A Quantitative Method for the Inoculation of Wheat Seedlings with Pycnidiospores of Septoria nodorum

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ABSTRACT

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A quantitative inoculation method was developed that permits the evaluation of symptoms produced by pycnidiospores of *Septoria nodorum* on wheat cultivars in the seedling stage. The following parameters were quantified: number of spores per suspension volume; volume sprayed onto wheat seedlings; and the speed at which plants were revolved during inoculation. The number of spores deposited on glass slides placed among the wheat seedlings gave an estimate of the number of spores deposited on leaf surfaces. The relationships between numbers of spores reaching seedling leaves and symptoms produced (number of lesions and percent necrosis) were evaluated for four spring wheat cultivars. The wheat cultivar, Fortuna, was very susceptible to the disease, whereas Manitou was relatively resistant. The use of our method to detect, measure, and screen for resistance to *S. nodorum*, and its possible use in genetic studies on the inheritance of resistance is discussed.

Additional key words: *Triticum aestivum*, glume blotch.

Septoria glume blotch of wheat, which is caused by *Septoria nodorum* (Berk.) Berk. (perfect state = *Leptosphaeria nodorum* Müller), reduces yields in many parts of the world (1, 2, 3, 4, 5, 11). Brönnimann (3) reported losses in experimental plots of 46.4% in winter wheat and 40.4% in spring wheat. The disease is associated mainly with humid conditions, but the proliferation of susceptible wheat cultivars also has exacerbated its importance in dryland wheat-growing areas (4, 5, 11).

Germplasm with resistance to Septoria glume bloth is scarce, and the mode of inheritance of the known resistance is not well understood (1, 4, 5, 11). Tolerance to the disease limits yield losses and is manageable in breeding programs (3). A major obstacle in breeding for resistance to the disease has been the lack of reliable screening and selection methods at an early stage of plant development. Several investigators have suggested means to inoculate and screen cereal cultivars based on foliar symptoms on seedlings or adult plants, or on yield comparisons (1, 2, 5, 6, 7, 11). The lack of a quantitative inoculation method also has been an impediment to studies of the disease under controlled conditions.

The objective of this study was to devise a method for quantitative inoculation of wheat seedlings to measure the response of cultivars to the parasite.

MATERIALS AND METHODS

The isolates of *S. nodorum* used in this study originally were collected from wheat fields in Montana. Only the most virulent isolates were kept for the study. Cultures were grown on yeast-malt agar in petri dishes for 7 days at 20 C under constant light from 20W cool-white fluorescent lamps (6.6×10^3 ergs/cm^2/sec) (8, 9, 10). The plates were flooded with 3-5 ml of sterile water and the cirrhi that contained pycnidiospores were removed by gently rubbing the agar surface with a glass rod. A spore suspension from several agar plates was used to inoculate the seedlings.

Four spring wheat cultivars that varied in their resistance to *S. nodorum* were used: DeKalb's SB-8, Fortuna (C.I. 13596), Manitou (C.I. 13775), and World Seeds' WO 1812 (C.I. 14585). Seeds of all cultivars were sown in straight lines and at equal spacing on the periphery of 20 × 20 × 6.5 cm-square plastic containers (30-40 seeds/cultivar) 10 days before the inoculation. The plants were kept in growth chambers at 15 C night/23 C day temperatures, and a 12-hr day length with a light intensity of 1.7×10^4 ergs/cm^2/sec. Each plastic container was placed on a phonograph turntable equipped with a variable speed mechanism [33, 45, and 78 revolutions per minute]. Two glass slides (7.5 × 1.25 cm) were placed back to back and held in a clothes pin standing upright among the wheat seedlings. Two of these spore catchers were placed on opposite sides of the container.

Fifteen ml of spore suspension was sprayed onto plants

in the revolving container from an atomizer attached to a pressure-vacuum diaphragm pump held at a distance of 20 cm from the seedlings. In no case was the volume of inoculum sufficient to cause run-off. Preliminary tests showed that run-off began after 20 ml had been applied.

The number of spores per suspension volume, volume of inoculum sprayed onto wheat seedlings, and revolving speed, all were regulated. We concluded from preliminary tests that 45 rpm was best; therefore, all subsequent experiments involving the turntable were done at that speed. Immediately after inoculation, the glass slides were placed in a duplicate container that contained soil, but no plants. This container was rotated on the turntable and the slides were sprayed with lactophenol cotton blue (12). The slides were then stored in a box for later microscopic examination. Pycnidiospores deposited on each slide were counted in 10 equally-spaced microscopic fields. The inoculated seedlings were moved into a moisture chamber where they were misted by two cold-water humidifiers for 48 hr, at 22 ± 2 C, and then they were returned to the growth chamber.

Eight days after inoculation, each leaf was marked at its base with a nonphytotoxic felt-tip marking pen (Sanford's Rub-a-Dub) to delimit the area to be considered in subsequent measurements. The number of lesions on each leaf was counted and the length and width of each leaf was recorded. Fifteen days after inoculation, the amount of necrotic tissue on infected leaves was estimated by measuring the length of the necrotic region of each leaf and relating it to the length and width of leaf previously recorded. Necrotic tissue was not always solid or continuous, but sometimes was interspersed with chlorotic or green tissue. The number of lesions/cm^2 and the percentage of necrosis was calculated for each leaf.

Two different kinds of experiments were used to characterize the technique: (i) a dilution series of spores with a constant 15 ml of inoculum applied at each spraying; and (ii) a dilution of spores applied in various volumes so that various total numbers of spores accumulated on the seedling leaves. Each series of experiments was repeated three times. The results were subjected to a regression analysis and an analysis of variance by the use of transformations best fitted to the data.

RESULTS AND DISCUSSION

The symptoms observed with four cultivars subjected to the various inoculation methods conformed to distinct mathematical functions for the two experimental series: (i) dilution of a fixed volume of inoculum; and (ii) increasing total numbers of spores applied by increasing volume. Examples of the two different inoculation series as they affected susceptible cultivar Fortuna are illustrated in Fig. 1 and 2. When 15 ml of spore suspension was sprayed on the plants, a threshold of 10^4 spores/ml had to be applied to obtain measurable infection, and 10^7 spores/ml were required for maximum infection (Fig. 1). Furthermore, when increasing volumes of inoculum containing n × 10^8 spores were applied to Fortuna seedlings, a gradual increase in numbers of lesions/cm^2 and percentage of necrosis was observed, but the increase in symptoms was not proportional to the increased number of spores applied per inoculation (Fig. 2). Necrosis was severe on Fortuna and often resulted in massive collapse of leaf tissue in the area surrounding a lesion. Therefore, estimation of lesions at 1 wk after

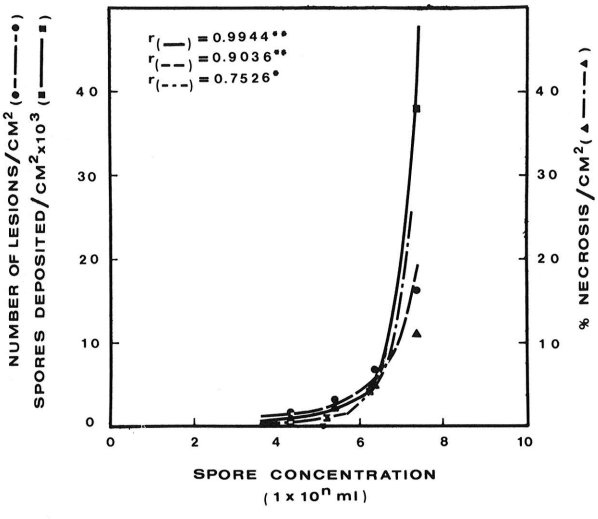

Fig. 1. The relation between pycnidiospores per milliliter of applied inoculum suspension in a fixed volume of 15 ml and symptoms produced by *Septoria nodorum* on seedlings of the spring wheat cultivar Fortuna. Spore concentrations are related to symptoms in the mathematical function $\hat{Y} = a + b_1X + b_2X^2$ where X = spore concentration; and $\hat{Y}$ = number of spores deposited on glass slides (■ —— ■) with regression coefficient (r) of 0.994 ($P < 0.01$), or number of lesions per cm^2 (· — — ·) with r = 0.9036 ($P < 0.01$), or percentage of necrosis (▲—·—▲) with r = 0.7526 ($P < 0.05$).

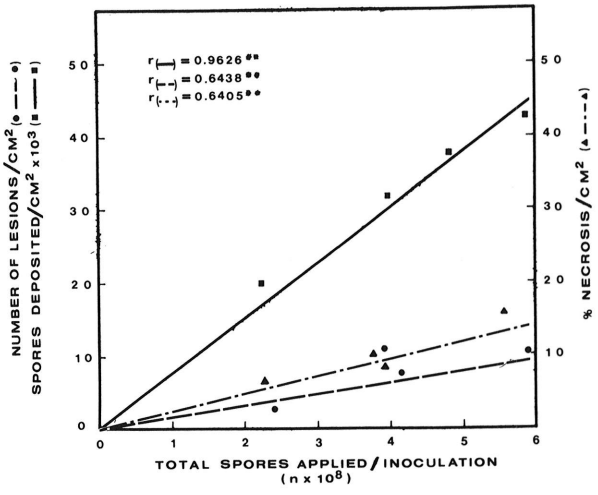

Fig. 2. The relationship between total pycnidiospores applied in inoculum of a fixed concentration in various volumes and symptoms produced by *Septoria nodorum* on seedlings of the spring wheat cultivar Fortuna. The affects of increasing spore numbers on the following parameters are shown: (i) number of spores deposited on glass slides per cm^2 (■ —— ■) with correlation coefficient (r) of 0.9626 ($P < 0.01$); (ii) number of lesions per cm^2 (· — — ·) with r = 0.6438 ($P < 0.01$); and (iii) necrosis (▲ — · —▲) r = 0.6405 ($P < 0.01$).

TABLE 1. Regression coefficients of responses of four wheat cultivars to different levels of inoculation with *Septoria nodorum*

Wheat cultivar	C.I. or P.I. number	Regression coefficients of lesions per cm^2 vs. spore concentration[y]	Regression coefficients of percentage of necrosis vs. spore concentration[y]
DeKalb SB-8		0.0918 b[z]	0.1207 b
Fortuna	13596	0.0998 b	0.1166 b
Manitou	13775	0.0541 a	0.0778 a
WO 1812	14585	0.0808 b	0.0921 ab

[y]Regression coefficients (b) of the linear regression [$\text{Log}\hat{Y} = a + b(\log X)$] for each wheat cultivar. X = spores/ml, $\hat{Y}$ = lesions/cm^2, or percentage of necrosis.

[z]Values followed by the same letter are not significantly different, $P = 0.05$, by the Student-Newman-Keuls multiple range test (13).

TABLE 2. The relation between number of pycnidiospores deposited[a] to symptoms produced by *Septoria nodorum*

Wheat Cultivar	Ratio spores deposited/cm^2 lesions/cm^2	Ratio spores deposited/cm^2 % necrosis
DeKalb SB-8	4,425.8	2,788.8
Fortuna	4,756.6	3,139.3
Manitou	10,613.4	5,501.8
WO 1812	7,080.1	4,415.2

[a]Spores counted on glass slides that were exposed to the same inoculum received by the wheat leaves.

inoculation and necrosis at 2 wk after inoculation, followed by consideration of both parameters together, gives more reliable information than either considered separately.

The regression coefficients (b) of the function $\hat{Y} = a + bX$, in which $\hat{Y}$ = lesions/cm^2 or percentage of necrosis and X = spores/ml, differed for each of the wheat cultivars studied (Table 1). The slope of the regression line representing the data for cultivar Manitou was significantly lower than that of the three susceptible spring wheat cultivars, for both symptom parameters. The magnitudes of receptiveness to infection of the susceptible wheat cultivars varied, with Fortuna being more susceptible than DeKalb SB-8 and WO 1812. This confirms with seedlings the results obtained by other workers with adult plants in the field (4, 11). The method, therefore, can be used to detect and measure the relative resistance to *S. nodorum* of different wheat cultivars in the seedling stage.

The relationship between the number of pycnidiospores deposited and symptoms produced by *S. nodorum* on seedlings of four spring wheat cultivars is presented in Table 2. The establishment of only one lesion per 1 cm^2 on the susceptible wheat cultivar Fortuna requires more than 4,750 deposited pycnidiospores, in an initial spore concentration of 10^7 pycnidiospores/ml. In the same manner, 10,000 pycnidiospores are required to produce a single lesion in the more resistant cultivar, Manitou. A somewhat smaller ratio was established for the relationship between spores deposited and percentage of necrosis.

The revolving inoculation technique may serve as a reliable method of screening wheat seedlings for resistance to Septoria glume blotch in breeding programs, and for genetical studies that deal with the inheritance of resistance. For these purposes, we recommend application of 15 ml of inoculum that contains 10^7 spores/ml to about 200 seedlings revolving on a turntable. The technique also may be used to evaluate the effect of environment on symptom expression of various wheat cultivars. Also, the revolving inoculation method is being used to quantitate symptoms produced by *S. tritici*.

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