A Quantitative Method for Estimating Density of Septoria tritici Pycnidia on Wheat Leaves

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Research supported by grant from Deutsche Welthungerhilfe, Germany.

Accepted for publication 23 May 1975.

ABSTRACT

Thirty-five-millimeter photographic transparencies of cleared wheat leaves heavily infected with pycnidia of *Septoria tritici* were scanned with a television beam. The signal which measures the opacity of the transparency was sampled at a dense grid of points and the digitized output was stored in a computer. The processed output was used to estimate the relationship between pycnidial number, pycnidial mean area, and percent coverage. The mean area of the pycnidia is related to pycnidial density; therefore, as the density increases, the size of the pycnidia decreases. The area of the available leaf region, in which each pycnidium is embedded, limits the maximum actual coverage by pycnidia per leaf unit area. The maximal actual coverage of heavily infected leaves was 22.87%. This was rescaled to give coverage of 100%. A diagrammatic scale for estimating pycnidial coverage was based on five grades: 12, 25, 50, 75, and 87 percent pycnidial coverage of wheat leaves.

Phytopathology 66:11-14

Additional key words: Septoria leaf blotch of wheat, epidemiology, disease assessment.

Septoria leaf blotch of wheat caused by *Septoria tritici* Rob. ex Desm. can assume epidemic proportions and cause severe reductions in yields (4, 8, 9, 18). The increased importance of this disease is associated with the rapid replacement of local wheat cultivars by the high-yielding, short-strawed cultivars which are susceptible to Septoria leaf blotch of wheat (4, 8, 9, 18).

Extensive disease resistance breeding programs are underway (4, 18). Disagreement exists concerning the measurement of the disease in relation to incidence, severity, intensity and progress of the disease (14). Assessment of Septoria leaf blotch severity is based on pycnidial density (8, 17), percentage of lesions on wheat leaves (2, 6, 11), or on scales used for other foliar diseases (1). Cultivars are scored on the relationship between severity and incidence of the disease (4, 9).

Jones and Cooke (13) estimated the relationship between disease intensity value and percent disease coverage of wheat heads infected by pycnidia of *S. tritici*. The relationship between percent coverage and actual rust coverage was established by Melchers and Parker (15). The authors modified the Cobb scale (5) by removing a fraction of the shaded area to leave 37% of the total area shaded. This actual rust coverage was arbitrarily selected as 100%. The Cobb scale was further modified to fit new relationships between percent coverage and actual rust coverage (16). Peterson et al. (16) used different diagrams based on size and density of rust pustules. This enables the scoring of rust coverage on different plant parts or the evaluation of the percent coverage of different rusts.

The work reported in this paper was undertaken primarily to estimate the relationship between pycnidial number, pycnidial mean area and percent coverage of Septoria leaf blotch of wheat.

MATERIALS AND METHODS.—*Wheat leaves.*—Wheat flag leaves were collected from plants heavily infected with Septoria leaf blotch. The green leaves were cleared for 12 hours with 5% KOH, washed and cleared further with 70% ethyl alcohol. Cleared leaf segments covered by varying pycnidial densities were recorded on 35-mm transparencies. Each picture contained a ruler for reference.

Digitization of the 35-millimeter transparencies.—Each transparency was scanned and projected onto a television screen. The cleared leaf image on the television screen was scanned and the generated beam was used as an input to an analog device. The electrical potential of the output signal was proportional to the degree of opacity of the scanned leaf image. This signal was then digitized by an analog-to-digital converter in which each sampling of the signal was assigned one of 64 values. Because of the limited capacity of the computer (2000 Digital Computer Family, Armament Development Authority, Ministry of Defense, Israel), a maximum of 25,344 digital points could be generated from the transparency (or portion of the transparency). Therefore, each time one-fourth of a transparency was scanned. The output consisted of a matrix containing 192 columns by 132 lines (a total of 25,344 points). This output was printed on a high-speed printer and/or punched on paper tape for processing. The printed matrix contained superimposed letters to indicate the degree of opacity.

Measurements of pycnidia.—The area of each pycnidium was defined as the six darkest levels of printed letters or letter combinations. The number of these letters was designated as the size of the pycnidium. Each pycnidium was approximately an elliptical shape (7), which can be parameterized by the lengths of the major and minor axes. One-half the lengths of these axes is referred to as a and b, respectively.

A total of 19 leaves (four segments per leaf) containing 6,012 individual pycnidia was measured.

RESULTS.—*The shape of the pycnidium.*—The area of an ellipse is πab where a and b are one-half the lengths of the major and minor axes, respectively. The circle is a special case of this formula when $a = b$. Therefore, we estimated the slope k in the equation Area $= k \cdot a \cdot b$., where the data consisted of the 6,012 pycnidia measured. The constant k was estimated to be 3.19 with a standard deviation of 0.11. This is quite similar to the value of π (= 3.14).

The relationship between the number of pycnidia and percent coverage.—The percent coverage of each leaf segment is plotted against the number of pycnidia per unit area in the leaf segment (Fig. 1-A). The choice of unit is arbitrary. The higher percent coverage is associated with lower density. This relationship is not linear. In addition, the variability in percent coverage for low density is much higher than for high density.

Various data transformations were performed in order to make the relationship linear and the variability about the line more homogeneous. Among those attempted for pycnidial coverage was Sin^{-1} (square root of pycnidial coverage). This transformation is often successful when the data consists of probabilities (percentages). The square root of the density was also tried as this transformation is often successful for counts. The data in Fig. 1-A are replotted in Fig. 1-B using these transformed scales. A straight line was now fitted to the data:

$$y = 0.43114 - 0.01029\ x$$

where $y = \mathrm{Sin}^{-1}$ (square root of pycnidial coverage), and x = square root of pycnidial number per unit area. The correlation coefficient (r) between x and y is -0.567 ($P < 0.001$). The straight line plotted in Fig. 1-B is transformed back to the original scale and plotted in Fig. 1-A. Note, that this results in a curved line in Fig. 1-A.

The negative slope of the equation indicates that low pycnidial density is associated with high coverage. This means that the size of a pycnidium is reduced as the density increases. However, in the present sample of heavily infected leaves, the maximum coverage was less than 25%. Therefore, the area covered by a pycnidium may not represent the whole region belonging to the pycnidium.

The relationship between pycnidial mean area and the available leaf surface area.—Consider the possibility that the pycnidium is embedded within a region as suggested above. Since these wheat leaf segments are heavily infected, a measure representing the maximal area occupied by a pycnidium is: size of leaf/number of pycnidia. This is equivalent to the converse of the density. Therefore, a regression relationship was derived relating (1/density) and pycnidial mean area. The resulting equation was: $d = 2.09\ h + 0.035$; where d = square root (size of leaf/number of pycnidia), and h = square root (mean area). The correlation coefficient (r) was 0.036 ($P < 0.001$). Here h is the length of the side of a square of area h^2 containing the pycnidium. If, for any mean area, we can now find the region in which the pycnidium is embedded, then we can estimate the region used by the pycnidium and relate it to the region available. That is,

$$\%\ \text{possible coverage} =$$

$$100 \times \frac{d^2}{(\text{size of leaf/number of pycnidia})}$$

$$100 \times \frac{(2.09\ (\text{square root mean area}) + 0.0346)^2}{(\text{size of leaf/number of pycnidia})}$$

If we disregard the constant 0.0346, then

$$\%\ \text{possible coverage} =$$

$$100 \times 2.09^2\ \frac{(\text{mean area})}{(\text{size of leaf/number of pycnidia})}$$

$$4.37 \times (\%\ \text{observed pycnidial coverage}).$$

Hence, the maximum observed pycnidial coverage is $100/4.37 = 22.87\%$.

A diagrammatic scale for estimating pycnidial coverage.—Based on the relationship between the possible percent coverage and the observed pycnidial coverage, a diagrammatic scale for estimating coverage by pycnidia of *S. tritici* can be proposed (Fig. 2). The scale defines 50% coverage as the natural midpoint of the categories. The limits of the grades are obtained by the Horsfall and Barratt (10) grading system by progressive halving above and below 50%, e.g., 12, 25, 50, 75, and 87 percent coverage. The Septoria grading method is illustrated for an actual wheat leaf based on actual observed coverage by pycnidia.

DISCUSSION.—Scoring of Septoria leaf blotch of wheat is based on estimating the formation of lesions, pycnidial density, and in some cases on chlorotic areas associated with the disease (3, 6, 8, 12). The severity of Septoria leaf blotch appears to commensurate with the density of pycnidia (8, 17). Wheat cultivars which exhibited low pycnidial density were rated as resistant to the disease (8, 17). A standard diagram for assessing infection by *S. tritici* on wheat heads, but not for foliage, was drawn by Jones and Cooke (13).

The relationship between pycnidial number and mean area on heavily infected wheat leaves reveals that the size of pycnidia is dependent on the density (Fig. 1-A). High density is associated with small pycnidia. The estimated maximum limit for pycnidial coverage is 22.87%. This percentage differs from the 37% selected by Melchers and Parker for rust (15). The maximal actual percentage for the elliptically ruptured epidermis serving as an index of pustule periphery of *Puccinia recondita* on wheat leaves was 24% (Eyal and Caldwell, *unpublished*).

The different methods of estimating the upper limit of percent coverage are not comparable because the techniques differed and no comparable standards for defining the periphery of the affected area were used.

There is a limit to the wheat tissue which is available for colonization by fruiting bodies. The relationship among pycnidial density, parasite mycelial colonization area, and available wheat tissue is not known. The present

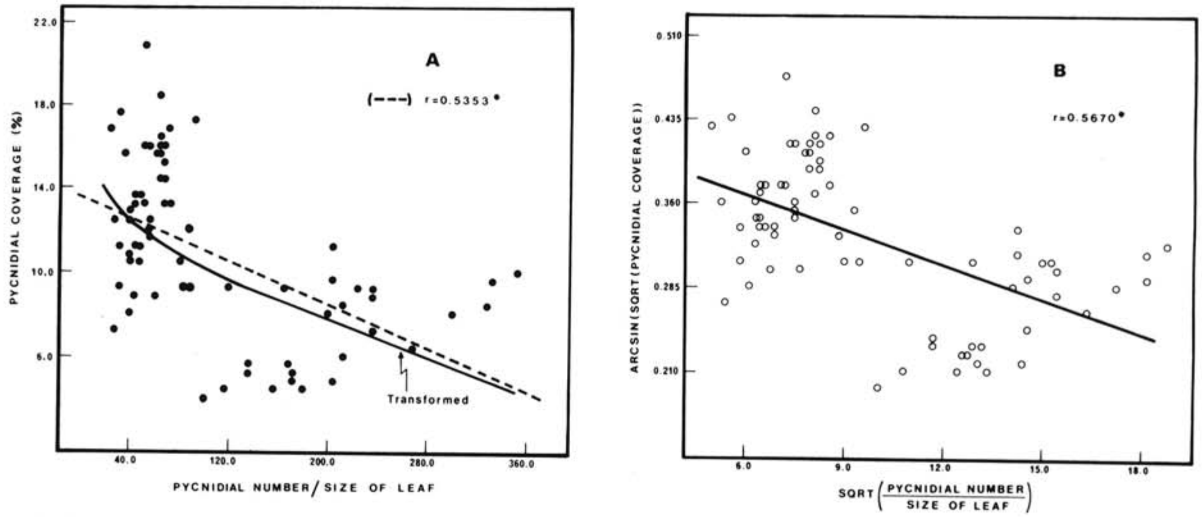

Fig. 1-(A, B). The relationship between the number of pycnidia of *Septoria tritici* and percent coverage on wheat leaves. **A)** Percent coverage related to the number of pycnidia per unit area (----) with correlation coefficient (r) of 0.5353 ($P = 0.05$); and the transformed curve. **B)** The relationship between SQRT (square root) of pycnidial number/size of leaf and Arcsin (Sin^{-1}) of the square root of pycnidial coverage, with correlation coefficient (r) of 0.5670 ($P < 0.05$).

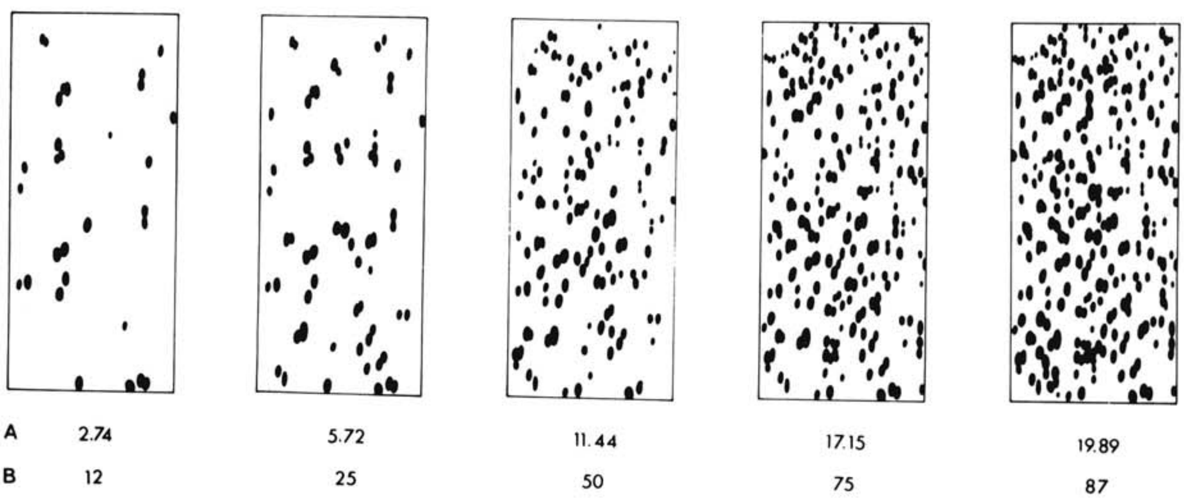

Fig. 2-(A, B). A diagrammatic scale estimating pycnidia coverage of *Septoria tritici* on wheat leaves. **A)** Actual observed pycnidial coverage (%). **B)** Scaled possible pycnidial coverage (%).

method is useful for quantitative estimates and observation of stained mycelial masses.

The standard diagram (Fig. 2) and the scheme proposed for calculating the Septoria progress coefficient (9), could serve as a reliable quantitative method for assessment of Septoria severity and disease progress.

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