Evaluation of Field Sampling Techniques for Estimation of Disease Incidence

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ABSTRACT

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Disease incidence and disease aggregation were varied in computer-simulated field tests to determine their effects on sampling techniques. Fields with 0.01, 0.1, 1.0, and 10% disease incidence were simulated. Five degrees of disease aggregation were simulated at each disease incidence level except at 0.01%, for which only three were simulated. Ten fields were generated for each of the 18 field types. Each field was sampled with five sampling designs (left and right diagonal, left and right W, and stratified random) at three sample sizes (20, 30, and 40 plants per sample site) and seven sample intensities (0.05, 0.1, 0.2, 0.4, 1.1, 2.2, and 4.4% of the plants sampled from the entire field). There were no significant differences between the left and right diagonal sampling designs or the left and right W sampling designs. Sample size had no apparent effect unless disease was random at the lowest disease incidence, where increasing sample size from 20 to 40 plants per sample site increased percent error. This was a result of decreased number of sample sites and sample site dispersal. In all fields, percent error of the disease incidence estimates and standard deviation of percent error were lowest with the stratified random sampling design if sample intensity was $\geq 0.2\%$ and highest with the diagonal if sample intensity was $\geq 0.4\%$. Percent error for all designs decreased as sample intensity increased from 0.05 to 0.2%. When sample intensity was $\geq 0.2\%$, the percent error for the diagonal and W designs achieved a minimum plateau; however, percent error for the stratified random sampling design continued to decrease as sample intensity increased if disease was aggregated. Percent error was inversely related to disease incidence and directly related to disease aggregation. The stratified random sampling design required the least number of samples and the lowest sample intensity to estimate disease incidence within a 95% confidence interval for all field types.

A common technique to assess crops for plant disease is to sample plants at random or uniform intervals along a path of a predetermined design. This technique is used to obtain samples from a field within a reasonable time. Conventional sampling designs include the diagonal, W, V, and X (2,7), which cover an entire field or are restricted to subdivisions of a field. These will be referred to as whole-field and partial-field designs, respectively. Lin et al (7) examined five sampling designs under random and aggregated disease distributions using simulation. Test sampling designs included entire-field X, W, and diagonal designs and partial-field X and W designs. They reported that sample size was more important than sampling design for disease estimation if disease was randomly distributed in a field. Sampling design was more important if disease was aggregated, that is, maximum dispersion of sample sites along the sampling design was the critical factor. They concluded that the entire-field X and W designs were equivalent to one another and were the most precise, having the least amount of variance; the diagonal design was intermediate; and the partial-field designs were the least precise.

Samples collected along predetermined designs, as above, provided estimates of disease incidence; however, they were biased considerably if diseased plants were aggregated (3). Cochran (4) discussed the stratified random sample design (SRSD) in which the entire population within a field was divided into uniform strata or sectors. These sectors were nonoverlapping, and together they composed the entire field. Once the sectors were determined, a randomly located sample was collected from within each sector. A feature of this sampling design was that every plant in the field had an equal likelihood of being sampled. This technique provided an unbiased estimate of disease incidence. An additional advantage of the SRSD was that sectors were uniform and independent. Thus, they could be compared with variance analyses. The SRSD and subsequent variance analyses were used to determine the disease incidence and distribution of lettuce anthracnose and drop caused by *Marssonina panattoniana* (Berl.) Magn. and *Sclerotinia minor* Jagger, respectively (5).

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Despite its advantages, the SRSD has had limited use because it was cumbersome. The advent of the portable microcomputer, however, has permitted the application of the SRSD (5). Thus, it is now pertinent to compare this technique with those examined previously. Computer simulation has several advantages in this regard. First, simulated fields with known disease incidence levels and distributions can be generated and sampled. From these, the actual bias of each sampling technique can be determined with a variety of disease conditions. Second, multiple sampling can be done to a degree not practical in actual field conditions. Third, a large number of factors can be tested in a relatively short time.

This simulation study examined five sampling designs, at three sample sizes and seven sample intensities for four disease incidence levels and five degrees of disease aggregation. The objectives were: 1) to determine the effects of sampling design on the accuracy and variability of disease incidence estimates; 2) to determine the effects of the sample size and sample intensity on accuracy of disease incidence estimates; and 3) to study these effects in relation to various disease incidence levels and degrees of disease aggregation. Disease detection was assumed to be perfect for the purpose of the simulation. The theoretical and practical considerations of disease sampling with imperfect detection have been addressed by Seem et al (9).

MATERIALS AND METHODS

Definitions and parameters. Sample size was the number of plants or plant units evaluated at each sample site. Sample intensity was the percentage of plants sampled from the entire field. Disease incidence was defined as the percentage of diseased plants in the entire field. The degree of aggregation was described with the variance-to-mean ratio where the variance was calculated from the observed number of diseased plants at each sample site and the mean was the average number of diseased plants per sample site.

Lin et al (7) examined sampling designs using simulated fields with 10–50% disease. However, most management decisions regarding plant disease must be made at much lower disease incidence levels, often below 1% (1,6,10). Because early detection gives more time to consider and use all of the available disease control options, disease incidence levels of 0.01, 0.1, 1.0, and 10% were selected for this simulation study to examine detection of low disease levels.

Diseases exhibit a variety of distributions in the field, dependent on the biology of the host and pathogen, weather conditions, cultural practices, etc. Therefore, it is important to examine the various sampling designs over a range of disease aggregations. Five degrees of disease aggregation were simulated at each disease incidence level except at 0.01%, for which only three were simulated. The 18 disease field types (combinations of disease incidence and aggregation) are summarized in Table 1. Ten fields of each type were generated for a total of 180 fields.

Field simulation. Simulated fields (hereafter referred to as fields) consisted of 180 by 180 arrays. Each array element represented the disease condition of 10 plants for a total of 324,000 plants per field. Fields with random disease distributions (random fields) were simulated by generating array coordinates with a pseudorandom number generator and adding a diseased plant to that array element. The total number of diseased plants in an element could not exceed 10. The disease incidence was defined by the number of coordinates generated. The true mean disease incidence was determined by sampling all array elements.

Fields with aggregated disease distributions (aggregated fields) were simulated by randomly locating disease loci and generating a normally distributed disease gradient around each locus, thus creating clusters of diseased plants. The degree of aggregation was defined by the steepness of the gradient. The disease incidence was defined by the total number of loci and the average number of plants per cluster. No restrictions were placed on the location of loci and the clusters could overlap. Various degrees of disease aggregation are illustrated in Figure 1.

Sampling factors. Five sampling designs were examined: a right diagonal, a left diagonal, a right W, a left W, and the stratified random (Fig. 2).

Sample sizes examined were 20, 30, and 40 plants per sample site. These were represented by two, three, and four vertically adjacent array elements, e.g., the number of diseased plants in a sample site located at the array element with x,y coordinates (53,61) and sample size of 30 would be the the sum of the array elements (53,61), (53,62), and (53,63).

Sample intensities examined were 0.05, 0.1, 0.2, 0.4, 1.1, 2.2, and 4.4% of the plants from the entire field. The number of sample sites was a function of sample intensity and sample size.

A sampling technique was defined as a specific sampling design, sample size, and sample intensity, e.g., the SRSD at sample size 20 and sample intensity 1.1%.

Sampling method. Fields were sampled with the diagonal and W sampling designs (Fig. 2A–D) by dividing each design into uniform sections, one for each sample site. A section was the linear set of array elements intersected by the sampling design. Sample sites were randomly located on each section within four array elements above or below the actual sampling design, e.g., if a section of a diagonal sampling design intersects array elements (42,42) to (53,53) then the sample site could be located in the area defined by array elements from (42,38) to (42,46) and (53,49) to (53,57). This simulated the sampling variability that could occur under actual field conditions.

Fields were sampled using the SRSD by dividing the field into sectors of uniform size, one for each sample site. A sector was a two-dimensional area with size defined as the total field area divided by the number of sample sites (Fig. 2E). Sample sites were randomly located in each sector. Because a sample site could be located anywhere in a sector, simulated sampling variability was not required.

A field was sampled with each sampling technique a minimum of 25 times or until the average bias stabilized. Average bias was the difference between the average estimated mean and the true mean. Stability was realized when the average of the three previous average bias values was within 0.01% of the current average bias. Each of the 180 fields was sampled with each of the 105 sampling techniques an average of 30 times for a total of about 567,000 field samples.

TABLE 1. Parameters used to generate simulated fields

Disease incidence	Disease distribution					
	1[a]	3	6	8	11	14
0.01[b]	+[c]	+	+			
0.1	+	+	+	+	+	
1.0	+	+		+	+	+
10	+	+	+	+	+	

[a] Approximate variance to mean ratio.
[b] Disease incidence expressed as a percentage of the total plant population.
[c] + Indicates that simulated fields were generated in this class.

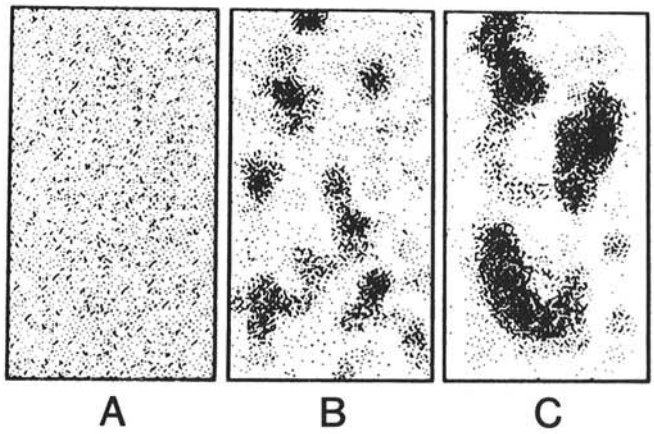

Fig. 1. Example of various degrees of aggregation from **A**, near-random to **C**, highly aggregated.

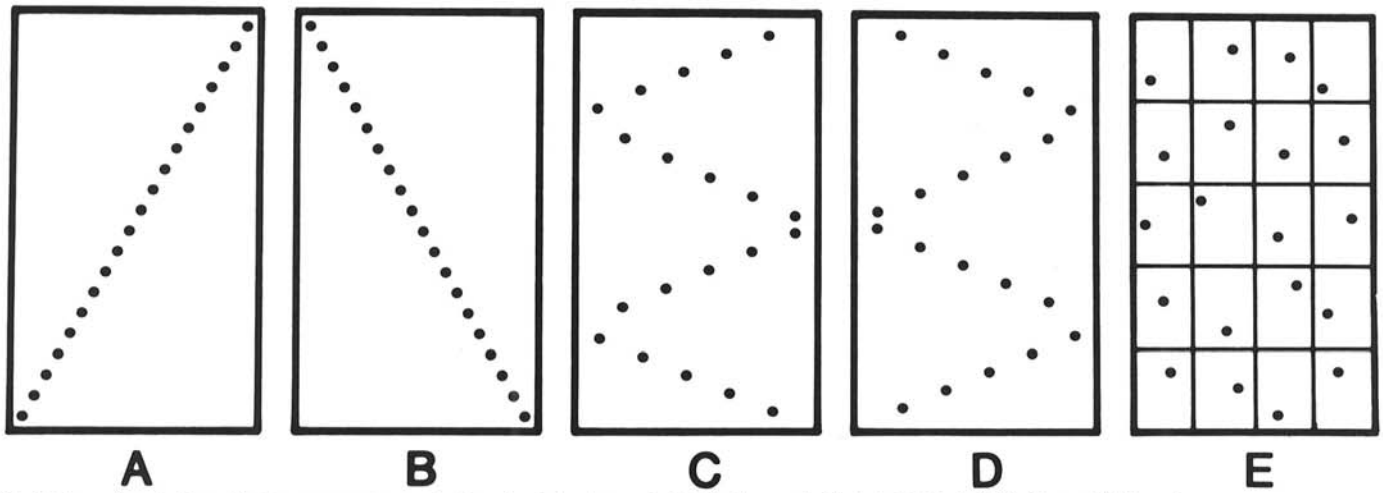

Fig. 2. Sampling designs. Points represent sample sites. **A**, right diagonal. **B**, left diagonal. **C**, right W. **D**, left W. **E**, stratified random.

Statistics. The true mean (the average number of diseased plants per sample site) and true variance (among sample sites) of each field were determined empirically by sampling each entire field with every possible sample size. The standard deviation of the true means from the 10 replicates of each of the 18 field types was calculated to test the reliability of the field simulation procedure.

Percent error was defined as:

$$\text{Percent Error} = 100\,|\,\mu - x\,|$$

where $|\,\mu - x\,|$ is the absolute value of the difference between the true disease mean and the disease mean estimated with a sampling technique. Percent error was determined for each sampling technique. The standard deviation of percent errors from the 10 replicates was calculated to determine the inherent variability of a sampling technique.

The relative cost of each sampling technique was computed with the formula

$$Ct = Cd(D) + Cp(P) + Cf$$

where Ct =the total relative cost, Cd =the cost to travel a distance unit, D =the number of distance units, Cp =the cost to evaluate a single plant, P =the number of plants, and Cf =the fixed costs. The value of D was fixed for the diagonal and W sampling designs and was computed for each sample intensity for the SRSD.

RESULTS

The presentation of results is based on data from all field types, sampling intensities, and sample sizes. Figures, however, are limited to data from typical examples of these data. The left and right diagonal sampling designs were equivalent with regard to percent error, as were the left and right W sampling designs. This result was implicit in the design of the experiment and was used as a check of the simulation and sampling system. Therefore, the presentation of results and discussion will treat only three sampling designs: the diagonal, the W, and the SRSD.

For each of the 18 field types, the variance of the true mean from the 10 simulations of a specific field type was less than 1% of the average of the true mean. It was assumed that any variance within a sampling technique was due to the bias and variability of that technique.

The effects of sampling design on percent error were pronounced and consistent. The lowest percent error was obtained with the SRSD in all field types if the sample intensity was 0.2% or greater. The highest percent error was obtained with the diagonal sampling design if the sample intensity was 0.4% or greater. The standard deviation of percent error followed similar trends. Typical results from four fields at a sampling intensity of 1.1% are in Figure 3. Both the percent error and standard deviation of percent error were lowest for the SRSD in all field types and highest for the diagonal sampling design in all but the random field with 0.01% disease incidence.

The effect of sample intensity on percent error was related to the sampling design. In general, the percent error for all sampling designs decreased as sample intensity increased from 0.05 to 0.2% (Fig. 4). Percent error for the diagonal and W sampling designs achieved a minimum plateau at sampling intensity 0.2–0.4% with no further decrease as sampling intensity increased. This was also true for the SRSD if the disease distribution was random. However, percent error continued to decrease as sample intensity increased if the disease distribution was aggregated as in Figure 4B and D.

The effects of sample size on percent error were examined only for the SRSD. There was little or no effect of sample size on percent error with most disease conditions (Fig. 5). The only apparent effects occurred in random fields with low disease incidence (0.01–0.1%) (Fig. 5B). In these conditions, percent error increased as sample size increased if the sample intensity was greater than 1.1%.

The effects of disease incidence on percent error were partially affected by the degree of disease aggregation. Percent error decreased as disease incidence increased for all degrees of disease aggregation and all sample intensities of 0.2% or greater (Fig. 6). In random fields, the decrease was more rapid at low disease incidence (0.01–0.1%) with the diagonal and W sampling designs (Fig. 6A and B). The decrease of percent error was more uniform with the diagonal and W designs in aggregated fields. The decrease of percent error with increase in disease incidence was similar for all disease distributions with the SRSD.

The effects of disease distribution on percent error were variable and partially dependent on disease incidence and sampling design. In general, percent error increased as disease aggregation increased at most disease incidence levels (Fig. 7). Percent error for the diagonal and W sampling designs increased as disease became slightly aggregated but did not continue to increase as disease became more aggregated in fields with 0.01% disease incidence (Fig. 7A and B). Percent error for these sampling designs continued to increase as disease aggregation increased at all higher disease incidence levels. With the SRSD, the increase of percent error with increased disease aggregation became less pronounced as disease incidence increased (Fig. 7A and B). These trends occurred at all sample intensities.

Figure 8 illustrates the relationship between relative sampling cost and percent error in a field with 1% disease incidence and intermediate aggregation (variance/mean = about 8). In this example, the values for fixed costs (Cf), cost per distance unit (Cd), and cost per plant (Cp) were 200, 1 and 2, respectively. The relative sampling cost at a particular sample intensity was least for the diagonal sampling design and greatest for the SRSD. However, the

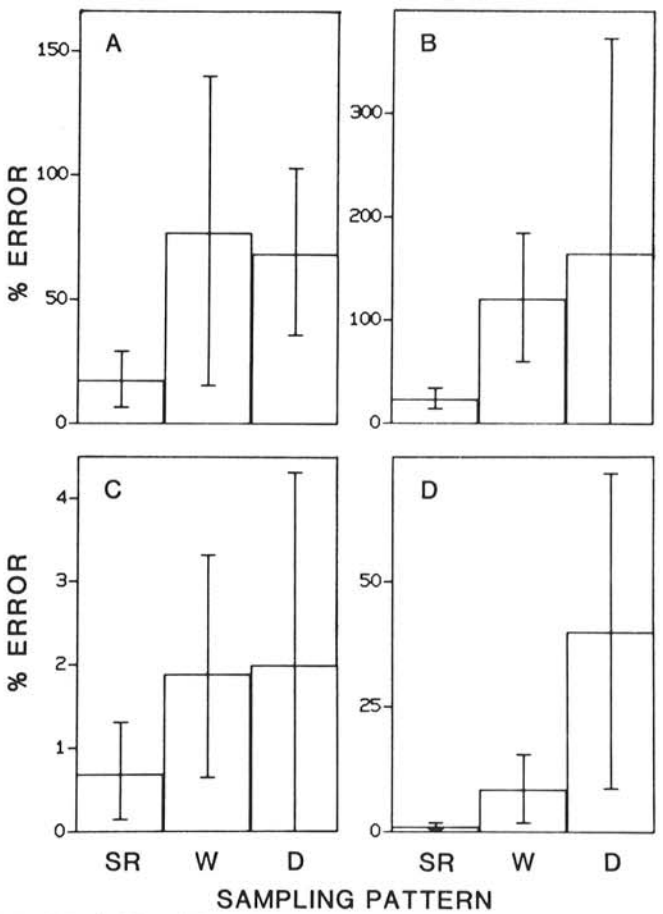

Fig. 3. Variability of disease incidence (DI) estimates for the diagonal (D), W, and stratified random (SR) sampling designs in random and aggregated disease distributions. Error bars represent percent error of the DI estimates plus or minus one standard deviation at sample intensity 1.1%. **A,** DI =0.01%, variance/mean (V/M) =1 (random). **B,** DI =0.01%, V/M =6 (aggregated). **C,** DI =10%, V/M =1 (random). **D,** DI =10%, V/M =11 (aggregated).

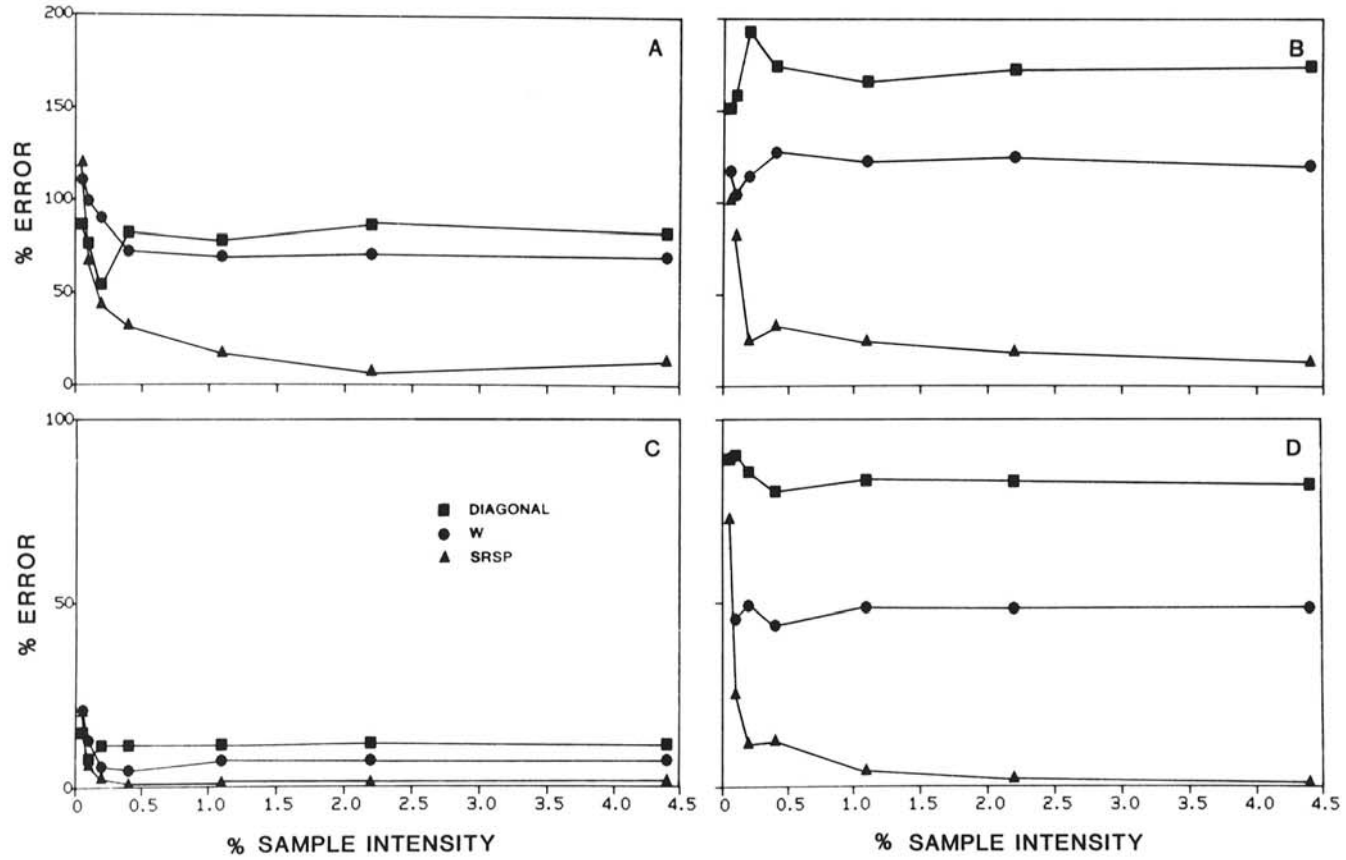

Fig. 4. Comparison of diagonal, W, and stratified random (SRSD) sampling designs in random and aggregated disease distributions. Effects of sample intensity on percent error of disease incidence (DI) estimates at sample size 20. **A,** DI=0.01%, variance/mean (V/M)=1 (random). **B,** DI=0.01%, V/M=6 (aggregated). **C,** DI=1%, V/M=1 (random). **D,** DI=1%, V/M=14 (aggregated).

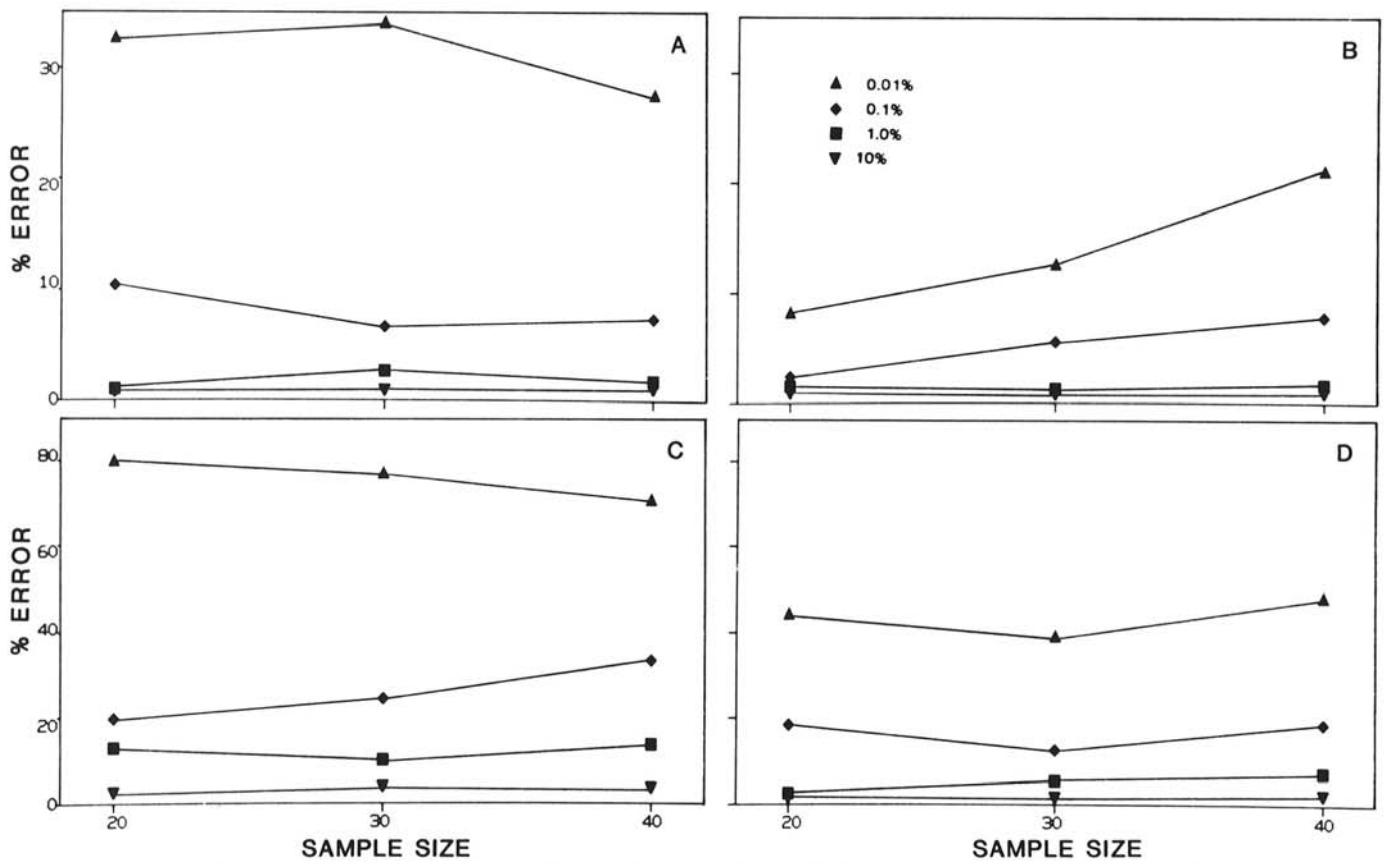

Fig. 5. Effects of sample size on percent error of disease incidence estimates for the stratified random sampling design at various disease incidence levels (0.01–10%) in random and aggregated disease distributions. **A,** Variance/mean (V/M)=1 (random), sample intensity (SI)=0.4%, **B,** V/M=1, SI=2.2%. **C,** V/M=11 (aggregated), SI=0.4%. **D,** V/M=11, SI=2.2%.

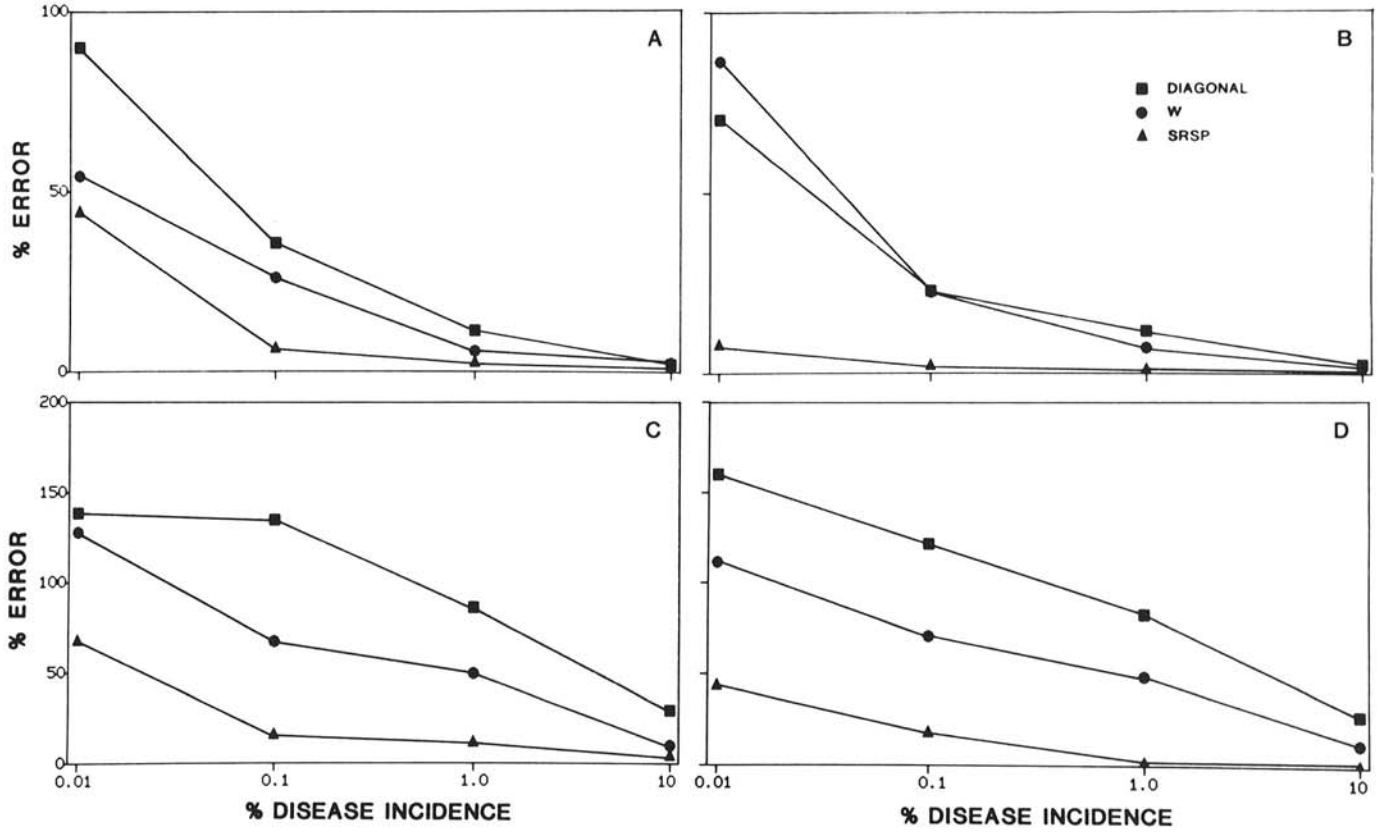

Fig. 6. Comparison of diagonal, W, and stratified random (SRSD) sampling designs in random and aggregated disease distributions. Effects of disease incidence on percent error of disease incidence estimates at sample size 20. **A,** Variance/mean (V/M) = 1 (random), sample intensity (SI) = 0.2%. **B,** V/M = 1, SI = 2.2%. C, V/M = 11 (aggregated), SI = 0.2% **D,** V/M = 11, SI = 2.2%.

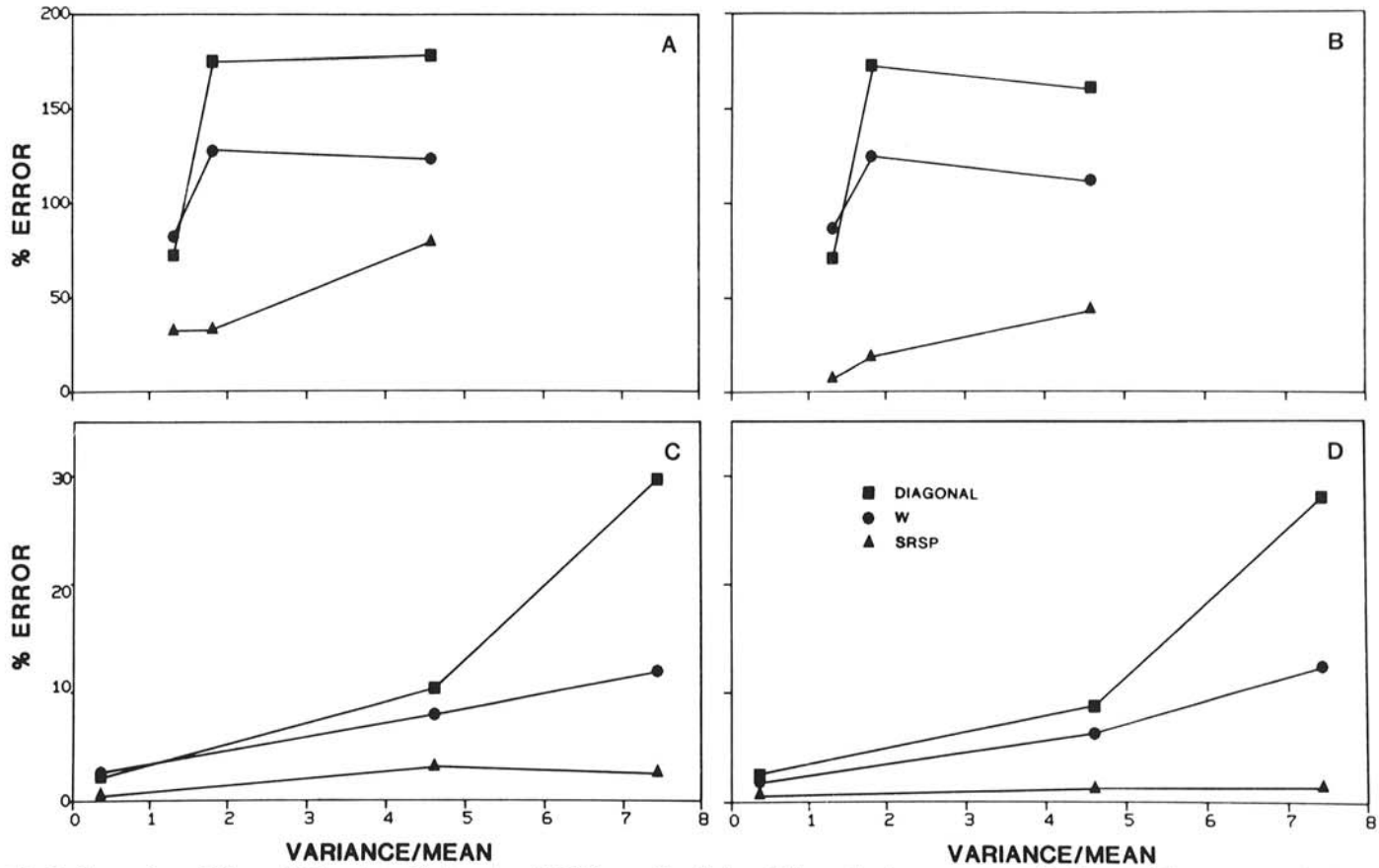

Fig. 7. Comparison of diagonal, W, and stratified random (SRSD) sampling designs. Effects of variance to mean ratio (degree of disease aggregation) on percent error of disease incidence (DI) estimates at sample size 20. **A,** DI = 0.01%, sample intensity (SI) = 0.4%. **B,** DI = 0.01%, SI = 2.2%. **C,** DI = 10%, SI = 0.4%. **D,** DI = 10%, SI = 2.2%.

cost to obtain a disease estimate below a specified percent error was lowest for the SRSD, i.e., the relative sampling cost to obtain a disease estimate with less than 32% error was 1,962 for the W sampling design and 1,540 for the SRSD. It is important to note that a slight increase in sampling cost resulted in only a minor decrease in percent error with the W sampling design, however, percent error with the SRSD decreased significantly. The value and relationship of relative sampling costs were dependent on the values assigned to Cf, Cd, and Cp.

Table 2 lists the optimum sampling techniques for each field type at thresholds of 10 and 20% error. The optimum sampling technique was defined as that which required the least number of sample sites at the lowest sample intensity to acquire a disease estimate with less than a designated percent error within a 95% confidence interval. Number of sample sites and sample intensity were used rather than relative sampling cost because relative sampling cost varies greatly with the values assigned to Cf, Cd, and Cp. In general, higher sample intensities were required as disease aggregation increased and lower sample intensities were required as disease incidence increased.

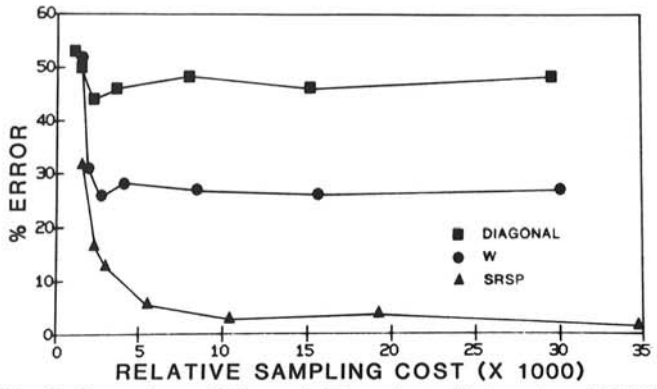

Fig. 8. Comparison of diagonal, W, and stratified random (SRSD) sampling designs. Effects of relative sampling cost of the percent error of disease incidence estimates in a field with disease incidence =1% and variance/mean =8.

The SRSD was the optimum sampling design for all field types at the thresholds of 10 and 20% error. It was not possible to achieve less than 10% error with the sample intensities tested for any of the fields with 0.01% disease incidence. Depending on the the degree of aggregation, either it was not possible or it required the maximum sample intensity (4.4%) to obtain less than 10% error for aggregated fields with 0.1% disease incidence. A threshold of 20% error could not be achieved for aggregated fields with 0.01% disease incidence. All sampling designs achieved the 10% threshold at the minimum sample intensity for random fields with 10% disease incidence. However, the percent error and standard deviation of the percent error were lowest for the SRSD.

DISCUSSION

The purpose of sampling design is to increase the accuracy, precision, speed, and scope of a sample for a minimum cost (4). In other words, sampling techniques are designed to reduce bias (percent error) and variance. Reduction of these parameters not only increases the accuracy of disease estimates, it also increases the power of means separation analyses that may be applied to the collected data. The stratified random sampling design was superior with regard to low bias and low variance for practically all field types and sample intensities. This may be true for the following reasons. First, Lin et al (7) stressed the importance of sample site dispersal to reduce variance. The SRSD disperses sample sites better than either the diagonal or W sampling designs. Second, every plant in the entire field has an equal likelihood of being sampled with the SRSD, whereas only plants located on or near a designated sampling path are sampled with the diagonal or W sampling designs. Therefore, only the SRSD provides information from the entire field and results in an unbiased estimate of disease incidence. The SRSD not only provides more accurate disease estimates, it also permits analysis of within-field variance (5) and spatial relationship (8). This is not possible with data collected from a specific predetermined path (X, V, W, and diagonal) unless the potential bias is determined and accounted for (8).

The dependence of sample intensity effects on sampling design is explained by the fact that the diagonal and W designs sample a subpopulation in the field. The subpopulation contains a finite

TABLE 2. Optimum sampling techniques to acquire disease estimates with less than 10 and 20% error

		10% Error threshold[a]					20% Error threshold				
DI[b]	DA[c]	Design[d]	Size[e]	Intensity[f]	% Error[g]	Std dev[h]	Design	Size	Intensity	% Error	Std dev
0.01	1	...[i]	...	...	...	...	SRSD	20	2.2	8.2	8.2
0.01	3	...	...	...	...	...	...	...	...	...	...
0.01	6	...	...	...	...	...	...	...	...	...	...
0.1	1	SRSD	20	1.1	4.2	3.8	SRSD	20	0.2	6.9	6.4
0.1	3	SRSD	20	4.4	4.8	4.1	SRSD	20	1.1	8.8	7.7
0.1	6	SRSD	40	4.4	5.1	4.2	SRSD	20	1.1	10.8	7.1
0.1	8	...	...	...	...	...	SRSD	20	0.2	6.9	6.4
0.1	11	...	...	...	...	...	SRSD	20	4.4	8.5	9.2
1.0	1	SRSD	40	0.2	4.9	3.2	SRSD	30	0.05	11.6	6.3
1.0	3	SRSD	40	1.1	5.4	3.2	SRSD	20	0.2	9.5	7.9
1.0	6	SRSD	30	1.1	4.1	3.4	SRSD	20	0.2	11.3	7.1
1.0	11	SRSD	20	1.1	3.7	2.8	SRSD	30	0.2	11.7	7.2
1.0	14	SRSD	30	1.1	5.2	3.8	SRSD	30	0.4	10.0	4.9
10	1	SRSD	20	0.05	2.6	1.7	SRSD[j]	20	0.05	2.6	1.7
10	3	SRSD	40	0.1	5.7	3.4	SRSD	20	0.05	10.1	5.9
10	6	SRSD	40	0.2	5.5	3.4	SRSD	20	0.1	7.8	6.0
10	8	SRSD	40	0.4	3.3	2.1	SRSD	20	0.1	8.0	6.7
10	11	SRSD	20	0.2	4.9	4.2	SRSD	40	0.1	12.2	6.9

[a] Percent error of the estimated disease incidence +/− one standard deviation.
[b] Disease incidence as a percent of the total plant population.
[c] Disease aggregation expressed as the approximate variance to mean ratio.
[d] Sampling design: SRSD = stratified random sampling design.
[e] Sample size = the number of plants sampled at a sample site.
[f] Sample intensity = the number of plants sampled in a field expressed as a percent.
[g] Percent error of the disease incidence estimated by a particular sampling technique.
[h] The standard deviation of the percent errors from 10 replicates.
[i] It was not possible to achieve the desired threshold with any sampling technique.
[j] Percent error of disease incidence estimates for the diagonal and W sampling designs were also less than the designated error threshold. However, percent errors and standard deviations were lowest for the SRSD.

amount of information about the disease incidence in the whole population. Once all of this information is collected, no further gain in information can be achieved by increasing the sampling intensity. Thus, percent error of these sampling designs reaches a minimum and no further improvement is possible with increased sample intensity. The SRSD samples from the whole population in a field. An increase of sample intensity will provide an increase of information. This is more pronounced if samples are collected from fields with aggregated disease distributions. In these fields, the average disease incidence of the entire field may not be accurately represented by a restricted portion of the field such as that along a predetermined path. Therefore, the percent error of the SRSD will decrease as the sample intensity increases until the whole population is sampled.

The apparent lack of sample size effects observed in this research was unexpected. A decrease of sample size within a given sample intensity results in a greater number of sample sites and, therefore, a higher degree of sample site dispersal. This should result in reduced percent error of the disease incidence estimates. The expected trend only occurred in fields with low disease incidence and random disease distribution. It is possible that the effects of sample size within the range examined (20–40 plants per sample site) are only apparent under the most adverse sampling conditions. An examination of a greater range of sample sizes could provide more information than available at present.

In conclusion, the SRSD is the most accurate sampling design for virtually all disease distributions. The question that remains is how does one determine an appropriate sample intensity for fields with unknown disease incidence and distribution. In general, disease estimation is more difficult in fields with low disease incidence or very aggregated disease. Therefore, it is helpful to know the approximate values of these parameters before sampling. Although this is rarely possible, one approach is to become familiar with the biology of the host and pathogen. General assumptions and predictions about the probable degree of disease aggregation are possible. A second approach is to conduct preliminary samples at a high sample intensity to determine empirically the typical distribution of a disease, then establish a disease incidence threshold defined as the lowest level of disease that must be accurately estimated. Once the approximate disease distribution and incidence threshold are established, the sample intensity to achieve a desired percent error can found in Table 2. This sample intensity could be used for all fields subsequently sampled.

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