The Effect of Postinoculation Periods of Leaf Wetness on the Response of Wheat Cultivars to Infection by Septoria nodorum

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ABSTRACT

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The length of postinoculation wet periods had varying effects on symptom expression in spring and winter wheat cultivars inoculated at the seedling stage of growth with pycnidiospores of *Septoria nodorum*. A progressive increase in the wetting period had a marked effect on the number of lesions and area of necrosis produced per leaf of the spring wheat cultivars, with each cultivar responding in a characteristic manner. Winter wheats showed more resistance to infection and less response to length of wet period than spring wheats, but differences in response to infection by *S. nodorum* also were observed among the winter wheat cultivars. The differences in disease damage among spring and winter wheat cultivars in response to prolonged postinoculation wet periods can be used to detect resistant germplasm and for screening segregating populations in breeding material.

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

Septoria glume blotch of wheat, caused by *Septoria nodorum* (Berk.) Berk. (perfect state = *Leptosphaeria nodorum* Müller) can become epidemic and reduce yields significantly (1, 2, 13, 19). Alternate wet and dry cycles are necessary for the production of new pycnidia on infected straw, and for the replenishment of pycnidiospores in older fruiting bodies (15). Rainfall, accompanied by wind, moves pycnidiospores about in water droplets and accomplishes vertical and horizontal spread of the pathogen under field conditions (5, 14, 20). High incidences of the disease are dependent upon timely rainfall for spore dissemination, followed by high humidity to promote spore germination and leaf infection (1, 2, 13, 14, 17).

There are conflicting reports as to the length of the dew period needed for spore germination and leaf infection, and the subsequent period of high humidity and/or free moisture necessary to support disease development under artificial as well as natural inoculation conditions (1, 2, 16, 19, 20). Thomas (19) reported that for seedlings of wheat cultivar Atlas 66 inoculated with *S. nodorum*, exposure to a wet period of 92 hr resulted in greater leaf damage than exposure to the same conditions for 24 or 48 hr. Shearer (16) showed that the degree of leaf injury was maximal after exposure to a 72-hr wet period. On the very susceptible spring wheat cultivar Fortuna, a 2-hr wet period was reportedly sufficient for a severe disease reaction (47.2% average yield loss per head) (2). Holmes and Colhoun (6) inoculated the winter wheat cultivar Cappelle-Desprez and the spring wheat cultivar Cardinal with *S. nodorum*, then provided periods of high humidity of various lengths after inoculation. Cardinal showed a trace of infection 15 days after inoculation when the postinoculation period of high humidity had been only 3 hr. However, in order for as much as 20% of the inoculated leaf area to show lesions after 25 days, a postinoculation period of high relative humidity of 50-70 hr was needed. Cappelle-Desprez showed no lesions 25 days after inoculation if the postinoculation period of high humidity was less than 20 hr. Fifty hr of postinoculation high humidity resulted in lesions covering 20% of the inoculated leaf area 25 days after the inoculation. Rapilly et al. (11, 12) reported that infective *S. nodorum* became dormant, but did not die, if bright sunshine and low humidity followed a short period of rain during which spore dissemination and infection took place. When such conditions were known to have occurred, plants were transplated from the field to a mist chamber where lesions developed in about 2 wk; plants in the field remained free of lesions, although they were known to have dormant infections (F. Rapilly, *personal communication*). After infection was established, further development of *S. nodorum* within the host plant

remained dependent upon external environmental conditions, especially humidity.

The objective of this study was to determine the effects of wet periods of various durations on the development of lesions and necrosis in several spring and winter wheat cultivars quantitatively inoculated with pycnidiospores of *S. nodorum*.

MATERIALS AND METHODS

The isolates of *S. nodorum* used in this study were originally collected from wheat fields in Montana, and all were selected for high virulence. Cultures were grown on yeast-malt agar for 7 days at 20 C under constant light of 20w cool-white fluorescent lamps (6.6×10^3 ergs/cm^2/sec). Pycnidiospore suspensions washed from the surface of several agar plates and adjusted with sterile water to a concentration of 10^7 spores/ml were used for seedling inoculations (9).

Seed of spring and winter wheat cultivars (identified in Tables 1 and 2) were sown in straight lines and at equal spacing on the periphery of square plastic containers filled with steamed composted soil without added nutrients. The plastic containers were $20 \times 20 \times 6.5$ cm and 30-40 seeds per cultivar were planted. One container was planted per wet period per trial. The spore suspension was sprayed (15 cc/ container) onto the wheat seedlings (10 days after sowing) as they were revolving on a phonograph turntable at 45 rpm (4). Immediately following inoculation, the containers were moved into a

TABLE 1. Relationship of postinoculation wet period to number of lesions and area of necrosis incited by *Septoria nodorum* on several wheat lines

Wheat cultivar	C. I. or P. I.[v] number	Lesions/cm^2 (no.)[w]		Necrosis/cm^2 (%)[w]	
		Regression coefficients[x]	Correlation coefficients	Regression coefficients[x]	Correlation coefficients
DeKalb's SB-8	...	0.56 ab[y]	0.97**[z]	0.72 ab	0.97**
Fletcher	13985	0.58 ab	0.98**	0.78 ab	0.99**
Fortuna	13596	0.65 a	0.98**	0.85 a	0.98**
Manitou	13775	0.49 b	0.95**	0.63 b	0.97**
Norana	15927	0.49 b	0.88**	0.66 b	0.96**
Shortana	15233	0.41 b	0.88**	0.63 b	0.98**
World Seed 1812	14585	0.54 ab	0.97**	0.72 ab	0.85**

[v]Cereal Introduction (C. I.) or Plant Introduction (P. I.) numbers.

[w]Data were from three replicated experiments.

[x]Statistical analysis conducted on the regression coefficients (b) of the linear (log-log) regression, where X = log wet periods; Y = log number of lesions/m^2, or log % necrosis/cm^2.

[y]Values followed by the same letter are not significantly different at $P = 0.5$, by the simultaneous test procedure (18).

[z]Double asterisks (**) indicate significant positive correlation (r) at $P = 0.01$.

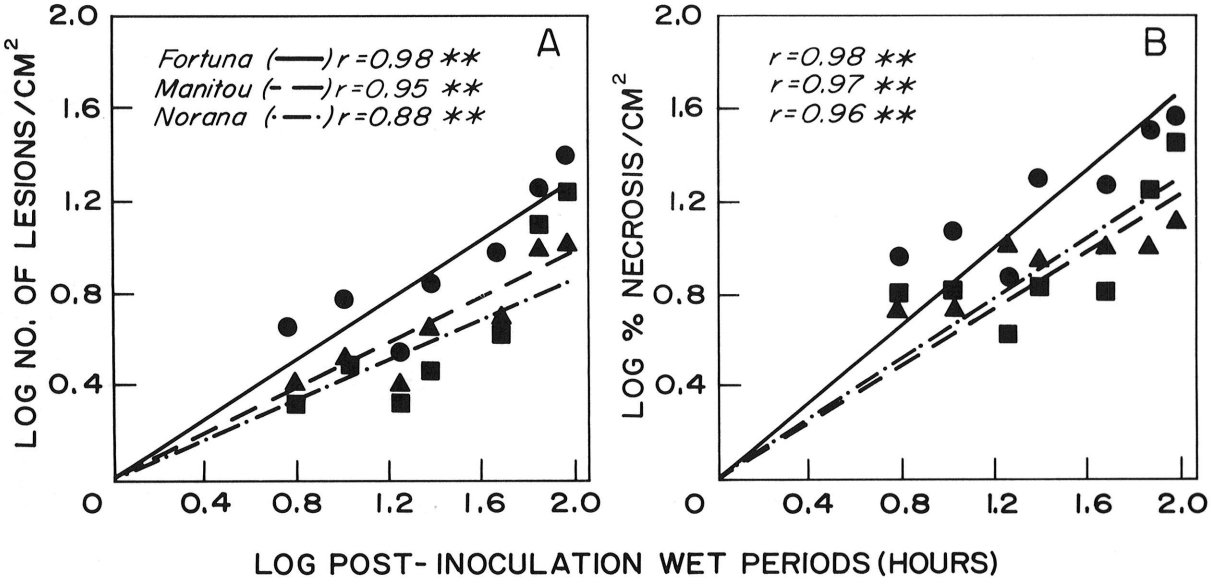

Fig. 1-(A, B). The response of three spring wheat cultivars, Fortuna, Monitou, and Norana, to different postinoculation wet periods, expressed in the function log $\hat{Y} = a + b \log X$, where $\hat{Y}$ = log of symptoms, and X = log of wet periods (hr). Correlation coefficients (r) (wet periods with lesions or necrosis) are given for each wheat cultivar. Graph **A)** represents lesions (no.)/cm^2; and **B)** necrosis (%)/cm^2.

mist chamber, and misted by two cool-water humidifiers for 6, 12, 18, 24, 48, 72, and 96 hr with air temperature at 22 ± 2 C. Upon removal from the chamber, each inoculated container and noninoculated check was dried for about 5 min with a fan and returned to the controlled-environment condition in which they were grown originally (15 C night/23 C day, a 12-hr day, and light intensity of 1.7×10^4 ergs/cm^2/sec).

Eight days after inoculation, each leaf to be considered was marked at its base with a nonphytotoxic felt tip pen 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 the leaf was measured. Fifteen days after inoculation, the amount of necrotic tissue on infected leaves was estimated by measuring the length of the necrotic region and relating it to the length and width of leaf previously recorded. Lesion counts and estimates of necrotic tissue were recorded separately for each centimeter of leaf length. The number of lesions per square centimeter and percentage of necrosis per square centimeter then were calculated for each entire leaf.

Two experimental series were conducted to evaluate the effect of wet period duration on disease damage to wheat seedlings inoculated with *S. nodorum*: a spring wheat cultivar series, and a group of resistant spring and winter wheat lines selected in screening experiments. Each series of experiments was repeated three times, and the results were subjected to statistical analysis (18). After several statistical transformations were attempted, the logarithm and square-root transformations were selected as best fitting the data (Fig. 1 and 2). Regression coefficients (b) and correlation coefficients (r) were calculated and are presented in tabular form rather than as additional figures (Tables 1 and 2).

RESULTS

Several spring and winter wheat cultivars differed in number of lesions and areas of necrosis when infected by *S. nodorum*. The differences were accentuated by extending periods of leaf wetness after inoculation. The relationships between periods of leaf wetness and increased symptoms (manifestations of disease damage) followed linear functions. A log-log linear regression of length of wet period on number of lesions and area of necrosis best fitted the data from the spring wheats. All of the spring wheats suffered increasing amounts of disease damage as the postinoculation wet period was extended (Fig. 1). The increases in disease damage (lesions and necrosis) were not identical for all cultivars, and extending the wet period increased the differences between cultivars. Linear regressions of lesions or necrosis on length of postinoculation wet period were calculated, then a statistical analysis of the slopes (b) of the regression lines was done. The analysis showed that the cultivar Fortuna had significantly more lesions and necrosis than did Manitou, Norana, and Shortana (Table 1). Cultivars DeKalb's SB-8, Fletcher, and World Seeds 1812 were intermediate, but not significantly different from Fortuna, in their response to infection by *S. nodorum*. Correlations between length of postinoculation wet period and symptoms (number of lesions and area of necrosis) were calculated. Coefficients (r) were highly significant for each cultivar (Table 1).

The effects of progressively longer periods of leaf wetness after inoculation with *S. nodorum* were studied on selected resistant spring and winter wheat cultivars. Extension of the wet period resulted in only a few more lesions and a relatively small increase in the area of necrosis when the more resistant winter wheat cultivars

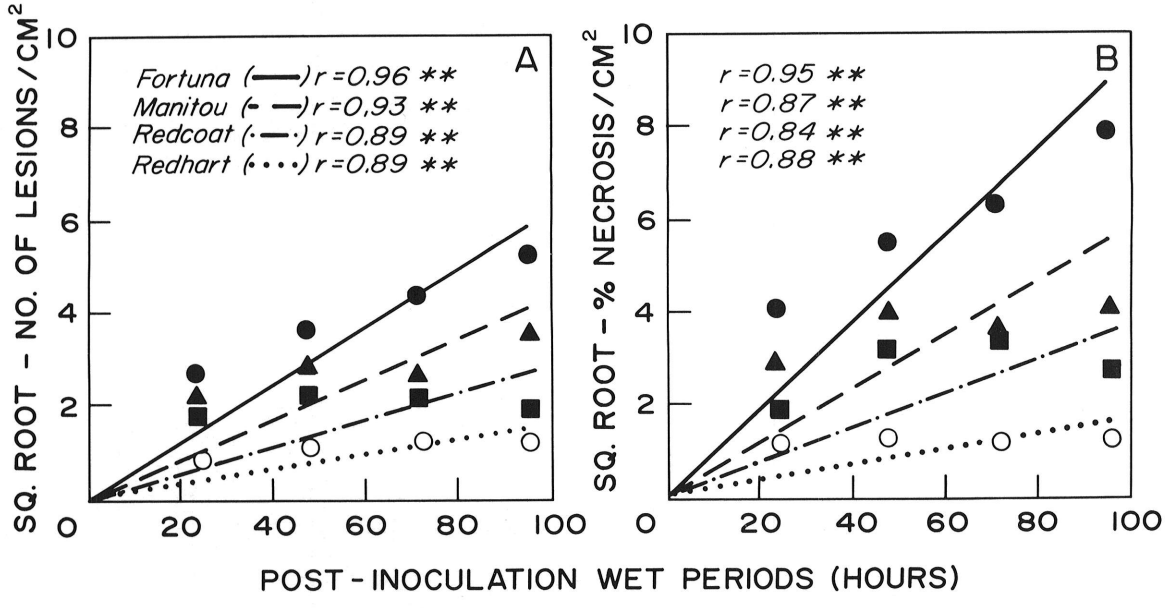

Fig. 2-(A, B). The response of two spring wheat cultivars, Fortuna and Manitou, and two winter wheat cultivars, Redcoat and Redhart, to different postinoculation wet periods, expressed in the function square root $\hat{Y} = a + bX$, where $\hat{Y}$ = square root of symptoms, and X = wet periods (hr). Correlation coefficients (r) (wet periods with lesions or necrosis) are given for each wheat cultivar. Graph **A)** represents lesions (no.)/cm^2; and **B)** necrosis (%)/cm^2.

TABLE 2. Relationship of postinoculation wet period to number of lesions and area of necrosis incited by *Septoria nodorum* on several wheat lines

Wheat cultivar	C. I. or P. I.[v] number	Lesions/cm^2 (no.)[w]		Necrosis/cm^2 (%)[w]	
		Regression coefficients[x]	Correlation coefficients	Regression coefficients[x]	Correlation coefficients
Spring wheats:					
Era	13986	0.048 b[y]	0.93**[z]	0.058 b	0.93**
Fortuna	13596	0.062 a	0.96**	0.093 a	0.95**
Manitou	13775	0.043 b	0.93**	0.057 b	0.87**
Svenno	13642	0.034 bc	0.94**	0.058 b	0.91**
Winter wheats:					
Coker 68-8	...	0.013 f	0.84**	0.015 d	0.87**
Hadden	13488	0.022 def	0.91**	0.028 d	0.94**
Harvest Queen	5314	0.022 de	0.92**	0.023 d	0.94**
Hybrid 143	4160	0.015 ef	0.86**	0.018 d	0.91**
Moking	12556	0.022 de	0.91**	0.018 d	0.88**
Redcoat	13170	0.028 cd	0.89**	0.036 c	0.84**
Red Chief	12109	0.016 ef	0.89**	0.016 d	0.88**
Redhart	8898	0.013 f	0.87**	0.016 d	0.89**
Turkey Sel	11984	0.027 cd	0.89**	0.025 d	0.85**

[v]Cereal Introduction (C. I.) or Plant Introduction (P. I.) numbers.
[w]Data were from three replicated experiments.
[x]Statistical analysis conducted on the regression coefficients (b) of the linear regression, where x = wet periods, y = square root of lesions/cm^2, or square root of % necrosis/cm^2.
[y]Values followed by the same letter are not significantly different at $P = 0.5$, by the Simultaneous Test Procedure (18).
[z]Double asterisks (**) indicate significant positive correlation (r) at $P = 0.01$.

were inoculated. Linear regressions were calculated and transformations were tried for best fit to the data. In this case, a square root transformation gave the best fit (Fig. 2). Regression coefficients for each cultivar were calculated, and a statistical analysis of slopes of the regression line (b) is shown in Table 2. With the exception of one spring wheat (Svenno) and two winter wheats (Redcoat and Turkey Sel), the spring wheats had significantly more lesions per square centimeter than did the winter wheats. As a result of infection by *S. nodorum* all spring wheats had significantly more necrosis than the winter wheats (Table 2). Among the winter wheats, only Redcoat had significantly less necrosis than the others, but there was considerable variation in the number of lesions per unit area. Since the differences reported were based upon the slope of the regression line, the actual differences between cultivars became greater with increased length of postinoculation wet period. Again, in every case there was a significant positive correlation (r) between length of wet period and symptoms.

DISCUSSION

Postinoculation leaf wetness is a critical factor affecting whether or not symptoms will develop on wheat leaves inoculated with *S. nodorum* (1, 3, 14). We have confirmed the observations of Holmes and Colhoun (6) that wheat cultivars vary in the length of postinoculation wet period required for any symptoms to appear at all. Additionally, we have shown that longer postinoculation wet periods support the development of some symptoms on resistant cultivars, and many symptoms (lesions and areas of necrosis) on susceptible cultivars. Hosford has reported similar results with other wheat leaf-spotting organisms (7, 8). We have shown that spring wheats generally are more heavily damaged in an environment favorable for disease development than are winter wheats. We conclude, therefore, that among the cultivars studied, the winter wheats are more resistant to *S. nodorum* than are the spring wheats. A similar conclusion was reached in Israel where higher levels of resistance to *S. tritici* were observed in vernalized winter wheats than in spring wheats grown in field nurseries (Eyal and Wahl, *unpublished*).

In all of our experiments, the expression of cultivar resistance to infection by *S. nodorum* as measured by the development of lesions and necrosis was related to the duration of postinoculation wet periods. Resistance was not altered, nor was ranking of cultivars changed by exposure to extended periods of leaf wetness; but, numbers of lesions and areas of necrosis increased at a greater rate in some cultivars than in others when they were subjected to prolonged periods of leaf wetness. Thus, long periods of postinoculation wetness had the effect of spreading cultivar reactions further apart and making it easier to determine which ones were more or less resistant to *S. nodorum*. Differences in the response of cultivars and lines of wheat to various postinoculation wet periods, linked with high spore concentrations in inoculum, are being used to detect resistant germplasm and for screening breeding populations at the seedling stage of growth for resistance to *S. nodorum* and *S. tritici* (Scharen and Krupinsky, *unpublished*).

Our study, and others we have cited, indicate that the effect of postinoculation wet periods on the development of disease in wheat due to infection by *S. nodorum* is a multi-phase process. Fungal spore germination and penetration of the host leaves constitutes the first phase, whereas the establishment of the host-parasite

relationship is the second phase. But, even after establishment of the pathogen within host-tissue, the progress of the disease and the eventual appearance of symptoms remains dependent upon external environmental conditions, notably moisture and temperature (6, 10, 11, 12). Disease progress and damage to the crop can be arrested at any stage of development by the onset of warm, dry weather that is unfavorable to the disease. By exploiting the sources of resistance identified in this study, and by using these methods to find other resistance sources, we expect that wheat cultivars may be developed that can withstand environmental conditions favoring infection by *S. nodorum*, and thus sustain less damage to yield than is suffered by most presently grown cultivars.

We also encourage future investigations into the nature of the effects of postinoculation leaf wetness upon disease development. The phenomenon of external environmental influence on the speed and extent of disease development, especially in leaf-spot diseases caused by facultatively parasitic fungi, may be widespread. Some reevaluation of etiological concepts historically related to leaf-spot diseases may be warranted.

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