# Dispersion Statistics and Sequential Sampling Plan for Leaf Blight Caused by *Botrytis squamosa* in Onions

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## ABSTRACT

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The spatial distribution of *Botrytis squamosa* on onions was studied by using Iwao's patchiness regression technique. The basic components of the Botrytis leaf blight lesion population were aggregates that were distributed contagiously in onion fields. Fungicide treatment did not alter this spatial distribution. This information was used to prepare a sequential sampling plan with one-half lesion per leaf as the economic threshold. At least ten one-leaf samples were needed to detect significant differences from the critical threshold of one-half lesion per leaf and up to 54 leaves were needed when the disease level was at or near the half-lesion-per-leaf threshold.

*Additional key words*: economic threshold, mean crowding.

Botrytis leaf blight caused by *Botrytis squamosa* J. C. Walker is a major foliar disease of onions (*Allium cepa* L.) in Québec. It causes reduction of bulb size and yield, especially when periods of warm, wet weather prevail during the growing season. Most growers control Botrytis leaf blight with weekly applications of fungicide starting early in July. In an integrated pest management (IPM) program where treatments are started only when the disease has reached a predetermined threshold and repeated only when the weather conditions are favorable for infection (3,13), unnecessary fungicide applications are avoided. An IPM program was initiated in the muck soil onion-growing region of southwestern Québec in 1982 (12), but disease sampling problems were encountered because no reliable technique was available for determining when the threshold of Botrytis leaf blight had been reached.

The number of samples necessary to obtain an estimate of the density of diseased plants with a known precision varies with the spatial distribution of the disease in a field (14); therefore, dispersion statistics are required to establish sampling procedures. Relatively few studies have dealt with the spatial distribution of disease (1,4,11,15,18). Lorbeer and Jares (9) reported that a single sampling transect across the field is the most efficient sampling pattern for Botrytis leaf blight; however, the spatial distribution of this disease has never been studied.

Sequential sampling allows the observer to estimate the population density of a disease after each sample, instead of taking a fixed number of samples (14). Such a procedure enables a rapid classification of population levels relative to a predetermined threshold (6) and can reduce by 47–63% the number of samples needed to achieve a given level of precision (19). Because the evaluation of disease density before and after fungicide treatments involves an important part of the human and economic resources of an IPM program, efficient sampling is essential.

## MATERIALS AND METHODS

This study was conducted in the muck soil region of Ste-Clotilde and Napierville in southwestern Québec, Canada. Six commercial fields of onions (cultivars Taurus, Sentinel, Exporter, and Trapp 8) were chosen and a 0.5–1.0-ha area in each field was chosen for sampling. At weekly intervals from the end of June to mid-August 1982, fifty samples were taken at random by walking 20–50 paces at random and sampling the onion nearest to the right foot. The sample unit consisted of the oldest erect leaf of the onion plant and the datum recorded was the number of Botrytis lesions on it.

**Dispersion statistics.** At each sampling, the mean number of lesions per leaf was determined and used to calculate the mean crowding of the sample, $\overset{*}{x}$ (8).

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

in which $\overset{*}{x}$ = the mean crowding of the sample, $\bar{x}$ = the mean number of lesions per leaf, and $s^2$ = the variance of the number of lesions per leaf.

These statistics show the intensity of the interactions between individuals as they express the degree of "crowding" in a habitat unit. The patchiness regression, which is the simple linear regression of $\overset{*}{x}$ on $\bar{x}$, quantifies the aggregation of individuals (5,7).

In the regression equation, $\overset{*}{x} = b_0 + b_1 \bar{x}$, $b_0$ = the index of basic contagion (intercept) and $b_1$ = the density-contagiousness coefficient (slope).

When $b_0 = 0$, a single individual is the basic component of the distribution and when $b_0$ is $>0$ or $<0$, there is a positive or negative association between individuals. The density-contagiousness coefficient ($b_1$) indicates how the basic components are distributed in space. When $b_1 < 1$, the distribution is regular in space; when $b_1 = 1$, the basic components are randomly distributed in space and when $b_1 > 1$, the basic components are distributed contagiously in space. In the latter case, the value of $b_1$ indicates the degree of aggregation of the basic components. The possible distributions and their ecological implications are discussed by Iwao (7).

The regression of $\overset{*}{x}$ on $\bar{x}$ was calculated for each onion field and the values of $b_0$ and $b_1$ were tested for significant differences from 0 and 1 by means of $t$ tests (2).

**Sequential sampling.** A sequential sampling procedure proposed by Iwao (6) was used. Many other sequential sampling procedures are based upon the tentative fitting of sample data to theoretical distribution models such as the Poisson or negative binomial. Iwao's method of sequential sampling does not rely on fitting sample data to theoretical distributions. Iwao's method can be used

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with no restrictions on the distribution patterns; the only requirement is that the regression of $\overset{*}{x}$ on $\bar{x}$ for the species has a significant correlation coefficient.

The curves for the upper and lower acceptance limits for the sequential sampling were obtained by using:

$$T_{\text{unper}} = nx' + t[n(b_0 + 1)x' + (b_1 - 1)x'^2]^{1/2} \quad (2)$$

and

$$T_{\text{lower}} = nx' - t[n(b_0 + 1)x' + (b_1 - 1)x'^2]^{1/2} \quad (3)$$

in which $T$ = the total count of lesions, $n$ = the number of samples taken, $x'$ = the economic threshold, and $t$ = the value of Student's $t$ test at chosen level of significance for a two-sided test and an infinite number of degrees of freedom.

The maximum number of samples to be taken was determined by

$$n_{\max} = t^2\,[(b_0 + 1)x' + (b_1 - 1)x'^2]/d^2 \quad (4)$$

in which $d^2$ = the confidence interval allowable for the estimation of density when $x$ exactly equals $x'$.

When the maximum number of samples is reached before a sample stop line is crossed, the disease level is $x = x' \pm d$.

These equations can provide sampling aids that enable a scout to classify, with a known precision, the disease level present in a field with regard to a predetermined critical level.

TABLE 1. Statistics of the mean crowding ($\overset{*}{x}$) on mean ($\bar{x}$) regression for each of six onion fields and for data pooled from all fields

| Field | Intercept ($b_0$) | Slope ($b_1$) | Correlation coefficient ($r$)[a] | Number of sampling occasions ($n$) |
|---|---|---|---|---|
| 1 | 1.2843 | 2.0567 | 0.7575* | 7 |
| 2 | 0.8694 | 2.2570 | 0.8587** | 8 |
| 3 | 1.0541 | 2.1649 | 0.7295* | 9 |
| 4 | 0.9727 | 2.9565 | 0.8961** | 6 |
| 5 | 0.9939 | 1.0197 | 0.8710* | 6 |
| 6 | 1.6051 | 0.9968 | 0.8892** | 7 |
| 1–6 | 1.3036 | 1.4244 | 0.8212** | 43 |

[a] Statistically significant at $\alpha = 0.05$ (*) and at $\alpha = 0.01$ (**).

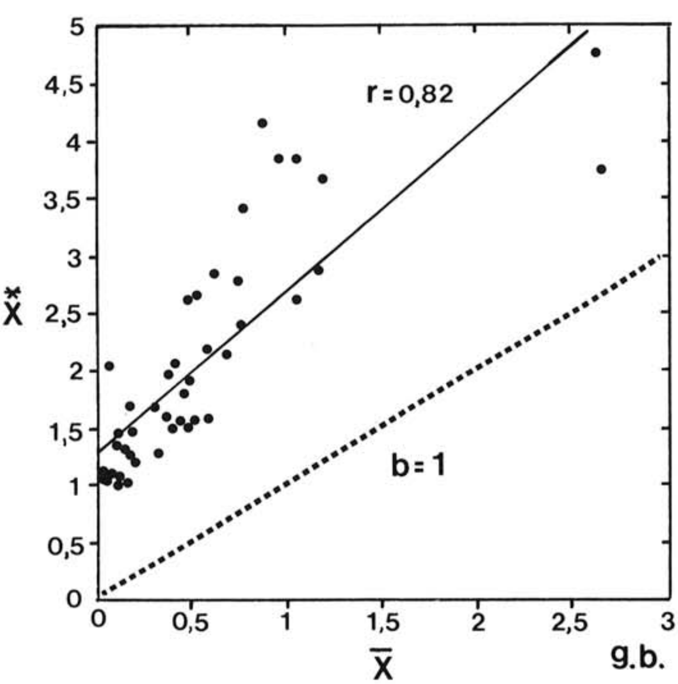


**Fig. 1.** Regression of mean crowding ($\overset{*}{x}$) on mean density ($\bar{x}$) of Botrytis leaf blight lesions on onion leaves.

## RESULTS AND DISCUSSION

**Spatial dispersion of Botrytis leaf blight.** The regressions of $\overset{*}{x}$ on $\bar{x}$ were significant for all fields (Table 1) confirming the existence of a relation between $\overset{*}{x}$ and $\bar{x}$ for the incidence of Botrytis leaf blight on sampled onion leaves. Similar relations exist for a wide variety of animals and plants partly because the basic component of the population, the distribution of which is measured here, should reflect the species-specific way of utilizing the habitat or, in the case of a disease, of spreading in its habitat (7).

The data from all fields were pooled and the regression $\overset{*}{x} = 1.3036 + 1.4244\,\bar{x}$, was obtained (Fig. 1). This regression is highly significant (Table 1) and as it covers the range of disease intensity that is of interest in an IPM program, these parameters were used in the subsequent calculations. The $b_0$ values of 1.3036 was significantly different from 0 ($P < 0.005$) and the $b_1$ value of 1.4244 was significantly different from 1 ($P < 0.005$). These results indicate that aggregates were the basic component of the population of Botrytis leaf lesions when all fields were considered and that these aggregates were contagiously distributed in the onion fields. A contagious, rather than random, distribution can be expected for many polycyclic plant diseases, because a new lesion is more likely to form near an inoculum source. Thus, once a lesion is present on an onion leaf, there is a high probability that more lesions will appear on that leaf and that plants near the infected ones have more chance of being infected as wind, water, and other mechanical transportation factors are responsible for inoculum spread (10).

Fungicide treatments could alter the spatial distribution of Botrytis leaf blight thus modifying subsequent sampling procedures. The sequential sampling method was designed not only to estimate the disease level in the field before the onset of a control program, but also to follow the progress of disease once fungicides have been applied, as a means of evaluating their effectiveness. We wished to determine if the dispersion of the disease is modified by fungicide treatments. To do this, the $\overset{*}{x}$ on $\bar{x}$ regressions of pre- and postfungicide treatment samples were plotted. Regression analysis and $t$ tests were used to test the differences among intercepts and slopes of these regressions. No significant differences ($P < 0.05$) were observed between regression parameters, indicating that fungicide applications did not affect the pattern of dispersion of Botrytis leaf blight in onion fields.

**Sequential sampling procedures.** The $b_0$ and $b_1$ values obtained were used in the sequential sampling procedure together with two other elements: a critical disease level, or economic threshold, and an acceptable error level. The economic threshold for Botrytis leaf blight on onions, ie, the level at which fungicide applications should be initiated, has been fixed at the first lesion detected in a field (16), at one lesion per 10 leaves (13), at one lesion per leaf (9), and as high as five to ten lesions per leaf (17). For the purpose of this experiment, a conservative economic threshold of one-half lesion

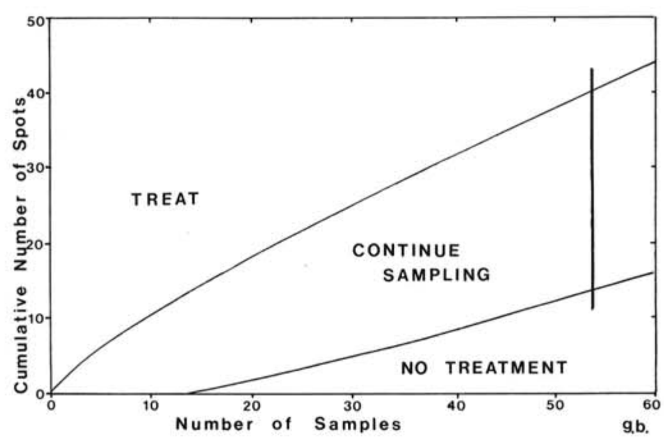


**Fig. 2.** Sequential sampling plan for Botrytis leaf blight lesions on onion leaves.

per leaf was chosen. By accepting a less conservative and thus higher economic threshold and with all other parameters being equal, the mean number of samples necessary to reach one of the acceptance limits would decrease. The Student's $t$ test for an error level of 0.1 was used to calculate the stop lines of the sequential sampling plan.

The upper limit of the sequential sampling plan was determined by

$$T_{\text{upper}} = n\,0.5 + 1.64[n(1.2579)]^{1/2}$$

and the lower limit by

$$T_{\text{lower}} = n\,0.5 - 1.64[n(1.2579)]^{1/2}.$$

The sequential sampling plan generated by these equations (Fig. 2) calls for a maximum number of 54 samples, as determined by equation 4 when the fixed level of precision, $d$, is 0.25.

To use this sequential sampling plan, Botrytis leaf blight lesions are counted on the oldest erect onion leaves on onions sampled at random in the field. The cumulative number of lesions is plotted on Fig. 2 after each sample. If the upper acceptance limit is crossed upward, the population density of the disease is significantly greater than one-half lesion per leaf and a fungicide treatment is warranted. If the lower acceptance limit is crossed downward, the population density is significantly lower than one-half lesion per leaf and no treatment is necessary. If the sampling continues until 54 leaves are taken and no acceptance limit is bridged, the population density of Botrytis leaf lesions would not be significantly different from one-half lesion per leaf. With the disease level near the critical density, fungicide treatments could be started or more sampling could be done later, depending on the weather forecast and the phenological stage of the crop. In all cases, it is recommended that at least 10 leaves be sampled before a decision is made.

Sequential sampling can reduce time and labor costs associated with disease monitoring by deciding whether or not control is needed, while staying within a predefined error level, instead of giving an exact estimate of the disease level. With a practical sampling procedure, the disease population density can be followed throughout the season. The first fungicide treatment can be delayed until a critical threshold is reached and thereafter treatments can be applied when the population density and the weather forecasts indicate an epidemic. These steps will reduce the amount of pesticide used in onion fields and improve the crop quality by enabling inefficient treatments to be detected early.

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