Use of Disease Reactions to Identify Resistance in Wheat to Bacterial Streak

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ABSTRACT

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An inoculation technique capable of uniformly infiltrating portions of leaves and rating scales for categorizing disease reactions were used to differentiate levels of resistance in wheat to bacterial streak, caused by *Xanthomonas campestris* pv. *translucens*. An inoculum concentration of 1×10^6 colony-forming units (cfu) per milliliter gave the best differentiation of resistance on flag leaves of adult plants, and concentrations of 1×10^4 to 1×10^5 cfu/ml gave the best differentiation of resistance on primary leaves of seedlings. Seven categories (rating scale 0–6) of disease reaction could be discerned on primary leaves, but only five categories (rating scale 0–4) could be discerned on flag leaves. Disease reactions were positively correlated with pathogen population size in inoculation sites on primary leaves ($r = 0.67$, $P \leq 0.0001$) and flag leaves ($r = 0.82$, $P \leq 0.0001$). For the cultivars tested, disease reactions on primary leaves were similar to disease reactions on flag leaves. Cultivars FFR 525W and Coker 983 had the highest disease reactions; Terral 101 and Twain had the lowest disease reactions; and Florida 302 and Keiser were intermediate. Disease reactions were associated with bacterial streak severities observed in the field. The inoculation technique and disease reaction scales should be useful for evaluating wheat cultivars and breeding lines for resistance, and for assessing pathogen strains for host range, virulence, and aggressiveness.

Bacterial streak, caused by *Xanthomonas campestris* pv. *translucens* (Jones et al) Dye, is an important disease of soft red winter wheat (*Triticum aestivum* L.) in the southeastern United States (6,8,9,11). Water-soaked lesions usually appear on leaves after the heading stage and may coalesce to kill entire leaves. Lesions become dry and necrotic after several days and may be confused with symptoms of leaf blotch caused by *Septoria tritici* (11). The pathogen also causes lesions on peduncles and glumes (black chaff) of some cultivars, but foliar symptoms appear to be the most damaging (6,8,11).

Differences in bacterial streak severity among cultivars under natural conditions in field tests have been recorded (1,8,9). However, the disease is seedborne (17), and ratings made under natural epidemics probably are influenced by levels of seedborne inoculum among the cultivars as well as by the resistance of the cultivars. A method of evaluating bacterial streak resistance under greenhouse or growth chamber conditions would be useful for determining the relative resistance of wheat cultivars and for screening breeding lines. Several methods have been used to inoculate wheat and other small grains with *X. c. translucens* (2,4,6,7). Each technique was reported to be useful for infecting plants or determining host range of *Xanthomonas* strains on small grains. However, these techniques were not quantitative enough to differentiate wheat cultivars for resistance (E. A. Milus, *unpublished*).

The objective of this study was to develop a procedure for differentiating levels of resistance among wheat cultivars to *X. c. translucens* under controlled conditions. A preliminary report has been published (12).

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MATERIALS AND METHODS

Inoculation procedure. A previously described rifampicin-resistant strain (88-14[Rif]) of *X. c. translucens* (13) was used in all experiments. 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 and 100 mg of rifampicin per liter (NDA-R). Inoculum was prepared in sterile deionized water (SDW) using 2-day-old cultures. Concentration was adjusted using a spectrophotometer at 590 nm and verified by dilution plating.

Bacterial suspensions were infiltrated into leaves using a disposable 1-ml syringe with a piece of soft rubber tubing (3 mm inside diameter and 6 mm outside diameter) extending 1 mm beyond the blunt end of the syringe. Inoculations were done in the greenhouse between 1200 and 1500 hr under natural light supplemented with light from 400-watt metal halide lamps on a 14-hr photoperiod to ensure that stomata were fully open. Experiments were conducted on adult plants and seedlings. Circular portions of flag leaves in contact with the rubber tubing (approximately 5 mm in diameter) were uniformly infiltrated. Primary leaves were less than 5 mm in width, and inoculum tended to spread beyond the edge of the tubing. Therefore, the infiltrated area on primary leaves was the width of the leaf by 5–8 mm long.

Adult-plant experiments. Seedlings were vernalized by germinating seeds at room temperature in petri dishes lined with moistened germination paper and then incubating seedlings at 2–5 C in darkness when the coleoptiles were approximately 0.5 cm long. After 8 wk, vernalized seedlings were transplanted to 15-cm pots 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. Plants were fertilized every 2 wk with Peters 20-20-20 (NPK) fertilizer.

The effects of mist treatment after inoculation, cultivar, inoculum concentration, and leaf position on disease reactions were determined in a split-split-split-split plot design in which run (repetition) was the whole plot factor, mist treatment was the split plot factor, cultivar was the split-split plot factor, inoculum concentration was the split-split-split plot factor, and leaf position was the split-split-split-split plot factor. Flag and flag-1 leaves of Florida 302, FFR 525W, Keiser, Terral 101, and Twain were inoculated with 1×10^5, 1×10^6, or 1×10^7 colony-forming units (cfu) per milliliter of strain 88-14[Rif]. Plants were inoculated at boot stage, except for FFR 525W, which was inoculated at early heading stage because of its earlier maturity. Leaves on one main tiller per pot were inoculated with each concentration. Four sites were inoculated on each leaf. The sites were identified by small ink marks on the leaf margin. Two pots (replications) of each cultivar were incubated on a greenhouse bench equipped with a mist system (model A Flora-mist nozzles operating for 12 sec every 12 min from 600 to 1800 hr), and two pots of each cultivar were incubated on an adjacent bench without mist. Greenhouse conditions were as described previously. The experiment was performed twice.

Inoculation sites were rated for disease reaction within the 5-mm-diameter infiltrated areas 7, 9, and 11 days after inoculation on a 0–4 scale (0 = no visible symptoms, 1 = chlorosis but no water-soaking, 2 = water-soaking less than

25%, 3 = water-soaking 25–100%, and 4 = water-soaking extending beyond the infiltrated area). Disease reactions of the four inoculation sites on each leaf were averaged.

In a separate experiment, the effect of cultivar on population size of the pathogen in inoculation sites was determined. Strain 88-14^{Rif} (1×10^6 cfu/ml) was used to inoculate flag leaves of Florida 302, FFR 525W, Keiser, and Terral 101, as described previously. Only four cultivars could be used because of a space constraint in the growth chambers. After inoculation, plants were moved to one of two growth chambers at 25 C and a 12-hr photoperiod. Inoculation sites on one flag leaf from each of two plants per cultivar (one plant from each growth chamber) were sampled at 0.5, 24, 48, 96, and 144 hr after inoculation. Two of the inoculation sites on each leaf were rated for disease reaction, and sections of leaf containing each inoculation site were sliced to allow bacteria to diffuse using a tool holding 11 scalpel blades 1.2 mm apart. The leaf sections with sliced inoculation sites were incubated in tubes of antibiotic-amended medium, and the bacterial population size was determined as described previously (13). Briefly, the test tube assay accurately estimated the population size by determining the time until initial turbidity of the medium. The experiment was repeated once, with one difference: in the second run leaves were sampled at 0.5, 24, 72, 120, and 168 hr after inoculation. Data for each run were analyzed as a split plot in which the whole plot factor was a randomized complete block with four cultivars and two blocks (growth chambers). Sampling time was the split plot factor.

Seedling experiments. Seedlings were grown in six-pack containers (8.9×13.3 cm) filled with potting mixture and kept in a greenhouse as described previously. Each cell of the six-pack was planted to a different cultivar. Seedlings were fertilized once after emergence with Peters 20-20-20 fertilizer and thinned to three uniform plants per cell before inoculation. Primary leaves were inoculated as described previously when second leaves were 2–4 cm long, except that there was only one inoculation site per leaf.

To determine the effect of inoculum concentration on disease reaction, primary leaves of Coker 983, Florida 302, FFR 525W, Keiser, Terral 101, and Twain were inoculated with 1×10^3, 5×10^3, 1×10^4, 5×10^4, 1×10^5, or 5×10^5 cfu/ml of strain 88-14^{Rif} and incubated at 25 C as described previously. Disease reactions within the infiltrated areas were rated 6, 8, and 10 days after inoculation on a 0–6 scale (0 = no visible symptoms, 1 = chlorosis but no water-soaking, 2 = water-soaking less than 10%, 3 = water-soaking 10–30%, 4 = water-soaking 31–70%, 5 = water-soaking 71–100%, and 6 = water-soaking extending beyond the infiltrated area). Disease reactions on the three leaves per cell were averaged. The experiment was performed twice, and each run was a 6×6 factorial involving concentration and cultivar with six replications. Run was considered a random effect.

The population size of strain 88-14^{Rif} in inoculation sites was determined by the tube assay as described previously. Seedlings of Florida 302, FFR 525W, Keiser, Terral 101, and Twain were inoculated with 1×10^4 cfu/ml of strain 88-14^{Rif} and incubated in a growth chamber at 25 C. Six primary leaves per cultivar were sampled at random 0.5, 48, 96, 144, 192, and 240 hr after inoculation to determine the population size of the bacterium. The experiment was done twice. Each run of the experiment was a split plot in which the whole plot structure was a randomized complete block with five cultivars and six replications, and sampling time was the split plot factor. Run was considered a random effect.

RESULTS

Adult-plant experiments. Disease reactions on flag leaves were differentiated best at 9 days after inoculation (*data not shown*) and ranged from 1 to 4 for the cultivars and strain evaluated (Fig. 1A). Cultivar ($P = 0.01$), inoculum concentration ($P \leq 0.0001$), and leaf position ($P \leq 0.0001$) had a significant effect on disease reaction. Run ($P = 0.14$), mist treatment ($P = 0.14$), and interactions among main effects ($P = 0.06$ to 0.99) were not significant. Average disease reactions on flag and flag-1 leaves were 2.6 and 1.8, respectively; and disease reactions on flag leaves better differentiated cultivars than reactions on flag-1 leaves. Although there was not a significant cultivar $\times$ inoculum concentration interaction, a concentration of 10^6 cfu/ml gave the best differentiation of disease reaction among cultivars (Fig. 2). Florida 302 and FFR 525W had the highest disease reactions; Terral 101 and Twain had the lowest; and Keiser was intermediate.

Trends in population size of strain 88-14^{Rif} in flag leaves were similar for both runs, but results of each run were analyzed and presented separately because samples were taken at different times (Fig. 3A and B). Cultivar ($P \leq 0.02$), sampling time ($P \leq 0.0001$), and the interaction between cultivar and sampling time ($P \leq 0.0001$) had a significant

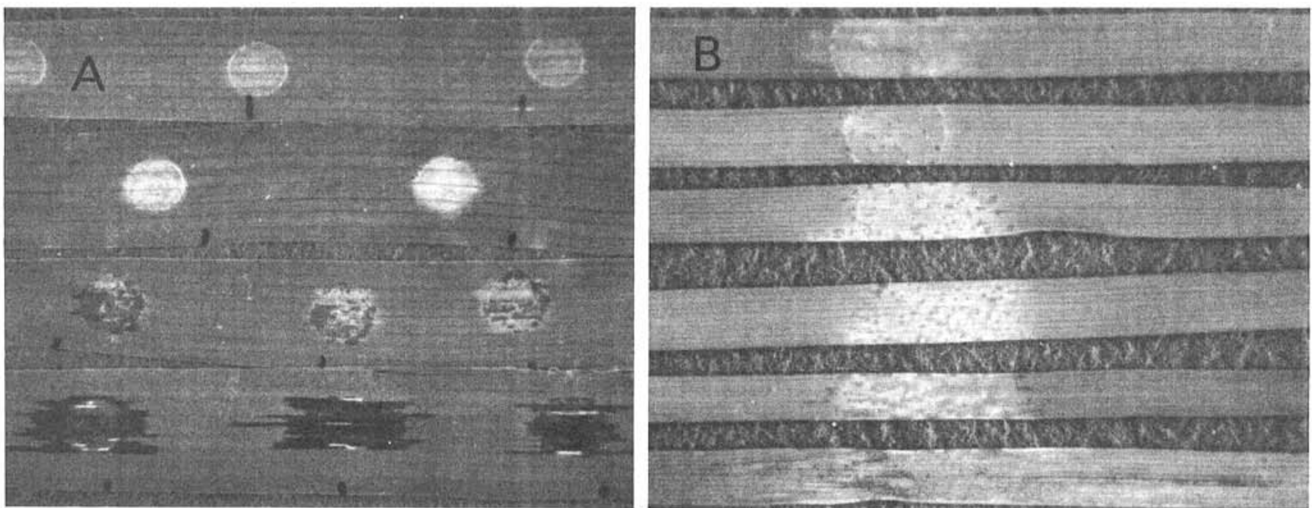

Fig. 1. Disease reactions on wheat cultivars caused by *Xanthomonas campestris* pv. *translucens* **(A)** on flag leaves of adult plants inoculated with 1×10^6 cfu/ml and incubated in a greenhouse at 15–25 C for 9 days (top to bottom: disease reactions 1–4) and **(B)** on primary leaves of seedlings inoculated with 5×10^4 cfu/ml and incubated in a growth chamber at 25 C and 12-hr photoperiod for 8 days (top to bottom: disease reactions 1–6).

effect on population size. Population size in all cultivars at 0.5 hr was approximately 3×10^3 and 1×10^4 for runs 1 and 2, respectively. Population size was lower on Terral 101 than on other cultivars at all sampling times after 24 hr. By 168 hr (run 1) or 144 hr (run 2) after inoculation, population size was greater than or equal to 1×10^8 cfu per inoculation site for Florida 302, FFR 525W, and Keiser, compared to about 10^7 cfu per inoculation site for Terral 101. Disease reactions were positively correlated with population size in inoculation sites over the course of the experiment ($r^2 = 0.83$ and 0.81 [$P \leq 0.0001$] for runs 1 and 2, respectively). Water-soaking was observed in inoculation sites with approximately 10^7 cfu or more per site (*data not shown*).

Seedling experiments. Disease reactions on primary leaves of seedlings were differentiated best at 8 days after inoculation (*data not shown*) and ranged from 1 to 6 for the cultivars and strain evaluated (Fig. 1B). Inoculum concentration, cultivar, and the interaction between inoculum concentration and cultivar had a significant ($P \leq 0.0001$) effect on disease reaction. A broad range of inoculum concentrations produced similar rankings for the cultivars (Fig. 4). However, concentrations from 1×10^4 to 1×10^5 were the most practical for differentiating cultivars. Coker 983 and FFR 525W had the highest disease reactions, and Terral 101 and Twain had the lowest disease reactions at most inoculum concentrations. Florida 302 and Keiser had intermediate disease reactions, but disease reactions generally were higher on Florida 302.

Time of sampling ($P \leq 0.0001$), cultivar ($P = 0.0002$), and the time of sampling × cultivar interaction ($P \leq 0.0001$) had a significant effect on population size. Population size reached a maximum at 192 hr after inoculation (Fig. 5). At 192 hr, FFR 525W had the highest and Terral 101 and Twain had the lowest population sizes. Population size was intermediate in Florida 302 and Keiser, but population size in Florida 302 was higher than in Keiser. Maximum population size in FFR 525W was approximately 1×10^9 per inoculation site, and maximum population sizes in Terral 101 and Twain were less than 1×10^7 per inoculation site. Population size in all cultivars at 0.5 hr was approximately 3×10^2 per inoculation site. Disease reactions were positively correlated with population size in an inoculation site over the course of the experiment ($r^2 = 0.67$, $P \leq 0.0001$). Water-soaking was observed in inoculation sites with approximately 10^7 or more cfu per site (*data not shown*).

DISCUSSION

In preliminary tests, inoculations using scissors (10) or a double sewing needle (3) dipped in inoculum did not result in bacterial streak symptoms on wheat. Inoculations using vacuum infiltration (4), a syringe with needle (6), blunt syringe (14), or syringe with needle through a rubber stopper (7) generally caused bacterial streak symptoms; but these techniques were not useful for differentiating resistance in wheat because precise amounts of inoculum could

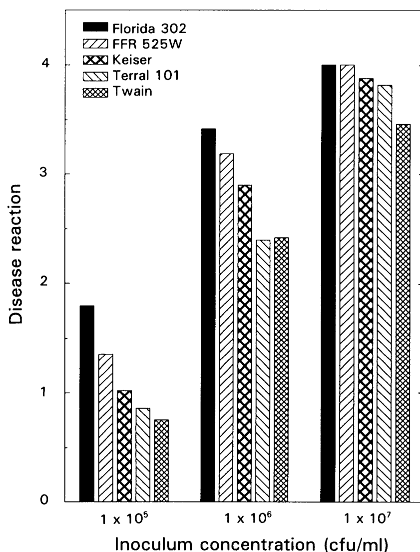

Fig. 2. Disease reactions (0–4 scale) on flag leaves of wheat cultivars 9 days after inoculation with *Xanthomonas campestris* pv. *translucens* at three inoculum concentrations. Inoculated plants were incubated in a greenhouse at 15–25 C. LSD ($P = 0.05$) to compare differences between cultivars within an inoculum concentration was 0.4.

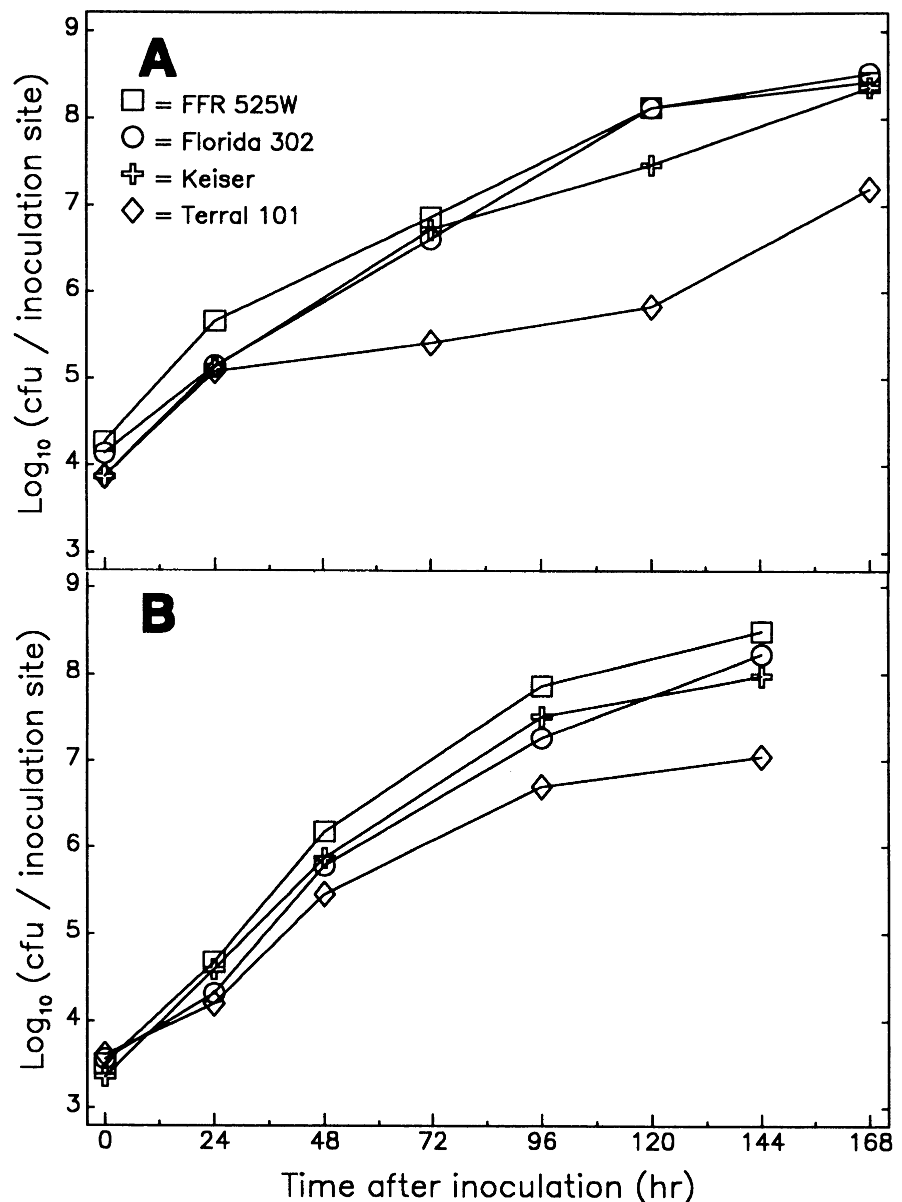

Fig. 3. Population size of *Xanthomonas campestris* pv. *translucens* in inoculation sites on flag leaves of wheat cultivars inoculated with 1×10^6 cfu/ml and incubated in a growth chamber at 25 C. **(A)** run 1, LSDs ($P = 0.05$) to compare differences within a cultivar and among cultivars were 0.5 and 0.6, respectively; and **(B)** run 2, LSDs ($P = 0.05$) to compare differences within a cultivar and among cultivars were both 0.3.

not be infiltrated into confined portions of leaves. Inoculations using an atomizer or high-pressure paint sprayer also usually caused disease, but these techniques were not useful because variability was too great within and among experiments.

In the current study, infiltrating bacterial suspensions into leaves using a syringe tipped with rubber tubing was an effective method for reliably differentiating wheat cultivars for bacterial streak resistance under controlled conditions. This technique was similar to one developed by Shane and Baumer (15) for *Pseudomonas syringae* pv. *syringae* on wheat. Best differentiation of resistance was obtained when inoculations were done when leaves just reached their fully expanded size. Inoculations at earlier or later times may result in increased susceptibility or resistance, respectively (*data not shown*). This phenomenon also was reported for rice inoculated with *Xanthomonas campestris* pv. *oryzae* (10). It was not necessary to provide a moist environment for disease development after inoculation.

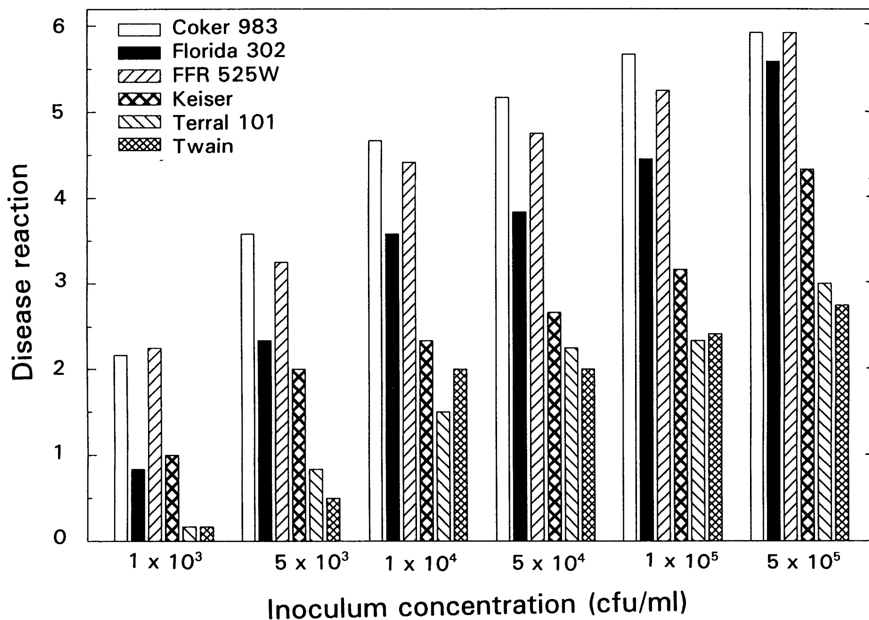

Fig. 4. Disease reactions (0–6 scale) on primary leaves of wheat cultivars 8 days after inoculation with *Xanthomonas campestris* pv. *translucens* at six inoculum concentrations and incubated in a growth chamber at 25 C with a 12-hr photoperiod. LSDs ($P = 0.05$) to compare differences within a cultivar and among cultivars were both 0.6.

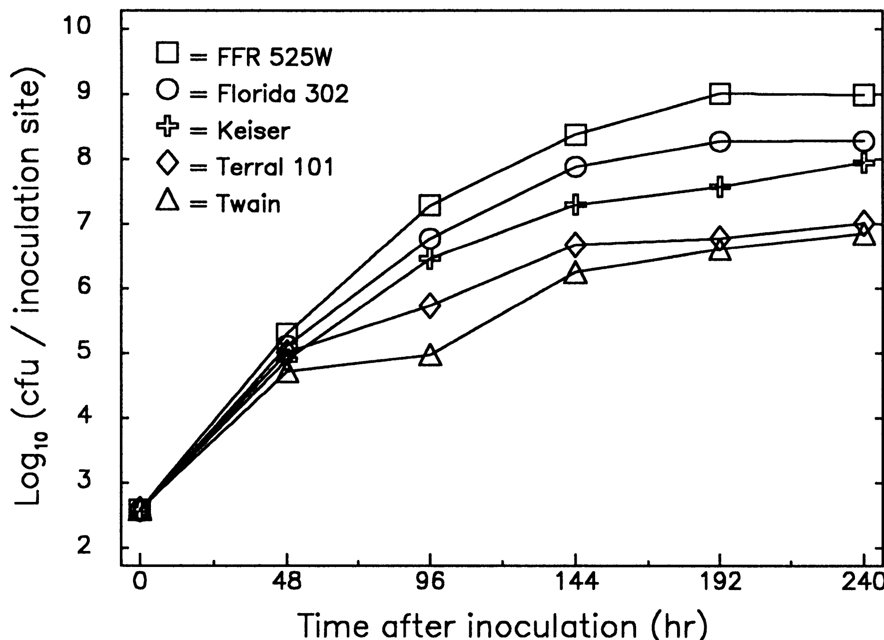

Fig. 5. Population size of *Xanthomonas campestris* pv. *translucens* in inoculation sites on primary leaves of wheat cultivars inoculated with 1×10^4 cfu/ml and incubated in a growth chamber at 25 C with a 12-hr photoperiod. LSDs ($P = 0.05$) to compare differences within a cultivar and among cultivars were 0.6 and 0.4, respectively.

The most practical inoculum concentrations for differentiating levels of resistance were 1×10^4 to 1×10^5 cfu/ml for primary leaves and 1×10^6 cfu/ml for flag leaves. Higher or lower concentrations produced disease reactions that were too high or low, respectively, for differentiating levels of resistance. Because resistances in the cultivars tested could be overcome by high inoculum concentrations, they are different from the hypersensitive, incompatible types of resistance described for *X. c. oryzae* (14).

Rankings of disease reactions on primary leaves of seedlings and flag leaves of adult plants were similar. However, there was a greater number of distinct categories of disease reaction on primary leaves than on flag leaves. Disease reaction 0 generally was observed only with inoculum concentrations lower than the recommended concentrations. Disease reaction 0 on the seedling scale was useful for other research on the host range of *Xanthomonas* strains, even though this category was not critical to the research reported here. Thus, a 0–6 scale and a 0–4 scale were used for rating disease reactions on primary and flag leaves, respectively.

For disease reactions with less than 30% water-soaking, the water-soaking generally was confined to small, pinpoint areas in the inoculation sites. As the percentage of water-soaked area increased, the water-soaked areas coalesced. It was unusual for water-soaking symptoms to spread more than 5 mm beyond the inoculated area on flag leaves (Fig. 1A). However, a water-soaked streak occasionally would extend the length of a primary leaf (Fig. 1B). It appears that systemic spread of *X. c. translucens* in wheat leaves usually is restricted, even in susceptible cultivars. This is in contrast to bacterial blight of rice, where water-soaking commonly spreads systemically within leaves from the point of inoculation (10).

Disease reactions on primary and flag leaves were positively correlated with population size of the pathogen in inoculation sites. The association of low disease reactions with low pathogen populations indicates that the disease reactions developed for bacterial streak are reliable for categorizing resistance. Similar associations between resistance and population size of the pathogen have been reported for *X. c. oryzae* on rice (3,14), *Xanthomonas phaseoli* on beans (5), *Xanthomonas campestris* pv. *vesicatoria* on tomato (16), and *P. s. syringae* on wheat (15).

Rankings of cultivars for disease reaction in both seedling (Fig. 4) and adult-plant (Fig. 2) stages were similar to bacterial streak severities observed in the

field (E. A. Milus, *unpublished*). Therefore, for the cultivars tested, disease reaction at the seedling or adult-plant stage was a good predictor of disease severity in the field. The inoculation technique and disease reaction scales developed in this study should be useful for screening wheat cultivars and breeding lines for bacterial streak resistance, and for evaluating strains of the pathogen for host range, virulence, and aggressiveness.

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