Virulence of *Xanthomonas campestris* pv. *translucens* on Selected Wheat Cultivars

E. A. MILUS, Assistant Professor, and D. B. CHALKLEY, Research Specialist, Department of Plant Pathology, University of Arkansas, Fayetteville 72701

ABSTRACT

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In this study, strains of *Xanthomonas campestris* pv. *translucens* from North and South America were examined for differences in virulence on a set of potential differential wheat cultivars. Seedlings of 19 wheat cultivars were inoculated with 81 strains of the pathogen isolated from wheat, barley, rye, or triticale and rated for percent water-soaking in the inoculated area 8 days after inoculation. Averaged over all cultivars, 69 virulent strains caused 14.4–38.1% water-soaking, and 12 weakly virulent strains caused 1.1–4.5%. Averaged over the 69 virulent strains, five susceptible cultivars had 43.4–75.0% water-soaking, and 17 resistant cultivars had 5.5–22.8%. The cultivar × strain interaction was highly nonsignificant ($P \geq 0.9999$) when data for the 69 virulent strains on the 19 cultivars were analyzed. Thus, there was no evidence for races among the strains that best represent strains capable of causing bacterial streak on wheat in the field. Cultivars rated as resistant to bacterial streak in the field are likely to remain resistant relative to susceptible cultivars, although actual disease severities may vary due to differences in inoculum level or environmental conditions.

Xanthomonas campestris pv. *translucens* (syn. *X. translucens* var. *undulosa*) causes bacterial streak (stripe) and black chaff of wheat (*Triticum aestivum* L.) and other small grains (22). Bacterial streak refers to water-soaked lesions on leaves, which are the most important disease symptom; black chaff refers to black lesions on glumes (4). Strains of *X. c. translucens* differ in their host range on small grains (wheat, barley, rye, and triticale) (3,5,8,9), but this study only examined strains that were pathogenic on wheat.

Although cultivars of several different market classes of wheat varied in resistance to bacterial streak under field conditions (1,2,6,10,11,13,20,21), there has been no definitive evidence for the existence of races of *X. c. translucens*. In a study with 20 hard red spring wheat cultivars inoculated with four strains of *X. c. translucens*, Bamberg (2) reported that several cultivars were resistant to certain strains but susceptible to others. Cunfer and Scolari (5) inoculated 14 wheat cultivars with six strains of *X. c. translucens* and found that 12 cultivars had differential susceptibility to the strains. Mellano and Cooksey (14) found evidence for strain–cultivar specificity among four wheat cultivars and four strains of *X. c. translucens*.

The existence of races capable of overcoming resistance identified in certain wheat cultivars could compromise the use of these cultivars for disease control. The objective of this study was to determine if races of *X. c. translucens* could be identified among strains from North and South America on a selected set of potential differential wheat cultivars.

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MATERIALS AND METHODS

Eighteen cultivars of soft red winter wheat and one cultivar of spring wheat (Pavon 76) were selected as potential differential cultivars. The soft red winter wheats are representative of the cultivars currently grown in the soft red winter wheat region of the United States. Field observations in Arkansas indicated that they represent a range in bacterial streak resistance (1). Pavon 76 was reported to be resistant under field conditions in Mexico (6). 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 15–25 C. Natural light was supplemented with light from high-intensity metal halide lamps operating from 0600 to 1800 hr. Plants were fertilized after emergence and after inoculation with Peters 20-20-20 (NPK) fertilizer.

A total of 105 strains of *X. c. translucens* isolated from wheat, barley, rye, or triticale was obtained. In addition to originating from diverse small-grain hosts, these strains were recovered from five countries and nine states. Strains Xt-12 (PDDCC 5749) and Xt-13 (PDDCC 5755) are the neopathotype strains for *X. c. secalis* and *X. c. undulosa*, respectively (7). All strains were characterized by fatty acid analysis by L. W. Barnes, Texas A&M University, by using the Microbial Identification System (MIDI, Newark, DE). Those with a similarity index >0.50 were considered good matches to the strains of *X. c. translucens* used to make the library, and those with indices 0.30–0.49 were good matches but possibly atypical strains (15). All strains were tested for pathogenicity on wheat as described previously (16), and those that were pathogenic under these optimum conditions were tested on each of the 19 wheat cultivars.

Cultures were stored in 15% dimethyl sulfoxide at −80 C and grown on nutrient agar (Difco Laboratories, Detroit, MI) amended with 5 g of dextrose per liter. Inoculum was prepared in sterile deionized water using 1-day-old cultures. Concentration was adjusted to approximately 5×10^4 cfu/ml using a spectrophotometer at 590 nm and verified by dilution plating. Bacterial suspensions were infiltrated into primary leaves of seedlings when second leaves were 2–4 cm long using a disposable 1-ml syringe as described previously (17). Three primary leaves of each cultivar were inoculated for each replication.

Inoculations were done over 10 mo from August 1992 until May 1993. Each strain was tested in at least two inoculations. Each inoculation included two replications of the 19 cultivars, five test strains, and strain 88-14^{Rif}, described previously (16), as a control. Reactions of strain 88-14^{Rif} were used to verify that conditions were conducive for disease development. No attempt was made to standardize the test strains to strain 88-14^{Rif}. Inoculated plants were incubated in a growth chamber at 25 C and a 12-hr photoperiod. Disease reactions in the inoculation sites (average of three inoculation sites per replication) were recorded after 8 days using a 0–6 scale (0 = no symptoms, 1 = chlorosis but no water-soaking, 2 = less than 10% water-soaking, 3 = water-soaking 10–30%, 4 = water-soaking 31–70%, 5 = water-soaking 71–100%, and 6 = water-soaking extending beyond the inoculated area) described previously (17). 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 0–6, respectively) and then transformed using the arcsine square root transformation.

Transformed data for all strains pathogenic on wheat were analyzed using a completely randomized two factor fac-

torial design to determine whether there was a significant cultivar × strain interaction that would be an indication of races. Because there was an obvious difference in virulence among the pathogenic strains, the means for the strains were compared using a protected least significant difference procedure (LSD) to separate the pathogenic strains into distinct virulent and weakly virulent groups, and data for strains in each group were analyzed separately. Box plots (18) were used to visually compare the distribution of percent water-soaking for the virulent strains on each cultivar. Box plots were generated using the boxplot macro program (19).

RESULTS AND DISCUSSION

All 105 strains were identified as *X. c. translucens* with similarity indices ranging from 0.43 to 0.90 according to the MIDI library (*data not shown*). Twenty-four strains, including the neopathotype strain for *X. c. translucens,* PDDCC 5752 (7), were clearly nonpathogenic on wheat and therefore not used in this study. Of the 81 pathogenic strains, 12 weakly virulent strains had mean water-soaking values (range 1.1–4.5%) that were significantly less ($P = 0.05$) than the other 69 virulent strains (range 14.4–38.1%). There was no statistically significant overlap between the weakly virulent and virulent groups.

Virulent strains included 51 strains from wheat, 10 from triticale, four from rye, and four from barley (Table 1). Of the weakly virulent strains, six were isolated from rye, five from wheat, and one from triticale. Boosalis (3) also noted that strains from rye were less virulent on wheat than on rye. Strains from wheat that were weakly virulent may have lost virulence during culturing and storage, as was reported for *X. c. glycines* (12). There was no relationship between virulence and similarity index.

The cultivar × strain interaction was highly nonsignificant when data from the 69 virulent strains were analyzed (Table 2). Thus, there was no evidence for races

Table 1. Geographic origin, original host, and virulence as measured by the mean percent water-soaking of *Xanthomonas campestris* pv. *translucens* strains on primary leaves of 19 wheat cultivars after inoculation with 5×10^4 cfu/ml and incubation at 25 C

Geographic origin	Strain[a,b]	Host[c]	Water-soaking (%) Mean	Std. err.
United States				
Alabama	Xt-113[2]	T	32.3	5.8
	Xt-114[2]	T	28.6	5.1
Arkansas	88-7	W	20.0	5.9
	88-11	W	23.0	5.1
	88-14[Rif]	W	26.8	5.4
	88-15	W	24.7	6.3
	89-26	W	18.5	4.8
	90-4	W	29.4	6.9
	90-6	W	23.6	6.3
	90-20	W	36.4	5.8
	90-21	W	30.5	5.8
	90-22	W	30.8	4.9
	90-23	W	20.3	4.3
	90-24	W	14.4	3.5
	90-25	W	19.0	5.5
	90-26	W	24.8	6.4
	90-27	W	16.6	3.5
	90-28	W	18.7	4.8
	90-29	W	18.0	4.6
	90-30	W	31.9	5.6
	90-31	W	32.0	5.7
	90-32	W	33.3	6.2
	90-33	W	35.8	5.3
	91-1	W	19.3	4.8
	91-2	W	20.9	4.9
	91-3	W	19.7	4.8
	92-1	W	30.4	5.7
	92-2	W	30.0	6.1
	92-3	W	32.6	5.9
	92-4	W	30.4	5.9
	92-5	W	36.2	6.3
	NK-1[4]	W	24.6	4.8
Florida	Xt-128[2]	T	21.9	5.4
	Xt-129[2]	T	34.1	5.3
	Xt-130[2]	R	30.5	6.2
	Xt-132[2]	B	34.8	6.0
Georgia	Xt-102[2*]	R	2.0	0.8
	Xt-109[2*]	R	2.0	0.8
	Xt-110[2]	T	27.1	7.3
	Xt-112[2*]	R	2.1	0.9
	Xt-122[2]	T	20.1	3.9
	Xt-126[2*]	R	2.2	1.6
	Xt-131[2]	W	30.7	5.3
Idaho	215A[3]	W	25.6	5.3
	222[3]	W	26.8	4.9
	248A[3]	W	20.9	3.9
Louisiana	Xct-030[1]	W	18.8	5.7
	Xct-034[1]	W	18.8	5.2
	Xct-037[1*]	W	3.4	1.3
	Xct-042[1]	W	18.7	5.2
	Xct-044[1]	W	20.2	5.3
	Xct-90-01[1]	W	33.3	4.1
	Xct-90-06[1]	W	20.9	5.7
Montana	Xt-121[2]	B	33.4	6.1
South Dakota	549[7*]	W	4.0	1.7
Brazil				
Parana	6712[8*]	W	3.2	1.4
	8685[8]	B	26.4	4.3
	7531[8]	R	19.6	3.4
	9217[8]	W	18.3	4.7
	9249[8]	W	19.8	4.3
	8687[8]	W	35.4	5.8
	7392[8]	R	28.8	5.2
	6667[8*]	W	2.4	0.8
	9242[8]	W	19.6	3.7
	8689[8]	W	19.4	3.9
	7385[8*]	W	1.6	0.6
	9231[8]	W	31.2	5.7
Canada				
	PDDCC5749[*]	R	3.6	1.0
	PDDCC5755	W	38.1	6.5
Mexico				
Mexico	CB75[6]	W	25.5	5.0
	CB2[6]	T	26.2	4.3
	CB64[6]	T	25.7	5.2
	CB182[6]	W	14.8	3.5
	CB74[6*]	R	1.1	0.3
	X-106[5]	T	20.5	4.1
Michoacan	CB69[6]	W	26.2	4.7
Sonora	CB118[6]	R	35.9	6.1
	CB4[6]	T	27.7	4.0
	X-56[5*]	T	4.5	1.9
Paraguay	224[3]	W	19.8	4.4
Unknown	X-26[5]	B	18.3	4.2

[a] Numerical superscript identifies the source of strains: [1] Christopher A. Clark and Wendy Kursell, Louisiana State University; [2] Barry M. Cunfer, University of Georgia; [3] Robert L. Forster, University of Idaho; [4] Luis Lazo-Anaya, Northrup-King Seed Co., Inc.; [5] David C. Sands, Montana State University; [6] Etienne Duveiller, CIMMYT; [7] Jack D. Otta, South Dakota State University; and [8] Y. R. Mehta, IAPAR. Strains without numerical superscripts were collected by the senior author.

[b] * = Weakly virulent strains; other strains were virulent.

[c] B = barley (*Hordeum vulgare* L.); R = rye (*Secale cereale* L.); T = triticale (× *Triticosecale* Wittmack); and W = bread wheat (*Triticum aestivum* L.).

among the strains that best represent strains capable of causing bacterial streak on wheat in the field. Furthermore, the mean square for cultivar (Table 2) was much greater than the mean square for strain, indicating that there was considerable variation for resistance among cultivars but little variation for virulence among strains.

When inoculated with each of the 69 virulent strains, cultivars Coker 983, FFR 525W, Wakefield, Coker 9835, and Florida 302 had mean water-soaking greater than 40% (Fig. 1) and were considered susceptible. Other cultivars had mean water-soaking less than 25% and were considered resistant. Although the ranges of some resistant cultivars overlapped with the ranges of some susceptible cultivars, disease reactions within individual inoculations usually differed consistently between resistant and susceptible cultivars. Whenever there was an indication that a strain may have an atypical disease reaction on any cultivar, the strain was retested in another inoculation in an effort to document differences large enough to be considered evidence for races.

Although the 12 weakly virulent strains produced a positive reaction in the pathogenicity test done under near-optimum conditions for disease development (high inoculum level on water-soaked and wounded wheat leaves of a susceptible cultivar at approximately 100% relative humidity), they caused little disease on any cultivar under the conditions used in this study. Susceptible cultivars had the highest percentage of water-soaking, but the mean percent ranged only from 5 to 16%. Resistant cultivars had mean water-soaking ranging from 0 to 3%. It is unlikely that any of these strains would be capable of causing much disease on wheat under field conditions.

Resistance to *X. c. translucens* among wheat cultivars appears to be quantitative rather than qualitative. Duveiller et al (6) came to a similar conclusion based on field experiments involving wheat cultivars and segregating populations inoculated with one strain of the pathogen. Cultivars rated as resistant to bacterial streak in the field are not likely to be attacked by new races. Although actual disease severities may vary due to differences in inoculum level or environmental conditions, resistant cultivars should continue to have low disease severities relative to susceptible cultivars.

These results demonstrated that there was no evidence for races among the virulent *X. c. translucens* strains and wheat cultivars used in this study. However, these results do not preclude the identification of races among other strains or with additional host genotypes.

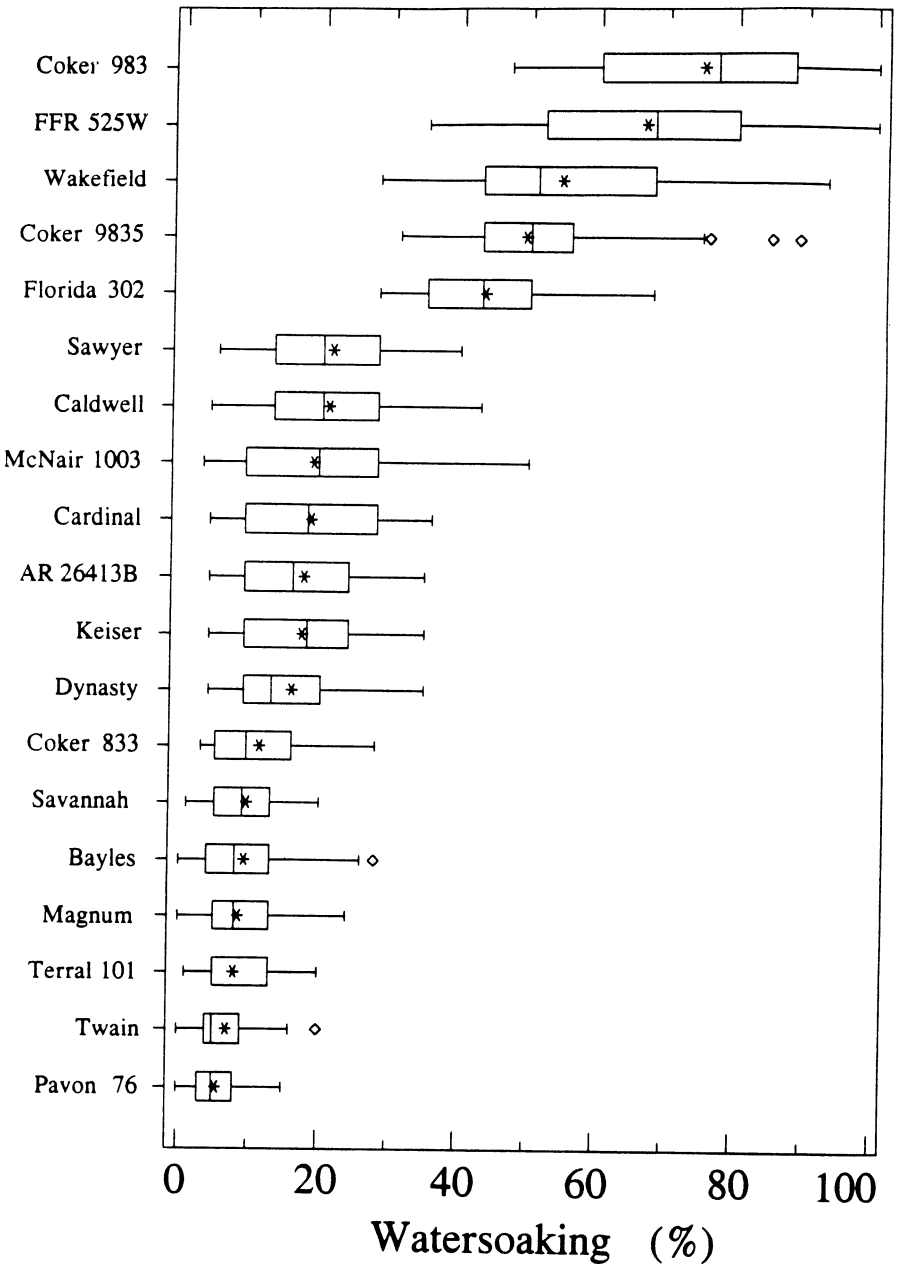

Fig. 1. Percent water-soaking caused by 69 virulent strains of *Xanthomonas campestris* pv. *translucens* on primary leaves of 19 wheat cultivars expressed as box plots showing the mean, median, and distribution of the 69 means for each cultivar. Primary leaves were inoculated with 5×10^4 cfu/ml, and plants were incubated at 25 C and a 12-hr photoperiod for 8 days. The length and position of a box represents the middle 50% of the scores in a distribution, with 25% on either side of the median line inside the box and an asterisk indicating the mean. Lines on either end of a box represent the range of the lower and upper 25% of the scores except for occasional scores that are outliers (points plotted as diamonds).

Table 2. Analysis of variance table for percent water-soaking[a] caused by 69 virulent strains of *Xanthomonas campestris* pv. *translucens* on 19 wheat cultivars

Source	df	Mean square	*F* value	Prob. > *F*
Strain	68	0.28	9.33	≤0.0001
Cultivar	18	9.95	333.64	≤0.0001
S × C	1,224	0.02	0.79	≥0.9999
Error	1,375	0.03	...	...

[a] Data transformed by arcsine square root transformation.

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