Reduced Infectability and Inoculum Production as Factors of Slow Mildewing in Knox Wheat

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ABSTRACT

Powdery mildew development on 'Knox' and 'Vermillion' wheat was compared to determine the basis of Knox's slow mildewing resistance. The upper three leaves of mature plants were inoculated with conidia of *Erysiphe graminis*. Fewer colonies developed on Knox than on Vermillion. The average colony size, and number of conidial chains per unit area of colony, were less on Knox than on Vermillion. The latter parameters were converted to a "sporulation index", a measure of spore-producing capacity of a colony. The combined effect of reduced colony formation and reduced spore-producing capacity indicate that mildew should spread one-third as fast on Knox as on Vermillion. This agrees with differences in infection rate in the field.

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'Knox' wheat (*Triticum aestivum* L. em. Thell), which is fully susceptible to *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal in the seedling stage, has effective mature plant resistance (6, 8). By the time stems are elongating in the spring, Knox has less mildew than other cultivars which also lack specific genes for mildew resistance. The rate of disease spread is less on Knox, which causes differences in severity to increase with time (8). The stability of Knox's resistance (1, 6, 8), and slower rate of disease spread, suggest that the resistance is of a general type (2, 10).

One can hypothesize several mechanisms for Knox's resistance, based upon findings with other diseases (10). Reduced spore production per unit area of lesion, lower infectability, longer latent period, smaller lesions, and a shorter infectious period, would all slow the rate of disease spread. In the field, colonies of *E. graminis* on Knox are smaller, on the average, than those on other susceptible cultivars (8). Smaller colonies produce fewer spores (7). Reduced infectability contributes to the resistance of 'Maldwyn' oats (5). After a single inoculation with *E. graminis*, percent severity on greenhouse-grown Maldwyn was less than that on 'Milford' oats. Resistance mechanisms in Knox were determined by comparing colony characteristics and capabilities and mildew severity after inoculation of greenhouse-grown Knox and 'Vermillion'. Field studies were conducted to establish the relationship between greenhouse and field results.

MATERIALS AND METHODS.—Experiments

were done during the winters of 1969-70 and 1970-71. During the first year, seed of each cultivar was obtained from a bulk supply. In the second year, plants of each cultivar were the progeny of a single plant grown the previous year in the greenhouse.

Knox (CI 12798) and Vermillion (CI 12748) were grown in the greenhouse after vernalization for 70 days at 3 C. When the flag leaves were fully expanded, the first (flag), second, or third leaves of plants were inoculated (3) by shaking mildew infected Knox seedlings over them in a settling tower. The culture of *E. graminis* came from an adult Knox plant at the Purdue University Agronomy Farm. Plants were arranged in the settling tower in a randomized complete block design, replicated four times the first year and eight times the second. After inoculation, the plants were placed in a greenhouse (1969-70) or a growth room (1970-71) maintained at 22.5 C with a 12-hr photoperiod of about 10,760 lx (1,000 ft-c) illumination.

Before conidial chains formed (5 to 7 days after inoculation), the number of colonies in nine equidistant holes (28 mm^2 each) of a template placed over the leaf were counted with the aid of 10 X hand lens. After colonies were fully developed (12 or 13 days after inoculation), frequency of colony type was determined by assigning each of 20 colonies per leaf to one of four conidial chain density classes (7) based on observations at 40 X magnification. The area covered by *E. graminis* colonies was estimated in each of the nine holes of the template using the Horsfall-Barratt scale (4). These readings were converted to percent severity for the whole leaf using the Elanco Conversion Tables for the Barratt-Horsfall Scale (Elanco Products Co., Indianapolis, Ind.). Spore density on leaves was estimated on the basis of spores deposited on eight microscope slides, placed at the midpoint of the leaves between each replication.

Seed from some plants used in these experiments was sown in 1-m rows at the Purdue University Agronomy Farm in the fall of 1971. Progeny of plants that were paired in the same replication in the greenhouse were planted next to one another in the field. Mildew severity was assessed on 22 May 1972 by using the Horsfall-Barratt scale on the third leaf from the top on five stems in each row.

RESULTS.—*Colony numbers and percent severity.*—In 10 of 16 comparisons, more mildew developed on Vermillion than on Knox. Although these differences were substantial in the 1969-70 experiments (experiments 1 through 4, Table 1), they were statistically significant in only three comparisons. With more genetically uniform material and eight replications in 1970-71, the differences between colony numbers or percent severities on Knox and Vermillion were generally significant, but no larger than in the previous year. In experiments 5 through 10, Vermillion plants were progeny of a single plant. The Knox plants of experiments 5 through 7 were progeny of one plant; those in experiments 8 through 10 were progeny of another. In the remaining experiments, progeny of three other plants of Knox and Vermillion were used. In none of these was there a significant difference between the cultivars in colony number or percent severity.

The amount of mildew on both Knox and Vermillion varied greatly, but the amount of mildew on Knox and Vermillion was linearly related. This indicated that differences in inoculum level and infection conditions affecting both cultivars equally, were responsible for the variation. For colony number, the relation between cultivars is:

$$N_V = 1.16\, N_K + 19.17 \qquad (r = 0.85757)$$

where N_V and N_K are the numbers of colonies per cm^2 on Vermillion and Knox, respectively. For percent severity the regression equation is:

$$S_V = 0.96\, S_K + 23.74 \qquad (r = 0.95311)$$

where S_V and S_K are percent severities on Vermillion and Knox, respectively. These equations indicate that, within the range of inoculum levels employed,

TABLE 1. Colony number and percent severity of powdery mildew on 'Vermillion' and 'Knox' wheats following uniform inoculation

Experiment	Conidia applied per cm^2	Leaf inoculated[a]	Colonies/cm^2[b]		Severity, %	
			Vermillion	Knox	Vermillion	Knox
1	2,030	1			78.9	57.7
2	344	1	38.3	16.4*	41.8	10.7**
3	1,118	1	14.6	6.5	17.9	4.9
4	409	1	63.4	55.8	55.2	20.7*
5	1,481	2	105.2	60.4**	64.2	38.2*
6	621	1	31.6	19.3**		
7	3,285	3			77.8	59.7***
8	1,742	2	84.2	27.4**	43.7	10.0**
9	2,038	2	80.9	55.8	54.4	34.6*
10	2,495	1	99.2	73.1**	74.9	51.8***

[a] Leaf 1 is uppermost (flag leaf).

[b] Tests of significance were made for each experiment and parameter separately. The symbols *, **, *** indicate significant differences at $P = .10, .05$ and $.01$, respectively.

mildew severity on Vermillion exceeds that on Knox by about 20 colonies per cm^2 or percent. As the level of initial severity increases, the ratio of severity on Vermillion to severity on Knox decreases, suggesting that infection efficiency decreases as inoculum level increases.

Colony size.—Percent severity divided by the number of colonies per cm^2 estimates colony area. The average colony area on Vermillion was 0.82 mm^2; that on Knox was 0.58 mm^2 (Table 2). Colony area on Vermillion tended to decrease as the number of colonies per cm^2 increased. Colony area and number were not related on Knox. At the higher densities of colonies on Vermillion, colony areas on the two cultivars were similar, but at low densities the colony area on Vermillion greatly exceeded the maximum area on Knox.

TABLE 2. The relation between the number of *Erysiphe graminis* colonies per cm^2 and colony area, on 'Knox' and 'Vermillion' wheats

Knox			Vermillion		
Experiment[a]	Colony number per cm^2	Colony size, (mm^2)	Experiment[a]	Colony number per cm^2	Colony size, (mm^2)
3	6.5	0.75	3	14.6	1.23
2	16.4	0.65	2	38.3	1.09
8	27.4	0.36	4	63.4	0.87
9	55.8	0.62	9	80.9	0.67
4	55.8	0.37	8	84.2	0.52
5	60.4	0.63	10	99.2	0.76
10	73.1	0.71	5	105.2	0.61
	$r = 0.0676$[b]			area = 0.00714 × number + 1.317 $r = -0.90852$[b]	

[a] Experiment numbers correspond to those in Table 1.
[b] Correlation coefficient for regression of colony area on number of colonies per cm^2.

In one experiment the length and width of the conidial chain-bearing portion of colonies were measured with a disk micrometer in a dissecting microscope and colony area was calculated as an ellipse [A = (½ l × ½ w)]. Based on 10 colonies on each of four leaves, mean colony area on Vermillion was 0.212 mm^2 and on Knox was 0.111 mm^2. The small size of colonies compared to those calculated above was probably due to differences in colony age. Severities from which areas were calculated were estimated 11 to 13 days after inoculation, whereas these direct measurements were made 7 days after inoculation.

Colony types.—Frequencies of colony types for the Knox and Vermillion selections (group 1) which differed significantly in mildew severity after uniform inoculation (Table 1) and for selections (group 2) which did not differ in severity following uniform inoculation are in Table 3. Within both groups, there were more class 3 colonies on Vermillion than on Knox. A chi-square test for homogeneity of the frequency distributions for Knox and Vermillion within each group yielded highly significant values of χ^2 ($P<.005$). Distributions among groups for each cultivar also differed ($P<.010$). There were more class 3 colonies on Knox in group 2 than in group 1. The frequency of class 3 colonies on Vermillion in both groups was the same, but there were fewer class 2 colonies and more class 0- and class 1 colonies in group 2 than in group 1.

The effect of these differences in colony type frequencies on inoculum potential was calculated using the relation between colony type and spore production. By sampling individual colonies, class 0, 1, 2, and 3 colonies yielded 0, 7.2, 21.3, and 95.6 conidia per mm^2 of the spore-trapping surface, respectively (7). Dividing each value by 95.6 conidia/mm^2 gives 0, 0.075, 0.223, and 1. The sum of each of these relative values, multiplied by the relative frequency of colonies in that class, yields a sporulation index. When colony type frequency is

TABLE 3. Frequency (%) of *Erysiphe graminis* colonies in each of three conidial chain density classes[a] on adult plants of 'Knox' and 'Vermillion' wheats following uniform inoculation

		Knox						Vermillion					
		Colony type				Colonies examined (no.)	Sporulation index[d]	Colony type				Colonies examined (no.)	Sporulation index[d]
		0	1	2	3			0	1	2	3		
Group 1[b]	mean	10.7	19.0	42.0	28.3			3.1	4.7	23.3	68.9		
	S.E.[e]	3.4	4.8	4.2	6.3	661	39.06	1.4	1.3	3.9	4.9	660	74.46
Group 2[c]	mean	8.4	11.4	38.6	41.6			6.2	8.0	17.3	68.5		
	S.E.	1.8	1.5	4.5	4.1	958	51.06	1.7	1.3	3.5	5.1	940	72.92
Groups 1 & 2	mean	9.5	15.2	40.4	34.9			4.6	6.4	20.3	68.7		
	S.E.	1.9	2.6	3.0	4.1	1,619	45.06	1.2	1.0	2.7	3.4	1,600	73.69

[a] Class 0 = no conidial chains, class 1 = few conidial chains, class 2 = moderately dense conidial chains, class 3 = dense conidial chains (7).
[b] The Knox selection in these experiments developed significantly fewer colonies than the Vermillion selections following uniform inoculation. See Table 1.
[c] These Knox and Vermillion selections did not differ significantly in number of colonies following uniform inoculation.
[d] Sporulation index = $(P_1 \times 0.07531) + (P_2 \times 0.22280) + (P_3 \times 1)$ where P_1, P_2, and P_3 are the percent of colonies in conidial chain density classes 1, 2, and 3, respectively.
[e] Standard error of the mean.

expressed as percent, 100 is the maximum value of the sporulation index. This index measures the capacity of *E. graminis* to produce spores on the plants for which colony type frequencies are known (Table 3). For each group of Knox and Vermillion, as well as for the combined data, the sporulation index for Vermillion significantly exceeded (1%) the sporulation index for Knox. Differences were not significant between groups of either cultivar. Thus, the differences in colony type frequencies between groups have no effect on inoculum productio

Behavior of progeny in the field.—Knox plants of experiments 5 through 10 (Table 1), consisting of 22 rows in the field, had a mean mildew severity of 3.14 ± 0.36% (C.V. = 54.12%). The severity on Vermillion was 22.33 ± 2.69% (C.V. = 56.47%). This difference was highly significant ($P<.005$). Considering the laboratory data of all plants as a group, the severities on Knox and Vermillion were 33.27 ± 4.30% (C.V. = 60.60%) and 54.17 ± 4.48% (C.V. = 35.79%), respectively. Again the difference was highly significant ($P<.025$).

Unlike laboratory experiments, there was no linear relation (r = 0.02051) between the severities of mildew on adjacent rows of Knox and Vermillion. Severity ranged from 2.8 to 51.2% among Vermillion rows, while severity on Knox was consistently low. Of the 22 rows, 17 had severities less than 3%.

Table 2 presents colony type frequencies for a group of experiments in which the Knox and Vermillion selections did not differ in mildew severity following inoculation in the settling tower. Progeny from plants used in one of these experiments were planted in the field. The plants behaved there as they did in the laboratory. Severity on Vermillion was 6.87 ± 2.64% (C.V. = 94.08%) and severity on Knox was 3.75 ± 0.63% (C.V. = 41.08%). The difference was not significant, evidently because the Vermillion selection was resistant.

DISCUSSION.—The comparison of severities of mildew in the laboratory after uniform inoculation suggests that reduced infectability is one component of Knox's slow mildewing resistance. This may be due to a reduction in the number of primary infections or to a failure of some primary infections to develop into visible colonies. Likewise, the greater frequency of colonies with low inoculum production potential on Knox would slow the rate of disease spread in the field. The smaller colonies on Knox are a third expression of resistance. At the higher colony densities in these experiments, colony sizes on Knox and Vermillion were the same. But as the number of colonies per cm^2 decreased, colony size increased substantially on Vermillion. Since colonies on Knox did not respond to reduced competition, something other than density of colonies restricts colony size on this cultivar.

Do these differences in the laboratory fully account for differences measured in the field? Consideration of the total effect of these three factors for resistance, which complement one another, should answer this. In all experiments, the sporulation index for Knox was less than that on Vermillion, with average values of 45.06 and 73.69, respectively. Thus, Vermillion has 1.64 times the per colony inoculum potential of Knox. This figure accounts for both conidial chain density and colony area since the sporulation index is based on conidia production of the four conidial chain density classes each averaged over all colony sizes (7). Since colony area and conidial chain density were positively correlated (7), it is reasonable to combine the two parameters.

Knox had less mildew than Vermillion (Table 1) in some laboratory experiments, while in other experiments it had as much. When I compared the progeny of the first group in the field, the severities on the two cultivars were nearly identical to severities on four-row 2.4-m-long plots of Knox and Vermillion grown from bulk seed during 1971-72. Thus, these progeny appeared typical of their respective cultivars. The plants of the second group did not differ in mildew severity in the field. The Vermillion was unusually resistant. In large field plots there are occasional Vermillion plants which have very little mildew, indicating that there is genetic diversity, either from seed mixing, outcrossing, or residual genetic variability in this old cultivar. It is reasonable, therefore, to take account of the difference in severity on the two cultivars following uniform inoculation measured in the first group of experiments. The average of the ratios of colony number on Vermillion to colony number on Knox in Table 1 is 1.85. That is, for a given level of inoculum 1.85 times more colonies form on Vermillion than on Knox. The combined effect of colony formation and sporulation capacity differences is therefore 1.85 × 1.65 = 3.03. On this basis, Vermillion is 3.03 times more susceptible than Knox.

The number of spores produced per unit area of colony, and the proportion of conidia which infect and give rise to lesions, are the two factors which determine the magnitude of van der Plank's basic infection rate (9). I calculated basic infection rates on Knox and Vermillion in the field (8). For the upper four leaves the ratio of these rates on Vermillion to those on Knox ranged from 1.98 to 5.40 with a mean of 3.18. The 3.03 value calculated above compares well with this ratio. Thus, the differences observed in the laboratory seem to account for the differences observed in the field. Other possible mechanisms, such as longer latent periods or shorter infectious periods apparently do not contribute to Knox's resistance. In preliminary observations in the laboratory, no differences between Knox and Vermillion were found in these characters.

Knox is a less congenial host for *E. graminis* than Vermillion. Although some colonies are as large and produce as many spores on Knox as on Vermillion, the average of a population of colonies is lower on Knox. It is the average that governs the rate of disease spread in the field. Knox's lower infectability may be the one extreme of the colony size spectrum. A greater proportion of colonies on this cultivar may never become big enough to see with the aid of a 10 × lens. Knox's lower infectability may also result

from fewer primary penetrations.

Even without understanding the histological and physiological bases of Knox's resistance, the information available is of practical use. Wheats with other resistance mechanisms, such as a longer latent period could be combined with wheats possessing the Knox-type resistance with the expectation of achieving even higher levels of resistance.

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