Optimum Sampling Size for Determining Different Aspects of Alternaria Blotch of Apple Caused by *Alternaria mali*

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ABSTRACT

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The optimum number of leaves and terminals to sample to estimate the incidence and severity of Alternaria blotch, caused by *Alternaria mali*, of apple (*Malus* × *domestica*) and associated defoliation was derived from data collected between 1989 and 1992 in three locations in North Carolina. On the basis of estimates of variance and cost, the mean optimum number of leaves per terminal was 16.88 for evaluating disease severity and 19.37 for determining disease incidence. The optimum numbers of terminals per tree for assessing severity, incidence, and defoliation were 1.2, 1.18, and 2.04, respectively. The variation in disease among leaves and among terminals contributed equally to overall estimates of variance. With the lower cost of sampling leaves compared to sampling terminals, increasing the number of leaves sampled would improve sampling efficiency more than increasing the number of terminals sampled.

Alternaria blotch, caused by *Alternaria mali* Roberts, of apple (*Malus* × *domestica* Borkh.) is a serious disease that primarily affects strains of the cultivars Delicious and Indo. It has been an important disease in Japan and other Asian countries since the late 1950s but was identified for the first time in the United States in North Carolina in 1988 (7). Since that time, it has been reported throughout the southeastern United States. The primary sites of infection are leaves, although lesions occur on fruit of susceptible cultivars such as Indo (7). Extensive infection can result in up to 57% defoliation and reduced yield (8).

As the disease spread to new areas and intensified from 1989 to 1992, more extensive research was needed to gain a better understanding of the epidemiology of the disease and develop an effective management plan. Previously, we investigated different aspects of the pathosystem such as chemical control (8,13,14) and the interaction of *A. mali* with different arthropod pests (9), but we always used a similar sampling design. In those studies (8,9,13,14), only two to four replications per treatment were used because of the number of trees available and/or lack of time and personnel to make disease assessments. The disease intensity (severity, incidence, and defoliation) was recorded on all expanded leaves from 10 arbitrarily selected terminals per tree (replication). With the number of replications fixed by physical constraints, we chose to seek the most efficient sampling method for disease assessment through sampling optimization procedure.

Other studies concerned with determining the optimum sample size in different pathosystems have been conducted for apple scab (2), foliar diseases and nematodes of alfalfa (6,10,15), soilborne pathogens (5), and *Leucostoma* spp., the cause of peach canker (1). Analytis and Kranz (2) suggested 14 leaves on each of 21 terminals per tree as an optimum sample size to determine the differences in severity of apple scab caused by *Venturia inaequalis* (Cooke)

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G. Wint. The objective of our study was to determine the optimum number of terminals per tree and leaves per terminal necessary to assess disease intensity and still obtain the same information about the pathosystem as in previous studies (8,9,13,14). We also wanted to compare the sources of variation (leaves, terminals) among these measures of disease (severity, incidence, defoliation) for the purpose of deciding how to allocate sampling resources in future studies.

MATERIALS AND METHODS

Experiments were conducted at three locations in North Carolina over 4 yr. Although the purpose of each experiment varied, disease assessment procedures were similar in all cases.

1989. This study was conducted at the Central Crops Research Station (CCRS), Clayton, to evaluate various fungicides for the control of Alternaria blotch. Fungicides evaluated were captan, mancozeb, captan + benomyl, and mancozeb + benomyl. There were five treatments, including a nontreated control, and three replications with two trees (subsamples) per replication. Treatments were assigned at random to five-tree groups (replications) grown in two rows containing 10 five-tree groups each; the second and fourth trees (subsamples) from each group were evaluated for disease severity, incidence, and defoliation. The disease assessments were conducted on four different occasions (8).

1990. This fungicide evaluation study was conducted in the same orchard at CCRS as in 1989. The number of treatments was expanded to seven but with only two replications (two five-tree groups and two trees per group) per treatment except for the untreated control, which had four replications. Two rates of iprodione were added to the treatments from the previous year, and disease assessments were made five times (8).

1991. *CCRS.* Seven different fungicide treatments were evaluated at CCRS, using the same trees as in 1990. Treatments were randomly assigned to five-tree groups. Fungicides evaluated were captan + benomyl, triflumizole (three rates), iprodione (three rates), and EXP 10064B (experimental compound, Monsanto Agricultural Company, St. Louis, MO). Three replications (three five-tree groups and two trees per group) per treatment were used. The disease assessments were made four times (13).

McKay. This study was designed to investigate the interaction between *A. mali* and the European red mite (*Panonychus ulmi* (Koch)) and was conducted in the McKay orchard in Henderson County in western North Carolina. Twenty-four trees from a single row were used. Six treatments with different combinations of Alternaria blotch intensity (low and high, maintained with biweekly sprays of iprodione) and mite population levels (low, moderate, and high, maintained with propargite) were randomized within four six-tree blocks (9). Disease assessments were made five times.

1992. *CCRS.* Eight fungicide treatments were evaluated on the same trees as before and treatments were randomly assigned to five-tree groups. Three rates of fluazinam, two rates of iprodione, and their combinations with captan and a spreader-sticker (Latron CS7, Rohm & Haas, Philadelphia, PA) and captan alone were evaluated. There were two replications per treatment (two five-tree groups and two trees per group) except for the control, which had four replications. Disease severity, incidence, and defoliation were assessed once, on 29 August (14).

McKay and Staton. The interaction study initiated at McKay in 1991 was expanded to examine the influence of white apple leafhoppers (*Typhlocyba pomaria* McAtee) and the green apple aphid (*Aphis pomi* De Geer)/spirea aphid (*Aphis spiraecola* Patch) complex, in addition to European red mites, on disease severity, incidence, and defoliation at four different levels of Alternaria blotch. Thirty-six treatments (three arthropod species × three population levels for each arthropod × four disease levels) with three replications were established at McKay and Staton orchards, both in Henderson County. Levels of Alternaria blotch and mites were maintained as in 1991, and three levels of white apple leafhopper populations and three levels of aphid populations were maintained with appropriate applications of methomyl and phosphamidon, respectively. Disease incidence and severity were recorded eight times at each location. In this study, all arthropod treatments were grouped within the four levels of Alternaria

Table 1. Variances for severity (scale 0–5) associated with different combinations of sampling sizes (number of leaves per terminal and number of terminals per tree) averaged over all sampling dates at different years and locations

Year	No. of leaves per terminal	No. of terminals sampled per tree					
Location[z]		2	3	4	6	8	10
1989							
CCRS	3	0.102	0.068	0.048	0.032	0.023	0.019
	5	0.097	0.057	0.037	0.024	0.017	0.014
	10	0.051	0.031	0.021	0.013	0.010	0.008
1990							
CCRS	1	0.113	0.065	0.053	0.030	0.027	0.021
	3	0.066	0.025	0.017	0.011	0.007	0.005
	5	0.021	0.009	0.007	0.005	0.003	0.003
	10	0.013	0.009	0.006	0.003	0.002	0.001
	15	0.007	0.004	0.003	0.002	0.001	0.001
	20	0.004	0.002	0.002	0.001	0.001	0.001
	25	0.003	0.002	0.001	0.001	0.000	0.000
1991							
CCRS	3	0.034	0.015	0.024	0.012	0.010	0.009
	5	0.033	0.017	0.023	0.015	0.011	0.008
	10	0.040	0.019	0.012	0.012	0.008	0.006
	15	0.036	0.017	0.010	0.008	0.006	0.005
	20	0.022	0.010	0.007	0.006	0.005	0.004
	25	0.022	0.009	0.007	0.006	0.005	0.004
McKay	3	0.564	0.259	0.172	0.092	0.058	0.043
	5	0.387	0.166	0.115	0.079	0.051	0.040
	10	0.117	0.051	0.045	0.042	0.027	0.023
	15	0.052	0.026	0.027	0.030	0.021	0.019
	20	0.032	0.022	0.021	0.019	0.014	0.014
	25	0.032	0.017	0.015	0.008	0.005	0.004
1992							
McKay	3	0.237	0.194	0.165	0.086	0.056	0.045
	5	0.202	0.136	0.112	0.069	0.047	0.040
	10	0.093	0.061	0.046	0.032	0.024	0.020
	15	0.056	0.039	0.028	0.020	0.016	0.013
	20	0.041	0.024	0.017	0.014	0.012	0.010
	25	0.033	0.020	0.014	0.011	0.009	0.007
Staton	3	0.134	0.115	0.059	0.051	0.033	0.029
	5	0.105	0.069	0.038	0.038	0.026	0.024
	10	0.092	0.056	0.042	0.032	0.021	0.017
	15	0.048	0.032	0.033	0.022	0.014	0.011
	20	0.036	0.024	0.020	0.014	0.011	0.010
	25	0.036	0.019	0.013	0.010	0.009	0.009

[z] CCRS = Central Crops Research Station, Clayton, North Carolina; McKay = McKay orchard, Henderson County, North Carolina; Staton = Staton orchard, Henderson County, North Carolina.

blotch, because in the preliminary analysis we found that means for optimum sample size calculated for nine individual treatments within four groups were similar to results obtained for single blocks consisting of the same treatments.

Data collection. Trees of the cultivar Delicious were used in all the experiments described above. In each experiment, disease severity was recorded on all unfolded leaves from 10 arbitrarily selected terminals using the lower portion of the Horsfall-Barratt scale (11) of 0–5, where 0 = no symptoms and 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, and 5 = 26–50% of the leaf area covered with lesions. The data on disease incidence for leaves and terminals were derived from severity records. Defoliation was recorded on the final one to three sampling dates of the 1989–1992 growing seasons by calculating the percentage of nodes (on the same 10 terminals per tree) where leaves had abscised. In all experiments conducted at CCRS between 1989 and 1992, the two trees (second and fourth) from each five-tree group (replication) were assumed to be different replications for the purpose of this study.

Statistical analysis. The optimum number of leaves per terminal and number of terminals per tree to be sampled for assessment of disease severity and incidence were determined for each treatment in each experiment and averaged for each location by year. The general linear model (GLM) procedure from SAS (12) was used to determine the variances and mean square errors among sampling elements, subunits, and units (leaves, treatments, and trees, respectively) (12). Data were analyzed as in a three-stage sampling design (leaves within terminals, terminals within trees, and trees within the orchard). The equations suggested by Campbell and Madden (4), adopted from Analytis and Kranz (2), were used to calculate the optimum number of leaves and terminals. Equation 1 was $n_{ss}(opt) = [(MSE \times n_{ss})/(MST_e - MSE)]^{1/2} \times (C_s/C_e)^{1/2}$, and equation 2 was $n_s(opt) = \{[(MST_e - MSE) \times n_s)]/(MST_r - MST_e)\}^{1/2} \times (C_u/C_s)^{1/2}$. In these equations, $n_{ss}(opt)$ = optimum number of leaves per terminal, MSE = mean sampling error (the error associated with variation among leaves on the same terminal), n_{ss} = actual number of leaves per terminal sampled, MST_e = mean sampling error associated with variation among terminals on the same tree, C_s = cost of selecting and moving to a new subunit (in our case a terminal) expressed in seconds, C_e = cost of locating and sampling an element (leaf) (seconds), $n_s(opt)$ = optimum number of terminals per tree, MST_r = mean sampling error associated with variation among trees (replications) within an orchard, n_s = actual number of terminals per tree sampled, and C_u = cost of selecting a new unit (tree) for

Table 2. The optimum sample size for Alternaria blotch of apple obtained from three locations during 1989–1992

Year Location[y]	Date	No. of treatments	Disease component	Optimum no. of leaves per terminal	Optimum no. of terminals per tree
1989					
CCRS	20 July	5	Severity	8.60	1.65
			Incidence	17.36	1.23
	14 Aug.	5	Severity	17.49	0.72
			Incidence	24.94	0.82
			Defoliation		1.07
1990					
CCRS	21 May	7	Severity	24.95	1.69
			Incidence	24.95	1.69
	8 June	7	Severity	46.68	1.14
			Incidence	46.68	1.14
1991					
CCRS	31 May	7	Severity	22.28	1.21
			Incidence	23.83	1.19
	15 July	7	Severity	11.48	1.53
			Incidence	11.48	1.53
	8 Aug.	7	Severity	9.60	2.82
			Incidence	11.20	1.40
			Defoliation		3.04
McKay	31 May	6	Severity	27.79	0.91
			Incidence	27.79	0.91
	17 June	6	Severity	16.94	1.26
			Incidence	20.81	2.29
	24 July	6	Severity	10.69	2.92
			Incidence	21.34	3.16
	22 Aug.	6	Severity	8.64	2.12
			Incidence	16.98	1.65
			Defoliation		3.97
1992					
CCRS	29 Aug.	9	Severity	15.44	1.22
			Incidence	14.56	1.35
			Defoliation		2.76
McKay	29 June	4	Severity	24.40	0.51
			Incidence	32.56	0.15
			Defoliation		2.56
	14 July	4	Severity	17.49	0.43
			Incidence	17.17	1.23
			Defoliation		1.41
	30 July	4	Severity	9.94	0.68
			Incidence	11.73	0.64
			Defoliation		1.70
	18 Aug.	4	Severity	11.37	0.61
			Incidence	15.38	0.58
			Defoliation		1.47
	4 Sept.	4	Severity	8.86	0.64
			Incidence	12.11	0.83
			Defoliation		1.49
Staton	30 June	4	Severity	19.82	0.87
			Incidence	22.56	0.97
			Defoliation		3.37
	15 July	4	Severity	16.96	0.85
			Incidence	17.26	0.78
			Defoliation		1.65
	31 July	4	Severity	11.90	0.87
			Incidence	11.82	0.81
			Defoliation		1.70
	19 Aug.	4	Severity	16.57	0.73
			Incidence	11.16	0.85
			Defoliation		1.18
	5 Sept.	4	Severity	13.43	0.96
			Incidence	12.37	0.79
			Defoliation		1.19
Mean			Severity	16.88 a[z]	1.20 b
			Incidence	19.37 a	1.18 b
			Defoliation		2.04 a

[y] CCRS = Central Crops Research Station, Clayton, North Carolina; McKay = McKay orchard, Henderson County, North Carolina; Staton = Staton orchard, Henderson County, North Carolina.

[z] Means followed by the same letter within a column are not significantly different at $P = 0.05$ according to the Waller-Duncan k-ratio t test.

sampling (seconds).

When defoliation was assessed, only an optimum number of terminals per tree was calculated because there is only one value (percent defoliation) per terminal. The calculations for this part of the study were conducted as in a two-stage sampling design (2,4). The equation used was that recommended by Campbell and Madden (4) to calculate the optimum number of terminals per tree for each treatment. Equation 3 was $n_s(opt) = (\sigma_e/\sigma_u) \times (C_e/C_{eu})^{1/2}$, where $n_s(opt)$ = the optimum number of terminals per tree, σ_e = (mean square sampling error associated with variation among elements (terminals))$^{1/2}$, σ_u = (mean square error associated with variation among units (replications))$^{1/2}$, C_e = the cost of obtaining the defoliation assessment from a single element (terminal), and C_{eu} = the cost of selecting the first element (terminal) on the next unit (tree).

The variance around the mean was determined for each treatment in each experiment using equation 4: $Var = MST/(n_s \times n_{ss})$, where Var = variance around overall mean for each treatment, MST = mean square error associated with variation among terminals, n_s = number of terminals per tree sampled, and n_{ss} = number of leaves per terminal sampled.

The GLM procedure from SAS (12) was used to calculate the coefficient of variation (CV) and the least significant difference (LSD) for all the above sampling dates and locations. All the individual treatments were utilized with the exception of 1992, when four groups of nine treatments from the McKay and Staton orchards were analyzed as described previously. The values for CV and LSD were obtained for different number of leaves on the optimum number of terminals per tree, using the exclusion statements in SAS (12). The relationship between CV and LSD and the number of leaves sampled were described with power (equation 5) and logarithmic (equation 6) functions. Equation 5 was $Y = a(x)^b$, with $a > 0$, and equation 6 was $Y = a + b(\ln x)$, where Y = CV or LSD, a and b = coefficients, and x = number of leaves sampled per terminal.

The cost functions were expressed as time (seconds). All other costs, such as

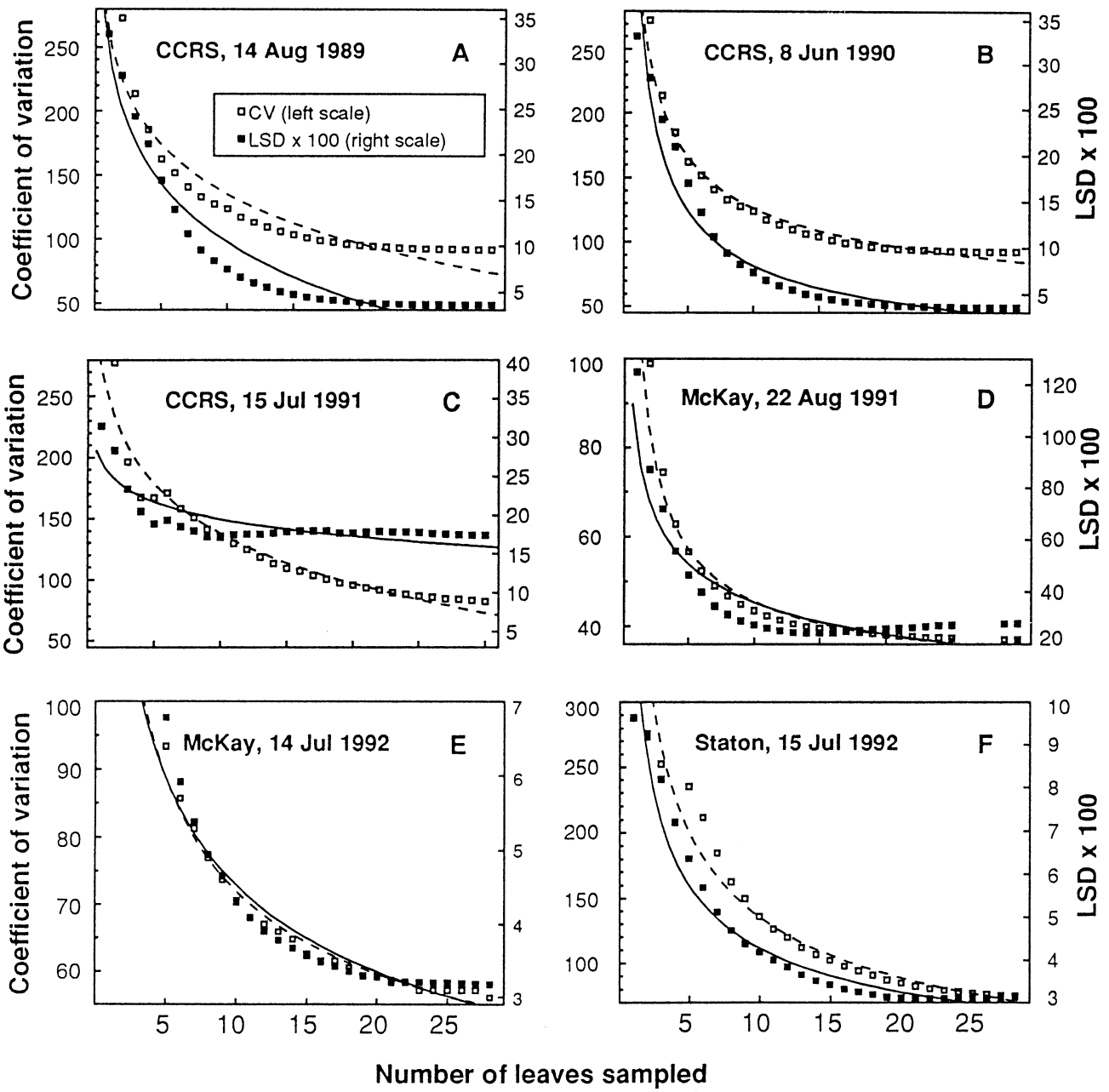

Fig. 1. The relationship between CV and LSD for severity (0–5 scale) and number of sampled leaves from two terminals per tree: **(A)** CCRS 1989, **(B)** CCRS 1990, **(C)** CCRS 1991, **(D)** McKay 1991, **(E)** McKay 1992, and **(F)** Staton 1992. CCRS = Central Crops Research Station, Clayton, North Carolina; McKay = McKay orchard, Henderson County, North Carolina; Staton = Staton orchard, Henderson County, North Carolina.

expense of travel to the orchards, spraying and maintenance of the orchards, and input of data to the computer, were disregarded (6). All data in 1992 were recorded on a tape recorder. To determine the cost functions, the tape was played after the data were taken and a stopwatch was used to calculate the time needed to complete disease assessment for each sample component (individual leaves, terminals, time to select new terminal and tree, etc.). The cost function $(C_s/C_e)^{1/2}$ in equation 1 was assumed to be equal to three, because the time required to select a new terminal was approximately nine to 10 times as long as the time required to assess the disease on the individual leaf. In equation 2, the cost function $(C_u/C_s)^{1/2}$ was assumed to be equal to one, because the cost of selecting a new tree for sampling was approximately equal to the time required to select a new terminal on the same tree. From equation 3, the cost function (C_e/C_{eu}) was approximately equal to one, because the time required to select the first terminal on the next tree for sampling, on average, was about the same as the time required to assess defoliation on a single terminal.

RESULTS

Variances for severity on the 0–5 scale calculated for each sampling date generally declined linearly as the number of terminals and leaves increased, except for a large decrease when 10 leaves per terminal were sampled compared to five leaves and when three terminals per tree were sampled compared to two (Table 1). However, when the cost function was included in the analysis, the optimum number of leaves was higher because of the very low cost associated with obtaining the disease assessment from a single leaf (Table 2). The optimum numbers of leaves per terminal to estimate severity and incidence and terminals per tree to estimate severity, incidence, and defoliation averaged over all sampling dates, locations, and experiments were 18.1 and 1.5, respectively. The mean optimum numbers of leaves per terminal and terminals per tree were higher, but not significantly ($P = 0.05$), when the disease incidence was assessed as opposed to disease severity (19.37 leaves and 1.18 terminals for incidence compared with 16.88 leaves and 1.2 terminals for severity). The mean optimum numbers of terminals per tree for severity and incidence assessment were lower than the optimum number for defoliation assessment (2.04). Results were similar for all the years and locations (Table 2).

The CV and LSD values for severity increased as the number of sampled leaves and terminals decreased (Fig. 1). The relationship between CV and LSD and number of leaves sampled per terminal was described by power and logarithmic functions. The coefficients a and b from equations 5 and 6 and the coefficients of determination are summarized in Tables 3 and 4.

The increase of CV and LSD values, when plotted against the number of leaves sampled on two terminals, usually occurred near values for the number of leaves sampled that were similar to those calculated for the optimum number of leaves when cost was taken into account (Fig. 1, Table 2).

DISCUSSION

The optimum number of terminals to sample for estimation of incidence, severity, and associated defoliation caused by *A. mali* from our analysis was lower than that obtained by Analytis and Kranz (2) for apple scab. They suggested sampling 13.2 leaves from 20.2 terminals per tree. However, in the case of Alternaria blotch, increasing the number of terminals per tree did not significantly reduce variation. The differences in results between the two studies may be a result of differences in the cost function $(C_s/C_e) = 2$ that Analytis and Kranz (2) used for selecting terminals as opposed to $(C_s/C_e) = 9$ in our analysis. Also, the value of the cost function $(C_u/C_s) = 1$ was applicable for our study because trees were located near one another, so it did not take long to walk to the next tree and select the first terminal for sampling. However, if trees were located farther from one another, this function would be larger and would result in an increase in the optimum number of terminals per tree. Other possibilities are that it actually took Analytis and Kranz (2) longer to assess disease on a leaf or they were able to locate new terminals more quickly. We have confidence in the cost

Table 3. The coefficients (a and b) from equation 5 (power function) and coefficients of determination (R^2) calculated for disease severity (scale 0–5)

Year Location[y]	Parameter[z]	a	b	R^2
1989				
CCRS	CV	321.51	−0.60	0.96
	LSD	42.36	−0.47	0.97
1990				
CCRS	CV	2,826.64	−0.73	0.89
	LSD	9.75	−0.24	0.88
1991				
CCRS	CV	197.86	−0.13	0.74
	LSD	54.49	−0.53	0.94
McKay	CV	80.14	−0.25	0.82
	LSD	156.18	−0.63	0.96
1992				
McKay	CV	139.79	−0.28	0.92
	LSD	11.81	−0.42	0.98
Staton	CV	365.38	−0.52	0.92
	LSD	15.11	−0.48	0.99

[y] CCRS = Central Crops Research Station, Clayton, North Carolina; McKay = McKay orchard, Henderson County, North Carolina; Staton = Staton orchard, Henderson County, North Carolina.
[z] CV = coefficient of variation, LSD = least significant difference among treatments ($P = 0.05$).

Table 4. The coefficients (a and b) from equation 6 (logarithmic function) and coefficients of determination (R^2) calculated for disease severity (scale 0–5)

Year Location[y]	Parameter[z]	a	b	R^2
1989				
CCRS	CV	248.44	−65.58	0.93
	LSD	33.60	−7.80	0.89
1990				
CCRS	CV	1,693.06	−464.95	0.77
	LSD	8.42	−1.24	0.75
1991				
CCRS	CV	199.03	−21.96	0.72
	LSD	39.56	−9.58	0.88
McKay	CV	79.67	−14.19	0.79
	LSD	112.92	−30.63	0.82
1992				
McKay	CV	120.45	−20.23	0.89
	LSD	8.66	−1.78	0.94
Staton	CV	295.87	−73.80	0.89
	LSD	11.27	−2.58	0.90

[y] CCRS = Central Crops Research Station, Clayton County, North Carolina; McKay = McKay orchard, Henderson County, North Carolina; Staton = Staton orchard, Henderson County, North Carolina.
[z] CV = coefficient of variation, LSD = least significant difference among treatments ($P = 0.05$).

functions we used because they were obtained by recording actual disease ratings on a tape recorder and calculating costs from a series of measurements from the readings. Adams et al (1) also reported that increasing the number of terminals per tree did not have a large influence on variation in detecting the virulence of different isolates of *L. cincta* and *L. persooni* on peach.

We used both formal and informal methods to estimate the optimum sample size (3). The informal method, where CV and LSD were plotted against number of leaves sampled on two terminals, provided us with a graphic indicator of an optimum sampling size. To conduct the formal methods for estimating optimum sampling size, we included a cost function and conducted a calculation as in two- and three-stage sampling designs. Because our results were similar for three different locations with different levels of the disease (8,9,13,14) and over a period of 4 yr, we suggest that in our case a sample of two terminals per tree with not more than 20 leaves per terminal will provide a satisfactory assessment of the incidence, severity, and defoliation of Alternaria blotch of apple necessary to detect treatment differences. This sampling design will enable us to significantly reduce the cost of sampling and to further expand our studies to include more locations and/or to increase the number of sampled trees (replications) where possible.

Our findings should be applicable for different levels of the disease, since the data we utilized in our study were obtained from experiments conducted over a range of different conditions. For example, very low levels of disease, induced by artificial inoculation with *A. mali* spore suspensions, was present at CCRS from 1989 to 1992 (8,14), whereas high levels of disease, with up to 57% defoliation, were present at the McKay orchard in 1991 and 1992 (9; *unpublished*). Furthermore, the environmental conditions at these two locations differ considerably; CCRS is located in the coastal plain in North Carolina and is warmer and drier than the McKay and Staton orchards located in the mountains.

The mean optimum number of leaves per terminal when the disease incidence was assessed was higher than that for disease severity. This result was expected because in the later part of the growing season, when disease incidence approaches 100%, it is easier to detect treatment differences when disease severity is assessed on a scale of 0–5 than when disease incidence is assessed. For example, in the case of the McKay orchard in 1992, disease severity increased dramatically in the later part of the summer, and the value for optimum number of leaves per terminal was lower in July, August, and September than in June. A similar trend was observed for the optimum number of terminals per tree when defoliation was assessed. The optimum number of terminals per tree to sample was lower for both the McKay and Staton orchards in the later part of the 1992 growing season, approximately mid-July, after defoliation became more apparent (Table 1). Similarly, the optimum numbers of leaves per terminal and terminals per tree to sample were greatest at CCRS, which had the lowest disease level. For example, over all years, in the early part of the season (May-June), optimum numbers of leaves and terminals to assess severity at CCRS were 31.3 and 1.38, respectively, whereas at McKay and Staton combined, the values were 22.2 and 0.84, respectively. This suggests that the optimum sampling design for Alternaria blotch does not necessarily need to be the same for the entire season or for every orchard. A more conservative approach is needed in the beginning of the season, when differences among treatments are not as evident. Because the number of new leaves is limited at that time, the number of terminals sampled per tree should be increased to three to five until the number of leaves per terminal reaches 20 and/or disease symptoms increase and defoliation becomes noticeable. Thus, it is very important to clearly determine the objectives of a study (i.e., how small a difference among treatments needs to be detected) before the decision on optimum sample size is made.

The different measures of the disease (severity, incidence, and defoliation) are useful for different purposes within this pathosystem. For epidemiological studies, disease severity may have more utility than incidence, as it relates better to defoliation and yield loss (*unpublished*). However, severity assessment requires greater skill and may be too time-consuming for growers, at least if the 0–5 scale is used. Disease incidence can be assessed fairly quickly and accurately and should be sufficient when considering decisions on whether or not to apply fungicides.

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