Sequential Sampling Plan for Timing Initial Fungicide Application to Control Botrytis Leaf Blight of Onion

P. C. Vincelli and J. W. Lorbeer

Research assistant and professor, respectively, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853.

This research was supported in part by the Cornell University Integrated Pest Management Program and the Orange County Vegetable Improvement Cooperative Association, Inc.

We thank J. Nyrop, D. S. Robson, and S. McAllister for helpful comments.

Accepted for publication 26 February 1987.

ABSTRACT

Vincelli, P. C., and Lorbeer, J. W. 1987. Sequential sampling plan for timing initial fungicide application to control Botrytis leaf blight of onion. Phytopathology 77:1301-1303.

A sequential sampling plan (SSP) that optimizes sampling intensity for determining disease levels of Botrytis leaf blight in onion fields was developed. With it, lesion counts are made on 15–50 onion plants per field to determine if the mean disease level has reached the critical disease level (CDL) of 1.0 lesion per leaf, an action threshold that calls for the initiation of a fungicide spray program. The SSP was validated by sampling from 15 to 50 computer-simulated lesion counts representing sequentially sampled plants until it was concluded that the disease level was either above or below the CDL. Five hundred conclusions were tested at each of nine disease levels (μ), where μ ranged from 0.2 to 1.8 lesions per leaf at 0.2 lesion per leaf increments. For $\mu \geqslant 1.0$ and $\leqslant 0.6$ lesions per leaf, correct conclusions were generally made that the CDL had ($\geqslant$ 99.8% accuracy) and had not ($\geqslant$ 97.8% accuracy) been reached, respectively. For $\mu = 0.8$ lesions per leaf, the SSP often led to the conclusion that the CDL had been reached, indicating the conservative nature of the SSP. For $\mu \leqslant 0.6$ and $\geqslant 1.4$ lesions per leaf, conclusions were generally reached after sampling 15–35 plants, thereby reducing sampling intensity from the current 50 plants. Similar results were obtained with field data.

Botrytis leaf blight of onion (*Allium cepa* L.), caused by *Botrytis squamosa* Walker (anamorph of *Botryotinia squamosa* Viennot-Bourgin), is an important foliar disease of onion in New York. Although sanitation practices can reduce disease development (4), a regular spray schedule of protectant fungicides is the principal means of controlling the disease. Growers using fixed spray schedules typically initiate their spray programs in mid-June and make subsequent applications at 7–10-day intervals until late August. An alternative to a fixed spray program is to apply the first fungicide application when leaf blight reaches a critical disease level (CDL) (8,10). This tactic involves walking onion fields and counting lesions caused by *B. squamosa* on the three oldest leaves on 50 plants. Thus, fungicide sprays are withheld until an action threshold, the CDL of 1.0 lesion per leaf, is reached in that field (8). Subsequent applications generally are made according to a fixed schedule.

Although the CDL method has been successfully used as a standard part of the Cornell Onion Integrated Pest Management (IPM) program for several years (1), sampling to determine the level of leaf blight is the most time-consuming part of IPM scouting procedures for onion. A sequential sampling plan (SSP) could optimize scouting time by allowing the scout to estimate the disease level after each sample rather than taking a fixed number of samples, thereby reducing the number of plants sampled when disease levels are well above or below a specified action threshold (12). Although an SSP is available for *B. squamosa* (2), this plan is based on a different action threshold and a different sampling procedure than those currently used in New York. In this study an SSP for Botrytis leaf blight was developed, and its performance was assessed through computer simulation.

MATERIALS AND METHODS

A total of 50 leaf blight surveys were made in 16 commercial onion fields located in Orange County, NY. Twenty-five surveys were made during the 1983–1985 growing seasons, and 25 surveys were made during the 1986 season. The fields sampled were up to 2 ha in size, sampled between 14 June and 5 August, and planted to the cultivars Downing Yellow Globe, Early Yellow Globe, Sentinel, or Spartan Banner. Lesions caused by *B. squamosa* were counted on the three oldest leaves ($\geqslant$ 80% green) on 50 randomly selected plants per field. In 47 of the 50 surveys, lesion counts were made before fungicide treatments or from plants sampled from several randomly placed 12-m^2 areas not treated with fungicide. No significant effect of fungicide treatment on the variance-to-mean ratio of lesion counts was observed.

An SSP was developed according to a procedure outlined by Iwao (6). Three parameters were calculated for each sampling occasion at each field: $\bar{x}$ = the mean number of lesions per plant, s^2 = the variance of number of lesions per plant, and $\overset{*}{x}$ = the mean crowding of the sample. Mean crowding was calculated according to the formula

$$\overset{*}{x} = \bar{x} + (s^2/\bar{x}) - 1 \quad (1)$$

and is a measure of the degree of crowding per habitat unit (7). For calculation of dispersion statistics, a linear regression of $\overset{*}{x}$ on $\bar{x}$ (5) was fitted to data from the 25 field surveys made during 1983–1985. The intercept (b_0) and slope (b_1) from this regression were used to determine upper and lower sampling limits for the SSP, using the following formulae (6):

$$T_{upper} = n(\mathrm{CDL}) + t[n(b_0 + 1)\mathrm{CDL} + n(b_1 - 1)\mathrm{CDL}^2]^{1/2} \quad (2)$$

and

$$T_{lower} = n(\mathrm{CDL}) - t[n(b_0 + 1)\mathrm{CDL} + n(b_1 - 1)\mathrm{CDL}^2]^{1/2} \quad (3)$$

where T = total number of lesions, n = number of plants sampled, t = value of Student's t at $\alpha = 0.025$ (two-sided test, df $= \infty$) (11), and CDL = critical disease level of 3.0 lesions per plant (average of 1.0 lesion per leaf on the three oldest leaves $\geqslant$ 80% green).

A computer simulation was conducted to assess sampling intensity and to evaluate error rates (9) over a range of lesion densities. In the simulation experiments, the protocol of the sampling plan was applied to simulated lesion counts that represented individual sequentially sampled plants with a random number of lesions. These lesion counts were obtained by generating negative binomially distributed random variates, with μ = mean number of lesions per plant and with the aggregation parameter (k) of the negative binomial distribution given by the formula

$$k = \mu^2/(\sigma^2 - \mu). \quad (4)$$

Values of σ^2 for the simulations were obtained by substituting the regression equation $\overset{*}{x} = b_0 + b_1 \bar{x}$ for $\overset{*}{x}$ in equation 1 and solving for s^2. Five hundred conclusions, each representing a simulated field survey, were generated at each of nine mean disease levels examined, ranging from 0.6 to 5.4 lesions per plant (average of 0.2–1.8 lesions per leaf on three leaves). Because individual plants are the sampling unit, the dispersion statistics and sequential sampling limits described above, as well as lesion counts for the simulated surveys, were generated on a lesions-per-plant basis. Results are presented on a lesions-per-leaf basis to conform to current practice (1,2,8,10).

RESULTS

The regression of $\overset{*}{x}$ on $\bar{x}$ yielded the equation $\overset{*}{x} = 1.323 + 1.394\,\bar{x}$ ($P < 0.0001$, $r^2 = 0.935$). Confirming previous work (2), the intercept value $b_0 = 1.323$ was significantly greater than zero ($P < 0.05$), and the slope value $b_1 = 1.394$ was significantly greater than one ($P < 0.001$). These results indicate that lesions caused by *B. squamosa* tend to be aggregated in the field (2,5). Kolmorgorov's goodness-of-fit test (3) was used to compare observed lesion counts to expected values that fit a negative binomial distribution model. Failure to reject ($P > 0.10$) the null hypothesis of agreement between observed and expected values indicated that lesion counts of *B. squamosa* followed a negative binomial distribution. It was found that a minimum sample of 15 plants is necessary to meet the assumption of normality, a critical assumption in Iwao's procedure (9). This was determined by randomly generating 10 means of $n = 15$ from field surveys for each of five fields. The Shapiro-Wilks Test for normality (3) then was used to determine that these means conformed to the null hypothesis of a normal distribution model ($P > 0.10$).

To use the SSP generated with equations 2 and 3, *B. squamosa* lesions are counted on a minimum of 15 plants. If the total number of lesions counted is less than or equal to the lower limit for 15 plants (Table 1), then sampling is ended and the conclusion drawn is that μ is below 1.0 lesion per leaf, where μ is the true mean lesion count for the field in question. The first fungicide application could safely be postponed in this situation. Likewise, if the total number of lesions counted is greater than or equal to the upper limit for 15 plants (Table 1), then sampling is ended, and the conclusion drawn is that μ is above 1.0 lesion per leaf and that a fungicide program should be initiated. If the total number of lesions counted lies between the upper and lower limits, then sampling continues on a plant-by-plant basis until either a conclusion is reached or 50 plants have been sampled. An arbitrary sampling limit of $n = 50$ plants was selected based on our estimate of the maximum time that could be reasonably spent sampling an onion field for leaf blight. If 50 plants are sampled and no conclusion is reached, there is insufficient evidence to conclude that μ is significantly different from 1.0 lesion per leaf. In such cases, it is recommended that a fungicide spray program be initiated.

It was found in simulation experiments that, over most disease levels, the SSP correctly indicated when the disease level was above or below the CDL (Table 2). A correct conclusion was reached at least 97.8% of the time for simulated field surveys where μ was equal to or below 0.6 lesions per leaf, and a conclusion was often reached after sampling 30 plants or less. For $\mu = 0.8$ lesions per leaf, 93.6% of the surveys resulted in the decision that μ was not significantly different from the 1.0 lesion per leaf level. For $\mu \geqslant 1.0$ lesion per leaf, at least 99.8% of the simulated surveys led to the decisions that $\mu = 1.0$ lesion per leaf or that $\mu > 1.0$ lesion per leaf, both of which call for the initial fungicide application. Sampling became less intensive as μ exceeded 1.2 lesions per leaf (Table 2).

The SSP was applied to all 50 surveys of lesion counts collected from commercial onion fields. Conclusions reached using the SSP were compared to conclusions reached using the mean number of lesions per leaf ($\bar{x}$) from a fixed sample of $n = 50$. Confirming the simulation results, complete agreement was found between the

TABLE 1. Upper and lower sampling limits of a sequential sampling plan for timing the initial fungicide application for control of Botrytis leaf blight of onion

Plants sampled (no.)	Lesions counted (total no.)	
	Lower limit	Upper limit
15	17	73
16	19	77
17	21	81
18	23	85
19	25	89
20	28	92
21	30	96
22	32	100
23	34	104
24	36	108
25	39	111
26	41	115
27	43	119
28	46	122
29	48	126
30	50	130
31	53	133
32	55	137
33	57	141
34	60	144
35	62	148
36	64	152
37	67	155
38	69	159
39	72	162
40	74	166
41	76	170
42	79	173
43	81	177
44	84	180
45	86	184
46	89	187
47	91	191
48	94	194
49	96	198
50	99	201

TABLE 2. Evaluation of a sequential sampling plan for *Botrytis squamosa* using computer-simulated lesion counts[a]

Lesions/leaf[b] (mean no.)	Probability of reaching specified conclusion[c]			Plants sampled[d] (mean no.)
	$\mu < 1.0$ lesion/leaf	$\mu = 1.0$ lesion/leaf	$\mu > 1.0$ lesion/leaf	
0.2	1.000	0.000	0.000	15.0
0.4	1.000	0.000	0.000	17.1
0.6	0.978	0.022	0.000	30.5
0.8	0.064	0.936	0.000	49.2
1.0	0.002	0.998	0.010	49.6
1.2	0.000	0.828	0.172	46.7
1.4	0.000	0.744	0.256	32.4
1.6	0.000	0.014	0.986	20.0
1.8	0.000	0.004	0.996	17.5

[a] Five hundred simulated field surveys per mean disease level.
[b] Lesion counts in each simulated survey were negative binomially distributed random variates with μ = mean number of lesions per leaf and $k = \mu^2/(\sigma^2 - \mu)$.
[c] Number of surveys where specified conclusion is reached divided by total number of surveys.
[d] Mean number of plants sampled $= 1/n\, \Sigma_{i=1}^{n} x_i$, where x_i = number of plants sampled in the ith survey, and n = total number of surveys.

sequential sampling plan and a fixed sample plan for $\bar{x} < 0.8$ and $\bar{x} > 1.0$ lesion per leaf. Conclusions generally were reached within 35 plants, although up to 50 plants were sampled using the SSP as $\bar{x}$ became close to 1.0 lesion per leaf. For $0.8 \leqslant \bar{x} < 1.0$ lesion per leaf, the conclusion generally reached was that the CDL had been reached and that a fungicide application was necessary, in agreement with the simulation results.

DISCUSSION

The overall performance of the SSP in both simulation experiments and with field data indicates that correct conclusions can be expected over most levels of disease intensity found in the field. Incorrect conclusions were reached in some simulated surveys and field surveys in which it was concluded that μ had reached the 1.0 lesion per leaf action threshold when in fact μ was below the CDL. However, this should not constitute an important error in practice because it only occurred at disease levels that approached the 1.0 lesion per leaf level. The alternative error—concluding that a fungicide application was unnecessary when in fact the CDL had been reached—only occurred once in 2,500 simulated field surveys and not at all with field data, indicating that conclusions reached with the SSP are desirably conservative.

Although an SSP for *B. squamosa* had been developed previously (2), no assessment was made of the accuracy of conclusions reached using that sampling plan. In this study, computer simulation of leaf blight field surveys permitted a detailed assessment of the reliability of conclusions reached with the SSP over a range of disease levels. The value of simulation in evaluating the SSP was confirmed by the complete agreement of field and simulation results. Previous work on sequential sampling for *B. squamosa* (2) has not taken into account the assumption of normality of lesion counts that is inherent in Iwao's sequential sampling procedure (9). It was found that at least 15 plants should be sampled to meet this assumption and to invoke the Central Limit Theorem (11), on which Iwao's statistics are based (9).

The SSP developed in this study offers a time-saving alternative to a fixed sample plan of $n = 50$ by allowing less intensive sampling when disease levels are well above or below the CDL of 1.0 lesion per leaf. Use of the SSP should reduce both scouting time and fatigue, because it will often be possible to examine fewer plants for lesions of *B. squamosa*. However, it is important that plants included in the sample be from widely scattered areas of the field to obtain a representative sample, and scouts should include the full length of a field as part of their normal procedure. It is suggested that the SSP be applied to areas of no greater than 2 ha that are homogenous with respect to onion type (early vs. late-maturing varieties, yellow vs. red onions, etc.), planting date, proximity to sources of primary inoculum, and other factors which might influence development of Botrytis leaf blight.

LITERATURE CITED

1. Andaloro, J. T., and Lorbeer, J. W. 1982. Implementation of an integrated pest management pilot program for onions in New York. (Abstr.) Phytopathology 72:257.
2. Boivin, G., and Sauriol, P. 1984. Dispersion statistics and sequential sampling plan for leaf blight caused by *Botrytis squamosa* in onions. Phytopathology 74:1385-1387.
3. Conover, W. J. 1980. Practical Nonparametric Statistics, 2nd ed. J. Wiley & Sons, New York. 493 pp.
4. Ellerbrock, L. A., and Lorbeer, J. W. 1977. Sources of primary inoculum of *Botrytis squamosa*. Phytopathology 67:363-372.
5. Iwao, S. 1968. A new regression method for analyzing the aggregation pattern of animal populations. Res. Popul. Ecol. 10:1-20.
6. Iwao, S. 1975. A new method of sequential sampling to classify populations relative to a critical density. Res. Popul. Ecol. 16:281-288.
7. Lloyd, M. 1967. Mean crowding. J. Anim. Ecol. 36:1-30.
8. Lorbeer, J. W., and Jares, T. W. 1981. Field sampling patterns for determining the critical disease level for initiating fungicidal control of Botrytis leaf blight of onion. (Abstr.) Phytopathology 71:238.
9. Nyrop, J. P., and Simmons, G. A. 1984. Errors incurred when using Iwao's sequential decision rule in insect sampling. Environ. Entomol. 13:1459-1465.
10. Shoemaker, P. B., and Lorbeer, J. W. 1977. Timing initial fungicide application to control Botrytis leaf blight epidemics on onions. Phytopathology 67:409-414.
11. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, Iowa. 507 pp.
12. Wald, A. 1947. Sequential Analysis. J. Wiley & Sons, New York. 212 pp.