A Sampling Procedure To Detect Grapevine Downy Mildew

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ABSTRACT

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A sampling procedure was developed that would detect primary infections by *Plasmopara viticola* in commercial vineyards with 99% confidence when the incidence of infected leaves was as little as 0.01%. The probability of detecting disease, if present, is the product of the probability of finding at least one diseased leaf (given that disease is present) and the probability of selecting a diseased sampling unit. Disease was simulated with artificial lesions and four to six incidence levels were assigned to half-vine sampling units consisting of 7.3 meters of vine row. Based on experimental results, probabilities of detecting at least one lesion within a sampling unit (disease incidence of 0.01–0.07%) were determined for different sampling period durations, times of season, and observers on cultivars Rhine Riesling, Muscat Gordo, Sultana (synonym Thompson Seedless), and Shiraz. An analysis of variance with a linear logistic model indicated that only disease incidence and cultivar accounted for significant variation in the probability of detecting disease. In vineyards with known distribution of disease, the minimum numbers of sampling units per vineyard, based on 2-min sampling periods per sampling unit and a 99% confidence level for detection were 13 for Riesling, 31 for Gordo, 39 for Sultana, and 73 for Shiraz; when the likely distribution of disease in a vineyard is not known, more sampling units are required.

Additional key words: disease management, scouting, *Vitis vinifera*.

Few sampling procedures have been developed for plant diseases in commercial crops though they are an important component of disease management programs. Because management decisions must often be based on the knowledge of either the incidence of a pathogen or intensity of disease within a crop (14), effective sampling procedures must provide information of known accuracy. Minimum expenditure of time and financial resources are frequent constraints.

For maximum vineyard productivity, grapevine downy mildew, which is caused by *Plasmopara viticola* (B. & C.) Berl. & Det., must be effectively managed. Economically, it is the most important of Australia's viticultural diseases, resulting in losses estimated at $9 million ($A, 4% of production value) in dry years, and more than $30 million ($A) in wet years (P. A. Magarey, *unpublished*). Losses in individual vineyards range from insignificant to 100%.

In vineyards of the River Murray irrigation districts near Loxton, South Australia, downy mildew occurs sporadically (9) but often enough to warrant the establishment of a management program (8). Such a program might delay any fungicide program until the disease was observed in vineyards because, historically, the frequency of severe disease years is low and weather conditions favorable for disease development can not be predicted from long-range forecasts. However, due to the ability of *P. viticola* to multiply and spread rapidly, any management program would require a sampling procedure for detecting a very low incidence of disease.

The rationale for this procedure is that, once detected, the disease can be controlled by well-timed protective fungicide programs. Spray applications could be delayed until disease was detected. Thus, in dry years, fungicide sprays against downy mildew may never be applied in vineyards with irrigation practices unfavorable for disease development. This contrasts with current practice in which three to four "insurance" sprays are applied annually, despite an absence of disease. Often, in wet years, eight or more sprays are applied; early detection would allow accurate scheduling of sprays before inoculum levels increased and the disease became difficult to manage. As a result, spray efficiency would be improved and the loss from disease lessened.

While most disease sampling procedures are designed to provide an estimate of disease intensity, disease detection sampling is designed to determine only incidence (the presence or absence of disease). Thus, detection sampling must be biased (or stratified) in favor of finding the pathogen. In the case of grapevine downy mildew, the procedure would bias the sampling to those areas of the vineyard and vine canopy where disease is most likely to occur.

A preliminary report of a sampling procedure for detecting downy mildew has been published (11). We now describe its further development for use in commercial vineyards. This specialized procedure can, in principle, be applied to other similar detection sampling problems.

MATERIALS AND METHODS

Assumptions. To establish a working procedure, preliminary constraints and assumptions were identified for the sampling unit and the threshold disease incidence. The sampling unit was defined as the lateral half of vine canopy (vine rows are physically divided by trellis wires) that extended between two trellis support posts. This length-of-row was 7-8 m long and contained four vines. The disease incidence threshold, or maximum allowable disease, was set in the range of one to five downy mildew lesions per sampling unit because a detectable disease incidence greater than this would likely be difficult to control with fungicides. Based on the theory of multiple infection process (7), it was further assumed that up to five lesions per sampling unit would always occur on separate leaves; so disease was expressed as incidence of infected leaves. All constraints and assumptions were applied to the four grape cultivars used in this study: Muscat Gordo, Shiraz, Sultana (syn. Thompson Seedless), and Rhine Riesling.

Simulated epidemics. To determine how well disease can be detected at various levels of infection, the exact prevalence must be known. This can be done by either examining every leaf or by

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simulating the disease. The latter method was chosen because disease was not present at the time of the study due to unfavorable natural conditions for disease development. Consequently, for sampling purposes disease was simulated with artificial lesions. Artifical lesions consisted of either pale-yellow, transparent, adhesive-backed plastic vinyl disks (12 mm in diameter), or bright-yellow, opaque, adhesive-backed paper disks (14 mm in diameter). The transparent disks closely resembled the "oilspot" stage of early downy mildew infections and the opaque disks provided vivid contrast to the leaf surface (Fig. 1). To mimic natural sites of primary infection, disks were placed on the upper leaf surfaces, usually on leaves within the lower two-thirds of the canopy. The location of each disk was recorded according to vine and shoot number to enable us to check that the disks remained attached. All studies were conducted in vineyards at the Loxton Research Centre.

Sampling theory and methods. The detection of downy mildew in a vineyard is hindered by two sampling problems: first, the detection of the disease, when it is present in selected sampling units at a level equal or greater than the threshold value; and second, the selection of enough sampling units with the disease.

The problem of detecting disease (given that disease is present) was addressed first. Various factors influencing the ability of observers to detect simulated disease were tested in the field by using two to five replicates for each treatment allocated to randomly selected sampling units (Table 1).

In all tests, sampling units with no disks were included to reduce observer bias in expectation of positive outcome for each sampling unit and to better simulate field conditions.

Each observer, usually the first, second, and fourth authors, used his own inspection method although it usually consisted of peering into the canopy at different angles while gently moving leaves and shoots. Small, electronic pocket timers which had switch selectable intervals from 0.5 to 7.5 min, in 0.5 min increments, were constructed for each observer (construction details are available from the first two authors). Once started, the timers gave a short beep every 30 sec until the end of the interval when they buzzed continuously until turned off. This facilitated maximum time efficiency while sampling and allowed observers to pace themselves during the time intervals considered in this study.

The variable for analysis was the number of disks detected by an observer, relative to the number of disks in the sampling unit. The effects of length of sampling time, disk color, cultivar, disk count, and observer, were tested by analysis of variance conducted according to a linear logistic model, e.g., for test 1 (Table 2):

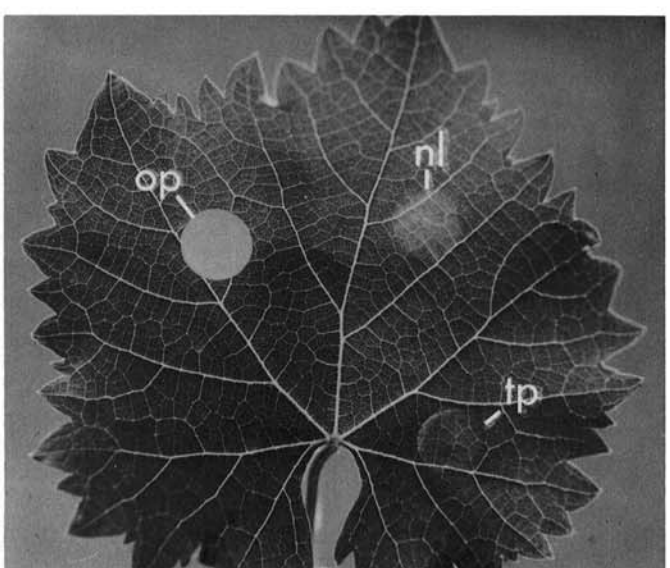

Fig. 1. Comparison of two artificial lesions, a transparent plastic disk (tp) and an opaque paper disk (op), and a natural grape downy mildew lesion (nl) in the "oilspot" stage.

$$\ln\left(P_{ijklm}/[1-P_{ijklm}]\right) = \mu + \phi_i + \beta_j + \gamma_k + \delta_l + \tau_m + \epsilon_{ijklm}$$

in which $P_{ijklm} = Y_{ijklm}/N_{ijklm}$, is the observed probability of detecting a disk at the ith time, $i = 1, 2, 3, 4, 5$; jth disk type, $j = 1, 2$; kth cultivar, $k = 1, 2$; lth disk count, $l = 1, 2, 3, 4, 5$; and mth observer, $m = 1, 2, 3$. Further, μ is the grand mean, $[\phi_i]$ are time effects, β_j are disk color effects, γ_k are cultivar effects, δ_l are disk count effects, τ_m are observer effects, and ϵ_{ijklm} are approximately normally distributed residuals with zero means and equal variances.

The models for tests 2 and 3 were similar and all were fitted with the statistical package GLIM (2); this calculated the likelihood ratio statistic which is asymptotically chi-square (3).

To determine the number, n, of sampling units to sample so that the disease can be detected with some confidence, it is assumed that when the disease incidence is very low it is randomly distributed (Poisson distribution) in the vineyard. Let P_I be the probability of detecting the disease in one sampling unit, given the true incidence, I, in the vineyard. P_I depends on measurement sensitivity and disease incidence. The probability of zero detections in n attempts is $(1 - P_I)^n$, (5). Thus, the probability, P_d, of detecting at least one disk or lesion in n sampling units is

$$P_d = 1 - (1 - P_I)^n. \quad (1)$$

TABLE 1. Factors and factor levels used in tests to assess the ability to detect downy mildew lesions simulated by small yellow disks in standard sampling units in vineyards[a]

Factor	Test 1[b]	Test 2	Test 3
Time[c]	1, 2, 3 min[d]	2 min[e]	2 min[e]
Disk color	Transparent, opaque	Transparent[e]	Transparent[e]
Cultivar	Muscat Gordo, Shiraz	Muscat Gordo, Shiraz, Sultana	Muscat Gordo, Rhine Riesling
Disk count[f]	0, 1, 2, 3, 4, 5	0, 1, 2, 3	0, 1, 2, 3
Observer	A, B, C	A, B, C, D	Experienced (ABC), inexperienced (F-J)
Date[g]	—[h]	Day 0; 6 days later	—[h]

[a] A sampling unit was 7.3 m of vine row containing four half-vines (eight arms).
[b] Two replications of each disk count per sampling unit were used in Test 1; five replications were used in tests 2 and 3. Test 1: 10, 15, 22 November 1983; Test 2: 10, 16 December 1983; test 3: 9, 10, 11 March 1983.
[c] Minutes allotted to examine each sampling unit.
[d] Each time interval was tested on a separate date.
[e] Factor held at single level and not included in test analysis.
[f] Total number of disks per sampling unit.
[g] Repeat of complete test after the elapse of time.
[h] Factor not considered in test experimental design.

TABLE 2. Analysis of variance for test 1 with a linear logistic model of factors influencing the ability to detect downy mildew lesions simulated by small yellow disks in standard sampling units in vineyards[a]

Factor	Levels	Degrees of freedom	Chi-square[b]
Time	1, 2, 3 min	2	3.3 N.S.
Disk color	Transparent, opaque	1	0.4 N.S.
Cultivar	Muscat Gordo, Shiraz	1	0.1 N.S.
Disk count	1, 2, 3, 4, 5	4	75.0 **
Observer	A, B, C	2	12.0 **
Residual[c]		79	68.9 N.S.
Total		89	159.7

[a] A sampling unit was 7.3 m of vine row containing four half vines (eight arms).
[b] Reduction in deviance due to the particular factor. The deviance is approximately distributed as chi-square. N.S. = not significant; ** = highly significant at $P = 0.01$.
[c] Contains all replications and factor interactions. Factor interactions were not significant.

If we require this probability of detecting the disease to exceed a chosen level (e.g., $\alpha = 0.95, 0.99$), then we must set

$$P_d \geqslant \alpha. \tag{2}$$

If we assume that our measurement sensitivity is adequate and fixed, then to ensure that equation 2 holds, we must select n from equation 1 such that

$$n \geqslant \ln(1-\alpha)/\ln(1-P_I). \tag{3}$$

In practice, for each disease incidence (I) we can only estimate P_I with: $\hat{P}_I$ = (number of sampling units in which disks were detected)/t, in which t = the number of sampling units which were searched. But $\hat{P}_I$ has a standard error, Σ, of

$$\Sigma = \hat{P}_I(1-\hat{P}_I)/t$$

and a 99% confidence interval of $\hat{P}_I$ is:

$$\hat{P}_I \pm 2.58$$

in which $P[Z < 2.58] = 0.495$, and Z has a standard normal distribution. As a rough guide, t should be near or greater than 100 when the normal approximation of the binomial is used (12).

A 99% confidence interval for $\hat{n}$, our estimate of n, is

$$\ln(1-\alpha)/\ln(1-[\hat{P}_I + 2.58\Sigma]) \leqslant n \leqslant \ln(1-\alpha)/\ln(1-[\hat{P}_I - 2.58\Sigma]) = \hat{n}_u \tag{4}$$

For maximum rigor, we then use $\hat{n}_u$, the upper bound of the 99% confidence interval for $\hat{n}$ as our estimate of n.

Thus, by using the estimates of our measurement sensitivity we can determine the number of sampling units that need to be assessed to detect disease in each cultivar, given that it is present in the sampling units. This is directly applicable in vineyards with known disease distribution patterns; we would allocate $\hat{n}_u$ sampling units to regions of the vineyard where disease is known to occur first. This addresses the first of our sampling problems, but by not considering the second (i.e., selecting diseased sampling units in vineyards where the incidence of disease is not known) we risk the disease being present in sampling units other than those we search.

The second sampling problem requires further consideration to ensure that sufficient diseased sampling units are selected. Think of a vineyard as a population of n_0 sampling units, n_1 of which are diseased, and n_2 are not ($n_0 = n_1 + n_2$). If we choose r sampling units at random from the vineyard, then the probability, q_k, that the group of r will contain exactly k diseased sampling units is:

$$q_k = \binom{n_1}{k}\binom{n_0 - n_1}{r - k} \Big/ \binom{n_0}{r} = \binom{n_1}{k}\binom{n_2}{r-k} \Big/ \binom{n_0}{r}, \tag{5}$$

$k = 0, 1, 2, \ldots n_1$, in which $\binom{n_0}{r} = n_0!/r!(n_0 - r)!$.

This family of probabilities is called the hypergeometric distribution (5).

Now we choose $r_{\hat{n}_u}$ so that the probability of k (the number of diseased sampling units being equal to or greater than $\hat{n}_u$) is 0.96;

$$P[k \geqslant \hat{n}_u] = q_{\hat{n}_u} + q_{\hat{n}_u+1} + \ldots + q_{n_1} = 0.96. \tag{6}$$

By estimating n_1, the number of diseased sampling units, we can determine the number of randomly selected sampling units needed to be 96% sure of searching greater than or equal to n diseased sampling units. If $n_0 \leqslant \hat{n}_u$ then the entire vineyard must be searched.

When we combine the two sampling methods, the probability of detecting the disease in vineyards is the product of the probability of detecting disease given disease is present, and the probability of selecting sufficient diseased sampling units, viz: $0.99 \times 0.96 = 0.95$. We defined this as an acceptable level of confidence on which to base a sampling procedure.

RESULTS

The zero disk count was always scored correctly and thus was omitted from our analysis. Counts were made at the end of the experiment to determine that all disks were still in place.

For the initial test, the chi-square values obtained with the linear logistic model (Table 2) indicated that examination time per sampling unit, disk color, and cultivar did not significantly affect disk detection, but disk count and observer had significant influence. None of the interaction terms were significant.

Empirical analysis of the test indicated the 1-min time interval was too short to adequately cover the entire sampling unit whereas the 3-min interval was excessive. Because disk detection was not different between time intervals, the 2-min interval was used in subsequent tests. Similarly, because both types of disk were equally detectable, subsequent tests were conducted with the more realistic-appearing transparent disks.

The variation in the ability of observers to detect disks was attributed to one observer's lack of experience in the likely location

TABLE 3. Analysis of variance for test 2 with a linear logistic model of factors influencing the ability to detect downy mildew lesions simulated by small yellow disks in standard sampling units in vineyards[a]

Factor	Levels	Degrees of freedom	Chi-square[b]
Cultivar (CUL)	Muscat Gordo,	2	10.17**
Disk count (DIS)	1, 2, 3	2	7.92 **
Observer	A, B, C, D,	3	2.12 N.S.
Date (DAT)	10 Dec, 16 Dec 1982	1	0.52 N.S.
Interactions	CUL.DIS	4	15.06 **
	CUL.DAT	1	1.67 N.S.
	DAT.DIS	2	2.37 N.S.
Residual[c]		32	41.66 N.S.
Total		47	81.49

[a] A sampling unit was 7.3 m of vine row containing four half-vines (eight arms).
[b] Reduction in deviance due to the particular factor. The deviance is approximately distributed as chi-square. N.S. = not significant; ** = highly significant at $P = 0.01$.
[c] Contains all replication and nonsignificant factor interactions.

TABLE 4. Characteristics of the canopy structure for four grapevine cultivars at Loxton, South Australia

	Cultivar			
	Muscat Gordo	Shiraz	Sultana	Rhine Riesling
Leaf area (cm^2)[a]	78.7	163.1	123.1	96.5
	(22.0)[b]	(43.9)	(47.9)	(23.7)
Leaf number[c]				
Mid-season[d]	1,340	2,176	3,024	–
	(284)	(244)	(348)	
Harvest[e]	4,324	5,565	8,250	4,996
	(237)	(213)	(224)	(256)
Internode length (cm)[f]	2.8	6.1	5.2	4.7
	(0.6)	(1.2)	(1.8)	(0.8)
Canopy volume (m^3)[g]	3.59	6.81	4.93	3.22

[a] Average area of 20 fully expanded leaves selected at random: Muscat Gordo and Shiraz—9 November 1983; Sultana and Riesling—15 May 1984.
[b] Standard deviation of the mean.
[c] Number of leaves per sampling unit of four half-vines (eight arms).
[d] Averaged based on leaf counts of four arms per cultivar; 10 December 1982.
[e] Average based on leaf counts of 10 arms per cultivar; 10 March 1983.
[f] Average based on 30–50 counts of numbers of nodes and shoot length per cultivar; 10 March 1983.
[g] Canopy volume per sampling unit based on half vine cross-sectional area × 7.32 m of vine row; 10 March 1983.

of downy mildew lesions. As a result, a more clearly defined observation method was used in subsequent tests.

The second test included four factors: cultivars, disk count, observer, and date of inspection. The results (Table 3) indicated that observer was no longer a significant factor in detecting disks and that a 6-day difference in date of inspection also had no influence. However, the detection of disks in this test was significantly affected by cultivar and disk count. These two factors had significant interaction, meaning the detection of different numbers of disks in a sampling unit was affected by the particular cultivar under test. All other interaction terms were not significant.

In an attempt to characterize the interaction between cultivar and disk count, we compared the vine canopy structure for each cultivar (Table 4). Gordo vines had smaller and fewer leaves than Shiraz. The average leaf area of Gordo was less than half that of Shiraz and, at midseason, Gordo had 38% fewer leaves than Shiraz although this difference had decreased to 23% by harvest. In addition, sampling units in Gordo occupied only about half the volume of those in Shiraz and the internode length of Gordo shoots was less than half that of Shiraz. Although the leaf area, canopy volume, and internode length of Sultana and Riesling were intermediate to Gordo and Shiraz, sampling units in Sultana contained nearly 50% more leaves than those in Shiraz and 200% more than in Gordo.

The third field test of the detection procedure was conducted primarily to test the extent of observer training necessary to produce uniform detection ability. The test employed experienced observers from the previous tests and inexperienced observers (students from the Waite Institute, University of Adelaide) who received brief instruction in the sampling method. Each group attempted to detect disks in the cultivar Muscat Gordo. The test also included attempts by the trained observers to detect disks in the cultivar Rhine Riesling. Results of the linear logistic model analysis (Table 5) show that the detection procedure was easily learned because the chi-square for level of training was not significant. This supported our findings in the second test in which an inexperienced fourth observer had been included. The training level was then collapsed into the remaining factors. The detection of disease by the scouts was similar in Muscat Gordo and Rhine Riesling at $P=0.05$. The number of disks in a sampling unit had a significant effect on the likelihood of detection. The interaction of cultivar and disk was also significant. The effect occurred because Gordo detection rates improved as disk count increased while the Riesling detection rates remained more or less constant over disk count.

TABLE 5. Analysis of variance for test 3 with linear logistic model of factors influencing the ability to detect downy mildew lesions simulated by small yellow disks in standard sampling units in vineyards[a]

Factor	Levels	Degrees of freedom	Chi-square[b]
Disk count	1, 2, 3	2	15.85 **
Observer[c]	Experienced, inexperienced	1	2.82 N.S.
Residual[d]		2	3.65 N.S.
Total		5	22.31
Cultivar (CUL)	Muscat Gordo, Rhine Riesling	1	3.68 N.S.
Disk count (DIS)	1, 2, 3	2	20.67 **
CUL.DIS		2	6.79 *
Residual[e]		6	5.56 N.S.
Total		11	36.70

[a] A sampling unit was 7.3 m of vine row containing four half-vines (eight arms).

[b] Reduction in deviance due to the particular factor. The deviance is approximately distributed as chi-square. N.S. = not significant; * = significant at $P = 0.05$; ** = highly significant at $P = 0.01$.

[c] Data from three experienced and five inexperienced observers.

[d] Contains interaction term and collapsed form of five replications.

[e] Contains collapsed observer term and collapsed form of five replications.

Sample size calculation. Based on the measured ability to detect the presence of disks in a sampling unit (Table 6) and by setting $\alpha = 0.90, 0.99$, equation 3 was used to calculate the number of sampling units that need to be observed in order to achieve a 90 or 99% confidence of detecting at least one disk if disease was present in those sampling units. Confidence intervals for this number were calculated from equation 4. The results are summarized in Fig. 2 for $\alpha = 0.90$ and 0.99.

Detection rates for Gordo and Shiraz (Fig. 2A and B) improved as the number of disks per sampling unit increased; thus, the number of sampling units needed to achieve a given level of confidence decreased as disk count increased. In Sultana, however, the detection rate (Fig. 2C) was not consistent with disk count so we chose the lowest detection rate, viz. for disk count level two, to calculate the number of sampling units to be observed.

Thus, in vineyards where we can preselect sampling units that would be the first to become diseased if downy mildew were to occur, the maximum number, $\hat{n}_u$, of sampling units for 99% confidence that at least one diseased leaf would be detected, and the time to survey those sampling units are 31 (62 min), 73 (146 min), 39 (78 min), and 13 (26 min) for Gordo, Shiraz, Sultana, and Riesling respectively (Fig. 2). For 90% confidence, the numbers and survey times are 16 (32 min), 36 (72 min), 20 (40 min), and 7 (14 min), respectively (Fig. 2).

The number of sampling units in Shiraz exceeds that in Gordo despite a higher measured disease detection rate because total replication in Shiraz was less than that in Gordo (Table 6, footnote a).

For vineyards in which likely disease distributions were not known, equations 5 and 6 were used to calculate the maximum number of randomly selected sampling units required to ensure sufficient sampling units were selected for 95% confidence of detecting disease if present. As examples, data from Gordo and

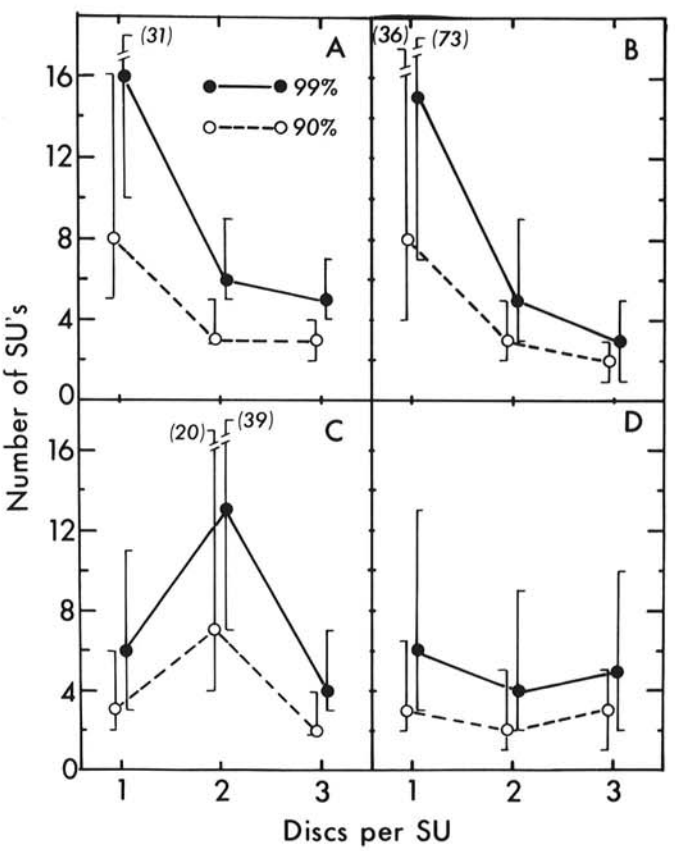

Fig. 2. Number of observed sampling units (four half-vines) and the 99% confidence interval needed to achieve 90 and 99% confidence levels in detecting grapevine downy mildew given various known levels of infection (one, two, or three disks per sampling unit). Cultivars evaluated: **A,** Muscat Gordo; **B,** Shiraz; **C,** Sultana; and **D,** Rhine Riesling.

Riesling (with the highest and lowest measurement sensitivities) are provided for different threshold disease incidences (Table 7).

DISCUSSION

Development of statistical procedures for the detection of plant disease has been limited mainly to quality assurance procedures for seedborne pathogens (6). The concept of a sampling procedure to detect disease within a population before it has reached an epidemic threshold level has been developed recently for an animal disease (10). Some general methods for presence or absence sampling have been presented by Southwood (13) for situations in which the dispersion of the organism can be described by a negative binomial distribution.

Disease detection. The method followed during this investigation was similar to that used by Geng et al (6) for an indirect bioassay. We assumed primary infections of grape downy mildew were randomly distributed and recognized that our detection ability was not perfect. Thus, we needed a measure of the probability that disease would be detected if it were present (i.e., measurement sensitivity [Table 6]), and this formed the basis of the detection procedure. However, we extended the work of Geng et al (6) by further considering optimal sampling unit number when incidence was unknown (Table 7).

Many sampling procedures have a cost factor directly included in the equation (1,4). The cost factor for the downy mildew detection procedure is time and it is indirectly considered in the measurement sensitivity. Although time spent sampling was analyzed, no difference was found between 1, 2, or 3 min per sampling unit (Table 2); participants in test 1 felt that 1 min was not enough time to adequately examine a sampling unit. On the other hand, the observers felt that 3 min per sampling unit was too much time and that if a disk had not been observed during the original examination, it would not be found during any brief reexamination. Thus, a 2-min interval (i.e., 30 sec per vine) was adopted.

The bright-yellow, opaque disk produced a striking contrast with the green leaf background compared to the pale-yellow, transparent disk (Fig. 1), but the ability of observers to detect disks was not influenced by disk type (Table 2). Thus, as with natural disease, the major criterion for observation is the presentation of the disk or lesion in the observer's line-of-sight. The contrast of the disk or lesion with its background is less important. Consequently, most observers adopted the method of gently moving leaves with their hands in order to open the canopy and allow the disks to enter within view. Adoption of this procedure by all observers and an understanding of where disease was most likely to occur, probably accounted for the loss of significance between observers from the first test (Table 2) compared to subsequent tests (Table 3). Because the third test demonstrated that the general procedure can be easily taught, observers need not be highly trained scouts but could be extension officers or growers. In addition, the procedure may also be applicable to other grapevine diseases that have distinct circular leaf lesions, e.g., caused by black spot or anthracnose *Elsinoë ampelina* Shear.

The effect of cultivar on detection rate is not clear. The three tests (Tables 2, 3, and 5) showed, respectively: no significant difference between detection rates of 0.64 (58 in 90 attempts) for Gordo and 0.66 (59 in 90 attempts) for Shiraz; an uncertain result; and a significant difference ($P > 0.06$) between detection rates of 0.50 (60 in 120 attempts) for Gordo and 0.65 (39 in 60 attempts) for Rhine Riesling. In test 2 (Table 3), the difference among cultivars was due to some interactive influence of cultivar and disk count. We could not identify a canopy characteristic that accounted for this interaction.

In addition, a similar inconsistency occurred in our detection ability on both days of observation in Sultana at disk count level two. In 35 attempts, 11 were detected, compared to 20 and 24 detections at levels one and three respectively. These variations make the significant cultivar difference in test 2 difficult to interpret. Further replication of disk count and placement in this test would be useful. We conclude however that the detection rates for Gordo, Shiraz, and Sultana are similar and that it is easier to detect disease in Riesling vineyards.

Because one disk on a leaf simulated disease, one disk per sampling unit equated to 0.07, 0.05, and 0.03% disease incidence for Gordo, Shiraz, and Sultana, respectively, at midseason, and 0.02, 0.02, and 0.01%, respectively, and 0.02% for Riesling, at harvest. There was no difference in detection rate for any cultivar, between the first and third tests during which time the relative disease incidence declined as leaf number increased (Table 4). Thus, our detection procedure may be effective at even lower disease incidences.

Implementation of the procedure. The detection procedure was developed as part of a total management program for downy mildew of grapevines (8). When fully implemented, the program will use a combination of continuous environmental monitoring and disease modeling to identify primary infection periods. After a predicted incubation period (5–11 days, P. A. Magarey, *unpublished*), scouts will be sent into vineyards to observe a preselected number of sampling units to confirm the presence of disease. If disease is detected, then a fungicide spray program will be initiated. If disease is not detected, no control action will be taken. Scouts will return to the vineyard and repeat the detection procedure after the incubation period following the next identified primary infection period.

In large vineyards or in vineyards where areas of high disease risk can not be identified, a quantitative method of determining the selection of sampling units allows operation of the detection procedure at an acceptable, though reduced, level of confidence. We would avoid random selection of sampling units wherever possible and aim to maximize the probability of finding disease by stratifying the vineyard into areas of differing disease risk and

TABLE 6. Pooled estimates[a] from tests 1, 2, and 3, of the probability of detecting downy mildew lesions simulated by small yellow disks in standard sampling units in vineyards[b]

	Disk count		
Cultivar	1	2	3
Muscat Gordo	0.26	0.55	0.63
Shiraz	0.28	0.66	0.83
Sultana	0.57	0.31	0.69
Rhine Riesling	0.60	0.70	0.65

[a] Estimates ($\hat{P}_1$) are data from three tests excluding observer B in test 1 on 10 and 15 November 1982, in Shiraz; and excluding test 2 on 10 December 1982, at level three of disk count in Gordo. $\hat{P}_1$ = (number sampling units in which lesions were detected)/(number sampling units searched). Number sampling units searched: Gordo, 88 for disk counts 1 and 2, 73 for disk count 3; Shiraz, 29; Sultana, 35; Riesling, 20.

[b] A sampling unit was 7.3 m of vine row containing four half-virus (eight arms).

TABLE 7. Maximum number of sampling units[a] to be searched to ensure 96% confidence that grapevine downy mildew will be detected in vineyards where disease distribution patterns are not known

			Number of sampling units in vineyard			
Cultivar	$\hat{n}_u^b$	Disease incidence[c]	25	75	250	750
Riesling	13	0.001%	25	75	206	243
		0.003%	25	53	59	61
		0.007%	22	27	28	28
		0.010%	13	13	13	13
Muscat Gordo	31	0.001%	25	75	250	473
		0.003%	25	53	119	124
		0.007%	25	54	58	59
		0.010%	25	31	31	31

[a] A sampling unit is 7.3 m of vine row containing four half-vines (eight arms).

[b] $\hat{n}_u$ = upper bound of the 99% confidence interval of n, the number of sampling units to ensure 99% confidence in detecting at least one lesion of downy mildew.

[c] Disease incidence = 100 × (number diseased leaves per sampling unit)/(number leaves per sampling unit).

searching those with highest risk, e.g. low, wet areas or those with overlapping irrigation from adjacent crops.

In developing the detection procedure for application in commercial vineyards, we have included a 99% confidence level to provide for the option of a high level of precision in management decisions (Fig. 2). However, because grape grower's attitudes to risk varies, we also included the lower confidence levels (Fig. 2). The decision about the number of sampling units to survey in each vineyard will depend on these attitudes and on the time required to complete the survey (14 to 146 min).

Recent observations at Loxton have shown that focal development of downy mildew can occur with a leaf disease incidence of 0.001%. Further sampling tests have been conducted to determine detection ability at levels lower than our assumed threshold disease incidence. Threshold values in Table 7 therefore are examples of interim application of our detection procedure in vineyards where disease history is not known.

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