sampling were not considered explicitly. Both costs and variances were used to evaluate efficiency in survey samples of intensity of foliar diseases (3,22) and density of inoculum in field soil (8,10,18). Estimates of optimal sampling rates from empirical studies of multistage sampling are applicable in future experiments when costs of sampling and/or components of error variance are likely to be similar. Costs and variances in experiment A and experiment B in the present study probably were affected by such factors as the sizes of sampling units, experimental design, and assessment scales used to measure disease. For example, in experiment B, variance among plots was reduced but costs of sampling were increased by arranging plots in blocks and by assessing variance among subplots within each plot. Therefore, results for these two experiments provided estimates for two distinct sets of experimental conditions. In addition, our results illustrate several features of multistage sampling to estimate intensity of alfalfa leaf spot diseases diseases and other similar diseases in field experiments.

In general, precision and the total cost of sampling are directly proportional to the number of primary sampling units, i.e., the largest sampling units from which subsamples are taken. If total cost is constrained, the optimal number of primary units, but not the optimal rate of sampling within a primary unit, is affected. Optimal rates of sampling within a primary unit depend only on the costs and variances associated with these individual units. If the rate of sampling within primary units is optimal, then the optimal number of primary units is simply the maximum number that can be sampled given the total cost. When total cost is doubled, sampling is most efficient when the number of primary units also is doubled but the rate of sampling within primary units is unchanged. Similarly, precision is doubled (i.e., the vari-

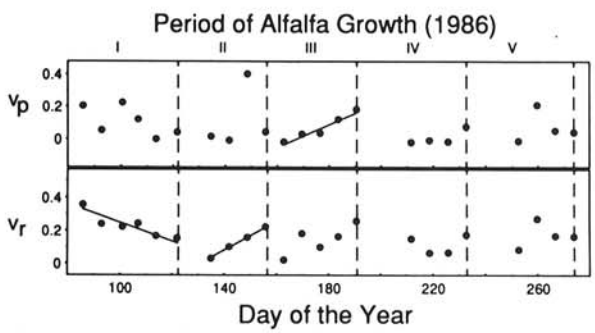

Fig. 1. Estimates of variance among plots (v_p) and variance among canopy sections (v_r) during five periods of alfalfa growth (I to V) in 1986 (experiment A). Best fitting regression lines are shown for those periods during which there was a significant ($P = 0.05$) effect of time (day of the year) on estimates v_p *and* v_r.

ance of a treatment mean is halved) when the number of primary units is doubled.

As a consequence of the relationships among precision, cost, and number of primary sampling units in some epidemiological studies, multistage sampling often is most efficient with a large number of primary units but with as few measurements per primary unit as possible. For example, for estimates of severity of Cytospora canker on peach, variation among branches was very small compared to variation among trees (1). Therefore, Adams et al (1) recommended that a large number of trees should be sampled but that severity be measured on only one branch per tree. Similarly, Campbell and Nelson (8) reported that the cost of collecting samples, which consisted of approximately 150 ml of field soil, was much smaller than the cost of assaying density of sclerotia of *Macrophomina phaseolina* in 10-g subsamples. In addition, variation among subsamples was smaller than variation among samples. Therefore, sampling was most efficient with a large number of samples but with only one subsample per sample. This was not the case with estimates of intensity of leaf spot diseases of alfalfa in our present study. The cost of increasing the number of measurements of leaf spot intensity per plot was small in relation to the cost of increasing the number of plots per treatment. Furthermore, variation among canopy sections, which consisted of measurement error (i.e., observational error) as well as true variation among canopy sections, was the greatest component of experimental error. Therefore, sampling was most efficient when resources were allocated to permit several measurements of leaf spot intensity within each plot.

Analytis and Kranz (3) and Thal and Campbell (22) reported results similar to ours when disease intensity was assessed visually on individual leaves of apple and alfalfa, respectively. In both studies, costs of measuring disease on leaves was small but variation among leaves was large. Consequently, sampling was efficient when disease was measured on several leaves in each primary sampling unit. For example, Analytis and Kranz (3) recommended measuring disease on 13 leaves per branch to estimate severity of Venturia scab in an apple tree. Thal and Campbell (22) recommended measuring disease on three to four leaves per stem to estimate severity of leaf spot diseases in a 1-m^2 quadrat of alfalfa.

The total time available for comparing intensity of leaf spot diseases of alfalfa will vary among experiments. However, based on the costs of sampling in the present study, a relatively large number of replicates should be sampled. For example, when the time available for sampling in experiment A is limited to only 20 min per treatment, which we regard as a minumum, eight replicate plots is optimal. Within a reasonable amount of time, say 25 min per treatment, 10 replicates could be sampled. Twenty replicates could be sampled if 1 h per treatment was allocated. However, our analysis accounts for costs due to time needed to sample but not for other costs. Costs of replication due to such factors as the cost of additional land also must be considered, particularly when plots are large.

Further work is needed to evaluate efficiency of sampling when other foliar diseases are assessed visually on sections of canopy in replicated field plots. However, our results and the results of Analytis and Kranz (3) and Thal and Campbell (22) suggest that variation within primary units may tend to be greater than variation among primary units. In addition, one of the main reasons for visual assessment of disease is to reduce the time required for measurements. Thus, in other foliar pathosystems, costs of sampling within plots also will tend to be smaller than costs of sampling additional plots. Therefore, multistage sampling to estimate foliar diseases may be most efficient, in general, with several measurements of disease per plot.

Estimates of components of error variance differed among sampling dates during periods of alfalfa growth but changes over time were inconsistent. Factors responsible for changes were not determined. The size of plants and defoliation of plants both increased monotonically with time. Therefore, there was no simple relationship between these factors and changes in components of error variances.

Differences in error variances over sampling dates have important implications for efficiency of sampling. Estimates of components of variance from a single preliminary sample may not be reliable. Because estimates of components of variance were calculated as means over a large number of sampling dates in our experiments, they should be more reliable. On individual sampling dates, sampling rates based on these means may be suboptimal, but on average, over a number of sampling dates, the efficiency of sampling should be improved compared with sampling without any knowledge of estimates of components of variance.

Changes in the magnitude of error variances during epidemics also have important implications for comparison of progress curves for leaf spot epidemics. Common methods of comparing progress curves, such as ordinary least squares regression are based on the assumption of constant variance over sampling dates (15). Therefore, methods of comparing disease progress curves that do not require this assumption, such as profile analysis (13), may be more appropriate for comparing leaf spot epidemics.

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