Estimation of Conidia Production by Individual Pustules of Erysiphe graminis f. sp. tritici

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ABSTRACT

Considerable variation was found in pustule area and density of conidial chains of *Erysiphe graminis* f. sp. *tritici* on individual leaves of mature wheat. Descriptive scales were devised to estimate these two characters independently. Pustule area was estimated on an A to C scale (class C having the largest pustules). Conidial chain density was estimated on a 0-3 scale (class 3 having the greatest number of conidial chains per unit area). Inoculum potentials associated with the various scale values were determined by measuring pustule area and harvesting spores from representative pustules of each area class and conidial chain density class. Class 3 pustules produced 4.5 and 13 times as many conidia as class 2 and class 1 pusutles, respectively. Class 0 pustules produced no conidia. Class C pustules produced 3.4 and 9.8 times as many conidia as class B and class A pustules, respectively. Class 3 pustules were generally larger than class 2 pustules which in turn were generally larger than class 1 pustules.

Since pustule area and conidial chain density are related, only one of these need be estimated as a measure of inoculum potential. This potential may be used as one criterion for selecting or evaluating wheat for "slow mildewing" resistance.

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There is renewed interest in general (horizontal) types of resistance to disease in plants (2, 10), because race-specific resistance is often short-lived. Powdery mildew of cereals, caused by *Erysiphe graminis* DC, is an example of a disease that has been controlled only for relatively short periods by race-specific, major genes (7). A type of adult plant resistance to powdery mildew has been identified in wheat (*Triticum aestivum* L. em. Thell.) (7, 8), and oats (*Avena sativa*) (4, 5) which appears to be general resistance. Cultivars possessing this "slow mildewing" (7) type of resistance show a lesser severity of mildew in the field than do highly susceptible cultivars. This resistance may also be seen in the greenhouse on uniformly inoculated plants (5, 7). The slow mildewing in wheat appears to be related in part to reduced numbers of infections and smaller pustules (7). Smaller pustules produce fewer spores, which presumably reduces the rate of pathogen spread (10, p. 176). Pustule size of *E. graminis* f. sp. *tritici* influences the number of spores produced per pustule (6). However, results were based on observations of only four pustules, produced on plants of unspecified age. The pustules were all much larger than any pustules observed on adult plants in the present study.

In greenhouse experiments, I examined pustules on wheat cultivars and lines which differ in their level of slow mildewing resistance, to determine whether resistance was due to differences in pustule characteristics. Rating scales are presented herein for describing two pustule characters: area and conidial chain density. The numerical relations between scale values have been calculated so that these scales can be used in quantitative analyses of slow mildewing.

MATERIALS AND METHODS.—Soft red winter wheat cultivars 'Knox' (C.I. 12798) and 'Vermillion' (C.I. 13080), and experimental lines Purdue 5724B3 selection, Purdue 582D4 selection, and Purdue 60909C2 selection were used in this study. Wheat plants were vernalized (70 days at 3 C) and grown in the greenhouse. The leaf just below the flag leaf was inoculated in a settling tower (3) by shaking mildewed seedlings over it. Plants were inoculated when they reached the boot stage (Feeke's growth stage 10), then placed in a growth chamber at a constant 22.5 C and 12-hr photoperiod. Illumination, by incandescent and fluorescent (cool white) lamps, was measured at 10,760 lx (1,000 ft-c) at plant height. Pustules were examined 10 days after inoculation. Numerous pustules were examined at ×40 magnification and a four-class scale was devised for making visual estimates of the density of conidial chains (Table 1). The classes were easily distinguishable when pustules were examined under magnification and most pustules could be confidently assigned to a class. Pustules were assigned to a density class without regard to the area of the pustule. Relative area of pustules was also estimated, on a scale of A to C (class C having the largest pustules), without regard to density of conidial chains. Then the length and width of the area covered by the mass of conidia of the pustule were measured with a disc micrometer. Although mycelium extended beyond

TABLE 1. The four classes of powdery mildew pustules based on the density of conidial chains

Class	Description
0 =	Mycelium only, no conidia produced
1 =	Few conidial chains (less than 15)
2 =	Numerous conidial chains but individual chains distinct from the base up.
3 =	Abundant conidial chains, so dense that individual chains cannot be followed to the base.

the conidial mass, only the conidia-producing area was measured to compute pustule size.

Initially, conidia production potential was estimated by determining the density of conidia within a pustule "print" obtained by lightly pressing a microscope coverslip against a pustule so that conidia adhered to it, and examining the coverslip under the microscope. In later experiments the orifice of a spore sampling device was placed over a pustule and air was drawn through the sampler for 15 sec (Fig. 1). Conidia were conducted into the chamber of the sampler chamber where they impacted on a microscope slide coated with silicone grease. The density of conidia in the print, expressed as conidia per mm^2, was estimated by counting conidia in five microscope fields. Since spore production by *E. graminis* on wheat does not exhibit diurnal periodicity (6), no attempt was made to standardize daily spore-sampling times. In any one experiment, pustules of all sizes and conidial chain densities were randomly sampled during a 2-hr period.

RESULTS.–Pustule size and conidial chain densities varied among pustules on individual leaves. Representatives of at least three classes were usually seen among 20 pustules on individual leaves. All four classes of pustules were commonly found on a single leaf. Proximity of pustules to one another did not affect the density of conidial chains in a pustule.

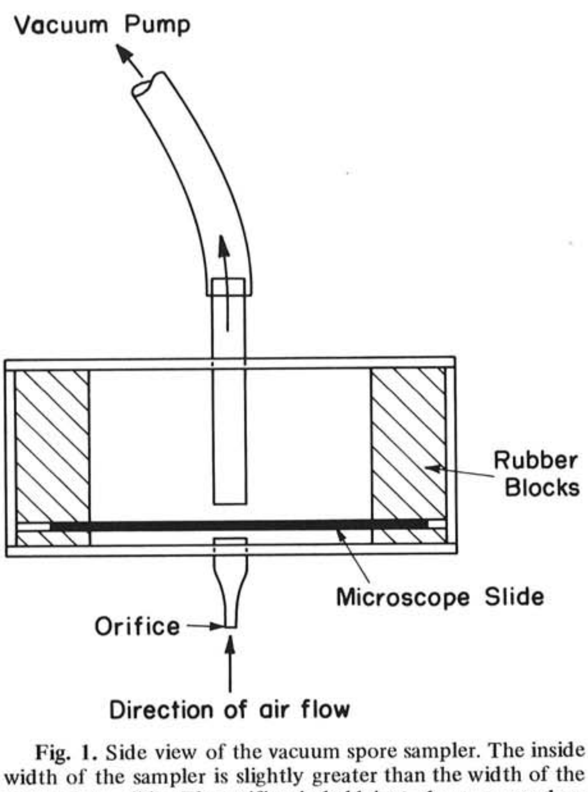

Fig. 1. Side view of the vacuum spore sampler. The inside width of the sampler is slightly greater than the width of the microscope slide. The orifice is held just above a powdery mildew pustule. When air is drawn through the sampler, conidia are carried from the pustule and impacted on the greased microscope slide.

TABLE 2. Conidia production and area of the conidia-bearing portion of *Erysiphe graminis* pustules in each conidial chain density class

Density class[a]	Conidia/mm^2	Conidia density[b]	Pustule area, mm^2
1	7.2 ± 3.5[c] (6)[d]	0.075	0.039 ± .016[c] (3)[d]
2	21.3 ± 2.4 (37)	0.223	0.279 ± .049 (29)
3	95.6 ± 12.7 (48)	1.000	1.005 ± .098 (35)

[a] For explanation of class values see Table 1.
[b] Number of conidia produced per pustule relative to a class 3 pustule.
[c] Standard deviation of the mean.
[d] Number of pustules sampled.

Classifying the pustules from the base to the tip of the leaf blade did not result in a gradient of conidial chain density classes.

The size of the pustule had no effect on the density of conidia deposited on coverslips pressed onto pustules. Since only conidia near the center of the pustule imprint were counted, the density of conidial chains on the pustule was reflected by the density of conidia deposited. Fifteen pustules of each size class were examined. The mean density of conidia in the imprint on the coverslip was 323 and 64 conidia/mm^2 for class 3 and class 2 pustules, respectively. Too few conidia were deposited from class 1 pustules to obtain reliable results.

A similar ratio of conidia production by class 3 and class 2 pustules was obtained with the vacuum spore sampler. Class 3 pustules yielded 4.5 times as many conidia as class 2 pustules and 13.3 times as many conidia as class 1 pustules (Table 2).

Two pustule characteristics, the density of conidial chains and pustule size, determined how many conidia were caught by the vacuum spore sampler. Therefore, the effect of pustule size on conidia production within each density class was examined (Fig. 2). Regardless of the class, conidia were produced only in the central portion of the mildew colony. Since the boundary of this mass of conidial chains was approximately elliptical with the long axis parallel to the long axis of the wheat leaf, area = π (1/2 length × 1/2 width) was used to calculate the area covered by this portion of the pustule. There was little variation in the area of class 1 pustules. However, there was considerable variation in the area of class 2 and of class 3 pustules; class 3 pustules usually were larger than class 2 pustules, but the ranges overlapped.

To determine whether class 3 pustules produced more conidia than class 2 pustules because of larger pustule area alone or because of both larger area and greater conidial chain density, linear regression lines were fitted to each set of data (Fig. 2). The null hypothesis, that for any pustule area X, an equal number of conidia would be produced by class 3 and class 2 pustules, was tested with the statistic:

$$t = \frac{\hat{Y}_3 - \hat{Y}_2}{s^2_{\hat{Y}_3} + s^2_{\hat{Y}_2}},$$

in which $\hat{Y}_3$ and $\hat{Y}_2$ are conidia numbers estimated from regression for class 3 and class 2 pustules and $s^2_{\hat{Y}_3}$ and $s^2_{\hat{Y}_2}$ are their respective variances (9, p. 170). This approach, rather than analysis of covariance, was used because the slopes of the two lines were sufficiently different (significant at the 10% level) when compared with a t-test.

Values for $\hat{Y}$ and $s^2_{\hat{Y}}$ were calculated for various values of X. At X = 0.5 mm^2, which was about the maximum area of class 2 pustules; a class 3 pustule produced significantly more conidia than a class 2 pustule. At X = 0.2 mm^2, the lower limit of class 3 pustules, the numbers of conidia produced by the two classes were not significantly different. At X = 0.4 mm^2, the level of significance for declaring $Y_3 > Y_2$ fell between $P = 0.10$ and 0.05.

The pustule area scale (A-C) was quantified by calculating the mean area for each scale value (Table 3). Class C pustules were four times larger than class B pustules and 11 times larger than class A pustules. Conidia production for each area class (without regard to conidial chain density class) was also calculated. Class A, B, and C pustules produced approximately the same numbers of conidia as density class 1, 2, and 3 pustules, respectively.

DISCUSSION.–Scales were devised to describe two pustule characters: area and density of conidial chains. A three-class scale described pustule area. This is analogous to a scale proposed by Browder (1) for *Puccinia recondita* in which pustule area is estimated independently of other lesion characters. Rather than using Browder's nine scale values, only three were used to describe *E. graminis* pustules, because it was felt that the additional scale values would only increase observer error. When the subjective area class values were compared with actual pustule area measurements, it was found that pustules could be assigned to one of three area classes by visual inspection with reasonable accuracy.

A four-class scale was used to distinguish conidial chain density. The scale values reflected substantial differences in the number of conidia produced per pustule.

The two scales were designed to measure different pustule characters and they did do so, as indicated by the comparison of regression lines for spore production on pustule area for class 3 and class 2 pustules. Over a portion of the area range common to both class 3 and class 2 pustules, the former produced significantly more spores than the latter. Despite the independence of the scales, pustule area and conidial chain density were related. Larger pustules tended to have more conidial chains per unit area than smaller pustules. Factors in the host which restrict pathogen development apparently affect both of these characters. The expression of the genes controlling these factors is evidently modified from cell to cell in the leaf, since pustules varied considerable in area and conidial chain density on an individual leaf.

Because 1, 2, and 3 pustules produced approximately the same number of conidia as A, B, and C pustules, the scales are directly interchangeable. That is, either area or conidial chain

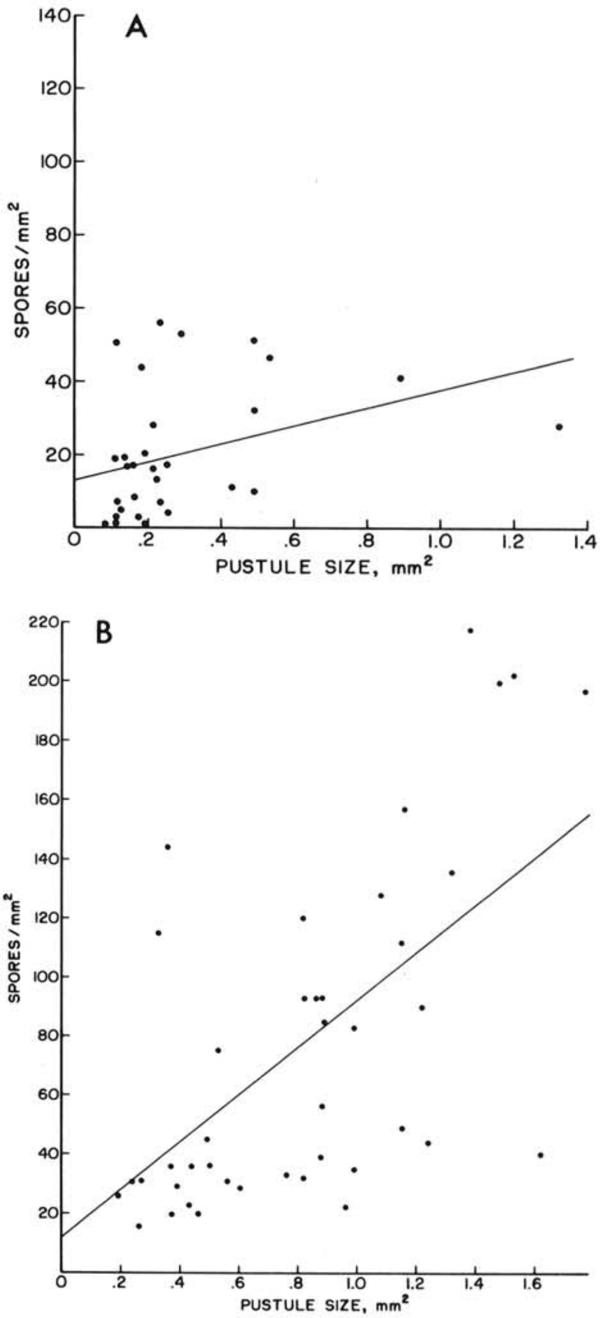

Fig. 2. The relationship between the area of a pustule of *Erysiphe graminis* f. sp. *tritici* and the number of spores collected from it with the vacuum spore sampler. **A)** Conidial chain density class 2 pustules. **B)** Conidial chain density class 3 pustules.

TABLE 3. Area of the conidia-bearing portion and relative conidia production of *Erysiphe graminis* pustules in each subjective area class

Pustule area class[a]	Pustule area, mm^2	Conidia/mm^2	Conidia density[b]
A	0.100 ± .014[c] (12)[d]	10.9 ± 1.2[c] (12)[d]	0.120
B	0.255 ± .023 (23)	27.0 ± 1.3 (23)	0.290
C	1.102 ± .092 (33)	93.0 ± 2.6 (33)	1.000

[a] Letters indicate relative pustule area. Smaller pustules are in class A, intermediate pustules are in class B, larger pustules are in class C.

[b] Number of conidia produced per pustule relative to a class C pustule.

[c] Standard error of the mean.

[d] Number of pustules measured.

density can be used to describe pustules with the same result. Which character is used to describe pustules depends upon the circumstances. When evaluating wheats in the greenhouse for slow mildewing, conidial chain density is more easily estimated than pustule area, since many individual pustules may be rapidly examined under a dissecting microscope. It is not practical to estimate conidial chain density in the field, where time will not permit examination of individual pustules on numerous breeding lines. However, a plant or line may be characterized by the predominant pustule area which in turn serves as an index of conidium-producing capacity.

When individual pustules are examined, the number of pustules in each conidial chain density class can be multiplied by the relative spore-producing capacity of that class (Table 2). The sum of these products is an index of the spore-producing capacity of a cultivar. Such indices can be used in interpreting the relative performance of highly susceptible and slow mildewing cultivars in the field (Shaner, *unpublished*).

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