Relationships Between Disease Reactions under Controlled Conditions and Severity of Wheat Bacterial Streak in the Field

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ABSTRACT

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Bacterial streak of wheat is best controlled by resistant cultivars, but there are difficulties associated with screening for resistance under field conditions. Screening for resistance under controlled conditions could facilitate the development of resistant cultivars if there is a good relationship between disease reaction and disease severity. The objective of this study was to determine the relationships between bacterial streak reactions on primary leaves of seedlings or flag leaves of adult plants and bacterial streak severity on adult plants in the field. Fifty spring wheats and 24 winter wheats were evaluated. Except for spring wheat cultivar reactions on primary leaves, reactions (ranks) of greenhouse-grown cultivars were significantly and positively related (r ranged from 0.60 to 0.64 with $P \leq 0.0012$) to ranks of cultivars evaluated for bacterial streak severity. However, selection for low percentages of water-soaking under controlled conditions would keep a few wheats that were susceptible in the field and discard several wheats that were resistant in the field. Because of these discrepancies we cannot recommend inoculation under controlled conditions as a reliable means of screening a random sample of wheats for bacterial streak resistance.

Additional keywords: *Xanthomonas campestris* pv. *translucens*, *Xanthomonas campestris* pv. *undulosa*

Bacterial streak of wheat (*Triticum aestivum* L.) foliage and black chaff of wheat heads and peduncles are caused by *Xanthomonas campestris* pv. *translucens*. These diseases occur worldwide in most wheat production areas (2,5) and in some areas may be attributed to infections by *X. campestris* pv. *undulosa*. Bacterial streak symptoms in the field are rare until after heading and are characterized by narrow, brown, water-soaked streaks that turn necrotic after a few days (1,17). Foliar infections have the greatest impact on grain filling (7).

Seedborne cells of the pathogen appear to be the most important source of inoculum (16); however, control by eradication of the pathogen from large quantities of seed is unlikely (9,10). Growing resistant cultivars appears to be the most feasible means of control (1,3,11), and races capable of overcoming resistance have not been identified (14).

Evaluating disease severity in the field is currently the only means of identifying resistance in wheat. Severe epidemics are sporadic, and it can be difficult to obtain sufficient disease pressure even in inoculated field tests (7,17). In addition, accurate evaluations of quantitative levels of resistance under field conditions are expensive and time-consuming. Because of the problems associated with field evaluations, it would be advantageous to screen for bacterial streak resistance under controlled conditions, especially in the seedling stage, provided the results predict bacterial streak severity under field conditions.

Milus and Mirlohi (15) demonstrated that, for a limited number of wheat cultivars, the disease reactions in inoculation sites on primary and flag leaves were associated with the population size of the pathogen in the sites and with the relative level of resistance observed in the field. The objective of this study was to determine the relationship of bacterial streak reaction on primary leaves of seedlings and flag leaves of adult plants with bacterial streak severity in the field based on a larger, more diverse group of wheat genotypes.

MATERIALS AND METHODS

A group of 24 soft red winter wheat genotypes (winter wheats) and a group of 50 spring wheat genotypes (spring wheats) (Table 1) were tested. The winter wheats were representative of cultivars grown in the soft red winter wheat region of the United States. The spring wheats were mostly CIMMYT genotypes and represented hexaploid spring wheat grown on five continents. Except for field tests, the procedures were similar for both winter and spring wheats.

Seedling experiments. Seedlings were grown in six-pack containers (8.9 × 13.3 cm) filled with potting mixture (peat, vermiculite, loam soil, sand, and perlite in a 6:4:3:3:2 ratio) and kept on a greenhouse bench at 15 to 25°C. Natural light was supplemented with light from high-intensity metal halide lamps operating from 0600 to 1800 hours. Plants were fertilized after emergence and after inoculation with dilute (1 g/liter) Peters 20-20-20 (NPK) fertilizer.

Primary leaves of winter wheats were infiltrated with a suspension (5×10^4 CFU/ml) of *X. campestris* pv. *translucens* strain 88-14^{Rif} when second leaves were 2 to 4 cm long, as described by Milus and Mirlohi (15). Inoculum concentration for experiments with spring wheats was 5×10^4 CFU/ml except for two experiments at 3×10^5 CFU/ml. Inoculated plants were kept in a growth chamber (Conviron model E7, Controlled Environments Inc., Pembina, ND) at 25°C and 12-h photoperiod of mixed fluorescent and incandescent light.

Disease reactions in the inoculation sites (average of three leaves per replication) were recorded 8 days after inoculation on a 0 to 6 rating scale (0 = no symptoms; 1 = chlorosis but no water-soaking; 2 = less than 10% water-soaking; 3 = water-soaking

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10 to 30%; 4 = water-soaking 31 to 70%; 5 = water-soaking 71 to 100%; and 6 = water-soaking extending beyond the inoculated area) as described previously (15). Disease reactions were transformed to the mean of the range in percent water-soaking (i.e., 0, 0, 5, 20, 50, 85, and 100% for disease reactions 0 to 6, respectively) for data analysis.

Experiments were conducted between October 1993 and July 1994 except for two experiments with spring wheats conducted in February and March 1993. A randomized complete block design was used for each experiment. Experiments with winter wheats had six replications and were conducted four times. Experiments with spring wheats had four replications and were conducted eight times.

Adult-plant experiments. Seedlings of winter wheats were vernalized in petri dishes and then grown in a greenhouse at 15 to 25°C as described by Milus and Mirlohi (15). Spring wheats were sown directly into 15-cm pots and grown in a greenhouse as described for winter wheats. At Feekes's (13) growth stage (GS) 10 (swollen boot), one flag leaf of each of three plants per pot was inoculated at three sites with a suspension of strain 88-14^{Rif} (1×10^6 CFU/ml) as described by Milus and Mirlohi (15). Because cultivars within an experiment reached boot stage at different times, inoculations were done over 5 to 10 days and 12 to 18 days, depending on the experiment, for winter and spring wheats, respectively. Fresh inoculum was prepared for each inoculation.

Disease reactions (average of three inoculation sites per leaf) were recorded 11 days after inoculation on a 0 to 4 scale (0 = no visible symptoms; 1 = chlorosis but no water-soaking; 2 = water-soaking less than 25%; 3 = water-soaking 25 to 100%; and 4 = water-soaking extending beyond the infiltrated area) as described by Milus and Mirlohi (15). Disease reactions were transformed to the mean of the range in percent water-soaking (i.e., 0, 0, 12, 67, and 100% for disease reactions 0 to 4, respectively) for data analysis.

Experiments with spring wheats were conducted twice between April and June 1993. Each experiment was a randomized complete block with two replications (pots). The percent water-soaking for the three flag leaves per pot were averaged. Tests with winter wheats were conducted four times between February and April 1994. Each experiment was a randomized complete block with two replications as described for spring wheats.

Field experiments with winter wheats. Soft red winter wheat genotypes were evaluated for bacterial streak severity in the field during the 1991 to 1994 seasons (year of harvest). All 24 cultivars in this study were included in the 1993 and 1994 experiments, but only 20 of the 24 cultivars were included in the 1991 and 1992 experiments.

Each experiment was a randomized complete block with four replications. Plots were planted in October by means of a small plot drill and were 7 rows (1.25 m) × 4.25 m long. Seeds of a susceptible cultivar (FFR 525W) were vacuum infiltrated with a suspension (10^7 CFU/ml) of *X. campestris* pv. *translucens* strain 88-14^{Rif} in 1991 and five strains (88-7, 88-11, 88-15, 90-4, and 90-6) in 1992 to 1994. Infested seeds were air dried on screens in a fume hood and then planted (seven rows wide) across the ends of individual plots and around the entire experiment as a source of early inoculum. Additional inoculum (approximately 10^{10} CFU/m^2) of the five strains was applied to the foliage three times between flag leaf emergence and early heading in 1993 and once at flag leaf emergence in 1994.

Propiconazole (Tilt 3.6E) fungicide was applied to suppress foliar fungal diseases that might have confounded ratings of bacterial streak severity. Except in 1994 when only an early application was used, applications (126 g a.i./ha) were made when most cultivars were at GS 8 (flag leaf emergence) and again at GS 10.5 (fully headed). Recommended fertility and weed control practices were used as needed.

Cultivars were rated for percent foliage with bacterial streak symptoms at GS 10.54 (watery ripe). Bacterial streak severities (and their ranges) that were recorded were as follows: 0; 2 (trace to 4%); 7 (5 to 10%); 15 (11 to 20%); 30 (21 to 40%); 50 (41 to 60%); 70 (61 to 80%); 85 (81 to 90%); 93 (91 to 96%); and 98 (>96% of the foliage with symptoms).

Field experiments with spring wheats. The 50 spring wheats were planted in a randomized complete block design with three replications at El Batan in the Mexican highlands (2,250 m above sea level) during the summer seasons (May to September) in 1991 and 1993. Each plot consisted of two rows 1.5 m long. Plants were inoculated at GS 3 (fully tillered) by spraying a suspension of *X. campestris* pv. *translucens* strain CFBP3085 (10^9 CFU/ml plus 0.02% Tween 20) on the foliage. Plants were sprayed every 15 days after booting with tebuconazole (Folicur 250 EC, 300 g a.i./ha) and oxydemeton-methyl (Metasystox R25, 500 g a.i./ha) to protect against leaf rust (*Puccinia recondita* f. sp. *tritici*) and the Russian wheat aphid (*Diuraphis noxia*), respectively. Bacterial streak severity was recorded at GS 10.54 with an assessment key developed specifically for bacterial streak of wheat (4).

Data analysis. Spearman's correlations (r) were derived by the PROC CORR procedure of SAS (Statistical Analysis Systems, SAS Institute, Cary, NC) to determine consistency of cultivar ranks for percent water-soaking and disease severity. A significant positive correlation among experiments indicates nonsignificant experiment × cultivar interaction and was used as the basis for pooling data across experiments. Spearman's correlations were then used on the pooled data to determine if the rankings of cultivars were consistent for percent water-soaking on primary leaves, percent water-soaking on flag leaves, and bacterial streak severity in the field.

Cultivar means for bacterial streak severity in the field were plotted versus means for percent water-soaking on primary and flag leaves to show the average values for each cultivar and to show the relationship between the variables.

Table 1. Winter and spring wheat genotypes in this study and the letters or numbers designating them in the figures

Winter wheats		Spring wheats			
Letter / Genotype		Number / Genotype		Number / Genotype	
A	Coker 983	1	Alondra	26	IAPAR 28
B	Magnum	2	Anahuac F75	27	Inia F66
C	Florida 302	3	Anza	28	Juriti
D	Cardinal	4	Bacanora T88	29	LfN/1158.57...
E	Terral 101	5	Batuira	30	Macuco
F	Bayles	6	Angostura F88	31	Maringa
G	FFR 525W	7	BH 1146	32	Morocco
H	Dynasty	8	Buck Ombu	33	Nacozari F76
I	Sawyer	9	Cacatu	34	Nainari 66
J	Savannah	10	Caete	35	Nanjing 8331
K	AR 26413B	11	Candeias	36	Opata M76
L	Coker 833	12	Ciano 79	37	Parula
M	Coker 9835	13	Turaco	38	Pavon F76
N	Caldwell	14	Cocoraque F75	39	Potam S70
O	Keiser	15	Corderilla 3	40	Mochis T88
P	Twain	16	Cucurpe S86	41	Seri M82
Q	McNair 1003	17	Dove	42	Siete Cerros T66
R	Wakefield	18	Esmerelda M86	43	Sonalika
S	Saluda	19	Veery 10	44	Sonora 64
T	Hazen	20	Galvez S87	45	Tapejara
U	Verne	21	Genaro T81	46	Thornbird
V	Andy	22	Glennson M81	47	Trigo Br1
W	Gore	23	GZ156/NAC...	48	Tucano
X	Florida 304	24	HD 2329	49	Tui
		25	IAC835	50	Ures T81

RESULTS

Winter wheats. Cultivar ranks were consistent among experiments for percent water-soaking on primary leaves (r ranged from 0.88 to 0.94 with $P \leq 0.0001$), for percent water-soaking on flag leaves (r ranged from 0.69 to 0.83 with $P \leq 0.0002$), and for bacterial streak severity (r ranged from 0.59 to 0.80 with $P \leq 0.017$) in the field. Thus, percent water-soaking and disease severity values were pooled across experiments for further comparisons.

With the pooled data, the rankings of cultivars for percent water-soaking on primary and flag leaves were positively correlated ($r = 0.64$, $P = 0.0008$, and $r = 0.62$, $P = 0.0012$, respectively) with the rankings for bacterial streak severity in the field. The rankings of cultivars for percent water-soaking on primary leaves also were positively correlated ($r = 0.73$, $P \leq 0.0001$) with the rankings of cultivars for percent water-soaking on flag leaves.

Most cultivars could be classified as either resistant or susceptible for both disease severity and percent water-soaking on primary leaves (Fig. 1A). Three cultivars (Savannah [J], Andy [V] and Florida 304 [X]) were more susceptible in the field than expected based on percent water-soaking and could be considered outliers. Five cultivars (Gore [W], Hazen [T], Saluda [S], Verne [U], and Wakefield [R]) were more resistant in the field than expected based on their percent water-soaking.

Similarly, most cultivars could be classified as either resistant or susceptible based on both disease severity in the field and percent water-soaking on flag leaves (Fig. 1B). In the field, Savannah (J) was more susceptible, and seven cultivars (Sawyer [I], Verne [U], Saluda [S], Wakefield [R], Gore [W], Caldwell [N], and Hazen [T]) were more resistant in the field than expected based on their percent water-soaking on flag leaves.

Spring wheats. Cultivar ranks were consistent among experiments for percent water-soaking on primary leaves (r ranged from 0.63 to 0.87 with $P \leq 0.0001$), for percent water-soaking on flag leaves ($r = 0.59$ with $P \leq 0.0001$), and for bacterial streak severity ($r = 0.51$ with $P \leq 0.0001$). Thus, percent water-soaking and disease severity values were pooled across experiments for further comparisons.

With the pooled data, all cultivars averaged less than 60% water-soaking on primary leaves (Fig. 2A). The rankings of cultivars for percent water-soaking on primary leaves were not related ($r = 0.12$, $P = 0.41$) to the rankings of cultivars for bacterial streak severity in the field. The rankings for percent water-soaking on primary leaves were also poorly related ($r = 0.37$, $P = 0.009$) to the rankings for percent water-soaking on flag leaves.

The rankings of cultivars for percent water-soaking on flag leaves were positively correlated ($r = 0.60$, $P \leq 0.0001$) with the rankings of cultivars for bacterial streak severity in the field.

Percent water-soaking on flag leaves (Fig. 2B) differentiated genotypes better than percent water-soaking on primary leaves. All genotypes with less than 50% water-soaking had disease severities ≤25%; however, genotypes with greater than 50% water-soaking had disease severities that spanned nearly the entire observed range in disease severity (approximately 10 to 60%. Morocco spring wheat (no. 32) had the highest flag leaf disease reaction of all wheats in this study and was the only wheat that had obvious bacterial exudate associated with the inoculation sites.

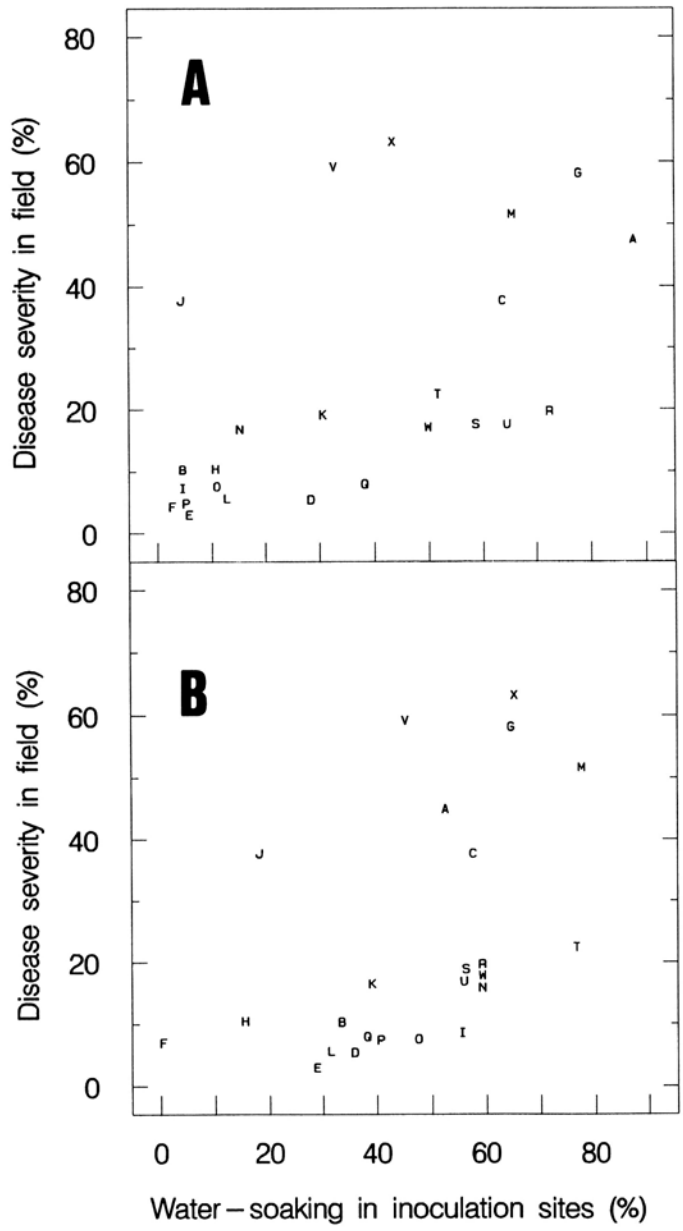

Fig. 1. Relationship between disease severity on adult plants in the field and percent water-soaking in controlled tests on **(A)** primary leaves and **(B)** flag leaves of 24 winter wheat genotypes (A to X as listed in Table 1).

DISCUSSION

This study demonstrated that the percentages of water-soaking on primary leaves of winter wheats and on flag leaves of both winter and spring wheats under controlled conditions were related to the percentage of foliage diseased in the field. However, selection for low percentages of water-soaking under controlled conditions would keep a few wheats that were susceptible in the field and discard several wheats that were resistant in the field. Because of these discrepancies we can not recommend inoculation under controlled conditions as a reliable means of screening a random sample of wheats for bacterial streak resistance.

The three winter wheat cultivars (Savannah [J], Andy [V], and Florida 304 [X]) that were more susceptible than expected in the field based on their percent water-soaking (Fig. 1A,B) matured much earlier than other cultivars in the study. Tillman

(18) found significant negative correlations between heading date (maturity) and bacterial streak severity for a group of 428 wheat lines. The early maturity of these three cultivars may have predisposed them to higher disease severity under field conditions.

Spring wheats in this study rarely exhibited a high percentage of water-soaking on primary leaves even though susceptible winter wheats included in experiments as checks had high values (>70%) of water-soaking. In the two experiments with higher inoculum concentration (3×10^5 CFU/ml), percent water-soaking tended to increase on all cultivars, and cultivars were no better differentiated. Duveiller (3) found that lesion length (length of water-soaking) on inoculated seedlings of the same cultivars also did not correlate with bacterial streak severity in the field.

Even though bacterial streak symptoms usually develop in the field after heading stage, disease reaction on flag leaves under greenhouse conditions was not always a good predictor of bacterial streak severity. Factors other than ability of the pathogen to multiply in leaves after syringe inoculation appear to affect disease development. As noted above, very early cultivars tend to be susceptible and very late cultivars tend to be resistant. Duveiller (3) noted a trend for taller cultivars to be more resistant.

X. campestris pv. *translucens* was found to be associated with symptomless wheat in Mexico, which suggests that the pathogen has an epiphytic phase (6). The pathogen also appears to be a well-adapted epiphyte under Arkansas conditions (E. A. Milus, unpublished data). Genotypes may differ in ability to support epiphytic populations of the pathogen prior to symptom expression, and thus low epiphytic populations may be an important component of resistance that is circumvented by syringe inoculation.

Although bacterial streak severity under field conditions is the ultimate measure of resistance, it is difficult and time consuming to obtain reliable field data. For winter wheats, similar field experiments were planted at two locations from 1991 to 1994, but only one location each year provided useful data. In noninoculated field tests, sufficient disease may not develop or varying levels of seedborne inoculum among the different entries may obscure differences in levels of resistance. Obtaining reliable bacterial streak data from field plots requires the plots to be inoculated uniformly even in disease-prone environments (3).

Results of this study may be useful for elucidating mechanisms that contribute to resistance. Winter wheats could be placed in four groups depending on their percentage of water-soaking and disease severity. Wheats with low water-soaking and low disease severity may have resistance due to inability of the pathogen to multiply to high populations in leaf tissue, because percent water-soaking has been shown to be related to population size of the pathogen (15). Wheats with low water-soaking and high disease severity may be predisposed by factors, such as early maturity, that overcome resistance to multiplication in leaf tissue. Wheats with high water-soaking and low disease severity may have mechanisms other than resistance to multiplication in leaves, such as resistance to epiphytic growth of the pathogen, that reduce disease severity. Wheats with high percent water-soaking and high disease severity may have no mechanisms for resistance.

Disease reactions on primary leaves or flag leaves were more consistent and more easily obtained than disease severity ratings in the field. Results presented here suggest that disease reaction on primary leaves may be a useful criterion for selecting resistant progeny from crosses involving one or more parents in which low bacterial streak severity is associated with low disease reaction. For example, Magnum (B), Terral 101 (E), Bayles (F), Sawyer (I), and Twain (P) winter wheats had both low disease reaction on primary leaves and low bacterial streak severity in the field. If these cultivars were used as sources of resistance, these types of resistance possibly could be selected based on disease reactions on primary leaves.

There have been few studies on the genetics of bacterial streak resistance in wheat (8), perhaps because of the difficulty in classifying progeny. Resistance in wheat is relative, and no cultivar is immune (1,8). Johnson et al. (12) used a needle inocula-

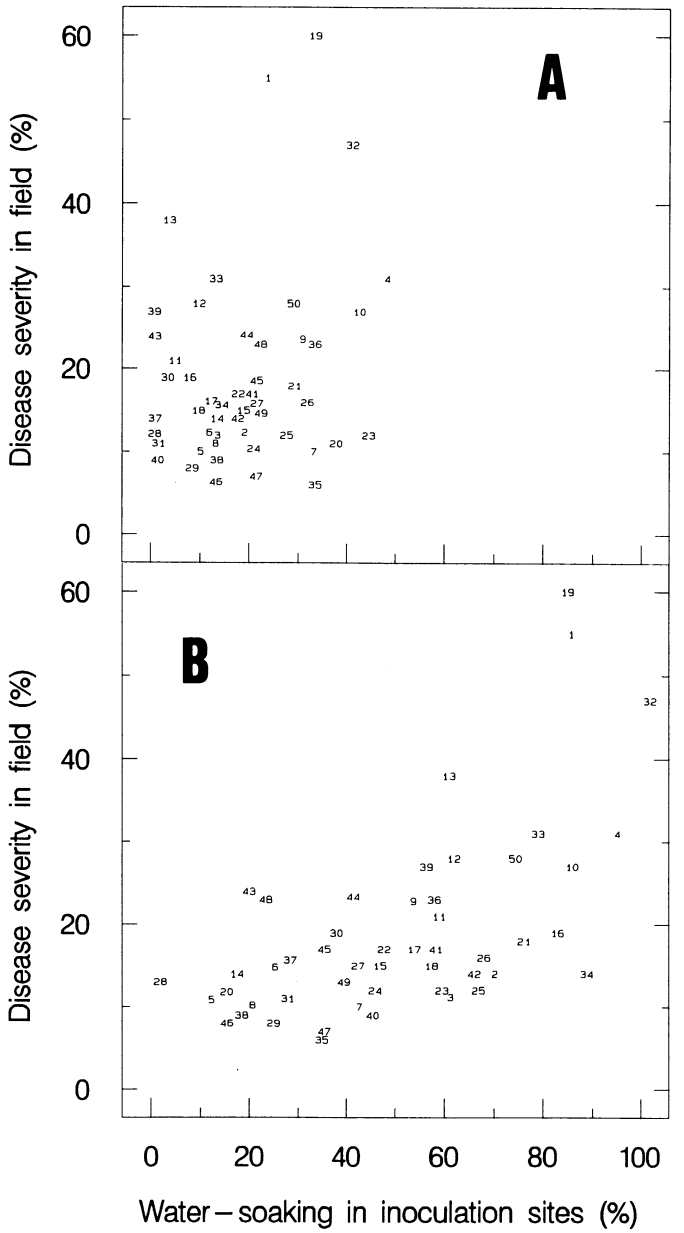

Fig. 2. Relationship between disease severity on adult plants in the field and percent water-soaking in controlled tests on **(A)** primary leaves and **(B)** flag leaves of 50 spring wheat genotypes (1 to 50 as listed in Table 1).

tion technique to accurately identify triticale plants completely resistant to bacterial streak and to determine the inheritance of the resistance. Use of disease reactions on primary leaves may simplify studies on the inheritance of certain types of bacterial streak resistance in wheat.

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